



**Optimization of Extraction Process Parameters and Characterization
of Gin Flavor from Juniper Berries (*Juniperus Communis*)**

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This is to certify that thesis prepared by Meroda Tesfaye, entitled: Optimization of Extraction Process Parameters and Characterization of Gin flavor from Juniper Berries and Submitted in Partial Fulfillment of the requirements for the degree of Master of Science (Process Engineering Stream) complies with the regulations of the University and meet the accepted standards with respect to originality and quality.

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Abstract

The aim of the thesis work was focused extraction, optimization and characterization of the essential oil from the berries of juniper to Gin flavor. A Juniper Berries of community family was the raw material of the experimental research which was collected from Chilimo forest 70 Km North West (NW) of Adiss Ababa. First, the juniper berries was dried through sun drying. Then the dried juniper berries were crushed in jaw crusher with sieve size and the samples were sieved using a set of sieves sizes arranged. Then after the experimental research activities were 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. In the extraction experiment, the minimum oil yield of 0.487% was obtained at 2 hours and 1.4-2.5 mm and maximum oil yield of 2.27% was obtained 4 hours and 0.355 - 1mm. This shows that, increasing extraction time and decreasing particle size. Increases oil yield, The volatiles juniper berries essential oil in vapour form released from the berries were condensed and characterized their Physico - chemical properties of the berries oil studied for the optimum yield show that the oil is a solidifying oil at room temperature with having yellow colour, 1.50° refractive index value, 2.25mpas dynamic viscosity, 170°C boiling point, 0.8584g/cm³ specific gravity, 4.7 pH, 0.5° optical rotation, 1.4 ml/g acid value, 10.23ml/g iodine value, 47.69ml/g saponification value, 0.704% free fatty acid and 71.7° Brix. The chemical composition of essential oil extracted from Juniper berries using steam distillation was identified by GC-MS have α -pinene (73.12%), β -myrcene (4.29%), β -pinene (2.15 %), methylene (3.15%), phenylephrine (1.09%), and carene (4.74 %) and Evaluate sensory quality of Dry Gin liquor product. Therefore, particle size and extraction time have greater effect on the quality and quantity of juniper berries essential oil the findings of this study help to indicate the quality of the Juniper Berries oil which is important in the production of high value gin flavor.

Key Words: *Juniper berries, Essential oil acceptability, Steam distillation, Extraction process, Gin Flavor.*

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Lists of Abbreviations

AAiT	Addis Ababa Institute of Technology
AAU	Addis Ababa University
JB	Juniper Berries
AV	Acid Value
ANOVA	Analysis of variance
EO	Essential Oils
GC	Gas chromatography
GC-MS	Gas chromatography Mass spectroscopy
ISO	International Standards Organization
IV	Iodine Value
OAA	Over all acceptability
OH	Hydroxile Group
LIDI	leather Industry Development Institute
NALF	National Alcohol Liquor Factory
SC – CO ₂	Supercritical carbon dioxide Extraction
SFE	Supercritical Fluid Extraction
SD	Steam Distillation

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Chapter One

1. Introduction

1.1 Background

The word *Gin* is derived from the French word for Juniper Berries, *genièvre*, which is the name for Gin in France. It originates in Holland back in 1550, we can credit Dutchman *Franciscus de la Boe Sylvius*, a professor of medicine who was experimenting with juniper as a diuretic infused juniper into white spirit and it was sold in chemists as a treatment for stomachaches, gout and gallstones. During the Thirty Years War (1618-1648) Dutch soldiers in the chilly trenches were fed Gin for its warming properties. When Dutchman William of Orange became King of England he took Gin with him. At the time England was at war with France so they had stopped buying French brandy and wine and started distilling gin. Gin is one of the broadest categories of spirits, represented by products of various origins, styles, and flavour profiles that all revolve around juniper as a common ingredient (Sylvius, 2013).

In Ethiopia alcohol is an integral part of peoples' lives, especially during holidays, celebrations and recreations. These Alcoholic beverages are drinks containing ethyl alcohol or ethanol which is an intoxicating ingredient it is produced by the fermentation of yeast, sugars and starches mainly derived from cereal grains. It often slow down the function of the central nervous system and can impair judgment and alter a person's emotions, abilities and behaviors. It plays an important role in social events and interactions mainly because when consumed in small amounts, it can help a person feel relaxed and less anxious. But if consumed in a larger amount, alcoholic beverages may have a negative impact on one's health as well as social and financial well-being (Virendra, 2009).

Gin can be made from any spirit alcohol. This spirit is from a base of either grain or molasses and has no flavour. The flavour for Gin comes from Botanicals (coriander, lemon peel, cinnamon, nutmeg, orange peel, angelica and cardamom) to these vary from producer to producer and regarded as very much of a trade secret. One of the primary sources of raw material being investigated for gin flavor is juniper berries; it is the predominant flavour along with other natural flavour (Murtagh, 2012).

Juniper Berries is evergreen shrubs or tree growing up to eighteen feet with narrow, stiff, prickly needles and little brown cones called berries. Given the widespread occurrence of Junipers is found in Ethiopia like Chilimo forest and different rural and urban area. Essential oil can be extracted in many methods from those Solvent –Extraction, Cold Pressing, Super Critical CO₂ Extraction, Microwave Extraction, Soxhlet Extraction, Steam Distillation and others. From this select steam distillation because it was a special type of distillation relatively cheap process to operate at a basic level, useful and selective separation process for temperature sensitive volatile compounds that are not or only poorly miscible with water and it was relatively high purity essential oil and lesser fuel. Extracted essential oil was volatile, aromatic penetrating characteristics properties (Virendra, 2007). The yield and the composition of the essential oils of the juniper berries were depends on the geographical origin of the plant, the maturity of the berries, the age of the plant, the meteorological condition or temperature, insolation.

Aromatic oils from junipers have been used since antiquity for fragrance, flavoring, Medicinal, insecticidal, and cosmetic purposes. In this study only use extracted essential oil for Gin flavor due to lake of juniper berries flavor in our country minimizing the imported flavor from other country and upgrading of the farmer life who supplies raw material to the juniper berries oil extraction factory. It was investigate as a pathway to couple use of new and established technologies for possible application to the growing gin flavor market in Ethiopia.

1.2 Statement of the problem

Alcoholic beverages are divided into three groups: malt liquors, fermented wines and distilled liquors. Gin is common distilled liquors. The source of alcoholic liquors supply in Ethiopia is both local production and import, although there are a number of small to medium factories that produce alcoholic liquors. The NALF is a government-owned factory. It has four Branch factories namely, Maichew Liquor Factory, Sebeta Alcohol & Liquor factory, Mekanisa Alcohol Factory and Akaki Alcohol Factory and is the largest producer and distributor of various liquors such as, Gin, Ouzo, Double Ouzo, Cognac, Brandy, Perno, Orange, Coffee, Fernit, Bitter, Aperitif, Lemon and Pineapple Liquors throughout the country (Tibebe , 2012).

Apart from domestic production, different types and brands of alcoholic beverages are imported to the county through legal and illegal means. The major legally imported alcoholic liquors include Gin is the one. The name Gin is derived from either the French *genièvre* or the Dutch *jenever*, which both mean juniper. All gins must be primarily flavor by juniper berries, juniper berries is a coniferous plant distributed throughout the Artic and found at different part Ethiopia. These juniper berries is use for Gin flavor after extraction of oil. There are many types of extraction. The extraction can be conducted with or without solvent. But, to get the essential oil, extraction through steam distillation is the most used method. Without any solvent, pure water is used at its boiling point as steam to extract the essential oil from juniper berries.

Potential of Juniper Berries oil for Gin flavor production not received much attention in Ethiopia, Currently industrial demand for essential oil is met through import from other country. So, all liquor factories are exposing to unnecessary expenditure due to expensiveness of oil to exploit the potential of Juniper Berries oil for Gin flavor production, which will minimize the imported Gin flavor, produce and supply enough oil of juniper berries for all liquor factories, the other one is juniper found at different place of Ethiopia this coniferous plant is cut by people use for wood, particularly fuel wood, furniture (bed, box etc) during this barrier is not considered. Extract oil from juniper berries use for Gin flavor is an effective solution to save the exposed juniper berries.

1.3 OBJECTIVE

✓ **General Objective**

The main objective of this research was optimization of extraction process parameters and characterization of gin flavor from Juniper Berries.

✓ **Specific objective**

The specific objectives were:-

- ✚ Optimize essential oil yield through evaluation of extraction parameters (extraction time and particle size) of juniper berries.
- ✚ Characterize physicochemical property of juniper berries oil, which is used as a feedstock for production of Gin flavor.
- ✚ Sensory analysis

1.4 Significance of the study

The significance of extraction of Gin flavor from juniper berries can be seen from different perspectives.

- ✚ This study was contributed to a significant improvement of unnecessary expenditure that import Gin flavor from other country. Or To meet the demand of Ethiopian liquor factory and Transformation Plan on the extraction of gin flavor.
- ✚ This work was show the possibility of get the oil and this can be used us an additive In Gin. Industries to improve the performance quality of the product.
- ✚ The experimental work showed that the possibility of getting the appropriate operating conditions for extraction of juniper berry.
- ✚ Upgrading of the farmers life who supplies raw material for the production of berries oil.

Chapter Two

2. Literature review

2.1 Over View of Essential Oil Manufacturing

It is estimated that there are 391,000 species of plants on Earth. The largest number of plants with a documented use is those that have been utilized as medicines. The next most common usage groups are those plants used in materials – this includes plants used for textiles and building materials. Plant species recorded as useful environmentally include those used to restrict erosion, as firebreaks or in agroforestry. There are Currently 8,000 different plant species in this category. Around 5,000 plant species provide human food; a further 5,000 are possible gene sources – species that are potentially useful in the genetic improvement of crops. The next most common category of useful plants comprises non-human foods; these include invertebrate food plants, such as those eaten by insects (e.g. silkworms) that are used by people, plants that encourage pollinators (e.g. bee plants), and those eaten by animals. Plant species recorded as being used as poisons, fuel or for social uses (including plants used as intoxicants or for religious reasons) number the fewest, but there are still more than 1,300 plant species in each of these (Floresha 2012).

It is possible that even more are used for medicinal purposes depending on taxonomic view point. between 50 and 67 species of juniper are widely distributed throughout the northern hemisphere, from the arctic, south to tropical Africa, from Ziarta, Pakistan east to eastern Tibet in the old world and in the mountains of central America. The highest-known juniper forest occurs at an altitude of 16,000 feet (4,900m) in south-eastern Tibet and the northern Himalayas, creating one of the highest tree-lines on earth.

Plant oils and extracts have been used for a wide variety of purposes. These purposes vary from the use of rosewood and cedar wood in perfumery, to flavoring drinks with lime, 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 (Carlos, 2007).

2.2 Natural Products for Manufacturing Essential Oils

Essential oils are natural, volatile, complex plant compounds, oily or lipid-like in nature and frequently characterized by a strong fragrance they have a low solubility in water but are soluble in fats, alcohol, organic solvents and other hydrophobic substances and are generally liquid at room temperature. They are stored in specialized plant cells, usually oil cells or ducts, resin ducts, glands or trichomes and may be extracted from the leaves, flowers, buds, seeds, fruits, roots, wood or bark of plants by a variety of methods, including solvent and supercritical fluid extraction, expression under pressure, fermentation or effleurage, but either low- or high-pressure steam distillation were used predominantly for commercial production. Essential oils make up only a small proportion of the wet weight of plant material, usually approximately 1% or less. The presence, yield and composition of essential oils may be influenced by many factors, including climate, plant nutrition and stress, the term 'essential oil' groups together a wide range of chemical compounds on the basis of their historic use and method of extraction, usually steam distillation, and belies the variety and complexity of compounds found within them.

Essential oils are often described as secondary plant metabolites. Traditionally, secondary plant metabolites have been all those compounds synthesized by the plant which do not appear to be essential for plant growth and development of those compounds without an obvious function. They are also not universally synthesized in all plants. In contrast, primary metabolites are produced by all plants, usually have an obvious function and are part of the essential metabolic processes of respiration and photosynthesis. This artificial and rather simplistic division is also difficult because the natural functions of many secondary plant metabolites are unknown simply because they have never been investigated; this lack of evidence or knowledge is then interpreted as a lack of function. Greater interest in investigation of secondary metabolites in recent years has led to the discovery that they have roles in defense, signaling and as intermediates in secondary metabolism. Oil is "essential" in the sense that it carries a distinctive scent, or essence, of the plant. Each of these complex precious liquids is extracted from a particular species of plant life this plant species originates in certain regions of the world, with particular

environmental conditions and neighboring fauna and flora. These oils were often used for their flavor and their therapeutic or odoriferous properties, in a wide selection of products such as foods, medicine, and cosmetics. Only pure oils contain a full spectrum of compounds that cheap imitations simply cannot duplicate (Virender, 2009.)

2.2.1. Chemistry of Essential Oils

Essential oils are not simple compounds or even simple mixtures of several individual compounds. They may contain up to approximately 100 components, although many contain about 20 to 60. The compounds found in essential oils are from a variety of chemical classes, predominantly terpenes, but phenylpropanoids and other compounds also occur although at lesser frequency and often, but not always, in smaller proportions. They are all hydrocarbons and their oxygenated derivatives, and they may also contain nitrogen or sulfur. They are generally low-molecular-weight compounds with limited solubility in water. The classification and nomenclature of essential oil compounds is complicated by the fact that many were isolated and studied before the investigation of systematic chemical nomenclature. Consequently known by nonsystematic or trivial or common names this was sometimes but not always based on their source, juniper berries which hint at historical botanical origins of this compound. In terms of shedding light on their chemistry, the long history and widespread use of this nonsystematic name further obfuscates the chemical nature and characteristics of essential oils and their components (Karchesy, 1998).

2.2.2 Sources of Natural Essential Oils

Essential oils are generally derived from one or more plant parts, such as fruits (bergamot, orange, lemon, juniper), leaves (e.g. Eucalyptus, mint, Ocimum spp., lemongrass, jamrosa), leaves and stems (e.g. geranium, patchouli, petitgrain, verbena, cinnamon), wood (e.g. cedar, sandal, pine), roots (e.g. angelica, saffron, vetiver, saussurea, valerian), flowers (e.g. rose, jasmine, carnation, clove, mimosa, rosemary, lavender) seeds (e.g. fennel, coriander, caraway, dill, nutmeg), rhizomes (e.g. ginger, calamus, curcuma, orris) and gums or oleoresin exudations (e.g. balsam of Peru, Myroxylon balsamum, storax, myrrh, benzoin).

2.3 Juniper Tree

2.3.1 Overview Juniper

It is a coniferous tree which belongs to the family of Cupressaceae. It has which bluish – green branches and bears small yellow-purplish flowers and three cornered seeds. It was a wonderful and a versatile garden plant. It can tolerate a wide range of growing and soil conditions, it was certain extent of drought; juniper has thick foliage, commonly used as strewing herb to freshen the stale air, due to its air-cleansing fragrance. The juniper trees vary in size, ranging from small to tall trees. Its seeds are cone like and very fleshy, due to which they appear as berries. The foliage of the tree comes in many colors, ranging from silvery-gray to mauve and purple in some seasons (Floresha, 2012).

Tree can grow anywhere between sit to 25 feet high. The juniper barriers, which grow in whorls of three on the woody twigs, emit a lemon-or apple-like fragrance when crushed. Both the branches and the berries have been used, since ancient times for medicinal and spiritual purposes. The evergreen tree is also popular as a residential it is ornament plant, often used in wildlife plantings and shelterbelts. The aromatic wood is also for making furniture, fence posts and pencils (Karchesy, 1998).

2.3.2 Juniper barrier

Juniper berries comes from ancient Egypt, The Egyptians most likely obtained their juniper, which is not native to Egypt, from the Greeks who used it as a medicine and stamina enhancer, for which it was closely associated with usage in the ancient Olympic Games. In the Middle Ages juniper berries found use as a Cure for flatulence, something for which juniper oil is still utilized for today Culinary uses for juniper date back to the time of the Roman Empire, Today, the most widely known use for juniper is as the primary flavoring agent in Gin. British, French, and Dutch laws require that juniper is the only primary botanical used to flavor beverages that are classified as Gin (Avinash, 2016).

The berries are fleshy and globular, measuring ¼ -1/3 inches in diameter. They contain three sticky, brown irregular shaped seeds. The fruits should be used when ripe, that is, and after the

skin has turned blue. On drying and storing, the smooth shiny skin darkens to a purple black and becomes slightly wrinkled or indented. The unripe fruit is green. The interior flesh of the mature Berries are brown yellow and the brown seeds are crunching but not hard. The oil is obtained mainly from the berries, it is volatile, odorous. Steam distillation is used in the extraction of Essential oil from the plant material. Essential Oil extracted by using Steam distillation is a special type of distillation or a separation process for temperature sensitive materials like oils, resins, hydrocarbons, etc. The yield and the composition of the essential oils of the juniper berries depends on the geographical origin of the plant, the maturity of the berries, the age of the plant, the meteorological condition or temperature, insolation, *etc.* and other environmental factors (Sela, 2012).

2.3.2.1 Local Names of Juniper Berries

Juniper berries are known by several names in different places:- Amharic (tid); india (dhup,shur);France (Geniever);Italian (Ginepro); Spanish (Enebro); Germa (Waccholer); oromic (Gatira).

2.3.3 Juniper berries growing

The juniper tree and shrubs grow mostly in all parts of northern hemisphere; all the species of juniper tree produce berries. These plants grow from direct planting of berries and also from the cutting from new growth. The trees can grow very well in light drained, impoverished soil. The soil to be selected can be alkaline or acidic. This plant is effective against gravel much and paving. The berries are picked when they are fully ripe sometime in late summer or early autumn. When ripe these berries are very dark in color (Avinash, 2016).

2.3.4 Juniper berries harvesting

When the juniper berries are ready to be harvested, you need to spread them on a flat ground in sunny place. You can also dehydrate the berries or place them in an oven. But you should make sure that these berries are used within one year of harvesting. Harvesting is the primary process of collecting the target crop product from the field, where it is open to the vagaries of the climate and the growing environment, and placing that product in controlled processing and stable storage conditions. The harvesting requirements was differ for the final product sought, and there

are specific needs such as maturity and evenness, that will dictate the harvesting management and timing. Plants should be harvested during the optimal season or time period to ensure the production of plant materials and finished spice products of the best possible quality. The time of harvest depends on the plant part to be used. Detailed information concerning the appropriate timing of harvest was often available in published standards, official monographs and major reference books. However, it is well known that the concentration of target constituents varies with the stage of plant growth and development (Stoilova, 2014). The best time for harvest should be determined according to the quality and quantity of target constituents. During harvest, care should be taken to ensure that no foreign matter was mixed with the harvested plant materials. When possible, plant parts should be harvested under the best conditions, avoiding dew, rain or exceptionally high humidity. If harvesting occurs in wet conditions, the harvested material should be transported immediately to a drying shed and drying started in order to prevent microbial fermentation and mould. They should be stored in an uncontaminated dry place (Khilender, 2010).

2.3.5 Juniper berries nutrients

Juniper berry is rich in nutrients and provide many health benefits to the people. They possess many important nutrients which were as follows. Vitamins and minerals-juniper berries are rich in vitamin C and vitamin B. they contain small amount of calcium. Volatile oils-it also contains a substantial amount of volatile oil.

2.3.6 Ripening cycles

The selection of seed or fruit to harvest which was at the correct maturity or ripeness, based on colour, is critical to obtaining a product of even high quality. The correct harvest maturity is often dependent on the choice of cultivar. Juniper berries ripen on a two-to-three year cycle. In the first year the plant forms flowers that eventually produce berries, in the second year of the cycle the berries often remain hard and green in color. By the third year the berries develop a rich purplish blue color, which signifies that they are ripe. Fruit over the entire plant is always in different stages. Plants have a combination of all three stages of berry on them at any given time. When harvesting your berries only remove the ripe (Svetomir, 2007).

Only ripe berries should be collected, which are smooth, plump and purple-black in appearance. Harvesting is done when strongly ripen every two years at different times so that blue (ripe) and green (unripen) berries are found on the same tree.

2.4 Properties Essential oils

A. Essential oils are volatile

Essential oils are volatile fragrant components from various indigenous and exotic plants which have been traded internationally for several centuries, 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 (Kabuba, 2009)

B. Essential oils are aromatic

Essential oils are highly aromatic and therefore, many of the benefits can be obtained by simply inhaling them. This can be done by breathing in the fragrance from the bottle or they can be diffused into the room (Kabuba, 2009).

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 (Kabuba J. T., 2009).

2.5 Juniper berry oil

It is generally obtained from raw fruit or from the berries. This oil occurs as a pale green or yellowish limip liquid having a terebinthic odor. This oil serves many medical purposes. It acts as a diuretic as a carminative and for curing certain diseases of kidney, used in combination with lard to prevent irritation from flies and used for the expulsion of uric acids in the joint and gout used to detoxify and clear congested skin. Some times this juniper berries essential oil is also used as a local stimulant (Benedikt , 2013). The essential oil extract from juniper berry is superior in quality; it is yellow oil with a watery viscosity, and a fresh, clean and slightly woody and fruity fragrance. The fresh and calming aroma of juniper berry oil is widely renowned for relieving stress and anxiety, when diffused it can also cleanse and purify the air. It contains

compounds with an extremely broad range of biochemical effects as well as odor, flavor and functional properties. Potential for Juniper berry oil because it mainly uses berries which do not compete with existing usage. The essential oil of juniper berry has diuretic properties, gastrointestinal irritant and antiseptic properties. The diuretic action of juniper is primarily due to its essential oil, which contains terpinen-4-ol. The content of essential oil in the berries ranges from 0.2 to 2.0 and its main compounds are terpen hydrocarbons such as α - and β -pinene, myrcene, sabinene, thujone, limonene, etc. Oil also contains sesquiterpene hydrocarbons and terpen alcohols (tshilenge, 2009).

The use of juniper berries has been approved for dyspepsia and, with other plants for bladder and kidney conditions. The berry has digestive, stomachic, and antirheumatic properties. Because of these antiseptic properties, we decided to investigate the antimicrobial activity of the essential oil from juniper berries (Avinash, 2016).

Table 2.1 Applications of juniper berry oil

Segment	Application	Benefits
Fragrance	Used in men's perfumes and aftershaves used in fragrance diffusers and added in masking sprays	Aromatic properties fresh, spicy woody masking properties.
Cosmetics	Topical application i.e. used in a broad range of products(especially) soaps Used in skin care products (i.e. acne cremes)	Aromatic properties:fresh,clear,and slightly woody aroma Anti-septic properties
Aromatherapy	Used in fragrance diffusers, incense product, steam inhalation product, massaging oil bath oils.	Relaxing properties
Pharmaceutical	Preparation based on essential oils on the German market includes bath additives, soft capsules.	Blood circulation, anti-rheumatic, diuretic and minor digestive problems
Foods	Flavour application to flavour foods and drinks i.e. alcoholic drinks	Flavouring properties a distinctive clean and tangy taste

2.6 Methods of Extraction

2.6.1 Solvent-Extraction

Solvent extraction is the process of separating one constituent from a mixture by dissolving it into a solvent in which it is soluble but in which the other constituents of the mixture are not, or at least less soluble. The separation process is performed by the process of percolation, where a solvent is passed through the mixed solids and, thus, the analyte which is soluble in the solvent is extract into that solvent. If the solvent used is water, then this process can be termed leaching. When the term solvent extraction is used, it is usually taken as meaning extraction involving two or more liquids, i.e. liquid-liquid extraction. In liquid-liquid extraction, the solution containing the desired constituent must be immiscible with the liquid used to extract the desired constituent. When the extraction process has occurred, the phase which contains the extracted analyte is known as the extract phase; while the sample from which the analyte has been removed is named the refined phase. The extraction of an analyte from one phase into a second phase is dependent upon two main factors: solubility and equilibrium. The principle by which solvent extraction is successful is that 'like dissolves like'. That was, to remove a polar solute from a solution a polar solvent should be used. Qualitative predictions can be made on the likely success of an extraction by considering the polarity of the analyte of interest and of the two solvents used. Uncharged solutes are more easily extracted into non polar organic solvents and the less polar the solute the more efficient the extraction process. If the solute is charged, then it was usually best to form an ion-pair with a counter ion and extract the newly formed neutral complex into a non-polar solvent. Therefore, with solvent extraction effective separation of the extracted oil from the solvent is necessary to remove any solvent which may contaminate the essential oils (Kabuba, 2009).

2.6.2 Cold Pressing

Another expression method of extracting Essential Oils is Cold Pressing expression, or scarification method. It was used to obtain citrus fruit oil such as orange, lemon, lime, mandarin, grapefruit, tangerine 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 (Keresa, 2014).

2.6.3 Super Critical CO₂ Extraction

A supercritical fluid is any substance at a temperature and pressure above its thermodynamic critical point. It can diffuse through solids like a gas, and dissolve materials like a liquid. Additionally, close to the critical point, small changes in pressure or temperature result in large changes in density, allowing many properties to be "tuned". Supercritical fluids are suitable as a substitute for organic solvents in a range of industrial and laboratory processes. Carbon dioxide and water are the most commonly used supercritical fluids, being used for decaffeination and power generation respectively. SFE is an efficient alternative for the extraction of natural substances from foods. Supercritical fluids possess excellent extractive properties such as high compressibility, liquid-like density, low viscosity, and high diffusivity. They have been widely used in many industrial applications, i.e. the decaffeination of coffee, the extraction of hops, the synthesis of polymers, the purification and the formation of nano particles for extraction of natural substances, supercritical carbon dioxide (SC-CO₂) is generally used. It has a greater ability to diffuse through the ultra-fine complex matrix than conventional organic solvents and can be easily separated from the products by depressurizing process. Furthermore, low critical temperature of carbon dioxide means that the SC-CO₂ system could be operated at moderate temperature, preventing the degradation of the substance due to heat induction. As a result, the obtained product is pure and of great quality, and thus safe for use as nutritional additives and for pharmaceutical. One compound, carbon dioxide, has so far been the most widely used, because of its convenient critical temperature, cheapness, chemical stability, non-flammability, stability in radioactive application and non-toxicity. Large amount CO₂ released accidentally could constitute a working hazard, given its tendency to blanket the ground, but hazard detectors are available. It is an environmentally friendly substitute to other organic solvents. The CO₂ that was used obtained in large quantities as a by-product of fermentation, combustion and ammonia synthesis and would be released into the atmosphere sooner rather than later, if it were not used as a supercritical fluid its polar character as a solvent, intermediate between a truly non polar solvent such as hexane and weakly polar solvents. Because the molecule is non-polar, it often classified as a non-polar solvent, but it has some limited affinity with polar solutes because of its

large molecular quadrupole (Sela, 2012). It has a particular affinity for fluorinated compounds and is useful for Working with fluorinated metal complexed and fluoropolymers Carbon Dioxide was not such a good solvent for hydrocarbon polymers and other hydrocarbons of high molar mass. Ethane, ethene and propane become alternatives for these compounds, although they have the disadvantages of being hazardous because of flammability and of being somewhat less environmentally friendly. However, small residues of lower hydrocarbons in foodstuffs and pharmaceuticals are not generally considered a problem. Water has good environmental and other advantages, although its critical parameters are much less convenient and it gives rise to corrosion problems. Supercritical water is being used, at a research level, as a medium for the oxidative destruction of toxic waste. There were particular interests in both supercritical and near-critical water because of the behavior of its polarity. Ammonia has similar behavior, is often considered and discussed, but not often used. Many halocarbons have the disadvantage of cost or of being environmentally unfriendly. Xenon is expensive, but is useful for small-scale experiments involving spectroscopy because of its transparency in the infrared (tshilenge, 2009).

2.6.4 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 (Khilendra, 2010).

2.6.5 Soxhlet Extraction

A Soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet. It was originally designed for the extraction of a lipid from a solid material. However, a Soxhlet extractor is not limited to the extraction of lipids. Typically, a Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity was insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. Normally a solid material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. Soxhlet equipped with a condenser, the solvent was heated to reflux. The solvent vapour travels up a distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapors cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days. During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled after extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and was usually discarded (Chandra, 2008).

2.6.6 Steam distillation

Steam distillation was 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 natures of steam distillation were that it enables a compound or mixture of compounds to be distilled at a temperature substantially below that of the boiling point of the individual constituent. Essential oils contain substances with boiling points up to 200°C or higher temperatures. In the presence of steam or boiling water,

however, these substances are volatilized at a temperature close to 100°C, at atmospheric pressure. Fresh, or sometimes dried, botanical material is placed in the plant chamber of the still and the steam is allowed 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 evaporate 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 was 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 in certain situations, floral water may be preferable to be pure essential oil, such as when treating a sensitive individual or a child, or when a more diluted treatment is required. Rose hydrosol, for example, is commonly used for its mild antiseptic and soothing properties, as well as its pleasing floral aroma (KUMAR, 2010).

A number of factors determine the final quality of a steam distilled essential oil. Apart from the plant material, most important are time, temperature and pressure, and the quality of the distillation equipment. Essential oils are very complex products. Each is made up of many, sometimes hundreds, of distinct molecules which come together to form the oil's aroma and therapeutic properties. Some of these molecules are fairly delicate structures which can be altered or destroyed by adverse environmental conditions. So, much like a fine meal was more flavorful when made with patience, most oils benefit from a long, slow 'cooking' process. It was possible that longer distillation times may give more complete oil and 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 improve the odor, as sometimes when materials that have a larger number of components are sniffed, the perception is often of slightly increased sophistication, added fullness and character, and possibly, and extra pleasantness (KUMAR, 2010).

2.6.7 Water distillation

In this method, the material is completely immersed in water, which is boiled by applying heat by direct fire, steam jacket, closed steam jacket, closed steam coil or open steam coil. The main characteristic of this process is that there is direct contact between boiling water and plant material. When the still is heated by direct fire, adequate precautions are necessary to prevent the charge from overheating. When a steam jacket or closed steam coil is used, there is less danger of overheating; with open steam coils this danger is avoided. But with open steam, care must be taken to prevent accumulation of condensed water within the still. Therefore, the still should be insulated. The plant material in the still must be agitated as the water boils, otherwise agglomerations of dense material will settle on the bottom and become thermally degraded. Certain plant materials like cinnamon bark, which are rich in mucilage, must be powdered so that the charge can readily disperse in the water; as the temperature of the water increases, the mucilage was leached from the ground cinnamon. This greatly increases the viscosity of the water-charge mixture, thereby allowing it to char. Consequently, before any field distillation done, a small-scale water distillation in glassware should be performed to observe whether any changes take place during the distillation process. From this laboratory trial, the yield of oil from a known weight of the plant material can be determined. The laboratory apparatus recommended for trial distillations is the Clevenger system. During water distillation, all parts of the plant charge must be kept in motion by boiling water; this is possible when the distillation material is charged loosely and remains loose in the boiling water. For this reason only, water distillation possesses one distinct advantage, i.e. that it permits processing of finely powdered material or plant parts that, by contact with live steam, would otherwise form lumps through which the steam cannot penetrate. Other practical advantages of water distillation are that the stills were inexpensive, easy to construct and suitable for field operation. These were still widely used with portable equipment in many countries. The main disadvantage of water distillation was that complete extraction was not possible. Besides, certain esters are partly hydrolyzed and sensitive substances like aldehydes tend to polymerize (KUMAR, 2010)

Water distillation requires a greater number of stills, more space and more fuel. It demands considerable experience and familiarity with the method. The high-boiling and somewhat water soluble oil constituents cannot be completely vaporized or they require large quantities of steam.

Thus, the process becomes uneconomical. For these reasons, water distillation is used only in cases in which the plant material by its very nature cannot be processed by water and steam distillation or by direct steam distillation.

Disadvantages of Water Distillation

- Oil components like esters are sensitive to hydrolysis while others like acyclic monoterpene hydrocarbons and aldehydes are susceptible to polymerization (since the pH of water is often reduced during distillation, hydrolytic reactions are facilitated).
- Oxygenated components such as phenols have a tendency to dissolve in the still water, so their complete removal by distillation is not possible.
- As water distillation tends to be a small operation (operated by one or two persons), it takes a long time to accumulate much oil, so good quality oil is often mixed with bad quality oil.
- The distillation process is treated as an art by local distillers, who rarely try to optimize both oil yield and quality.
- Water distillation is a slower process than either water and steam distillation or direct steam distillation.

A. Water distillation

B. Water and steam distillation

C. Direct steam distillation

2.6.8 Direct Steam Distillation

As the name suggests, direct steam distillation was the process of distilling plant material with steam generated outside the still in a satellite steam generator generally referred to as a boiler. As in water and steam distillation, the plant material was supported on a perforated grid above the steam inlet. A real advantage of satellite steam generation was that the amount of steam can be readily controlled. Because steam generated in a satellite boiler, the plant material heated no higher than 100 and, consequently, it should not undergo thermal degradation. Steam distillation was the most widely accepted process for the production of essential oils on large scale. Throughout the flavor and fragrance supply business, it was a standard practice. An obvious drawback to steam distillation, much higher capital expenditure needed to build such a facility. In some situations, such as the large-scale production of low-cost oils (e.g. rosemary, Chinese

cedarwood, lemongrass, litsea cubeba, juniper berries, citronella, cornmint), the world market prices of the oils are barely high enough to justify their production by steam distillation without amortizing the capital expenditure required to build the facility over a period of 10 years or more (Kabuba, 2009)

Advantages of Direct Steam Distillation

- Amount of steam can be readily controlled.
- No thermal decomposition of oil constituents.
- Most widely accepted process for large-scale oil production, superior to the other two processes.

Disadvantage of Direct Steam Distillation

- Much higher capital expenditure needed to establish this activity than for the other two Processes.

2.7 Important Physical and Chemical properties of Essential Oils

The chemical properties of essential oils depend on the natural factors such as type of species, the geographical origin and location of the plant, time of harvesting, plant parts from which the oils are extracted, Essential oils components and percentage are different from oil to oil even for the same botanic plant due to: Weather and planting time, Soil elements, Irrigation and Time of harvest (Igbokwe, 2015).

2.7.1 Physical properties of Essential Oils

❖ Specific gravity

Specific gravity is an important criterion of the quality and purity of an essential oil. Values for essential oils vary between the limits of 0.854 and 0.879 at 25°C, in general, the specific gravity is less than 1.000 Hence essential oil can be collected over (floating on) water (Kabuba, 2009).

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 (Kabuba, 2009).

Refractive index

When a ray of light passes from a less dense to a more dense medium, it was bent or "refracted" toward the normal. If e represents the angle of refraction and i the angle of incidence, according to the law of refraction (Kabuba, 2009).

$$\frac{\sin I}{\sin e} = \frac{N}{n} \quad (2.1)$$

Where n is the index of refraction of the less dense and N is the index of refraction of the denser medium. Refractometer offer a rapid and convenient method for the determination of this physical constant.

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.

2.7.2 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, Sulphur and nitrogen compounds are sometimes also present (Kabuba, 2009).

Terpenes

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 anti- inflammatory and bactericidal properties.

Ketones

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

Esters

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

Alcohols

It is hydroxile group (OH) bonded to a C₁₀ skeleton. Their names end in- ol. They are highly sought after for their aroma. Linalool, for example, has two forms. R-linalool is found in roses and lavender and is the main component of *Mentha arvensis*. S-linalool found in lavender oil at > 5% indicates adulteration. Linalool gives tea, thyme, and cardamom leaves their taste. Menthol, another compound found in this group, is responsible for the smell and taste of mint. Mint essence may contain up to 50% of this component. Geraniol, from scented geraniums, citronelol, from roses (*Rosa gallica*), borneol from rosemary, and santalol from sandalwood (*Santalum album*, Santalaceae family).

Phenols

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

Aldehydes

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

Esthers

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

2.8 Parameters Affecting Yield and Quality of Essential Oil

Yield and quality of essential oil from steam distillation was affected by the various process Parameters. It was advisable to keep them in mind while designing such systems. Some of the important parameters were being listed below.

Improper Design of Equipment

Improper designing of condenser or separators, of tank can lead to loss of oil and high capital Investments. The design of the furnace and chimney affects the firing and heat control of the Distillation rates. Tank height diameter ratio is important. Similarly the use of a condenser with an improper design and without calculating the heat transfer areas based on the steam generation Areas lead to improper condensation and loss of oil.

Mode of Distillation

The way for distillation should be chosen considering the boiling point of the essential oil and the nature of the herb, as the heat content and temperature of steam can alter the distillation characteristics. Since the heat content and temperature of steam depend upon its pressure, a change in steam pressure can alter the distillation characteristics. High-boiling constituents of essential oils normally require high pressure steam to distill over.

Nature of raw material

Nature 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.

Time for Distillation

Different constituents of the essential oil get distilled in the order of their boiling points. Thus, the highest boiling fractions will be last to come over when, generally, very little oil is distilling. If the distillation is terminated too soon, the high boiling constituents will be lost.

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.

Material of Fabrication of Equipment

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

Particle Size of the raw material loading to the chamber

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

2.9 Concluding remarks

Gin originates in Holland back in 1550, we can credit Dutchman *Franciscus de la Boe Sylvius*, a professor of medicine who was experimenting with juniper as a diuretic infused juniper into white spirit and it was sold in chemists as a treatment for stomachaches, gout and gallstones. The flavour for Gin comes from difference Botanicals (coriander, lemon peel, orange peel, etc) to these vary from producer to producer. Juniper Berries is the primary sources of raw material being investigated for Gin flavoring and it is predominant flavour along with other natural flavourings.

Juniper Berries is a coniferous evergreen shrub widely distributed throughout at different part Ethiopia. The berries are fleshy and globular, measuring $\frac{1}{4}$ - $\frac{1}{3}$ inches in diameter. They contain three sticky, brown irregular shaped seeds. The fruits should be used when ripe, that is, and after the skin has turned blue. On drying and storing, the smooth shiny skin darkens to a purple black and becomes slightly wrinkled or indented. There are many types of extraction. The extraction can be conducted with or without solvent. But, to get the essential oil, extraction through steam distillation is the most used method. Without any solvent, pure water is used at its boiling point as steam to extract the essential oil from juniper berry. The essential oil extract from juniper berries is superior in quality it is yellow oil with a watery viscosity, and a fresh, clean and slightly woody and fruity fragrance. The yield and the composition of the essential oils of the juniper berries depends on the geographical origin of the plant, the maturity of the berries, the age of the plant, the meteorological condition (temperature, insolation, *etc.*) and other environmental factors.

The importance of juniper berries oil is use which minimize the imported Gin flavour, by produce to supply enough oil of juniper berries for all liquor factories and save the berries because people only use the wood for different purpose during this the berries is exposed to the environment. Therefore, extraction of juniper berries oil and supply Gin flavor to enhancing the potential alcohol factory that produce Gin.

Chapter Three

3. Materials and methods

3.1 Materials Collection, Transportation, Sample Preparation and Storage

The raw material (Juniper Berries) get from oromia region of Chilimo forest it was located in west showa zone in Dendi woreda about 70km away from Addis Ababa in the Northwest direction. The collected raw material was separate the barrier from needles then transported by car to Addis Ababa then the berries was dry by using sun and remove the seed from the berries after that transport from the home to AAiT School of Chemical and Bio Engineering Research Laboratory. Sample (Juniper berries) were cleaned properly after that crushed using jaw crusher then it was sifted to pass through sive test at different size all samples were put in a plastic bags and stored at cool, dry and dark place. For further laboratory analysis and extraction experiment was conducted

3.1.1 Material and Chemicals used for the Research

Table 3.1 material equipment and chemical

Material and equipment	Chemical
juniper berries (Raw material)	Na_2SO_4 (Anhydrous sodium sulfate)
Oven dryer	NaCl
Steam generator Boiler , Extraction chamber	NaOH
Jaw crusher	Distilled water
Power supply	KOH
Filter, sieve	phenol phathaline indicator
Black bottle	oxalic acid,
Plastic bag	HCl
Separating funnel	Ethanol
Viscometer	oxalic acid,
Gas chromatography - Mass spectroscopy	NaS_2O_3
density separator	
Flask, 500 ml and 1000ml Beakers ,PH mete	

3.1.2 Framework of the Research

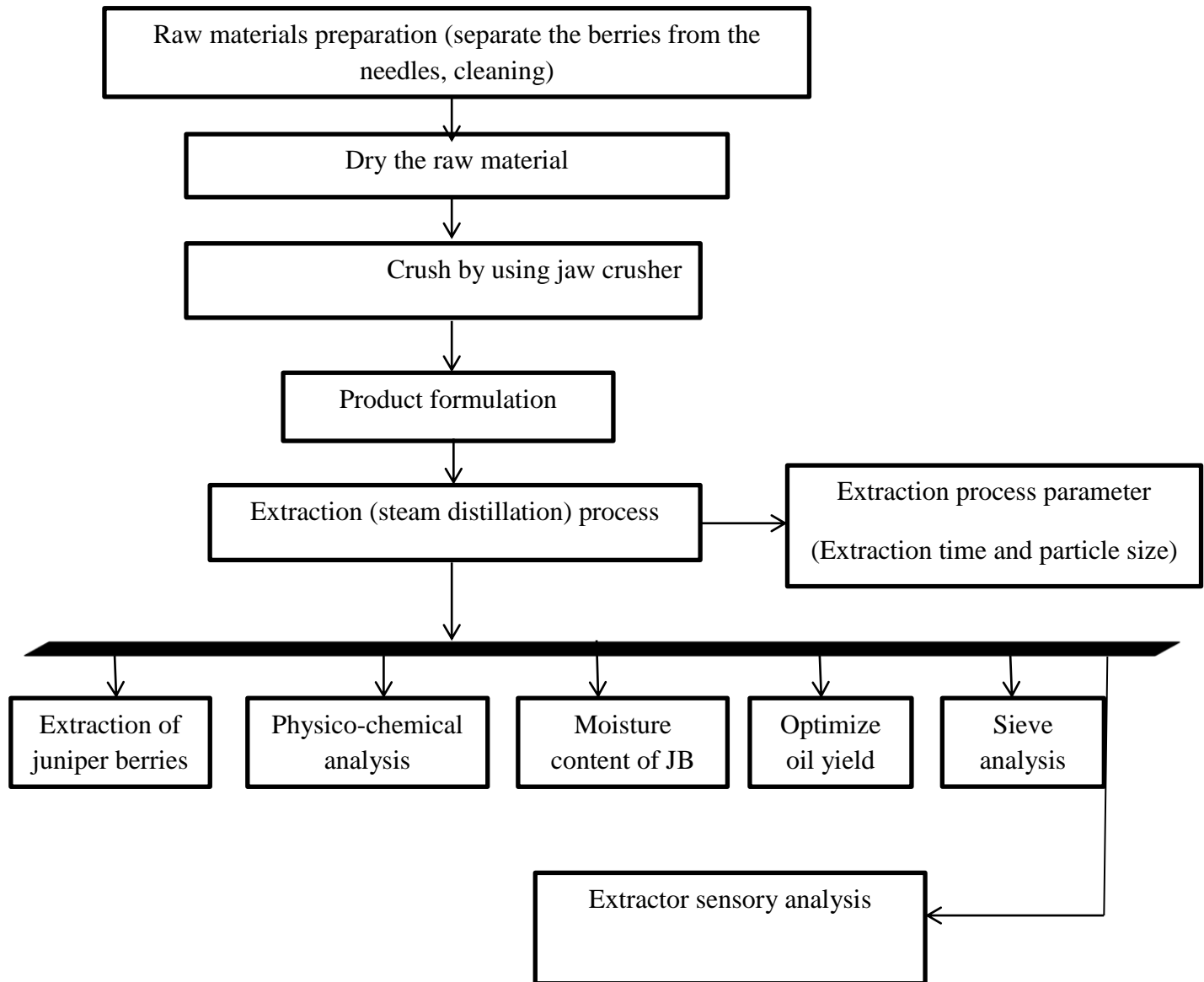


Figure 3.1 Framework of the research

3.2 Methods of oil extraction

The extraction of juniper berries oil was conducted using steam distillation method with different extraction time and with different particle size ranges at temperature of 98.5°C ,96.5 but high extraction of oil was obtained at 98.5 temperature and atmospheric pressure of 1atm. For the current experimental work, 100gram of juniper berries used at three different extraction time intervals; 2 hours, 3 hours and 4hours with different particle size ranges of 0.355-1 mm, 1-1.4 mm and 1.4-2.5 mm with three replications to get maximum yield. Before the process started the column was cleaned then the juniper berries is 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 (Brewer, 2011).

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.



Figure 3.2 photograph of steam distillation equipment

3.2.1 Components of the steam distillation plant

I. Boiler

Produce steam by boiling water which can help the extraction process by entering to the extraction chamber. For this experimental work the boiler was operates at atmospheric pressure and temperature 98.5 and 96.5.

II. Extraction chamber

This facilitated primary as a container and as a vessel in which the steam contacts the plant material and vaporize its oils. The plant material was pack in to the extraction chamber so that distillation commence. Proper charging was very important otherwise the steam channels

through the plant material and low yield results. The first load was contact to the format 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.

IV Separator

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

3.3 Sample Analysis

3.3.1 Determination of moisture content of the berries

Four samples of the juniper berries were randomly selected from each group weighted and dried in oven at 105°C and the weight was measured every two hours. The procedure was repeated until a constant weight was obtained and the percentage moisture content of the juniper berries was determined (Avinash, 2016).

$$\text{Moisture\%} = \frac{W_1 - W_2}{W_2} * 100 \text{-----} (3.1)$$

Where:

W_1 = Original weight of the sample before drying

W_2 = Weight of the sample after drying.

3.3.2 Size reduction and sieve analysis of the berries

The moisture was removed by placing the sample in sun dry. The dry Juniper berries are crushed by jaw crusher. The sample was sieve use with set of sieves sizes arrange in descending order to obtain particle size of 0.355-1mm, 1-1.4 mm, 1.4-2.5 mm. This is because to investigate the effect of particles size on yield and quantity of oil.



Figure 3.3 sieve and jaw crusher



Figure 3.4 juniper berries before size reduction and after size reduction

3.3.3 Determination of the yield of juniper berries oil extraction

100g (W_1) of the sample was placed in the round flask and 1000 mL of distilled water was poured into the round bottom flask for each run repeatedly. The apparatus was heated and allowed for 2hrs, 3hrs and 4hrs continuous extraction using Clevenger apparatus. The experiment was repeated for 0.355-1mm, 1-1.4mm and 1.4-2.5mm respectively for each run.

$$\% \text{ oil yield} = \frac{\text{weight of oil}}{\text{weight of juniper berries}} * 100 \quad (3.2)$$

3.4 Characterization of the extracted Juniper berries oil

Physical properties of extracted oil some of these properties are specific gravity, refractive index, boiling point, specific gravity, viscosity, PH value and chemical properties like saponification value, iodine value, acid value and free fatty acid were determined for the oil which was extracted using optimum operating parameters (Hailemariam, 2016).

3.4.1 Characterization of the Physical Properties of Essential Oil

3.4.1.1. Specific gravity of essential oil

The density of the juniper berries oil was determined by using density bottle. A clean and dry empty bottle of 25ml capacity was weighed (W_0) and then the bottle was filled with the oil, stopper inserted and reweighed to give (W_1). The oil was substituted with water after wash and dries the bottle and weight to give (W_2) the expression for specific gravity (Sp.gr) is:

$$\text{Sp.gr} = \frac{W_1 - W_0}{W_2 - W_0} = \text{-----} \quad (3.3)$$

3.4.1.2. pH value of essential oil

2g of the juniper berries was taken and putted in a clean dry 25ml beaker and 13ml of hot distilled water was added to the oil in the beaker and stirred slowly. Then it was cooled in cold-water bath to 25. First, the pH electrode was standardized with a buffer solution and then immersed in the sample and the pH was read.

3.4.1.3. Determination of Refractive Index

The Refractive Index of the juniper berries was measured by Refracto meter at 25 in Adiss Ababa University (Arat kilo) Department of Physics laboratory. First the cuvet was cleaned next filled with juniper berry oil then put on the prism for refractive index determination, the value is calculated as:

$$N = \tan (\theta b) \text{-----} \quad (3.4)$$

Where:-

N= refractive index and

θb = angle of rotation.

3.4.1.4. Determination of optical rotation

The optical rotation of the juniper berries oil is measure using Polari meter with a 2 dm length Polari meter tube where the angle of rotation in degrees are read at 25°C using D-line of

polarized sodium light. The specific optical rotation was measured in Addis Ababa University (Arat Kilo) in Department of Physics laboratory

3.4.1.5. Boiling Point of essential oil

25 ml of juniper berries essential oil poured in to beaker and a thermometer was inserted and placed on a heating mantle, it was observed that the oil in the beaker started circulating leading to boiling of oil and read temperature on thermometer.

3.4.2 Characterization of the chemical Properties of Oil

3.4.2.1. Acid value

Acid value was the mass of potassium hydroxide (KOH) in mg that was required to neutralize one gram of chemical substance, the acid value is a measure of the amount of carboxylic acid groups in a chemical compound. The acid number was used to quantify the amount of acid present, in juniper berries essential oil. Acid value was determined according to the method of European Pharmacopeia. Juniper berries essential oil (8g) was accurately weighed and dissolved in 10ml of 95% ethanol and 2-3 drops of phenolphthalein indicator was added. The free acid was then titrated with standard 0.1 N aqueous sodium hydroxide solutions by adding the alkali dropwise at a uniform rate of about 30 drops per minute. The content of the flask was continuously agitated. The primary manifestation of the red coloration that did not fade within 10 seconds was considered the end point. Afterward, the acid value was determined using the following equations:

$$\text{Acid value} = \frac{5.16 * (\text{number of ml of 0.1N NaOH})}{\text{Weight of sample in gram}} \quad (3.5)$$

3.4.2.2. Saponification Value

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. Juniper berries essential oil (1g) was accurately weighed and dissolved in 10ml of 70% ethanol and then 10ml of 2.5N KOH solution was added. This procedure was performed together with blank experiment which was also performed omitting the oil. The mixture was refluxed for two hours then cooled. The

unreacted KOH was titrated with standard N/2 oxalic acid by adding 2-3 drops of phenolphthalein indicator. After that, the saponification value was determined using the following equation:-

$$\text{Saponification value} = \frac{56.1 (V_1 - V_2)}{2 * M} \quad \text{-----} \quad (3.6)$$

Where:

V_1 = volume of 0.5 Normality of oxalic acid for blank

V_2 = volume of 0.5 Normality of oxalic acid for sample

M = Mass in gram of the oil

3.4.2.3. Iodine value

0.31g of juniper berries essential oil was weighed into a conical flask. 10ml of carbon tetrachloride and 20ml of the wij's solution were added to the flask and the solution was kept in dark for 30min at room temperature. 15ml of 10% potassium iodide solution with 100ml of distilled water were added to the flask. The resulting solution was titrated against 0.1M sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$), using starch as indicator till the end point where the blue black coloration becomes colorless. A blank titration was carried out at the same time starting with 10ml carbon tetrachloride. Iodine value was then calculated by the following formula.

$$\text{Iodine number} = \frac{[(B-S)*N*12.69]}{\text{Weight of the sample}} \quad \text{-----} \quad (3.7)$$

Where:

B = 0.1 N Sodium Thiosulfate required (ml) by blank

S = 0.1 N Sodium Thiosulfate required (ml) by sample

N = Normality of Sodium Thiosulfate solution

3.4.2.4 Chemical characterization of juniper berry oil

Determination of the chemical composition of the extracted essential oil from juniper berries was carried out by Gas Chromatography-Mass Spectroscopy. Gas Chromatographic (GC) analysis were performed with a Hewlett Packard 5973 Series equipped with a HPChemstation data processor, fitted with a HP-5MS capillary column (30 m x 0.25 mm i.e., 0.25 μm film thickness column temperature, 45°C (8 min) to 230°C at 2 °C/min, 180°C , 230°C (15 min); injector temperature 250°C ; detector temperature 260°C ; split ratio 1:20; carrier gas N₂. The oven temperature was programmed from 60°C to 220°C rate of 6°C/ min. Helium gas was employed as the carrier gas at 1 mL/min flow rate; source 70 eV. Juniper Berries oil sample was diluted with normal n-hexane and 0.1 μL was injected into the GC-MS. The detected compounds of oil were identified by processing the raw GC-MS data that obtained from 4 kilo was comparing with National Institute of Standard and Technology mass spectral database and from retention times and mass spectra of standard compounds.

3.5 Experimental design

3.5.1 Full Factorial design and Data analysis

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 juniper berries 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 three replications and three levels for each. Extraction time with three levels (2hr, 3hr and 4hr) and particle size three levels (0.355-1mm, 1-1.4 mm & 1.4-2.5 mm) This design of the experiment helps us to differentiate the significance of the main and the interaction factors.

Chapter Four

4. Results and Discussion

4.1 Quantitative Analysis of the essential oil

30Kg of the juniper berries was collected on December, 2017 get from oromia region of Chilimo forest it is located in west showa zone in Dendi woreda. samples of each kind these were fresh, and sundried juniper berries were taken for this analysis. The samples were taken randomly and average moisture content was calculated using equation (3.1). Table 4.1 indicates fresh juniper berries moisture content.

Table 4.1 Moisture content of fresh juniper berries

Sample mass in gram (g)				
	Sample 1	sample2	Sample 3	Sample 4
Time (Hr.)	16.7	21.7	54.91	70.6
2	13.2	18.5	49.81	65.1
4	11.52	15.1	42.41	54.5
6	9.67	12.9	38.31	47.5
8	7.36	12.2	29.91	44.3
10	7.36	12.2	29.91	44.3
Average Moisture	55.93	43.78	45.53	37.25
contain in %				

The wet basis moisture content of the four juniper berries sample were 55.93%, 43.78%, 45.53%, and 37.25%. Thus, the mean average moisture content of the four juniper berries samples was 44.6%. This result is somehow excess than a study on fresh juniper berries that reported a

moisture content of 25-36 % this is because of so many factors such as difference in geographical locations, production techniques, and plant collection time.

Table 4.2 Moisture content of sun dry juniper berries

	Sample mass in gram (g)			
	Sample 1	Sample 2	Sample 3	Sample 4
Time (Hr.)	27.00	36.00	48.00	55.00
2	26.86	35.69	47.58	54.72
4	26.65	35.24	47.21	54.31
6	26.43	34.91	46.83	53.98
8	26.21	34.75	46.73	53.74
10	26.21	34.75	46.73	53.74
Average Moisture	2.93	3.47	2.64	2.29
contain in %				

the moisture content of the four sun dried juniper berries samples having mass of 27, 36, 48, and 55, grams were 2.93%, 3.47%, 2.64% and 2.29% respectively. Thus, the average moisture content of the four samples was 2.83%. This result agrees to the moisture content of the dried juniper berries.

4.1.1. Analysis on factors affecting essential oil yield

The experimental design selected for this study is the general factorial design and the response variable is the Yield of juniper berries Essential oil. Three levels for particle size and three levels for extraction time and two factors general factorial design was applied. And then 27 experiments were performed for this research work. Optimization of extraction of essential oil by the classical method involves changing one independent variable while maintaining all others at a fixed level. This procedure is extremely time consuming and expensive for a large number of variables. To overcome this difficulty, the experimental design software can be employed to investigate the extraction parameters of juniper berries 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 work, 100g of juniper berries were used for each experiment. The pressure in the boiler for consecutive tests was set at one atm and temperature 98.5 and 96.5°C. 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 100g of juniper berries.

Table 4.3 oil yield by steam distillation and result using Design Expert standard method

Std	Run	Block	Factor1	Factor 2	Response 1
			A:time (min)	B:size (mm)	Yield %
1	17	Block 1	2	0.355-1	1.51
2	20	Block 1	2	0.355-1	0.92
3	1	Block 1	2	0.355-1	1.03
4	8	Block 1	3	0.355-1	1.43
5	3	Block 1	3	0.355-1	1.52
6	25	Block 1	3	0.355-1	1.32
7	24	Block 1	4	0.355-1	1.75
8	12	Block 1	4	0.355-1	2.05
9	6	Block 1	4	0.355-1	2.27
10	5	Block 1	2	1-1.4	0.51
11	10	Block 1	2	1-1.4	0.69
12	16	Block 1	2	1-1.4	0.93
13	4	Block 1	3	1-1.4	0.98
14	11	Block 1	3	1-1.4	1.06
15	14	Block 1	3	1-1.4	0.87
16	18	Block 1	4	1-1.4	1.31
17	15	Block 1	4	1-1.4	1.25
18	23	Block 1	4	1-1.4	1.55
19	27	Block 1	2	1.4-2.5	0.487
20	26	Block 1	2	1.4-2.5	0.52
21	21	Block 1	2	1.4-2.5	0.63
22	7	Block 1	3	1.4-2.5	0.89
23	2	Block 1	3	1.4-2.5	0.91
24	19	Block 1	3	1.4-2.5	1.01
25	22	Block 1	4	1.4-2.5	0.989
26	13	Block 1	4	1.4-2.5	0.95
27	9	Block 1	4	1.4-2.5	1.12

Extraction time, Particle size and Yield found, the maximum extraction of juniper berry oil was 2.27% at particle size ranges from 0.355- 1mm for the extraction time of 4 hours and the minimum yield of essential oil obtained was 0.487% 1.4- 2.5 mm at minimum extraction time that is at 2 hours.

4.1.2. Development of regression model equation

The model equation that correlates the response (yield of essential oil) to the extraction process variables in terms of actual value after excluding the insignificant terms was given below. The predicted model for percentage of yield of juniper berries essential Oil in terms of the coded factors is given in equation (4.1).

$$\text{Yield of essential oil} = +1.13 - 0.33 *A [1] - 0.018 *A [2] + 0.41 *B [1] - 0.11*B [2] \text{—————(4.1)}$$

Where:-

A is extraction time

B is size of particle

The result indicates that the yield of juniper berries essential oil was dependent only on the linear terms. Based on the coefficients in equations (4.1) it was evident that the percentage of essential oil yield increases with the particle size (A) and extraction time (B).

4.1.3. Model adequacy check

The model was tested for adequacy by analysis of variance. The regression model was found to be reasonably significant with the correlation coefficients of determination of R-Squared, adjusted R-Squared and predicted R-Squared having a value of 0.8503, 0.8231 and 0.7746 respectively. The quality of the model developed could be evaluated from their coefficients of correlation. The value of R-squared for the developed correlation is 0.8503. It implies that 85.03% of the total variation in the yield of essential oil was attributed to the experimental variables studied. The “Pred R-Squared” of juniper berries essential oil 0.7 is 7467 close to the “Adj R-Squared” of 0.8231 that is less than 0.2 differences as one might expect. The “Adeq Precision” measures the signal to disturbance ratio due to random error. A ratio greater than four

is desirable. Juniper berries essential oil has ratio of 16.973, which indicates an adequate signal. Therefore, this model can be used to navigate the design space.

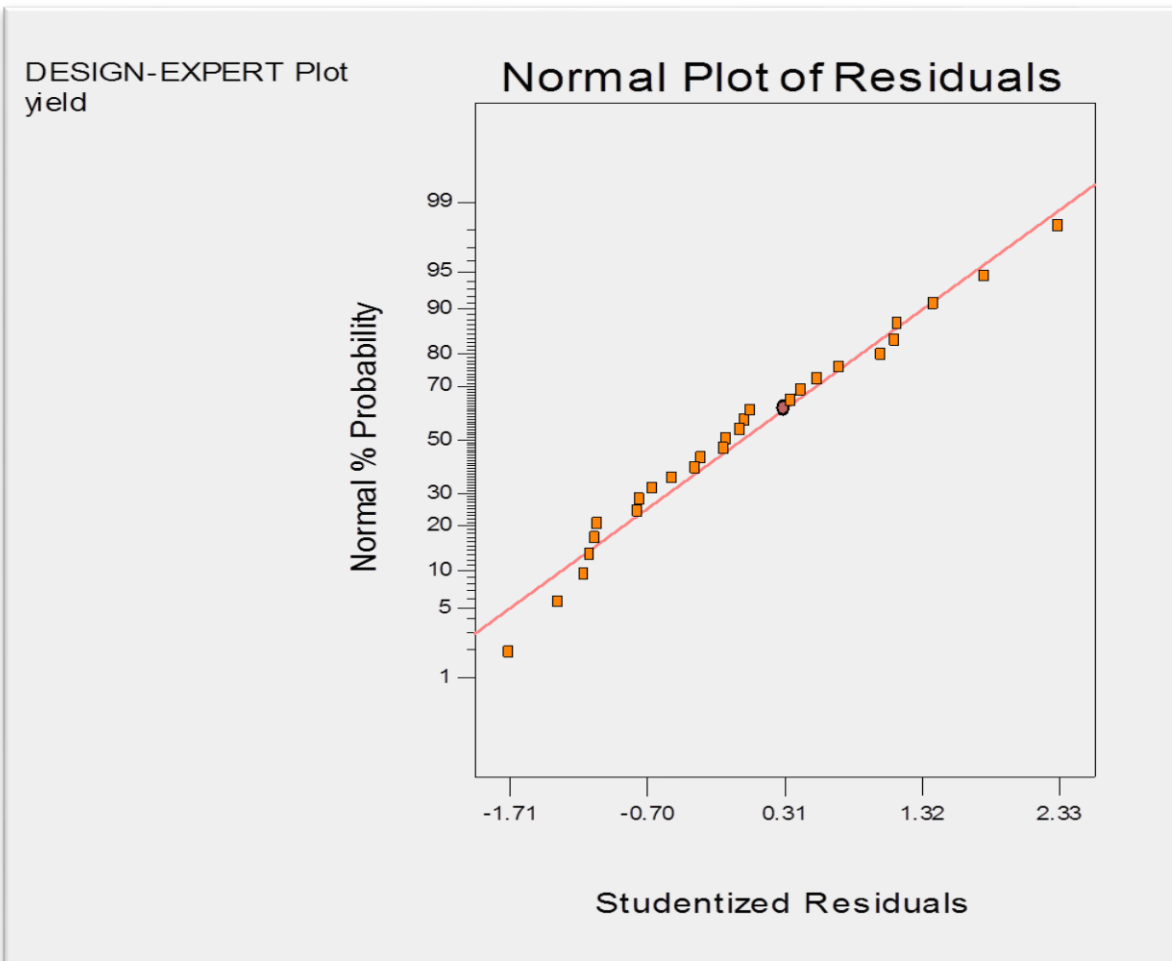


Figure 4.1 Normal plots of residuals of juniper berries essential oil

the normal probability plot indicates the residuals following a normal distribution, in the case of this experiment the points in the plots shows fit to a straight line in the figure i.e. the error distribution is approximately normal. This indicates the model satisfies the assumption of ANOVA.

The adequacy of the model was further checked by analysis of variance (ANOVA) as shown in Table 4.4 Based on a 95% confidence level; F-value is a test for comparing model variance with residual (error) variance. If the variances are close to the same, the ratio will be close to one and it is likely that any of the factors have a significant effect on the response with the P-value less than 0.05. It was calculated by model mean square divided by residual mean square. Here the model F-value of 31.25 implies the model is significant. There is only a 0.01% chance that a “Model F-Value” this large could occur due to personal error or disturbance.

Table 4.4 ANOVA for the regression model equation and coefficients

Source	Sum of Square	Df	Mean Square	F-value	P-value Prob > F	Remark
Model	4.38	4	1.10	31.25	< 0.0001	significant
A-extraction time	2.01	2	1.01	28.71	< 0.0001	
B- particle size	2.37	2	1.18	33.79	< 0.0001	
Residual	0.77	22	0.035			
<i>Lack of Fit</i>	<i>0.23</i>	4	0.056	1.86	0.1623	<i>not significant</i>
Pure Error	0.55	18	0.030			
Cor Total	5.15	26				

The above figure shows Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. This shows that the particle size and extraction time are significantly affect the essential oil yield but the interaction effect is not as such effect on juniper berries essential oil yield. If there are many insignificant model terms ,model reduction may improve your model. The "Lack of Fit F-value" of 1.86 implies the Lack of Fit is not significant relative to the pure error. There is a 16.23% chance that a lack of fit F-value this large could occur due to noise. Non-significant lack of fit is good because we want the model to fit.

4.1.4 Effect of Individual Process Variables on the yield of extracted oil

Established on the analysis of variance juniper berries 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 juniper berries essential oil.

4.1.4.1. Effect of extraction time on the percent yield of JB oil

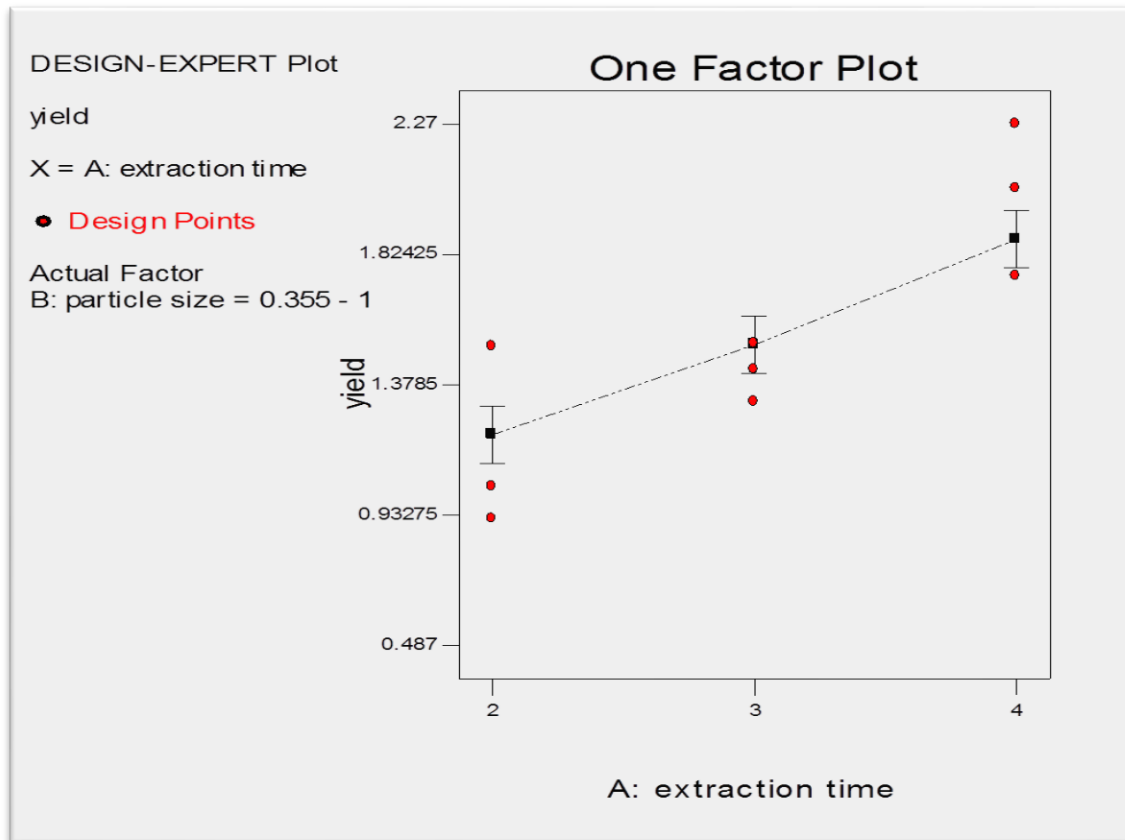


Figure 4.2 Effect of time on the yield of extracted oil

Extraction time plays unlimited role on the percentage yield of juniper berries oil using steam distillation. In figure above shows that as contact time increase the oil yield also increase till transfer of oil from the berries 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.

4.1.4.2. Effect of particle size on the yield of extracted oil

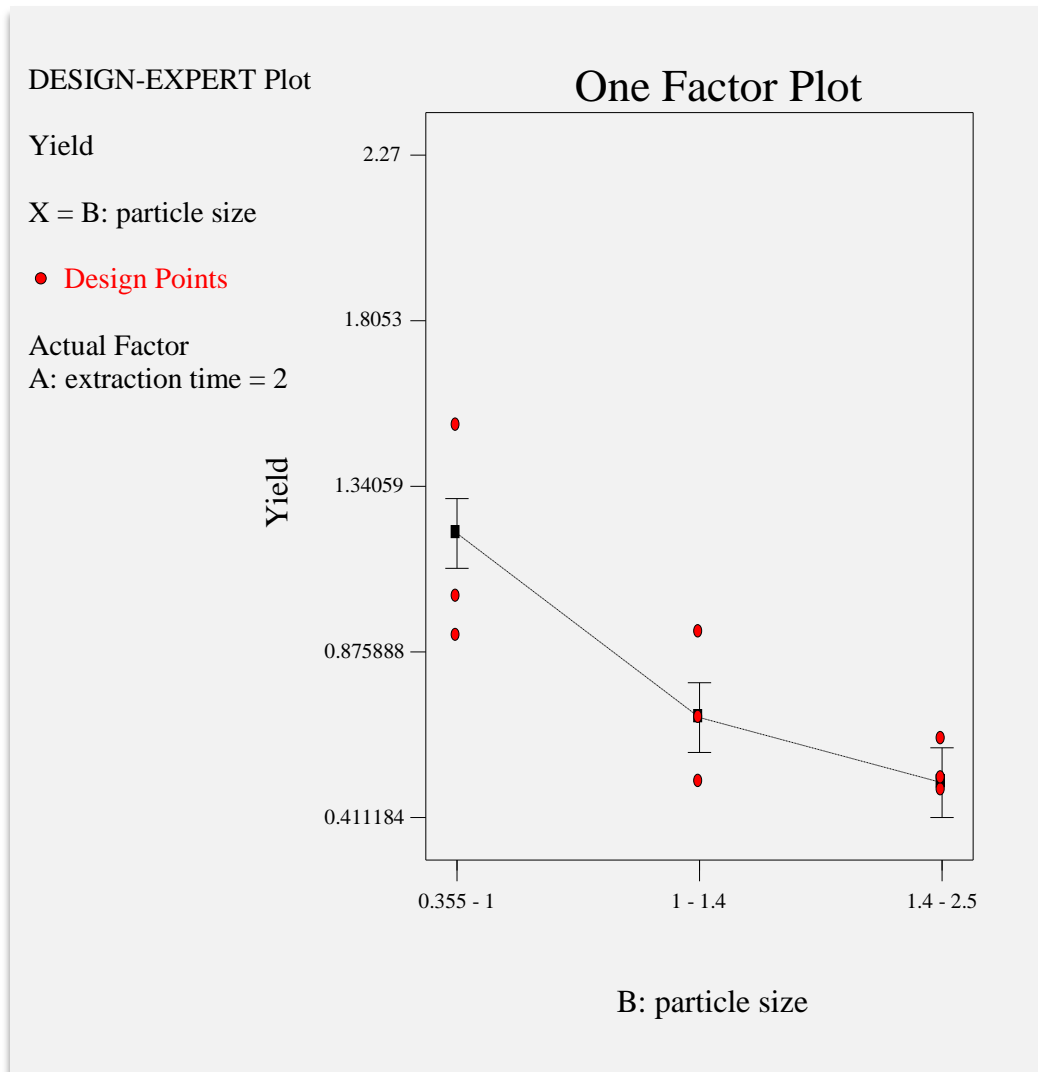


Figure 4.3 Effect of particle size on the yield of extracted oil

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

4.1.4.3. 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

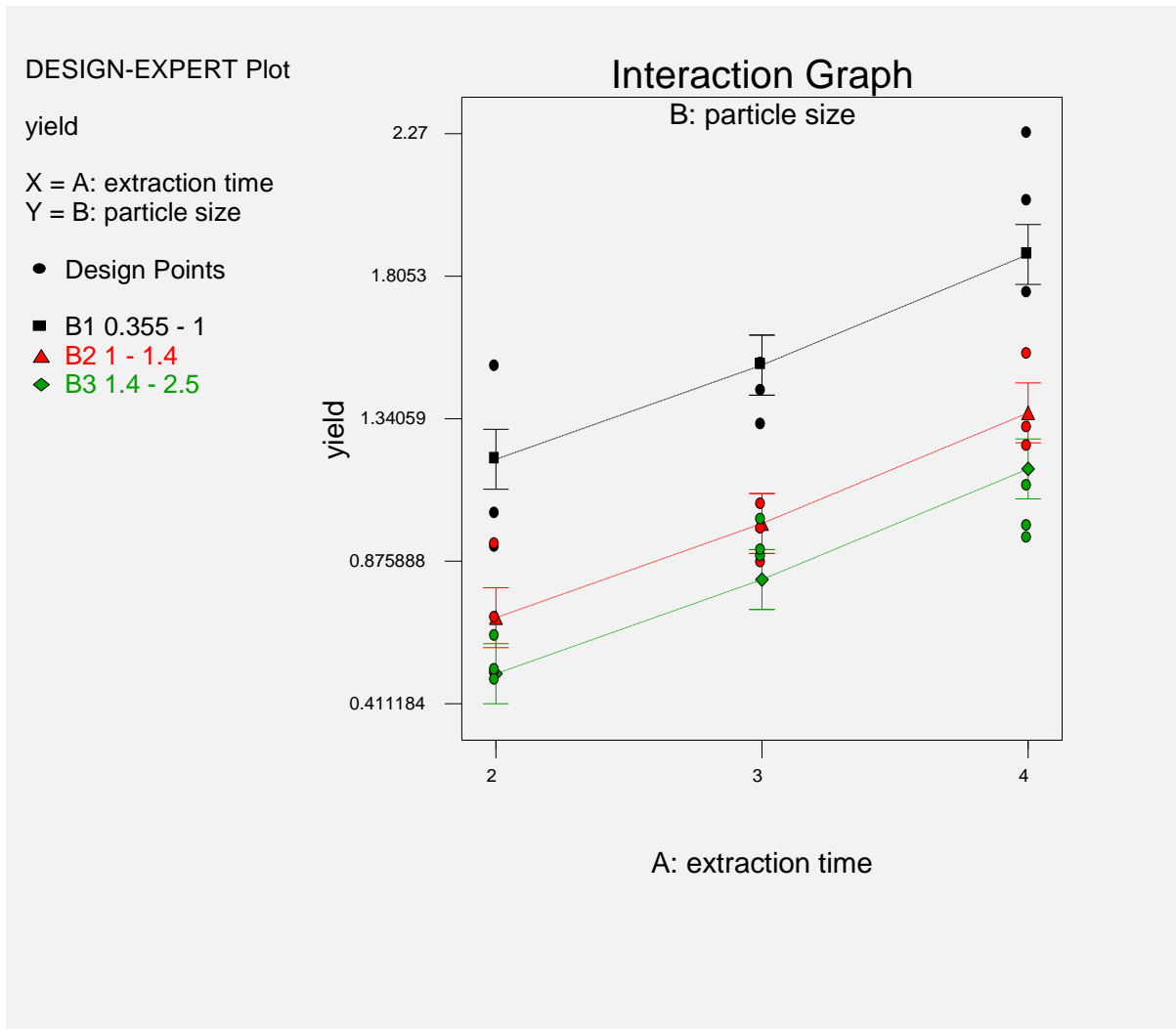


Figure 4.4 Effects of time, particle size and their interactions on juniper berries oil yield

Interactions graph shows that there is no interaction among each factor. This shows us an increment in time will increase the quantity of juniper berries oil extracted. Extraction beyond 4 hours didn't gives a significant change on oil yield. On Figure above it was observed that as time increase and particle size decrease yield increase.

4.1.5 Optimization of Juniper Berries Oil Extract Using Steam Distillation.

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

Table 4.5 Optimum Desirability results of oil yield

Number	Extraction time	Particle size	yield	Desirability	
1	4	<u>0.355 - 1</u>	<u>2.02</u>	<u>0.86</u>	<u>Selected</u>
2	3	<u>0.355 - 1</u>	<u>1.42</u>	<u>0.53</u>	
3	4	<u>1 - 1.4</u>	<u>1.37</u>	<u>0.49</u>	
4	2	<u>0.355 - 1</u>	<u>1.15</u>	<u>0.37</u>	
5	4	<u>1.4 - 2.5</u>	<u>1.02</u>	<u>0.29</u>	
6	3	<u>1 - 1.4</u>	<u>0.97</u>	<u>0.27</u>	
7	3	<u>1.4 - 2.5</u>	<u>0.94</u>	<u>0.25</u>	
8	2	<u>1 - 1.4</u>	<u>0.71</u>	<u>0.13</u>	
9	2	<u>1.4 - 2.5</u>	<u>0.55</u>	<u>0.03</u>	

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 attractiveness 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 attractiveness is to 1. Based on the above analysis best local maximum for juniper berries oil yield of 2.0233 % was found at a time 4 hrs, particle size 0.355-1mm and the value of desirability obtained was 0.862%.

4.2. Quality Analysis of the juniper berries oil

4.2.1. Physical characteristics of juniper berries essential oil

4.2.1.1. Specific gravity

Density bottle method was used to determine the specific gravity of the essential oil as detail experimental procedure were stated in chapter three

W_0 is weight in gram of empty Pycnometer @ 25 = 25.947 g

W_1 is weight in gram of Pycnometer with juniper berries oil @ 25 = 46.315g

W_2 is weight gram of water in Pycnometer @ 25 = 49.673g

Substituting in to the equation below

$$\text{Sp.gr} = \frac{46.315 - 25.947}{49.673 - 25.947} = 0.8584 \text{ g/ml}$$

4.2.1.2. pH value

The pH of juniper berries essential oil was measured by pH meter in Leather industry development institute (LIDI). Its value was recorded as 4.7.

4.2.1.3. Refractive index of juniper berries 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 (HeNe laser wave length) calculated as:

$$(360^\circ - 312^\circ 20') = (360 - 321.33) + 17.67$$

$$N = \tan (56.34) = 1.50^\circ$$

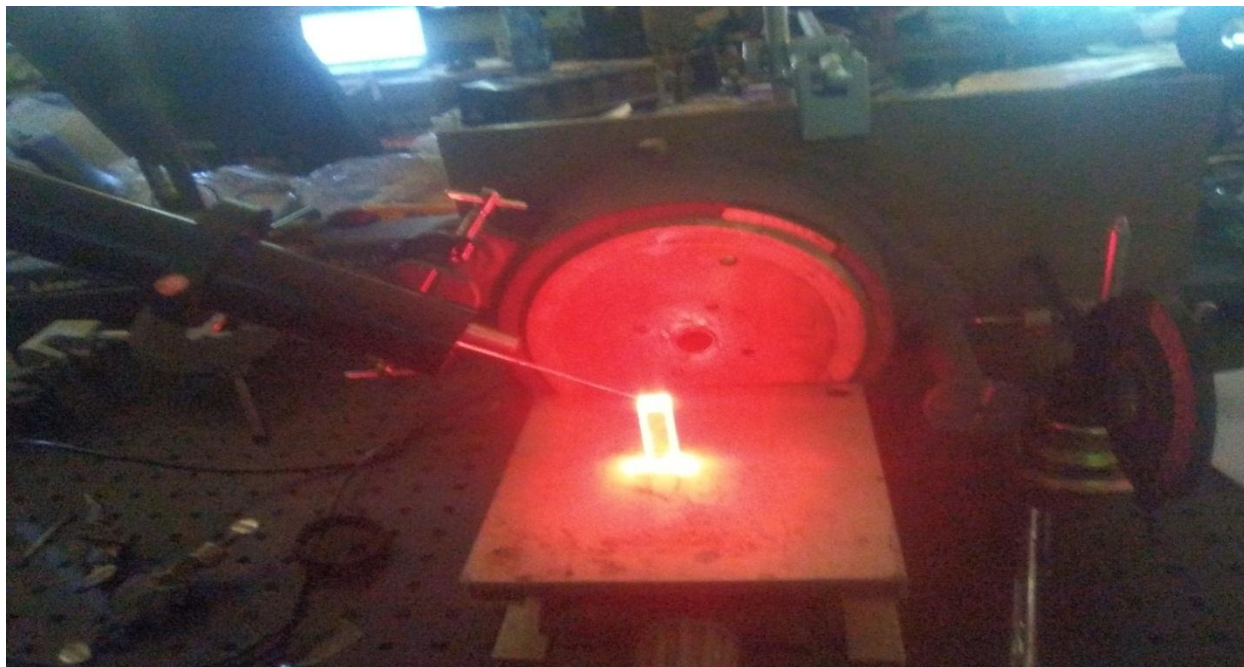


Figure 4.5 Refractive index measurements

4.2.1.4. Optical rotation of juniper berries 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 juniper berries oil was measured by Polari meter in AAU (Arat Kilo) Department of Physics. The Polari meter recorded was 0.5 degree in 1cm path length.

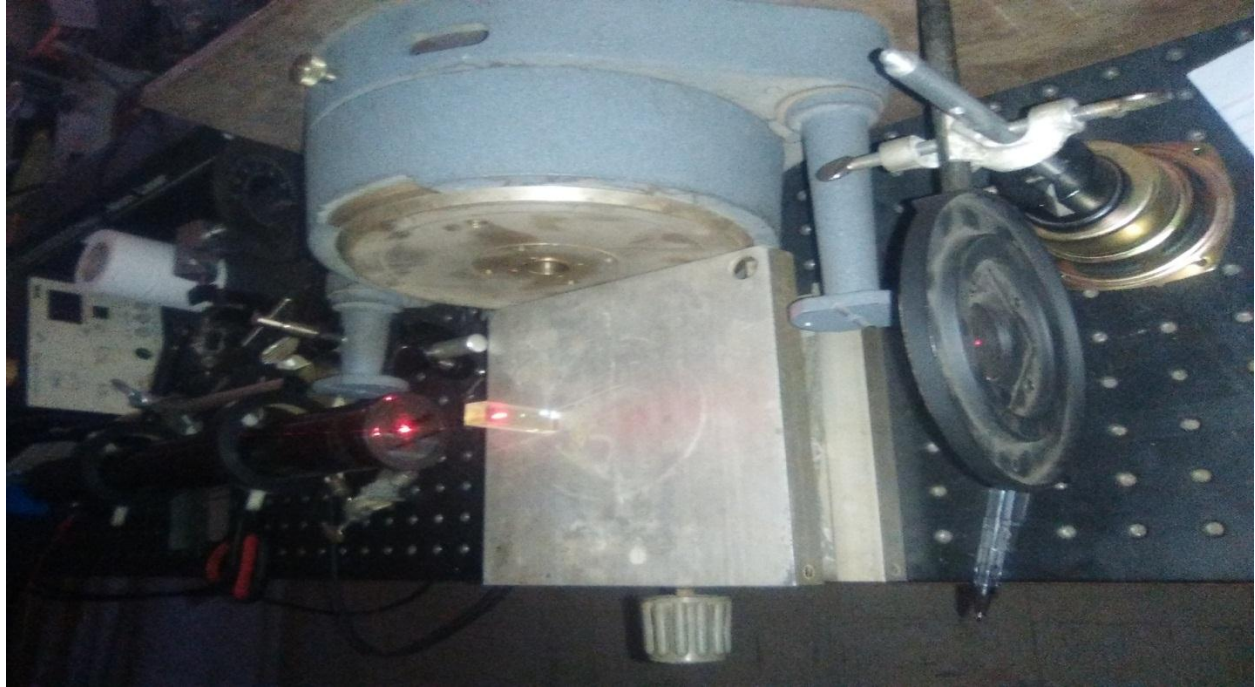


Figure 4.6 Spectrometer, optical rotation measurement

4.2.1.5. Viscosity of juniper berries oil

Dynamic Viscosity (μ) of juniper berries oil was measured by viscometer (Brookfield) at AAU (4killo) food science department laboratory. The value was 2.22 mili Pascal second (mpas).

4.2.1.6. Boiling point of juniper berries oil

The boiling point of juniper berries oil was measure by the procedure described on the methodology. Its value was 170°C.

4.2.1.7. Solubility of juniper berries oil

The juniper berries oil was soluble in alcohol, fixed oils and insoluble in water, propylene glycol. Generally the physical properties of juniper berries oil extracted using steam distillation were summarized in the Table 4.6.

Table 4.6 Summary of physical characteristics juniper berries oil

Physical properties	Results	Unit	European standards
Specific gravity	0.8584	g/cm ³	0.8456 - 0.890
Refractive index @ 25°C	1.50	-	1.4750 - 1.4880
Optical rotation	0.5	°	-3° - 20°
pH at 25°C	4.7	-	-
Dynamic viscosity	2.25	mPa.s	-
Boiling point	170	°C	131 - 172
Colour	yellow	-	Colorless to yellow
Solubility in alcohol	Soluble	-	Soluble in alcohol
Solubility in water	Insoluble	-	Insoluble in water
Brix	71.7	°	71.2 -73

4.2.2. Chemical characteristics of juniper berries essential oil

4.2.2.1. Acid value

Table 4.7 Acid value of juniper berries oil

Raw material conditions	Weight of sample(g)	Volume of Sodium Hydroxide used(ml)	Acid value
Extracted Oil	8	2	1.40ml/g

The acid values are in the range, if use sample weights 8-10 get 1 to 4 acid values.

4.2.2.2. Percentage of free fatty acid (% FA)

Where:

AV= is acid value of the oil

% FA= 0.503* AV

0.503*1.4 = 0.7055 %

4.2.2.3. Saponification value determination

$$\frac{56.1(15.2 - 13.5)\text{ml}}{2 * 1\text{gram}} = \frac{47.69 \text{ ml}}{\text{gram}}$$

4.2.2.4. Iodine number determination

The iodine value is used to determine the unsaturated of oils and in assessing the stability of oil in industrial application .The lower the iodine value of oil, which reflects its characteristics such as higher resistance to oxidation, the longer shelf life and higher quality. Whereas the higher the iodine value of oil, the lower the quality. Iodine values of juniper berries oil calculated by used below equation

$$\text{Iodine number} = \frac{1.269(14.5-12)}{0.31} = 10.23 \text{ ml/g}$$

Table 4.8 Summary of chemical characteristics

Chemical properties	Value	Unit
Acid value	1.4	ml/g
Saponification value	47.69	ml/g
iodine number	10.23	ml/g
free fatty acid	0.704	%

4.2.2.5. Gas Chromatography- Mass Spectroscopy

Juniper berries was extracted with water to get yellow oil. Components of the oil were determined using GC-MS analysis. The GC-MS spectrum of the seed oil was shown and its components were identified as shown in Table 4.9. A total of thirty components, with different retention times, were eluted from the GC column as indicated by the chromatogram and were further analyzed with an electron impact MS voyager detector. Identification of constituents was done on the basis of their retention time and mass spectra library search. MS also equipped with capillary flow technology which enables simultaneous analysis of the sample on both detectors. The relative amount of individual components was calculated based on GC peak areas. Comparison of the GC-MS spectrograph obtained with the instruments data bank together with

NIST MS data demo version revealed that the essential oil of juniper berries contained a mixture of terpenes that eluted at different retention times depending on the boiling point of the eluted component (Stoilova, 2014).

Table 9.4 show major component obtained by GC-MS analysis of the essential oils of juniper berries 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, it can be seen that these oils were characterized by the presence of monoterpene 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. The predominant fraction of the oils was monoterpene hydrocarbons. Great variability in the chemical composition and especially in the content of some components was observed. The most variable were α -pinene (73.12 %) followed by β -myrcene (4.29%). The major component was α -pinene (73.12%). Other predominant components were β -myrcene (4.29%, β -pinene (2.15 %), methylene (3.15%), phenylephrine (1.09%), and carene (4.74 %). The chemical compositions revealed that these berry had composition one way or another closed to those of other juniper berries essential oils GC-MS analyzed data. For example International Journal of Pharmacy and Pharmaceutical Sciences reported the following values α -pinene (76.23%), β -myrcene (5.89%), β -pinene (3.21%), D-limonene (2.83%), phenylephrine (0.97%), methylene (4.51%), and cymene (2.4%).

Table 4.9 Chemical composition of juniper berries oil

No.	Compound	Library Match Quality (%)	Retention Time(min)	Area (%)
1	2,6,6-Trimethylbicyclo [3.1.1] hept-2 ene	96	4.950	73.12
2	Acetamide,N-(aminocarbonyl) - 2-chloro-	64	5.453	0.21
3	Beta pinene	94	5.510	2.15
4	Beta myrcene	93	5.623	4.74
5	(+) -3-carene	94	5.902	4.74
6	1,2,5 – oxadiazol -3 carboxamide, 4, 4 ‘ –azobis - ,2,2 ‘ – dioxide	64	6.957	0.47
7	1 – (3,5 – Dimethyl -1 – adamantanoyl) semicarbazide	62	3.379	0.46
8	2 – Amino – 1 – (o - hydroxyphenyl) propane	62	8.063	0.48
9	Benzeneethanamine, 4 – methoxy -.alph a. –methyi-	64	9.504	0.17
10	1 – Octanamine , N –methyi -	64	14.966	0.22

File :D:\MassHunter\GCMS\1\5977\April 25 2017\00101001.D
Operator : Estif
Acquired : 25 Apr 2017 14:49 using AcqMethod Faty acids 31 minsplittless.M
Instrument : AAU berries
Sample Name: juniper berries
Misc Info : Sunitel
Vial Number: 1

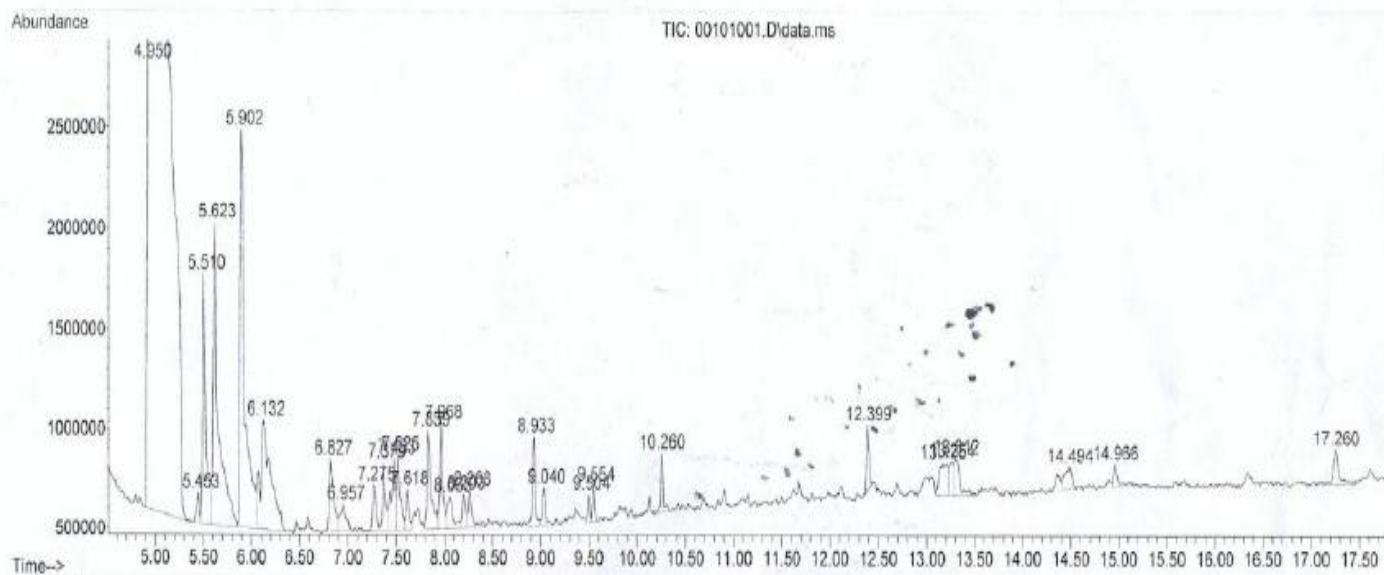


Figure 4.7 Chromatogram of juniper berries

4.2.3. Overall acceptability of juniper berries oil

The study concludes that juniper berries can be considered as an alternative essence for Gin flavor. The Gin that used juniper berries oil was comparable to that of imported from other country. The oil extracted in this and imported juniper berries oil was tested for some parameters like pH value, brix, specific gravity and it was observed that not that much variation in the quality of the oil extracted in this and imported during used for Gin flavor even the color is not changed both was the known Gin color white. Test the essence (sample) in to the Gin at NALF. Prepare 500 ml of flask, essence 0.6ml (juniper berries oil) ethanol (96.6%) soft water and 4g sugar. mix 51.1mm soft water and ethanol 207 ml in 500ml of flask and put in to shaker for 45min then add 0.4 ml of essence and 4 gram sugar.

Table 4.10 Acceptability parameters of dry Gin essence

Parameters	NALF sample	Extracted sample
Brix	71-73	71.7
Specific gravity	0.8475-0.954	0.8584
pH	4.5-7.98	4.7
Refractive index	0.475-0.498°	0.5°
Colour	yellow	Yellow

Finally when check alcoholic content by use alcoholic analyzer it drops from 96.6 to 40% of Gin alcoholic content and also colour is white. From this result the essence (juniper berries oil) was acceptability in NALF for Gin flavor.

4.3 Sensory quality evaluation

Sensory evaluation is an important criterion for quality assessment in new product development and to meet the consumer requirements. Any new product must give satisfaction and pleasure to the consumers. For this reason the extracted oil from juniper berries for Gin flavor were evaluated for various sensory attributes. A 9-point hedonic scale quality analysis was used to

evaluate the characteristics of two dry Gin samples which were extracting at pH, specific gravity and brix and refractive index for visual color, flavor, aroma, and test and over all acceptability analysis using 12 experienced panelists. Comparison among the averages of sensory attributes (visual color, flavor, aroma, and test and over all acceptability) control sample (sample1) is bought from market and sample 2 is extracted from juniper berries using steam distillation. Two equal litter of dry Gin samples were served with biscuit and water, with which the panelists could preceding the biscuit before quality attribute then panelists could rinse their mouth after each quality attribute.

Table 4.11 Evaluation of dry Gin samples

Sensory attributes	Sample 1(Control)	Sample 2
Visual color	8.92±0.29 ^a	8.91±0.29 ^a
flavor	8.17±0.72 ^{bc}	8.3±0.65 ^{cde}
aroma	8.42±0.52 ^{ab}	8.1±0.66 ^{de}
test	8.17±0.58 ^{bcd}	7.41±0.9 ^d
OAA	8.5±0.5 ^{bc}	8.25±0.75 ^{abc}

The values within the same row sharing same subscript letters are not significantly different. From table 4.11, it is evident that the visual color, flavor, aroma, test, and OAA of the two samples varied from 8.92 to 8.91, 8.17 to 8.3, 8.42 to 8.1, 8.17 to 7.41, and 8.5 to 8.25 respectively. The visual color and OAA of the two samples were not significantly different ($p>0.05$). Moreover aroma, test, and OAA of controlled sample1 were greater than sample2 from this the two samples is not that much difference, it was almost the same.

Chapter Five

5. Conclusion and Recommendations

5.1. Conclusion

This work was aimed at extraction, optimization and characterization of juniper berries oil for gin flavor. The extraction was done by steam distillation using water as a solvent extraction time and particle size as factors 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 98.5°C 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 juniper berries. 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 juniper berries oil was 0.49% obtained extraction time 2hr and particle size 1.4-2.5mm followed by a yield of 0.51% extraction time 2hr and particle size 1-1.4. The maximum yield of juniper berries oil was 2.27% at 4 of extraction time and 0.355-1mm particle size followed by yield of juniper berries oil 2.05% at particle size of 0.355-1mm and 4hr extraction time. It was clearly seen in design expert software that decreasing particle size and increasing extraction time content of juniper berries would increase the yield.

The linear model selected for optimizing the experimental variables shows that the optimum yield if found to be 2.27% with the desirability criteria of 0.862 which corresponds to the maximum yield obtained. The optimum value of the process parameters optimized were extraction time 4hr and particle size 0.355-1mm keeping the criteria of the optimization in the range of the level selected for experimental variable.

Physical and chemical properties like specific gravity at 25 °C, viscosity, pH value, boiling point, refractive index, optical rotation, solubility in ethanol, brix, acid value, Saponification value, iodine value and free fatty acid content of the juniper berries 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 juniper berries essential oil were determined using Gas Chromatography -Mass Spectroscopy (GC-MS) the identified major components were α -pinene (73.12%), β -myrcene (4.29%), β -pinene (2.15 %), methylene (3.15%), and carene (4.74 %). Finally, the oil is accepted in NALF depending on parameters like Brix, PH, Specific gravity and colour and sensory analyses also done to meet consumer requirements. Finally extraction of essential oil from Juniper Berries using Steam distillation can be used in any alcoholic and liquor factory's scale for flavor of Gin.

5.2. Recommendations

It was known that many juniper berries found in rural and urban region but the society not take attention to use for Gin flavor only use the needle for furniture, fuel wood therefore giving a better attention to extract oil from juniper berries and use Gin flavor unquestionable, because dry Gin was candidate liqueur drink. Therefore, based on the findings of this research work a further because study on:-

- ❖ Studies on evaluation of juniper berries essential oil extracted by supercritical CO₂ extraction for the production of perfume, cosmetics formulation, drug formulation and other relevant products.
- ❖ Government and private sectors should talk emphasis to create awareness to the society about industrial utilization, economic values of juniper berries essential oil use Gin flavor and promote the plantation of juniper berries.
- ❖ Further study should be conducted on the quality of the juniper berries essential oil obtained at each operating conditions using GC-MS. This will provide alternative to manufacturers on the minimum conditions required to get a specific quality of juniper berries essential oil.
- ❖ Studies on Techno – Economic evaluation of juniper berries essential oil production

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Appendices

Appendix –A: Quantitative Analysis Result of juniper berries essential oil

Factor		Yield of essential oil (g)			
Extraction Time (min)	Particle size (mm)	Replicates (g)			Average
		1	2	3	
2	0.355 – 1	1.51	0.92	1.03	1.15
3	0.355 – 1	1.43	1.52	1.32	1.423
4	0.355 – 1	1.75	2.05	2.27	2.02
2	1 – 1.4	0.501	0.69	0.93	0.707
3	1 – 1.4	0.98	1.06	0.87	0.97
4	1 - 1.4	1.31	1.25	1.55	1.37
2	1.4 - 2.5	0.487	0.52	0.63	0.546
3	1.4 - 2.4	0.89	0.91	1.01	0.94
4	1.4 - 2.5	0.989	0.95	1.25	1.063

Appendix - B: Summary of statistics executed for expansion index

Std. Dev.	0.19	R-Squared	0.8503
Mean	1.13	Adj R-Squared	0.8231
C.V. %	16.60	Pred R-Squared	0.7746
PRESS	1.16	Adeq Precision	16.973

Appendix-D: Integration peak list of juniper berries essential oil

Peak	Start	RT	End	Height	Area	Area %
1	4.904	4.935	5.164	32882059.07	113129309.1	100
2	5.476	5.5	5.567	1719436.8	2717160.37	2.4
3	5.567	5.608	5.77	1706699.8	5188411.61	4.59
4	5.866	5.89	6.052	3117062.8	6398940.25	5.66
5	6.092	6.119	6.28	623760.6	3023863.46	2.67
6	6.771	6.819	6.886	488583.13	1257491.5	1.11
7	7.411	7.465	7.567	326336	1577537.27	1.39

Appendix-E: Constraints of yield of Juniper berries essential oil

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
extraction time	is the range	2	4	1	1	3
particle size	is in range	0.355 - 1	1.4 - 2.5	1	1	3
yield	maximize	0.487	2.27	1	1	3

Appendix-F: Experimental Design Summary

Study Type	Factorial		Experiments 27				
Initial Design	Full Factorial		Blocks		No Blocks		
Center Points	0						
Design Model	2FI						
Response	Name	Units	Obs	Minimum	Maximum	Trans	Model
Y1	yield	%	27	0.49	2.27	None	Main effects
Factor	Name	Units	Type	Low Actual	High Actual		
A	Extraction Time	min	Categorical	2	4	Levels	3
B	Particle size	mm	Categorical	0.355 -1	1.4 -2.5	Levels	3

Appendix-G search report of GC-MS for essential oil of dry juniper berries

Library Search Report

Data Path : D:\MassHunter\GCMS\1\5977\April 25 2017\
 Data File : 00101001.D
 Acq On : 25 Apr 2017 14:49
 Operator : Estif
 Sample : Juniger berreds
 Misc :
 ALS Vial : 1 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L Minimum Quality: 90

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

PK#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	4.950	73.12	D:\MassHunter\Library\NIST14.L (1S)-2,6,6-Trimethylbicyclo[3.1.1] hept-2-ene	16223	007785-26-4	96
			(1R)-2,6,6-Trimethylbicyclo[3.1.1] hept-2-ene	16224	007785-70-8	96
			(1R)-2,6,6-Trimethylbicyclo[3.1.1] hept-2-ene	16226	007785-70-8	95
2	5.453	0.21	D:\MassHunter\Library\NIST14.L Acetamide, N-(aminocarbonyl)-2-chl oro- Acetamide, 2-chloro- 4-Iodo-3-methoxyamphetamine	16309	004791-21-3	64
				2508	000079-07-2	59
				150328	1000124-02-9	43
3	5.510	2.15	D:\MassHunter\Library\NIST14.L .beta.-Pinene .beta.-Pinene .beta.-Pinene	16059	000127-91-3	94
				16055	000127-91-3	93
				16060	000127-91-3	92
4	5.623	4.29	D:\MassHunter\Library\NIST14.L .beta.-Myrcene .beta.-Pinene .beta.-Phellandrene	16066	000123-35-3	93
				16055	000127-91-3	83
				16088	000555-10-2	58
5	5.902	4.74	D:\MassHunter\Library\NIST14.L 3-Carene (+)-3-Carene 3-Carene	16028	013466-78-9	95
				16050	000498-15-7	94
				16036	013466-78-9	93
6	6.132	3.15	D:\MassHunter\Library\NIST14.L Cyclohexane, 1-methylene-4-(1-meth ylethenyl)- Cyclohexane, 1-methylene-4-(1-meth ylethenyl)- Cyclohexene, 4-methylene-1-(1-meth ylethyl)-	16233	000499-97-8	46
				16228	000499-97-8	46
				16216	000099-84-3	45
7	6.827	0.79	D:\MassHunter\Library\NIST14.L Benzenepropanamine, .alpha.-methyl Acetamide, N-(aminocarbonyl)-2-chl oro- N-acetyl-3-fluoroamphetamine	24045	022374-89-6	59
				16309	004791-21-3	53
				60532	1000379-09-7	52
8	6.957	0.47	D:\MassHunter\Library\NIST14.L 1,2,5-Oxadiazol-3-carboxamide, 4,4 '-azobis-, 2,2'-dioxide Amphetamine Benzeneethanamine, N-methyl-	144528	1000294-17-4	64
				15918	000300-62-9	64
				15957	000589-08-2	64
9	7.275	0.53	D:\MassHunter\Library\NIST14.L Acetamide, N-(aminocarbonyl)-2-chl	16309	004791-21-3	59

			oro-					
			1-(3,5-Dimethyl-1-adamantanoyl)sem	125446	351327-47-4	58		
			icarbazide					
			Acetamide, 2-chloro-	2507	000079-07-2	53		
10	7.379	0.46	D:\MassHunter\Library\NIST14.L					
			1-(3,5-Dimethyl-1-adamantanoyl)sem	125446	351327-47-4	62		
			icarbazide					
			dl-Phenylephrine	37706	001477-63-0	53		
			1,2,5-Oxadiazol-3-carboxamide, 4,4	144528	1000294-17-4	53		
			'-azobis-, 2,2'-dioxide					
11	7.483	0.76	D:\MassHunter\Library\NIST14.L					
			Benzeneethanamine, 4-fluoro-.alpha	27377	000459-02-9	53		
			.-methyl-					
			4-Fluoroamphetamine	27371	064609-06-9	53		
			Acetamide, N-(aminocarbonyl)-2-chl	16309	004791-21-3	50		
			oro-					
12	7.525	0.74	D:\MassHunter\Library\NIST14.L					
			4-Fluoroamphetamine	27371	064609-06-9	47		
			1-(3,5-Dimethyl-1-adamantanoyl)sem	125446	351327-47-4	46		
			icarbazide					
			Norpseudoephedrine	25677	036393-56-3	43		
13	7.618	0.33	D:\MassHunter\Library\NIST14.L					
			1,3-Dioxolane-4-methanol	4769	005464-28-8	50		
			Acetic acid, hydroxy[(1-oxo-2-prop	21889	006737-24-2	49		
			enyl)amino]-					
			4-Fluoroamphetamine	27371	064609-06-9	47		
14	7.839	1.09	D:\MassHunter\Library\NIST14.L					
			Acetamide, N-(aminocarbonyl)-2-chl	16309	004791-21-3	50		
			oro-					
			dl-Phenylephrine	37708	001477-63-0	47		
			Phenylephrine	37699	000059-42-7	47		
15	7.968	0.78	D:\MassHunter\Library\NIST14.L					
			Acetamide, N-(aminocarbonyl)-2-chl	16309	004791-21-3	50		
			oro-					
			1,2,5-Oxadiazol-3-carboxamide, 4,4	144528	1000294-17-4	35		
			'-azobis-, 2,2'-dioxide					
			Alanylglycine, TMS derivative	81934	1000333-70-1	30		
16	8.063	0.48	D:\MassHunter\Library\NIST14.L					
			2-Amino-1-(o-hydroxyphenyl)propane	25738	073470-00-5	62		
			(-)-Norephedrine	25674	000492-41-1	59		
			Norpseudoephedrine	25677	036393-56-3	59		
17	8.203	0.33	D:\MassHunter\Library\NIST14.L					
			Norephedrine, (.+/-.)-	25687	014838-15-4	53		
			Ethylamine, 2-(adamantan-1-yl)-1-m	58724	1000310-59-0	53		
			ethyl-					
			3-Ethoxyamphetamine	46993	135014-86-7	53		
18	8.268	0.43	D:\MassHunter\Library\NIST14.L					
			Acetamide, N-(aminocarbonyl)-2-chl	16309	004791-21-3	59		
			oro-					
			2-Amino-1-(o-hydroxyphenyl)propane	25738	073470-00-5	53		
			Benzenemethanol, .alpha.-[(methyla	25766	006589-55-5	53		
			mino)methyl]-					
19	8.933	0.70	D:\MassHunter\Library\NIST14.L					
			2-Ethoxyamphetamine	46994	135014-84-5	38		
			3-Hydroxy-N-methylphenethylamine	25720	1000335-06-7	38		
			3-Methoxyamphetamine	35663	017862-85-0	35		
20	9.040	0.33	D:\MassHunter\Library\NIST14.L					

			N-Methyl-2-phenyl-1-propylamine	24026	1000379-96-9	58
			Amphetamine	15918	000300-62-9	53
			Benzeneethanamine, 4-methoxy-.alph a.-methyl-	35730	000064-13-1	52
21	9.504	0.17	D:\MassHunter\Library\NIST14.L Benzeneethanamine, 4-methoxy-.alph a.-methyl- dl-Phenylephrine Tocainide	35727	000064-13-1	64
				37708	001477-63-0	59
				57432	041708-72-9	59
22	9.554	0.21	D:\MassHunter\Library\NIST14.L Amphetamine Acetic acid, [(aminocarbonyl)amino]oxo- Chlorodifluoroacetamide	15918	000300-62-9	52
				14446	000585-05-7	50
				13030	000354-28-9	50
23	10.260	0.42	D:\MassHunter\Library\NIST14.L (-)-Norephedrine 2,4-Dimethylamphetamine (-)-Norephedrine	25675	000492-41-1	59
				34153	075659-61-9	59
				25674	000492-41-1	59
24	12.399	0.43	D:\MassHunter\Library\NIST14.L Imidazole-5-carboxylic acid, 2-ami no- Amphetamine Benzenemethanol, .alpha.-[(methyla mino)methyl]-	11942	1000126-50-5	59
				15918	000300-62-9	58
				25766	006589-55-5	53
25	13.175	0.69	D:\MassHunter\Library\NIST14.L R-(-)-Cyclohexylethylamine 2,5-Dimethoxy-4-(methylsulfonyl)am phetamine Amphetamine	12150	005913-13-3	53
				133280	146724-75-6	53
				15918	000300-62-9	53
26	13.264	0.39	D:\MassHunter\Library\NIST14.L Amphetamine 2-Ethoxyamphetamine 3-Methoxyamphetamine	15918	000300-62-9	53
				46994	135014-84-5	53
				35666	017862-85-0	53
27	13.312	0.59	D:\MassHunter\Library\NIST14.L Amphetamine Phenethylamine, p,.alpha.-dimethyl 2-Ethoxyamphetamine	15918	000300-62-9	53
				24044	000064-11-9	53
				46994	135014-84-5	53
28	14.494	0.44	D:\MassHunter\Library\NIST14.L 2-Pentanamine 2-Ethoxyamphetamine 2,5-Dimethoxy-4-(methylsulfonyl)am phetamine	1930	063493-28-7	59
				46994	135014-84-5	59
				133280	146724-75-6	59
29	14.966	0.22	D:\MassHunter\Library\NIST14.L 1-Octanamine, N-methyl- Octodrine 1-Propanamine, N,2-dimethyl-	20905	002439-54-5	64
				13312	000543-82-8	59
				1956	000625-43-4	50
30	17.260	0.58	D:\MassHunter\Library\NIST14.L 2-Pentanamine Benzeneethanamine, 3-fluoro-.beta. ,5-dihydroxy-N-methyl- 2-Butanamine, (S)-	1930	063493-28-7	59
				52380	1000116-56-0	59
				764	000513-49-5	45

Appendix-H: Actual, predict and Residual

Standard order	Actual value	Predicted	Residual
1	1.51	1.21	0.30
2	0.92	1.21	-0.29
3	1.03	1.21	-0.18
4	1.43	1.52	-0.085
5	1.52	1.52	4.667E-003
6	1.32	1.52	-0.20
7	1.75	1.88	-0.13
8	2.05	1.88	0.17
9	2.27	1.88	0.39
10	0.51	0.69	-0.18
11	0.69	0.69	-1.667E-00
12	0.93	0.69	0.24
13	0.98	1.00	-0.019
14	1.06	1.00	0.061
15	0.87	1.00	-0.13
16	1.31	1.36	-0.050
17	1.25	1.36	-0.11
18	1.55	1.36	0.19
19	0.49	0.51	-0.022
20	0.52	0.51	0.011
21	0.63	0.51	0.12
22	0.89	0.82	0.074
23	0.91	0.82	0.094
24	1.01	0.82	0.19
25	0.99	1.18	-0.19
26	0.95	1.18	-0.23
27	1.12	1.18	-0.057

Appendix-I: Laboratory Equipment's and Samples Photos



Figure C₁ juniper berries



Figure C₂ Weight balance and Oven drying apparatus



Figure C₃:- steam distillation and juniper berries essential oil



Figure C₄:-Acid value and iodine value determination



Figure C₅ :- Dry Gin Juniper Berries and essence tests of juniper berries liqueur

Declaration

I, the undersigned, declare that this thesis is my original work and has not been presented for a degree in any other University, and that all sources of materials used for the thesis have been duly acknowledged.

Name: **Meroda Tesfaye**

Signature: _____

Place: **Addis Ababa, Ethiopia**

Date of submission: _____

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

Name: **Dr.Eng. Shimelis Admassu (Associate Prof.)**

Signature: _____