ADDIS ABABA UNIVERSITY

ADDIS ABABA INSTITUTE OF TECHNOLOGY (AAIT)

SCHOOL OF CHEMICAL AND BIO-ENGINEERING

EXRTACTION AND CHARACTERIZATION OF ESSENTIAL OIL FROM EUCALYPTUS LEAVES USING STEAM DISTILLATION

BY:

MEAZA KIDANE

JUNE 15, 2016

ADDIS ABABA, ETHIOPIA

ADDIS ABABA UNIVERSITY
ADDIS ABABA INSTITUTE OF TECHNOLOGY (AAIT)
SCHOOL OF CHEMICAL AND BIO-ENGINEERING

EXRTACTION AND CHARACTERIZATION OF ESSENTIAL OIL FROM EUCALYPTUS LEAVES USING STEAM DISTILLATION

A thesis Submitted to the Research and Graduate School of Addis Ababa University, Addis Ababa Institute of Technology, Department of Chemical Engineering in partial fulfillment of the requirements for the attainment of the Degree of Masters of Science in Chemical Engineering under Process Engineering Stream.

BY:

MEAZA KIDANE

THESIS ADVISOR

Mr. TESHOME WERKU (Asis. Prof.)

JUNE 15, 2016

ADDIS ABABA, ETHIOPIA,

ADDIS ABABA UNIVERSITY

ADDIS ABABA INSTITUTES OF TECHNOLOGY

SCHOOL OF CHEMICAL AND BIO ENGINEERING

PROCESS ENGINEERING STREAM

EXRTACTION AND CHARACTERIZATION OF ESSENTIAL OIL FROM EUCALYPTUS LEAVES USING STEAM DISTILLATION

A thesis Submitted to the Research and Graduate School of Addis Ababa University, Addis Ababa Institute of Technology, Department of Chemical Engineering in partial fulfillment of the requirements for the attainment of the Degree of Masters of Science in Chemical Engineering under Process Engineering Stream.

By: Meaza Kidane

June, 2016

Addis Ababa, Ethiopia

Approved by the Examining Board:

Pro. Whasik Min	_____	_____
Chairman, Department's Graduate Committee	Signature	Date
Mr. Teshome Werku (Asis.Prof)	_____	_____
Advisor	Signature	Date
Mr. Adamu Z.	_____	_____
External Examiner	Signature	Date
Dr. Belay Woldeyes	_____	_____
Internal Examiner	Signature	Date

Acknowledgments

Praise God, the Most Merciful and Compassionate, for giving me the strength in completing this thesis research.

Next and for most, I would like to express my deepest gratitude to my advisor, Ato Teshome Worku (Asso.Prof) from Addis Ababa University, Addis Ababa Institute of Technology (AAIT), Department of Chemical Engineering for his tireless advice, supervision, guidance and patience throughout my research work.

My great thanks and appreciation also goes to Addis Ababa University for the provision of financial support so as to accomplish my research work, all laboratory assistant members in AAIT Chemical Engineering Department Mr. Hintsa, Mr. Samsom, Mr. Nebyu and others who were helping me throughout the work.

I also would like to say thank you to lab assistants of Adiss Ababa University (Arat kilo) Department of Biology and Biodiversity management, Department of Chemistry and Physics Department for their help on characterization of the raw material and the sample.

My friends, Mebrhit G/Mariam, Sami, Sol, and Hareg and others who help me are well acknowledged for the moral, technical and material contributions they made.

Last but not least, I want to acknowledge my family who were on my side in the whole my life in education in general and my graduate studies in Addis Ababa University in particular.

Table of Contents **Page No.**

LIST OF TABLES

List of Tables **Page No.**

LIST OF FIGURES

List of Figures **Page No.**

LIST OF ACRONOYMS

EGEO	Eucalyptus Globulus Essential oil
GC	Gas chromatography
MS	Mass-spectrometer
GC-MS	Gas chromatography- Mass-spectrometer
SCFE	Supercritical fluid extraction
ANOVA	Analysis of variance
JACAF	Japan Association for International Collaboration of Agriculture and Forestry

Abstract

The overall objective of this study was the extraction and characterization of the essential oil from the leaves of Eucalyptus globulus. Eucalyptus globulus of Myrtaceae family was the raw material of the experimental research which was collected from Entoto forest, 15 Km North West (NW) of Adiss Ababa. First, the leaf of Eucalyptus globulus was dried through partial sun drying. Then the dried Eucalyptus leaves were crushed in cross beater mill with sieve size of 10 mm and by cutting mill. The sample was sieved using a set of sieves sizes. Next to this, the experimental work was carried out by steam distillation set up. A general factorial design was employed to the extraction process using DESIGN EXPERT 7.0 software and linear regression model. This design helped to identify individual effects of extraction time, and particle size, as parameter and their interaction in the entire extraction process. In the extraction experiment, the minimum oil yield of 0.05% was obtained after the extraction time of 1 hour with particle size ranges of 12.5-20 mm and maximum oil yield of 1.19 % was obtained at optimum extraction time of 3 hours with particle size ranges of 5-8 mm. This shows that, increasing extraction time (optimum of 3hrs) and decreasing particle size increases yield. Characterization of the oil was also carried out. Accordingly the yield was found with 1.4463 refractive index value, 0.90841 specific gravity, and 2° optical rotation, 6.31 pH, 3.15 mpas dynamic viscosity, 174℃ boiling, 31.725ml/g iodine value, 14 ml/g saponification value, 19.635 acid value and 1,8 cineol(99%) component were obtained. Finally, the findings of this study will help to indicate the quality of the Eucalyptus oil which is important in the production of high value essential oils.

Key Words: *Eucalyptus Globules, Steam distillation, Eucalyptus oil, Gc-Ms, 1, 8 Cineol Addis Ababa, Entoto*

1. INTRODUCTON

1.1 Back ground

Essential oils contain highly volatile substances that are isolated by a physical method or process from plants of a single botanical species. The oils normally bear the name of the plant species from which they are derived. Essential oils are so termed as they are believed to represent the very essence of odor and flavor (Weiss, 1997). Essential oil plants and culinary herbs include a broad range of plant species that are used for their aromatic value as flavorings in foods and beverages and as fragrances in pharmaceutical and industrial products. Essential oils derive from aromatic plants of many genera distributed worldwide (Panda, 2011).

Essential oils are used in the embalming process, in medicine and in purification rituals. There are also over 200 references to aromatics, incense and ointments in the Old and New Testaments. Research has confirmed centuries of practical use of Essential Oils, and we now know that the 'fragrant pharmacy' contains compounds with an extremely broad range of biochemical effects. There are about three hundred essential oils in general use today by professional practitioners. Continual bombardment of viral, bacterial, parasitic and fungal contamination occurs in our body. Essential oils are a great benefit to help protect our bodies and homes from this onslaught of pathogens. Immune system needs support and these essential oils can give the required endorsement (pandey and Virendra, 2006-2007).

The Eucalyptus, a native genus from Ethiopia, belongs to Myrtaceae family and comprises about 900 species (Weiss, 1997). More than 300 species of this genus contain volatile oils in their leaves. Fewer than 20, within these species, known for their high content of 1, 8-cineole (more than 70%), have been commercially used for the production of essential oils in pharmaceutical and cosmetic industries. Over the past few years, the interest in natural medicine has been increasing in industrialized societies particularly against microbial agents because of the ever growing problem of antibiotic resistance (Panda, 2011).

1.2 Statement of the Problem

In Ethiopia, the increased demand for wood, particularly fuel wood, construction material and the government plan of reforestation have led to a rapid expansion of plantations of fast-growing species of Eucalyptus globulus, and more than 127,000 hectares of land have been planted in the last decades and usually harvested at the age of 5 - 7 years (kidanu *et al.*, 2004; hunde *et al.*, 2007; and dagne *et al.*, 2000). The eucalyptus species growing in Ethiopia have been the subject of only botanical studies. The essential oils of some of these eucalyptus species found in the central parts of the country were studied (nyssen et al., 2005 as cited in Gebrekidan *et al*., 2012). But to the best of our knowledge, no reports are available on the optimum condition of operating parameters (extraction time and particle size of the leaves) and composition of the eucalyptus oil of central, Ethiopia. Extraction and marketing of the eucalyptus oil is not also widely known in most regions of the country. It is therefore necessary to optimize operating conditions of the eucalyptus oil extraction process and to investigate the chemical composition of the essential oil extract from eucalyptus leaves. The leaves contain essential oils of medicinal importance, but variation in the contents and composition of its oils may occur due to different soil natures and climatic conditions. The abundant availability of eucalyptus trees in the country, it can also lay down a foundation for small-scale industries.

1.3 Objectives

1.3.1 General Objective

The general objective of the study was to extract and characterization essential oil from Eucalyptus leaves using steam distillation.

1.3.2 Specific Objectives

The specific objectives were:

- ✓ To investigate the effect of extraction time and particle size on the yield of extracted oil
- ✓ To determine the optimum condition of extraction time and particle size, and
- ✓ To characterize eucalyptus oil.

1.4 Significance of the study

This study will contribute to development extraction technology for the production of essential oil from eucalyptus leaves this can be used us an additive in soap, in pharmaceutical and cosmetic industries to improve the performance quality of the product.

2. LITERATURE REVIEW

2.1 Introduction

It is estimated that there are 250,000 to 500,000 species of plants on Earth. A relatively small percentage (1 to 10%) of these is used as foods by both humans and other animal species. It is possible that even more are used for medicinal purposes (Moerman, D. E. 1996). Moerman (1996) reported that while 625 species of plants have been used by various Native American groups as food, 2,564 have found use as drugs. According to his calculations, this leaves approximately 18,000 species of plants which were used for neither food nor drugs. Plant oils and extracts have been used for a wide variety of purposes for many thousands of years (Jones, 1996). These purposes vary from the use of rosewood and cedar wood in perfumery, to flavoring drinks with lime, fennel or juniper berry oil, and the application of lemongrass oil for the preservation of stored food crops. In particular, the antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies. Since ancient times, herbs and their essential oils have been known for their varying degrees of antimicrobial activity. More recently, medicinal plant extracts were developed and proposed for use in food as natural antimicrobials (Kumar, 2010).

2.2 Essential oils

An essential oil is a concentrated, hydrophobic liquid containing volatile aroma compounds from plants. Essential oils are also known as volatile, ethereal oils or aetherolea, or simply as the "oil of" the plant from which they were extracted, such as oil of clove. Oil is "essential" in the sense that it carries a distinctive scent, or essence, of the plant (Sewanu, 2012).

Essential oils are frequently referred to as the “life force” of plants. These "essential" oils are extracted from flowers, leaves, stems, roots, seeds, bark, and fruit rinds. The amount of essential oils found in these plants can be anywhere from 0.01 percent to 10 percent of the total. These oils have potent antimicrobial factors, having wide range of therapeutic constituents. These oils are often used for their flavor and their therapeutic or odoriferous properties, in a wide selection of products such as foods, medicine, and cosmetics. Only pure oils contain a full spectrum of compounds that cheap imitations simply cannot duplicate (Asaad, 2014).

2.2.1 Sources of natural essential oils

Essential oils are generally derived from one or more plant parts, such as flowers (e.g. rose, jasmine, carnation, clove, mimosa, rosemary, lavander), leaves (e.g. Eucalyptus , 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) (Trieste, 2008).

2.3 Eucalyptus Tree

2.3.1 Overview of eucalyptus in the world

In World large numbers of aromatic and medicated plants are available in most of the region. In which Eucalyptus plant is most common among them. Eucalyptus is recognized today as a natural product which has much to offer in solving global agricultural, environmental and public health problems (Chaitanya *et al.*, 2011). Natural properties of eucalyptus do not have any toxic reactions, so they are helpful in plant protection and management. All the parts of plant like seed, flowers, bark, and leaf can be used to produce high quality product (Pollack, 2010).

Eucalyptus is an evergreen tall tree, native to Australia, effectively introduced worldwide, now extensively cultivated in many other countries including Portugal. In Portugal, the planting of *Eucalyptus globulus* occupies about 20% of the forest area and is mainly used by the pulp industries, as source of cellulosic fiber, but some parts of the plant (principally leaves and bark) continue to be rejected by the paper industry (Pombal S. etal., 2014).

2.3.2 Overview of eucalyptus in Ethiopia

2.3.2.1 History of afforestation

The history of afforestation with eucalyptus species in Ethiopia goes back as early as the era of Emperor Menelik II at the end of the 19th century. The Imperial Court until that time used to lead a nomadic life, in which it simply repeated the cycle of exhaustion of wood vegetation around the court, followed by migration to a next place. The living style changed as a result of the

afforestation with eucalyptus, and it enabled Addis Ababa to become the permanent capital city. When Emperor Menelik II introduced eucalyptus, he tested more than ten varieties, and among them two varieties currently have been cultivated widely, Eucalyptus globules (common name in Ethiopia, white eucalyptus) and E. camaldulensis (red eucalyptus) (Jagger and Pender ., 2000).

2.3.3 Local Names

Amharic (nech bahir zaf); Creole Patois (kaliptis); English (turpentine gas, Tasmanian blue gum eucalypt, Tasmanian blue gum, southern blue gum, fever tree,bluegum eucalyptus, blue gum); Japanese (yukari-no-ki); Spanish (eucalipto); Swahili (mkaratusi); Tigrigna (tsaeda-kelamitos); Trade name (blue gum) (Orwa *et al.*, 2009).

2.3.4 Botanic Description

Eucalyptus globulus ssp. globulus is a large to very large evergreen tree, 40-55 (max. 60) m tall, with straight, massive trunk 0.6-2 m in diameter; narrow, irregular crown of large branches and drooping aromatic foliage; crown of open-grown trees broadly rounded or irregular with branches nearly to the ground; bark smoothish, mottled grey, brown, and greenish or bluish, peeling in long strips, at base becoming grey, rough and shaggy, thick and finely furrowed; root system deep and spreading. Leaves alternate, drooping on flattened, yellowish leafstalks of 1.5-4 cm, narrowly lance shaped, 10-30 cm long, 2.5-5 cm wide, mostly curved or sickle shaped, long-pointed at tip, short-pointed at base, not toothed on edges, hairless, thick, leathery, with fine, straight veins and vein inside margin, shiny, dark green on both surfaces, aromatic with an odour like that of camphor when crushed (Orwa *et al.*, 2009).

Flowers 1 (rarely 2-3) at leaf base on very short, flattened stalk or none, more than 5 cm across the very numerous, spreading, white stamens about 12-15 mm long, with odour of camphor; buds top-shaped, 12-15 x 12-25 mm; base (hypanthium) 4 angled, very warty, whitish bloom, with 2 lids. Fruits or seed capsules single at leaf base, broadly top-shaped or rounded, 1.5-5 x 2-2.5 cm, 4-angled, warty, with whitish, broad, thick, flat or convex disc and 3-5 slits; seeds many and irregularly elliptical, 2-3 mm long, dull black; many small, sterile seeds. The genus Eucalyptus was described and named in 1788 by the French botanist l'Héritier. The flowers of the various Eucalyptus species are protected by an operculum, hence the generic name, which

comes from the Greek words ‘eu’ (well), and ‘calyptos’ (covered) therefore eucalyptus means well covered (ibid).

2.3.5 Leaves constituents

The herbal substance (dried leaves) contains 1-3.5% volatile oil (Blaschek *et al.* 2007, Wichtl, 2004). The oil contains as a major constituent 1, 8-cineole in an amount of 54-95% (WHO monographs, 2002; Betts, 2000). The oil derived from fresh leaves consists of 45-75% 1, 8-cineole. Other authors stated a 1, 8-cineole content of 70-85% for the volatile oil (Wichtl, 2004). Beside 1, 8-cineole, the oil contains monoterpenes such as cymene, α-pinen, β-pinen and small amounts of myrtenol, pinocarveol, aliphatic aldehyde, flavonoids such as rutin, hyperoside and quercitrin (Blaschek *et al.*, 2007). The concentration of α-terpineol was estimated to be 28% (WHO monographs, 2002). Takasaki *et al*., 1990 isolated 12 compounds with acylphloroglucinol-monoterpene or -sesquiterpene structures, euglobals from the leaves. The herbal substance also contains gallotannins and smaller amounts of procyanidines, triterpenoids (ursolic acid derivates) and flavonoids as well as phloroglucinol derivates such as euglobals and macrocarpals (Wichtl, 2004). The leaves of *Eucalyptus globulus* have smaller amounts of tannins than many other Eucalyptus species (Duke, 1985). Tannin content can depend on the methods of drying leaves (Cork & Krockenberger, 1991).

1, 8-cineole is also known as eucalyptol. Some authors classified eucalyptol as the active ingredient in Eucalyptus oil. Aside from medicinal use, 1, 8-cineole is used as a flavouring agent for lozenges, as a fragrance as well as in cosmetics (Clare, 2010).

2.4 Eucalyptus oil

The essential oil extract from the Eucalyptus leaves which contain compounds with an extremely broad range of biochemical effects as well as odor, flavor and functional properties. Antimicrobial, analgesic and anti-inflammatory properties of E. citriodora, E. globulus and E. teretcorni have been reported from different parts of the world (Ramezani *et al.*, 2002; Silva, 1997). Eucalyptus has a potential for eucalyptus oil because it mainly uses leaves which do not compete with existing usage. Considering that eucalyptus in Ethiopia (particularly, E. globules) is not currently suffering from diseases or insect pests, it has high potential for organic oil (Jaicaf, 2008).

2.5 Uses of Eucalyptus oil

2.5.1 Medicinal and Antiseptic characteristics

The cineole-based oil is used as component in pharmaceutical preparations to relieve the symptoms of influenza and colds, in products like cough sweets, lozenges, ointments and inhalants. Eucalyptus oil has antibacterial effects on pathogenic bacteria in the respiratory tract (Salari *et al.,* 2006). Inhaled eucalyptus oil vapor is a decongestant and treatment for bronchitis. Cineole controls airway mucus hyper secretion and asthma via anti-inflammatory cytokine inhibition (Juergens *et al.,* 2003) (Juergens *et al.,* 2004). Eucalyptus oil also stimulates immune system response by effects on the phagocytic ability of human monocyte derived macrophages (Serafino *et al.,* 2008).

Eucalyptus oil also has anti-inflammatory and analgesic qualities as a topically applied liniment ingredient (Göbel *et al.,* 1994; Hong and Shellock, 1991).

Eucalyptus oil is also used in personal hygiene products for antimicrobial properties in dental care and soaps. It can also be applied to wounds to prevent infection (Nagata *et al.*, 2008).

2.5.2 Repellent and Bio pesticide

Cineole-based eucalyptus oil is used as an insect repellent and bio pesticide. In the U.S., eucalyptus oil was first registered in 1948 as an insecticide and miticide (Flower and Vegetable Oils).

2.5.3 Flavouring

Eucalyptus oil is used in flavouring. Cineole-based eucalyptus oil is used as flavouring at low levels (0.002%) in various products, including baked goods, confectionery, meat products and beverages. Eucalyptus oil has antimicrobial activity against a broad range of foodborne human pathogens and food spoilage microorganisms (Zhao and Agboola, 2007). Non-cineole peppermint gum, strawberry gum and lemon ironbark are also used as flavoring.

2.5.4 Fragrance

Eucalyptus oil is also used as a fragrance component to impart a fresh and clean aroma in soaps, detergents, lotions and perfumes. It is known for its pungent, intoxicating scent (Kabuba, 2009).

2.5.5 Industrial

Research shows that cineole-based eucalyptus oil (5% of mixture) prevents the separation problem with ethanol and petrol fuel blends. Eucalyptus oil also has a respectable octane rating and can be used as a fuel in its own right. However, production costs are currently too high for the oil to be economically viable as a fuel. Phellandrene- and piperitone-based eucalyptus oils have been used in mining to separate sulfide minerals via flotation (Boland *et al.,* 1991).

2.6 Methods of Extraction

The following are the methods of extraction of essential oil and their drawbacks.

2.6.1 Solvent-Extraction:

In the Solvent-Extraction method of Essential Oils recovery, an extracting unit is loaded with perforated trays of essential oil plant material and repeatedly washed with the solvent. A hydrocarbon solvent is used for extraction. All the extractable material from the plant is dissolved in the solvent. This includes highly volatile aroma molecules as well as non-aroma waxes and pigments. The extract is distilled to recover the solvent for future use. The waxy mass that remains is known as the concrete. The concentrated concretes are further processed to remove the waxy materials which dilute the pure essential oil. To prepare the absolute from the concrete, the waxy concrete is warmed and stirred with alcohol (ethanol). During the heating and stirring process the concrete breaks up into minute globules. Since the aroma molecules are more soluble in alcohol than the waxes, an efficient separation of the two results. This is not considered the best method for extraction as the solvents can leave a small amount of residue behind which could cause allergies and effect the immune system (Pandey, 2006-2007).

2.6.2 Maceration:

Maceration actually creates more of “infused oil” rather than an Essential Oil. Plant matter is soaked in vegetable oil, heated and strained which point it can be used for massage. This method is not desirable because it changes the composition of oil (Kumar, 2010).

2.6.3 Cold Pressing:

This method is used to extract the Essential Oils from citrus rinds such as orange, lemon, grapefruit and bergamot. This method involves the simple pressing of the rind at about 120 degrees F to extract the oil. The rinds are separated from the fruit, are ground or chopped and are then pressed. The result is a watery mixture of essential oil and liquid which will separate given time. Little alteration from the oil's original state occurs – these citrus oils retain their bright, fresh, uplifting aromas like that of smelling a wonderfully ripe fruit. The drawback of this method is, oils extracted using this method have a relatively short shelf life (Li Y. *et al*., 2014).

2.6.4 Effleurage:

This is one of the traditional ways of extracting oil from flowers. The process involves layering fat over the flower petals. After the fat has absorbed the essential oils, alcohol is used to separate and extract the oils from the fat. The alcohol is then evaporated and the Essential Oil is collected (Mukhtar *et al.,* 2009).

2.6.5 Super Critical CO_2 Extraction:

Supercritical CO_2 extraction (SCO_2) involves carbon dioxide heated to 87 degrees F and pumped through the plant material at around 8,000 psi, under these conditions; the carbon dioxide is likened to a 'dense fog' or vapor. With release of the pressure in either process, the carbon dioxide escapes in its gaseous form, leaving the Essential Oil behind. The usual method of extraction is through steam distillation. After extraction, the properties of a good quality essential oil should be as close as possible to the "essence" of the original plant. The key to a 'good' essential oil is through low pressure and low temperature processing. High temperatures, rapid processing and the use of solvents alter the molecular structure, will destroy the therapeutic value and alter the fragrance (Singh *et al.,* 2007).

2.6.6 Microwave Extraction

Microwave energy is a superior alternative to several thermal applications owing to its efficient volumetric heat production. The volumetric heating or heating of the bulk as opposed to transferring heat from the surface, inwards, is more efficient, uniform and less prone to overkill or supererogation. Controllability is by far the greatest advantage of microwaves over

conventional thermal technologies. In processing applications, the ability to instantaneously shut the heat source makes enormous difference to the product quality and hence the production economics. The raw material is heated directly by microwaves and this brings about quality consistency and minimizes the impact on the environment as opposed to using fossil fuels or less efficient, indirect electrical heating systems. Specifically in the essential oil extraction, microwave mediated processes are highly desirable due to their small equipment size (portability) and controllability through mild increments of heating. However, so far the microwave technology has found application in very few industrial bio-processing installations due to the lack of available data on microwave interaction with heterogeneous natural raw materials. The sensing and close control of microwave process is a challenging science and there seems to be insufficient literature in this regard (Kabuba, 2009).

2.6.7 Turbo Distillation Extraction:

Turbo distillation is suitable for hard-to-extract or coarse plant material, such as bark, roots, and seeds. In this process, the plants soak in water and steam is circulated through this plant and water mixture. Throughout the entire process, the same water is continually recycled through the plant material. This method allows faster extraction of essential oils from hard-to-extract plant materials (Kumar, 2010).

As cited in Kumar (2010), Steam Distillation is a special type of distillation or a separation process for temperature sensitive materials like oils, resins, hydrocarbons, etc. which are insoluble in water and may decompose at their boiling point. The temperature of the steam must be high enough to vaporize the oil present, yet not so high that it destroys the plants or burns the essential oils. The experiment has been carried out for the extraction of oil from Eucalyptus which has high essential oil content. Such Eucalyptus essential oil, which have been used as perfume and chemical raw materials for a long time, are now been studied as renewable sources of energy.

Anitescu *et al* have studied that ripe fruits of Coriander sativum L. were extracted by steam distillation and by supercritical fluid extraction (SFE), using CO_2 in a two-stage separation system. An inexpensive thermal expansion procedure for supercritical fluid delivery has been developed. The identification of components was performed by gas chromatography and mass

spectrometry (GC±MS). The percentage composition of the 40 identified compounds was compared with the composition of commercial coriander oil extracted by hydro distillation.

Roy Teranishi et al have studied that system combines steam distillation and liquid-liquid extraction to recover volatiles from fats and oils. Oil is pumped in at the top of a spinning-band distillation column, in which the oil is heated to100 ℃ and spread to a thin film. As the oil film drops down to the pot, steam, which is introduced at the bottom, travels upward to strip the volatiles from the oil. The steam distillate is extracted in liquid-liquid extractor incorporated in the system, and the extracted water is recycled as steam. Stripped oil in the pot serves as a liquid seal to force steam up the column. The level of the oil in the pot is maintained automatically by an overflow system. Many liters of oil can be pumped through this system to be stripped of volatiles by steam. The volatiles can be isolated easily from the small amount of solvent recycled in the liquid-liquid extractor.

Referring to the above literature review, it was found that Steam Distillation method is an appropriate and economical method for extraction of Essential Oil.

2.6.8 Extraction of Essential Oils Using Steam distillation Method:

Steam distillation is a special type of distillation or a separation process for temperature sensitive materials like oils, resins, hydrocarbons, etc. which are insoluble in water and may decompose at their boiling point. The fundamental nature of steam distillation is that it enables a compound or mixture of compounds to be distilled at a temperature substantially below that of the boiling point(s) of the individual constituent(s). Essential oils contain substances with boiling points up to 200℃or higher temperatures. In the presence of steam or boiling water, however, these substances are volatilized at a temperature close to $100°C$, at atmospheric pressure.

Fresh, or sometimes dried, botanical material is placed in the plant chamber of the still and the steam is allows to pass through the herb material under pressure which softens the cells and allows the Essential Oil to escape in vapor form. The temperature of the steam must be high enough to vaporize the oil present, yet not so high that it destroys the plants or burns the Essential Oils. Besides the steam tiny droplets of Essential Oil evaporates and travel through a tube into the still's condensation chamber. Here Essential Oil vapors condense with the steam. The essential oil forms a film on the surface of the water. To separate the Essential Oil from the water, the film is then decanted or skimmed off the top. The remaining water, a byproduct of

distillation, is called floral water, distillate, or hydrosol. It retains many of the therapeutic properties of the plant, making it valuable in skin care for facial mists and toners (A solution containing chemicals that can change the color of a photographic print). In certain situations, floral water may be preferable to be pure essential oil, such as when treating a sensitive individual or a child, or when a more diluted treatment is required. Rose hydrosol, for example, is commonly used for its mild antiseptic and soothing properties, as well as its pleasing floral aroma (Kumar, 2010).

A number of factors determine the final quality of a steam distilled essential oil. Apart from the plant material, most important are time, temperature and pressure, and the quality of the distillation equipment. Essential oils are very complex products. Each is made up of many, sometimes hundreds, of distinct molecules which come together to form the oil's aroma and therapeutic properties. Some of these molecules are fairly delicate structures which can be altered or destroyed by adverse environmental conditions. So, much like a fine meal is more flavorful when made with patience, most oils benefit from a long, slow 'cooking' process (http://www.bellevuemassagetherapy.com/methods-of-extracting-essential-oils.html).

It is possible that longer distillation times may give more complete oil. It is also possible however, that longer distillation time may lead to the accumulation of more artifacts than normal. This may have a curious effect of appearing to improving the odor, as sometimes when materials that have a larger number of components are sniffed, the perception is often of slightly increased sophistication, added fullness and character, and possibly, and extra pleasantness (Kumar,2010).

2.6.8.1 Advantages of using Steam Distillation:

The advantage of Steam Distillation is that it is a relatively cheap process to operate at a basic level, and the properties of oils produced by this method are not altered. As steam reduces the boiling point of a particular component of the oil, it never decomposes in this method. This method apart from being economical is also relatively faster than other method (Kumar, 2010).

2.6.9. Methods for Distillation

Three are three types of distillation for isolating essential oils from plant materials:

A. Water distillation

B. Water and steam distillation

C. Direct steam distillation

A. 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 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 is 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. For this reason only, water distillation possesses one distinct advantage, i.e. that it permits 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.

Disadvantages of Water Distillation

• Oil components like esters are sensitive to hydrolysis while others like acyclic monoterpene hydrocarbons and aldehydes are susceptible to polymerization (since the pH of water is often reduced during distillation, hydrolytic reactions are facilitated).

• Oxygenated components such as phenols have a tendency to dissolve in the still water, so their complete removal by distillation is not possible.
• As water distillation tends to be a small operation (operated by one or two persons), it takes a long time to accumulate much oil, so good quality oil is often mixed with bad quality oil.
• The distillation process is treated as an art by local distillers, who rarely try to optimize both oil yield and quality.
• Water distillation is a slower process than either water and steam distillation or direct steam distillation.

B. Water and Steam Distillation

In water and steam distillation, the steam can be generated either in a satellite boiler or within the still, although separated from the plant material. Like water distillation, water and steam distillation is widely used in rural areas. Moreover, it does not require a great deal more capital expenditure than water distillation. Also, the equipment used is generally similar to that used in water distillation, but the plant material is supported above the boiling water on a perforated grid. In fact, it is common that persons performing water distillation eventually progress to water and steam distillation.
It follows that once rural distillers have produced a few batches of oil by water distillation, they realize that the quality of oil is not very good because of its still notes (subdued aroma). As a

result, some modifications are made. Using the same still, a perforated grid or plate is fashioned so that the plant material is raised above the water. This reduces the capacity of the still but affords a better quality of oil. If the amount of water is not sufficient to allow the completion of distillation, a cohobation tube is attached and condensate water is added back to the still manually, thereby ensuring that the water, which is being used as the steam source, will never run out. It is also believed that this will, to some extent, control the loss of dissolved oxygenated constituents in the condensate water because the re-used condensate water will allow it to become saturated with dissolved constituents, after which more oil will dissolve in it.

Advantages of Water and Steam Distillation over Water Distillation

• Higher oil yield.
• Components of the volatile oil are less susceptible to hydrolysis and polymerization (the control of wetness on the bottom of the still affects hydrolysis, whereas the thermal conductivity of the still walls affects polymerization).
• If refluxing is controlled, then the loss of polar compounds is minimized.
• Oil quality produced by steam and water distillation is more reproducible.
• Steam and water distillation is faster than water distillation, so it is more energy efficient.
Many oils are currently produced by steam and water distillation, for example lemongrass is produced in Bhutan with a rural steam and water distillation system.

Disadvantages of Water and Steam Distillation

• Due to the low pressure of rising steam, oils of high-boiling range require a greater quantity of steam for vaporization - hence longer hours of distillation.
• The plant material becomes wet, which slows down distillation as the steam has to vaporize the water to allow it to condense further up the still.
• To avoid that the lower plant material resting on the grid becomes waterlogged, a baffle is used to prevent the water from boiling too vigorously and coming in direct contact with the plant material.

C. Direct Steam Distillation

As the name suggests, direct steam distillation is the process of distilling plant material with steam generated outside the still in a satellite steam generator generally referred to as a boiler. As in water and steam distillation, the plant material is supported on a perforated grid above the steam inlet. A real advantage of satellite steam generation is that the amount of steam can be readily controlled. Because steam is generated in a satellite boiler, the plant material is heated no higher than 100 ℃ and, consequently, it should not undergo thermal degradation. Steam distillation is the most widely accepted process for the production of essential oils on large scale. Throughout the flavor and fragrance supply business, it is a standard practice.

An obvious drawback to steam distillation is the much higher capital expenditure needed to build such a facility. In some situations, such as the large-scale production of low-cost oils (e.g. rosemary, Chinese cedarwood, lemongrass, litsea cubeba, spike lavender, eucalyptus, citronella, cornmint), the world market prices of the oils are barely high enough to justify their production by steam distillation without amortizing the capital expenditure required to build the facility over a period of 10 years or more.

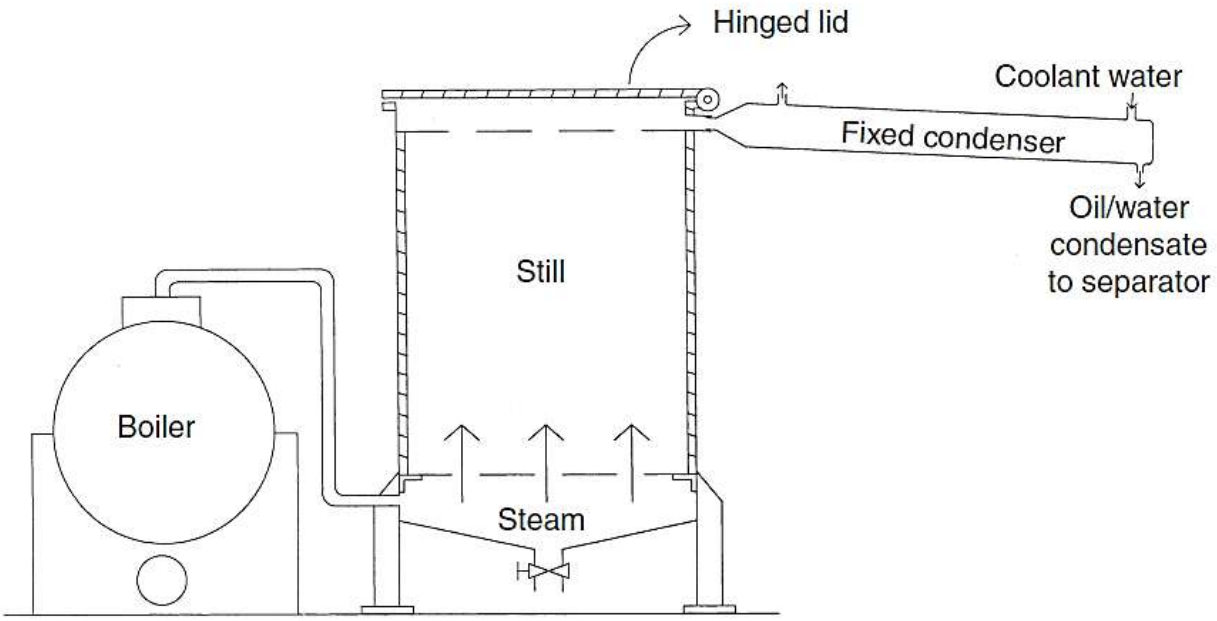


Figure 2.1 Steam distillation process with a separate boiler for extraction of Eucalyptus globulus Essential oil (Coppen J. J, 2003).

Advantages of Direct Steam Distillation

• Amount of steam can be readily controlled.

• No thermal decomposition of oil constituents.

• Most widely accepted process for large-scale oil production, superior to the other two processes.

Disadvantage of Direct Steam Distillation

• Much higher capital expenditure needed to establish this activity than for the other two processes.

2.7 Important Physical and Chemical properties of Essential Oils

The chemical properties of essential oils depend on the natural factors such as type of species, the geographical origin and location of the plant, time of harvesting, plant parts from which the oils are extracted, etc. (Dey, 1996).

Essential oils components and percentage are different from oil to oil even for the same botanic plant due to:

- ✓ **Weather and planting time**

Most of herbs are planted but small amounts could also be wild grown or collected plants. By means of an example with spearmint, the oil percentage from a summer crop is double that from a winter crop. The oil percentage from a given summer could be different from a previous summer even from the same field. The component analysis of the oil could also be different from one season to another.

- ✓ **Soil elements**

The B-phellanderene percentage increases in marjoram oil with the higher levels of molybdenum manganese, copper, calcium, zinc or iron in the soil.

- ✓ **Irrigation**

The highest yield of plant material results from increasing the leaf area. For example, this will happen if a basil field is irrigated every 4 days. The essential oil is highest at medium levels of soil moisture.

- ✓ **Time of harvest**

The peppermint oil yield increases as the herb approaches maturity in the full bloom stage.

2.7.1 Physical properties

- ✓ **Specific gravity**

Specific gravity is an important criterion of the quality and purity of an essential oil. Values for essential oils vary between the limits of 0.696 and 1.188 at 15 °C, in general, the specific gravity is less than 1.000 (Guenther, 1960). Hence essential oil can be collected over (floating on) water.

- ✓ **Optical rotation**

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

- ✓ **Refractive index**

When a ray of light passes from a less dense to a more dense medium, it is bent or "refracted" toward the normal. Refract meters offer a rapid and convenient method for the determination of this physical constant.

- ✓ **Molecular refraction**

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

- ✓ **Solubility**
  - **Solubility in Alcohol**

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

- **Solubility in water**

Most of essential oils of commercial interest are steam volatile, reasonably stable to action of heat and practically insoluble in water and hence suitable for processing by steam distillation.

- ✓ **Boiling range**

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

- ✓ **Evaporation residue**

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

- ✓ **Flash point**

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

2.8 Chemical constituents of essential oils

Essential oil components are divided into terpenoids and non-terpenoids.

i. **Non-terpenoids**: This group contains short-chain aliphatic substances, aromatic substances, nitrogenated substances, and substances with sulphur. They are less important than terpenoids in terms of uses and applications.

ii. **Terpenoids:** These are more important commercially and in terms of their properties. Terpenes derive from isoprene units (C_5) bonded in a chain. Terpenes are a type of chemical substance found in essential oils, resins, and other aromatic plant substances, (pines, citrus fruits…). They are usually found in monoterpene oils (C_{15}) and diterpenes (C_{20}). They may be aliphatic, cyclic, or aromatic.

According to their function group they can be:

- ✓ Alcohols (menthol, bisabolol) and phenols (timol, carvacrol)
- ✓ Aldehydes (geranial, citral) and cetones (camphor, thuyone)

- ✓ Esthers (bornile acetate, linalile acetate, methyl salicilate, anti-inflammatory compound similar to aspirin)
- ✓ Ethers (1.8 - cineol) and peroxides (ascaridol)
- ✓ Hydrocarbons (limonene, pinene α and β)

a. Monoterpenic hydrocarbons

These are the commonest compounds in essential oils, and precursors of the more complex oxidised terpenes. Their names end in –ene. Limonene, for example, is the precursor to the main components of mint essences (*Mentha* spp, Lamiaceae Family) such as carvone and menthol. Limonene is also found in citric plants and in dill (*Anethum graveolens*, Apiaceae family). Pinene α and β are also widely present in nature, especially in trementine essence of the *Pinus* genre (Pinaceae family).

b. Alcohols

Alcohols have the hydroxile group (OH) bonded to a C_{10} skeleton. Their names end in –ol. They are highly sought after for their aroma.

Linalool, for example, has two forms. R-linalool is found in roses and lavender and is the main component of *Mentha arvensis*. S-linalool found in lavender oil at > 5% indicates adulteration. Linalool gives tea, thyme, and cardamom leaves their taste. Menthol, another compound found in this group, is responsible for the smell and taste of mint. Mint essence may contain up to 50% of this component.

Geraniol, from scented geraniums (Pelargonium spp), citronelol, from roses (Rosa gallica), borneol from rosemary, and santalol from sandalwood (Santalum album, Santalaceae family).

c. Aldehydes

Aldehydes are highly reactive compounds. Their names end in –al. Many of them, such as those found in citrus fruits, match their respective alcohol. For example: geraniol – geranial, and citronelol – citronelal. They are found in abundance in citrus plants, and are responsible for their characteristic smell, particularly the isomers geranial (α citral) and neral (β citral) known as citral in combination. In addition to its characteristic aroma, citral has anti-viral, antimicrobiotic, and sedative properties. But many aldehydes, including citral, cause irritation to the skin and cannot

be used externally. Another important group is the aromatic aldehydes, such as benzaldehyde, main ingredient of bitter almond oil and cause of their typical aroma.

d. Phenols

They are only found in a few species but are very powerful and irritating. The most important are timol and carvacrol, which are found in thyme (Thymus) and oregano (Origanus), both of the Labiatae family. Another important phenol is eugenol, which is found in many species, for example, clove essence. It is both a powerful bactericide and also anaesthetic, and is used in dentistry.

e. Phenolic Ethers

These are the main components of species such as celery and parsley (apiol), aniseed (anetol), basil (metilchavicol), and estragon (estragol). Safrol is a component which is used extensively in the perfume industry and is found in the bark of the sassafras tree (Sassafras albidum Lauraceae family).

f. Ketones

These are produced by the oxidisation of alcohols and are fairly stable molecules. They end in –one. Carvone is found in Mentha spicata. Tuyone -first isolated in Tuya- (Thuja occidentalis Cupressaceae family) and pulegone are fairly toxic and should never be used during pregnancy.

Tuyone is found in plants of the Artemisia genus (Artemisia absinthium with which absinthe and vermouth are made), and in salvia (Salvia officinalis). Pulegona was first isolated in Mentha pulegium.

g. Ethers

Ethers or monoterpenic oxides are reactive and unstable. One example is bisabolol oxide found in camomile (Matricaria chamomilla). Another common ether is 1.8 –cineol (also known as eucaliptol), which is the main component of eucalyptus oil. It is an expectorant and mucolitic, and the main component of cough medicines. The aroma of eucalyptus oil varies depending on

1.8 –cineol content: the oil with a high content (Eucalptus globulus) is used for medicinal purposes, whereas that with a lower content (Eucaliptus radiata) is used in aromatherapy.

h. Esthers

Most esthers are formed from a reaction of a terpenic alcohol with an acetic acid. Their aroma is characteristic of the oils in which they are found. Lavender oil, for example, contains linalool in its Esther, linalile acetate. The relative abundance of both these components is a sign of good quality. Methyl salicilate, a derivate of salicylic acid and methanol, is an anti-inflammatory compound similar to aspirin and is found in a certain type of heather (Gaultheria procumbens Ericaceae family). It is used externally in liniments (Onyinyechi, 2012).

2.9 Parameters Affecting Yield and Quality of Essential Oils

The yield and quality of essential oil from steam distillation is affected by the various process parameters. It is advisable to keep them in mind while designing such systems. Some of the important parameters are being listed below.

2.9.1 Mode of Distillation

The technique for distillation should be chosen considering the boiling point of the essential oil and the nature of the herb, as the heat content and temperature of steam can alter the distillation characteristics. For high boiling oils such as woody oils (e.g. sandalwood, cedar wood) and roots (e.g. Cyperus), the oil should be extracted using boiler-operated steam distillation. Since the heat content and temperature of steam depend upon its pressure, a change in steam pressure can alter the distillation characteristics. High-boiling constituents of essential oils normally require high pressure steam to distill over. For oil of rose and other florals, the material is generally immersed in water, i.e. Hydrodistillation, as flowers tend to aggregate and form lumps which cannot be distilled using water and steam distillation or direct steam distillation.

2.9.2 Improper Design of Equipment

Improper designing of tank, condenser or separators can lead to loss of oil and high capital investments. The design of the furnace and chimney affects the firing and heat control of the distillation rates. Tank height: diameter ratio is important. Similarly the use of a condenser with

an improper design and without calculating the heat transfer areas based on the steam generation areas will lead to improper condensation and loss of oil.

2.9.3 Material of Fabrication of Equipment

Essential oils which are corrosive in nature should be preferably distilled in stills made of resistant materials like aluminum, copper or stainless steel. The tank still can be made from a cheaper metal like mild steel or galvanized iron, and the condenser and separator can be made from a resistant material like stainless steel. As only vapor is present in the tank still, the rust and other products of corrosion may not be carried over into the oil. This can result in considerable savings in the capital cost of the equipment. Expensive, high-value essential oils like rose, agarwood, kewda, sandalwood and lavender should be distilled in stainless steel systems.

Although copper was the most common material of fabrication of distillation stills since ancient times, its availability is getting reduced and with the arrival of superior alloys like stainless steel, it is slowly disappearing from the scene.

2.9.4 Condition of Raw Material

The condition of the raw material is important because some materials like roots and seeds will not yield essential oil easily if distilled in their natural state. These materials have to be crushed, powdered or soaked in water to expose their oil cells. Chopping of plants will also change the packing density of the material when placed in the distillation still. One can pack up to 50% more plant material in the same still after chopping of some aromatic herbs like mint. Air drying and wilting the herb prior to distillation also has considerable effect on distillation. If required, drying of the herbs prior to distillation should be done in shaded areas and the dried material should not be kept in heaps.

2.9.5 Time for Distillation

Different constituents of the essential oil get distilled in the order of their boiling points. Thus, the highest boiling fractions will be last to come over when, generally, very little oil is distilling. If the distillation is terminated too soon, the high-boiling constituents will be lost. In many aromatic plants, like vetiver, patchouli, chamomile, sandalwood and agarwood, these high-

boiling fractions are valuable due to the quality of their aromas. Thus, the time of distillation must be chosen with due care.

2.9.6 Loading of Raw Material and Steam Distribution

Improper loading of the herb may result in steam channeling, causing incomplete distillation. The herb should be evenly and uniformly loaded in the tank without leaving any voids. Excessive filling of plant material may also lead to formation of “rat holes” which may allow steam to escape without vaporizing the oil. For powdered herbs, a proper stainless steel wire mesh or muslin cloth should be put at the false bottom to prevent plant material from falling into the tank base.

2.9.7 Operating Parameters

Proper control of injection rates and pressure in boiler-operated units is necessary to optimize the temperature of extraction for maximal yield. Generally, high-pressure steam is not advisable for the distillation of essential oils. The temperature of the condensate should not be high, as it can result in oil loss due to evaporation. In directly fired-type FDUs, the firing of the furnace should be well controlled as it can result in high flow rates and high condensate temperatures.

2.9.8 Condition of Tank and Equipment

The tank and other equipment should not be rusted. If rusted, the tank should be cleaned with dilute caustic solutions. The perforated grids should not be corroded or have large gaps permitting the plant material to settle to the bottom of the tank and emit a burnt odor. The distillation tanks should be well steamed prior to distillation for multiple crop distillation (Kabuba, 2009).

2.9.9 Particle Size of the raw material loading to the chamber

The size of the leaves has its own contribution on the yield of the extract oil. The particle size should be optimum in order to steam is distributed properly trough the chamber.

3. MATERIALS AND METHODS

The experimental work was done in the laboratory of Addis Ababa University Institute of Technology, School of Chemical and Bio Engineering.

3.1 Material and Equipment

The materials and equipment needed for the experiment work were plastic bag, eucalyptus leaves, crucibles, oven, cross beater mill, sieve, electronic balance, steam distillation set up, flask, separating funnel, black bottle, Anhydrous sodium sulfate, filter, heater, thermometer, pH meter, viscometer, Pycnometer, spectrometer, Polari meter and Gc-Ms.

On the other hand chemicals needed for characterization of eucalyptus oil were, Potassium hydroxide, ethanol, sodium hydroxide, phenolphthalein, oxalic acid, chloroform, iodine bromide solution, potassium iodide and sodium thiosulphate.

3.1.1. Raw Material preparation

Eucalyptus leaves were collect from Entoto forest. The family of eucalyptus was Myrtaceae and the botanical name eucalyptus E. globulus. The authenticity of variety was verified in Addis Ababa University Department of Biology and biodiversity management (Arat kilo).The impurities were removed. The eucalyptus leaves were collected as fresh leaves then partial sun drying was used to dry the leaves.

3.2. Sample Analysis

3.2.1. Moisture Content Determination

35.48 g, 43.4 g, and 46.49 g of the eucalyptus leaves was weighed and dried in an oven at 103℃and the weight was measured every 2 hrs. The procedure was done repeatedly until a constant weight was obtained. The percentage moisture in the leaves was calculated using the following formula:

$$\text{Moisture } \% = \frac{w_1 - w_2}{w_1} x100 \qquad (3.1)$$

Where: W_1 is original weight of the sample before drying; and W_2 is Weight of the sample after drying.

3.2.2. Size reduction and Sieve analysis of the leaves

The moisture was removed by partial sun drying. The dried Eucalyptus leaves were crushed in cross beater mill with sieve size of 10 mm and by cutting mill. The sample was sieved using set of sieves sizes arranged in descending order 5 mm, 8 mm, 12.5 mm and 20 mm to obtain particular sizes of 5- 8 mm, 8- 12.5 mm, and 12.5 -20 mm. This was aimed to investigate the effect of particles size on yield.

Figure 3.1 Cutting mill and Cross beater mill

Figure 3.2 Eucalyptus (globulus) leaves before size reduction and after size reduction

3.3 Extraction method of Eucalyptus oil

The extraction of eucalyptus oil was conducted using steam distillation method with different extraction time and with different particle size ranges at constant temperature of 94.5℃ and atmospheric pressure of 1atm. For the current experimental work, one kg of eucalyptus leaves was used at three different extraction time intervals; 1 hour, 2 hour and 3 hour with different particle size ranges of 5-8 mm, 8-12.5 mm and 12.5-20 mm with three replications to get maximum yield. Before the operation started the column was cleaned then the eucalyptus leaves was added to the column (extraction chamber). After feeding the raw material in to the column and opening the water valve of condenser, the boiler was made to generate steam and let to enter into the column to extract the required yield i.e. eucalyptus oil. Finally collection of the condensate mixture and then separation of the required oil was done.

The experimental work was carried out by the following steam distillation set up shown in the figure 3.3.The steam distillation set up shown below has three separate components namely boiler, extraction chamber and condenser.

Fig.3.3. steam distillation set up of school of Chemical and Bio-Engineering

3.3.1. Components of the extraction plant are:

I. Boiler

Generate steam by boiling water which can facilitate the extraction process by entering to the extraction chamber. For this experimental work the boiler was operates at atmospheric pressure and constant temperature.

II. Extraction chamber

This served primary as a container and as a vessel in which the steam contacts the plant material and vaporize its oils. The plant material was pack in to the extraction chamber so that distillation commence. Proper charging was very important otherwise the steam channels through the plant material and low yield results. The first load was contact to the set-up and establishes the procedure determines processing parameters.

III. Condenser

A coil flow condenser was used to convert all the steam and the accompanying oil vapors from the top of the extraction chamber in to liquid. Water was feed to the overhead reservoir and this permitted the water to trickle over the entire length of the condenser tubes. It was noted that the condenser tubes all sloped down ward slightly, to ensure proper drainage of the condenser oil and steam. The cooling medium used in this device was cooling water drawn from a running tap.

3.3.2 Separator

Essential oil extracts and water condensate were known to have different densities and also from an immiscible two liquid phases mixture at low room temperature conditions. The separation of essential oils from the condensation hence utilizes the density and immiscibility advantage for the two be isolated each other. This phenomena was the oil extract float on the water layer due to being dense than water. In separation the water from the oil, the water layer is carefully run out from the bottom of decanter by opening the tap until its meniscus was just at the calibration mark. The contents that remained inside the decanter are the oil layer and the water between the tap bridge and the bottom of the calibration mark.

Fig.3.4 shows separation process carried out in the experimental work done to separate eucalyptus oil and hydrosol based on their density difference.

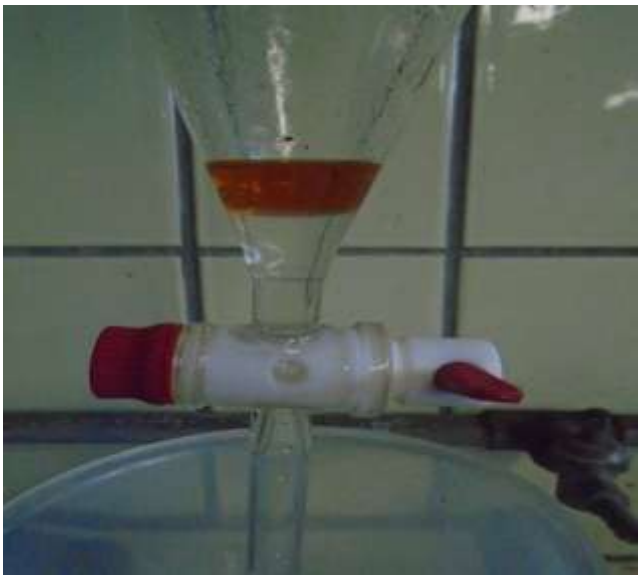


Fig.3.4 separating process of essential oil and water (hydrosol) mixture

3.4 Determination of the yield of eucalyptus oil

$$\% \text{ age yield of oil} = \frac{\text{Weight of oil x100}}{\text{Weight of Eucalyptus leaves}} \qquad (3.2)$$

3.5 characterization of eucalyptus oil

3.5.1 Physicochemical properties of eucalyptus oil

Physical characterization is determining the physical properties of the extracted oil. Some of these properties are specific gravity, refractive index, flash point, evaporation residue etc... Chemical properties of eucalyptus oil are such as acid value, saponification value, iodine number and composition of the oil that can be identify by Gc-Ms. Physicochemical properties were used to determine the quality of EGEO extracted. All the parameters are determined according to the method of European Pharmacopeia (European Pharmacopoeia Commission, 2001).

- **Determination of pH Value**

2 g of the eucalyptus oil was poured into a clean dry 25ml beaker next 13ml of hot distilled water was added in to the beaker and stirred slowly. It was then cooled in a cold-water bath to 25℃.

The pH electrode was standardized with buffer solution then the electrode was immersed into the sample finally the pH value was read and record.

- **Specific gravity determination**

A tube (Pycnometer) of known weight (W) filled was first with essential oil and then with water and the respectively weight w_1 and w_2 was determined. Then, the specific gravity was calculated using the following formula:

$$\text{Sp. gr} = \frac{w_1 - w}{w_2 - w} \qquad (3.3)$$

- **Determination of Refractive Index**

The Refractive Index of the Eucalyptus oil was measured by Abbe Refractometer at 25℃ in Adiss Ababa University (Arat kilo) Department of Physics laboratory. First the cuvet was cleaned next filled with eucalyptus oil then put on the prism for refractive index determination. The set up shows in the following figure and the value is calculated as:

$$N = \tan(\theta b) \qquad (3.4)$$

Where: N is refractive index and θb is angle of rotation.

Figure 3.5 Refractive index measurement

- **Determination of optical rotation**

The optical rotation of the eucalyptus globulus essential (EGEO) was measured using Polari meter with a 2 dm length Polari meter tube where the angle of rotation in degrees are read at 25 ℃ using D-line of polarized sodium light. The specific optical rotation was measure in Adiss Ababa University (Arat Kilo) in Department of Physics laboratory by the set up shows below in the figure.

Figure 3.6 Optical rotation measurement

- **Determination of viscosity of the eucalyptus oil**

35m1 of eucalyptus oil was poured into a test tube and a viscometer was used to measure the viscosity at a temperature of 19℃.

Figure 3.7 Shows measurement of dynamic viscosity of eucalyptus oil using viscometer.

Figure 3.7 Viscosity measurements by viscometer

- **Determination of boiling temperature of the eucalyptus oil**

25 ml of eucalyptus oil was poured in to Borosilicate glass and a thermometer was inserted and placed on a heating mantle or heater, it was observed that the oil in the Borosilicate started

circulating leading to boiling of oil and read temperature on thermometer then recorded. The measurement carried out is shown in Figure 3.8.

Figure 3.8 Boiling point measurement of eucalyptus oil

- **Evaporation residue of eucalyptus oil**

37 grams of eucalyptus oil was poured into Borosilicate glass, put it on heater then thermometer was inserted into the Borosilicate glass until it reads temperature of 100 c^0 to determine volatile matter.

$$\text{Percentage of the oil} = \frac{W_2}{W_1} \text{x } 100\% \qquad (3.5)$$

Where:

W_1 is weight before evaporation and W_2 is weight after evaporation

- **Flash point of eucalyptus oil**

The flash point of eucalyptus oil was measure by adding 25 milliliter of sample in to Borosilicate glass and then put it on heater next thermometer was immersed in to the glass containing the sample to read the minimum temperature of first flame. Finally the temperature was read and then recorded.

- **Solubility of eucalyptus oil**

Solubility of eucalyptus oil has been seen by adding 2 grams of sample into 10 milliliter of alcohol and water.

- **Acid value determinations**

(2g) of eucalyptus oil was accurately weighted and dissolved in 10 ml of 95 % ethanol and 2-3 drops of phenolphthalein indicator was added. The free acid was then titrated with standard 0.1 Normality of aqueous sodium hydroxide solutions by adding the alkali drop-wise at a uniform rate of about 30 drops per minute. The content of the flask was continuously agitated. The primary manifestation of the red coloration that did not fade within 10 seconds was considered the end point. Afterward, the acid value is determined using the following equations (Boukhatem *et al*., 2014) :

$$\text{Acid value} = \frac{5.61 \text{ x (number of mL of 0.1 N NaOH)}}{\text{Weight of sample in gram}} \qquad (3.5)$$

Where N is Normality

- **Saponification value determination**

EGEO (1g) was accurately weighed and dissolved in 10 ml of ethanol and then 10 ml of 2.5 Normality KOH solutions was added.

This procedure was performed together with blank experiment which was also performed omitting the oil. The mixture was refluxed for two hours then cooled. The unreacted KOH was titrated with standard 0.5 Normality of oxalic acid by adding 2-3 drops of phenolphthalein indicator until became colorless. After that, the saponification value was determined using the following equation:

$$\text{Saponification value} = \frac{56\ (v_1\ -\ v_2)}{2 \text{ x W}} \qquad (3.6)$$

Where W is the weight of oil, V_1 is the volume of 0.5 Normality of oxalic acid for blank; V_2 is the volume of 0.5 Normality of oxalic acid for sample (Boukhatem *et al*., 2014).

- **Iodine number determination**

EGEO (0.1g) was dissolved in 10 ml of chloroform. Then 25 ml of iodobromide solution was added and allowed to stand for 30 minutes in dark. Again 30 ml of 1 N potassium iodide and 100 ml of distilled water were added and the liberated iodine was titrated with 0.1 normality solution

of sodium thiosulphate with constant shaking. When iodine color became quite pale, 1 ml of 1 % starch solution was added and the titration was continued until the blue color was discharged. A blank test was also carried out parallel under identical condition. The iodine number was determined using the formula:

$$\text{Iodine number} = 1.269 \frac{(V_1 - V_2)}{W} \quad (3.7)$$

Where:

W is the weight of sample,

V_1 is the number of ml of thiosulphate consumed by the blank; and

V_2 is the number of ml of thiosulphate consumed by the test sample (Boukhatem, *et al., 2014).*

3.5.2 Chemical characterization of eucalyptus oil

Determination of the chemical composition of the extracted EO from Eucalyptus globulus was carried out by Gas Chromatography-Mass Spectroscopy. Gas Chromatographic (GC) analysis were performed with a Hewlett Packard 6890 Series equipped with a HPChemstation data processor, fitted with a HP-5MS capillary column (30 m x 0.25 mm i.d., 0.25 μm film thickness (Hewlett Packard, Palo Alto, USA); column temperature, 45 ℃ (8 min) to 230 ℃ at 2 ℃/min, 180℃, 230 ℃ (15 min); injector temperature 250℃; detector temperature 250℃; split ratio 1:20; carrier gas N_2. Volume injected 1 μL. The Gas Chromatography-Mass Spectrometry (GC MS) analysis were performed in a HP 7820 GC using a mass selective detector Hewlett Packard 6890/MSD5977, equipped with HP Chemstation software and Wiley 275 spectra data. A fused silica capillary column HP-5MS (30 m x 0.25 mm), 0.25 μm film thickness (Hewlett Packard, Palo Alto, USA) was used. The temperature program was the same used in the gas chromatography (GC) analysis: interface 280℃; split ratio 1:20; carrier gas He; flow rate: 1.0 mL/min; ionization energy 70eV; volume injected 1 μL.

The detected compounds were identified by processing the raw GC-MS data and comparing with National Institute of Standard and Technology (NIST, USA) mass spectral database and from retention times and mass spectra of standard compounds. Relative amounts of detected compounds were calculated based on GC peak areas (Akolade, Jubril Olayinka *et al., 2012).*

3.6 Design of the Experiment

Data analysis has performed by design-expert 7.0 software using general factorial design method. For steam extraction there were two factors; time and particle size with three levels and three replications each. This design of the experiment helps us to differentiate the significance of the main and the interaction factors. This program software also used to develop the mathematical model that will describe the effects of the main and interaction factors on the response.

4. RESULT AND DISCUSSION

4.1 Determination of Moisture Contents

The fresh leaves was collected on November, 2015 after drying by taking 35.48g, 43.4g and 46.49g the moisture content of the sample was obtained in the following table.

Table 4.1 Moisture content determination of the dry eucalyptus leaves

	Time for drying							Moisture Content (%)
	0	2	4	6	8	10	12	
Sample Weight (g)	35.48	35.07	35.04	35.02	34.97	34.95	34.95	1.493
	43.4	42.98	42.97	42.94	42.93	42.92	42.92	1.105
	46.49	45.92	45.88	45.87	45.83	45.82	45.82	1.441

The moisture content of the leaves of 35.48, 43.4 and 46.49 grams was 1.493, 1.105 and 1.441 % respectively. The average moisture content of the three samples will be 1.3463 %.

4.2 Percent yield

Table 4.2 shows that the run order, extraction time, particle size and yield found. The maximum extraction of eucalyptus oil was 1.19% at particle size ranges from 5-8mm for the extraction time of 3 hours and the minimum yield obtained was at maximum particle size and minimum extraction time. Balascheck et al (2007) was also found a yield with range of 1-3.5%. The result of the current yield was calculated by Eq 3.2.

Table 4.2. Experimental results of eucalyptus oil yield extracted using steam distillation

Std	Run	Block	Factor 1 A : Time	Factor 2 B: particle size	Response 1 Yield %
27	1	Block 1	3	12.5-20	0.59
17	2	Block 1	3	8-12.5	1.13
8	3	Block 1	3	5-8	0.51
6	4	Block 1	2	5-8	1.07
21	5	Block 1	1	12.5-20	0.23
19	6	Block 1	1	12.5-20	0.05
4	7	Block 1	2	5-8	0.78
1	8	Block 1	1	5-8	0.24
10	9	Block 1	1	8-12.5	0.57
22	10	Block 1	2	12.5-20	0.37
18	11	Block 1	3	8-12.5	0.86
11	12	Block 1	1	8-12.5	0.32
20	13	Block 1	1	12.5-20	0.22
13	14	Block 1	2	8-12.5	0.8
23	15	Block 1	2	12.5-20	0.12
7	16	Block 1	3	5-8	1.01
26	17	Block 1	3	12.5-20	0.25
3	18	Block 1	1	5-8	0.95
15	19	Block 1	2	8-12.5	0.61
9	20	Block 1	3	5-8	1.19
16	21	Block 1	3	8-12.5	0.85
25	22	Block 1	3	12.5-20	0.26
14	23	Block 1	2	8-12.5	0.6
24	24	Block 1	2	12.5-20	0.18
2	25	Block 1	1	5-8	0.9
12	26	Block 1	1	8-12.5	0.31
5	27	Block 1	2	5-8	0.85

4.3 Statical Analysis

Table 4.3: Analysis of variance for eucalyptus oil extraction using steam distillation

Sourse	Sum of squares	df	Mean square	F-value	p-value pro>F
Model	2.08	4	0.52	11.46	< 0.0001*
A-time	*0.46*	*2*	*0.23*	*5.04*	*0.0158*
B-particle size	*1.62*	*2*	*0.81*	*17.88*	*< 0.0001*
Residual	1.00	22	0.045		
Lack of Fit	*0.14*	*4*	*0.035*	*0.73*	*0.5805***
Pure Error	*0.86*	*18*	*0.048*		
Cor Total	3.07	26			
*significant			** not significant		

The Model F-value of 11.46 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The "Lack of Fit F-value" of 0.73 implies the Lack of Fit is not significant relative to the pure error. There is a 58.05% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

Table 4.4: Model adequacy measures

Std. Dev.	0.21	R-Squared	0.6757
Mean	0.59	Adj R-Squared	0.6167
C.V. %	36.32	Pred R-Squared	0.5115
PRESS	1.50	Adeq Precision	9.814

Coefficent of varation, the standard deviation expressed as a percentage of the mean; predicted Redual Error sum of squares, which is the a measure of how the model fits each piont in the design; the R- squared, measure of the amount of varian around the mean explained by the model; Adj R- squared, a measure of the amount of variation in new data explained by the model, and Adquate precision, this is a signal to disterbance ratio due to random error, presented in Table 4.4, below, are used to decide whether the model can be used or not. The "Pred R-Squared" of 0.5115 is in reasonable agreement with the "Adj R-Squared" of 0.6167. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Here the ratio of 9.814 indicates an adequate signal. This model can be used to navigate the design space.

Final Equation in Terms of Coded Factors:

$$\text{yield} = 0.59 - 0.16\,A[1] + 0.012 * A[2] + 0.25 * B[1] + 0.086 * B[2] \qquad (4.1)$$

Diagonostic Plots

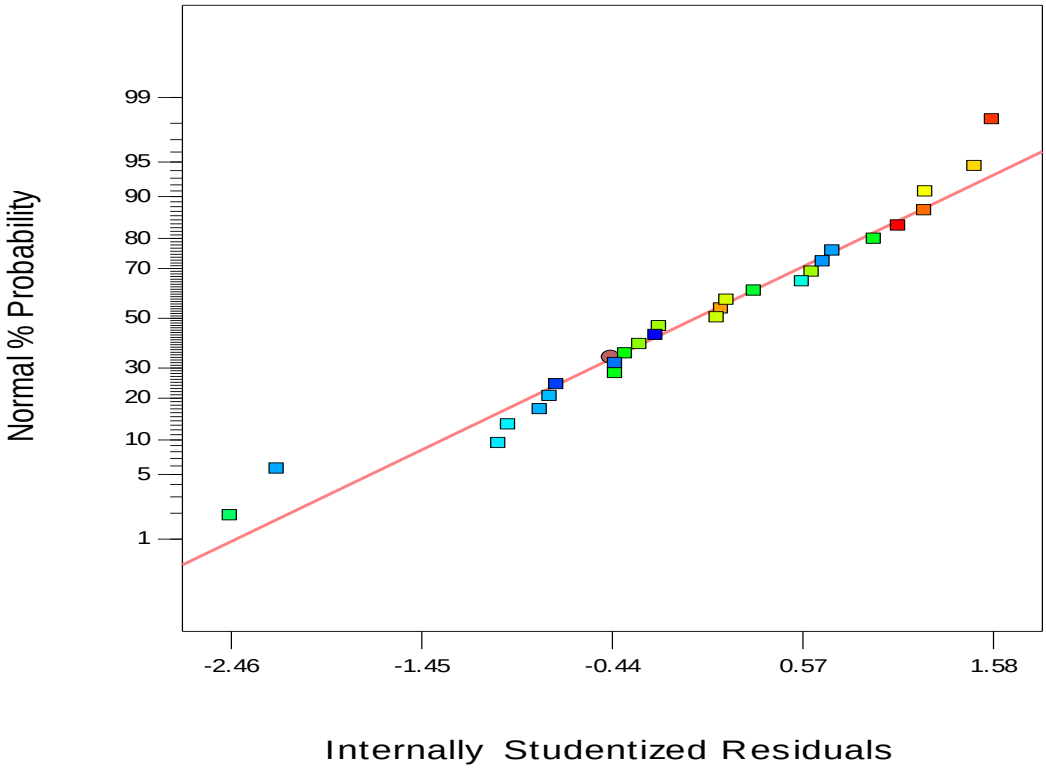


Figure 4.1 Normal plots of residuals

The normal probability plot, (Figure4.1), indicates the residuals following a normal distribution, in which case the points follow a straight line. This indicates the model satisfies the assumption of ANOVA.

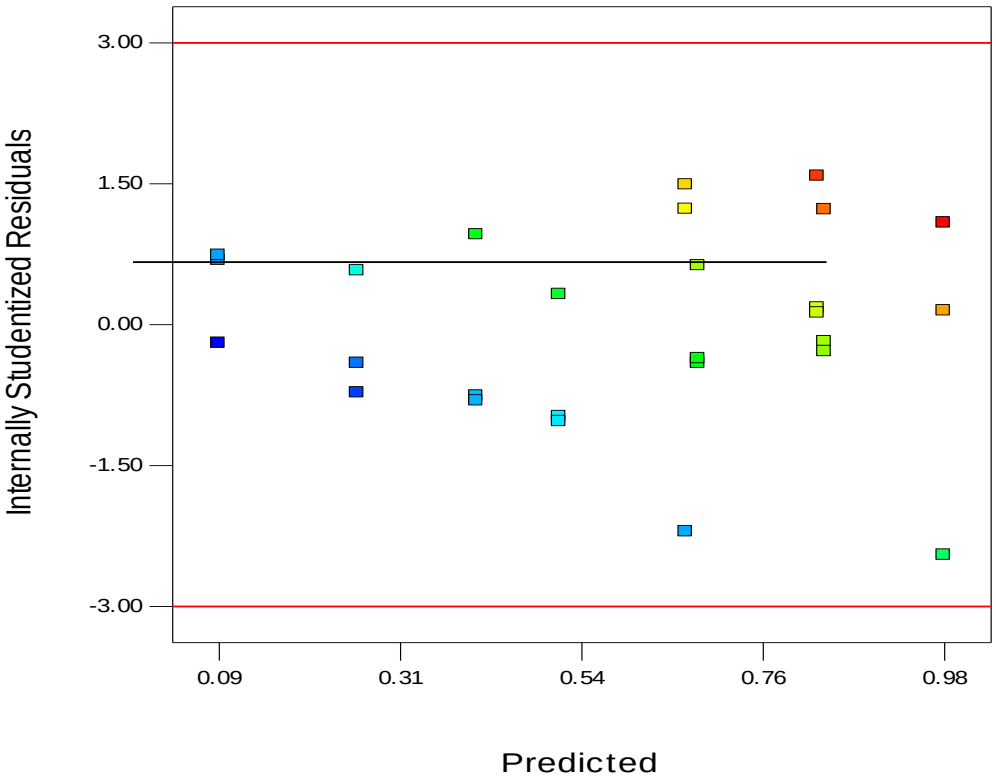


Figure 4.2 Plot of residuals versus model predicted values

The plot of the residuals versus the predicted response values (Figure 4.2), tests the assumption of constant variance. The plot shows constant range of residuals across the graph which is welcome deserving no need for a transformation to minimize personal error.

4.4 Individual effects of each factor on the yield of extracted oil

4.4.1 Effect of extraction time

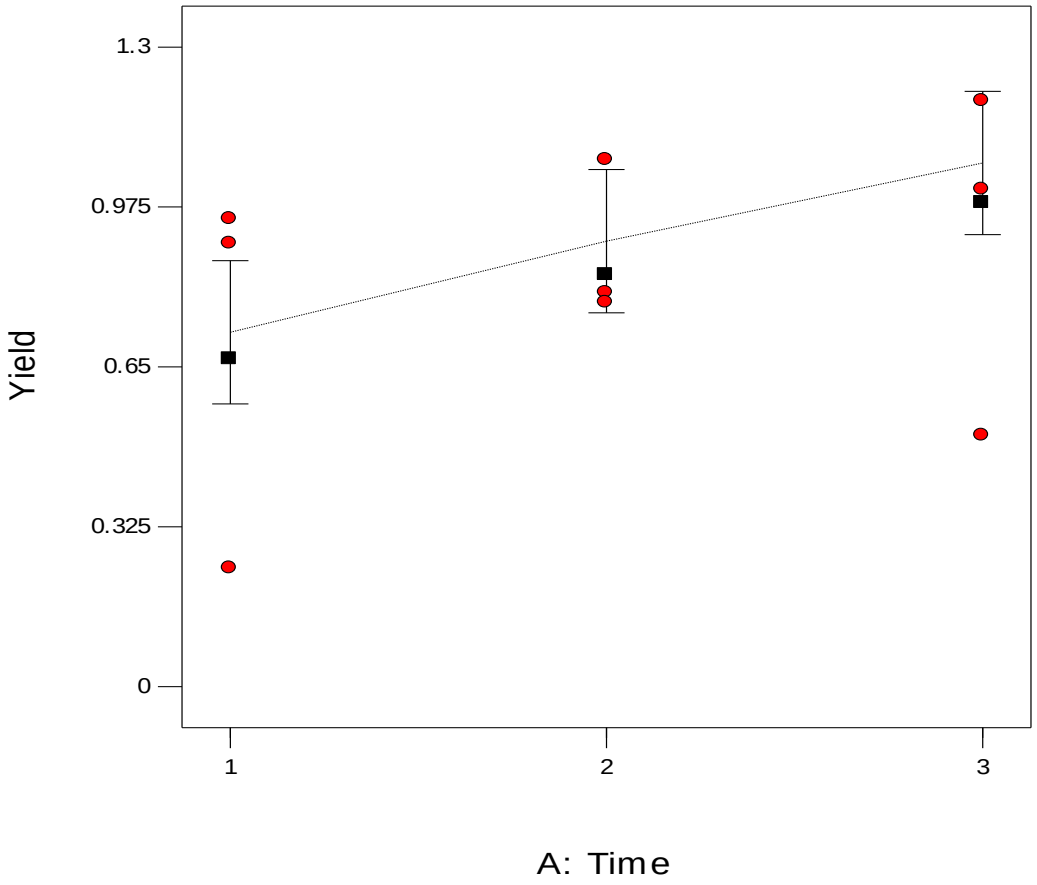


Figure 4.3 Effect of time on the yield of extracted oil

Extraction time plays a great role on the percentage yield of eucalyptus oil using steam distillation. In figure 4.3 Shows that as contact time increase the oil yield also increase till transfer of oil from the leaves to the steam attains zero. When the maximum amount of extractable oil is obtained, the oil yield level remains invariable even by extending the reaction time. So that in the steam distillation extraction the maximum oil yield could be finding at an extraction time of 3 hours.

4.4.2 Effect of particle size on the yield of extracted oil

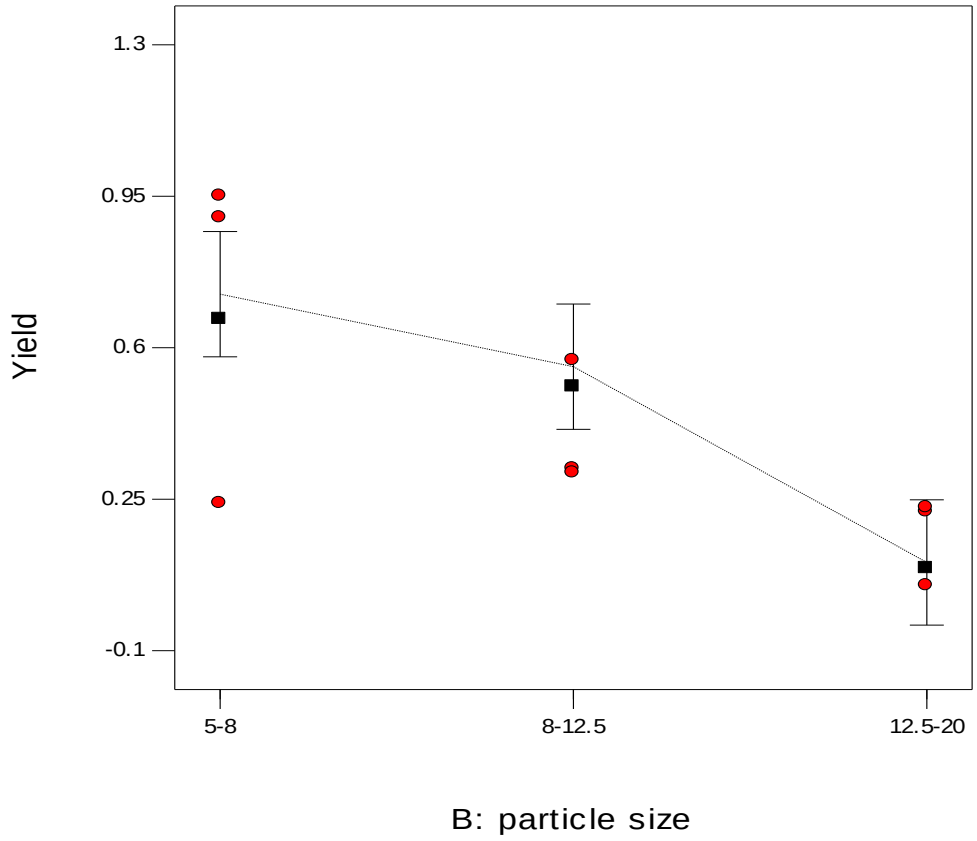


Figure 4.4 Effect of particle size on the yield of extracted oil

The effect of increasing and decreasing particle size on oil yield has been shown on Figure 4.4 above. It was quite clear that there was an increase in the oil yield to a maximum value due to reduce in particle size and a further increase in the particle size results in a drop in oil yield. In the figure 4.4 it was observed that the minimum particle size has had maximum oil yield whereas the maximum particle size has had minimum oil yield.

4.5 Interaction effect of factors on the yield of extracted oil

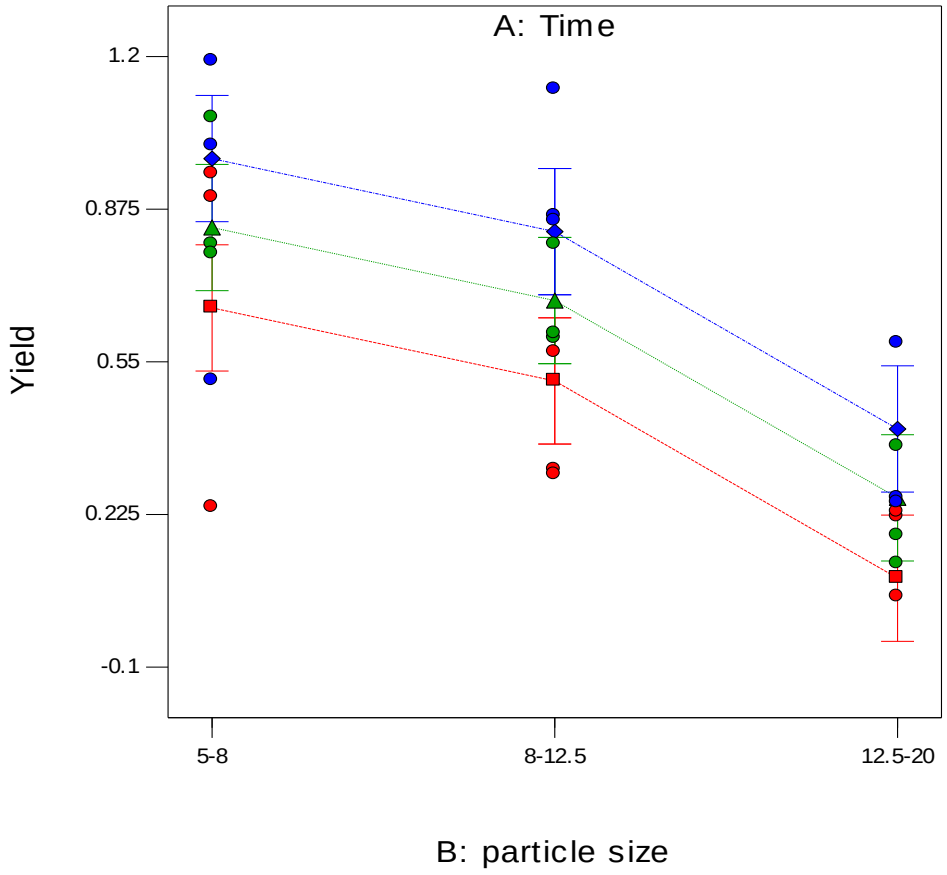


Figure 4.5 Effects of time, particle size and their interactions on eucalyptus oil yield

Figure4.5 shows that there is no interaction among each factor. This shows us an increment in time will increase the quantity of eucalyptus oil extracted. Extraction beyond 3 hours didn't give a significant change on oil yield.

Where: A_1, A_2 and A_3 are codes for time of 1, 2, and 3 hours respectively.

B_1, B_2 and B_3 are ranges of particle size from 5-8, 8-12.5, and 12.5-20 mm respectively.

On Figure 4.6 it was observed that as time increase and particle size decrease yield increase. While particle size increase yield decreases.

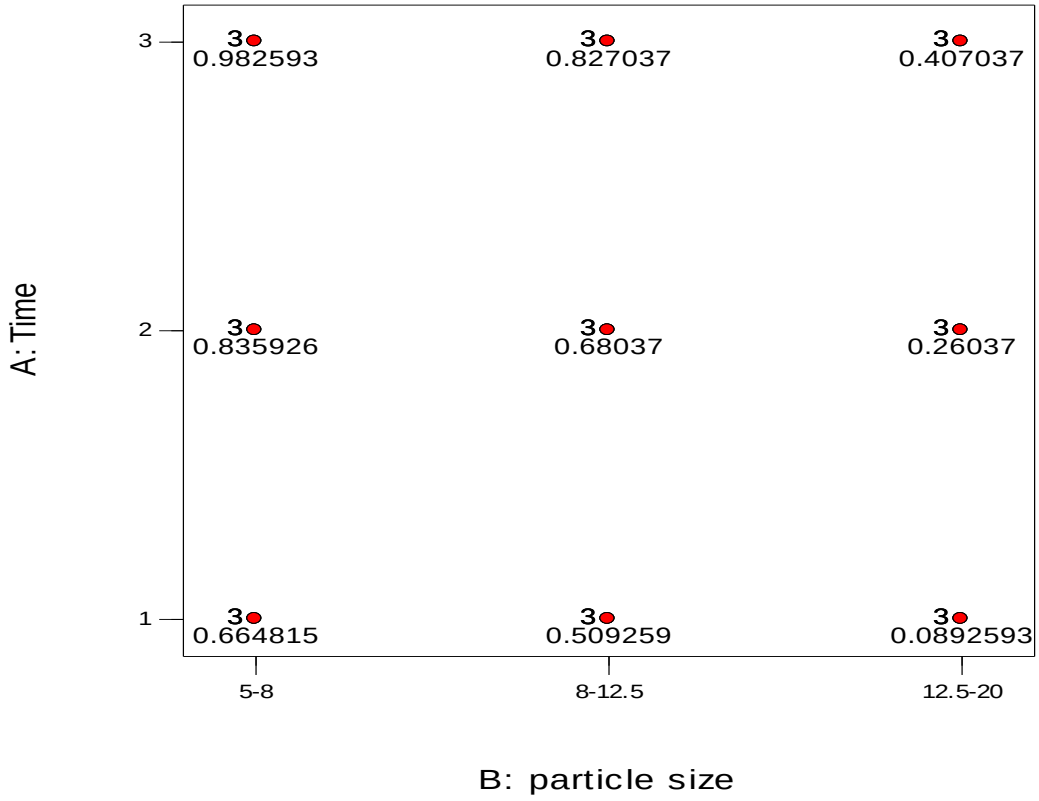


Figure 4.6 contour plot of time, particle size and yield.

Figure 4.7 Shows that yield increase as time increase and particle size decrease on three dimensional surface planes.

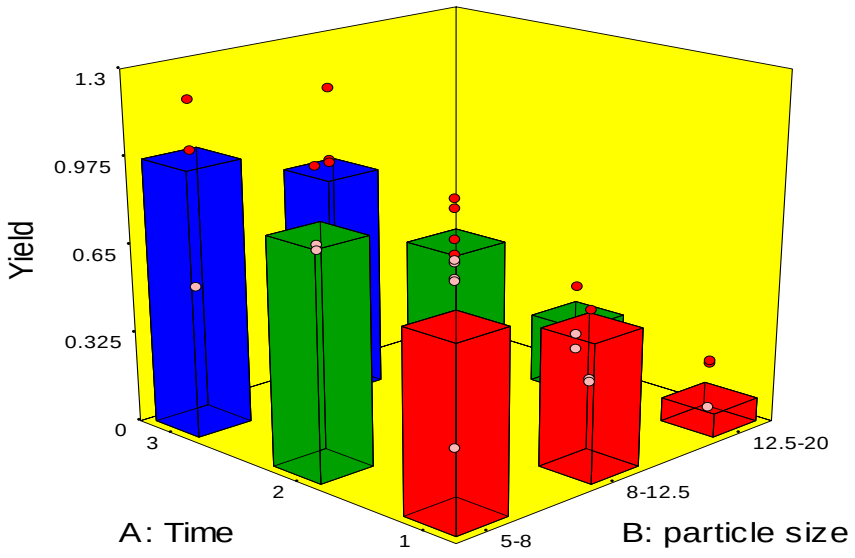


Figure 4.7: 3D surface plot of time, particle size and yield.

4.6 Optimization

The goal of optimization is to maximize economic benefit or increasing the yield of eucalyptus essential oil by minimizing process cost.

To investigate the optimum values of eucalyptus oil extraction from eucalyptus (globulus) leaves using steam distillation are summarized as follows:

Table 4.5 Optimum possible solutions

Number	Time	particle size	yield	Desirability	
1	3	5-8	0.986296	0.821	Selected
2	2	5-8	0.845185	0.698	
3	3	8-12.5	0.825185	0.680	
4	2	8-12.5	0.684074	0.556	
5	1	5-8	0.668519	0.543	
6	1	8-12.5	0.507407	0.401	
7	3	12.5-20	0.405185	0.312	
8	2	12.5-20	0.264074	0.188	
9	1	12.5-20	0.0874074	0.033	

The desirability lies between 0 and 1 and it represents the closeness of a response to its ideal value. If a response falls within the unacceptable intervals, the desirability is 0, and if a response falls within the ideal intervals or the response reaches its ideal value, the desirability is 1. Meanwhile, when a response falls within the tolerance intervals but not the ideal interval, or when it fails to reach its ideal value, the desirability lies between 0 and 1. The more closely the response approaches the ideal intervals or ideal values, the closer the desirability is to 1. Based on the above analysis best local maximum for eucalyptus oil yield of 0.9863 % was found at a time 3 hr, particle size 5-8 mm and the value of desirability obtained was 0.821%.

4.7 Physical properties

- ✓ **pH determination of eucalyptus (globulus) oil**

The pH of eucalyptus globulus essential oil was measured by pH meter in environmental laboratory of Chemical Engineering Department. Its value was recorded as 6.31.

- ✓ **Specific gravity calculation of eucalyptus (globulus) oil**

Weight of Pycnometer (W) at 25℃ = 24.3994 gram

Weight of Pycnometer with eucalyptus oil (W_1) at 25℃= 46.212 gram

Weight of Pycnometer with eucalyptus water (W_2) at 25℃ = 49.512 gram

$$\text{Specific gravity} = \frac{W_{1-W}}{W_{2-W}}$$

$$= \frac{(47.212 - 24.994)\ \text{gram}}{(49.512-24.994)\text{gram}} = 0.903957$$

- ✓ **Refractive index of eucalyptus (globulus) oil**

Refractive index of eucalyptus (globulus) oil was measured by Refracto meter (spectrometer) setup in AAU (Arat Kilo) Department of Physics. The refractive index was measure at wave length of 632.8 Nano meter calculated as:

N = tan (θb)

θb= Rotation with sample – initial Rotation=

= (252-196.66) = 55.34

N = tan (55.34) = 1.4463

- ✓ **Optical rotation of eucalyptus (globulus) oil**

Optical rotation of eucalyptus (globulus) oil was measured by Polari meter in AAU (Arat Kilo) Department of Physics. The Polari meter recorded was 2 degree.

- ✓ **Viscosity of eucalyptus (Globulus) oil**

Dynamic Viscosity (μ) of eucalyptus (Globulus) oil was measured by viscometer at 19.7℃ in chemical engineering department laboratory. The value was 3.15 mili Pascal second (mpas).

- ✓ **Boiling point of eucalyptus (globulus) oil**

The boiling point of eucalyptus oil was measure by the procedure described on the methodology. Its value was 174℃.

- ✓ **Evaporation residue of eucalyptus (globulus) oil**

The percentage of eucalyptus oil which does not evaporate at 100℃ was 98.65%. Figure 4.10 shows evaporation residue measurement i.e mass of eucalyptus oil that evaporates at a temperature of 100℃ is 1.35%.

$$\% \text{ age of oil not volatile at } 100℃ = \frac{W_2}{W_1} \text{X } 100 = \frac{36.5}{37} X \ 100 = 98.65\%$$

- ✓ **Flash point of eucalyptus (globulus) oil**

Flash point of eucalyptus oil was measured as 50℃.

- ✓ **Solubility of eucalyptus (globulus) oil**

The eucalyptus oil was soluble in alcohol and insoluble in water.

Generally the physical properties of eucalyptus (globulus) oil extracted using steam distillation were summarized in the Table 4.6.

Table 4.6 Values and unit of physical properties of eucalyptus oil.

Physical properties	Results	unit	AFNOR standards
pH at 25 ℃	6.31	-	-
Specific gravity at 25℃	0.90841	-	0.906 to 0923
Refractive index at 25 ℃	1.4463	-	1.459 to 1.467
Optical rotation at 25℃	2	degree	0° to + 10°
Dynamic Viscosity at 19.7 ℃	3.15	mpas	-
Flash point	50	℃	49
Boiling point	174	℃	176
Evaporation residue	98.648	%	-
Colour	pale yellow liquid	-	Colorless to pale yellow
odor	Characteristic odor	-	Characteristic odour
Solubility in alcohol	soluble	-	Soluble in alcohol
Solubility in water	insoluble	-	Insoluble in water

4.8 Quality Evaluation of the of eucalyptus oil

- ✓ **Acid value determination**

The acid value of was determined by equation.3.5 as follows:

$$\text{A.V} = \frac{56.1(\text{volume of alcoholic sodium hydroxide solution x normality})}{\text{mass of sample}}$$

$$= \frac{56.1(\ 7\text{mlx}0.1)}{2gram} = \quad 19.635 \text{ ml/gram}$$

- ✓ **Saponification value determination**

The saponification of eucalyptus oil was calculated by equation.3.6.

$$\text{S.V} = 56\left(\frac{V_1 - V_2}{2xW}\right)$$

$$= \frac{56(11.5-11)\text{ml}}{2x1gram} = 14 \text{ ml/gram}$$

- ✓ **Iodine number determination**

The iodine number calculated by equation 3.7.

$$\text{I.N} = 1.269\left(\frac{V_1 - V_2}{W}\right)$$

$$= \frac{1.269(\ 10.5-8)}{0.1} = 31.725 \text{ ml/g}$$

Chemical properties of eucalyptus oil such as acid value, saponification value and iodine number are summarized in table 4.7.

Table 4.7 chemical properties of eucalyptus oil extracted using steam distillation.

Chemical properties	**V**alue	unit
Acid value	19.635	ml/g
saponification value	14.00	ml/g
iodine number	31.725	ml/g

4.9 Gc-ms Analysis

A total of eighteen components, with different retention times, were eluted from the GC column as indicated by the chromatogram and were further analyzed with an electron impact MS voyager detector. Identification of constituents was done on the basis of their retention time and mass spectra library search. The mass spectrographs of the identified constituents are given in Table 4.8. The relative amount of individual components was calculated based on GC peak areas. Comparison of the GC-MS spectrograph obtained with the instruments data bank together with NIST MS data demo version revealed that the essential oil of 1, 8 cineol contained a mixture of terpenes that eluted at different retention times depending on the boiling point of the eluted component. The GC chromatogram obtained revealed a high concentration of 1, 8 cineol indicated by presence of two large peaks which eluted at 27.239 and 33.025 minutes with peak areas of 31469347.42 and 2966067.98 respectively. The presence of these two peaks may be due to the two isomers of cineol that is geranial and neral, which may be difficult to distinguish with GC-MS.

Table 4.8 Chemical composition of eucalyptus (Globulus) oil

Chemical compound	Library Match Quality (%)	Retention time (min)	Area (%)
Eucalyptol	99	9.845	3.99
Terpineol	90	16.575	1.16
1H-cycloprop[e] azulene	99	26.009	4.76
1H-cyclopropa[a] naphthalene	97	26.921	2.35
Aromadendrene	99	27.239	30.24
(1s,4aR,7R)-1,4a-Dimethyl	94	27.396	0.97
Aromadendrene	99	28.113	8.75
1H-Cycloprop[e] azulene, 1a,2,3,5,6,7,7a,7b- octahydro	99	29.516	4.26
Azulene,1,2,3,3a,4,5,6,7-octaoctahydro-1,4-dimethyl-7-(1-methylethenyl)	96	32.069	4.60
1,1,4,7-Tetramethyl-1a,2,3,4,6,7,7a,7b-octahydro-1H-cycloprppa[e] azulene	96	32.343	1.17
Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methenyl)	98	33.025	19.90
Azulene	97	33.314	5.57
Naphthalene, decahydro- 4a-methyl-1-methylene-7-(1-methenyl)	95	33.716	2.12
Hexadecanoic acid ,methyl ester	99	45.127	1.59

5. CONCLUSION AND RECOMENDATION

5.1 Conclusion

In this work, extraction eucalyptus oil was carried out. Extraction time and particle size were considered as factors to see their effects on the yield of eucalyptus oil extracted using steam distillation. Volume or mass of eucalyptus oil increase as extraction time increase and particle size decrease. The maximum yield was found at extraction time of 3 hours and particle size range of 5-8 mm which is 1.19 %. The effect of extraction time and particle size was analysis by using DESIGN EXPERT 7.0 software with three levels and three replications each. Chemical analysis of eucalyptus oil was carried out by GC-MS. The 1, 8 Cineole (eucalyptol) component found was 99%. Most physical properties and chemical of eucalyptus oil were between the ISO standards but some were not this could be due to different factors such as duration of characterization, place to put the oil. Finally, that was concluded extraction of essential Oil from eucalyptus (Globulus) leaves using Steam distillation can be used on industrial scale to make various finished products which includes body oils, cosmetic lotions, baths, hair rinses, soaps, room sprays etc.

5.2 Recommendation

- In this research work, the effects of temperature and pressure were not studied due to the lack appropriate equipment. Therefore, further study is need on these effects.
- A further study can also be conducted in focusing on the quality of the eucalyptus essential oil obtained at each operating conditions using GC-MS. This will provide alternative to manufacturers on the minimum conditions required to get a specific quality of essential oil with minimum capital.
- Study can be carried on converting the residue or the waste to valuable product and using the hydrosol as integrated small scale industry.

6. REFERENCES

AFNOR (2000) Association Française deNormalization.

Akolade, Olayinka J., Olajide… (2012). Chemical composition, antioxidant and cytotoxic effects of Eucalyptus globulus grown in north-central Nigeria

Asaad A., S.,R., (2014) A practical study for new design of essential oils extraction apparatus using ohmic heating; *International Journal of Agricultural Sciences ISSN: 2167-0447 Vol. 4(12), pp. 351-366*

Boland, D.J., Brophy, J.J., and A.P.N. House, *Eucalyptus Leaf Oils*, 1991, p. 8 ISBN 0-909605-69-6

Boukhatem N., M., Amine M., F., Kameli A., Saidi F., Walid K., Mohamed B. S. (2014). Quality assessment of the essential oil from Eucalyptus globulus Labill of Blida (Algeria) origin; *International Letters of Chemistry, Physics and Astronomy*

Coppen, J. J., CRC Press (2003) 112-145.

Dagne E., Bisrat D., Alemayehu M., Worku T. (2000), Essential Oils of Twelve Eucalyptus Species from Ethiopia, J. Essent. Oil Res.; 12: 467-70.

European Pharmacopoeia Commission, (2001) Council of Europe.

Flower and Vegetable Oils, R.E.D. Facts, EPA

Göbel, H; Schmidt, G; Soyka, D (1994). "Effect of peppermint and eucalyptus oil preparations on neurophysiological and experimental algesimetric headache parameters". *Cephalalgia: an international journal of headache 14 (3): 228–34; discussion 182. Doi: 10.1046/j.1468-2982.1994.014003228.x. PMID 7954745.*

Guenther, E (1960). The Essential oils: History –origin plant production – analysis, vol.1. Fourth printing, D.van Nostrand company, Inc., prenceton, New Jersey, USA

H. J. Williams, Ahmed, Mahmoud, A. I. Scott, J. H. Reibenspies, and T. J. Mabry. 1993. New sesquiterpene a-methylene lactones from the Egyptian plant Jasonia candicans. J. Nat. Prod. 56:1276–1280.

Harborne, J.B., Baxter, H., *Chemical Dictionary of Economic Plants*, ISBN 0-471-492264

Hong, CZ; Shellock, FG (1991). "Effects of a topically applied counterirritant (Eucalyptamint) on cutaneous blood flow and on skin and muscle temperatures. A placebo-controlled study". *American journal of physical medicine & rehabilitation / Association of Academic Physiatrists 70 (1): 29–33. Doi: 10.1097/00002060-199102000-00006. PMID 1994967.*

Hunde, T., Gizachew, B., Harwood, C. (2007), Genetic variation in survival and growth of Eucalyptus globulus ssp. globulus in Ethiopia Australian Forestry; 70, 48.

Jagger P. and Pender J. (2000)**.**The role of trees for sustainable management of less-favored lands: the case of eucalyptus in Ethiopia.

Jaicaf, 2008 "Medicinal crops" in Ethiopia - Current status and future potentials-

Juergens, U.R; Dethlefsen, U; Steinkamp, G; Gillissen, A; Repges, R; Vetter, H (2003). "Anti-inflammatory activity of 1.8-cineol (eucalyptol) in bronchial asthma: A double-blind placebo-controlled trial". *Respiratory Medicine 97 (3): 250–6. Doi: 10.1053/rmed.2003.1432. PMID 12645832.*

Juergens, Uwe R.; Engelen, Tanja; Racké, Kurt; Stöber, Meinolf; Gillissen, Adrian; Vetter, Hans (2004). "Inhibitory activity of 1, 8-cineol (eucalyptol) on cytokine production in cultured human lymphocytes and monocytes". *Pulmonary Pharmacology & Therapeutics 17 (5): 281–7. Doi: 10.1016/j.pupt.2004.06.002. PMID 15477123.*

Kabuba Tshilenge john (2009) steam extraction of essential oils: investigation of process parameters

Kidanu, S., Mamo, T., Stroosnijder, L. (2004), Eucalyptus–wheat interaction on Ethiopian Nitosols Agricultural Systems; 80, 151.

Kumar K.satish, 2010 extraction of essential oil using steam distillation: *National Institute of Technology, Rourkela*

Li Y. et al., (2014). Essential Oils: From Conventional to Green Extraction: *Essential Oils as Reagents in Green Chemistry*, SpringerBriefs in Green Chemistry for Sustainability, DOI 10.1007/978-3-319-08449-7_2

Nagata, Hideki; Inagaki, Yoshika; Tanaka, Muneo; Ojima, Miki; Kataoka, Kosuke; Kuboniwa, Masae; Nishida, Nobuko; Shimizu, Katsumasa; Osawa, Kenji; Shizukuishi, Satoshi (2008). "Effect of Eucalyptus Extract Chewing Gum on Periodontal Health: A Double-Masked, Randomized Trial". *Journal of Periodontology 79 (8): 1378–85. Doi: 10.1902/jop.2008.070622. PMID 18672986.*

Nyssen, J., H. Vandereyken, J. Poesen, J. Moeyersons, J. Deckers, Mitiku Haile, C. Salles & G. Govers (2005),Orwa C, Mutua A, Kindt R, Jamnadass R, Simons A. 2009. Agroforestree Database: a tree reference and selection guide version 4.0 (http://www.worldagroforestry.org/af/treedb/)

Orji Onyinyechi A. (2012). Extraction and formulation of perfume from lemongrass leaves.

Panda, H., Essential Oils Handbook, National Institute of Industrial Research, Delhi.

Pandey D. and Virendra Rao P.S, Extraction of essential oil from Eucalyptus leaves (B-tech project), NIT Rourkela.

Pant Chaitanya, Thomas J. Sferra, Abhishek Deshpande, Anil Minocha, *European Journal of Internal Medicine* 7 (2011) 210-222.

Pollack A., Rising Threat for Infections Unfazed by Antibiotics. *The New York Times*, February 27 (2010) B1.

Pombal S., Rodilla J., Gomes A., Silva* L., Rocha P. (2014) Evaluation of the antibacterial activity of the essential oil and antioxidant activity of aqueous extracts of the *Eucalyptus globulus* Labill Leaves.

Salari, M. H.; Amine, G.; Shirazi, M. H.; Hafezi, R.; Mohammadypour, M. (2006). "Antibacterial effects of Eucalyptus globulus leaf extract on pathogenic bacteria isolated from specimens of patients with respiratory tract disorders". *Clinical Microbiology and Infection* ***12*** *(2): 194–6. Doi:10.1111/j.1469-0691.2005.01284.x. PMID 16441463.*

Serafino, A; Sinibaldi Vallebona, PS; Andreola, F; Zonfrillo, M; Mercuri, L; Federici, M; Rasi, G; Garaci, E; Pierimarchi, P (2008). "Stimulatory effect of Eucalyptus essential oil on innate cell-mediated immune response". *BMC Immunology 9: 17. Doi: 10.1186/1471-2172-9-17. PMC 2374764. PMID 18423004.*

Sewanu Oluwagbemiga Soyingbe (2012).The chemical composition, antimicrobial and antioxidant properties of the essential oils of Tulbaghia violacea Harv. and Eucalyptus grandis W. Hill ex Maiden.

Trieste (2008) Extraction Technologies for Medicinal and Aromatic Plants: *International center for science and high technology*, page 42-47.

Weiss, E.A., 1997. Essential Oil Crops, CAB International, USA.

Zewdie, M., Olsson, M., Verwijst, T. (2009), Aboveground biomass production and allometric relations of Eucalyptus globulus Labill. Coppice plantations along a chronosequence in the central highlands of Ethiopia Biomass and Bioenergy; 33, 421.

Zhao, J., Agboola, S., Functional Properties of Australian Bushfoods - A Report for the Rural Industries Research and Development Corporation, 2007*, RIRDC Publication No 07/030*

Zhongguo Zhong Yao za zhi = Zhongguo zhongyao zazhi = China journal of Chinese materia medica 29 (2): 168–71. PMID 15719688.

APPENDICES

Appendix A

Table.A1.Library search report of GC-MS for essential oil of dry eucalyptus leaves.

Library Search Report

Data Path : D:\MassHunter\GCMS\1\5977\April 27\
Data File : 00201002.D
Acq On : 27 Apr 2016 16:19
Operator : Estif
Sample : Eca-5
Misc :
ALS Vial : 2 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

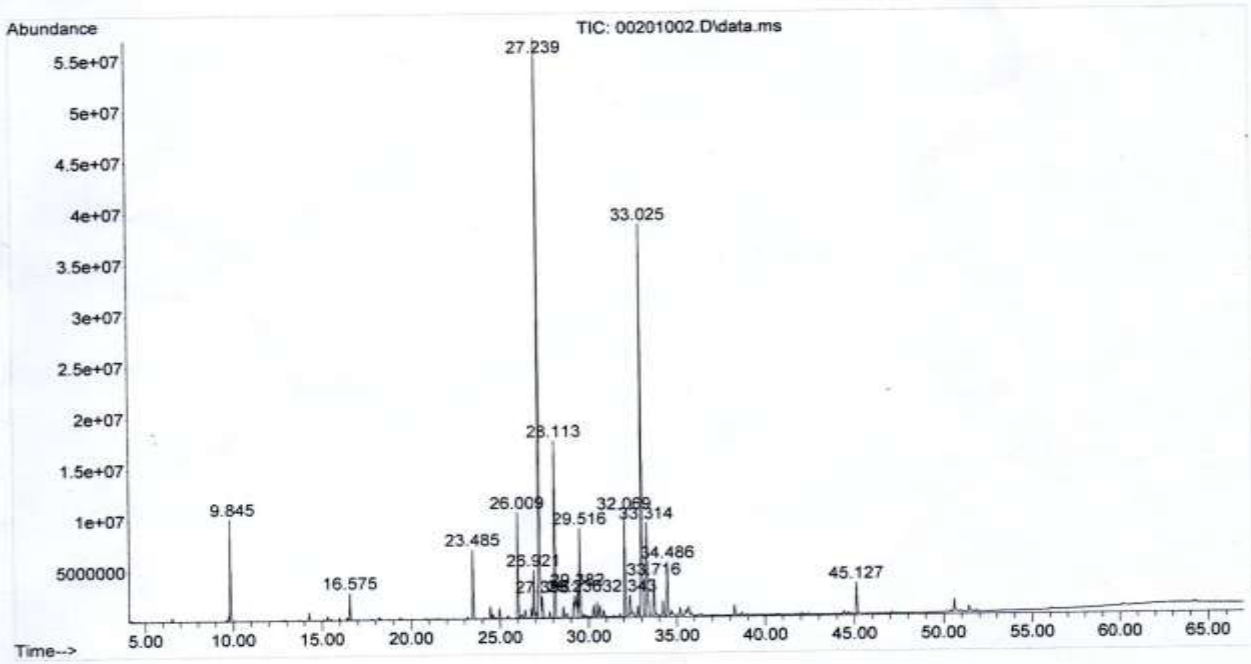
Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	9.845	3.99	D:\MassHunter\Library\NIST14.L			
			Eucalyptol	27458	000470-82-6	99
			Eucalyptol	27464	000470-82-6	95
			Eucalyptol	27467	000470-82-6	91
2	16.575	1.16	D:\MassHunter\Library\NIST14.L			
			Terpineol	27454	1000411-59-6	90
			.alpha.-Terpineol	27528	000098-55-5	87
			(+)-4-Carene	16052	029050-33-7	83
3	23.485	2.94	D:\MassHunter\Library\NIST14.L			
			3-Methyl-trans-3a,4,7,7a-tetrahydroindane	16201	1000145-84-3	78
			Camphene	16040	000079-92-5	72
			Cyclohexene, 3-methyl-6-(1-methylethylidene)-	16239	000586-63-0	64
4	26.009	4.76	D:\MassHunter\Library\NIST14.L			
			1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]-	68931	000489-40-7	99
			1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]-	68924	000489-40-7	99
			1H-Cyclopropa[a]naphthalene, 1a,2,3,3a,4,5,6,7b-octahydro-1,1,3a,7-tetramethyl-, [1aR-(1a.alpha.,3a.alpha.,7b.alpha.)]-	68920	000489-29-2	99
5	26.921	2.35	D:\MassHunter\Library\NIST14.L			
			1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)]-	68940	017334-55-3	97
			1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)]-	68936	017334-55-3	95
			1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)]-	68938	017334-55-3	93
6	27.239	30.24	D:\MassHunter\Library\NIST14.L			
			Aromandendrene	68525	000489-39-4	99
			Aromandendrene	68532	000489-39-4	98
			1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-	68717	072747-25-2	97

7	27.396	0.97	D:\MassHunter\Library\NIST14.L				
			(1S,4aR,7R)-1,4a-Dimethyl-7-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalene	68817	052026-55-8	94
			(1R,4aS,8aR)-1,4a-Dimethyl-7-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octahydronaphthalene	68827	194607-93-7	91
			Alloaromadendrene	68577	025246-27-9	91
8	28.113	8.75	D:\MassHunter\Library\NIST14.L				
			Alloaromadendrene	68580	025246-27-9	99
			(1R,9R,E)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	68724	068832-35-9	99
			Aromandendrene	68514	000489-39-4	99
9	29.236	1.69	D:\MassHunter\Library\NIST14.L				
			Alloaromadendrene	68577	025246-27-9	89
			(1R,9R,E)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	68724	068832-35-9	89
			Alloaromadendrene	68580	025246-27-9	87
10	29.382	1.34	D:\MassHunter\Library\NIST14.L				
			Butanoic acid, 2-methyl-, 2-phenylethyl ester	70530	024817-51-4	80
			Octanoic acid, 2-phenylethyl ester	109808	005457-70-5	72
			Butanoic acid, 2-methyl-, 2-phenylethyl ester	70526	024817-51-4	72
11	29.516	4.26	D:\MassHunter\Library\NIST14.L				
			1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.beta.,7b.alpha.)]-	68928	021747-46-6	99
			1H-Cyclopropa[a]naphthalene, decahydro-1,1,3a-trimethyl-7-methylene-, [1aS-(1a.alpha.,3a.alpha.,7a.beta.,7b.alpha.)]-	68918	020071-49-2	90
			Neoisolongifolene	68570	1000156-12-4	86
12	32.069	4.60	D:\MassHunter\Library\NIST14.L				
			Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,3a.beta.,4.alpha.,7.beta.)]-	68912	022567-17-5	96
			Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]	68864	017066-67-0	94
			Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,3a.beta.,4.alpha.,7.beta.)]-	68914	022567-17-5	86
13	32.343	1.17	D:\MassHunter\Library\NIST14.L				
			1,1,4,7-Tetramethyl-1a,2,3,4,6,7,7a,7b-octahydro-1H-cyclopropa[e]azulene	68772	405112-35-8	96
			Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)-(.+/-.)-	68889	005951-61-1	95
			1H-Cyclopropa[a]naphthalene, 1a,2,3,3a,4,5,6,7b-octahydro-1,1,3a,7-tetramethyl-, [1aR-(1a.alpha.,3a.alpha.,7b.alpha.)]-	68921	000489-29-2	95
14	33.025	19.90	D:\MassHunter\Library\NIST14.L				
			Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)	68912	022567-17-5	98

			-, [1R-(1.alpha.,3a.beta.,4.alpha.,7.beta.)]-			
			(-)-Globulol	85692	000489-41-8	96
			1,1,4,7-Tetramethyl-1a,2,3,4,6,7,7a,7b-octahydro-1H-cyclopropa[e]azulene	68772	405112-35-8	94
15	33.314	5.57	D:\MassHunter\Library\NIST14.L			
			Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,3a.beta.,4.alpha.,7.beta.)]-	68912	022567-17-5	97
			Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]-	68870	003691-11-0	91
			.beta.-Panasinsene	68592	1000159-39-0	90
16	33.716	2.12	D:\MassHunter\Library\NIST14.L			
			Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]	68863	017066-67-0	95
			Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]	68864	017066-67-0	95
			Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]	68856	017066-67-0	94
17	34.486	2.59	D:\MassHunter\Library\NIST14.L			
			1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.beta.,7b.alpha.)]-	68922	021747-46-6	59
			1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.beta.,7b.alpha.)]-	68927	021747-46-6	49
			Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]-	68873	003691-11-0	42
18	45.127	1.59	D:\MassHunter\Library\NIST14.L			
			Hexadecanoic acid, methyl ester	130813	000112-39-0	99
			Hexadecanoic acid, methyl ester	130818	000112-39-0	97
			Pentadecanoic acid, 14-methyl-, methyl ester	130841	005129-60-2	97

Essential oil.M Thu Jun 02 16:08:30 2016

File :D:\MassHunter\GCMS\1\5977\April 27\00201002.D
Operator : Estif
Acquired : 27 Apr 2016 16:19 using AcqMethod Essential oil.M
Instrument : AAU
Sample Name: Eca-5
Misc Info :
Vial Number: 2



Appendix B

Table b.2 Actual, predict and Residual

Standard order	Actual value	Predicted	Residual
1	0.24	0.67	-0.43
2	0.90	0.67	0.23
3	0.95	0.67	0.28
4	0.78	0.85	-0.07
5	0.85	0.85	4.815E-003
6	1.07	0.85	0.22
7	01.01	0.99	0.02
8	0.51	0.99	-0.48
9	1.19	0.99	0.2
10	0.57	0.51	0.06
11	0.32	0.51	-0.19
12	0.31	0.51	-0.2
13	0.80	0.68	0.12
14	0.60	0.68	-0.08
15	0.61	0.68	-0.07
16	0.85	0.83	0.02
17	1.13	0.83	0.3
18	0.86	0.83	0.03
19	0.05	0.87	-0.82
20	0.22	0.87	-0.65
21	0.23	0.87	-0.64
22	0.37	0.26	0.11
23	0.12	0.26	-0.14
24	0.18	0.26	-0.08
25	0.26	0.41	-0.15
26	0.25	0.41	-0.16
27	0.59	0.41	0.18

Table B.1 Design summary

Study type	Factorial
Initial point	Full Factorial
Center point	0
Design Model	2FI
Run	27
Blocks	No

Appendix C

Laboratory equipment's and samples photos

Eucalyptus Leaves (E. globulus)

Sieve arrangement

Different particle size sample

Eucalyptus leaves inside oven

Steam distillation set up

density separator funnel

Refractive index measurement

Optical rotation measurement

Acid value determination

Blank and sample for saponification value Before titration

Sample after titration for saponification value Determination

Blank after titration for saponification value determination