ADDIS ABABA UNIVERSITY

ADDIS ABABA INSTITUTE OF TECHNOLOGY

SCHOOL OF CHEMICAL AND BIOENGINEERING

Extraction, characterization and optimization of Botanical Gin flavor from Juniper berries, Cassia bark and Ginger

By

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A Thesis submitted to Addis Ababa Institute of Technology, School of Chemical and Bio Engineering in partial fulfillment of the requirement for the Degree of Master of Science in Chemical Engineering (process engineering)

Addis Ababa, Ethiopia

September, 2023

Addis Ababa University

Addis Ababa Institute of Technology

School of Chemical and Bio Engineering

This was to certify that the work in this thesis study entitled "**Extraction, Characterization and Optimization of Gin Botanical from Juniper berries, Cassia bark and Ginger**" submitted by Melkamu Getaneh in partial fulfillment of the requirements for the degree of Masters Science (Process stream) in Addis Ababa Institute of Technology complies the regulation of the University and meets with the standard quality.

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Acknowledgements

Above all, I would like to thank God Almighty for giving me the strength and wisdom to successfully complete this thesis.

Next, I want to express my sincere gratitude to my advisor, Dr. Shegaw Ahmed, for excellent scientific guidance, support in providing ideas and editing the thesis material.

I appreciate and proud of the owner and general manager of Balezaf Alcohol and Liquor Factory, Ato Belay Tekle who supported me to learn by covering tuition fee, advising me in title selection and allowing to use Balezaf Alcohol and Liquor Factory LAB instrument for laboratory analysis. I would also like to thank all the staff at Balezaf Alcohol & Liquor Factory for their unwavering support, knowledge sharing and deep scientific discussion. All colleagues and friends, chemists, quality controllers, supervisors, technical managers and production managers, especially Asefa Yeshaneh (quality manager) were greatly thanked for the enthusiastic studying atmosphere during the entire stay throughout the program duration and lab analysis. It was my greatest pleasure to thank for their unreserved assistance and sharing of knowledge during my design and preparation of small scale distillation. I would also like to thank the management of Balezaf Alcohol and Liquor Factory for giving me the chance to attend my MSc. study. I thank Tigst Tesfay (Bottling supervisor) for allowing me to prepare the sample and taste the flavor. Without this support, the chance couldn't successful.

My deepest gratefulness goes to my beloved wife Mrs. Yenework Meselu, for her understanding love, patience and shouldering all the responsibilities with baby Edemikael Melkamu. It was her greatest encouragement and sacrifices that brought this work to a successful end. Finally, I would like to thank JIJE LAB GLASS pvt. Limited company, Ethiopian conformity assessment enterprise and Ethiopian food and drug authority to conducting additional lab analysis of my product.

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List of abbreviation

AOAC= Association of official analytical collaboration

ABV = Alcohol by volume

ANOVA = Analysis of variance

BALF = Balazaf alcohol and liquor factory

^{o}Brix = Percent by weight of soluble solid in solution

E.U = European Union

Kg = Kilo gram

QDA = Quantitative descriptive analysis

FAO = Food and agriculture organization of the united nation

GNS = Grain neutral spirits

^{o}C = Degree Celsius

PH = Negative logarithm of concentration of hydrogen ion

RI = Refractive index

IWSC = International wine and spirit competition

UNIDO = United nation industrial development organization

QDA = Quantitative descriptive analysis

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Abstract

The botanical gin flavor was extracted from juniper berry as the main ingredient in a variety of ways. Quality was never stays the same and the yield of flavor and quality was influenced by different factors. The cheapest approach for flavor extraction in this instance was the water distillation using ethanol as a solvent method. The factor variable which affect the extraction process was botanical weight and alcohol concentration and the response was yield and sensory evaluation analysis. At 0.5 kg of botanical weight and 35% alcohol concentration in the extraction experiment, the minimal oil yield of 0.78% was obtained. A 1.5 kg botanical weight and a 50% alcohol concentration produced the highest oil yield of 2.76%. The physico chemical properties of the flavor studied for the optimum yield showed that the flavor was odorous and colorless, having 1.36619 refractive index value at 25^{o}c, 1.108cp dynamic viscosity, 75^{o}c boiling point, 0.8569 specific gravity, 4.84 pH**,** 0.307 degree optical rotation, 0.16 ml/g acid value, 2.44ml/g iodine value, 0.08% free fatty acid and 21.4 ^{o}Brix. The chemical composition of the flavor identified by GC-MS had β-pinene (4.89%), Terpinolene (8.57%), Cyclopropane, trimethyl (2-methyl-1-propnylidene) (8.35%), citronellol (6.34 %), γ-terpinene (1.60%), β-humulene (4.35%), globulol (4.23 %) and citral (2.90%). Gin liquor's sensory qualities was also assessed and obtained a good taste at 1.5kg botanical weight and 50% alcohol concentration at which both response were having 95.5% desirability. The findings of this investigation showed that the gin flavor extracted from procera juniper berry, cassia bark and ginger satisfy the requirement.

Key words; botanical gin, flavor extraction, herb and spice, hydro distillation, maceration procera juniper berry.

CHAPRET ONE

Introduction

1.1 background

Either the Dutch term "junever" or the French word "genievre," both of which indicates juniper berries, were the sources of the name "gin". Gin was a tasty alcoholic beverage made by distilling spirit with juniper berries and other botanicals at a high proof level. Gin liquor was first made more than three centuries ago in Holland by Franciscus "de la Boe" Syrvius (1614–1672), a physician at the University of Leiden in the Netherlands, who combined alcohol and juniper berries to treat kidney compliance (Clutton, 1978). The British soldiers drank gin before battle, which gave them bravery. They developed a taste for it and introduced the process of making this beverage to England. In the eighteenth century, gin became a cheap beverage for the underprivileged in London, and it was produced without limitations anywhere in the city. As was the case with many other spirit categories that were controlled throughout the EU at the same time, regulations for the category had to take into account the production and significant requirements that were in place at the time because to its lengthy and varied history. Gin was one of the broadest categories of spirits; it included beverages with a wide range of styles, provenance, and flavor profiles, all of which had juniper as a main ingredient (Matthew, 1966).

Traditional alcoholic beverages were extensively enjoyed throughout Ethiopia by various ethnic groups as a significant component of local customs surrounding various social events, including as public holidays, weddings, funerals, and other celebrations. Tella, Shamita, Tej, Borde, and Areki were some of the most popular home-brewed drinks. Tella and Areki were the most popular fermented Tej beverages to be consumed on major holidays (Dersehilign, 2017). This traditionally made and distilled alcoholic beverage's production and consumption volumes were not recoded (WHO, 2014).

Many African nations experienced contamination and impurities (Mureithi, 2002; Riley, 1999). Regular customers in the study cities complained that particular vending machines utilised cement and specific plant roots to boost the alcohol content of their drinks (Yohannes, 2013). Such a problem was generally more prevalent in major cities like Addis Ababa, in the drunk of Areki and Tej. Methanol, light oil, fusel oil, alcohol content, pH and sensory evaluation were not

known in traditionally fermented and distilled beverage. Duo to this and other (like insufficient production process) the market of alcoholic beverage in Ethiopia gone towards industrially produced alcoholic beverage.

Gin was an industrially distilled alcoholic drunk whose dominant flavor comes from juniper berries; the alcohol content of bottled gin in the EU was at least 37.5 % ABV. There were two types of gin, distilled gin and compounded gin. Distilled gin was made by steeping the botanicals in neutral unflavored alcohol and distilling it in a Carter still, while Compound gin was simply made by flavoring neutral alcohol with essences and/or other "natural flavorings" without re-distillation.

The main herbs used to make gin were juniper berries, coriander, angelica, citrus peels, nutmeg, ginger, cardamom, cassia bark, cinnamon, orris root, licorice and seeds of paradise. The number and combination of substances used was usually a closely guarded secret among top distillers. (Gin Bible, 2011). Most distilleries had their own recipes, choosing from the above or other plants. From this study the three species and herb were selected which was available and grow easily in Ethiopia. These include juniper berries, cassia bark and ginger.

Aromatic oils from juniper berries, ginger, cassia bark, nutmeg, cardamom and others had been used since ancient times as fragrances, spices, medicines, pesticides and cosmetics. In this study only use extracted essential oil with alcohol for Gin flavor due to lake gin flavor in our country to minimizing the imported flavor from other country and upgrading of the farmer life who supplies raw material to the flavor extraction factory, by creating job opportunity in plant and contributing to decrease warming of earth by planting this gin flavor feed stack plant.

1.2 Statement of the problem

Ethiopia's supply of alcoholic beverages came from both domestic and imported sources. Although there were several small and medium-sized factories that produce alcoholic beverages such as; National alcohol, Balzaf Alcohol and Liqueur Factory, Rorank, Viv, etc. Balzaf Alcohol and Liquor Factory was one of the largest private distilleries that produces and distributes various liquors such as dry gin, ouzo, cognac, super mint, zebibia extra, Pernod, Fernet, Bitter, Aperitif, lemon, vodka, mini gin and pineapple liqueurs over the country. All liquor factories used the gin flavor by importing.

All distilleries making gin must be flavored with various herbs and spices, commonly known as botanicals. These botanicals were distributed worldwide and found in various regions of Ethiopia and could be used to flavor gin after the essential oil had been extracted. There were many types of extraction. The extraction was done with or without a solvent. However, the most commonly used method to obtain essential oil was extraction by water distillation with a solvent. To extract essential oil from selected plant materials, alcohol was used as a solvent, RO water and steam. The potential of this botanical oil to flavor gin had not received much attention in Ethiopia. Currently, industrial demand for gin flavor was covered by imports from other countries.

Distilleries in Ethiopia face unnecessary costs because the flavor were expensive. To utilize the potential of juniper berries, ginger and cassia bark gin flavor should produce and supply enough gin flavor to all distilleries which minimizes or ceased the flavor of imported. On the other hand, juniper procera was found in different places in Ethiopia, this coniferous plant was cut by people to make wood, especially firewood, and furniture, but during this activity the berries were not considered. Extract Gin flavor from this plant berries were an effective solution to save the berries. This study focused on improving the flavor of gin using ginger and cassia bark and increasing the yield using ethanol as a solvent.

1.3 Objective

1.3.1 General objective

The main objective of this research was to extract, characterize and optimize the flavor parameter of gin flavor from *Juniperus procera* berries, Ginger and Cassia bark.

1.3.2. The specific objectives

- To determine the optimal flavor yield by evaluating extraction parameters (botanical ratio and ethanol concentration).
- To characterize the physical-chemical properties of the flavor of gin.
- To Enhances the taste of gin by using ginger and cassia bark.

1.4 Scope of the study

This study was a year-long research project supported by laboratory results. The research provides insight into the current demand for gin flavors and their sources, as well as the benefits of using spices and botanical aromas. Additional discussion of the aromatic herbs available in Ethiopia also included. State-of-the-art technology and operational stages of gin flavor production (e.g. raw material preparation, extraction and concentration) were described. In addition, it considers the possibility of obtaining liquor flavors based on theory and laboratory-based analysis. Finally, conclusions were reached after checking the properties of gin flavor.

1.5 Significant of study

The production of gin flavor had different merit

- ✓ This study was gone a long way in stopping or reducing the amount of imported gin flavoring.
- ✓ Easily meet the demand for gin flavor with timely delivery
- ✓ The study shows the possibility of obtaining a gin flavor using locally available botanicals
- ✓ This work serves as a benchmark for making other distilled spirits, soft drinks or food flavors using locally available herbs and spices.
- ✓ Improves the life of the society that sold the raw material and engaged in aromatic extraction.

Chapter Two

Literature review

2.1 Overview of Botanical Gin Flavor Manufacturing

Historically, there was little literature in the public domain on food flavors until the mid-1970s. While flavor research had existed for well over 100 years, the vast majority of early flavor research was done within companies and held secret. The industry generally did not even disclose findings via patents but chose to hold findings as trade secrets, which was still common today. At the time of the publication, most flavors were based on essential oils, and this collection was regarded as the source of information on flavorings (Reineccius, 2006). A cold deprives the brain of aroma stimuli and leaves flavor perception to the basic tastes and chemesthetic response. Without aroma, it was very difficult to identify the flavor of a food product. The task of identifying volatile flavor components (aroma compounds) particularly in a food matrix was one of the most formidable tasks faced by an analytical chemist. Ernest Guenther was one of the first individuals to publish a significant work in the public domain. He published a six-volume collection on essential oils.

Schwab (2008) published on the Biosynthesis of plant-derived flavor compounds. The other document published in titled of Biotechnology in Flavor Production by Frenkel, 2008. The researchers who published specifically related to gin flavor were Meroda, 2017 Optimization of Extraction Process Parameters and Characterization of Gin Flavor from Juniper Berries *(Juniperus Communis)*. While these flavorings were primitive by today's standards, this book provided a view of how flavor companies created flavorings. The next significant work was that of Hodel, 2020. Hodel describes detailed the influence of distillation parameters on the extraction of juniperus communis L. in vapor infused gin. Based on his result the greatest influence was botanical ratio, whilst the least influential was alcohol concentration for all compound except terpinen-4-ol.

The above-mentioned texts had been used to extract flavor for different purposes from different kind of aroma containing plants. But the last two researcher were extract such volatile aroma for specifically flavored gin liquor. But both used only one plant and the plant species was juniperus communis L., which was not found in Ethiopia. So it needs to additional researches in Ethiopia

to use locally available raw material and used other aroma containing herbs or spice to enhance the flavor quality.

2.1.1 Gin

Beverages were a drunk which was alcoholic or non-alcoholic. Alcoholic beverage also could be fermented (wine, beer, Perry and cider) and distilled. Distilled beverage also subdivided into two; spirit/unflavored (vodka, brandy, whiskey…) and liquor/flavored (gin, rum, ouzo...). Non-alcoholic beverage was drink which had zero alcohol content that could be carbonated (flavored fizzy drink) and non-carbonated (tee, coffee, mineral water, milk beverage).

Gin was distilled liquor and had been known for hundreds of years. Most people know that this was a flavored with botanical spirit, with the main botanical being juniper berries. Franciscus "de la vaux" Silvius (1614-1672), a medicine at the University of Leiden in the Netherlands, was widely credited as the originator of the botanical-flavored spirit called gin. The product made by him, pot distilled from rye, and redistilled with juniper and other botanical, was called by the French name essence de Genièvre owing to its strong juniper aroma. This later appeared as the Dutch "Geneva" and eventually became the English "gin". Gin was a distilled spirit that draws out its flavor through a balanced blend of carefully selected botanicals. The minimum ABV for bottling gin in the EU was 37.5ABV, and the botanical recipe, which must include juniper berries, was a closely guarded secret of certain distilleries. There was two types of gin: distilled gin and compound gin. Distilled gins were made by maceration of botanicals with unflavored, neutral spirits and distilled in a Carter still, while compound gins were made neutral with essences and other "natural flavors" without re-distillation. It was made simply by adding flavor to a distilled spirit. Dutch gin, Plymouth gin, London dry gin, Old tom gin were some known gin brand which produced thought the world. (Clutton, 1978).

Dutch gin

This was sometimes called Holland, Geneva, or Genever. Geneva even now he resembles the original gin of the 17th century. The flavor, reminiscent of almond, was derived from the botanical ingredient and the source of spirit used to make it. Geneva production was concentrated in the town of Schiedam near Rotterdam, where local grain supplies were readily available. Over three centuries, the production method hadn’t changed much. Geneva was distilled in pot stills and usually heated over charcoal. This method was called an aude system.

Barley grains were saturated with water and allowed to germinate inside the malt house. The diastase enzyme converts malted barley into sugars. The malt was then milled and mashed with up to 30% of the vat of corn, rye or both. Add yeast to the mixture and let it ferment. After 12-14 hours, a layer of white foam covers each vat. This was called 'Dutch yeast' and was skimmed, dry-pressed and shipped to local bakeries. The resulting liquid was distilled in a pot still with low rectification. Repeat this process two more times to obtain Spirits. Malt wine was redistilled with juniper berries and other botanicals to make Geneva. It must be 35ABV. Dutch gin was slightly sweet.

Plymouth gin

The Old Refectory (1793) of the Dominican Convent (built around 1425) was originally used as a distillery for the production of Plymouth Gin. The gin, with its unique flavor, was very popular with the Royal Navy. In fact, they were the first to add Angostura bitters (from Trinidad) to Plymouth gin to create the famous 'pink gin'. Plymouth Gin production was very local. Messre, coats and co. (Plymouth) Ltd. were the sole agents for its manufacture. Spirits were sourced from Grain Whiskey Distillery in Strathclyde or in some cases from supplier of Grain Spirits in London.

Grain alcohol was pumped into a still and diluted with water. The dilution would rectified. The spirit was then injected into the still and mixed with the herbal ingredients. The Centre part of the distillation was done at reduced intensity, used for binding and bottling as Plymouth gin. It was slightly sweet and had a strong citrus flavor.

London dry gin

This was Britain's most famous and popular gin and now made all over the world. This description was about the manufacturing process and not the geographic location of the distillery. London Dry Gin was made by redistilling rectified spirits with certain botanicals added. The term 'dry' in the context of London Dry Gin means a low overall flavor level. This was because gin distilled from extremely pure spirits and small amounts of herbal ingredients, and the amount of sugar used was very small, about 0.1-0.5 g per liter of liquor (Simpson, 1966).

Old tom gin

The origin of the term "Old Tom" was somewhat obscure. It was known so as far back as 1730 in England. Owing the Gin craze, the British government tried to stem the flow of gin with prohibitive taxes and licensing, which drove the scene underground. Captain Dudley Bradstreet obtained gin from the distillery and sold it to the public. He rented a house, nailed a cat sign to the window, and stuck flexible tubes in the cat's legs. Passers-by were asked to put money in the cat's mouth and say "puss!" 2 days please worth the gin. ’ Bradstreet then pours some of the gin from the tube into the customer’s bottle. The practice was illegal, but he was making over $200 a month.

Further explanation of this term was provided by Board's (Distillers) Ltd. Given from London (estimated 1726). They noted that Old Tom refers to Hodges' old Thomas Chamberlain distillery. He experimented with gin flavors and even added sugar syrup to his London gin.

Old Tom was a gin that after distillation, sweetened with 3-6% w/v sugar (sometimes glycerin). Sometimes the sugar syrup was flavored with orange blossom water. Old Tom gin was now known to be sweeter than standard gin.

Table 2.1 Most gin producing company and awarded gold around the world 2022.

Rank	Company	Gin	Alcohol %	Bottle size/ml/	Country	Point out of 100
1	Mt. Uncle Distillery	Botanic Australis Navy strength gin	57	700	Australia	99
2	Herno, Distillery	Herno gin /London dry gin category/	57	500	Sweden	98
3	Revsunds Distillery	Mylta cask edition 2021 gin	43	500	Sweden	98
4	Skrea Backe	Ocean strength gin	57	500	Sweden	98
5	Triple eight distillery	Gale force gin	44.4	750	USA	98

6	Matsui Shuzo	The Hakuto premium Matsui gin	47	700	Japan	98
7	Garage 22 Prague distillery	Gin 22/ London dry gin /	42	500	Czech republic	96

Source: IWSC-2022

2.2 Botanicals

Botany was the scientific study of plants and plant-like organisms. It helps us understand why plants were so important to the world. Plants were at the beginning of most food and energy chains, providing us with oxygen, food and medicines (Alexey Shipunov, 2021). Plants were the primary raw material not only for gin manufacturing, but also for a variety of other products such as nutritional supplements, cosmetics, pharmaceuticals, essential oils, food and beverages. Today, most of the gin "core" botanicals (juniper berry, cassia bark, etc.) used by various gin makers come from overseas. Cultivation in Ethiopia had the potential to shorten supply chains, create value for Ethiopian growers, and provide opportunities for provenance, quality assurance and market differentiation desired by the gin industry. Gin botanicals were high-quality raw materials and provide good yields for small-scale producers. Ethiopia had the ability to grow a wide variety of gin botanicals well and sustainable alternative. Ethiopia grown gin botanicals could be grown organically and create market interest here and overseas.

Table 2.2 List of some herbs and spice containing essences in Ethiopia.

Botanical name	**Scientific name**	**Local name**
Ginger	Zingiber officinal	ዝንጅብል
Juniper berries	Juniperus procera	የፅድ ፍሬ
Garden cress	Lipidium sativum	ፌጦ
Vernonina amygdalina	Vernonina Amygdalina	ግራዋ
Turmeric	Curcuma longa	እርድ
Fenugreek	Trigonella Foenum Graceum	አብሸ
Black Cardamom	Amomum Sublatum	ኮረሪማ

Cinnamon	Cinnamomum Zelanicum	ቀረፋ
Moringa	Moringa Oleifera	ሽፈራው
Koseret	Lippa Abyssinica	ኮሰረት
Piper cubeba	Piper Nigrum	ቁንዶ በርበሬ
Cardamom		ሀሌ
Fennel seed	Foeniculum Vulgare	እንስላል
Black Mustard	Brassian Nigra	ሰንስፍጭ
Rue	Ruta Chalepensis	ጤናዳም
Basil	Ocimum Basilicum	በሶብላ
Coriander	Coriandrum Sativum	ድምብላል
Rosemary	Rosmarinus Officinalis	ሮዝመሪ
Thyme	Thymus Vulgaris	ጦሲኝ

(Source: Endashaw Bekele, 2007; Atey G/medhin, 2008; UNIDO and FAO, 2005)

2.2.1 Juniper

The first key requirement for gin was juniper. While all gin categories establish a basic juniper factor "dominant flavor", this flavor profile factor remains purely subjective. There was no definitive test of flavor/strength of the juniper as the lead botanical. This had been the cause of much debate, especially given the growing number of gins with strong additional flavor profiles. The strength of juniper varies from brand to brand and also on the type and geographic origin of this natural product (Gin guild, 2021).

Juniper (Juniperus procera) was a slow-growing coniferous shrub of the Cupressaceae family, native to the mountains of East Africa and a characteristic coniferous species of the Afromontane flora (White 1978). This tall (up to 50 m high) evergreen tree was Africa's only tropical juniper and grows on the Ethiopian plateau with an average annual rainfall of 500 to 1100 mm, preferably in dry forests at altitudes of 2300 to 3200 m (Gardner, 1926). Juniper was a wind-pollinated dioecious plant whose early widespread distribution had been associated with rich folklore and diverse ethnobotanical uses, including medicinal, veterinary and culinary. Oils from leaves, shoots and fruits and resins from woody parts were some of the important ingredients used in various pharmaceutical applications. For example, cedar oil was used in microscopy,

soaps, perfumes, medicines, and abortion (Jansen, 1981). Berry oil was used medicinally for flavoring alcoholic beverages and other foods (Zaman et al., 1968).

(a) (b)

Figure 2.1 (a). Juniper procera tree, (b) Juniper berries

2.2.2 Juniper berries

Today juniper was mainly used as the main flavor in gin. British, French and Dutch legislation stipulates that juniper was the only primary plant used to flavor beverages classified as gin (Avinash, 2016). The fruit was fleshy and spherical, 1/4 to 1/3 inch in diameter. It contain three sticky, brown, irregularly shaped seeds. Fruits should be used when ripe, that was after the skin turns blue. On drying and storing, the smooth shiny skin darkens to a purple black and becomes slightly wrinkled or indented. Immature fruits were green. The interior flesh of the mature berries were brown yellow and the brown seeds were crunching but not hard. The oil extracted mainly from berries, was volatile, and had a pleasant fragrance. Steam distillation with solvents was used to extract essential oils from plant material. Essential oils obtained by steam distillation were a special type of distillation or separation process that separates temperature sensitive substances such as oils, resins and hydrocarbons. The yield and composition of juniper berry essential oil vary depending on the geographic origin of the juniper plant. Fruit ripeness, plant age, weather conditions or temperature, insolation, etc., and other environmental factors (Sela, 2012). This oil had antioxidant and anti-inflammatory properties, was antidiabetic, promotes heart health, and also had antibacterial and antifungal properties (Emami, 2007).

2.2.3 Juniper berries harvesting

Harvesting was the primary process of gathering the desired crop from fields exposed to changes in climate and growing conditions and exposing it to controlled processing and stable storage

conditions when the juniper berries were ripe for the crop. Harvest requirements depend on the desired end product, with specific requirements such as maturity and uniformity that determine harvest management and timing. Plants should be harvested during the optimal season or period to ensure the production of the highest possible quality plant material and final spice products. Harvest time depends on the part of the plant used. Detailed information on proper harvest times was often available in published standards, official monographs, and key references. However, it was well known that the concentration of target components varies according to the stage of plant growth and development (Stoilova, 2014). The optimal time for harvesting should be determined according to the quality and quantity of target constituents. When harvesting, be careful not to contaminate the harvested plant material with foreign matter. Whenever possible, plant parts should be harvested under optimal conditions, avoiding dew, rain, or unusually high humidity. If harvesting takes place in damp conditions, the harvested material should be transported immediately to the drying shed and drying should begin to prevent microbial fermentation and mold development. They should be stored in a dry, uncontaminated area (Khilender, 2010).

2.2.4 Cassia bark

Cassian bark was an evergreen tree that grows mainly in China (cinnamon) and South and Southeast Asia. Aromatic bark oils had healed properties that make them useful as alternative medicines (Bousbia, 2009). Essential oil had been found to be an important component of cassia, and the oil extracted from different parts of the plant such as leaves, calyx, twigs, seeds and bark (Revindran, 2004). Cinnamon oil contains large amounts of terpenes and other aromatic compounds that had been observed to exhibit diverse activities such as antioxidant, antiallergic, anticancer and antibacterial properties (Prasad, 2009, Unlu, 2010). Cassia bark was an important source of commercial raw materials due to its high content of essential oils and transcinnamaldhyde (Commission, 2010; Geng, 2011, 2013a).

Figure 2.2 Cassia tree

2.2.5 Ginger

Ginger (Zingiber officinale Rosc.) was actually a member of the Zingiberaceae family. The name "zingiber" derived from the Greek word "zingiber" and the Sanskrit word "singabella" meaning horn, because the shape of the ginger rhizome resembles the antlers of a deer derived from "Oficina" meant to be used in medicine or pharmacy (Vasala, 2012). Ginger rhizomes was used in a variety of foods and drinks. That's because ginger was a spice that gives spiciness and a savory sensation. Ginger was also used in various food and beverage applications, where its bioactive compounds provide specific functional properties (Srinivasan, 2017). Ginger rhizome products also used in the form of fresh ginger, preserved ginger, dried ginger, ginger powder, ginger essential oil, ginger oleoresin, and ginger paste (Vasala, 2012). In traditional medicine, ginger rhizomes had long been used to treat colic, diarrhea, and nausea, as well as various foods to aid digestion (Sharifi-Rad, 2017). Water-ethanol ginger extract now produces oleoresins and

essential oils containing many phenolic compounds. The extracted compounds had functional and medicinal properties such as antioxidant, antihyperglycemic, antibacterial, anticarcinogenic, anti-inflammatory, immunomodulatory, antilipidemic, antitumor, and antimutagenic effects (Ali, 2008; Arablou and Aryaeian, 2018; Mahboubi, 2019). Essential oil obtained different part of the herb or spice.

Table 2.3 Source of natural essential oil

Leaves	**Flower**	**Peel**	**Seed**	**Wood**
Cinnamon	Chamomile	Bergamot	Almond	Camphor
Eucalyptus	Clary	Grape fruit	Anise	Cedar
Lemon Grass	Sage	Lemon	Celery	Rosewood
Melaleuca	Clove	Lime	Cumin	Sandalwood
Oregano	Geranium	Orange	Nutmeg	
Patchouli	Hyssop	Tangerine		
Peppermint	Jasmine			
Pine	Lavender			
Rosemary	Manuka			
Spearmint	Marjoram			
Tea Tree	Orange			
Wintergreen				
Thyme				
Berry	**Bark**	**Resins**	**Rhizomes**	**Root**
Juniper	Cassia	Frankincense	Ginger	Valerian
Allspice	Cinnamon	Myrrh		

(Source: ICS-UNIDO, 2008)

2.3 Aromatherapy

Fragrance based treatment was utilize of fragrant basic oils to advance mental and physical wellbeing and physical excellence. Science knows that our sense of scent plays a vital part in our generally wellbeing. Numerous of the common basic oils had therapeutic properties and had been utilized in pharmaceutical since antiquated times and still broadly utilized nowadays. For illustration, numerous basic oils had sterile properties, a few more grounded than others. In

expansion, diverse fundamental oils had distinctive properties, numerous of which were utilized elevating the intellect. The unstable component confines commercially accessible from fragrant plants were fundamental oils, concretes, absolutes, greases, and resinoids. Fundamental oils were isolated from plant fabric by refining, whereas other unstable confines were gotten by dissolvable extraction. This was since liquor was utilized as the dissolvable in this think about (Handa, 2008).

2.3.1 Concrete

It was an extract of fresh flowers, herbs, leaves and flower tips of plants obtained using hydrocarbon solvents such as butane, pentane, hexane and petroleum ether. Concrete was rich in hydrocarbon-soluble substances and contains no water-soluble components. It was usually a waxy, semi-solid, dark substance that does not contain the original solvent. In practice, concrete produced in static extraction equipment. These extractors were equipped with numerous perforated bowls so that the flowers do not compact under their own weight. Each perforated tray had spacers so the number and spacing of holes was fixed.

2.3.2 Absolutes

Concrete not widely used in perfumery as it was, but commonly converted into alcohol-soluble, volatile concentrates called absolutes. I.e. it had to been extracted with alcohol. To produce an absolute, concrete mixed with absolute alcohol and thoroughly stirred in a vessel equipped with an agitator. Keep the temperature between 40-60°C during stirring to soak the concrete into the solution. Waxes were generally insoluble in alcohol below -1 °C, so the solution was cooled to -5 to -10 °C to precipitate the wax. Precipitated wax removed by passing the solution through a rotary filter. The filtrate from the rotary filter was pumped to the primary evaporator where it was concentrated to approximately 10% alcohol. Finally, the concentrated extract was pumped into a stirred evaporator to carefully remove the alcohol under high vacuum.

2.3.3 Resinoids

Resinoids were extracts of natural resinous materials made with hydrocarbon solvents. Resinoids usually obtained from dry materials. The extraction process was the same as in concrete production, except that perforated discs were not used to stack the material. Instead, a powder made from dried plant material was fed into the extractor.

2.3.4 Pomades

Pomade obtained through a process called effleurage, a method of cold fat extraction. The fat was spread out on a wooden-framed glass plate, leaving a clear border around the edges. The surface grooving with a wooden spatula increases the fat absorption area. Fresh flowers were spread out on a thick surface and frames piled up. After the balm had been absorbed by the flowers, remove the faded flowers by hand. Fresh flowers were again laid out on the surface of the fat. This was repeated until the fat surface completely enriched with balm. The pomade thus obtained ready for cold alcohol extraction.

2.4 Essential oil and its component

Basic oils were utilized in a assortment of customer items such as cleansers, cleansers, toiletries, beauty care products, pharmaceuticals, fragrances, confectionery, delicate drinks, refined alcoholic refreshments (difficult drinks) and pesticides. Worldwide generation and utilization of basic oils and aromas was expanding exceptionally quickly. Generation innovation was an basic calculate in making strides the in general abdicate and quality of basic oils. Conventional strategies for preparing fundamental oils were of extraordinary significance and still utilized in numerous parts of the world. A unadulterated basic oil was a blend of over 200 components, more often than not a blend of terpene or phenylpropane subordinates, with negligible chemical and auxiliary contrasts between compounds. They might essentially classified into two bunches:

Unstable division:

Basic oils, which account for 90-95% of the oil weight, contain monoterpene and sesquiterpene hydrocarbons and their oxygenated subordinates, aliphatic aldehydes, alcohols and esters.

Nonvolatile buildup:

It makes up 1-10% of the oil and contains hydrocarbons, greasy acids, sterols, carotenoids, waxes and flavonoids (Handa, 2008).2.4.1 Hydrocarbon Essential oils were composed of compounds with hydrogen and carbon as building blocks. A basic hydrocarbon present in plants was isoprene with the following structure:

2.4.2 Terpenes

Generally, the name ends with "ene". For example: limonene, pinene, piperene, camphene, etc. Terpenes had anti-inflammatory, antiseptic, antiviral and bactericidal properties. Terpenes was further divided into monoterpenes, sesquiterpenes and diterpenes. Coming back to the isoprene

units under the "hydrocarbon" heading, two of these isoprene units linked together give a monoterpene, three linked gives a sesquiterpene, and four linked isoprene units give a diterpene.

2.4.3 Alcohols

Feature: Antiseptic, antiviral, fungicide, fungicide. Alcohol compound contained hydroxyl compounds. Alcohols occur naturally as free compounds or in combination with terpenes or esters. An alcohol was formed when a terpene combines with an oxygen atom and a hydrogen atom. If the terpene was a monoterpene, the resulting alcohol called a monoterpenol. Alcohol had little or no toxic effects on the body or skin. Therefore, they could considered safe to use.

2.4.4 Aldehydes

Feature: Antifungal, anti-inflammatory, antiseptic, antiviral, bactericidal, disinfectant, soothing. From a medical point of view, essential oils containing aldehydes were effective in treating candida and other fungal infections.

2.4.5 Acids

Feature: Anti-inflammatory. Organic acids were usually found free in very small amounts in essential oils. Vegetable acids act as ingredients or buffer systems to control acidity.

2.4.6 Esters

Esters were formed by the reaction of alcohols and acids. Essential oils containing esters used for their calming and balancing effects. Since it contains alcohol, it acts as an effective antibacterial agent. From a medical point of view, esters were thought to have antifungal and sedative properties and to help balance the nervous system. They generally had no precautions, except for the toxic methyl salicylate in birch and wintergreen.

2.4.7 Ketones

Feature: Anticatarrhal, cytoproliferative, expectorant, fragility.

Ketones were commonly found in plants used to treat upper respiratory ailments. Supports mucus flow and relieves congestion. Essential oils containing ketone bodies promote wound healing and promote the formation of scar tissue. Ketones were usually (but not always) highly toxic. The most toxic ketone was thujone, found in mugwort, sage, tansy, thuja, and worm wood oil. Other toxic ketones found in essential oils include pulegone in pennyroyal and pinocampfone

in hyssop. Non-toxic ketones include jasmon from jasmine oil, fenson from fennel oil, carvone from spearmint and dill oils, and menthone from peppermint oil.

2.4.8 Lactones

Feature: Anti-inflammatory, anti-inflammatory, expectorant, antipyretic.

Lactones were known to be particularly effective due to their anti-inflammatory effects, possibly due to their role in reducing prostaglandin synthesis and expectorant activity. It was more potent expectorants than ketones (Fermeglia, 2008).

2.5 Base Neutral Spirit

The second most important requirement for gin was the base alcohol used for distillation. Most distilleries buy the base neutral spirit (GNS) needed to make their gin before adding the botanicals needed to make their gin and redistilling it. Regulations reflect high purity requirements of 96ABV or higher. This proof requires a complex and expensive manufacturing process to achieve this basic purity. The requirement was not met without first correcting all base alcohols to 96%ABV.

There were now several gin makers looking to differentiate their products by adding an element of making base spirits in-house to their manufacturing process. Not all use grains (usually wheat) as a base, but potatoes, molasses, grapes and apples were used. By producing base spirits, brands claim the local origin and distinctive character of their base spirits and, in turn, gin. This gives companies control over the entire process, potentially allowing them to claim super-premium or exclusive production processes for their products, as well as legal use of fields all the way to bottle labels. The purity of the base spirit was very important for the distilled gin category. The purity of the base spirit was key to the distilled gin categories, so a key point that should be emphasized was that, under the Regulations applying to the Distilled and Ethiopian Dry gin categories, any subsequent dilution of the initial distillation (itself using 96% ABV spirit), other than by water, i.e. by the subsequent addition of spirit, which was obviously the case in multi-shot production, requires and restricts such additional spirit to being of ‘the same composition purity and alcoholic strength’ as that initially used. Using other spirit/alcohol, in all or part, would not be compliant with the Regulations (Walker, 2023).

2.6 Extraction Method

Traditional techniques for processing essential oils were of great importance and still used in many parts of the world. There were various methods to extract essential oils. Timid essential oil processing technology was very important but still underdeveloped in many parts of the world. Hydrogen distillation (HD), steam distillation (SD), solvent extraction, Soxhlet extraction, and cold pressing were the most commonly used methods.

Maceration could be used if distillation yields had low oil. Distillation was suitable for powdered almonds, rose petals and rose petals, while solvent extraction was suitable for expensive, delicate and thermally labile materials such as jasmine, tuberose and hyacinth. Hydro distillation was the most common method of producing citronella oil from plants (Singh. 2008).

2.6.1 Hydrodistillation:

Hydrodistillation was the conventional strategy of expelling fundamental oils. Water or hydrogen refining was one of the most seasoned and least difficult strategies (Meyer-Warnod, 1984) utilized to extricate fundamental oils. It was commonly utilized to isolated basic oils and scents from plants. The routine strategy for the extraction of fundamental oils was hydro refining (HD), in which the basic oils were vanished by warming a blend of water or other dissolvable and plant materials taken after by the liquefaction of the vapors in a condenser. In this strategy, the fabric totally inundated in water and connected to bring it to a bubble. Warm from an open fire, steam coat, closed steam coat, closed steam coil, or open steam coil. The most highlight of this handle was coordinate contact between bubbling water and the plant fabric.

When the still was warmed by coordinate fire, satisfactory safety measures were essential to anticipate the charge from overheating. When a steam coat or closed steam coil was utilized, there was less threat of overheating; with open steam coils this peril was dodged. But with open steam, care must be taken to anticipate aggregation of condensed water inside the still. In this manner, the still ought to be well protects. The plant fabric within the still must be disturbed as the water bubbles, something else agglomerations of thick fabric will settle on the foot and gotten to be thermally debased. Certain plant materials like cinnamon bark, which were wealthy in mucilage, must be powdered so that the charge was promptly scatter within the water; as the temperature of the water increments, the mucilage will be filtered from the ground cinnamon.

This incredibly increments the thickness of the water-charge blend, subsequently permitting it to char. Subsequently, sometime recently any field refining was done, a small-scale water refining in dish sets ought to be performed to watch whether any changes take put amid the refining handle. Amid water refining, all parts of the plant charge must be kept in movement by bubbling water; this was conceivable when the refining fabric was charged loosely and remains free within the bubbling water.

2.6.1.1 Mechanism of Hydrodistillation

Hydrodistillation of plant material involves the following main physicochemical processes:

i) Hydrodiffusion

The movement of essential oils or hot water through plant membranes was called hydrodiffusion. In steam distillation, the vapor does not actually pass through the dry cell membrane. Therefore, dried plant material were vacuum stripped with dry steam after the plant had been thoroughly ground to remove all essential oils from the oil-bearing cells material. However, when the plant was saturated with water, vapor exchange occurs within the tissue based on permeability during swelling. Plant cell membranes were almost impermeable to essential oils. So in the actual process at the temperature of boiling water some of the essential oil dissolves in the water inside the gland the oil-water solution permeates, by osmosis, the swollen membranes and finally reaches the outer surface, where the oil was vaporized by passing steam.

ii) Hydrolysis

In the present context hydrolysis was understood to mean a chemical reaction between water and certain constituents of the essential oil. Esters were components of essential oils and tend to react with water to form acids and alcohols in the presence of water, especially at elevated temperatures.

iii) Decomposition by heat

Almost all components of essential oils become unstable at high temperatures. Distillation must be done at low temperatures in order to obtain the highest quality oil. The temperature of steam distillation was entirely determined by the operating pressure, but the operating pressure for water distillation, water and steam stripping was usually atmospheric pressure. All three effects

described above - hydrogen diffusion, hydrolysis and thermolysis - occur simultaneously and influence each other. Diffusion rates typically increase with temperature, and so does the water solubility of essential oils. The same applies to the rate and extent of hydrolysis. However, better oil yield and quality could be obtained by:

(1) Keep the temperature as low as possible

(2) Steam distillation uses as little water as possible, and

(3) Before distillation, the plant material was thoroughly ground and uniformly packed.

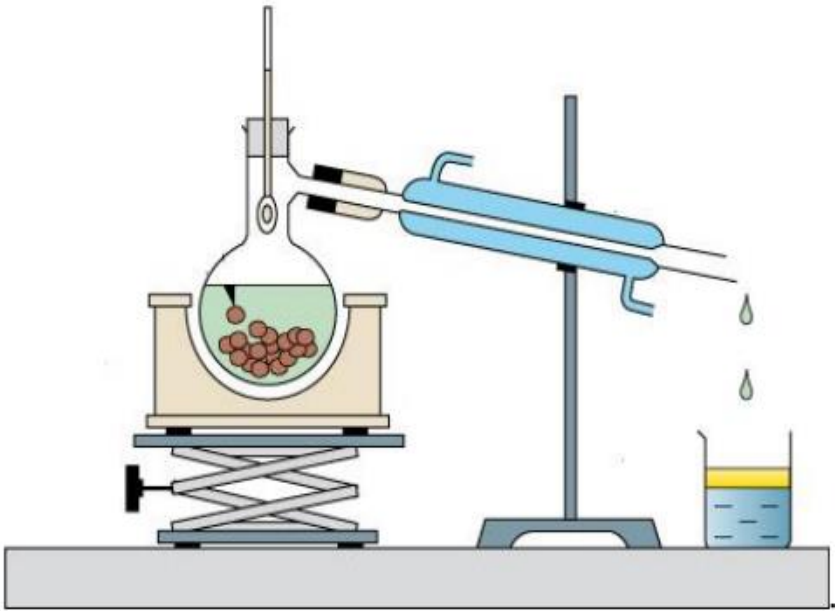

Figure 2.3. The schematic subsidize apparatus for hydrodistillation.

Advantages of Solvent Hydrogen Distillation

- Higher oil yield
- Controlled reflux minimizes loss of polar compounds
- Good flavor quality
- It was loosely packed with distillation ingredients and will remain loose in boiling water.
- Distillers were inexpensive, easy to assemble and suitable for on-site use. They were still commonly used in portable devices in many countries.

Disadvantage of hydro distillation

- Oil components such as esters were sensitive to hydrolysis, but acyclic monoterpene hydrocarbons and aldehydes were more likely to polymerize (during distillation, the pH of the water was often lowered, which accelerates the hydrolysis reaction).
- Oxygen-containing components such as phenol tend to dissolve in still water and couldn't be completely removed by distillation.
- Since water distillation was usually a small-scale operation (performed by one or two people), it takes a long time for large amounts of oil to accumulate
- The distillation process was treated as an art by local distillers, who rarely try to optimize both oil yield and quality.
- Water distillation was a slower process than either water and steam distillation or direct steam distillation.

2.6.2 Steam Distillation:

Steam distillation was a type of distillation (separation or extraction process) for temperature-sensitive plants such as natural aromatic compounds. Once a common laboratory method for purifying organic compounds, it had now been superseded by vacuum distillation. Steam distillation remains important in certain industries (Fahlbusch, 2003). It was one of the oldest officially recognized methods for isolating essential oils from plant material. The plant material loaded into the distiller exposed to steam without being macerated in water. The injected steam flows through the plant from the bottom to the top of the alembic. Steam distillation was the process of passing steam through a raw material. This vapor acts as an agent to break up the pores of the raw material and release the essential oil through them. This system produces a mixture of steam and the desired essential oil. This vapor was further condensed and essential oils were collected (Rai and Suresh, 2004).

Advantages of Direct Steam Distillation

- Could easily adjust the amount of steam.
- No thermal decomposition of oil components.

- The most popular method for large-scale oil production,

Disadvantage of Direct Steam Distillation

- Establishing this activity requires significantly higher capital expenditure than the other processes.

2.6.3 Solvent Extraction:

Solvent extraction, also known as liquid-liquid extraction or partitioning, was a method of separating compounds based on the solubility of each part. This was done using two immiscible liquids, such as water and an organic solvent. In the solvent extraction method of obtaining essential oils, the essential oil plant material placed in an extraction unit with perforated trays and repeatedly washed with a solvent. Solvent extraction used in the processing of perfumes, vegetable oils and biodiesel. It also used in sensitive plants to produce large quantities of essential oils at low cost (Chrissie, 1996).

2.6.4 Soxhlet Extraction:

The Soxhlet extractor was an experimental device invented by Franz von Soxhlet in 1879 (Soxhlet, 1879). It was originally developed for the extraction of lipids from solid substances. Soxhlet extraction was typically used when the compound of interest poorly soluble in a solvent and the impurities were insoluble in that solvent. This allows for unsupervised or controlled operation while efficiently reusing small amounts of solvent to dissolve large amounts of material. It was a solid-liquid contact technique used to remove one or more compounds from a solid by dissolving the solid in the refluxing liquid phase. In a conventional Soxhlet apparatus, a solid matrix placed in a cavity and the cavity gradually filled with the extracted liquid phase by condensing vapor from the still. When the liquid reaches a preset level, a siphon draws the contents of the cavity back into the still, transferring the extracted analytes to the bulk liquid (Schantz, 1998). This process repeated until almost complete extraction was achieved. Soxhlet extraction had several advantages. Most importantly, the sample was repeatedly exposed to new portions of solvent. This method avoids the possibility of saturating the solvent with extractables and improves analyte removal from the matrix.

2.6.5 Cold Pressing Method:

The term "cold pressed" theoretically means that the oil was pressed at low temperatures and pressures. It was one of the best ways to extract essential oils. This process used for most carrier oils and many essential oils. This process ensures that the resulting oil was 100% pure and retains all the botanical properties. It was a mechanical extraction method that reduces and minimizes heat throughout the batch of ingredients. The cold press method also called the scarification method. Cold pressing was mainly used to extract essential oils from vegetable, flower, seed, lemon and tangerine oils (Arnould, 1981). During this process, the oil-bearing outer layer of the plant was scraped away. The whole plant then pressed to squeeze the material out of the pulp and release the essential oil from the sac. The essential oil floats to the surface of the material and separated from the material by centrifugation.

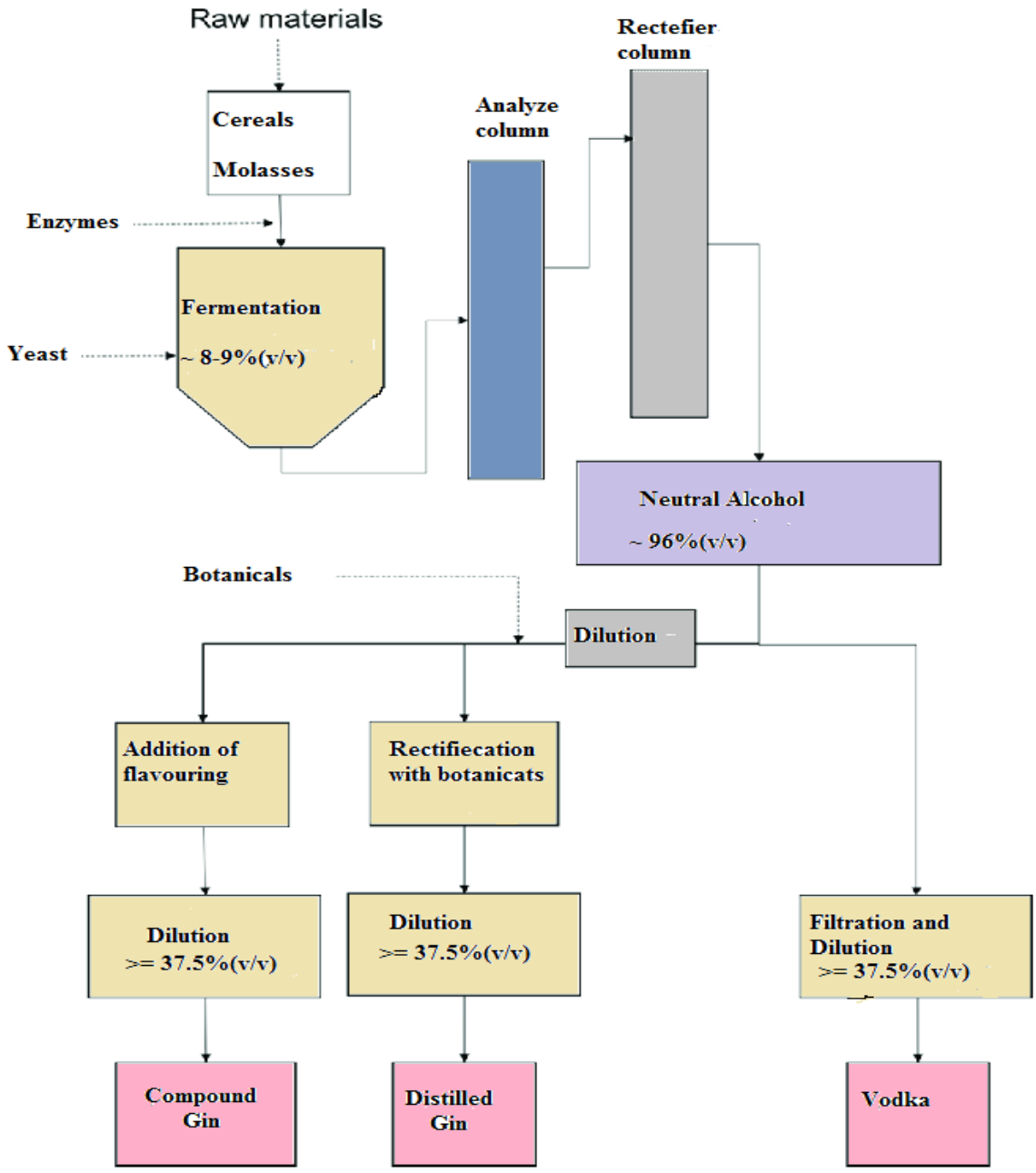

Figure 2.4 Process flow diagram for gin production

2.7 Sensory Evaluation

Methods for sensory evaluation provide a structured manner to gather data on samples' sensory properties as perceived by the human senses. These techniques were used for marketing goals (such as competition monitoring) as well as product creation and reformulation, on- and offline

quality control. Samples assessed by sensory panels were related to various experiment results, such as various product compositions, manufacturing processes, etc. A person who could identify the sensory characteristics that would help to characterize the product was called the panelists. (Watts, 1989).

2.7.1 Flavor

The combination of smell and taste with mouthfeel generally results in a sensation called taste. Taste was characterized as a set of sensory impressions (feelings) observed when food or drink was placed in the mouth. Taste was the sound of foods and potions. No taste was detected when the nose or breath was held. Perfumes should be sniffed or sniffed quite slowly but strongly as breathing slows down. There was no clear sense of smell when exhaling. Odor was also visible by diffusion, but it was exceptionally moderate and no sense of smell was detected while holding the breath. During mastication, molecules of aromatic substances move from the mouth to the olfactory region. Gentle exhalation through the nose during tasting has been found to be beneficial because it forces volatile molecules to the olfactory epithelium. The main condition for tasting a substance was its solubility in water. The two most primitive tastes, salty and sour, show a good relationship with atomic structure. The slower responding sweet and bitter tastes were associated with less chemical composition. Sweetness was characteristic of sugars and was related to the possession of hydroxyl groups (OH). Bitterness was not effectively related to any of the structural properties. A truly countless number of different flavors could be felt rather than tasted. For example, real tastes can be felt when the nose is blocked. In fact, when a person was cold, the taste response other than the specific sensation of the food in the mouth was sometimes the only taste component that the person perceived (Lawless, 1999).

2.7.2 Fundamentals of sensory assessment

Effective use of a sensory assessment operation requires three key components i.e. adequate laboratory facilities, sensory panels and rigorous training programs.

- **Preparation of laboratory and equipment**

Advanced research facilities include classrooms, conference rooms, boardrooms and planning rooms. Sensory assessment should be performed in a clean, quiet, and well-lit room. The main purpose behind the structural details is comfort for lasting flavor and ease of cleaning. It is good to maintain neutral tones and a good temperature and humidity throughout the premises, or at

least in the reading room. The test area where the cabin is located must be separate from the sample preparation and the washrooms, which must be clearly separated.

Sampling fundamentals

- Sampling should be done by a trained and experienced person and it was important that the sample was representative of the set**.**
- A sample preparation method should be chosen that is most likely to reveal a difference in the particular quality characteristic being evaluated. It must be ensured that the flavor is not lost and that no foreign flavors or odors have been introduced during preparation, storage, serving, etc. Foods such as hot sauce, spices, vinegar, etc. may need to be diluted with some medium due to their strong physiological effects.
- Sufficient samples were needed to draw conclusions. If you don't sample, you won't get the right amount of food, even if the ingredients are available.
- The temperature of the food must be close to the recommended temperature for consumption. Samples must be provided in containers of the same type, size, shape and color and the sample must be free of taste or smell. Tests should be done at least 1 hour before or after lunch.
- The operator uses substances that may interfere with the results, such as smoking, chewing tobacco, or taking drugs, the test must be performed at least 30 minutes before the test. You should avoid using chemicals with strong smells, such as cosmetics, flowers or hair oils.
- The number of samples presented in a session depends on the nature of the test product and the evaluation method used. Different strength levels or food quality can affect the testing process. If interest persists, panel members will review a number of samples per category, particularly for mild or mildly flavored foods. Ideally, the number of students should be limited to six.
- Coding should be done to hide the identity of the sample, so a multi-digit code generated from a table of random numbers should be used. Don't always use a specific code or combination of codes to quickly index results.
- The assessment sheets must be clearly written and the questions must be arranged in a logical order for the tests provided for each test. Make sure you use the right words without confusion. Your review card should be simple, short, and easy to follow and

write exactly what you want. All sensory attributes should be given due weight (Rochmawati, 2019). Many new testing methods were still being developed. These are grouped into three broad categories:

A. Discriminative testing

It has been one of the most useful analytical tools available to sensory professionals. The change to a descriptive test is justified based on the observed difference between the two products. The main purpose of all these methods was to answer a simple question. "Were the products considered different"? Obviously, the answer to this question had important consequences. If discriminant test findings were to be accepted by management as reliable, valid, and reliable, it was essential that each test be conducted with due consideration of all aspects of test design, product preparation and manipulation, implementation, data analysis, and interpretation.

B. Descriptive analysis

Relatively few examined judges participate in the descriptive test product category. The training focused mainly on the development of descriptive language, which was the basis for the scoring of the product. Other important training activities include grouping attributes (i.e., appearance attributes, aroma attributes, etc.), developing definitions for each attribute, and identifying useful guidelines during training, and familiarizing subjects with the scoring procedure. Descriptive analysis has had many applications including competition monitoring, storage stability, product development, quality control, physical/chemical and sensory correlation, etc. Depending on the experimental methods used, the training was quite different. Some of the descriptive methods reported in the literature are summarized here.

Profiles of flavor

The flavor profile method was the only formal qualitative descriptive method and probably the best known of the sensory testing methods. This method uses a panel of four to six screened and selected subjects who first study the product and then discuss it in an open session. Once agreement is reached on the product description, the panel leader summarizes the result in a report. The method was very attractive because the results were obtained quickly and the need for statistics would disappear. Only repeated analysis (10, 15 or 20 times) with the same test product gives consistent results for the entire panel. The profile method focuses on the overall taste of the product and the individual characteristics of the taste in relation to each other. The

dimensions of taste analysis in the profile method are: Perceived smell, taste, smell, taste and emotional factors (called character notes).

Table 2. 5The degree of intensity of each factor scale after taste.

O	Not taste
X	Threshold
1 or +	Slightly
2 or ++	Moderate
3 or +++	Very Strong

Amplitude of flavor, graded on following scale.

Table 2.6 Order of flavor taste graded scale

X	Very low
1	Low
2	Medium
3	Very high

C. Affective testing

Receptive preference was a valuable and necessary part of any sensory program at the consumer level. It refers to the measurement of liking or preference for a product. Recommendation was measured by directly comparing two or more products. An indirect measure of preference was obtained by determining which product received a significantly higher rating than another product in a multi-product test, or which product received a higher rating from significantly more people. The two most commonly used methods to measure liking and acceptance were the paired comparison test and the nine-point hedonic scale. Other methods were either variations of these two methods or types of quality scales, such as excellent to poor and palatable to unpleasant (Sidel, 1993).

Hedonic scale

Since its development, the Hedonic Scale 9 has been widely used in a variety of products with great success. Customers were able to easily understand scale with minimal training and were

able to produce product variations across project teams. Results using this measure are very informative because the statistics provide subject-specific means, variances, and frequency distributions.

2.7.3 Selection and training of sensory panel

Selection

By selecting and training the most stable and responsive members, one can hope to have a small but effective team. The choice is important because each person has different characteristics, needs, preferences and ability to resolve differences. Being good at tasting wine doesn't make you a good judge of chocolate, so your identification skills may not be very broad. Few of the judges were able to taste all the characteristics and flavors of the food. The judges were selected based on the following criteria (Singh, 1995):

A. Identifying

A few sorts of screening prepare were required for selecting board individuals counting particular tests based on: Discriminating contrasts between arrangements or substances of known chemical Composition □Ability to recognize flavors and odors. □Performance in comparison with other board members. □Ability to separate diverse tests to be utilized afterward within the test.

B. Responsiveness to stimuli

For general panel selection the candidates could be eliminated on the basis of lack of sensitivity to the senses, attributes, because of poor memory, slow recovery from stimulation and failure to understand the test. Sensitivity to taste or odor appears to be only one of the factors influencing discrimination. In most cases, elaborate tests based on acuity seem unnecessary.

C. Factors affecting sensitivity

Imperative components in fruitful judging were intrigued, inspiration, information and comparison of results, alteration to the test circumstance, memory etc. the board individuals ought to be given as much information as conceivable on the reason and require of examination. In any case, when this data might impact their judgment it ought to be withheld. A rewards framework for keeping up intrigued was regularly suggested. This may take the shape of uncommon pay, time off, uncommon benefits, giving refreshments after board sessions etc

D. Numbers of Panel

The number of judges required would shift agreeing to the changeability of people and of the product. For distinctive testing with prepared lab judges the board ought to comprise of 10 to 20 people with at slightest 3 or 4 replications per judge per treatment. In arrange to attain more prominent control of the board, it was conceivable that 10 judges superior than 20. Master boards of 5 to 10 were palatable. Customer type's tests require more than 80. Organized of nourishment technologists (USA) suggests 3 to 10 for prepared; 8-25 for semi-trained and 80 for untrained.

E. Coaching

Preparing ought to be recognized from involvement. Preparing steps may be taken intentionally to increase the viability and rate at which the person acclimatizes unused information. Preparing was coordinated towards getting bored individuals to ignore their individual inclinations. It might too be coordinated to secure acknowledgment of little contrasts. One of the imperative points of preparing was to get homogeneity of reaction. Affectability to essential tastes and odors increments due to preparing. Preparing makes a difference judges learn to compare flavors and flavor qualities in show disdain toward of time slack between tests.

F. Environment of taste

Control of environment variables and tests was all around recognized for tangible assessment of nourishments and drinks. Intrusions and diversions ought to be dodged amid testing. Consistency, quietness, comfortable environment, efficiency and smoothness of introduction of tests were critical. Judges ought to record their outcomes about freely and ought to not be able to communicate their impressions to those entering the lab afterward either verbally or through facial expressions.

G. Taste time

Tests ought to be organized when the judges feel their best. For the most part a time of 10:15 to 11:45 AM and 3:00 PM had been suggested. At these times the impact of breakfast had passed but the subject was not hungry however. No noteworthiness contrasts had been watched when the tests were conducted at 11 am or 3 pm.

H. Hiding

Veiling alludes to purposefulness minimizing of colors, taste or odor properties so that the differences between tests were assessed with less impedance from the variable which had been minimized i.e. utilize of colored holders, colored lights etc.

2.7.4 Sensory analysis preparation

It was relevant to talk about appropriate planning of nourishment, cooking methods, strategies of detecting, flavor choose up and the benchmarks utilized. The typical method to test the nourishment beneath conditions approximately the same as found beneath ordinary utilization i.e. bread ought to be dry, butter strong, vegetables entirety etc. inside a set of tests, the standard ought to moreover be displayed as an obscure. The tests displayed must be precisely alike. Among the components to be considered were visual appearance, test measure, temperature, utensils, pouring, coding, arrange, informational and washing. The capacity to taste may be influenced by substances earlier to flavor assessment. Tests ought to be the same in frame, consistency, color and appearance. Test measure ought to be adequate for judges to taste with certainty. Adequate test to donate a feeling of sizable chunk ness was suggested. Consistency of temperature was exceptionally imperative. All tests ought to be served within the holders of the same estimate, shape and color. The utensils ought to not give a taste or odor to the tests. Numerous cleansers take off an odor (since they were perfumed) unless completely washed. Samples should be put within the holder in a uniform/ tasteful way. All tests displayed ought to be coded to dodge giving data to the board. Three digit arbitrary numbers were perfect. Whereas a few agents had found no impact from arrange of serving but as a rule the primary test was expected to be standard against which the rest of the tests were appraised. In a perfect world, solid flavored tests ought to be served final as they as often as possible over sensitize the taste buds and recuperation was not fast. It may not be vital to deliver enlightening to a prepared board but this was not genuine for all boards. Questions emerge as to strategy of whirling the glass, chewing, noticing, to swallow or not, how numerous tests to warm up, to wash between tasting or not and the time permitted for each of these. The most excellent hone appears to be permit the judges to utilize his favored strategy (Szabó, 2014).

2.8 Toxicity Determination

Toxicology was the subjective and quantitative consider of the unfavorable or poisonous impact of chemicals and other anthropogenic materials or xenobiotics on organism's /poisons/ and their impacts on individuals, creatures, natural life and the environment. It moreover bargains with nourishment and beauty care products for open utilization both in lively or dead casualties. It was as it were as of late that consider of harms gets to be really logical & within the past it was basically a commonsense craftsmanship utilized by killers & professional killers. Harm had

played a vital portion in human history. Nourishment toxicology was consider of the nature, properties, impacts and discovery of harmful substances in nourishment and their malady appearance in people.

Harmfulness was the capacity of a chemical operator to cause damage. It was a subjective term which depends on the sum of chemical ingested, seriousness of the introduction, measurements & others. It can be intense (harmful occasion which happens before long after intense or constrained introduction), or persistent (apply to an occasion which happens numerous weeks, months or a long time after introduction). Inveterate poisonous quality: Poisonous impact that requires a few time to create, e.g. cancer. Testing for inveterate harmfulness include nonstop nourishing of the test substance to rodents for 20-24 months. (Biruh Alemu and Mistire Wolde, 2007) In this investigate the intense harmfulness was decided. The intense harmfulness of a substance was characterized by its LD50 / deadly measurements that would slaughter 50 of a gather of uncovered creatures (Deadly dosage fifty) (Henshaw, 2012)

Table 2. 8 Determination of acute toxicity level.

LD_{50}	**Levels of toxicity**
≤ 1mg	Extremely Toxic
1-50mg	Highly toxic
50-500mg	Moderately toxic
>500mg	Free of Toxic

Chapter Three

Materials and Methods

3.1 Determine Optimum Yield of Flavor

3.1.1 Raw Materials Collection

Juniper Berries were collected from Suba Menagesha National Forest Park. It was found in Oromia region, Sebeta, West of Addis Ababa, about 35km away from Addis Ababa and transported to Balezaf alcohol and liquor factory, Sebesta. The fresh ginger and cassia bark were purchased from market. Samples (Juniper berries, ginger and Cassia bark) was dried using sun light and cleaned properly. After that the sample was crushed using jaw crasher. All samples were put in plastic bags and stored at cool, dry and dark place separately for further laboratory analysis and extraction experiment.

Figure 3.1 Hammer mill.

3.1.2 Chemicals

Phenolphthalein indicator, oxalic acid, Chloroform/$CHCl_3$/, Ethanol, Iodine Monochloride (Wijs Solution), NaS_2O_3, RO water. All the chemicals were laboratory grade.

3.1.3 Equipment

Steam generator boiler, Extraction chamber, Jaw crusher, Power supply, bottle, Plastic bag, Viscometer, Gas chromatography - Mass spectroscopy, 500 ml and 1000ml Beakers ,PH meter was used for the study.

3.1.4 Research Frame work

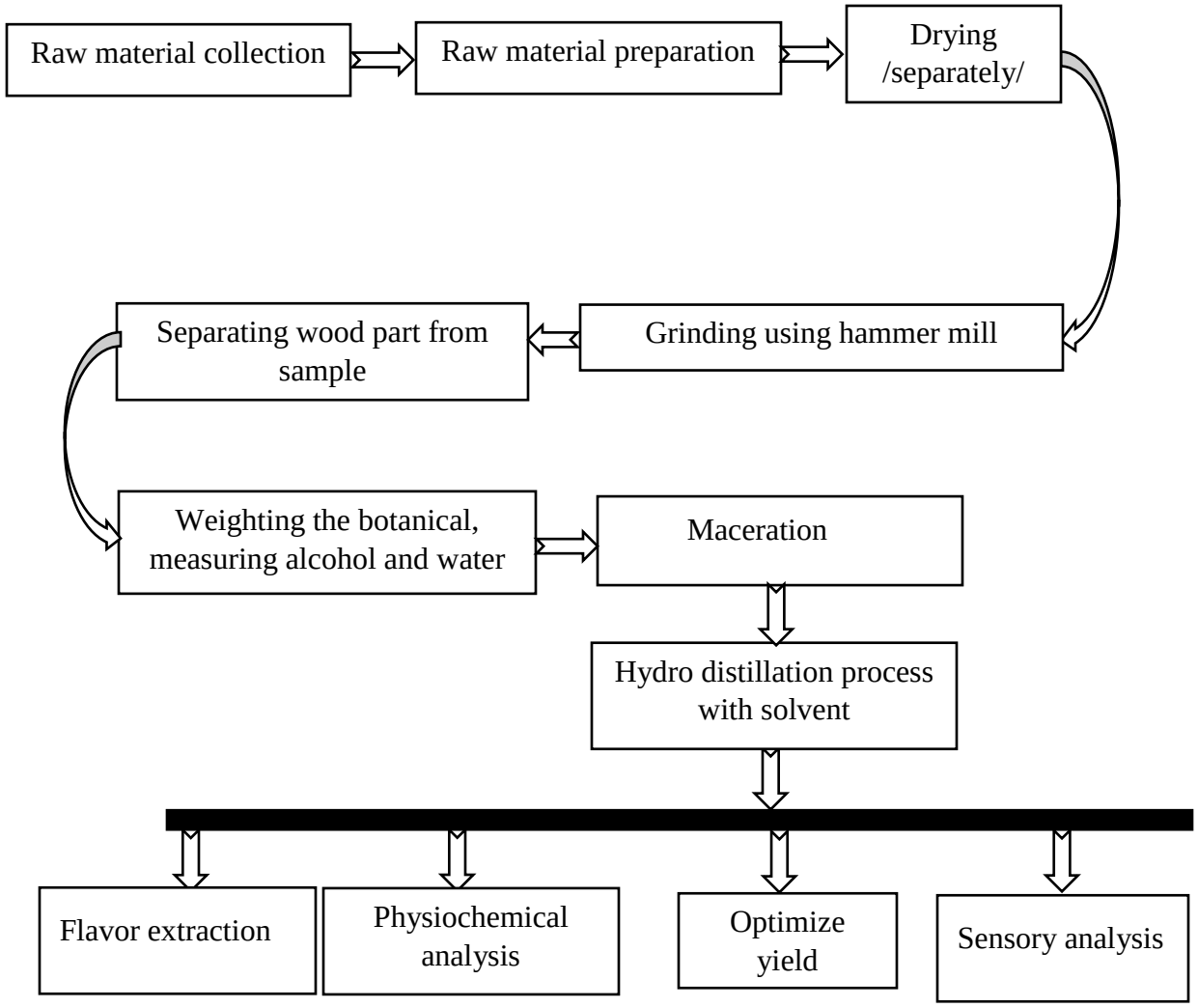

Figure 3.2 Framework of the research

3.1.5 Extraction Method

The extraction of gin flavor was conducted utilizing hydro distillation/water refining strategy with diverse liquor concentration (35%, 50%, and 65%) with 96V verification and diverse botanical weight (0.5kg, 1kg and 1.5kg). In this strategy, the fabric was totally submerged in water, which was bubbled by applying warm by open steam coil, from numerous of strategies

(coordinate fire, steam coat, closed steam coat and closed steam coil). The most characteristic of this handle was that coordinate contact between bubbling water and plant fabric. Amid water refining, all parts of the plant charge must be kept in movement by bubbling water; this was conceivable when the refining fabric charged loosely and remains free within the bubbling water. For this reason as it were, water refining has one particular advantage, i.e. that it grants handling of finely powdered fabric or plant parts that, by contact with live steam, would something else shape protuberances through which the steam was not enter. Other commonsense focal points of water refining were that the stills was reasonable, simple to develop and appropriate for field operation. These still broadly utilized with versatile gear in numerous nations. (Taxus baccata L.-2008). The botanical weight composition proportion, RO water and ethanol was set within the plant chamber of the pot still which was made from stainless and the steam was permits pass through closed steam coil which mellows the cells and permits the fundamental oil to elude in vapor frame with ethanol. Ethanol would utilize to break up rapidly and increment the sum of flavor extricate. The temperature of the steam must be tall sufficient to vaporize the oil show, however not so tall that it crushes the plants or burns the basic oils. The flavor extricate from this distinctive botanical would be vaporize with ethanol and the blend condense at cross stream. condenser and collect with bottle.

a)  b) 

Figure 3.3. Extraction of flavor with (a) Glass in heat mantle and (b) Pot still using steam

3.2 Characterization Techniques

3.2.1 Characterization of the Physical Properties

1. Refractive index

The sample of gin flavor was placed in the prism and illuminated by pointing the additional reflector to a suitable light source. Adjusting the achromatizing prisms by turning the dispersion knob ensures a reading at the correct wavelength (589 nm for standard measurement). The draft was then observed through one of the eyepieces and a reflectance index or Brix scale reading was taken from the internal scale. The refractometer reading of the gin taste sample at 20 ^{o}c. Refractive index of juniper berries was measured using a refractometer at 40o^{c} at JIJE LAB GLASS Pvt and also at Balezaf Alcohol and Liqueur. JIJE LAB used AOAC Official Method 920.160. The refractive index was the ratio of the Sine of the Angle of Incidence to sine of the Angle of Refraction as a ray of light passes from air to gin flavor kept at constant temperature.

$$N = \left(\frac{\sin i}{\sin r}\right) \quad \text{3.1}$$

Where: - N= refractive index, i = angle of incidence, r = angle of reflection.

2. Optical rotation

The tube was reins and the tube was placed in the Kruss Polari meter II which filled by extracted gin flavor 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. After the instrument door closed and the Polari meter run. The direction of rotation was determined. Optical rotation of gin flavor was measured by Polari meter. This measurement was taken place in Ethiopian conformity assessment enterprise and the formula was given as (Laurence A. 2017)

$$\text{Specific rotation } [\alpha] = \frac{\alpha}{cxl} \quad \text{3.2}$$

Where: $[\alpha]$ = specific rotation (o)

α = observed rotation

c = concentration (g/ml)

l = path length (dm)

3. Specific gravity

The specific gravity of gin flavor measured by a cleaned and dried empty bottle with a capacity of 150ml and weigh the empty bottle (W_0). The bottle would fill with the gin flavor and weigh (W_1). The water would be replaced with water after washing and drying the bottle and the weight will be (W_2). The expression for the specific gravity was: (ratio of material density with the water)

Specific gravity = $(W1 - W_0)/ (W2 - W_0)$ …….3.3

4. PH value

The PH value of gin flavor was measured in PH meter in Balezaf Alcohol and Liquor Factory laboratory.

5. Boiling point

100 ml of gin flavor poured in to beaker and a thermometer was inserted and placed on a heating mantle to heat until vigorous stream of bubble emerges and read temperature on thermometer.

6. Moisture content (raw material)

Five samples were taken randomly from each of the berries and spice collected. The samples were dried and the moisture content was measured using a common hot air oven. The 10 gram samples of juniper berries, ginger, cassia bark were taken in to a crucible and dried at 105^{o}c. The measurement was taken at one hour intervals until a constant weight was reached (Altuntas et al., 2007). The following formula shows how the moisture content calculated:

Moisture content % = $[(W_1 - W_2)/ W1] x100$…… 3.4

Where: W_1 = weight of sample before drying

W_2 = weight of sample after drying

7. Yield

The amount of flavor that was obtained by water distillation using ethanol as solvent was determined by weight difference as shown in the formula below (weight difference).

Yield% = $[(W_1 - W_2)/ W1] x100$ …… 3.5

Where: W_1 = weight of sample before extracting essential oil

W_2 = weight of sample after extracting essential oil

8. Toxicity level determination of gin flavor

The acute toxicity of gin flavor was tested at Ethiopian food and drug authority, food quality and safety control directorate case team section. 10 male Albino mouse of one month age was selected 5 of them was for control and 5 of them for test. The physical appliance was observed and the weight of each mice was measured. The weight was measured at beginning, at seventh and fourteenth day. Since the sample was 81.3%ABV, this was diluted to 15% to feed the mouse. The para meter for this measurement was body weight, irritation, gross observation necropsy (skin, eye…) .The age, sex and weight of were determined and feed directly for 14 days.

3.6.2 Characterization of the chemical Properties

1. Acid value

The Acid content was determined by direct titration of oil/fat in alcoholic medium against potassium hydroxide/sodium hydroxide standard solution, each oil sample (1.0 g) was weighed and dissolved in 50 mL of ethanol in an Erlenmeyer flask. Two drops of phenolphthalein indicator were added and titrated with 0.1 N potassium hydroxide (KOH) solution to the pink end point (which lasted for 15 minutes). The acid value was calculated (Equation 3.6) (Okpuzor et al., 2009):

$$\text{Acid value} = \frac{56.1xV\ xN}{W} \quad \text{……..3.6}$$

Where

V = Volume in mL of standard potassium hydroxide or sodium hydroxide used

N = Normality of the potassium hydroxide solution or Sodium hydroxide solution; and

W = Weight in gm of the sample

56.1 = molecular weight of KOH

2. Percentage of free fatty acid (% FA)

$$\%\ FA = 0.503 * AV \quad \text{……..3.7}$$

Where: AV= was acid value of the oil

3. Iodine value

The value of iodine was defined as one gram of iodine combined with one hundred grams of oil. This was a direct chemical measurement of the degree of unsaturation. 0.31 g of gin aroma was weighed into an Erlenmeyer flask. A solution of 10 ml of carbon tetrachloride and 20 ml of Wij was added to the flask and the solution was kept in the dark for 30 minutes at room temperature. 15 ml of 10% potassium iodide solution in 100 ml of distilled water was added to the flask. The resulting solution was titrated with 0.1 M sodium thiosulfate (Na2S2O3) using starch as an indicator to the end point where the blue-black color changes to colorless. A zero titration was performed simultaneously, starting with 10 mL of carbon tetrachloride. The iodine value was then calculated using the following formula (Wijs , AOAC Official Methods of Analysis (1984).

$$\text{Iodine number} = \frac{12.69\text{x } N\text{x}(B-S)}{W} \quad \text{.....3.8}$$

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

W = weight of sample

3.7 Experimental design

3.7.1 General factorial design and Data analysis

Design Expert software (version 6.0.8) was used for data analysis; General structural design was chosen over other design methods. As this allows the evaluation of the effects of multiple process parameters and their interactions on the response variable, gin flavor production was recorded for each combination of process settings. The software was also used to characterize how significant factors affect the response (for optimization), to show non-significant factors, and to identify significant factors. To distill water with alcohol as a solvent, in this study, two factors, three levels and three repetitions was used. Botanical ratio/weight/ in three levels (0.5 kg, 1 kg and 1.5 kg). This level h.as the same botanical ratio and different weight. Alcohol content in

three levels (35%, 50% and 65%). This experimental design helps to distinguish between the significance of main and interaction factors.

Table 3.1. Classification of factor and level

Factor	Level		
	1	2	3
Botanical weight(kg)	0.5	1	1.5
Concentration (%)	35	50	65

3.8 Sensory evaluation preparation

The sensory evaluation was performed in Balezaf Alcohol and Liquor Factory bottling laboratory class. Ten panelist were selected from chemist, quality controller and shift supervisor for competition of produced gin flavor sample with existing one or gin liquor which were produced from imported flavor by using discrimination test. Four panelist were selected for evaluation of the gin flavor profile that was produced locally from *junipers procera* berry, ginger and cassia bark itself using affective test. The blended sample was prepared in ten sample testing coded beakers with the same volume for both control and extracted sample. The panelist was grouped in to two with five member. Five chair had for each panelist and the presentation was given for them how to taste the liquor sample. It was not allowed them to talk each other and there was one meter distance between the chairs. The panelist was reins their mouth with water before taste the first sample, after tasted the first sample eat bread and reins with water to taste the next sample. The gin flavor profile was also performed with four panelist with the same procedure above.

Chapter four

Results and Discussion

4.1 Determine Optimum Yield of Flavor

4.1.1 Moisture content analysis

The average wet and dry base moisture content for five samples of *juniper procera* berries was 47.43±1.92% and 9.48±0.44% respectively. The average moisture content of wet base for five sample ginger was 79.39±1.64% and the average moisture content of dry base ginger was 10.89±0.08%. The average moisture content of dry base cassia bark for five sample was 16.16±0.38%. The fresh cassia bark was not found in the market due to this reason moisture content of wet base cassia bark was not calculated. The moisture content calculation of this spice and herb used to know and compare the cost of raw material and product obtained since the raw material was mostly purchased in fresh.

Table 4.1. The average dry and wet base moisture content of the samples compared with literature value.

Herb/spice	Average moisture content (%wet base)		Average moisture content (%dry base)	
	Sample	literature	Sample	Literature
Procera Juniper berry	47.43±1.92	44.6%(Meroda, 2017)	9.48±0.44	14.07%(Altunats,2007)
Ginger	79.39±1.64	88.5(Haozhe, 2016)	10.89±0.08	10(Haozhe, 2016)
Cassia bark	-		16.16±0.38	11.1(Goswami, 2016)

4.1.2 Analysis of factors that affect the product

A common factorial plan with two reactions was chosen for this think about. The primary reaction variable was execution and the moment reaction variable was tactile examination. The surrender was gotten by calculating the weight distinction utilizing condition 3.4. The result of the tangible examination was too done agreeing to area 3.5. A common factorial plan was

connected for diverse botanical weight with three levels and two variables at constant charge volume. And after that 27 tests were done for this inquire about. Plan tests were carried out to portray the quantitative impact of two parameters, to be specific liquor substance and botanical weight of the test. Extraction yields were calculated from the proportion of the weight of the gotten fundamental oil to the weight of the crude fabric utilized within the extraction. The surrender bend of the mass of the extricated basic oil was created in connection to the liquor concentration utilized and the botanical mass. In this consider result 35%, 50%, 65% liquor concentration and 0.5kg, 1kg, 1.5kg botanical weight product utilized. The evaporator weight for progressive tests was set to climatic weight and the temperature was 75oC. Plan Master Computer program (Form 6.0.8.1) was utilized for relapse and graphical examination of information gotten by successive F-test, lack-of-fit test and other satisfactory measures to choose the best show for execution (amounts) and tactile esteem of extricated gin flavor of three botanicals.

Table 4.2. Flavor yield and sensory analysis grade by water distillation with solvent and result using Design Expert standard method.

		Factor 1	Factor 2	Response 1	Response 2
Std	Run	A:botanical weight Kg	B:alcohol concentration %	Yield %	sensory analysis Grade
1	23	0.5	35	0.78	1
2	11	0.5	35	0.84	1
3	2	0.5	35	0.8	1
4	17	1	35	0.91	2
5	1	1	35	0.88	3
6	14	1	35	0.89	2
7	7	1.5	35	1.41	2
8	8	1.5	35	1.09	2
9	16	1.5	35	1.35	3
10	4	0.5	50	1.14	2

11	5	0.5	50	1.35	2
12	13	0.5	50	1.52	2
13	19	1	50	1.98	3
14	22	1	50	2.58	3
15	12	1	50	2.76	3
16	6	1.5	50	2.65	4
17	26	1.5	50	2.58	4
18	24	1.5	50	2.67	4
19	15	0.5	65	2.16	3
20	21	0.5	65	2.71	3
21	3	0.5	65	2.56	3
22	27	1	65	1.86	3
23	20	1	65	1.78	3
24	25	1	65	1.99	3
25	10	1.5	65	1.5	2
26	9	1.5	65	1.48	2
27	18	1.5	65	1.74	2

The maximum yield was 2.76% was obtained from alcohol concentration (50%) and 1kg botanical weight.

4.1.3 Development of regression model equation for yield

Below was a model equation that correlates the reaction (essential oil yield) with the extraction process variables as a true value after removing pale terms. The predictive model of the gin flavor performance rate of the coded factors was given in equation (4.1).

Final Equation in Terms of Coded Factors

yield = +1.70-0.16*A[1]+0.034*A[2]-0.71*B[1]+0.43*B[2]-0.026*A[1]B[1]-0.14*A[2]B[1]-0.64 * A[1]B[2]+0.27* A[2]B[2]……..4.1

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

Where: A = botanical weight

B = alcohol concentration

The result indicates that the yield of juniper berries essential oil was dependent only on the terms indicated above. Based on the coefficients in equations (4.1) it was evident that the percentage of essential oil yield decrease with the botanical weight (A) and alcohol concentration (B) at constant charge.

4.1.4 Adequacy of the model for yield

Show fit was tried by examination of fluctuation. The relapse demonstrate was found to be tolerably critical with determinant relationship coefficients in R-squared, balanced R-squared, and anticipated R-squared of 0.9427, 0.9173, and 0.8712, individually. The quality of the created show was assessed by these relationship coefficients. The R-squared esteem of the created relationship was 0.9427. This implies that 94.27% of the full variety in flavor execution was due to the explored test factors. Pred R-squared of 0.8712 was in sensible assention with Adj R-squared of 0.9173 i.e. the distinction was less than 0.2. Adeq Exactness measures the signal-to-noise proportion. A proportion more prominent than 4 was alluring. A proportion of 16.081 demonstrates a adequate flag. This layout was utilized to explore in plan mode.

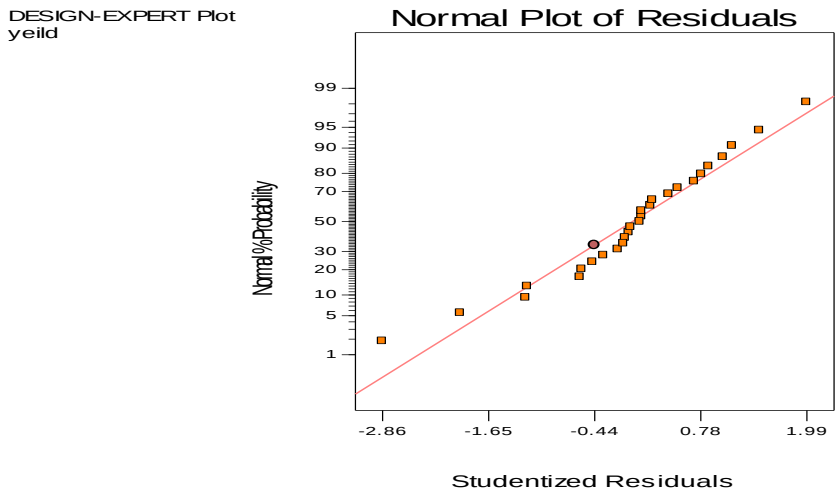

Figure 4.1. Normal plots of residuals of flavor yield

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 was 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 4.3 Based on a 95% confidence level; F-value was a test for comparing model variance with residual (error) variance. If the variances were close to the same, the ratio would be close to one and it was likely that any of the factors had 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 37.04 implies the model was significant. There was only a 0.01% chance that a"Model F-Value" this large could occur due to personal error or disturbance.

Table 4.3 ANOVA for the regression model equation and coefficients of yield

ANOVA for selected factorial model

Response 1: yeild

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	11.47	8	1.43	37.04	< 0.0001	significant
A-botanical weight	0.3945	2	0.1972	5.10	0.0176	

B-alcohol concentration	6.88	2	3.44	88.87	< 0.0001	
AB	4.20	4	1.05	27.10	< 0.0001	
Pure Error	0.6967	18	0.0387			
Cor Total	12.17	26				

Factor coding was coded.

Sum of squares was Type II Classical

The Model F-value of 37.04 implies the model was significant. There was only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms were significant. In this case A, B, AB were significant model terms.

Fit Statistics

Table.4.4 Result of fit statistics for yield

Std. Dev.	0.20	R^2	0.9427
Mean	1.7	Adjusted R^2	0.9173
C.V. %	11.56	Predicted R^2	0.8712
Press	1.57	Adeq Precision	16.081

The "Pred R-Squared" of 0.871 was in reasonable agreement with the "Adj R-Squared" of 0.9427. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 was desirable. Your ratio of 16.081 indicates an adequate signal. This model was used to navigate the design space.

4.1.5 Effect of Individual Process Variables on the yield of extracted flavor

Different process variables significantly affected the extraction of gin flavor from different plant materials. Significant individual process variables affecting yield were alcohol content, botanical

weight and charge or amount of liquid used, but in this study charge was held constant to reduce the amount of the variable.

1. **Effect of botanical weight**

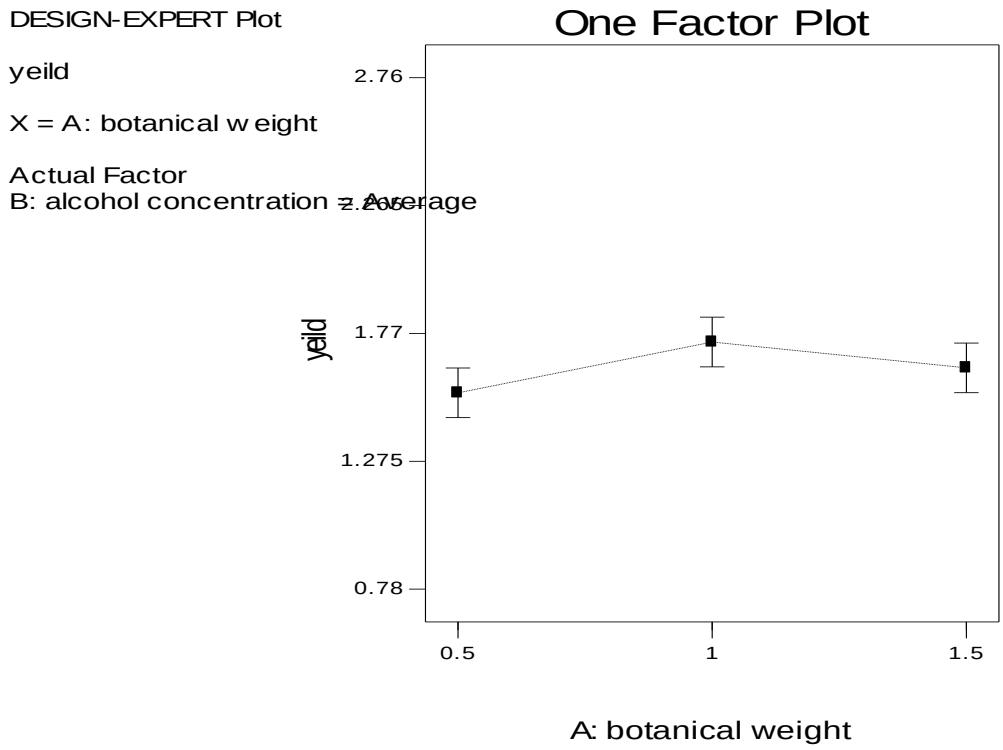

Figure 4.2. Effect of botanical weight on the yield of extracted flavor

Botanical weight was the main factor that control the amount of flavor to produce. In figure above shows that as the botanical weight increase the flavor yield increase till transfer of flavor from the soaked botanical to the steam becomes bitter or zero and decrease when the maximum amount of botanical weight used which was above the critical point in which material does not easily move inside the still and the flavor also not escape to the steam since the volume charge held constant. So that in the hydro distillation extraction the maximum flavor yield could be finding at a 1kg botanical weight.

2. Effect of alcohol concentration

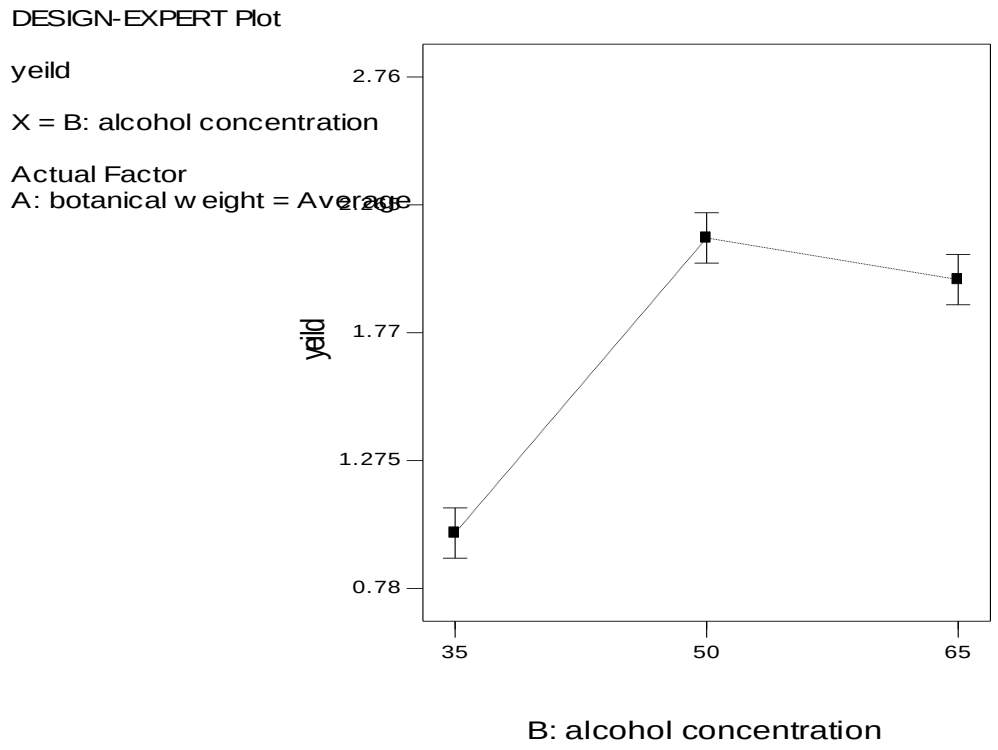

Figure 4.3. Effect of alcohol concentration on the yield of extracted flavor.

As shown in the above figure the increment of alcohol concentration the amount of average flavor yield decreased due to decreased in polarity.

3. Interaction effect of factors on the yield of extracted flavor

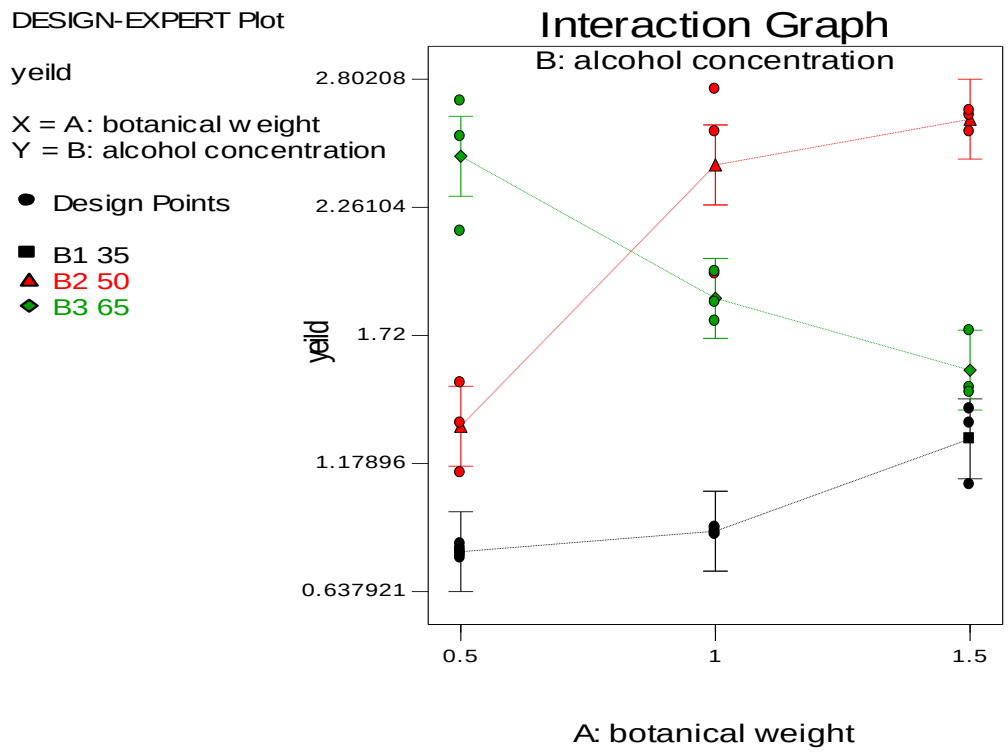

Figure 4.4 Effects of interaction between botanical weight and alcohol concentration for yield.

The graph shows that there was interaction factor even though the interaction was occurred at minimum yield. This shows us an increment in botanical weight and alcohol concentration the quantity of flavor extracted decreased. On Figure above it was observed that the maximum yield 2.76% was obtained at 1kg botanical weight and 50% alcohol concentration and the minimum yield was 0.78 at 0.5kg botanical weight and 35% of alcohol concentration.

4.1.6 Adequacy of the model for sensory evaluation

ANOVA for selected factorial model

Response 2: sensory analysis

Table 4.5 ANOVA for the regression model equation and coefficients of sensory analysis

Source	Sum of Squares	df	Mean Square	F-value	p-value	

Model	17.41	8	2.18	29.37	< 0.0001	Significant
A-botanical weight	3.63	2	1.81	24.50	< 0.0001	
B-alcohol concentration	5.85	2	2.93	39.50	< 0.0001	
AB	7.93	4	1.98	26.75	< 0.0001	
Pure Error	1.33	18	0.0741			
Cor Total	18.74	26				

Factor coding was coded.

Sum of squares was Type II Classical

The Model F-value of 29.37 implies the model was significant. There was only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms were significant. In this case A, B, AB were significant model terms. Values greater than 0.1000 indicate the model terms was not significant. If there were many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Fit Statistics

Table 4.6 Result of fit statistics for sensory analysis result

Std. Dev.	0.2722	R^2	0.9289
Mean	2.52	Adjusted R^2	0.8972
C.V. %	10.81	Predicted R^2	0.8399
		Adeq Precision	19.0919

The Predicted R^2 of 0.8399 was in reasonable agreement with the Adjusted R^2 of 0.8972; i.e. the difference was less than 0.2. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 was desirable. Your ratio of 19.092 indicates an adequate signal. This model was used to navigate the design space.

Final Equation in Terms of Coded Factors:

sensory analysis = +2.52-0.52 *A[1]+0.26*A[2]-0.63*B[1]+0.48*B[2]-
0.37*A[1]B[1]+0.19*A[2]B[1]-0.48 * A[1]B[2]-0.26* A[2]B[2]……4.2

1. **Effect of botanical weight on sensory evaluation**

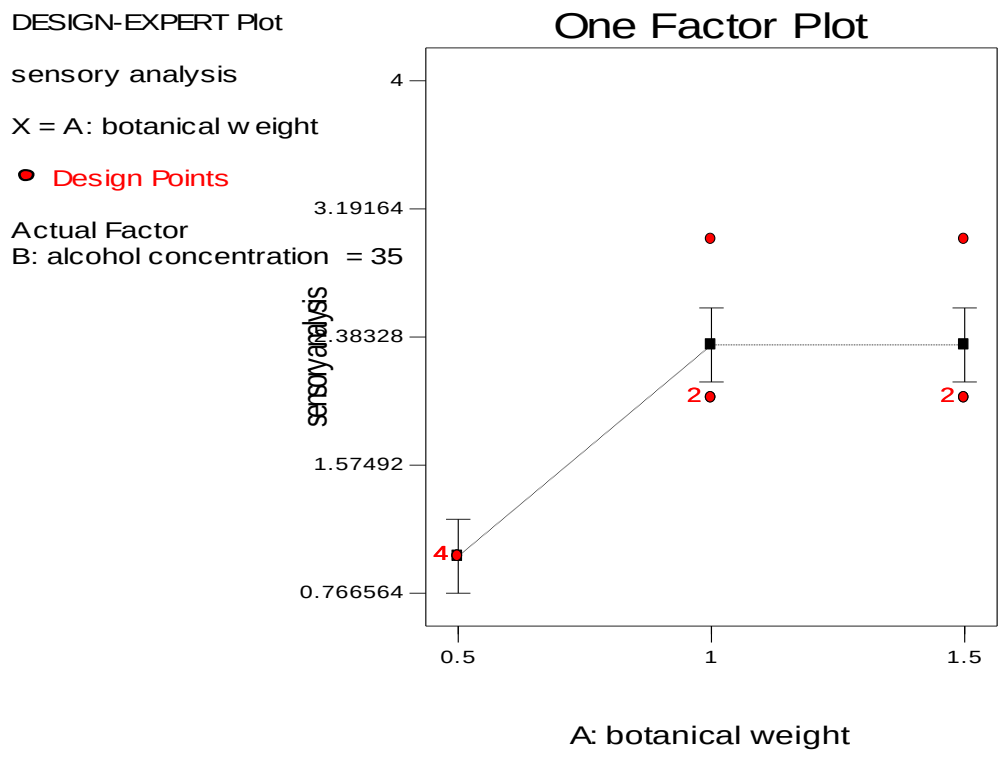

Figure 4.5 Effect of botanical weight on the sensory analysis of extracted flavor

The first and the main factor that affect the gin flavor taste was the amount of botanical weight and botanical ratio used. Because the amount of dry mass which used in flavor extraction was directly proportional to the amount of essential oil or essence produced up to 1kg botanical weight. When the right amount of botanical weight as well as botanical ratio would not use, even if the botanical ratio in this research was held constant, the right value of sensory result would not obtained. As shown from the graph for constant botanical ratio the sensory evaluation result increase with botanical weight up to 1 kg and decrease above 1kg botanical weight.

2. Effect of alcohol concentration on sensory evaluation

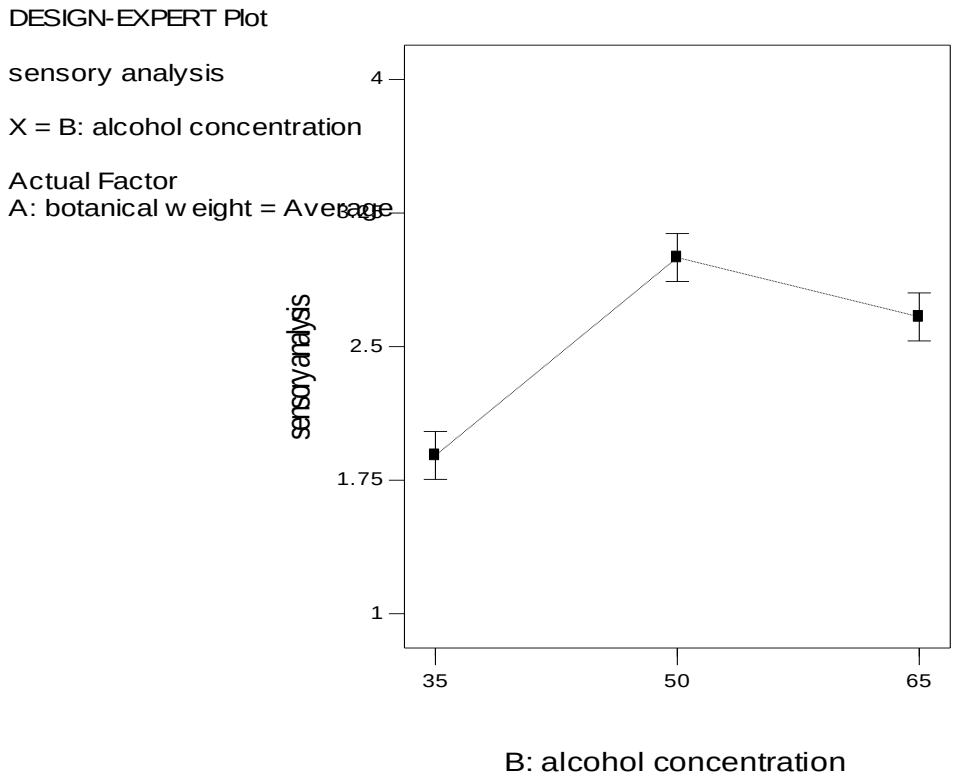

Figure 4.6 Effect of alcohol concentration on the sensory analysis of extracted flavor

The second factor that effect on gin flavor taste quality was the amount of alcohol concentration. As shown the above figure when the amount of alcohol aoncentration increase the amount of avarage sensory evaluation of flavor decrease this was because of when the alcohol concentration increas the flavor taste become bitter. In this reaserch the good flavor taste was obtained at 50%.

3. Interaction effect of factors on the sensory evaluation of extracted flavor

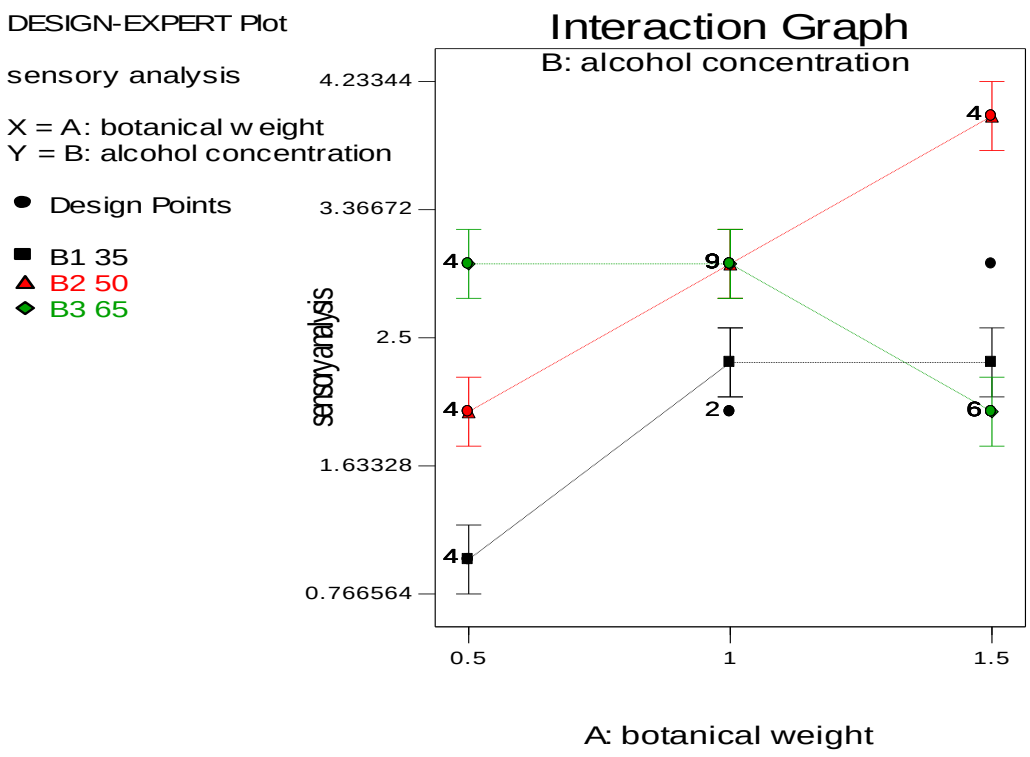

Figure 4.7 Effect of intraction between botanical weight and alcohol concentration for sensory analysis

The objective of gin extraction was to balance of extracting the right amount of desirable quality flavor without excessive extraction of essential oil or essences. During production process mostly the first and last product which was called heads and tails were removed to avoid light and heavy oil from flavor. From the above graph when alcohol concentration increases with botanical weight the flavor would become bitter and felt not good since excess oil extracted i.e at 65% alcohol concentration. Therefore the appropriat amount of botanical weight and alcohol concentration should be selected during gin flavor extraction process which was 1.5kg botanical weight and 50% alcohol concentration.

4.1.7 Optimization of flavor Extract Using hydro Distillation

The goal of optimization was to maximize financial benefits or increase the yield of juniper essential oil by minimizing process costs. To find out the optimal values of juniper berries, the aromatic extract of ginger and cassia bark was concentrated using water distillation with ethanol as a solvent as follows.

Table 4.7 Optimum Desirability results of gin flavor production

Number	Botanical Weight	Alcohol Concentration	Yield	Sensory Result	Deseriability	
1	1.5	50	2.63333	4	0.967	Selected
2	0.5	65	2.47667	3	0.756	
3	1	50	1.87667	3	0.554	
4	1	65	1.67	3	0.449	
5	1.5	65	1.57333	2	0.401	
6	0.5	50	1.33667	2	0.281	
7	1.5	35	1.28333	2.33333	0.254	
8	1	35	0.893333	2.33333	0.057	
9	0.5	35	0.806667	1	0.013	

The desirability of the design expert software ranges from 0-1 and indicates how close the response was to its ideal value. If the response falls within the unacceptable ranges, the attractiveness was 0, and if the response falls between the ideal ranges or the response reached the ideal value, the desirability was 1. Meanwhile, if the response falls within the tolerance ranges but not within the ideal or if it does not reach its ideal value. The closer the answer was to the ideal ranges or ideal values, the more the attraction force was to 1. Based on the above analysis, the best local flavor yield of a maximum of 2.63% and a flavor score of 4 (excellent) was found in the botanical study. Weight 1.5kg and alcohol content 50% a. At this stage, a desirable value of 96.7% was obtained.

4.2 Physical characteristics of gin flavor

4.2.1 Refractive index

Refractive index of gin flavor was 1.36619 or 21.4 ^{o}Brix. The value of refractive index which imported from England in Balezaf Alcohol and Liquor factory was 1.36653 or 21.6 ^{o}Brix as stated in summary table. The higher the refractive index, the greater the risk of spoilage caused by oxidation. The result obtained was in the standard range of refractive index of 1.3329-1.5320 International scale (1936).

4.2.2 Optical rotation

The Polari meter of gin flavor measured was 0.307 degree in 200mm path length and at wave length of 589nm. The specific rotation was also calculated as follow.

Specific rotation = 0.307/0.0103*2 = 14.9^{o}

Actually the units was $^{o}cm^{2}g^{-1}$. But the specific rotation usually reported in degree. From instrument read value of specific rotation at Ethiopian conformity assessment enterprise was 14.365. The difference encountered was due to inaccuracy concentration measurement of gin flavor. It shows that the value was found in the range -2.5 – 3.5 (Specification of gin flavor hydrosol).

4.2.3 Specific gravity

The value of specific gravity of gin flavor was 0.8569 as calculated below.

I.e. Wo/weight of empty Pycnometer = 45.0735

W1/ weight of Pycnometer filled with flavor = 130.0748

W2/ weight of Pycnometer filled with water = 144.2768

$$S.g = (W1 - W_0)/ (W2 - W_0) = \frac{130.0748-45.0735}{144.2768-45.0735} = \frac{85.0013}{99.2033} = 0.8569$$

The result show that the specific gravity of gin flavor was in the range between 0.8456 - 0.890(European standard).

4.2.4 PH value

The value of gin flavor was recorded as 4.84. The PH value of gin flavor mostly falls 4 to 5.5 when that flavor extract using ethanol as a solvent. If the flavor extract using steam it falls 9 to 11 which was measured by pH meter.

4.2.5 Viscosity

The viscosity of extracted gin flavor measured by viscometer was 1.108cp. The result shows that the low viscosity of gin flavor was low which was near to the standard found in literature of 1.1cp at 25^{o}c.

4.2.6 Boiling point

The boiling point was measure by the procedure described on the methodology and its value was 75^{o}c. The value was the optimum temperature at which good result of sensory analysis was obtained.

4.2.7 Flavor toxicity determination

The flavor toxicity was determined after fourteenth day as observed the physical appearance was good and the body weight was increase as controlled mouse. The value of $\%DL_{50}$ was 0% that means no death count from tested mouse. Therefore the gin flavor was nontoxic.

Table 4. 8 Summary of physical characteristics of gin flavor

Physical property	Imported flavor/BALF	Sample
Color	Colorless	Colorless
Boiling point	75^{o}c	75^{o}c
^{o}Brix	21.6	21.4
Refractive index	1.36653	1.36619
Optical rotation	-	0.307
PH	4.72	4.84
Specific gravity	0.8608	0.8569
Viscosity	-	1.108cp

4.3 Chemical characteristics of gin flavor

4.3.1 Acid value

The acid value of gin flavor was obtained as follow.

Acid value = mg of alcoholic ether solution X normality of alcoholic KOH solution X 56.1/g of sample.

= 0.0571x0.1x56.1/2

= 0.16(mgKOH/g)

4.3.2 Percentage of free fatty acid

%FA = 0.503Xav

= 0.503x0.16

= **0.08%**

The value shows that the acid value of gin flavor was decreased compared to 1.4(mgKOH/g) (Meroda), and free fatty acid 0.704%, because the value obtained from pure juniper berry only and the method of extraction was using water only. The other produced of gin flavor was mostly secure for such information. The increase acid value was taken as an indicator of oxidation of the oil which lead to gum or sludge formation.

4.3.3 Iodine number determination

Based on Based on AOAC official method iodine number was calculated as follow. 1g of sample accurately weight and placed in flask. A blank solution was prepared with 15ml cyclohexane acetic acid solvent and swirled it to completely dissolve. Dispense 25ml wejis solution in to flask containing test sample. 20ml KI solution and 150ml water was added and titrate with 0.1N of standard $Na_2S_2O_3$ solution. Titration continued until yellow color disappeared and 2ml starch indicator was added and also continued titration until blue color had just disappeared.

$$\text{Iodine number} = \frac{12.69\text{x Nx}(B-S)}{W}$$

Where:

B = 0.1 N Sodium Thiosulfate required (ml) by blank = 13.9

S =0.1 N Sodium Thiosulfate required (ml) by sample = 12

N = Normality of Sodium Thiosulfate solution = 0.1

W = weight of sample = 1

$$\text{Iodine number} = \frac{12.69x\ 0.1x(13.9-12)}{1}$$

= 2.44ml/g

The iodine number of extracted gin flavor also decreased compared to lecture found. This was happen due to extraction process method and the literature cited taken was only from pure juniper berry essential oil. The iodine value indicates unsaturation of oil. The higher of unsaturation the grater the possibility of the oil to go rancid. So the value was below from literature cited and it indicates good result.

Table 4.9 Summary of chemical property of gin flavor

Chemical properties	Sample result	Result from Literature/for juniper berry only/
Acid value	0.16(mgKOH/g)	1.4(mgKOH/g) (meroda, 2017)
Fatty acid value	0.08%	0.704%(meroda, 2017)
Iodine number	2.44ml/g	10.23ml/g(meroda, 2017)

4.3.4 Gas Chromatography- Mass Spectroscopy

Developmental methods of analysis of essential oils with GC-MS was used for identification chemical compositions of essential oils, regardless of the type of essential oils, and analyze the authenticity of essential oils. The essential oils used in this study were juniper berries, ginger and cassia bark oil/flavoring as they were the main herbs and spices used to flavor gin. GC-MS was performed at JIJE LAB GLASS pvt. Company. The GC instrument was a 2.6 µm column with helium carrier gas at 150 °C and detected by FID at 250 °C. The GC-MS spectrum of the seed oil was displayed and its components identified as shown in Table 4.7. According to the chromatogram, a total of 28 components with different retention times were eluted from the GC column. Components were identified based on their retention time and mass spectral library

search. The relative amount of individual components was calculated from the GC peak areas. Table 4.10 shows the main components obtained by GC-MS analysis of aroma of juniper berries, ginger and cassia bark. To identify the components of essential oil extracted by water distillation, their mass spectra and retention times were compared with those of the reference standards, it was seen that these spices were characterized by the presence of monoterpene hydrocarbons, oxygenated monoterpenes and sesquiterpenes, but quantitative differences were observed in the concentrations of these components.

Table 4.10 Chemical component of flavor obtained from GC/MS.

Component		Sample/junipers procera/ %Area	Literature/junipers communis/ %Area –as reference
From Juniper	β- penine	4.89	1.26
	3-carene	3.48	0.51
	γ- terpinene	1.60	6.58
	Terpinolene	8.57	0.19
	α-penine	0.25	10.56
	Bornylene	0.96	1.40
	Myrtenyl acetate	0.75	4.98
	Longifolene/ tricyclic sesquiterpene/	7.88	2.02
From cassia bark	3- careen	3.48	0.16
	γ- terpinene	1.60	0.01
	Terpinen -4 ol	7.35	0.5
	Terpinolene	8.57	0.01
	β-Humulene	4.35	0.01
	α- penine	0.25	
From ginger	Citral	2.90	2.68
	Terpinolene	8.57	14.93
	Octanal	1.19	-

(Literature cited from journal of Medicinal and Aromatic Plant science 32(3) (2010) 199-201, open chemistry 2021; 214-227 and Nutrition and Food Science Technology volume 9- 2022.)

The table above shows that the results of this study and the literature were not the same due to geographical location, atmospheric weather and distillation process, and basically the juniper type was not the same as the highlighted procera juniper result. In addition, in this study, flavor extraction was done by combining three species, where each plant could have the same chemical components such as carene, terpinolene, α- and β-penine, γ-terpine, and the combined ratio also affects the amount of the result. Overall, the gas chromatography result shows that African native procera berries had their own flavor identity, which mainly consisted of cyclopropane (8.35%), cyclohexane (7.91%), terpinolene (8.57%), citronellol (6.34%) β-penis (4.89%), α-pinene (0.25%), γ-terpene (1.60%), etc.

4.4 Sensory evaluation

Based on discussed in chapter three section 2.6 the sensory evaluation was done by using both Descriptive analysis method for the product itself to determine the profile of produced flavor and Affective testing method(Hedonic scale) for comparison with the existing gin liquor which was produced from imported gin flavor.

4.4.1 Gin flavor profile

The gin flavor profile was performed by selecting four panelists which were two from chemist and two from quality controller and panel leader was selected from them to summarize report the result the panelist was well trained. The sample was taken as ‘sample 1’ from prepared one and after panelist checked the following gin flavor profile result was obtained.

Table 4.11 Extracted gin flavor profile

Sensory attributes	Sample 1
Flavor	2.25
Odor	2
Aroma	2
Test	2.75
over all acceptability	2.5

The amplitude of overall gin flavor grade result was 2.5, i.e. high.

4.4.2 Sensory evaluation by comparison

Acknowledgment inclination a important and essential component of each tangible program was performed at consumer's levels. It alludes to measuring enjoying or inclination for a item. Inclination was measured specifically by comparison of two or more items with each other.A 9-point hedonic scale quality analysis was used to evaluate the characteristics of two dry Gin samples for visual color, flavor, aroma, and test and overall acceptability analysis using 10 experienced panelists which were selected from Balezaf alcohol and liquor factory. Comparison among the averages of sensory attributes (visual color, flavor, aroma, taste and overall acceptability) control sample (sample1) was obtained from Balzaf product and sample 2 was extracted from juniper procera berries, cassia bark and ginger. Two equal amount of dry Gin samples were served with bread and water, with which the panelists could preceding the bread before quality attribute then panelists could rinse their mouth after each quality attribute.

Table 4.12 Evaluation of dry Gin liquor samples

Sensory attributes	Sample 1(control)	Sample 2
Color	8.7	8.4
Odor	8.6	7.9
Aroma	8.4	8.2
Flavor	8.6	8.2
Test	8.2	8.2
Over all acceptability	8.8	8.1

Chapter five

Conclusion and Recommendation

5.1 Conclusion

The liquor gin was produced either compound gin or distilled gin with the first main ingredient of juniper berries based flavor and the second ingredient of high proof spirit /ethanol/. Botanical gin flavor was extracted, optimized and characterized which obtained from locally available botany and spice procera junipers berries, cassia bark and ginger. The extraction process was done through hydro distillation with ethanol as a solvent to enhance the quality and amount of product. Botanical weight and alcohol concentration was the factor variable and charge volume of liquid, temperature, pressure and the ratio of botanic and spice was held constant to reduce variability and uncontrollability of the equipment. The temperature was held at 75^{o}c with atmospheric pressure. The charge was 4.4 ml per gram of dry weight which taken from literature. The charge amount was inversely proportional to the amount of product yield and directly proportional to quality or flavor concentration. This was because when the amount of botanical weight increase at constant charge the dry mass was not completely immerse or maceration not completely taken place, this decrease the amount of yield, and the flavor concentration was maximize even the small amount of flavor was obtained.

The amount of gin flavor yield was calculated by mass difference before and after extraction was taken place. The minimum yield was 0.78% which obtained at 0.5kg botanical weight and 35% alcohol concentration. The maximum amount of gin flavor was 2.76% which obtained at 1kg botanical weight and 50% alcohol concentration. It was clearly states that at low alcohol concentration and low botanical weight the amount of flavor yield was low and at high alcohol concentration and botanical weight the amount of yield not high rather at 1kg botanical weight and 50% alcohol concentration was obtained. This was because when the amount of botanical weight increase there was lack of total maceration and when the alcohol concentration increase the polarity decreases and the flavor not completely escape during extraction process.

Design experiment version 6.0.8.1 with full factorial was used analysis checking like ANOVA, adequacy, desirability and etc. the linear fit modal was used to optimizing the experimental variables shows that the optimum yield found to be 2.67% with the desirability criteria of 96.7%

at 1kg botanical weight, 50% alcohol concentration and 4 sensory evaluation. This shows that phenolic and other flavor component compound did not permit extract with alcohol concentration < 50%. The highest amount of flavor component extract 50% of ethanol concentration. When the alcohol concentration increase to above 70% the polarity was decreased and the flavor component not dissolve and extract easily during hydro distillation with solvent extraction process.

In this work the physical properties refractive index, optical rotation, specific gravity, PH value, viscosity, boiling point and toxicity was determined and chemical properties like acid value, percentage of free fatty acid, iodine number and GC-MS value was obtained from Balezaf alcohol and liquor factory laboratory, JIJE lab glass pvt. Ltd, Ethiopian food and drug authority and Ethiopian conformity assessment enterprise. The major component which identified by Gas Chromatography -Mass Spectroscopy (GC-MS) were β-pinene (4.98%), Terpinolene (8.57%), Cyclopropane, trimethyl (2-methyl-1-propnylidene) (8.35%), citronellol (6.34 %), γ-terpinene (1.60%), β-humulene (4.35%), globulol (4.23 %) and citral (2.90%).

Sensory analysis evaluation was performed in Balezaf alcohol and liquor factory bottling section by selecting professionals as a panelist from blending chemist, bottling supervisor, quality control chemist, distillery supervisor, production manager and other group of people in the company who were good in tasting alcohol drink. The nine point hedonic scale (expressive strategy) had been utilized broadly its improvement with a wide assortment of item, significant victory and may caught on effectively. The affective method also used for determined the profile of the product itself. The graded system divided in to four as 4(excellent), 3(very good), 2(good) and 1(poor). From the panelist result at 1.5kg botanical weight and 50% ethanol concentration excellent result was obtained.

5.2 Recommendation

This study showed the possibility of extracting botanical gin flavor using lacily available raw material and can be ceased or reduced the imported flavor. The study can be advanced to the next stage of investigation of botanical gin flavor by varying the *juniperus procera* at different location of Ethiopia since at different location medley content of essence can be obtained due to weather and soil variation that the flavor quality equivalent to the imported one. Botanical gin flavor basically extracted not only from three species rather can be produced up to 20 and more different botanical species even though the main ingredient was juniper berries, to enhance the flavor taste. So the study was indicates to the future researcher to found out the idle resource of aroma containing herb that found locally.

The study shows the future potential of Produce different liquor flavor like; ouzo, lemon, aperitif, etc., and also Produce soft drink, cakes and biscuit flavors. Produce juniper berries essential oil's cosmetics, drugs and perfumes.

Therefore; the concerned government official body should give attention and informed to the society towards juniper berries nutritional, economical and medical value and the flavor extractor work with Ethiopian forestry development to use the seed, speared from berries which was 14.3% of the seed, for planting the procera juniper.

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Appendices

APPENDIX A. moisture content

A.1 Moisture content of fresh procera juniper berry.

	Samples in gram				
Time/hr.	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Initial	11.0808	7.9790	9.5902	8.6906	9.1589
4	9.5641	6.3951	8.0735	7.1739	7.6401
8	6.5412	5.0843	6.8950	5.5840	6.0012
12	5.9781	4.5640	5.3504	5.0051	5.5014
16	5.8981	4.0756	5.2015	4.7024	5.2890
20	5.8713	4.0050	5.2406	4.5151	5.0026
24	5.8712	4.0048	5.2403	4.5150	5.0023
Moisture %	47.01	49.80	45.35	49.61	45.38
Seed content %	13.97	13.92	15.23	14.81	13.45

A.2 Moisture content of dried procera juniper berry.

	Samples in gram				
Time/hr.	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Initial	10.5422	10.6580	10.4504	10.2401	10.4104
1	9.7861	9.9030	9.6853	9.4600	9.6523
2	9.7178	9.8381	9.5901	9.3812	9.5614
3	9.6889	9.7979	9.4814	9.2541	9.4432
4	9.6002	9.7179	9.4145	9.2350	9.4152
5	9.5915	9.7074	9.4043	9.2339	9.4054
6	9.5915	9.7073	9.4040	9.2337	9.4054
Moisture %	9.01	8.92	10.01	9.82	9.65

A.3 Moisture content of fresh ginger

	Samples in gram				
Time/hr.	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Initial	10.2140	10.0841	10.4150	10.2401	10.4910
4	6.5410	6.2548	6.8751	6.5402	6.7421
8	2.9801	2.8040	2.9048	2.8452	2.9410
12	2.4146	2.0017	2.1802	2.0091	1.9907
16	2.4145	2.0017	2.1801	2.0089	1.9906
Moisture %	76.36	80.15	79.06	80.38	81.02

A.4 Moisture content of dried ginger

	Samples in gram				
Time/hr.	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Initial	10.0220	10.4102	10.0012	10.0005	10.0048
1	9.0372	9.3980	8.9967	8.9987	9.0021
2	8.9980	9.3526	8.9466	8.9673	8.9511
3	8.9868	9.3245	8.9342	8.9456	8.9348
4	8.9465	9.2834	8.9145	8.9041	8.9118
5	8.9464	9.2834	8.9143	8.9041	8.9116
Moisture %	10.73	10.82	10.86	10.96	10.92

A.5 Moisture content of dried cassia bark

	Samples in gram				
Time/hr.	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Initial	10.0016	10.0041	10.0002	10.0023	10.0008
1	8.6477	8.5956	8.6514	8.6312	8.6417
2	8.5487	8.4871	8.5493	8.5318	8.5320
3	8.4861	8.3912	8.4863	8.4945	8.4840
4	8.4592	8.3656	8.3478	8.3821	8.3714
5	8.4591	8.3656	8.3477	8.3820	8.3712
Moisture %	15.42	16.37	16.52	16.20	16.29

Appendix- B. Laboratory equipment and samples image.

Appendix-B1; Optical rotation measurement.

Figure B1; Polari meter.

Appendix-B2. ºBrix and refractive index measurement

International scale (1936) of refractive index of sucrose solution at 20^{oc}.

$$n_D^{20}$$

The italicized n denotes refractive index, the superscript indicates the temperature in degrees Celsius, and the subscript denotes the wavelength of light (in this case the D indicates the sodium D line at 589 nm).

%	n_D^{20}	%	n_D^{20}	%	n_D^{20}	%	n_D^{20}	%	n_D^{20}
0	1.33299	20	1.36384	40	1.39986	60	1.44193	80	1.49071
1	1.33442	21	1.36551	41	1.40181	61	1.44420	81	1.49333
2	1.33586	22	1.36720	42	1.40378	62	1.44650	82	1.49597
3	1.33732	23	1.36889	43	1.40576	63	1.44881	83	1.49862
4	1.33879	24	1.37060	44	1.40776	64	1.45113	84	1.50129
5	1.34026	25	1.37233	45	1.40978	65	1.45348	85	1.50398
6	1.34175	26	1.37406	46	1.41181	66	1.45584	86	1.5067
7	1.34325	27	1.37582	47	1.41385	67	1.45822	87	1.5094
8	1.34477	28	1.37758	48	1.41592	68	1.46061	88	1.5122
9	1.34629	29	1.37936	49	1.41799	69	1.46303	89	1.5149
10	1.34782	30	1.38115	50	1.42009	70	1.46546	90	1.5177
11	1.34937	31	1.38296	51	1.42220	71	1.46790	91	1.5205
12	1.35093	32	1.38478	52	1.42432	72	1.47037	92	1.5234
13	1.35250	33	1.38661	53	1.42647	73	1.47285	93	1.5262
14	1.35408	34	1.38846	54	1.42863	74	1.47535	94	1.5291
15	1.35568	35	1.39032	55	1.43080	75	1.47787	95	1.5320
16	1.35729	36	1.39220	56	1.43299	76	1.48040		
17	1.35891	37	1.39409	57	1.43520	77	1.48295		
18	1.36054	38	1.39600	58	1.43743	78	1.48552		
19	1.36218	39	1.39792	59	1.43967	79	1.48811		

Table B2; International scale (1936) of refractive index of sucrose solution at 20^{oc}

Temperature Corrections of RI

Unless the correction factors were specified in the detailed specification, approximate corrections shall be made using the following equation:

$R = R' + K (T' – T)$

Where, R = the reading of the refractometer reduced to the specified temperature, T°C;
R'= the reading at T''°C; K = constant, 0.000365 for fats, and 0.000385 for oils (if Abbe refractometer was used), or = 0.55 for fats and 0.58 for oils (if Butyro refractometer was used);

T’ = the temperature at which the reading R’ was taken; and T = the specified temperature (~40.0°C).

Figure B2; Refracto meter.

Appendix B3; PH measurment

Figure B3; PH meter.

Appendix B4; Sample of liquor and flavor

a)  b) 

Figure B4; a). Liquor sample and b). Flavor sample

Appendix B5; Herb/spice sample

Figure B5 (1). Ginger sample raw and chopped

Figure B5 (2); Procera juniper berries raw and grinded

Figure B5 (3). Cassia bark

Appendix B6; GC-MS Measurement

Figure B6. Gas chromatography

a) 

b) 

Figure B7. a).Hot air oven and b) Mice for toxicity determination

Appendix C; Chromatogram peaks

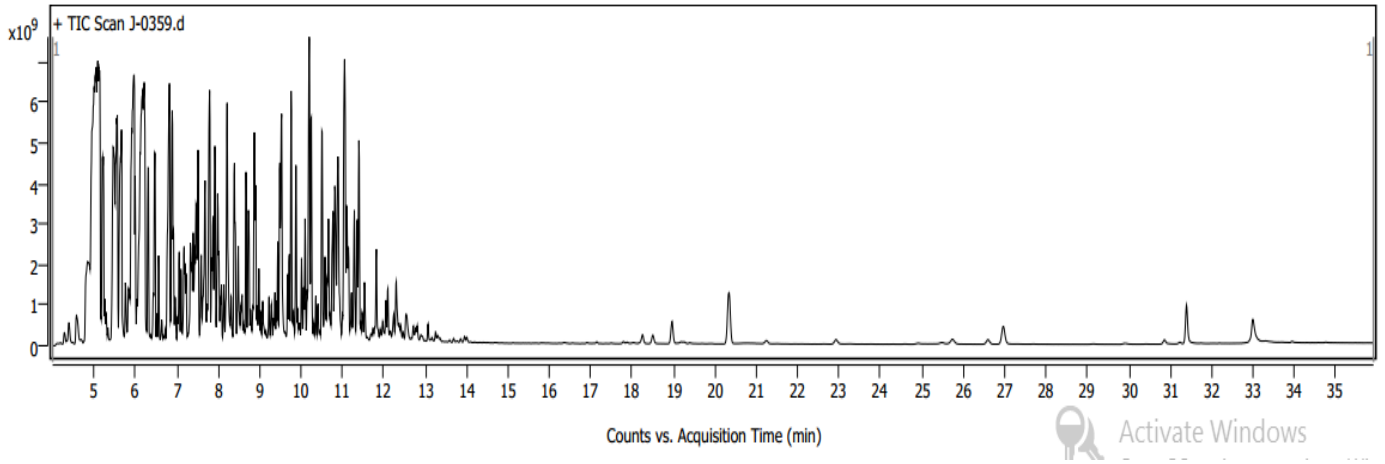
Analysis Report

Agilent | Trusted Answers

Sample Information

Name	J-0359	**Data File Path**	D:\MassHunter\GCMS\1\data\FAME\120_Oil\J-0359.D
Sample ID		**Acq. Time (Local)**	4/29/2023 1:43:31 AM (UTC+03:00)
Instrument	GC-TQ (JIJE LABOGLASS PLC_LABORATORY)	**Method Path (Acq)**	D:\MassHunter\GCMS\1\methods\FAME\FAME_Meat.M
MS Type	QQQ	**Version (Acq SW)**	MassHunter GC/MS Acquisition 10.0.384.1 14-Feb-2019 Copyright © 1989-2018 Agilent Technologies, Inc.
Inj. Vol. (ul)	1	**IRM Status**	
Position	2	**Method Path (DA)**	D:\MassHunter\GCMS\1\data\FAME\120_Oil\J-0359.D\Results\Qual\Version4\Default.m
Plate Pos.		**Target Source Path**	D:\MassHunter\PCDL\default.csv
Operator	DESKTOP-3TUI5GU\Admin	**Result Summary**	119 identified (119 found)

Sample Chromatograms



Peak	Start	RT	End	Height	Area	Area %
1	4.253	4.288	4.327	243643414	579077918	1.13
2	4.330	4.389	4.445	456593448	1216564279	2.37
3	4.547	4.575	4.711	616078133	2258062134	4.40
4	4.769	4.845	4.905	1949721280	12749278375	24.83
5	4.905	5.057	5.065	6752496594	51347692669	100.00
6	5.065	5.090	5.175	6901002299	34678717390	67.54
7	5.175	5.225	5.276	4541222368	14453618559	28.15
8	5.276	5.285	5.318	696702104	1239843781	2.41
9	5.386	5.462	5.513	4779531970	19638316266	35.25

Appendix D; Chromatograph analysis condition

- ✓ Chromatographic system: 8890B GC
- ✓ Inlet: MM
- ✓ Detector: 7000D TQ
- ✓ Column: HP-5 UI, 30mX0.25mmX0.25µm
- ✓ Oven temperature: 60^{o}c hold for 2 minutes, then 15^{o}c/min to 180^{o}c for 0 minutes, by 5^{o}c/minto 190^{o}c hold for 13min, then by 10^{o}c/min to 202^{o}c hold for 3min, 15^{o}c/min to 250^{o}c hold for 3 min.

GC- experimental condition

- ✓ Inlet temperature: 280^{o}c
- ✓ Injection volume: 1µL
- ✓ Split ratio: 20
- ✓ Carrier gas: Helium
- ✓ Column flow: 1ml/min

MS-experimental condition

- ✓ Ionization mode: EI
- ✓ EMV mode: gain factor
- ✓ Gain factor: 1
- ✓ Transfer line temperature: 270^{o}c
- ✓ Ion source temperature: 230^{o}c
- ✓ Quad temp.: 230^{o}c
- ✓ Solvent delay: 3 minutes
- ✓ Acquisition mode: Scan, 50-550amu

Appendix E; Chemical component of gin flavor from GC-MS result.

	Compound name	Formula	Retention time	%Area
1	α-pinene	C10H16	4.29	0.25
2	Styrene	C8H8	4.39	0.52
3	Bornylene	C10H16	4.58	0.96
4	Cyclopropane, trimethyl(2-methyl-1-propnylidene)	C10H16	5.46	8.35
5	Cyclohexane, butnyl-	C10H16	5.56	7.91
6	Octanal	C8H16O	5.76	1.19
7	γ-terpinene	C10H16	5.83	1.60
8	3-carene	C10H16	6.31	3.48
9	β-pinene	C10H16	6.47	4.98
10	4-thujanol	C10H18O	6.56	1.18
11	Terpinolene	C10H16	6.82	8.57
12	Fenchol	C10H18O	7.10	1.06
13	Isoborneol	C10H18O	7.59	1.72
14	Terpinene-4 ol	C10H18O	7.79	7.35
15	2-pinen-4-one	C10H14O	8.14	1.02
16	Citronellol	C10H20O	8.22	6.34
17	Cis-Genariol	C10H18O	8.49	2.08
18	Citral	C10H16O	8.67	2.90
19	Idanone	C9H8O	8.73	3.34
20	Myrtenyl acetate	C12H18O2	9.23	0.75
21	α-Terpinyl acetate	C12H20O2	9.44	1.57
22	Cyclosativene	C15H24	9.76	4.70
23	Longifolene	C15H24	10.20	7.88
24	Caryophyllene	C15H24	10.36	0.71
25	β- humulene	C15H24	10.50	4.35
26	Isoledene	C15H24	10.58	1.77

27	Globulol	C15H26O	11.40	4.23
28	Juniper camphor	C15H26O	13.07	0.30
29	Verticiol	C20H34O	18.25	0.37
30	Manoyl oxide	C20H34O	18.50	0.35
31	Geranyl linalool isomer	C20H34O	18.97	0.85
32	aR-Abietatriene	C20H30	20.34	2.59
33	Kaurenal	C20H30O	25.73	0.32
34	Phenanthrene, 1,2,3,4a,10a-hexahydro-7-methoxy-1,1,4a-trimethyl-8-(1-methylethyl)-	C21H30O	26.60	0.26
35	Trachyloban-18-al, (4 alpha.)-	C20H30O	26.96	1.06
36	Ferruginol	C20H30O	31.39	1.58
37	Tetracosanoic acid, methyl ester	C25H50O2	32.99	1.64

Appendix F; Diagnostics Case Statistics for yield

Standard Order	Actual Value	Predicted Value	Residual	Leverage	Student Residual	Cook's Distance	Outlier t	Run Order
1	0.78	0.81	-0.027	0.333	-0.187	0.002	-0.182	23
2	0.84	0.81	0.033	0.333	0.234	0.003	0.228	11
3	0.80	0.81	-6.667E-003	0.333	-0.047	0.000	-0.046	2
4	0.91	0.89	0.017	0.333	0.117	0.001	0.114	17
5	0.88	0.89	-0.013	0.333	-0.094	0.000	-0.091	1
6	0.89	0.89	-3.333E-003	0.333	-0.023	0.000	-0.023	14
7	1.41	1.28	0.13	0.333	0.890	0.044	0.884	7
8	1.09	1.28	-0.19	0.333	-1.358	0.102	-1.393	8
9	1.35	1.28	0.067	0.333	0.468	0.012	0.458	16
10	1.14	1.34	-0.20	0.333	-1.381	0.106	-1.420	4

11	1.35	1.34	0.013	0.333	0.094	0.000	0.091	5
12	1.52	1.34	0.18	0.333	1.288	0.092	1.313	13
13	1.98	1.67	0.31	0.333	2.178	0.263	2.466	19
14	1.58	1.67	-0.090	0.333	-0.632	0.022	-0.621	22
15	1.45	1.67	-0.22	0.333	-1.545	0.133	-1.613	12
16	2.76	2.67	0.090	0.333	0.632	0.022	0.621	6
17	2.58	2.67	-0.090	0.333	-0.632	0.022	-0.621	26
18	2.67	2.67	0.000	0.333	0.000	0.000	0.000	24
19	2.16	2.23	-0.070	0.333	-0.492	0.013	-0.481	15
20	1.97	2.23	-0.26	0.333	-1.826	0.185	-1.966	21
21	2.56	2.23	0.33	0.333	2.318	0.299	2.690	3
22	1.86	1.88	-0.017	0.333	-0.117	0.001	-0.114	27
23	1.78	1.88	-0.097	0.333	-0.679	0.026	-0.669	20
24	1.99	1.88	0.11	0.333	0.796	0.035	0.788	25
25	1.50	1.57	-0.073	0.333	-0.515	0.015	-0.504	10
26	1.48	1.57	-0.093	0.333	-0.656	0.024	-0.645	9
27	1.74	1.57	0.17	0.333	1.171	0.076	1.184	18

Proceed to Diagnostic Plots (the next icon in progression). Be sure to look at the:

1) Normal probability plot of the studentized residuals to check for normality of residuals.

2) Studentized residuals versus predicted values to check for constant error.

3) Outlier t versus run order to look for outliers, i.e., influential values.

4) Box-Cox plot for power transformations.

If all the model statistics and diagnostic plots were OK, finish up with the Model Graphs icon.

Appendix G; Diagnostics Case Statistics for sensory analysis

Standard Order	Actual Value	Predicted Value	Residual	Leverage	Student Residual	Cook's Distance	Outlier t	Run Order
1	1.00	1.00	0.000	0.333	0.000	0.000	0.000	23
2	1.00	1.00	0.000	0.333	0.000	0.000	0.000	11
3	1.00	1.00	0.000	0.333	0.000	0.000	0.000	2
4	2.00	2.33	-0.33	0.333	-1.500	0.125	-1.558	17
5	3.00	2.33	0.67	0.333	3.000	0.500	4.123 *	1
6	2.00	2.33	-0.33	0.333	-1.500	0.125	-1.558	14
7	2.00	2.33	-0.33	0.333	-1.500	0.125	-1.558	7
8	2.00	2.33	-0.33	0.333	-1.500	0.125	-1.558	8
9	3.00	2.33	0.67	0.333	3.000	0.500	4.123 *	16
10	2.00	2.00	0.000	0.333	0.000	0.000	0.000	4
11	2.00	2.00	0.000	0.333	0.000	0.000	0.000	5
12	2.00	2.00	0.000	0.333	0.000	0.000	0.000	13
13	3.00	3.00	0.000	0.333	0.000	0.000	0.000	19
14	3.00	3.00	0.000	0.333	0.000	0.000	0.000	22
15	3.00	3.00	0.000	0.333	0.000	0.000	0.000	12
16	4.00	4.00	0.000	0.333	0.000	0.000	0.000	6
17	4.00	4.00	0.000	0.333	0.000	0.000	0.000	26
18	4.00	4.00	0.000	0.333	0.000	0.000	0.000	24
19	3.00	3.00	0.000	0.333	0.000	0.000	0.000	15
20	3.00	3.00	0.000	0.333	0.000	0.000	0.000	21
21	3.00	3.00	0.000	0.333	0.000	0.000	0.000	3

22	3.00	3.00	0.000	0.333	0.000	0.000	0.000	27
23	3.00	3.00	0.000	0.333	0.000	0.000	0.000	20
24	3.00	3.00	0.000	0.333	0.000	0.000	0.000	25
25	2.00	2.00	0.000	0.333	0.000	0.000	0.000	10
26	2.00	2.00	0.000	0.333	0.000	0.000	0.000	9
27	2.00	2.00	0.000	0.333	0.000	0.000	0.000	18

* Case(s) with |Outlier T| > 3.50

Proceed to Diagnostic Plots (the next icon in progression). Be sure to look at the:

1) Normal probability plot of the studentized residuals to check for normality of residuals.

2) Studentized residuals versus predicted values to check for constant error.

3) Outlier t versus run order to look for outliers, i.e., influential values.

4) Box-Cox plot for power transformations.If all the model statistics and diagnostic plots were OK, finish up with the Model Graphs icon.

Appendix H; Effect summery of yield and sensory analysis respectively

	Term	DOF	SumSqr	MeanSqr	F Value	Prob>F	% Contribtn
Require	Intercept						
Model	A	2	0.838489	0.419244	13.7909	0.0002	9.00342
Model	B	2	4.84202	2.42101	79.63855	< 0.0001	1.9921
Model	AB	4	3.08529	0.771322	33.1288	< 0.0001	25.3724
Error	Lack Of Fit		0.000				0.000
Error	Pure Error	18	0.5472				5.87566
	Residuals	18	0.5472	0.0304			

Term		DOF	SumSqr	MeanSqr	F Value	Prob>F	%Contribtn
Require	Intercept						
Model	A	2	3.62963	1.81481	24.5	< 0.0001	19.3676
Model	B	2	5.85185	2.92593	39.5	< 0.0001	31.2253
Model	AB	4	7.92593	1.98148	26.75	< 0.0001	42.2925

Error	Lack Of Fit			0.000
Error	Pure Error	18	1.33333	7.11462
	Residuals	18	1.33333	0.074074

Appendix-I; Summary of experimental design

Appendix I1; Build Information

File Version	6.0.8.1		
Study Type	Factorial	**Subtype**	Randomized
Design Type	Full Factorial	**Runs**	27
Design Model	2FI	**Blocks**	No Blocks
Center Points	0		

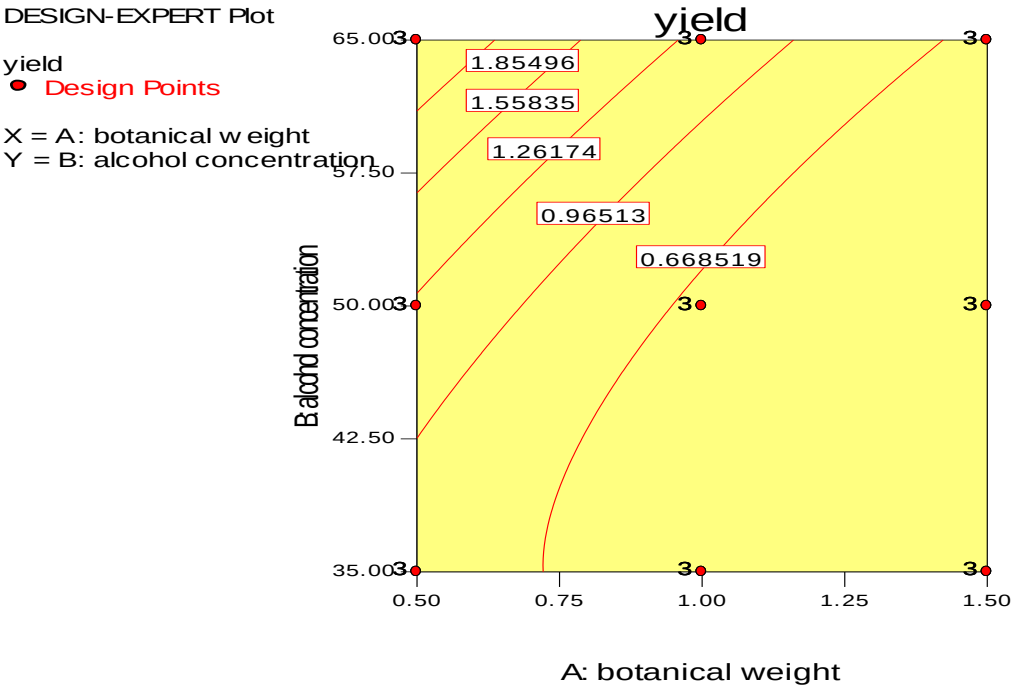
Appendix I2; Factors

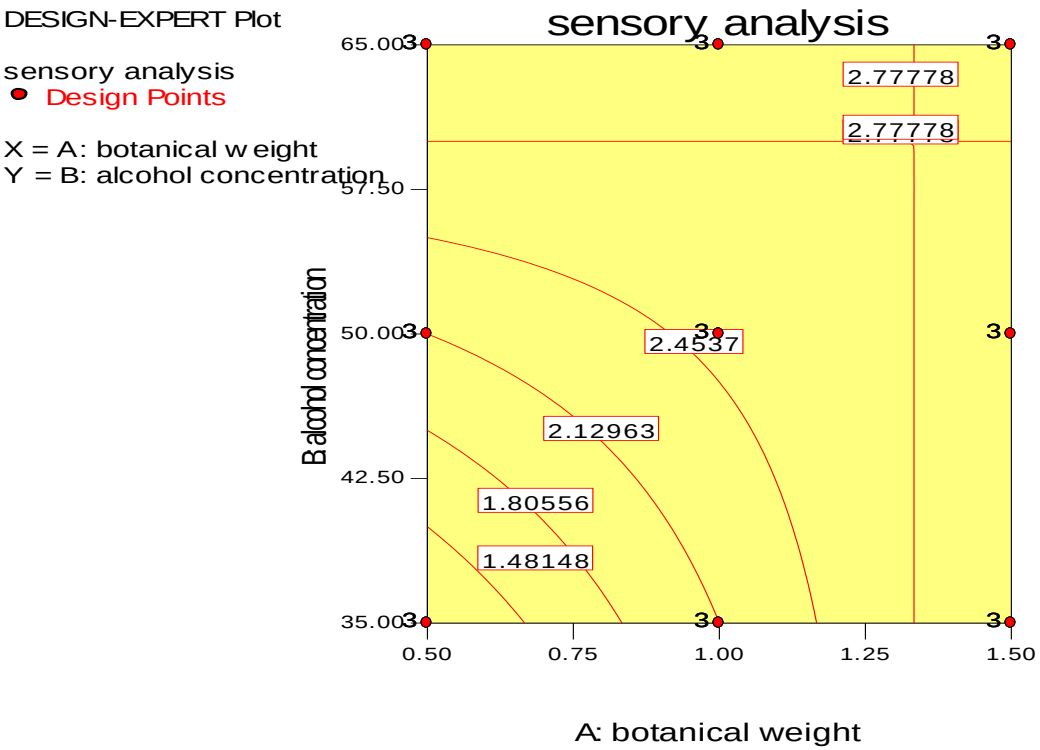
Factor	**Name**	**Units**	**Type**	**Minimum**	**Maximum**	
A	botanical weight	Kg	Categoric	0.5	1.5	**Levels:** 3
B	alcohol concentration	%	Categoric	35	65	**Levels:** 3

Appendix I3; Responses

Response	**Name**	**Units**	**Observations**	**Analysis**	**Minimum**	**Maximum**	**Mean**	**Std. Dev.**	**Ratio**	**Transform**	**Model**
R1	Yield	%	27	Factorial	0.78	2.76	1.59	0.5985	3.54	None	2FI
R2	sensory analysis	grade	27	Factorial	1	4	2.52	0.8490	4.00	None	2FI

Appendix I4; contour and of yield and sensory analysis



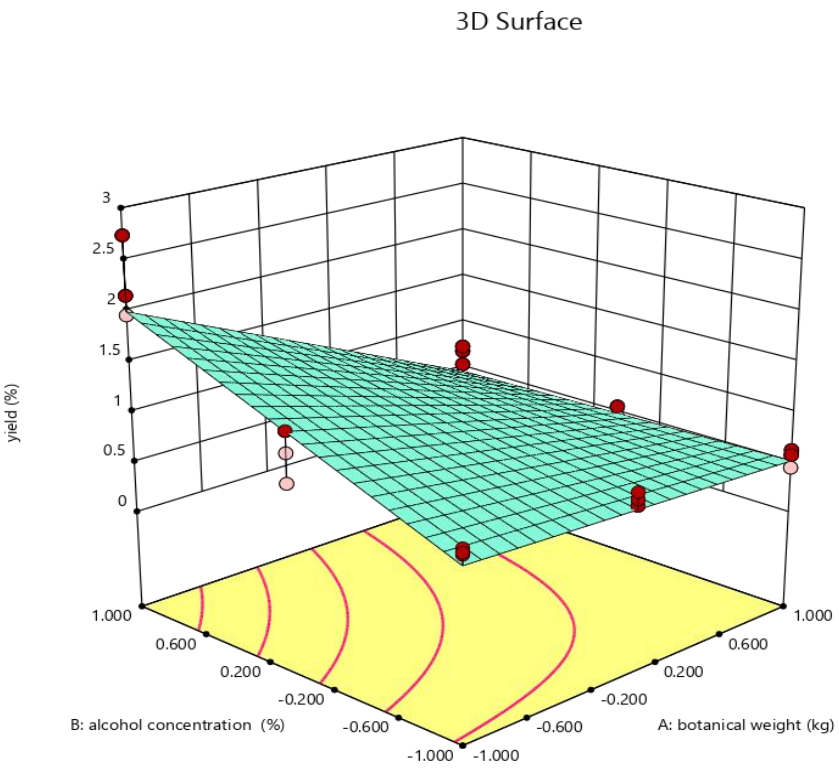


Appendix I5; 3D surface of yield and sensory analysis

Design-Expert® Software
Factor Coding: Coded

yield (%)
Design Points:
Above Surface
Below Surface

X1 = A: botanical weight
X2 = B: alcohol concentration



Factor Coding: Coded

sensory analysis (grade)
Design Points:
Above Surface
Below Surface

X1 = A: botanical weight
X2 = B: alcohol concentration