

ADDIS ABABA UNIVERSITY
ADDIS ABABA INSTITUTE OF TECHNOLOGY
SCHOOL OF CHEMICAL AND BIOENGINEERING



Extraction, Characterization and Optimization of the Sweet Basil
(*Ocimum Basilicum*) Essential Oil

A Thesis submitted to the school of Graduate Studies of Addis Ababa University in partial fulfillment of the Requirements for Degree of Master of Science in Chemical Engineering (process Engineering stream)

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The undersigned have examined the thesis entitled '**extraction, characterization and optimization of essential oil from sweet basil (*Ocimum basilicum*)**' presented by SEBLEWONGEL GASHAW, a candidate for the degree of **Master of Science** and here by certify the valuable of acceptance.

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DECLARATION

I certify that research work titled “extraction, characterization and optimization of sweet basil (*Ocimum basilicum*) essential oil” is my own work. The work has not been done elsewhere for assessment. Where the material that has been used from other sources it has been properly referred.

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ABSTRACT

Essential oils are aromatic substances that found in the immune system of the plant and it is very interesting natural product in their variety application. Ethiopia has also spending a huge foreign currency to import the essential oil for its different application. This study has been carried out to extraction of essential oil from the leaves of *Ocimum basilicum* L. by using steam distillation extraction method. The Sweet basil (*Ocimum basilicum* L.) is a native plant in Ethiopia locally named as “*Bessobela*” (in Amharic). This study is more important in investigation of sweet basil as assume the one of the economy source crop for extraction of essential oil and decreasing a country foreign currency spending to import EO. A general factorial design was applied to in the analysis of results using DESIGN-EXPERT 6.08 software and linear regression model. In this study, the minimum and maximum oil yield are 0.12% and 0.8% respectively. Thus, minimum was obtained at 2hrs in the extraction of fresh sweet basil leaves and maximum oil yield of 0.8 % was obtained at a time of 4hrs in extraction of the sweet basil dried in atmospheric air. Characterization of the oil at the optimum condition yield was also carried out and also the results are shown as 0.8481g/cm³ specific gravity, 1.49 refractive index value, 3.5 pH, 6.02 mpas dynamic viscosity, 205°C boiling point temperature, 78°C flash point, 5.04 mgKOH/g acid value, 56 mgKOH/g saponification value, 3.17 ml/g iodine value and 50.93 mgKOH/g ester value. The essential oil was analyzed by using GC-MS and FTIR. Then, Twenty-five components were obtained and it has been reached in monoterpene hydrocarbons, oxygenated monoterpenes, sesquitrpene and alcohol group. Generally , according to its chemical constituents , the sweet basil essential oil exhibits potent antimicrobial activity due to its richness in linalool (oxygenated monoterpene), and the Methyl chavicol (estragol) constituents of *O. basilicum* oil implies that EO extensively used as a flavoring, antimicrobial, antioxidant and anti-inflammatory agent.

Key words: Essential oil, GC-MS, FTIR, *Ocimum basilicum* L, steam distillation

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TABLE OF CONTENTS

DECLARATION	III
ABSTRACT	IV
ACKNOWLEDGMENTS	V
TABLE OF CONTENTS	VI
LIST OF FIGURES	IX
LIST OF TABLES.....	X
LIST OF SYMBOL AND ACRONYM.....	XI
1. INTRODUCTION.....	1
1.1 Background	1
1.2 Problem Statement	4
1.3 Objective	5
1.3.1 General Objective	5
1.3.2 Specific Objectives	5
1.4 Significance of the Study	5
1.5 Scope of the Study	5
2. LITERATURE REVIEW	6
2.1. Essential Oil	6
2.1.1. Physical and Chemical Properties of Essential Oil.....	6
2.1.2. Chemical Compositions of Essentials Oil	7
2.1.3. Essential Oil Production Globally	9
2.1.4. Some Application of Essential Oil	10
2.1.5. Studies on Essential Oil.....	12
2.2. Reviews on Sweet Basil	14
2.2.1. Types of Basil	16
2.2.2. Sweet Basil in Ethiopia.....	18
2.3. Applications of Sweet Basil Essential Oil	20
2.4. Extractions Technology	22

2.4.1.	Conventional Extraction Methods	22
2.4.2.	Innovational Extractions Methods	25
2.5.	Clarification and Storage of Essential Oils	26
2.6.	Quality Assessment of Essential Oils	27
2.6.1.	NMR Spectroscopy.....	27
2.6.2.	High-Performance Liquid Chromatograph (HPLC).....	27
2.6.3.	Gas Chromatography- Mass Spectroscopy.....	28
2.6.4.	Fourier-Transform Infrared Spectroscopy (FTIR).....	29
2.7.	Factors That Affecting the Extraction of Essential Oil Yield.....	30
2.7.1.	Intrinsic Factor.....	30
2.7.2.	Extrinsic Factor	30
3.	MATERIALS AND METHODS	33
3.1.	Materials and Equipments.....	33
3.2.	Methods.....	33
3.2.1.	Raw Material Collection and Preparation.....	34
3.2.2.	Extraction of Sweet Basil Using Steam Distillation.....	35
3.2.4	Determination of Percentage Yield of Essential Oil Extracted	36
3.2.5.	Physio-Chemical Property Determination.....	36
3.2.6.	Compositional Analysis of Essential Oil.....	40
3.3.	Experimental Design and Frame Work.....	41
3.3.1.	Experimental Design	41
3.3.2.	Experimental Framework	43
4.	RESULT AND DISCUSSION.....	44
4.1.	Proximate Analysis Result of Sweet Basil Leaves	44
4.2.	Physical Properties of Sweet Basil Essential Oil.....	44
4.3.	The Chemical Properties of Sweet Basil Essential Oil	46
4.3.1.	pH Value	46
4.3.2.	Saponification Value	46
4.3.3.	Acid Value	47

4.3.4.	Iodine Value.....	47
4.3.5.	Ester Value.....	47
4.4.	Gas Chromatograph- Mass Spectroscopy	47
4.5.	FTIR Analysis of Essential Oil	50
4.6.	Steam Distillation Extraction of Essential Oil Yield	51
4.7.	Experimental Analysis and Adequacy Check for Full Factorial Model	52
4.7.1.	Effects of the Parameters on Yield of Essential Oil	55
4.7.2.	Interaction Effects of Extraction Time and Drying Condition of Sweet Basil on Essential Oil Yield.....	56
4.8.	Process Variables and Response Factor Optimization.....	57
5.	CONCLUSIONS AND RECOMMENDATIONS.....	59
5.1.	Conclusions.....	59
5.2.	Recommendations	60
	REFERENCES	61
	APPENDICES	66
	Appendix A: Formulas and equations in characterization of essential oil.....	66
	Appendix B: Functional Group Properties with Wavenumber Interval.....	67
	Appendix C: GC-MS result of sweet basil essential oil.....	70
	Appendix D: Laboratory Equipment and sample photo	71

LIST OF FIGURES

Figure 2-1. Global Essential Oils Market	9
Figure 2-2. (a) two species of Ocimum plant and (b) sweet basil seeds	17
Figure 2-3 steam distillation extraction setup.....	23
Figure 3-1 Experimental Frame Work.....	43
Figure 4-1 FTIR Analysis of Sweet Basil Essential Oil	50
Figure 4-2 Predicted Vs Actual Value of Yield.....	55
Figure 4-3 Interaction Effect of Extraction Time and Drying Condition of sweet	56
Figure C-1 Retention versus abundance graph.....	70
Figure D-2 (a) fresh sweet basil (b) essential oil (c) steam distillation with condenser (d) decantation and separation (e) refractometer (f) PH meter (g),(h),(i) &(j) chemicals for characterization.....	72

LIST OF TABLES

Table 2-1. Physical and Chemical Properties of Sweet Basil.....	7
Table 2-2. The Essential Oils of Import and Export Data	10
Table 2-3. Scientific Classification of Basil	15
Table 2-4. Nutritional value of basil per 100 grams	15
Table 2-5 The Average Basil Export Volume and Value.....	19
Table 4-1 Proximate Analysis of Basil Leaves.....	44
Table 4-2 The Major Property of Sweet Basil Essential Oil	46
Table 4-3 Chemical Properties of Sweet Basil Essential Oil	47
Table 4-4 Chemical Composition of Sweet Basil Essential Oil.....	49
Table 4-5 Experimental Sweet Basil Essential Oil Yield.....	51
Table 4-6 Comparison of Studies on Extraction of Sweet Basil EO	52
Table 4-7 Model Summary of Statistics in Essential Oil Yield.....	53
Table 4-8 Analysis of Variance for The Response Yield of Basil Essential Oil	53
Table 4-9 Modified Analysis of Variance for the Response Yield of Basil EO.....	54
Table 4-10 Model Adequacy Measures for Basil Essential Oil Yield.....	54
Table 4-11 Summary on Factors, Response (EO Yield) And Goals of Optimization.....	57
Table 4-12 Solution of an Extracting Condition at Maximum Yield	57
Table 4-13 Result of Optimization and Model Validation	58
Table B-0-1 Functional group properties with wavenumber.....	67

LIST OF SYMBOL AND ACRONYM

g	Gram
ml	Milliliter
hr	Hour
°C	Degree centigrade
µm	Micro Meter
ANOVA	Analysis of Variance
AOAC	Association of official analytical chemists
AOCS	American Oil Chemical Society
AV	Acid Value
EO	Essential oil
FT-IR	Fourier transform infrared
GC-MS	Gas chromatograph- Mass spectroscopy
HARC	Hawasa agricultural research center
HPLC	High-performance liquid chromatography
M	Molarity
N	Normality
QC	Quality control
RT	Retention time
UNCTAD	United nation conference on trade and development
WGARC	Wondo Genet Agricultural Research Center

1. INTRODUCTION

1.1 Background

Essential oils are aromatic substances present in the specialized cells of certain plants used by them to protect themselves from predators and pests, but also to attract pollinators. Essential oils are part of the immune system of the plant. The world famous alchemist, physician, physicist, astrologer, theologian and philosopher of Switzerland, Paracelsus called distilled oils from herbs—quintal essential—the quintessence of the plant, and hence the name of essential oils. It is acquired from the essence rich in natural flavors and active ingredients that it secretes the cells of specific parts of the plant. valuable liquids are obtained by distilling or pressing the secretory organs. The content in essential oils of plants is often below 1 %, rarely reaching 15 % or even more, in the dry product of some plants (Monica Butnariu¹, 2018)

Essential oil is a volatile oils obtained from plants, having the odor and good fragrance which used mainly in the manufacture of perfumes, flavors, and pharmaceuticals. The extracted Essential oils from plants are in a composite mixture of oxygenated derivatives, terpenes and other aromatic compounds.

Nowadays, EOs are charming substantial interest because of their use in the treatment of particular infectious diseases for which artificial antibiotics are becoming less and less active for preserving food against oxidation as alternatives to man-made chemicals (Mohammed Chenni , Douniazad El Abed , Njara Rakotomanomana , Xavier Fernandez , 2016). Interest in the application of essential oils in various fields is increasing continuously, and with their significant pharmacological activities, Various studies have been conducted to examine the antimicrobial activities of essential oils against multiple bacteria. Furthermore, Essential oils are employed by psychiatrists for their psychic patients because of the amiable odor extracted by the aromatic plant's oils. (Perry N. , Perry E., 2006)

The essential oils are applicable in numerous fields such like food, pharmaceutical, and cosmetic industries. They are still being investigated so as to achieve formulate and produce more efficient essential oil-based products. In pharmaceutical industries, medicines in the form of capsules, syrups, ointments, creams and sprays involved some compositions of essential oils and their production quantities are continuously growing.

The aromatic medicinal plants are also being applied in pharmaceutical industries as antibacterial, anti-inflammatory, antiviral, and antifungal agents. (Ramsey Jt, Shropshire Bc, Nagy Tr, Chambers Kd, Li Y, Korach K., 2020)

Plants used to extract essential oil

Essential oils are derived from sections of plants and it have various fragrances.

Flowers: cannabis, chamomile, clary saga, clove, hops, hyssop, jasmine, lavender, Manuka, orange, rose, plumier

Leaves: basil, bay leaf, buchu, cinnamon, common saga, eucalyptus, guava, lemon grass, oregano, peppermint, patchouli, pine, rosemary, spearmint, tea tree, thyme, wintergreen peel, grape fruit, bergamot, lemon, lime, orange, tangerine,

Resin: benzoin, copaiba, frankincense, labdanum, myrrh

Rhizome: galangal, ginger

Seeds: anise, buchu, celery, cumin, flax, nutmeg oil

Woods: agarwood, cedar, rosewood, camphor, sandalwood

Basil (*Ocimum basilicum*) is among the most vital herbs across the world and it has the acronym of 'King of herbs.' The original home of the typical basil is India, where it has grown under cultivation since 3000 B.C. Basils (*Ocimum spp.*, *Lamiaceae*) essential oils are rich in phenolic compounds and a wide array of other natural products including polyphenols like flavonoids and anthocyanin. (Joshi V. , Bothara B. , Surana J., 2011)

The Basil oil has large economic value due to the presence of particular substances like estragol, linalool, eugenol, methyl cinnamate, limonene and geraniol. Furthermore, Sweet basil (*Ocimum basilicum L.*) is one of the most frequently used culinary herbs (family of *Lamiaceae*), known to have strong antioxidant and antimicrobial activities because of its phenolic acids and aromatic compounds. so, that the demands for essential oils as food preservatives is growing because of their biological action against food-borne pathogens like salmonella typhimurium, clostridium per fringes, listeria mono-cytogenesis, Pseudomonas putida, and Staphylococcus aureus. The pathogens in food and meat products has been addressed by low dosage application of essential oils, as indicated by numerous researches. commonly, the basil leaves are employed in folk medicine as a treatment for a lot of diseases, including cancer, convulsion, diarrhea, epilepsy, gout, nausea, sore throat, toothaches, and bronchitis. (Duke, James A., 2002)

EXTRACTION, CHARACTERIZATION AND OPTIMIZATION OF ESSENTIAL OIL FROM SWEET BASIL

In Ethiopia, this subspecies named as Sacred basil and the local name 'bessobela'. It has grown in the wild for long generations, with locals harvesting it by hand before sun-drying it which used for spicing meals like pasta and leguminous dishes. The tender stems, leaves, and flowers are dried, ground, and added to sauces either alone or mixed within other spices to allow a fine flavor to stews. Traditionally, it is a vital ingredient in berbere and shiro powders and the preparation of spiced butter. The dried leaves can be applied for preparing roast beef locally known as "tibs" and both dried and fresh inflorescences and leaves are used as flavoring agents in the preparation of all forms of "wote". According to a report (yimer, 2010), Ethiopia has exported 68,786 kg of basil leaves to Sudan, Israel and the USA, in which a total foreign currency of \$54,991, \$4,425 and \$746 respectively.

1.2 Problem Statement

Essential oils are so named as to indicated the very essence of odor and flavor that extracted from plant. A wide range of Essential oil plant species are used for their aromatic value as flavorings in foods and beverages and as fragrances in pharmaceutical and industrial products. Nowadays, in the world Growing inclination of consumers towards natural and organic products for personal care and medical treatments. The increasing reliability over natural & organic ingredients in many process industry is also leading to increased usage of essential oils in cosmetics, pharmaceutical, food and beverages purposes. Ethiopia has also import the essential oil from foreign countries and it was spending 62,101; 50,397; 50,135 thousand of USD at 2016, 2017, 2018 respectively (Addis Ababa development study association ,2018). It indicating that Ethiopia is spending huge foreign currency for importation of the essential oils for their industries.

Several studies in the literature have seen that world science approves that natural essential oil is extracted from the different aromatic herbs. From those herbs, the sweet basil is one of the source of essential oil extraction and traditionally applicable for flavoring purpose. Unlike its flavoring purpose, Basil (*Ocimum basilicum*) is also applicable for production of cosmetics and perfumes, as well as for its antioxidant, antimicrobial effect etc. (Duke, James A., 2002) Ethiopia is one of the center of primary diversity for sweet basil, diverse agro-ecological conditions and market availability, sweet basil need to be considered as one of the economy source crop in Ethiopia.

According to a report (yimer, 2010) Ethiopia has exported 68,786 kg of basil leaves to Sudan and the USA, from which a total foreign currency of \$54,991.20 and \$746.00 respectively. but, it can extract more concentrated essential oil from *Ocimum basilicum*. Despite its vast potential of this plant as a sources of essential oils, our country is not utilizing this resource. This may be due to lack of adequate research and knowledge about its potential application in industrial scale. Thus, this research is intended to contribute in reducing the huge amount of money allocated for importation of essential oil and also expected that it more profitable of exporting the extracted sweet basil essential oil rather than exporting the sweet basil herbs for foreign countries.

1.3 Objective

1.3.1 General Objective

The general objective of this study was the extraction of essential oil from sweet basil (*Ocimum basilicum*) leaves using steam distillation method.

1.3.2 Specific Objectives

The specific objectives of this study were:

- Proximate analysis of sweet basil (*Ocimum basilicum*) leaves.
- Analysis the effect of the extraction time and the drying conditions of the fresh sweet basil leaves in the steam distillation extraction.
- Characterization, component analysis of EO using Gas chromatography mass spectrometry (GC-MS) and structural determination using Fourier transform infrared spectroscopy (FTIR) of sweet basil essential oil.

1.4 Significance of the Study

The application of this study can be applied for both generating knowledge and solving the current problems which is currently prevailing on the utilization of essential oils and reducing foreign currency for importation of essential oil in our country. It is paramount important in investigating oils and essential oils present in the *Ocimum basilicum* and brings a great opportunity for strategic solution of changing agriculturally produced plants for industrial application. Organizations like that of cosmetics and perfume industry, pharmaceutical industry, medicine and other food processing sector will be benefited a lot from this study since the expected result is cross linked with these organizations and they improved expenditure of the foreign currency from the importing the essential oil for their product.

1.5 Scope of the Study

The scope of the study includes to analysis the sweet basil leaves used for extraction of essential oil with steam distillation methods, perform the optimum conditions of the extractions of leaves for good yields and characterize the basil essential oil. Also perform quality assessment of those products such like composition and structural determination.

2. LITERATURE REVIEW

2.1. Essential Oil

An essential oil is described as a concentrated hydrophobic liquid containing chemical constituent from plants. Essential oils are volatile oils or just the oil of the plant because of it contains the "essence of" the plant's fragrance. volatile oil is not actually an oil because it doesn't contain fatty substance. It is essence rich in natural flavors and active ingredients that it secretes the cells of certain parts of plants (Monica Butnariu¹, 2018).

An herbal extraction from a botanical material is introduced with a solvent within which number of the raw material components dissolve. Ultimately, the solvent becomes infused with the botanical materials that its pulled from the source plant, and this is often called as the "extract." The tip product of the extraction is liquid, or the liquid is removed to show the remnants of the botanical into a solid. The solvents can used as preservatives or as agents that help plant cells to interrupt down and release their contents. (Atish A. Yadav, Sushmita S. Chikate, Rebika B. Vilat, Mahesh A. Suryawanshi, Vijay B. Mane, 2017)

2.1.1. Physical and Chemical Properties of Essential Oil

The physio-chemical properties of the essential oils are listed as follows and also specifically for the sweet basil essential oil physiochemical properties has shown in Table 2-1.

- Essential oils are soluble in alcohol, ether, and glued oils, but insoluble in water.
- The volatile oils are generally liquid and colorless at room temperature.
- They have a characteristic odor.
- They are usually liquid at room temperature.
- They have a density but unity, with the exception of some cases (cinnamon, saffras, and vetiver).
- They have a refractive index and a very high optical activity.

Table 2-1. Physical and Chemical Properties of Sweet Basil

Botanical name	<i>Ocimum basilicum</i>
Source	Leaves, cultivated
Methods of distillation	Steam distillation
Odor	Sweet basil and typical aromatic odor
Appearance	Pale yellow liquid
Solubility	Soluble in all Proportion of 90% alcohol and insoluble in water
Boiling point	215±2°c
Specific gravity	0.876 to 0.920 at 20°c
Refractive index	1.469 to 1.495 at 20°c
Optical rotation	-2 to -15at 20°c
Main constituents	Linalool: 40 to 60%, Eugenol: 8 to 18%, 1-8 Cineole: 7 to 15%, Camphor: 0.1 to 1.5%, Trans- beta-Ocimene: 0 to 2%, Terpinen-4-ol: 0 to 3%, Methyl Chavicol: 0 to 1%

Source: International journal of pharmaceutical science and research

2.1.2. Chemical Compositions of Essentials Oil

Essential oils are complex mixtures (5000–7000 chemical constituents) during which mono- and sesquiterpene constituents predominate, but also contain aromatic compounds, often phenyl-propane derivatives, and infrequently meet diterpenes. The most constituents are Linalool, 1,8, cineol, eugenol, methyl cinnamate, camphor, methyl eugenol, methyl chavicol, β -elemene, β ocimene, camphene, carvacrol, α -bergamotene, α -cadinol and geranial. (Debjoy Bhattacharjya, Sinchan Adhikari, Arijit Biswas, Anil Bhumali, Parthadeb Ghosh and Soumen Saha, 2019)

The constituents of essential oils may be classified as volatile and non-volatile fractions. The general chemical components of the aromatic oil volatile fraction include mono- and sesqui-terpene components, and other oxygenated derivatives together with alcohols, aliphatic aldehydes, and esters. The remaining 1-10% from the isolated essential oil are non-volatile residues like carotenoids, fatty acids, flavonoids and waxes.

➤ **Hydrocarbons**

Hydrocarbon is a chemical compound which found in essential oils with their building blocks connected by hydrogen and carbon bonding. An example of basic hydrocarbons found in essential oils is isoprene.

▪ Terpenes

Terpenes' group are classified as Mono-, sesqui-, and diterpenes. Monoterpene is that the result of combining two isoprene units from head to tail, whereas sesqui-terpenes are formed if three isoprene join together, while diterpenes structured from four linked isoprene units. The most properties of monoterpenes are anti-bacterial, analgesic, stimulant, and expectorant.

▪ Sesqui-terpenes

Sesqui-terpenes are biogenetically isolated from farnesyl pyrophosphate, an intermediate in both the mevalonate and non-mevalonate pathways employed by organisms within the biosynthesis of terpenoids, sterols, and terpenes.

▪ Diterpenes

These constituents are combining four units of isoprene. They are considered as too heavy components which cannot be easily evaporate during the extraction process using steam distillation; hence, it is not possible to be found in isolated aromatic oils. Diterpenes are found in plants with C₂₀ chemical structures.

➤ **Alcohols**

Alcohols in essential oils provide some excellent properties like anti-septic, anti-viral, anti-bacteria, and germicidal. Alcohols naturally occur as one component or combined with a terpene or ester. The attachment of terpenes with oxygen and hydrogen atom may result within the formation of alcohols.

➤ **Esters**

The formation of esters between alcohols and acids. The ester component inside essential oils offers soothing and balancing effects. Because of the presence of alcoholic groups inside esters, they are able to provide anti-inflammatory activities. In bioscience, esters are considered to own antifungal and sedative properties, within by balancing action on the nervous system. They are generally free from precautions with the exception of methyl

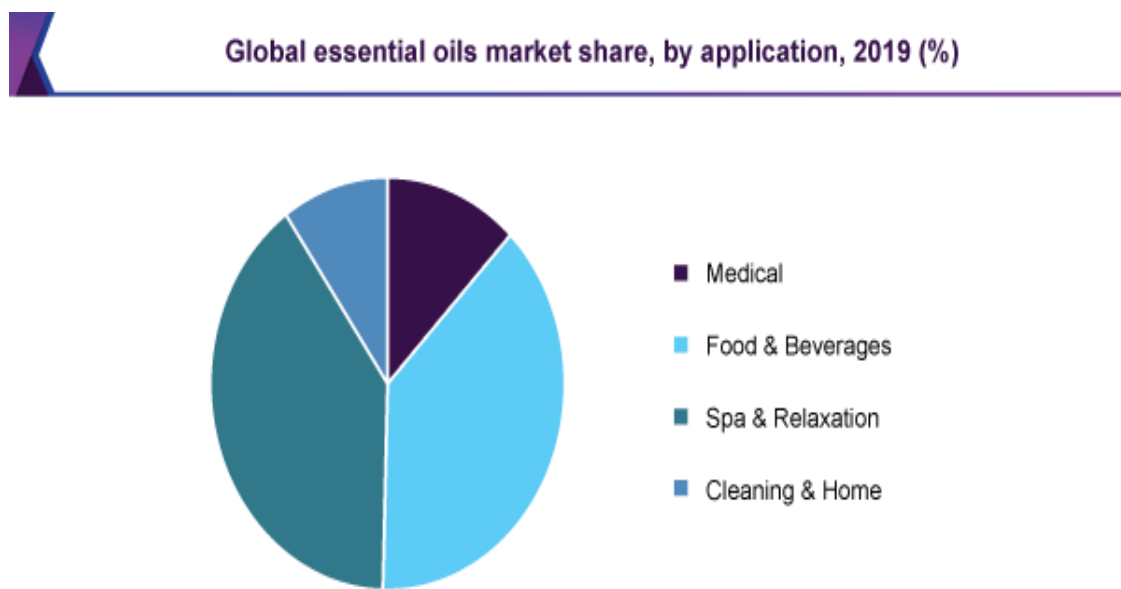
salicylate found in birch and wintergreen which is toxic within the system. The most common esters found in essential oils are linalyl-acetate and geranyl formate which are present in bergamot and lavender, and geranium essential oils.

➤ **Ketones**

Ketones have properties of anti-catarhal, cell proliferate, expectorant, and vulnerary. It is often found in plants and used for upper respiratory tract complaints. Essential oils with ketone group are provided for wound healing and improve scar tissue.

2.1.3. Essential Oil Production Globally

The practical use of Essential Oils that the 'fragrant pharmacy' contains compounds with an especially broad range of biochemical effects. Essential oils have a great benefit to our bodies and prevent homes from pathogens. The human Immune system needs support to kept personal health so, the essential oils can give the desired endorsement. (Agnes Peterfalvi, Eva Miko, Tamas Nagy, Barbara Reger, Diana Simon, Attila Miseta, Boldizsár Czéh, and Laszlo Szereday, 2019).



Source: www.grandviewresearch.com

Source: Essential oils market size, share, analysis report 2020-2027

Figure 2-1. Global Essential Oils Market

There are 80 varieties of essential oils within the international market, Patchouli Oil, Vertiver Oil, Cintronella Oil, and Cloves Oil are some examples. In line with report presented at figure2-1, Food and beverage emerged together as the major application

EXTRACTION, CHARACTERIZATION AND OPTIMIZATION OF ESSENTIAL OIL FROM SWEET BASIL

segments in 2019 with a volume share of 38.6% and anticipated to expand at the fastest CAGR from 2020 to 2027. Spa and relaxation held the biggest volume share in 2019 and it can have projected to witness significant growth over the forecast period. The expansion of this segment has been primarily attributed to the changing lifestyle of consumers a round the world. Busy and hectic lifestyle has created needs of stress-relieving therapies, which is driving the demand for aromatherapy. Robust growth of end-use industries like food & beverage, personal care & cosmetics, and aromatherapy has translated into an increasing the demand for the product. (Essential Oils Market Size, Share, Analysis Report, 2020).

Table 2-2. The Essential Oils of Import and Export Data

Ethiopia’s Agriculture Imports (CIF Value - thousands of USD)								
Commodity	2011	2012	2013	2014	2015	2016	2017	2018
Essential Oils, perfume & flavor mat’ls	35,780	35,689	46,481	37,670	48,090	62,101	50.397	50,135
Ethiopia’s export data (CIF Value – thousands of USD)								
Commodity	2011	2012	2013	2014	2015	2016	2017	2018
Essential Oils, perfume & flavor mat’ls	174	134	55	103	448	196	303	310

Source: UNCTAD Stats, 2011-2018

2.1.4. Some Application of Essential Oil

Essential oil has many varieties which extracted from different source of herbs, thus essential oils have huge applications. some applications are:

➤ **Aromatherapy:**

Aromatherapy is another medicine that uses volatile matter called essential oils and other aromatic compounds for the aim of altering some body mood or health. when volatile oil is inhaled it and goes directly from olfactory system to limbic system of the brain. Brain responds to the actual scent affecting our emotions and analytical balance. Essential Oils

also absorbed by the skin and carried throughout the body via the circulatory system to succeed in all internal organs (Kumar, K. Satish, 2010) .

➤ Antimicrobial Activity

The antimicrobial effect of volatile oil depends on concentration, content and interactions between the most active compounds. It employed in food preparation, forming the sensory profile, and increasing the period of time of the resulting foods. Effective preservatives are characterized by a broad spectrum of antimicrobial activity at a minimum concentration. Formulations containing both varieties of preservatives like essential oil and an artificial one are tested and proposed as a compromise that enables for reducing concentration of both components because of their synergistic activity. Although ,some of EO may cause risk of contact allergy or phototoxic reaction. (Joshi, 2014)

➤ Antibacterial Activity

An important feature of essential oils is their hydrophobicity, which enables them to partition into lipids of the plasma membrane of bacteria, disrupting the structure, and making it more permeable. This will then cause leakage of ions and other cellular molecules. Although a particular amount of leakage of bacterial cells is tolerated without loss of viability, greater lose cell contents or critical output of molecules and ions can result in cell death. (Filomena Nazzaro, Florinda Fratianni, Laura De Martino, Raffaele Coppola, and Vincenzo De Feo, 2013). The EOs containing mainly aldehydes or phenols, like cinnam aldehyde, citral, carvacrol, eugenol, or thymol were characterized by the best antibacterial activity, followed by EOs containing terpene alcohols. Some EOs, containing ketones or esters, like β -myrcene, α -thujone, or geranyl acetate, had weaker activity, while volatile oils containing terpene hydrocarbons were usually inactive. Generally, essential oils are characterized by a high level of phenolic compounds, like carvacrol, eugenol, and thymol and it have important antibacterial activities. (Wissal Dhifi, Sana Bellili, Sabrine Jazi, Nada Bahloul and Wissem Mnif, 2016)

➤ Anti-Inflammatory Activity

Inflammation defined as a standard protective response induced by tissue injury or infection and functions to combat invaders within the body (microorganisms and non-self-cells) and to get rid of dead or damaged host cells. Recently, essential oils are employed

in clinical settings to treat inflammatory diseases, like rheumatism, allergies, or arthritis (Naho Maruyama 1, 2005).

➤ Repellent and Insecticidal Activity

Essential oils constitute a fashionable in structurally-diverse compounds with a range of insecticidal and repellent mechanisms. The toxic effect of essential oils is not suitable for granary insects and for flying insects. The fumigant toxicity of essential oils and their main components, the volatile monoterpenes (Catherine, Roger, 1997).

2.1.5. Studies on Essential Oil

(Danso-Boateng, 2013) studied on the effect of drying methods on nutrient quality of Basil leaves that cultivated in Ghana, Basil leaves were dried using five different drying methods: drying using microwave at power 3, drying with oven at 110°C, hot-air-drying at 100°C, drying by sun at 33°C and drying at ambient air 28°C. Moisture content of the fresh and dried leaves were determined using laboratory oven kept at 105±3°C for twenty-four hrs. Extractions from fresh and dried leaves samples were analyzed for protein, iron (using UV spectrophotometer) and carbohydrates (using handheld refractometer). Finally, they conclude that Microwave-drying was the optimum method for basil leave drying due to it taken shorter treatment time of 4 min and gave the simplest retention of protein and carbohydrates when put next to oven-drying which required 17 min, preserving 42% (wt) of iron.

(Tambun R., Purba H., Ginting k., 2015) studied on the extraction of basil leaves oleoresin with ethyl acetate solvent by using soxhlation method with both effects of extraction time and ratio from the material with a solvent. The analysis consists of oleoresin yield, density, index of refraction and volatile oil content. They got 20.152% yield of basil oleoresin, 0.9688 g/cm³ of density, 1.502 of refractive index, 15.77% of volatile oil content, and dark-green oleoresin product at ratio of material and solvent 1:6 (w / v) for six hrs extraction time.

(Aswati Mindaryani and Suprihastuti Sri Rahayu, 2017) studied on the Basil essential oil extraction using hexane and ethanol solvent and compared to extraction using steam distillation then their resulted showed that n- Hexane was a for better solvent for extraction of sweet basil than Ethanol at the higher the extraction temperature, the perfect the extraction process, so that the desired solvent was less. Also, the GC-MS analysis showed

that leaves and mixture of leaves and stems gave the similar compounds within the volatile oil produced, but the mixture of leaves and stems gave a far better product in terms of appearance although leaves gave higher yield.

In the study of Chemical composition and antimicrobial activities of leaves of sweet basil (*Ocimum basilicum* L.) herb within the eastern a part of Ethiopia (H. Gebrehiwot, R. K. Bachetti, A. Dekebo, 2015). A little amount of powder leaves of the plant were entered to hydro distillation to extract the volatile oil and also the components of the oil were evaluated by gas chromatography-mass spectroscopy instrument. The extracted volatile oil was tested in vitro for its antibacterial activity against two bacteria; *Escherichia coli* and *Staphylococcus aureus* and antifungal activity against two fungi; *Aspergillus niger* and *Rhizoctonia bataticola* by paper disc diffusion method. Then their result showed that the hydro distilled oil was effective against all the tested bacterial and fungal strains. However, as their results shows that the crude extract was found to not have antimicrobial activity toward the tested bacteria and fungi which study justified the uses of the sweet basil plants in traditional medicine practice as a therapeutic agent and it emphasize must make on the antimicrobial activities of the plant during the time of drug extraction.

The study that was undertaken at the research Laboratory of Wondo Genet Agricultural Research Center(WGARC) (2018), Leaves, umbrellas, and stems were separated manually to organize samples for oil distillation. The composite of leaves and umbrellas (herbage yield) was mixed evenly and weighed employing using a sensitive balance and A 300-g composite sample was packed into a polythene container and stored in an ice box to conserve the moisture within the fresh herbage. The volatile oil was extracted by a hydro-distillation method by employing using a Clevenger-type apparatus. When the water was boiling, steam was injected into a part of the apparatus containing the plant sample. The vapor mixture of water and oil was condensed with cold water. The distillate was then directed into a separator where the oil separated from the distillate water.

2.2. Reviews on Sweet Basil

Basil belongs to *Ocimum* and may be a member of the mint family (*Lamiaceae*). The genus *Ocimum* contains between 50 and 150 species of herbs and shrubs that were found within the tropical regions of Asia, Africa, and Central and South America. Basil is home ling to areas in Asia and Africa and grows wild as a perennial on some pacific islands. Basil was brought from India to Europe through the center East within the 16th century and subsequently to America within the 17th century. (Javanmardi et al. 2002)

Physically, basils are characterized by square, branching stems, opposite leaves, brown or black seeds (also called nettles) and flower spikes, but flower color and therefor the size, shape, and texture of the leaves vary by species. Leaf color can also vary, from green to blue/purple and plants can grow to from 1 to 2 feet in height and it is varies depending on the species. (The Herb Society of America, 2003). The sweet basil types are including Lemon Basil, Italian or Curly Basil, and Lettuce-leaf Basil; the names of these cultivars give way to their variances.

Several religions and spiritual beliefs practice rituals are importance of the use of Basil. In Judaism, traditional stories agree in which the use of Basil for increased strength during times of fasting. Also, in various Orthodox churches, Basil is usually wont to sprinkle and for prepare holy water. As well, pots of the herb are often positioned below church altars to pay reverence to the idea that it had been found growing round the grave of Christ. (Basil Sweet Oil - Benefits & Uses of Energizing & Stimulating Oil, 2018)

According to various other belief systems, such as those of Europe, India, the ancient civilizations of Egypt and Greece, Basil was buried with those who die, because it was believed that own protective qualities that would help keep them secure Afterlife and believed that is a guarantee their safe arrival at the gates of Heaven. (Basil Sweet Oil - Benefits & Uses of Energizing & Stimulating Oil, 2018).

Throughout history, the Basil herb is believed to own fortifying effect on a person's mind and emotions. The basil has been used for multiple applications within multiple forms, including teas, dried powders, and oils. In Asia, such as in India, where it absolutely was referred to as "Tulsi" and was thought of as sacred. And it is a typical component in the traditional medical practices like for anti-inflammatory, antioxidant, antibiotic, antiviral and anti-depressant.

EXTRACTION, CHARACTERIZATION AND OPTIMIZATION OF ESSENTIAL OIL FROM SWEET BASIL

Basil and its derivatives ingredients were used popular in beverages and Italian foods, like pizza, pasta, and salads. They still be a popular ingredients in pickled or fermented foods, sauces, pastes, condiments, and meats. (Olsen, 2019)

Table 2-3. Scientific Classification of Basil

Scientific Classification	
Kingdom	Plantae
Phylum	Magnoliophyte
Class	Magnolijopsida
Order	Lamiales
Family	Lamiaceae
Genus	Ocimum
Species	<i>O. basilicum</i>

Source: Wikipedia (Ocimum basilicum)

Basil are become as worth of the daily nutritional value with a magnesium content of 16 percent and 17 percent of iron. 17 percent of Calcium are needs in daily nutrients while vitamin C are used for reinforcing immunity and stands at 30 percent of the daily requirements. Each serving of the herb have not saturated fat and cholesterol, which helps to the heart healthy. With a dietary fiber content of 1.6 grams, basil provides 6% of the daily required for this fiber that assists in digestion (Basil Sweet Oil - Benefits & Uses of Energizing & Stimulating Oil, 2018).

Table 2-4. Nutritional value of basil per 100 grams

Energy-94kilojoules (22 kilocalorie)	Carbohydrates - 2.65 g
Dietary fiber - 1.6 g	Fat - 0.64 g
Protein - 3.15 g	Water - 92.06 g
Vitamin A - 264 mg	Thiamine - 0.034 mg
Riboflavin - 0.076 mg	Niacin - 0.902 mg
Vitamin B6 - 0.155 Mg	Folate - 68 Mg
Choline - 11.4 mg	Vitamin C - 18.0 mg
Vitamin E - 0.80 mg	Vitamin K - 414.8 Mg
Calcium - 177 mg	Iron - 3.17 mg
Magnesium - 64 mg	Manganese - 1.148 mg

Source: USDA Nutrient Database

Sweet basil seeds contain a reasonable amount of hemicellulose and cellulose, accounting for their hydrophilic character. They are high in fiber and associated nutritional properties, and may be considered as a brand new non-conventional source of fiber which helps prevent rapid blood sugar elevations after meals (Javad Khazaei 2006).

Among the species of the genus *Ocimum*, *Ocimum basilicum L.* is that the one of volatile oil crop and cultured commercially in the round world. Aburigal et al. (2016) Reported that content of essential oils was higher in basil leaves than in inflorescences. May et al. (2008) Also reported that basil essential oil is principally within the leaves aside from plant branch, it is insignificant in amount of essential oil.

Sweet basils tend to possess a higher percentage of methyl chavicol while spicy of the basils get from their large amounts of eugenol (herb society of America guide, 2003). Sweet basil has low in calories and almost no fat, but it is a good source of vitamin A. The basil herb contains up to 1.5% of essential oil, within the composition of which the foremost precious compounds are linalool and eugenol. Besides it have saponines, tannins, flavonoids and glycosides, enzymes and organic acids. (Dzida, 2010)

2.2.1. Types of Basil

Basil has power to synthesize and convert phenyl propene in determining the flavor of herbs which may also act as cultivator attractants or herbivore deterrents. The flavor and smell of basil varieties is basically determined by their chemical components, basil varieties contain the subsequent oils in varying quantities: cinnamate, citronellal, geraniol, linalool, methyl chavicol, myrcene, pinene, ocimene, terpineol. (Sullivan, Herbs Basil (*Ocimum basilicum*), 2009)

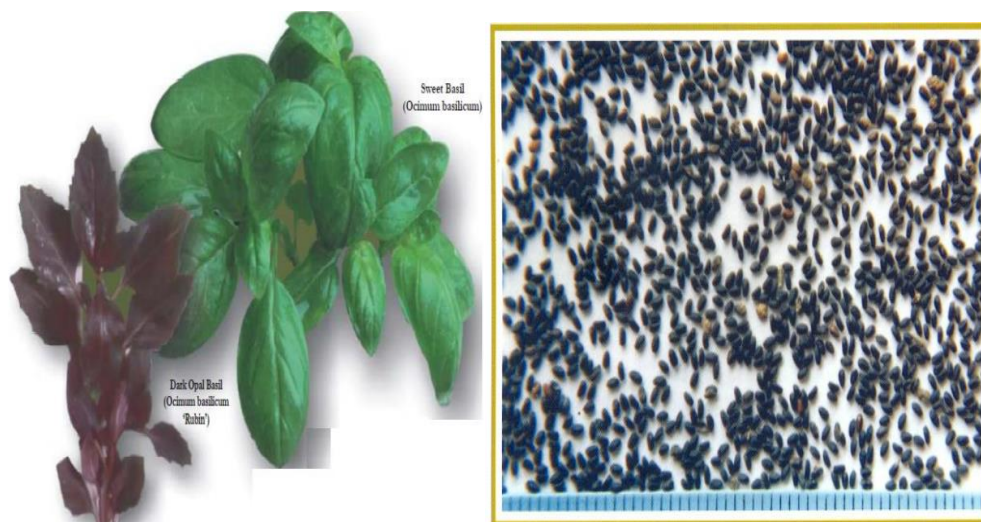


Figure 2-2. (a) two species of *Ocimum* plant and (b) sweet basil seeds

An herb like basil, at just once described as a ‘protection against evil’, or ‘good for the heart’ whose scent ‘taketh away sorrowfulness’. Most basil contains methyl chavicol, eugenol and linalool. The number of every of those chemical constituents varies looking on the species or variety. Methyl chavicol provides a sweet flavor that has been compared to anise and French tarragon, linalool produces a floral scent, and eugenol is paying homage to cloves. (Vieira, 2006). over 60 kinds of basil, all of which differ somewhat in appearance and taste. A number of are described below:

➤ Genovese Basil

It is difference from sweet basil, because Genovese is flatter and pointier than traditional sweet basil. It also includes a more aromatic flavor.

➤ Napolitano Basil

This is another kind of the sweet basil. It still seems as the traditional basil flavor, though it is a little better spicier than the other kinds of sweet basil.

➤ Italian Large Leaf Basil

A type of basil that sweeter smell and taste than Genovese basil.

➤ Lettuce Leaf Basil

It is one of the largest kind of sweet basil and since of this, it makes it the foremost productive.

➤ Dark Opal Basil

This basil has a dark coloring. Because this kind of basil could be a great one to use in an exceedingly decoration to feature color.

➤ Purple ruffle basil

This form of basil is extremely like dark opal basil. The most difference that it is refilled, but it carries the similar colors. So this type is another good one to feature a decoration to use in dish as garnish.

➤ Lemon Basil

This is one that more common basil. It is like traditional basil and due to its lemon flavor, it is great incorporates in both salads and fish recipes.

➤ Lime Basil

Lime basil is comparable to lemon basil because it seems like a traditional type of basil. Yet, it too packs a distinct flavor.

➤ Christmas Basil

This basil is pleasant and it commonly connect us with Christmas as fond memories at good times. it is super excited when it tastes the fruity flavor of this basil variety.

➤ Holy Basil

This Basil actually incorporate unique history behind its name and deeply respected within the Hindu religion. This is the reasons of its name “Holy Basil”, also it stated as Sacred Basil. it can be placed inside a tea and drank regularly to strength your immune system.

2.2.2. Sweet Basil in Ethiopia

Ethiopia is one among of the middle of primary diversity for sweet basil, diverse agro-ecological conditions and market availability, sweet basil have to considered collectively the economy source crop in Ethiopia. In Ethiopia, basil is locally called “Bessobela” in Amharic, “sikakime” or “duguno” in Afan-oromo, “seseg” in Tigrigna, “Gimenja” in Hadiya, “qantalama” in sidamigna, “Kepowa” in Wolayita and different Ethiopian ethnosc group has different name for sweet basil (H. Gebrehiwot, R. K. Bachetti, A. Dekebo, 2015)

In Ethiopia, the tender stems, leaves, and flowers are dried, ground, and added to sauces either alone or mixed with other spices to supply a fine flavor to stews. It is a vital ingredient in berbere and shiro powders and for the preparation of clarified (spiced) butter. The dried leaves are often used for preparing beef roast locally called “tibs” and both dried and fresh inflorescences and leaves are used as flavoring agents within the preparation of all types of “wote”.

EXTRACTION, CHARACTERIZATION AND OPTIMIZATION OF ESSENTIAL OIL FROM SWEET BASIL

According to the Wondo Genet Agricultural Research center 2017, Morphological quality traits resolve for Ethiopian sweet basil accessions are; leaf color is light green, deep green, blue-green and green. Stem color is red violet and green. Major (82.14 %) of Ethiopian basil flower color is pink and a few (17.86%) are white. Main Economic traits of sweet basil are leaf and inflorescence which may be used fresh or dried for direct local consumption as spice or which is used as raw material for extraction of volatile oil which will be used for food, beverage flavoring and used for medicinal purpose. Inflorescence essential oil yield/ha is smaller amount leaf essential oil yield at both location this was because of leaf weight and leaf essential oil content is greater than inflorescence weight and inflorescence essential oil content. Total volatile oil yield per hectare was a sum of oil yield per hectare extracted from both leaf and inflorescence separately. In keeping with research the ranges of total volatile oil yield in Ethiopia of sweet basil accession from 3.00 to 53.33 kg/ha/ harvest at WGARC and 0.865 to 13.47 kg/ha/harvest HARC.

According to a report (yimer, 2010) ,Ethiopia has exported 68,786 kg of dried basil leaves to Sudan and the USA, from which a complete foreign currency of \$54,991.20 and \$746.00, respectively that was obtained back in 2009. The export volume accounted 19.77% between 2006 - 2009 and exhibited 0.15% and 0.14% both volume and share value of the overall spice export. Export of Basil was mainly destined to Sudan with 91.4% share of total export value of basil from Ethiopia, and therefore the remainder export visiting to Israel (7.4%), and USA (1.2%).

Table 2-5 The Average Basil Export Volume and Value

Destination	Volume in Kg	Value in USD	% share of Value
Israel	2,000.00	4,425.00	7.4
United States	1,130.00	746.00	1.2
Sudan	65,656.00	54,991.20	91.4
Total	68,786.00	60,162.20	100.0

Source: market profile on spices Ethiopia for the period 2005-2009/10

2.3. Applications of Sweet Basil Essential Oil

Sweet Basil Essential Oil is understood to give off a warm, sweet, freshly floral and crisply herbaceous odour that has been depicted as being airy, vibrant, uplifting, and evocative of the scent of licorice. This fragrance can be blended well with citrusy, spicy, or floral essential oils, like Bergamot, Grapefruit, Lemon, Black Pepper, Ginger, Fennel, Geranium, Lavender, and Neroli for a better fragrance. Its aroma is further characterized as somewhat camphoraceous with nuances of spiciness that energize and stimulate the body and mind to push mental clarity, enhance alertness, and calm the nerves to reduce stress, promote deep sleep, and anxiety-free. Emotionally, sweet basil essential oil is calming stress and uplifting to moods and helps improve mental clarity, memory, and sharpens the senses (Basil Sweet Oil - Benefits & Uses of Energizing & Stimulating Oil, 2018).

➤ Anti-Inflammatory Effects

The eugenol component of basil's essential oil has been improving it for Anti-Inflammatory Effects. Many non-steroidal over-the-counter anti-inflammatory medications, including aspirin and ibuprofen, also because the commonly used medicine acetaminophen, work by inhibiting this same enzyme. This enzyme-inhibiting effect of the eugenol that is found in basil qualifies it as an "anti-inflammatory" effect of food that may provide important healing benefits together with symptomatic relief for people with inflammatory health problems like rheumatoid arthritis or inflammatory bowel conditions. Its digestive properties offer relief for symptoms of metabolic malfunctions, like nausea, hiccups, vomiting, and constipation. Sweet Basil Essential Oil's anti-inflammatory effect is reputed to assist calm skin that is afflicted with complaints, like acne or eczema, and to soothe sores as well as minor abrasions. (Basil Sweet Oil - Benefits & Uses of Energizing & Stimulating Oil, 2018)

➤ Nutrients Essential for Cardiovascular Health

Basil may be a superb source of vitamin A (through its concentration of carotenoids such as beta-carotene). Beta-carotene is a more powerful anti-oxidant than vitamin A, but not only protects epithelial cells from free radical damage and also it assists to prevent free radicals from oxidizing cholesterol within the blood stream.

Basil is additionally a decent source of magnesium, which raise cardiovascular health by prompting muscles and blood vessels to relax, thus improving blood flow and the danger of irregular heart rhythms or a spasm of the heart muscle or a blood vessel.

In addition to the above health benefits, it also emerged from our food ranking system as a wonderful source of vitamin K , manganese, an awfully source of copper, vitamin C, a decent source of calcium, iron, folate and omega-3 fatty acids (Duke, James A., 2002).

➤ Antibacterial and antioxidant effect

The phenolic compounds of sweet basil make it the most contribution to the antioxidant properties of basil leaf extracts and it have high value for the food and pharmaceutical application of this plant. Almost all essential oils have bactericidal properties by promoting the assembly of white blood cells and it will prevent and treat infectious illness. According to this property, the aromatic herbs and oils gave as high repute with relation to infections such as malaria, typhoid within the tropics and epidemics of plague in the Middle Ages. Bactericidal and antiviral agents (prophylactics) also used for cover against colds flu and Febrifuge agents for reducing fever and temperature (Filomena Nazzaro, Florinda Fratianni, Laura De Martino, Raffaele Coppola, and Vincenzo De Feo, 2013) .

➤ Used in aromatherapy applications

Basil Essential Oil is ideal giving relief from headaches, fatigue, sadness, and also the discomforts of asthma, as well as for initiating psychological endurance. Furthermore, the fragrance of Sweet Basil helps to repel insects and bacteria that cause unpleasant room odors and uncomfortable to live and work, thus effectively deodorizing stale indoor environments, including cars, further as foul-smelling fabrics, including furniture (Medically reviewed by Arno Kroner, DAOM, LAc, 2020) .

➤ For Cosmetically

Basil Essential Oil is more applicable in refresh, nourish, and provide support the repair of damaged skin. It is often wont to balance oil production, calm acne breakouts, alleviate dryness, soothe symptoms of skin infections and other topical ailments by supporting the skin's suppleness and resilience. When combined with a carrier oil (such as jojoba, sweet almond, or avocado), basil essential oil are often applied on to the skin or added to bath. (Medically reviewed by Arno Kroner, DAOM, LAc, 2020)

In hair, Sweet Basil Oil is thought for contributing a light and refreshing scent to any regular shampoo or conditioner in addition as for excite stimulating circulation, regulating the scalp's oil production, and facilitating healthy hair growth to reduce or slow the speed of hair loss. By hydrating and then cleansing the scalp, it effectively to eliminate any accumulation of dead skin, dirt, grease, environmental pollutants, bacteria and stimulating, dandruff and other topical conditions in skin. In exhibiting these cleansing and clarifying properties, Sweet Basil Oil are applied to promote the look soft, shine and lustrous strands of skin. Furthermore, they need come to be common additions to perfumes further as oral hygiene products.

➤ For Medicinally

Sweet basil (*Ocimum basilicum* L.), with it's wonderful aroma and flavor, is one amongst the foremost most famous medicinal plants within the world which is employed in medical treatments like for headaches, coughs, diarrhea, worms, and kidney malfunctions. (Telci et al. 2006) When diluted and added to a warm bath, Basil Oil is said to soothe menstrual cramps, joint pain, muscular aches and spasms, gout, insect bites, and exhaustion. Since the oil is purifying, it is going to help the reduction of cellulite. Basil has analgesic properties and may lessen pain. It is a natural remedy used to soothe insect bites. This oil has also been accustomed to increase lactation. Today, they continue to be ideal applicable for supporting skin, hair, and kidney health, soothing headaches, head colds, coughs, stomach spasms, diarrhea, and addressing a loss of appetite, intestinal worms, and fluid retention (Medically reviewed by Arno Kroner, DAOM, LAc, 2020).

2.4. Extractions Technology

The essential oil yield of medicinal and aromatic plants is influenced by drying, whereas looking on the extraction time and temperature also because the method, the oil yield may be increased and/or decreased. There are existing different extraction methods in different conditions.

2.4.1. Conventional Extraction Methods

Conventional techniques applied to extract essential plant oils are supported water distillation by the heating process. Conventional extraction techniques such as solvent extraction and steam distillation need longer time to undergo the extraction process and an oversized of organic solvents are required. Additionally, the disadvantages of those

techniques like various volatile components losses, poor efficiency of oils extraction, degradation of unsaturated compounds, and toxic residues from extraction process must be encounter.

➤ **Steam Distillation**

Steam Distillation is that the most well- liked extraction method by isolate essential oils from plants to be used in natural products. The process is applying when the steam vaporizes the plant material's volatile compounds, then it eventually undergoes a condensation and collection process. The contents of essential oils that will being extracted by this system is 93% and also the rest 7% can be further extracted by other methods. this method started by heating of plant material using steam which is supplied from steam generator. Heat is that the main factor in determining the effectively break down of the plant material structures and release of the aromatic components or essential oils.

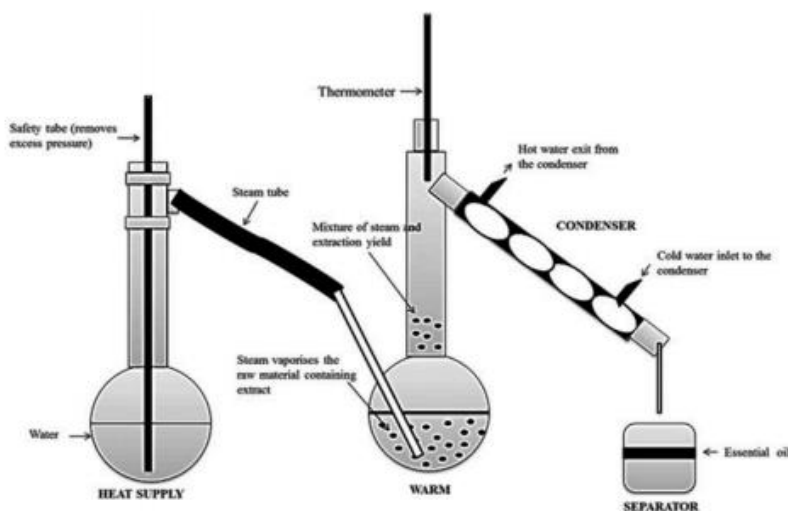


Figure 2-3 steam distillation extraction setup

➤ **Solvent Extraction**

Solvent extraction is that the process of diffusion of solvent into the oil-bearing cell of plant and resulting a solution of oil within the solvent. This method used a solvent of food grade solvents like hexane and ethanol to isolate essential oils from plant material. And also other Ordinary solvents like acetone, petroleum ether or methanol are implemented by this system to extract fragile or delicate flower materials which cannot be extracted using heat or steam supplied. It is best suited to plant materials that yield small amounts of essential oil, that are largely resinous, or that are delicate aromatics unable to resist the

pressure and distress of steam distillation. Through this process, the non-volatile components of the plant material like waxes and pigments are also extracted and then it removed through other processes.

Once the plant material has been treated with the solvent, it produces a waxy aromatic compound which known as "concrete." When this concrete substance is mixed with solvents to be extracted by mildly heating the mixture, and therefore the process is followed by filtration and evaporation of the solvents. The filtrate contains a resin or the mixture of wax, fragrance, and essential oil. Therefore, mentioned chemicals utilized in the process then remain within the oil and therefore the oil is employed in perfumes by the perfume industry or for aromatherapy purposes.

➤ **Hydro Diffusion**

Hydro diffusion extraction method is an extraction process during which steam is supplied to a container which holds plant materials. This system is just applied on dried plant samples which will be damaged at boiling temperature. In the hydro diffusion method, steam is supplied underside of the generator as well as in the steam distillation process, steam is applied at the bottom of the steam generator. This process was carried out at low pressure or vacuum and steam temperature are often reduced below 100°C. Today, the steam diffusion method was further promoted by adding microwave technology.

➤ **Hydro Distillation**

Hydro distillation is that the oldest and simplest oils extraction method. Rose was the primary extracting plant by using this method. The procedures will take from immersing the plant materials directly into water inside the alembic (vessel) and boiled the mixture. The Equipments used in this methods are including a heating source, vessel (Alembic), a condenser to convert vapor from vessel onto liquid, and a decanter to gather the condensate and to separate essential oils with water.

This extraction technique is taking account as a novel method to extract plant materials like wood or flower and is often involving hydrophobic natural plant material with a high boiling point. Because the oils are surrounded by water, this method is ready to shield essential oils to be extracted at a particular degree without being overheated. The most advantageous of this extraction technique is its ability to in isolate plant materials below 100°C.

2.4.2. Innovational Extractions Methods

It is the modifying extraction techniques due to the existing of various disadvantages of conventional methods which improves the essential oils to undergo chemical alteration like hydrolysis, isomerization, and oxidation. These processes involve warmth and affect the standard of essential oils, at the identical time prolonging the extraction period. Within the field of essential oils extraction process, it is very important to keep up the oils chemical constituents and natural proportion at its original state. The parameters that required to be considered in new extraction techniques are reduction of extraction period, energy consumption, solvent used and greenhouse gas emission.

➤ Supercritical Fluid Extraction

Supercritical fluid extraction (SFE) is thought to be a promising alternative technique to steam distillation (or hydro distillation) and solvent extraction for the extraction of flavor and fragrance compounds from natural materials. Selection of the extraction pressure and temperature range is a crucial factor affecting the final composition of the extract and process yield, since the solubility of each component within the supercritical fluid will primarily rely on these parameters.

Fluids with these critical parameters exhibit very interesting properties like low viscosity, high diffusivity, and density closer to liquids. CO₂ is employed as a supercritical solvent for the extraction of essential oils because of its numerous attractive properties:

- (i) easily reach critical point (low critical pressure 72.9 atm, and temperature 31.2°C);
- (ii) unaggressive for thermos labile molecules of the plant essence;
- (iii) chemically inert and non-toxic;
- (iv) non-flammable;
- (v) relatively low cost in high purity;
- (vi) easily removable from the product;
- (vii) its polarity just like pentane which implies that is suitable for extraction of lipophilic compounds.

Generally, the principle of supercritical fluid extraction process involves the utilization and recycling fluid in repeated steps of compression/decompression. The supercritical state of CO₂ can be achieved by highly compressing and heating this fluid. Then, it passes through the raw plant material to load volatile matter and extracts plant. The method

followed by decompression steps to separate the obtained extracted oil from the mixture of CO₂ and plant. The CO₂ is released from second separator and recycled into vessel and no solvent residue remains in.

➤ **Subcritical Extraction Liquid**

subcritical stage of liquid is that the time in which the liquid reaches pressure above the critical pressure, P_c and not up to the critical temperature. The fluids that are required to extract essential oils using this method are water and CO₂. The subcritical state of fluid offers several superior characteristics like lower viscosity, lower density, and enhanced diffusivity between gas and liquids. This extraction method is a fast essential oil isolation process, and preferable approach in conducted at a low working temperature. Moreover, it is a cost efficient extraction, simple and environmental friendly process.

In this process, the specified duration of extraction is simply 15min compared to 3h required to extract essential oils by using conventional methods. Essential oils with more valuable properties which are a higher amount of oxygenated components with no significant presence of terpenes can be obtained and allow substantial cost saving in terms of both energy and plant materials.

➤ **Solvent Free Microwave Extraction**

This technique is an expeditious isolation of essential oils from spices, aromatic herbs, and dry seeds. Mostly it used to obtain essential oils with high yield and selectivity, shorter extraction time, and environmentally friendly process. This approach is a mix of two techniques which are heating plant samples using microwave technology then followed by dry distillation which operates at an atmospheric pressure within the absence of any solvent, while the volatile oil yields were determined looking on each different microwave power used.

2.5. Clarification and Storage of Essential Oils

The extracted essential oils must be dry with adding some gram of anhydrous sodium sulfate to get rid of the water content of it. And store the essential oils in amber or dark colored glass container with Teflon applicator but not store concentrated oils in plastic because it will leach into the oil and alter the chemistry. it will maintain their potency for many years when stored properly, keep bottles tightly capped and kept far away from

sunlight and heat to prevent evaporation and oxidation. If stored properly the essential oil does not change its flavor in any condition.

2.6. Quality Assessment of Essential Oils

Quality control (QC) and assessment of essential oils can be performed on variety techniques, the physio-chemical properties are determined by standardized methods like measuring the ester, acid or carbonyl index, the refraction, density, optical rotation, freezing or boiling points, or quantification of ethanol or moisture. It is then necessary to use more powerful analytical techniques: separation techniques (GC, liquid chromatography (LC), high-performance thin-layer chromatography (HPTLC)) and spectroscopic techniques (vibrational techniques, and nuclear magnetic resonance) (T.Tiên ,F. Hadji-Minaglou,S. Antoniott, 2014) .

The analysis of qualitative and quantitative composition is detected by GC to providing its quality. In this regard, comparisons use the chromatographic profile obtained by GC equipped with a flame-ionization detector (FID), which provides a large range of linearity and has relatively simple maintenance requirements.

2.6.1. NMR Spectroscopy

NMR spectroscopy is a spectroscopy which provides an information for the control of authenticity by determining stable-isotope ratios, affording a method for measuring isotopic patterns within natural and artificial molecules for the needs of differentiation.

2.6.2. High-Performance Liquid Chromatograph (HPLC)

High-performance LC (HPLC) is not widely employed in essential oils, but is a quite away of choice for analyzing less volatile or non-volatile constituents. HPLC highlights non-volatile markers of adulteration like synthetic compounds or vegetable oils. This technique was used to detect a mixture of essential oils (e.g., adding orange oil in lemon oil). HPLC enables the mobile phase to progress by capillarity over a plate charged with a stationary phase together with compounds from a mixture. Different lengths of migration are observed for every compound, looking on mechanisms of partition between the mobile and stationary phases and adsorption phenomenon.

The most recent advances during this technique were mainly observed within the quality of stationary phases and also the efficiency of detection techniques. High-performance

stationary phases, characterized by smaller particle sizes with narrow size distribution, were developed. These increased the resolution and also the reproducibility of LC analysis. Similarly, new stationary phases like chiral phases, were developed and are now commercially available. The detection systems were also considerably improved (e.g., the setup of scanners for densitography for the range 190–900 nm).

2.6.3. Gas Chromatography- Mass Spectroscopy

Gas chromatography mass spectroscopy (GC/MS) are a materials analysis methods that employs gas chromatographs (GC) fitted with mass selective detectors called mass spectrometers (MS). GC/MS analysis is a perfect tool for identifying unknown substances or contaminants that are present in very low quantities.

A GC-MS report is that the fingerprint of any particular batch of essential oil. The novel properties of the oil are deduced from its chemical composition and GC-MS is in a position to point the purity of essential oils in most cases. The gas chromatograph utilizes a capillary column which depends on the column's dimensions (length, diameter, film thickness) additionally because the phase properties (e.g. 5% phenyl poly-siloxane). The difference within the chemical properties between different molecules in an exceedingly mixture and their relative affinity for the stationary phase of the column will promote separation of the molecules because the sample travels the length of the column. The molecules are retained by the column then elute (come off) from the column at different times (called the retention time), and this enables the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer is broken each molecule into ionized fragments and detecting these fragments using their mass-to-charge ratio.

The mass spectrum is employed to spot the components by comparing each to reference libraries of over 275,000 unique spectra. To quantify the compounds within the analyzed sample, analysts establish a standard curve of known concentrations of every material.

Analysis in the Mass Spectrometer (MS): The separated components flow directly out of the column and into the MS which has three internal steps:

(a) Ionization source – components are blasted with electrons, causing them to interrupt and switch positively charged ions.

(b) Filter – the ions submit to an electromagnetic field and filter through supported mass. Analysts set a predetermined range of masses to be allowed through as they pass from the ionization source.

(c) Detector – counting the amount of filtered ions, the information was send to a computer and a mass spectrum, a distribution of ions of various sizes was generated.

Combining the 2 processes reduces the like hood of error, because it is extremely unlikely that two different molecules can behave within the same way in both a gas chromatograph and a mass spectrometer. Therefore, when an determining mass spectrum appears at a characteristic retention time in a GC-MS analysis, it typically increases certainty that the analyze of interest is within the sample. (Mathias, 2014)

2.6.4. Fourier-Transform Infrared Spectroscopy (FTIR)

Fourier-transform infrared spectroscopy (FTIR) is conducted to confirm the potency and consistent quality of a batch of oil. It identifies the structural components of essential oil compounds. In an FTIR scan, infrared light of various frequencies is shined through a sample of volatile oil and therefore the amount of light absorbed by the sample is measured. The standard quality of the sample is decided by comparing the results from an FTIR reading to a historical database with absorption patterns of high-quality samples recorded.

In short, IR spectrum is classified into three wavenumber regions: far-IR spectrum ($<400\text{ cm}^{-1}$), mid-IR spectrum ($400\text{-}4000\text{ cm}^{-1}$) and near-IR spectrum ($4000\text{-}13000\text{ cm}^{-1}$). The mid-IR spectrum is the most generally employed in the sample analysis, but far- and near-IR spectrum also contribute within providing information about the samples analyzed. This study focused on the analysis of FTIR in the mid-IR spectrum. The mid-IR spectrum is split into four regions:

- (i) The single bond region ($2500\text{-}4000\text{ cm}^{-1}$)
- (ii) The triple bond region ($2000\text{-}2500\text{ cm}^{-1}$)
- (iii) The double bond region ($1500\text{-}2000\text{ cm}^{-1}$) and
- (iv) The fingerprint region ($600\text{-}1500\text{ cm}^{-1}$)

2.7. Factors That Affecting the Extraction of Essential Oil Yield

Essential oils have a really high variability of their composition, both in qualitative and quantitative terms. Various factors are chargeable for this variability and might be grouped into two categories:

2.7.1. Intrinsic Factor

Essential oil production and its concentration within the herb is clearly influenced by genetic, on to genetic, and environmental effects. The intrinsic factors associated with the plant, and interaction with the environment (soil type and climate, etc.) and therefore the maturity of the plant concerned, even at harvest during the day. The interaction between genetic and environment effects causes secondary metabolites to be synthesized and essential oils are produced in response to worry. increases its shelf life. Basil plants were harvested when the plants at the flowering stage, when the contents and quality of volatile oil is that the highest.

2.7.2. Extrinsic Factor

The extrinsic factors are associated with the extraction method and also the environment just like the following.

➤ Drying condition and Drying temperature

The extractable herbal plant should be immediately dried after harvesting and washing it, as long-term exposure outside ends up in darkening of leaves because of oxidation reaction. Drying is preferably done at temperature below 40°C to reduce the loss of highly volatile oil and every one constituent bioactive compounds (Werker et al., 1993). A Madeline Hill and Gwen Barclay don't recommend oven drying since high temperatures can cause a loss of essential oils and reduced flavor.

➤ Extraction time

It will be explained that extraction time is required to get the upper oleoresin/ volatile oil yield during prolong contact between basil leaves and solvent and giving basil leaves an opportunity to contact with solvent in order that the oleoresin may be extracted optimally.

➤ Extraction temperature

Moreover, the temperature of the system is near to the boiling point of the solvent. This excess energy within the variety heat helps to extend the extraction kinetics of the system.

➤ Sample particle size

For instance, grinding of plant sample creates more broken cells, which facilitates the elution of bioactive compounds to extraction solvent. The limitation is that breaking the plant particle to very fine sizes requires huge energy consumption and complex machinery. Needless to say, more bioactive compounds will be extracted from plant sample with smaller particle sizes because of broken cells. This effect is amplified when the particle size of the plant sample is reduced below the leaf thickness (average value of 240 μm) of the plant sample, as indicated by size D (<180 μm). Nevertheless, excessive grinding of plant particles to realize powder-like sizes is not feasible as only small portion of the plant samples may be converted into the powder form, to not mention the method requires high energy consumption and long duration.

➤ Solvent choice

Variety of solvent are used for soxhlet extraction with different polarities and mixture too. The acceptable selection of the solvent in solvent extraction is that the prior criteria to urge the expected products and its yield. The choice is maintained by their polarity properties of solvent.

Hexane is non polar solvent and suitable for extraction of non-polar compounds like aliphatic hydrocarbons, neutral lipids. ethanol is polar and stronger so it employed for the neutral and polar lipids. benzene is applicable for aromatic compounds. ether and ethyl acetate are applicable for relatively polar compounds containing oxygen. Dichloromethane has high extraction efficiency for a large range of non-polar to polar compounds and suitable for simultaneous analysis because of the subsequent advantages: its boiling point is low and simple to re-concentrate after extraction and to separate from water because of its higher specific gravity and it is non-flammable.

However, dichloromethane, like benzene, is carcinogenic, and up to date trend are to refrain from using these solvents in liquid-liquid extractions. It is sometimes possible to selectively extract semi-volatile compounds from water by changing the character of samples, not changing solvents. As an example, by changing the pH of samples, only acid or basic substances is extracted. When pH of the water is smaller amount than 2, basic compounds become fully ionized and don't seem to be extracted by the solvent, allowing selective extraction of acidic and neutral compounds.

➤ Solid to solvent ratio

To increase the oleoresin extract, much solvent should be available to maximize the diffusion of product yield. The increasing in the ratio of material and solvent with basil leaves affect the diffusion of oleoresin from basil leaves to the solvent, more solvent making diffusion of oleoresin are going to be greater, because of solvent distribution to basil leaves will be greater. Equitably solvent distribution to basil leaves will increase the yield of oleoresin produced. The more solvents used will reduce the saturation level of the solvent, so that the extracted component may be maximized.

In this study, the extraction of essential oil from sweet basil was performed in steam distillation and analysis the consequence of two extrinsic factors, the sweet basil drying condition and extraction time on the response of sweet basil essential oil yield.

3. MATERIALS AND METHODS

The experimental work has been done in the Laboratory of School of Chemical and Bioengineering at Addis Ababa Institute of Technology and College of Natural Sciences Chemistry Department at Addis Ababa University. During this research, sweet basil of local origin was characterized for their property, its essential oils, and explored further for characterize the essential oil. Materials and Equipment used and procedures followed are described below:

3.1. Materials and Equipments

The major raw material that utilize in this study to extraction of essential oil was sweet Basil and it obtained from local market from sebeta, Oromia.

The chemicals that used for characterization of the essential oil were (Potassium hydroxide(KOH), Phenolphthalein indicator solution, Ethyl Ethanol, Hydrochloric acid) for determination of saponification value, (Sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$), Carbon tetra chloride, Potassium iodide, Starch soluble-ex potato indicator and wiji solution (40% iodine mono-chloride in acetic acid)) for determination of iodine value, (Sodium hydroxide, distilled water and ethanol) for determination of acid value of EO and Anhydrous sodium sulfate to dried the essential oil.

The equipment that was used for the extraction and characterization of the essential oil were Digital balance, oven dryer, furnace, measuring cylinder, Micropipette, burette, cylindrical flask stirrer, thermometer, sealer and elemental analyzer, pH meter, (Boiler, steam distillery, condenser, separator funnel) for extraction, Refractometer (measuring refractive index), UV spectrophotometer (measuring the protein contents of basil leaves), Rotary-viscometer (measuring the dynamic viscosity), Gas-chromatography with mass spectrometry and Fourier transform infrared ray spectrometer.

3.2. Methods

Several advanced (supercritical fluid extraction, subcritical extraction liquid, solvent-free microwave extraction) and conventional (hydro distillation, steam distillation, hydro diffusion, solvent extraction) methods for the extraction of essential oils are discussed within the literature review parts of paper. Advanced methods are considered as the most abundant extraction techniques because of less extraction time, low energy consumption,

low solvent used and less carbon dioxide emission. but due to the lack of accessibility use of the advanced extraction method in our country. During this study used the steam distillation method for extraction and different equipment /measurements are used for analysis and characterization of the product. This study has seen the consequence of the two factors where extraction time and also the drying conditions of the sweet basil leaves that are fresh, atmospheric air dry and sundry sweet basil. Optimization of extraction of essential oil by the classical method involves changing one independent variable while maintaining all others at a fixed level.

3.2.1. Raw Material Collection and Preparation

The sweet basil leaves were collected from Oromia sebeta market and washed using tap water. The leaves of Basil were dried in sun light and in atmospheric air to minimize the loss of highly volatile essential oil and all constituent bioactive compounds. After the drying, the leaves samples were weighted in a digital balance with four decimal points. firstly, Proximately Analysis of fresh sweet basil leaves has been done.

➤ Moisture content

The amount of water present in the sweet basil leaves sample considered to be equal to the loss of weight after drying. Sample dried in oven at 110°C for 17 minute and reweight according to Method AOAC. Formula show below.

$$\text{moisture content \%} = \left(\frac{(W_1+W_2)-(W_2+W_3)}{(W_1+W_2)-W_3} \right) * 100\% \dots\dots\dots\text{Equation 3-1}$$

Where: W_1 = Weight of wet sample, W_2 = pan weight, W_3 = weight of sample after dried

➤ Total Ash content

The total ash method is intended to measure the total amount of material remaining after ignition. Place about 5g of the ground air-dried leaves in an exceedingly a previously ignited and tared crucible (usually of platinum or silica). Spread the leaves in an even layer and ignite it by gradually increasing the warmth to 500–600 °C until it become white, it implies that the absence of carbon. Cool in a desiccator and weigh. If carbon-free ash cannot be obtained during this manner, cool the crucible and moisten the residue with about 2 ml of water. Dry on a water-bath, then on a hot-plate and ignite to constant weight. Allow the residue to chill in a suitable desiccator for half-hour, then weigh without once. Calculate the content of total ash in mg per g of air-dried leaves.

$$\text{Ash content}(\%) = \frac{W_3 - W_1}{W_2 - W_1} * 100 \quad \dots\dots\dots \text{Equation 3-2}$$

Where, W_1 = Weight of crucible

W_2 = Weight of crucible + Weight of sample before ashing

W_3 = Weight of crucible + Weight of sample after ashing

3.2.2. Extraction of Sweet Basil Using Steam Distillation

The fundamental nature of steam distillation is that it enables a compound or mixture of compounds to be distilled at a temperature substantially below that of the boiling point(s) of the individual constituent(s). Essential oils contain substances with boiling points up to 200°C or higher temperatures. within the presence of steam or boiling water, however, these substances are volatilized at a temperature near to 100°C, at atmospheric pressure. During the extraction process, the system temperature is adequate to the boiling temperature of water at atmospheric pressure. To attain this temperature and thus obtain the distillation of the first EO droplet.

In the extraction of essential oil in steam distillation method after pretreatment of the raw material. The weight sample sweet basil leaves were kept within the plant chamber of the still and waters are continuously fill in to the boiler and connected to the water condenser. After check the whole pre operation view, the power supply become turn on. When the temperatures of water reached 100°C it became a vapor steam then the pressurized steam was injected through the bottom part of the apparatus containing the plant sample and circulated through the plant material. The heat of the steam forces the tiny intercellular pockets that hold the volatile oils to open and release them. The temperature of the steam must be high enough to open the pouches, yet not so high that it destroys the plants or burns the volatile oils. As the steam is released, the tiny droplets of essential oil evaporate and together with the steam molecules, travel through a tube into the still's condensation chamber. As the steam cools, it condenses into water. The volatile oil forms a film on the surface of the water in the separator funnel. To separate the essential oil from the water, the film is then decanted or skimmed off at the top. The remains water or a byproduct of distillation, is named floral water, distillate, or hydrosol. In the most case those floral water is applicable for the aromatherapy for child. The oil was collected and dried over anhydrous sodium sulfite and stored in sealed vials at 4°C until started analysis.

A number of factors are considered in determining the final quality of extracted essential oil. Apart from the selecting raw material, the most significant factors (extraction time and drying condition of raw material were studied in this study.

3.2.4 Determination of Percentage Yield of Essential Oil Extracted

The total oil yield depends on the operating parameters. Designed experiments were administered to map quantitative effects of these parameters. The yield of extraction is calculated from the relation between the essential oil mass obtained and the raw material mass employed in the extraction. The mean yield (%) table is built from oil extracted in relevance the quantity of sample employed in each run. during this work 2000 grams of the plant materials was used for every batch and yield (%) is calculated using equation listed below. The extraction parameters were very important to provide both a decent quality and an affordable amount of essential oils. To attain this objective, different parameters with different levels were used during the extraction process and obtained the optimum condition.

$$\text{Yield of oil} = \left(\frac{\text{oil weight}}{\text{sample weight}} \right) * 100\% \quad \dots\dots\dots \text{Equation 3-3}$$

3.2.5. Physio-Chemical Property Determination

The physio-chemical properties of the sweet basil essential oil were determined and then results were shown as follow:

➤ **Solubility of sweet basil essential oil**

Most essential oils are slightly soluble in water and with absolute alcohol. Solubility of sweet basil essential oil has been Tested by adding 4 grams of sample into 20 milliliters of alcohol and water in separately. Shake well and check whether it can soluble or not.

➤ **Determination of boiling point temperature**

5mL of the essential oil was placed in an exceedingly small test tubing. A capillary sealed at one end and it placed open-end down into the essential oil. The test tube is firmly attached to a thermometer with a rubber band and this entire assembly immersed in an oil bath at half-filled 100ml beaker. Because of the increasing temperature slowly, a rapid evolution of bubbles from the top of the tube will begins. Heating was continued for about 5-10 seconds to make certain all of the air has been expelled from the capillary and therefore the vapors of the volatile oil become up to the atmospheric pressure. As well as

the temperature decreases, the bubbles slowed down and the volatile oil starts rising into the capillary. At that point when the bubble stops, the thermometer is read and recorded. This process was repeated twice, and the temperature reading in each case was recorded.

➤ **Determination of Flash point temperature**

The flash point may prove useful within the valuation of an essential oil. The flash point has value as a sign of adulteration whereas additions of adulterants like alcohol and low boiling mineral spirits may greatly lower the flash point. The flash point of sweet basil essential oil was measured by adding 10 milliliter of sample in to Borosilicate glass and so kept it on heater next thermometer was inserted in to the glass containing the sample to read the minimum temperature of first flame. Finally, the temperature was read then recorded.

➤ **Determination of Specific gravity**

For the determination of specific gravity of oils, the density of the oil was measured by using density bottle. A clean and dry bottle of 25ml capacity was weighed (W_0) and then the bottle stuffed with the oil, stopper inserted and reweighed (W_1). The oil is substituted with water after washing and drying the bottle and weigh (W_2) (Middle-East J. Med. Pl. Res. 2012).

$$S. g = \frac{\text{mass of substance}}{\text{mass of an equal volume of water}} = \frac{W_2 - W_0}{W_1 - W_0} \quad \dots \text{Equation 3.4}$$

➤ **Determination of pH value**

The sample was taken and putted in a clean dry 25ml beaker and the hot distilled water was added to the sample within the beaker and stir slowly. Then it cools in cold water bath to 25^oc. The pH electrode is standardized with a buffer solution first and then immerse in the sample and the pH is read (AOAC official method analysis 960.19, 2000).

➤ **Determination of kinematic viscosity**

Viscosity is one of the vital physical characteristics of volatile and fixed oil. It is referred as resistant to flow and sear due to gravity. Viscosity of any liquid is constant at a particular temperature and also as index of its composition. So it is used for standardization of particles oils. The sample oil is poured into a test tube and a viscometer is used to measure the viscosity at a temperature of 20^oc. A dynamic viscosity of the essential oil is measured directly using rotary-viscometer. Then the kinematic viscometer was estimated as follows:

kinematic viscosity = $\frac{\text{dynamic viscometry of the sample}}{\text{density of sample}}$Equation 3-4

➤ **Determination of Refractive index**

Refractive index is that the ratio of velocity of light in vacuum to the velocity in the substance or the ratio of the sine of the angle of incidence to sine of the angle of reflection. Essential oils carry its many various compounds. Their varying composition allows for them to be characterized by refractive index measurement. These measurements can therefore also provide insight into the standard quality of essential oils, as any change in their optimal composition may affect the refractive index. the refractive index is well determined by refractometer.

Formula for RI (n) = c / v Equation 3-5

Where: c= the speed of light in vacuum

V= the phase velocity of light in medium

➤ **Determination of saponification value**

The sample is taken and placed in to flask. The alcoholic potassium hydroxide solution is added in to the flask and connected to reflux condenser and kept on the water bath and boiled gently for 30 min. After the flask and the condenser washed with of hot ethyl alcohol. Then of phenolphthalein indicator were added and regularly rotating the contents of the flask, the excess potassium hydroxide was titrated with hydrochloric acid to the top point, until the pink color of the indicator just disappears. The identical procedure will conduct for the blank (without the substance being tested) and in both case recorded the ml of HCL for calculation saponification value using the subsequent formula (The International Pharmacopoeia -Ninith Edition, 2019)

Saponification value SV = $\frac{5.6N(B-S)}{W}$ Equation 3-6

Where: B = the volume of the solution used for blank test;

S = the volume of the solution used for determination S.V;

N= Actual normality of the HCl used;

W = Weight of oil taken in gram.

➤ **Determination of Iodine value**

The iodine value of the sample was determined by A.O.A.C Official Method 993.20, for iodine values of oil. The system specified by ISO 3961 (1989) was used. The sample is weighed into a conical flask and the carbon tetra chloride was added to dissolve the oil. Then the iodine mono chloride solution in glacial acetic solution is added to the flask using a safety pipette in fume chamber. Stopper was then inserted and also the content of the flask was vigorously swirled. The flask is then placed within the dark for two and half hour. After the time arrived, 10% aqueous potassium iodide and water is added using a measuring cylinder. The content was titrated with 0.1M sodium-thiosulphate solutions until the yellow color almost disappeared. Few drops of 1% starch indicator is added and the titration continued by adding thiosulphate drop wise until blue coloration disappeared after vigorous shaking. similar procedure is employed for blank test and other samples. The iodine value (I.V) is given by the expression:

$$\text{Iodine value (IV)} = \frac{12.96 * C * (V_1 - V_2)}{w} \quad \dots\dots\dots\text{Equation 3-7}$$

Where: C = Concentration of sodium thiosulphate used;

V1 = Volume of sodium thiosulphate used for blank;

V2 = Volume of sodium thiosulphate used for determination,

W = Mass of the sample.

➤ **Determination of acid value**

Acid value is a numerical value equivalent to the number of milligrams of sodium hydroxide required to neutralize the free acids present in 1 gram of the oil. The determination was performed as follows.

- Chemical preparation

The phenopetaline indicator solution was Prepared by dissolved the phenopetaline indicator powder into 100 ml of ethanol and mixed well by shaking. And also 0.1N sodium hydroxide solution was Prepared by dissolves 4gm sodium hydroxide pellet within 900ml distilled water and stirred well and make final volume 1000ml.

- Preparation of sample

Taking the ten ml essential oil sample b/c the expected acid value of essential oil is 1-5. Then measured the 50 ml of ethanol (99%) and poured into 250 ml conical flask. After neutralize the ethanol, it added into the flasks that contain the essential oil sample and

boiled it until the sample completely dissolved .it cool in few minutes and added few drops of phenopetaline indicator before start the titration with 0.1N sodium hydroxide solution.

- Titration

Taking 0.1 N Sodium hydroxide filled in the burette and noted the initial volume reading. And Started the titration after adding some drop of phenopetaline indicator with vigorous agitation until the solution pink color was observed and volume of 0.1M KOH was noted.

$$\text{acid value (AV)} = 56.1 * N * \frac{V}{W} \dots\dots\dots\text{Equation 3-8}$$

Where: V = volume of standard sodium hydroxide (ml),

N = Normality of sodium hydroxide solution

W = sample weight (in g)

➤ **Ester Value Determination**

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

$$\text{Ester value} = \text{Saponification value} - \text{acid value} \dots\dots\dots \text{Equation 3-9}$$

3.2.6. Compositional Analysis of Essential Oil

➤ **Gas Chromatography-Mass Spectrometry (GC-MS)**

This method is employed for determining constituents present in essential oils. It is simple, allows fast responses, efficient, and it used analytical technique for the determination of essential oils constituents. For conducting GC-MS Analysis the subsequent procedure was taken as follows; The sample was injected into a port of GC which is heated to up to 300°C where the material is then volatilized. Separation of gaseous components as they flow through the column. The column is wound within a special oven which controls temperatures from -20°C to 32°C. The column surface is coated within a material which can separate the different chemical compounds in the sample based on size and/or polarity. Sample components that are more volatile and smaller in size will travel through the column more quickly than others.

➤ **Fourier transform infrared spectroscopy (FTIR)**

Fourier Transform Infrared (FTIR) spectroscopy is a dynamical instrumentation technique that is useful for examination of varied variety of matter. When a sample is analyzed using infrared radiation, the FTIR spectra provide information about the vibrational and rotational energies of a molecule. The infrared radiation is absorbed at specific frequencies characteristic of the molecular species, whereby the FT-IR spectrum may be a true signature of the molecule. Knowing the radiation intensity as a function of the infrared frequency, one can specify the kinds of chemical functional groups.

Fourier Transform Infrared Spectroscopy was employed to investigate changes that occur within the chemical structure of essential oil from sweet basil after was identify using Fourier transform infrared spectroscopy (FTIR) equipped with origin JASCO. This technique was used to manipulate structural changes in samples and to examine the changes in functional groups induced by various treatments as a result of chemical modification. Where the spectra are performed at room temperature in the range of 400 to 4000 cm^{-1} with the resolution of change X cm^{-1} and a total of 3736 points for the sample (Lun, Anas, Gunny, & Kasim, 2017).

3.3. Experimental Design and Frame Work

3.3.1. Experimental Design

Factorial experiment is an experiment whose design consists of two or more factors, each factor with discrete possible value or levels. Factorial design is employed to research the effect of every factor. In a factorial experiment all the possible combination factor level was tested in which determining the effect of individual factors and to assessing the interaction effect of change on two or more variable at a time. The analysis was performed by using design expert software and then selected general full factorial design method. This method of experiment design helps to differentiate the significance of the main and the interaction factors on the yield of product. The software also used to develop the mathematical model that was indicate the main and interaction effects of the factors on the response.

Factor: two factors were investigated as mentioned earlier, these are: drying condition of sweet basil and the extraction time.

Factors levels: for every factors, three levels were considered.

Replicates: Each independent experiment was repeated three times.

Number of runs: For “m” level, “n” factors, and “k” replicate, the number of experimental runs that required to be performed is adequate to $k \cdot m^n$ (Zivorad, 2004).

In this research $m=3$, $n=2$, and $k=3$ thus, $3 \cdot 3^2 = 27$ experimental runs were performed.

Generally, during the study, the Parameters where extraction time and the drying condition of sweet basil leaves was accustomed to examined the optimum condition for best yields and quality of basil essential oil. The experiments that were conducted in three times for more accuracy. The drying conditions were wet basil, atmospheric air dry basil and sundry basil and extraction time (2, 3 and 4hrs) are factors that was examined. So it needs 9 experiments in this study for all determinations of effects of factors on the extraction of sweet basil essential oil. After replication 3 times, totally it needs 27 experiments.

3.3.2. Experimental Framework

The overall experimental work in this study were described as follow:

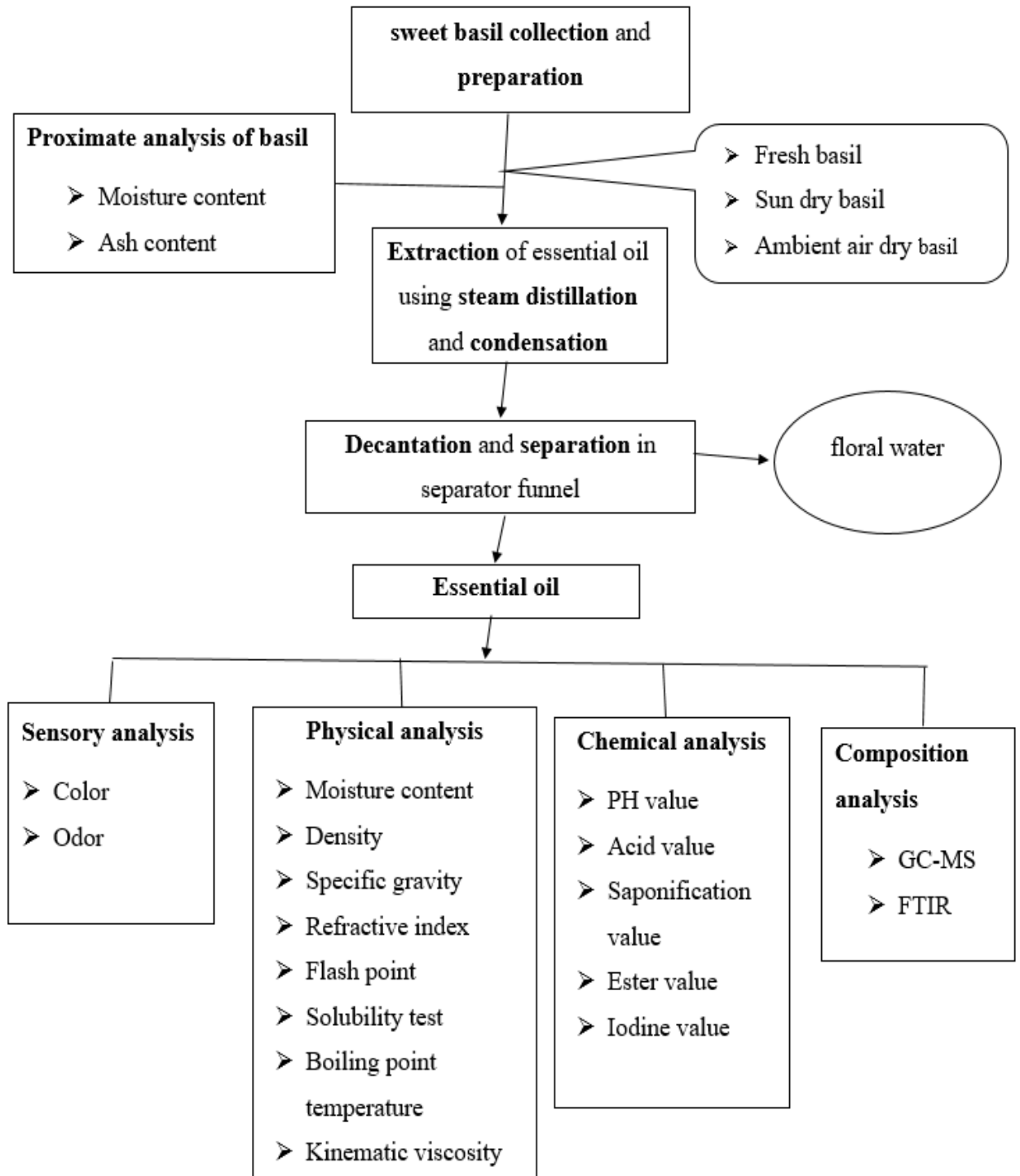


Figure 3-1 Experimental Frame Work

4. RESULT AND DISCUSSION

The results of this work are presented and discussed below. It includes proximate analysis of the sweet basil leaves, characteristics, the chemical composition and structural of essential oil from sweet basil at the optimum extraction time and drying conditions of sweet basil.

4.1. Proximate Analysis Result of Sweet Basil Leaves

The moisture contents of the sweet basil that were used in the extraction of essential oil is within the range of 70- 80%, which described that 70-80% water content of the total raw material were removed under sun drying and air drying of it in the pretreatment process for extraction of essential oil in the steam distillation methods. This is often varying due to factors such as variance in geographical locations, production techniques and plant collection time. And the ash contents that got from this study were 0.32 g from the first 25g of dry sweet basil leaves.

Table 4-1 Proximate Analysis of Basil Leaves

Properties	Average value
Moisture content	70-80%
Ash content	0.32g

4.2. Physical Properties of Sweet Basil Essential Oil

Some physical properties of the sweet basil essential oil were characterized and also the results are shown below:

- solubility of sample

Approximately eight drops of water were added to the test tube containing four drops of sweet basil volatile oil. The test tube was stirred well thoroughly with a glass stirring rod. Two separate phases were observed. It indicated that it does not soluble in water. And the same procedure also taken in the testing of sample solubility in ethanol. Thus, the sweet basil volatile oil is insoluble compound in water, but the sample sweet basil essential oil was soluble in ethanol.

- Specific gravity

Specific gravity is that the ratio of the density of a substance to the density of a reference substance; equivalently is the ratio of the mass of a substance to the ratio of the mass of a

reference substance for a given volume. It helps in obtaining important information of a substance like concentration.

Weight of Pycnometer (W) at 25°C = 16.5728 g

Weight of Pycnometer with essential oil (W₁) at 25°C = 28.4465g

Weight of Pycnometer with water (W₂) at 25°C = 30.5728g

$$\begin{aligned}\text{Sp. gr} &= (w_1 - w) / (w_2 - w) \\ &= (28.4465 - 16.5728) / (30.5728 - 16.5728) \\ &= 0.8481\end{aligned}$$

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

$$= 0.8481 \times 1 \text{ g/ml}$$

$$= 0.8481 \text{ g/ml}$$

➤ Refractive index

The refractive index provides an information about the behavior of light. It is mostly applied for identifying a selected substance, confirm its purity or measure its concentration. From the experimental result, the refractive index of sweet basil essential oil was within the range of 1.49-1.5.

➤ Boiling point temperature

The boiling point of organic compound provided important information about their physical properties and structural characteristics. Boiling point helps identify and characterize a compound. The normal boiling point of a compound is an indicator of the volatility of that compound. The high boiling implies that the faster volatile.

➤ Flash point temperature

The flash point of the sweet basil essential oil was 78°C. it implies that at 78°C the volatile materials starts to its vapor ignite if a given an ignition source and provide a vital information about storing and usage of the product.

➤ Kinematic Viscosity

Dynamic viscosity of essential oil was read from vibrio-viscometer and its value 6.02 m pas at temperature 20°C. Substituting the dynamic viscosity of the oil which is 6.02 maps (6.02 × 10⁻³ kg/m. s) and density = 848.1 kg/m³ in equation 3.5, then kinematic viscosity is 7.09 × 10⁻⁶. it implies that the sweet basil oil is less viscous liquid.

The physical properties of sweet basil essential oil were determined and discussed in the above and are summarized in the subsequent table 4.2.

Table 4-2 The Major Property of Sweet Basil Essential Oil

Properties	Result
Solubility	Soluble in ethanol, not in water
Color	pale-yellow liquid
Odor	floral, spicy odor
boiling point temperature (°C)	210
flash point temperature(°C)	78
specific gravity	0.8481 gm/cm ³
refractive index	1.49

4.3. The Chemical Properties of Sweet Basil Essential Oil

4.3.1. pH Value

The pH value of sweet basil essential oil was tested in triplicated and the results taken as an average. Then, the pH value of sweet basil essential oil was slightly acidic. In preparation of skin and hair care materials, the preferable pH value within the range of 3.5-6.5 (Mueller et al., 2000). The obtained pH value of sweet basil oil is within the range to be employed in producing cosmetic materials.

4.3.2. Saponification Value

The saponification value (SV) expressed as the number of milligram of KOH required to saponify 1 g of fat and calculated (A.O.A.C official method of analysis 920.160, 2000). In this study the saponification value of sweet basil essential oil at the optimum condition were calculate after the noted the quantify of HCL and its value 112 mgKOH/gm. It is a measure of the average molecular weight (a chain length) of all the fatty acids present. The saponification number measures the bounded and unbounded acids present in oil or fat. The smaller the molar mass of the fat, the higher the saponification number. It is very important characteristics considered in soap production.

4.3.3. Acid Value

It is the amount of base expressed in milligrams of potassium hydroxide that may needed to neutralize the acidic constituents in 1 g of sample. The acid number is a measure of the amount of carboxylic acid groups in a chemical compound, like a fatty acid, or in a mixture of compounds. Then, the acid value of the sweet basil was calculated using equation 3.9 and also the results was 5.04 mgKOH/gm.

4.3.4. Iodine Value

The iodine value is used to determine the unsaturated of oils and in assessing the stability of oil in industrial application. The lower the iodine value of oil, which reflects its characteristics such as higher resistance to oxidation, the longer period and better quality. Whereas the higher the iodine value of oil, the lower the quality. during this study the Iodine values of sweet basil essential oil was 3.172.

4.3.5. Ester Value

The ester value of the sweet basil volatile oil was the difference between the saponification value and acid value of the sweet basil oil. Then its value was 50.96 mgKOH/gm. Table 4-3 shows that the summary of the results obtained in the determination of chemical properties of sweet basil essential oil that described in the above sub sections.

Table 4-3 Chemical Properties of Sweet Basil Essential Oil

Properties	Experimental Results
pH value	5
Saponification value (mgKOH/g)	56
Acid value (mg /g)	5.04
Iodine value (mg /g)	3.17
Ester value (mg KOH /g)	50.96

4.4. Gas Chromatograph- Mass Spectroscopy

The identification of the components of the essential oil extracted by steam distillation process was dispensed by comparison of their mass spectra and retention times to those of reference standards. The 25 components that existing within the sweet basil oil are presented in table 4.4. It is often seen that these oils were characterized by the presence of

monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene and alcohol. but the quantitative differences were observed within the contents of those components.

The main constituents are 1H-Benzocycloheptene, α -octahydro-3,5,5-trimethyl-9-methylene- (25.28 %), Cyclohexene, 4-[(1E)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methyl- (21.44), 1,3,6-Octatriene, 3,7-dimethyl-, (Z)- (18.015%), Estragol (11.84), Humulene (4.7845). Methyl chavicol (estragol), one among the main *O. basilicum* oil constituents, is extensively used as a flavoring, antimicrobial, antioxidant, anti-edematogenic and anti-inflammatory agent. Basic chemical composition of the linalool chemo type of the *O. basilicum* oil is linalool, epi- α -cadinol, c-cadinene, Germacrene D , and camphor. Methyl-chavicol chemo type of *O. basilicum* oils contains high percentage of methyl chavicol, 1,8-cineole, *trans*-ocimene, etc. Due to its richness in linalool (oxygenated monoterpene), the *O. basilicum* essential oil exhibits potent antimicrobial activity. Phenolic components are the most contributors to the oil antimicrobial properties, causing the leakage of intracellular ATP and potassium ions of bacterial cells, which ends up in death of bacterial cell (Kumar, 2016).

The chemical compositions revealed that these leaves had composition somehow relative closed to those of other *Ocimum Basilicum* L essential oils GC-MS analyzed data in numerous research article GC-MS results such like (Joshi, 2014) , (Angel calin-sanchez, krzysztof Lech, Antoni szumny, Adam Figie, Angel A. carbone-Barrachina, 2012), (katarzyna Baczek, olga Kosakowska, Malgorzata Gniewosz, Iwona Gientka and Zenon Weglarz, 2019) and mostly similar with (Heri Septya Kusuma1, Ditta Kharisma Yolanda Putri1, 2018).

This significant difference is due to the fact that the chemical compounds of any plant essential oil can vary greatly depending on geographical region, the age of the plant, local climate; seasonal variations, experimental conditions. Genetic difference is additional accountable for the changes in the types of chemical compounds.

EXTRACTION, CHARACTERIZATION AND OPTIMIZATION OF ESSENTIAL
OIL FROM SWEET BASIL

Table 4-4 Chemical Composition of Sweet Basil Essential Oil

PK	RT	Area Pct.	Library/ID	Qual
1	5.921	1.5956	beta.-Pinene	93
2	7.1027	18.0169	1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	96
3	10.21	11.8408	Estragol	98
4	10.3588	0.2149	alpha.-Terpineol	93
5	11.7498	0.2305	Copaene	99
6	11.975	0.3061	(-).beta.-Bourbonene	95
7	12.0865	0.1756	1HCyclopenta[1,3]cyclopropane[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3As (3a.alpha.,3b.beta.,4.beta.,7.alpha.,7aS*)]-	96
8	12.1676	0.1598	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	93
9	12.4581	1.6507	beta.-ylangene	99
10	12.5344	3.4222	trans-.alpha.-Bergamotene	99
11	12.6257	3.9099	Caryophyllene	98
12	12.7845	0.7146	(1R,5R)-4-Methylene-1-((R)-6-methylhept-5-en-2-yl)bicyclo[3.1.0]hexane, (relative configuration)	70
13	12.8675	2.3031	(1S,5S,6R)-6-Methyl-2-methylene-6-(4-methylpent-3-en-1-yl)bicyclo[3.1.1]heptane	93
14	12.9584	0.2105	cis-.beta.-Farnesene	76
15	13.0273	0.473	Naphthalene, decahydro-1,6-bis(methylene)-4-(1-methylethyl)-, (4.alpha.,4a.alpha.,8a.alpha.)-	96
16	13.1227	4.7845	Humulene	95
17	13.194	0.6843	Germacrene D	90
18	13.3144	0.8473	cis-.beta.-Farnesene	96
19	13.5319	25.2817	1H-Benzocycloheptene, 2,4a,5,6,7,8,9,9a-octahydro-3,5,5-trimethyl-9-methylene-	92
20	13.6599	0.4851	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	98
21	13.7565	0.2908	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	99
22	13.9175	21.4466	Cyclohexene, 4-[(1E)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methyl-	95
23	14.7551	0.319	1,5,6,7-Tetrahydro-4-indolone	46
24	15.5437	0.2891	p-Cymene	59
25	16.4207	0.3474	beta.-Bisabolene	70

4.5. FTIR Analysis of Essential Oil

The following FTIR result was done at Addis Ababa university, Department of Chemistry. Spectrum 65 FT-IR (PerkinElmer) in the range 400-4000 cm^{-1} (resolution 4 cm^{-1} , number of scans:4) is obtained using ATR. The peaks of the curve indicating the functional groups that exist and different component were displayed. The graphs curve made with frequency vs transmittance. The point of the curve reads with reader in the origin software.

The peak of the FTIR result curve were stretching from 400-4000 cm^{-1} . The stretching of the curve makes it difficult to read it easily, because the peaks were not sharp to identify which peaks, including the functional groups.

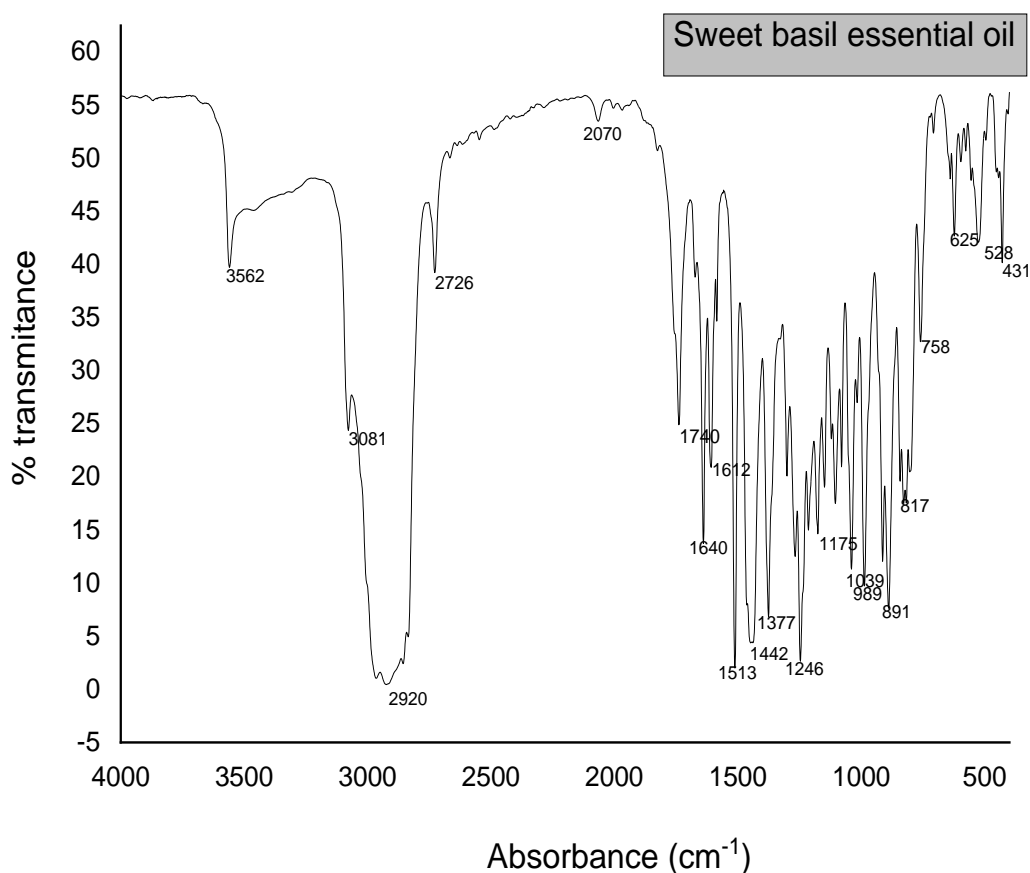


Figure 4-1 FTIR Analysis of Sweet Basil Essential Oil

FTIR spectra of essential oil from sweet basil are 40 peaks as shown in figure 4-1. The absorption bands are observed in two wavenumber regions of 400 - 2000 cm^{-1} and 2000 - 4000 cm^{-1} . For the single bond area of wavenumber 2000-4000 cm^{-1} there are 5 peaks in the single bond area. The first peak at 3562 cm^{-1} is characterized for Internally bonded OH

stretch, Tertiary alcohol OH stretch, Phenols OH stretch and found on alcohol and hydroxyl compound class. The peak at 3081 cm^{-1} is represented C-H stretch and Olefinic (alkene) compound. The peak at 2920 cm^{-1} is characterized for stretching Methylene C-H asym. /sym. The peak at 1640 cm^{-1} is characterized for stretching vibration Alkenyl C=C stretch and Olefinic (alkene). The peaks at 1612 cm^{-1} and 1513 cm^{-1} are characterized for stretching vibration in Aromatic ring (aryl) and also found in C=C-C Aromatic ring stretch. The band at 1442 cm^{-1} is characterized for stretching of Methyl C-H asym. /sym. Bond. The peaks at 1377 , 1246 and 1176 cm^{-1} are characterized for stretching Aromatic C-H out-of-plane bend, C-H 1,3-Disubstitution (meta). The peak at 1039 and 989 cm^{-1} are characterized for stretching Aromatic C-H in-plane bend, also at 817 and 891 cm^{-1} are characterized for stretching Aromatic C-H out-of-plane bend and C-H 1,3-Disubstitution (meta).

4.6. Steam Distillation Extraction of Essential Oil Yield

The percentage oil yield was calculated by using equation 3.3 then the obtained result is shown below within the table 4.5:

Table 4-5 Experimental Sweet Basil Essential Oil Yield

Run	Factor 1: extraction time (hr)	Factor2: drying condition	Replicate			Average Percent of oil yield
			Oil yield percent			
1	4	dry with sun	0.45	0.45	0.6	0.5
2	3	dry with sun	0.41	0.35	0.5	0.42
3	2	without drying	0.05	0.15	0.16	0.12
4	2	dry with sun	0.3	0.25	0.35	0.3
5	4	Dry with atmospheric air	0.75	0.8	0.85	0.8
6	3	Dry with atmospheric air	0.72	0.68	0.76	0.72
7	3	without drying	0.1	0.17	0.21	0.18
8	2	Dry with atmospheric air	0.45	.35	0.6	0.55
9	4	without drying	0.15	0.22	0.2	0.2

Table 4-6 Comparison of Studies on Extraction of Sweet Basil EO

no	Extraction method	Parameter	Obtained yield (%)	Reference
1	Supercritical fluid extraction	Extraction temperature and pressure	0.719 to 1.483%	(Zoran Z., Snezana F., Senka V. and Ahmed M., 2015)
2	Hydro-distillation	Broad and narrow leaves	1.3 to 2.1 % And 1.5 to 1.8 %	(Eleni W., 2011)
3	Solvent extraction	Extraction time and ratio from the material with a solvent	20.15%	(Tambun R., Purba H., Ginting k., 2015)
4	Steam distillation	Extraction time and drying	0.12 to 1.8%	This study

The maximum extraction of sweet basil EO was 0.8% obtained for atmospheric air dried sweet basil leaves at extraction time of four hrs and the minimum yield of essential oil obtained was 0.12 % from fresh sweet basil leaves at the minimum extraction time that is at two hr.

The essential oil yield in this study is relatively similar with the results of study obtained by WGARC with the percent yield of the sweet basil essential oil 0.167 to 1.09 % dry weight base and with HARC its percent yield varied from 0.061 to 1.136%.

4.7. Experimental Analysis and Adequacy Check for Full Factorial Model

It has been shown that the extraction time and drying condition of the sweet basil leaves has been a significant effect on the yield of essential oil. Then, the resulted data was inserted as an input to the software DESIGN-EXPERT 6.08 to be analyzed in determine the significant factors of the experimental work.

EXTRACTION, CHARACTERIZATION AND OPTIMIZATION OF ESSENTIAL OIL FROM SWEET BASIL

The Sweet basil EO yield at different level of factors which obtained from the experimental work was analyzed by DESIGN-EXPERT and it gives up model type, F value, P-value, R² and the general yield formula.

Table 4-7 Model Summary of Statistics in Essential Oil Yield

Source	Sum of squares	DF	Mean squares	F Value	Prob>F	
Mean	1.6	1	1.6			
Linear	0.46	3	0.15	80.31	0.0001	
2FI	7.63E-03	2	3.82E-03	6.08	0.088	
Quadratic	1.61E-03	1	1.61E-03	11.56	0.0001	Suggested
Cubic	2.78E-04	2	1.39E-04			Aliased
Residual	0	0				
Total	2.06	9	0.23			

From the above table, the suggested model is quadratic to get the optimum yield of essential oil and the F value is 0.0001 which is less than alpha. Then the quadratic model was used.

Table 4-8 Analysis of Variance for The Response Yield of Basil Essential Oil

Source	Squares	DF	Square	Value	Prob > F	
Model	0.47	6	0.078	561.37	0.0018	Significant
A	0.047	1	0.047	337.08	0.003	
B	0.41	2	0.21	1482.32	0.0007	
A ²	1.61E-03	1	1.61E-03	11.56	0.0767	
AB	7.63E-03	2	3.82E-03	27.48	0.0351	
Residual	2.78E-04	2	1.39E-04			
Cor	0.47	8				
Total						

Where: A: extraction Time

B: dry condition of sweet basil

The Model F-value of 561.37 represent the model is significant. There is only a 0.18% chance that a "Model F-value" this large could occur due to noise. Values of "Prob > F"

EXTRACTION, CHARACTERIZATION AND OPTIMIZATION OF ESSENTIAL OIL FROM SWEET BASIL

below 0.0500 indicate model terms are significant. In this case, A, B and AB are significant model terms. Values above 0.1000 indicate the model terms are not significant. Based on the upper software analysis A^2 is no significant effects of the model this means there is no effect of extraction time is doubled. it can upgrade the model by ignoring the above A^2 value. After modified the value of R- square and mean value as shown below. Temperature more effect on the yield of essential oil based on the F- value. Determine the exact value of essential oil yield more economical value.

Table 4-9 Modified Analysis of Variance for the Response Yield of Basil EO

Source	Squares	DF	Square	Value	Prob > F	
Model	0.47	5	0.093	148.53	0.0009	Significant
A	0.047	1	0.047	74.58	0.0033	
B	0.41	2	0.21	327.95	0.0003	
AB	7.63E-03	2	3.82E-03	6.08	0.008	
Residual	1.88E-03	3	6.28E-04			
Cor Total	0.47	8				

From the above tables 4-8 and 4-9, the one original quadratic model and modified model F value is 561.37 and 148.53 are shown respectively. it indicated the modified model is best to analyze the data and the effect of the factor on the yield of essential oil was minimum.

Table 4-10 Model Adequacy Measures for Basil Essential Oil Yield

Std. Dev.	0.012	R-Squared	0.9994
Mean	0.42	Adj R-Squared	0.9976
C.V.	2.8	Pred R-Squared	0.982
PRESS	8.44E-03	Adeq Precision	66.227

Unmodified equation of yield

Final equation in Terms of Actual Factors:

$$\text{yield} = 0.44 + 0.088 * A - 0.014 * B_{[1]} + 0.27 * B_{[2]} - 0.028 * A^2 + 0.012 * AB_{[1]} + 0.037 * AB_{[2]}$$

Modified equation of yield

Final Yield Equation In Terms of Actual Factor:

$$\text{yield} = 0.42 + 0.088 * A - 0.014 * B_{[1]} + 0.27 * B_{[2]} + 0.012 * AB_{[1]} + 0.037 * AB_{[2]}$$

Where: A: Extraction time

B: Drying condition

As mentioned in the model equation of essential oil yield, it can get the value of yield at different extraction parameter level and easily determine the optimum extraction condition and economical feasible value related to cost.

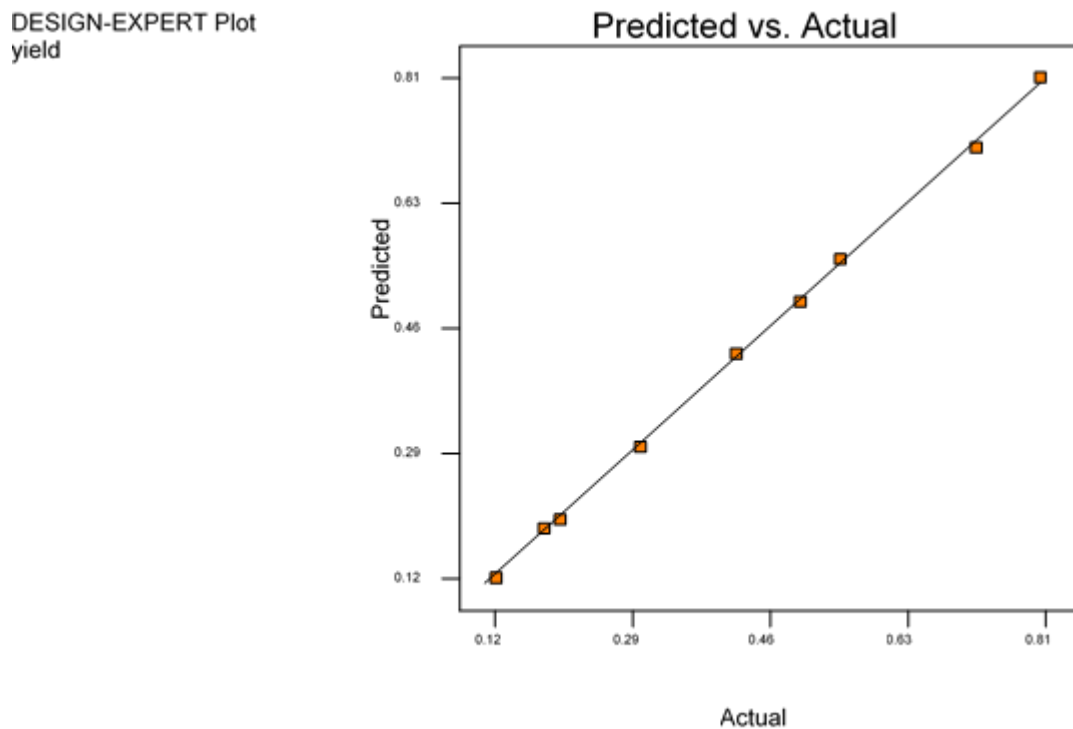


Figure 4-2 Predicted value Vs Actual Value of Yield

From the above figure 4-2 show that the predicted and actual value lay on the same line. Therefore, the experiment performed properly and the extraction parameter (time and drying condition of raw material) are fit to the experimental prediction value.

4.7.1. Effects of the Parameters on Yield of Essential Oil

- Effects of extraction time on yield

Extraction time plays a great role on the percentage yield of sweet basil essential oil using steam distillation. It shows that as contact time increase the oil yield also increase till transfer of oil from the leaves to the steam attains zero. When the maximum amount of extractable oil is obtained, the oil yield level remains invariable even by extending the

reaction time. In order that within the steam distillation extraction the maximum oil yield may be finding at an extraction time of 4hr.

- Effects of drying condition on the essential oil yield

The yield of the essential oil increased as the drying conditions of sweet basil leaves move from fresh to sun dried then to atmospheric air dried sweet basil leaves. At this drying condition, maximum average yield of sweet basil essential oil was obtained from atmospheric air dried sweet basil leaves, which is 0.8%. Whereas the minimum average yield of essential oil was obtained from fresh sweet basil leaves. when the herbs leaves were dried more in sun dry the volatile components of it can be slightly evaporated through sun light.

4.7.2. Interaction Effects of Extraction Time and Drying Condition of Sweet Basil on Essential Oil Yield

The graph placed in below expressed the interaction effect of extraction time and drying condition of sweet basil and identified the extraction condition on the yield of essential oil. The extraction conditions which was at time four hrs when the sweet basil drying with the atmospheric air and yield of sweet basil essential oil at this extraction parameter 0.8%.

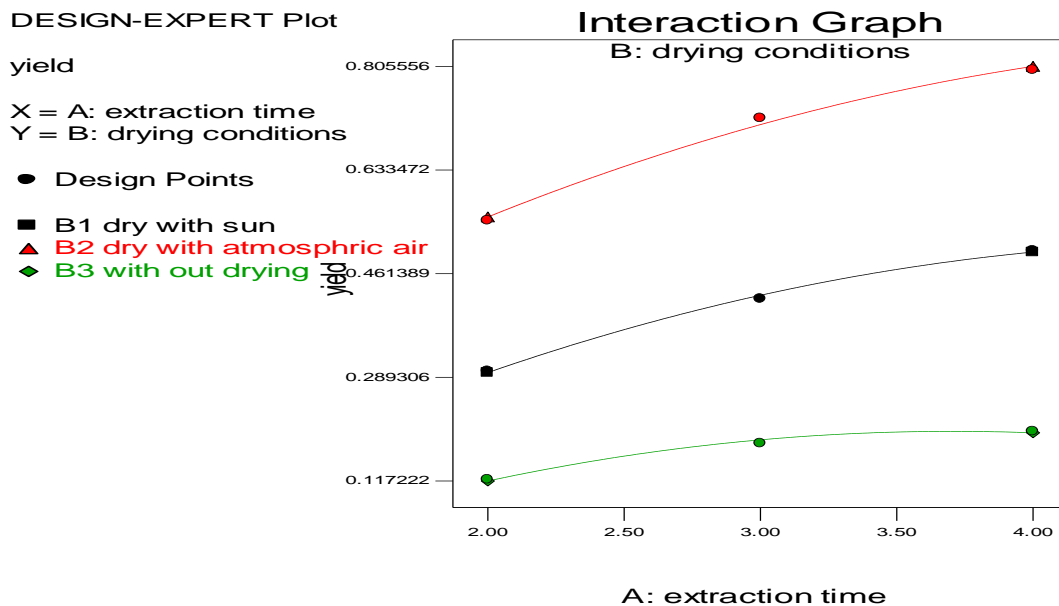


Figure 4-3 Interaction Effect of Extraction Time and Drying Condition of sweet basil on the Yield

4.8. Process Variables and Response Factor Optimization

This study investigated the optimum process variables within a maximum yield of sweet basil essential oil also it implies that will get maximum economical benefit as sweet basil used as input material for the extraction of essential oil. Optimization able to generate the predicted response(s) for any set of factors. The process variables which are extraction time and drying condition of sweet basil have to optimized for maximizing the economic benefit by minimizing process cost and maximizing the desire product.

Table 4-11 Summary on Factors, Response (EO Yield) And Goals of Optimization

Name	Goal	Lower Limit	Upper Limit	Weight	Importance
extraction time	is in range	2	4	1	3
drying conditions	is in range	without drying	Drying with atmospheric air	1	3
yield	maximize	0.12	0.8	1	3

For the maximum yield, the extraction condition sets at minimum economical consideration. also it considered as cost and consumption of complete extraction of the its essential oil of herbs. Finally, the experimental optimization set at the optimum extraction condition and related cost, minimum cost, maximum yield or maximum final output related to good quality. From table 4-11 optimum goals, eights solutions were founding and then the optimum extraction condition at maximum yield and minimum time at good desirability as shown below table.

Table 4-12 Solution of an Extracting Condition at Maximum Yield

No	extraction time	drying conditions	Yield (%)	Desirability	
1	3.91	dry with atmospheric air	0.804175	1	Selected
2	3.94	dry with atmospheric air	0.80735	1	
3	3.97	dry with atmospheric air	0.811375	1	
4	3.89	dry with atmospheric air	0.80155	1	
5	3.99	dry with atmospheric air	0.81405	1	
6	3.93	dry with atmospheric air	0.805675	1	
7	4	dry with sun	0.506666	0.569	
8	4	without drying	0.206667	0.127	

EXTRACTION, CHARACTERIZATION AND OPTIMIZATION OF ESSENTIAL OIL FROM SWEET BASIL

In the above eight solutions, the maximum yield got based on the set value approximately 0.8041% with a better Desirability value at one. this optimum value gets at extracting parameter which was 3.91 hrs when the sweet basil dried with atmospheric air.

To confirm the model, the actual validation experiment was carried out at optimum conditions in a design expert. Then, to validate the optimum conditions predicted by the full factorial methodology model results, triplicate experiments were done at the specified optimum process conditions predicted by the model.

After triplicate experiments were performed at the optimized process conditions, the mean percentage of essential oil yield obtained was 0.75%. As shown in table 4-10 and related to the data obtained from optimization analysis using the desirability function.

Table 4-13 Result of Optimization and Model Validation

	extraction time(hrs)	drying conditions	yield (%)
Predicted	3.91	dry with atmospheric air	0.804175
Experimented	3.91	dry with atmospheric air	0.75

The mean percentage of yield obtained by triplicate experiments 0.75% which is not significantly varies from the predicted value of 0.8% yield at the optimal conditions are time 3.91 hrs and the sweet basil dried in atmospheric air.

Therefore, the model was capable for predicting the maximum essential oil yield. i.e. numerical optimization can be taken as an optimal value because the predicted value was close enough to the experimental value. In summary, the extraction time and drying condition could be used for the optimize the sweet basil essential oil yield by tuning it.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

The sweet basil essential oil is a pale-yellow liquid with a floral, spicy odor. It is soluble in ethanol and insoluble in water. The extraction of the sweet basil leaves is a one source of the supply of essential oil for its huge application. The factors that has shown in this study was extraction time and drying conditions of sweet basil and got the optimum condition with the maximum yield 0.8% from air dried sweet basil leaves at four hrs extraction time. The minimum yield was 0.12% from fresh sweet basil leaves at two hrs of extraction time. It has seen in design expert software that reducing moisture content of sweet basil leaves would increase the yield at increasing the extraction time.

In summary, Air drying is mostly preferred instead of sun drying, as intense heating in sun can cause loss of some volatile components and also the of fresh basil was not gave effective yield. Accordingly, at the optimum condition yield was found with 0.8481g/cm^3 specific gravity, 1.49 refractive index value, 3.5 pH, 6.02 mpas dynamic viscosity, 205°C boiling point temperature, 78°C flash point, 5.04 mg KOH/g acid value, 56 mg KOH/g saponification value, 3.17 ml/g iodine value and 50.93 mg KOH/g ester value. The essential oil was characterized by combined Gas Chromatography and Mass Spectroscopy then resulted as the presence of monoterpene hydrocarbons, oxygenated monoterpenes, sesquitrpene and alcohol. The *Ocimum Basilicum* L. essential oil was subjected to GC-MS analyses, twenty-five components representing 96.9 % of the total oil composition. The high concentrations of Linalool (Germacrene D), β -ylangene, trans- α -Bergamotene, Caryophyllene, β -Pinene , α -Terpineol , β -Bisabolene, cis- β -Farnesene, Copaene, methyl chavicol (estragol), β -elemene, β -ocimene were found. Therefore, due to its richness in linalool (oxygenated monoterpene), the *O. basilicum* essential oil exhibits potent antimicrobial activity and Methyl chavicol (estragol) constituents of *O. basilicum* oil implies that EO extensively used as a flavoring, antimicrobial, antioxidant and anti-inflammatory agent.

5.2. Recommendations

Based on this research findings, the following recommendations are forwarded.

- In this research the effects of temperature and pressure factors were not studied due to uncontrollability of the steam distillery. Therefore, further studies should be done in order to investigate better quantity and quality of sweet basil essential oil based on those factors.
- *O. basilicum* leaves should be harvested early in the morning after evaporation of dew drops and just before intense sunshine to achieve maximum essential oil contents. During the drying process, leaves should not be shredded and not long drying in sun as a huge amount of essential oil can be lost along with some major chemical constituents.
- Further research is required on solvent extraction of sweet basil. Before conducting this study in selection of extraction methods, I have seen the Soxhlet extraction and got other products from sweet basil. So it needs to study on those extra products in the extraction of essential oil from sweet basil.
- Further study should be conducted on the quality of the sweet basil essential oil obtained at each operating condition using GC-MS. This will provide an alternative to manufacturers on the minimum conditions required to get a specific quality of sweet basil essential oil.
- Further detailed research on feasibility studies are required for implementing this research. Extracting essential oils rather than exporting the raw plant materials can save the country's foreign currency and hence the production of essential oils still needs to be needed as a good source of foreign exchange revenue for our country.

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Appendices

Appendix A: Formulas and equations in characterization of essential oil

$$\text{moisture content \%} = \left(\frac{(w_1 + w_2) - (w_2 + w_3)}{(w_1 + w_2) - w_2} \right) * 100\%$$

$$\text{Ash content(\%)} = \frac{w_3 - w_1}{w_2 - w_1} * 100$$

$$\text{Yield of oil} = \left(\frac{\text{oil weight}}{\text{sample weight}} \right)$$

$$\text{s.g} = \frac{\text{mass of substance}}{\text{mass of an equal volume of water}} = (w_2 - w_0)/(w_1 - w_0)$$

$$\text{kinematic viscometry} = \frac{\text{dynamic viscometry of the sample}}{\text{density of sample}}$$

$$\text{Saponification value SV} = \frac{5.6N(B-S)}{W}$$

$$\text{Iodine value (IV)} = \frac{12.96 * C * (V_1 - V_2)}{W}$$

$$\text{acid value (AV)} = 56.1 * N * \frac{V}{W}$$

Ester value = Saponification value - acid value

Appendix B: Functional Group Properties with Wavenumber Interval

Table B-0-1 Functional group properties with wavenumber

Functional group/assignment	Wavenumber (cm ⁻¹)
1. Saturated Aliphatic (alkene/alkyl)	
a) Methyl (-CH₃)	
Methyl C-H asym./sym. Stretch	2970–2950/2880–2860
Methyl C-H asym./sym. Bend	1470–1430/1380–1370
gem-Dimethyl or “iso”- (doublet)	1385–1380/1370–1365
Tri methyl or “tert-butyl” (multiplet)	1395–1385/1365
b) Methylene (>CH₂)	
Methylene C-H asym./sym. Stretch	2935–2915/2865–2845
Methylene C-H bend	1485–1445
Methylene —(CH ₂) _n — rocking (n ≥ 3)	750–720
Cyclohexane ring vibrations	1055–1000/1005–925
c) Methyne (>CH-)	
Methyne C-H stretch	2900–2880
Methyne C-H bend	1350–1330
Skeletal C-C vibrations	1300–700
d) Special methyl (-CH₃) frequencies	
Methoxy, methyl ether O-CH ₃ , C-H stretch	2850–2815
Methyl amino, N-CH ₃ , C-H stretch	2820–2780
2. Olefinic (alkene)	
Alkenyl C=C stretch	1680–1620
Aryl-substituted C=C	1625
Conjugated C=C	1600
Terminal (vinyl) C-H stretch	3095–3075 3040–3010
Pendant (Vinylidene) C-H stretch	3095–3075
Medial, cis- or trans-C-H stretch	3040–3010

EXTRACTION, CHARACTERIZATION AND OPTIMIZATION OF ESSENTIAL
OIL FROM SWEET BASIL

Vinyl C-H in-plane bend	1420–1410
Vinylidene C-H in-plane bend	1310–1290
Vinyl C-H out-of-plane bend	995–985 + 915–890
Vinylidene C-H out-of-plane bend	895–885
trans-C-H out-of-plane bend	970–960
cis-C-H out-of-plane bend	700 (broad)
3. Olefinic (alkene)	
Alkenyl C=C stretch	1680–1620
Aryl-substituted C=C	1625
Conjugated C=C	1600
Terminal (vinyl) C-H stretch	3095–3075 3040–3010
Pendant (Vinylidene) C-H stretch	3095–3075
Medial, cis- or trans-C-H stretch	3040–3010
Vinyl C-H in-plane bend	1420–1410
Vinylidene C-H in-plane bend	1310–1290
Vinyl C-H out-of-plane bend	995–985 + 915–890
Vinylidene C-H out-of-plane bend	895–885
trans-C-H out-of-plane bend	970–960
cis-C-H out-of-plane bend	700 (broad)
4. Aromatic ring (aryl)	
C=C-C Aromatic ring stretch	1615–1580 1510–1450
Aromatic C-H stretch	3130–3070
Aromatic C-H in-plane bend	1225–950 (several)
Aromatic C-H out-of-plane bend	900–670 (several)
C-H Mono substitution (phenyl)	770–730 + 710–690
C-H 1,2-Disubstitution (ortho)	770–735
C-H 1,3-Disubstitution (meta)	810–750 + 900–860
C-H 1,4-Disubstitution (para)	860–800
Aromatic combination bands	2000–1660 (several)
5. Acetylenic(alkyne)	
C≡C Terminal alkyne (mono substituted)	2140–2100
C≡C Medial alkyne (di substituted)	2260–2190

EXTRACTION, CHARACTERIZATION AND OPTIMIZATION OF ESSENTIAL
OIL FROM SWEET BASIL

Alkyne C-H stretch	3320–3310
Alkyne C-H bend	680–610
Alkyne C-H bend	630 (typical)
6. Aliphatic organ halogen compound	
Aliphatic fluoro compounds, C-F stretch	1150–1000
Aliphatic chloro compounds, C-Cl stretch	800–700
Aliphatic bromo compounds, C-Br stretch	700–600
Aliphatic iodo compounds, C-I stretch	600–500
7. Alcohol and hydroxyl compound	
Hydroxyl group, H-bonded OH stretch	3570–3200 (broad)
Normal “polymeric” OH stretch	3400–3200
Dimeric OH stretch	3550–3450
Internally bonded OH stretch	3570–3540
Non bonded hydroxyl group, OH stretch	3645–3600 (narrow)
Primary alcohol, OH stretch	3645–3630
Secondary alcohol, OH stretch	3635–3620
Tertiary alcohol, OH stretch	3620–3540
Phenols, OH stretch	3640–3530

Appendix C: GC-MS result of sweet basil essential oil

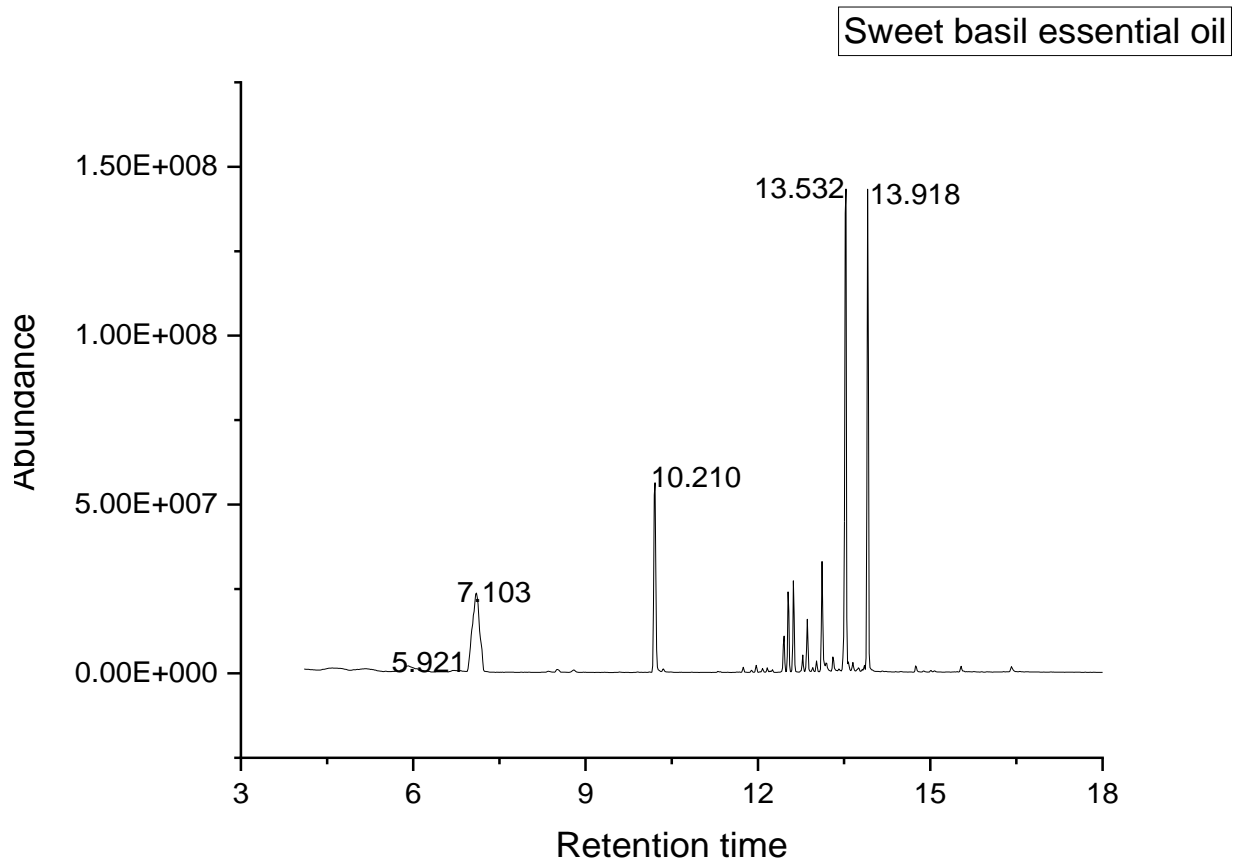


Figure C-1 Retention versus abundance graph

EXTRACTION, CHARACTERIZATION AND OPTIMIZATION OF ESSENTIAL OIL FROM SWEET BASIL

Appendix D: Laboratory Equipment and sample photo



(a)



(b)



(c)



(d)



(e)



(f)

EXTRACTION, CHARACTERIZATION AND OPTIMIZATION OF ESSENTIAL OIL FROM SWEET BASIL



Figure D-2 (a) fresh sweet basil (b)essential oil (c)steam distillation with condenser (d) decantation and separation (e) refractometer (f) PH meter (g),(h),(i) &(j) chemicals for characterization