ADDIS ABABA UNIVERSITY
ADDIS ABABA INSTITUTE OF TECHNOLOGY
SCHOOL OF CHEMICAL AND BIO ENGINEERING

Extraction of Essential Oil from Ocimum Lamiifolium Using Steam Distillation

Zelalem Belay

A Thesis Submitted to

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Advisor: Prof. Dr. Ing. Belay Woldeyes Signature ____________ Date ___________

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DECLARATION

I declare that this thesis for the M.Sc. Degree at Addis Ababa University, hereby submitted by me, is my original work and has not previously been submitted for the degree at this or any other university, and that all resources of materials used in this thesis have been duly acknowledged.

Name: Zelalem Belay

Signature: _________

Date of Submission: ___________

This thesis has been submitted for examination with my approval as University Advisor.

Name: Prof. Dr. Ing. Belay Woldeyes

Signature: _________

Date: _________

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List of Acronyms

AAiT = Addis Ababa Institute of Technology

ANOVA = Analysis of Variance

AOAC = Association of official analytical chemists

AOCS = American Oil Chemical Society

AV = Acid Value

DPPH = Di-Phenyl Picryl Hydrazyl

EBI = Ethiopian Bio-Diversity Institute

EO = Essential Oils

FFA = Free Fatty Acid

FT-IR = Fourier Transform Infrared

GC-MS = Gas Chromatography with Mass Spectroscopy

LIDI = Leather Industry Development Institute

N = Normality

O.L = *Ocimum Lamiifolium*

P.S = Particle Size

RT = retention time

SG = Specific Gravity

WHO = World Health Organization

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Abstract

Essential oils are very interesting natural plant products, and among other qualities they possess various biological properties. This study has been carried out to extract the essential oil from the leaves of Ocimum Lamiifolium using steam distillation. Ocimum Lamiifolium is an indigenous plant in Ethiopia and is locally called "Dama-Kesse'' (in Amharic). It is among the commonly occurring Ocimum species that distributed in different regions of Ethiopia.

The average optimum yield of the essential oil in percentage from 100 g of plant materials is 0.4133 ± 0.0208% (v/w) and extracted at a temperature of 85ºC, 4 h extraction time and <1mm particle size of plant materials. The essential oil was analysed by combined Gas Chromatography and Mass Spectroscopy unit, and it was found to been riched in Sesquiterpenes (93.69%) and oxygen containing components. Among the major components from the GC-MS analysis which accounted 69.83% were β-Bisabolene (29.52%), caryophyllene (14.57%), Elemicin (14.29%) and Germacrene D (11.45%).

The main and interaction effects of the process parameters (temperature, extraction time and particle size) and the optimum conditions were examined using design expert 7.0.0 software. In the investigation all the main factors had a significant effect, but no significant interaction effects of the parameters had shown on the yield of the essential oil. The model adequacy also investigated by normal probability plot of residuals and plot of residual verses predicted value. Both plots have shown that the model was acceptable.

Finally, the optimum conditions of the process parameters for the extraction were optimized and the optimal treatments of each factor to maximize the yield were 87.24°C of temperature, 4.85 hours (4 hours and 51 minutes) of extraction time and <1 mm of particle size. The maximum yield that was obtained at these optimum conditions using 100 g of dried leaves of the plant materials was 0.418748 ± 0.0208% (v/w).

Key words: *Composition, essential oil, extraction, GC-MS, Ocimum Lamiifolium, steam distillation,yield.*

1. INTRODUCTION

1.1. Background

Essential oils are very interesting natural plant products and among other qualities they possess various biological properties. The term “biological” comprises all activities that these mixtures of volatile compounds (mainly mono- and sesquiterpenoids, benzenoids, phenylpropanoids, etc.) exert on humans, animals, and other plants.

Essential oils are complex mixtures of volatile compounds produced by living organisms and isolated by physical means only (pressing and distillation and/or extraction) from a whole plant or plant parts of known taxonomic origin (Husnu and Gerhard, 2010).

Essential oils are generally derived from one or more plant parts, such as flowers, leaves, leaves and stems, bark, wood, roots, seeds, fruits, rhizomes or gums or oleoresin exudations. Essential oils are used widely by the pharmaceutical and cosmetic/perfumery industries as well as in aromatherapy and alternative medicines. And also they are used in a wide variety of consumer goods such as detergents, soaps, toilet products, confectionery food products, soft drinks, distilled alcoholic beverages (hard drinks), insecticides, antibacterial, antioxidant, antifungal, antimicrobial and anti-inflammatory activities. Essential oils have distinctive characteristics, which make them a very valuable commodity with many industrial uses and applications. Their aromatic value enables them to be used as flavourings in both the food and beverage industries. These oils are also widely used in both the cosmetic and pharmaceutical industries. With such applications, there is a huge demand for essential oils worldwide and hence they have been traded internationally for several centuries. There is hence a need to improve the quality and quantity of essential oils produced as they have a very competitive and profitable market worldwide (Worwood, 1990). The chemical composition of the essential oils is important in determining their quality and consequently price in the market (Learmonth, et al. 2002). It is therefore important to note and understand the effects of some of the parameters such as temperature, pressure, particle size, steam rate, time of extraction etc., that might be affect the quality and yield of the essential oil extracts. Essential oils can be extracted using a variety of methods, although some are not commonly used today. Currently, the most popular method of extraction is steam extraction, but as technological advances are made, more efficient and economical methods are being developed. These include solvent extraction, supercritical fluid extraction, cold pressing and microwave extraction. The suitability of extraction method varies from plant to plant and there are significant differences in the capital and operational costs associated.

This research seeks to provide import information about extracting essential oils from local and indigenous plant *Ocimum Lamiifolium* and determine the optimum operating conditions of process parameters in small-scale using steam distillation. Essential oils obtained by steam distillation from aromatic plant materials are complex mixtures of compounds of widely differing in composition and boiling points and form very important constituents of cosmetics, perfumes, spices which contribute to fragrance, flavour and preservation of foods. Commercial essential oils are obtained mainly by steam distillation. The methods and equipments employed for the recovery of essential oils in developing countries are often obsolete, producing in poor and inconsistent quality of essential oils resulting in poor returns.

1.2. Statement of problem

A huge range of medicinal plants parts are used as raw traditional drugs and they possess varied medicinal properties. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used. Many other raw drugs are collected in larger quantities and traded in the market as the raw materials for many herbal industries. Nowadays, many medicines that have been widely used come from plant resources. Traditionally, the vast majority of people rely on traditional plant based medicines for primary health care. In Ethiopia there are many medicinal plants which are used simply in raw forms to treat different diseases. Essential oils can be extracted from these traditionally used medicinal plants that are available in different parts of Ethiopia. Among many traditionally used medicinal plants, *O. Lamiifolium* which is one of the well-known indigenous plants in Ethiopia is used as a raw form to treat different diseases in different parts of the country. Why this plant is medicinal is a critical question and opens the door for studies to be done on it. Therefore, extract the essential oil from leaves of *O. Lamiifolium* to be used for the treatment of different diseases rather using it in raw forms, detect the composition of the essential oil extracted and investigate the optimum operating condition for the extraction process were needed.

1.3. Objectives

1.3.1. General Objective

The general objective of this work was to extract essential oils from *Ocimum Lamiifolium* using steam distillation for antimicrobial activity.

1.3.2. Specific objectives

The specific objectives were to:

- Extract the essential oils from leaf of *Ocimum Lamiifolium* using steam distillation.
- Characterize some physic- chemical properties of the essential oil extracted at the optimum conditions of the operating parameters.
- Determine the effect of various operating parameters such as temperature, Extraction time and particle size of the plant materials on the yield of the essential oil.
- Analyze the quantity (yield) and quality (composition) of the essential oil.

1.4. Scope of the Study

This research was an experimental study to show the yield and composition of the essential oil from the leaves of *Ocimum Lamiifolium* as a raw material using steam distillation method. The scope of this study was from extraction and analysis of the chemical composition of the essential oil up to optimize the parameters of the extraction process for the optimum yield of the extracted essential oil.

1.5. Significance of Study

First of all, the purpose of this research paper was to analyze the composition and investigate the optimum conditions of process parameters to extract essential oils from *Ocimum Lamiifolium* to get optimum yield and best quality.

The essential oil obtained from this plant has many applications. However, its increased use has not been accompanied by an increase in the quantity and the quality of the essential oil.

The uses of plants for medicinal activities can be diversified in Ethiopia and other countries in the world. But in different parts of the glob this medicinal plants are used simply in raw forms by cutting the different parts of the plant. For example, in Ethiopia many plant including *O. Lamiifolium* are used simply by squeezing the leaves with water and simply drink to treat different diseases like cough/cold, fever etc.

The essential oil from this plant is very useful because it has extraordinary medicinal properties and that contains several antioxidant compounds. The traditional uses of *O. Lamiifolium* may be attributed to its essential oil content, therefore, isolating the oil from the plant that made it useful in pharmaceuticals and cosmetic/perfumery industries as well as in aromatherapy, alternative medicines. Therefore, this study is important to commercialize and supply high amount of products with the best quality that are used for the treatment of different ailments by determining the optimum conditions of the process parameters.

2. LITERATURE REVIEW

2.1. Review of Essential Oils from *Ocimum* Species

2.1.1. Reviews on Different Species of Ocimum Extracts

Different researchers worked on different species of the genera *Ocimum* in *Lamiaceae* family for the purpose of different activities of the extracts by different extracting methods. Some of those works are mentioned below.

The Chemical Composition and Antibacterial activity of Essential oil of *Ocimum basilicum* of Northern Ethiopia that isolated by hydro distillation shows that a total of 76.7% of components was identified in its essential oil and the oil showed considerable antibacterial activity against gram positive bacteria (S. aureus) than gram negative (E. coli) bacteria. The major compounds of the essential oil from this plant are copaene (25.5%), p-menth-2-en-1-ol (7.7%), eugenylacetae (4.8%), bornyl acetate (4.0%), himachalene (3.6%), rosifoliol (3.0%) and cubebene (2.5%) (Unnithan et al., 2013).

Essential oils isolated by hydro distillation of the over ground parts of *Ocimum basilicum L.* and *Ocimum minimum L.* in Turkey and examined by GC-MS. A total of 49 and 41 components, respectively, were identified accounting for 88.1% and 74.4% of the oils of *O. basilicum* and *O. minimum*, respectively. As conclude by the researchers, the results were generally different, according to literature findings, as concerns the major compounds and the observed differences may be probably due to different environmental and genetic factors, different chemo-types and the nutritional status of the plants as well as other factors that can influence the oil composition. These results show that *O. basilicum* and *O. minimum* are remarkably variable species. Actually, the high quantities of methyl eugenol and geranyl acetate, respectively, make them a most interesting species from the economic point of view (Ozcan and Chalchat, 2002).

The study on the Essential oil composition of *Ocimum basilicum L., O. gratissimum L. and O. suave L.* in the Republic of Guinea extracting by Steam distillation using a steam cooker and a hot plate. From the result of the study the maximum essential oil yield from leaves of *O basilicum* was

1.8% (v/w) compared with 1.0% (v/w) for *O. gratissimum* and 1.7% (v/w) for *O. Sauve* and concludes that in addition to the essential oil yield, it is important to consider biomass abundance. From this standpoint, *O. gratissimum* is the most important species. Determining the extraction yield of essential oil and comparing the chemical composition of the different species and chemo-types is an important step in promoting the use of essential oils as insecticides (Sekou et al., 2012). The study on composition of three sweet basil (*Ocimum basilicum L.*) cultivars the oil content of the plant (0.38–0.55%) was similar to several literature reports. Suchorska and Osinska studied five forms of sweet basils from Germany, Romains, Hungary and Egypt and reported that the oil content varied from 0.1 to 0.55%. A study by Marotti et al. showed that the content of essential oil in herb of 10 Italian basil cultivars ranged from 0.3 to 0.8%. Galambosi and Szebeni reported oil contents in basil herb from 0.38 to 1.29%, while Seidler-Lozykowska from 0.23 to 1.67%. In a large study on 270 sweet basil accession in Germany, oil content varied from traces to 2.65%. Such variations in the essential oil content of basil across countries might be attributed to the varied agro-climatic conditions of the regions. Due to the high content of linalool, methyl chavicol and methyl cinnamate, the studied cultivars may become applied in food and perfume industries (Wesolowska et al., 2012).

2.1.2. Some of the Studies on *O. Lamiifolium* Extracts

There are many indigenous plants in the world which have high medicinal activities. To mention some of them *A. Corrorima*, *C. Myrrha*, *N. sativa*, *O. Lamiifolium*, and *O. Europaea* are among very well- known plants in serving the people as spice, flavor, fumigants, and traditional medicines. Some of their biological activities have been corroborated by scientific researches and have shown antibacterial, antioxidant, antifungal, antimicrobial and anti-inflammatory activities.

A research which was done in Sheko ethnic group in Southwest Ethiopia, *O. Lamiifolium* is used to treat skin and gastro-intestinal ailments. In this study the plant has been assigned with the highest fidelity level values, a possible indication of their better healing potential. An ethno-medicinal survey that was conducted on the investigation of knowledge and practice of traditional anti-malarial plants, the leaf part of *O. Lamiifolium* has been traditionally used for anti-malarial activity. The thermal expulsion and direct burning of the leaves part revealed significant repellency against the main vectors of malaria. The Ethno-botanical uses of this plant were also corroborated by a scientific laboratory based research (Getasetegn and Tefera, 2016).

As the study about the use of medicinal plants in self-care in rural central Ethiopia revealed that *O. Lamiifolium* is among the most frequently used plants. In vitro research was done by agar disc diffusion method on the aqueous, ethanol and methanol extracts of *O. Lamiifolium*. The result revealed that the three extracts have antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Shigellaboydii*. Evaluation research was done on the antipyretic effects of aqueous and ethanol extracts in mice and the result revealed that the extracts have antipyretic property. These extracts were also investigated on anti-inflammatory activities. Both have significant activity but greater anti-inflammatory activity was observed for the aqueous extract. Aqueous extracts of the plant allowed animals to recover barbiturate sleep duration in proportions of 88%. The dried methanolic extract was also tested in vitro for its protection activity against acetaminophen-induced hepatotoxicity. The result of this study revealed that the plant has activity (Getasetegn and Tefera, 2016).

O. Lamiifolium extracts are also known to have insecticidal and insect repellent and anti-inflammatory activities (Kashyap et al., 2011). The in vitro antimicrobial activity of the oil was studied against 24 medically important pathogens including Gram-positive and Gram-negative bacteria as well as some fungal strains using standard disc diffusion technique. The essential oil was shown to possess a broad-spectrum bactericidal activity. Among the bacterial strains tested, Gram-negative bacteria such as *Escherichia coli* were more potently inhibited than the Gram-positive ones. The essential oil also exhibited an excellent fungicidal activity on fungal strains including *Candida albicans* and *Aspergillus Niger*. In addition, the oil was demonstrated to have a good free-radical scavenging potential in 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay (Kashyap et al., 2011).

A study on the larvicidal properties of the essential oils that were extracted from leaves or seeds of the test plants by hydro-steam distillation of some aromatic plants against larvae of *A. arabiensis* and *A. aegypti* in the laboratory and anopheles in simulated field condition stated that *O. Lamiifolium* showed highest larvicidal activity and conclude that it is the most toxic of all was O. *Lamiifolium* essential oil (Gelila, 2015).

The results of the study by Behailu Etana showed that some medicinal plants are popular than others, in this study the highest informant consensus goes to *O. Lamiifolium* which is cited by 89 informants. The popularity of this medicinal plant is due to the preference of the species for treating fibril illness in the community rather than going to modern medication for the disease and its easy access in the home gardens of many people (Behailu , 2010).

Essential oils were extracted from leaves of O. *Lamiifolium* by hydro-distillation method using a Clevenger-type apparatus for 3 hours (Guenther, 1960). *C. Ambrosioides* was also extracted by ethanol in Soxhlet apparatus. The production and quantification of essential oils contents of the test plants ranged from 0.44 to 2.8% (w/w) based on dry weight ratios. The highest oil yield was harvested from the seeds of *S. molle* (2.8%), which was followed by *P. nigrum* (1.8%), *C. ambrosioides* (1.6%) and *S. molle* (leaves) (1.6%). The lowest oil yield was recorded from distillation of *O. Lamiifolium* (0.44%). In simulated field conditions, essential oils of *C. ambrosioides*, *O. Lamiifolium*, *S. molle* (leaves), *O. suave* (fresh) and *P. nigrum* were evaluated against field population of anopheles' larvae in Sille, South of Arba-Minch. Essential oil of *O. Lamiifolium* showed the highest larvicidal activity against the field population of anopheles' mosquitoes (Fekadu, 2006).

Lino and Deogracious also reported difference in antibacterial activity when two extraction methods were used. The present study shows that the maximum effect was exhibited by *O. Lamiifolium*. These results are in parallel with the previous report on *Ocimum* species. However, in the present study the maximum effect of tested plant extracts was showed on *E. coli* followed by *S. aureus* and *P. aeruginosa*. This result is in contradiction with the report by Pankaj and Puroshottam (2011), revealed that *S. Aureus* (a Gram-positive bacterium) was observed as most susceptible bacterium as it was inhibited by extracts of *Ocimum s*pecies. *Streptococcus* pyogenes and *Salmonella typhi* was found to be resistant to all the extracts tested.

The results of the study on the traditional usage of *O. Lamiifolium* and *Amaranthusdubius* plants extracts which possess compounds with antibacterial properties that can be used as antibacterial agents in new drugs for the therapy of infectious diseases caused by pathogens and further work may be carried out for pharmacological evaluation. The development of drug resistance in recent years that use medicinal plants to evaluate plants possessing antibacterial activity for various diseases is growing. The present investigation revealed that the extract of *O. Lamiifolium* and *Amaranthusdubius* leaves have potent antimicrobial activity which explains its use in traditional system of medicines. Hence, *O. Lamiifolium* and *Amaranthusdubius* can be employed as a source of natural antimicrobials that can serve as an alternative to conventional medicines (Amare et al., 2013).

In light of the results obtained from the above works, it could be concluded that some of the traditional uses of *O. Lamiifolium* may be attributed to its essential oil content. Researchers have been done worldwide on the use of medicinal plants to cause human diseases. And extracts of many plant species have been found to be active against many pathogenic bacteria. Aqueous extract of some plants have been reported from time to time to demonstrate antimicrobial activity. The efficacy of various species of medicinal plants against a variety of pathogens has been reported by a number of workers. Essential oils have increasing importance in flavour and fragrance industries. They are obtained by distillation techniques. But in order to produce oil with market potential, its optimum production parameters have to be well known prior to its commercial production. Determination of the steam distillation parameters of commercially available Laurel leaves oil in pilot plant scale is described. The effect of steam rate and processing time play a major role in distillation of essential oils. Distillation speed was high in the beginning of the process, and then gradually reduced as the distillation proceeded. Although, distillation is a well-known and simple process, data on optimum conditions such as time of distillation, steam rate, temperature, particle size of the plant materials etc. need to be worked out on individual raw materials, since every plant material is unique by it-self and the conditions cannot be generalized. So, the influence of distillation parameters on essential oil extraction for the specified plant materials should be studied (Satish, 2010).

From above none of the studies had mentioned the optimum condition for the extraction of the essential oil and the composition of the product from the leaves of *O. Lamiifolium*. Therefore, the gap to be filled by this work was to investigate the optimum conditions of the parameters that considered in this study (temperature, extraction time and particle size of the plant materials) to extract the optimum yield and best quality of essential oils from dried leaves of *O. Lamiifolium* as well as to know the composition of the essential oil extracted using steam distillation extraction method by means of experimental study.

2.2. Essential oils

An essential oil is a concentrated, hydrophobic liquid containing volatile aroma compounds from plants. Essential oils are also known as volatile, ethereal oils or aetherolea, or simply as the oil of the plant from which they were extracted, such as oil of clove. Oil is "essential" in the sense that it carries a distinctive scent, or essence of the plant. The essence or aromas of plants are due to volatile or essential oils. Many of which have been valued since antiquity for their characteristic odors. The essential oils have characteristic fragrances and tastes. They are mixtures of known and unknown compounds. They may contain hydrocarbons, terpene alcohols, aldehydes, ketones, phenols and esters (Denston, 1939).

Essential oils are frequently referred to as the “life force” of plants which are extracted from flowers, leaves, stems, roots, seeds, bark, and fruit rinds that are widely used in foods, cosmetics, and pharmaceuticals. An estimated 3000 essential oils are known, of which about 300 commercially important are destined chiefly for the flavors and fragrances market (Bizuneh, 2014). Generally, essential oils are complex mixtures of hydrocarbon monoterpenes, oxygenated monoterpenes, hydrocarbon sesquiterpenes, oxygenated sesquiterpenes, and related compounds that are derived from the secondary metabolism of plants. The oils are formed in green (chlorophyll bearing) parts of the plant and, with plant maturity are transported to other tissues particularly the flowering shots (Galadima et al., 2012). The amount of essential oils found in these plants can be anywhere from 0.01% to 10% of the total. 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, medicine, and cosmetics. Only pure oils contain a full spectrum of compounds that cheap imitations simply cannot duplicate (Satish, 2010).

2.2.1. Essential oils in tropical countries

Developing countries are endowed with vast resources of aromatic plants which have been used by their people for centuries as food, health care products, flavour and fragrances. Apart from the traditional ways of using these plants, many are exported to industrialized countries as raw materials for drugs, fragrances and flavour. The value-added products are then imported back costing the countries several times more than the original revenue gained from exporting the raw materials. The actual value of these resources has not been retained in many countries for want of know-how and trained manpower.

Today the promotion and development of plant based products have gained momentum due to certain ground realities:

- Green consumerism and the current resurgence on the use of "Naturals" in developed countries which has given a fresh impetus to the development of plant based products.
- Free market economy bringing in more openness and expanding markets and demand for new resources, materials and products.
- A growing acceptability of the social responsibility of minimizing socio-economic inequalities in favour of rural people resulting in opening up of additional job and income opportunities for the poor people.
- Poor economic conditions in developing countries restricting imports thereby placing increasing reliance on substitutes using indigenous plant resources.
- Increasing awareness regarding biodiversity conservation and therefore sustainable and protective use of plant resources.

Even those countries which are producing essential oils have many constraints to be competitive in the world market.

Some of the problems associated with the essential oils industry in developing countries are:

- Poor propagation, agricultural practices and raw materials due to indiscriminate harvesting and poor post-harvest treatment.
- Lack of research on development of high yielding varieties, domestication etc. and inefficient processing techniques leading to low yields and poor quality products.

- Lack of quality control of raw materials and finished products, high energy losses during processing, insufficient Research & Development on product and process development and difficulties in marketing.
- Lack of downstream processing facilities, trained personnel, equipment and up-to-date technologies and non-availability of locally fabricated equipment
- Lack of commitment and support from governments and lack of financial resources, loans, credit facilities

Synthetic substitutes have been produced from petrochemicals to compete with plant products such as resins, aroma chemicals, phyto-chemicals, rubber, leather etc. In the case of essential oils and flavors, real substitutes having all aroma characteristics of the natural products have not been fully accomplished. Nevertheless, the low cost and certain improvements on the natural, have given the synthetic chemicals a major share of the aroma chemicals market. Back to nature movement which has revived the interest on natural products will be beneficial for the developing countries which are endowed with vast resources of aromatic plants.

The entry into world markets depends not only on the demand but on the competitive price of production, quality and the ability to provide the quantities required by the purchaser. Of course the cost of production of the various products will vary according to the raw materials, facilities for processing and labor and energy costs. The bottleneck in many developing countries has been the lack of sufficient raw materials to supply the ordered quantities. The expansion of the resource base is therefore essential which may in certain instances mean large scale cultivations (Tuley, 1995). The world production and consumption of essential oils and perfumes are increasing very fast. Production technology is an essential element to improve the overall yield and quality of essential oil. The traditional technologies pertaining to essential oil processing are of great significance and are still being used in many parts of the globe.

2.2.2. Chemical Constituents of Essential Oils

An essential oil contains more than 200 chemical components, but some are many times more complex. Essential oils consist of chemical compounds which have hydrogen, carbon and oxygen as their building blocks. They can be essentially classified into two groups:

Volatile fraction: Essential oils constituting of 90–95% of the oil in weight, containing the monoterpenes and sesquiterpenes hydrocarbons, as well as their oxygenated derivatives along with aliphatic aldehydes, alcohols, and esters.

Nonvolatile residue: This comprises 1–10% of the oil, containing hydrocarbons, fatty acids, sterols, carotenoids, waxes, and flavonoids. However, the properties of these components can change. For example, the components from the oils extracted from plants can change according to how, when and where these plants are grown and harvested. The constituents can be again subdivided into two groups, such as the hydrocarbons which are made up of mostly terpenes and the oxygenated compounds which are mainly alcohols, aldehydes, esters, ketones, phenols and oxides (Satish, 2010).

2.2.3. Uses of Essential Oil

Aromatherapy: Aromatherapy is a form of alternative medicine that uses volatile plant materials, known as essential oils, and other aromatic compounds for the purpose of altering a person's mood, cognitive function or health. Science has discovered that our sense of smell plays a significant role in our overall health. Since ancient times, essential oils have been used in medicine because of their medicinal properties, for example some oils have antiseptic properties. In addition, many have an uplifting effect on the mind.

Importance of Essential Oil in pharmaceutics: Essential Oils have versatile applications in pharmaceutics. Some of the applications are listed below.

Antiseptics: The antiseptic properties of Essential Oil make them active against wide range of bacteria as on antibiotic resistant strains. In addition to this they are also against fungi and yeasts.

Expectorants and diuretics: When used externally, essential oils like (L'essence de terebenthine) increase microcirculation and provide a slight local anesthetic action. Till now, essential oils are used in a number of ointments, cream and gels, whereby they are known to be very effective in relieving sprains and other particular pains. On the renal system, these are known to increase vasodilation and in consequence bring about a diuretic effect**.**

Spasmolytic and sedative: Essential oils from the *Umbelliferae* family, *Mentha* species and verbena are reputed to decrease or eliminate gastrointestinal spasms. These essential oils increase secretion of gastric juices. In other cases, they are known to be effective against insomnia (Satish, 2010).

2.3. *Ocimum Lamiifolium* (*O. Lamiifolium*)

A wide variety of indigenous and minor crops have been utilized for daily consumption since ancient times. The family *Lamiaceae* is one of the largest families, which comprises the larger proportion of medicinal plant species. *Ocimum* is one of the important genera of family *Lamiaceae* which is often referred to as the "king of the herb. The genus *Ocimum* comprises more than 150 species and it is considered as one of the largest genera of the *Lamiaceae* family. It is widely distributed in tropical and warm temperate regions of the world specially, in tropical America, Africa and Asia. Unlike several other economic *Lamiaceae, Ocimum* requires warmth for growth and should be protected from frost. The typical characteristics of this family are a square stem, opposite and decussate leaves with many gland dots. The flowers are strongly zygomorphic with two distinct lips. Many of the family, particularly sub-family *Nepetoideae,* to which *Ocimum* belongs, are strongly aromatic due to essential oils which consist of monoterpenes, sesquiterpenes and phenylpropanoids. It is a versatile aromatic genus well known for medicinal properties and for economically important essential oils.

The genus is very variable and possesses wide range of intra and inter-specific genetic diversity. It is cultivated for its extraordinary essential oil which display many therapeutic usages such as in medicinal application, herbs, culinary, perfume for herbal toiletries, aromatherapy treatment and as flavoring agent. It has wide range of therapeutic effect like antimicrobial, antispasmodic, bactericide, carminative, anthelmintic, hepatoprotective, antiviral, larvicidal, remedy of coughs, colds, measles, abdominal pains, diarrhea, insect repellent, particularly against mosquitoes and storage pest control (Wano, 2006). Generally, leaves of *Ocimum* species contain essential oil from 0.5 to 1.4%. In addition to essential oil the herb also contains 2, 5-dimethoxybenzoic acid (ocimol) and gratissimin (α-truxillic acid dimethylester). Thermal expulsion of *Ocimum* species showed significant repellency against the main vectors of malaria in Africa (Seyoum et al., 2003). Some of the *Ocimum* species that exist in Ethiopia are *O. Basilicum, O. Lamiifolium, O. Somaliense, O. Circinatum, O. Kilimandscharicumlinn* and etc. *O. Lamiifolium* is an indigenous and one of the most widely used medicinal herbs in Ethiopia, but little seems to be known about the uses of the remaining species of subgenus *Nautochilus* (Gayatri, 2011). Its leaves are squeezed and sniffed to treat coughs and colds. They are also used to treat eye infections and to stop nose bleedings. Studying the use of medicinal plants in self-care in rural central Ethiopia by Gedif and Hahn (2003) reported that out of 25 plant species belonging to 21 families had medicinal values and *O. Lamiifolium* is mentioned among the most frequently used plants.

Figure 2.1: Upper parts of *Ocimum Lamiifolium (Dama-Kesse)* plant

O. Lamiifolium is an erect, robust branching sub shrub or shrub growing about 0.7-3m tall and indumentums of simple hairs. Leaves are ovate and opposite, and flowers are pinkish in racemes. It grows beside roads and streams, in bush land and at forest edged and on grassland between 1200-2900 m. It has petiolate leaves; which is 0.1-0.5 times as long as the blade, up to 40 mm long (Hedberg et al., 2006; Giday et al., 2010). It is a perennial evergreen shrub having oblong, ovate green colored leaves (0.5-5 m), oppositely arranged having pubescent leaf surface, narrow at the base and deeply serrated (Getasetegn and Tefera, 2016). The essential oils obtained from this plant as repellent against nuisance biting insects and malaria vector. However, its use has not been accompanied by less quantity, quality and accessibility of clinical evidence to support traditional medicine practitioner's claims (Getasetegn and Tefera, 2016).

2.3.1. *Ocimum Lamiifolium* in Ethiopia

O. Lamiifolium is an indigenous plant in Ethiopia and locally is called *"Dama-Kesse''* (in Amharic). It is among the commonly occurring *Ocimum* species that distributed in different regions of Ethiopia. It is also cultivated and used as medicine in East Africa from Kenya to Malawi, Democratic Republic Congo, Cameroon and many other countries. It is one of the highly regarded and most widely used medicinal plants in the Ethiopian traditional medicine. Among others, it is used for the treatment of inflammatory conditions and infections. The juice is also used as eye rinse to treat eye infections. At the same time, the crushed leaves are put in the nostrils to stop nose bleeding (Asfaw and Demissew, 2009). Atsebha (2005) investigated and reported the repellency of *O. Lamiifolium* by thermal expulsion and direct burning of the leaves.

Traditionally, the fresh leaves are squeezed and the juice is sniffed to treat cough and cold and drunk to treat diarrhea, amoeba (diarrhea with blood). The juice is also used as eye rinse to treat eye infections and headaches. Its use for pain and fever has already been justified by scientific studies (Makonnen et al., 2003a; Makonnen et al., 2003b). Even if it is possible to deduce the anti-inflammatory activities of this plant based on its action on pain and fever, there are no, however, scientific studies justifying the optimum parameters for the extraction of best quality and maximum yield of its essential oil.

2.3.2. Uses of *O. Lamiifolium*

Ethno botanical Uses: Traditionally, the leaves part of *O. Lamiifolium* is used against eye diseases and headaches. It is also used to relieve pain and fever in a special local preparation in Ethiopia known as "*Yemich medhanit*'' (Hedberg et al., 2006; Giday et al., 2010).

The fresh leaves water extract is drunk to treat diarrhea, amoeba (diarrhea with blood) and cough/cold (Gedif and Hahn, 2003). The plant has synergic activity on these diseases when the water extracts of fresh leaves of *Vernonia amygdalina* and *Clutia abyssinica* mixed with root of *O. Lamiifolium* (Stark et al., 2013). An ethno-medicinal survey that was conducted on the investigation of knowledge and practice of traditional anti-malarial plants, the leaf part of *O. Lamiifolium* has been traditionally used for anti-malarial activity (Karunamoorthi and Tsehaye, 2012). The thermal expulsion and direct burning of the leaves part revealed significant repellency against the main vectors of malaria (Wano, 2006).

Medicinal uses: As the study about the use of medicinal plants in self-care in rural central Ethiopia revealed, *O. Lamiifolium* is among the most frequently used plants (Gedif and Hahn, 2003). The above ethno-botanical uses of this plant were also corroborated by a scientific laboratory based research. *In vitro* research was done by agar disc diffusion method on the aqueous, ethanol and methanol extracts of *O. Lamiifolium*. The result revealed the three extracts have antibacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Shigellaboydii* (Gebrehiwot et al., 2015). Evaluation research was done on the antipyretic effects of aqueous and ethanol extracts in mice and the result revealed the extracts have antipyretic property (Makonnen et al., 2003). These extracts were also investigated on anti-inflammatory activities. Both have significant activity but greater anti-inflammatory activity was observed for the aqueous extract.

Aqueous extracts of the plant allowed animals to recover barbiturate sleep duration in proportions of 88% (Woldesellassie et al., 2011). The dried methanolic extract was also tested *in vitro* for its protection activity against acetaminophen induced hepatotoxicity. The result of this study revealed the plant has activity (Mukazayire et al., 2010). *Ocimum* species and their essential oils have been traditionally used to kill or repel insects, and also to flavor foods and oral products, in fragrance, in folk medicine and as condiments (Padilha et al., 2003). Nerio et al. studied and reported that *Ocimum* species have promising essential oils and used as insect repellents (Nerio et al., 2010). *O. Lamiifolium* extracts are also known to have insecticidal and insect repellent (Dagne, 2009), anti-inflammatory (Kashyap et al., 2011) activities. The *in vitro* antimicrobial activity of the oil was studied against 24 medically important pathogens including Gram-positive and Gram-negative bacteria as well as some fungal strains using standard disc diffusion technique. The essential oil was shown to possess a broad-spectrum bactericidal activity.

2.4. Methods of Extraction

2.4.1. Introduction

The vast majority of essential oils are produced by steam distillation. There are, however, different processes that are used for essential oils extraction. In all of them, water is heated to produce steam, which is used to extract the most volatile aromatic chemicals. The steam is then cooled (in a condenser) and the resulting distillate is collected. The essential oil will normally float on top of the hydrosol (the distilled water component) and may be separated off. The following techniques are used mainly for trapping small amounts of volatiles from aromatic plants in research laboratories and partly for determination of the essential oil content in plant material. The vast majority of essential oils are produced from plant material in which they occur by different kinds of distillation or by cold pressing in the case of the peel oils from citrus fruits. In water- or hydro distillation, the chopped plant material is submerged and in direct contact with boiling water.

In steam distillation, the steam is produced in a boiler separate of the still and blown through a pipe into the bottom of the still, where the plant material rests on a perforated tray or in a basket for quick removal after exhaustive extraction. In addition to the aforementioned distillation at atmospheric pressure, high-pressure steam distillation is most often applied in European and American field stills and the applied increased temperature significantly reduces the time of distillation. Generally, the process of steam distillation is the most widely accepted method for the production of essential oils on a large scale. The length of distillation depends on the plant material to be investigated; however, it is usually fixed to 3-4 h (Hüsnü and Gerhard, 2010). The following are the methods of extraction of essential oil and their drawbacks.

2.4.2. Solvent-Extraction

In the solvent-extraction method of essential oils recovery, an extracting unit is loaded with perforated trays of essential oil plant material and repeatedly washed with the solvent. A hydrocarbon solvent is used for extraction. All the extractable material from the plant is dissolved in the solvent. This includes highly volatile aroma molecules as well as non-aroma waxes and pigments. The extract is distilled to recover the solvent for future use. The waxy mass that remains is known as the concrete. The concentrated concretes are further processed to remove the waxy materials which dilute the pure essential oil. To prepare the absolute from the concrete, the waxy concrete is warmed and stirred with alcohol (ethanol). During the heating and stirring process the concrete breaks up into minute globules. Since the aroma molecules are more soluble in alcohol than the waxes, an efficient separation of the two results. This is not considered the best method for extraction as the solvents can leave a small amount of residue behind which could cause allergies and effect the immune system.

2.4.3. Maceration

Maceration actually creates more of "infused oil" rather than an essential oil. Plant matter is soaked in vegetable oil, heated and strained at which point it can be used for massage. This method is not desirable because it changes the composition of oil.

2.4.4. Cold Pressing

This method is used to extract the essential oils from citrus rinds such as orange, lemon, grapefruit and bergamot. This method involves the simple pressing of the rind at about 120°F to extract the oil. The rinds are separated from the fruit, are ground or chopped and are then pressed. The result is a watery mixture of essential oil and liquid which will separate given time. Little alteration from the oil's original state occurs; these citrus oils retain their bright, fresh, uplifting aromas like that of smelling a wonderfully ripe fruit. The drawback of this method is, oils extracted using this method have a relatively short shelf life.

2.4.5. Effleurage

This is one of the traditional ways of extracting oil from flowers. The process involves layering fat over the flower petals. After the fat has absorbed the essential oils, alcohol is used to separate and extract the oils from the fat. The alcohol is then evaporated and the essential oil is collected (Satish, 2010).

2.4.6. Super Critical CO_2 Extraction

Supercritical CO_2 extraction (SC-CO_2) involves carbon dioxide heated to 87°F and pumped through the plant material at around 8,000 psi, under these conditions; the carbon dioxide is likened to a 'dense fog' or vapor. With release of the pressure in either process, the carbon dioxide escapes in its gaseous form, leaving the essential oil behind. The usual method of extraction is through steam distillation. After extraction, the properties of a good quality essential oil should be as close as possible to the "essence" of the original plant. The key to a 'good' essential oil is through low pressure and low temperature processing. High temperatures, rapid processing and the use of solvents alter the molecular structure, will destroy the therapeutic value and alter the fragrance (Satish, 2010).

2.4.7. Turbo Distillation Extraction

Turbo distillation is suitable for hard-to-extract or coarse plant materials, such as bark, roots, and seeds. In this process, the plants soak in water and steam is circulated through this plant and water mixture. Throughout the entire process, the same water is continually recycled through the plant material. This method allows faster extraction of essential oils from hard-to-extract plant materials.

2.4.8. Steam Distillation

The fundamental nature of steam distillation is that it enables a compound or mixture of compounds to be distilled at a temperature substantially below that of the boiling point(s) of the individual constituent(s). Essential oils contain substances with boiling points up to 200°C or higher temperatures. In the presence of steam or boiling water, however, these substances are volatilized at a temperature close to 100°C, at atmospheric pressure. Steam distillation is a special type of distillation or a separation process for temperature sensitive materials like oils, resins, hydrocarbons, etc. which are insoluble in water and may decompose at their boiling point. The 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. Steam distillation is the most commonly used method for extracting essential oils. Many traditional distillers favour this method for distilling most oils as they claim that none of the newer methods produce better quality oils (Boucard et al., 2005). Steam distillation, as described by Boucard et al., (2005), is done in a still in which fresh or dried plant material is placed in a chamber of the still. Pressurized steam, generated in a separate chamber, is then circulated through the plant material. The heat of the steam forces opens the tiny intercellular pockets in which the essential oils are contained releasing the oils. During steam distillation, the temperature of the steam must be moderated so that it is high enough to open the oil pouches without destroying the plants, fracturing or burning the essential oils as has been recommended in the literature (Sheridan, 2000). Some or most essential oils have been found to be heat sensitive and hence thermos-degradable.

As the tiny droplets of the essential oils are released, they evaporate and mingle with the steam, travelling through a pipe into a condenser. Here essential oil vapors condense with the steam. The steam and oil vapour are then condensed to a liquid mixture. The essential oil forms a film on the surface of the water. As the oil-water mixture has been found to be nearly immiscible at a temperature lower than about 65^oC in previous work done by Sheridan (2000), and the mixture can be separated using various gravity related techniques. Due to the immiscibility of the oil and water at low temperature, the essential oil can be separated from the water by either decanting water or skimming of the oil from the top as the oil is less dense than water at this conditions.

The density of some essential oils such as lavender oil has been reported to average 0.89g/l, as opposed to 1g/l (Ndou, 1986) for water at room temperature and atmospheric pressure conditions. The remaining water, a byproduct of distillation is referred to as 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). In certain situations, floral water may be preferable to be pure essential oil, such as when treating a sensitive individual or a child, or when a more diluted treatment is required. Rose hydrosol, for example, is commonly used for its mild antiseptic and soothing properties, as well as its pleasing floral aroma (Sheridan, 2000).

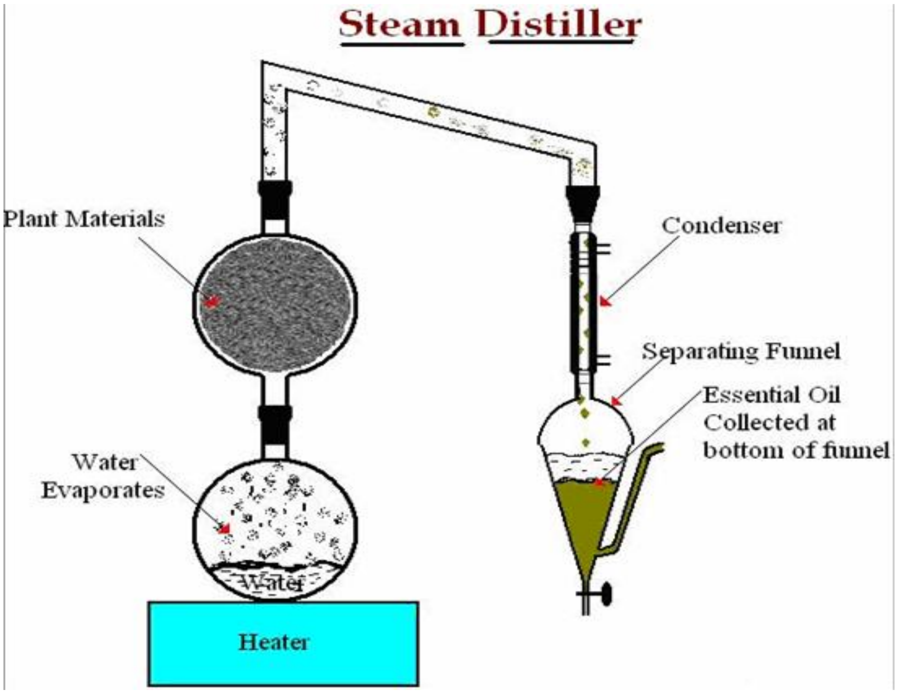

Figure 2.2: Flow sheet for Extraction of Essential oils using Steam Distillation Method

Steam distillation also carried out by passing steam into a one-liter round-bottomed flask containing the dried plant material and of distilled water for different time levels and collecting the condensate (water and oil) in an Erlenmeyer flask. This method of obtaining essential oil is recommended by Simon (1990). The condensate will be saturated with sodium chloride and extract three times with methylene chloride to extract the essential oil completely. Anhydrous sodium sulphate will be added to the methylene chloride in order to remove moisture. Subsequently, the solvent will be removed by rotary evaporation. Extractions will be performed at least three times, and the mean values will be reported. The essential oil content will be calculated based on dry weight of plant materials (Wesolowska, 2012). Referring to the above literature review, it was found that Steam Distillation method is an appropriate and economical method for extraction of essential oil. The steam distillation is a relatively cheap process to operate at a basic level, and the properties of oils produced by this method are not altered. As steam reduces the boiling point of a particular component of the oil, it never decomposes in this method. This method apart from being economical, it is also relatively faster than other methods.

2.4.9. Factors in extracting of essential oils using steam distillation

Essential oils have increasing importance in flavour and fragrance industries. They are obtained by distillation techniques. In order to produce oil with market potential its optimum production parameters have to be well known prior to its commercial production. Essential oils are very complex products. Each is made up of many, sometimes hundreds, of distinct molecules which come together to form the oil's aroma and therapeutic properties. Some of these molecules are fairly delicate structures which can be altered or destroyed by adverse environmental conditions. So, much like a fine meal is more flavorful when made with patience, most oils benefit from a long, slow 'cooking' process (Satish, 2010). A number of factors determine the final quality of a steam distilled essential oil. Apart from the plant material, most important factors are extraction time, steam temperature, operating pressure, the steam flow rate, the quality of the distillation equipment, the particle size and the condition of plant materials.

It is possible that longer distillation times may give more complete oil. It is also possible however, that longer distillation time may lead to the accumulation of more artifacts than normal. This may have a curious effect of appearing to improving the scent, as sometimes when materials that have a larger number of components are sniffed, the perception is often of slightly increased sophistication, added fullness and character, and possibly, and extra pleasantness. The effect of steam rate and processing time play a major role in distillation of essential oils. Distillation speed was high in the beginning of the process, and then gradually reduced as the distillation proceeded. Although, distillation is a well-known and simple process, data on optimum conditions such as time of distillation, steam rate, temperature, particle size of the plant materials etc. need to be worked out on individual raw materials, since every plant material is unique by it-self and the conditions cannot be generalized (Satish, 2010). So, the influence of distillation parameters on essential oil extraction for the specified plant materials should be studied. Therefore, the main aim of this paper is to present the experimental study of parameters to extract optimum yield and quality of essential oils from dried leaves of one of the well-known indigenous plant in Ethiopia such as *O. Lamiifolium* using steam distillation. The main parameters considered for this study are the extraction time, the size of the plant materials and the temperature of the steam.

3. MATERIALS AND METHODS

3.1. Materials, chemicals and equipment

The required materials and equipment that were needed for the extraction of the essential oil in this study were three-way round-bottomed flasks, rotary evaporator, Erlenmeyer flasks, heating mantle, electronic analytical balance, Measuring cylinder, Conical flasks, the plant materials (dried leaves of *O. Lamiifolium*), distilled water, anhydrous sodium sulphate, Methylene chloride and Sodium chloride. All chemicals used in this study were analytical graded and obtained from the chemical suppliers in Addis Ababa.

3.2. Methods

Collection of the plant materials

A total of 12kg of the leaves of the plant material were collected from their growing habitats from different localities in Ethiopia (from home gardens of Addis Ababa and from Amhara region, Bahir Dar area and Gondar area). The plant was named *O. Lamiifolium* scientifically and locally called *Dama-Kesse* in Amharic. Taxonomic identification of this plant was confirmed by professionals at the Ethiopian Biodiversity Institution (EBI) in Addis Ababa.

Treatment and preparation of the plant materials

After collecting the fresh leaves of the plant, a sample was immediately loading on an electronic balance to known the initial mass before drying to investigate the moisture content of the plant materials. Prior to drying the plant materials in a shaded environment and prepared for the extraction of the essential oils, the moisture content of the sample was determined by using oven drying method at 100°C for 1 day. On completion of the drying process, the leaves were re-weighted using the analytical balance to determine any potential mass loss as a result of the evaporation of both moisture and volatile oil components due to the drying effect. The remaining sample was then spread in a shaded environment and left to air dry for 48 hours at room temperature. The dusts and unwanted materials present on the plant material should be remove before drying for the oil extraction.

Size reduction and sieve analysis

After the remaining moisture was removed by placing in an oven at 50°C for 18 hours, the dried leaves of *O. Lamiifolium* was milled in cross beater miller with a size of 1mm in thermal laboratory of AAiT and then the sample was shaken using vibrating shaker for 5 minutes with amplitude of 10mm and 60rpm to get the required particle size of the plant materials. For this specific study the particle size of the plant materials was arranged in sizes of less than 1 mm [0.25-1mm) and greater than or equal to 1mm [1-2.5mm]. This particular size range was selected because literature reveled that to have a higher yield of essential oil particle size should be less than 5mm and higher than 0.2mm (Henry, 1983).

Factors selection and Response variable of the process

The dependent (response) variable for this experiment was the yield of essential oil extracted from the leaves of *O. Lamiifolium*. The yield of the extraction process was dependent on several conditions. Among these many factors, the extraction process parameters were the main one. This is why this paper was mainly focused on the extraction process factors or parameters. There were many factors which affect the extraction of the essential oils from the given plant materials. These are temperature, distillation time, pressure, particle size, method of extraction and so on. Temperature, distillation time and particle size were taken for this specific study. Temperature and extraction time had three levels of each and particle size of the plant material had two levels. Temperature was investigated at levels of 75°C, 85°C and 95°C. The distillation time considered was in levels of two hours, four hours and six hours. The third independent variable which was particle size was investigated in particle size less than 1mm and particle size greater than or equal to 1mm. If the number of independent variable was several for the given experiment then, the conclusion drawn from the analysis would be incorrect. As a result, as much as possible large numbers of factors in the experiment are not advisable that was why in this experiment considered only the factors that would affect the product highly. This does not mean that the rest of the independent variable does not affect the product both quantitatively and qualitatively. It is obvious that there are so many predictors that were affect the response variable but taking the major one is the great task of the experimenter.

3.3. Extraction of essential oil from *O. Lamiifolium* using steam distillation

Experimental Setup

Generally, in steam distillation the plant material was packed into the extraction chamber so that distillation could commence. For each load, plant materials were placed into the extraction chamber. The first load was conducted to set-up and establishes the procedure and determines processing parameters. The experiments of this study was conducted in the laboratory of leather industry development institute (LIDI), Addis Ababa.

The modified experimental setup of the apparatus that was used for the extraction of the essential oil from the given plant materials by means of steam distillation method for this specific study is shown in figure 3.1 below. The experiment was conducted in a Clevenger's type apparatus. Apparatus consist of one round bottom flask of 500ml which is connected with another conical flask which holds raw materials. The top flask is connected with condenser through the connecter. The separating funnel is used for the separation of essential oil and water.

Figure 3.1: Experimental set-up for steam distillation of essential oil

Experimental Procedure

In this research, the essential oils were extracted from leaves of the plant by steam-distillation method using a Clevenger-type apparatus until oil distillation ceased after 2-6 hours. For this specific study, the same mass (100grams) but different particle sized plant materials (leaves) were placed in three similar 500ml conical flasks. For steam generation the same volume (300ml) of distilled water was added in two similar round bottomed flasks and boiled with two separated heating mantles at atmospheric pressure. After the water in round bottomed flasks was boiled, the temperature of the steam was set at the required value and allowed to channel into the 500ml conical flask containing the plant materials. The particle sizes of the plant materials and the temperature of the steam of the two experimental set ups was recorded. The steam ruptures the leaves and expels the oil held within the cells of the leaves and both the steam and the oil were passed through a conical flask and cooled in the condenser and returned to the glass cylinder.

3.4. Determination of the percentage yield of the essential oil extracted

Product Collection

The condensate (steam & oil) was collected in a separating measuring cylinder where the essential oil and water were separated. The water was gently drained off and the oil was collected in a 10ml measuring cylinder and measured. The measurement was taken at an interval of 2 hours for 6 hours and the cumulative volume of the oil was measured and recorded. The essential oils in the distillate were dried over anhydrous sodium sulphate (Na_2SO_4) and stored in refrigerator at 4°C. The same procedure was repeated for the other combination of the process parameters that are considered for this specific study. Three tests were conducted for each of the process parameters at the same levels in order to confidential on the results.

Isolation of the Oil Extracts from the Steam Condensation

After the steam produced by heating the distilled water in the round-bottomed flask with the heating mantle, it was allowed to make contact and pass through the plant materials in the conical flask, the essential oil with the steam flows into the condenser through the connector glass which able to collect and measure the liquids that were returned from the condenser and measure the volume of both the liquids with the scale written on it as measuring cylinder which was connected to the conical flask that holds the plant materials. After the completion of each batch of the extraction process, the yellow liquid essential oil extracts and water condensate (hydrosol) are known to have different densities and also form an immiscible two liquid phase mixture at room temperature conditions. The separation of essential oils from the condensate hence utilizes this density and immiscibility advantages for the two liquids to be isolated from each other. The yellow liquid essential oil with a pungent scent was collected with a separated test tube for each experimental runs and put in a refrigerator until the next analysis.

Percentage Yield Determination

The total oil yield depends on the operating parameters. Designed experiments were carried out to map quantitative effects of these parameters. The yield of extraction is calculated from the relation between the essential oil mass obtained and the raw material mass used in the extraction. The mean yield (%) table is constructed from oil extracted in relation to the amount of sample used in each run. In this work 100grams of the plant materials was used for each batch and yield (%) is calculated using equation C.1 in Appendix C. The extraction parameters were very important to produce both a good quality and a reasonable amount of essential oils. As a result, to achieve these objectives different parameters with different levels were used during the extraction process. The parameters were temperature, distillation time and particle size of the plant materials (leaves of *O. Lamiifolium*).

3.5. Determination of the effect of various operating parameters

In this section the main effects of the individual factors and the interaction effect of the factors considered for this study (temperature, extraction time and particle size) on the yield of the essential oil and the optimum value of the parameters were investigated. The main effects of the parameters, the interaction effects of the parameters and the optimization of process conditions for the maximum yield of the essential oil were performed using the design expert 7.0.0 software. By selecting the general factorial with three categorical factors of three levels of the first and the second factors (temperature and extraction time respectively) and two levels of the third factor which was particle size and replicated three times with a single response of percentage yield of the essential oil. With the above information of the design, a total of 54 experimental runs were expected to be conducted. After the design 54 raw experimental data were filled in the response column and made randomized on the design expert software. The ANOVA, the normal plot of the residuals, residuals vs. predicted and predicted vs. actual plots in the diagnostics part and the plots of the individual factor and their interactions of the model in the analysis part of the design and the numerical solutions of the optimization in a report and ramps form of the results of the study were presented in the results and discussion section.

3.6. Qualitative analysis of the essential oil at the optimum conditions

Qualitative analysis of the essential oil was done using Gas Chromatography with Mass spectrometer to know the composition of the oil and the quantity of each component.

Identification of Components of the essential oil using GC-MS

The GC-MS is composed of two major building blocks; the gas chromatograph and the mass spectrometer. The components of essential oil were identified on the basis of comparison of their retention time and mass spectra with published data (Massda, 1976; Adams, 2001) and computer matching with WILEY 275 and National Institute of Standards and Technology (NIST3.0) libraries provided with computer controlling GC-MS system.

These two components, used together, allow a much finer degree of substance identification than either unit used separately. It is not possible to make an accurate identification of a particular molecule by gas chromatography or mass spectrometry alone. The mass spectrometry process normally requires a very pure sample while gas chromatography using a traditional detector (e.g. Flame Ionization Detector) detects multiple molecules that happen to take the same amount of time to travel through the column (i.e. have the same retention time) which results in two or more molecules to co-elute. Sometimes two different molecules can also have a similar pattern of ionized fragments in a mass spectrometer (mass spectrum). Combining the two processes makes it extremely unlikely that two different molecules will behave in the same way in both a gas chromatograph and a mass spectrometer. Therefore, when an identifying mass spectrum appears at a characteristic retention time in a GC-MS analysis, it typically lends to increased certainty that the analytes of interest is in the sample (Kasumba, 2003). The spectrum of the unknown component was compared with the spectrum of the known components stored in the library. The Name, Molecular weight and Structure of the components of the tested materials were ascertained.

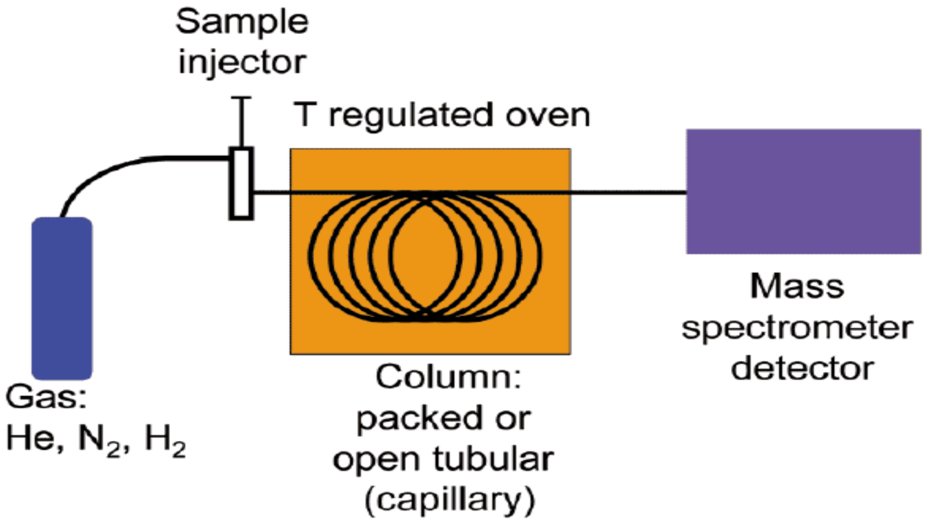

Figure 3.2: Gas Chromatography-Mass spectrometer schematic diagram

The gas chromatograph utilizes a capillary column which depends on the column's dimensions (length, diameter, film thickness) as well as the phase properties (e.g. 5% phenyl polysiloxane). The difference in the chemical properties between different molecules in a mixture will separate the molecules as the sample travels the length of the column. The molecules take different amounts of time (called the retention time) to come out of (elute from) the gas chromatograph, and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass to charge ratio (Hites, 2006). Figure 3.2 above shows a schematic diagram of Gas Chromatography-Mass Spectroscopy unit.

The component identification was achieved by the GC-MS analysis using HP 5890 series Gas Chromatogram equipped with Mass selective detector (MSD), HP 5972 series (German) in Addis Ababa University, institute of natural science, chemistry department, Addis Ababa, Ethiopia. Helium was used as carrier gas at a constant flow of 1ml/min and an injection volume of 1µl was employed. The injector and Ion-source temperature were 250°C and 280°C respectively. The oven temperature was programmed from 50°C (isothermal for 4 minutes), with an increase of 3°C/min, to 280°C and held for 10minute isothermal at 280°C. Total GC running time was 90.67 minutes.

3.7. Characterization of the product

3.7.1. Characterization of the physical properties of the oil

A). Determination of moisture content of the product

The moisture content of the oil is the loss in mass of the sample on heating at 105±1°C under operating conditions specified. Moisture content determination of an essential oil can be determined by Karl-Fischer titration. Drying of an essential oil is accomplished by the addition of or filtering through desiccating agents such as anhydrous sodium sulphate. A simple way to check the presence of moisture in an essential oil is carried out by mixing 0.5ml essential oil with 1ml carbon disulphide (CS_2). A clear solution indicates the absence of moisture. The proximate analyses were done using the method of Association of Official Analytical Chemists. For this specific study the moisture content of the essential oil extract was determined by air-oven drying method after placing the sample in the oven and drying it to constant weight (A.O.A.C, 2000 official method 925.10).

The procedure was; 5 grams of oil was weighted and putted in a dish and then dried in an oven at 105°C for 1 hour. The dish was removed from the oven and cooled in a dissector and weighed. The process was repeated until a constant weight was observed. After three repetitions the mass of the essential oil constantly became 4.89 grams and the moisture and volatile matter of the oil was determined (Singh R., 1984) by weight difference using formula of the moisture content (A.O.A.C, 2000). The value of the moisture content is calculated using equation C.2 in Appendix C.

B). Determination of Specific Gravity

The specific gravity of oil is the weight of a given volume of oil at a specified temperature compared with the weight of an equal volume of water at the same temperature, all weightings being taken in air. A pycnometer is used for this determination. The density of oil was determined using density bottle method. A clean and dry density bottle of 25ml capacity at 30°C was weighed in gram (W_0). Then the bottle was filled with water and reweighed at 30°C (W_1). Melted oil was brought to 30°C and the water was substituted with this oil after drying the density bottle and weighted again (W_2) and the specific gravity was determined (A.O.A.C 920.212, 2000).

The process of determining the value was measure 5ml of oil and became 4.55 grams and the same volume of water was measured and it became 5 grams then the specific gravity of the essential oil equals to density of the substance divided by the density of the reference substance (water). The value of the specific gravity is calculated using equation C.3 in Appendix C.

C). Determination of kinematic viscosity of oil

A kinematic viscosity of oil was measured indirectly using Viscometer model. Initially, a sample was heated at a temperature of 30°C. A sample of 5 ml oil was measured and fed to a sample holder of the Vibro Viscometer. A sensor of the viscometer was immersed the oil and the dynamic Viscosity of oil was displayed on the Vibro Viscometer screen at a temperature of 30°C. Then the Kinematic Viscosity was calculated using equation C.4 in Appendix C.

D). Determination of pH

For p^H determination, 2grams of the sample was taken and placed in a clean dry 25ml beaker and 13ml of hot distilled water was added to the sample in the beaker and stirred slowly. Then it was cooled in a cold water bath to 25°C. The pH electrode was standardized with a buffer solution first and then the electrode immersed in to the sample, the value was read and recorded (A.O.A.C 960.19, 2000).

3.7.2. Characterization of the chemical property of the essential oil

A). Determination of Saponification value

The Saponification value (SV) is expressed as the number of milligram of KOH required to Saponifying 1gm of oil. By principle the oil sample is saponified by refluxing with a known excess of alcoholic potasium hydroxide solution. The alkali required for saponification is determined by titration of excess potasium hydroxide with standard hydrochloric acid. The saponification value is an index of mean molecular weight of the fatty acids of glycerides comprising a fat (A.O.A.C 920.160, 2000). The procedures for preparing the reagents and measuring the saponification value was conducted by 2 grams of the sample was taken and placed in to 250ml capacity conical flask. 25ml of alcoholic potassium hydroxide solution was added in to the flask and connected to reflux condenser and kept on the water bath and boiled gently for 1 hour. After the flask and the condenser were cooled, the inside of the condenser was washed with 10ml of hot ethyl alcohol. Then few drops of phenolphthalein indicator were added and the excess potassium hydroxide was titrated wit 0.5 N hydrochloric acid to the end point, until the pink color of the indicator just disappears. The same procedure was conducted for the blank. The value was calculated using the equation C.5 in Appendix C.

The required solutions were prepared with the required concentration as below.

- **Preparation of 0.5 N alcoholic potassium hydroxide solutions:** to prepare 0.5N of ethanolic KOH solution, 14.027grams of KOH was dissolved in 500ml of ethanol.
- **Preparation of 0.5N hydrochloric acid solutions:** Stock solution of 13.7ml of hydrochloric acid was poured in 500ml distilled water. After that 2grams of oil was dissolved in alcoholic KOH and heated gently for 1 hour. It was titrated with HCL to the end point. Similar titration was done for the blank. In both cases the value of HCL was recorded.

B). Determination of Acid Value

Acid value is a numerical value equivalent to the number of milligrams of potassium hydroxide required to neutralize the free acids present in 1 gram of the oil. The determination was performed as follows. 25ml of Toluene and 25ml of ethanol was mixed in a 250ml beaker. The resulting mixture was added to 2grams of oil in a 250ml conical flask and few drops of phenolphthalein were added to the mixture. The mixture was titrated with 0.1M KOH to the end point with consistent shaking for which a dark pink color was observed and the volume of 0.1M KOH (V_0) was noted. The required solutions were prepared with the required concentration as shown below.

- **Preparation of 80 percent ethanol:** 19.6ml distilled water was added into 80.4ml 99.5 percent absolute ethanol.

- **Preparation of 0.5N sodium hydroxide solutions:** 10.1grams of 99 percent NaOH was dissolved in 500ml distilled water.

At the end of the procedure the acid value and FFA value were calculated using equations C.6 and C.7 in Appendix C respectively.

3.8. Experimental design and Statistical data analysis

3.8.1. Experimental design

As it was stated above in section 3.2 the variable of interest in this research was temperature, distillation time and the particle size of the plant materials. The effects of each variable and their interaction effects on the yield were investigated. To investigate such like effects, and the most efficient and advisable method is factorial design. By the factorial design, in each complete trial or replication of the experiment all possible combination of the levels of the factors is investigated. If the levels of the factors are two we can use 2^k factorial design or if the levels of the factors are three we can use 3^k factorial design so in this experiment the levels of the factors are three as a result, the general factorial designs with three factors is advisable and could be applied. Full factorial design is used to investigate the effect of each factor. In a factorial experiment all the possible combination factor level would be tested and it would be possible to determine the effect of individual factors and to assess the effect of change of two or more variable at a time (Zivoad, 2004). The analysis was performed by utilizing design expert 7.0.0 software using general full factorial design method. This method of experiment design helps to differentiate the significance of the main and the interaction factors. The software also used to develop the mathematical model that will describe the effect of the main and interaction factors on the response. Three factors were selected as mentioned earlier, these are: temperature, extraction time and particle size. For temperature and extraction time, three levels of each were considered. And two levels for particle size. Each independent experiment was repeated three times. Therefore, the numbers of experimental runs that were needed to be performed in this study were fifty-four. The factors, number of levels and the selected values of the factors were shown in table 3.1 below.

Table 3.1: The process parameters/factors and their levels of the experiment

Factors/ Parameters	No. of levels	Levels of the factor
Temperature (°C)	3	75 85 95
Extraction time (hours)	3	2 4 6
Particle size (mm)	2	< 1 ≥1

3.8.2. Model development of the data took from the laboratory experiment

Finally, regression models were established for the dependent variables to fit the experimental data for the response using design expert 7.0.0 software.

3.8.3. The analysis of variance (ANOVA)

Usually, the analysis of variance computations would be done using a statistical software package. However, the manual computing formulas are occasionally useful. The error sum of square may be found by subtracting the sum of squares for each main effect and interaction from the total sum of squares.

4. RESULTS AND DISCUSSION

4.1. The Quantitative Analysis of the Essential Oil Extracted

Since all interaction of the level of factors should be considered in factorial design, there were fifty-four total runs to be performed during the laboratory experiments. The yield (%) was obtained by dividing the liquid essential oil (ml) extracted by the plant materials used for each specific runs (100 grams). Therefore, the percentage yield here is expressed with volume by weight (v/w or ml/g). The volume of essential oils obtained from a given plant material at different operating parameters was different and the mean values were tabulated in table 4.1 below. The essential oil volume was less in comparison to hydrosol of the plant material. The yield (%) obtained from the experiment was calculated by the appropriate equation of percentage yield in Appendix c and tabulated in table A.1 (Appendix A).

Table 4.1: The Mean Values of the Raw Yield (%) of Experimental Results

Time (hours)	**Temperature (°C)**	**Mean ± SD (%)**	
Particle size		**<1mm**	**≥1mm**
	75	**0.3133±0.0252**	**0.2733±0.0252**
2	**85**	**0.3400±0.0200**	**0.3100±0.0300**
	95	**0.3867±0.0252**	**0.2833±0.0351**
	75	**0.3667±0.0351**	**0.2933±0.0306**
4	**85**	**0.4133±0.0208**	**0.3367±0.0351**
	95	**0.3867±0.0252**	**0.3200±0.0458**
	75	**0.3667±0.0351**	**0.2933±0.0306**
6	**85**	**0.4000±0.0100**	**0.3367±0.0252**
	95	**0.3867±0.0252**	**0.3367±0.0513**

From the tabulated data shown above, what observed was that the highest yield in average was observed around temperature level of 85°C, extraction time level of 4 h and particle size level of <1mm particle size. As observed from the raw data of table 4.1 the highest average yield is 0.43% and in average 0.4133 ± 0.0208% and obtained at the temperature level of 85°C, 4h extraction time and at <1mm particle size of plant materials. Whereas the lowest value of the essential oil yield was 0.25% and in average it is 0.2733 ± 0.0252. This value was obtained at the temperature level of 75°C, 2 h extraction time and at >=1mm of particle size. From this result it can be noted that the optimal extraction time and particle size for all temperatures of this study are about 4 h and <1mm particle size respectively. The extractable oil decreases as the temperature is above and/or below 85°C. The quantitative study can be used to give an insight on the effect of the temperature, extraction time and particle size on the extraction process.

4.2. The Qualitative Analysis of the Yield

As indicated in section 3.3 the qualitative (composition) analysis of the essential oil was inspected by GC-MS.

Determination of Major Component of the essential oil using GC-MS

The retention times and chemical composition of the major phyto-components present in *O. Lamiifolium* essential oil were presented in table 4.2 below. There are 19 total components of the essential oil extracted in this study which can be compliance with minimum quality of 90. These are presented in table A.2 (Appendix A).

Table 4.2: The major components and composition (%) of *O. Lamiifolium* essential oil

RT	Name of the Compound	Chemical Formula	Molecular Weight	Composition %
13.851	β-Bisabolene	$C_{15}H_{24}$	204.3511	29.52
12.828	Caryophyllene	$C_{15}H_{24}$	204.3511	14.57

14.371	Elemicin	$C_{12}H_{16}O_3$	208.2536	14.29
13.612	Germacrene D	$C_{15}H_{16}$	204.3511	11.45

A total of nineteen components, with different retention times, were identified from the GC column as indicated by the chromatogram in figure 4.1 and there were further analyzed with an electron impact MS voyager detector. Identification of constituents was done on the basis of their retention time and mass spectra library search. The mass spectrographs of the identified constituents are given in Appendix B. The instruments data bank was also able to identify the presence of β-Bisabolene (29.52%), Caryophyllene (14.57%), Elemicin (14.29%) and Germacrene D (11.45%) with the respective retention time of 13.851, 12.828, 14.371 and 13.612 minutes respectively.

4.3. Physicochemical Characterization of the Essential Oil Extracted

4.3.1. The Moisture Content

After the completion of the procedures and used the formula in Appendix C.2 for the calculation of the moisture content, the calculated value of the moisture content of the essential oil extracted was became 2.2 percent. This result indicated that 2.2 percent from the total essential oil extracted was moisture and the rest was moisture free essential oil.

4.3.2. The Specific Gravity Value

From the reading of the densitometer, the density of the essential oil extracted from leaves of *O. Lamiifolium* became 0.88 g/ml and the density of water is 1000kg/m^3 which are equal to1g/ml then by the formula in the annex that used for calculating the specific gravity, the value of the specific gravity of the extracted essential oil was became 0.88. The equation is shown in Appendix C.3. Since the density of water is 1 g/ml and the density of the essential oil extracted was 0.88, the essential oil was less dense than water and it could be separated simply by decanting because it was floated above the water surface.

4.3.3. Kinematic Viscosity

Dynamic viscosity of the essential oil extracted, which was read from vibro viscometer, was 6.7mpa/sec at a temperature of 29.9°C, which is equal to $26.7*10^4$ kg/m.s and the density from the above densitometer reading was 880 kg/m^3 substituting the dynamic viscosity and the density value of the essential oil in equation used for the determination of the kinematics viscosity in the annex, the calculated value of the kinematics viscosity was became 303.4m^2/s. The equation is shown in Appendix C.4. The result indicated that the essential oil could be able to move and cover 303.4 m^2 area with in a second.

4.3.4. P^H Value

The p^H value of *O. Lamiifolium* leaf oil was measured using p^H meter three times and the results obtained are summarized in table 4.3 below. As shown in the table the pH value of *O. Lamiifolium* leaf essential oil was slightly basic. Therefore, in preparation of medicines for the treatment of different diseases, the preferable pH value is in the range of 7.5-10.5 (Mueller *et al*., 2000). The obtained pH value of *O. Lamiifolium* leaf essential oil is in the range to be used in producing medicines.

Table 4.3: The p^H Value of *O. Lamiifolium* Leaf Essential Oil

Run No.	p^H values
1	8.21
2	8.22
3	8.23
Mean ± SD	**8.22±0.01**

4.3.5. Saponification Value

Using the equation C.5 for the calculation of the Saponification value in Appendix C the values for three replicates and the mean value were tabulated in the table 4.4 below. Lower the saponification value, larger the molecular weight of fatty acids in the glucerides and vice-versa.

Table 4.4: Saponification value of *O. Lamiifolium* leaf essential oil

Run No.	Volume of HCL for the blank (ml)	Volume of HCL for the sample (ml)	Mass of sample (gm.)	SV
1	37.6	18.3	2	194.9
2	37.6	18.4	2	193.5
3	37.6	18.7	2	189.3
		Mean		**192.6±2.9**

Hence, the Saponification value of *O. Lamiifolium* leaf oil was 192.6±2.9mg KOH/g of essential oil. High Saponification value implies greater proportion of fatty acids of low molecular weight. The values obtained for Saponification value of *O. Lamiifolium* leaf oil was favorably comparable with the Saponification value of olive oil (185-196) which is a well-known vegetable oil in cosmetics industry (A.O.A.C. 2000).

4.3.6. Acid Value

Using equations C.6 and C.7 in Appendix C for the calculations of acid values, the triplicated results for *O. Lamiifolium* leaf essential oil obtained is summarized in table 4.5 below.

Table 4.5: Acid values for *O. Lamiifolium* leaf essential oil

Run No.	Titration volume of oil (ml)	AV of oil	% FFA of oil
1	0.11	0.21	0.105
2	0.14	0.32	0.19
3	0.08	0.11	0.12
	Mean ± SD	**0.2133 ± 0.105**	**0.1383 ± 0.0454**

From the table above the average acid value of *O. Lamiifolium* leaf essential oil is 0.2133±0.105 which is relatively small. The low acidity of the essential oil is an indication of the essential oil which is free from hydrolytic rancidity and enables the direct use of such oil without further neutralization (Arogba, 1999). Therefore, the result obtained indicated that *O. Lamiifolium* leaf essential oil can be used directly without further neutralization. The low free fatty acids content (0.1383±0.0454) was indicative of low enzymatic hydrolysis. This can be an advantageous that *O. Lamiifolium* leaf essential oil cannot develop off (rancidity) flavor during storage.

4.3.7. Iodine Value

The iodine value (IV) is the amount of iodine (in gram) necessary to saturate 100 grams of oil sample. The iodine value is used to determine the unsaturation of oils and in assessing the stability of oil in industrial application (Xu*et al*., 2007). The lower the iodine value indicates the higher resistance to oxidation, the longer shelf life and higher quality of the oil. Whereas the higher the iodine value of oil, the lower the quality. Testing of iodine value of *O. Lamiifolium* leaf oil has been conducted at food laboratory of AAiT and it was found to be 118 ±0.922g/100g of oil. The result indicated that *O. Lamiifolium* leaf oil has high iodine value, which indicates high resistance to oxidation and longer shelf life. The oil can be classified as a nondrying oil since its iodine value is higher than 100.

4.4. Experimental Design and Statistical Data Analysis

4.4.1. Analysis of Variance for the Data (ANOVA)

The table 4.6 below shows analysis of variance (ANOVA) obtained from design expert software, which tells as the significance of different factors. The study was interested in testing the hypotheses of no main effect for factor *A*, no main effect for *B*, no main effect for factor C, no AB interaction effect, no AC interaction effect and no BC interaction effect on the yield of *O. Lamiifolium* essential oil. The analysis of variance (ANOVA) would be used to test these hypotheses. The analysis of variance (ANOVA) can be computed manually but it can be also computed by using different software. For this cause design expert 7.0.0 software were used and the output of the data was discussed. The following table 9 shows the analysis of variance for the data using $\alpha = 0.05$.

Table 4.6: Analysis of variance (ANOVA) for the Selected Factorial Model

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.090	8	0.011	13.54	< 0.0001	significant
A-Temperature	5.378E-003	1	5.378E-003	6.48	0.0144	
B-Extraction time	0.011	1	0.011	13.71	0.0006	
C-Particle size	0.055	1	0.055	66.81	< 0.0001	
AB	2.667E-004	1	2.667E-004	0.32	0.5736	
AC	4.444E-005	1	4.444E-005	0.054	0.8180	
BC	4.444E-005	1	4.444E-005	0.054	0.8180	
A^2	0.014	1	0.014	16.61	0.0002	
B^2	3.559E-003	1	3.559E-003	4.29	0.0441	
Residual	0.037	45	8.296E-004			
Lack of Fit	3.000E-003	9	3.333E-004	0.35	0.9512	not significant
Pure Error	0.034	36	9.537E-004			
Cor Tota	l0.13	53				

The Model F-value of 13.54 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, A^2, B^2 are significant model terms.Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy) model reduction may improve your model. The "Lack of Fit F-value" of 0.35 implies the Lack of Fit is not significant relative to the pure error. There is a 95.12% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

The "Pred R-Squared" of 0.5711 is in reasonable agreement with the "Adj R-Squared" of 0.6543. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 12.205 indicates an adequate signal. This model can be used to navigate the design space.

4.4.2. Regression Model Equation

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

Final Equation in Terms of Coded Factors:

% yield = + 0.38+0.012 * A+0.018 * B - 0.032 * C + 3.333E-003 * A * B+1.111E-003 * A * C - 1.111E-003 * B * C-0.034 * A^2-0.017 * B^2

Where: A is temperature level, B is extraction time level and C is particle size level

Considering the ANOVA table above, the model terms A, B, C and A^2were significant model terms and none of the interaction terms were significant. Often we think about removing non-significant model terms or factors from a model in this case all interaction terms were removed in the model. But in this case, removing AB, AC and BC and retaining A, B and C will result in a model that is not hierarchical. The hierarchy principle indicates that if a model contains a higher order term, it should contain all lower-order terms that compose it. Hierarchy promotes a type of internal consistency in a model, and many statistical model builders rigorously follow the principle (Douglas, 2001). Therefore, the final equation in terms of coded factor with the interaction effect is given by a second order regression equation.

Final Equation in Terms of Actual Factors:

% Yield = - 2.10699 + 0.057833 * Temperature + 0.030833 * Extraction time - 0.078519 * Particle size + 1.66667E-004 * Temperature * Extraction time + 2.22222E-004 * Temperature * Particle size - 1.11111E-003 * Extraction time * Particle size - 3.38889E-004 * Temperature^2 –4.30556E-003*Extractiontime^2

4.4.3. Model Adequacy Check

Before the model implemented for different applications it should satisfy different criteria such as the normal distribution of the error term (residuals) and the residuals verses predicted value of the model data. Unless the model should not satisfy these criteria it is not advisable to use the model for different purpose. Figures 4.2 and 4.3 are shown the experimental criteria plotted by the model equation that developed by the design expert 7.0.0 software.

The normal probability plot of the residuals in figure 4.2 below showed that, it follows almost a straight line which implies residuals are approximately normally distributed that satisfy the most important assumption in any model.

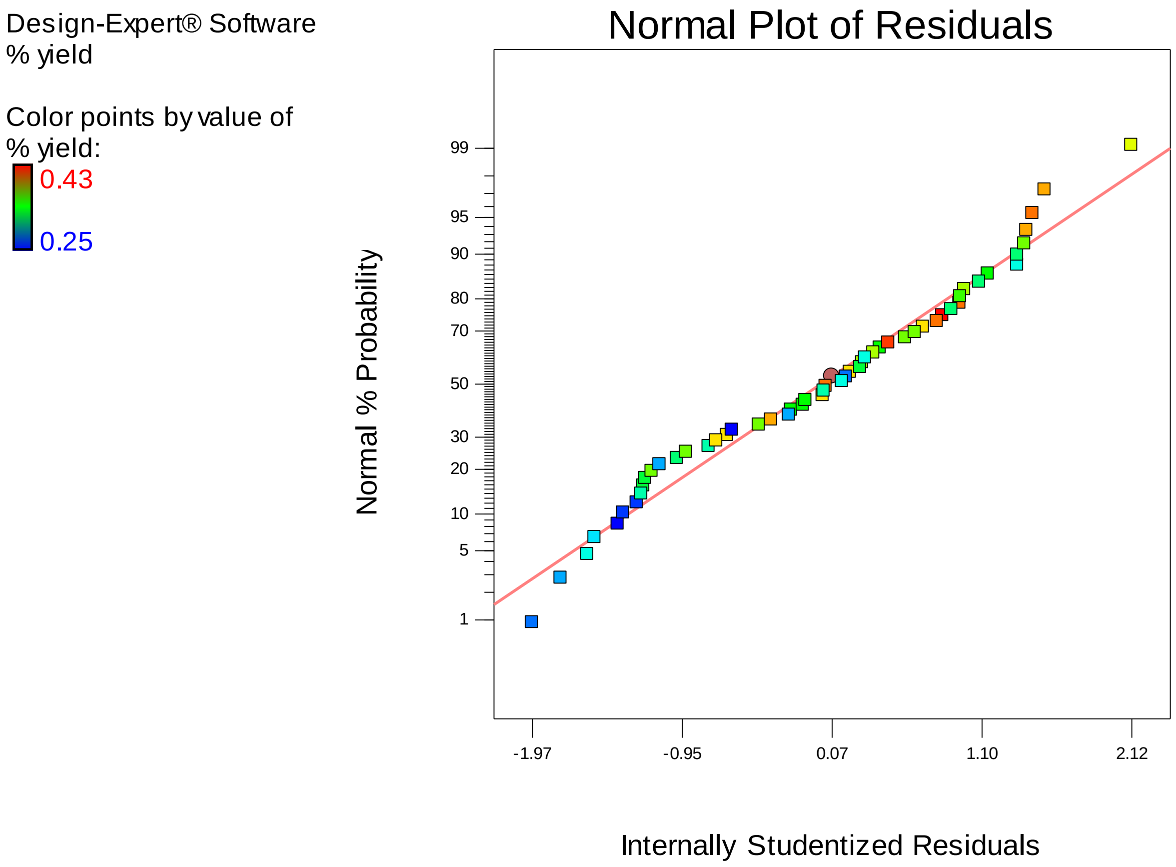

Figure 4.2: Normal probability plot of the studentized residuals

The plot of residuals verses predicted value in figure 4.4 below showed that the residuals did not follow any pattern that means no serious deviation from the assumptions. The randomness of the plot implies that the model is adequate and there is no indication of a severe problem. Therefore, the model equation may fit to design the model.

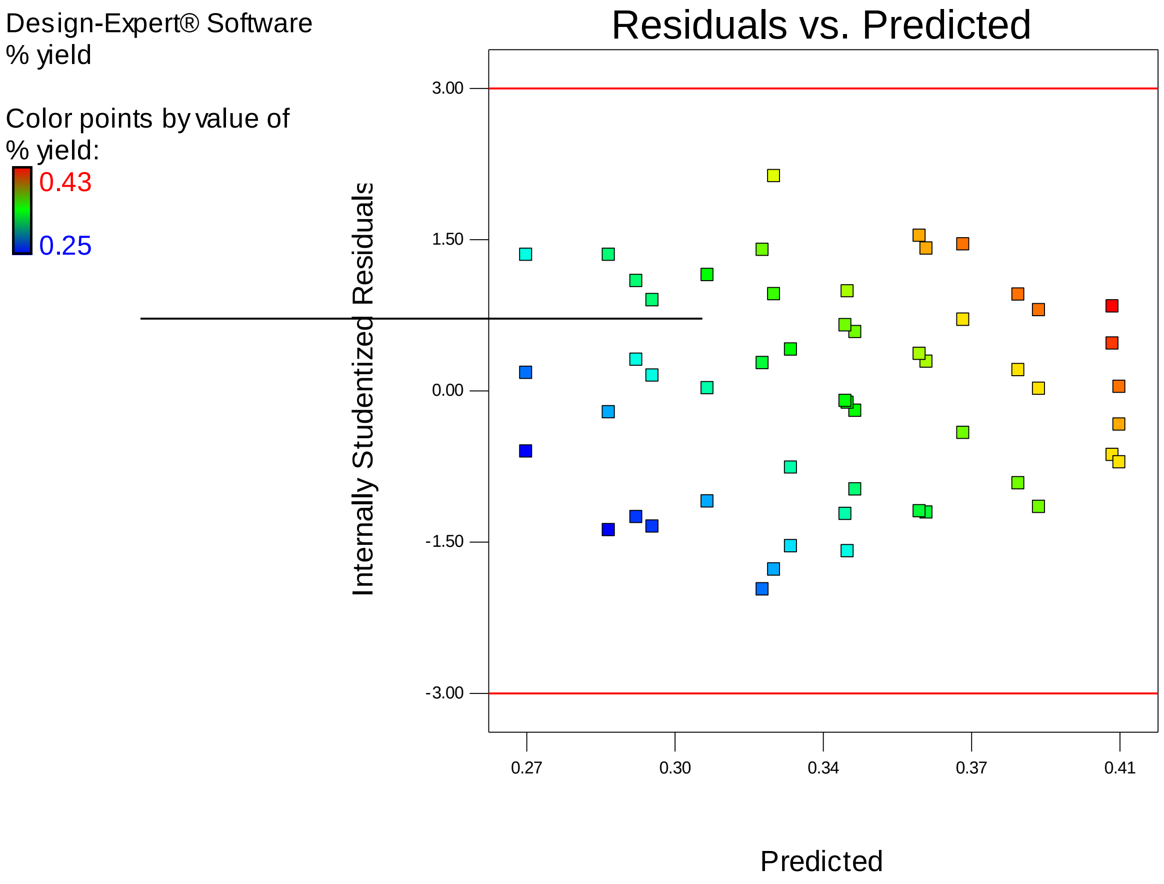

Figure 4.3: Residual value versus predicated value of percentage oil yield

Adequacy precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 33.941 indicates an adequate signal. This model can be used to navigate the design space.

4.5. Effects of the Factors/ Process Parameters on the Yield

In this section the effects of the factors on the yield of essential oil and the optimal levels of the parameters were investigated. The plots used for discussion here is that the one which were gave maximum yield during the extraction process. Some of the plots are shown in Appendix D.

4.5.1. The Main Effects of Process Parameters

I). Effect of Temperature on Essential Oil Yield

The effect of temperature and the percent yield of the essential oil are shown in figure 4.4 below. As shown in the figure the yield of essential oil increased gradually with rise in temperature in the range between 75ºC to 85ºC and then seems like no further increments on yield up to 95°C. Further rise in temperature above 95°C the yield showed decreased. This may be due to the greater speed of the steam resulting less contacting time of the plant material with the steam. It makes difficult to separate the oil molecule in higher temperature so that less essential oils are diffused from the plant material to the feed carrier.

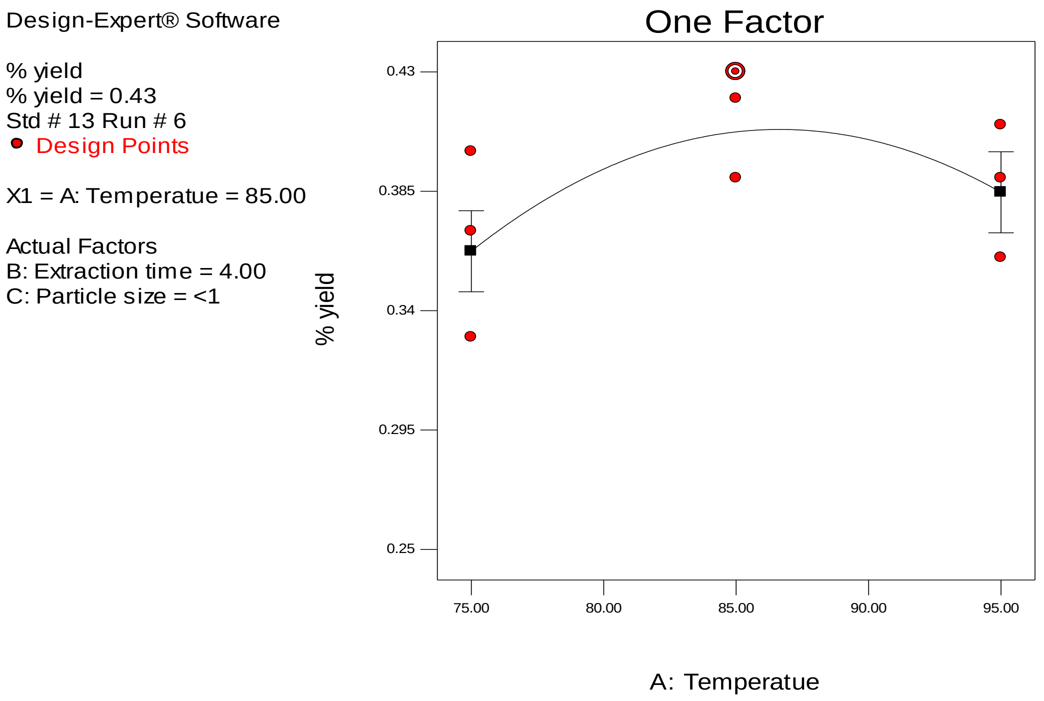

Figure 4.4: Effects of temperature on yield at 4 hours and <1 mm particle size

Temperature effects on extraction are dual. Higher temperature can accelerate the steam flow and thus increase the essential oils. Higher temperature can also decrease the fluid density that may reduce the extraction efficiency (Guo-Qing *et al*., 2005). High temperature also affects the purity of the oil recovered in the extract because at higher temperature more unwanted compounds of the leaf were extracted. Hence, it was found that 85ºC was the optimum temperature for extracting the essential oils from the leaf of *O. Lamiifolium*.

II). Effect of Extraction time on Essential Oil Yield

The influence of extraction time on the yield obtained at different particle size is presented in figure 4.5. From the figure it can be seen that at the initial stage of the extraction process (1-2 hours) there was insignificant essential oil yield but beyond 2 hours, the essential oil yield increased with increase in time of extraction until it reached about 4 hours for all temperatures and the considered particle size of the plant materials. Beyond 4 hours insignificant amount of essential oil was obtained. For smaller particle size the yield rose rapidly with time up to 4 hour and then after it was not varying (it was constant). In 4 hours the oil in the plant material was almost exhausted hence negligible oil yields beyond this time.

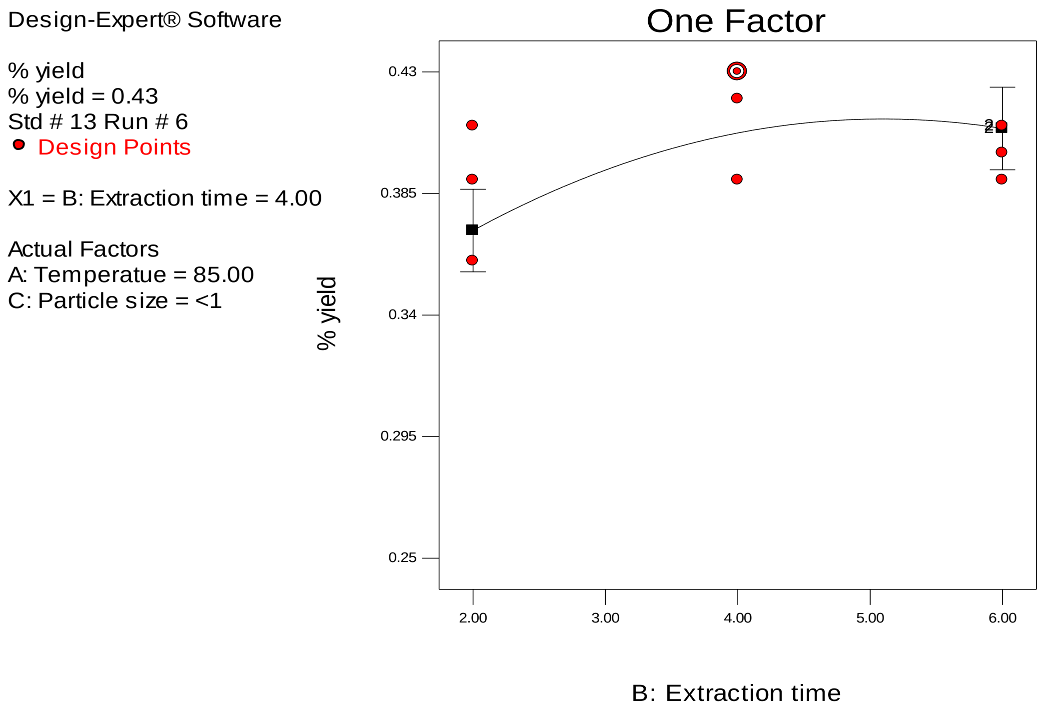

Figure 4.5: Effect of extraction time on yield of essential oil at 85°C and <1 mm particle size

This showed that the rate of extraction was high at the beginning (2-4 hours) and gradually decreased slowly with time. At 85ºC and <1 mm particle size the yield increased by 17.74%, as the extraction time increased from 2 to 4 hours and it decreased by 2.73% as the time increased from 4 to 6 hours. However, for larger particle size the yield was lower at the beginning of the extraction and increased gradually as the extraction time increased. The yield increased by only

7.93% as the time increased from 2 to 4 hours while by 1.92% as the extraction time increased from 4 to 6 hours.

The result obtained in this research indicates that smaller particle size needs small extraction time to start the extraction and obtained maximum yield in comparison to large particle size. According to this study the maximum oil yield is obtained at 4 h extraction time and at lower particle size and since at 4 h extraction time 0.4133 ± 0.0208% of the maximum yield was obtained, so extraction time above 4 h is wastage of time and cost. Hence, it was found that four hours was the optimum extraction time for this study. The percentage oil yield was directly related to extraction time i.e. the yield increases as extraction time increases.

III). Effect of Particle Size on Essential Oil Yield

The effect of particle size on the yield of the essential oil shown in figure 4.6 indicates that the particle size plays the biggest role on yield of the essential oil. It is quite clear that there is an increase in the oil yield as the particle size decreased and an increase in the particle size results a drop in oil yield. Thus, the percentage essential oil yield was inversely related to the particle size i.e. smaller size gives high yield while larger size results a lower yield. The reason is that larger particles have smaller surface area of contact and larger distance to steam entrance. The highest percentage of oil yield was obtained with the smaller particle size.

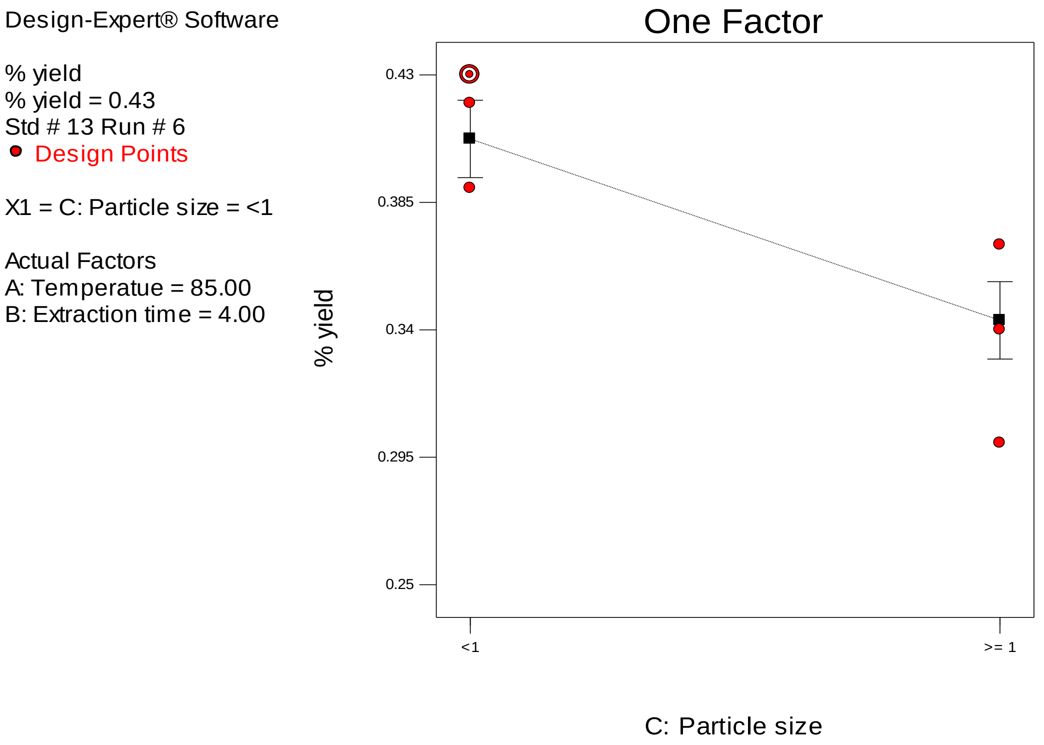

Figure 4.6: Effect of the particle size on yield of the essential oil at temperature of 85°C and extraction time of 4 h

4.5.2. Interaction Effects among the Factors of the Process on the Yield

I). The Interaction Effect of Temperature with Extraction time

As it has seen from figures 4.7 (a, b and c) below with different plots, there were no interactions of the two factors at a constant level of particle size. This means that temperature and extraction time have related neither directly nor inversely during the extraction process of the essential oil according to this study. Consequently, the interaction effect of temperature with extraction time on the yield of essential oil from *O. Lamiifolium* using steam distillation extraction was insignificant.

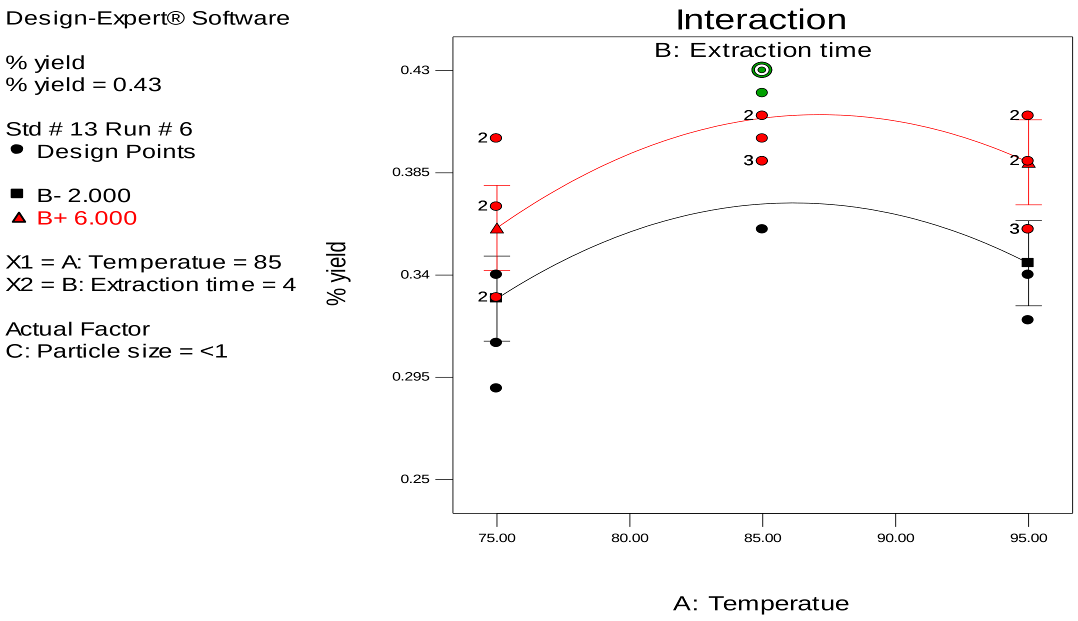

Figure 4.7a: The interaction effects of temperature with time on the yield at <1 mm particle size using line plot

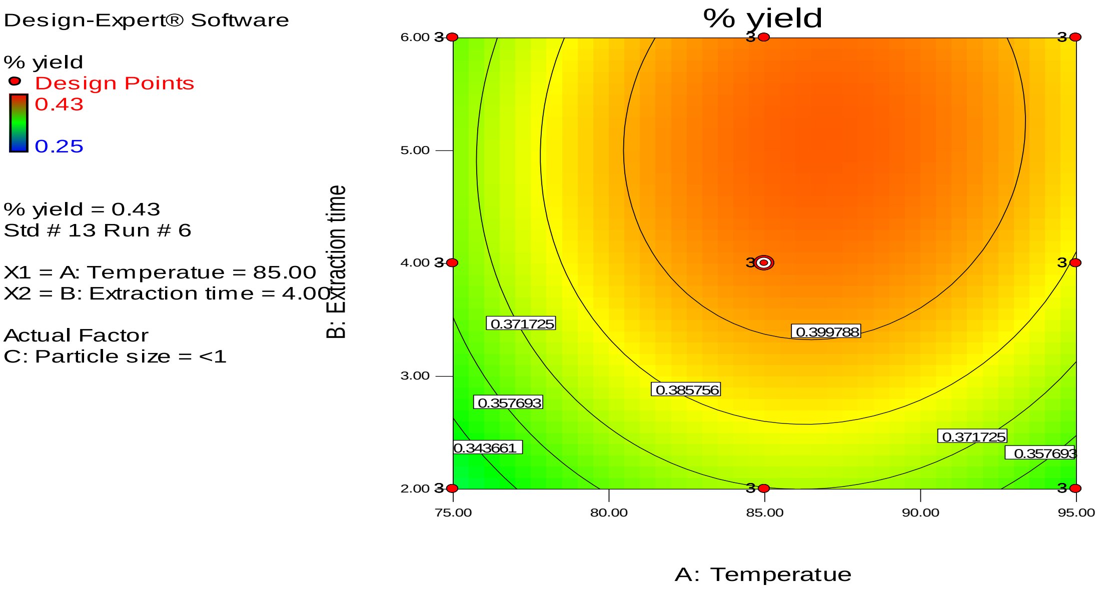

Figure 4.7b: The interaction effects of temperature with time on the yield at <1 mm particle size using contour plot

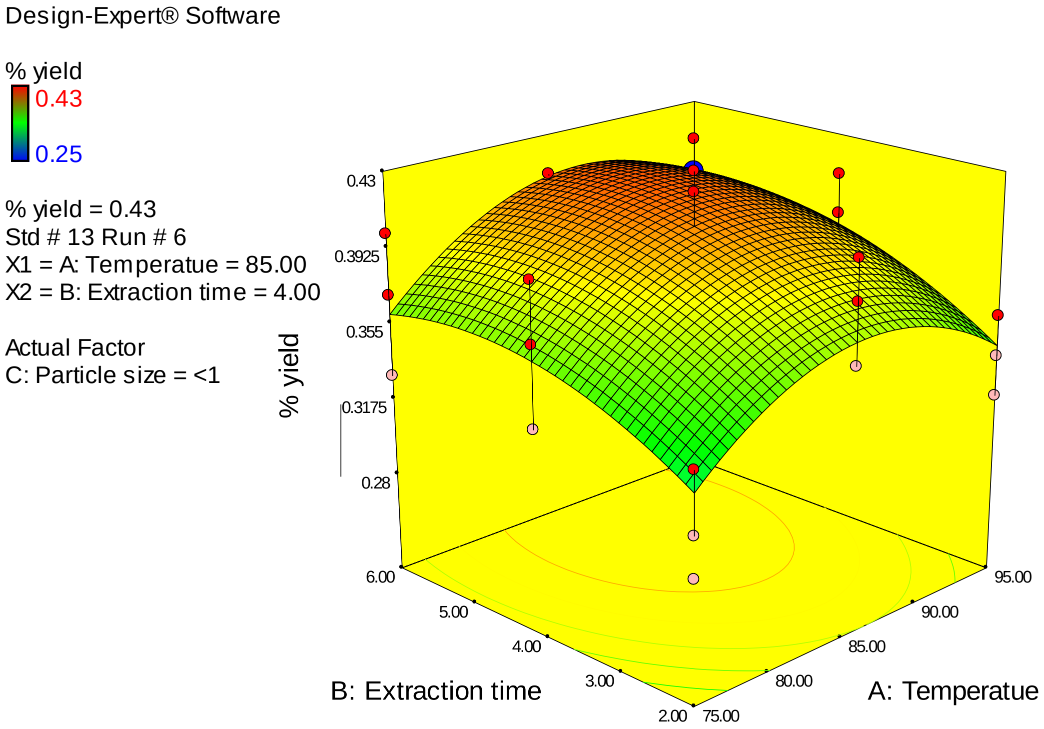

Figure 4.7c: The interaction effects of temperature with time on the yield at <1 mm particle size using response surface plot

In fact, the interaction effect of temperature and extraction time is also the same for larger particle size plant materials except the yield of the essential oil which is smaller than the yield for smaller particle size plant materials as discribed above in main effects of the process parameters on the yield. The figures 4.8 (a, b and c) below shows the simiilarity of the shape of the plots with figures 4.7 (a, b and c) of the interaction of temperature with extraction time.

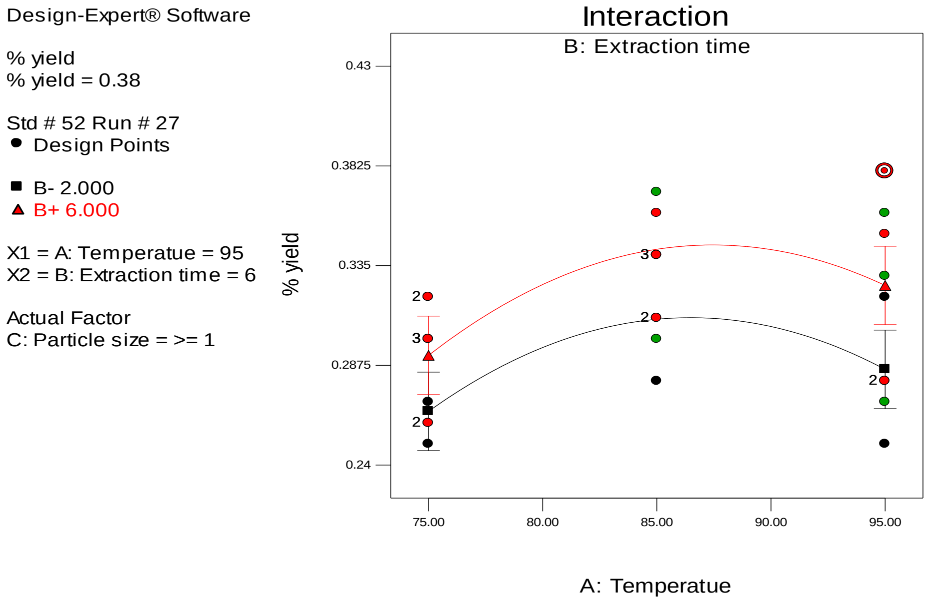

Figure 4.8a: The interaction effects of temperature with time on the yield at >=1 mm particle size using line plot

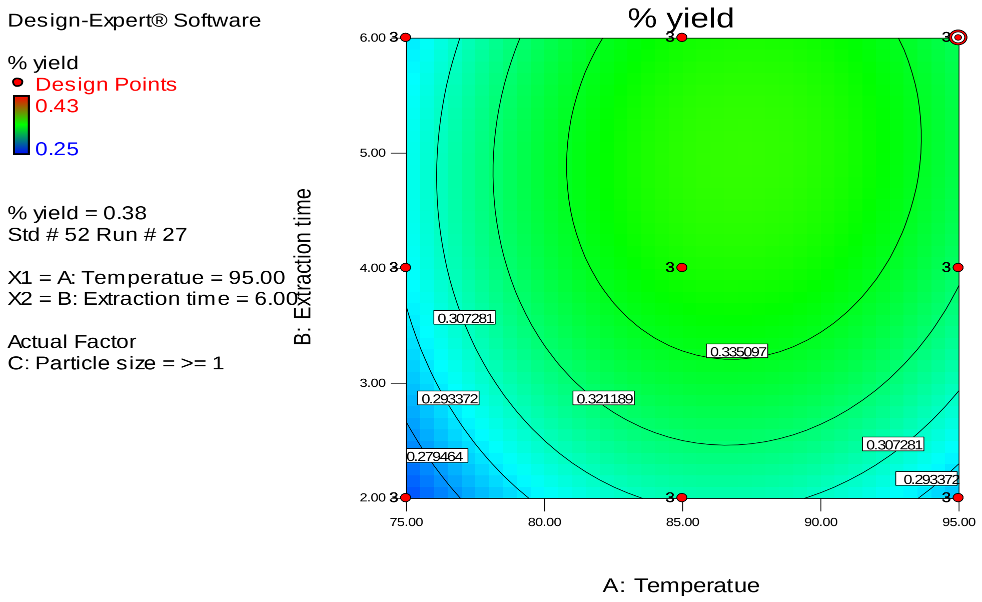

Figure 4.8b: The interaction effects of temperature with time on the yield at >=1 mm particle size using contour plot

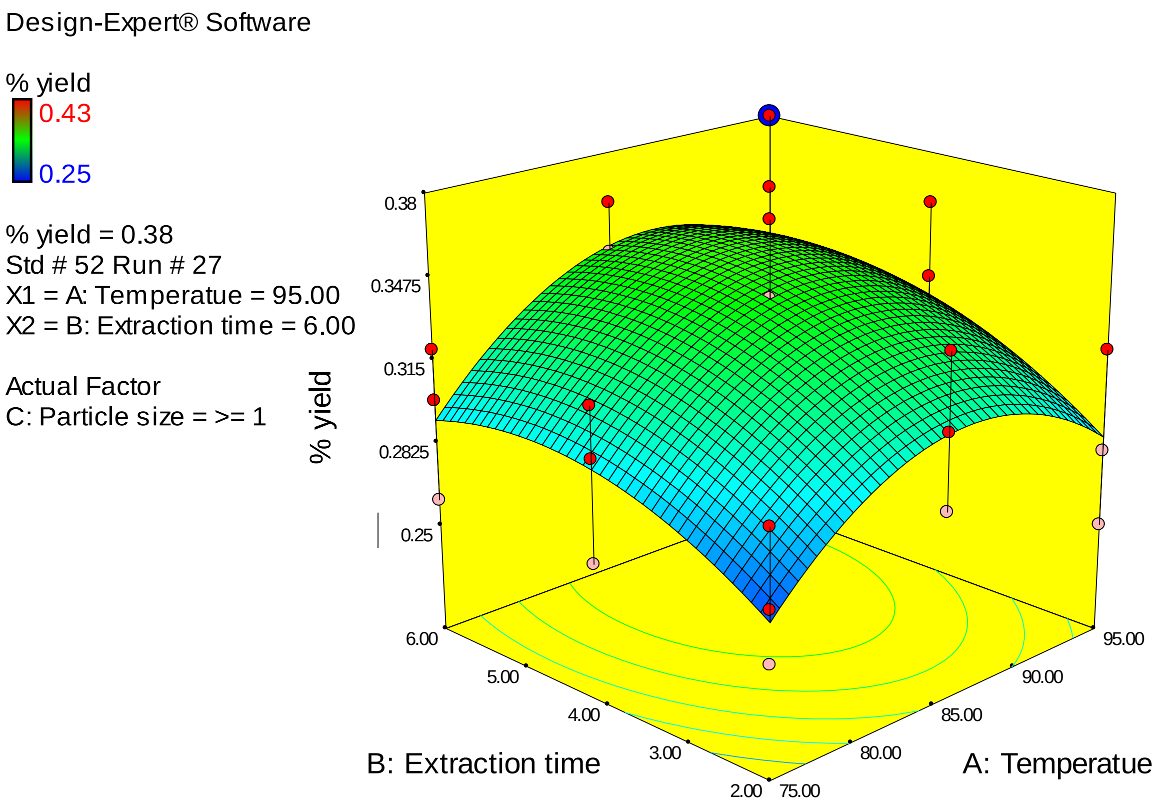

Figure 4.8c: The interaction effects of temperature with time on the yield at >=1 mm particle size using response surface plot

II). The interaction effect of temperature with particle size on yield

Figure 4.9 below showed that the interaction effect of temperature with particle size on the yield of the essential oil. Here, there would be no possible interactions in a constant level of extraction time. As observed from the figure all treatments of temperature could give the same yield throughout all level of particle size. As a result, there was no an interaction effect of temperature and particle size on the yield of the essential oil. The highest yield also obtained at the <1mm of particle size and 85°C of temperature at constant extraction time, 4 hours.

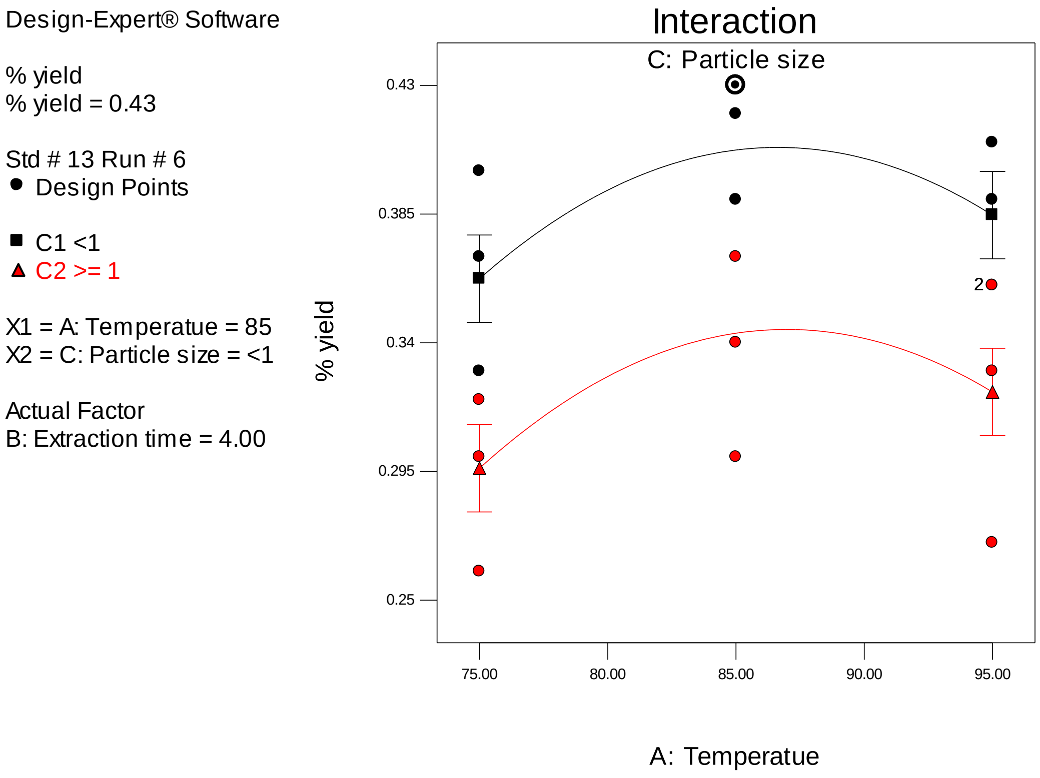

Figure 4.9: The interaction effects of temperature with particle size on the yield

III). The interaction effect of extraction time with particle size on the yield

There were no interactions between particle size and extraction time as depicted by similar shape of the curves in figure 4.10 below. This shows that irrespective of extraction time, lower particle sizes give a higher yield and irrespective of particle size, higher extraction time can give higher yield. Similarly, as can be noticed from the figure, there was no interaction effect between particle size and extraction time. Where: C1 and C2 are codes for particle sizes <1 mm and >=1 mm respectively. B1, B2 and B3 are codes for extraction time two, four and six hours, respectively. Design points are points on the graph which helps to develop mathematical model of the predicted response based on these points.

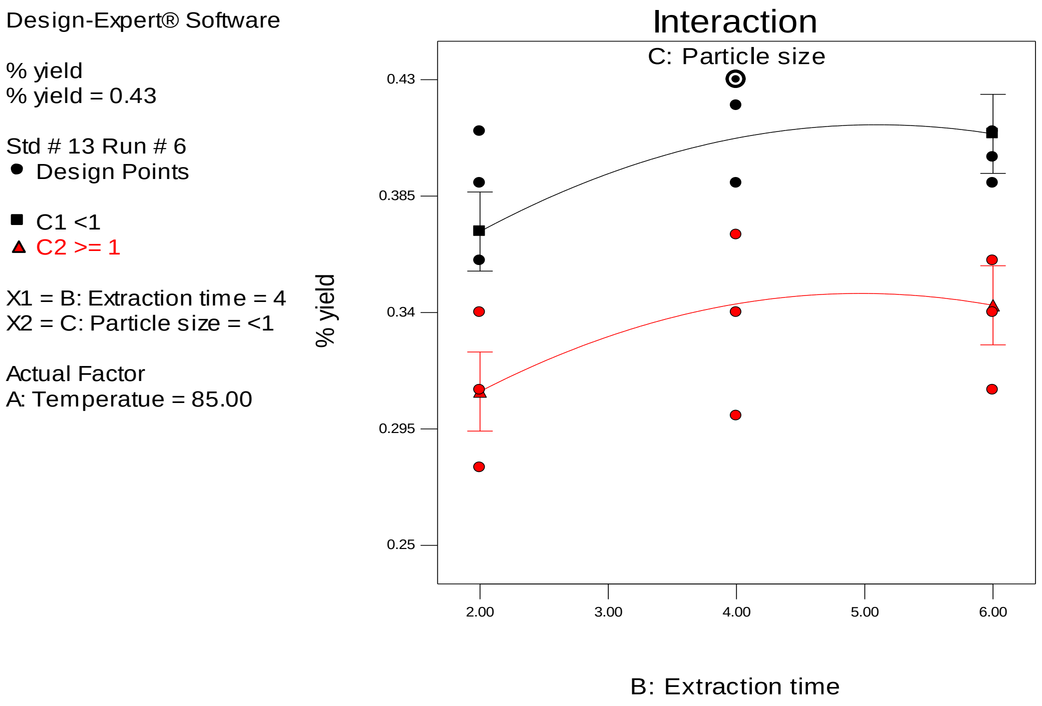

Figure 4.10: The interaction effects of temperature with particle size on the yield

4.6. Optimization of the yield of the essential oil

Using optimization functional in design expert software 7.0.0, the predicted maximum yield solutions at the <1mm and >=1mmparticle size with the corresponding operating conditions are tabulated in table 4.7 below which was in agreement with the experimental value because the value of the essential oil extracted subtract from the value of optimized value is less than 0.05.

Table 4.7: The two maximum possible solutions of the essential oil yield

No.	Temperatue	Extraction time	Particle size	% yield	Desirability
1	88.95	5.04	<1	0.408275	0.879
2	87.74	4.93	≥ 1	0.341248	0.507

The objective here was to obtain maximum yield in the given interval of the investigated independent variables. Using design expert software, the maximum yield of essential oil was achieving at the combination of 88.95°C, 5.04 hours and <1mm levels with the desirability equals to 0.879. The maximum yield at the particle size of >=1mm was obtained at the combination of the 87.74°C and 4.93 hours with the desirability equals to 0.507.

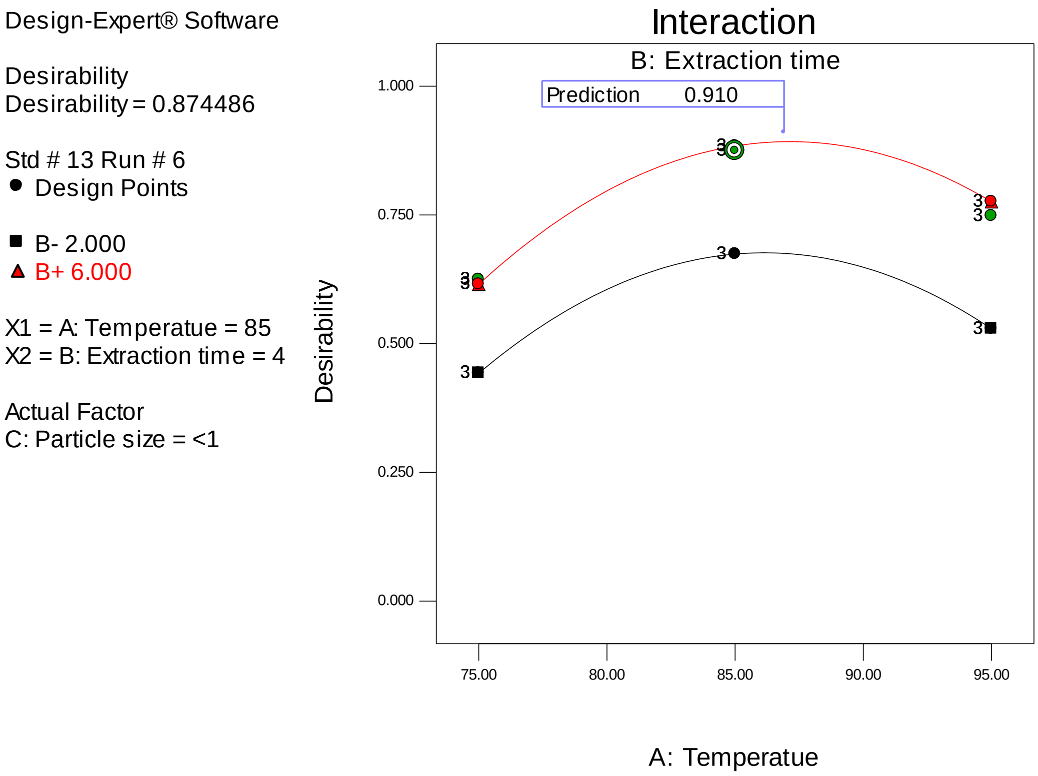

Figure 4.11a: The desirability of the highest yield offering combination of the treatments at particle size <1 mm in line plot

The other analysis such as main and interaction effect analysis also enforce this conclusion. But desirability less than 70 % combination of factors were not advisable. As a result, even if, it is a maximum yield relative to the other yields, the desirability of the larger particle size is not advisable solution. The two solutions of the yields at the two different particle sizes offering the combinations of the treatments were shown by different plots in figures 4.11 and 4.13 below.

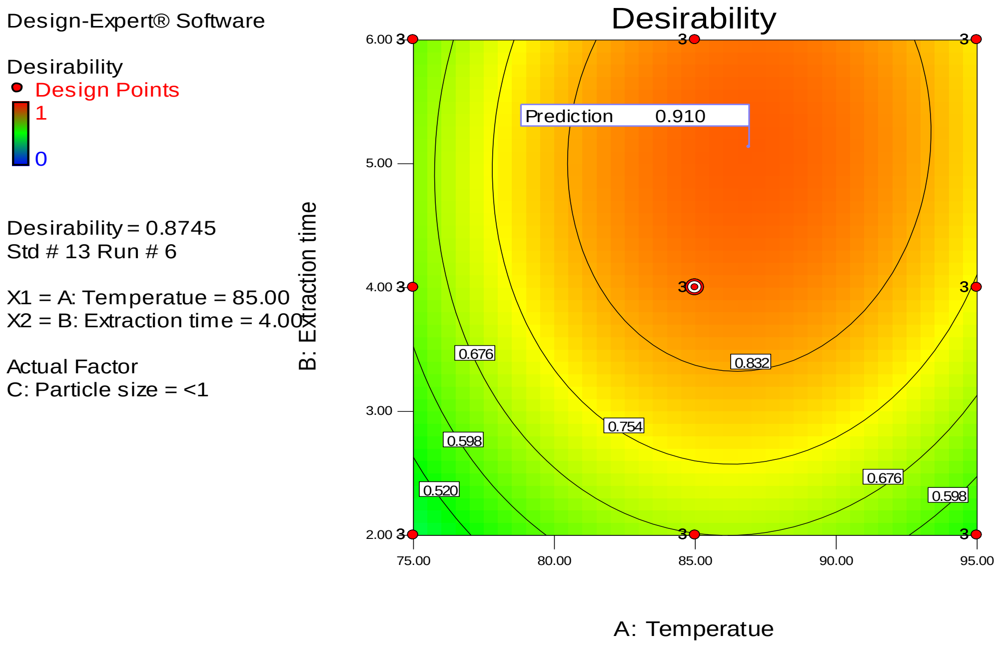

Figure 4.11b: The desirability of the highest yield offering combination of the treatments at particle size <1 mm in contour plot

The two possible solutions of highest yield offering possible combination of the treatments in ramps form shown in figures 4.12 (a, b, c) and 4.14 (a, b, c) were strengthening the above expression (report form).

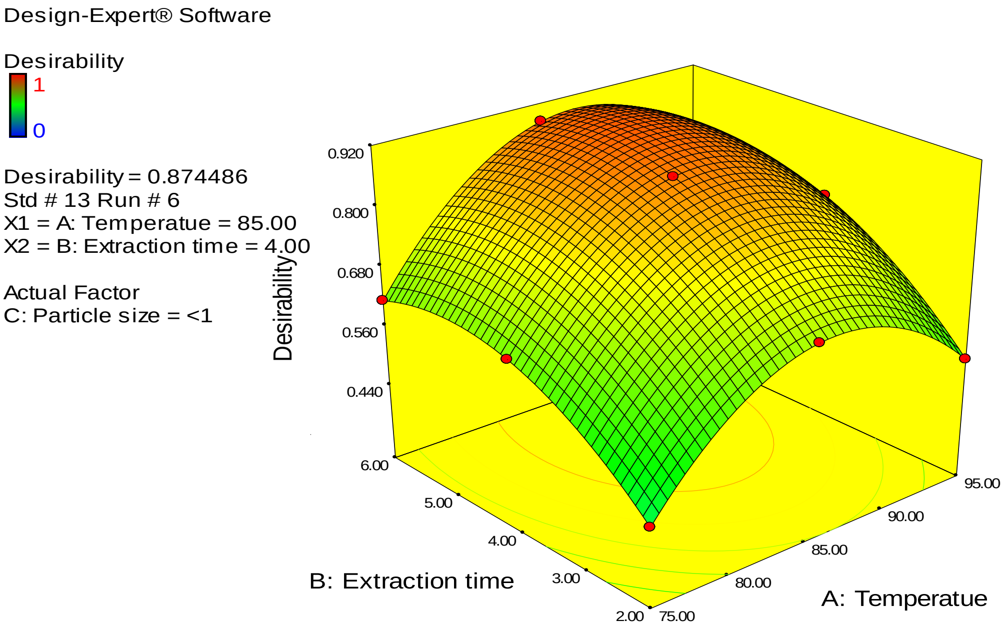

Figure 4.11c: The desirability of the highest yield offering combination of the treatments at particle size <1 mm in response surface plot

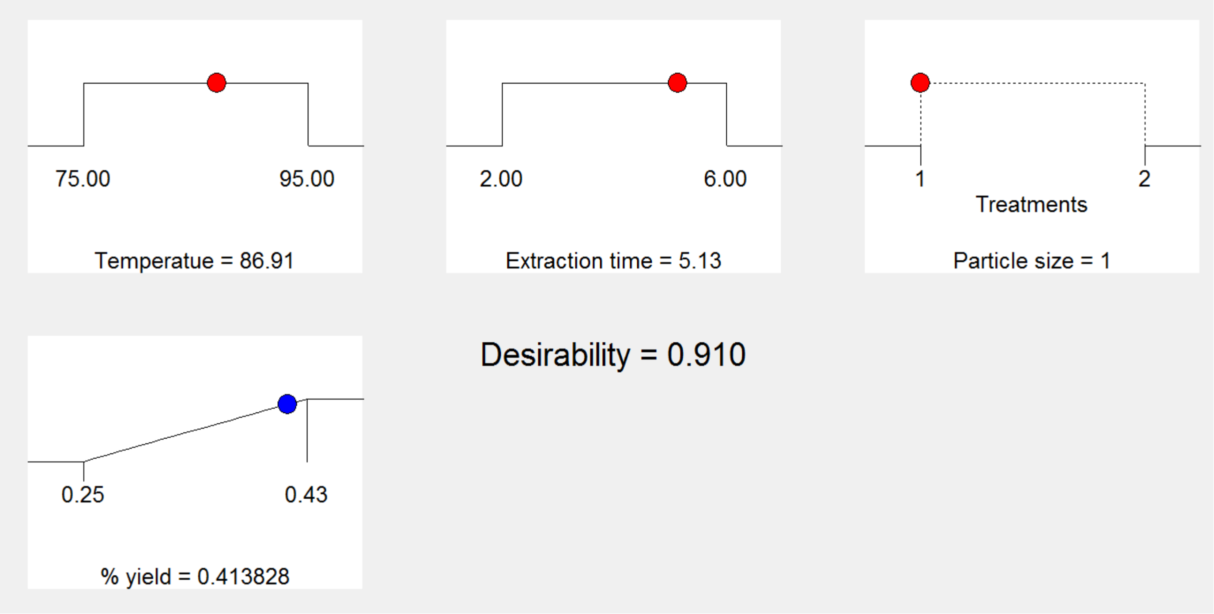

Figure 4.12: The highest yield offering treatments at particle size <1 mm in ramp form

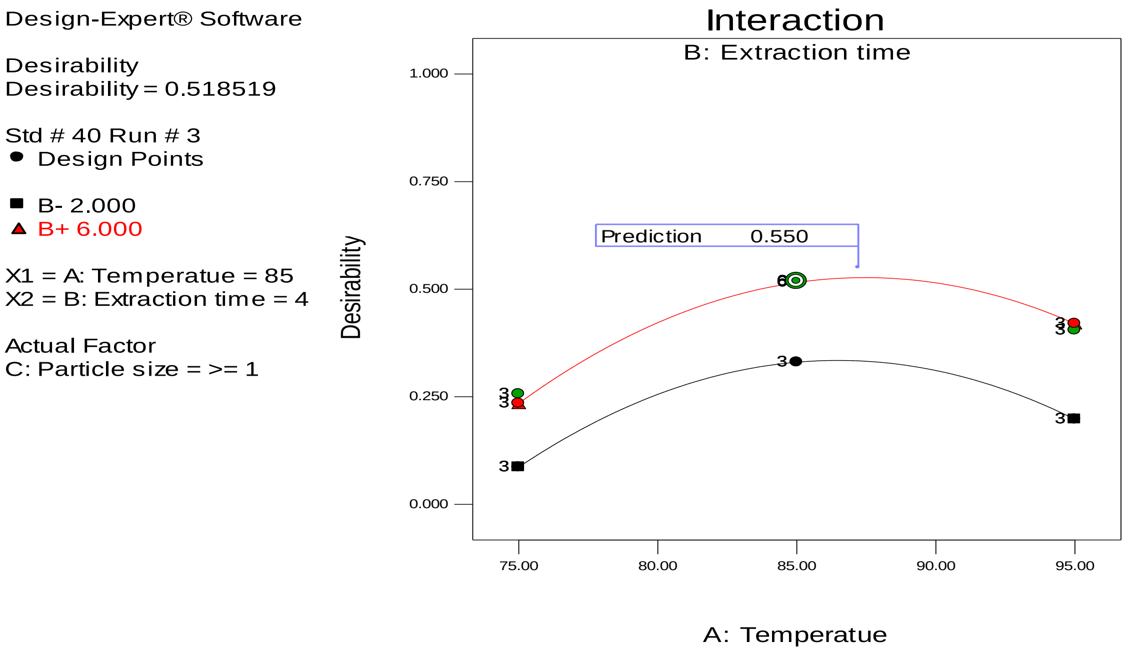

Figure 4.13a: The desirability of the highest yield treatments at >=1 mm particle size in line plot

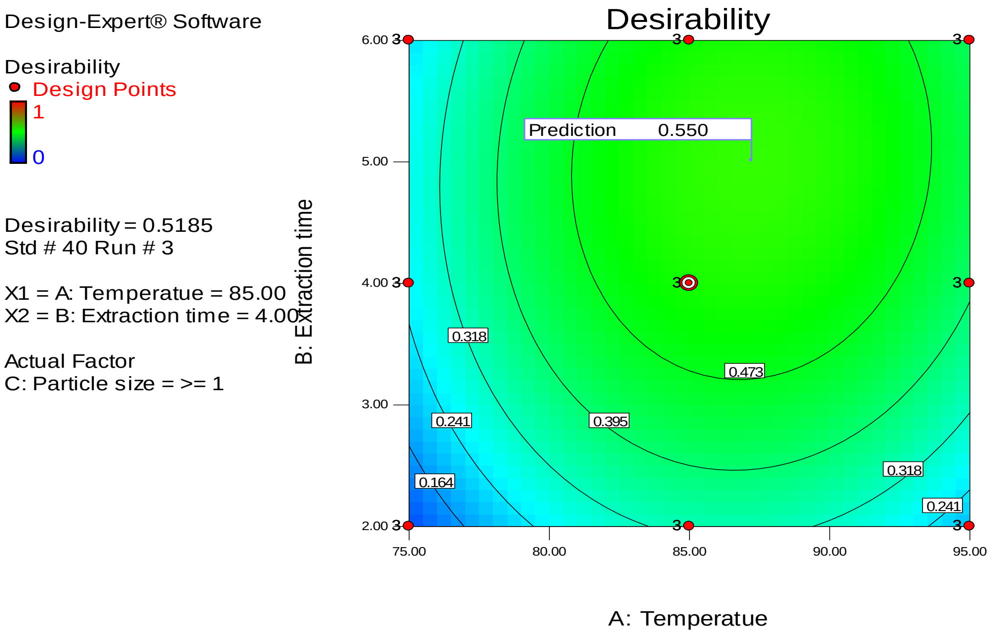

Figure 4.13b: The desirability of the highest yield treatments at >=1 mm particle size in line counter plot

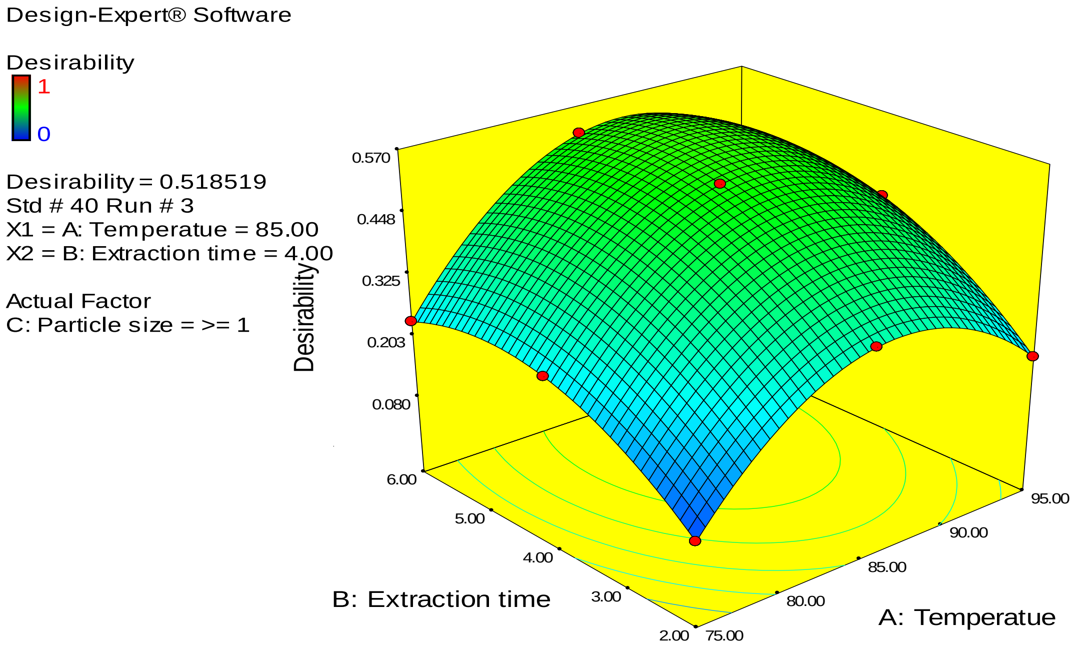

Figure 4.13c: The desirability of the highest yield treatments at >=1 mm particle size in line response surface plots

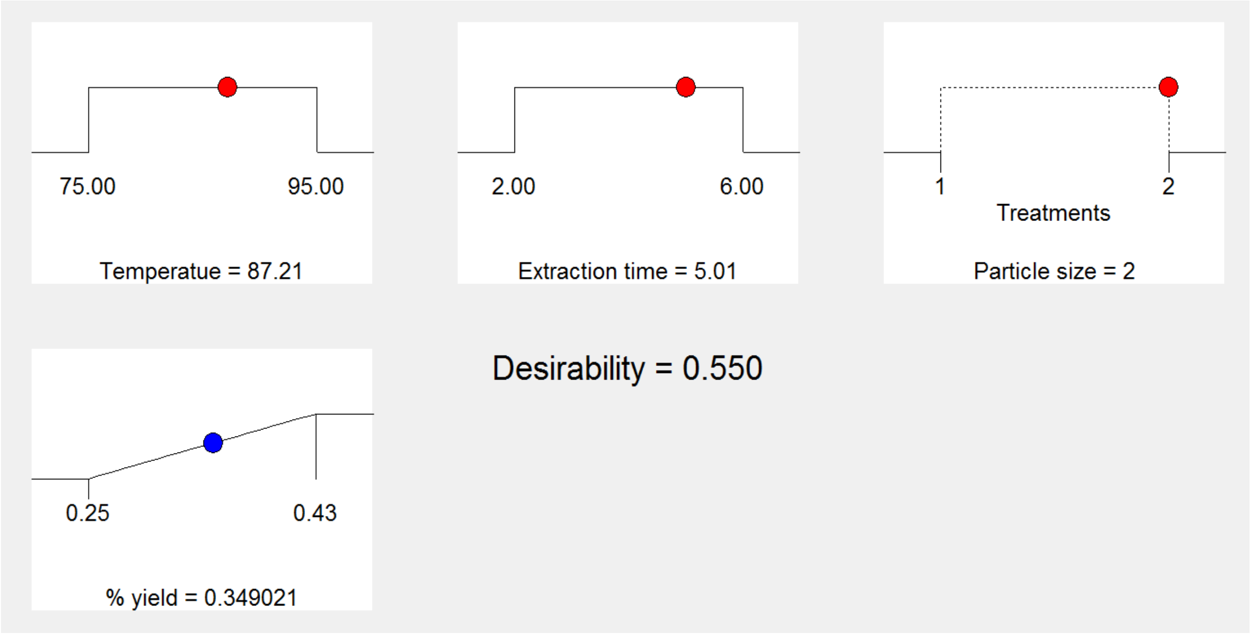

Figure 4.14: The highest yield offering treatments at particle size >=1 mm

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

This work was intended to study the influence of different factors (temperature, extraction time and Particle sizes) on the quality and quantity of essential oil extracted from leaves of *Ocimum Lamiifolium*. There are different methods of essential oil extraction from plant materials. In this work, steam distillation extraction method was used. The yield was determined in percentage of the essential oil recovered from an initial mass of 100 grams of the plant material.

From the experimentation it was found that maximum essential oil yield of 0.4133±0.0208% (v/w) was obtained at 85°C, 4 hours and <1 mm particle size and a minimum yield of 0.2733±0.0252% (v/w) was obtained at temperature of 75 °C, 2 hours of extraction time and >=1 mm particle size. The observed quantitative variability of the yield was due to temperature, extraction time as well as particle size difference. Thus, determination of optimal temperature, extraction time and appropriate size of the plant material needs to have consideration to get the maximum amount of the required product.

The P value of < 0.0001 from the analysis of variance (ANOVA) table of the design expert indicated that operating parameters have significant effect on the essential oil yield. The quality of the essential oil could be affected due to several reasons like operating conditions, maturity stage, drying condition, and type of extraction methods.

Optimization model was also developed using design expert 7.0.0 software for prediction of essential oil yield. The model fits the experimental data. From the design expert, temperature, extraction time, and particle size were the major process parameters found to significantly influence the essential oil yield. No interactions effects of the parameters were observed on the yield of the essential oil. Findings of this paper showed that 87.24°C of temperature, 4.85 hours of extraction time and <1mm of particle size were the optimum conditions which gives 0.418748±0.0208% (v/w) essential oil. It was shown that an increase in temperature and extraction time increases the extraction rate but until some temperature level and extraction time. In general, the yield was decreased when the particle size of the plant material became large.

The quality of the essential oil extracted was examined over the entire 4 hours of extraction time using Gas Chromatography-Mass Spectroscopy unit. GC-MS analysis revealed that four major chemical components were identified in the essential oil of *Ocimum Lamiifolium* leaves, including β-Bisabolene (29.52%), caryophyllene (14.57%), Elemicin (14.29%) and Germacrene D (11.45%). As it has been observed from the above list, β-Bisabolene comprises 29.52% of the total yield, and it is the compound most responsible for the aroma/ fragrant of *O. Lamiifolium* essential oil. The ingredients obtained from this study indicated that the oil can be fully utilized for the manufacture of antioxidant, antimicrobial and antiseptic agents.

5.2. Recommendations

Production technology is an essential element to improve the overall yield and quality of essential oil. Therefore, using more efficient methods of extraction is recommended to extract more yields with good quality.

After accomplishing the required tasks of the extraction process, further treatments should take on the extracts before using the essential oil for the treatment of different diseases.

Extracting essential oils rather than exporting the raw plant materials can be save the country's foreign currency and hence the production of essential oils could still be a good source of foreign exchange revenue for our country.

Many plant species have been lost and some are in danger of extinction. It has also caused biodiversity conservation problems. It is therefore vital that systematic cultivation of these plants be introduced in order to conserve the biodiversity and protect endangered species.

Furthermore, the requirement of essential oils for use in aromatherapy is increasing and creating a demand for organically produced exotic oils. The development of the essential oils industry is therefore important to our country which has rich resources of raw materials (medicinal plants) or the climatic conditions for the initiation of cropwise cultivation programs.

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APPENDIX

Appendix A: Experimental Result Analysis of the Essential Oil

A.1 Quantitative Analysis of the Results

Table A.1: Essential oil yield (%) extracted

Time (hours)	Temperature (°C)				Mean	SD	Mean ± SD
Particle size <1mm							
2	**75**	0.31	0.34	0.29	0.3133	0.0252	0.3133±0.0252
	85	0.36	0.39	0.41	0.3867	0.0252	0.3867±0.0252
	95	0.34	0.36	0.32	0.3400	0.0200	0.3400±0.0200
4	**75**	0.37	0.33	0.4	0.3667	0.0351	0.3667±0.0351
	85	0.43	0.39	0.42	0.4133	0.0208	0.4133±0.0208
	95	0.39	0.36	0.41	0.3867	0.0252	0.3867±0.0252
6	**75**	0.37	0.33	0.4	0.3667	0.0351	0.3667±0.0351
	85	0.4	0.39	0.41	0.4000	0.0100	0.4000±0.0100
	95	0.39	0.36	0.41	0.3867	0.0252	0.3867±0.0252
Particle size =<1mm							
2	**75**	0.27	0.3	0.25	0.2733	0.0252	0.2733±0.0252
	85	0.31	0.28	0.34	0.3100	0.0300	0.3100±0.0300
	95	0.28	0.25	0.32	0.2833	0.0351	0.2833±0.0351
4	**75**	0.3	0.26	0.32	0.2933	0.0306	0.2933±0.0306
	85	0.34	0.3	0.37	0.3367	0.0351	0.3367±0.0351
	95	0.36	0.27	0.33	0.3200	0.0458	0.3200±0.0458
6	**75**	0.3	0.26	0.32	0.2933	0.0306	0.2933±0.0306
	85	0.34	0.31	0.36	0.3367	0.0252	0.3367±0.0252
	95	0.38	0.28	0.35	0.3367	0.0513	0.3367±0.0513

A.2 Qualitative Analysis of the Results

Table A.2: Library Search Report from GC-MS Analysis

Table A.3: The Components which complains with minimum quality

S/No.	RT	Name of the compound	Chemical Formula	Molecular Weight	Peak area %	Quality
1	6.012	Sabinene	$C_{10}H_{16}$	136.2340	1.12	95
2	7.015	(R)-α-Pinene	$C_{10}H_{16}$	136.2340	1.43	90
3	10.313	D-Carvone	$C_{10}H_{14}O$	150.22	1.13	96
4	11.658	1,5,5-Trimethyl-6-methylene-cyclohexne	$C_{10}H_{16}$	136.2340	1.24	91
5	11.888	Eugenol	$C_{10}H_{12}O_2$	164.204	2.92	98
6	12.210	α-Copaene	$C_{15}H_{24}$	204.3511	1.99	99
7	12.355	1,5,5-Trimethyl-6-methylene cyclohexenes			3.59	98
8	12.828	Caryophyllene	$C_{15}H_{24}$	204.3511	14.57	99
9	12.939	(1R, 2s, 6s, 7s, 8s) -8-Isopropyl-1-methyl-3-methylenetricyclo [4.4.0.02,7] decane-rel-,			1.48	98
10	13.271	Humulene	$C_{15}H_{24}$	204.3511	1.80	95
11	13.375	Bicyclosesquiphellandrene	$C_{15}H_{24}$	204.3511	0.75	95
12	13.612	Germacrene D	$C_{15}H_{24}$	204.3511	11.45	98
13	13.851	β-Bisabolene	$C_{15}H_{24}$	204.3511	29.52	98
14	14.020	Cis-Muurola-3,5-diene	$C_{15}H_{24}$	204.3511	1.27	95
15	14.087	δ-Cadinene	$C_{15}H_{24}$	204.3511	2.24	99
16	14.371	Elemicin	$C_{12}H_{16}O_3$	208.2536	14.29	99
17	14.776	γ-Cadinene	$C_{15}H_{24}$	204.3511	0.96	96
18	15.993	Heptadecane	$C_{17}H_{36}$	240.4677	1.24	98
19	18.907	Palmitic acid	$C_{16}H_{32}O_2$	256.4241	0.97	96

Appendix B: Plots of GC-MS Analysis of the Essential oil at different retention time

User Chromatograms

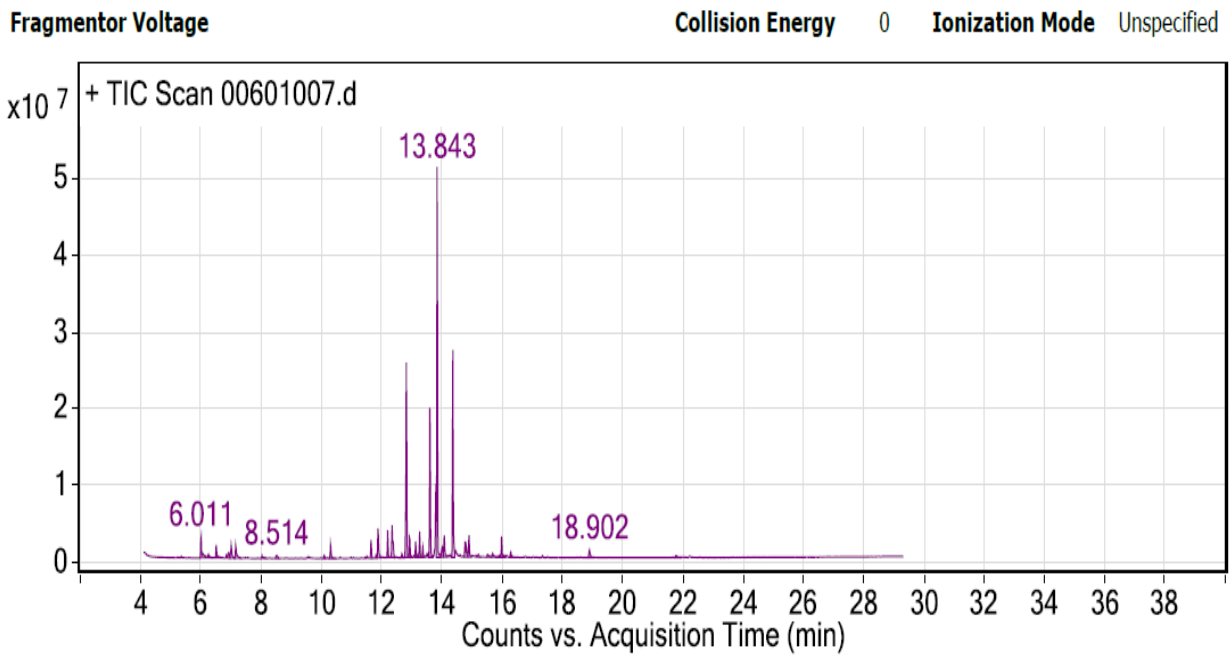


Figure B.1: TIC at first peak Retention time from 6.011-18.902 min

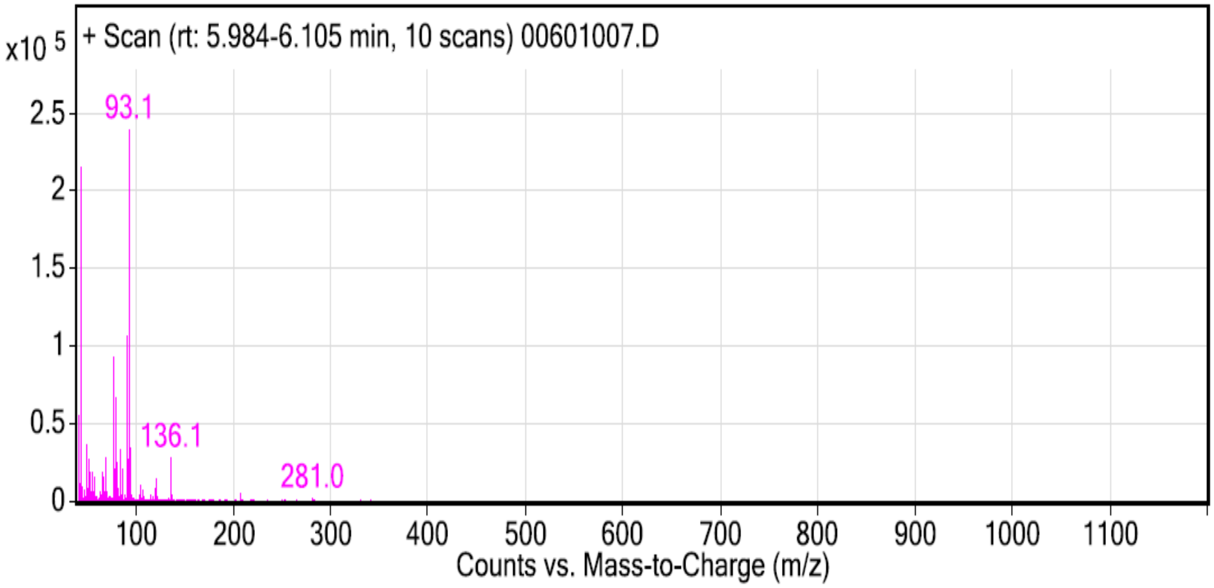


Figure B.2: TIC of Retention Time of = 5.984 – 6.105 min

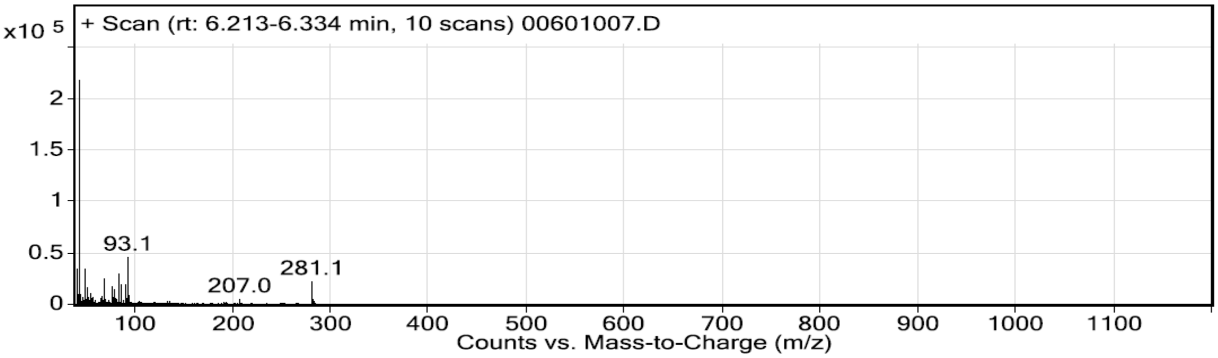


Figure B.3: Retention time of 6.213- 6.334 min

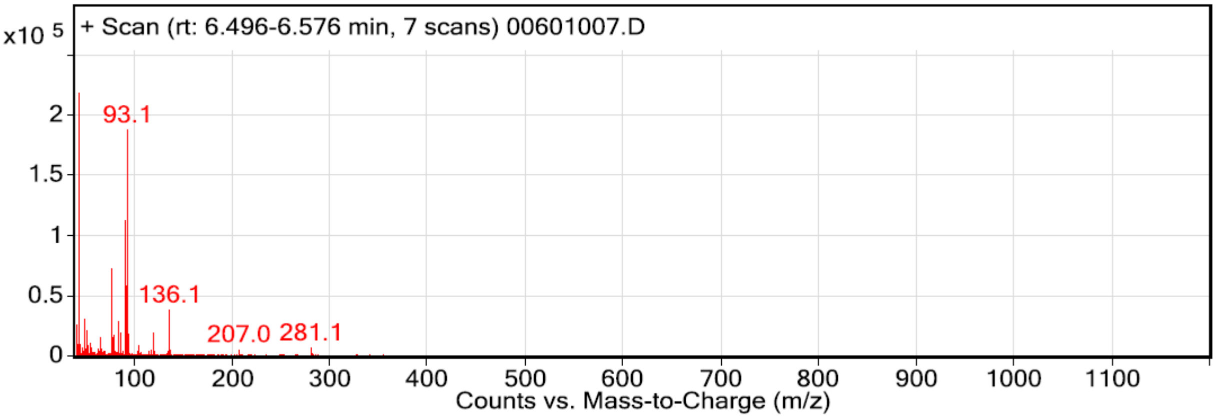


Figure B.4: Retention time of 6.496- 6.576 min

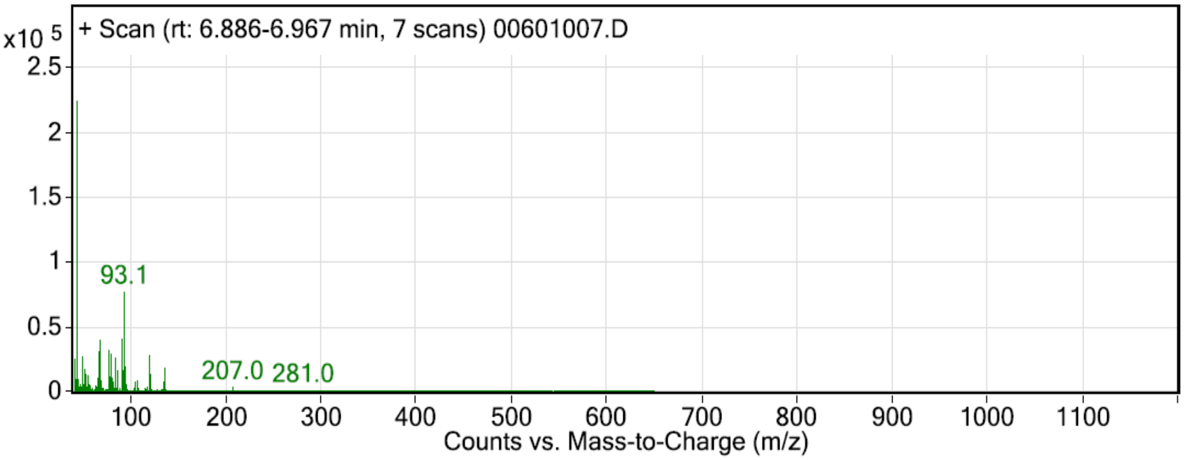


Figure B.5: TIC at first peak Retention time from 6.886-6.967 min

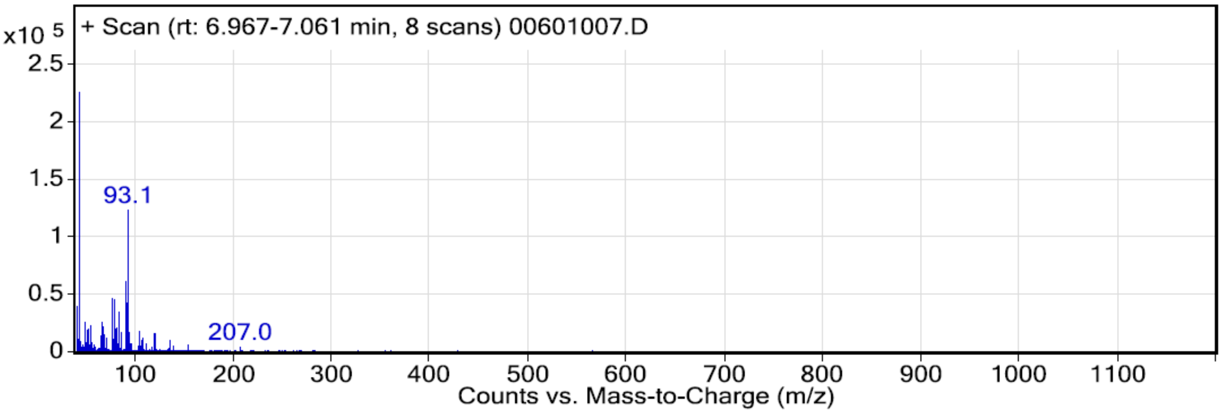


Figure B.6: TIC at first peak Retention time from 6.967-7.061 min

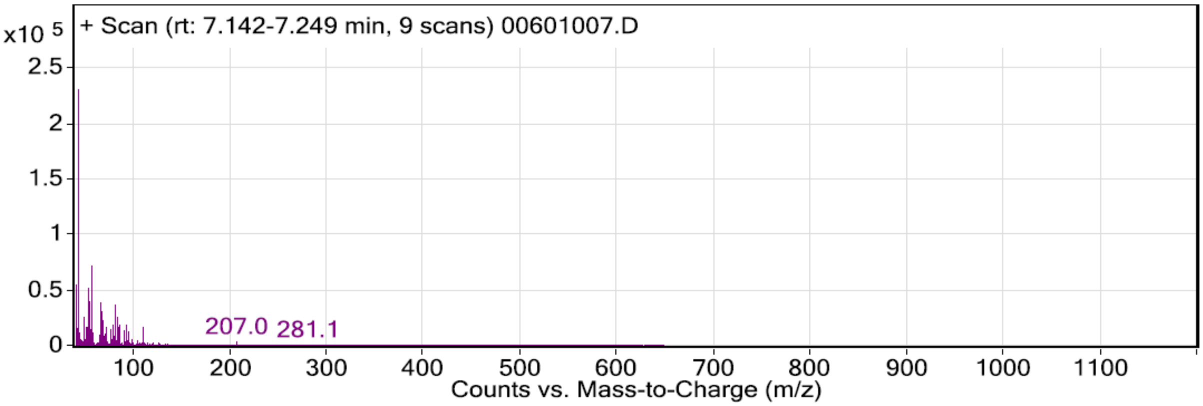


Figure B.7: TIC at first peak Retention time from 7.142-7.249 min

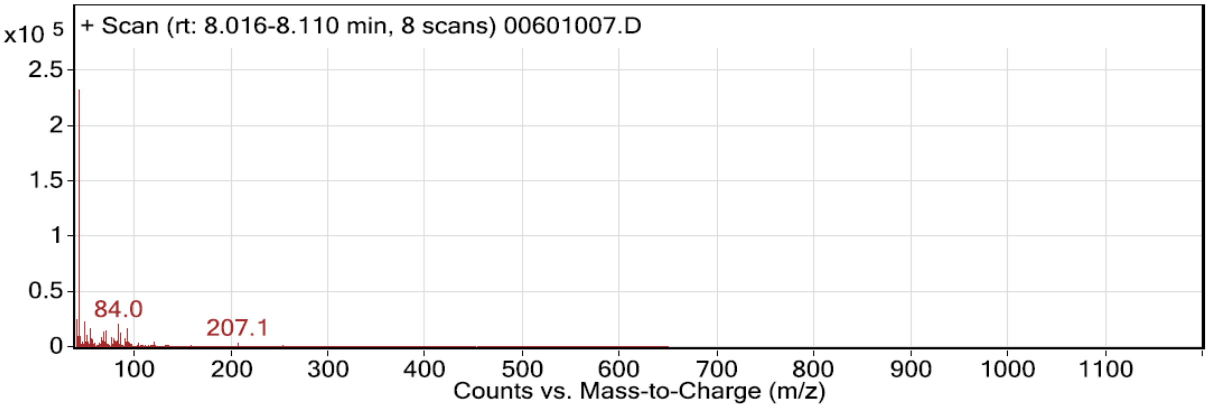


Figure B.8: TIC at first peak Retention time from 8.01-8.110 min

Table B.1: Integration peak list

Peak	Start	RT	End	Height	Area	Area %
1	5.972	6.011	6.159	2704919.22	7596877.92	9.1
2	6.213	6.253	6.388	458776.67	1558897.42	1.87
3	6.469	6.509	6.671	1653933.24	3610924.17	4.33
4	6.886	6.913	6.967	699639.42	2398239.59	2.87
5	6.967	7.007	7.128	1695435.85	4743278.46	5.68
6	7.128	7.155	7.33	1678060.07	4769473.81	5.71
7	7.989	8.03	8.184	329289.64	938835.42	1.12
8	8.466	8.514	8.597	370013.97	1419469.68	1.7
9	10.055	10.089	10.156	392533.6	864532.29	1.04
10	10.25	10.304	10.479	1813862.02	3899942.26	4.67
11	11.61	11.649	11.716	2371225.26	4148634.78	4.97
12	11.838	11.878	12.05	3831762.35	8869248.7	10.62
13	12.171	12.201	12.252	3506376.06	5742891.07	6.88
14	12.282	12.349	12.43	4167185.92	11023663.1	13.21
15	12.618	12.672	12.753	518666.78	1177692.39	1.41
16	12.753	12.82	12.901	25354653.5	48999715.79	58.7
17	12.901	12.928	13.049	2573224.5	5400997.87	6.47
18	13.103	13.13	13.17	2102398.5	3552793.54	4.26
19	13.224	13.264	13.305	3385619.5	6000942.47	7.19
20	13.305	13.372	13.412	1284360.5	2615111.92	3.13
21	13.574	13.601	13.708	19463015.5	37593837.3	45.03
22	13.816	13.843	13.964	50722279.88	83480659.89	100
23	13.964	14.018	14.045	1229507.81	3704798.61	4.44
24	14.045	14.085	14.173	2758769.72	6703308.93	8.03
25	14.327	14.368	14.448	26903723	47130870.64	56.46
26	14.731	14.771	14.866	1991802.38	6527032.68	7.82
27	14.866	14.906	14.987	2786394.2	5883207.24	7.05
28	15.162	15.215	15.269	327620.29	967861.31	1.16
29	15.465	15.525	15.646	322865.26	1320751.68	1.58
30	15.646	15.686	15.807	464483.44	1550236.88	1.86
31	15.807	15.929	15.955	264729.3	900385.28	1.08
32	15.955	15.982	16.117	2696785.75	5587437.74	6.69
33	16.225	16.292	16.386	672129.18	1712911.77	2.05
34	18.849	18.902	19.064	978202.05	3317349.92	3.97
35	21.715	21.782	21.944	236747.8	1150915.34	1.38

Appendix C: Formulas used to characterize the physic-chemical properties

C.1: Percentage yield from 100 grams of sample

$$\text{Percentage yield of extracted oil} = \frac{\text{mass of oil (in gm)}}{\text{mass of sample (in gm)}} * 100\%$$

C.2: Moisture content

$$\text{Moisture Content (\%)} = \frac{\text{W2}}{\text{W1}} * 100$$

Where: W1= weight of the sample feed into the oven

W2 = loss of weight

C.3: Specific gravity

$$\text{Specific Gravity} = \frac{\text{density of the oil}}{\text{density of the water}}$$

C.4: Kinematics viscosity

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

Where μ = Dynamic Viscosity and

ρ = Density of oil

C.5: Saponification value

$$\text{Saponification value (S.V)} = 56.1 * \frac{N * (B - S)}{W}$$

Where: B = the volume in ml of standard HCl required for blank test

S = the volume in ml of standard HCl required for the Sample

N = Actual normality of the HCl used

W = Weight of oil taken in gram

C.6: Acid value

$$\text{Acid value (AV)} = 56.1 * \frac{N * V}{W}$$

Where: V = volume of standard potassium hydroxide (ml),

N = Normality of potassium hydroxide solution

W = sample weight (in g)

C.7: Free Fatty Acid

$$\text{FFA as oleic acid} = 28.2 * \frac{N * V}{W} \text{ percent by weight}$$

Where: V = volume of standard potassium hydroxide (ml),

N = Normality of potassium hydroxide solution

W= sample weight (in g)

C.8: Iodine value

$$\text{Iodine value (I.V)} = 12.69 * \frac{N * (B - S)}{W}$$

Where: N = Normality of standard sodium thiosulphate used;

B = Volume in ml of sodium thiosulphate used for blank;

S = Volume in ml of sodium thiosulphate used for determination,

W = Mass of the sample.

Appendix D: Tables from Design Expert

Table D.1: The design layout of the experiments designed by design expert software

Std.	Run	Block	Factor 1 A:Temperature ◦C	Factor 2 B: Extraction Time Hours	Factor 3 C:Particle size mm	Response 1 % yield
52	1	Block 1	95.00	6.00	2.00	0.38
35	2	Block 1	95.00	2.00	2.00	0.25
48	3	Block 1	75.00	6.00	2.00	0.32
33	4	Block 1	85.00	2.00	2.00	0.34
45	5	Block 1	95.00	4.00	2.00	0.33
1	6	Block 1	75.00	2.00	1.00	0.31
20	7	Block 1	75.00	6.00	1.00	0.33
32	8	Block 1	85.00	2.00	2.00	0.28
8	9	Block 1	95.00	2.00	1.00	0.36
43	10	Block 1	95.00	4.00	2.00	0.36
49	11	Block 1	85.00	6.00	2.00	0.34
25	12	Block 1	95.00	6.00	1.00	0.39
18	13	Block 1	95.00	4.00	1.00	0.41
6	14	Block 1	85.00	2.00	1.00	0.41
17	15	Block 1	95.00	4.00	1.00	0.36
53	16	Block 1	95.00	6.00	2.00	0.28
13	17	Block 1	85.00	4.00	1.00	0.43
28	18	Block 1	75.00	2.00	2.00	0.27
21	19	Block 1	75.00	6.00	1.00	0.4
10	20	Block 1	75.00	4.00	1.00	0.37
27	21	Block 1	95.00	6.00	1.00	0.41
24	22	Block 1	85.00	6.00	1.00	0.41
5	23	Block 1	85.00	2.00	1.00	0.39
2	24	Block 1	75.00	2.00	1.00	0.34
42	25	Block 1	85.00	4.00	2.00	0.37
34	26	Block 1	95.00	2.00	2.00	0.28
14	27	Block 1	85.00	4.00	1.00	0.39
15	28	Block 1	85.00	4.00	1.00	0.42
11	29	Block 1	75.00	4.00	1.00	0.33
38	30	Block 1	75.00	4.00	2.00	0.26
3	31	Block 1	75.00	2.00	1.00	0.29
9	32	Block 1	95.00	2.00	1.00	0.32
54	33	Block 1	95.00	6.00	2.00	0.35
40	34	Block 1	85.00	4.00	2.00	0.34
22	35	Block 1	85.00	6.00	1.00	0.4

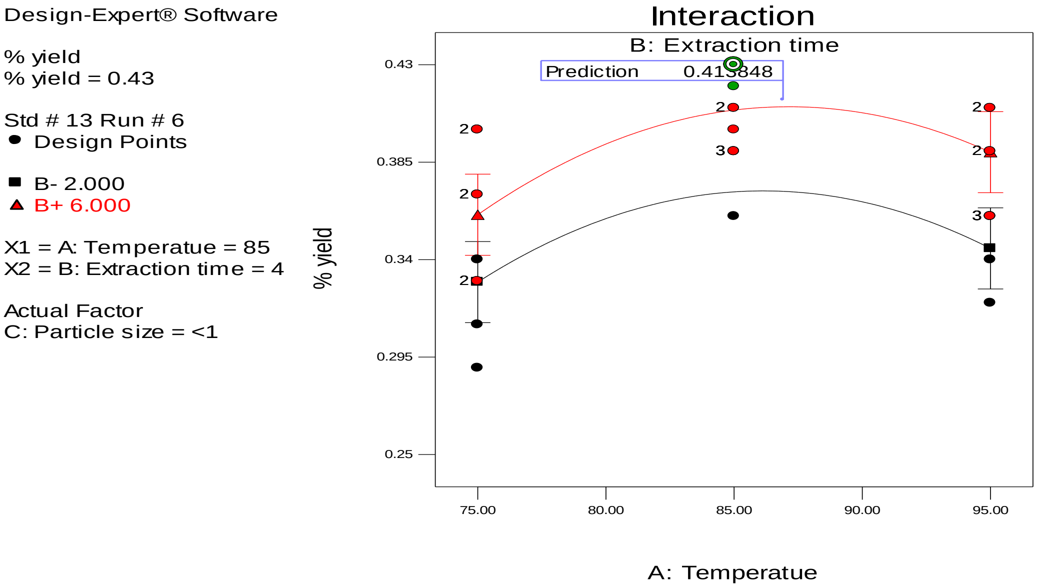
47	36	Block 1	75.00	6.00	2.00	0.26
12	37	Block 1	75.00	4.00	1.00	0.4
36	38	Block 1	95.00	2.00	2.00	0.32
41	39	Block 1	85.00	4.00	2.00	0.3
30	40	Block 1	75.00	2.00	2.00	0.25
31	41	Block 1	85.00	2.00	2.00	0.31
7	42	Block 1	95.00	2.00	1.00	0.34
46	43	Block 1	75.00	6.00	2.00	0.3
50	44	Block 1	85.00	6.00	2.00	0.31
37	45	Block 1	75.00	4.00	2.00	0.3
23	46	Block 1	85.00	6.00	1.00	0.39
4	47	Block 1	85.00	2.00	1.00	0.36
44	48	Block 1	95.00	4.00	2.00	0.27
29	49	Block 1	75.00	2.00	2.00	0.3
51	50	Block 1	85.00	6.00	2.00	0.36
16	51	Block 1	95.00	4.00	1.00	0.39
26	52	Block 1	95.00	6.00	1.00	0.36
39	53	Block 1	75.00	4.00	2.00	0.32
19	54	Block 1	75.00	6.00	1.00	0.37

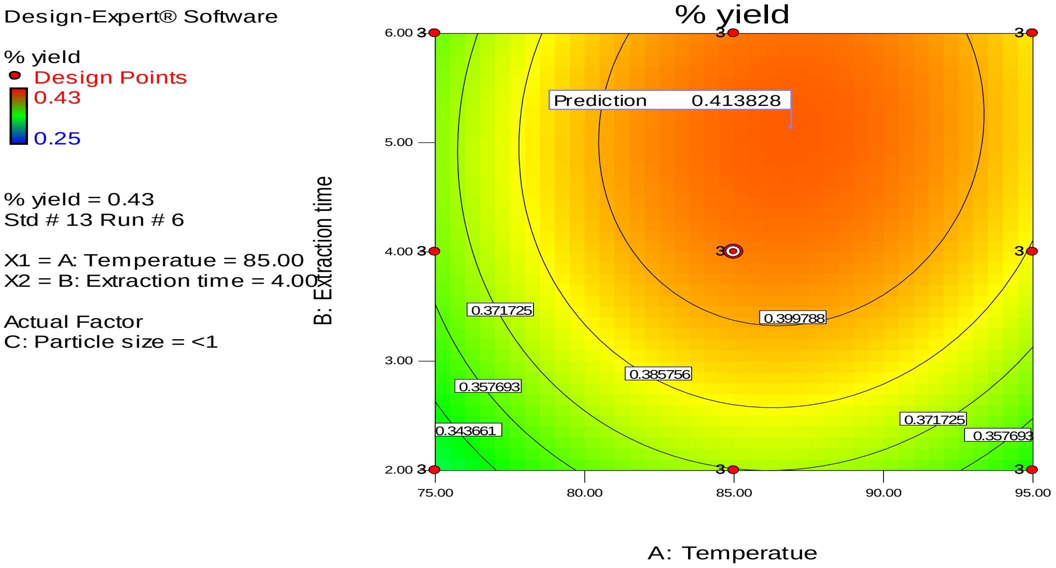
Table D.2: Difference between the experimental (actual) value and predicated value

Standard Order	**Actual Value**	**Predicted Value**	**Residual**
1	0.31	0.33	-0.020
2	0.34	0.33	9.907E-003
3	0.29	0.33	-0.040
4	0.40	0.39	8.519E-003
5	0.43	0.39	0.039
6	0.37	0.39	-0.021
7	0.36	0.38	-0.018
8	0.39	0.38	0.012
9	0.41	0.38	0.032
10	0.37	0.36	0.012
11	0.33	0.36	-0.028
12	0.40	0.36	0.042
13	0.43	0.41	0.016
14	0.39	0.41	-0.024
15	0.42	0.41	5.741E-003
16	0.39	0.40	-5.648E-003
17	0.36	0.40	-0.036
18	0.41	0.40	0.014

19	0.37	0.36	7.685E-003
20	0.33	0.36	-0.032
21	0.40	0.36	0.038
22	0.43	0.41	0.016
23	0.39	0.41	-0.024
24	0.42	0.41	6.296E-003
25	0.39	0.39	-9.259E-005
26	0.36	0.39	-0.030
27	0.41	0.39	0.020
28	0.27	0.26	7.870E-003
29	0.30	0.26	0.038
30	0.25	0.26	-0.012
31	0.31	0.31	-4.074E-003
32	0.28	0.31	-0.034
33	0.34	0.31	0.026
34	0.28	0.29	-0.011
35	0.25	0.29	-0.041
36	0.32	0.29	0.029
37	0.30	0.29	6.759E-003
38	0.26	0.29	-0.033
39	0.32	0.29	0.027
40	0.34	0.34	-1.852E-004
41	0.30	0.34	-0.040
42	0.37	0.34	0.030
43	0.32	0.31	7.870E-003
44	0.28	0.31	-0.032
45	0.35	0.31	0.038
46	0.30	0.30	-1.019E-003
47	0.26	0.30	-0.041
48	0.32	0.30	0.019
49	0.34	0.34	-2.963E-003
50	0.31	0.34	-0.033
51	0.38	0.34	0.037
52	0.32	0.31	0.010
53	0.28	0.31	-0.030
54	0.35	0.31	0.040

Appendix E: Plots from design expert software





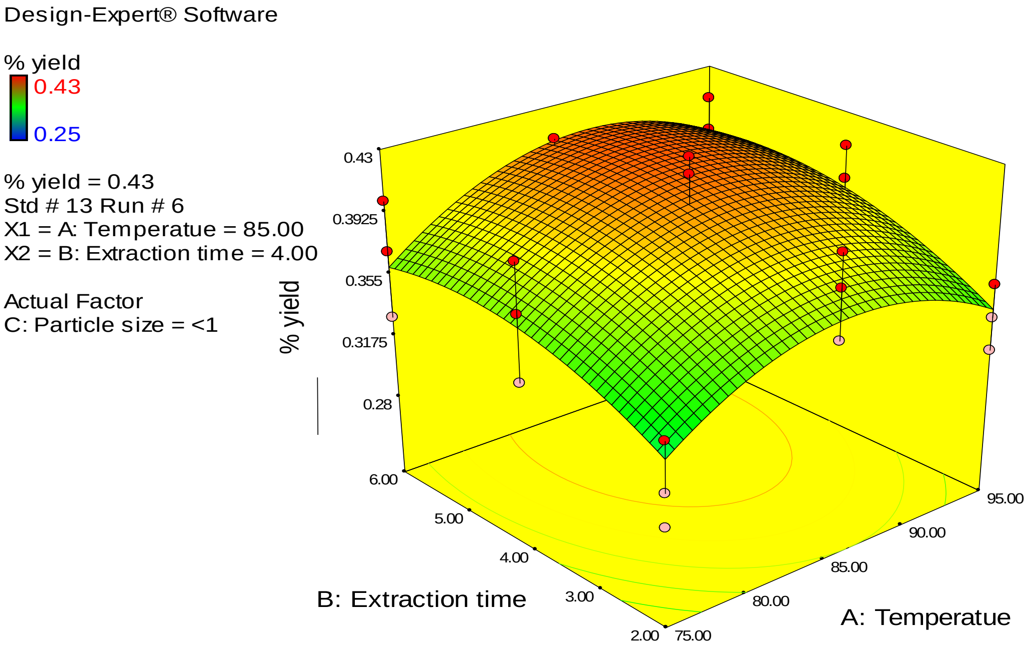
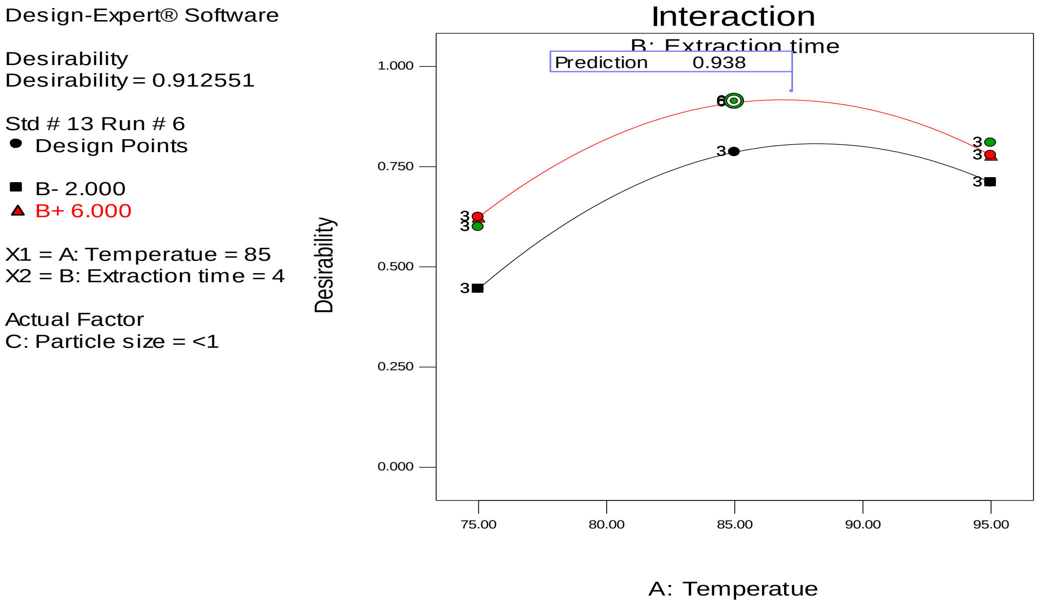


Figure E.1: Plot of Optimum Yield Prediction



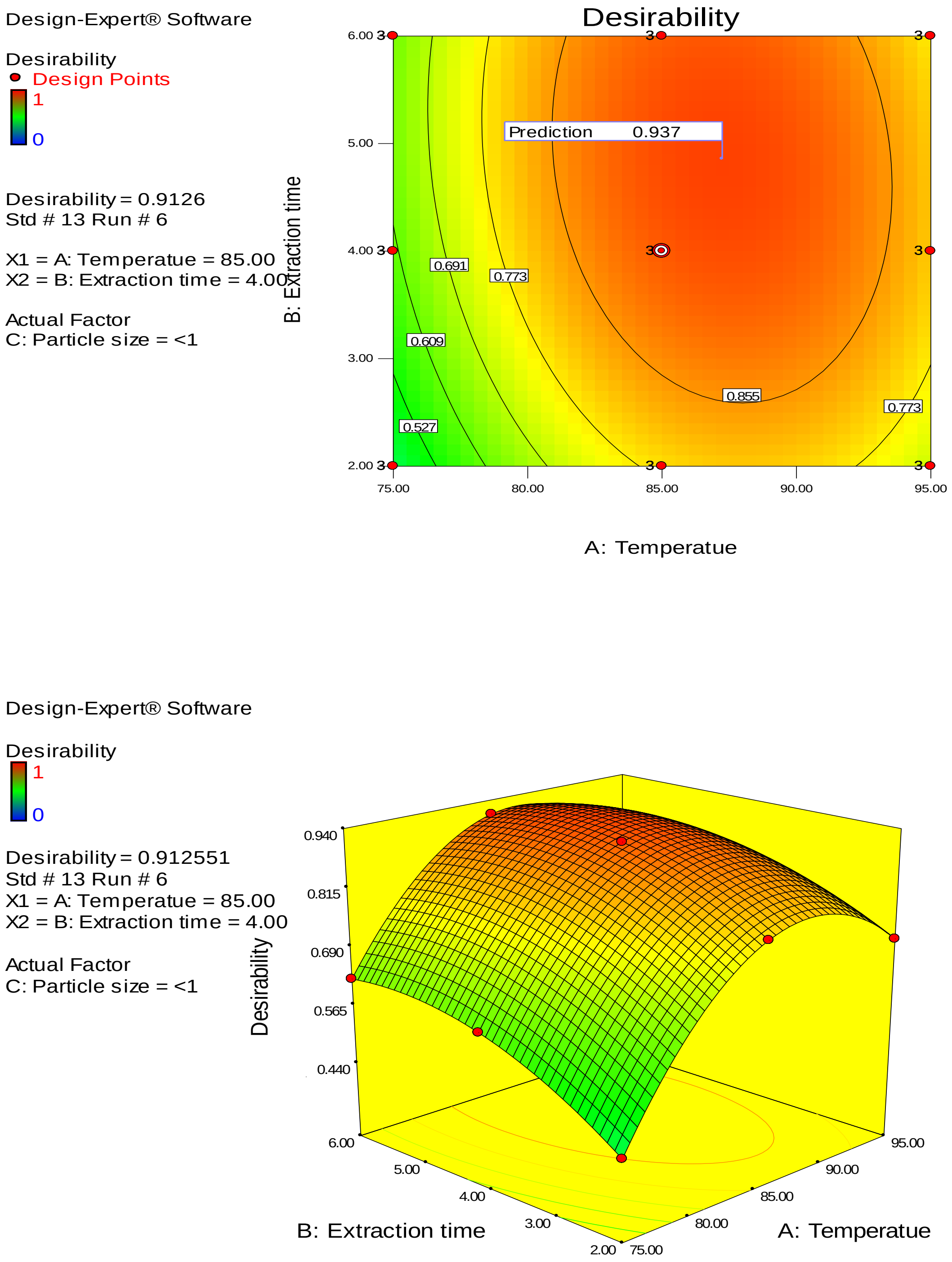


Figure E.2: Plot of Optimum Desirability Prediction

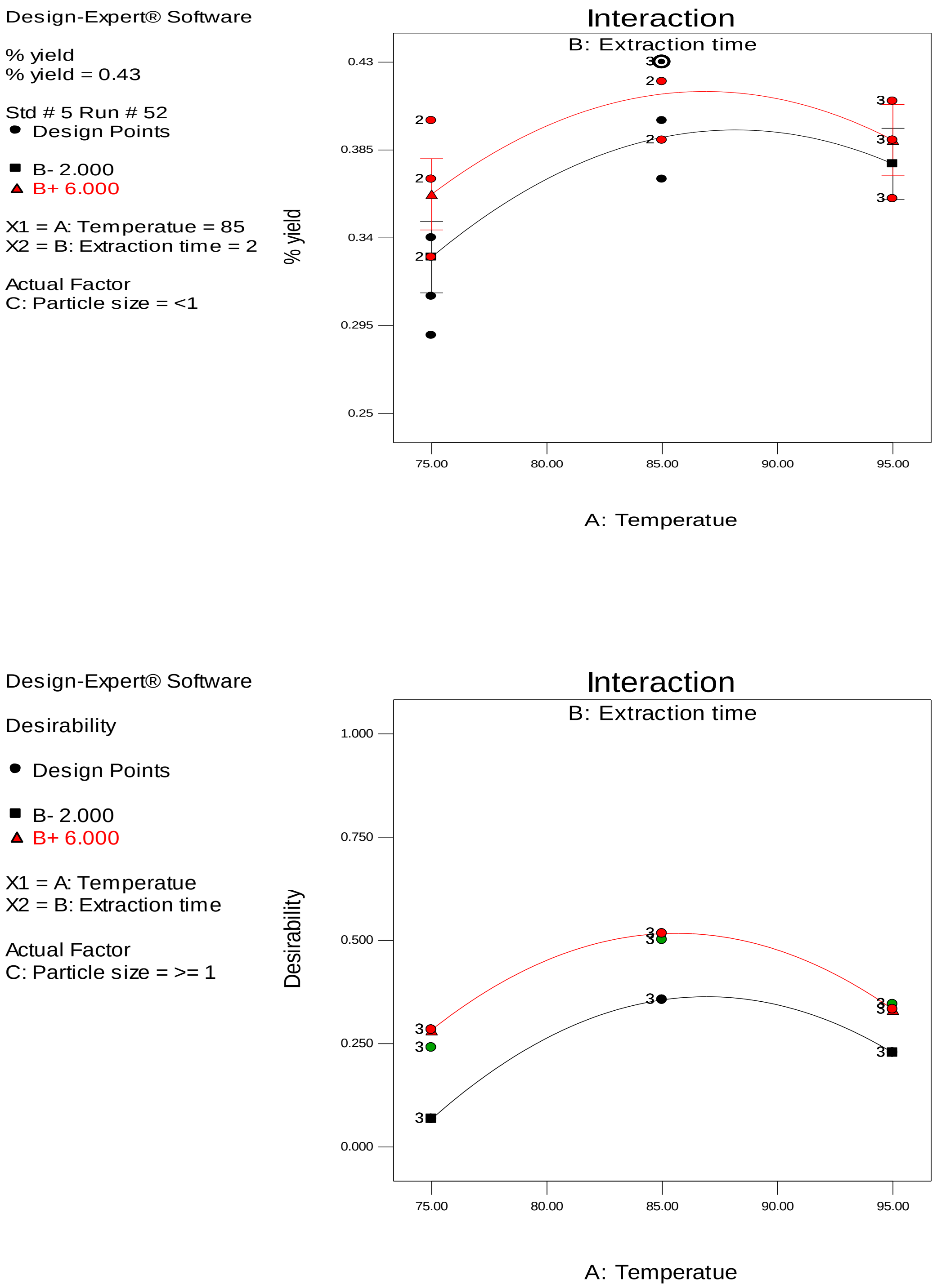


Figure E.3: Yield and Desirability of the Interaction Effects of process parameters

Appendix F: Experimental conducted Report from LIDI

Appendix G: Samples photos and Laboratory equipments

Figure G.1: Plant material & cross beater miller

Figure G.2: Sieves analysis and samples of different particle size for extraction

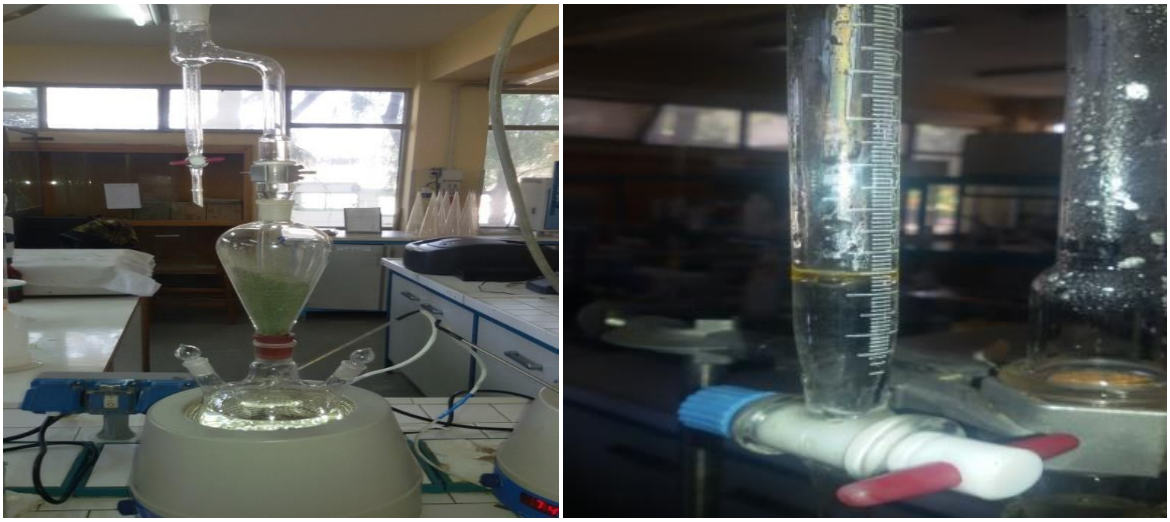


Figure G.3: Laboratory set up and product collection

Figure G.4: Essential oil extracted from leaves of *Ocimum Lamiifolium*