Adama Science and Technology University

Bio-lubricant Synthesis from *Ocimum lamiifolium* and Lactic Acid: A Study on Its Anti-microbial Activities

By: Dereje Ayele

A Thesis Submitted to

The Department of Chemical Engineering School of Mechanical, Chemical and Materials Engineering

Presented in Partial Fulfillment of the Requirements for the Degree of Master's in Chemical Engineering (Specialization in Process Engineering)

Office of Graduate Studies

Adama Science and Technology University

Adama, Ethiopia, Dec/2020

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Declaration

I hereby declare that this thesis entitled "**Bio-lubricant Synthesis from *Ocimum lamiifolium* and Lactic Acid: A Study on Its Anti-microbial Activities**" submitted in partial fulfillment of the requirements for the award of MSc. degree in Adama Science and Technology University, School of Mechanical, Chemical and Materials Engineering, Department of Chemical Engineering is an authentic work carried out by me under my advisor's guidance. The matter embodied in this project work has not been submitted earlier for the award of any degree or diploma to the best of my knowledge and belief.

Author: Dereje Ayele Signature, Date:

_____

This MSc Thesis has been submitted for examination with my approval as thesis advisors

Advisor: Dr. Melaku Tesfaye

Signature_____

Co-Advisor: Dr. Alemu Gonfa

Signature _____

Date of submission _____

Approval of Board of Examiners

This is to certify that the thesis prepared by **Mr. Dereje Ayele** entitled "**Bio-lubricant Synthesis from *Ocimum lamiifolium* and Lactic Acid: A Study on Its Anti-Microbial Activities** and submitted as a partial fulfillment for the award of the Degree of Master of Science in chemical engineering (specialization in process engineering) complies with the regulations of the university and meets the accepted standards with respect to originality, content and quality.

Signed by the Examining Committee:

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List of Abbreviation and Acronyms

AV- Acidic value

ANOVA- Analysis of Variance

FTIR- Fourier Transfer Infra-Red

GC/MS- Gas Chromatography-Mass Spectrometer

IV - Iodine Value

RSM- Response Surface Methodology

OLLA- Lactic Acid Oligomer

OLLA-g-OCL- In Situ Synthesized Bio- Lubricant

SCFE- Supercritical Fluid Extraction

SFA- Saturated Fatty Acid

SV - Saponification Value

UV- VIS- Ultraviolet-Visible Spectrophotometer

WHO - World Health Organization

ABSTRACT

Plant oils, which contain high amount of bioactive constituents, were perceived to be a viable alternatives for bio lubricant applications due to their biodegradability and high antimicrobial efficacy. They can also be used to formulate biodegradable lubricants for skin care applications. The present study was to extract, optimize extraction parameters, characterize Ocimum lamiifolium leaves oil, synthesize bio lubricant, and study antimicrobial activities for application of human skin. The extraction was carried out using Soxhlet method by using hexane as a solvent and separated by a rotary evaporator. Experimental analysis was performed by RSM box-Behnken method with 17 runs. The primary and interaction effects of the process parameters (temperature, extraction time, and particle size) and the optimum conditions were examined using design expert software. In the investigation, all the main factors had a significant effect, but no significant interaction effects of the parameters had shown on the oil yield. The model adequacy was also investigated by normal probability plot of residuals and plot of residuals versus predicted value. Both plots have shown that the model was acceptable. Finally, the optimum conditions of the process parameters for the extraction were optimized. The average optimum yield of the essential oil in percentage from 100 g of plant materials was 12.4% (w/w) and extracted at an extraction time of 4.5 hrs., solute to solvent ration ratio1.38 g/mL, and <1mm particle size of plant materials. The oil and synthesized bio lubricant for proximate analysis and instrumental analysis result found to be 15%, 25%, 4.2-5.91, 0.92g/ml, 11.5%, 19.7gKOH, 8.9mg/KOH, 35.3gL, 23.7gcm/sec,1160cp and 95% respectively to moisture content, the volatile component of lubricant, p^H, specific gravity, ash value, saponification value, acid value, iodine value, spreadability, a viscosity of lubricant and diffusion. Instrumental analysis was carried out to characterize functional group present in, identify bioactive compound present in O. lamiifolium oil, and analyze absorption ability of the compound found in the product using FTIR, GC-MS, and UV-Vis analytical techniques, respectively. UV-Vis spectrophotometer analysis result shows the characteristic peaks in the range (200-700 nm), which supports the absorption of the results for UV-Vis light and colors. The FTIR analysis spectrum confirmed the presence of phenolic and alcoholic compounds that shows a shifting of peaks due to the interaction of lactic acid with O. lamiifolium during in situ polycondensation reaction, which was responsible for the characteristic change. The GC/MS analysis results of O. lamiifolium plant extract provide different peaks depicting the presence of 32 phytochemical compounds. The major 7 phytoconstituents were (phenol, Isocaryophyllene, Elemicin, methyl ester, methyl linolenate, Eicosyne, and squalene). Crude extract and synthesized bio-lubricant were screened for antibacterial activities against Staphylococcus aureus*, bacillus subillitls, Salmonella typhi, and Escherichia coli (E.coli). The plant extracts of O. lamiifolium and synthesized bio lubricant showed maximum activity against 2 pathogens,* Staphylococcus aureus*, Escherichia coli, and moderate to a minimum against Salmonella typhi and bacillus subillitls. The results obtained in the present study suggest that the extracts of O. lamiifolium revealed a significant scope to develop a novel antibacterial herbal formulation/bio-lubricant.*

Keywords: *Ocimum lamiifolium, RSM box-Behnken, GC-MS, FTIR, UV-Vis, Soxhlet extraction, Bio-lubricant, phytoconstituents.*

CHAPTER ONE

INTRODUCTION

1. Background

Traditional medicine is the total of knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures used to maintain health and prevent, diagnose, improve, or treat physical and mental illnesses [1]. Drugs of natural origin now play an ever more important role in medical and healthcare services because metabolites produced by plants constitute a major source of bioactive substances which can be used as an alternative for cheap and effective herbal drugs against common infections [2]. About 85 % of the world population uses herbal medicines to prevent and treat diseases, and the demand is increasing in developed and developing countries because modern medicine is either unobtainable or prohibitively expensive [3]. The trend is likely to continue despite advances in modern medicine and the establishment of the state of the art health institutions.

Medicinal plants, and the drugs derived from them, are the most important and readily available source of healthcare remedies to rural people in Africa. In eastern Africa, many biological resources are used to obtain pharmaceuticals with a high national and international economic value [4,5].

About 80 % of the African population relies on traditional medicine for healthcare needs. Some people use traditional medicine only, while others combine it with conventional drugs. The use of medicinal plants by local people accounts for 70% or more of basic healthcare treatments in Africa. Traditional medical practitioners (TMPs) are a crucial component of the healthcare delivery system [6]. Medicinal plants are used mainly in local, traditional medicine rather than exported to foreign markets. Demand is increasing and often exceeds supply [7].

The situation is the same in Ethiopia, where traditional medicine has been in use since time immemorial and found to be culturally entrenched in all communities [8]. About 95% of traditional medicine preparations in the country are of plant origin as evidenced by the wealth of indigenous knowledge on the utilization of medicinal plants for treating human and livestock ailments [9].

The majority (80%) of Ethiopian people depend on traditional medicine for their health care[10]. Ethiopia is also a home for many languages, cultures, and beliefs that have

contributed to the high diversity of traditional knowledge and practice of the people, including the use of medicinal plants [4].

O. lamiifolium belongs to the family *Lamiaceae* is a well-known plant in serving people as spice, flavor, fumigants, and traditional medicines [11]. Some of their biological activities have been corroborated by scientific researches and have shown anti-microbial activities such as antibacterial, antioxidant, antifungal, and anti-inflammatory. It is widely distributed in tropical and warm temperate regions, especially in tropical America, Africa, and Asia. The typical characteristics of this family are a square stem, opposite and decussate leaves with many gland dots. The flowers are strongly zygomorphic with two distinct lips [12]. *O. lamiifolium* is an indigenous and one of Ethiopia's most widely used medicinal herbs [9].

Local people claim the benefit of certain natural or herbal products; however, it needs much technological trial and advanced look should be considered to demonstrate the effectiveness of a bioactive compound which acts as a remedy for diseases, bioavailability, biodegradability, bio computability, efficacy, safety and drug interactions of newly developed bioactive compounds and their formulations (extracts) require a careful evaluation of immediate and long-term side effects [13]. The premier steps to utilize are extraction, isolation, and characterization of a bioactive compound, using chromatographic techniques, such as UV-VIS and Fourier Transform infrared radiation (FTIR) and GC-MS [14].

Using identified active components, the further process can be done to come with the best product of medicinal plant to overcome the weak side of plant-based medicine to use in medicine with its efficiency and simple delivery without toxic side effects. Bio-based and biodegradable polymers are the best options for this new drug delivery system of herbal drugs, it has a potential future for enhancing the activity and overcoming problems associated with plant medicine. This study, therefore, contributes additional new knowledge to the use of *O. lamiifolium* active constituents in the form of bio-lubricant by grafting bioactive compounds with L-lactic acid oligomer. The analysis of chemical composition and the antimicrobial study was also conducted.

1.1. Statement of Problem

In Ethiopia, various traditionally used indigenous medicinal plants have impressive healing power from different diseases [15]. *O. lamiifolium* is one of the most useable and it is indigenous to the country [16]. But the way of collection, preparation, and delivery to users are still less effective and traditional. Nowadays, researchers of medicinal plants trying to improve traditional medicine due to modern medicine are failing to effectively treat the most common health conditions, widespread antibiotic resistance, the emergence of new pathogens, and the lack of effective new therapeutics exacerbate the problems. The researches aimed at evaluating the antimicrobial potential of extract leaf of *O. lamiifolium* and isolated compounds has been reported. But, this phytomedicines needs further improvements to use in different application, hence, the motivation for the current research work.

Biodegradable polymers are getting attention in the current world in replacing conventional materials [17]. Due to its bioavailability, biodegradability, bio computability, efficacy, safety, low cost, and drug interactions of newly developed bioactive compounds and their formulations. In addition, incorporating this material in the drug delivery system reduces the side effects and improves the therapeutic efficacy and ultimately, patient compliance [18]. Significant steps were gone in some countries to produce biodegradable polymer grafted drugs and got an appreciable result. This helps potentially produce more effective drug products, like bio-lubricant with good quality and low cost for replacing traditional formulation.

Synthesis of biodegradable lubricant has not been synthesized yet in our country cause. Thus, this study was planned to introduce biodegradable polymer grafted bio lubricant that produced from lactic acid oligomer grafted *O. lamiifolium* by incorporating the leaf extract in the backbone of lactic acid oligomer (OLA) to attain demand of medicine for anti-bacterial and skin care applications.

1.2. Objectives

1.2.1. General Objective

The overall objective of the research was to extract *O. lamiifolium* leaf oil, optimize extraction parameters, and synthesize bio-lubricant by grafting with lactic acid: A study on its anti-bacterial activities.

1.2.2. Specific Objectives:

- To extract oil from the leaves of *O. lamiifolium* plant**.**
- To optimize the different parameters such as extraction time, solute to solvent ratio, and particle size on the oil yield.
- To synthesis the bio lubricant through grafting of lactic acid oligomer on the identified *O. lamiifolium* oil components.
- To study the physicochemical and instrumental properties of the products
- To study anti-microbial activities of the *O. lamiifolium* oil and bio lubricant.

1.3. Scope of the Study

This study was full of experimental work and mainly focused on the synthesis of bio lubricant by grafting lactic acid oligomer with *O. lamiifolium* extract for application of skincare and antimicrobial activity. And it was supported by different works of literature and experimental work to study the antimicrobial activity of the plant by identifying its components using different analytical techniques like FTIR, UV-VIS, and GC-MS with Soxhlet extraction at optimum conditions. Extracted crude oil with a high amount of bioactive components was used to synthesize Bio lubricant for skincare application.

The major objective of this project was to optimize Soxhlet extraction parameters for *O. lamiifolium* oil and synthesis bio-lubricant for the application of skincare delivery. The scope of this research was from the extraction of oil and optimization of extraction parameters up to the study of anti-microbial activity of the bio lubricant for skincare application**.**

1.4. Significance of Study

This work aimed to synthesize bio lubricant through grafting lactic acid oligomer with O. *lamiifolium* leaf extract and a study of the anti-microbial property. The extract of this plant has numerous applications. However, delivery methods of this herbal medicine in the application are still under expectation. In our country Ethiopia, countless plants are used by the traditional way for different medicinal therapy, like anti-bacteria, anti-malaria, anti-inflammatory and in general as anti-microbial purposes, etc. *O. lamiifolium* is one of the well-known medicinal plants in Ethiopia, which has been used traditionally for various purposes; local healers are using a different part of this plant to mitigated illness of local people. The oil from this plant is very useful because it has extraordinary medicinal properties and that contains several bioactive components. The traditional uses of *O. lamiifolium* may be attributes to its oil content; therefore, extracting the oil from the plant that made it useful was the first core line for further study in alternative medicines. Thus, this research focused on synthesizing biodegradable lubricant and anti-bacterial activities of drugs from the plant using optimized parameters of Soxhlet extraction methods for the extraction and characterization of physicochemical parameters. This will contribute to the enhancement of Ethiopia's economic and health status to boost the foreign currency by exporting the product of *O. lamiifolium* plant.

CHAPTER TWO

2. Literature Review

As the study about the use of medicinal plants in self-care in rural central Ethiopia revealed that *Ocimum lamiifolium* is among the most frequently used plants [19]. In vitro research was done by the agar disc diffusion method on the aqueous, ethanol, and methanol extracts on *O. lamiifolium* [20]. From this, the result revealed that the three extracts have antibacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* and *Shigellaboydii*. Evaluation research was done on the antipyretic effects of aqueous and ethanol extracts in mice and the result revealed that the extracts have antipyretic properties [21]. These extracts were also investigated on anti-inflammatory activities. Both have significant activity but greater anti-inflammatory activity was observed for the aqueous extract [22]. Aqueous extracts of the plant allowed animals to recover barbiturate sleep duration in proportions of 88%. The dried methanolic extract was also tested in vitro for its protective activity against acetaminophen-induced hepatotoxicity. The result of this study revealed that the plant has activity against hepatotoxicity [23]. Essential oils isolated by hydrodistillation of the overground parts of *Ocimum basilicum* L. and *Ocimum minimum L.* in Turkey and examined by GC-MS, A total of 49 and 41 components, respectively, were identified accounting for 88.1% and 74.4% of the oils of *O. basilicum and O. minimum*, respectively [24]. As concluded by the researchers, the results were generally different, according to literature findings, as concerns the major compounds and the observed differences may be probably due to different environmental and genetic factors, different chemo-types and the nutritional status of the plants as well as other factors that can influence the oil composition. These results show that *O. basilicum and O. minimum* are remarkably variable species. The high quantities of methyl eugenol and geranyl acetate, respectively, make them a most interesting species from the economic point of view [25].

Research which was done in Shako ethnic group in Southwest Ethiopia, *O. lamiifolium* is used to treat skin and gastro-intestinal ailments [10]. In this study, the plant has been assigned with the highest fidelity level values, a possible indication of their better healing potential. An ethnomedicinal survey that was conducted on the investigation of knowledge and practice of traditional antimalarial plants, the leaf part of *O. lamiifolium* has been traditionally used for anti-malarial activity. The thermal expulsion and direct burning of the leaves part revealed

significant repellency against the malaria-causing mosquito. From this concluded that the Oil from O. *lamiifolium* using steam distillation is the main vector of malaria [26].

The Ethno-botanical uses of this plant were also corroborated by scientific laboratory-based research. *O. lamiifolium* extracts are also known to have insecticidal and insect repellent and anti-inflammatory activities. The in vitro antimicrobial activity of the oil was studied against 24 medically important pathogens including Gram-positive and Gram-negative bacteria as well as some fungal strains using the standard disc diffusion technique [9]. The essential oil was shown to possess a broad-spectrum bactericidal activity. Among the bacterial strains tested, Gram-negative bacteria such as Escherichia coli were more potently inhibited than the Gram-positive ones. The essential oil also exhibited an excellent fungicidal activity on fungal strains including Candida albicans and Aspergillus Niger [27]. In addition, the oil was demonstrated to have a good free-radical scavenging potential in 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay [28].

A study on the larvicidal properties of the essential oils that were extracted from leaves or seeds of the test plants by hydro-steam distillation of some aromatic plants against larvae of Amphibians, insects in the laboratory, and anopheles in simulated field conditions stated that *O.lamiifolium* showed the highest larvicidal activity and conclude that it is the most toxic of all [29].

From above none of the studies had mentioned the optimum condition for the extraction of the leaves of *O. lamiifolium* using Soxhlet. Therefore, the gap was filled by this work. to investigate the optimum conditions the parameters that considered in this study was (temperature, extraction time, and solute to solvent ratio of the plant materials) to extract the optimum yield and best quality of oil from dried leaves of *O. lamiifolium* as well as to know the composition of the oil extracted using Soxhlet extraction method through experimental study.

2.1. *O. lamiifolium*

The family *Lamiaceae* is one of the largest families, which comprises the larger proportion of medicinal plant species. *Ocimum* is one of the family Lamiaceae's important genera, which is often referred to as the "king of the herb" [30]. The genus *Ocimum* comprises more than 150 species and it is considered as one of the largest genera of the *Lamiaceae* family [30]. It is widely distributed in tropical and warm temperate regions, especially in tropical America, Africa, and Asia. Unlike several other economic *Lamiaceae,* Ocimum requires warmth for growth and should be protected from forest [31]. The typical characteristics of this family are a square stem, opposite and decussate leaves with many gland dots. The flowers are strongly zygomorphic with two distinct lips. Many of the family, particularly sub-family Nepetoideae, to which Ocimum belongs, are strongly (Diversity of the genus *Ocimum* aromatic due to essential oils, which consist of monoterpenes, sesquiterpenes, and phenylpropanoids). It is a versatile aromatic genus well known for medicinal properties and economically important oils [32].

The genus is very variable and possesses a wide range of intra-specific and inter-specific genetic diversity. It is cultivated for its extraordinary essential oil, which displays therapeutic usages in medicinal application, culinary, perfume for herbal toiletries, aromatherapy treatment, and as a flavoring agent. It has a wide range of therapeutic effects like antimicrobial, antispasmodic, bactericide, carminative, anthelmintic, hepatoprotective, antiviral, larvicidal, the remedy of coughs, colds, measles, abdominal pains, diarrhea, insect repellent, particularly against mosquitoes and storage pest control. Generally, leaves of Ocimum species contain essential oil from 0.5 to 1.4 %. In addition to essential oil, the herb also contains 2, 5-dimethoxybenzoic acid (ocimol) and *gratissimin* (α-truxillic acid dimethyl ester) [33]. Thermal expulsion of *Ocimum* species showed significant repellency against the main vectors of malaria in Africa. Some of the *Ocimum* species that exist in Ethiopia are *O. basilicum, O. lamiifolium, O. somaliense, O. circinatum, O. kilimandscharicumlinn,* etc. *O. lamiifolium* is an indigenous and one of the most widely used medicinal herbs in Ethiopia, but little seems to be known about the uses of the remaining species of subgenus Nautochilus. Its leaves are squeezed and sniffed to treat coughs and colds. They are also used to treat eye infections and to stop nose bleedings. Studying the use of medicinal plants in self-care in rural central Ethiopia reported that out of 25 plant species belonging to 21 families had medicinal values [34]

2.1.1. *O. lamiifolium* in Ethiopia

O. *Lamiifolium* is an indigenous plant in Ethiopia and locally is called "Dama-Kesse'' (in Amharic). It is among the commonly occurring *Ocimum* species distributed in different regions of Ethiopia [35]. It is also cultivated and used as medicine in East Africa from Kenya to Malawi, Democratic Republic Congo, Cameroon, and many other countries. It is one of the highly regarded and most widely used medicinal plants in Ethiopian traditional medicine. Among others, it is used for the treatment of inflammatory conditions and infections; the juice was also used as an eye rinse to treat eye infections. At the same time, the crushed leaves are put in the nostrils to stop nose bleeding. Traditionally, the fresh leaves are squeezed and the juice is sniffed to treat cough and cold and drunk to treat diarrhea, amoeba (diarrhea with blood). Scientific studies have already justified its use for pain and fever.

2.1.2. *O. lamiifolium* in SNNPR Gede'o Zone

The exact location of the Gedeo zone lies between 5 50 26 to 6 12 48 N Latitude and 38 12 48 to 38 13 02 E Longitude. Gede'o is very well known for its Indigenous Knowledge-based self-sustaining land-use system. The Gede'o cultural landscape has multiple facets. The Agroforestry system developed and adopted plants locally to sustain lively hood and demand the medicinal value of the plants [36]. *O. lamiifolium* is traditionally used to relieve pain, wound, fever, malaria, and inflammatory disorders in local healers of Gede'o. It is also used for the treatment of intestinal disorders, eye disease, and cough. Different parts of the plant were widely used against malaria, helminthic infections, fever, dysentery, and other disorders.

O. lamiifolium is an indigenous plant in Ethiopia and locally in Gede'o called "Madda fayisacho" Traditionally, the fresh leaves are squeezed and the juice is sniffed to treat cough and cold and drunk to treat diarrhea, amoeba (diarrhea with blood). The juice is also used as an eye rinse to treat eye infections and headaches, scientific studies have already justified its use for pain and fever figure-1 a and b shows *O. lamiifolium* leaf and flower, respectively [37].

a) b)

Figure-1 a) *O. lamiifolium* plant, b) *O. lamiifolium* flower

2.2. **Uses of *O. lamiifolium***

Ethnobotanical Uses: Traditionally, the leaves part of *O. lamiifolium* is used against eye diseases and headaches. It is also used to relieve pain and fever in a special local preparation in Ethiopia known as "Yemich Medhanit''.

The fresh leaves water extract is drunk to treat diarrhea, amoeba (diarrhea with blood), and cough cold. The plant has synergic activity on these diseases when the water extracts of fresh leaves of *Vernonia amygdalina* and *Clutia abyssinica* are mixed with the leaves of *O. lamiifolium* [38].

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

Aqueous extracts of the plant allowed animals to recover barbiturate sleep duration in proportions of 88% [23]. The dried methanol extract was also tested in vitro for its protective activity against acetaminophen-induced hepatotoxicity. The result of this study revealed the plant has activity. *Ocimum* species and their essential oils have been traditionally used to kill or repel insects, and also to flavor foods and oral products, in fragrance, in folk medicine, and

as condiments [39]. Studied and reported that *Ocimum* species have promising essential oils and are used as insect repellents.

O. lamiifolium extracts are also known to have insecticidal, insect repellent, and anti-inflammatory activities. The in vitro antimicrobial activity of the oil was studied against 24 medically important pathogens, including Gram-positive and Gram-negative bacteria as well as some fungal strains using the standard disc diffusion technique. The essential oil was shown to possess a broad-spectrum bactericidal activity.

2.2. Methods of Plant Oil Extraction

Natural plant oils are used in a wide variety of consumer goods such as detergents, soaps, toilet products, cosmetics, pharmaceuticals, perfumes, food products. The need and consumption for plant oil are hardly increasing so that, producing it in modified is essential. The traditional technique of oil processing is of great significance and is still being used in many parts of the universe. Cohobating, maceration, effleurage, water distillation, water, and steam distillation, steam distillation are the most traditional and commonly used methods. Distillation methods are good for powdered almonds, rose petals, and rose blossoms, whereas solvent extraction is suitable for expensive, needed for identification of bioactive components without denatures delicate and thermally unstable materials [40]. Medicinal and cosmetics Plant oil can be found from one or more plant parts, such as flowers (e.g. rose, jasmine, carnation, clove, mimosa, rosemary, lavender), leaves (e.g. *mint, Ocimum spp., lemongrass, jamrosa*), leaves, and stems (e.g. *geranium, patchouli, petitgrain, verbena, cinnamon*), bark (e.g. *cinnamon, cassia, canella*), wood (e.g. *cedar, sandal, pine*), roots (e.g. *angelica, sassafras, vetiver, Saussurea, valerian*), seeds (e.g. *fennel, coriander, caraway, dill, nutmeg*), fruits (*bergamot, orange, lemon, juniper*), rhizomes (e.g. ginger, calamus, Curcuma, orris) and gums or oleoresin exudations (e.g. balsam of Peru, *Myroxylon balsamum, storax, myrrh,* benzoin [40].

Extraction is the first step of any medicinal plant study and plays a significant and crucial role in the outcome of the study. The most common factors affecting extraction processes are the properties of the plant part, the solvents, and temperature and extraction time. It is only possible to conduct further separation, identification, and characterization of bioactive compounds if the extraction process has been appropriately done. Bioactive compounds from plant materials can be extracted by various classical extraction techniques. Most of these techniques are based on the extracting power of different solvents used and the application of

heat and/or mixing. The commonly used classical techniques are Soxhlet extraction, maceration, and hydrodistillation to obtain crude extract which is then concentrated using a rotary evaporator [14].

2.2.1. Traditional and Modern Methods of Plant Oil Extraction

2.2.1.2. Pelatrice Process

In the pelatrice process, citrus fruits are fed from a hopper into the abrasive shell of the machine. The fruits are rotated against the abrasive shell by a slow-moving Archimedean screw whose surface rasps the fruit surfaces causing some of the oil cavities on the peel to burst and release their oil-water emulsion. This screw further transports the fruit into a hopper in which rollers covered with abrasive spikes burst the remaining oil cavities. The oil and water emulsion is washed away from the fruit by a fine spray of water. The emulsion next passes through a separator where any solids are removed, after which it passes through two centrifugal separators working in series to yield the pure oil. Most bergamot oil and some lemon oil are produced this way in Italy.

Figure 2 Pelatrice for the extraction of citrus oil

2.2.1.3. Sfumatrice Process

The sfumatrice equipment consists of a metallic chain that is drawn by two horizontal ribbed rollers. The peels are conveyed through these rollers during which time they are pressed and bent to release their oil. As in pelatrice, the oil is washed away from the sfumatrice rollers by fine sprays of water. Again, the oil is initially passed through a separator before being sent to two centrifuges in series, so that purified oil can be produced. At one time, sfumatrice was

the most popular process for citrus oil isolation in Italy; however, today the pelatrice method appears more popular.

2.2.1.4. Enfleurage

Enfleurage is not commonly used today, but it is one of the oldest medicinal plant or vegetable oil extraction methods that implement the use of fat. Either vegetable fat or animal fat becomes infused with the flower's fragrance compounds by the end of this process. The fats that are used are odorless and solid at room temperature. The enfleurage process can be done either "hot" or "cold." In both instances, the fat that is saturated with fragrance is called "enfleurage pomade."

2.2.1.5. Cold Enfleurage

Despite the introduction of the modern process of extraction with volatile solvents, the old-fashioned method of enfleurage, as passed on from father to son and perfected in the course of generations, still plays an important role. Enfleurage on a large scale is today carried out only in the Grasse region of France, with the possible exception of isolated instances in India where the process has remained primitive. The principles of enfleurage are simple. Certain flowers (e.g., tuberose and jasmine) continue the physiological activities of developing and giving off perfume even after picking. Every jasmine and tuberose flower resembles, so to speak, a tiny factory continually emitting minute quantities of perfume. Fat possesses a high power of absorption and readily absorbs the perfume emitted when brought in contact with fragrant flowers. This principle, methodically applied on a large scale, constitutes enfleurage. During the entire period of harvest, which lasts for eight to ten weeks, batches of freshly picked flowers are strewn over the surface of a specially prepared fat base (corps), let there (for 24 h in the case of jasmine and longer in the case of tuberose), and then replaced by fresh flowers. At the end of the harvest, the fat, which is not renewed during the process, is saturated with flower oil. Thereafter, the oil is extracted from the fat with alcohol and then isolated. The success of effleurage depends to a great extent upon the quality of the fat base employed. Utmost care must be exercised when preparing the corps. It must be practically odorless and of proper consistency. If the corps is too hard, the blossoms will not have sufficient contact with the fat, curtailing its power of absorption and resulting in a subnormal yield of flower oil. On the other, if it is too soft, it will tend to engulf the flowers and the exhausted ones will adhere; when removed, the flowers will retain adhering fat, resulting in

considerable shrinkage and loss of corps. The consistency of the corps must, therefore, be such that it offers a semi-hard surface from which the exhausted flowers can easily be removed. The process of effleurage is carried out in cool cellars, and every manufacturer must prepare the corps according to the prevailing temperature in the cellars during the months of the flower harvest. Many years of experience have proved that a mixture of one part of highly purified tallow and two parts of lard is eminently suitable for enfleurage. This mixture assures a suitable consistency of the corps in conjunction with high power of absorption. The fat corps thus prepared is white, smooth, absolutely of uniform consistency, free of water, and practically odorless. Some manufacturers also add small quantities of orange flowers or rose water when preparing the corps. This seems to be done for the sake of convention. Such additions somewhat shade the odor of the finished product by imparting a slight orange blossom or roses note.

2.1.1.6. Maceration

Macerated oils are also referred to as infused oils. They are created when carrier oils are used as solvents to extract therapeutic properties from plant material. The benefit of macerated oil above distilled oil is that more of a plant's essence is captured in the oil, because it captures heavier, larger plant molecules than the ones captured in the distillation process. This keeps the product closer to retaining more of the plant's valuable offerings [40].

2.2.2. Modern (Non-Traditional) Methods of Extraction of Plant Oils

Traditional methods of extraction of essential oils have been discussed and these are the methods most widely used on a commercial scale. However, with technological advancement, new techniques have been developed which may not necessarily be widely used for commercial production of essential oils but are considered valuable in certain situations, such as the production of costly essential oils in a natural state without any alteration of their thermosensitive components or the extraction of essential oils for micro-analysis. These techniques are as follows: Headspace trapping techniques; Static headspace technique, Vacuum headspace technique, Dynamic headspace technique, Solid-phase micro-extraction (SPME), Supercritical fluid extraction (SFE) Phytosol(phytol) extraction, Protoplast technique, Simultaneous distillation extraction (SDE) Microwave distillation, Controlled instantaneous decomposition(CID), thermo micro distillation Micro distillation, Molecular spinning band distillation, and Membrane extraction**.**

2.2.2.1. Steam Distillation Process

Steam Distillation is the most popular method used to extract and isolate essential oils from plants for use in natural products. This happens when the steam vaporizes the plant material's volatile compounds, which eventually go through a condensation and collection process [40].

2.2.2.2. Water Distillation

In this method, the material is completely immersed in water, which is boiled by applying heat by direct fire, steam jacket, closed steam jacket, closed steam coil, or open steam coil. The main characteristic of this process is that there is direct contact between boiling water and plant material. When the still is heated by direct fire, adequate precautions are necessary to prevent the charge from overheating. When a steam jacket or closed steam coil is used, there is less danger of overheating; with open steam coils, this danger is avoided. But with open steam, care must be taken to prevent the accumulation of condensed water within the still. Therefore, the still should be well insulated. The plant material in the still must be agitated as the water boils, otherwise, agglomerations of dense material will settle on the bottom and become thermally degraded. Certain plant materials like cinnamon bark, which are rich in mucilage, must be powdered so that the charge can readily disperse in the water; as the temperature of the water increases, the mucilage will be leached from the ground cinnamon. This greatly increases the viscosity of the water-charge mixture, thereby allowing it to char. Consequently, before any field distillation is done, a small-scale water distillation in glassware should be performed to observe whether any changes take place during the distillation process. From this laboratory trial, the yield of oil from a known weight of the plant material can be determined. The laboratory apparatus recommended for trial distillations in the Clevenger system. During water distillation, all parts of the plant charge must be kept in motion by boiling water; this is possible when the distillation material is charged loosely and remains loose in the boiling water [42]. For this reason, only water distillation possesses one distinct advantage, i.e., that it permits the processing of finely powdered material or plant parts that, by contact with live steam, would otherwise form lumps through which the steam cannot penetrate. Other practical advantages of water distillation are that the stills are inexpensive, easy to construct, and suitable for field operation. These are still widely used with portable equipment in many countries. The main disadvantage of water distillation is that complete extraction is not possible. Besides, certain

esters are partly hydrolyzed and sensitive substances like aldehydes tend to polymerize. Water distillation requires a greater number of stills, more space, and more fuel. It demands considerable experience and familiarity with the method. The high-boiling and somewhat water-soluble oil constituents cannot be completely vaporized or they require large quantities of steam. Thus, the process becomes uneconomical. For these reasons, water distillation is used only in cases in which the plant material by its very nature cannot be processed by water and steam distillation or by direct steam distillation [42].

2.2.2.3. Solvent Extraction Method

The quality, flavor, and nutritional and medicinal value of essential oils are directly related to the way the oil is extracted and processed. The highest quality oils are exposed to the least amount of heat, light, pressure, and chemicals in the extraction and refining process, the yield and composition of essential oils also depend on the extraction method.

Solvent extraction is adapted in producing essential oils generated by some flowers (Rose, Violets, and Geranium), gums, and resins. The raw material is placed in a glass, vessel and soaked with a suitable solvent (petroleum, hexane, ether, or benzene). After the extraction, the solids are separated from the liquid mixture. The latter is heated so that the more volatile essential oils can be evaporated to be subsequently condensed. Alternatively, if the solvent is more volatile, such as ethanol, it could then be vaporized leaving behind the essential oils. As solvent extraction uses very little heat, it is found to be advantageous in producing essential oils with whole fragrances that would otherwise be destroyed or altered during steam distillation. Therefore, this extraction technique can be used to extract essential oils from very delicate plants to produce higher amounts of essential oils at lower costs. Therefore, with solvent extraction effective separation of the extracted oil from the solvent is necessary to remove any solvent which may contaminate the essential oils. This process also sometimes yields an aromatic resinous product known as oleoresin, which is more concentrated than essential oils with an even wider application in food and other industries.

2.2.2.4. Soxhlet Extraction

Sox let extractor was first proposed by German chemist Franz Ritter VonSoxhlet (1879). It was designed mainly for the extraction of lipid but now it is not limited to this only. Soxhlet extraction has widely been used for extracting valuable bioactive compounds from various

natural sources. It is used as a model for the comparison of new extraction alternatives. In this method, the finely ground crude drug is placed in a porous bag or “thimble” made of strong filter paper, which is placed in the chamber of the Soxhlet apparatus. The extracting solvent in a flask is heated, and its vapors condense in the condenser. The condensed extract drips into the thimble containing the crude drug and extracts it by contact. When the level of liquid in the chamber rises to the top of the siphon tube, the liquid contents of the chamber siphon into a flask. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave a residue when evaporated. The advantage of this method, compared to previously described methods, is that large amounts of the drug can be extracted with a much smaller quantity of solvent. This affects a tremendous economy in terms of time, energy, and consequently financial inputs. On small scale, it is employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on a medium or large scale figure-3 shows double effect Soxhlet extraction set up [43].

Figure 3: Soxhlet apparatus

2.3. Analytical Method Used In Identification and Characterization

Analysis of quality and quantity found in medicinal plant active components is mostly studied by different analytical methods and it depends on the selection of proper method for a given plant material [44].

2.3.1. Identification and Characterization

Since plant extracts usually occur as a combination of various types of bioactive compounds or phytochemicals with different polarities, their separation remains a big challenge for the process of identification and characterization of bioactive compounds. It is a common practice in isolation of these bioactive compounds that several different separation techniques such as UV-visible, FTIR, GC-MS, and NMR, should be used to obtain pure compounds. The pure compounds are then used for the determination of structure and biological activity. Besides that, non-chromatographic techniques such as immunoassay, which uses monoclonal antibodies (MAbs), phytochemical screening assay, Fourier-transform infrared spectroscopy (FTIR), can also be used to obtain and facilitate the identification of the bioactive compounds[14].

2.3.2. Ultraviolet-Visible (UV-VIS) Spectrophotometry Analysis

Ultraviolet-visible spectrophotometry is related to the spectroscopy of photons in the UV-visible region. UV-visible spectroscopy uses light in the visible ranges or its adjacent ranges. The color of the chemicals involved directly affects the absorption in the visible ranges. Molecules undergo electronic transitions in these ranges of the electromagnetic spectrum. UV-Vis Spectrometric is important in the analysis of some herbal raw materials for understanding qualitative and quantitative parameters without markers and comparison on different parametric products.

2.3.3. High-Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography is a highly improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it move faster. It also allows the use of smaller particle sizes for the column packing material which gives it a greater surface area for interactions between the stationary phase and the molecules flowing through it. This allows a much better separation of the components of the mixture [14]. Preparative high-performance liquid chromatography (HPLC) has become a favorite method

of natural product isolation and purification. The various modes available (e.g., normal-phase, reversed-phase, size exclusion, and ion-exchange) to date can be used to purify most classes of natural products. Although preparative HPLC is very similar to analytical HPLC, instead of injecting a small amount of sample to maximize the resolution, the amount of feed is very high to maximize the purification productivity and minimize the amount of solvent used.

2.3.5. Gas Chromatography-Mass Spectroscopy (GC-MS)

It is an analytical technique for separating compounds based primarily on their volatilities. GC provides both qualitative and quantitative information for individual compounds present in a sample. The gas phase is flowing and the liquid phase is stationary. The rate of migration for the chemical species is determined through its distribution in the gas phase. For example, a species that distributes itself 100% into the gas phase will migrate at the same rate as the flowing gas, whereas, a species that distributes itself 100% into the stationary phase will not migrate at all. Species that distribute themselves partly in both phases will migrate at an intermediate rate [45]. Gas chromatography involves a sample being vaporized and injected onto the head of the chromatographic column. The sample is then transported through the column by the flow of the inert, gaseous mobile phase. The column itself contains a liquid stationary phase, which is adsorbed onto the surface of an inert solid.

2.3.6. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier- transform infrared spectroscopy is a valuable tool for the identification of functional groups present in the plant extract. It helps for the identification and structure determination of the molecule. It is a high-resolution analytical tool to identify the chemical constituents and elucidate the structural compounds. FTIR offers a rapid and non-destructive investigation of fingerprint herbal extracts or powders [47].

2.3.7. Nuclear Magnetic Resonance Spectroscopy (NMR)

Nuclear Magnetic Resonance Spectroscopy gives physical, chemical, and biological properties of matter. One dimensional technique is routinely used but the complicated structure of the molecules could be achieved through two-dimensional NMR techniques. Solid-state NMR spectroscopy is used for the determination of the molecular structure of

solids. Radiolabeled 13C NMR is used to identify the types of carbon are present in the compound. 1 H-NMR is used to find out the types of hydrogen are present in the compound and to find out how the hydrogen atoms are connected.

2.4. Biodegradable Materials

Biodegradable materials are materials, which can be degraded or break down naturally, enzymatic action of living organisms. Such as bacteria, fungus, and yeasts decomposed by bacteria or other natural organisms into none toxic and re-usable substances not to adding to pollution and ultimate end product of degradations process, these being CO_2, H_2O, and biomass under aerobic condition and hydrocarbons, methane, and biomass under anaerobic conditions. Almost all biodegradable materials are made of polymers.

2.4.1. Biodegradable Polymers

Biodegradable polymers are a special class of polymer that breaks down after its intended purpose by bacterial decomposition process to result in natural byproducts such as gases (CO_2, N_2), water, biomass, and inorganic salts. The biodegradation process takes place the long polymer molecules are reduced to shorter and shorter lengths and undergo oxidation (oxygen group attached themselves to the polymer molecules). Biodegradability depends strongly on environmental conditions: temperature, presence of microorganisms, presence of oxygen, and water. So both the biodegradability and the degradation rate of a biodegradable plastic product may be different in the soil, on the soil, in a humid or dry climate, in surface water, in marine water, or human-made systems like home composting industrial composting, or anaerobic digestion **[**48**].** This process is triggered by heat, UV light, which is a component of sunlight, mechanical stress (e.g. Wind or compaction in landfills). Oxygen causes the molecule to become hydrophilic (water-attracting) and small enough to be ingestible by microorganisms setting the stage for biodegradation to begin. These polymers are found both naturally and synthetically made, and largely consist of ester, amide, and ether functional groups. Their properties and breakdown mechanism are determined by their exact structure. These polymers are often synthesized by condensation reactions, ring-opening polymerization, and metal catalysts.

2.4.2. Applications of Biodegradable Polymers in Medicinal Area

Biodegradable polymers have innumerable uses in the biomedical field, particularly in the fields of tissue engineering and drug delivery. For a biodegradable polymer to be used as a therapeutic, it must meet several criteria: 1) be non-toxic to eliminate foreign body response; 2) the time it takes for the polymer to degrade is proportional to the time required for therapy; 3) the products resulting from biodegradation are not cytotoxic and are readily eliminated from the body; 4) the material must be easily processed to tailor the mechanical properties for the required task; 5) be easily sterilized, and 6) have an acceptable shelf life. Biodegradable polymers are of great interest in the field of drug delivery and Nanomedicine. The great benefit of a biodegradable drug delivery system is the ability of the drug carrier to target the release of its payload to a specific site in the body and then degrade into nontoxic materials that are then eliminated from the body via natural metabolic pathways. The polymer slowly degrades into smaller fragments, releasing a natural product. The drug slowly releases as the polymer degrades. For example, polylactic acid, poly (lactic-co-glycolic) acid, and poly (caprolactone), all of which are biodegradable, have been used to carry anti-cancer drugs. Encapsulating the therapeutic in a polymer and adding targeting agents decreases the toxicity of the drug to healthy cells. Biodegradable polymers and biomaterials are also of significant interest for herbal instated therapy and regeneration. Tissue engineering is the ability to regenerate tissue with the help of artificial materials. The perfection of such systems can be used to grow tissues and cells in vitro or use a biodegradable scaffold to construct new structures and organs in vitro. For these uses, a biodegradable scaffold is preferred as it reduces the risk of immunological reaction and rejection of the foreign object. While many of the more advanced systems are not ready for human therapeutics, there is significant positive research in animal studies. For example, it was possible to successfully grow rat smooth muscle tissue on a polycaprolactone/polylactide scaffold. Further research and development may allow for this technology to be used for tissue replacement, support, or enhancement in humans. One of the ultimate goals of tissue engineering is the creation of organs, such as the kidney, from basic constituents[49] Scaffolding is necessary to grow the entity into a functioning organ, after which the polymer scaffold would degrade and be safely eliminated from the body. There are reports of using polyglycolic acid and polylactic acid to engineer vascular tissue for heart repair. The scaffold can be used to help create undamaged arteries and vessels[50]**.** In addition to tissue engineering, biodegradable polymers are being used orthopedic applications, such as bone and joint replacement. A wide variety of non-

biodegradable polymers have been used for orthopedic applications including silicone rubber, polyethylene, acrylic resins, polyurethane, polypropylene, and polymethyl methacrylate. The primary role of many of these polymers was to act as biocompatible cement in the fixation of prostheses and the replacement of joints. Newer biologically compatible synthetic and natural biodegradable polymers have been developed; these include polyglycolide, polylactide, polyhydroxobutyrate, chitosan, hyaluronic acid, and hydrogels. In particular, poly (2-hydroxyethyl-methacrylate), poly (ethylene glycol), chitosan, and hyaluronic acid have been used extensively in the repair of cartilage, ligaments, and tendons. For example, poly (L-lactide) (PLA) is used to make screws and darts for meniscal repair and is marketed under the trade name Clear fix Meniscal Dart/Screw. PLA is a slow degrading polymer and requires times greater than two years to degrade and be absorbed by the body.

Among the biomaterials (biopolymers) used in the medical field, polylactic acid (PLA) has received significant attention because it can be produced from lactic acid, a naturally occurring organic acid that can be produced by fermentation. PLA is considered both biodegradable (e.g. adapted for short-term packaging) and biocompatible in contact with living tissues (e.g. for biomedical applications such as implants, sutures, drug encapsulation, etc.). PLA can be degraded by abiotic degradation (i.e. simple hydrolysis of the ester bond without requiring the presence of enzymes to catalyze it). During the biodegradation process, and only in a second step, the enzymes degrade the residual oligomers till final mineralization (biotic degradation)[51].

2.5. Polylactide

Poly (lactic acid): PLA is biodegradable thermoplastic aliphatic polyester derived from renewable resources, such as corn starch, tapioca roots, or sugarcane. It has been extensively studied for several applications such as packaging, orthopedics, drug delivery, sutures, and scaffolds, and other biomedical applications. It possesses better mechanical properties and is relatively easy to process using conventional methods like thermoforming, injection, and blow molding without releasing toxic products. To meet the performance requirements, PLA can be synthesized by various methods such as poly-condensation reaction, ring-opening polymerization (ROP), and solid-state polymerization using different catalysts **[**51**].** Polycondensation reaction requires continuous removal of water under vacuum and the final molecular weight is highly dependent on the water removal efficiency [52].

Polylactide (PLA): PLA is usually obtained from polycondensation of D- or L-lactic acid or ring-opening polymerization of lactide, a cyclic dimer of lactic acid. Two optical forms exist D-lactide and L-lactide. The natural isomer is L-lactide and the synthetic blend is DL-lactide. Other different synthetic methods have been studied too. They have been reported in detail. PLA is a hydrophobic polymer due to the presence of –CH3 side groups. It is more resistant to hydrolysis than PGA because of the steric shielding effect of the methyl side groups. The typical glass transition temperature for representative commercial PLA is 63.8 °C, the elongation at break is 30.7% and the tensile strength is 32.22 MPa. Regulation of the physical properties and biodegradability of PLA can be achieved by employing a hydroxy acids comonomer component or by the racemization of D- and L- isomers [53]. Among the biomaterials (biopolymers) used in the medical field, poly (lactic acid) (PLA) has received significant attention. It is produced from lactic acid, a naturally occurring organic acid that can be produced by fermentation. The attractive price and commercial availability of lactic acid are important reasons for this application. LA and its copolymers are being used in the biomedical area in the form of implants or devices due to its excellent biocompatibility and biodegradability [54]

2.6. The Oligomer

An oligomer is a molecular complex of chemicals that consists of a few repeating units, in contrast to a polymer, where the number of monomers is, in principle, infinite. Dimers, trimers, and tetramers are, for instance, oligomers composed of two, three, and four monomers, respectively [55].

Oligomer Molecule: A molecule of intermediate relative molecular mass, the structure of which essentially comprises a small plurality of units derived, actually or conceptually, from molecules of lower relative molecular mass.

Oligomerization: The process of converting a monomer or a mixture of monomers into an oligomer. An oligomerization by chain reaction carried out in the presence of a large amount of chain-transfer agent so that the end-groups are essentially fragments of the chain-transfer agent is termed oligomerization [56]. Lactic acid oligomers are formed from lactic acid, a renewable material produced by the fermentation of sucrose or glucose. Due to their production method and origin, oligomers formed from lactic acid are completely natural and fit perfectly within the framework of green chemistry. Lactic acid is a chiral

molecule with two optical isomers (L (+) and D (-)) so lactic acid oligomers will also present these two forms. L (+) lactic acid is the biologically important isomer. Only the L (+) form is presented here, although everything also applies to the D (-) form. Lactic acid polymers are present in the following chemical form [57]. In the case of lactic acid, because the molecule is bi-functional (it is a hydroxyl acid), it will be the sole reagent of the poly condensation. The polymerization of lactic acid leads to linear chains of oligomers with an average molecular weight that depends on process conditions. The general lactic acid polycondensation process looks like this:

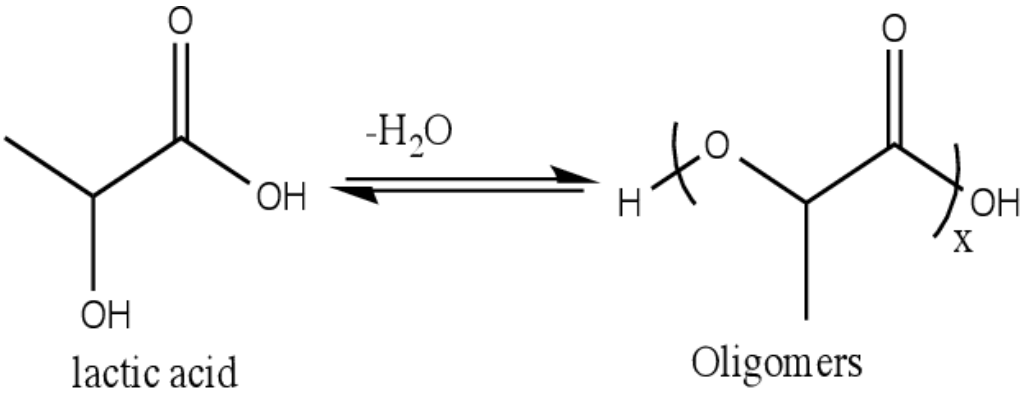


Figure 4: Lactic Acid Poly Condensation Process

To obtain oligomers of lactic acid of sufficient molecular weight, it is important to apply the process conditions that facilitate the elimination of water molecules.

Lactic acid or lactide oligomers are used in the following added-value domains:

• Substitution of waxes, oils, and oligomers currently used in the formulation domain.

• Polymerization or copolymerization of new and existing polymers.

• Synthesis of new products in such sectors as binding agents and inks and to form a polymer-drug conjugate.

2.7. Polymer-Drug Conjugates

Polymer-therapeutics is a field that has progressed a lot over the last decade. Polymer-drug conjugates, arising from polymer therapeutics, comprise water-soluble polymer being conjugated to a drug chemically with the help of a biodegradable coupler. Helmut Ringsdorf, in 1975, first provided a model for pharmacologically active polymers. He gave the idea that polymer drug-conjugates can be used for the delivery of small hydrophobic molecules. Owing to their colloidal nature, these conjugates are stable to sustain in the

circulation for extended periods. The major difference between these conjugates and drug delivery vehicles with the drug entrapped by physical means in them (e.g. liposomes), is that the polymer-drug conjugates are conjugated chemically and this makes them new chemical entities (NCE). A multitude of drug conjugates, employing linear polymers, have been manufactured. The most widely explored ones are those made using polyethylene glycol (PEG) and N-(2-hydroxypropyl) methacrylamide (HPMA) copolymers. PEG-protein conjugates are of significant interest since PEG can provide protection against enzymatic degradation of proteins and also it lowers the absorption by the reticuloendothelial system.

2.8. Lactic acid

Lactic acid is low molecular weight organic acid produced mainly by fermentation using lactic acid bacteria or synthetically. It is considered one of the main well-known organic acids with a wide range of industrial applications.

2.8.1. **Properties, Uses, and Applications**

Lactic acid is a three-carbon organic acid: one terminal carbon atom is part of an acid or carboxyl group; the other terminal carbon atom is part of a methyl or hydrocarbon group, and a central carbon atom having an alcohol carbon group. Lactic acid (LA), is the smallest optical active organic compound present in nature. Due to the presence of a chiral carbon, LA exists in the two optical isomers, L (+) and D (-).

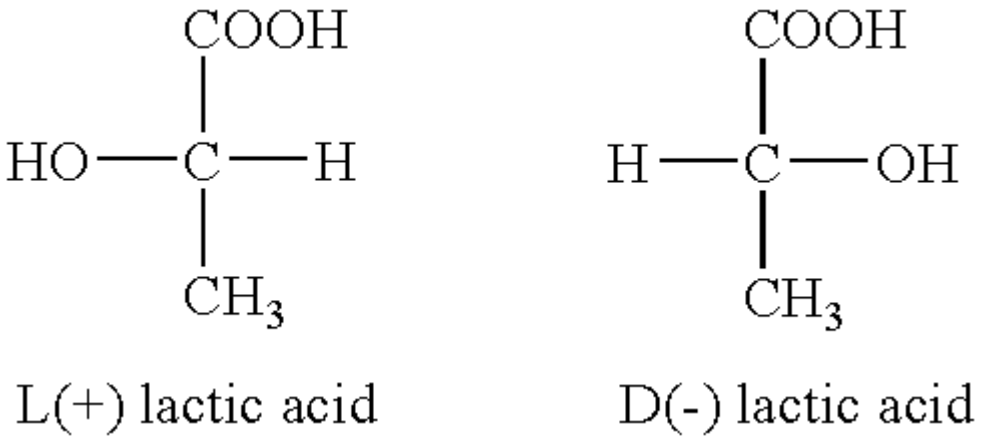


Figure- 5 Lactic Acid Isomers

Pure lactic acid is a colorless and hygroscopic liquid; it can be defined as a weak acid because of its partial dissociation in water and water-miscible organic solvents but insoluble in other organic solvents. It exhibits low volatility.

Technical grade lactic acid is used as an acidulant in vegetable and leather tanning industries. Various textile finishing operants and acid dying of food require low-cost technical grade lactic acid to compete with a cheaper inorganic acid. Lactic acid is being used in many small-scale applications like pH adjustment hardening baths for cellophane used in food packaging, terminating agent for phenol-formaldehyde resins, alkyd resin modifier, solder flux, lithographic and textile printing developers, adhesive formulations, electroplating and electropolishing baths, detergent builders. Lactic acid has many pharmaceutical and cosmetic applications and formulations in topical ointments, lotions, anti-acne solutions, humectants, parenteral solutions, and dialysis applications, for anti carries agents. Calcium lactate can be used for calcium deficiency therapy and as an anticaries agent. Its biodegradable polymer has medical applications as sutures, orthopedic implants, controlled drug release, etc. Polymers of lactic acids are biodegradable thermoplastics. These polymers are transparent, biodegradable and their degradation can be controlled by adjusting the composition, and the molecular weight. Their properties approach those of petroleum-derived plastics. Lactic acid esters like ethyl/butyl lactate can be used as green solvents. They are high boiling, non-toxic and degradable components.

2.9. Lactic Acid Oligomer as Antimicrobial Agent

Antimicrobial polymers (polymeric biocides) are polymers with antimicrobial activity or the ability to inhibit the growth of microorganisms such as bacteria, fungi, or protozoans. Currently, polymers are being invented to mimic antimicrobial peptides that are used by the immune systems of living things for killing bacteria. In general, such polymers are synthesized by attaching or inserting an antibacterial drug onto a polymer backbone via an alkyl or acetyl linker. Antimicrobial polymers increase the efficiency and selectivity of antimicrobial agents together with decreasing environmental hazards. Poly (lactic-co-glycolic acid) (PLGA) and poly (lactic acid) (PLA) based polymers have been used as effective antimicrobial polymers.

PLA provides promising results for the local drug delivery of linezolid in cases of chronic osteomyelitis. The antimicrobial effect of olive leaf extract can be incorporated into PLA films. These films are effective in cases of Staphylococcus aureus bacterial strains.

2.10. Bio-lubricant

A lubricant is a material used to facilitate the relative motion of solid bodies by minimizing friction and wear between interacting surfaces, carry and releasing some additives. In addition to the primary purposes of reducing friction and wear, the term bio lubricant applies to all lubricants that are easily biodegradable and non-toxic to humans and the environment. The uses of bio-lubricants are still very limited when compared to those of mineral oils, although this trend is increasing and depends on investment in research and development. The increase in demand for biodegradable lubricants is related to the evolution of environmental regulations, with more restrictive rules being implemented to minimize the environmental impact caused by inappropriate disposal. Bio lubricants are classified as either natural or synthetic oils according to chemical composition.

2.10.1. Classification and Importance of Bio lubricants

Bio lubricants can be classified according to their chemical fluid composition in natural and synthetic oils. Natural oils are made using plant oils or animal fats, while synthetic oils use natural oils as starting materials to form more advanced bio-lubricants. Among them, it was reported that ester synthesis, involving modification by microorganisms, alcohols, polyalcohols, polyglycols, perfluoroalkyl ethers, and other species, can graft to natural oil. In such a way that the synthesized bio lubricant exhibited thermo-oxidative stability, wear resistance, and, anti-microbial, sun radiation resistance, color resistance, and lubricity properties even greater than those exhibited by mineral oils. However, synthetic esters also display several limitations, since chemical modification raises the price of the lubricant, slightly increases the volatility and toxicity, diminishes the friction tolerance, and the esters do not work well with mineral oils in comparison to unmodified plant oil oils.

Plant oils possess excellent biodegradability, renewability, and lubricating performance in lubricant, cosmetic and pharmaceutical applications. Many researchers and scientists have begun to consider different types of natural materials such as plant oils, animal fats, and organic waste oil as the starting materials for the synthesis of bio-lubricants for applications

in the food industry, skincare, pharmaceutical air conditioning, biodegradable grease, and others.

Lubricating oils are also required to carry out a range of other functions, including the removal of heat, prevention, and the transfer of power. Additionally, lubricants provide a liquid seal at moving contacts and remove wear particles. To perform these roles, lubricating oils must have specific physical and chemical characteristics.

2.11. Lactic Acid and Skin Relation

Lactic acid is very good at penetrating the skin. Because of this, it is used in several products for skin peels, and in formulations for transdermal delivery of medications, such as topical ointments and extended-release patches for delivery through the skin that makes it easy to penetrate.

2.12. Human Skin and Skin Infection Treatments

Human skin, the outer covering of the body, is the largest organ in the body. It also constitutes the first line of defense. The skin contains many specialized cells and structures. It is divided into three main layers, viz. epidermis, dermis, and hypodermis.**b** Skin diseases present a major health concern worldwide [58]. Skin problems significantly affect the quality of health and are difficult to treat due to persistence [58]. The skin is an external organ covering the body and serves many important functions, including percutaneous absorption, organ protection, fluid preservation, body shape maintenance, temperature regulation, and eliminating toxins from the body by sweat excretion [59].

Skin diseases affect people of all age groups and gender [60]. Skin ailments or infectious dermatological diseases are particularly present in tropical areas of the Globe [61] Skin diseases constitute about 34% of all the ailments and are supposed to be the most common disease among rural people. Skin ailments such as boils, itching, dermatitis, eczema, scabies, skin allergy swelling, and psoriasis are caused by a variety of microorganisms common bacterial infections are cellulitis, abscess, and impetigo [62]. Previous reports found that wound healing; eczema, dermatitis, fungal diseases, pyoderma, scabies, and skin allergies are the largest group of skin diseases that occur in most countries. Most of the plants used for treating skin disorders possibly have other additional properties like anti-inflammatory, anti-microbial, anti-viral, cicatrizant, hemostatic, analgesic effects that require scientific confirmation [63].

CHAPTER THREE

3. Materials and Methods

3.1.1. Equipment and Materials

The equipment and materials used for this study were, oven drier, crusher, viscometer, distiller, three-necked round flasks, Watt-man filter papers, rotary evaporator, Erlenmeyer flasks, heating mantle, electronic analytical balance, magnetic stirrer(85-2 magnetic stirrer), thermometer, thermostatic oil bath, Measuring cylinder, Conical flasks, water distiller, separator funnel and sieves

3.1.2. Instruments used

The Instruments used were Soxhlet full apparatus, rotary evaporator, Chiller, Ovendryer, muffle furnace, Reactor, Ultraviolet-visible spectrophotometry (UV-Vis), Fourier Transfer Inferred Radiation Spectroscopy (FTIR), and Gas Chromatography-Mass Spectrometer (GC-MS)

3.1.3. Chemicals and Solvents used in the process

The chemicals used were, Diethyl ether and lactic acid (ultra-pure 98 %), ethanol pure (98 %), hexane (99.9 %), silicon oil, potassium hydroxide, carbon tetrachloride, iodine chloride, potassium iodide, sodium thiosulfate aluminum foil, All chemicals used in this study were analytical graded and obtained from chemical suppliers in Addis Ababa (yeshadam and Alchem import plc.

3.1.4. Solvents Used

Distilled water, Hexane, Ethanol, and Toluene were the solvents that used in the process.

3.2. Methods

3.2.1. Raw Material Collection and Preparation

Enough amount of *Ocimum Lamiifolium* leaves (15 kg) was collected by purchasing from Gede'o Zone local market and growing habitat field in Adama Fentale Woreda.

Sample preparation was followed the procedures done by Kasiramar G et al., 2019 [64]. The measuring of sample weight was done by using digital balance AU101 model mettle Toledo mark (USA) to investigate the moisture content and volatile components of the plant materials, oven-dry was used at 100 ^{o}C for 1 day and natural drying by sunlight for 48 hr. The potential mass loss was determined as a result of the loss of both moisture and volatile oil

components due to the drying effect. The dust and unwanted materials were removed before drying for extraction, finally, the dried leaf was milled with a size of (0.25-2.5 mm) [65]. And the sample was kept at 4 ^{O}C for extraction.

3.2.2. Size Reduction

Dried leaf of *O. lamiifolium* was crushed using a high-speed multi-function crusher, MFC 90D model (Kinematica industrial and trade Co., Ltd, (Switzerland) with the size of 0.25 mm, 0.38 mm, 0.5, 2.5 mm. Sieve analysis was done to attain the desired size of 0.25-2.5[65] for maximum yield.

Figure 6 Size reduction equipment

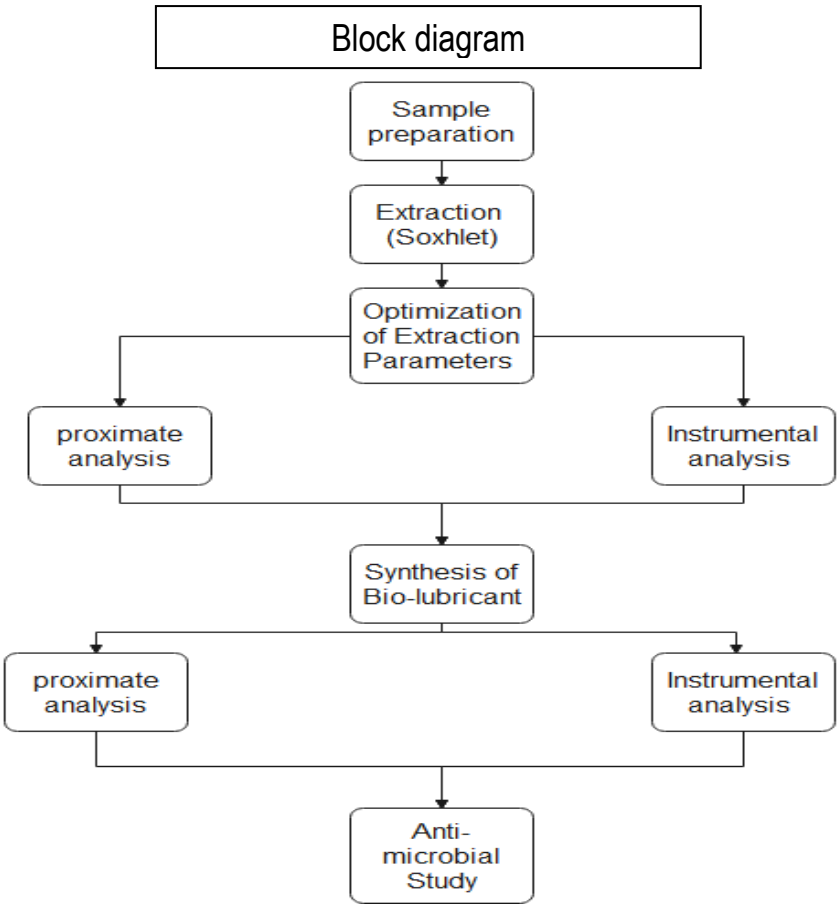


Figure-7 Block Diagram of the total process

3.2.3. Extraction of O. *lamiifolium* Leaves Oil

Extraction processes were carried out according to Alemu Belay (2017). A 30 gm sample was used for extraction. The extraction of oil from O. *lamiifolium* plant leaves was started by filling powder into a porous bag (thimble). Experimental work was conducted using Soxhlet extractions [66]. The Soxhlet was heated to 70 ^{o}C. The extraction temperature (70 ^{0}C) was chosen to avoid the thermal degradation of bioactive compounds like phenolic compounds in the extract. The experiment was duplicated for the following particle sizes (2.5 mm, 1.5 mm, 0.5 mm, and 0.25 mm), solvent to solute ratio, and extraction time. The resulting extracts mixture (crude oil and hexane) was separated by a rotary evaporator (EYELA SB-1100, rotational speed 8 rpm) and the solvent was recovered. Then, the weight of extracted oil was determined for each run hour at the end of the separation. The products were weighed and kept at 4 ^{O}C for further analysis of physicochemical properties and other parameters [66].

Figure-8 Soxhlet extraction set up

3.2.4. Determination of Oil Yield by Percentage

The yield of extraction was calculated from the relation between the oil mass obtained and the mass of raw materials used in the extraction, according to the method followed in AOAC (2000) and the previous work of [68].

$$\textbf{\% total oil yield} = \frac{\boldsymbol{Mol}}{\boldsymbol{Ms}} * \textbf{100}$$

Where: Mol – the mass of oil extracted

Ms- the mass of the sample (crushed leaf)

3.2.5. Raw Material and Product Characterization

Successful extraction was performed by the Soxhlet extraction system, however, parameters determine product quantity and quality. The main effects of the individual factors and the interaction effect of the operating parameters were considered (extraction time, solute to solvent ratio, and particle size) on oil yield [69]. The optimum values of the parameters were investigated by the method of RSM Box Behnken. The main effect of the parameters and the

optimization of process conditions for the maximum oil yield was performed using a design expert [70].

3.3. Optimization of Extraction Parameters

The optimization was performed by utilizing Design Expert® software using response surface Based on the Box-Behnken method design was used to design the experiment and analysis of variance (ANOVA) was used for statistical significance of the experimental data. The RSM approach using the 3-factors, 2 levels, and 17 experiments was applied to obtain the optimum oil yield from optimizing the three most important variables including extraction time, particle size, and solvent to solute ratio. The 2 levels of three independent variables used in RSM are given in Table-3.1. The independent variables are designated as A, B, and C respectively.

Table3. 1 Actual values for the level of independent variables used in optimization.

Independent variables with actual values			**Level**	
Symbols	**Independent variables**	**Units**	**Max**	**Min**
A	Extraction time	hr	6	3
B	Particle size	mm	2.5	0.25
C	Ratio	w/w	0.2	0.05

The combined effect of these variables on the extraction of oil was studied with different combinations of the input variables (factors). The experimental design and the results of the oil yield (response) extracted from *O. lamiifolium*.

3.4. Synthesis of Drug Lubricant (Graft Co-Polymerization of Lactic Acid with *O. lamiifolium*)

The Oligomerization reaction of Lactic acid was performed in the absence of catalyst by removing water from 88 wt. % aqueous solution of lactic acid using a microwave oven. A 250 ml three-necked flask was placed in a thermostatic oil bath equipped with a condenser. Continuous experiments were done enough amount of aqua L-lactic acid solution was charged into a three-necked flask and temperature was raised to 120 °C to remove water at an optimized time (60 min) at atmospheric pressure. Different molecular weight oligomers were prepared at different temperatures (120 to 180 °C). Lactic acid grafted *O. lamiifolium* drug conjugate was also developed through the same procedure, *O. lamiifolium* oil was grafted with in situ polycondensation grown lactic acid oligomer, the experiments were carried out by varying parameters like reaction time, lactic acid to *O. lamiifolium* oil ratio, temperature, and other parameters kept constant.

Loading the sample with variable concentration (5 % w/v, 10 % w/v, 15 % w/v) the method of grafting was done. The lactic acid oligomer and aqueous O. *lamiifolium* oil component were initially taken into a 250 mL flask in different ratios and were mixed gently using manual mixing. The mixture was poured into three necks 250 mL bottles and equipped with a thermostatic oil bath, the temperature was maintained from 120-180 ^{O}C to get its optimum. the internal temperature was measured by a thermometer until it reaches equilibrium with the external temperature of the reactor. During the reaction, both Oligomerization and grafting took place, as result; bio-lubricant (OLLA-g-OC) was synthesized after completion of the reaction. The condensed water as a byproduct was received by mounting bath with cold water chiller into the outlet of the reactor**.** The product was kept in a 4 ^{o}C aluminum foil sealed bottle to further analysis.

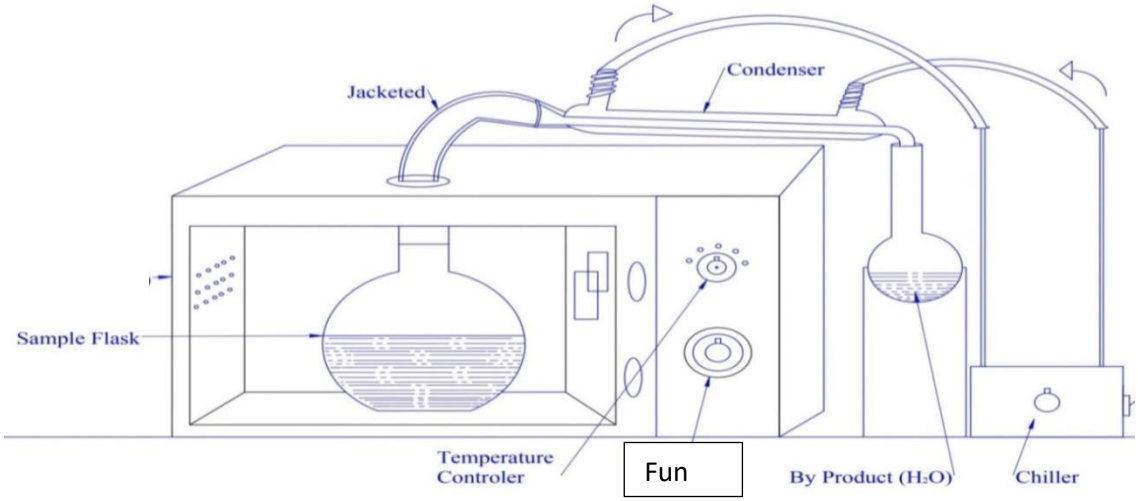


Figure 9. Oven reactor for in situ polycondensation reaction (grafting)

3.4.1. Proximate Analysis of *O. lamiifolium* Oil and synthesized Bio-lubricant

3.4.1.1. **Determination of Moisture**

A. Moisture for *O. lamiifolium*

Moisture content was determined by the oven drying method as mentioned in moisture analysis in pharmaceutical(May 14/2019). A 10 gm. of the sample was weighed and dried in an oven at 105 ±1 °C for 1 hr. The sample was removed from the oven and cooled for 20 min in a dissector and reweighed. The process was repeated until a constant weight was observed. The process was repeated six times. This process was determined by weight difference.

The moisture content of *O. lamiifolium* dried leaf by percent (%) $= \frac{W1-w2}{W1} * 100\%$

Where: W1= initial weight

W2: weight after oven drying

B. Moisture Content Determination For Bio lubricant (LOD)

Percentages (%) of water were measured by loss on drying according to R. K. Tsatsop, G. Djiobie (Sep 09/2017). A 10 gm. of bio-lubricant was taken place in the petri dish on a water

bath and kept in drying oven RS513-A Model (Germany) at 105 ^{O}C for 5 hrs, and weighed at the interval of every 1 hrs until the difference between three successive weighings corresponds to not more than 0.25 %.

3.4.1.2. **Determination of Specific Gravity of Oil**

The specific gravity was determined by the weight of a given volume of oil at a specified temperature compared with the weight of an equal volume of water at the same temperature. All weighing was done in the air. A pycnometer was used for this determination. The density of oil was used to get the specific gravity [71]

A clean and dry density bottle of 25 mL capacities at 30 °C was weighed in grams as (W_0). Then, the bottle was filled with water and reweighed as (W_1), the water was replaced by O. *lamiifolium* oil. The bottle was weighted again as (W_2) and finally, the specific gravity of the oil was calculated by the density of the reference substance (water) [71].

$$\text{Specific gravity} = \frac{W1-W0}{W2-W0} = \frac{density\ of\ oil}{density\ of\ water}$$

where W_0 = weight of dry empty density bottle

W_1 = weight of density bottle with oil

W_2 = weight of density bottle with distilled water.

3.4.1.3. Determination of PH

A. Determination of pH for extracted *O. lamiifolium* crude oil

For pH determination, 2 gm of the sample was placed in a clean dry 25 mL beaker and 13 mL of hot distilled water was added to the sample in the beaker and stirred manually. The solution was cooled in an ice bath to 25 °C. The pH-meter was calibrated with a buffer solution of pH 4.00, 7.00, and 10.00. The electrode was immersed into the sample, the value was read and recorded according to [72]

B. Determination of pH for Bio-Lubricant

The pH of synthesized drug lubricant was measured by the digital pH meter Model (country). The equipment was calibrated by using a buffer solution of pH 4.00, 7.00, and 10.00. One gram drug lubricant was dissolved in 100 mL of distilled water and set aside at 4 °C for two hours. The P^H was determined in triplicate and an average value was calculated.

3.4.1.4. Determination of Iodine Value of *O. lamiifolium* Crude oil

The iodine value, of *O. lamiifolium* oil, was determined based on the method specified by ISO 3961 (1989). A 200 gm of the oil was accurately weighed into a conical flask and 20 mL of carbon tetrachloride (CCl_4) was added to dissolve the oil. A 25 mL of iodo-chloride solution in glacial acetic (Wijs solution) was accurately measured from a burette and added to the flask using a safety pipette in a fume chamber. The stopper was then inserted and the content of the flask was securely shacked. The flask was then allowed to stand at 30 ^oC for 2 hrs and protected from light. A 20 mL of 10 % aqueous potassium iodide and 100 mL recently boiled and cooled water was added to the mixture using a measuring cylinder. The liberated ion was titrated with 0.1 M sodium-thiosulphate solutions shaking until the color changed into quite pale. A 1 mL of 1 % starch indicator was added and the titration continued by adding thiosulphate dropwise until blue coloration discharged after vigorous shaking. The same procedure was used for blank sample[73]

The iodine value (I.V) was given by the expression:

Iodine value (I.V) = 12.96*CST*(Vst1-Vst2)/ W

Where: Cst = Concentration of sodium thiosulphate used

Vst1 = Volume of sodium thiosulphate used for blank

Vst2 = Volume of sodium thiosulphate used for determination

W = Mass of the sample (oil).

3.4.1.4. Determination of Saponification Value

An American Standard for Testing Material (ASTM) method- (D 5558-95) was used for the determination of the Saponification Values of the *O. Lamiifolium* oil. A 2 gm of the oil was

weighed into a 250 ml conical flask. A 25 mL of 0.5 M mixture of equal volumes of ethanol and potassium hydroxide was added and the resulting mixture was refluxed for 30 min while being stirred continuously. The resulting solution was subsequently titrated against 0.5 M HCl with 1 mL phenolphthalein as an indicator. The resulting endpoint was obtained when the pink color changed into colorless. The same procedure was used for the blank sample. The Saponification value (SV) was then calculated using the expression:

Saponification value (S.V.) = 56.1 (B-S) ×N of HCl / W

Where: B – ml of HCl required by blank

S – ml of HCl required by the sample

N – Molarity of HCl

56.1– A molar mass of KOH

W- the weight of the sample

3.4.1.5. Determination of Ash Content

The weight of the clean empty crucible was measured after heating in a muffle furnace and cooled in a dissector for 30 min (W_1). Two gm of the sample was taken in a crucible (W_2). The crucible was placed in a muffle furnace at 550 °C for 3 hrs. The appearance of gray-white ash indicates the complete oxidation of all organic matter in the sample. After ashing the furnace was switched off, the crucible was cooled and weighed (W_3). The experiment was in triplicate to check error value and percent ash was calculated by following formula [74]

Ash content by percent % = $\frac{W3-W1}{W2} * 100$

Where: W_1- the weight of the empty crucible

W_2- the weight of the crucible with a sample

W_3- the weight of the crucible with a sample after ashing

3.4.1.6. Determination of Acid Value

The acid value was determined according to the method applied by A.O.A.C. (2000). O. *lamiifolium* oil 2 gm was accurately weighed and dissolved in 250 mL conical flask with10 mL of neutralized ethanol and 2-3 drops of phenolphthalein indicator were added. The free acid was titrated with standard 0.1 N aqueous potassium hydroxide solutions by adding the alkali drop-wise at a uniform rate of about 30 drops per minute and the solution was titrated against 0.1 M KOH using 2-3 drops of phenolphthalein. The content of the flask was continuously agitated. Dark Pink coloration that did not fade within 10 sec was considered the endpoint. Afterward, the acid value was determined using the following equations:

$$\text{Acid Value} = \frac{56.1 * V * 0.1N\ KOH}{W}$$

Where: V = ml of 0.1N KOH consumed by sample oil

N = normality of KOH

W = weight in grams of the oil

Then, Free fatty acid = Acid value/2

3.5. Physicochemical Characterization of Bio lubricant

3.5.1. Organoleptic Test

The prepared formulations/plant-based drug lubricant was inspected visually for their physical appearance, color, odor, consistency and phase separation, homogeneity. Except for homogeneity, all the characteristics were inspected visually; however, the homogeneity was tested by pressing a small quantity of the formulated product between thumb and index finger. The consistency of the formulation and the presence of coarse particles were used to evaluate the homogeneity of the formulation. In this test color, odor, and consistency of the sample were determined by taking about 1 gm. of the sample were checked by 10 panelists from ASTU.

3.5.2. Diffusion study

The diffusion study was carried out by preparing petri dish glass with known diameter, based on the procedure used by Chen, meix. Ale 2016 [75]. A hole has made on a paper board at the center and an herbal-based drug lubricant was placed in it. The time taken by drug lubricant to get diffused through was noted after 1 hr with this the area covered was measured.

3.5.3. Determination of Viscosity

The viscosity of the drug lubricant should be comparable with the product that can be easily removed from the container and easily applied to the skin. The viscosity should remain constant. The measurement of viscosity of the synthesized drug lubricant was measured by using Viscometer, ASTM D562 VK-200 Model (India) attached with the spindle. Bio-lubricant was filled in standard beaker 250 mL and spindle was lowered perpendicularly, rotated in the bio-lubricant container and the operating method was based on KREBS, also operating with a fully automatic way with specification speed of 200 rpm, accuracy 1 % of full scale and repeatability ± 0.2 %. After the triplicate measurement, the average viscosity value was noted at 25± 1 °C.

Figure 10 Viscosity measuring device (Viscometer)

3.5.4. Determination of Solubility

Solubility of *O. lamiifolium* oil and bio-lubricant were important parameters to achieve the desired concentration of drug in systemic circulation for desired application to check the cleansing properties. Solubility study of materials was carried out in the water, boiling water, distilled water, alcohol, ether, and chloroform [75].

3.5.5. Stability Study

A physical stability test of bio-lubricant was carried out for four weeks at various temperature conditions (4, 25, and 37 ^{o}C). The physical properties were checked by retesting all tested physical parameters (color, phase separation, viscosity, spreadability, and diffusion, etc.) [76]

3.5.6. Wash ability

Bio-lubricant was applied on the skin and keep for 24 hrs. Then ease extends of washing with water was checked by applying it on the hand skin of willing 10 ASTU students as panelists.

3.5.7. Spreadability

It gives the spreading capacity of formulated bio- lubricant when it is applied on the skin or bacterially affected area of skin. It is expressed as time in seconds taken by two slides to slip off from drug conjugate. Spreading value is important for knowing the therapeutic potency of a topical formulation. The ability was determined by an apparatus constructed in our laboratory as described by [76]**.** A 1 gm. Bio-lubricant was placed in between the two Petri dish slides under the two slide glass. The drug conjugate was sandwiched between these plates. A 1 kg weight was placed on the top of the two plates for 5 minutes to expel air and provide a uniform film of the drug lubricant between the plates. Excess of cream was scraped off from the edges. The top plate was then subjected to a pulley of a certain 14.2 gm. With the help of string attached to the hook, the time (in seconds) required by the top plate to cover a distance of 10 cm be noted. The lesser the time taken for separation of two slides results in better spreadability.

Spread ability was given in unit gm. cm/sec calculated by the following formula:

$$\mathbf{S} = \frac{\mathbf{M} * \mathbf{L}}{\mathbf{T}}$$

Where: S = spreadability

M = weight tide to the upper slide,

L = length of a glass slide,

T= time taken to separate the slides

Figure-11 Spreadability test of bio-lubricant

3.6. Compositional Characterization

3.6.1. GC-MS Analysis

The component identification was achieved by the GC-MS analysis using HP 5890 series GC equipped with mass selective detector (MSD), HP 5972 series (German). Helium was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 1 µl was employed, injector temperature 250 ^{o}C and Ion-source temperature 280 ^{o}C. The oven temperature was programmed from 50 (isothermal for 4min.), with an increase of 3 min, to 280 min, and held for 10 min. isothermal at 280. Total GC running time was 90.67 min [77].

3.6.2. UV-Vis Analysis

The extract was centrifuged at 3000 rpm for 10 min and filtered through Whatman No.1 filter paper. The sample was diluted to 1:10 with Ethanol. The extract was scanned at a wavelength ranging from 200 to 800 nm using Double beam UV-Vis Spectrometer of Shimadzu model UV1800 (China) along with UV Probe software was used for analysis. The characteristic peaks were detected. The peak values of the UV-Vis were recorded [78].

3.6.3. FTIR Analysis

The FTIR spectrum model JASCO FTIR-6600-A (USA) was used to identify the functional groups of the active components present in the extract based on the peaks values in the region of IR radiation. When the extract was passed into the FTIR, the functional groups of the components were separated based on the ratio of their peak. The results of FTIR analysis confirmed the presence of alcohol, alkanes, aldehyde, aromatic compound, aromatic amines, and halogen compound

3.7. Antimicrobial Study of *O. lamiifolium* oil and Bio lubricant

I. Bioactivity Test

The biological activity of the essential oil was tested against two gram-positive and two gram-negative bacteria by the Disk-diffusion method. Reference drug, chloramphenicol was used side by side to compare the antimicrobial effectiveness. Four microorganisms (Gram-negative bacteria; Escherichia coli and Salmonella typhimurium and Gram-positive bacteria; Staphylococcus aureus, Bacillus subtilis) were selected and administered the isolated oil [79].

II. Preparation of Inoculums

The test bacterial strains, Escherichia coli (Gram-negative) and Staphylococcus aureus, (Gram-positive) were transferred from the stock cultures and streaked on Mueller Hinton plates, and incubated for 24 hrs. At 37 ^{0}C well separated bacterial colonies were then used as inoculums. Bacteria were transferred using a bacteriological inoculating loop to autoclaved Mueller Hinton agar that was cooled to about 45 °C in a water bath and mixed by gently swirling the flasks. The medium was then poured into sterile Petri dishes, allowed to solidify, and used for the biotest [80].

III. Biological Activity of Crude *O. lamiifolium* and Bio- lubricant

The antimicrobial study was employed by agar disc diffusion method with Whatman filter paper, Petri discs were used, being impregnated with the crud extract of the Ocimum Lamiifolium plant and then the discs were placed to the agar plate of Muller Hinton agar (MHA) of which the bacterial cell suspension (concentration of 1.3×10^{8} colony-forming unit (CFU). The test organisms were inoculated in Nutrient broth and incubated overnight (24 hrs.) at 37 ^{o}C. To adjust the turbidity to 0.5 McFarland standards giving a final inoculum of 1.3×10^{8} CFU/mL 25 mg/mL concentration sample was prepared in MHA, the plate was lawn cultured with standardized microbial culture broth. After incubation, Petri plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The test was performed on four bacteria, Escherichia coli, S.thy, Bacillus subtilis, and Staphylococcus aureus each anti-microbial agent was tested in triplicate, and each zone of inhibition was measured twice. This type of experiment has repeated the average of the readings was recorded and the zone of inhibition (ZOI) was observed and measured in mm.

CHAPTER FOUR

4. Results and Discussions

The present research work was planned to optimize extraction parameters, study the effect of the process parameters on the yield, optimizing the yield of *O. lamiifolium* crude oil, characterizing physicochemical properties, evaluating total major components and antibacterial activities of extract using Soxhlet extractor and Hexane as a solvent, characterizing synthesized bio lubricant for its physicochemical properties and evaluating antimicrobial activities. The oil was extracted using a Soxhlet extractor with optimized parameters and the essential oil component was obtained using Analytical and chromatographic analysis. The extracted crude oil component was characterized from the essential oil yield obtained from the Soxhlet extraction.

4.1. Physicochemical Characterization of Extracted Oil

Using process parameters that gave a maximum oil yield oil was extracted and physical and chemical properties were studied. The physicochemical properties were studied showed in range standard results of moisture content, P^H, viscosity, specific gravity, solubility, and others

4.1.1. Moisture components

The moisture content of the dry leaf of *O. lamiifolium* was determined using equation-1 at 3.4 and summarized in table 4.1. The calculated value of the moisture component become 15 % of of the *O. lamiifolium* and the rangining showed 85% moisture free of O. lamiifolium. This less amount of moisture helps to achieve Soxhlet extraction neglecting interaction of moisture. The amount of yield summary was comparably supported as reported by Zhang, Q.-W et al 2018.

4.1.2. Specific Gravity of the Oil

The specific gravity determined using pycnometer method, the density of oil calculated as 0.92 g/ml at the temperatures of 25°C-300C. Calculating with water, whose density is 1.00 g/ml. by the formula in the material and method that used to calculate the specific gravity, the value of the specific gravity of the extracted essential oil resulted 0.90±0.02g/ml.

4.1.3. Viscosity of the oil

Viscosity of O. *lamiifolium* oil was recorded from Viscometer, ASTM D562 VK200 Model (India) the recording takes place in triplicate and the average value calculated as 0.96 cp at room temperature .

4.1.4. pH Value of the oil

The pH value of oil was measure using pH meter, after repeated measuring until reach constant value. The average value became 4.5. From this result the oil found in O. lamiifolium is slightly acidic and it is comparable with literature reported in [82]

4.2. Chemical characterization of oil.

4.2.1. Total ash by percent (%)

From table-2. The value of total ash after the appearances of gray white color ash, measured as 6.5% which is less than 10%. This indicates complete oxidation of all organic matter in the sample and the left minimum amount may be potassium and calcium based inorganic matters[83].

4.2.2. Acid value

Acid value represents the mg KOH required to neutralize the free fatty acid in 1 g of oil. Therefore, acid value is a good indicator of oil degradation caused by hydrolysis[84] and calculated as 8.9 mg KOH/g. [84]

4.2.3. Saponification Value

Saponification value is an indication of the size or nature of fatty acid chains esterified to glycerol. And gives a measure of the average length of the fatty acid chain that makes up a fat in combination with acid values, saponification values are useful in providing information as to the quantity, type of glycerides and mean weight of the acids in a given sample of oil. Saponification is only of interest if the oil is for industrial purposes, as it has no nutritional significance. But due to the fact that each fat has within the limits of biological variation, a constant fatty acid composition, determination of the saponification value is a reasonable means of characterizing the fat [9]. The results showed that minimum saponification value and recorded with the oil from Ocimum. L with 19.7 mg KOH/g, that the oil indicating low fat content.

Table4. 1 Physicochemical properties of *Ocimum lamiifolium* leaf oil

Physical Parameters	Values	Chemical parameters	Values
Moisture content	15%	Iodine value	35±0.3 g/L
pH value	4.5	Acidic value	8.9 mg KOH/g
Specific gravity	0.92 g/mL	Saponification mg/g	19.7
		Ash content	6.5%

4.3. Physicochemical Analysis Result of Bio lubricant

The organoleptic properties, including physical appearance, color, odor, phase separation, and homogeneity of the drug conjugate lubricant formulations, are shown in the table blow (Table 4.2). Results showed that the formulation had a good appealing appearance and smooth texture, and it is found to be homogenous with no signs of phase separation. It is brown black in color and characteristic of aromatic odor.

4.3.1. Stability study

Physical stability test of the herbal based drug/ lubricant was carried out for four weeks at various temperature conditions like 4oC, 25oC and 37oC. The drug lubricant showed to be physically stable at different temperature i.e. 4oC, 25oC and 37oC within four weeks. No changes observed in physical quality color, phase separation spread ability, diffusion, PH and viscosity.

4.3.2. pH value determination

PH of prepared herbal based drug lubricant was measured by using digital pH meter. As mentioned in the methodology part. The pH of O. lamiifolium based lubricant calculated in the normal pH range of human skin and found to be 5.91. The result depicts the value lies in the normal pH range of the human skin 5.4-6.8. PH of O.lamiifolium showed slightly acid. After reaction (synthesis of bio lubricant), it became in the near range of normal human skin which is suitable to apply in human normal skin. An interaction of irritancy with acidity with normal body skin rang shows no expectation of irritation as the acidity decrease near to normal skin pH, the skin irritancy decreases. This was confirmed by study made by Amgad A, Awad etal.2015.

4.3.3. Spreadability

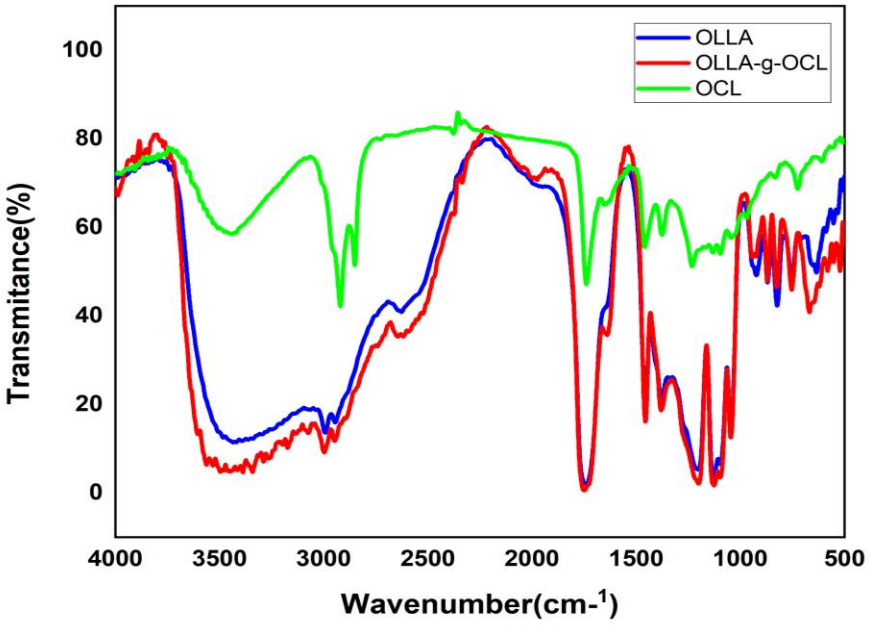
The spread ability was determined by placing excess of sample in between two slides, which was compressed to uniform thickness, by placing 1 kg for definite time. The time required to separate the two was found to be 6 sec and calculated spared ability showed26.7 gcm/sec. This number confirmed that bio-lubricant synthesized from O. Lamiifolium is readily spreadable as compared with herbal based biolubricant synthesized as evaluated by Abhijeet P. pandey et al. (2010). Comparative study on viscosity and spredablity depicts the viscosity of the bio lubricant increases, the spread ability decrease. One has interactive effect in to another.

Table-4. 2 Physicochemical characteristics for *O. lamiifolium* plant-based bio- lubricant

Physicochemical parameters	**Results**
Color	Dark brown
Odor	Characteristic
pH	5.91
Loss on drying(moisture and volatile)	25%
Solubility	Soluble in water, hexane, alcohol chloroform
Viscosity	1160 (cp)
Washability	Good
Diffusion study	>95% (in 1hr) in petri dish
Spread ability	26.7 gcm/sec
Stability	Stable at 4, 25 and 37 ^{0}C

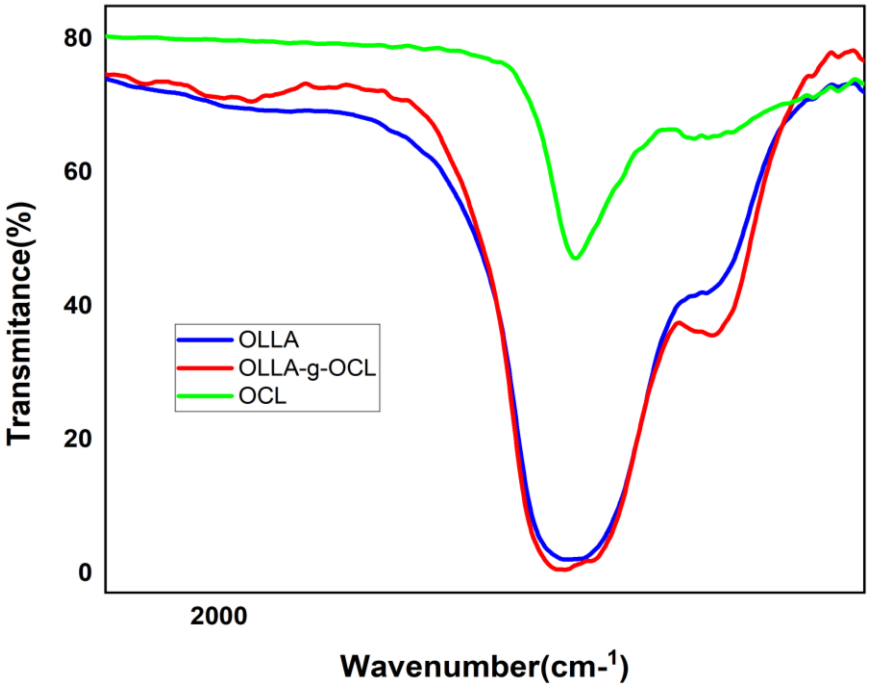
4.4. FTIR Analysis for *O. lamiifolium* Oil (OCL) and Grafted Copolymer (OLLA-g-OCL)

The spectra were used to identify the chemical structure, functional groups and intermolecular interaction of grafted conjugate, lactic acid oligomer and O. lamiifolium extract. The peaks of OLLA-g-OCL at 634 cm-1, 754 cm-1, 824 cm-1 and 869 cm-1 showed the presence of -C-C stretching, peaks at 1046 cm-1,1128 cm-1, represents C-O bond stretching in LA, OLLA, and OLLA-g-OL drug lubricant of the ester groups;1378 cm-1 and 1455 cm-1 depicts to CH3 angular deformation and 1745 cm-1 due to the -C=O stretching band of the carbonyl groups. peaks at 2943 cm-1 and 2990 cm-1 attributes to asymmetric and symmetric stretch of CH2, the intensity of the peak ranging from 1086 cm-1 to 1745 cm-1 attributed to the C-O and C=O starching vibrations. Peaks at 1752 cm-1 shifted to 1745 cm-1 with addition of OC.L and decreased the intensity of OLLA-g-OC.L due to C=O stretching band resulted due to the presence of the same peak at 1743 in OC.L that gives strength to generate the amide which indicated the grafting between OC.L and OLLA. The peaks at 1201 cm-1 and 1120 cm-1 shifted in to 1205 cm-1 and 1128 cm-1 respectively, this gives additional conformation that the incorporation of OC.L in to OLLA contributes to the formation of OLLA-g-OCL by involving in the reaction. This indicated the grafting between GA and OLLA. The reduction in the intensity was observed with addition of Ocimum lamiifolium, which clearly indicated the consumption of O-H groups in the reaction and supported the formation of OLLA-g-OCL occurred at O-H and N-H groups.



A)

Figure-12 A: FTIR Spectrum for OLLA, OLLA-g-OCL, and OCL



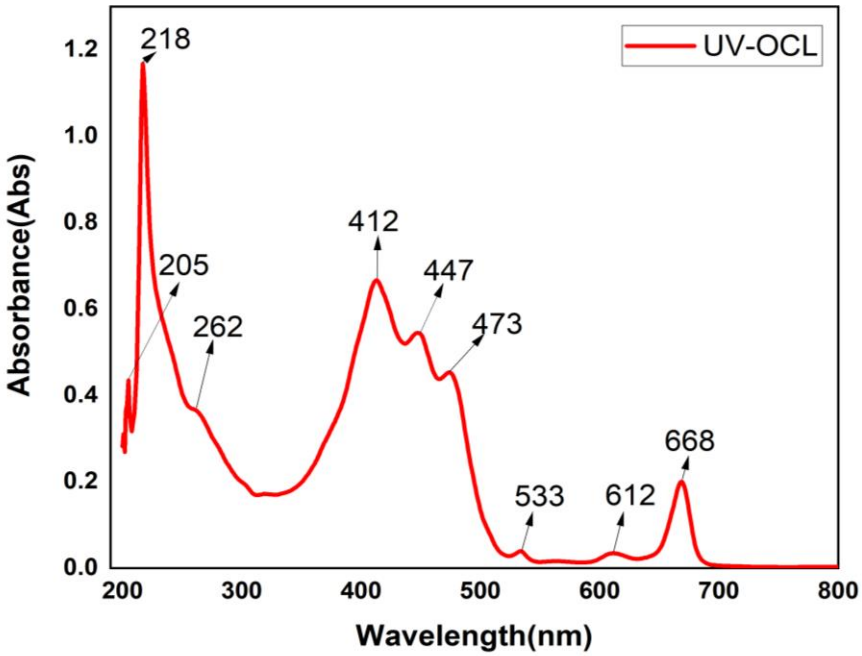
B)

Figure-13 B: FTIR Spectrum for OLLA, OLLA-g-OCL, and OCL Major Peaks

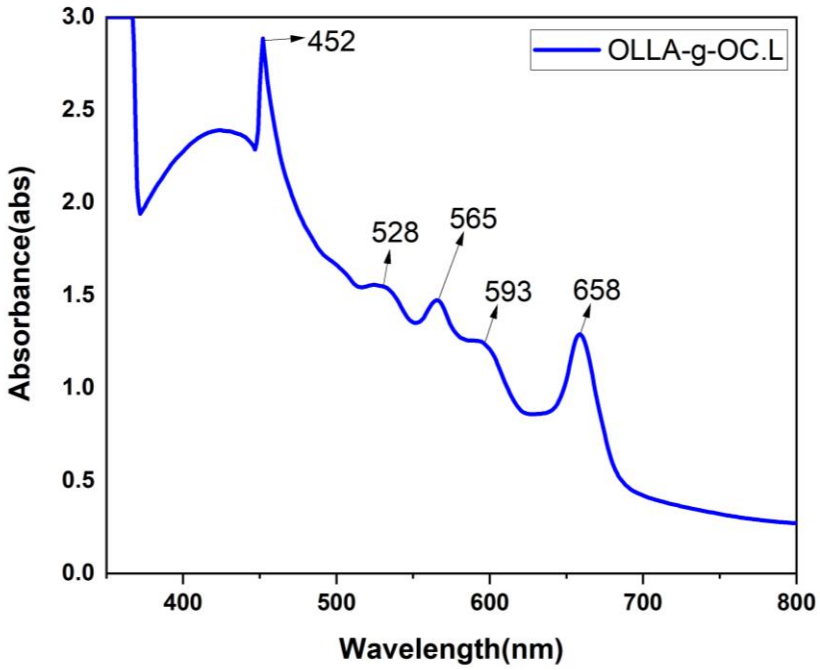
4.5. Results on UV-Vis Analysis for *O. lamiifolium* crude oil and Synthesized Bio lubricant

The UV-VIS absorption graph showed a wonderful absorption results in UV-VIS of different range. O. lamiifolium oil indicated good absorption from 200 nm to 800 nm. From figure-13, it can be seen that both O. lamiifolium oil and synthesized bio lubricant showed their transmission in UV-rays from 200 nm to 400 nm and absorption of UV rays from the sun. from the study of UV-VIS transmission through O. lamiifolium oil and the synthesized bio lubricant showed peaks at 205 nm, 218 nm, 262 nm, 412 nm, 447 nm, 473 nm, 533 nm, 612 nm and 668 nm, and peaks of bio lubricant at 200 nm, 2015 nm, 220 nm, 230 nm, 237 nm, 286 nm, 366 nm, 415 nm, 565 nm, 658 nm and 660 nm showed both can absorb ultraviolet and visible light which resulted in the range of 200-700 nm. From this, it can be concluded that the formulation can be applicable in skin protection and skin softener to suppress the influence of the sun light on human skin that can cause sunburns, wrinkles, lower immunity against infection and also high dosage of UV leading to skin reddening with dangerous sun

stroke. The literature by Radovan R korac etal. (2011) confirmed that the absorption ability of plant with their absorbance peaks led them to transmit in to and absorbs different color depending on their rand. O. lamiifolium based bio lubricant shows characteristic peak at 412nm that can absorb violet color, 447nm blue,533 nm green, 612 nm orang and 668 nm can absorbs red. Thus, this herbal based bio-lubricant has potential protective agent from photo damaging.



A)



B)

Figure-14 A and B, UV-Vis Spectrum for OCL and bio- lubricant Respectively

4.6. GC-MS Analysis for *O. lamiifolium* Oil

Chemical composition of O. lamiifolium obtained from Soxhlet extraction was analyzed by GC-MS and put in figure-14. 32 components were found and 7 of it identified as major constituents that all are responsible for anti- microbial properties of OC.L. The major analyzed constituents were phenol, 2-methoxy(2-propenyl), Isocaryophyllene, benzene 1,2, 3- thimethoxy-5-(2-propeny-Elemicin, hexadecanoin acid, methyl ester neophytodien and squalene. From literature all major compounds where comparable for their anti-microbial activities [85]. Different researchers works show positive argument with antimicrobial, ethno, modern, and food flavoring, preserving and packaging applications of major constituents in O. *lamiifolium*. Eugenol was identified from three plants of lamacia families for their antimicrobial and anti-oxidant study and revealed that eugenol has shown excellent antmicrobial activities. From the report of HP Hormann etal.2013, repored that supports Isocaryophyllene was identified as an alternative synthetic anti-inflammatory agent with potential application in food and pharmaceutical. Elemicin, hexadecanoic acid, squalen were listed under plant sourced highly inabitory chemicals for bacteria fungi, inflammatory wound and has been reported by Mahivachegam Chandra etal 2008.

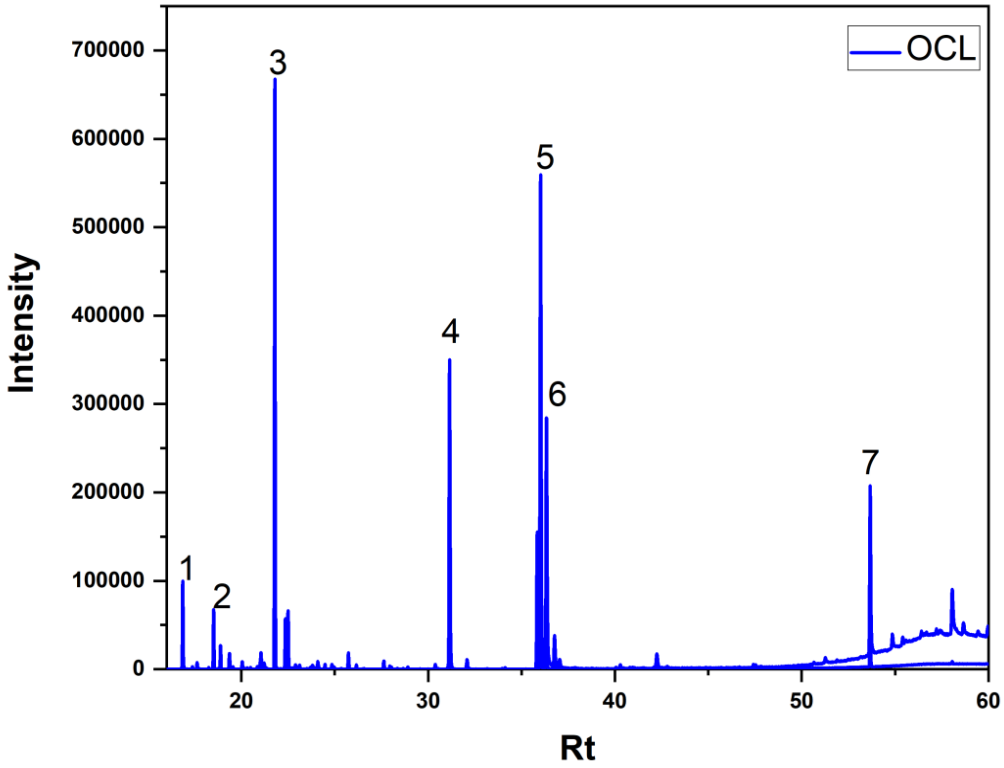


Figure-15 GC-MS Spectrum for *O. lamiifolium*

Table4. 3 Major Chemical Compound Identified by GC-MS

NO	**Compound Name**	**Rt (min)**	**Peaks area (%)**
1	Phenol (Eugenol)	16.85	3.26
2	Isocaryophyllene	18.513	1.40
3	Elemicin (benzene)	21.79	33.21
4	Hexadecanoic Acid, methyl ester	31.14	16.03
5	Octadecanoic acid, methyl ester	36.01	21.21
6	Neophytadiene	36.33	14.05
7	Squalene	53.67	10.79

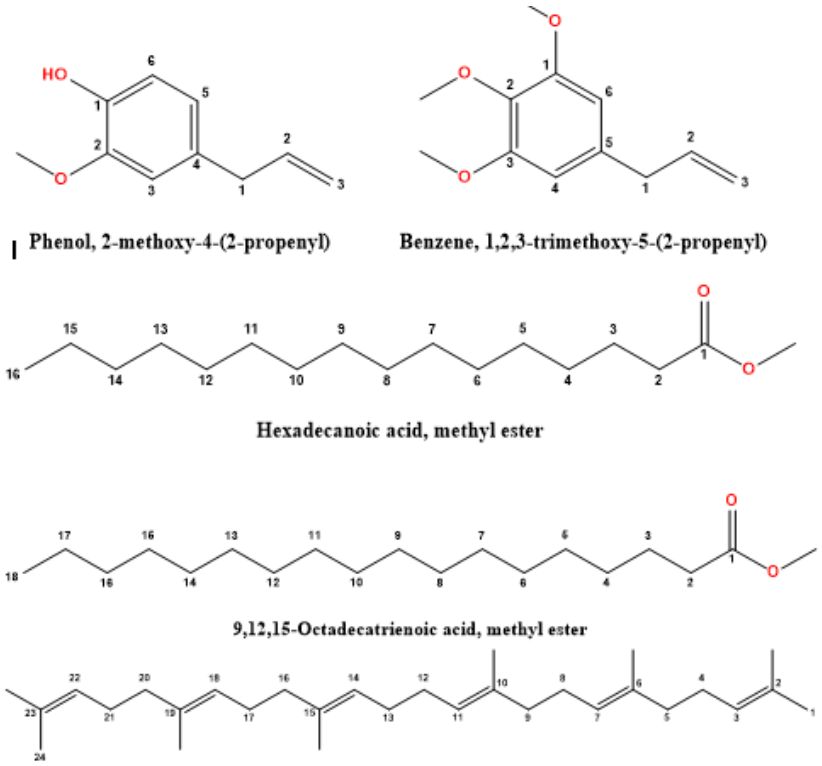


Figure-16 Structure of Five major Chemical Compound in *O. lamiifolium*

4.7. Experimental Design (DOE) Results for Optimization

4.7.1. Statistical analysis

In this study, the program was employed using a design experiment with box-Behnken under RSM and used in the analysis of variance (ANOVA). The statistical software program was used to generate the model equation, interaction effects of the independent variables, and surface plots using the fitted equation obtained from the regression analysis holding one of the independent variables constant.

4.7.2. Result Obtained from Soxhlet Extraction of *O. lamiifolium*

The extraction parameters selected for the entire work were particle size, extraction time, and solid mass (solute) to solvent ratio some of the factors which were very important to produce the optimum amount of crude oil. Using these parameters the extraction yield was used to evaluate the performance of Soxhlet extraction for the extraction of *O. lamiifolium* crude oils. Extract yield was the ratio of the extracted weight to the dry sample weight. Selecting these three parameters, there were about 17 numbers of experimental runs as per by the Box Behnken of the optimization process of RSM (response surface methodology). This optimization process is the best method for optimization than the other when there are

constraints in raw materials used and the expensive market price of the chemical reagent used to do all the experimental runs [86]. These 17 numbers of experimental runs with their level were performed as duplicates. The yield obtained for maximizing the yield was taken as the mean of these two replicates plus standard deviations. The individual replicates of raw data were written in appendix B and one can refer to them. The yield from these two replicates by different combinations as per the Design Expert output by AOAC (2000) official method was tabulated in the table below. The figures obtained on the yield from this experimental result were show maximum and minimum values. The following table shows the sum of squares, mean square, Adjusted R-square, Predicted R-square, F value, P-value (prob>F), significance model, and lack of fitness of different models when the response (yield) is evaluated within their respective ANOVA analysis.

The result of *O. lamiifolium* oil is presented in Table Appendix C-3. The yield for each run of the experiment was determined using Equation 1. The varying oil yield values are indications that the extraction parameters considerably affect the oil yield. It was observed that the yields obtained compared well with the predicted yield by the Design-Expert software. The maximum oil yield of 12.4 % (w/w) was obtained from the extraction of the corresponding solute/solvent ratio of (0.28% w/v), particle size (1.38 mm), and extraction time of (4.5 hrs) using Soxhlet extraction and n-hexane as a solvent. Also, the percentage oil yield compared well with the yield obtained from *O. lamiifolium* extraction being reported to have an oil yield of steam distillation 0.28 % (w/w) it may be suggested that the variation in the oil yield of OC.L may be due to the differences in the extraction method, the species, and the environmental conditions. This is an indication that the oil yield from the extraction of oil from OCL was affected by the process parameters or conditions.

4.7.3. Statistical Analysis for Extraction Parameters of *O. lamiifolium* Oil

The statistical analysis for oil extraction from *O. lamiifolium* was done using analysis of variance (ANOVA). Table-4 shows the ANOVA results of oil extraction. The multiple regression analysis of the experimental data gives a second-order polynomial equation, which was modified to discard the insignificant AC model term. The quadratic model developed describes the interaction between the dependent oil yield (Y) and the coded values of the independent variables A, B, and C (extraction time, particle size, and solute to solvent ratio). This is shown in the equation below.

I. The final equation in terms of actual factors

***O. lamiifolium* Oil Yield** = +12.40 + 0.0571 (**extraction time)** + 0.3076 (**particle size)** + 0.2310 (**ratio**) + 0.1150 (**extraction time*particle size)** - 0.0812 (**particle size*ratio) -** 1.03 (**extraction time**)^2 **-** 1.31 (**particle size)²** -1.05 (**ratio)^2**

II. Final Equation in Terms of Coded Factors

***O. lamiifolium* Oil Yield** = +12.40 + 0.0571 (**A)** + 0.3076 (**B**) + 0.2310 (**C)** + 0.1150(**AB -**) 0.0165 (**AC)** - 0.0812 (**BC)** -1.03 (**A²**) - 1.31 (**B²)** -1.05 (**C²)**

The equation in terms of coded factors can be used to make predictions about the response for 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.

Where: Y represents response variable oil yield measured in (%). The significance and adequacy of the model were tested by using ANOVA. It was observed from the table that the linear interaction effects are due to the Coded Factor A, corresponding to extraction time, B-particle size, and C-solute to the solvent ratio which was a significant factor. The quadratic effects of extraction time (A^2) particle size (B^2) and solute to a solvent ratio (C^2). Interactions of Extraction time with particle size (AB) and particle size with solute to the solvent ratio(BC) are all significant being less than 0.05, while the values greater than 0.05 means not significant the only value which was greater than 0.05 (AC) and it is omitted to reduce the model.

Table4. **4 ANOVA for Statistical Analysis**

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	27.74	9	3.08	2596.11	<0.0001	significant
A-extraction time	0.0255	1	0.0255	21.51	0.0024	
B-particle size	0.7426	1	0.7426	625.41	<0.0001	
C-ratio	0.4436	1	0.4436	373.63	<0.0001	
AB	0.0529	1	0.0529	44.55	0.0003	

AC	0.0017	1	0.0017	1.46	0.2667	
BC	0.0420	1	0.0420	35.38	0.0006	
A^2	4.47	1	4.47	3761.97	<0.0001	
B^2	7.23	1	7.23	6085.32	<0.0001	
C^2	10.31	1	10.31	8680.74	<0.0001	
Residual	0.0083	7	0.0012			
Lack of Fit	0.0083	3	0.0028			
Pure Error	0.0000	4	0.0000			
Core Total	27.75	16				

4.7.4. ANOVA for Quadratic Mode

The ANOVA result shows that the interaction factor (AC) is not significant. The interaction effects of solute to solvent ratio, extraction time, and particle size on the yield were studied using the interaction plot and 3D surface plot of RSM. From Figure-16, the combined effect of extraction time and solute to solvent ratio was studied using the interaction plot of RSM, and it was observed that the oil yield increased with an increase in extraction time and solute to solvent ratio. Also, it can be deduced from Figure-17 that an increase in solute to solvent ratio and extraction time favors the extraction rate as much solvent is needed to ensure that vapor rose through the bottom conical flask into the Soxhlet chamber at the top just as the solvent boiled. However, the oil yield was observed to decrease with higher extraction time, particularly at the extraction time higher than 5 hrs.

From Table 4 standard deviation of 0.0345, mean of 10.52 C.V. of 0.3276, R^2 of 0.9997, adj. R^2 of 0.9993, and adeq. A precision of 11.264 was obtained for crude oil yield. The fitness of the polynomial model was expressed by the coefficient of determination of R^2 and the coefficient of adjusted R^2, which were obtained as 0.9997 and 0.9993, respectively. It is suggested that these values should be at least 0.80 for the good fit of the model. Therefore, the predicted R^2 value of 0.9951 is in reasonable agreement with the adjusted R^2 value of 0.9993 and the difference is less than 0.2. This indicates indicate that the regression model is acceptable. The lack of fit value of 0.0028 implies non-significance; this is relative to the pure error. This was desirably low for the model, which shows an adequate representation of the interaction by the model. None significant lack of fit of the model is good as the model could be used for theoretical prediction of the oil extraction. It can be deduced from the F-

value of 2596.1 with a correspondingly low probability value of 0.0001, which is less than 0.05, that the model terms are significant. In this case, the adequate precision value (P adeq = 133.2) is greater than 4, which is desirable for the model showing an adequate signal-to-noise ratio of the model. Table 5 shows the assessment of experimental errors and the confidence interval (CI) of the experimental variables. The standard errors are analyzed based on the differences between the predicted oil yields and the experimental values recorded as shown in

4.7.5. Effects of Solute to Solvent Ratio and Extraction time on the Oil Yield

The effect of solute to solvent ratio and extraction time was studied using the interaction plot in Figure 16, which depicts the interaction between solute to solvent ratio and extraction time. The extraction efficiency increases with the increases in extraction time (duration), until a certain time range, further increment of the extraction time may not affect the extraction after the equilibrium of the solvent is reached inside and outside the material to be extracted. It can be deduced from the analysis of variance (ANOVA) results in Table 4 that the combined effects of solute to solvent ratio and extraction time have no a significant effect on the oil yield from *O. lamiifolium* because the solute to the solvent ratio is too high may cause excessive extraction solvent and requires a long time for concentration.

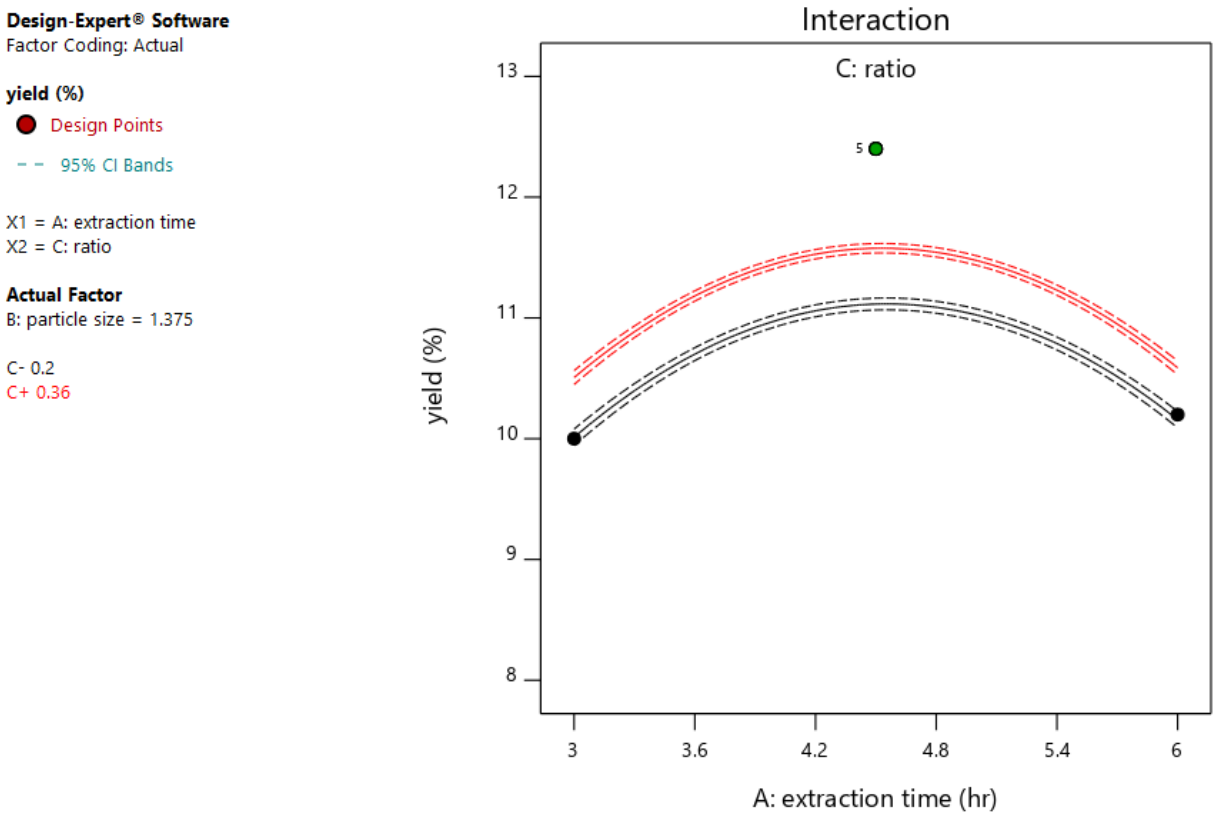


Figure-17 Yield (%) versus Extraction time with Ratio

4.7.6. Effect of Extraction Time and particle size on the Oil Yield from *O. lamiifolium*

The graphical representation showed the extraction time and particle size indicated that the extraction time and particle size had a direct significant effect on the yield of oil. At higher particle size, an increase in extraction time from a lower limit (3 hrs) to a higher limit (6 hrs) increased the extraction yield. The same is true for lower particle size (0.25 mm); an increase in extraction time from (3 hrs) to (6 hrs) increased the extraction yield. This shows that higher extraction time with lower particle sizes give a higher yield and higher particle sizes with lower extraction time can give a lower yield. It was noticed that an increase in time favored the extraction of oil until it got close to the moderate reaction time and increasing reaction time above this level tends to produce lower oil yields. The oil yield was observed to decrease with higher particle size so, as can be noticed from Figure there was no interaction effect is between particle size and extraction time.

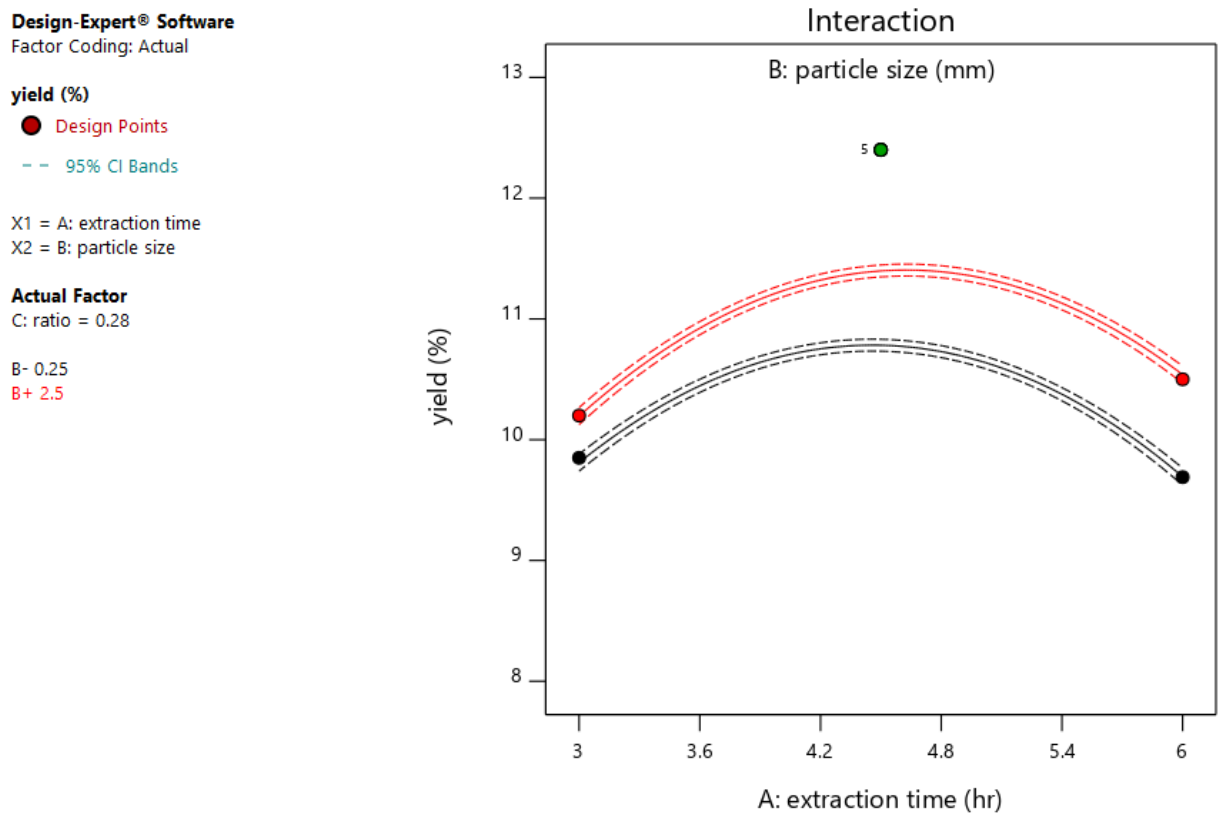


Figure-18 Yield (%) versus Extraction time with constant particle size

4.7.7. Effect of the particle size and Solute to Solvent Ration on the Oil Yield from *O. lamiifolium*

The 3D surface plot interaction from the Design Expert presented in Figure-18 represents the effects of solute to solvent ratio and Particle size. Interactions on the oil yield for extraction were studied. It was observed that an increase in solute to solvent ratio favors the extraction rate as much solvent is needed for extraction; it showed that the particle size and meal to solvent ratio have a significant effect on the extraction yield. In the graphical representation, there was no interaction between particle size and solute to solvent ratio as depicted by the similar shape of the curves in figure-14. At a higher particle size (2.5 mm), a decrease in solute to solvent ratio increased the extraction yield. The same is true for lower particle size (0.25 mm); a decrease in solute to solvent ratio hours increased the extraction yield. This shows that a lower solute to solvent ratio with lower particle sizes gives a higher yield and a higher particle size with a higher solid to solvent ratio can give a lower yield.

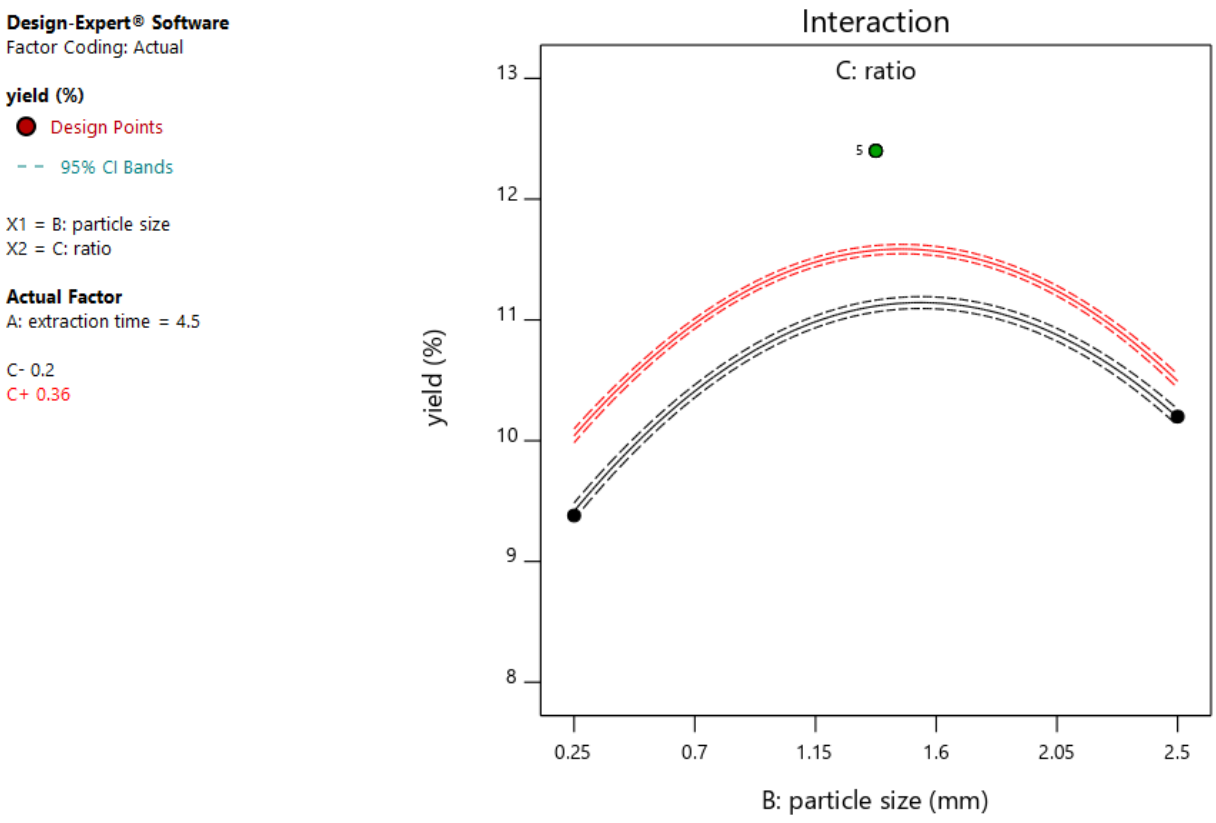


Figure-19 Yield(%) Versus Particle Size with a constant ratio

4.7.8. Optimization of Processing Parameters for Maximum *O. lamiifolium* Oil Yield

Determination of the optimum yield was based on numerical optimization using Design-Expert software. The maximum recovery of oil was obtained at the solute to solvent ratio, particle size, and extraction time of 0.28 g/ml, 1.375 mm, and 4.5 h, respectively, with the oil yield. The surface plot of the optimum value was illustrated in Figure-16. Under these optimum conditions, the oil recovery was predicted as 12.4 % with a desirability of 1.000 %. These optimum conditions compared well the experimental value, which gives the optimum oil yield of 12.34 %, a value slightly lower than the predicted value of 12.4 %. To verify and confirm the agreement between the results obtained from the model, the optimum conditions obtained were checked by a laboratory to validate the prediction of the model. A confirmatory experiment was done in triplicate using the optimal conditions, and the average OC.L oil yield obtained was 12.346 %. The result obtained from the experiment has established the optimal conditions for solvent extraction of oil from *O. lamiifolium* using response surface methodology for optimization.

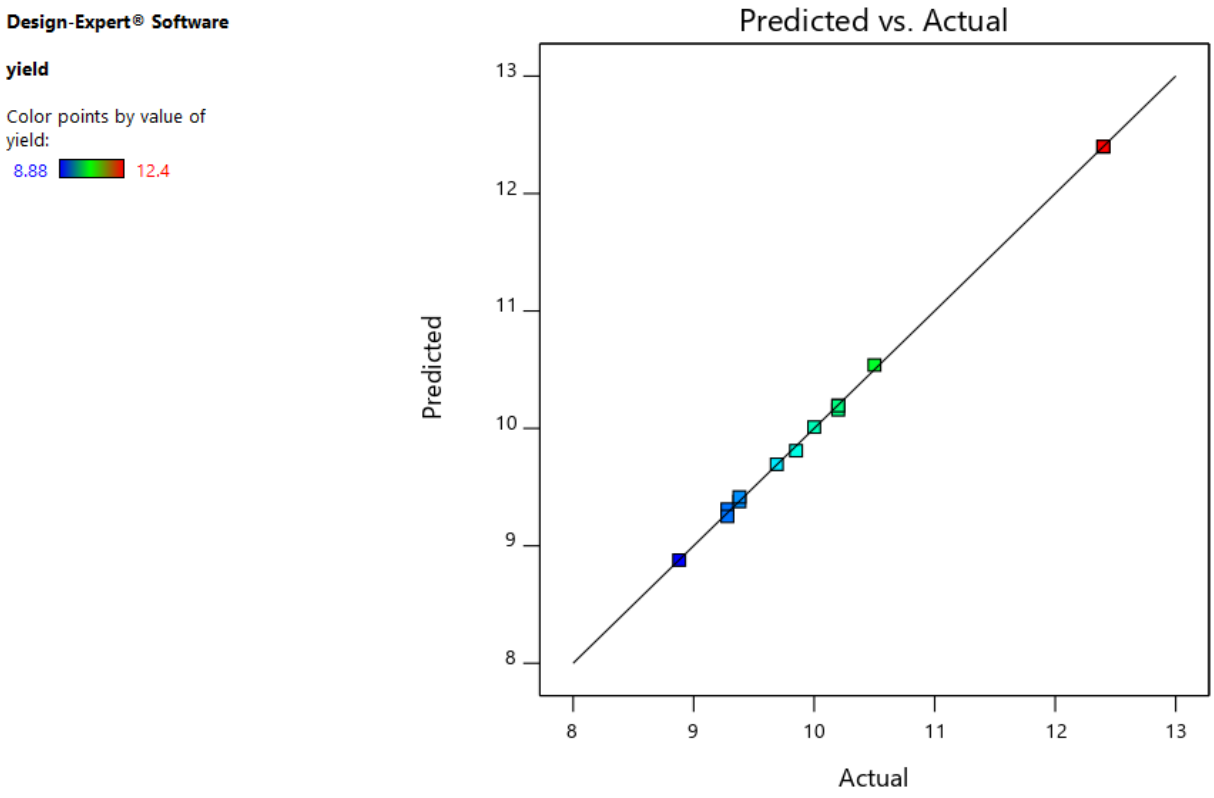


Figure-20 Predicted Versus Actual Values Graph

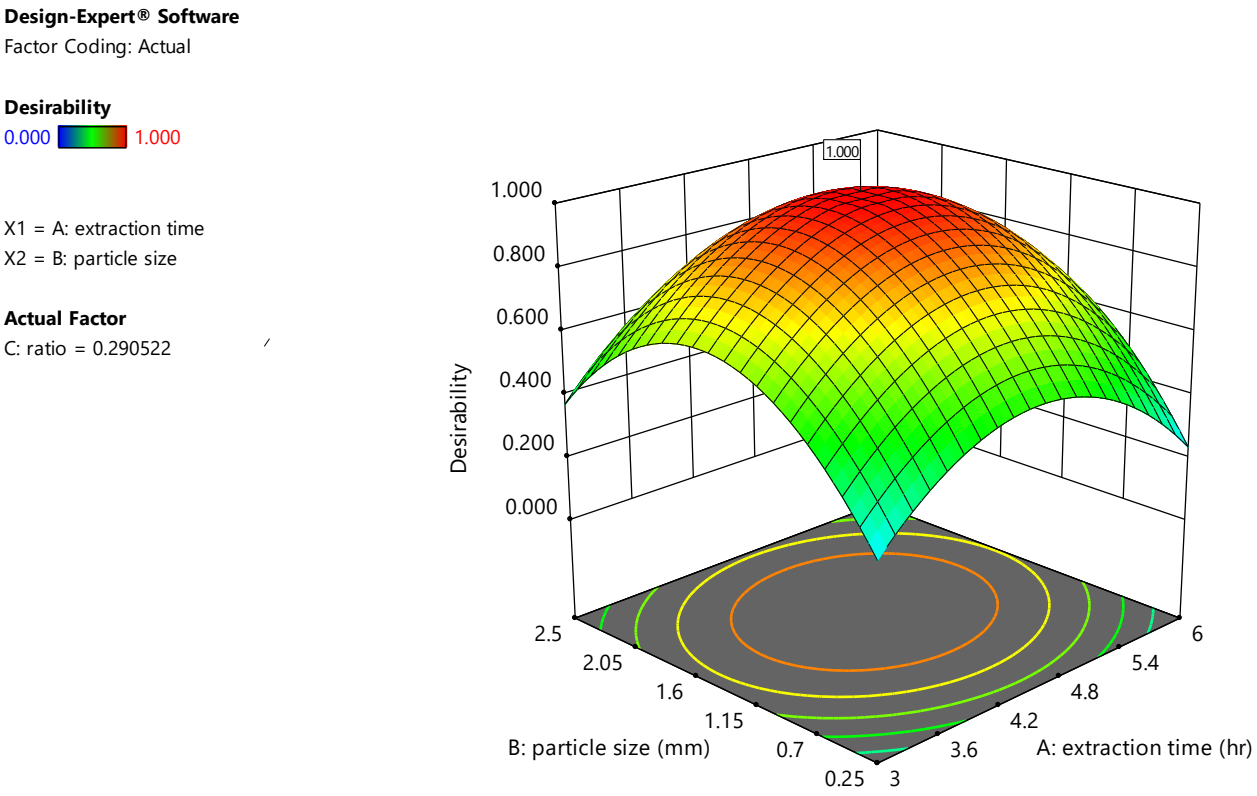


Figure-21 3D surface of desirability with particle size and extraction time

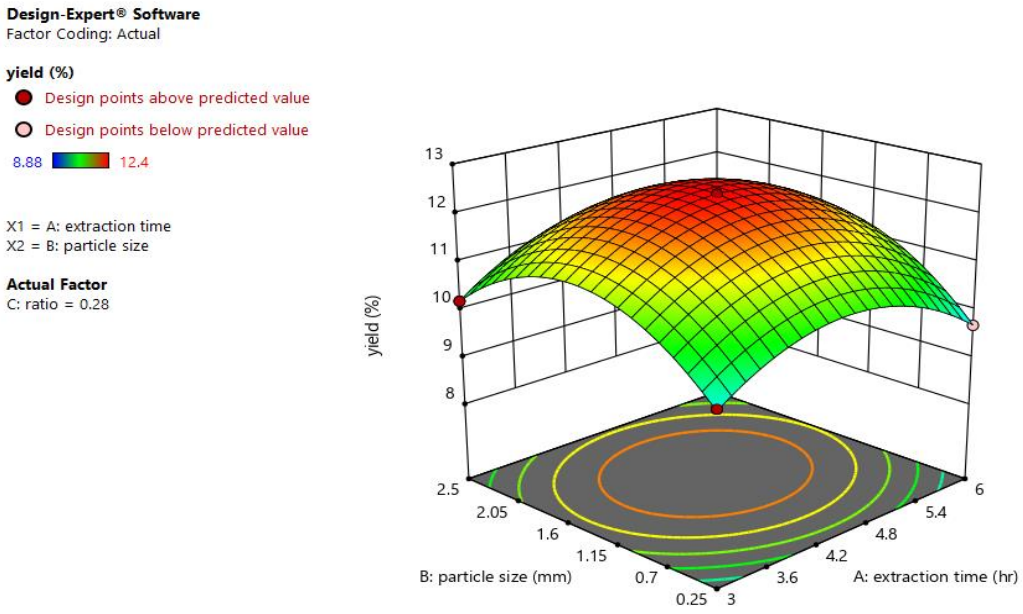


Figure-22 3D surface for yield(%) versus particle size and extraction time on

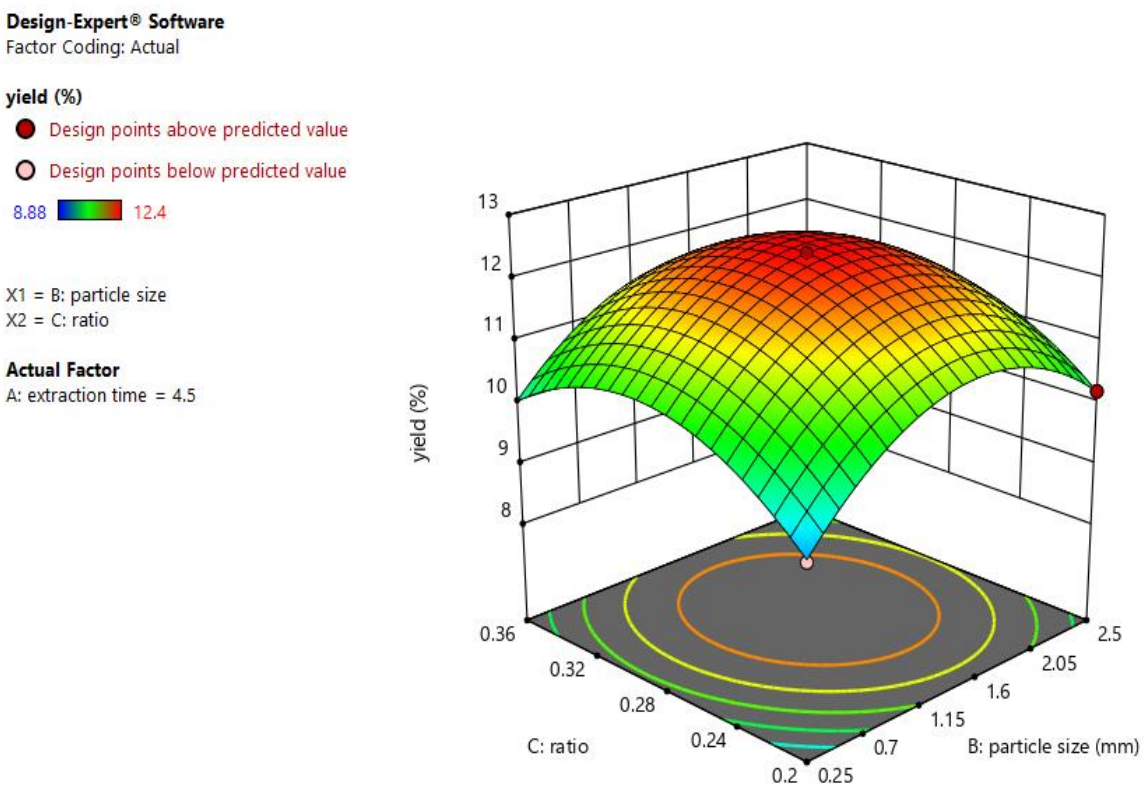


Figure-23 3D for yield(%) versus Solute to Solvent Ratio and Particle Size

4.8. Synthesis of Bio lubricant, Physical Evaluation, and Anti-Bacterial Study

The organoleptic properties, including color, texture, phase separation, homogeneity, and immediate skin feel of the ointment formulations, Results showed that the ointments had a good appealing appearance and smooth texture, and they were all homogenous with no signs of phase separation. All formulations were dark brown or brownie black in color and characteristic/ aromatic odor. Most Bio lubricants are advisable to be stored at temperatures below 30 °C to avoid some softening and liquefying properties of the formulation.

A)

B)

C)

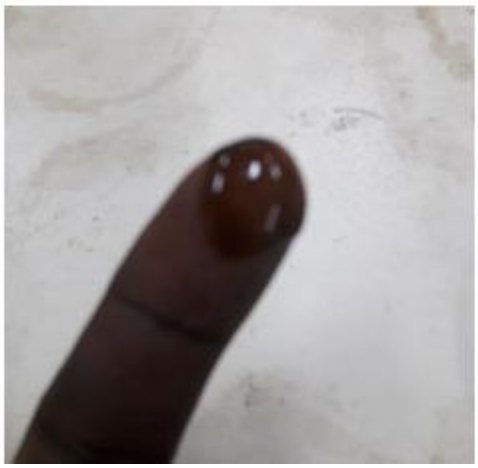


Figure-24. A, B, and C Bio lubricant Result After Four Weeks for Stability Tests

Figure-25 Bio-lubricant Synthesis process flow (Grafting Copolymerization)

4.8.1. Antimicrobial Activity Study

The antimicrobial activities of O. *Lamiifolium* oil and bio lubricant against the microorganisms examined in the present study, their potency, were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and zone diameter. The results are given by the following table [87].

Table 4. 5 Shows Zone of Inhibition for Gram-Positive and Gram-Negative Bacterias

Compounds (Code)	**Concentration (mg/mL)**	**Zone of inhibition in (mm)**			
		Gram-positive bacteria		**Gram-negative bacteria**	
Agr.	cfu	Escherichia coli	Bacillus subtilis	Salmonella typi	Staphylococcus aureus
O.CL	25,50 and 75	13	24.5	14 15	24
Bl- at 165 ^{o}c	25,50 and 75	30.5	20.4		24.6
Ciproflaxin	0.001	25	20	22	30.67

The result showed that the potential antibacterial effects of *O. lamiifolium* extract and lubricant synthesized by grafting *O. lamiifolium* with OLLA exhibited against two gram-positive and gram negative bacterial strains. Table 5. Shows dose-dependent potential activity (2.5 w/v, 5.0 w/v and 7.5 w/v) that affected the tested pathogens. The maximum inhibition zone (25.5 mg/mL and 26.4 mg/mL) observed on S. Aueres gram-positive as compared the three tested bacterium that is followed by S. thy (24.5 mg/mL and 20.4 mg/mL), E.coli (23.8 mg/mLand11 mg/mL) gram-negative and B.subtillis (17.2 and14 mg/ mL). This revealed that S. aureus was observed as the most susceptible bacterium that was inhibited by both extract and bio-lubricant. It showed a zone of inhibition (ZOI) against all bacteria tested *O. lamiifolium* and synthesized bio-lubricant was found to be effective against both gram-positive and gram-negative bacteria. The plant extracts of *O. lamiifolium* and synthesized bio-lubricant showed maximum activity against 2 pathogens, Staphylococcus aureus, Escherichia

coli, and moderate to a minimum against Bacillus subtilis and Bacillus subtilis. It has been widely observed and accepted that the medicinal value of plants lies in the bioactive phytocomponents present in the plants that dissolve in different solvent systems.

Disc diffusion assay of different bacterial strains shows a good inhibition response zone on incorporating *O. lamiifolium* oil and synthesized bio-lubricant.

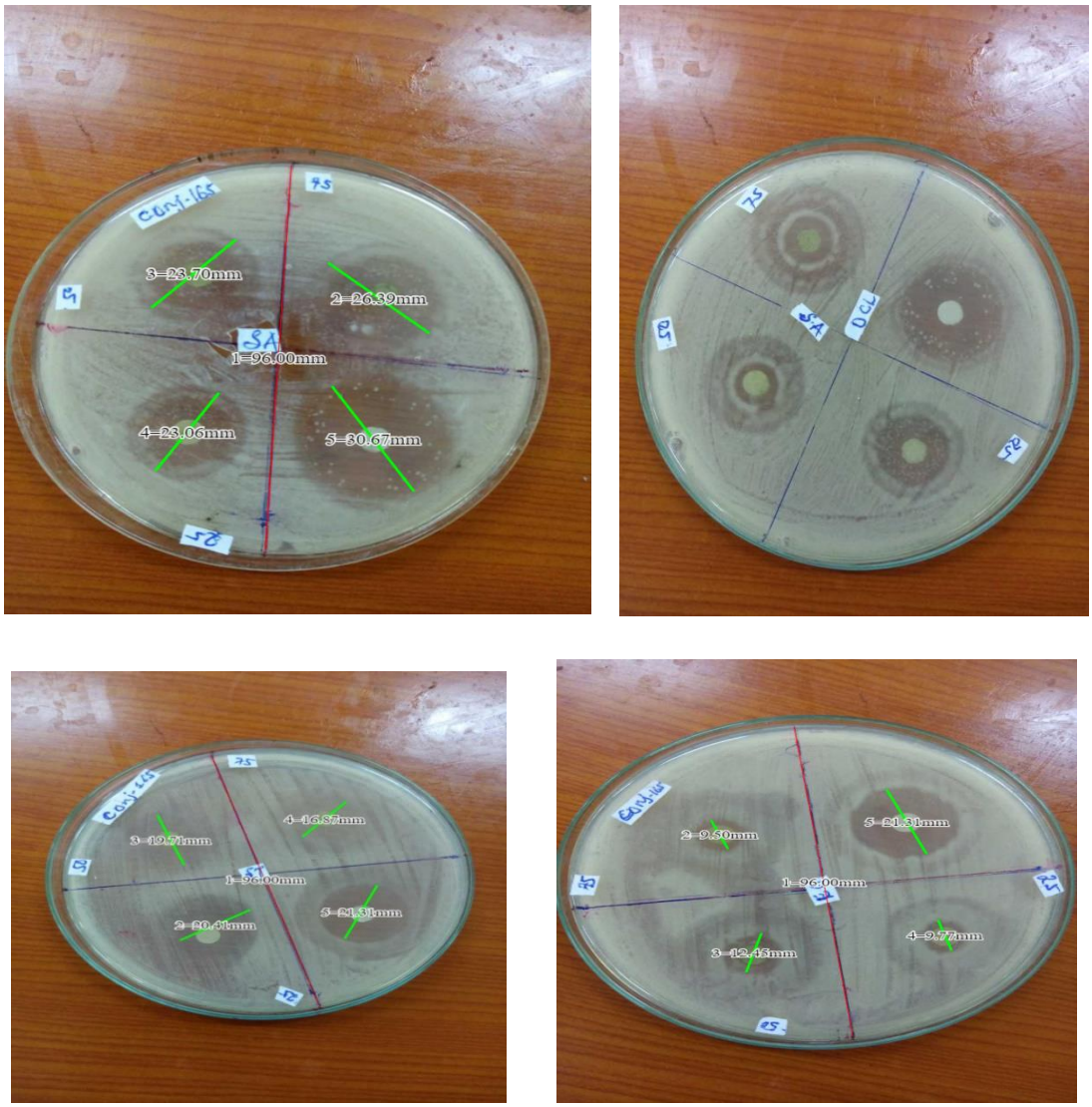


Figure-26. Anti-Bacterial Result of Synthesized Bio lubricant

CHAPTER FIVE

5. Conclusion and Recommendation

5.1. Conclusion

This work was intended to optimize the influence of different parameters (extraction time, solute to solvent ratio, and particle size) on the extraction quantity (yield) of the *O. lamiifolium* and an interaction effects of one another on the Oil yield, proximate and analytical studies, and anti-bacterial assay to synthesis bio lubricant for skin application. The optimization result was taken for extraction maximum oil yield to synthesize plant-based drug lubricant for application of bacterial caused skin infections. There are different methods of oil extraction from *O. lamiifolium* leaf. In this thesis, Soxhlet extraction was used. The solvent used for the extraction was n-hexane with a maximum oil yield obtained was 12.4 % at the extraction time of 4.5 hrs., particle size 1.375 mm, and solute to solvent ratio 0.28 w/v. The observed quantitative difference in the quantity of the oil was due to these three factors. The proximate analysis results revealed characteristics in range for pharmaceutical drug lubricant standards as shown in table -5. The instrumental analysis was analyzed by FTIR (to identify functional groups found and responsible for the antimicrobial properties of the product) GC-MS (to know the chemical composition and major organic compound responsible for the inhibition of bacterial activities) and UV-Vis analysis to check the absorption related to sunlight during application. The present investigation revealed that the extract of *O. lamiifolium* leaves has potent antimicrobial activity. Hence, *O. lamiifolium* can be employed as a source of natural antimicrobials that can serve as a medicinal ingredient incorporating into different skincare formulations. In general, the phytochemical analysis of *O. lamiifolium* species extracts has led to the identification of 32 organic compounds and 7 as major Phenol, Isocaryophyllene, 1, 2, 3-thimethoxy-5-(2-propenyl Elemicin, hexadecanoic acid, methyl ester, methyl linolenate, Eicosyne, and Squalene), Furthermore, both the plant extracts and synthesized bio lubricant was found to exhibit good antibacterial activity in the selected in two gram-negative (Bacillus subtilis and Staphylococcus aureus) two gram-positive (*Escherichia coli* and Salmonella typhi) bacteria assays. Different formulations of bio-lubricants were carried out by polycondensation reaction with 120-180 ^{O}C with 10 % w/v mixing ratio for five hrs, was considered for optimum desirable characteristics like compatibility with extracts, diffusion, PH, spreadability, and viscosity. From the above consideration 10 % w/v was selected as the final for the preparation of bio-lubricant because

found to be compatible with the extracts at 10 % concentration as well as possessing other optimum characteristics such as anti-bacterial potent effect. The ointment readily spread when applied on the skin topically and rubbed gently. The product was well diffused in the media around the cup of agar media and the diffusion of the product was comparable to similar work on eucalyptus plant Hayam, Saad Ali, et al 2012. Similarly, the ointment was found to be physically stable at different temperatures i.e. 2 ^{O}C, 25 ^{O}C, and 37 ^{O}C within a test period of four weeks. There were no changes in the spreadability, diffusion even after the exposure to different temperatures. Therefore, from the above experimental results, it can be concluded that the oil extracted from *O. lamiifolium* leave can be a good ingredient for the formulation of bio-based lubricants for skin care applications. It also holds for the production of novel drugs with the isolation of specific compounds [88].

5.2. Recommendation

Comparison of different extraction technology such as steam distillation, supercritical fluid extraction and cold press with Soxhlet extraction are suggested. Optimization of the extraction process of the essential oil was done on the three parameters extraction time, particle size, solute to solvent ratio while other parameters like temperature and solvent type were not considered. In fact These parameters could have an impact on the process to optimize the yield, therefore; further research work is recommended on the other variable parameters using the Soxhlet extraction method. Samples for the research work were collected from one area from Gede'o southern Ethiopia, but there could be a possibility that yield can be affected by different seasons, geographical areas, and its origins. Therefore, future study is recommended on those factors. The result of this research work proved that the produced oil serves as an additive, food preservative, flavoring, anti-oxidant and anti-fungal effect in the production of skincare bio lubricant giving it more anti-microbial efficiency. Study absorption and penetration of the *O. lamiifolium* bio-lubricant is one outlook for future work to be studied. Based on this one can produce better skincare products for this, further study should be done.

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Reference

1. Ndhlala, A.R., et al., 16 - Antibacterial, Antifungal, and Antiviral Activities of African Medicinal Plants, in Medicinal Plant Research in Africa, V. Kuete, Editor. 2013, Elsevier: Oxford. p. 621-659.
2. Atanasov, A.G., et al., Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnology Advances, 2015. 33(8): p. 1582-1614.
3. Abera, B., Medicinal plants used in traditional medicine by Oromo people, Ghimbi District, Southwest Ethiopia. Journal of Ethnobiology and Ethnomedicine, 2014. 10(1): p. 40.
4. Mahomoodally, M.F., Traditional Medicines in Africa: An Appraisal of Ten Potent African Medicinal Plants. Evidence-Based Complementary and Alternative Medicine, 2013. 2013: p. 617459.
5. Bekalo, T.H., S.D. Woodmatas, and Z.A. Woldemariam, An ethnobotanical study of medicinal plants used by local people in the lowlands of Konta Special Woreda, southern nations, nationalities and peoples regional state, Ethiopia. Journal of Ethnobiology and Ethnomedicine, 2009. 5(1): p. 26.
6. Sen, S. and R. Chakraborty, Revival, modernization, and integration of Indian traditional herbal medicine in clinical practice: Importance, challenges, and future. Journal of Traditional and Complementary Medicine, 2017. 7(2): p. 234-244.
7. James, P.B., et al., Traditional, complementary and alternative medicine use in Sub-Saharan Africa: a systematic review. BMJ global health, 2018. 3(5): p. e000895-e000895.
8. Abera, B., Medicinal plants used in traditional medicine by Oromo people, Ghimbi District, Southwest Ethiopia. Journal of ethnobiology and ethnomedicine, 2014. 10: p. 40-40.
9. Lulekal, E., et al., Antimicrobial activity of traditional medicinal plants from Ankober District, North Shewa Zone, Amhara Region, Ethiopia. Pharmaceutical Biology, 2014. 52(5): p. 614-620.
10. Giday, M., Z. Asfaw, and Z. Woldu, Ethnomedicinal study of plants used by Sheko ethnic group of Ethiopia. Journal of Ethnopharmacology, 2010. 132(1): p. 75-85.
11. Kefe, A., et al., Antimalarial properties of crude extracts of seeds of Brucea antidysenterica and leaves of Ocimum lamiifolium. BMC Complementary and Alternative Medicine, 2016. 16(1): p. 118.
12. Nahak, G., R. Mishra, and R. Sahu, Taxonomic distribution, medicinal properties and drug development potentiality of Ocimum (Tulsi). Drug invention today, 2011. 3(6).
13. Garrison, T.F., A. Murawski, and R.L. Quirino, Bio-Based Polymers with Potential for Biodegradability. Polymers, 2016. 8(7): p. 262.
14. Sasidharan, S., et al., Extraction, isolation and characterization of bioactive compounds from plants' extracts. African journal of traditional, complementary, and alternative medicines : AJTCAM, 2011. 8(1): p. 1-10.

15. Araya, S., B. Abera, and M. Giday, Study of plants traditionally used in public and animal health management in Seharti Samre District, Southern Tigray, Ethiopia. Journal of Ethnobiology and Ethnomedicine, 2015. 11(1): p. 22.
16. Chekole, G., Ethnobotanical study of medicinal plants used against human ailments in Gubalafto District, Northern Ethiopia. Journal of Ethnobiology and Ethnomedicine, 2017. 13(1): p. 55.
17. Mohanty, A.K., M. Misra, and L.T. Drzal, Sustainable Bio-Composites from Renewable Resources: Opportunities and Challenges in the Green Materials World. Journal of Polymers and the Environment, 2002. 10(1): p. 19-26.
18. Harsha, K., B. Kumud, and D.P. Katare, Biodegradable polymers, role in enhancing bioavailability of drug. Asian Journal of Biomedical and Pharmaceutical Sciences, 2012. 2(5): p. 1-6.
19. Sahalie, N.A., L.H. Abrha, and L.D. Tolesa, Chemical composition and antimicrobial activity of leave extract of Ocimum lamiifolium (Damakese) as a treatment for urinary tract infection. Cogent Chemistry, 2018. 4(1): p. 1440894.
20. Amabye, T. and S. Mussa, In Vitro Antimicrobial Efficacy of Fractions from Demakese (Ocimum lamifolium) Leaves Extract from Mekelle Tigray, Ethiopia. Nat Prod Chem Res 3: 196. doi: 10.4172/2329-6836.100019 6 Page 2 of 3 by Ocimum lamifolium. 2015, These results are in parallel with the previous report on Ocimum spp. by ref ….
21. Makonnen, E., et al., Antipyretic properties of the aqueous and ethanol extracts of the leaves of Ocimum suave and Ocimum lamiifolium in mice. J Ethnopharmacol, 2003. 88(1): p. 85-91.
22. Woldesellassie, M., M. Eyasu, and U. Kelbessa, In vivo anti-inflammatory activities of leaf extracts of Ocimum lamiifolium in mice model. J Ethnopharmacol, 2011. 134(1): p. 32-6.
23. Mukazayire, M.-J., et al., Evaluation of the hepatotoxic and hepatoprotective effect of Rwandese herbal drugs on in vivo (guinea pigs barbiturate-induced sleeping time) and in vitro (rat precision-cut liver slices, PCLS) models. Experimental and toxicologic pathology : official journal of the Gesellschaft fur Toxikologische Pathologie, 2010. 62(3): p. 289-299.
24. Brada, M., et al., Essential Oil Composition of Ocimum basilicum L. and Ocimum gratissimum L. from Algeria. Journal of Essential Oil Bearing Plants, 2011. 14(6): p. 810-814.
25. Safari Dolatabad, S., M. Moghaddam, and H. Chalajour, Essential oil composition of four Ocimum species and varieties growing in Iran. Journal of Essential Oil Research, 2014. 26(4): p. 315-321.
26. Alebie, G., B. Urga, and A. Worku, Systematic review on traditional medicinal plants used for the treatment of malaria in Ethiopia: trends and perspectives. Malaria Journal, 2017. 16(1): p. 307.
27. Kamble, V.A. and S.D. Patil, Spice-Derived Essential Oils: Effective Antifungal and Possible Therapeutic Agents. Journal of Herbs, Spices & Medicinal Plants, 2008. 14(3-4): p. 129-143.

28. Ololade, Z., et al., Ocimum basilicum var. purpureum Floral Essential Oil: Phytochemicals, Phenolic Content, Antioxidant, Free Radical Scavenging, Antimicrobial Potentials. Life Science Journal, 2017. 14(9).
29. Kaliyaperumal, K., A. Girmay, and S.F. Hayleeyesus, Larvicidal efficacy of Ethiopian ethnomedicinal plant Juniperus procera essential oil against Afrotropical malaria vector Anopheles arabiensis (Diptera: Culicidae). Asian Pacific Journal of Tropical Biomedicine, 2014. 4: p. S99-S106.
30. Raja, R., Medicinally Potential Plants of Labiatae (Lamiaceae) Family: An Overview. Research Journal of Medicinal Plant, 2012. 6: p. 203-213.
31. Agostini, F., et al., Essential oil yield and composition of Lamiaceae species growing in southern Brazil. Brazilian Archives of Biology and Technology, 2009. 52: p. 473-478.
32. Tangpao, T., H.-H. Chung, and S.R. Sommano, Aromatic Profiles of Essential Oils from Five Commonly Used Thai Basils. Foods (Basel, Switzerland), 2018. 7(11): p. 175.
33. De Lima, V., et al., Chemical composition and free radical-scavenging, anticancer and anti-inflammatory activities of the essential oil from Ocimum kilimandscharicum. Phytomedicine, 2014. 21(11): p. 1298-1302.
34. Gedif, T. and H.J. Hahn, The use of medicinal plants in self-care in rural central Ethiopia. J Ethnopharmacol, 2003. 87(2-3): p. 155-61.
35. Teklehaymanot, T. and M. Giday Ethnobotanical study of medicinal plants used by people in Zegie Peninsula, Northwestern Ethiopia. Journal of ethnobiology and ethnomedicine, 2007. 3, 12 DOI: 10.1186/1746-4269-3-12.
36. Mesfin, F., S. Demissew, and T. Teklehaymanot, An ethnobotanical study of medicinal plants in Wonago Woreda, SNNPR, Ethiopia. Journal of Ethnobiology and Ethnomedicine, 2009. 5(1): p. 28.
37. Mesfin, F., S. Demissew, and T. Teklehaymanot, An ethnobotanical study of medicinal Plants in Wonago Woreda, SNNPR, Ethiopia. Journal of ethnobiology and ethnomedicine, 2009. 5: p. 28.
38. Sarin, R.V., S. Narwal, and P.A. Bafna, Anti-diarrhoeal activity of aqueous extract of Ocimum kilimandscharicum. J Ethnopharmacol, 2013. 148(1): p. 223-8.
39. Beatovic, D., et al., Chemical Composition, Antioxidant and Antimicrobial Activities of the Essential Oils of Twelve Ocimum basilicum L. Cultivars Grown in Serbia. Records of Natural Products, 2015. 9: p. 62-75.
40. Zhang, Q.-W., L.-G. Lin, and W.-C. Ye, Techniques for extraction and isolation of natural products: a comprehensive review. Chinese Medicine, 2018. 13(1): p. 20.
41. Elyemni, M., et al., Extraction of Essential Oils of Rosmarinus officinalis L. by Two Different Methods: Hydrodistillation and Microwave Assisted Hydrodistillation. The Scientific World Journal, 2019. 2019: p. 3659432.

42. Taraj, K., et al., Water Distillation Extraction of Essential Oil from Sideritis Raeseri Herb. Environmental Processes, 2019. 6(4): p. 1051-1058.
43. Redfern, J., et al., Using soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. Journal of microbiology & biology education, 2014. 15(1): p. 45-46.
44. Sathish, S., J. Narayanan, and M. Johnson, Phytochemical Analysis of Vitex altissima L using UV VIS,FTIR and GC-MS. International Journal of Pharmaceutical Sciences and Drug Research, 2012. 4: p. 56-62.
45. Elumalai, D., et al., Gas chromatography mass spectrometry analysis and larvicidal activity of leaf essential oil extract of Leucas aspera against dengue, malaria and filariasis vectors. The Journal of Basic and Applied Zoology, 2018. 79(1): p. 49.
46. Wulandari, d., chromatography (hplc) lab report. 2017.
47. Manilal, A., et al. In vitro antibacterial activity of medicinal plants against biofilm-forming methicillin-resistant Staphylococcus aureus: efficacy of Moringa stenopetala and Rosmarinus officinalis extracts. Heliyon, 2020. 6, e03303 DOI: 10.1016/j.heliyon.2020.e03303.
48. Van den Oever, M., et al., Bio-based and biodegradable plastics – Facts and Figures. Focus on food packaging in the Netherlands. 2017.
49. Chen, F.-M. and X. Liu, Advancing biomaterials of human origin for tissue engineering. Progress in polymer science, 2016. 53: p. 86-168.
50. Lipsa, R., N. Tudorachi, and C. Vasile, Poly(α-hydroxyacids) in biomedical applications: synthesis and properties of lactic acid polymers. e-Polymers, 2010. 10(1): p. 087.
51. Jain, M., S. Zinjarde, and D. Gokhale, Poly-Lactic acid (PLA): synthesis and biomedical applications. Journal of Applied Microbiology, 2019. 127.
52. Lopes, M., A. Jardini, and R.M. Filho, Synthesis and Characterizations of Poly (Lactic Acid) by Ring-Opening Polymerization for Biomedical Applications. Chemical engineering transactions, 2014. 38: p. 331-336.
53. Chen, G.-X., et al., Synthesis of high-molecular-weight poly(L-lactic acid) through the direct condensation polymerization of L-lactic acid in bulk state. European Polymer Journal, 2006. 42: p. 468-472.
54. Makadia, H.K. and S.J. Siegel, Poly Lactic-co-Glycolic Acid (PLGA) as Biodegradable Controlled Drug Delivery Carrier. Polymers, 2011. 3(3): p. 1377-1397.
55. Schneider, R.V., et al., Sequence-definition in stiff conjugated oligomers. Scientific Reports, 2018. 8(1): p. 17483.
56. Ambrosio-Martín, J., et al., Melt polycondensation to improve the dispersion of bacterial cellulose into polylactide via melt compounding: enhanced barrier and mechanical properties. Cellulose, 2015. 22(2): p. 1201-1226.

57. Pohanka, M., D-Lactic Acid as a Metabolite: Toxicology, Diagnosis, and Detection. BioMed Research International, 2020. 2020: p. 3419034.
58. Pereira, R.F. and P.J. Bártolo, Traditional Therapies for Skin Wound Healing. Advances in wound care, 2016. 5(5): p. 208-229.
59. Chuong, C.M., et al., What is the 'true' function of skin? Experimental dermatology, 2002. 11(2): p. 159-187.
60. Özçelik, S., et al., Distribution of childhood skin diseases according to age and gender, a single institution experience. Turk pediatri arsivi, 2018. 53(2): p. 105-112.
61. Malik, K., et al., An ethnobotanical study of medicinal plants used to treat skin diseases in northern Pakistan. BMC Complementary and Alternative Medicine, 2019. 19(1): p. 210.
62. Tabassum, N. and M. Hamdani, Plants used to treat skin diseases. Pharmacognosy reviews, 2014. 8(15): p. 52-60.
63. Tabassum, N. and M. Hamdani, Plants used to treat skin diseases. Pharmacogn Rev, 2014. 8(15): p. 52-60.
64. Kasiramar, G., significant role of soxhlet extraction process in phytochemical research. 2019. 7: p. 43-47.
65. Gupta, A., M. Naraniwal, and V. Kothari, Modern extraction methods for preparation of bioactive plant extracts. International Journal of Applied and Natural Sciences, 2012.
66. Piras, A., et al., Ocimum tenuiflorum L. and Ocimum basilicum L., two spices of Lamiaceae family with bioactive essential oils. Industrial Crops and Products, 2018. 113: p. 89-97.
67. Al-Sumri, A., et al., Study on the Extraction of Date Palm Seed Oil using Soxhlet Apparatus. International Journal of Scientific and Engineering Research, 2017. 7: p. 1266.
68. Costa, O.B.d., et al., Essential Oil Constituents and Yields from Leaves of Blepharocalyx salicifolius (Kunt) O. Berg and Myracrodruon urundeuva (Allemão) Collected during Daytime. International Journal of Forestry Research, 2014. 2014: p. 982576.
69. Uzoh, C.F., O.D. Onukwuli, and J.T. Nwabanne, Characterization, kinetics and statistical screening analysis of gmelina seed oil extraction process. Materials for Renewable and Sustainable Energy, 2014. 3(4): p. 38.
70. Anuar, N., et al., Optimization of extraction parameters by using response surface methodology, purification, and identification of anthocyanin pigments in Melastoma malabathricum fruit. TheScientificWorldJournal, 2013. 2013: p. 810547-810547.
71. Saha, S., et al., Effect of Methanolic Leaf Extract of Ocimum basilicum L. on Benzene-Induced Hematotoxicity in Mice. Evidence-Based Complementary and Alternative Medicine, 2012. 2012: p. 176385.
72. Kapilraj, N., S. Keerthanan, and M. Sithambaresan, Natural Plant Extracts as Acid-Base Indicator and Determination of Their pKa Value. Journal of Chemistry, 2019. 2019: p. 2031342.
73. Abdalaziz, M., et al. Evaluation of Fixed Oil, Seed Extracts, of Carum carvi L. 2017.

74. Okonkwo, S. and E. Okafor, Determination of the Proximate Composition, Physicochemical Analysis and Characterization of Fatty Acid on the Seed and Oil of Gossypium Hirsutum. International Journal of Chemistry, 2016. 8: p. 57.
75. Chen, M.X., K.S. Alexander, and G. Baki, Formulation and Evaluation of Antibacterial Creams and Gels Containing Metal Ions for Topical Application. Journal of Pharmaceutics, 2016. 2016: p. 5754349.
76. Sabale, V., H. Kunjwani, and P. Sabale, Formulation and in vitro evaluation of the topical antiageing preparation of the fruit of Benincasa hispida. Journal of Ayurveda and integrative medicine, 2011. 2(3): p. 124-128.
77. Awasthi, P.K. and S.C. Dixit, Chemical Compositions of Ocimum sanctum Shyama and 0cimum sanctum Rama Oils from the Plains of Northern India. Journal of Essential Oil Bearing Plants, 2007. 10(4): p. 292-296.
78. Navarra, G., et al., Simultaneous Determination of Caffeine and Chlorogenic Acids in Green Coffee by UV/Vis Spectroscopy. Journal of Chemistry, 2017. 2017: p. 6435086.
79. Gadisa, E., et al., Combined antibacterial effect of essential oils from three most commonly used Ethiopian traditional medicinal plants on multidrug resistant bacteria. BMC Complementary and Alternative Medicine, 2019. 19(1): p. 24.
80. Ali, I.E., et al., Asymptomatic bacteriuria and antimicrobial susceptibility pattern of the isolates among pregnant women attending Dessie referral hospital, Northeast Ethiopia: A hospital-based cross-sectional study. Turkish journal of urology, 2018. 44(3): p. 251-260.
81. Zhang, Q.-W., L.-G. Lin, and W.-C. Ye, Techniques for extraction and isolation of natural products: a comprehensive review. Chinese medicine, 2018. 13: p. 20-20.
82. Laaroussi, H., et al., Physicochemical Properties, Mineral Content, Antioxidant Activities, and Microbiological Quality of <i>Bupleurum spinosum Gouan Honey from the Middle Atlas in Morocco. Journal of Food Quality, 2020. 2020: p. 7609454.
83. Srivastava, S., et al., Nutritional quality of leaves of some genotypes of mulberry (Morus alba). International journal of food sciences and nutrition, 2006. 57(5-6): p. 305-313.
84. Kardash, E. and Y.I. Tur'yan, Acid value determination in vegetable oils by indirect titration in aqueous-alcohol media. Croatica Chemica Acta, 2005. 78(1): p. 99-103.
85. Sahalie, N., L. Abrha, and L. Tolesa, Chemical composition and antimicrobial activity of leave extract of Ocimum lamiifolium (Damakese) as a treatment for urinary tract infection. Cogent Chemistry, 2018. 4: p. 1-10.
86. Li, X., H. Yu, and M. Yuan, Modeling and Optimization of Cement Raw Materials Blending Process. Mathematical Problems in Engineering, 2012. 2012: p. 392197.
88. Ali, H., N. Ahmed, and E. Mohamed, formulation and evaluation of herbal cream from ziziphus spina leaves extract. international research journal of pharmacy, 2013. 4: p. 44-48.

Appendix

Appendix-A Proximate Analysis on Ocimum Lamiifolium Oil and Bio-Lubricant

A-1 Moisture content of Ocimum Lamiifolium dried leaf (%) = $\frac{W1-W2}{W1} * 100\%$

Were: W1= intial wegiht

W2: wegiht after oven drying

A-2. Percentage of extraction yield = $m(oil)/m(sample) * 100$

A-2. Specific gravity = $\frac{W1-W0}{W2-W0} = \frac{density\ of\ oil}{density\ of\ water}$

where W_0 = weight of dry empty density bottle

W_1 = weight of density bottle + oil

W_2 = weight of density bottle + distilled water

A-3. Iodine value (I.V) =12.96*CST*(Vst1-Vst2)/ W

Where: Cst = Concentration of sodium thiosulphate used

Vst1 = Volume of sodium thiosulphate used for blank

Vst2 = Volume of sodium thiosulphate used for determination

W = Mass of the sample(oil).

A-4. Saponification value (S.V.) = 56.1 (B-S) ×N of HCl / Weight of sample

Where: B – ml of HCl required by blank

S – ml of HCl required by sample

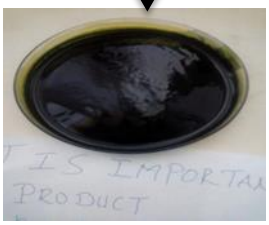
N – Molarity of HCl

Apendix-B. Raw Material Preparation and Oil Extractionn

(B1)

(B2)

(B3)



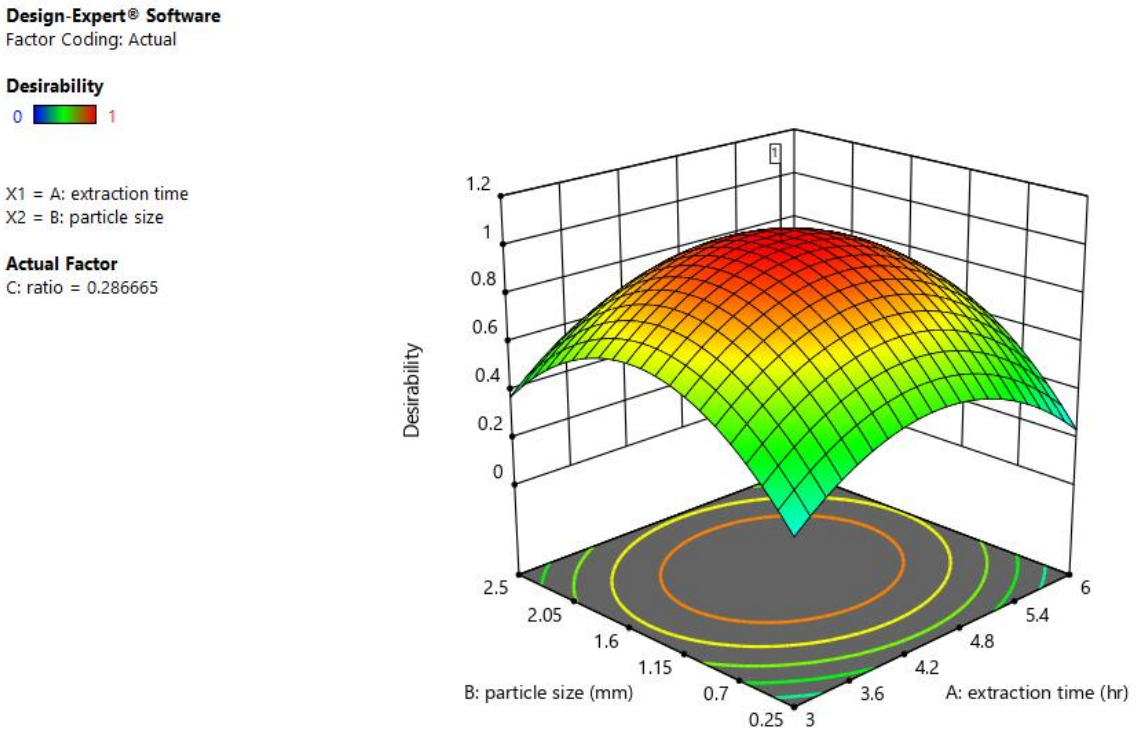
B1. Sample preparation

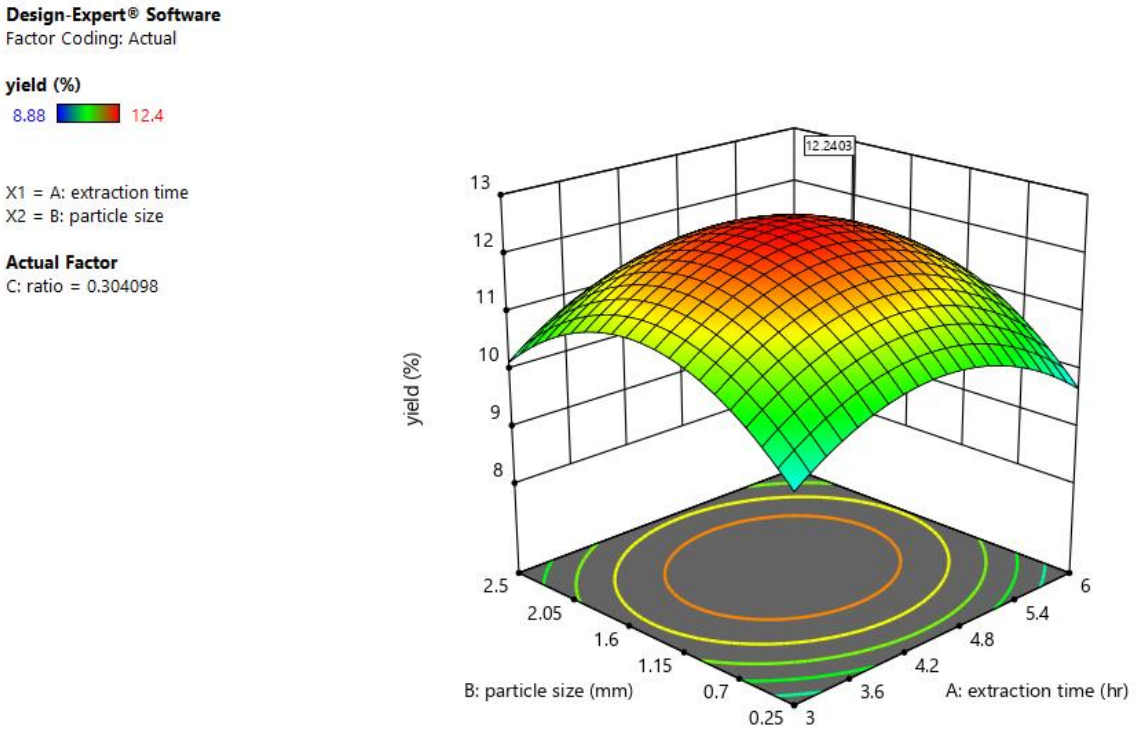
B-2 Oil extraction and separation

B-3 Extracted oil

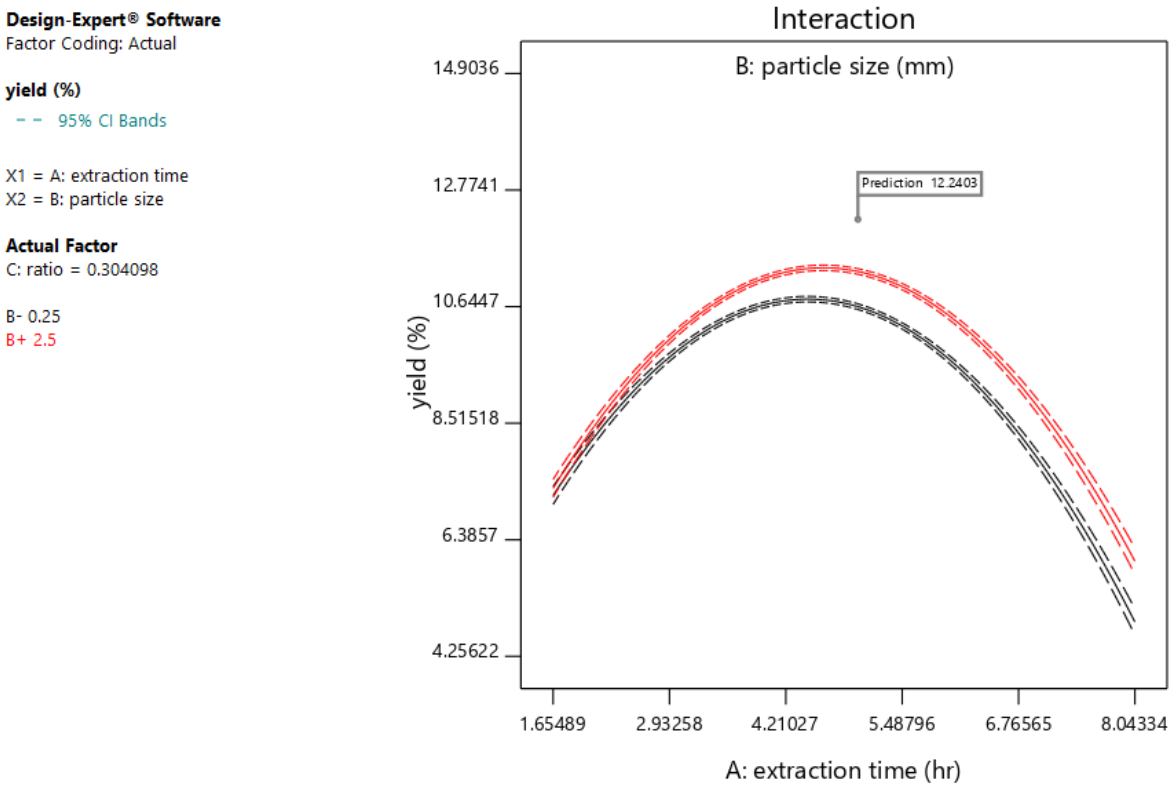
Appendix-C optimization process and graphical and tabular result data

C1- Desirability graph



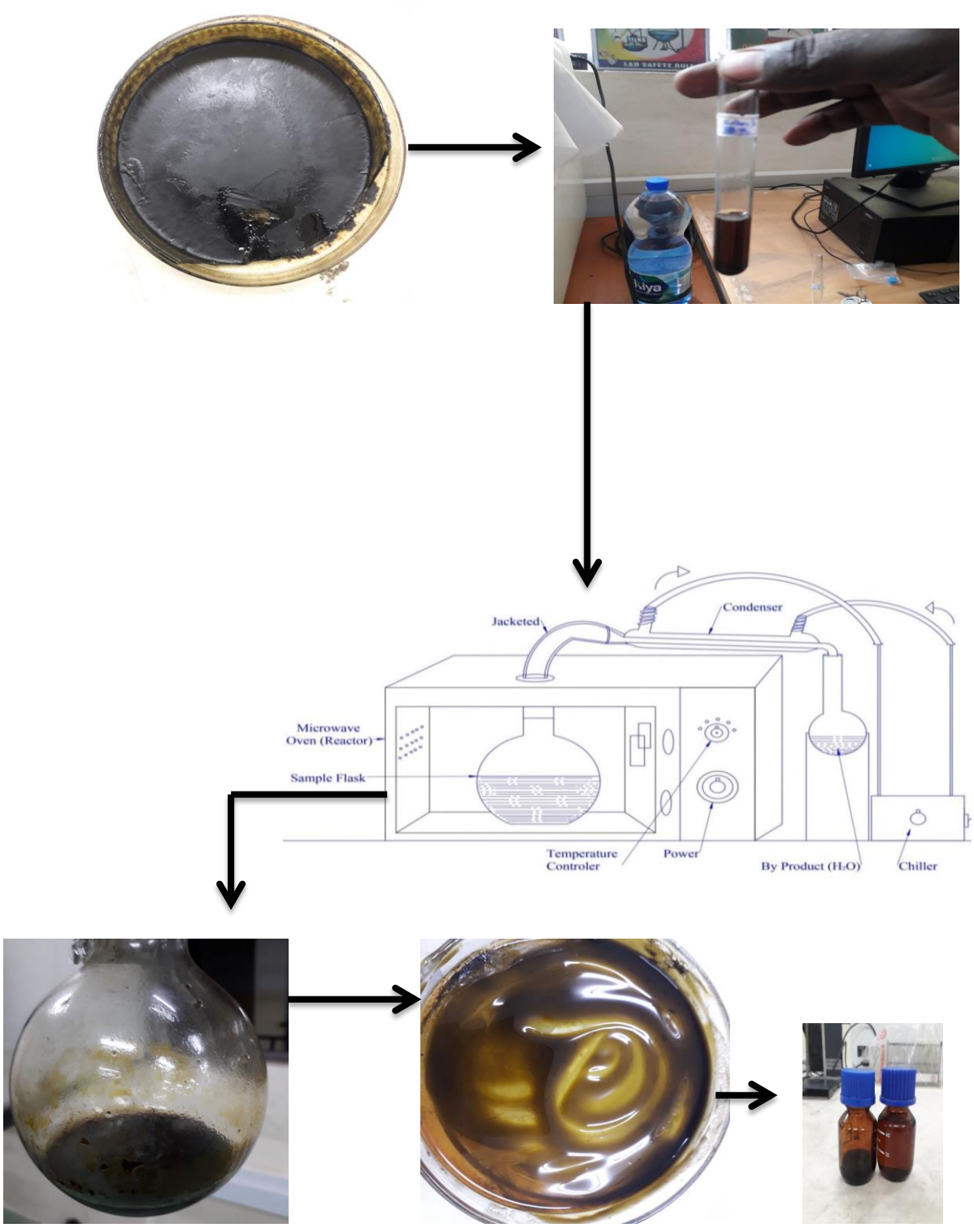


Appendix -C-2 3D of particle size versus extraction time



Appendix- D-1. Furnace for Ash content determination of OCL and bio -lubricant

Appendix –E synthesis of bio-lubricant



Appendix. E-1 Analytical Equipments

E-2. UV-VIS instrument

Appendix. E-3, Spreadablity test