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Extraction and Characterization of Essential oil from Thyme (Thymus Schimperi L.) Leaves by using Steam Distillation

TSIGE TSEGAY

A thesis submitted to the School of Chemical and Bio Engineering

Presented in Partial Fulfillment of the Requirements for the Degree of Master of Science (Process Engineering)

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DECLARATIO

I declare that this thesis entitled extraction and characterization of essential oil from Thyme (Thymus schimperi L.) leaves using steam distillation has not been submitted in any form for another degree, diploma or an award at any university or other institution of the tertiary education. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due reference to the literature and discussions. Information taken from published and unpublished work of others has been acknowledged in the text and a list of references is given. The work was under the guidance of Dr. Eng. Shimelis Kebede instructor in Addis Ababa University, School of Chemical and Bio Engineering.

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This thesis has been submitted to my Advisor for examination and approval

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Date: _____________________

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LIST OF ACROOYMS

AAIT	Addis Ababa Institute of Technology
ANOVA	Analysis of variance
AOAC	Associates of Analytical Chemistry
FID	Flame Ionization Detector
GC-MS	Gas chromatography- Mass-spectrometer
GC	Gas chromatography
MSD	Mass Selective Detector
MS	Mass-spectrometer
NIST	National Institute of Standard and Technology
RT	Retention Time
SCFE	Supercritical fluid extraction

ABSTRACT

Essential oils are very interesting natural plant products, and among other qualities they possess various biological properties. This study has been carried out to extract the essential oil from the leaves of thymus schimperi L. using steam distillation. Thyme (thymus schimperi L).is an indigenous plant in Ethiopia and is locally called "Tosign'' (in Amharic). It is among the commonly occurring thymus species that distributed in different regions of Ethiopia. In this research, thyme leaves were collected from debresina, Tarmaber Woreda. First, the leaf of thyme was dried through partial sun drying. Then the dried thyme leaves were crushed in cross beater mill with sieve size of 2mm 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 1.18% was obtained after the extraction time of 60 minutes with particle size of 2 mm and maximum oil yield of 2.12 % was obtained at optimum extraction time of 150 minutes with particle size of 0.5 mm. This shows that, increasing extraction time (optimum of 150 minutes) and decreasing particle size increases the yield of oil. Characterization of the oil was also carried out. Accordingly the yield was found with 1.54 refractive index value, 0.948$\frac{g}{cm3}$ specific gravity, and 0.42 degree optical rotation, 6.2 pH, 6.02 mpas dynamic viscosity, 1870C boiling, 101.512ml/g iodine value, 33.6 ml/g saponification value, 5.18 acid value, 28.42 ml/g ester value and 2.605% free fatty acid. The essential oil was analyzed by combined Gas Chromatography and Mass Spectroscopy unit, and it was found to been reached in monoterpenes alcohol and phenolic containing components. Among the major components from the GC-MS analysis were thymol (63.20 %), p-Cymene (11.112%), Carvacrol (8.62%) and Linalool (2.41 %).

Key words: *Composition, essential oil, extraction, GC-MS, thymus schimperi L., steam distillation, yield.*

1. INTRODUCTON

1.1. Background

The name thyme, in its Greek form, was first given to the plant by the Greek's as a derivative of a word which meant "to fumigate", either because they used it as incense, for its balsamic odor, or because it was taken as a type of all sweet smelling herbs. Other say the name derived from the Greek words, Thymol, meaning perfume or famous, signifying courage, the plant being held in ancient and medieval days to be a great source of invigoration, its pleasant qualities inspiring courage. The genus Thymus L. belongs to the family Lamiaceae, and consists of about 215 to 350 species, according to different literature data (Hagos Hailu Kassegn, 2016). They are usually herbaceous perennials, small shrubs occurring within the Mediterranean region, which is a center of the entire genus, and are also characteristic of Asia, Southern Europe and North Africa (Maksimovi ´c et al., 2008).

Thymus schimperi Ronniger Perennial herb, woody at the base, 5-40 cm long; Stems prostrate, sometimes erect in younger parts; leaves glandular on both sides, glabrous , ciliolate hairs at the margin, ovate to sub-orbicular, elliptic or lanceolate ; Corolla white to purple, 6-8mm long, tube 4-5mm long, 2-ped,the middle lobe of the lower lip slightly larger. Common in open grassland, on bare exposed rocks, on slopes and tops of mountains in afro montane and afroalpine vegetation zones; altitude: 2250-4000m (Zuberi et al., 2014).

Thyme is largely distributed in temperate zones and is uncommon in the African tropics. Ethiopia has considerably abundant Lamiaceae family herb growing at different regions and possesses a variety of the wild growing species of this family. Many species belonging to different genera of the family Lamiaceae have been reported to found in different parts of the country. The two species, T. schimperi Ronniger and T. serrulatus Hochst.ex Benth, both locally known as Tosign, are the endemic species represented in Ethiopia while T. vulgaris is a species, native to Southern Europe (Asfaw et al. , 2000). Thymus schimperi is wild growing species of thyme and comparatively well-known in Central, Eastern and Northern Ethiopia. Thymus serrulatus is growing in Tigray, and Bale, Showa, Gonder and Wollo are the major growing areas of thyme in Ethiopia. Wild thyme of T. Schimperi is harvested and dried by people living close to the town of Dinsho and near Menz (North Showa), put in plastic bags and sold to travelers on buses (Hailemariam & Emire, 2013).

The main uses of thyme in culinary and food process` sing are defined by the properties of thyme components for aroma and flavor, antioxidant and antimicrobial activities. The thymol and carvacrol, present in thyme essence, as well as the flavonoids and other polyphenols are considered to be involved in the antioxidant activity. Rosmarinic acid, hydroxycinnamic derivatives and flavonoid compounds showed important in vitro antioxidant activity by inhibiting iron-induced superoxide anion formation and lipid peroxidation in microsomal and mitochondrial systems. Furthermore, the thymol present in the essential oil showed in vitro antioxidant activity by neutralizing the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical (Descalzo & Sancho, 2008).

Thymus is an aromatic plant belonging to the Lamiaceae family, used for medicinal and spice purposes almost everywhere in the world. The non-medicinal use of thyme is worthy of attention, because thyme is used in the food and aroma industries; it is widely used as culinary ingredient and it serves as a preservative for foods especially because of its antioxidant effect. Ethanol extract or essential oil of thyme has a significant rate of antifungal and antimicrobial activities with strongly inhibited lipid peroxidation and high-OH radical scavenging (Bozin Biljana et al., 2006). The major phenolic components in thyme extracts, especially thymol and carvacrol, present higher antioxidant activity than the well-known BHT (butylated hydroxytoluene) and α- antioxidants (Lee & TAKAYUKI, 2002).

Essential oils and extracts from Thymus plants exhibit antioxidant, antibacterial, antifungal, antiviral, cytotoxicity, anti-parasitic and other properties. Oral treatment of coughs, upper respiratory infections, acute and chronic bronchitis, whooping cough, and catarrh are among the internal health problems that herbal medicines belonging to the genus thymus are used as treatment in traditional medicine. For external use, thyme is used as mouthwash to treat laryngitis, as anti-acne and anti-stomatitis agents, and in the topical treatment of minor injuries (Woldesemayate et al., 2016).

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(“Essential oils and waxes,” 1991). 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, 2003).

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 (Rao & Pandey, n.d.).

Essential oils are very interesting natural plant products and among other qualities they possess various biological properties. The term "biological" comprises all activities that these mixtures of volatile compounds (mainly mono- and sesquiterpenoids, benzenoids, phenylpropanoids, etc.) exert on humans, animals, and other plants. Essential oils are complex mixtures of volatile compounds produced by living organisms and isolated by physical means only (pressing and distillation and/or extraction) from a whole plant or plant parts of known taxonomic origin (Franz & Novak, 2010).

Essential oils are generally derived from one or more plant parts, such as flowers, leaves, leaves and stems, bark, wood, roots, seeds, fruits, rhizomes or gums or oleoresin exudations. Essential oils are used widely by the pharmaceutical and cosmetic/perfumery industries as well as in aromatherapy and alternative medicines. And also they are used in a wide variety of consumer goods such as detergents, soaps, toilet products, confectionery food products, soft drinks, distilled alcoholic beverages (hard drinks), insecticides, antibacterial, antioxidant, antifungal, antimicrobial and anti-inflammatory activities. Essential oils have distinctive characteristics, which make them a very valuable commodity with many industrial uses and applications. Their aromatic value enables them to be used as flavorings in both the food and beverage industries. These oils are also widely used in both the cosmetic and pharmaceutical industries. With such applications, there is a huge demand for essential oils worldwide and hence they have been traded internationally for several centuries. There is hence a need to improve the quality and quantity of essential oils produced as they have a very competitive and profitable market worldwide (Worwood & V.A., 1990).

The chemical composition of the essential oils is important in determining their quality and consequently price in the market (Learmonth, et al. 2002). It is therefore important to note and understand the effects of some of the parameters such as temperature, pressure, particle size, steam rate, time of extraction etc., that might be affect the quality and yield of the essential oil extracts.

Essential oils can be extracted using a variety of methods, although some are not commonly used today. Currently, the most popular method of extraction is steam distillation, but as technological advances are made more efficient and economical methods are being developed. These include methods such as solvent extraction, supercritical fluid extraction, cold pressing and microwave extraction. The suitability of extraction method varies from plant to plant and there are significant differences in the capital and operational costs associated.

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

1.2. Statement of problem

The increasing importance of essential oils as pharmaceutical and aromatherapy aid besides their traditional role in cosmetics not only as potent ingredient but also as a fragrance donor has opened up wide opportunities for global marketing. Ethiopia is endowed with a diversity of flora. Most of which has remained unexploited. Its application in antioxidant, aromatherapy, perfumery and other related industries are limited due to lack of adequate research on the chemical and biological potential of its raw materials. Among these plants, which are not well known about their valuable uses, Thyme is one of the most useful plants and so far, its many uses are not discovered.

Currently Ethiopia is importing phenolic compounds or essential oil from abroad for chemical and pharmaceutical uses although the main constitute of Thyme leaves oil is used as a raw material in different chemical industries. Thyme leaves is considered one of the most important sources for the extraction of phenolic compounds with strong antioxidant activity. Thyme extracts, enriched in phenolic compounds are effective antioxidants due to their phenolic hydroxyl groups but they also possess plenty of other beneficial effects like flavor in cosmetics, antimicrobial, antiviral, anti-inflammatory, antitussive, antifungal, ant oxidative, antiseptic, and antispasmodic activities. They are also used for aromatherapy, a natural remedy to cure psychological stress and poor physical conditions, and are also used as food flavorings to be added to beverages, confectionery and other processed foods, and as cosmetic fragrances for perfume products or toiletries.

1.3. Objectives

1.3.1. General Objective

The general objective of the study was to extract and characterization essential oil from Thyme (Thymus schmperi L.) Leaves using steam distillation.

1.3.2. Specific Objectives

- To characterize the raw material or thyme leaves in terms of moisture content, ash content and crude fat content by proximate analysis
- To study the effective parameters of extraction time and particle size of thyme leaves on the extracted oil yield
- To determine the optimum conditions for the extraction of essential oil from thyme leaves
- To characterize the physico-chemical properties of Thyme leaves extracted essential oils.

1.4. Significance of the study

Ethiopia currently imports essential oil from abroad for the pharmaceutical, cosmetics industries and other purposes. This study will contribute to provide import substitution by enabling the development of small scale process plants to extract essential oil from thyme leaves using steam extraction methods due to the availability and cheap of raw material. The technology will be transferred to interested private sectors or any other agencies. In addition this study will seeks to a significant improvement from traditional methods to technological manufacturing of the oil for the use of thyme leaves extracts.

2. LITERATURE REVIEW

2.1. Introduction

Recently there has been an increase in demand for essential oils extracted from plant material. This can be attributed to the fact that essential oils have multifunctional properties and hence are playing an increasing role in the food, fragrance, agricultural and pharmaceutical industries (Kim et tl., 2005). 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 (Alfal, 2018). Alfal, 2018 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 & F.A, 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 K.SATISH, 2010).

2.2. Essential oils

Essential oils are volatile oil of plant origin. Essentially the oil is named the plant from which it is derived. The oil can be recovered from plants by various methods, chiefly among them are steam distillation (including water and steam distillation) expression, solvent extraction, effleurage and maceration. These are all physical processes. Essential oils are derived from rosemary, almond (bitter) bay, beryamond, clover eucalyptus, lavender, lemon, orange, thyme, peppermint and rove. Physical processes mentioned above isolate most of these. However, some of these oils such as bitter bay and certain rose oils are steam distilled only after some fermentation has occurred.

Essential oils are concentrated volatile aromatic compounds produced by plants - the easily evaporated essences that give plants their wonderful scents. Each of these complex precious liquids is tracted from a particular species of plant life. Each plant species originates in certain regions of the world, with particular environmental conditions and neighboring fauna and flora. Essential oils are frequently referred to as the “life force” of plants. Unlike fatty oils, these "essential" oils are volatile, highly concentrated, substances extracted from flowers, leaves, stems, roots, seeds, bark, resin or fruit rinds. The amount of essential oils found in these plants can be anywhere from 0.01 percent to 10 percent of the total. That's why tons of plant material are required for just a few hundred pounds of oil. These oils have potent antimicrobial factors, having wide range of therapeutic constituents. These oils are often used for their flavor and their therapeutic or odoriferous properties, in a wide selection of products such as foods, medicines, and cosmetics. Beware of imitations. Essential oils cannot be substituted with synthetics. Only pure oils contain a full spectrum of compounds that cheap imitations simply cannot duplicate (Al-hilphy, 2015).

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 (KUMAR K.SATISH, 2010).

The essence or aromas of plants are due to volatile or essential oils. Many of which have been valued since antiquity for their characteristic odors. The essential oils have characteristic fragrances and tastes. They are mixtures of known and unknown compounds. They may contain hydrocarbons, terpene alcohols, aldehydes, ketones, phenols and esters (Denston & T.C, 1939) .

2.3. Uniqueness of essential oils

In early work, the term "essential oils" was defined as the volatile oils obtained by the steam distillation of plants. This definition was clearly intended to make a distinction between "fatty oils" and the oils, which are easily volatile. Gradually with the advance of science came improvements in the methods of preparing the oils, and parallel with this development a better knowledge of the constituents of the oils was gained. It was found that the oils contain many classes of organic substances with varying volatility. Although a list of all the known oil components would include a variety of chemically unrelated compounds, it is possible to classify these into four main groups of essential oils (Guenther & E., 1960):

- ✓ Terpenes, related to isoprene
- ✓ Straight-chain compounds, not containing any side branches
- ✓ Benzene derivatives

- ✓ Miscellaneous

Essential oils are different from other oils by their properties:

A. Volatile

Essential oils are the volatile fragrant components from various indigenous and exotic plants which have been traded internationally for several centuries (KABIJBA, 2009). All true essential oils are secondary metabolites of plant products and in some instances the oil extracted from one part of the plant is different from that extracted from other parts (KABIJBA, 2009).

B. Aromatic

Essential oils are highly aromatic and therefore, many of the benefits can be obtain by simply inhaling them. This can be done by breathing in the fragrance from the bottle, or they can be diffused into the room.

Essential oils, when diffused, can be the best air filtration system in the world.

They will:

- ✓ Purify the air by removing metallic particles and toxins from the air
- ✓ Increase atmospheric oxygen
- ✓ Increase ozone and negative ions in the house, which inhibits bacterial growth
- ✓ Destroy mold, cigarettes and animal odors

Fill the air with a fresh, herbal aromatic scent (Becker, 2005)

C. Penetrating characteristics

The penetrating characteristic of essential oils greatly enhances their ability to be effective. Essential oils will penetrate into the body when applied to the skin. Essential oils rubbed into the feet will be distributed to every cell in the body in minutes. They will even penetrate a finger or toe nail to treat fungal infection underneath. Other vegetable oils do not have this propensity to penetrate (Becker, 2005)

D. High frequency

The effectiveness of essential oils is sometimes also described in terms of frequency. It has been reported that the human body has an electrical frequency and that much about a person's health can be determined by frequency. In 1992, Bruce Tainio of Tanio Technology, an independent division of Eastern State University in Cheny, Washington, built the first frequency monitor in the world. Tainio has determined that the average frequency of the human body during the day time is 62-68 MHz (a healthy body frequency is 62-72). When the frequency drops, the immune system is compromised. If the frequency drops to 58 MHz, cold and flu symptoms appear, at 55 MHz, diseases like Candida take hold, at 52 MHz, Epstein bar and at 42 MHz, cancer. According to Dr. Royal R. Rife, every disease has a frequency (Becker, 2005). He found that certain frequencies can prevent the development of disease and that others would destroy disease. Substances with higher frequency will destroy diseases of a lower frequency. The study of frequencies raises important questions, concerning the frequencies of substances we eat breath and absorb. Many pollutants lower healthy frequency. Processed canned food has a frequency of zero. Fresh produce has up to 27 MHz Essential oil start at 52 MHz and go as high as 320 MHz, which is the frequency of Rose oil. Clinical research shows that essential oils have the highest frequency of any natural substance known to man, creating an environment in which disease, bacteria, virus, fungus, etc., cannot live (KABIJBA, 2009).

2.4. 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, lavender), leaves (e.g. Eucalyptus, mint, Ocimum spp., lemongrass, jamrosa, Neem, Thyme), leaves and stems (e.g. geranium, patchouli, petit grain, 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, curcumaorris) and gums or oleoresin exudations (e.g. balsam of Peru, Myroxylonbalsa mum, storax, myrrh, benzoin) (Trieste, 2008).

Table 2.1: Source of natural essential oil

Leaves	Flowers	Peel	Seeds	Woods
Basil Bay leaf Cinnamon Eucalyptus Lemon grass Melaleuca Oregano Patchouli Peppermint Pine Rosemary Spearmint Tea Tree Wintergreen Thyme	Chamome Clary Sage Clove Geranium Hyssop Jasmine Lavender Manuka Marjoram Orange Rose Ylang-Ylang	Bergamot Grape fruit Lemon Lime Orange Tangerine	Almond Anise Celery Cumin Nutmeg Oil	Camphor Cedar Rosewood Sandal wood
Berries	**Bark**	**Resins**	**Rhizome**	**Root**
Allspice Juniper	Cassia Cinnamon	Frankincense Myrrh	Ginger	Valerian

2.5. Uses of Essential Oil

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

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

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

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

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

2.6. Review of Essential Oils from Thymus Species

2.6.1. Reviews on Different Species of Thymus Extracts

Different researchers worked on different species of the genera Thymus in Lamiaceae family for the purpose of different activities of the extracts by different extracting methods. Some of those works are mentioned below. Chemical composition and antimicrobial activity of the essential oil of wild Thymus vulgaris grown in South Jordan that isolated by hydro distillation shows that a total of forty-eight components of the oil representing more than 97% of the oil contents, monoterpenes being most abundant (about 85%) with thymol (37.05%), cis- dihydrocarvone (9.34%), carvacrol (8.45%), hydroxy-3-(3-methyl-2-butenyl)-3-cyclopenten-1-one (8.41%), p-cymene (5.73%), cis-sabinene (4.42%), z-isoeugenol (3.342%), and aroma dendrene (3.42%) as major constituents. The essential oil from Thymus vulgaris was found to be active against all the tested bacteria except in Pseudomonas aeruginosa. The high concentrations of thymol and carvacrol may account for its high activity against resistant bacteria (Al-shuneigat et al., 2014).

Essential oils isolated by hydro distillation from Thymus serpyllum, Thymus algeriensis and Thymus vulgaris in Serbia and examined by GC-MS. In total, 48 compounds were identified. Results showed that oxygenated monoterpenes are the major portion of all EOs samples, with highest content observed in T. algeriensis (74.61%), and similar content in T. speryllum and T. vulgaris (54.49% and 58.11%, respectively). Twenty nine compounds were identified in T. serpyllum oil, which accounts for 99.98% of the total oil. The major constituent of the oil was thymol (56.02%), followed by carvacrol (14.00%) and p-cymene (6.27%). GC-MS analysis of T.

algreriensis oil showed 45 compounds representing 99.64% of the total oil. Thymol was the main constituent (38.50%) followed by p-cymene, terpinene and bornyl acetate and borneol (8.91%, 7.19%, 7.03% and 6.07%, respectively). In the oil of T. vulgaris, 26 constituents represented 99.06% of the total oil, with thymol also being the major constituent (49.10%) along with p-cymene (20.01%). According to presented results it is obvious that the oils from all the three Thymus species belong to “thymol chemo type”. Many studies on the chemical composition of the oils from the plants belonging to the genus Thymus were conducted, including T. serpyllum, T. algeriensis and T. vulgaris (Stahl-Biskup & E., 1991)

The- study on the Essential oil composition of Thymus serpyllum (wild thyme, mother-of-thyme),a s-mall perennial shrub, is the dominant Thymus species in Northern and Central Europa by hydro distillation using a Daring-type apparatus. The plant has been cultivated for centuries, due to its importance in pharmacology and decorative horticulture. From the result of the study the maximum essential oil yield from leaves of Thymus serpyllum was from 0.45- 0.55% (w/w).

There was no significant effect of distillation time on essential oil content found. These results are in agreement with the previous results of Pióro-Jabrucka and Osińska who found that the essential oil content among T. serpyllum populations in Poland, varied in the range of 0.21–0.60% (w/w). The relative amounts of the volatile components identified in the essential oils are a total of 40 compounds representing 97.47 and 99.57% of the oil were identified after 2 h of distillation. Carvacrol was the main constituent of volatile oil (32.43 and 44.00%), followed by p-cymene (6.16 and 12.04%), β-caryophyllene (7.68 and 9.68%) and γ-terpinene (6.47 and 9.09%). Carvacrol methyl ether (4.97 and 8.63%) and (-)-β-bisabolene (3.70 and 4.29%) were identified in significant amounts. Similarly, 40 components representing 97.4 and 99.53% of the oil were identified after three hours of distillation. The major constituents of the oil were carvacrol (32.05 and 46.16%), p-cymene (4.80 and 13.46%), β-caryophyllene (8.18 and 9.56%), carvacrol methyl ether (4.98 and 8.31%), γ-terpinene (5.73 and 7.68%). Forty compounds (97.05 and 98.87% of total oil) were also identified after 4 h of distillation. Carvacrol was predominant component (30.90 and 44.08%). Other abundant constituents were p-cymene (4.51 and 12.08%), β-caryophyllene (8.68 and 10.75%), γ-terpinene (5.72 and 8.33%), carvacrol methyl ether (4.93 and 8.21%).In general, the major components identified in the oil of wild thyme obtained at different distillation times were similar. Although, the highest amount of carvacrol (32.05 and 46.16%) was noticed in the oil obtained after 3 h of distillation (Wesołowska et at., 2012).

2.7. Thyme plant

2.7.1. Botanical Aspect of Thyme

The genus Thymus (labiate) includes about 350 species worldwide and is widely distributed in temperate zones (Demissew., 1993). The leaves are opposite, grayish-green, entire, linear or elliptic, up to 15 mm long, tomentose beneath. The flowers are small, pale-purple or white, arranged in terminal inflorescences that may be dense or loose. Flowers appear since the beginning of summer until the end of autumn. The fruit is an ovoid smooth achene. The two species, T. schimperi Ronniger and T. serrulatus Hochst.ex Benth are endemic to the Ethiopian highlands growing on edges of roads, in open grassland, on bare rocks and on slopes, between 2200-4000 m altitudes. Both species are perennial herbs, woody at the base and 5-40 cm high. The inflorescence is commonly crowded into globose and oblong heads with pink corollas (Asfaw et al., 2000). As it is economically important aromatic species as much in the market of products, it presents a challenge to maintain the organoleuptic qualities during the cultivation process. The vegetative propagation is important because it provides the maintenance of the characteristics of the mother plant, standing out via stakes propagation, for the small quantity necessary for the speed obtain the seeding (K. D. et al. , 2006).

2.7.2. Overview of thyme in the world

Thyme is native to the western Mediterranean area, especially the south of Italy, from where it spread to almost every region. This plant is cultivated in almost every country, as an aromatic for culinary uses (especially in the south of France, Spain, Morocco and North America ("WHO Monogr. Sel. Med. plants," 1999).Thyme is largely distributed in temperate zones and is uncommon in the African tropics. Ethiopia has considerably abundant Lamiaceae family herb growing at different regions and possesses a variety of the wild growing species of this family. Many species belonging to different genera of the family Lamiaceae have been reported to found in different parts of the (Hailemariam & Emire, 2013).

Thymus vulgaris (T. vulgaris) or common thyme is a low growing herbaceous plant which sometimes becomes somewhat woody. It is native to southern Europe, where it is often cultivated as a culinary herb. It typically grows as a sub-shrub, between 15 and 20 cm tall (Mohsenipour & Hassanshahian, 2015).Thymus species are considered as medicinal plants due to their pharmacological and biological properties. In native medicine, flowering parts and leaves of

Thymus species have been extensively used as herbal tea, tonic, carminative, antitussive, and antiseptic as well as for reating colds (Mohsenipour & Hassanshahian, 2015).

2.7.3. Overview of thyme in Ethiopia

Thyme is an indigenous plant in Ethiopia and locally is called Tosign (in Amharic). It is among the commonly occurring thymus species that distributed in different regions of Ethiopia. Ethiopian thyme (Thymus schimperi) is a wild-growing perennial herb which is rich in medicinally important metabolites. It belongs to the family Lamiaceae to the tribe Mentheae within the subfamily Nepetoideae. Lamiacae includes about 236 genera, 7173 species native to Europe, and grown in the Mediterranean basin and northern Europe, as well as other parts of the world such as Asia, South America, and Australia (Morales, 2002). The Mediterranean region, in particular the West Mediterranean region, can be described as the center of origin of the genus (Hardman et al., 2002). Thymus is considered a well-defined genus, based on the morphological and chemical features of its species, and is widely distributed throughout the Old World. Two species, T. Schimperi Ronniger and T. serrulatusHochst.exBenth, both locally known as Tosign, are endemic species represented in Ethiopia. Both species are perennial herbs, woody at the base and 5-40 cm high (Harley, et al .,1998). T. schimperi has ovate to elliptic leaves with entire margins. It occurs in open grasslands, between bare rocks, on slopes and tops of mountains between 2250-4000 m.a.s.l (Asfaw & Demissew, 2009).It is also well known in Central, Eastern and Northern highlands of Ethiopia and is harvested and dried by people living close to the towns of Dinsho (Bale Zone) and Menz (North Showa Zone) and sold at local markets (Demissew., 1993).

In Ethiopia, the dried leaves of T. schimperi are also used in traditional medicine for the treatment of headache, inflammation, spasm, thrombosis, urinary retention, mental illness, eye disease, toothache, stomachache, earache, liver disease, gonorrhea, leprosy, lung TB, and acne and as car is (Asfaw & Demissew, 2009). Tea made by the herb in water is also recommended as a local medicinal remedy for respiratory problems, gastrointestinal disorders, and liver disease (Dawit Abebe, 1993).The dried leaves are also used to flavor tea, coffee and different kinds of stew (Jansen, 1981).

Figure 2.1: Thymus Schimperi L. (Thyme) plant

2.7.4. Leaves constituents

Thyme leaves are very small, usually 2.5 to 5 mm in length and vary considerably in shape and hair covering, depending on the cultivar, with each species having a slightly different scent. *T. schimperi* leaves are oval to oblong in shape and somewhat fleshy while the leaves are almost stalk less with margins curved inwards and highly aromatic. The fragrance of its leaves is the result of an essential oil, which give its flavoring value for culinary purposes, and is the source of its medicinal properties (C.Rey, 1992).

Herbal thyme contains about 2.5% but not less than 1.0% of volatile oil. The composition of the volatile oil fluctuates depending on the chemo type under consideration. The principal components of Herbal thyme are thymol and carvacrol (up to 64% of oil), along with linalool, p-cymol, cymene, hymene, α- pinene, luteolin, and 6-hydroxyluteolin glycosides, as well as di-, tri- and tetramethoxylated flavones, all substituted in the 6-position (for example 5,4-dihydroxy-6,7-dimethoxyflavone, 5,4-dihydroxy-6,7,3-trimethoxyflavone and its 8-methoxylated derivative 5,6,4-trihydroxy-7,8,3-trimethoxyflavone. There are several reports in the literature on the chemical composition of T. vulgaris oil. Most reports indicate thymol or carvacrol to be the major compounds in the oil (Geneva, 1999). Thymol and γ-terpinene are found to be the major constituents of T. vulgaris cultivated in Wondo Genet by the Essential Oil Research Center (E. Dagne et at., 1998). Investigation of the chemical compositions of the two thymus species in Ethiopia (T. schimperi Ronninger and T. serrulatus Hochst.ex Benth) was limited except for the volatile oil constituents by Asfaw et al. (Asfaw et al., 2000) and Dagne et al. (Dagne & Bisrat, 1998) for essential oil constituents.

The remedial potential of Thymus is due to the presence of flavonoids, thymol, carvacrol, eugenol, phenols, luteolin and tetramethoxylated. Its controls numerous valuable effects, such as, antispasmodic, bactericides, antiseptics, antioxidants, anthelmintic properties and has late been recommended as substitute as cancer prevention agent (Monira, A., 2012).

Thymol is one of the most important essential oils found in Thyme and known for its antiseptic and antifungal properties. It also contains other volatile oils such as carvacolo, geraneol and borneol (Moghtader M., 2012). Thymol (2-isopropyl-5-methylphenol) is monoterpenoid phenol, major compound put forth in Thymus species and different plants having a place with the Lamiaceae family. Carvacrol (5-isopropyl-2-methylphenol; mol. wt. 150.21) is also monoterpenoid phenol present in essential oil of Thymus species and many other aromatic herbs and spices For carvacrol many biological effects are reported including antithrombotic. Linalool is monoterpenes alcohol compound which constitutes as major volatile component in several aromatic plant species essential oil and also present in Thymus species (Hina J et al., 2013).

Thyme oils are very complex natural mixtures which can contain about 30 –60 components at quite different concentrations. Generally, these major components determine the biological properties of the essential oils (AA., 2014).

Table 2.2: Chemical composition of thyme essential oil

Chemical constituent	**Chemical constituent Biological activities**
Thymol	Antiseptic, antibacterial, antifungal and antioxidant properties.
Carvacrol	Antimicrobial, antithrombotic, anti-inflammatory, acetyl cholinesterase inhibitory properties.
Linalool	Antiviral effect, anti-inflammatory, antioxidant, anti-nociceptive as well as analgesic activity. Anti-carcinogenic,

Source: (Hina J et al., 2013) .

2.8. Thyme oil

Essential oils and extracts from Thymus plants exhibit antioxidant, antibacterial, antifungal, antiviral, cytotoxicity, anti-parasitic and other properties (Ait M’Barek et al., 2007). Oral treatment of coughs, upper respiratory infections, acute and chronic bronchitis, whooping cough, and catarrh are among the internal health problems that herbal medicines belonging to the genus thymus are used as treatment in traditional medicine (Basch et al., 2004). For external use, thyme is used as mouthwash to treat laryngitis, as anti-acne and anti-stomatitis agents, and in the topical treatment of minor injuries. Thyme is one of the best herbs to use as a cough and cold remedy, addressing all the cold symptoms in a holistic way. Thyme acts both as and expectorant to clear the lungs of congestion and as an antitussive, calming coughing spasms. Thyme infusion tea will settle the stomach, help for better sleep, soothe a sore throat, relieve aches and pains, and encourage the body to sweat, helping to eliminate toxins and bring down a fever. Drinking a warm thyme tea sweetened is a pleasant and tasty way to get these benefits. Thyme essential oil can also be used in room diffusers. This long list of medicinal actions is attributed mainly to the essential oil components, Thymol and carvacrol which are highly antiseptic. Thyme oil can be used to treat cuts and wounds to prevent and treat infections when diluted with a carrier oil (H. D. et al, 2015). Recent data suggest that thyme's essential oil, and its main component carvacrol, show anti-inflammatory effects that have been attributed to the four inhibition of inflammatory edema and leukocyte migration (Fachini-Queiroz et al., 2012).

Some plant extracts have been known as antimicrobials as well as antioxidants in food systems. Thyme is the one among the potential herbs for extracting natural antioxidants. Ethiopia has abundant wild thyme (Thymus Schimperi) to extract natural antioxidant and antimicrobial from this potential herb. Thyme leaves are d in Ethiopia extensively as spice/ additive to flavor a wide range of food and beverage products. However, antioxidant potential of this herb was not yet well fully studied and exploited properly to improve the shelf-stability of food products. Therefore, the purpose of the research work was to evaluate the total antioxidant activity of Thymus Schimperi extract using lipid oxidation inhibition system and determine its preservative effect on oil, butter and meat (Hailemariam & Emire, 2013).

2.9. Uses of thyme oil

2.9.1. Medicinal and Antiseptic characteristics

According to the World Health Organization the definition of traditional medicine may be summarized as the sum total of all the knowledge and practical, whether explicable or not used in diagnosis, prevention and elimination of physical, mental or social imbalance and relaying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in written. Traditional medicine might also be considered as a solid, amalgamation of dynamic know how and ancestral experience (Geneva, 2002).The volatile oil from thyme was found to contain p-cymene, γ-terpine, carvacrol, rosmarinic acid, eugenol and Thymol (Asfaw et al., 2000). The volatile oil not only has carminative action, but also antiseptic, antimicrobial and antifungal activities (Debebe et al., 1993). Thyme is prepared as infusion to treat spasmodic cough, laryngitis, bronchitis and urinary infections. It is also used as a decongestant, as a cholagoge, to reduce flatulence and to fight parasites. External uses of thyme include preparations to wash skin wounds or infections (Debebe et al., 1993). In the Ethiopian traditional medicine the plant has many medicinal applications. Some of the reported applications are for the treatment of gonorrhea, cough, inflammation, spasm, thrombosis, urinary retention, mental illness, eye disease, toothache, stomach problems, leprosy lung TB, acne and as caris (Abebe, Ayehu, & Geneva, n.d.).

Antimicrobial activity

Thyme essence – especially the phenolic components thymol and carvacrol – show antibacterial activity against gram-positive and gram-negative bacteria, mainly due to their effects on the bacterial membrane. Since thymol and carvacrol are eliminated through the respiratory tract, these compounds have respiratory antiseptic action. Because of its antibacterial activity, thyme is also useful as an antiseptic for the urinary tract, mouth and skin wounds. Furthermore, thymol and carvacrol are antimitotic agents, effective against Candida albicans. Thyme water extract showed significant in vitro inhibitory effects on the growth of Helicobacter pylori and its powerful urease activity. Sebum excess promotes the growth of certain microorganisms on the skin and the scalp. Such microbial flora imbalance is one of the factors causing dandruff and acne.Thyme antimicrobial activity is of great use to formulate cosmetic products aimed at the regulation of sebum hyper secretion and treatment of related disorders. Therefore, thyme extract is recommended to formulate cosmetic products with purifying and antiseptic activity (Thyme (Thymus Vulgaris L.), Thymol, 2009).

Antioxidant activity

The Thymol and carvacrol, present in thyme essence, as well as the flavonoids and other polyphenols are considered to be involved in the antioxidant activity. Rosmarinic acid, hydroxycinnamic derivatives and flavonoid compounds showed important in vitro antioxidant activity by inhibiting iron-induced superoxide anion formation and lipid peroxidation in microsomal and mitochondrial systems. Furthermore, the thymol present in the essential oil showed in vitro antioxidant activity by neutralizing the DPPH (diphenylpicrylhydrazyl) radical.

Aged rats, which had been fed on a diet including thyme since young age, showed high proportions of antioxidant enzymes such as superoxide dismutase in liver and heart, as compared with a control group. Therefore, thyme extract is recommended to formulate cosmetic products aimed at the protection of skin and hair integrity against oxidative processes ("Thyme (Thymus Vulgaris L.), Thymol&Thyme tech Lit.," 2009).

Anti-inflammatory activity

In vivo tests of thyme ethanol extract on rats showed anti-inflammatory and analgesic activities. These activities could be related to carvacrol and thymol, which showed inhibitory effects on the enzyme cyclooxygenase in animal models, as well as inhibitory effects on the complement and on nitric oxide synthesis. Carvacrol has inhibitory action on prostaglandin synthesis. This action supports the use of thyme in ointments and other preparations to treat muscle and joint pain. The rosmarinic acid in this extract also has anti-inflammatory activity. Topical applications of thyme essential oil are rubefacient and generate analgesia, beneficial in cases for bruises or sprains. Antimatory action has been reported for the phenol compounds in thyme extracts. Therefore, thyme extract is recommended to formulate cosmetic products with anti-irritant activity("Cosmetic composition for the treatment of hair and skin…," 2009).

Anti-viral property

Silke Nolkemper et al. conducted an experiment with aqueous extracts from species of the Lamiaceae family were examined for their antiviral activity against Herpes simplex virus (HSV). Extracts from thyme (Thymus vulgaris) has shown inhibitory activity against Herpes simplex virus type 1 (HSV-1), type 2 (HSV-2) and an acyclovir-resistant strain of HSV-1 was tested in vitro on RC-37 cells in a plaque reduction assay (Silke Nolkemper et al., 2006).

Insecticidal activity

The insecticidal activity of thyme volatile oil, thymol and carvacrol was evaluated in laboratory against completely different larval stages of lesser mealworm. The sooner and later larval stages were reared on diets containing one or two acetone solutions of tested compounds. Insecticidal activity of thyme volatile oil and pure monoterpenes against A. diaperinus larvae relied on the dose and age of larvae. The growth of younger larvae was considerably affected, whereas those of older larval stage was less influenced and only by pure oil components. In young larvae the application 1% thyme oil, thymol and carvacrol, caused mortality of 50.0, 86.67 and 85%, respectively (Reddy & Biotech, 2016).

2.9.2. Food condiment

The fresh or dried leaves of both species are used locally as condiments in the preparation of chills powder, stew, bread and tea. Thyme has many uses: in chicken broth or stuffing; in clam chowder and marinades for meats or fish; in sauces; with onions, carrots or peas; in egg dishes with other sweet herbs; even in a baked apple dessert. The flavor can be captured in oils or butter. The caraway-scented form (or chemo type) of Thymus herb has a historical association with roast beef (baron of beef). Lemon thyme, T. xcitriodorus, is recommended for fish, for tea, and for salad dressings, or anywhere milder thyme is desired (Demsew & N. Asfaw, 1994).

2.9.3. Fragrance

Thyme is a culinary herb used in a wide range of cuisines. In French cuisine it is commonly used with meat dishes. Mediterranean and Asian cuisine also use fresh and dried thyme in sauces. The fastest growing market for thyme oil is cosmetics and in aromatherapy (Lawrence, 2002). It is also used as a topical treatment and in toothpastes. Various thyme species are also grown as ornamentals (Baker & Grant, 1813).

2.10. Factors affecting yield and quality of thyme essential oils

The yield and quality of essential oils have been known to vary due to a number of factors.

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.

2.10.1. Mode of distillation

Technique for the distillation should be chosen on basis of oil boiling point and nature of herb as the heat content and temperature of steam can alter the distillation characteristics.

2.10.2. Improper Design of Equipment

Improper designing of tank, condenser, or separators can lead to loss of oils 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.

2.10.3. Material of construction of equipment's

Essential oils which are corrosive in nature should be preferably distilled in stills made of resistant materials like Aluminum, Copper or Stainless Steel.

2.10.4. Condition of raw material

Condition of 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, dried, 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.

2.10.5. Filling of raw material / steam distribution

Improper loading of the herb may result in steam channeling causing incomplete distillation. Operating parameters like steam injection rate, inlet pressure, condensate temperature. Proper control of injection rates and pressure in boiler-operated units is necessary to optimize the temperature of extraction and for maximal yield. Temperature of condensate should not be too high as this can result in oil loss due to evaporation.

2.10.6. Distillation time

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

2.10.7. Pre-condition of tank and equipment's

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 (KABIJBA, 2009) .

2.10.8. 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.

2.10.9. 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.

2.11. Methods of essential oil extraction

Essential oils can be extracted using a variety of methods, although some are not commonly used today. The specific extraction method employed is dependent upon the plant material to be distilled and the desired end product. The essential oils from aromatic plants are for the most part volatile and thus, lend themselves to several methods of extraction such as steam distillation, solvent extraction, supercritical fluid extraction, etc.

2.11.1. 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 (Rao & Pandey, n.d.)

2.11.2. 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 (Al., 2014).

2.11.3. 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 (KUMAR K.SATISH, 2010).

2.11.4. 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 K.SATISH, 2010).

2.11.5. Super Critical CO2 Extraction

Supercritical CO2 extraction (SCO2) 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 (KUMAR K.SATISH, 2010).

2.11.6. 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 (KABIJBA, 2009).

2.11.7. 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 K.SATISH, 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 thyme which has high essential oil content. Such thyme 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 CO2 in a two-stage separation system. An inexpensive thermal expansion procedure for supercritical fluid delivery has been developed. The identification of components will performed by gas chromatography and mass spectrometry (GC±MS). 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.

2.11.8. Steam Distillation

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, 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 K.SATISH, 2010).

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.

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 K.SATISH, 2010).

2.11.8.1. 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 K.SATISH, 2010).

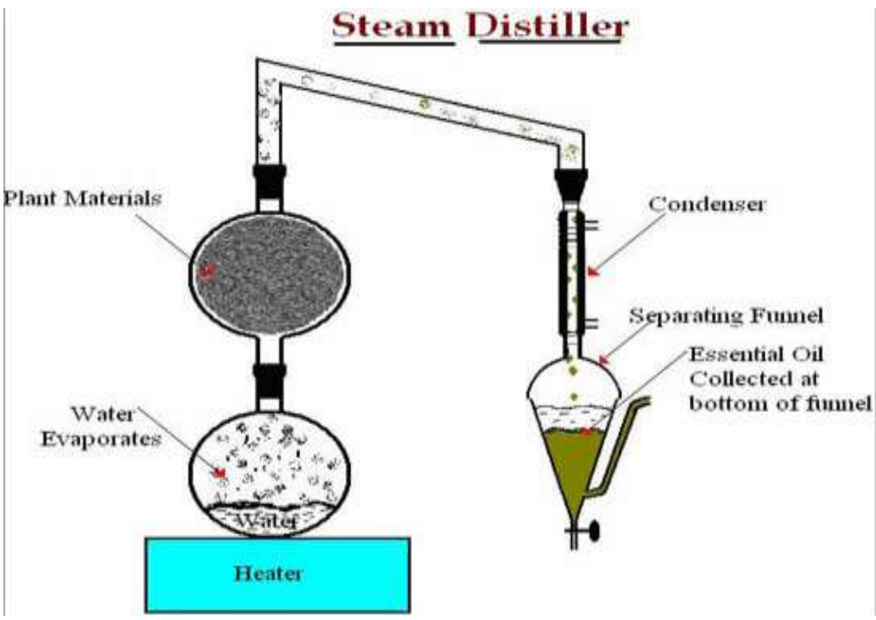

Figure 2.2: Steam distillation process with a separate boiler for extraction of thyme oil

2.12. 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 (D. & 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 four 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.

- **Length of distillation operation**

To specify time for distillation operation you must consider whether the herbs (species) are fresh, faded, or dry. It would take additional time for distillation if the herb is faded than if it is fresh. Leaves take less time than seeds because leaves are thinner than seeds and cells are more concentrated in leaves than seeds (Guenther & E., 1960).

2.12.1. Physical properties of essential oils

- **Specific gravity Specific**

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 & E., 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 odor 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.

2.12.2. Chemical properties of essential oils

In general, essential oils consist of chemical compounds that have hydrogen, carbon, and oxygen as their building blocks. These can be subdivided into two groups: the hydrocarbons, which are made up almost exclusively of terpenes (monoterpenes, sesquiterpenes, and diterpenes); and the oxygenated compound (mainly esters, aldehydes, ketones, alcohols, phenols) and oxides; acids, lactones, sulphat and nitrogen compounds are sometimes also present.

- **Aldehydes**

Citral, citronellal, and neural are important aldehydes found notably in lemon scented oils such as Melissa, lemongrass, lemon verbena, citronella, etc. Aldehydes in general have a sedative effect; citral has antiseptic properties.

- **Phenols**

These tend to have a bactericidal and strongly stimulating effect, but can be skin irritants. Common phenols include eugenol (found in clove and West India bay), thymol (found in thyme), carvacrol (found in oregano and savoury).

- **Alcohols**

Common terpene hydrocarbons include limonene (antiviral, found in 90 % of citrus oils) and pinene (antiseptic, found in high proportions in pine and turpentine oils). Sesquiterpenes have outstanding antiory and bactericidal properties.

- **Ketones**

Some of the most common toxic constituents are ketones, such as thujone found in mugwort, tansy, sage and wormwood; and pulegone found in pennyroyal and buchu. Non-toxic ketones include jasmine (in Jasmine) and fen hone (in fennel oil).

- **Oxides**

By far the most important oxide is cineol (or eucalyptol). It has an expectorant effect, and is well known as the principal constituent of eucalyptus oil. It is also found in a wide range of other oils, especially those of a camphoraceous nature such as rosemary, bay laurel, tea tree, and cajuput.

- **Esters**

Probably the most widespread group found in essential oils, which includes linalyl acetate (found in bergamot, clary sage, and lavender) and geranyl acetate (found in sweet marjoram). They are characteristically fungicidal and sedative, often having a fruity aroma.

- **Alcohols**

These compounds have good antiseptic and antiviral properties with an uplifting quality; they are also generally non-toxic. Among the most common terpene alcohols are linalool (in rosewood, linaloe, and lavender), citronellol (in rose, lemon, eucalyptus and geranium) and geraniol (found in palmarosa); also borneol, methol, terpineol, nerol, farnesol, vetiver, benzyl alcohol, and cedrol (KABIJBA, 2009).

3. MATERIALS AND METHODS

3.1. Material and Equipment

The materials and equipment used during the experiment work were thyme leaves, plastic bag, test tube, crucibles, beaker, oven, cross beater mill, sieve, electronic balance, steam distillation set up, flask, separating funnel, Anhydrous sodium sulfate, filter paper, heat mantel, thermometer, muffle furnace, desiccator, pH meter, viscometer, Pycnometer, spectrometer, Polari meter and Gc-Ms. On the other hand chemicals needed for characterization of thyme oil were, Potassium hydroxide, ethanol, sodium hydroxide, phenolphthalein, oxalic acid, chloroform, iodine bromide solution, potassium iodide and sodium thiosulphate. All the chemical and reagents were purchased and obtained from School of Chemical and Bioengineering of Addis Ababa Institute of Technology, Center of Food Science and Nutrition as well as the Chemistry Department, Addis Ababa University.

3.2. Framework of the Research

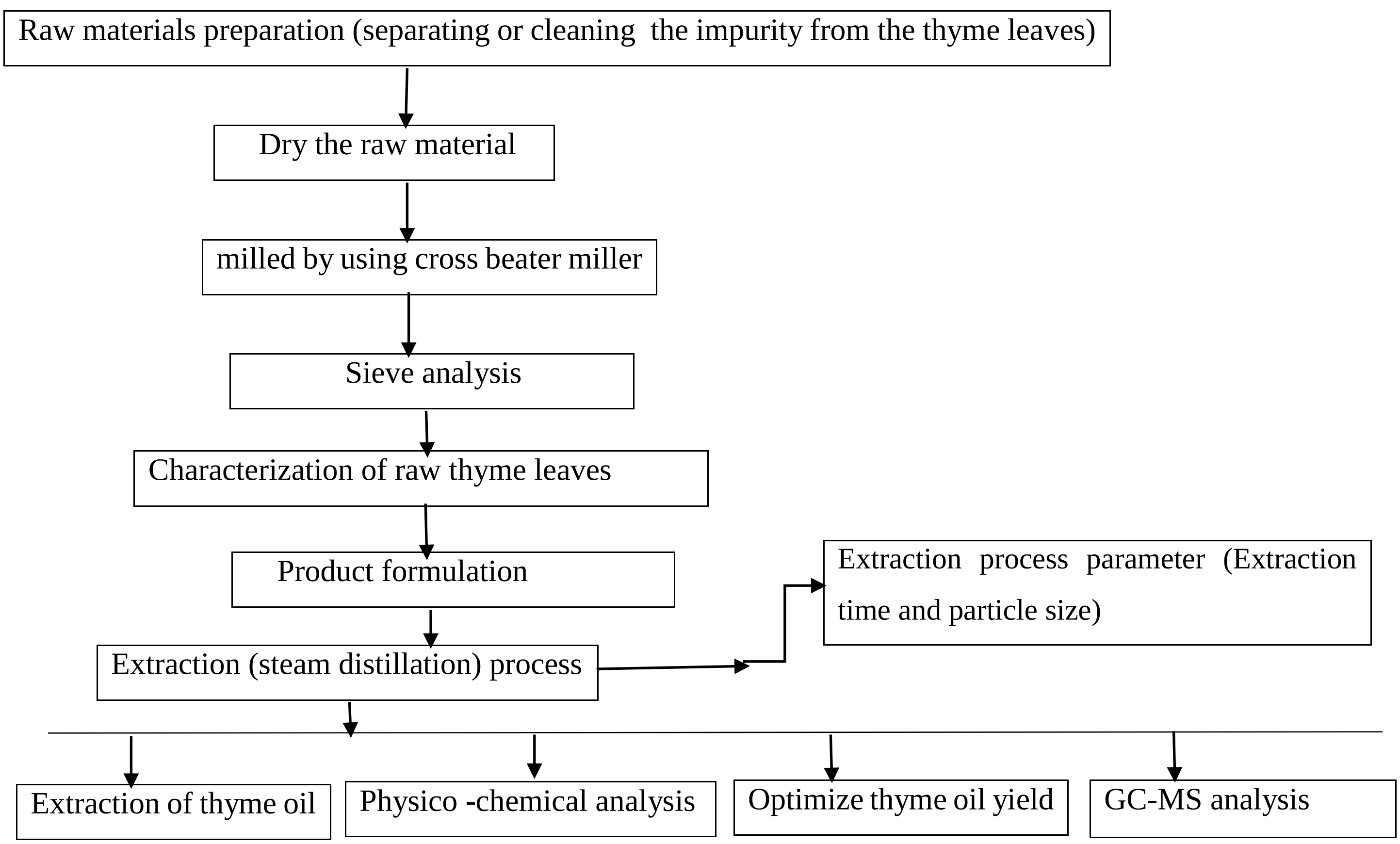

Figure 3.1: Framework of the research

3.3. Methodology

3.3.1. Raw Material preparation

Thyme samples or leaves were collect from the growing fields of Tarmaber which is 180km far away from Addis Ababa, Ethiopia. The dust, grass and other unwanted materials were removed by hand in order to obtain the pure thyme leaves. The thyme leaves was collected as fresh leaves then sun drying were used to dry the leaves. The sun dried thyme leaves was packed in plastic bags and taken to Addis Ababa University, Technology Institute, Food Chemistry and Analysis Laboratory for further analyses. The moisture content of the sample was determined by using oven drying method at 105°C for 24 hours.

3.3.2. Size reduction and Sieve analysis of the Thyme leaves

The moisture content was removed by sun drying and air drying, the dried leaves of Thyme was milled in cross beater miller with a size of 2 mm in thermal laboratory of AAiT and then the sample was shaken using vibrating shaker for 6 minutes with amplitude of 10mm and 60rpm to get the required particle size of the plant materials. The sieve size was arranged in descending order of mesh size 0.5 mm, 1 mm, 1.5 mm and 2 mm. This was aimed to investigate the effect of particles size on yield. This particular size was selected because literature reveled that to have a higher yield of essential oil particle size should be less than 5mm and higher than 0.2mm (Henry, 1983).

Figure 3.2: Cross beater mill and Sieving analysis

Figure 3.3: thyme leaves before size reduction and after size reduction

3.3.3. Characterization of raw material or thyme leaves

Proximate analysis was calculated based on the procedure of Association of Official Analytical Chemist (AOAC., 2000) to determine the moisture content, ash content and crude fat content. The procedures and equations are shown below in detail.

- **Moisture content determination of the Thyme leaves**

Moisture was determined according to (AOAC., 2000) Method by oven drying method. 5 gram of well-mixed sample was accurately weighed in clean, dried crucible and the weight of both recorded. The crucible was placed in an oven set at 105oC for 24 hours and allowed to dry overnight. Then the crucible with sample were removed and placed in the desiccators for 30 min to cool. After cooling, it was weighed again. The percentage moisture was calculated by using the following formula

$$\text{Moisture content } (\%) = \frac{W2 - W3}{W2 - W1} X100 \qquad 3.1$$

Where: W1 = Weight of crucible (g)

W2 = Weight of crucible and weight of sample as received (g)

W3=Weight of crucible and weight of dried sample (g)

- **Ash content determination of the Thyme leaves**

For the determination of ash content according to (AOAC., 2000), clean empty crucible was placed in a muffle furnace at 575 o C for an hour, cooled in desiccators and then weight of empty crucible was noted. 5 gram of sample was taken in crucible and reweighed. The sample was ignited over a burner with the help of blowpipe, until it is charred. Then the crucible was placed in muffle furnace at 575oC for 3 hours. The appearances of gray white ash indicate complete oxidation of all organic matter in the sample. After ashing furnace was switch off. The crucible was cooled in desiccators and weighed. Percentage ash content was calculated by following formula:

$$\text{Ash content (\%)} = \frac{W3 - W1}{W2 - W1} X100 \qquad 3.2$$

Where, W1 = Weight of crucible

W2 = Weight of crucible + Weight of sample before ashing

W3 = Weight of crucible +Weight of sample after ashing

- **Crude fat content determination of the Thyme leaves**

Dry extraction method for fat determination was implied It consisted of extracting dry sample with some organic solvent, since all the fat materials e.g. fats, phospholipids, sterols, fatty acids, carotenoids, pigments, chlorophyll etc. are extracted together therefore, the results are frequently referred to as crude fat. Fats were determined by intermittent soxhlet extraction apparatus. Crude fat was determined by hexane extract method using Soxhlet apparatus.

Seven grams of the dried thyme leaves powder was subjected to fat estimation by refluxing for 3 hours using Soxlet extractor and 250ml of hexane as the extracting solvent. After the extraction time was end the round-bottom flask containing a mixture of fat and hexane solvent was detached from the Soxlet extractor. The filter paper with the sample remove from the soxlet extractor and the filter paper and its content were heated to 105^{0}C in an oven for 2 houres and later cooled in a desiccator. The weight of the crude fat was determined and expressed as percentage fat using the formula:

$$\text{Crude fat content (\%)} = \frac{W1 - W2}{W} X100 \qquad 3.3$$

Where, W = Weight of sample

W1 = Weight of filter paper + Weight of sample before extraction

W2 = Weight of filter paper + Weight of sample after extraction

3.3.4. Factors selection and Response variable of the process

The dependent (response) variable for this experiment was the yield of essential oil extracted from the leaves of thyme. The yield of the extraction process was dependent on several conditions. Among these many factors, the extraction process parameters were the main one. This is why this paper was mainly focused on the extraction process factors or parameters. There were many factors which affect the extraction of the essential oils from the given plant materials. These are temperature, distillation time, pressure, particle size, method of extraction and so on. Distillation time and particle size were taken for this specific study. Extraction time and particle size of the plant material had four levels of each. The distillation time considered was in levels of 60 minute, 90 minute, 120 minute and 150 minute. The second independent variable which was particle size was investigated in particle size of 0.5 mm, 1 mm, 1.4 mm and 2 mm. If the number of independent variable was several for the given experiment then, the conclusion drawn from the analysis would be incorrect. As a result, as much as possible large numbers of factors in the experiment are not advisable that was why in this experiment considered only the factors that would affect the product highly. This does not mean that the rest of the independent variable does not affect the product both quantitatively and qualitatively. It is obvious that there are so many predictors that were affect the response variable but taking the major one is the great task of the experimenter.

3.4. Extraction method of Thyme using steam distillation

3.4.1. Experimental Setup

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

The modified experimental setup of the apparatus that was used for the extraction of the essential oil from the given plant materials by means of steam distillation method for this specific study is shown in figure 3.4 below. The experiment was conducted in a Clevenger's type apparatus. Apparatus consist of one round bottom flask of 1000 ml which is connected with another conical flask which holds raw materials. The top flask is connected with condenser through the connecter. The separating funnel is used for the separation of essential oil and water.

Figure 3.4: Experimental set-up of steam distillation

3.4.2. Components of the extraction plant are:

I. Boiler

The dictionary defines steam as "the gas or vapor into which liquid water is changed by boiling, especially when used under pressure as a source of energy". The primary-objective of a boiler operation is to provide a continuous supply of steam at atmospheric pressure and constant temperature are suitable for the experimental work.

II. Extraction chamber

The extraction chamber is simply a fixed bed where the hot steam helps to release the aromatic molecules from the plant material since the steam forces open the pockets in which the oils are kept in the plant material. The molecules of these volatile oils then escape from the plant material and evaporate into the steam. Proper loading 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.

3.4.3. Experimental Procedure

The extraction of thyme leaves oil was conducted using steam distillation method with different extraction time and with different particle size ranges at constant temperature of 105°C and atmospheric pressure of 1atm. For the current experimental work, 200 g of thyme leaves was used at four different extraction time intervals; 60 min, 90 min, 120 min and 150 min with different particle size of 0.5 mm, 1 mm, 1.4 mm and 2mm without replications to get maximum yield. Before the operation started the column was cleaned then the thyme 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. thyme oil.

Raw 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 then the oil is collected.

3.4.4. Isolation of the Oil Extracts from the Steam Condensation

After the steam produced by heating the distilled water in the round-bottomed flask with the heating mantle, it was allowed to make contact and pass through the plant materials in the conical flask, the essential oil with the steam flows into the condenser through the connector glass which able to collect and measure the liquids that were returned from the condenser and measure the volume of both the liquids with the scale written on it as measuring cylinder which was connected to the conical flask that holds the plant materials. After the completion of each batch of the extraction process, the pale yellow liquid essential oil extracts and water condensate (hydrosol) are known to have different densities and also form an immiscible two liquid phase mixture at room temperature conditions. The separation of essential oils from the condensate hence utilizes this density and immiscibility advantages for the two liquids to be isolated from each other. The pale yellow liquid essential oil with a pungent scent was collected with a separated test tube for each experimental runs and put in a refrigerator until the next analysis. Finally, the essential oils in the distillate were dried over anhydrous sodium sulphat (Na2SO4) and stored in refrigerator at 4°C.

Figure 3.5: separating process of essential oil and water mixture

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

The total oil yield depends on the operating parameters. Designed experiments were carried out to map quantitative effects of these parameters. The yield of extraction is calculated from the relation between the essential oil mass obtained and the raw material mass used in the extraction. The mean yield (%) table is constructed from oil extracted in relation to the amount of sample used in each run. In this work 200grams of the plant materials was used for each batch and yield (%) is calculated using equation 3.4. The extraction parameters were very important to produce both a good quality and a reasonable amount of essential oils. As a result, to achieve these objectives different parameters with different levels were used during the extraction process. The parameters were distillation time and particle size of the plant materials (leaves of thyme).

$$\text{yield of oil}(\%) = \frac{\text{wieghet of oil}}{\text{wieghet of thyme leave}} \text{x}100 \qquad (3.4)$$

3.6. Characterization of the extracted Thyme essential oil

3.6.1. Physical properties of Thyme essential oil

The physical properties of the extracted essential oil are such as, specific gravity, refractive index, flash point, evaporation residue, pH value, boiling point etc. All the parameters are determined according to the method of European Pharmacopeia (“European Pharmacopoeia Commission,” 2000).

- **Specific gravity determination**

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 & E., 1960).The density of the thyme leaves oil was determined by using tube (Pycnometer). A clean and dry empty Pycnometer of 25ml capacity was weighed (W) and then the Pycnometer was filled with the oil, stopper inserted and reweighed to give (W1). The oil was substituted by water after wash and dries the Pycnometer and weight to give (W2).Then, the specific gravity was calculated using the following formula:

$$\mathrm{Sp.gr} = \frac{\mathrm{w1 - w}}{\mathrm{w2 - w}} \qquad (3.5)$$

- **Determination of pH Value**

2 g of the Thyme oil was poured into a clean dry 25ml beaker and13ml of hot distilled water was added in to the beaker and stirred slowly. Then cooled in a cold-water bath to 25^0C.The pH electrode was standardized with buffer solution then the electrode was immersed into the sample finally the pH value was read and record.

- **Determination of Refractive Index**

The refractive index of a substance is the ratio of the speed of light in a vacuum to the speed of light in the substance. For practical measurements, including this method, the scales of standard instruments indicate refractive indices with respect to air rather than vacuum. Refractive Index of the sample was determined indirect method using spectrometer (He-Ne Leather). It was ascertained that the temperature of the spectrometer is 25^0C at room temperature, wave number 632.8nm and the prism was clean and completely dry. The prism with sample was placed in the cuvet and adjusted the instrument and light to obtain the most distinct reading and then determined the refractive index using Brewster's angle in Addis Ababa University (Arat kilo) Department of

Physics laboratory. Brewster's angle formula was used to calculate refractive indices of the interface the set up shows in the following figures.

$$N = \tan(\theta_b) \quad (3.6)$$

Where: N = refractive index and

θ_b = Angle of refraction, the refractive index of air is 1.

The index of refraction of oils is characteristic within certain limits for each kind of oil. It is related to the degree of saturation particularly to the extent of conjugation, but it is affected by other factors such as free fatty acid content, oxidation, and heat treatment.

Figure 3.6: Refractive index measurement

- **Determination of 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, or to the left. The degree of rotation and the direction are important indicators of purity. The optical rotation of the thyme essential was measured using Polari meter with a 2 dm length. Polari meter tube where the angle of rotation in degrees are read at 25^0C using D-line of polarized sodium light. Polari meter with a standard 10 mm tube (cuvet) and a sodium vapor lamp was adjusted and volatile oil sample and instrument temperature was 25^0C.The Polari meter tube was filled with oil and wipe off excess oil on the exterior. Then the tube is placed in the Polari meter and the analyzer is slowly turned until both halves of the field were viewed through the telescope, reading was observed to clockwise (+) and anti-clock wise (-

).The specific optical rotation was measure in Addis Ababa University (Arat Kilo) in Department of Physics laboratory by the set up shows below in the figure.

Figure 3.7: Optical rotation measurement

- **Determination of dynamic viscosity of the thyme oil**

A sample of 35ml oil was measured and fed to a sample holder of the viscometer. A sensor of the viscometer was immersed to the oil and then a dynamic viscosity of oil was displayed on the viscometer screen at a temperature of 21.7^0C. Figure 3.8: Shows that the measurement of dynamic viscosity of thyme oil using viscometer.

Figure 3.8: Dynamic Viscosity measurements

- **Kinematic viscosity of thyme essential oil**

Kinematic viscosity of the essential oil was measured indirectly using viscometer which is available in laboratory of school of chemical and bio engineering. Firstly, to determine the dynamic viscosity of thyme essential oil by using viscometer. Then the kinematic viscometer was calculated by the following formula.

$$\text{Kinematic viscosity } = \frac{\text{Dynamic Viscosity of thyme oil}}{\text{Density of thyme oil}} \quad (3.7)$$

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

In the case of isolates and synthetics, the boiling range is an important criterion of purity. 25 ml of thyme 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 3.9.

Figure 3.9: Boiling point measurement of thyme oil

- **Evaporation residue of thyme oil**

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 odor of oil as it volatilizes during the heating. 35 grams of thyme oil was poured into Borosilicate glass, put it on heater then thermometer was inserted into the Borosilicate glass until it reads temperature of 100^0C to determine volatile matter.

$$\text{percentage of the oil} = \frac{W2}{W1} x100 \quad (3.8)$$

Where:

W_1 is weight before evaporation and

W_2 is weight after evaporation

- **Flash point of thyme oil**

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. The flash point of thyme oil was measure by adding 20 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 thyme oil**

Most essential oils are only slightly soluble in water and are miscible with absolute alcohol. The solubility of oil may change with age. Solubility of thyme oil has been chacked by adding 4 grams of sample into 20 milliliter of alcohol and water.

3.6.2. Chemical properties of Thyme oil

Chemical properties of thyme oil are such as acid value, saponification value, ester value and iodine number. The composition and quality of the thyme essential oil that can be identify by using Gc-Ms. All the parameters are determined according to the method of European Pharmacopeia ("European Pharmacopoeia Commission," 2000).

- **Acid value determinations**

Acid value is the mass of potassium hydroxide or sodium hydroxide in mg that is required to neutralize one gram of chemical substance. The acid number is a measure of the amount of carboxylic acid groups in a chemical compound. The acid number is used to quantify the amount of acid present, in thyme leaves essential oil sample. Acid value was determined according to the method of European Pharmacopeia. 2.5gram of thyme 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. After that, the acid value is determined using the following equations:

$$\text{Acid value} = \frac{5.61x(\text{number of ml of N NaoH})}{\text{weight of sampel in gram}} \tag{3.9}$$

Where N is Normality

- **Saponification value determination**

Saponification value represents the number of milligrams of potassium hydroxide or sodium hydroxide required to saponify 1g essential oil under the condition specified. Saponification value was calculated by European Pharmacopeia standard procedure. 1.5gram of thyme essential oil and 10 ml of 70% ethanol were accurately measure and both poured in to beaker and then 10 ml of 2.5 Normality KOH solutions was added in to the beaker. Then, this procedure was the same for preparation of the blank experiment which was also performed omitting the oil. The mixture was refluxed for 25 minutes then cooled. The KOH was titrated with standard 0.5Normality of oxalic acid by adding 2-3 drops of phenolphthalein indicator until became colorless. After completed the process, the saponification value was calculated by using the following equation:

$$\text{Saponification value} = \frac{56(V1 - V2)}{2xW} \tag{3.10}$$

Where W is the weight of oil, V1 is the volume of 0.5 Normality of oxalic acid for blank;

V2 is 0.5 Normality of oxalic acid for sample.

- **Iodine number determination**

0.2 gram of thyme oil and 10 ml of chloroform were poured in to 250 conical flask. Then 25 ml of iodo bromide solution was added to the conical flask and allowed to stand for 30 minutes in dark. Again 30 ml of 1 N potassium iodide and 100ml 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:

$$\text{Iodine number} = 12.69\frac{(V2 - V1)}{W} \tag{3.11}$$

Where: W is the weight of sample,

V1 is the number of ml of thiosulphate consumed by the blank; and

V2 is the number of ml of thiosulphate consumed by the test sample

- **Ester value, content of esters and combined alcohols**

The determination of the ester content is of great importance in the evaluation of many essential oils. Most esters, which occur as normal constituents of essential oils, are esters of monobasic acids. Ester value may be defined as "the number of milligrams of potassium hydroxide required to neutralize the acids liberated by the hydrolysis of esters present in 1g of the essential oil materials". The value of ester can be calculated as follow:

$$\text{Ester value} = \text{Saponification value} - \text{acid value} \quad (3.12)$$

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

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

3.7.1. Identification of Components of the essential oil using GC-MS

The identification and analysis of the thyme leaves essential oil components were carried out by gas chromatography mass spectrometry (GC-MS). The operating conditions (refer to table 3.1) were applied to identify the components in the thyme leaves oil extracted by steam distillation method for this thesis. The chemical compositions of the oils were analyzed using GC-MS (Agilent; GC: 7890B Series II; MS: 5977A VL MSD) using an Agilent technology 5977A mass spectroscopy ion trap detector at the laboratory of Addis Ababa town JIJE LABO GLASS P.L.C. or JIJE Analytical Testing Service Laboratory. The sample was injected through the auto sampler and analyzed with DB-5MS, 30 m × 0.25 mm x 0.25μm column. The oven temperature was programmed as follows; 60 °C hold for 0 minutes, then 5 °C/min to 110°, by 2 ^{o}C/min to 119 °C hold for 6 min, at 4 °C/min to 140 ^{o}C hold for 3 min, 15°C/min to 200°C hold for 5 min, at 5°C/min to 235 ^{o}C, 10°C/min to 250°C hold for 2 min and 2 min solvent delays. Helium gas was used as a carrier gas at a flow rate or Column flow of 1ml/min. The mass spectrometry Transfer line temperature was 280°C and recorded at 70ev with scanning from 50 to 550amu at 3.35sec. The identification of the compounds was based on the retention index and retention time. The chemical structures of isolated the compounds were elucidated using mass spectroscopy (MS).

Table 3.1: GC-MS Method for chemical analysis of the Thyme leaves oil samples

Method	
Type	7890B GC
Column	DB-5MS(30m*0.25mm,coating thickness 0.25µm and Agilent technology 5977A mass spectroscopy ion trap detector)
Carrier gas	Helium
Linear Velocity	1ml/min
Detector Type	mass spectroscopy
Injection Volume	1Ml
Injection Type	Split ratio: 1:00
Transfer line temperature	280 °C
Injector or Inlet temperature	260 °C
Oven Temperature	60 °C hold for 0 minutes, then 5 °C/min to 110°, by 2 °C/min to 119 °C hold for 6 min, at 4 °C/min to 140 °C hold for 3 min, 15°C/min to 200°C hold for 5 min, at 5°C/min to 235 °C, 10°C/min to 250°C hold for 2 min.
Ion Source temp	230°C
Acquisition mode	Scan, 50–550 amu
Comparison of.	Retention Indices

3.8. Design of the Experiment

Design Expert Software (version 7.0.0) was used for analysis of the data; General Factorial Design had been chosen than other design methods. Because it enables evaluation of the effect of several process parameters and their interactions on the response variable, at each combination of the process setting, the yields of thymus schimperi essential oil were recorded. The soft-ware also used to characterize how the significant factors affects the response (for optimization purposes) screen out insignificant factors and identify significant factors. For steam distillation it was two factors and four levels for each. Extraction time with four levels (60min, 90min, 120min and 150min) and particle size four levels (0.5mm, 1mm, 1.5mm and 2mm.). This design of the experiment helps us to Optimize of Process Parameters. Significance of the result was set from analysis of variance.

Table 3.2: Time and particle size that can affect extraction process

Factors	Number of levels	Levels of the factor
Particle size (mm)	4	0.5
		1
		1.5
		2
Extraction time (minutes)	4	60
		90
		120
		150

4. RESULTS AND DISCUSSION

4.1. Characterization of raw thyme leaves

The experimental values of various physico-chemical characteristics of the raw thyme leaves are given in table 4.1: below.

For this study the raw material or thyme leaves moisture content of average sample was obtained (13.02%) which is slightly higher than the moisture content reported in literature of approximately (12%) (CXS, 2017). This is because of so many factors such as difference in geographical locations, production techniques, and plant collection time.

Ash content indicates the level of essential or non-essential mineral elements in the sample. The average ash content of the this study obtained was (11%), which is lower than the literature reported value of (12%) (CXS, 2017).

The average crude fat content in this study was found (2.14%) and slightly lower than the value reported by an earlier researcher (Hagos Hailu Kassegn, 2016) which is (2.75%).

Table 4.1: Laboratory result of raw thyme leaves characterization

Number	Properties	Average value	Other literatures values	Reference
1	Moisture content (% dry weight)	13.0%	12%	(CXS, 2017).
2	Ash content (% dry weight)	11%	12%	(CXS, 2017).
3	Fat content (% dry weight)	2.14%	2.75%	(Hagos Hailu , 2016)

4.2. Determination of percent yield

The experimental design selected for this study is the general factorial design and the response variable is the of thymus schimperi Essential oil. Four levels for particle size and four levels for extraction time and two factors general factorial design was applied. And then 16 experiments were performed for this research work. Optimization of extraction of essential oil by the classical method involves changing particle size and extraction time while temperature and pressure are at a fixed level. To overcome this difficulty, the experimental design software can be employed to investigate the extraction parameters of thymus schimperi essential oil.

Designed experiments were carried out to diagram quantitative effects of the two parameters these were time of extraction and particle size of the sample. The yields of the extraction is calculated from the relation between the essential oil mass obtained and the raw material mass used in the extraction ,The yield curve has constructed from essential oil mass extracted in relation to the amount of extraction time and particle size. In this study, 200g of thymus schimperi were used for each experiment. The pressure in the boiler for consecutive tests was set at one atm and at constant temperature 105^0C. When is much more pressures would have resulted and higher temperatures that would destroy components in the essential oil. Design Expert 7.0.0 Software program was used for regression and graphical analysis of the data obtained by incorporating the sequential F-test, lack-of-fit test and other adequacy measures for selecting the best model the yield (quantities) of the oil extracts from 200g of thymus schimperi. Table 4.2. Shows that the run order, extraction time, particle size and yield found. The maximum extraction of thyme leaves oil was 2.12% at particle size 0.5 mm for the extraction time of 150 minutes and the minimum thyme leaves oil yield 1.18% obtained was at maximum particle size 2mm and minimum extraction time 60 minutes. This result agrees to the average essential oil yield of thyme leaves ranges 1 to 3% of dry yield (Khalil et al., 2017). The result of the current yield was calculated by Equation 3.1.

Table 4.2: Experimental results of Thyme oil yield extracted using steam distillation

Std	Run	Factor 1 A: Particle size mm	Factor 2 B:Extraction time Minutes	Response 1 Yield %
1	6	0.50	60.00	1.72
2	8	1.00	60.00	1.70
3	13	1.50	60.00	1.54
4	5	2.00	60.00	1.18
5	15	0.50	90.00	1.77
6	16	1.00	90.00	1.73
7	11	1.50	90.00	1.70
8	9	2.00	90.00	1.45
9	3	0.50	120.00	2.05
10	1	1.00	120.00	1.88
11	10	1.5.0	120.00	1.81
12	4	2.00	120.00	1.52
13	14	0.50	150.00	2.12

14	7	1.00	150.00	1.89
15	12	1.50	150.00	2.03
16	2	2.00	150.00	1.67

4.3. Statically Data Analysis

4.3.1. Analysis of Variance for the Data (ANOVA)

The table 4.3 below shows analysis of variance (ANOVA) obtained from design expert software, which tells as the significance of different factors. The analysis of variance (ANOVA) would be used to test these hypotheses. The analysis of variance (ANOVA) can be computed manually but it can be also computed by using different software. For this cause design expert 7.0.0 software were used and the output of the data was discussed. The following table 4.3 shows the analysis of variance for the data.

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

Source	Sum of squares	Degree of freedom	Mean Square	F-Value	P value Prob > F
Model	0.75	2	0.38	42.32	< 0.0001
A	0.40	1	0.40	44.79	< 0.0001
B	0.35	1	0.35	39.85	< 0.0001
AB	2.704E-003	1	2.704E-003	0.29	0.6014
Residual	0.12	13	8.877E-003		
Cor Total	0.87	15			

The Model F-value of 42.32 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-particle size and B-extraction time are significant model terms of the two varieties. Values greater than 0.1000 indicate the model terms are not significant. The P-value of AB (interaction factor) is 0.6014 > P-value thus; the interactions of particle size and extraction time are not significant in the model terms. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The model adequacy can also be checked using the paramtes given in table shown below.

Table 4.4: Model adequacy measures

Std. Dev.	0.097	R-Squared	0.8700
Mean	1.73	Adj R-Squared	0.8375
C.V.	5.59	Pred R-Squared	0.7401
PRESS	0.23	Adeq Precision	16.964

Coefficient of variation, 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 point 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 Adequate precision, this is a signal to disturbance ratio due to random error, presented in Table 4.4, above, are used to decide whether the model can be used or not. And from the above table 4.4, it can be obserbed that The "Pred R-Squared" of 0.7401 is in reasonable agreement with the "Adj R-Squared" of 0.8375. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 16.964 indicates an adequate signal. This model can be used to navigate the design space. The R-square vlue of 0.87 indicats that 87% of the total varion in the yield of the oil was described by the developed model. Only 0.13% of the total varion was not described by the devolped model. More ever, the the effectives of the model indescribing the variaon in the yield of the oil can also be checked using the coefficient of regression and corrosponding high and low convidence interval.

4.3.2. The Regression model Equation

Design-expert was applied to analyze results on the extraction process and a first order regression equation, with the interaction terms, of the form, the final model equation in terms of coded factor was presented by equations representing the variation of percentage oil yield of thyme oil with independent factors. Generally, The regression model equation both in terms of the coded and actual factors are given below.

Final Equation in Terms of Coded Factors:

%Yield (Y) = +1.74-0.21* A+0.2* B+0.023 * A * B…………………………………………[A]

Where: A is particle size level and B is extraction time

The Final Equation in Terms of Actual Factors

%Yield(Y)=+1.71300-0.35480*A+3.56667E-003*B+6.93333E-004*A*B…………………...[B]

Where: A is particle size level and B is extraction time

From Equation (A) and (B) it canbe obserbed that the yield of the oil was linear function of the both particle size and extraction time. The yield was linearly increased with increasing extraction time but linearly decreased with increased plarticle size.

4.3.3. Model Adequacy Check

Before the model implemented for different applications it should satisfy different criteria such as the normal distribution of the error term (residuals) and the residuals verses predicted value of the model data. Unless the model should not satisfy these criteria it is not advisable to use the model for different purpose. The adequacy of the model which describes the variation of the the yield of the essential oil can also be checked using diagnotic plots as diccussed below.

Diagnatic plots

The most commonly dignatic plots used for assesing the adequacy of analysiof variance includes;

1. Normal probability plot of the studentized residuals to check for normality of residuals.
2. Studentized residuals versus predicted values to check for constant error.

Table 4.5: Difference between the experimental (actual) value and predicated value

Stan. Order	Actual value	Predicted Value	Residuals
1	1.72	1.77	-0.050
2	1.70	1.61	0.086
3	1.54	1.46	0.083
4	1.18	1.30	-0.12
5	1.77	1.89	-0.12
6	1.73	1.74	-0.012
7	1.70	1.60	0.10
8	1.45	1.45	8.000E-004
9	2.05	2.01	0.045
10	1.88	1.87	0.011
11	1.81	1.73	0.076
12	1.52	1.60	-0.078
13	2.12	2.12	-2.600E-003
14	1.89	2.00	-0.11
15	2.03	1.87	0.16
16	1.67	1.75	-0.076

a) Normal probablity plot of residuals

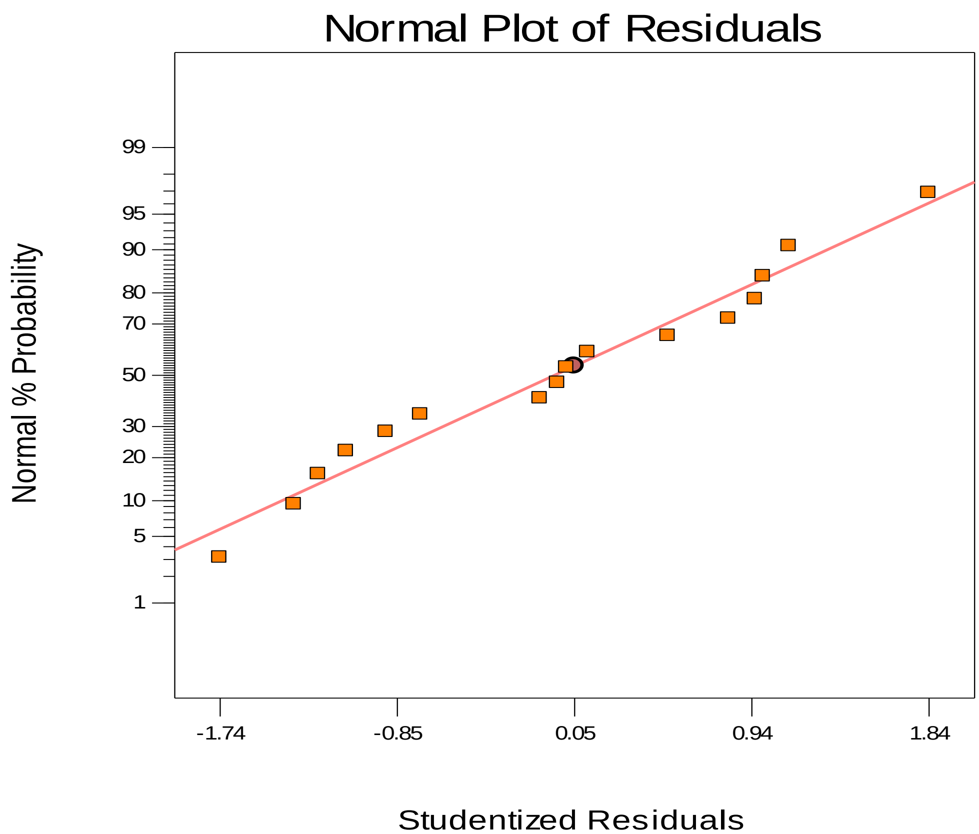

Figure 4.1: Normal probability plot of the Studentized residuals

The normal probability plot of the residuals in figure 4.1 above showed that, it follows almost a straight line which implies residuals are approximately normally distributed that satisfy the most important assumption in any model.

b) Residuals vs. Predicted values

The plot of residuals verses predicted value in figure 4.2 below showed that the residuals did not follow any pattern that means no serious deviation from the assumptions. The randomness of the plot implies that the model is adequate and there is no indication of a severe problem. Therefore, the model equation may fit to design the model.

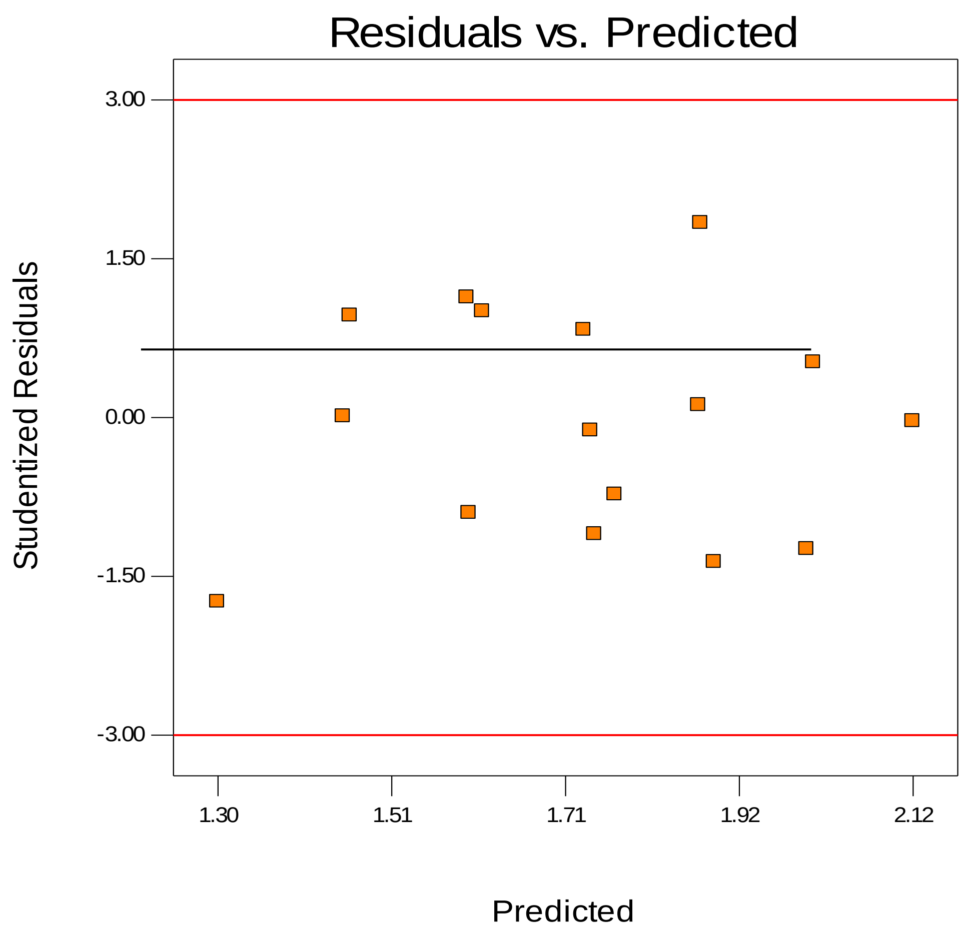

Figure 4.2: Residual value versus predicated value of percentage oil yield

Adequacy precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 16.964 indicates an adequate signal. This model can be used to navigate the design space.

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

Established on the analysis of variance thyme leaves oil yield was significantly affected by various process variables. Significant individual process variables that affect the yield were particle size and extraction time. These factors have influence on the yield of thyme leaves essential oil.

4.4.1. Effect of extraction time

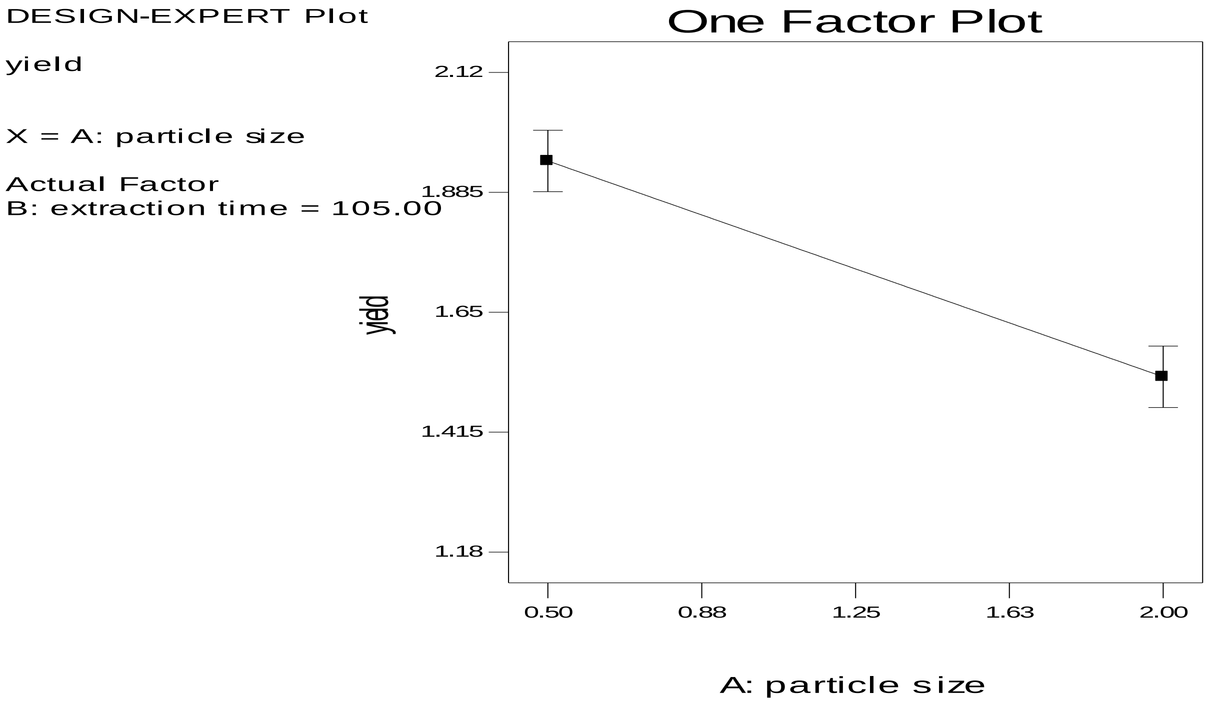

Figure 4.3: Effect of particle size on the yield of extracted oil

The particle size plays the biggest role on yield of thyme leaves essential oil (Fig 4.3). It is quite clear that there is an increase in the oil yield as the particle size decreased and an increase in the particle size results in a drop in oil yield. Thus, the percentage essential oil yield was inversely related to the particle size i.e. smaller size gives high yield while larger particle size results a lower yield. The reason is that larger particles have smaller surface area of contact and larger distance to solvent entrance. So, in the figure 4.3 it was observed that the minimum particle size has had maximum oil yield whereas the maximum particle size has had minimum oil yield.

4.4.2. Effect of extraction time on the percent yield of Thyme oil

Extraction time plays a great role on the percentage yield of thyme leaves oil using steam distillation. In figure 4.4 Shows below 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 150 minutes.

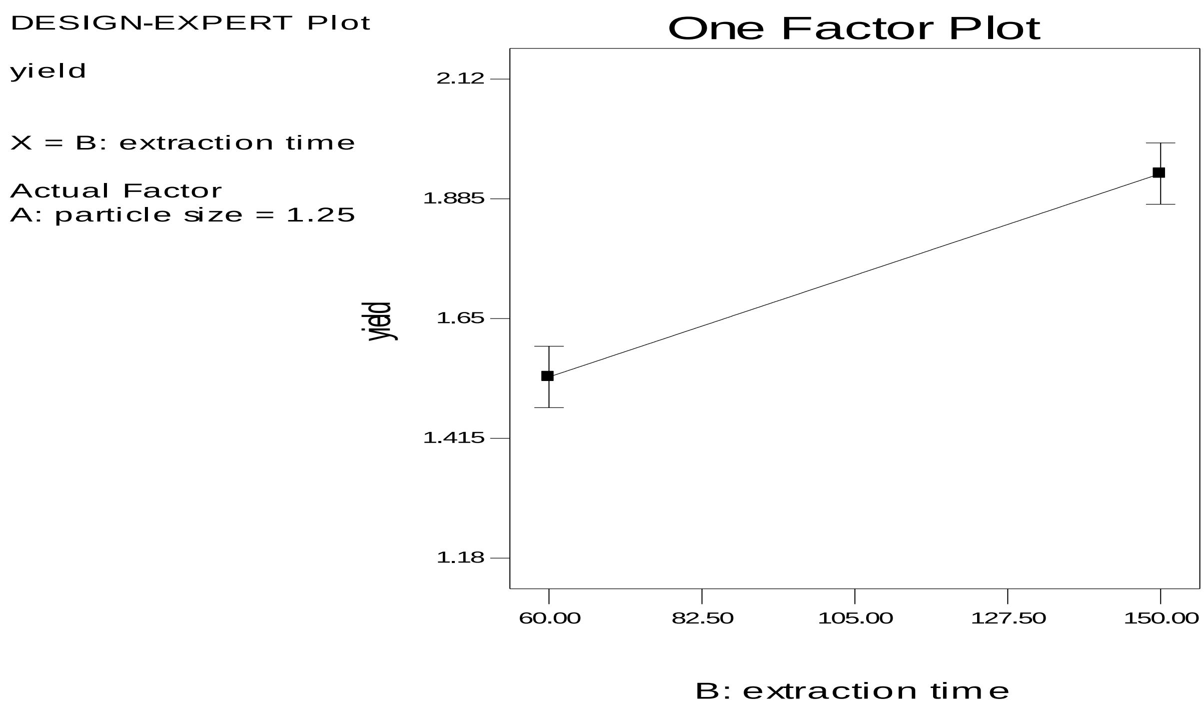

Figure 4.4: Effect of extraction time on the yield of extracted oil

4.5. Interaction effect of factors on the yield of extracted oil

From design, expert software the output of interaction effect between extracted time and particle size is shown below figure. Interactions graph shows that there is no interaction among each factor. This shows us an increment in time will increase the quantity of thyme leave oil extracted. Extraction beyond 150 minutes didn’t gives a significant change on oil yield. On Figure below it was observed that as time increase and particle size decrease yield increase.

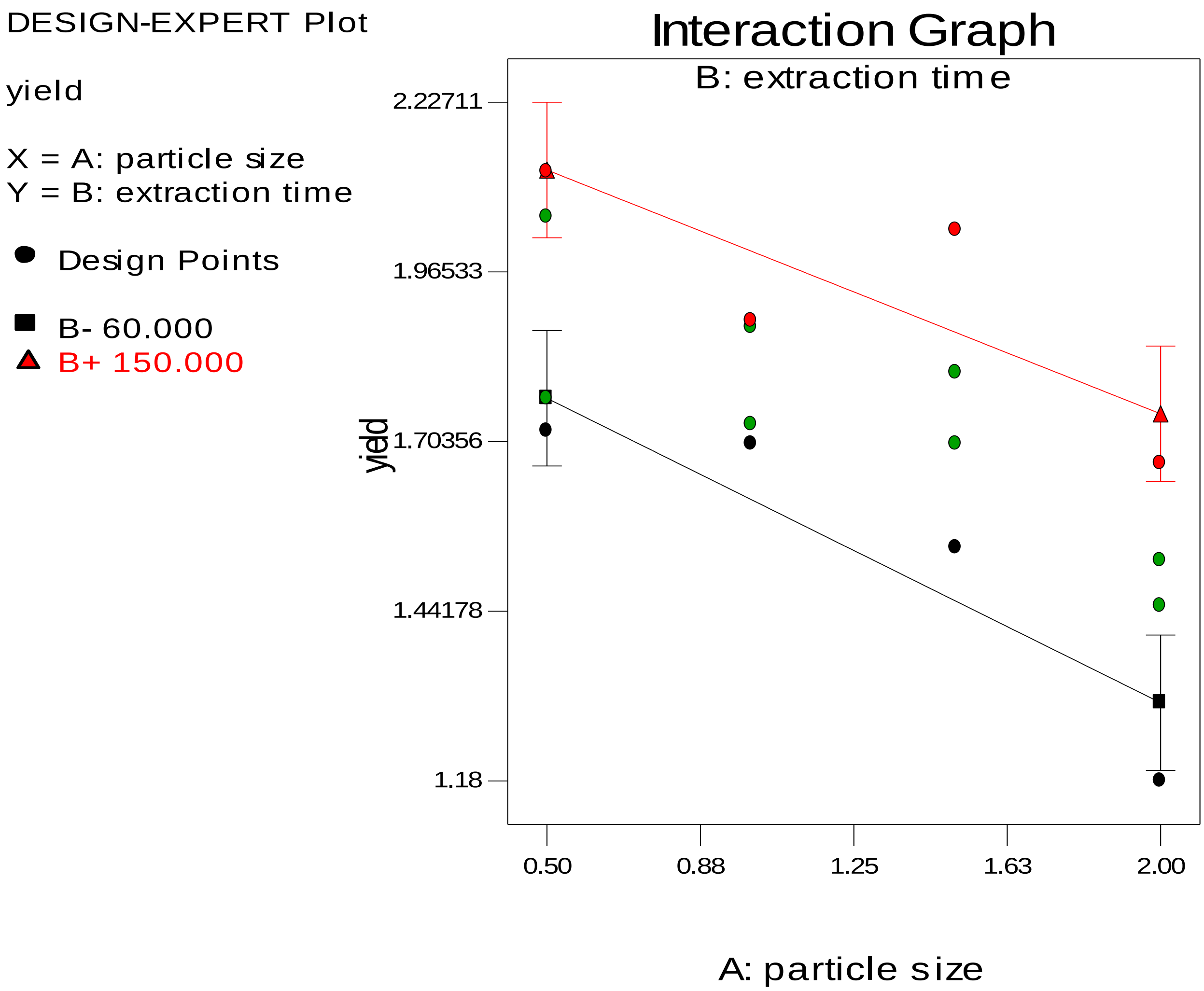

Figure 4.5: Effects of extraction time, particle size and their interactions on thyme oil yield

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

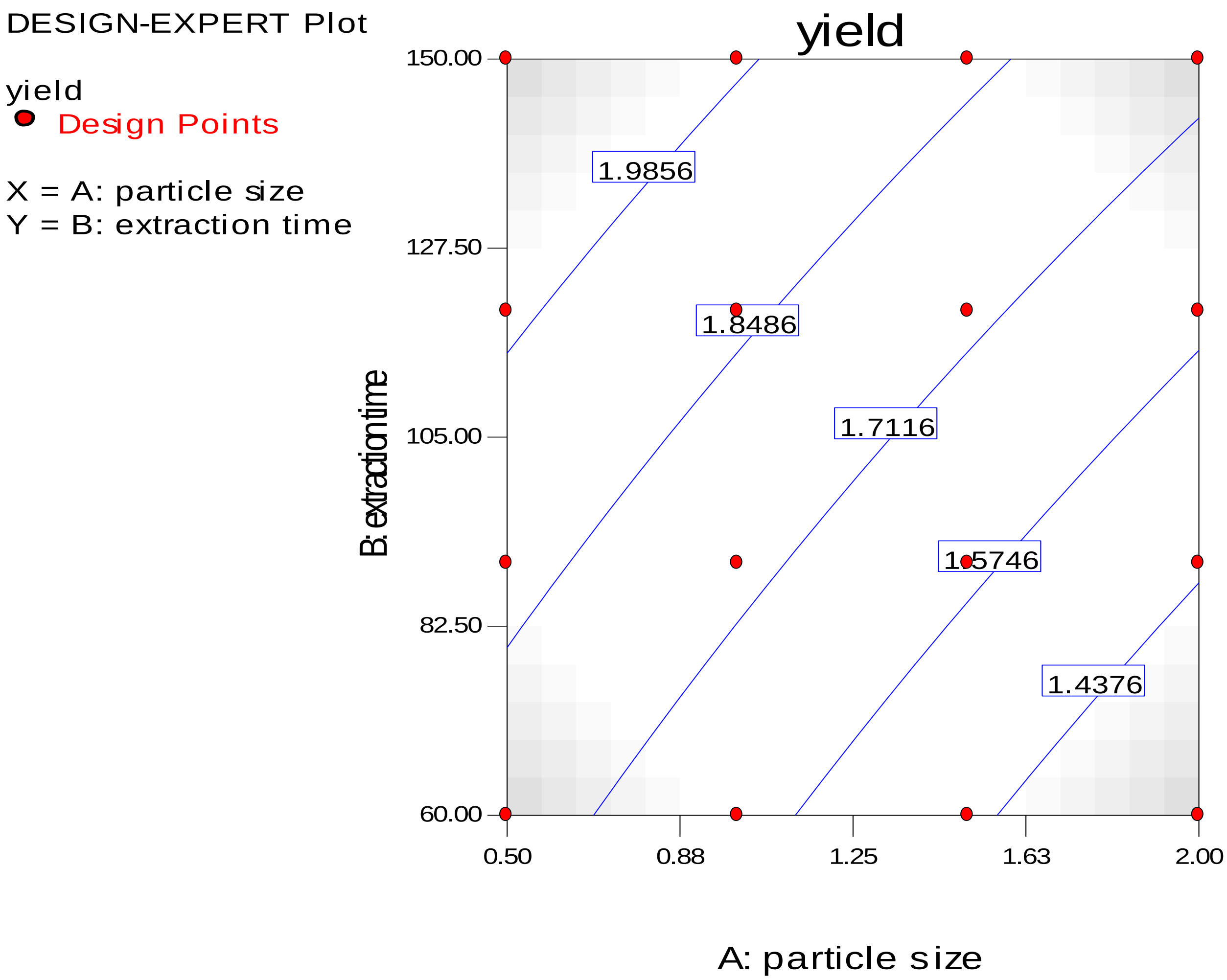

Figure 4.6 contour plot of extraction time, particle size and yield.

The figure below shows that the yield increase as extraction time increase and particle size decrease on three dimensional surface planes.

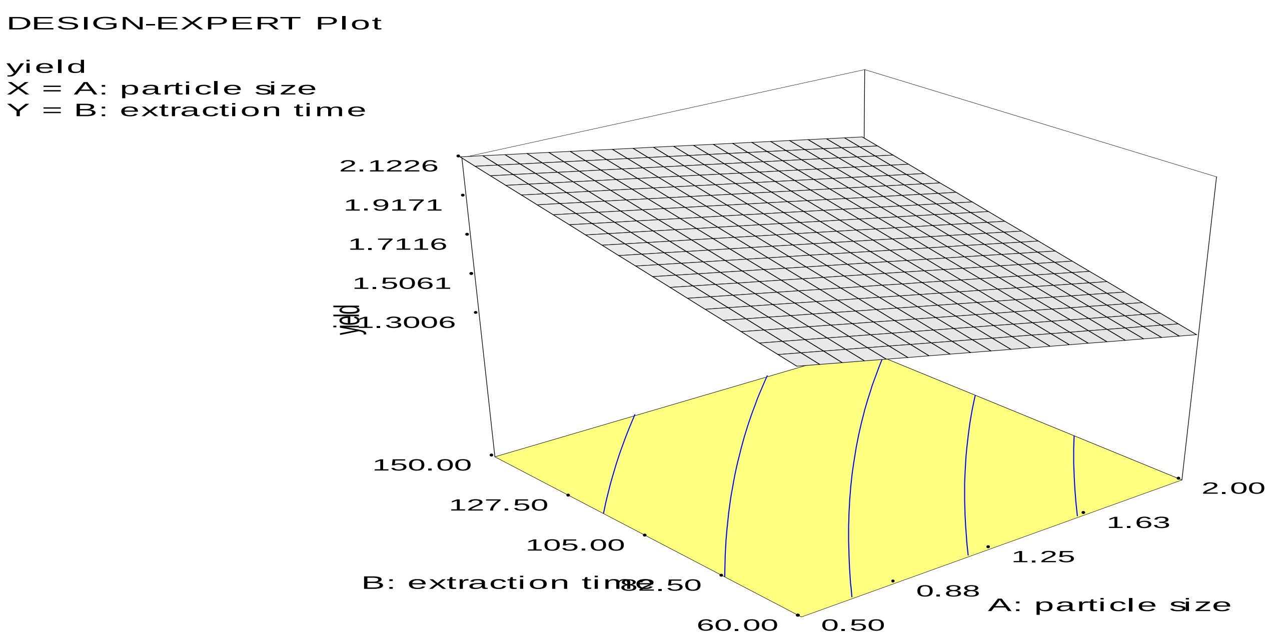

Figure 4.7: 3D surface plot of extraction time, particle size and yield.

4.6. Optimization of Thyme Oil Extract Using Steam Distillation.

The goal of optimization is to maximize economic benefit or increasing the yield of thyme leaves essential oil by minimizing process cost from un wanted expenditure during importing the thyme leaves essential oil from other country. To investigate the optimum values of thyme leaves oil extraction by using steam distillation are summarized as follows.

Constraints

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Particle size	is in range	0.5	2	1	1	3
Extraction time	is in range	60	150	1	1	3
Yield	maximize	1.18	2.12	1	1	3

Table 4.6: Optimum possible solutions

Number	particle size	extraction time	Yield	Desirability	
1	0.51	149.75	2.12018	1.000	Selected
2	0.53	150.00	2.11614	0.996	
3	0.64	150.00	2.0887	0.967	

Using optimization functional in design expert software 7.0.0, the predicted maximum yield one solutions founds at the 0.51 mm particle size and with the extraction time149.75 minutes as shown in table 4.6 above. 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 thyme oil yield of 2.12018 % was found at a time 149.75 minutes, particle size 0.51 mm and the value of desirability obtained was1.00 %.

4.7. Physical properties

- **Specific gravity calculation of thyme essential oil**

Weight of Pycnometer (W) at 25^0C = 16.5728 gram

Weight of Pycnometer with thyme oil (W1) at 25^0C = 42.0672 gram

Weight of Pycnometer with water (W2) at 25^0C = 43.4476 gram

$$\mathrm{Sp.gr} = \frac{\mathrm{w1} - \mathrm{w}}{\mathrm{w2} - \mathrm{w}}$$

$$= \frac{(42.0672-16.5728)g}{(43.4476-16.5728)g}$$

$$= 0.9486$$

Density of thyme essential oil = Specific gravity × density of water

$$= 0.9486 \times 1 g/ml = 0.94 g/ml$$

- **Refractive index of thyme oil**

Refractive Index or index of refraction of a substance or medium was a measure of the speed of light in that medium expressed as speed of light in vacuum relative to that in the considered medium determined by spectrometer using Brewster's angle.it is the angle at which the glare off the gels surface was the most polarized, thus allowing for a maximum amount of light to be removed by the polarizer. Setup in AAU (Arat Kilo) physics Department. The refractive index was measure at wave length of 632.8 Nano meter (He Ne leser wave length) calculated as:

$N = \tan({}^{\theta}b)$

${}^{\theta}b$ = Brewster angle or Rotation with sample – initial Rotation

= (253-196) = 57.00 degree

$N = \tan(57^0) = 1.54$

- **Determination of pH Value for thyme oil**

The pH value of thyme leaves essential oil determined by pH electrode as measuring experimental procedure was stated in method and its value was recorded as 6.20.

- **Optical rotation of thyme oil**

Polari meter tube containing essential oil was placed in the trough of the instrument between polarizer and analyzer. Care was taken in filling the tube to avoid the entrance of air bubble which could disturb the rotation of light. Analyzer was slowly turned until both the halves of the field were viewed through the telescope. The direction of rotation was determined. Optical rotation of thyme oil was measured by Polari meter in AAU (Arat Kilo) Department of Physics. The Polari meter recorded was 0.42 degree in 1cm path length.

- **Dynamic Viscosity of thyme oil**

Dynamic Viscosity (μ) of thyme oil was measured by viscometer at 21.7^{0}C in chemical engineering department laboratory. The value was 6.02 mili Pascal second (mpas).

- **Kinematic viscosity of thyme essential oil**

Dynamic viscosity of essential oil, which was read from vibro-viscometer, was 6.02 mpas at temperature 21.7^{0}C.Then Substituting the dynamic viscosity of the oil which is 6.02 maps = 6.02×10-3 kg/m.s and density = 948.6 kg/m3 in to equation (3.4) gives the kinematic viscosity.

$$\text{Kinematic viscosity } = \frac{6.02 \times 10 - 3\,\frac{kg}{m}.s}{948.6\,\frac{kg}{\text{m3}}}$$

$$= 6.346 \frac{m2}{s}$$

- **Boiling point of thyme oil**

Thyme essential oil boiling point was measure by using thermometer and by followed the procedure identified on the method and its value was recorded as 187^0C.

- **Evaporation residue of thyme oil**

The percentage of thyme essential oil was calculated as below formula.

$$\text{percentage of the oil} = \frac{\text{W2}}{\text{W1}} \text{x}100$$

Where: W_1 is weight before evaporation $(35g)$ and

W_2 is weight after evaporation $(34.2g)$

$$= \frac{34.2\ g}{35g} \times 100\% \ = 97.71\%$$

- **Flash point of thyme oil**

The flash point of thyme leaves essential oil was measured or recorde 62^0C by using thermometer.

- **Solubility of thyme oil**

The thyme oil was soluble in alcohol, fixed oils and insoluble in water. Generally the physical properties of thyme oil extracted using steam distillation were summarized below in the Table 4.7.

Table 4.7: Physical properties of thyme essential oil.

Physical properties	Results	Unit	Thyme and Thyme Oil Profile (Baker & Grant, 1813)
pH at 25^0C	6.2	-	Solution neutral to litmus test
Specific gravity at 25^0C	0.948 g/cm3	-	0.916 g/cm3
Refractive index at 25^0C	1.54	-	-
Optical rotation at 25^0C	0.42	Degree	-5° to + 5°
Dynamic Viscosity at 21.7^0C	6.02	Mpas	-
Flash point	62	^{0}C	60˚C
Boiling point	187	^{0}C	190˚C
Evaporation residue	97.71	%	-
Color	Pale yellow to reddish brown	-	Colorless to reddish brown

Smell	pleasant smell	-	Thyme smell, usually considered Pleasant
Solubility in alcohol	Soluble	-	Solubility in alcohol
Solubility in Water	Insoluble	-	Insolubility in Water

4.8. Chemical characteristics of thyme essential oil

➢ **Acid value determination**

The acid value of was determined by using equation.3.9 as follows:

$$\text{Acid value} = \frac{5.61\text{x(number of ml of N NaoH)}}{\text{weight of sampel in gram}}$$

$$= \frac{5.61\text{x}0.1\text{x}20.5\text{ml}}{2.5\text{g}}$$

$$= 5.049\, {}^{ml}/_{g}$$

➢ **Saponification value determination**

The saponification of thyme oil was calculated by using equation.3.10.

$$\text{Saponification value} = \frac{56(\text{V1} - \text{V2})}{2\text{xW}}$$

$$= \frac{56(16-14.2)}{2\text{x}1.5}$$

$$= 33.6\, {}^{\text{ml}}/_{\text{g}}$$

➢ **Iodine number determination**

The iodine number calculated by equation 3.11.

$$\text{Iodine number} = 12.69\frac{(\text{V2} - \text{V1})}{\text{W}}$$

$$= \frac{12.69(15-14.2)\text{ml}}{0.2\text{g}}$$

$$= 57.105\, {}^{ml}/_{g}$$

➢ **Ester value, content of esters and combined alcohols**

The ester value calculated by equation 3.12.

$$\text{Ester value} = 33.6\, {}^{\text{ml}}/_{\text{g}} - 5.05\, {}^{ml}/_{g}$$

$$= 28.55\, {}^{ml}/_{g}$$

➢ **Percentage of free fatty acid (% FA)**

Free fatty acid was determined using the following equation:

$\% \text{ FA} = k \times \text{Acid value(AV)}$

Where: K = Constant (0.503) (Soni, Dabhi, & Thomas, n.d.)

AV= is acid value of the thyme oil

$\% \text{ FA} = 0.503 \times 5.05$ =2.54

The Chemical properties of thyme leaves essential oil such as acid value, saponification value, Ester value, iodine number and free fatty acid are summarized in table 4.8.

Table 4.8: chemical properties of thyme oil extracted using steam distillation.

Chemical properties	**V**alue	Unit
Acid value	5.05	ml/g
Saponification value	33.6	ml/g
iodine number	57.12	ml/g
Ester value	28.55	ml/g
Free fatty acid	2.54	%

4.9. Gas Chromatography- Mass Spectroscopy analysis

The GC-MS library available in the laboratory at the Addis Ababa town JIJE LABO GLASS P.L.C. or JIJE Analytical Testing Service Laboratory is the NIST14 L Mass Hunter GC-MS (1) 5977 MS firm. A total of thirty seven components, with different retention times, were eluted from the GC column as indicated by the chromatogram and were further analyzed with an electron impact Mass Spectroscopy 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 4.9. 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 MS data demo version revealed that the essential oil of Thyme leaves contained a mixture of terpenes that eluted at different retention times depending on the boiling point of the eluted component.

Table 4.9: Chemical composition of thyme leaves essential oil steam distillation

Pick No.	Name of the compound	Chemical formula	RT(min)	Area	%Area
1	Bicyclo[3.1.0]hex-2-ene2-methyl-5-(1-methylethyl)-	C10H16	4.95	751846.00	0.22
2	Î±-Pinene	C10H16	5.12	372316.00	0.11
3	3-Octanone	C8H16O	6.13	2086501.00	0.61
4	Î²-Myrcene	C10H16	6.22	473210.00	0.14
5	3-Octanol	C8H18O	6.41	5553598.00	1.62
6	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	C10H16	6.92	1312813.00	0.38
7	p-Cymene	C10H14	7.12	38213390.00	11.12
8	D-Limonene	C10H16	7.22	403730.00	0.12
9	Eucalyptol	C10H18O	7.33	519689.00	0.15
10	.gamma.-Terpinene	C10H16	7.93	7196213.00	2.09
11	cis-Sabinene hydrate	C10H18O	8.28	3365507.00	0.98
12	Bicyclo[4.2.0]oct-1-ene, exo-7-(1,4-cyclohexadien-1-yl)-	C14H18	8.75	244451.00	0.07
13	Linalool	C10H18O	9.00	8278817.00	2.41
14	trans-Sabinene hydrate	C10H18O	9.10	1079007.00	0.31
15	Isopinocarveol	C10H16O	10.18	254783.00	0.07
16	Benzene acetic acid, methyl ester	C9H10O2	11.05	1085377.00	0.32
17	Terpinen-4-ol	C10H18O	11.29	1018237.00	0.30
18	3-Decanone	C10H20O	11.37	259243.00	0.08
19	p-Cymen-8-ol	C10H14O	11.48	667943.00	0.19

20	.alpha.-Terpineol	C10H18O	11.75	1280585.00	0.37
21	Cyclohexane, (2-nitro-2-propenyl)-	C9H15NO2	12.56	886516.00	0.26
22	Benzene, 2-methoxy-1-methyl-4-(1-methylethyl)-	C11H16O	13.07	1310296.00	0.38
23	3-Methyl-4-isopropylphenol	C10H14O	14.73	841768.00	0.25
24	Thymol	C10H14O	15.27	217123619.00	63.20
25	Carvacrol	C10H14O	15.52	29628871.00	8.62
26	Caryophyllene	C15H24	21.81	5046324.00	1.47
27	trans-.alpha.-Bergamotene	C15H24	22.62	783028.00	0.23
28	p-Cymene-2,5-diol	C10H14O2	23.24	3704270.00	1.08
29	cis-Î²-Farnesene	C15H24	25.25	331131.00	0.10
30	Î²-Bisabolene	C15H24	26.46	406945.00	0.12
31	Î²-Sesquiphellandrene	C15H24	27.25	1269720.00	0.37
32	cis-Î±-Bisabolene	C15H24	28.19	891342.00	0.26
33	Spathulenol	C15H24O	29.83	1333975.00	0.39
34	Caryophyllene oxide	C15H24O	29.98	2762482.00	0.80
35	12,15-Octadecadienoic acid, methyl ester	C19H34O2	40.40	269582.00	0.08
36	Oleic acid, methyl ester	C19H36O2	40.56	221873.00	0.06
37	Vitamin E	C29H50O2	46.15	2324687.00	0.68

Result obtained by GC-MS analysis of the essential oils of *Thymus schimperi* L. are presented .The identification of the components of the essential oil extracted by steam distillation process was carried out by comparison of their mass spectra and retention times to those of reference standards. From this table 4.9, it can be seen that these oils were characterized by the presence of monoterpenes hydrocarbons, oxygenated monoterpenes and sesquiterpenes, but the quantitative differences were observed in the contents of these components. The steam distillation oil composition is comparable to those reported in literatures. This oil has high monoterpenes hydrocarbons and other contents. Thirty-seven components were identified in the essential oil of Thymus schimperi. The major components were thymol (63.20 %), p-Cymene (11.112%), Carvacrol (8.62%). Other predominant components were Linalool (2.41 %), gamma.-Terpinene (2.09 %), 3-Octanol (1.62 %), Caryophyllene (1.47 %), p-Cymene-2, 5-diol (1.08 %), α-terpineol (0.37) and Caryophyllene oxide (0.80) .The chemical compositions revealed that these leaves had composition somehow closed to those of other thyme leaves essential oils GC-MS analyzed data. For example MOJ Toxicology Sciences reported the following values thymol 40.98%, p-cymene 15.32%, γ-terpinene 12.01%, carvacrol 2.63%, α-pinene 1.12% and α-terpineol 0.33% and traces components such as, linalool 0.7, cineole 0.41%,Caryophyllene oxide 0.45% and camphor 0.3% (Saqqa et al., 2018).

5. CONCLUSION AND RECOMENDATION

5.1. Conclusion

This study was aimed at extraction and characterization of thyme leaves essential oil majorly used for medicinal and antioxidant activity. The extraction was done by steam distillation using water as a solvent extraction time and particle size as factors or parameters and the response variables. Temperature, pressure and was maintained constant at their optimum conditions from the literature due to the uncontrollability of the equipment. Temperature was set at 105^0C pressure was at one atmosphere. Variability of these operating conditions is the pre-dominant factors for the quality and quantity of the essential oil. There are different methods of essential oil extraction from thyme leaves. However, in this study steam distillation extraction method was used due to availability of the equipment and it was recommended by so many literatures. The minimum yield of thyme leaves oil was 1.18%obtaind extraction time 60 minutes and particle size 2mm.The maximum yield of thyme leaves oil was 2.12% at 150 minutes of extraction time and 0.5mm particle size. The effect of extraction time and particle size were analysis by using design expert 7.0 software with four levels and without replications each. It was clearly seen in design expert software that decreasing particle size and increasing extraction time content of thyme leaves oil would increase the yield. Physical and chemical properties like specific gravity at 25^0C, viscosity, pH value, boiling point, refractive index, optical rotation, solubility in ethanol, acid value, Saponification value, ester value iodine value and free fatty acid content of the thyme leaves essential oil was determined and obtained comparable results from literatures some was not this could be due to time of collecting raw material. Furthermore, the components of thyme leaves essential oil were determined using Gas Chromatography -Mass Spectroscopy (GC-MS) the identified major components were thymol (63.20 %), p-Cymene (11.112%), Carvacrol (8.62%), Linalool (2.41 %), gamma.-Terpinene (2.09 %), 3-Octanol (1.62 %) and Caryophyllene (1.47 %). As it has been observed from the above list, thymol comprises (63.20 %) of the total yield, and it is the compound most responsible for the monoterpenoid phenol of thyme leaves essential oil. The ingredients obtained from this study indicated that the oil can be fully utilized for the manufacture of antioxidant, antimicrobial, antifungal and antiseptic agents or properties.

5.2. Recommendations

- ❖ Extracting essential oils rather than exporting the raw plant materials can save the country's foreign currency and hence the production of essential oils could still be a good source of foreign exchange revenue for our country.
- ❖ In this research or study the effect of temperature and pressure factors were not studied. This is due to uncontrollability of equipment of steam distillation that used for extraction of essential oil. Therefore, further study should be done in order to produce much butter quantity and quality of thyme leaves essential oil.
- ❖ Many plant species have been lost and some are in danger of extinction. It has also caused biodiversity conservation problems. It is therefore vital that systematic cultivation of these plants be introduced in order to conserve the biodiversity and protect endangered species.
- ❖ Study can be carried on converting the residue or the waste to valuable product and using the hydrosol as integrated small scale industry.
- ❖ Furthermore, the requirement of essential oils for use in aromatherapy is increasing and creating a demand for organically produced exotic oils. The development of the essential oils industry is therefore important to our country which has rich resources of raw materials (medicinal plants) or the climatic conditions for the initiation of crop wise cultivation programs.

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7. APPENDICES

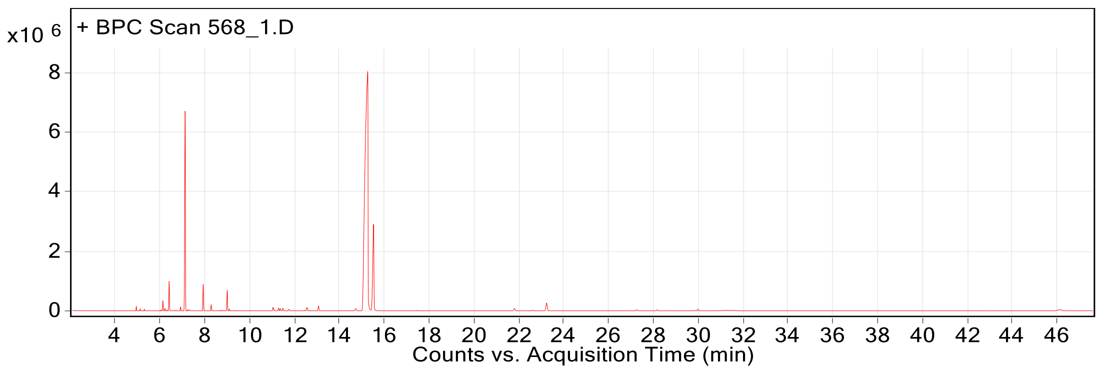
Appendix A: Qualitative analysis of thyme leaves essential oil

Data Filename	568_1.D	**Sample Name**	Thyme oil
Sample Type		**Position**	1
Instrument Name	GCMS	**User Name**	JIJE
Acq Method	Essential oil_Thyme.M	**Acquired Time**	5/31/2019 1:07:08 PM
IRM Calibration Status	Not Applicable	**DA Method**	Essential oil_Thyme.M
Comment			

Expected Barcode		**Sample Amount**	
Dual Inj Vol	1	**TuneName**	ATUNE.U
TunePath	D:\MassHunter\GCMS\1\5977\	**MSFirmwareVersion**	6.00.25
OperatorName	JIJE	**RunCompletedFlag**	True
Acquisition SW Version	MassHunter GC/MS Acquisition B.07.03.2129 18-May-2015 Copyright © 1989-2014 Agilent Technologies, Inc.		

User Chromatograms

Fragmentor Voltage **Collision Energy** 0 **Ionization Mode** Unspecifiec



Fragmentor Voltage **Collision Energy** 0 **Ionization Mode** Unspecifiec

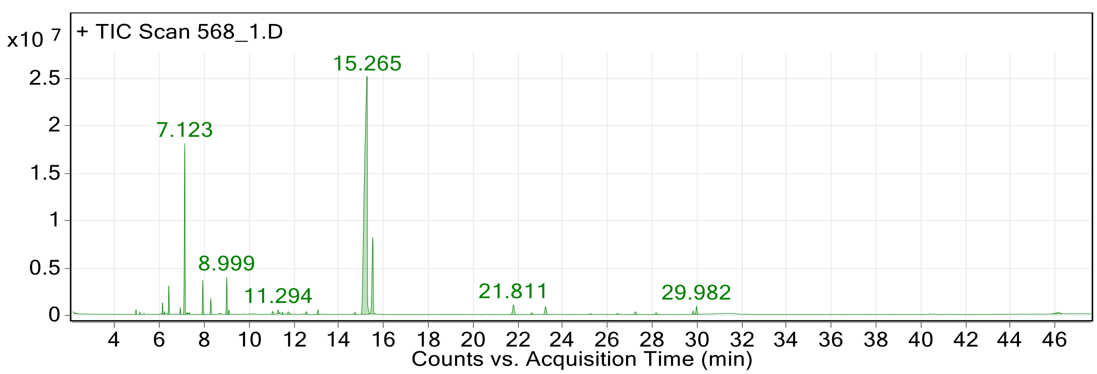


Table A1: Integration peak list of thyme leaves essential oil

Integration Peak List

Peak	Start	RT	End	Height	Area	Area %
1	2.19	2.213	2.419	97743.3	477270.68	0.22
2	4.908	4.948	4.987	468133.39	751846.08	0.35
3	5.085	5.12	5.161	228028.53	372316.14	0.17
4	6.093	6.133	6.172	1196188.05	2086501.15	0.96
5	6.179	6.218	6.26	276629.82	473209.67	0.22
6	6.365	6.413	6.522	3010126.01	5553598.08	2.56
7	6.88	6.922	7.006	691025.4	1312813.2	0.6
8	7.054	7.123	7.174	18028327.88	38213390.12	17.6
9	7.174	7.22	7.254	190775.48	403730.38	0.19
10	7.294	7.328	7.374	207161.11	519689.4	0.24
11	7.872	7.929	8.031	3590062.62	7196213.2	3.31
12	8.233	8.284	8.368	1626778.88	3365506.55	1.55
13	8.942	8.999	9.051	3920000.9	8278816.61	3.81
14	9.051	9.097	9.152	465748.44	1079007.44	0.5
15	10.991	11.048	11.139	308515.13	1085376.99	0.5
16	11.249	11.294	11.334	437612.91	1018236.66	0.47
17	11.434	11.483	11.547	238719.23	667942.89	0.31
18	11.69	11.746	11.918	266939.88	1280585.34	0.59
19	12.501	12.558	12.661	289272.05	886516.26	0.41
20	13.011	13.073	13.131	478853.84	1310295.94	0.6
21	14.653	14.727	14.816	209026.99	841768.21	0.39
22	14.992	15.265	15.374	25120113.13	217123619.5	100
23	15.408	15.522	15.659	8098556.95	29628871.06	13.65
24	21.679	21.811	21.934	998927.22	5046324.12	2.32
25	22.543	22.623	22.715	174529.72	783027.74	0.36
26	23.134	23.236	23.44	816360.34	3704269.62	1.71
27	25.193	25.25	25.324	75689.55	331130.92	0.15
28	26.389	26.457	26.548	102909.62	406944.84	0.19
29	27.157	27.247	27.367	277351.55	1269720.07	0.58
30	28.082	28.185	28.286	186551.41	891342.5	0.41
31	29.76	29.833	29.919	346105.77	1333974.98	0.61
32	29.919	29.982	30.171	833255.57	2762482.38	1.27
33	45.817	46.147	46.392	169774.61	2324686.79	1.07

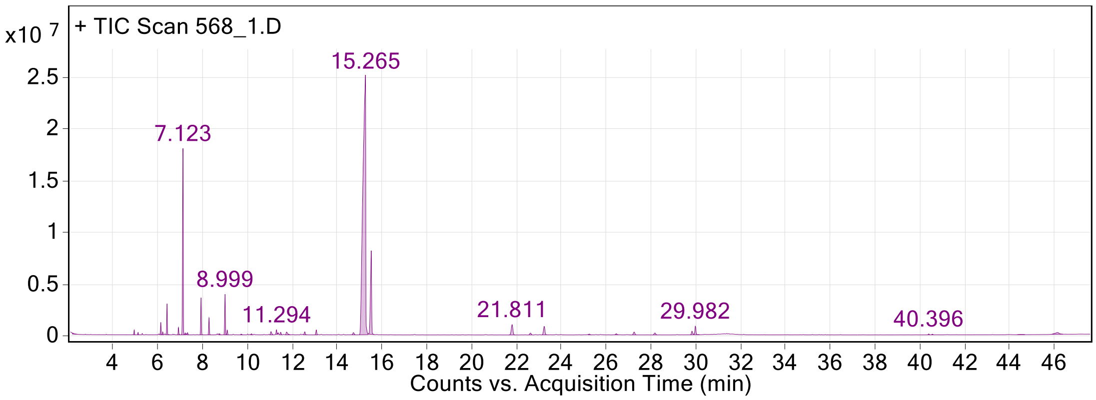


Table A2: Integration peak list of thyme leaves essential oil

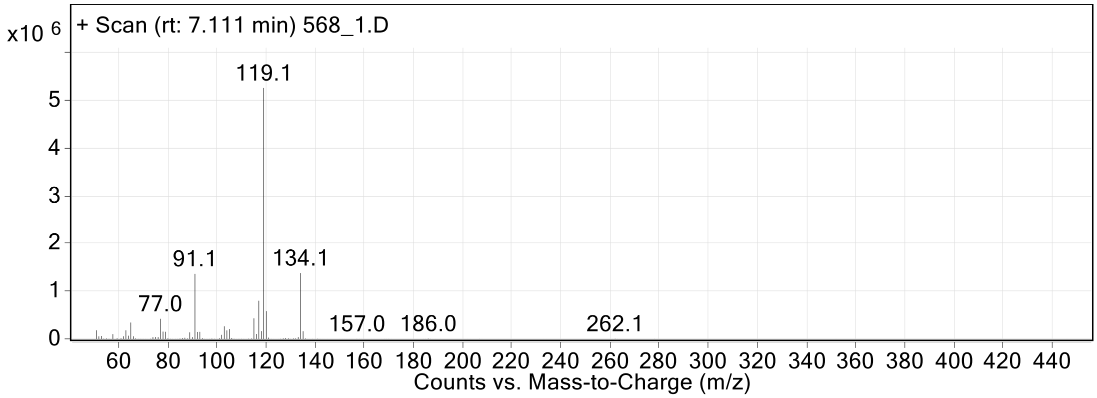
Integration Peak List

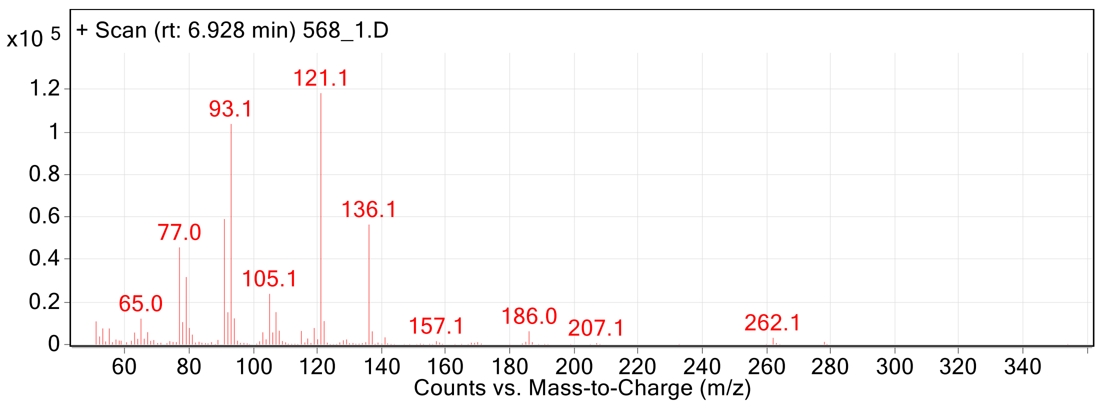
Peak	Start	RT	End	Height	Area	Area %
1	2.19	2.213	2.419	97743.3	477270.68	0.22
2	4.908	4.948	4.987	468133.39	751846.08	0.35
3	5.085	5.12	5.161	228028.53	372316.14	0.17
4	6.093	6.133	6.172	1196188.05	2086501.15	0.96
5	6.179	6.218	6.26	276629.82	473209.67	0.22
6	6.365	6.413	6.522	3010126.01	5553598.08	2.56
7	6.88	6.922	7.006	691025.4	1312813.2	0.6
8	7.054	7.123	7.174	18028327.88	38213390.12	17.6
9	7.174	7.22	7.254	190775.48	403730.38	0.19
10	7.294	7.328	7.374	207161.11	519689.4	0.24
11	7.872	7.929	8.031	3590062.62	7196213.2	3.31
12	8.233	8.284	8.368	1626778.88	3365506.55	1.55
13	8.719	8.753	8.798	115581.52	244451.49	0.11
14	8.942	8.999	9.051	3920000.9	8278816.61	3.81
15	9.051	9.097	9.152	465748.44	1079007.44	0.5
16	9.679	9.715	9.795	69566.28	222549.68	0.1
17	10.138	10.178	10.229	100360.61	254783.16	0.12
18	10.991	11.048	11.139	308515.13	1085376.99	0.5
19	11.249	11.294	11.334	437612.91	1018236.66	0.47
20	11.334	11.368	11.396	131915.15	259242.75	0.12
21	11.434	11.483	11.547	238719.23	667942.89	0.31
22	11.69	11.746	11.918	266939.88	1280585.34	0.59
23	12.501	12.558	12.661	289272.05	886516.26	0.41
24	13.011	13.073	13.131	478853.84	1310295.94	0.6
25	14.653	14.727	14.816	209026.99	841768.21	0.39
26	14.992	15.265	15.374	25120113.13	217123619.5	100
27	15.408	15.522	15.659	8098556.95	29628871.06	13.65
28	21.679	21.811	21.934	998927.22	5046324.12	2.32
29	22.543	22.623	22.715	174529.72	783027.74	0.36
30	23.134	23.236	23.44	816360.34	3704269.62	1.71
31	25.193	25.25	25.324	75689.55	331130.92	0.15
32	26.389	26.457	26.548	102909.62	406944.84	0.19
33	27.157	27.247	27.367	277351.55	1269720.07	0.58
34	28.082	28.185	28.286	186551.41	891342.5	0.41
35	29.76	29.833	29.919	346105.77	1333974.98	0.61
36	29.919	29.982	30.171	833255.57	2762482.38	1.27
37	40.333	40.396	40.47	85664.2	269582.42	0.12
38	40.493	40.562	40.636	67193.37	221872.99	0.1
39	44.35	44.476	44.688	20753.05	222539	0.1
40	45.817	46.147	46.392	169774.61	2324686.79	1.07

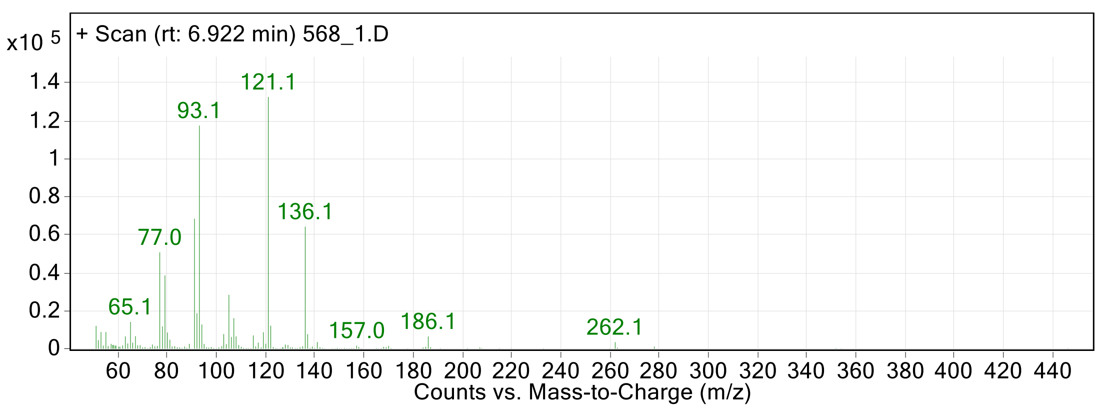
Appendix B: Plots of GC-MS Analysis of the Essential oil at different retention time

User Spectra

Collision Energy	Ionization Mode	Unspecified
0		







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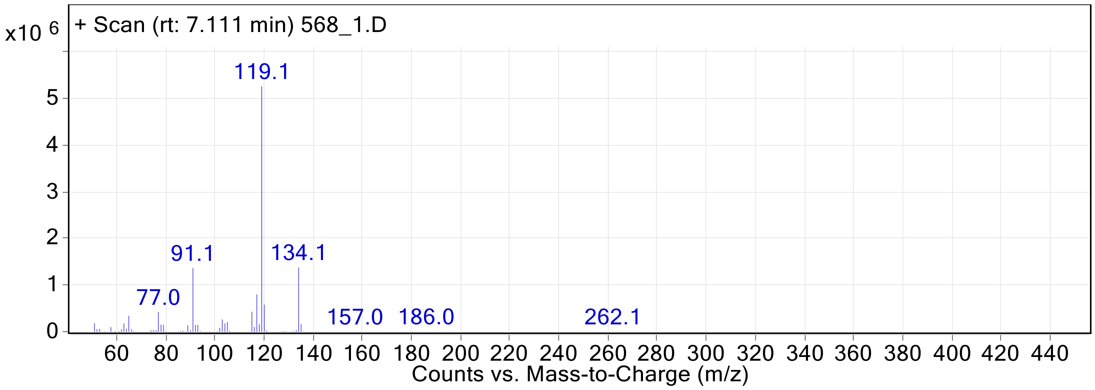

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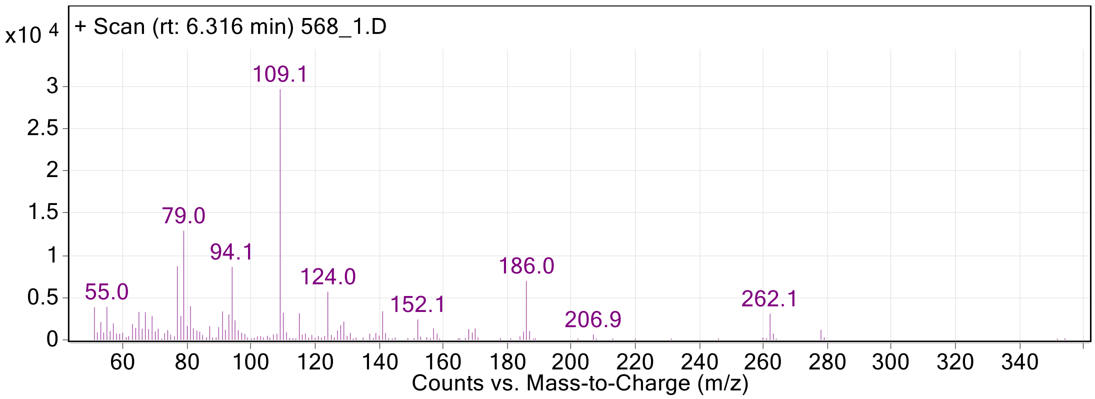

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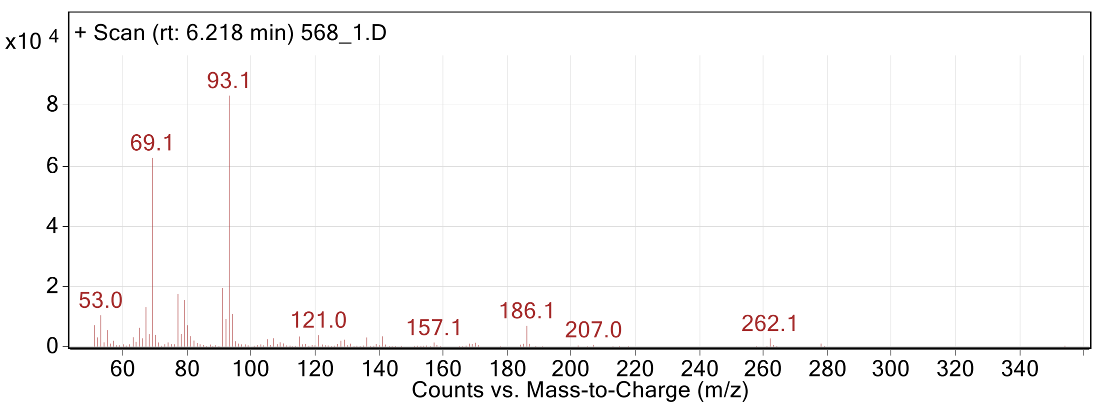

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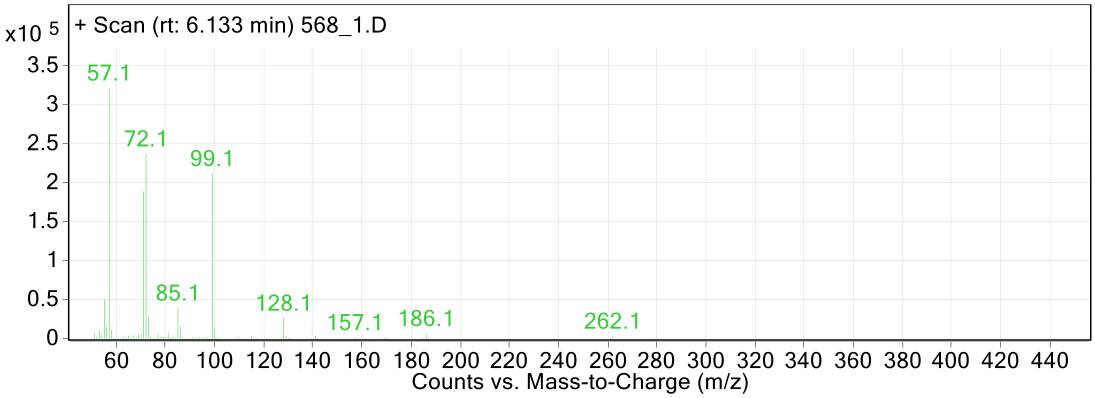

Collision Energy	Ionization Mode	Unspecified
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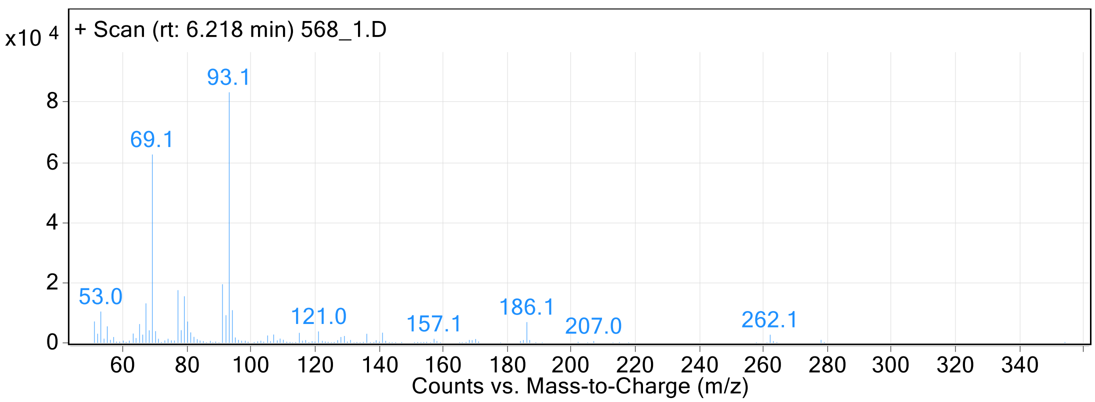

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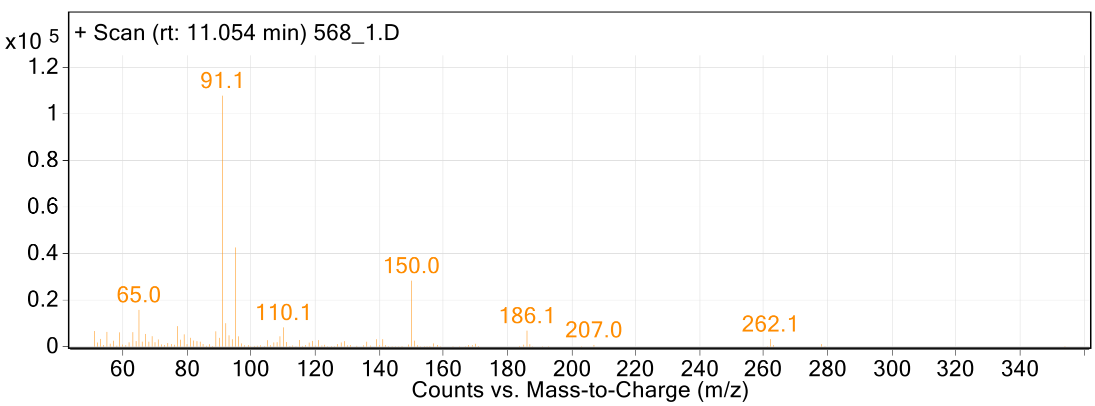

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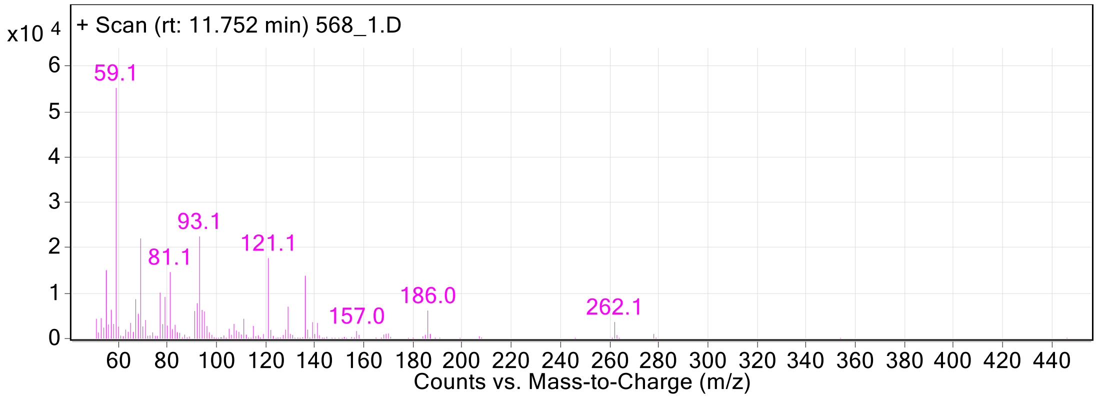

Appendix C: Laboratory equipment's and samples photos

Figure C, 1: Plant material & cross beater miller

Figure C, 2: Sieves analysis and samples of different particle size for extraction

Figure C, 3: Laboratory set up and product collection

Figure C, 4: Essential oil extracted from leaves of thyme leaves

Figure C, 5: Optical rotation and Refractive index measurement

Figure C, 6: Prepared solutions and titrated equipment used for determination of the acid value, saponification value and iodine number