



ADDIS ABABA UNIVERSITY

ADDIS ABABA INSTITUTE TECHNOLOGY

SCHOOL OF CHEMICAL AND BIO ENGINEERING

PROCESS ENGINEERING STREAM

**EXTRACTION, OPTIMIZATION AND CHARACTERIZATION OF
LEMONGRASS ESSENTIAL OIL**

BY

SOLOMON TADELE FEREDE

July, 2021

ADDIS ABABA, ETHIOPIA



ADDIS ABABA UNIVERSITY
ADDIS ABABA INSTITUTE TECHNOLOGY
SCHOOL OF CHEMICAL AND BIO ENGINEERING
PROCESS ENGINEERING STREAM
EXTRACTION, OPTIMIZATION AND CHARACTERIZATION OF
LEMONGRASS ESSENTIAL OIL

A Thesis Submitted to the Graduate Studies of Addis Ababa
University in Partial Fulfillments of the Requirements for the Degree
of Masters of Science in Chemical Engineering (Process Stream)

BY

Solomon Tadele Ferede

Advisor: Dr. Nurelegne Tefera (Ass. Prof)

July, 2021

ADDIS ABABA UNIVERSITY

ADDIS ABABA INSTITUTE TECHNOLOGY

SCHOOL OF CHEMICAL AND BIO-ENGINEERING

***EXTRACTION, OPTIMIZATION AND CHARACTERIZATION OF
LEMONGRASS ESSENTIAL OIL***

BY

SOLOMON TADELE

Approved by the examining committee members:

	Name	signature	date
Advisor	Dr. <u>Nurelegne Tefera (Ass. Prof)</u>	_____	_____
Internal Examiner	<u>Dr. Shegaw Ahmed</u>	_____	_____
External Exminer	<u>Prof. Belay Woldeyes</u>	_____	_____
Department Dean	<u>Dr. Abubeker Yimam (Ass. Prof)</u>	_____	_____

Abstract

*Herbal plant extracts are used in a variety of commercial sectors, including the pharmaceutical, food, and chemical industries, have received renewed attention in recent decades. The Ethiopian highlands have a suitable environment for the cultivation of essential oil herbs. Among essential oil source plants, lemongrass (*Cymbopogon citratus*) is one of the most widely cultivated in the country, which is readily available at the Wondogenet agricultural research center. This study mainly covers the extraction, optimization, and characterization of lemongrass essential oil. The extraction process was done by steam distillation with concentrations of yield and quality of products. Three selected factors: raw material particle size, weight of raw per batch and extraction time with constant temperature and steam supply were employed. The optimized result of the extraction process was a raw material particle size of 10cm, a raw material mass per batch of 1kg, an extraction time of 62 min, yield of 0.755% and the result was verified. The three lemongrass varieties, Wg lomisar java, Wg lomisra UA, and Wg lomisar (I), were used in the experiment. Among these, Wg Lomisar (I) was the best variety in terms of yield and quality. Physico-chemical characterization results: specific gravity of 0.907, refractive index of 1.4824, kinematic viscosity of 3.1643 μ , functional group identification by FT-IR, and compositional analysis was done by gas chromatography and mass spectroscopic (GC-MS) methods. Among the major components of the GC-MS analysis were Citral (28.4227%), Beta.-Myrcene (11.6291%), Heptadecane, 9-octyl (7.7451%), and Docosanoic acid, methyl and ester (5.701%) respectively. These physico-chemical result attributes indicate that the product would have a significant role in cosmetic and perfumery activities.*

Key words: *Citral, *Cymbopogon citratus*, lemongrass, steam distillation, Wg lomisar (I)*

Acknowledgment

First of all, I would like to express my special gratitude to my advisor, (Associate Professor) Nurelegne Tefera (PhD), for his valuable support, guidance, constructive criticism and encouragement during every stage of this worked thesis. I want to give my great respect to my family, friends and **Tana Drilling and Industry plc.** They gave me unreserved support to achieve my dream goal. I am grateful to Mr. Hintsu for his contribution to the successful laboratory session. I would like to express my heartfelt gratitude to Ethiopia's agricultural research institute and the wondogenet agriculture research center for providing an efficient and effective service with a critical variety of information and sufficient raw materials for the experiments' completion.

Table of Contents

Abstract.....	iv
Acknowledgment.....	vi
List of Tables	iv
List of figures.....	v
List of Abbreviations	vi
1. Introduction.....	1
1.1. Background.....	1
1.2. Problem Statement	2
1.3. Objective	3
1.3.1. General objective	3
1.3.2. specific objectives of the research.....	3
1.4. Scope of the study	3
1.5. Significance of the study.....	3
2. Literature review	5
2.1. General discription of essential oil.....	5
2.2. Application of essential oil	5
2.3. Uniqueness of essential oils	8
2.3.1. Volatile nature of essential oils	8
2.3.2. Aromatic nature of essential oils.....	8
2.4. Factors that affects essential oil quality and quantity	9
2.4.1. Essential oil extraction methods	9
2.5. Lemongrass essential oil attributes	15
2.5.1. Selected Lemongrass essential oil extraction method.....	16
2.5. 2. Important properties of lemongrass essential oil	17
2.5.3. Characterizations of lemongrass essential oils.....	19
2.6. Applications of lemongrass essential oil.....	21
2.7. Safety aspects for essential oil using.....	22
3. Materials and methods	23
3.1. Materials	23
3.2. Methods.....	23
3.2.1. Lemongrass raw material collection and preparation	23
3.2.2. lemongrass raw material particle size reduction	24
3.2.3. Lemongrass raw material moisture content determination	24
3.2.4. Lemongrass essential oil extraction Procedure	25

3.2.5.1. Physical property characterization	27
3.2.5.2. Chemical property characterization.....	28
3.3. Experimental design and statistical analysis	28
4. Result and discussion	31
4.1. Lemongrass raw material characterization.....	31
4.1.1. Moisture content determination	31
4.2. Wg Lemongrass Essential Oil Variety Selection	31
4.3. Lemongrass essential oil extraction	33
4.3.1. Model aduquacy check.....	33
4.3.2. Analysis of variance (ANOVA) response percent yield	35
4.3.4. The single and interaction effects of process variable	37
4.5.1. Determinations of functional group by FT-IR Analysis	47
5. Conclusion and recommendation.....	52
5.1 Conclusions.....	52
5.2. Recommendations.....	53
Reference	54
Appendix.....	58

List of Tables

Table 3.1: Factorial design fo lemongrass essential oil extraction.....	28
Table 4.1: Moisture content measurment results.....	30
Table 4.2: Wg Lemongrass Variety Extraction results.....	30
Table 4.3. Lemongrass essential oil extraction yield	32
Table 4.5: Analysis of variance (ANOVA) regression model equation and coefficients.....	35
Table 4.6: Input concestraints for optimzation of lemongrass essential oil extraction.....	43
Table 4.6. Verification of the optimized result	44
Table 4.7: Physical property standard comparison result summery.....	45
Table 4.8 :Functional group FT-IR Analysis result.....	46
Table 4.6: Lemongrass essential oil (Wg Lomisrar (I) main Chemical composition.....	48

List of figures

Figure 2.1: <i>Industries and product categories that use essential oils</i>	7
Figure 2.2: Block flow diagram extraction lemongrass essential oil.....	10
Figure 2.3: Solvent extraction process flow diagram.	13
Figure 2.4: The supercritical CO ₂ extraction flow diagram	15
Figure 3.1: Wg Lomisar varieties image.....	23
Figure 3.2: Lemongrass essential oil extraction process flow diagram.....	24
Figure 4.1. Predicted vs Actual plot lemongrass essential oil experiment results	33
Figure 4.1: The normal vs Residual plot lemongrass essential oil experimental results.....	34
Figure 4.3: The effect of raw material particle size on lemongrass essential oil.....	37
Figure 4.4: The effects of raw material weight per batch on lemongrass essential oil	38
Figure 4.5: The effect of extraction time on lemongrass essential oil.....	39
Figure 4.6: The interaction effects particle size and weight raw material	40
Figure 4.7: The interaction effects particle size and extraction time.....	41
Figure 4.8: The interaction effects of raw material weight and extraction time.....	42
Figure 4.9: Lemongrass essential oil FT-IR result.....	47
Figure 4.10: GC-MS analysis Lemongrass essential oil result.....	48

List of Abbreviations

BC	Before Birth of Christ
C. Citratus	<i>Cymbopogon citratus</i>
CCD	Central composite design
CI	confidence level
Dv	dynamic viscosity
FT-IR	Fourier transfer infrad radiation
GC- MS	Gas chromatography mass spectrometry
GTP	Growth and transformation plan
Kv	kinematic viscosity
LG	Lemongrass
RSM	Response Surface Methodology
SFE	supercritical fluid extraction
Sp.g	Specific gravity
TM	Traditional medicine
ρ	density

1. Introduction

1.1. Background

Extracting and using essential oil dates back to 2000 BC in China, Greece, Egypt, and Indian civilizations. People use essential oils for the emotional, physical and spiritual wellbeing of man (Hans, 2011). Herbal essential oils have recently gained popularity in aromatherapy. It is a field of alternative medicine that asserts essential oils and other aromatic compounds have therapeutic effects for a range of skin ailments, tissue, and have no adverse effects like synthetic medications. (Sarkic & Stappen,2018).

The lemongrass (*C.citrus*) comprises the aromatic therapeutic plant It is a hardy perennial tropical with a long, thin, and well-kept grass plant that thrives in warm, semi-warm, and temperate climates. *Cymbopogon* is derived from the Greek words "kymbe" (boat) and "pogon" (beard), which refer to the flower's spike. *Citrus* comes from the Latin word *citrus*, which meaning "lemon-scented leaves." *Cymbopogon citratus* is known in Mexico as "zacate limon" (lemongrass) or limon, and it is used as an infusion in water or milk simply because herb consumption is a social norm. (María del Carmen, et, al. 2015).

Lemongrass essential oil is collected from the leaves, which contain the majority of the oil glands, and is one of the most popular sources of essential oil. The quality and quantity of lemongrass essential oil yield and extraction processes are influenced by growing region, soil type, climatic conditions, species type, and maturity level. The extraction procedure, for example, has a significant impact on the output and quality of essential oils. The extraction process should be chosen depending on the product yield and quality requirements. Hydro and steam distillation, absolute, cold pressing, solvent, microwave assisted hydrodistillation, supercritical fluid carbon dioxide extraction and large scale chromatography are some of the essential oil extraction methods (Dinh Nhat Do, 2021).

Steam distillation is now the most prevalent method for extracting lemongrass essential oil. It is mostly used for temperature-sensitive compounds that are insoluble in water and may breakdown near their boiling point, such as oils, resins, and hydrocarbons. It is also commonly used to extract other essential oils. Steam is passed through the dried or fresh plant material. It has the advantage of softening the cells and allowing the essential oil to escape as a vapour. The steam temperature should be high enough for the oil to vapourize, but an

extreme rise in temperature could damage the plant material or cause the essential oils to burnt [Ewa Majewska,et al, 2019].

Lemongrass essential oil has a wide range of biological properties, including anti-inflammatory and antioxidant, anti-diabetic, hypolipidemic, renoprotective and cardio protective, anticancer, antiseptic, febrifuge, antidyspeptic, carminative, tranquilizer, pain balm, and stomachic properties. It's present in perfumes, cosmetics, soaps, detergents, and chocolates, as well as synthetic Vitamin A products. It has an industrial profile in beverages, food, fragrance, household products, personal care, such as deodorants, herbal tea, skin care, insect pesticides, plant pesticides, and other pharmaceutical products (Braithair, 2016).

The most important component of lemon grass (*Cymbopogon citratus*) is citral, a natural mixture of isomers Neral and geranial, two isomeric aldehydes (Muhammad, 2019). Due to the high citral concentration, the oil has a pronounced lemon-like odor (75-90 percent). Commercially, a minimum of 70 percent citral content is required. The lemongrass essential oil color ranges from reddish-yellow to reddish-brown (Vanish & Hena, 2012).

1.2. Problem Statement

Essential oil ingredients are being cultivated and exported commercially in Ethiopia, but due to a lack of knowledge and skill during cultivation and production, the sector has a smaller impact on the domestic market than it does on the international market. Despite the fact that essential oil-source raw materials are cultivated and oil is being produced in the country, the product is limited in quantity and quality, which is why the sector has no influence on private and government investment participation.

Essential oils are deeply incorporated into the lives of people in industrialized countries, and Ethiopia aims to be one of the world's most promising developing nations, as evidenced by the country's interest in planning two consecutive growth transformation plans (GTP I and II), which primarily concern the establishment of agricultural industries. To use such business opportunities, the involvement of capable researchers will help to line up the implementing mechanisms.

Ethiopians are fortunate to have a diverse flora, which is important in the manufacturing of essential oils. According to the study, there are between 6,500 and 7,000 plant species available. Among the 600-1000 aromatic plants that can be harvested and used in various

purposes, 10-12 percent are endemic. Lemongrass (*Cymbopogon citratus*) is one of the most widely available essential oil plants in the country with a similar large number of plants. However, the development of these types of industries, namely the food, therapeutic, soap, and perfumery industries has remained an untapped business area throughout the country; this work will empower the sector by providing organized chemical and biological potential information for investors.

1.3. Objective

1.3.1. General objective

The major goal of this research was extracting of essential oil from three different variety of lemongrass and characterization of the product from the selected variety.

1.3.2. specific objectives of the research

- To characterize three varieties of lemongrass raw materials
- To extract essential oil from lemongrass
- To optimize the three extraction factors, such as particle size of raw material, raw material weight per batch and extraction time,
- To characterize the selected essential oil using different characterization techniques.

1.4. Scope of the study

The primary focus of the study is extraction, optimization, and characterisation of lemongrass leaves gathered from a single area (Wondogenet). Steam distillation was used for extraction, and quantitative and qualitative methods were used to select the variety. Functional group and content analyses were performed, as well as qualitative characterisation using FT-IR and GC-MS. The maturity degree of lemongrass leaves was not investigated.

1.5. Significance of the study

The The finding of the research is essential in providing lemongrass essential oil information for government, investors, and beneficiary organs. In this research work, lemongrass variety selection was done based on the best quantitative and qualitative results. Wg Lemongrass (I) is the best intermes of quantity and quality of product, which can be cultivated in a commercial crop for lemongrass essential oil markets, either through contract farming or individual. This will encourage participation and give a clear insight into further research work with scholars by providing reliable data, information, and knowledge in the field.

Investors will be attracted to this unexploited business sector to establish small and large-scale lemongrass essential oil industries in the country.

2. Literature review

2.1. General description of essential oil

Essential oil is a highly concentrated, hydrophobic (impervious to water), lipophilic (oil or fat soluble) liquid for plants that contains very unstable aroma compounds and carries the scent, flavor, or essence of the plant. Ethereal oils, often known as volatile oils, are a broad category of oils. Essential oils can be found in many different areas of the plant. (Abdurahman, et.al, 2013). Essential oils are volatile oils obtained from plants and named by the plant from which they are taken. Plant oil can be extracted in a variety of methods. Expression, solvent extraction, effleurage, and steam distillation (both water and steam distillation). maceration, to name a few. All of these are physical processes. Lemongrass, rosemary, bitter bay, lemon, and clove are among the essential oils produced by these methods (Lamdi and colleagues. 2011).

Essential oils are made in the protoplasm of plant cells. They are chemicals excreted by plants that do not occur naturally in their metabolism. They are chemically active and have a lot of energy. As a result, it's difficult to understand why plants lose so much energy that they don't use. In the gland of the plant, the oils are stored as micro droplets. These aroma drops slowly pervades the air as it diffuses through the walls and evaporates. The most odoriferous regions are those in the tropics with the most sun radiation. (National Science Foundation and Industrial Technology Institute (CISIR), 1999).

Plants that are young produce more oil than those that are older. This is due to the oil's higher components continuing to evaporate. older plants is content increased The resinous and darker oil of. Although essential oils are normally insoluble in water but soluble in organic solvents, in some cases (such as rose water and dragger flower water), enough of the oil can be diluted with water to provide the desired effect. a strong odor in the solution (Tajidin, N. E, 2012).

2.2. Application of essential oil

For skin therapy, essential oils are preferred. Essential oils are important because skin ailments are often the surface representation of a deeper condition, such as a buildup of toxins in the blood, hormone imbalance, or neurological and mental issues. The flexibility of essential oils is especially crucial in this market because they can be used to treat a wide

range of skin diseases. They're useful because they can handle a wide range of problems. Essential oils are great for cosmetics and general skin care, as well as the treatment of specific ailments, because they are soluble in oil and alcohol and provide their aroma in water.

Essential oils are commonly used in antiseptics for cuts, insect bites, and spots. Thyme, sage, eucalyptus, tea tree, clove, lavender, and lemon are some of the herbs used. Eczema, infected sores, acne, and bruises can all benefit from anti-inflammatory oils. Lavender, yarrow, and chamomile (Yarrow, lavender, and German and Roman chamomile). Lavender, tea tree, myrrh, patchouli, and sweet orange can help with athlete's foot, candida, ringworm, and other fungal infections.

Burns, cuts, scars, and stretch marks can all benefit from granulation as a stimulating or curative (healing) agent. Lavender, chamomile, roses, neroli, frankincense, and geraniums are among the most popular scents. Excessive sweating and deodorants that clean wounds bergamot, lavender, thyme, juniper, cypress, lemongrass bergamot, lavender, thyme. The general essence of essential oil industrial integration is clearly shown in the following figure (2.1) below.

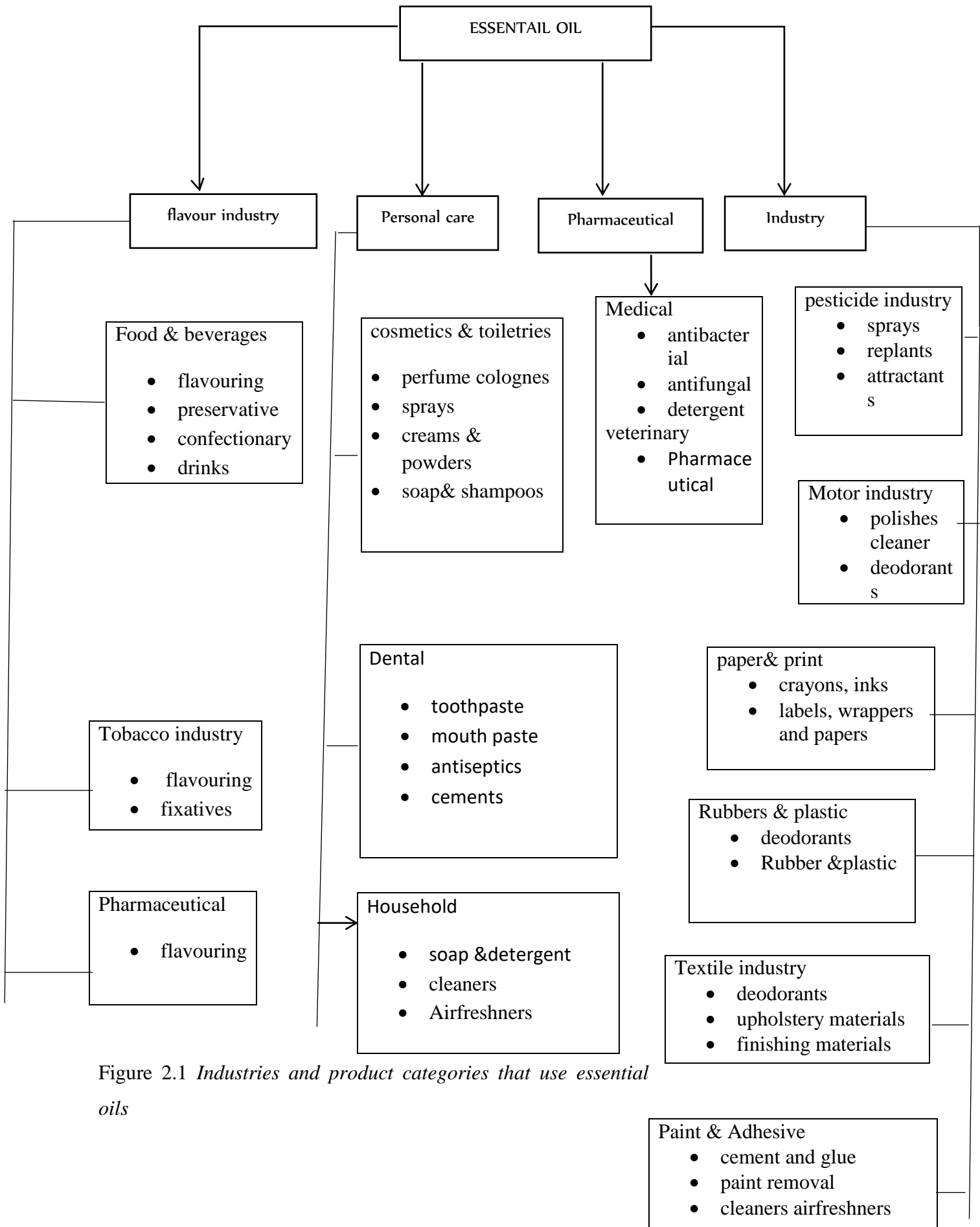


Figure 2.1 Industries and product categories that use essential oils

2.3. Uniqueness of essential oils

The term "essential oils" was first used to describe volatile oils extracted from plants using steam distillation. This definition clearly intends to distinguish between "fatty oils" and essential oils, which are easily volatile. As science evolved, there was a gradual shift in the methods for making the oils, as well as a shift in the ways of employing them. Getting the oils ready A wide spectrum of chemical compounds with varying degrees of volatility were identified in these oils. Essential oils can be categorized into four groups, despite the fact that a list of all known oil components would include a variety of chemically related molecules. Terpenes are a molecule with a straight chain that is linked to isoprene. is a compound with a straight chain not containing any side branches, a Benzene derivative (Guenther, et. al, 1960).

2.3.1. Volatile nature of essential oils

Essential oils are the volatile aromatic components of a broad range of indigenous and exotic plants that have been sold for ages around the world (Yesenofski, 2005). True essential oils are secondary metabolites of plant products, and oils isolated from different portions of the plant may differ in some circumstances (Learmoth et al, 2002).

2.3.2. Aromatic nature of essential oils

Aromatherapy is the therapeutic use of essential oils produced from plants. It enters the body through the olfactory system or the skin. Essential oils have been used commercially for hundreds of years and are commonly used to treat stress and minor illnesses. Numerous studies have shown that olfactory stimulus affects psychological indicators such as blood pressure, electroderm, and mood, as well as skin temperature, skin blood flow, pulse rate, and brain activity. Clinical research, on the other hand, do not back up aromatherapy's therapeutic effects. (Narisa, 2014).

Aromatherapy applications play a multipurpose role in human daily life, with the ability to have a specific, significant impact on human wellbeing, interaction, and relationships. Highly aromatic essential oils have numerous benefits of their own, which can be obtained by simply inhaling them. This can be accomplished by inhaling the aroma from the container or by diffusing it across the room. Diffusing essential oils might be the most effective air purification solution. It will clean the air by erasing metallic particles and toxins from the air, going to increase atmospheric oxygen, ozone, and negative ions in the house, inhibiting bacterial growth, destroying mold, cigarettes, and animals, and increasing atmospheric

oxygen, ozone, and negative ions in the house, as well as increasing atmospheric oxygen, ozone, and negative ions in the house. It will release a fresh herbal aromatic perfume into the air (Fentahun,2016).

2.4. Factors that affects essential oil quality and quantity

Extraction methods, agronomic location, species variety, harvesting season, maturity level, soil type, and temperature zone are just a few of the many elements that influence essential oil yield and quality.

2.4.1. Essential oil extraction methods

Essential oils can be extracted by a variety of methods, some of which are no longer in use. The extraction method employed is dictated by the final product quality demand., they can be extracted using a variety of methods, including steam distillation, solvent extraction, and supercritical fluid extraction are some of extraction methods.

Alcohol and a fermentation process were used in the early stages of extraction. It is necessary to develop new ways of doing things. Oil extraction is breaking into the mainstream of aromatherapy, bringing previously unavailable oils into the fold. A little education for the aromatherapy enthusiast can help with the new labels of CO₂ and Super Critical CO₂, as well as the traditional steam and hydro distillations, absolutes, and cold pressing. A little education for the aromatherapy enthusiast can go a long way in essential oil selection (Parikh,2011).

It is vital to know how oils are extracted from plants because certain methods utilize solvents that can degrade their therapeutic characteristics. Some plants, particularly flowers, are not suitable for steam distillation. They are either too delicate, or their aroma and therapeutic essences can not be released entirely by water. While these oils will not be tethered, they will be created as 'absolutes' (Mosquera, 2016).

2.4.1.1. Steam Distillation

Steam distillation accounts for the vast majority of essential oil use around the world. Different extraction methods, on the other hand, were used. Steam distillation is a process that involves heating water to create steam, which is then used to extract the most volatile aromatic compounds from all of them. Steam distillation is the process of distilling plant material using steam after it has been cooled (in a condenser). The plant material is supported on a perforated grid above the steam input for water and steam distillation. It is possible to vary the amount of steam produced by satellite steam generation, which is a big benefit.

Because steam is produced in a secondary boiler, the plant material is heated to more than 100° C at one atmosphere pressure. This will help in preventing thermal degradation essential oils (chemat, 2015).

The most widely established method for producing essential oils on a large scale is steam distillation. It is a normal procedure in the taste and fragrance supply industry. The substantially larger capital cost required to build a steam distillation unit is an obvious disadvantage. In some cases, such as large-scale production of low-cost oils (e.g., lemongrass, rosemary, Chinese cedar wood, lit sea cubeba, spiked lavender, eucalyptus), the world market prices of the oils are barely high enough to justify steam distillation without amortizing the capital expenditure required to build the facility over a 10-year or longer period. cosmetics, and a variety of other uses Steam is circulated through the plant material holding the desired oil in this procedure. It's also used to make complicated organic molecules. On a big scale, this process is utilized to create lavender and myrrh oil. Steam distillation methods are also employed in the petroleum industry and in the manufacturing of consumer food products. Distillation by steam distillation processes is also used in the petroleum industry and in the production of consumer food products. Steam distillation is primarily used to extract essential oils for perfumery and pharmaceuticals(S. Chanthal, 2012).

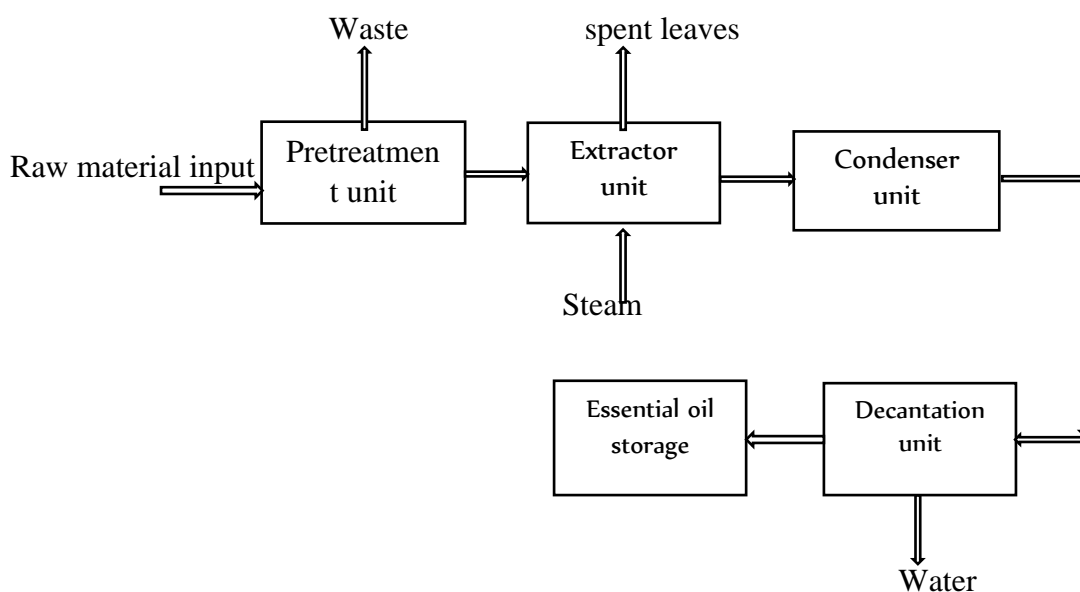


Figure 2.2 Block flow diagram extraction lemongrass essential oil

2.4.1.2. Water/Hydro *Distillation*

In this extraction process involves completely immersing the charge (which is generally comminuted) in warm water. Small-scale essential oil producers frequently use the most basic stills available. On an open fire, they are prepared..

Disadvantages of Hydro Distillation Because of the increased fuel consumption, the process is slow and the distillation time is significantly longer, making the approach less cost-effective. Due to difficult heating management, the herb extraction is not always thorough, as the building's bottom walls come into close contact with the furnace's fire. There's a chance that neighboring plant material will catch fire, giving the essential oil an unpleasant odor. Hot water can hydrolyze some essential oil components, such as esters, which react with water at high temperatures to produce acids and alcohol. Large-scale distillation (commercial scale distillation) or vigorous boiling of tough roots is not recommended. materials made of wood, e.g. sandalwood and cinnamon bark.

2.4.1.3. water/ Steam distillation

When a combination of two immiscible liquids is heated in the vapor phase while exposed to the surface of each liquid, each constituent exerts its own vapor pressure as a function of temperature, as if the other constituent were not there. As a result, the vapor pressure throughout the entire system rises. Boiling begins when the combined vapor pressures of the two immiscible liquids reach just above atmospheric pressure. Many insoluble organic compounds in water can be filtered in this method at temperatures well below the boiling point of disintegration. To address the drawbacks of water/hydro distillation, the technique was improved and wet steam distillation was established. Many chemical substances that are insoluble in water can be filtered in this way at temperatures much below the boiling point at which decomposition occurs.

Water distillation is risky and time-consuming due to the low steam pressure. Inadequate condensation, oil separation, and recovery are all issues. The quality of the yield is poor. Poor construction materials and a high level of pollution. In Indonesia, researchers investigated the impact of sample particle size and bed volume on the extraction of lemongrass essential oil using steam water distillation. It investigated the yield and quality of the citral oil produced. A two-hour drying and refining interval was chosen as a constant variable. With a sample

particle size of three cm and an 80 percent bed volume, a minimum oil yield of 0.53 percent was produced, while the maximum yield was 1.95 percent (Alam, 2018).

2.4.1.4. Distillation with cohobation

The solubility of most essential oils in water is restricted, although other oils, such as oil roses, have a high solubility. The amount of oil lost in the distillation's outgoing water can be surprisingly high in such a circumstance. The condensed water from The condensed water from the separator is returned to the extraction chamber; steam distillation is not an option since the water level in the extraction chamber would continue to rise due to the continuous steam injection.

Instead, water and steam are used in the distillation process. Condensed water from the separator can flow to the extraction chamber by gravity since the condenser is raised above the distillation still. By minimizing the overall amount of water utilized in this closed cycle operation, it is possible to create larger yields of more water soluble essential oils. Bulga and the Soviet Union (Johann, 2017).

2.4.1.5. Solvent Extraction

Distillation is used to separate a combination of multiple elements based on their varying volatility, or to separate volatile compounds from non-volatile parts of a raw material. Solvent extraction could be employed if the final product is too sensitive to heat or humidity. Essential oils produced by some flowers (Rose, Violeta) are extracted using a solvent extraction method. (Rose, Violeta, and Geranium), gums and resins. The raw material is steeped in a suitable solvent in a glass tank petroleum, ether or benzene. The particles are separated from the liquid mixture after extraction. The latter is heated in order to evaporate the more volatile essential oils, which are then condensed. If the solvent is more volatile, such as ethanol, it can be vaporized instead, leaving behind the essential oils.

Because it uses relatively little heat, solvent extraction is advantageous in getting essential oils with full fragrances that might otherwise be destroyed or altered during steam distillation. As a result, essential oils from exceedingly delicate plants can be extracted using this extraction process resulting in better yields at reduced prices. However, there are significant drawbacks to using the solvent extraction process. Solvent residues frequently contaminate the product, generating negative effects that make essential oil use unsuitable for skin

applications yet acceptable for aromas and perfumes. As a result, thorough separation of the extracted oil from the solvent is required when using solvent extraction. Any solvent that has the potential to taint the essential oils should be avoided. This technique can also produce oleoresin, an aromatic resinous substance that is more concentrated than essential oils and has a broader range of applications in food and other industries. Flow diagram of the solvent extraction process in fig (3.3) below (Luis F, 2015).

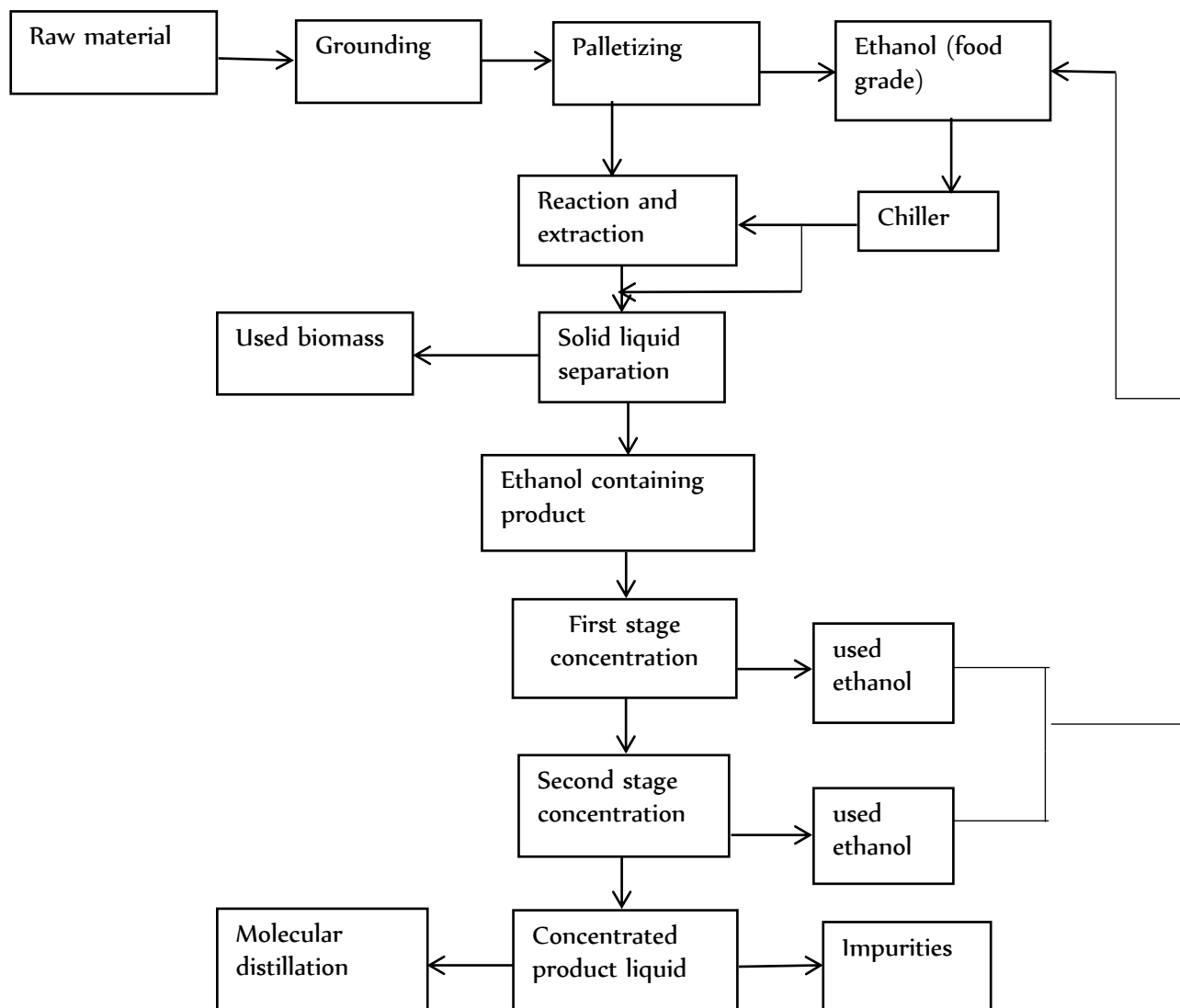


Figure 3.3.Solvent extraction block flow diagram.

2.4.1.6. Supercritical Fluid CO₂ Extraction

Temperature, pressure, sample volume, analyte collection, modifier (co-solvent) addition, flow and pressure control, and restrictors are all elements to consider when supercritical fluid extraction (SFE) is used to reduce the usage of organic solvents while enhancing sample throughput. For SFE, cylindrical extraction vessels are commonly utilized, and their performance is excellent in any doubt.

Another essential step after SFE is the collection of the extracted analyte, which means that analyst loss might occur during this phase, leading the analyst to assume that the real efficiency is low. The use of CO₂ as an extracting fluid has numerous advantages. Carbon dioxide is inexpensive, safe, and abundant, in addition to its good physical qualities. However, whereas carbon dioxide is a greenhouse gas, it is inexpensive, safe and abundant. But while carbon dioxide is the preferred fluid for SFE, it possesses several polarity limitations. Solvent polarity is important when extracting polar solutes and when strong analyte-matrix interactions are present. To overcome the polarity limits, organic solvents are commonly added to the carbon dioxide extracting fluid. In recent years, argon has been utilized instead of carbon dioxide since it is less expensive and more inert. Component recovery rates normally increase as pressure or temperature rises; for argon, the maximum recovery rates are found at 500 atm and 150° C.

The extraction technique has specific advantages: The extraction of ingredients at low temperatures, which strictly avoids damage from heat and some organic solvents. It is a solvent-free extraction method that is also environmentally beneficial. The rapid expansion of SFE's applications has been the most significant area of development. Pesticides, environmental samples, foods and fragrances, essential oils, polymers, and natural goods all benefit from SFE extraction. The extraction procedure's expensive capital is the main impediment to its practical deployment. The flow diagram for the process is illustrated in fig (3.4) below (Lisong Hu,2019).

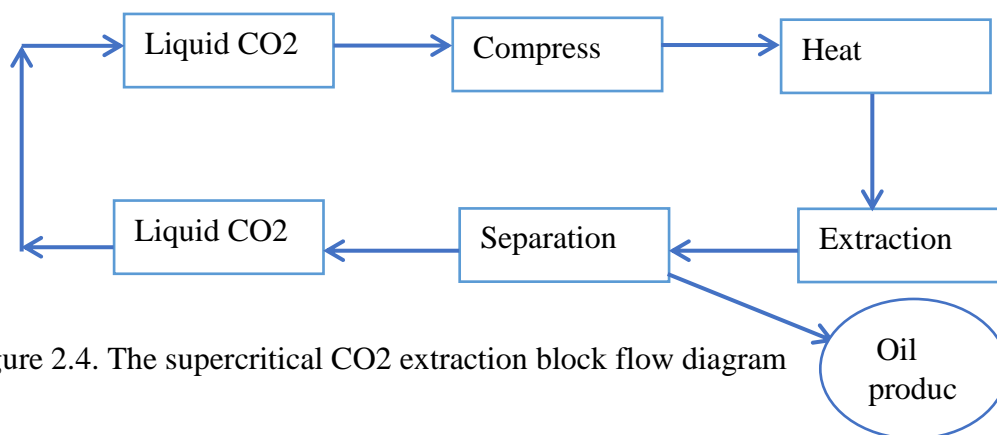


Figure 2.4. The supercritical CO₂ extraction block flow diagram

2.5. Lemongrass essential oil attributes

Lemongrass (*Cymbopogon citratus*) belongs to the Poaceae family, which contains more than 635 genera and 9000 species. This herb plant can be found all over the world. There are over 140 different cultivated species. Only 52 species of *Cymbopogon* are found in Africa, 45 in India, six in Australia, six in South America, four in Europe (only in Montenegro), two in North America, and the rest are found elsewhere in South Asia. The two primary species, *Cymbopogon exuosus* and *Cymbopogon citratus*, are widely farmed for their essential oils in many parts of the world (Ewa Majewska, 2019). Citral, beta-myrcene, and other nerol components are the major components of lemongrass essential oils. *Cymbopogon citratus* has a higher concentration, while *Cymbopogon flexuosus* has a lower concentration..

Lemongrass is grown in dense clumps up to 2 meters in diameter and has leaves up to one meter long. It is widely used in herbal teas, nonalcoholic beverages, baked goods and confectionary products. Lemongrass essential oil is used in fragrance, perfume, and cosmetic products such as soap and cream, while citrus extracted from lemongrass is used in flavoring, soft drinks, scented soap, detergents, and a variety of industrial products (Thabile et al, 2008)

The yield and quality of lemongrass (*cybopogo citratus*) essential oil is influenced by a variety of factors, as shown in the table above. Extraction methods, species type, agro-ecological location, extraction condition, soil type, and chemical fertilizer employed are only a few examples. According to a study conducted by the Wondogenet research center, the value of essential oil production and the value gained for various characteristics in various areas are significantly higher. Lemongrass genotyping indicated significant agronomic and chemical differences between test locations. Based on experimental results, superior genotypes such as WG- Lamisar-java, Lomisar EAS (I), and WG-Lamisar UA are chosen for lemongrass essential oil (Beemnet, 2014).

The study examines the effect of lemongrass (*Cymbopogon citratus*) essential oil on drying conditions, which is one of the most important elements affecting the quantity and quality of essential oil production. Sun drying for 36 hours, shade drying for 48 hours, and oven drying at 45 degrees for 7 hours are some of the settings and drying methods used. . The findings of the experiments demonstrate that the essential oil content varies significantly depending on the drying method used. In comparison to the other two drying methods, oven drying yielded the highest essential oil content (2014, Duta) .

2.5.1. Selected Lemongrass essential oil extraction method

Lemongrass essential oil is one of market affiliated product that can extracted by physical extraction methods from locally available raw materials; For large scale or commercial scale production needs special investigations of extraction mechanism interms of establishment capital cost, operating cost, yield, quality and byproducts that can sold. based on this criteria steam distillation has been selected fo this extraction process.

2.5.1.1. Steam distillation.

Steam distillation extraction was chosen for this study, because it promotes product quality, yield, low capital requirements, operational simplicity, and the low cost of quality control and assurance systems. The essential oil extraction process in steam distillation generally yields two products: a relatively pure essential oil and an aqueous condensate known as a hydrosol. The effectiveness of steam distillation is dependent on temperature, raw material size, and time and steam supply pressure.

Essential oil extraction by steam distillation is not so complex and is not so easy; it requires well studied material specification for the equipment's construction, best instrument integration, skillful operators and availability of utilities to get effective experimental results and to achieve the planned objectives successfully. Steam distillation is engineered to give excellent results and performs disturbance-free operations. It is designed based on a particular type of raw-material extraction. The process technology and engineering design ensure the consistency of the yield quality and quantity.

To achieve the intended experimental plan, the extraction process was carried out effectively and efficiently. After the raw material treatment has gone through, the raw material is placed inside the distillation column. The steam and lemongrass raw materials are in contact in the extraction chamber; during this, the steam drives the essential oil from the body of the leaves with the principle of surface diffusion. Lemongrass essential oil extraction at 94°C and

atmospheric pressure (best recommended temperature and pressure in the case of Ethiopia, Addis Abeba). The given temperature and pressure in this extraction process will cause thermal degradation of the essential oil components. The extraction was taking place in a thermal laboratory room at Addis Ababa Institute of Technology's school of chemical and bio engineering department.

2.5. 2. Important properties of lemongrass essential oil

Physical and chemical property characterization will aid in determining the quality and characteristics of the essential oil. Lemongrass essential oils have the following physicochemical properties: moisture content, density, specific gravity, optical rotation, refractive index, molecular refraction, solubility, boiling point, dynamic and kinematic viscosity, evaporation residue, flash point oxides, esters, alcohol, and aldehydes.

Moisture content: Drying could inhibit the growth of spoilage-causing microbes and reduce the occurrence of chemical reactions inside, extending the product's shelf life and resulting in a better economic value than raw material consumption. Thin lamination is one of many viable processes for removing moisture from materials (including convection drying, vacuum drying, freeze drying, and fluidized bed drying). The method of drying has been shown to be easy and cost-effective. However, determining optimal drying models for raw materials is essential in order to build an efficient drying system for a certain product. By forecasting the drying process of materials based on the moisture balance, accurate modeling of material behavior during the drying process aids in the development of dried goods. The material's balance, which can be used to make improvements to existing drying systems Although lemongrass has been thoroughly explored in terms of its biological activity and chemical composition, there is still a scarcity of information on its drying kinetics (Thi Van Linh Nguyen, et.al 2018).

Refractive index: When light travels from a less dense to a denser medium, it bends or "refracts" back to its original state. According to the law of refraction, e indicates the angle of refraction and the angle of incidence, where n is the index of refraction of the less dense medium and N is the index of refraction of the denser medium. Refractometers offer a rapid and convenient method for the determination of refractions. The experimental result ranged 1.4800 ± 0.0002 (Beriso M, et.al. 2020)

Molecular refraction: The index of refraction of a liquid varies with temperature and the wave length of the light. In order to compare the refractivity of different liquids, the use of molecular refractivity (molecular refraction) is useful.

Optical rotation: Most essential oils when placed in a beam of polarized light possess the property of rotating their plane of polarization to the right (dextrorotatory), or to the left (laevorotatory). The degree of rotation and the direction are important indicators of purity.

Specific gravity: The specific gravity of an essential oil is a significant indicator for its quality and purity. Specific gravity is less than 1.0 in most cases. As a result, essential oil can be obtained by (floating in) water. At 20°C, the specific gravity of lemongrass essential oil ranges from 0.895 to 0.908. (Merck, et al. 2015).

Solubility in Alcohol: Most essential oils are very mildly soluble in water, although they are miscible with pure alcohol. Lemongrass essential oil is known for its alcohol solubility, which is a major attribute of its.

Flash points: The flash point of an essential oil may be beneficial in determining its value. Adulteration is indicated by the inclusion of adulterants such as alcohol and low boiling minerals, thus the flash point is important. The flash point will be significantly reduced if you use spirits.

Aldehydes: The most prominent aldehyde components are citronellal and nerual, which are abundant in lemon-scented oils such as lemongrass, Melissa, lemon verbena, and citronella.

Phenols: These have a bactericidal and invigorating effect, although they might irritate the skin. Eugenol (found in clove and West Indian bay), thymol (found in thyme), and carvacrol are all examples of phenols (found in oregano and savoury).

Terpenes: Limonene (antiviral, found in citrus oils) and pinene are two common terpene hydrocarbons (antiseptic, found in high proportions in pine and turpentine oils).

Ketones: Ketones, such as tansy, sage, and wormwood; and pulegone, found in pennyroyal, are some of the most prevalent poisonous ingredients.

Oxide: is the most important of the oxides (or eucalyptol). It is well known as the main ingredient of eucalyptus oil and has an expectorant effect. It can also be found in a variety of other oils, particularly those with a camphoraceous aroma, such as rosemary and broom. other oils, especially those of a camphoraceous nature, such as rosemary, bay laurel, tea tree, and cajuput.

Alcohols: These chemicals have antibacterial and antiviral qualities, as well as an elevating effect, and are generally nontoxic. Linalool (found in rosewood, linaloe, and lavender), citronellol (found in rose, lemon, eucalyptus, and geranium), and geraniol (found in

palmarosa) are among the most prevalent terpene alcohols, as are borneol, methol, terpineol, nerol, farnesol, vetiverol, benzyl alcohol, and Cedrol.

2.5.3. Characterizations of lemongrass essential oils

Gas chromatography-mass spectrometry (GC-MS) and fourier transform infrared analysis (FT-IR) have been used to analyze the chemical content and quality of lemongrass essential oil and functional group of organic compound available in the extracted essential oil.

2.5.3.1. Gas chromatography-mass spectrometry (GC-MS) analysis

Lemongrass essential oil component identification is aided by GC-MS analysis, which necessitates particular instrument integration for accurate measurement findings. Perkin Elmer Turbo Mass Spectrophotometer with XLGC Perkin Elmer Auto Sampler HLs were performed using a Perkin Elmer Turbo Mass Spectrophotometer and a Perkin Elmer Auto Sampler XLGC. A Perkin Elmer Elite-5 column was used. 95 percent Dimethyl polysiloxane capillary column with a film thickness of 0.25 mm and a diameter of 30 m 0.25 mm. With a flow rate of 0.5 ml/min, helium was used as the carrier gas. The sample injection volume was 1 liter. The oven was preheated at 110°C for 4 minutes before being increased to 240°C. The temperature was then set to climb at a rate of 20°C per minute to 280°C.at a pace for the next 5 minutes. The program lasted a total of 90 minutes. The Mass Spectrometry transfer line was kept at a constant temperature of 200°C. The source was kept at a constant temperature of 180°C. Gas chromatography-mass spectrometry was studied using electron impact ionisation. To measure the peak regions and analyse the data, the Turbo-Mass-OCPTVS-Demo SPL software was utilized (Ojochenemi, E,2007).

According to the International Journal of Pharmacy and Chemistry. Hydro-distillation process for about four hours, the chemical constituents of the extracted oil were identified using GC-MS analysis. The major compounds in the oil were citral (34.8 percent), neral (30.72 percent), -myrecene (11.28 percent), geraniol (5.54 percent), 1,3,4-trimethyl 3-cyclohexene-1-Carboxaldehyde (2.20 percent), and citronellol (2.20 percent) (1.34 percent). D-Limonene (0.03 percent), Geranyl Acetate (0.57 percent), and Bicyclo [3.1.1] are the chemicals. A minor quantity of heptane-2-carboxaldehyde-6,6-dimethyl (0.23 percent) was found. The oil's antibacterial and antifungal properties were investigated. The microorganisms were sensitive, and there was no evidence of resistance, with all growth inhibition zones occurring in areas with high activity (Mahmoud Mohamed Ali, et.al, 2017).

2.5.3.2. Fourier transform infrared radiation(FT-IR) analysis

Fourier transform infrared radiation analysis (FT-IR) has a role in compound identification, such as organic compound functional group determination, inorganic compound molecular composition surface identification, and quantitative determinations of mixtures. The most important aspect of the FTIR analysis analysis is to figure out what something means. FTIR (Fourier transform infrared) spectrum. The spectrum is a broad term that refers to "absorption versus wave number" as a result of data on "transmission versus wave number.

Only "absorption" is discussed in this paper. When compared to curves that have a "wave number,"In short, the IR spectrum is divided into three wavenumber regions: far-IR spectrum ($<400\text{ cm}^{-1}$), mid-IR spectrum ($400\text{-}4000\text{ cm}^{-1}$), and near-IR spectrum ($4000\text{-}13000\text{ cm}^{-1}$). The mid-IR spectrum is the most widely used in extensive analysis, but the far- and near-IR spectrums also contribute to providing information about the samples analyzed. This study focused on the analysis of FTIR in The mid-IR spectrum is divided into four regions: the single bond region ($2500\text{-}4000\text{ cm}^{-1}$), the triple bond region ($2000\text{-}2500\text{ cm}^{-1}$), the double bond region ($1500\text{-}2000\text{ cm}^{-1}$) as well as the fingerprint region ($600\text{-}1500\text{ cm}^{-1}$) (Jl. Dr. Setiabudi,et.al, 2019).

To obtain meaningful information, many spectral interpretation procedures have been used. Based on FT-IR analysis, it entails the direct use of 1-band intensity, the determination of 2- or 3-band intensity ratios, and the use of a chemometrical or multivariate method known as principal component analysis. Examine the elements. The findings show that simple algorithms and PCA patterns may both be utilized to track the shift from PCW to SCW biosynthetics in cotton fibers. Simple algorithms were found to be capable of detecting small differences in fibers older than 25 DPA between respective fibers generated by the culture. In this study, FT-IR spectra represented developmental immature fiber (im) mutant fibers and their near-isogenic wild type Texas Marker-1 (TM-1) fibers using PCA and a simple algorithm, and compared the correlations between cellulose content determined by conventional chemical analysis and the respective ATR FT-IR spectral responses acquired by various spectral acquisition methods. Several spectral interpretation strategies have been proposed. Notably, a simple technique has been devised to estimate fiber cellulose age and crystallinity simultaneously using ATR FT-IR spectra (Yongliang Liu, 2017).

2.6. Applications of lemongrass essential oil

For many years, *Cymbopogon citratus* has been cultivated for medicinal purposes in various countries all over the world. Lemongrass is used in folk remedies for coughs, consumption, and indigestion, elephantiasis, malaria, ophthalmia, pneumonia and vascular disorders. Researchers have found that lemongrass is an antidepressant, antioxidant, antiseptic, astringent, bactericidal, fungicidal, It has both sedative and anxious qualities. Furthermore, many studies have shown that lemongrass oil has antibacterial action against a wide range of species, including gram positive and gram negative bacteria as well as yeast, and that gram positive bacteria are more sensitive to oil than gram negative bacteria. (Mohd Irfan, 2010).

Weed control will be restricted because lemongrass is such a robust grower, mainly consisting of hand hoeing or plucking out any broad-leaf weeds that may appear. Citral, a component of lemongrass oil, has a wide range of applications and can be processed into a violet-like smell for perfumery as well as a source of vitamins A and E. Deodorants, waxes, and polishes all include detergents and insecticides (Department of agriculture, forestry and fisheries, 2012).

Cosmetic: Lemongrass oil is used as a fragrance component in soaps, detergents, and other cosmetic products. It is also used in aromatherapy, and it improves circulation and muscle strength. Steam is used in a lemongrass facial to tone, astringent, and properties. Inhalation refines and firms up the sluggish, lacklustre, oily skin. It is used as a tissue toner in aromatherapy.

Therapeutic and Pharmaceutical uses: Lemongrass is used as a diuretic, tonic, antibacterial, stimulant, and diuretic. It aids digestion, and a peppered lemongrass concoction is used to alleviate menstruation issues and nausea. Perspiration is induced, the body is cooled, and the fever is reduced. Diarrhoea, stomachaches, headaches, and fevers are all treated with this medicine. It can aid with muscle discomfort, circulation problems, muscle tone and slack tissue. Athlete's foot and acne are treated with this antibacterial oil. When used in a vaporiser for cold and flu relief, lemongrass inhibits airborne microorganisms. The aroma of lemongrass in a foot bath soothes sweaty feet while its antibacterial capabilities protect against fungal illnesses. Lemongrass oil should be diluted and applied directly to the ringworm-affected skin. It's used in veterinary for dogs and cats as shampoos a repellent for fleas, lice and ticks.

Food and Flavouring : The bulbous stem and leaves, which have a characteristic lemon flavor, are utilized in oriental cookery and are in limited supply for a variety of meals. Tea made from the leaves can be consumed on its own, but it is more commonly mixed with other teas.

Perfumery uses: Bath salts, soap bases, blend well with aromas of rose, rose geranium or palmarosa, citrus, lime and orange.

Safety on using: Pure, undiluted lemongrass essential oil should not be applied to any part of the body due to its sensitizing potential. Lemongrass can be extremely acidic in containers with plastic tops, as well as on any material it comes into contact with. Because oil can be hazardous if swallowed, it should be kept out of the reach of children. The essential oils are shall be kept with appropriate care, the oil can last 6 months to 2 years and then the oil should be discarded.

2.7. Safety aspects for essential oil using

Herbal essential oils play a significant role in a person's daily life. However, employing essential oils, particularly while using traditional medicine, can be difficult (TM). Many years ago, human society began utilizing TM without first obtaining clinical evidence to prove its efficacy and safety. The main problem with the current scenario is the lack of an appropriate methodology and approach for evaluating and conducting TM research. The problem's complexity starts with a distorted view of society. "Natural" means "safe," and this mindset frequently leads to society's misapplication of TM for self-care. Accidents caused by unqualified practitioners are reported frequently using problems with essential oil products (Zhang,2009). Essential oils are widely available in pharmacies, supermarkets, and on the internet, and are used for a variety of purposes in society. In 2016, 11 percent of Australians used essential oils for therapeutic purposes, with the majority of them self-prescribed, according to a recent study. Essential oils' safety profile has yet to be fully determined, despite their widespread use and appeal. Because of the chemical complexity of essential oils, identifying the individual components can be challenging. Certain unfavorable impacts are attributed to it. However, some essential actions have been taken (Olivi Vostinaru).

3. Materials and methods

3.1. Materials

Raw materials: Three varieties of Lemongrass raw materials WG-Lomisar I, WG- Lomisar UA and WG- Lomosar java have been collected from Wondogenete agriculture research center.

Chemicals: Distilled water, Ether, Ethanol, Anhydrous sodium sulfate, Sodium Bisulphate, Sodium Hydroxide, potassium Hydroxide, Oxalic Acid and Water

Equipments: Steam generator boiler, extraction chamber (distiller), heat exchanger, separator funnel, and spectrophotometry, interferometer, Electric heater, digital weight balance, separation funnel, vibro-viscometer, density bottle (pycnometer), glass bottle, micro pipet, (250, 400 and 1000 ml) beakers and (250ml, 500ml and 1000 ml) flask, HP 5977 series.

3.2. Methods

3.2.1. Lemongrass raw material collection and preparation

Three varieties of Lemongrass raw materials, WG-Lomisar I, WG- Lomisar UA, and WG- Lomosar Java, were collected from Wondogenete agriculture research center. The location coordinates are 07° 192 'North latitude, 38° 382 East longitude, and 1876 m.a.s.l. The site location means an annual rainfall of 1000 mm with minimum and maximum temperatures of 12.02 and 26.72°C, respectively. The soil texture at the testing site was sandy clay loam with a pH of 6.4 (werotaw,2018). The Wondogenet agriculture research center has four lemongrass varieties, among them three species brought for this study. The center cultivated the plant through a procedure following agronomic growing mechanisms with the help of qualified professionals.

Herbs are particularly perishable due to their high moisture content and the fact that most herbs are chill-sensitive. They are dried to create shelf-stable products. Lemongrass is one of the sensitive perishable herbs. It must be preserved by lowering the moisture content, which inhibits microbial growth and chemical changes during storage (Grant Thamkaew, 2020). Therefore, drying should get attention. Lemongrass is a well-known, fragrant herb. It contributes to the formation of a number of volatile chemicals, including citral, beta – myrcene, and limonene, to protect against raw material pollution and product component degradation. Proper drying was implemented at the AAiT school of chemical and bio

engineering thermal lab. The raw material was dried properly, taking into account the perishability of large and fresh leaves stored in a small confiding area. The three raw materials brought for study were in the following fig (3.1) below.



Figure 3.1. Wg Lomisar varieties image

3.2.2. lemongrass raw material particle size reduction

The raw materials were fully dried and size reduction was done with the help of scissors; the selected raw material particle sizes to examine were 50 cm, 30cm and 10cm.

3.2.3. Lemongrass raw material moisture content determination

The moisture content determination was done by direct measurement method using moisture content analyzer. The procedure was: first adjustment of operational time was done, then from the display panel press tare to have effective mass measurement, then carefully measure one gram of sample, finally press start operation. A lemongrass sample was randomly chosen for measurement. The working temperature was $100 \pm 2^\circ\text{C}$. All measurements were taken on the same date. The determination was done by (Thi Van Linh Nguyen, 2021) moisture determination means and the measurement continued until the constant result was seen on the display monitor.

3.2.4. Lemongrass essential oil extraction Procedure

Before beginning the extraction process, the following checks and cleanings are required: the availability of electricity, the water supply system, and the main equipment parts functionality; each joint leakage; cleaning the extraction chamber and then properly putting raw material inside the extraction chamber in an appropriate manner; closing the extraction chamber, and finally, the three phase electric energy source. The temperature began to rise until it reached the maximum possible stage, which is displayed at 84°C in the display compartment, , but the temperature was 95°C in the extraction unit and the recording time started after the first drop of extract was seen. The steam rises and comes into contact with the raw material in the extraction chamber, and after a certain amount of time, the oil mixture will begin to drop into a temporary receiver flask at the bottom of the condenser. The receiver's stored oil mixture was separated with a separator funnel within a range of 10 minutes, and the yield was recorded. This procedure was followed until the final extraction process was completed and the product yield was calculated and converted to percent by using the following equation.

$$\text{Percent Yield} = \frac{\text{Mass oil}}{\text{Mass of raw material}} \times 100\% \quad \text{--- 3.1}$$

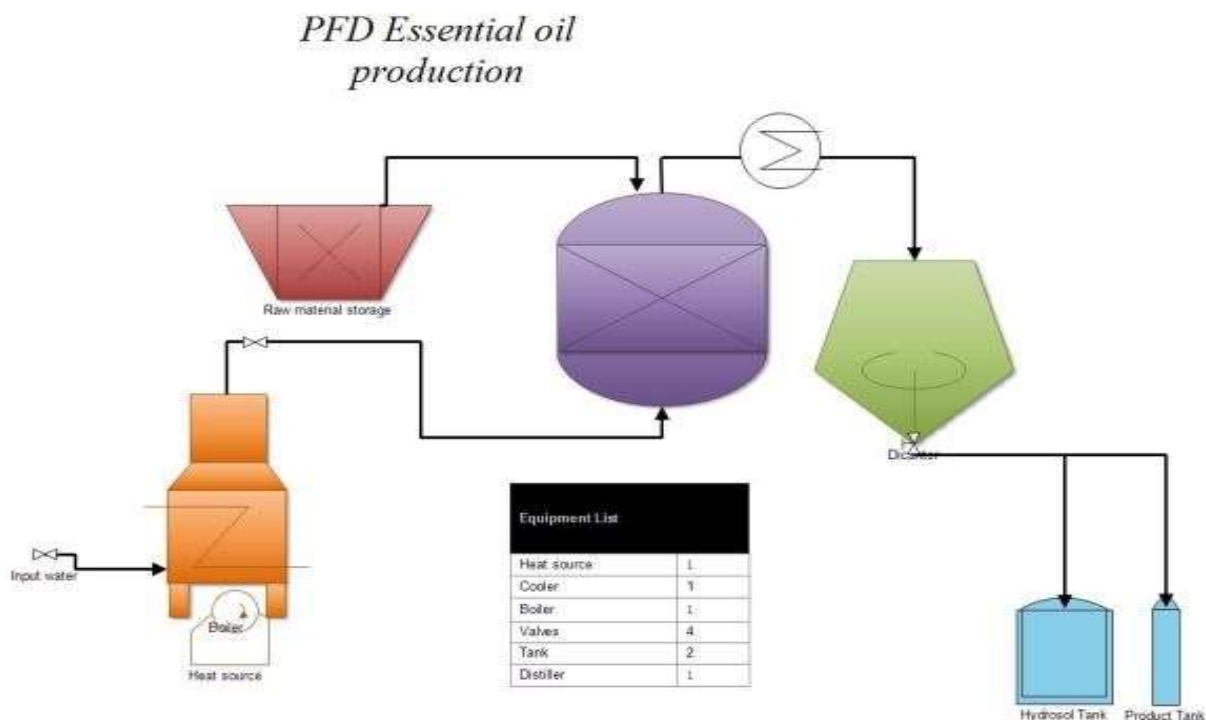


Figure 3.2. Lemongrass essential oil extraction process flow diagram

3.2.4.1. Steam source (conventional Boiler)

The steam source (conventional boiler) was connected to a three-phase power supply and could hold up to 24 liters of water. It was made of pressured air-tight stainless steel components. This was used to generate steam, which was then used to extract the plant materials. It contains water from the municipal water supply system automatically.

3.2.4.2. Essential Oil Extraction Chamber

The experiment was done on a pilot plant scale to examine the interaction effect of the three parameters; raw material particle size, weight of raw material per batch, and extraction time. Stainless steel was used to make the extraction equipment construction. material with a 60cm diameter and an elevation of 70cm, with movable sieved metal for better raw material setup and steam interaction. The extraction chamber cover is easily dismantled and returned to its original position by the operator without using any additional fixing tools. The chamber serves as a container for the plant material, and as a contact area for steam and raw materials inside. Extraction is effective in dragging the volatile compounds into the herbal body tissue by hot vapor and then the essential oil diffuses by the principle of the surface diffusion process and the volatility nature of essential oils. The pipe was connected to a condenser to drain the vapors into the condenser unit.

3.2.4.3. The condenser unit

The laboratory condenser was used to convert the incoming oil vapor mixture into liquid with the help of a tube and tube heat exchanger using the condensate material, tap water. The mixture and the tap water were never in contact with each other. The feed is sourced from the overhead product of the extraction chamber and the water is permitted to trickle over the entire length of the condenser tube, but the condenser and oil mixture never come in contact with each other. The heat exchanger's operational process is parallel with the vapor, and the condenser tube was formed to slant downward slightly to ensure proper condensation occurs; tap water drains continuously with carrying heat energy.

3.2.4.4. The separator funnel unit

It is used to separate two or more immiscible components based on density difference or the component's specific gravity, as the name implies. Products with fewer than one access ability will be at the top, and vice versa. In the case of lemongrass essential oil separation, the

extracted mixture was collected from the condenser drain part at the bottom and then poured into the separator funnel, where it was allowed to move until the boundary layer (desired and undesired product) was reached. The hydrosol drains until the essential component reaches the funnel bottle neck switch; then the receiver is replaced with a product collector tube. The proper separation has been performed on a fixed and well-adjusted laboratory stand, which facilitates proper settlement and effective boundary formation.

3.2.5. Physico-chemical characterization Lemongrass essential oil

3.2.5.1. Physical property characterization

3.2.5.1.1. Specific gravity determination

A density bottle was used to measure liquid oil's density. The measuring procedure: first, a carefully cleaned and dried 50 ml bottle was prepared, then the digital mass measuring unit weighed the result (W0), and the bottle was filled with oil until the bottle neck was reached. Then reweighed the essential oil bottle (W1). Finally, fill the cleaned bottle with distilled water and record the results (W2).

$$\text{Specific gravity} = \frac{\text{Measured mass of oil-bottle mass}}{\text{Measured mass of distil water - Mass of Bottle}}$$

$$\text{Sp. g} = \frac{W1-W0}{W2-W0} \text{-----} 3.1$$

Where as

Sp.g: Specific gravity

W0: empty bottle mass

W1: lemongrass essential oil filled bottle mass

W2: Distill water filled bottle mass

3.2.5.1.2. Kinematic viscosity determination

The dynamic viscosity of lemongrass essential oil was directly tested using a vibro-viscometer. The temperature was around room temperature during the measuring time. The measurement took place in the reaction lab room, school of chemical and bioengineering department. First, the sample handling was cleaned and then immersed in 35ml of essential oil extract sample handling. Finally, the sensor was in contact with the sample and the vibro-

viscometer displays on the screen the dynamic viscosity and temperature of 2.87 and 19.6°C respectively. The kinematic viscosity was determined by using dynamic viscosity measurement results.

3.2.5.2. Chemical property characterization

3.2.5.2.1. Fourier Transform Infrared (FTIR) Analysis

The lemongrass essential oil's FT-IR spectrum was obtained at the Addis Ababa University science campus using a Perkins Elmer Spectrum 65 FT-IR spectrometer, and the functional groups were determined by using IR correlation charts. The infrared spectral transmittance was expressed as a percentage. The wave number region used for the analysis was 4000-400 cm⁻¹ (mid-infrared). Fourier Transform-Infrared spectroscopy was performed on a Perkin Elmer FT-IR using spectrum software version 10.3.2 and a liquid sampler to show the functional groups and provide structural information obtained from the various varieties.

3.2.5.2.2. Gas Chromatography-Mass Spectroscopy Analysis

The GC-MS analysis with the HP 5977series was used to identify the components. Helium was used as a carrier gas at a constant flow of 1ml/min and an injection volume of 1μ was used by the injector, which was equipped with a mass selective detector (MSD).

3.3. Experimental design and statistical analysis

3.3.1. Experimental factor selection

Factor selection for this study this was a general factorial design and the response variable is the lemongrass essential oil yield. Three levels of extraction time, raw material particle size and raw material weight per batch. Factor selection was done based on the available and effective control units and the functionality of the equipment. In addition, the following factors were taken into account.

Extraction chamber size: extraction is taking place on a pilot plant scale; because dry leaves have a large surface area comparable to other solid materials, it requires a small amount of mass to operate efficiently and effectively; the selected mass was 0.5 and 1.0 Kg, respectively.

Applicability of the real scenario: size reduction is one of the challenges in lemongrass essential oil extraction; if size reduction goes down to 10 cm, the feasibility aspect will face

challenges or the cost of production will rise because of the size reduction cost increase unexpectedly. Therefore, the selected raw material particle sizes were 10 and 50 cm respectively.

Repeated experimental results: During preliminary experiments for the selection of the best yielding lemongrass variety, an unrestricted time of extraction was implemented, which gives an effective extraction time indication of around 90 minutes; based on this (90 ± 30 min) was chosen as input to study the effect of time on the extraction of lemongrass essential oil for this experiment. Almost all herbal essential oil extraction processes necessitate a limited amount of time in contact with heat; any longer will cause component degradation. As a result, the maximum time was set at 120 minutes.

3.3.2. Full factorial design

The experiment was carried out by design-expert software version (12.0.3.0). It goes over factorial design and outcome analysis in depth. The yield of lemongrass essential oil was tested in the response. The central composite design (CCD) of the experiment employs a three-level box-henken design for response surface methodology, permitting to investigate the combined effects of raw material particle size, raw materials weight per batch, and extraction time on lemongrass essential oil yield, which is suitable for quadratic response exploration. The factorial design is important since it just requires a few runs per study factor.

Analysis of variance (ANOVA) and regression are the fundamental frameworks for data interpretation. The primary distinction is the qualitative rather than quantitative nature of the explanatory elements. In anova, the explanatory variables are generally referred to as factors. A normality test or the evaluation of certain charts can be used to determine the residues' normality. In the case of this study, quadratic regression model was used to study the effects of the experimental results.

Table 3.1. Factorial design fo lemongrass essential oil extraction

Factor	Name	Units	Level		
			-1	0	1
A	Raw material particle Size	cm	10	30	50
B	Weight of raw material per batch	Kg	0.50	0.75	1.00
C	Extraction Time	min	60	90	120

The equation in terms of coded factors can be used to make predictions about the response to given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

4. Result and discussion

4.1. Lemongrass raw material characterization

4.1.1. Moisture content determination

Table 4.1 Moisture content measurement results

Sample	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	mean values
result (w/w)	11.00	10.85	13.40	13.20	13.35	13.45	10.50	10.90	11.50	13.25	12.14

Ten lemongrass samples were used to determine the moisture contents of the raw, by using a constant mass (one gram). The measurement results were ranged from 10.85 to 13.45. The mean value result was (12.14). The samples had an average moisture content of 12.14 percent. Due to different variations in the raw material location, the results obtained were different.

4.2. Wg Lemongrass Essential Oil Variety Selection

Table 4.2 Wg Lemongrass Variety Extraction results.

Type	Yield (%)	Yield (%)	average	Remark
Wg Lomisar Java	0.39	0.45	0.42	
Wg-Lomisar(UA)	0.69	0.65	0.67	
Wg-Lomisar (I)	0.79	0.82	0.805	selected for farther investigation

The extraction was carried out by measuring 1kg of each sample lemongrass species and the extraction time was 90 minutes, which was replicated. The result is shown above in table (4.2). Variety selection was done by quantitative and qualitative methods.

To compare and contrast the Lemongrass raw material variety, 1 kg of Wg Lomisa java was measured and the recorded essential oil yield was 0.39 and 0.45% respectively. In a similar manner, the second variety, Wg Lomiar UA, was measured and extracted, yielding 0.69 and 0.67%, respectively. Finally, Wg Lomisar (I), measured and extracted by similar

mechanisms, the yield were 0.79 and 0.82%. Constant mass (1kg) and time (90 minutes) were used to determine the highest yield throughout the extraction process, so a 0.80% product is satisfactory in comparison with.

The other selection criteria were the product component quality, which was determined based on the product component quality, which was determined by using GC-MS analysis results. The two highest yielding product samples (Wg Lomisar (I) and Wg Lomisar UA) were chosen for further quality investigation. Wg lomisar (I) yield was appreciable results compared to Wg lomisar (UA), as its identified essential chemical components were higher. Based on these quantitative and qualitative measurement results, Wg lomisar (I) is the selected strain among the three Wg lemoisar varieties and the overall extraction process was performed with the selected strain.

4.3. Lemongrass essential oil extraction

Extracting lemongrass essential oil by steam distillation requires control of extraction factors such as extraction temperature, pressure and steam supply to control the thermal degradation of the extract, In addition to selected factors. In the case of this, steady extraction was taking place on a pilot plant scale. The temperature was not excess 94°C and the pressure was atmospheric pressure and also the steam supply was constant through the extraction process.

Table 4.3. Lemongrass essential oil extraction yield

Std	Run	Factor 1	Factor 2	Factor 3	Response 1
		A:Raw material particle Size(cm) cm	B:Weight of raw material per batch(Kg) Kg	C: Extraction Time(min) minute	Yield actual value %
14	1	30	0.75	90	0.69
17	2	30	0.75	90	0.76
13	3	30	0.75	90	0.75
8	4	50	0.75	120	0.70
1	5	10	0.5	90	0.70
10	6	30	1	60	0.72
3	7	10	1	90	0.80
9	8	30	0.5	60	0.65
15	9	30	0.75	90	0.80
4	10	50	1	90	0.72
16	11	30	0.75	90	0.7900
7	12	10	0.75	120	0.80
2	13	50	0.5	90	0.68
11	14	30	0.5	120	0.69
5	15	10	0.75	60	0.75
12	16	30	1	120	0.780
6	17	50	0.75	60	0.60

Wg Lomisar was used for the overall extraction process. Based on the three input extraction factors such as: raw material particle size, raw material weight per batch, and extraction time the output percent yield were recorded in the above table (4.3).

4.3.1. Model aduquacy check

The regression model determination is tested by R-Squared, Adjusted R-squared and predicted R-squared. The values are 0.9342, 0.8496, and 0.7298, respectively, and an analysis of variance will be used to run the additional adequacy test model.

The **Predicted R^2** of 0.7298 is as close to the **Adjusted R^2** of 0.8496 as one might normally expect; i.e., the difference is less than 0.2. This may indicate it has no block effect or the model has no problem with the model and/or data.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 13.678 indicates an adequate signal. This model can be used to navigate the design space.

Design-Expert® Software

Yield

Color points by value of
Yield:
0.65 0.8

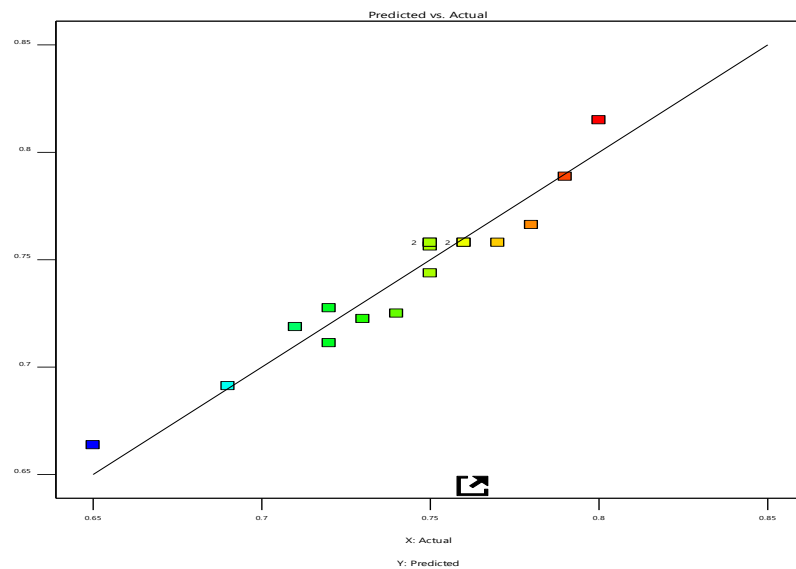


Figure 4.1. Predicted vs Actual plot experiment result

The above-mentioned fig (4.1) provided information about the actual and predicted equation. The experimental data was accurately described when all of the points were close to the line of perfect fit. This implies that the experiment result and the predicted successful capture of the correlation between the three experimental factors.

Design-Expert® Software

Yield

Color points by value of

Yield:

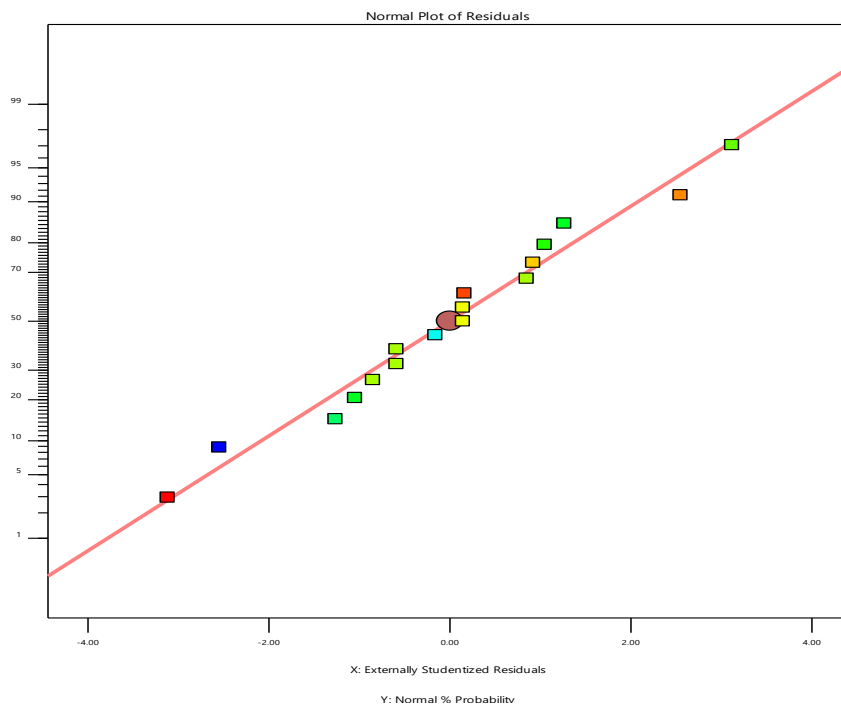
0.65  0.8

Figure 4.2. The normal vs residual plot of experimental results

The normal and residual plots of lemongrass essential oil experimental results are shown in Fig (4.2) above. How much of the design was precisely modeled, since almost all the points lined up and the deviation of points for the essential point from normality was insignificant and the normal probability plot indicates it. The residuals have a normal distribution, and the points in the plot in this experiment fit into a straight line. The error distribution is approximately normal. The quality of the model is further checked by analysis of variance (ANOVA) in table (4.2). Based on 95%CI (confidence level), the F-value is a test for comparing model variance with the residual (error) variance.

4.3.2. Analysis of variance (ANOVA) response percent yield

Design Expert 12.0.3.0 The software program was used for regression and graphical analysis of the data obtained by incorporating the sequential F-test, lack-of-fit test and other adequacy measures for selecting the best model. To decide on the adequacy of the model for the extraction of lemongrass essential oil, three different tests, such as the Sequential model, Sum of Squares, Lack of Fit Tests and Model Summary Statistics, were carried out and p-values for all the regressions were associated. The Prob > F value for the model is lower than 0.0500, which indicates that the model is considered to be statistically significant, but values greater than 0.1000 indicate the model terms are not significant. This means that at least one

of the terms in the regression equation had a significant correlation with the response variable.

Designed experiments were carried out to map the quantitative effects of the three parameters. These are extraction time, raw material particle size and the weight of the raw material per batch. The yields of the extraction are calculated from the relation between the essential oil mass obtained and the raw material mass input in the extraction.

Table 4.4. Analysis of variance (ANOVA) regression model equation and coefficients

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0207	9	0.0023	11.04	0.0023	significant
A-Size raw material	0.0036	1	0.0036	17.38	0.0042	significant
B-Weight of raw material per batch	0.0045	1	0.0045	21.71	0.0023	significant
C-Time	0.0061	1	0.0061	29.11	0.0010	significant
AB	0.0020	1	0.0020	9.74	0.0168	significant
AC	0.0001	1	0.0001	0.4811	0.5103	
BC	0.0000	1	0.0000	0.0000	1.0000	
A ²	0.0004	1	0.0004	1.93	0.2078	significant
B ²	0.0017	1	0.0017	8.31	0.0236	significant
C ²	0.0022	1	0.0022	10.48	0.0143	significant
Residual	0.0015	7	0.0002			
Lack of Fit	0.0012	3	0.0004	0.560	0.648	not significant
Pure Error	0.0003	4	0.0001			
Cor Total	0.0221	16				

Factor coding is Coded

The sum of squares is Type III – Partial. The Model F-value of 11.04 implies the model is significant. There is only a 0.23% chance that an F-value this large could occur due to noise.

P-values of less than 0.0500 indicate that the model terms are significant. In this case, A, B, C, AB, B², C² 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 the hierarchy), model reduction may improve your model.

The F-value for Lack of Fit of 0.560 indicates that the lack of fit is not significant in comparison to a pure error. There is a 64.8% chance that a lack of a Fit F-value this large could occur due to noise. Non-significant lack of fit is good –the goal of the model is to fit.

4.3.3. The development of regression model equation

After removing the unimportant elements, the model equation links the response (yield) to the extraction process variables in terms of actual value. For given values of each element, the equation in terms of actual factors can be used to create predictions about the response. For each factor, the levels should be specified in the original units. Because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design process, this equation can not be used to determine the relative impact of each factor; consequently, the concern predicted model for percentage yield of Lemongrass essential Oil in terms of the coded factors is given below.

$$\text{Yield} = +0.7580 - 0.0213 * A + 0.0097 A^2 + 0.0238 * B - 0.0203 B^2 + 0.0275 * C - 0.0228 C^2 - 0.0225 A * B \text{ -----} 4.1$$

A: Raw material particle size

B: weight of raw material per batch

C: Extraction time

4.3.4. The single and interaction effects of process variable

To study the single factor effect of lemongrass essential oil, the other remaining factors were kept constant, but the interaction effects one factor kept constant and the remaining two factors were continuously changed.

4.3.4.1. The effect of particle size on lemongrass essential oil extraction yield.

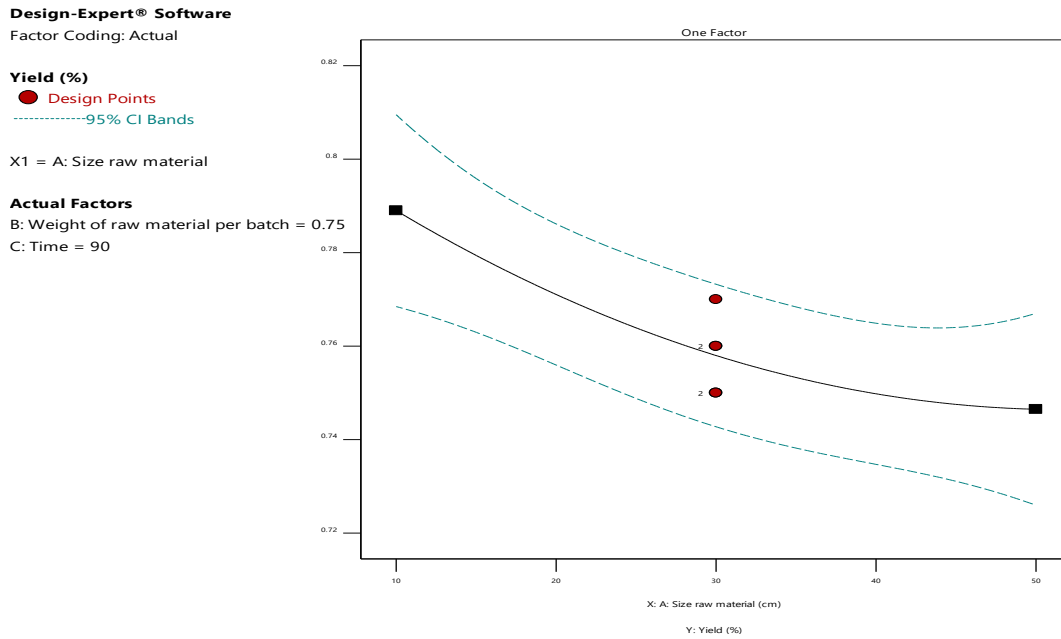


Figure 4.3. The effect of raw material particle size on lemongrass essential oil percent yield

The single factor that influences lemongrass essential oil extraction. Particle size is one of the selected effects that affect the extraction process. The effect is clearly seen in fig (4.3) above.

4.3.4.2. The effect of raw material weight per batch on lemongrass essential oil yield

Design-Expert® Software

Factor Coding: Actual

Yield (%)

● Design Points

-----95% CI Bands

X1 = B: Weight of raw material per batch

Actual Factors

A: Size raw material = 30

C: Time = 90

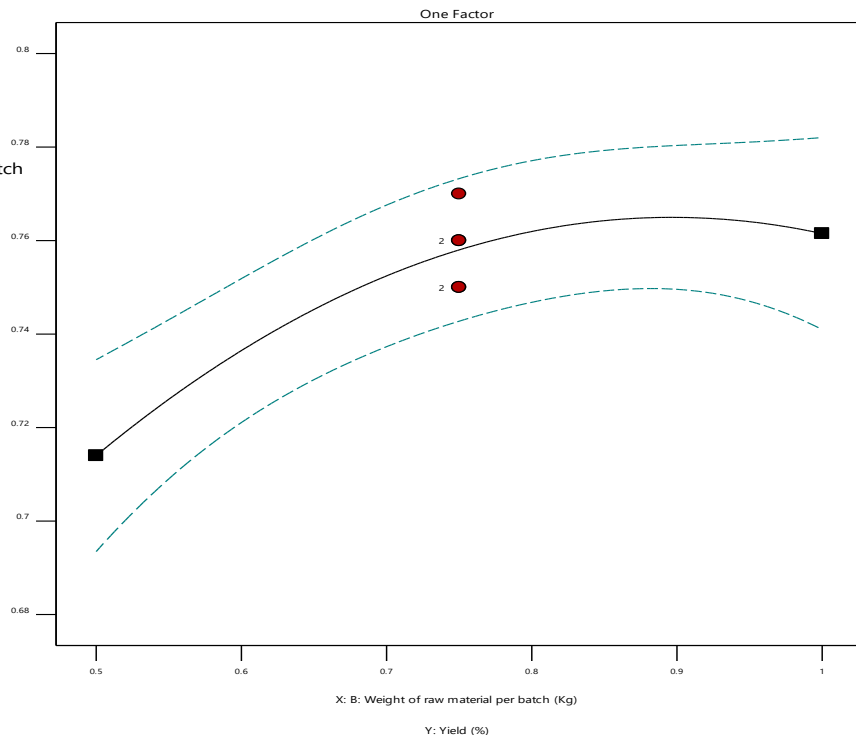


Figure 4.4. The effects of raw material weight per batch on yield

Lemongrass is a member of the Poaceae family, which has a large surface area compared to other solid matters. Therefore, input mass analysis was required to obtain the highest yield in a pilot plant scale extraction process.

4.3.4.3. The effects of extraction time on lemongrass essential oil yield

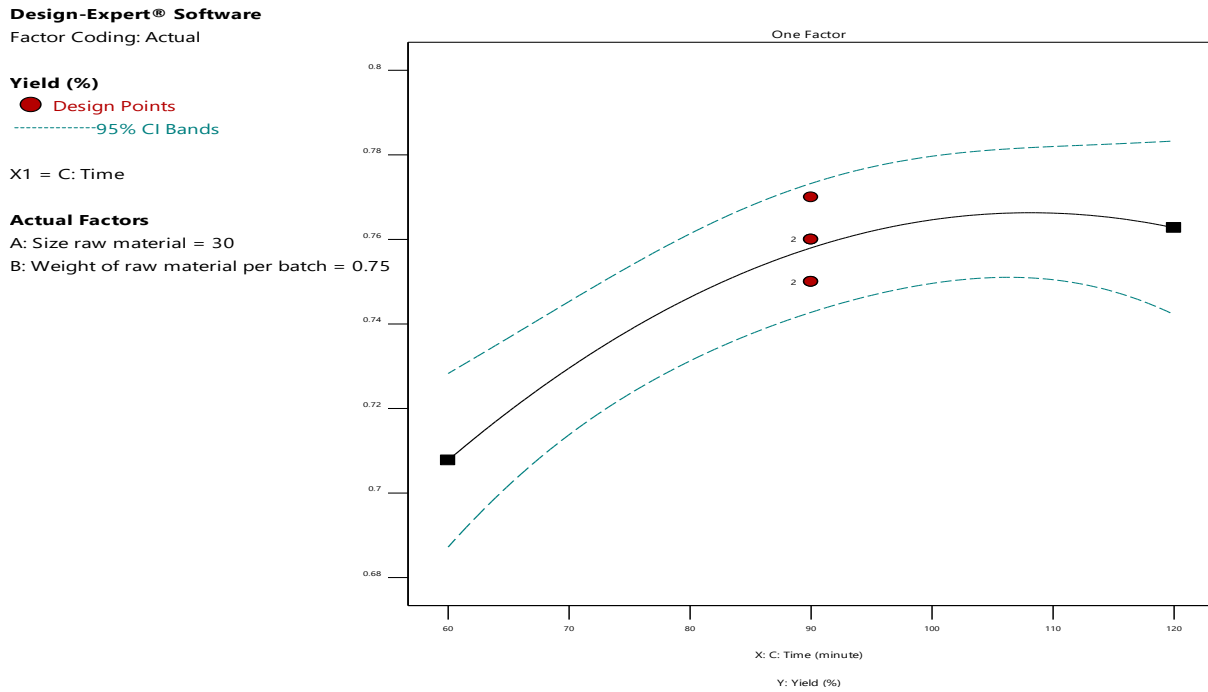


Figure 4.5. The effect of extraction time on lemongrass essential oil yield

Lemongrass essential oil extraction time was dependent on steam supply and extractor compartment quality. The extraction was carried out as mentioned above on a pilot plant scale, which is an efficient and effective extraction process, and the time distribution extraction process is similar to the above fig (4.5).

4.3.4.4. The interaction effects of particle size of raw material and weight of raw material per batch on lemongrass essential oil extraction

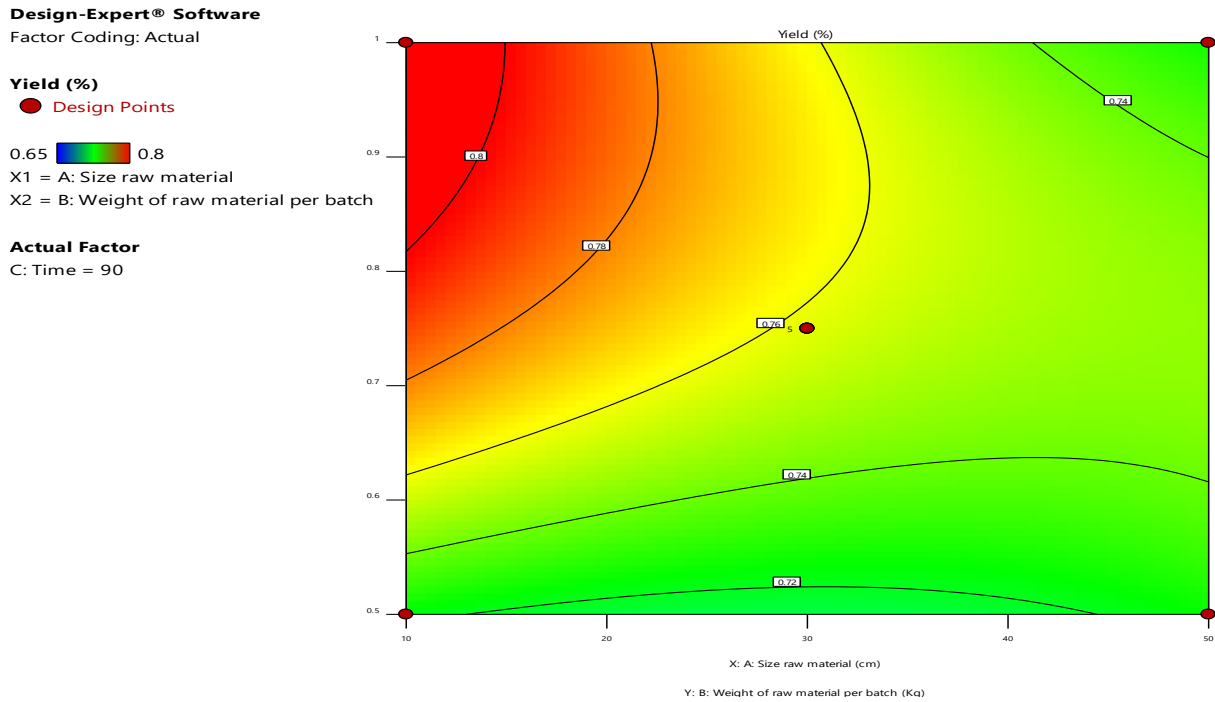


Figure 4.6. The interaction effects of particle size and weight of raw material

The interaction effect of raw material particle size and weight of raw material per batch is clearly seen in the above fig (4.6). This experimental result was shown at a constant time of 90 min or the effects of time were ignored. The yield increases as the weight of the raw material increases and the particle size of the raw material decreases. The highest yield was seen in the regions where raw material particle size was reduced and the weight of raw material per batch increased.

4.3.4.5. The interaction effects of particle size of raw material and extraction time on lemongrass essential oil extraction

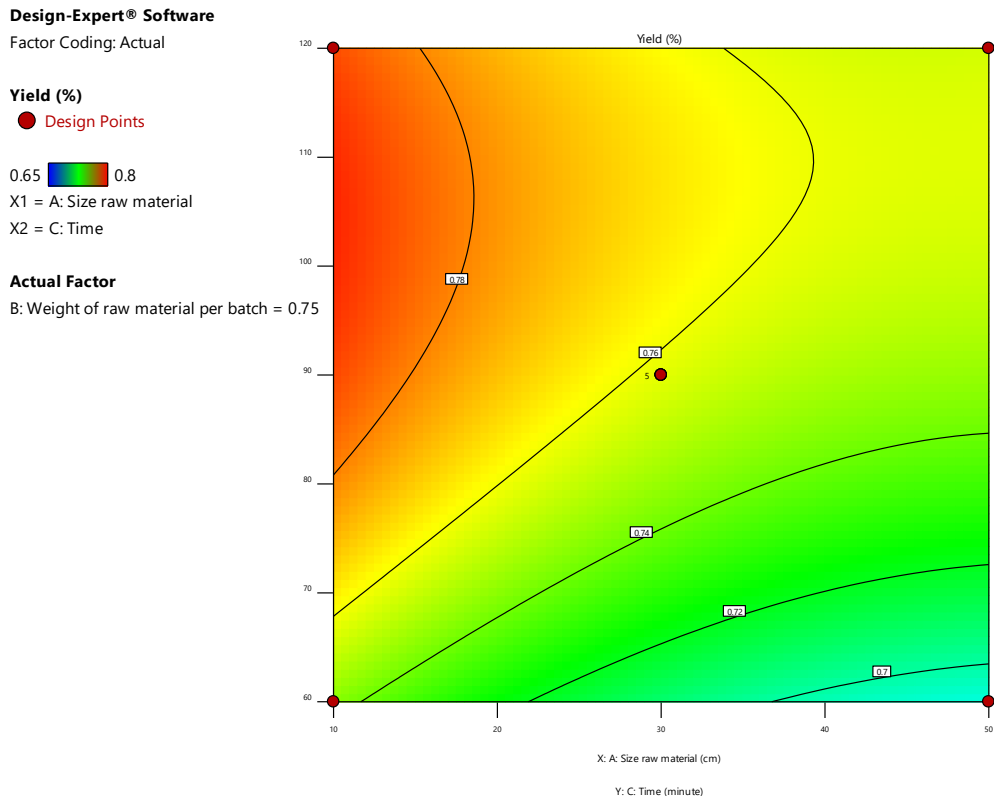


Figure 4.7. The interaction effects of particle size and extraction

The interaction effect of raw material particle size and extraction time was also seen with the fixing of the weight of raw material per batch, which is a constant of 0.75kg. Both effects are nearly balanced and similar, but the interaction effects show that size reduction has an impact comparable to extraction time in the above fig(4.7), since the extraction time after 60 min has not yet had a significant influence on the overall extraction process, comparable to the raw material's particle size.

4.3.4.5. The interaction effects of raw material weight per batch and extraction time on lemongrass essential oil extraction

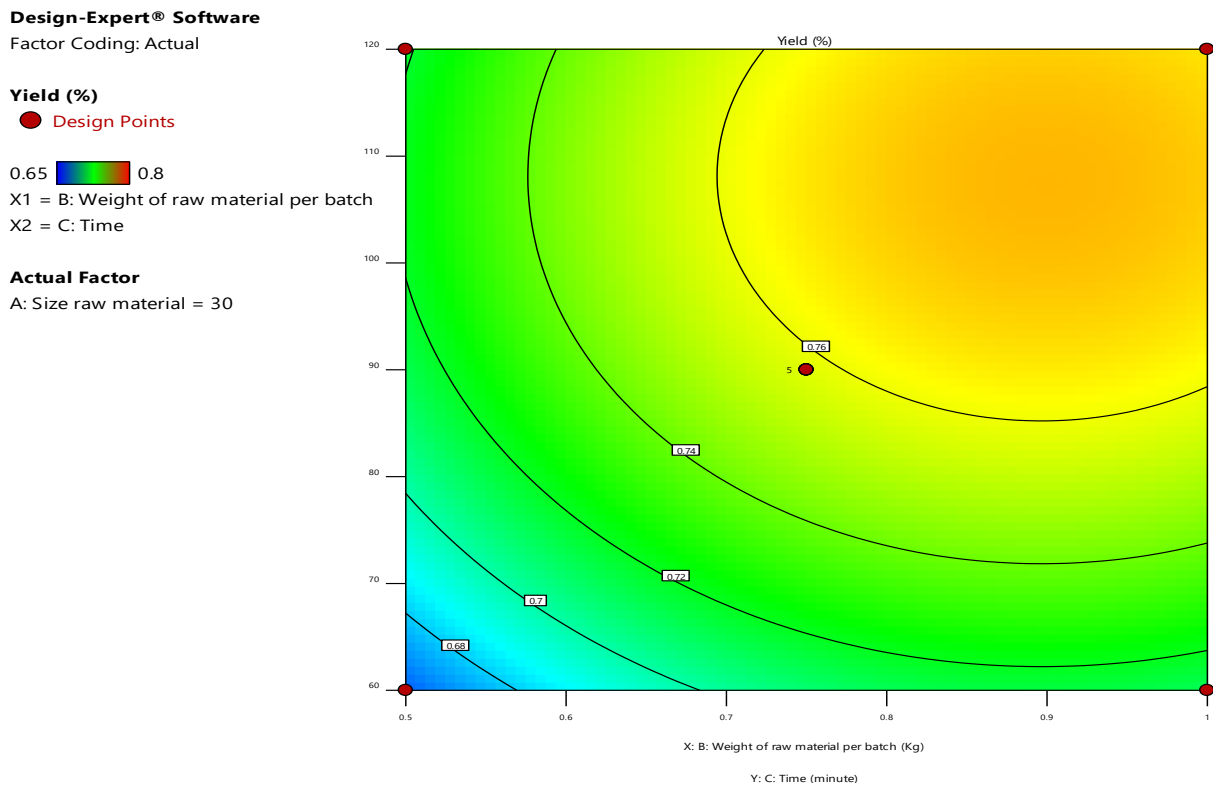


Figure 4.8. The interaction effects of weight of raw material and extraction time

The interaction effects of raw material weight per batch and extraction time are evaluated by ignoring the effects of raw material size, which was constant at 30 cm. According to the fig (4.8) above, the weight of raw material per batch increases, as does the extraction time, and the yield increases proportionally.

4.3.4.6. Optimization and verification of lemongrass essential oil extraction experimental result

Optimization was performed using input constraint goals determined by the study's interests, while raw material particle size reduction had little cost. Therefore, the input was in the range of values. The second weight of raw material per batch change has no effect on production costs. Its input value was within a given range. Extraction time has a notable impact on overall production costs. Therefore, the input value was minimized. Thermal degradation of oil components might occur and the cost of steam and other operational costs would rise. The final constraint was yield maximization, which is the primary and ultimate goal of this study. optimization was done by expert design software design. The use of statistical experiment design techniques in the extraction process may result in an increased product yield. Approximate conformation of the output response to nominal and targeted requirements, as well as reducing development time and overall costs..

Table 4.5. input constraints for optimization of lemongrass essential oil extraction

Constraints						
Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:Size raw material(cm)	is in range	10	50	1	1	1
B:Weight of raw material per batch(kg)	is in range	0.5	1	1	1	2
C:Time(min)	minimize	60	120	1	1	5
Yield (%)	maximize	0.65	0.8	1	1	5

The optimized output result was a raw material particle size of 10 cm, a raw material weight per batch of 1 kg, an extraction time of 62 minutes and a yield of 0.775. The detailed information is attached in the appendix part.

Verification: Empirical data, observations, and experiments result must be verified in order to validate the truth or provide a rational justification for a theory. In the case of steam distillation of lemongrass essential oil, process optimization has been thoroughly verified based on methodologies largely employed in experimentation and duplicated. The optimized result has been validated, and the result has been confirmed as the optimized result in Table(4.6) below.

Table 4.6. Verification of the optimized result

Ex.No	factor employed			Yield yield%	Rremarkemark
	particle size(cm)	weight raw material per batch(Kg)	extraction time (min)		
1 st	10	1	62	0.760	
2 nd	10	1	62	0.765	
Average	10	1	62	0.762 ≈ 0.775 ε _i % < 2	The conformation was successful.

4.4. Physical characterization of Lemongrass essential oil

4.4.1. Specific gravity determination Lemongrass essential oil

The specific gravity determination procedure was explained in section three, and the equation employed to calculate the specific gravity value.

Where: W₀–weight in grams of Empty Pycnometer at 20°C=24.5637g

W₁: weight in grams of Pycnometer with lemongrass oil at 20°C=69.4246

W₂: weight in grams of distilled water Pycnometer at 20=74.0513

$$\text{Sp.gr} = \frac{W_1 - W_0}{W_2 - W_0}$$

$$\text{Sp.gr} = \frac{69.4246 - 24.5637}{74.0513 - 24.5637} = 0.907$$

4.4.2. Kinematic viscosity of lemongrass essential oil

The dynamic viscosity was measured with Vibro viscometer directly, and the result was 2.87 mpa.s, at the temperature of 20°C. The dynamic viscosity correlation can be expressed in the following unit expression $Dv=2.87\text{mpa.s}=2.87 \times 10^{-3} \text{ kg/m.s}$ and the density of lemongrass essential oil is $=907 \text{ kg/m}^3$ by using equation (3.1) kinematic viscosity of the lemongrass essential oil calculated.

$$Kv = \frac{Dv}{\rho}$$

$$Kv = \frac{2.87 \times 10^{-3} \frac{\text{kg}}{\text{m}} \cdot \text{s}}{907 \text{ kg/m}^3} = 3.1643 \times 10^{-6}$$

Table 4.7 Physical property standard comparison result summery.

conditions	This study	Standard values	source
Appearance	Liquid	Liquid	(Merck 2015)
Colour	light Yellow	Reddish-yellow to reddish-brown	(Merck 2015)
Odour	Sweet, lemon like	Sweet, lemon like	(Merck 2015)
Relative Density/ specific gravit	0.907 @ 20°C	0.895-0.908 @ 20°C	(Merck 2015)
Refractive Index	1.4824 @ 20°C	1.4800±0.0002	Beriso M, 2020
kinematic viscosity	3.1643*10 ⁻⁶ @ 20°C	< 7 x 10 ⁻⁶ m ² /s at 40°C	(Moyler 2010)

The main physical characteristics of lemongrass essential oils are: relative density, refractive index, kinematic viscosity, and optical rotation, which can help to express the physical properties of product quality and the experimental results were supplemented with standardized results. As a result, this study was successful and achieved the intended goal.

4.5. Chemical characterization of Lemongrass essential oil

4.5.1. Functional group determination by FT-IR Analysis

The vibrational frequency in the wave numbers of the sample spectrograph obtained from an FT-IR spectrophotometer were compared to those of an IR correlation chart Lemongrass essential oil FT-IR absorption spectrum in the table (4.9) below to determine the functional group found in the lemongrass essential oil.

The dominant peak observed approximately 3500 (Dimeric O-H stretch), 2934 cm⁻¹, Alkene (C-H-stretch), 2862 alkene (C-H- weak vibration), 2801 cm⁻¹ aldehyde (CHO-), 1680 cm⁻¹ Aromatic (citral) (C-C cis and trans citral), 1465cm⁻¹ (bending of CH₃), 1452 (C-C), 1379, ester (C-O), 1197 cm⁻¹ (CH), 1124cm⁻¹ and (C-O stretching) of skeleton are observed.

The spectra of lemongrass essential oil were given as a percentages of transmittance versus wave number. The main transmittance region range was 400cm⁻¹- 400cm⁻¹. The peaks results are typically 1680, 2934, 452 and 3500 are observed. 1680 is approximated with aromatic component (citral) which is clearly seen in GC-MS analysis result it has higher amount with other components. Therefore, the approximation result is relatively effective. IR correlation chart is used (Asep Bayu, 2019). The Lemongrass essential oil functional group identification was done with coupling with GC-MS analysis.

Table 4.8 functional group FT-IR Analysis result

Wave number (cm-1)	peak assignment
3500	Dimeric O-H stretch
2934	C2H2 Alkene symmery
2862	C2H2 Alkene
2801	CHO Aldehyde
1724	C-O-Ester
1680	C=C stretch (citral)
1465	CH3 bending
1452	C-C (cis and trans)
1379	C-C and ester (C-O)
1197	CH-Alkene
1124	C-O stretching

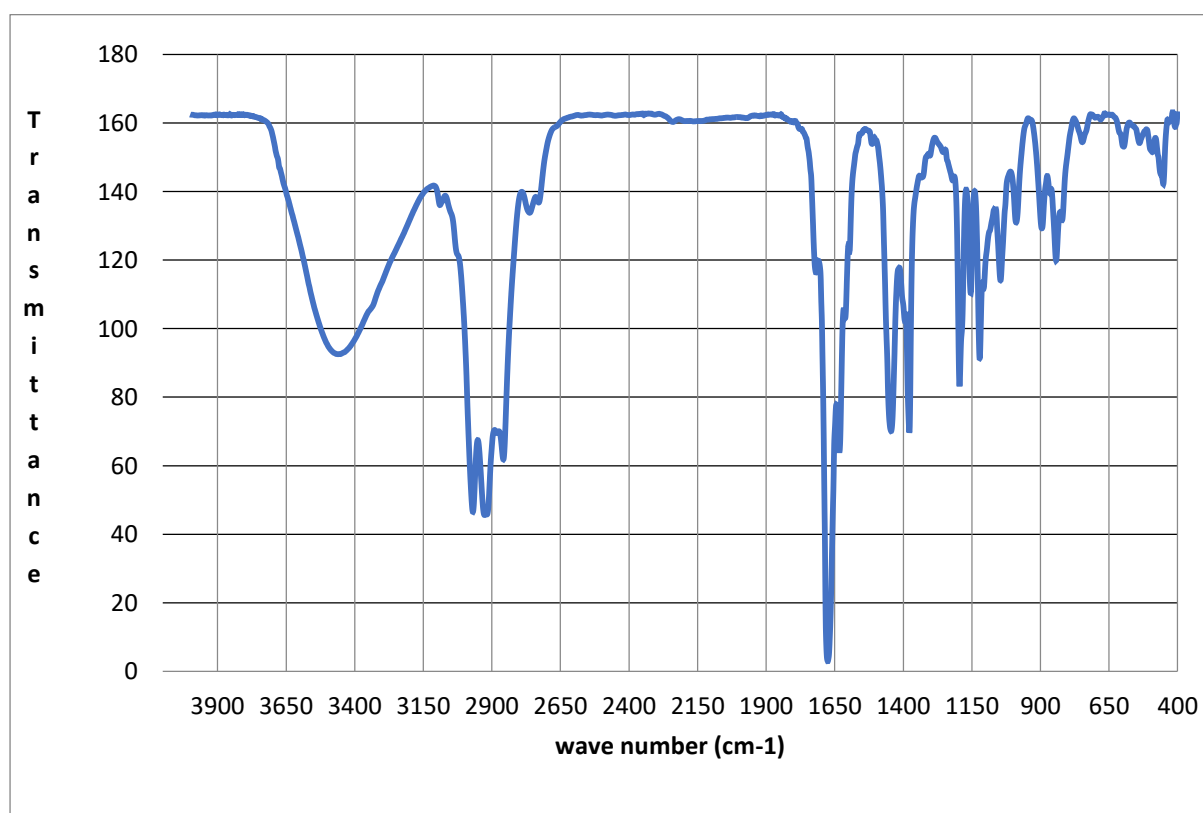


Figure 4.9 Lemongrass essential oil FT-IR result

4.5.2. Gas chromatography mass spectroscopy analysis

By using GC-MS Analyzer from Wg Lomisar (I), 13 main components are found, which is 65.5% of the total components. The remaining portion or (34.5%) components are lacking quality identification or those components quality indications are less than (80%). The chemical constituents of oil are presented in the given fig(4.10) and (Table 4.6). The major components of the essential oils are: Citral (28.4227%), Beta.-Myrcene (11.6291%), Heptadecane, 9-octyl- (7.7451%), Docosanoic acid, methyl ester (5.701%) respectively.

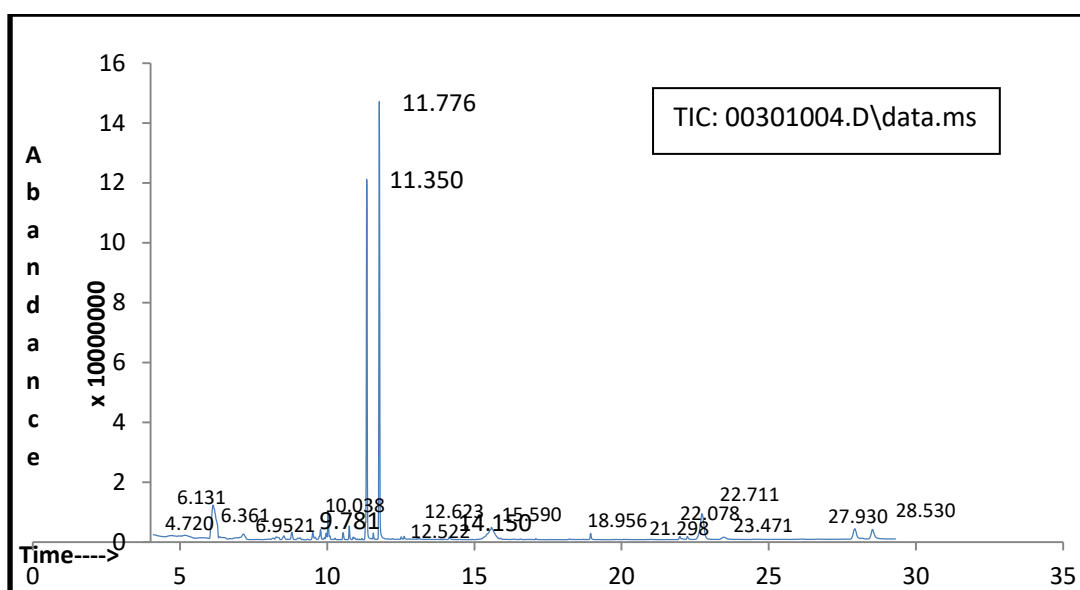


Figure 4.10 GC-MS Lemongrass essential (Wg lomisar I) oil component result

Table 4.9 Major chemical composition of lemongrass (Wg Lomisrar (I) essential oil.

Library/ID	Retention time(min)	Area (%)	Quality
Beta.-Myrcene	6.131	11.6291	96
Isoneral	9.7805	1.2132	95
Isogeranial	10.0385	2.3811	97
(-)-trans-Isopiperitenol	10.5466	0.6389	86
(-)-trans-Isopiperitenol	10.7557	0.9393	89
2-Undecanone	11.5726	0.5098	89
Citral	11.7758	28.4227	91
Docosanoic acid, methyl ester	15.5902	5.701	99
Hexadecanoic acid, methyl ester	18.956	0.5756	97
Heptadecanoic acid, 16-methyl-, methyl ester	22.2423	0.3886	95
Heptadecane, 9-octyl-	22.7374	7.7451	93
Octacosane	27.9302	2.7922	91
Tetracosanoic acid, methyl ester	28.5303	2.6782	99

Citral: Citral is the essential oil's principal terpenoid component, and it has antioxidant properties as well as co-oxidation with the target substrate and cross-termination of the oxidative chain (Ewa Majewska, 2019). In the form of gel or functional paper microencapsulation with oil, it is utilized in the perfume industry, as well as for wound cleaning and skin disease therapy, and it can also be used for hand hygiene. The content of citral, terpene, and aldehyde, which are primarily used in the manufacture of vitamins, determines the quality of lemongrass oil (A, E, and K). Temperature, light intensity, soil moisture, fertilizer, and maturity stages all influence the citral content of lemongrass essential oil (Getachew Jimayu, 2017). The commercial values of citral level of around 65-85 percent w/w (Wilson, 2002). When we compare the two products, Wg lomisar (I) and Wg Lomisrar (UA), Wg lomisar (I) oil species chemical content was appreciable.

β -myrcene: used as a flavoring additive in foods and beverages, for example. Cosmetics, soaps, and detergents, as well as other fragranced items, including air care products, polishes, wax mixes, adhesives, disinfectants, biocides, paints, plasters, fuels, inks, toners, and electronic cigarette liquids, use it extensively. β -Myrcene is a precursor to a number of industrially significant compounds, including menthol, geraniol, nerol, linalool, and isophytol. β -Myrcene is employed in the manufacture of terpene polymers, terpene-phenol

resins, and terpene–maleate resins, in addition to its major use as an intermediary in the creation of terpene alcohols.

Lemongrass essential GC-MS analysis results published in an international pharmacy and chemistry journal is identical to this experimental results. The content of β -Myrcene, in particular, was comparable with the research findings. They examined the efficacy of two microbes, bacteria and fungi, and the results showed a significant drop in microbes over time. If access to the efficacy test medium, this extract hopes to score appreciable results. (Mahmoud Mohamed Ali,et.al 2017).

5. Conclusion and recommendation

5.1 Conclusions

This study was aimed to give useful information for optimization and characterization of lemongrass essential oil extraction. Lemongrass essential extraction methods include steam distillation, solvents, and supercritical CO₂. Steam distillation has been selected for the extraction process. It has the best product quality, a small capital requirement for establishment, and low operational and quality control. Selected extraction parameters were raw material particle size, raw material weight per batch and extraction time with constant temperature, pressure and steam supply. The experimental analysis was done by using design software. The optimized yield was 0.75% and the input factors were particle size of 10 cm, raw material weight per batch of 1 kg, and extraction time of 62min. and varfication was done and successful.

The three different lemongrass varieties, Wg Lomisar java, Wg Lomisar UA and Wg Lomisa (I) were collected from Wondogenet agricultural research center and extracted in dried condition. Among these, Wg lomisar (I) has the highest yield and best quality among the three varieties. Physical and chemical properties characterization was done with a selected variety of some of them: density, specific gravity, dynamic viscosity, and kinematic viscosity, as well as FT-IR and GC-MS analysis. The GC-MS component analysis results were Citral (28.4227%), Beta.-Myrcene (11.6291%), Heptadecane, 9-octyl- (7.7451%), Docosanoic acid, methyl ester (5.701%) respectively. Despite the fact that the citral content was much lower than expected, the other physical and chemical characterization results were within the range of international standards. As a result, the ingredients obtained from this study can be fully utilized in the manufacture of antioxidants, antimicrobial, antifungal, antiseptic agents, insect repellants, and other cosmetic products.

5.2. Recommendations

- This research was conducted with a limited financial resources, but such a remarkable topic necessitates substantial financial support in order to conduct an adequate and effective study of the overall factors.
- The available laboratory equipment control system was out of phase, the main parameters of steam distillation extraction, such as temperature and steam control, were not investigated in this study. Therefore, the institute (AAiT) should install effective steam distillation equipment to improve the effectiveness of the steam distillation extraction study.
- Further research into the impact of maturity level on yield and quality is required to determine the lemongrass essential oil variety.
- Detailed research feasibility, such as marketing, demand and supply studies, are required to implement the research work into a real scenario.
- A material safety data sheet (MSDS) should be prepared, which contains aggregate information about a product.

Reference

Abdurahman Nour, Ranitha and Azhari, (2013), Extraction and Characterization of Essential Oil from from Ginger (*Zingiber Officinale Roscoe*) and Lemongrass (*Cymbopogon citratus*) by Microwave Assisted Hydrodistillation.

Alam P. N.,(2018), Extraction of citral oil from lemongrass (*Cymbopogon Citratus*) by steam-water distillation technique.

Asep Bayu, Dani Nandiyanto, Rosi Oktiani, Risti Ragadhita ,(2019), how to read and interpret FTIR spectroscopy of organic material.

Beemnet Mengesha Kassahun, Desta Fikadu Egeta, Wondu Bekele Yosef, Tewodros Lulsseged, Variability in Lemongrass (*Cymbopogon citratus* (DC) Stapf.) Genotypes for Agronomic and Chemical Characters in Ethiopia.

Beriso Mieso, Abdela Befu, (2020), Physical Characteristics of the Essential Oil Extracted from Released and Improved.

Braimah Soale Rashid,(2016) BSc. Thesis, Design and optimization of lemon grass oil extractor

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

Cultivars, (2011), Chemical Composition, Antimicrobial and Antioxidant Activities of Essential Oils from Organically Cultivated Fennel Cultivars,

Dinh Nhat Do, Dinh Phuc Nguyen, Viet-Duc Phung , Xuan-Tien Le, Tuan Minh Le, Van Manh Do Bui Quang Minh and Xuan Cuong Luu, (2021), Fractionating of Lemongrass (*Cymbopogon citratus*) Essential Oil by Vacuum Fractional Distillation.

Debashis Duta, Pawan Kumar, Nath, Verma, and Gangwar, Qualities of lemongrass (*Cymbopogon citratus*) essential oil at different drying conditions.

Ewa Majewska, Mariola Kozłowska, Eliza Gruczyńska-Skowska, Dorota Kowalska, Katarzyna Tarnowska, (2019), Lemongrass (*Cymbopogon citratus*) Essential Oil: Extraction, Composition, Bioactivity and Uses for Food Preservation a Review.

Farid Chemat:(2015) Extraction steam distillation.

Fentahun Mengistu (PhD), Medicinal and Aromatic plants research strategy, 2016.

Getachew Jimayu and Aynalem Gebre,(2017), Influence of harvesting age on yield and yield related traits of lemongrass (*Cymbopogon Citratus.*) varieties at Wondogenet, southern Ethiopia.

González-hernández,(2015) Chemical composition of scented extracts obtained from *calendula officinalis* by three extraction methods.

Grant Thamkaew, (2020) Ingegerd Sjöholm & Federico Gómez Galindo,2020, A review of drying methods for improving the quality of dried herbs

Guenther,(1960.) The essential oil History -origin in plant production -ananalysis. (fourth, Ed.) .

Hamid, Aiyelaagbe and Usman, (2011), essential oils: its medicinal and pharmacological uses.

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

Johann, (2017) International Journal of Homeopathy & Natural Medicine

Kpo Samsono,(2020), GC-FID and FT-IR characterization of Lemongrass Oil extracted With soxhlet extraction apparatus using ethanol as solvent.

Lisong, Menghao, Shaohai,(2019) Study on the Composition and Super Critical Carbon Dioxide Extraction of Chinese Hickory, (*Carya cathayensis* Sarg.) Kernel Oil.

Luis. Salomé-abarca, ramón m. Soto-hernández, nicacio cruz-huerta and víctor a.

María del Carmen Vázquez-Briones, Luis Ricardo Hernández & José Ángel Guerrero-Beltrán¹,(2015) physicochemical and Antioxidant Properties of *Cymbopogon citratus* Essential Oil.

Merck, (2015), The Merck Index Online. Cambridge, UK : Royal Society of Chemistry.

Muhammad, Supardan, Erni Misran Mahlizar, Satriana, Wan Aida w. Mustapha, (2019.)Effect of material length on kinetics of essential oil hydrodistillation from lemongrass (*cymbopogon citratus*).

Mosquera, Noriega, Cornejo and Pardo, (2016) Biological activity of *Cymbopogon citratus*.

Moyler, (2010), Citral from Lemongrass and Other Natural Sources: Its Toxicology and Legislation. In Essential Oil-Bearing Grasses: The Genus *Cymbopogon*.

Mahmoud Mohamed Ali, Mohammad Abdi Yusuf, Mohamed and Abdalaziz, (2017), GC-MS Analysis and Antimicrobial Screening of Essential Oil from Lemongrass (*Cymbopogon citratus*).

Narisa Kamkaen, Nijisiri Ruangrunsi, Natedaow Patalung, Rithn Watthanachaiyingcharo-en, (2014), Physiological and psychological effects of lemongrass and sweet almond massage oil lemongrass and sweet almond massage oil.

Nicola Wilson, Maria Ivanova, Robert Watt and Anthony Moffat, (2002) The quantification of citral in lemongrass and lemon oils by near-infrared spectroscopy.

Nguyen Dang ,(2018): Kinetics and modeling of oil extraction from Vietnam lemongrass by steam distillation.

Ojochenemi, Yakubu, Olawale Otitoju and Joshua Onwuka, (2007), Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Aqueous Extract of *Daniellia oliveri* Stem Bark.

Olivi Vostinaru, Simona Codruta Heghes and Lorena Filip , Safety Profile of Essential Oils.

Parikh, and Desai (2011), Hydrodistillation of essential oil from *Cymbopogon flexuosus*, *Int. Food Eng* .

Ranitha Mathialagan,(2014) Identification and optimization of lemongrass essential oils extracted by hydrodistillation and microwave assisted hydrodistillation methods.

Robert Becker, (1999), The Body Electric validates that the human body has an electrical frequency and that much about a person's health.

Sarkic, Stappen, (2018), Essential Oils and Their Single Compounds in Cosmetics A Critical Review

Tajidin, Ahmad, Rosenani, Azimah, and Munirah, (2012) Chemical composition and citral content in lemongrass (*Cymbopogon citratus*) essential oil at three maturity stages.

Thabile Precious Nkambule,(2008), Antimicrobial properties of selected asian herbs MSc.Thesis.

Vanisha Nambiar and Hema Matela,(2012) International Journal of Pharmaceutical and Biological Archives.

Werotaw Sisay Yeshitila, (2019), Herbage and Essential Oil Yield of Two Lemmon Grass (*Cymbopogon Citratus*) Varieties as Affected by Harvesting Cycle and Number of Tillers Per Planting Slips at Wondo Genet, Southern Ethiopia.

Yongliang Liu and Hee-Jin Kim , (2017), Fourier Transform Infrared Spectroscopy (FT-IR) and Simple Algorithm Analysis for Rapid and Non-Destructive Assessment of Developmental Cotton Fibers.

Setiabudi,(2019), How to read and interpret FTIR spectroscopy of organic material.

Thi Van Linh Nguyen, My Duyen Nguyen, Duy Chinh Nguyen, Long Giang Bach and Tri Duc Lam (2018), Model for thin Layer drying of lemongrass (*Cymbopogon citratus*) by hot air.

Wilson,(2002) The quantification of citral in lemongrass and lemon oils by near-infrared spectroscopy.

(2009) The Use of Herbal Medicines Report of the Regional Meeting 10–12 March, Yangon, Myanmar in Primary Health Care.

(2019).This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 6–13 June 2017 Lyon, France.

(2012), Citronella grass and lemongrass grown in Thailand,” Journal of AOAC International, vol. 95.

**Appendix
Appendix A**

Table A1. Summary information about lemongrass essential oil experiment

File Version	12.0.3.0								
Study Type	Response Surface	Subtype	Randomized						
Design Type	Box-Behnken	Runs	17						
Design Model	Quadratic	Blocks	No Blocks						
Build Time (ms)	30.00								
Factors and statistical data									
Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	Size raw material	cm	Numeric	10.00	50.00	-1 ↔ 10.00	+1 ↔ 50.00	30.00	14.14
B	Weight of raw...	Kg	Numeric	0.5000	1.0000	-1 ↔ 0.50	+1 ↔ 1.00	0.7500	0.1768
C	Time	minute	Numeric	60.00	120.00	-1 ↔ 60.00	+1 ↔ 120.00	90.00	21.21

Responses

Response	Name	Units	Observations	Analysis	Minimum	Maximum	Mean	Std. Dev.	Ratio	Transform	Model
R1	Yield	%	17	Polynomial	0.65	0.8	0.7424	0.0372	1.23	None	Quadratic

Constraints

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A: Size raw material	is in range	10	50	1	1	1
B: Weight of raw material per batch	is in range	0.5	1	1	1	2

C:Time	minimize	60	120	1	1	5
Yield	maximize	0.65	0.8	1	1	5

Table A2. The output solutions of the given experiment input constraints.

Number	Size raw material	Weight of raw material per batch	Time	Yield	Desirability	
1	10.000	1.000	62.172	0.775	0.895	Selected
2	10.000	1.000	62.354	0.775	0.895	
3	10.000	1.000	61.865	0.774	0.895	
4	10.000	1.000	62.615	0.776	0.895	
5	10.000	1.000	62.834	0.776	0.895	
6	10.000	1.000	61.455	0.773	0.894	
7	10.000	1.000	63.062	0.776	0.894	
8	10.000	0.997	62.870	0.776	0.894	
9	10.000	1.000	60.988	0.772	0.894	
10	10.000	1.000	63.547	0.777	0.894	
11	10.000	0.995	62.294	0.775	0.894	
12	10.000	0.994	62.697	0.776	0.894	
13	10.000	1.000	64.031	0.778	0.894	
14	10.000	0.990	62.837	0.776	0.894	
15	10.000	0.995	63.890	0.778	0.894	
16	10.000	1.000	64.422	0.779	0.893	
17	10.000	0.987	63.268	0.777	0.893	
18	10.048	1.000	60.000	0.770	0.893	
19	10.000	1.000	65.165	0.781	0.893	
20	10.000	0.981	63.315	0.776	0.892	
21	10.000	0.976	62.726	0.775	0.892	
22	10.000	0.987	66.059	0.782	0.890	
23	10.000	0.964	64.047	0.777	0.889	
24	10.312	0.996	60.000	0.769	0.889	
25	10.000	0.995	67.273	0.785	0.889	
26	10.000	1.000	67.976	0.786	0.887	

27	10.000	0.993	67.822	0.786	0.887	
28	10.000	0.940	60.331	0.768	0.884	
29	10.000	1.000	69.377	0.789	0.884	
30	10.326	0.949	60.000	0.767	0.882	
31	10.000	0.917	60.940	0.768	0.879	
32	10.000	0.912	62.750	0.771	0.878	
33	10.000	0.943	68.950	0.786	0.877	
34	10.000	1.000	71.845	0.793	0.875	
35	10.000	0.900	60.634	0.766	0.874	
36	10.000	0.891	69.202	0.782	0.863	
37	10.000	0.870	69.775	0.781	0.855	
38	10.000	0.814	76.634	0.785	0.807	

File:D:\MassHunter\GCMS\1\5977\may 17, 2021\01301001.D

Operator: Estif

Acquired: 17 May 2021 10: using Acq Method Essential oil 29.33 splitless.M

Instrument: AAU

Sample Name: SoleGI

Misc Info:

Vial Number: 13

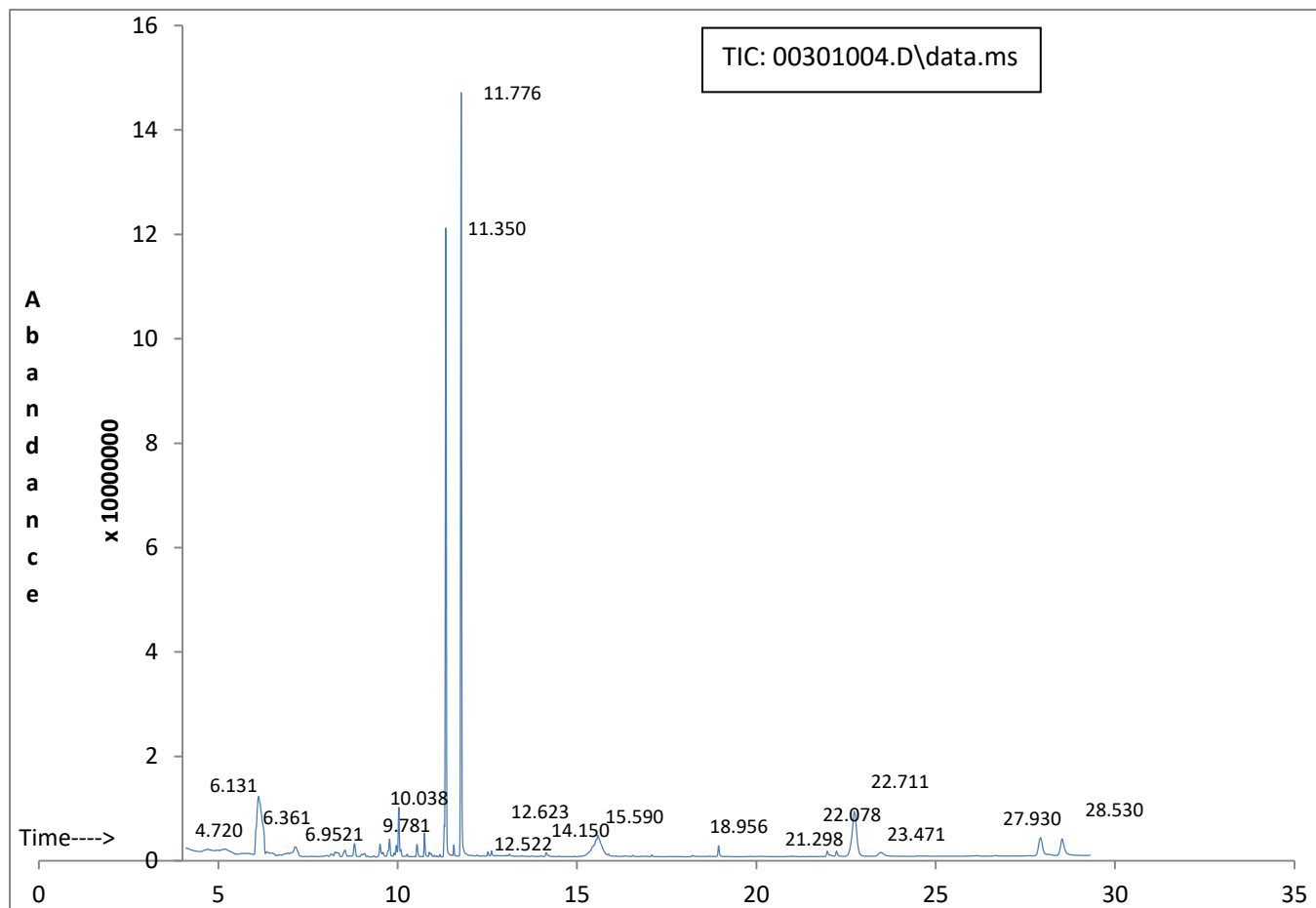


Figure A: Lemongrass essential oil (Wg lomisar I) GC-MS

Table A3: Wg lomisar (I) GC-MS all component result report

PK	RT	Area Percet	Library/ID	Ref	CAS	Quality
1	4.7202	0.4086	Methylene chloride	1544	000075-09-2	74
2	6.131	11.6291	.beta.-Myrcene	16066	000123-35-3	96
3	6.3609	0.3226	2-Chloroethanol	1054	000107-07-3	25
4	6.9524	0.4341	2-Chloro-N-ethylacetamide	9669	000105-35-1	28

5	7.1553	1.674	2-Chloro-N-ethylacetamide	9669	000105-35-1	20
6	8.1575	0.2405	Acetic acid, chloro-, ethyl ester	9933	000105-39-5	16
7	8.2693	0.3229	N-methyl-.alpha.methyl-3,4-methylenedioxyphenethylamine	58623	1000378-97-3	47
8	8.3223	0.366	2-Chloro-N-(hydroxymethyl)acetamide	10268	002832-19-1	30
9	8.5352	0.5988	Ethanone, 1-(4-pyridinyl)-, oxime	16546	001194-99-6	38
10	8.8044	0.9534	Linalool	27447	000078-70-6	55
11	9.0102	0.2137	p-Hydroxynorephedrine	37710	000552-85-2	46
12	9.0874	0.2565	Cathine	25667	000492-39-7	43
13	9.517	0.7439	6-Octenal, 7-methyl-3-methylene-	25938	055050-40-3	50
14	9.5976	0.2814	Acetamide, N-(aminocarbonyl)-2-chloro-	16309	004791-21-3	43
15	9.7805	1.2132	Isoneral	25795	1000414-18-0	95
16	9.914	0.1895	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	27823	000464-45-9	43
17	9.971	0.5269	3-Pyridinecarboxylic acid, 4-hydroxy-	18143	000609-70-1	59
18	10.0385	2.3811	Isogeranial	25818	1000414-18-1	97
19	10.5466	0.6389	(-)-trans-Isopiperitenol	25892	074410-00-7	86
20	10.7557	0.9393	(-)-trans-Isopiperitenol	25892	074410-00-7	89
21	10.8908	0.1886	1-(3,5-Dimethyl-1-adamantanoyl)semicarbazide	125446	351327-47-4	55
22	10.9386	0.171	1-Bromo-2-bromomethylcyclohexane	115639	052428-01-0	27
23	11.3507	24.6491	2,6-Octadienal, 3,7-dimethyl-, (Z)-	25967	000106-26-3	78
24	11.5726	0.5098	2-Undecanone	39885	000112-12-9	89
25	11.7758	28.4227	Citral	25780	005392-40-5	91
26	12.5224	0.1846	Pterin-6-carboxylic acid	71717	000948-60-7	59
27	12.623	0.2299	2,4-Dimethylamphetamine	34153	075659-61-9	46
28	14.1497	0.2773	3-Hydroxy-N-methylphenethylamine	25720	1000335-06-7	49
29	15.5902	5.701	Docosanoic acid, methyl ester	209113	000929-77-1	99

30	18.956	0.5756	Hexadecanoic acid, methyl ester	130813	000112-39-0	97
31	21.9853	0.3466	N-Desmethyltapentadol	71584	1246819-18-0	58
32	22.2423	0.3886	Heptadecanoic acid, 16-methyl-, methyl ester	157956	005129-61-3	95
33	22.7374	7.7451	Heptadecane, 9-octyl-	207503	007225-64-1	93
34	23.4712	0.805	DL-Alanine, N-DL-alanyl-	32141	002867-20-1	43
35	27.9302	2.7922	Octacosane	235615	000630-02-4	91
36	28.5303	2.6782	Tetracosanoic acid, methyl ester	228678	002442-49-1	99

File:D:\MassHunter\GCMS\1\5977\may 27, 15:09:09, 2021\01301001.D

Operator: Estif

Acquired: Thu May 27 15:09:09 2021, using Acq Method Essential oil 29.33 splitless.M

Instrument: AAU

Sample Name: WG LGUA

Misc Info:

Vial Number: 4

PK	RT(min)	Area Percent	Library/ID	Ref	CAS	Quality
1	4.7202	0.4086	Methylene chloride	1544	000075-09-2	74
2	6.131	11.6291	.beta.-Myrcene	16066	000123-35-3	96
3	6.3609	0.3226	2-Chloroethanol	1054	000107-07-3	25
4	6.9524	0.4341	2-Chloro-N-ethylacetamide	9669	000105-35-1	28
5	7.1553	1.674	2-Chloro-N-ethylacetamide	9669	000105-35-1	20
6	8.1575	0.2405	Acetic acid, chloro-, ethyl ester	9933	000105-39-5	16
7	8.2693	0.3229	N-methyl-.alpha.methyl-3,4-methylenedioxyphenethylamine	58623	1000378-97-3	47
8	8.3223	0.366	2-Chloro-N-(hydroxymethyl)acetamide	10268	002832-19-1	30
9	8.5352	0.5988	Ethanone, 1-(4-pyridinyl)-, oxime	16546	001194-99-6	38
10	8.8044	0.9534	Linalool	27447	000078-70-6	55
11	9.0102	0.2137	p-Hydroxynorephedrine	37710	000552-85-2	46
12	9.0874	0.2565	Cathine	25667	000492-39-7	43
13	9.517	0.7439	6-Octenal, 7-methyl-3-methylene-	25938	055050-40-3	50
14	9.5976	0.2814	Acetamide, N-(aminocarbonyl)-2-chloro-	16309	004791-21-3	43
15	9.7805	1.2132	Isoneral	25795	1000414-18-0	95
16	9.914	0.1895	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	27823	000464-45-9	43

17	9.971	0.5269	3-Pyridinecarboxylic acid, 4-hydroxy-	18143	000609-70-1	59
18	10.0385	2.3811	Isogeranial	25818	1000414-18-1	97
19	10.5466	0.6389	(-)-trans-Isopiperitenol	TIC: 00301004.D\data.ms)-7	86
20	10.7557	0.9393	(-)-trans-Isopiperitenol			89
21	10.8908	0.1886	1-(3,5-Dimethyl-1-adamantanoyl)semicarbazide	125446	351327-47-4	55
22	10.9386	0.171	1-Bromo-2-bromomethylcyclohexane	115639	052428-01-0	27
23	11.3507	24.6491	2,6-Octadienal, 3,7-dimethyl-, (Z)-	25967	000106-26-3	78
24	11.5726	0.5098	2-Undecanone	39885	000112-12-9	89
25	11.7758	28.4227	Citral	25780	005392-40-5	91
26	12.5224	0.1846	Pterin-6-carboxylic acid	71717	000948-60-7	59
27	12.623	0.2299	2,4-Dimethylamphetamine	34153	075659-61-9	46
28	14.1497	0.2773	3-Hydroxy-N-methylphenethylamine	25720	1000335-06-7	49
29	15.5902	5.701	Docosanoic acid, methyl ester	209113	000929-77-1	99
30	18.956	0.5756	Hexadecanoic acid, methyl ester	130813	000112-39-0	97
31	21.9853	0.3466	N-Desmethyltapentadol	71584	1246819-18-0	58
32	22.2423	0.3886	Heptadecanoic acid, 16-methyl-, methyl ester	157956	005129-61-3	95
33	22.7374	7.7451	Heptadecane, 9-octyl-	207503	007225-64-1	93
34	23.4712	0.805	DL-Alanine, N-DL-alanyl-	32141	002867-20-1	43
35	27.9302	2.7922	Octacosane	235615	000630-02-4	91
36	28.5303	2.6782	Tetracosanoic acid, methyl ester	228678	002442-49-1	99

Figure A2: (Wg Lomisar UA) GC-MS result

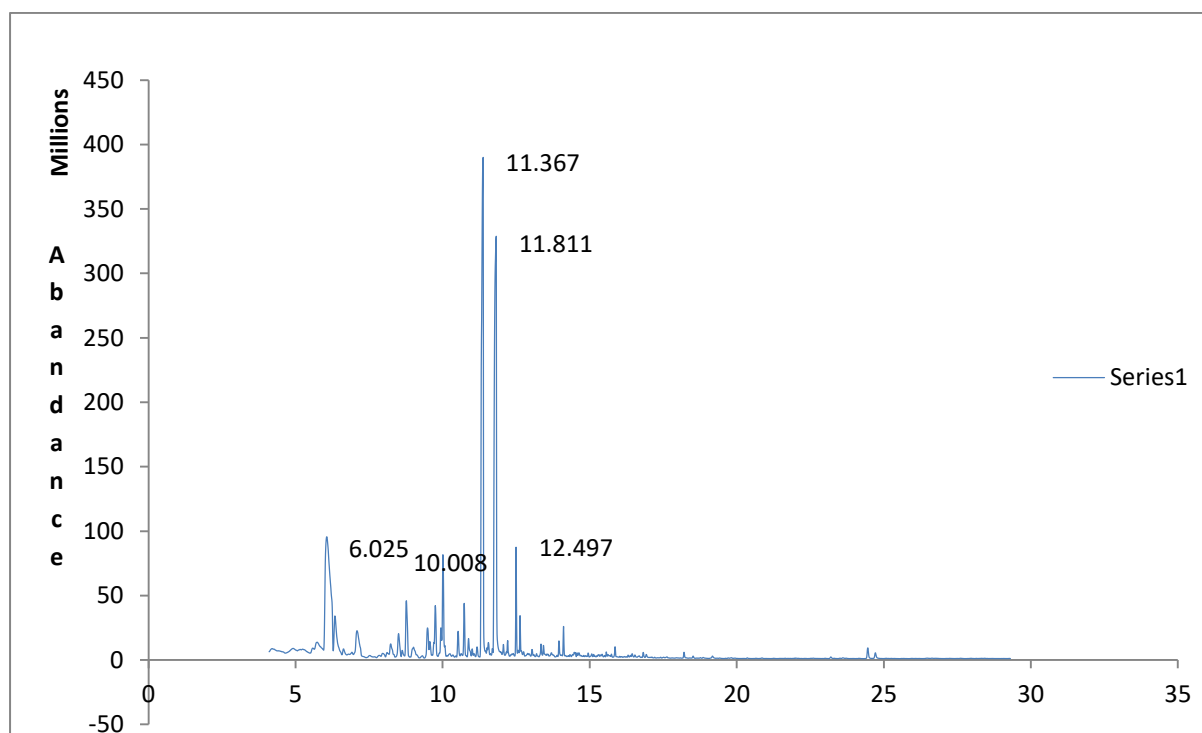


Table A4: Wg Lomisar UA GC-MS all component result report

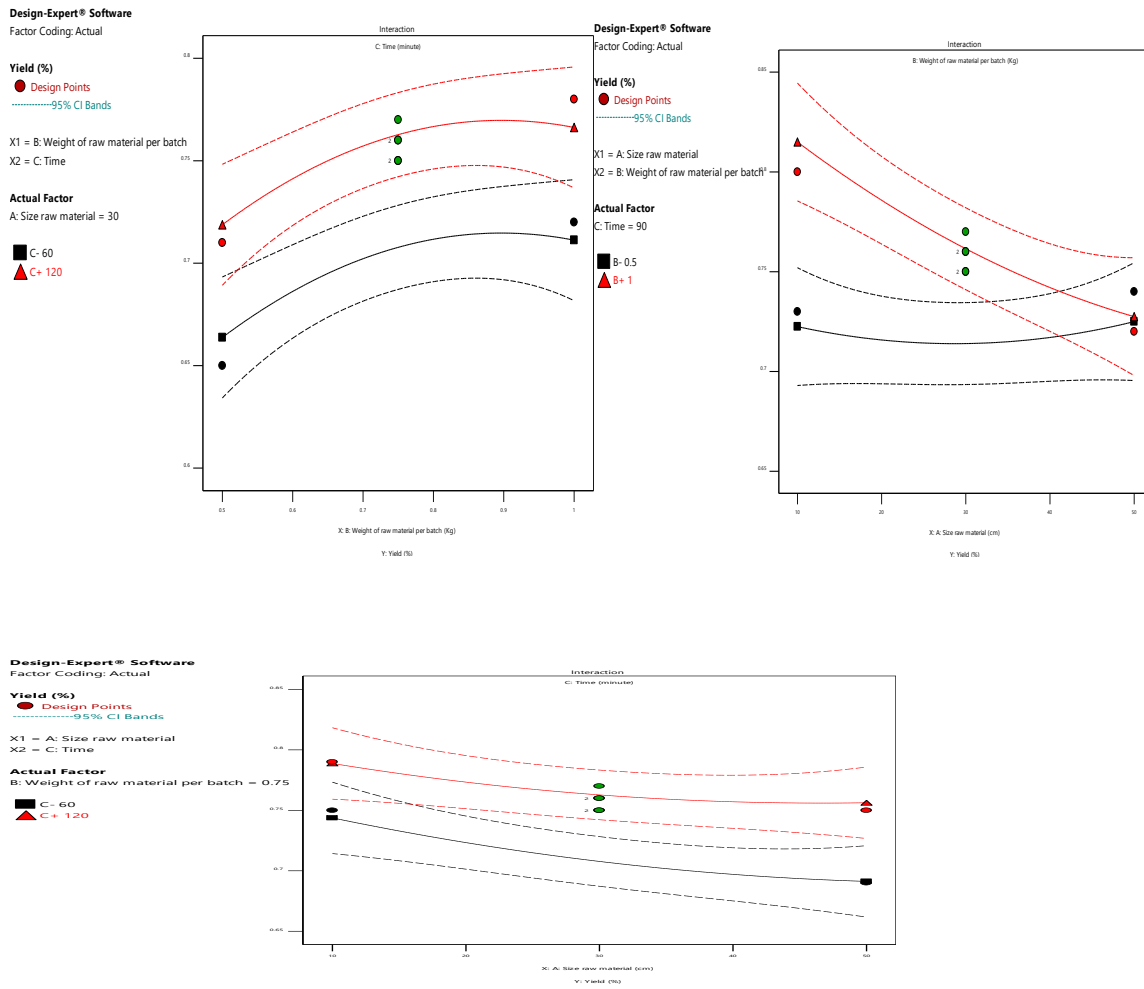


Figure A3: The three factor interaction effects

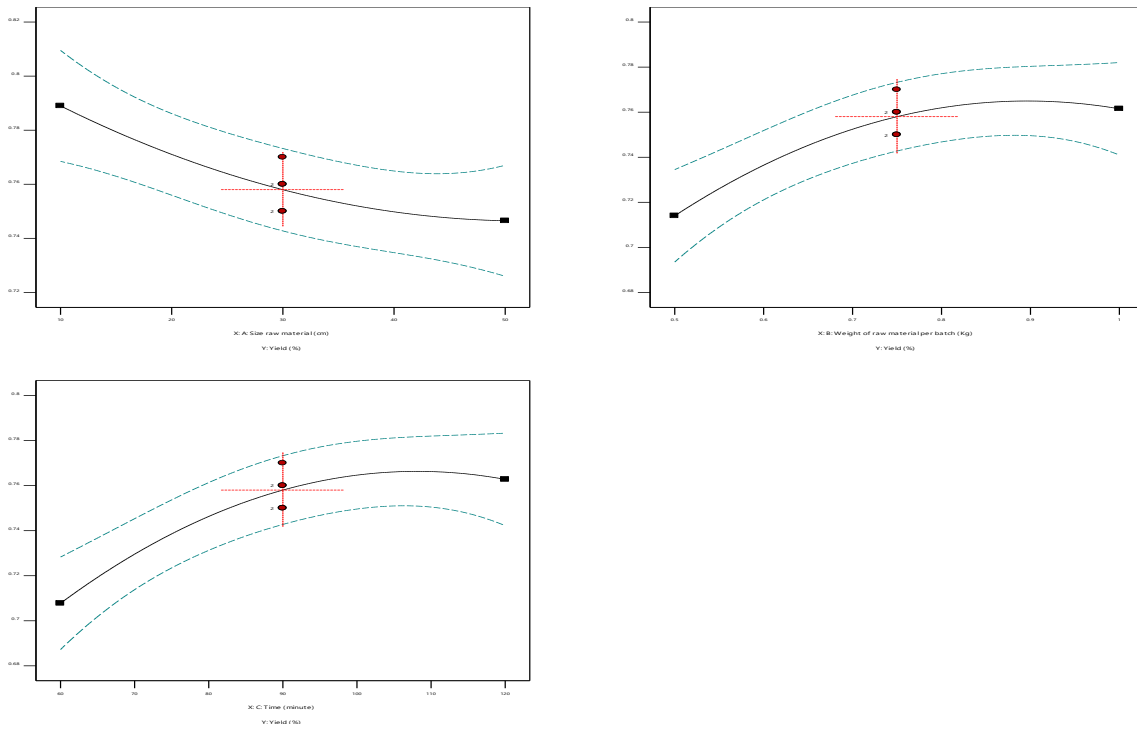


Figure A4: All single interaction effects

Table A5: The data design software analysis report

Run Order	Actual Value	Predicted Value	Residual	Leverage	Internally Studentized Residuals	Externally Studentized Residuals	Cook's Distance	Influence on Fitted	Standard Order
1	0.7800	0.7750	0.0050	0.750	0.270	0.251	0.022	0.435	12
2	0.8000	0.7838	0.0162	0.750	0.877	0.861	0.231	1.491	7
3	0.6000	0.6163	-0.0163	0.750	-0.877	-0.861	0.231	-1.491	6
4	0.6900	0.7580	-0.0680	0.200	-2.052	-3.011	0.105	-1.506	14
5	0.7000	0.7037	-0.0038	0.750	-0.202	-0.188	0.012	-0.326	8
6	0.6800	0.6588	0.0212	0.750	1.147	1.179	0.395	2.042	2
7	0.8000	0.7580	0.0420	0.200	1.268	1.337	0.040	0.669	15
8	0.8000	0.8213	-0.0213	0.750	-1.147	-1.179	0.395	-2.042	3
9	0.7900	0.7580	0.0320	0.200	0.966	0.960	0.023	0.480	16
10	0.7600	0.7580	0.0020	0.200	0.060	0.056	0.000	0.028	17
11	0.7500	0.7580	-0.0080	0.200	-0.241	-0.224	0.001	-0.112	13
12	0.6900	0.7075	-0.0175	0.750	-0.945	-0.937	0.268	-1.622	11
13	0.6500	0.6550	-0.0050	0.750	-0.270	-0.251	0.022	-0.435	9
14	0.7200	0.7025	0.0175	0.750	0.945	0.937	0.268	1.622	10
15	0.6500	0.6513	-0.0013	0.750	-0.067	-0.063	0.001	-0.108	4
16	0.7500	0.7463	0.0037	0.750	0.202	0.188	0.012	0.326	5
17	0.7000	0.6988	0.0012	0.750	0.067	0.063	0.001	0.108	1

Appendix B

Sample and Laboratory equipment photo.



B1:Wg Lomisar variety



B2: Lemongrass essential oil extraction by steam distillation setup



B3: Digital balance and Vibro-Viscomete



B4 : Moisture content analyzer



B5 Refractometer

Declaration

I, Solomon Tadele(candidate), declare that this thesis is my original work, has not been presented for a degree in other university and that all source of material used for the thesis have been duly acknowledged.

Solomon Tadele _____

Name

Signature

_____ Date

This Thesis has been submitted for examination with approved as university Advisor

Dr. Nurelegne Tefera (Ass. Prof) _____

Name:

Signature:

_____ Date