PHYSICOCHEMICAL ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF SWEET BASIL (*Ocimum basilicum* L.) LEAF AND INFLORESENCE ESSENTIAL OILS.

M. Sc. THESIS

GETACHEW YIBELTAL

JANUARY, 2021

HARAMAYA UNIVERSITY, HARAMAYA

Physicochemical Antioxidant and Antimicrobial Properties of Sweet Basil (*Ocimum basilicum*L.) Leaf and Inflorescence Essential Oil

A Thesis Submitted to the School of Biological Sciences and Biotechnology

Postgraduate Programs Directorate

HARAMAYA UNIVERSITY

In Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE IN BIOLOGY

Getachew Yibeltal

January, 2021

Haramaya University, Haramaya

APPROVAL SHEET

HARAMAYA UNIVERSITY

POSTGRADUATE PROGRAM DIRECTORATE

As thesis research advisors, we hereby certify that we have read and evaluated this thesis, prepared, under our guidance by Getachew Yibeltal entitled Physicochemical Antioxidant and Antimicrobial Properties of Sweet Basil (*Ocimum basilicum*L.) Leaf and Inflorescence Essential Oil

Zekeria Yusuf (PhD)	_____	_____
Major Advisor	Signature	Date
MulugetaDesta (PhD)	_____	_____
Co- Advisor	Signature	Date

As member of the Board of Examiners of the M.Sc. Thesis Open Defense examination, we certify that we have read and evaluated the Thesis prepared by GetachewYibeltal the candidate. We recommend that the thesis be accepted as fulfilling the thesis requirements for the degree of Master of Science in Biological Sciences.

_____	_____	_____
Chairperson	Signature	Date
_____	_____	_____
Internal examiner	Signature	Date
_____	_____	_____
External examiner	Signature	Date

Final approval and acceptance of the Thesis is contingent upon the submission of its final copy to the council of Graduate Studies (CGS) through the candidate's department or school graduate committee (DGC or SGC).

DEDICATION

This thesis work is dedicated to my mother Abebech Mitku and my father Yibeltal Adamu Who Encouraged And Strengthened Me In All My Life .

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this M.Sc Thesis is my own work. I have followed all ethical and technical principles of scholarship in conducting studies, data collection, data analysis, and compilation of this Thesis. Any scholar matter that is included in the Thesis has been given recognition through citation.

This thesis has been submitted in partial fulfillment of the requirements for the Degree of Master of Science in Biology at Haramaya University. The thesis is deposited at Haramaya University library and is made available to borrowers under rules of the Library. I solemnly declare that this thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

Brief quotations from this thesis may be made without special permission provided thataccurate and complete acknowledgment of sources is made. Requests for permission for extended quotations from or reproduction of this thesis in whole or in part may be granted by the Head of the School or Department when in his or her judgment the proposed use of the material is in the interest of scholarship. In all other instances, however, permission must be obtained from the author of the thesis.

Name: Getachew Yibeltal
Signature ----------
Place: Haramaya University
Date of Submission: January 2021

BIOGRAPHICAL SKETCH

The author was born in East Gojam ,Bichena Town , on 21 November, 1972. He attended his primary school education at Enemay Primary and Junior Secondary School. He completed his high school education in Belay Zeleke Senior Secondary School from1990 to1993. He joined Bahidar University in October 1994 to attend his B.Sc degree in Biology and graduated in July, 1997. Then in 1998, he was employed in Amhara regional state in Belay Zeleke Secondary and Preparatory School as Biology teacher. After 11 years of service, he joined the Postgraduate Program of Haramaya University to pursue Msc study in the field of biology in 2009.

ACKNOWLEDGEMENTS

First of all I would like to thank my major advisor Dr. Zekeria Yusuf and co-advisor Dr.MulugetaDesta for their valuable comments, suggestions, support and professional guidance which were instrumental in the completion of this thesis work. Moreover, I would like to acknowledge the Ministry of Education (MoE) for its budget support; and the School of Biological Sciences and Biotechnology, Haramaya University, as well for hosting me as a student.

Last but not least my deepest appreciation goes to all my parents, brothers, sisters, and friends for their relentless encouragement on my work.

ACRONYMS/ABBREVIATIONS

AA	Ascorbic acid
ACV	Acid value
DPPH	2, 2- Diphenyl-1-picrylhydrazyl
EO	Essential Oil
FFA	Free fatty acids
HPSA	Hydrogen peroxide scavenging activity
MBC	Minimum bactericidal concentration
MFC	Minimum fungicidal concentration
MIC	Minimum inhibitory concentration
PV	Peroxide value
Spgr	Specific gravity

TABLE OF CONTENTS

LIST OF TABLES

Table **Page**

LIST OF TABLES IN THE APPENDIX

Appendix Table **Page**

LIST OF FIGURES IN THE APPENDIX

Physicochemical Properties and Biological Activities of Sweet Basil (*Ocimumbasilicum*L.) Leaf and Infloresence Oils

ABSTRACT

The emergence of increased multidrug resistant bacteria has initiated development of new antimicrobial agents. Essential oils from aromatic plants and herbal extracts have attracted scientific interest due to their potential use as antimicrobial and/or antioxidant compounds in foods.The present study was aimed to examine physicochemical properties and biological activities of essential oils extracted from sweet basil leaves and infloresence Evaluation of the Physicochemical properties of the essential oil extracts of Ocimum basilicum (L) showed significant difference between leaf and infioresence in oil yield, specific gravity, acid value (ACV), and free fatty acid values. The antioxidant activities of Ocimum basilicum (L) leaf and infloresence oil extracts recorded significantly higher antioxidant activities with respect topersentage scavenging activity DPPH(86.45%) for leaf oil extracts. However, significantly higher ascorbic acid content (13.42%) and hydrogen peroxide scavenging activity (5.50) were observed for O. basilicum infloresence oil extract. The highest antibacterial activity with a zone of inhibition of (15.47mm) was obtained at the highest concentration (3µl/ml) of the oil extract from O. basilicum (L.) inflorescence against S. aureus while the lowest antibacterial activity (12.50mm) was recorded for leaf oil extract against E. coli indicating that S. aureus was more susceptible than E. coli. On the other hand, the strongest antifungal activity with maximum zone of inhibition (15.90mm) was recorded for leaf oil against C. albicans and the weakest antifungal activity with minimum zone of inhibition (11.97mm) was recorded for infloresence oil against A. niger. The highest bacteriostatic (bacterial inhibitory) concentration with a MIC of 0.09µl/ml and a corresponding MBC of 0.19 µl/ml was obtained from infloresence oil extract against S. aureus while the lowest bacteriostatic concentration with minimum inhibitory concentration(MIC) of 0.38 µl/ml, the largest value) and a minimum bactericidal concentration (MBC)of 0.75 µl/ml was recorded for leaf oil extract against E. coli. O. basilicum (L.) leaf oil extract has demonstrated the strongest fungal inhibitory concentration with a MIC of 0.125µl/ml,(the least value) and a minimum fungicidal concentration with MFCof 0.09µl/ml against C. albicans whereas the weakest fungal inhibitory concentration with MICof 0.63µl/ml and MFC of 1.25µl/ml was obtained for the inflorsence oil extract against A.niger showing that C. albicans was the most susceptible to the oil extract From the present study In can be concluded that the sweet basil infloresence and leaf oil extracts can be potential sources of antioxidant, antibacterial and antifungal agents which can be used as natural antioxidants and antimicrobials in medicine, food industry and cosmetics.

Keywords: Antioxidant, Bacteriocidal Activity, Fungicidal Activity, Oil extract, Scavenging activity, Zone of inhibition.

1. INTRODUCTION

Basil (*Ocimumbasilicum*L.) also known as sweet and garden basil, a member of the mint family is the most common species in Lamiaceae family. This family includes 3500 species distributed as herbaceous plants, sometimes shrubs, but rarely as trees, with a significant content of essential oils. *Ocimum* includes about 150 species (Pushpangadan&Bradu, 1995) and within the species *Ocimum basilicum*(L.) there are a number of varieties that differ in the general morphological structure and texture, and in the content of chemical parameters (Grayer *et al.,* 1996). The basil plant (*Basiliciherba*) is used in traditional and homeopathic medicine to treat a number of diseases. The essential oil of basil (*Basiliciaetheroleum*) is used in the food, perfumery and cosmetics industry and has expressed bactericidal, fungicidal, antiviral, repellent, antioxidant, antidiarrhoeal, chemopreventive and radioprotective activity (Runyoro*et al*., 2010).

The useful parts of the plants are leaves and infloresence. Hot tea of basil plant leaves is good for treating nausea, dysentery, and flatulence. Externally, basil formulations can be used for different skin infections such as treatment of acne, snake bites and insect stings. In addition to these, basil has been used as a remedy for an enormous number of ailments, including cancer, convulsion, deafness, diarrhea, epilepsy, insanity, sore throat, toothaches, and whooping cough (Khatri *et al.,* 1995). Many medicinal and aromatic plants, including basil, are typically consumed without further processing after harvest and it is important that chemicals are not present in or on any part of the plant (Banchio*et al.,* 2008).

The leaves and infloresence tops of sweet basil are used as a carminative, galactogogue, stomachic and antispasmodic in folk medicine (Sajjadi, 2006). Recently the potential uses of *O. basilicum*essential oils, particularly as antimicrobial and antioxidant agents, have also been investigated (Sajjadi;2006). The chemical composition of basil oil has been the subject of a considerable number of studies. There is extensive diversity in the constituents of the basil oils, and several chemotypes have been established from various phytochemical investigations (Sajjadi, 2006). However, methyl chavicol, linalool, methyl cinnamate, methyl eugenol,

eugenol and geraniol are reported as major components of the oils of different chemotypes of *O. basilicum*(Sajjadi, 2006).

Several studies have demonstrated that the supplementation of avilamycin or probiotics in broiler diets increased growth performance and reduced diseases and management problems (Wellenreiter*et al.,* 2000; Vahdatpour *et al.,* 2011; Yakhkesh i*et al.,* 2011). Also, many studies have also been conducted on the effects of dietary essential oils or combinations on the performance of poultry, but with varying and conflicting results. While some reports (Brenes and Roura, 2010; Bozkurt *et al.*, 2012) have demonstrated that essential oils improved animal performance, some researchers (Botsoglou *et al*., 2003, 2004) have reported that these additives were not effective in this regard. Besides that, essential oils enhance production of digestive secretions, stimulate blood circulation, exert antioxidant properties, reduce levels of pathogenic bacteria and may enhance immune status (Hosseini *et al.,* 2013).

Although new technologies (such as genetic engineering, irradiation of food and modified-atmosphere packaging) can improve food safety, microbiological hazards and the foodborne diseases they cause may lead to increasingly important public health problems. Over the past decades, a significant increase in the incidence of diseases caused by microorganisms transmitted mainly by food (such as *Salmonella* spp. and *Campylobacter* spp.) has been reported (Newell *et al.*, 2010). The emergence of increased antibiotic resistance in bacteria causing foodborne disease is an additional complicating factor. In fact, there is shortage of new antibiotics able to act against multidrug-resistant bacteria and thus innovative approaches are needed for the development of new antimicrobial agents. Some areas that are being investigated include natural produc screening, exploring novel chemical species and development of antibiotic potentiators (e.g. efflux-pump inhibitors) (Freire-Moran *et al.* 2011).

Essential oils and herbal extracts have attracted scientific interest due to their potential use as antimicrobial and/or antioxidant compounds in foods (Shan *et al.,* 2007; Tajkarimi *et al.*,2010). Essential oils (EOs) are the volatile oily liquid products of the secondary metabolism of aromatic plants, obtained from different plant parts. Several EOs have been studied for several types of biological effects among which antimicrobial, antiinflammatory

and antioxidant activities (Gutierrez *et al.,* 2009). In fact, there is a renewed interest for the possible commercial applications of EOs in sectors like the food and pharmaceutical industries. Concentrating in the food industry, there is a growing consumers' demand for the development of natural food additives, which will supply protection against spoilage, food-borne pathogens and oxidation processes thus reducing the use of synthetic food additives such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). There is experimental evidence, however not definitive, which associates the use of such additives with toxic effects in biological systems (Labrador *et al.,* 2007).

Basil (*Ocimumbasilicum* L.) is an aromatic plant used extensively to add aroma and flavor in food. Locally known as Beso Bila, Traditionally, beso bila has been used as a medicinal plant. The essential oil of the species contains a wide and varying array of chemical compounds, depending on variations in genotype, climatic conditions, geographical location, seasonal variation, agronomic treatment and stage of development (Zheljazkov*et al.,* 2008). Similar variation in chemical composition has been documented in the phenolic acid constituents present in extracts from *O. basilicum*accessions (Kwee and Niemeyer, 2011). An often used classification of basil based on its EO composition and according to geographical origin, involves the following four major chemotypes (Nguyen *et al.,* 2010), even though several chemotype variations have also been reported (Zheljazkov*et al.,* 2008): (i) European chemotype with EO having linalool and estragole as major components, (ii) Reunion chemotype with EO containing estragole as main component, (iii) Tropical chemotype with methylcinnamate as main component and (iv) Eugenol chemotype containing eugenol as main component.

Besides fresh leaves have been normally used *in natura*, one can extract a valuable essential oil from basil, used in the manufacture of perfumes and flavors for food and beverages (Marotti*et al.,* 1996). The basil essential oil also has insecticide and insect repellent properties (Umerie*et al.,* 1998). Maia *et al.* (1996) reported the potential uses of basil essential oil in order to replace molecules obtained from endangered species. Basil essential oil can be extracted from leaves and infloresence tops through hydrodistillation (Charles and Simon, 1990). The chemical composition of basil extracts reveals the presence of tanines, flavonoids, saponins, and volatile terpenes like camphor, tymol, methylchavicol, linalool, eugenol, 1-8-

cineol and pinenes. The composition essential oil may reveal several types of basil like the European, the French or so called sweet basil, the Reunion or Comores, the Bulgar, the Java or Methyl Cinnamante and the Eugenol (Simon *et al.*, 1990).

The antimicrobial activity of basil EOs and herb extracts isolated from different *Ocimum*species has been studied by several groups. In some cases, the reaction of basil EOs with both Gram-positive and Gram-negative bacteria has been probed and effort has been made to correlate the expressed antimicrobial activity with the EO chemical composition (Alves-Silva *et al.* 2013). One of the emerging research areas for improving the plant growth and yield, as well as for the control of different phytopathogenic agents, is the use of plant growth promoting rhizobacteria (PGPR). They are able to improve plant growth by increasing the rate of seed germination and seedling emergence, minimizing the adverse effects of external stress factors, and protecting plants from soil-borne pests and diseases. In this respect, different isolates of fluorescent *Pseudomonas* species take prominent place. Consequently, these isolates have been intensively studied (Mrkovački*et al.*, 2016).

The production of basil essential oil has increased the demand for basil vegetal matter to be extracted in specialized distilleries, thus creating a need of fresh plants from periodical harvest of semiperennial plants. Few works have been done about seasonal variation of basil oil yield during the year (Muni *et al.*, 2002) and about oil production as a function of the number of plants per area (Gill and Randhawa, 2000). The economical interest of some vegetable species for essential oil production has brought the need of studies about the capacity of them to bear intense exploitation when submitted to frequent harvesting, aiming to reach the maximum longevity of the plants. However, the knowledge about basil cultivation in this intensive system is almost inexistent (Fernandes*et al.*, 2004). Fernandes*et al.* (2004) has observed, in a study about hydropony basil cultivation on different substrates, in protected environments, that the major substances in basil essential oils were linalool, atrans-bergamoptene, germecrene-D, cubenol and g-cadinene. Linalool was the most abundant compound, both in the narrow leaf basil (44,3 to 59,8 %) and in the broad leaf basil (22,7 to 37,4 %).

In Ethiopia, the tender stems, leaves, and flowers are dried, ground, and added to sauces either alone or mixed with other spices to provide a fine flavour to stews. It is an important ingredient in berbere and shiro powders and in the preparation of clarified (spiced) butter. The dried leaves can be used for preparing roasted beef locally known as “tibs” and both dried and fresh inflorescences and leaves are used as flavoring agents in the preparation of all kinds of stew (Alemu*et al.,* 2018). On account of such justification the present study was aimed to examine physicochemical properties and biological activities of oil extracted from basil leaves and flowers.

Objectives

General Objectives

- To evaluate physicochemical properties, antioxidant and antimicrobial activities of oil extracted from basil leaves and infloresences.

Specific Objectives

- To determine essential oil yield extracted from basil leaves and infloresences;
- To determine oil quality with respect to acid value, free fatty acids and peroxide values;
- To determine the antioxidant activities of essential oil extracted from basil leaves and inflorescence;
- To determine the antibacterial and antifungal activities of essential oils with respect to minimum inhibitory concentrations and minimum bactericidal and fungicidal concentrations .

2. LITERATURE REVIEW

2.1. Botanical Description of Ocimum basilicum L.

The genus *Ocimum* L., comprises of more than 150 species and grows widely throughout temperate regions of the world (Buchbauer, 2012). Among the plants known for medicinal value, the members of genus *Ocimum*(family Lamiaceae) are very important for their therapeutic potentials. *Ocimum sanctum* L., *Ocimum gratissimum* L., *Ocimum canum*Sims, *Ocimumbasilicum*L., *Ocimum kilimandscharicum* Guerke, *Ocimum americanum* L., and *Ocimum micranthum* Willd are examples of important species of the genus *Ocimum*(Silva *et al.,* 2008). *Ocimumbasilicum*L. commonly called as Sweet Basil which belongs to family Lamiaceae is native to Indo-Malayan region. It is called the "king of herbs" which contains plenty of phytochemicals with significant nutritional as well as antioxidant capabilities and health benefits (Yayasinghe*et al.,* 2003).

Beso Bila is an erect branching herb that grows 0.3 to 1.3m high, with light green silky leaves. Its leaves are simple, opposite, 3 to 11 cm long, 1 to 6 cm wide, ovate, acute and usually toothed containing numerous oil glands which store essential oils. The infloresence of Beso Bila are white to purple in color and arranged in a terminal spike. It has long been utilized traditionally for curing a lot of troubles, such as anxiousness, stings, sickness, strong aching, gripe, pyrexia, platonic transmissions, infective diseases, headaches, coughs, acne, diarrhea, constipation, warts, worms and kidney malfunction (Lachowicz*et al*. 1996). Sweet basil is cultivated for the production of essential oils, dry leaves as a culinary herb, condiment/spice or as an ornamental plant. It is used as an ingredient in various dishes and food preparations, especially in the Mediterranean cuisine (Zheljazkov*et al.,* 2008).

Basil essential oils contain a broad array of chemical compounds depending on variations in chemo types, infloresence and leaf colors, aroma and particularly the origin of the plant. Moreover, the aromatic and morphological character of plants is greatly influenced by environmental conditions and agronomic techniques (Opalachenoiva and Obreshkova, 2003). The chemical composition of essential oils of *Ocimum* species has been well studied. Basil essential oils contained monoterpenes derivatives (camphor, limonene, 1, 8-cineole, linalool, geraniol) and phenyl propanoid derivatives (eugenol, methyleugenol, chavicol, estragole,

methyl-cinnamate) (Vina and Murillo, 2003). Different chemotypes of basil have been recognized based on the predominant essential oil constituents (e. g. linalool, methyl chavicol, methyl cinnamate, methyl eugenol, eugenol). Basil essential oil has been reported to contain various biological activities with beneficial effects on humans (*Grayer et al.,* 1996).

2.2. Chemical Compostion of Sweet Basil Essential Oils

The chemical composition of *O. basilicum*essential oil has been carried out in various parts of the world. Many authors isolated the essential oil from *O. basilicum*and reported that numerous volatile constituents. The main 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.

The composition of 18 Turkish basil essential oils was analyzed by GC and GC/MS (Telci*et al.*, 2006). Variation of essential oils in the landraces was subjected to cluster analysis, and seven different chemotypes were identified. They were linalool, methyl cinnamate, methyl cinnamate/linalool, methyl eugenol, citral, methyl chavicol (estragol), and methyl chavicol/citral. Methyl chavicol with high citral contents (methyl chavicol/citral) can be considered as a ‘‘new chemo type’’ in the Turkish basils. Methyl eugenol and methyl chavicol have a structural resemblance to carcinogenic phenylpropanoids, Chemo types having high linalool, methyl cinnamate or citral contents and a mixture of these is suitable to cultivate for use in industry. The content of methyl chavicol was 74.7%, followed by linalool 14.3% which were detected as major compounds (Saikia and Nath, 2003). The chemical variation of phenolic acids of 23 accessions of *O. basilicum*L. were studied in Iran. Morphological studies of accessions showed a high level of variability in recorded traits and showed drastic variations between accessions (Javanmardi *et al*., 2002).

2.3. Pharmacological Studies of the Essential Oils of *O. basilicum*L.

2.3.1 Antibacterial activity

Even though plenty of antimicrobial agents are commercially available due to their discriminate use in the day to day life, numerous new plant based antibiotics are emerging. In this series *Ocimum* species have excellent antimicrobial properties. To overcome the increasing resistance of disease causing bacterial strains, more effective antimicrobial agents with novel mode of action must be developed with cost effective manner. Essential oils derived from several *Ocimum* species have been reported to be active against several Gram-positive and Gram-negative bacteria due to their terpene constituents. In recent years essential oils and plant crude extracts are of certain plants have been shown to have antimicrobial effects (Manosroi*et al.,* 2006).

2.3.2. Antioxidant activity

Antioxidants are very important for reducing risk of cancer. Basil essential oils have moderate to good antioxidant properties (Manosroi*et al.,* 2006).

2.3.3. Insecticidal activity

The insecticidal activity of ocimen, cineole, linalool, methylcinnamate and methyl chavicol against stored grain insects (Triboliumcastaneum, Sitophilus oryzae, Stagobiompaniceum and *Bruchuschinensis*) was analyzed (Deshpande and Tipnis, 1997). Methyl cinnamate and methyl chavicol were found to be the most effective among these compounds.

2.3.4. Larvicidal activity

Mosquito repellant and larvicidal activities of the essential oil of *Ocimum* species was tested (Chokechaijaroeparn*et al.,* 1994) and observed that the strongest larvicidal activity of the essential oil of *O. basilicum*has shown (EC50 =81, EC90 =113) ppm. The mosquito repellent activities of essential oils from *H. Spicigera*, *S. hermonthica*and *O. basilicum*(Basil) against *Anopheles gambiae*and *Culexquinquefasciatus*was carried out under laboratory conditions. At 50% concentration, *O. basilicum*and *H.* s*picigera*oil exhibited higher repellant potential on *Anopheles gambiae*with a protection time of 183 and 120 min, respectively, while *H.*

*Spicigera*and *S. hermonthica*had a protection time of 180 and 175 min, respectively against *Anopheles gambiae.* At 100% concentration, *O. basilicum*oil exhibited the highest protection time against the two species of mosquito tested and at all the concentrations (Baba *et al*., 2012).

*O. basilicum*was equally potent against *Culexquinquefasciatus*with 180 min protection time and an LC (90) values of 23.44, 21.17 and 18.56 ppm, respectively. The basil oil was more effective natural larvicidal agents against Cx. *Tritaeniorhynchus,* Ae. *albopictus*and An. *Subpictus*(Oliveiraa*et al*., 2009).

2.3.5. Anticonvulsant effects

The possible CNS depressant and anticonvulsant effects of *O. basilicum*(access "Maria Bonita") leaf essential oil in different experimental models was analyzed and revealed the depressant CNS activity with the decrease of spontaneous activity, ptosis, ataxia, and sedation. Additionally, all doses of essential oil induced a significant increase of sleeping time ($p<0.05$) and decrease in the latency to sleep ($p<0.01$). Essential oil also increased the latency for development of convulsions in pentylenetetrazol (PTZ) and picrotoxin tests ($p<0.05$). For PTZ, the effect of essential oil was reversed by flumazenil. EO did not interfere with the convulsions induced by strychnine ($p>0.05$) (Sethi*et al.,* 2013).

2.3.6.Toxicity on Fungi

The toxicity of essential oils of twelve exotic collections belonging to nine *Ocimum*species in a pure state and four dilutions with dimethyl sulphoxide was evaluated (Sethi*et al.,* 2013). The pure oil had the maximum toxicity against *A. niger, A. flavus*Link, *F. oxysporum*Schl. ex Fries and *Penicilium* species. Another study showed that the basil essential oil exhibited different degrees of antifungal activity against the tested fungi with MIC in 125-250µg/ml (Manosroi*et al.*, 2006).

2.3.7. Cytotoxicity

The cytotoxic and apoptosis induction activity of essential oils from Thai medicinal plants on P388 (murine leukemia) and HeLa (human cervical adenocarcinoma) cell lines was evaluated.

Each cell line was treated with the concentration range of oil sample of 0.078-10.0μg/ml for 24h by MTT assay. The IC50 μg/ml on P388 and HeLa cell lines were Sweet Basil oil in 303.0μg/ml and 380.0μg/ml respectively. DNA fragmentation was detected by agarose gel electrophoresis and ethidium bromide staining method. Low molecular weight DNA fragmentation was observed at low concentration of 25 μg/ml of Sweet basil (Marotti*et al.*, 1996).

The methyl cinnamate and linalool rich basil essential oil were tested for *in vitro* cytotoxicity against the human cervical cancer cell line (HeLa), human laryngeal epithelial carcinoma cell line (HEp-2) and NIH 3T3 mouse embryonic fibroblasts by MTT assay and the results showed that the basil oil has potent cytotoxic nature and IC50 values were 90.5, 96.3 μg/ml, respectively in India. *O. basilicum*oil was less toxic to normal fibroblast (NIH-3T3) cell line with IC50 value of 120 μg/ml (Wierdak*et al.,* 2013). Further the cytotoxicity of Lamiaceae essential oils against MCF-7, LNCaP and normal fibroblast NIH-3T3 cell line was analyzed in Pakistan [12]. The IC50 values were found to be 260.3-270.7 μg/ml,170.1-172.1 μg/ml and 149.9-395.3 μg/ml respectively (Wierdak*et al.,* 2013).

2.3.8. Anti inflammatory activity

The anti inflammatory effect of *Ocimum basilicum*L. and *Ocimum gratissimum* L essential oils on xylene-induced ear edema as a model of inflammation was studied. At 50 μg/ear OBV and OGV, exhibited significant ($P<0.05$) topical anti-inflammatory effect with edema inhibitions of 50.0, 63.3, 62.7 and 80 % respectively. The effects were comparable ($P<0.05$) with that of 100 μg/ear hydrocortisone (% edema inhibition of 54.8) (Okoye*et al.,* 2014).

2.4. Nutritional Values of Sweet Basil Seeds

Sweet basil seeds are having many uses, nutritional facts and medicinal values and surprising health benefits It is used for weight management purposes (for weight loss) It is rich in fiber thus it makes your stomach full and you don’t feel hungry for a certain period of time. After soaking into water, sweet basil seeds have the capacity to expand to 30 times their original size making an excellent natural diet supplement. Sweet Basil seeds contain adequate amount of vitamin K, protein and iron which are important for hair nourishment. They are important

to make your hair healthy and shiny. Furthermore, sweet basil seeds when used along with coconut oil are effective to eliminate many skin related diseases (Copetta*et al.*, 2006). And hence they are important in ensuring healthy skin.

Sweet basil seeds also contain many polyphenolic flavonoids especially Orientin and Vicenin. These contribute to the antioxidant benefits properties of their essential oils. These compounds were tested in-vitro in the laboratory for their possible anti-oxidant protection against radiation-induced lipid per-oxidation in mouse liver. Rich in essential oils like eugenol, citronellol, linalool, limonene, citral and terpineol. These provide antibacterial and anti-inflammatory benefits (Manosroi*et al.*, 2006). High levels of beta carotene, lutein, zeaxanthin, Vitamin A and Vitamin K. These compounds help act as protective scavengers against oxygen-derived free radicals and reactive oxygen species (ROS) that play a role in aging and various disease processes. Zeaxanthin is a yellow flavonoid carotenoid compound, is selectively absorbed into the retinal macula lutea where it found to filter harmful UV rays from reaching the retina. Studies suggest that Sweet Basil Seeds which are rich in zeaxanthin anti-oxidant help to protect from *age-related macular disease* (AMRD), especially in the elderly (Manosroi*et al.*, 2006).

Basil seeds also ontain good amounts of minerals like potassium, manganese, copper, calcium and magnesium, and vitamins C and folates. Potassium is an important component of cell and body fluids, which helps control heart rate and blood pressure. Manganese is used by the body as a co-factor for the antioxidant enzyme, *superoxide dismutase.* Very good source of iron at 40% RDA per 100 grams. Iron, being a component of hemoglobin inside the red blood cells, is one of the chief determinants of oxygen-carrying capacity of the blood. Basil seeds contain 42% of carbohydrates, 20% proteins and almost 25% fats. High fiber content. 4 grams of Sweet Basil Seeds contains more fiber than an entire bulb of lettuce. Low in calories and have alpha-linolenic acid abbreviated as ALA acid. It is highly beneficial since it has Omega-3 fatty acids. High in calcium (244% of recommended daily dose (RDD)), magnesium (178% RDD), iron (499% RDD), potassium (56% RDD), folic acid (78%RDD) and vitamin E (53% of RDD) (Okoye*et al.*, 2014).

3. MATERIALS AND METHODS

3.1. Description of Study Area

The study was conducted in Bichena town that Located in Amhara Region West Gojjam Zone 256 Km Weast of Addis Ababa .the Basil Leaf and Infloresence Was Collected from home garden and dried out. Oil extraction was carried out in Animal Nutrition Laboratory, while oil quality analysis was conducted in Biotechnology laboratory in Haramaya University.

3.2. Plant Material and Extract Preparation

The sweet basil (*Ocimumbasilicum*L.) plant sample was collected from home garden,Bichena town, West Gojam, Ethiopia. The leaf and flower samples were manually washed with distilled water and residual moisture was evaporated at room temperature. Thereafter, the samples were ground in a grinder for 2 minutes, the process was stopped for 15 sec to avoid heating of sample using the standard methods of the Association of the Analytical Chemists (AOAC, 2000). Hexane was used as a solvent for oil extraction.

3.3. Research Design

The oil extraction was done in Soxhlet apparatus using hexane as a solvent. Then, physicochemical properties of the oil extract were assessed in terms of oil content, specific gravity, acid value, percent free fatty acid and peroxide value. The antioxidant activities were also investigated based on DPPH free radical and hydrogen peroxide scavenging activities . The antimicrobial experiment was arranged as 2x1x3x5x2 [2 source extracts (oil extract from leaves and inflorescence, 1 solvent system i.e. hexane,3 levels of concentration, 5 test organisms (2 bacteria: *Escherichia coli* and *Staphylococcus aureus;* three fungi *(Aspergillus versicolor, A. Niger, Candida albicans*), 2 number of replication]in a completely randomized factorial design with two replications. The antimicrobial activities were determined using disc diffusion method and broth dilution method. In addition, the minimum inhibitory concentration (MIC),the minimum bactericidal concentration (MBC) and the minimum fungicidal concentrations (MFC).were determined for each test Pathogen following standard methods.

3.4. Extraction of Essential Oils

Extraction of the essential oils was done by solvent extraction method in soxhlet apparatus using hexane as a solvent. For this purpose two flasks were kept in hot air oven for 2 hours. Then the flasks were kept in desiccators for 30 min to cool at room temperature. 20gm of powdered sweet basil leaf and flower samples were dissolved in 120ml hexane solution and kept in Soxhlet apparatus for 8 hours. The crude extract was concentrated in water bath by adding sodium sulfate.

3.5. Data Collection

Data were recorded for oil yield, specific gravity, acid value, free fatty acid value, peroxide value, DPPH and hydrogen peroxide scavenging activities, ascorbic acid(vitamin C), and antimicrobial activities including zone of inhibition, MIC, MBC and MFC values.

3.5.1. Determination of the Essential Oil Yield and Specific Gravity

The % oil yield of the sample was determined as:

$$\text{Essential Oil yield} = \frac{\text{oil weight (OW)}}{\text{sample weight (SW)}} \times 100$$

Where, oil weight= W2-W1: W1= Weight of the extraction flask (g); W2=Weight of the extraction flask plus the dried crude fat (g).

Specific gravity: the specific gravity of the oil was determined gravimetrically by employing the weight ratio of the oil to the equivalent amount of water according to the following formula: Specific gravity = $\frac{\mathbf{W2}}{\mathbf{W1}}$

Where, W2 and W1 are the weights of oil and equivalent amount of water, respectively.

3.5.2. Determination of Acid Value

The acid value was determined as per AOAC (1990) method. Briefly 2g of Essential oil sample was weighed into a 250ml conical flask and then, 25ml diethyl ether mixed with 25ml alcohol and 1ml of 1% phenolphthalein indicator were added to the mixture. The conical flask

was then placed on a hot water bath until the oil was completely dissolved in the solvent. The hot solution was then titrated with 0.1M KOH until a pink colour which persisted for 15 seconds was noticed. The acid value was calculated as:

$$\text{Acid value} = \frac{\text{Titre (ml)x 5.61}}{\text{Weight of sample used}}$$

Acid value was expressed as mg KOH /g of oil.

3.5.3. Estimation of Free Fatty Acid

The percentage free fatty acid (%FFA) was estimated by multiplying the acid value with the factor 0.503. Thus, %FFA = 0.503×Acid value.

3.5.4. Determination of Peroxide Value

To a weighed sample (1.0g) in a flask, powdered potassium iodide (1.0g) and solvent mixture (2: 1, glacial acetic acid:chloroform v/v) were added. The resulting solution was then placed on a water-bath to dissolve properly and 5% potassium iodide ($20cm^3$) was then added. The sample solution was then titrated with 0.002N sodium thiosulphate using starch as an indicator. The peroxide value of the samples was calculated using the following equation (Nielsen, 2002).and is expressed either in milliequvalents of peroxide Kg or millimoles of peroxide/L.

PV = 2 X V

Where: PV = Peroxide value, V = Volume of Sodium thiosulphate used, 2 = (N x 1000) / W,

N = Normality of Sodium thiosulphate used, W = Weight of sample used.

3.5.5. DPPH Radical Scavenging Activity

The DPPH radical scavenging activity (RSA) of the essential oil extract is used to measure antioxidant activity using the DPPH (2, 2- diphenyl-1-picrylhydrazyl) method(Loizzo*et al.*, 2016)**.** Briefly, 2 mL of essential oil extract (100µg/ml) was added to 2mL of DPPH (0.1 mM) solution. The mixtures was kept aside in a dark area for 30 min and absorbance was measured at λmax 517 nm against an equivalent amount of DPPH and methanol as a standard. The

percentage of DPPH radical scavenging activity DPPH- (RSA %) was estimated using the equation:

$$\text{DPPH radical scanveging activity}(\%) = \frac{(A_{control} - A_{sample})}{A_{control}} x100$$

where $\mathbf{A_{control}}$ is the absorbance of the standard solution and $\mathbf{A_{sample}}$ is the absorbance in the presence of the sample.

3.5.6. Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide radical scavenging activity of the essential oil extract was determined using the H_2O_2 method (Bozin*et al.*, 2008). Briefly, 2 mL of oil extract solution (100µg/mL) was added to 4.0 mL of H_2O_2 (20 mM) solution in phosphate buffer (pH 7.4). After 10 min, the absorbance was measured at λ_{max} 230 nm against the phosphate buffer control solution. The percentage scavenging of H_2O_2 was calculated using the equation:

$$\% \text{ scavenging of } H_2O_2 = \frac{(A_{control} - A_{sample})}{A_{control}} x100$$

Where $A_{control}$ = absorbance of the blank (phosphate buffer with H_2O_2) and A_{sample} = absorbance of the oil sample.

3.5.7. Ascorbic Acid Analysis

The ascorbic acid content was determined using the 2, 6- dichlorophenol indophenol (DCPIP) dye method (AOAC, 2000). Accordingly, 5ml of the standard ascorbic acid solution was pipetted into a 100 ml conical flask and then 5ml of 3% HPO_3 solution was added. The ascorbic acid solution was titrated with the dye solution to a pink colour, that persisted for 15sec. The titre value was recorded. Dye factor was expressed as mg of ascorbic acid per ml of the dye. Since 5ml of the standard ascorbic acid solution contains 0.5 mg ascorbic acid,

$$\text{Dye factor (mg ascorbic acid per ml of dye)} = \frac{0.5mg}{\text{titrant volume}}$$

One ml of the extracted essential oil was diluted to 5ml with 3% metaphosphoric acid in a 50 ml volumetric flask. The aliquot was then centrifuged (Model, Z300, 580W, 3052 Nm,

German) for 15 minutes and titrated with the standard dye to a pink end point (persisting for 15 seconds). The ascorbic acid content was calculated from the titration value, dye factor, dilution and volume of the sample as:

$$\% \text{ AA} = \frac{(\text{ABRsample})\text{x dye factor x volume of initial test solution}}{\text{volume of test solution titrated}} \text{ x } 100\%$$

Where: AA=Ascorbic Acid; ABR= Average Burette reading

3.6. Antimicrobial activity of the Essential oil extracts

The experiment was arranged in 2x1x5x3 factorial design. A completely randomized design (CRD) was used to determine the antimicrobial activities using disc diffusion and broth dilution methods. In addition, the least concentration of oil extract that showed microbial inhibition was considered as the MIC and used for further determination of the minimum bactericidal concentration (MBC) and minimum fungicidal concentrations (MFC).

3.6.1. Test Pathogens

Five test pathogens including two bacteria: *Escherichia coli* and *Staphylococcus aureus;* three fungi *(Aspergillus versicolor, A. niger and Candida albicans)* were obtained from Ethiopian Public Health Institution. The fungal and bacterial pathogens were subcultured and maintained on Potato Dextrose Agar (PDA) and Nutrient Agar, respectively. Then, the fungal and bacterial cultures were incubated for 72 h at 27 ºC and for 18-24 h at 37 ºC, respectively.

3.6.2. Media Preparation and Standardization of Inoculum

Nutrient Agar (NA), Potato Dextrose Agar (PDA), and Muller Hinton agar (MHA) were used for sub-culturing of bacterial test pathogens, fungal test pathogens, and determination of antimicrobial activities, respectively. These media were prepared and sterilized using an autoclave according to the manufacturers' instructions. Two to three bacterial colonies on the plate were picked up with a sterile inoculating loop and transferred into a test tube containing sterile normal saline and vortexed thoroughly. The spores of the test fungi were harvested by washing the surface of the fungal colony using 5mL of sterile saline solution. This procedure was repeated until the turbidity of each bacterial and fungal spore suspension matched the

turbidity of 0.5 McFarland Standards as described by the Clinical Laboratory Standards Institute (CLSI, 2015). The resulting suspension was used as inoculum for the pathogens in the antimicrobial susceptibility test.

3.6.3. Disc diffusion Method

Discs of 6 mm diameter was prepared from sterile filter paper cut into small, circular pieces of equal size by a perforator and impregnated each with 0.1 ml of the prepared test extract. The extract impregnated discs were placed onto MHA plates evenly inoculated with test pathogens (Hudzicki, 2009).

3.6.4. Inoculation of Mueller Hinton Agar (MHA) plates

After adjusting the turbidity of the suspension of inoculum within 15 minutes, a sterile cotton swab was dipped into adjusted suspension and rotated several times by pressing firmly on the inside wall of the tube above the fluid level. This removes excess fluid from the swab. Then, the dried surface of Mueller Hinton Agar plates were inoculated by streaking using the swab three times over the entire surface and rotating the MHA plates approximately 60° each time to ensure an even distribution of the inoculum. Then, the MHA plates were left open for three to five minutes to allow for any excess surface moisture to be absorbed (CLSI, 2012).

Following this step, the impregnated discs were dispensed onto the surface of the inoculated agar plates using sterile forceps. Each disc was pressed down to ensure complete contact with the agar surface. The discs were distributed evenly so they were not any closer than 24 mm from center to center (CLSI, 2015). Discs of commercial ampicillin (1µl/disc) and ketokonazole (1µl/disc) were used as positive controls for bacterial and fungal pathogens, respectively and the pure solvent (hexane) impregnated discs were used as negative controls.

Then the MHA plates were sealed with parafilm and incubated at 37°C for 24 hrs and 27°C for 72 hrs for bacterial and fungal pathogens, respectively. After incubation, the diameters of the zone of inhibition around each disc were measured to the nearest millimeter along two axes (i.e. 90° to each other) using a transparent ruler and the means of the two readings were recorded. For each selected pathogen the experiment was carried out in three replications.

3.6.5. Determination of Minimum Inhibitory Concentration (MIC)

The oil extracts that showed significant antimicrobial activity in the antimicrobial activity tests were selected for determination of MIC based on the method used by Morshed*et al* (2012) with slight modifications. The MIC of the oil extracts were determined by broth dilution method. In the broth dilution method, the oil extract solution for example at 1µl/ml was serially diluted in a two-fold dilution as 1 µl/ml, 0.50 µl/ml, and 0.25 µl/ml, 0.125 µl/ml, 0.0625 µl/ml concentrations. Two mililiter of nutrient broth and potato dextrose broth for bacteria and fungi, respectively, were added into all test tubes and 0. 1 ml of the prepared concentration of each oil extract was mixed with the nutrient broth and potato dextrose. Thereafter, standardized inoculums of 0.1 ml of the respective test pathogens were dispensed into the test tubes containing the suspensions of the broth and the oil extract. Then, all test tubes were properly corked and incubated at 37°C for 24 hrs for bacteria and 27°C for 72 hrs for fungi. After that, they were observed for absence or presence of visible growth. The lowest concentration at which no visible growth of organisms was regarded as the MIC. The experiment was carried out for each test organism in triplicates.

3.6.6. Determination of Minimum Bactericidal (MBC) and Fungicidal Concentrations (MFC)

For the determination of the MBC and MFC, fresh nutrient agar and potato dextrose agar plates were inoculated with one loop full of culture taken from each of the broth cultures that showed no growth in the MIC tubes. That is MBC/MFC values were determined by subculturing from respective MIC values if for example MIC=0.50 µl/ml (v/v) subculturing was performed as 0.50 µl/ml, 1.00 µl/ml, 1.50 µl/ml, 2.00 µl/ml up to four acceptable concentration levels. Since antibacterial agents are usually regarded as bactericidal if the MBC is no more than four times the MIC (CLSI, 2012). MBC/MFC is the amount of the extract that kills microbial growth. While MBC assay plates were incubated for 48 h, MFC assay plates were incubated for 3 days. After the incubation periods, the lowest concentration of the extract that did not allow any bacterial or fungal growth on solid medium was regarded as MBC and MFC for the extract (CLSI, 2015). This observation was matched with the MIC test tube that did not show evidence of growth after 48 h of incubation for bacteria or spore

germination after 3 days of incubation for fungi. ketokonazole (1µl/disc) disc was applied as positive control for incubation of fungi while ampicillin (1µl/disc) was served as positive control for bacterial pathogens.

3.7. Data Analysis

The experimental data were subjected to analysis of variance (one-way ANOVA) using SPSS for windows version 20 to investigate statistical significanct difference between the different essential oil quality parameters. Differences between means were considered statistically significant at $P< 0.05$.

4. RESULTS AND DISCUSSION

4.1. Physicochemical Properties of Essential oils from Sweet Basil (*Ocimum basilicum*L.) Leaf and Infloresence.

Physicochemical properties of *Ocimum basilicum*(L)leaf and infloresence oil extracts were determined with respect to essential on oil yield, specific gravity, acid value, free fatty acids and peroxide values as shown in Table 1. Data shows that Significant differences between leaf and infioresence essential oil extracts were obtained for oil yield, specific gravity, acid value (ACV), and free fatty acid values.However, there was no significant difference in peroxide values despite the fact that it is higher for leaf oil extract. Significantly higher oil yieldwas observed for leaf (62.50%) than inflorescence (48.50%). The essential oil is mainly concentrated in the leaves and that the oil amount in the branches is almost insignificant (May *et al*., 2008).

The major physicochemical properties of basil essential oil vary extensively, dependingon genetic factors, geographical origins, nutritional status,the extracted plant parts (stem, leaf, and flower), and the extraction methods used (Rattanachaikunsopon and Phumkhachorn, 2010). However, because of the variationsof the plant and the essential oil composition, several chemotypes have been described with the basic components of linalool and estragole, either alone or in the form of a mixture, as well as in combinations with linalool and eugenol or estragole and methyl eugenol (Villalobos-Pascualand Acosta-Ballesta, 2003).

Table 1. Physicochemical properties of sweet basil (*Ocimum basilicum*L.) leaf and flower oils

Source of Oil extract	Oil yield	Spgr	ACV	FFA	PV(meq of peroxide /Kg
Infloresence	48.50±2.12b	0.69±0.03b	4.35±0.20a	2.19±0.42a	2.30±0.10a
Leaf	62.50±3.53a	0.89±0.05a	3.09±0.40b	1.55±0.20b	2.90±0.14a

Means followed by same letter within a column were not significantly different at 0.05 probability level based on LSD (Least Significance difference) test. within Spgr: specific gravity; ACV: acid value; FFA: free fatty acids; PV: peroxide value.

Physical properties of lipids derive directly from their chemical structures and functional groups and greatly influence the functions of lipids in foods and the methods required for their manipulation and processing. They can also be used to assess the purity or quality of lipid materials in reference to known standards or preferred characteristics (Nichols and Sanderson, 2003).Specific gravity is a parameter used to identify, measure concentration and confirm purity of substances. In the present study, the values of specific gravity were less than 1, indicating that the oil is less dense than water suggesting that the oil composed of light molecular weight components and therefore volatile.

Moreover In the present study the contents ofacid value (4.35mg KOH/g for infloresence oil and 3.09mg KOH/g for leaf oil), free fatty acids for leaf oil (1.55% as oleic acid) and for flower oil (2.19%), and peroxide value for flower oil (2.30 meq O2/kg oil) and leaf oil (2.90meq O2/kg oil) (Table 1), the extracted sweet basil leaf and flower oils had an acceptable initial quality. The Codex Alimentarius Commission expressed the permitted maximum acid values of 10 and 4mg KOH/g oil for virgin palm and coconut oils, respectively (Alfawaz, 2004). It has been shown that oils become rancid when the peroxide value ranges from 20.0 to 40.0meq O2/kg oil (Ajayi*et al.*, 2006). On the other hand, according to the CodexAlimentarius Commission, the peroxide value for unrefined olive oil may be maximum 20 meq/kg oil (Markovic andBastic, 1975). Therefore, considering that the oil studied was unrefined and its initial quality indicators were within the reported limits, the sweet basil oils can be regarded as an edible oil with good quality.The acid value may be overestimated if other acid components are present in the system, e.g. amino acids or acid phosphates.

4.2. Antioxidant Activities of *Ocimum basilicum*(L.) Leaf and Infloresence Essential Oils

In this study Ocimum basilicum (L) leaf oil extracts showed significantly higher antioxidant activities with respect toDPPH radical scavenging activities (86.45) However, significantly higher ascorbic acid content (13.42%) and hydrogen peroxide scavenging activity (5.50)were observedfor *O.basilicum* flower oil extract.(Table 2). The higher DPPH radical scavenging activity (86.45±0.07) indicates higher antioxidant activities and the presence of higher essential omega-3 fatty acids in *O. basilicum*(L.) leaf oil. The antioxidant activities of leaf oil was found to be significantly higher than flower oil extract in *O.basilicum* indicating that leaf

oil might possess better biological activities, oil quality and pharmacological applications. This finding was in agreement with Eriotou *et al*. (2015) who reported that there is no strong correlation between antimicrobial and free radical scavenging activities

Table 2. Antioxidant activities of sweet basil (*Ocimum basilicum*L.) leaf and infloresence

Source of Oil extract	DPPH radical scavenging activity	HPSA	AA
Infloresence	1.95±0.07b	5.50±1.27a	13.42±2.23a
Leaf	86.45±0.07a	2.35±0.07b	8.28±1.08b

Means followed by same letter within a column were not significantly different at 0.05 probability level based on LSD (Least Significance difference) test DPPH: 2, 2- diphenyl-1-picrylhydrazyl; HPSA: hydrogen peroxide scavenging activity; AA: ascorbic acid.

Antioxidants refer to any substances in attendance at stumpy concentration infood stuffs and capable to significantly averting oxidation by playing a responsibilityin antioxidation as a free radical scavenger, chelator, reducing agent, and/or singlet oxygen scavenger (Cozzi *et al*., 1997). Only a few spices are allowed as food additives by the law because of their toxicity effects and other side effects afar registered synthetic antioxidants. Butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT),tertiary butylatedhydroquinone (TBHQ) and pueraria glycoside (PG) are typicalantioxidants permitted as the food additives. There is a need for food scientists to fervorof seeking natural antioxidants from the various sources because harms of human health caused by food additives (Dorman *et al*., 2008). It is emphasized that use of spices and herbs as antioxidants is a promising alternative to the use of synthetic antioxidants. Chemical constituents having antioxidant activity arefound in high concentration in plants. The benefits resulting from the use of naturalproducts rich in bioactive substances has promoted the growing interest of food industries(El-Ghorab *et al*., 2008). Herbs and spices are one of the important sources for search of natural antioxidants from safety point of view (Nadeem *et al*., 2013).

4.3. Antimicrobial Activities of Sweet Basil (*Ocimum basilicum*L.) Leaf and Infloresence oils

The antimicrobial activity of the oil extracts was evaluated against two bacteria: *E. coli* (gram negative), *S. aureus* (gram positive),and three fungal pathogens including *Aspergillus versicolor, A. niger*and *Candida albicans* by the disc diffusion assay. All test microbes were susceptible to all tested oil extracts, although the degree of inhibition varied among the oil extracts (Table 3).The mean zone of inhibition at highest concentration (3µl/ml) against bacterial test pathogens ranged from 12.50±0.50 mm to 15.47±0.50mm, while 11.97±0.45mmto 15.90±0.85mm against fungal test pathogens.

The strongest antibacterial activity with maximum zone of inhibition (15.47mm) at highest concentration (3µl/ml)of the oil was recorded for *O. basilicum* (L.) flower oil extract against *S. aureus* while the weakest antibacterial activity (12.50mm) was observed for leaf oil against *E. coli* indicating that *S. aureus* was more susceptible than *E. coli*. Hence flower oil has exhibited more antibacterial potential than leaf oil in sweet basil (*O. basilicum* L.). On the other hand, the strongest antifungal activity with maximum zone of inhibition (15.90mm) was recorded for leaf oil against *C. albicans* and the weakest antifungal activity with minimum zone of inhibition (11.97mm) was recorded for flower oil against *A. niger* indicating that leaf oil extract had more effective antifungal potential than leaf oil extract in *O. basilicum* (L.). Similar study was conducted by Sakkas and Papadopoulou (2017) suggested the basil essential oil's antibacterial activityis attributed to its high content in linalool and estragole,whereas the antimicrobial spectrum is restricted to specificbacteria (*Staphylococcus spp., Enterococcus spp., E. coli, P. aeruginosa, A. baumannii, A. hydrophila, B. cereus, Bacillussubtilis, Enterobacter spp., Listeria spp., Proteus spp., Salmonella spp., Serratiamarcescens, and Y. enterocolitica*) and fungi(*Candida spp., Rhodotorula spp., and Saccharomyces cerevisae*).Basil oils exhibitedsufficient to moderate effects against multi-resistant clinical isolates of *A. baumannii, E. coli, K. pneumoniae*, and *P. aeruginosa* (Sakkas *et al*., 2016).

Table 3. Antimicrobial activities essential oil extracts from sweet basil (*Ocimum basilicum*L.) leaf and flowers as mean diameter of zone of inhibition against test pathogenic microbial spp.

Test Pathogens	oil extract	Concentrations of the oil extract (v/v)			Ampicillin (1µl/ml)
		1µl/ml	2µl/ml	3µl/ml	
E. coli	Infloresence	9.47±0.50bD	12.03±0.55aC	14.57±0.40abB	18.87±0.32aA
E. coli	Leaf	0.00±0.00cD	8.77±0.80cC	12.50±0.50cB	19.03±0.47aA
S. aureus	Infloresence	10.57±0.51aD	12.60±0.36aC	15.47±0.50aB	18.90±0.26aA
S. aureus	Leaf	9.67±0.35bC	10.77±0.25bC	14.50±0.50bB	18.40±0.40aA
					Ketokonazole (1µl/ml)
A. versicolor	Infloresence	9.40±0.36bD	11.70±0.26bcC	14.03±0.45bB	18.00±0.50aA
A. versicolor	Leaf	10.10±0.35abD	12.00±0.50abC	15.50±0.40aB	17.33±0.76aA
A. niger	Infloresence	6.60±0.53dC	10.83±0.76cB	11.97±0.45cB	17.83±0.29aA
A. niger	Leaf	8.60±0.53cD	11.60±0.05bcC	13.50±0.25bB	17.33±0.29aA
C. albicans	Infloresence	10.00±0.47abC	12.00±0.46abB	12.50±0.22cB	17.50±0.50aA
C. albicans	Leaf	10.77±0.25aD	12.83±0.76aC	15.90±0.85aB	17.67±0.28aA

Means followed by same small letters within a column were not significantly different at 0.05 probability level based on LSD (Least Significance difference) test.Same Capital letters cross the raw show no significant difference. *E. coli*: *Escherichia coli; S.aureus*: *Staphylococcus aureus; A. versicolor: Aspergillus versicolor.C.albicans;Candida albicans*

4.4. Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC),and Minimum Fungicidal Concentration (MFC) of Oils from Sweet Basil (*Ocimumbasilicum*L.) Leaf and Infloresence

The effectiveness of the antimicrobial activities of *O. basilicum* (L.) leaf and flower oil extracts was assessed by MIC, MBC and MFC (Table 4). The strongest bactericidal activity with MIC (0.09µl/ml) and corresponding MBC (0.19 µl/ml) was exhibited by the flower oil extract against *S. aureus* while the weakest bactericidal activity with MIC (0.38 µl/ml, the largest value) and MBC (0.75 µl/ml) was recorded for leaf oil extract against *E. coli* indicating that *S.aureus* is more susceptible to the oil extract than *E.coli. Thus,* Infloresence oil extract possesses stronger antibacterial potential than leaf oil extract in sweet basil (*O. basilicum* L.).

Table 4. MIC, MBC, and MFC of sweet basil (*Ocimumbasilicum*L.) leaf and infloresence

Pathogens	Oil extract	MIC(µl/ml)	MBC/MFC (µl/ml)
E. coli	infloresence	0.19	0.38
E. coli	Leaf	0.38	0.75
S. aureus	infloresence	0.09	0.19
S. aureus	Leaf	0.12	0.25
A. versicolor	infloresence	0.25	0.50
A. versicolor	Leaf	0.19	0.38
A. niger	infloresence	0.63	1.25
A. niger	Leaf	0.38	0.75
C. albicans	Inflorescence	0.12	0.25
C. albicans	Leaf	0.09	0.19

Contrastingly, *O. basilicum* (L.) leaf oil extracthas demonstrated strongest antifungal activity with MIC (0.125µl/ml, the least value) and MFC (0.09µl/ml) against *C. albicans*whereas the weakest antifungal activity with MIC (0.63µl/ml) and MFC (1.25µl/ml) was obtained for the flower oil extract against *A.niger*showing that*C. albicans* was the most susceptible to the oil extract, while*A. niger*was the most resistant to the oil extract, and the leaf oil was more effective antifungal potential than the inflorescence oil in *O. basilicum*(L.). Similar study was conducted by Eriotou et al (2015). who demonstrated the measurement of minimum inhibitory concentration (MIC) of the EOs have variable levelsof inhibition. The lowest MIC value (0.039 ml/100 ml) was found for the small-leaved basil EO against *B. cereus* 10876 and the red basil EO against *M. luteus*49732, *B. cereus* 14579 and *B. cereus* 10876.

The essential oils, particularly those rich in phenolics,have the potential to alter both the permeability and the function of the cell membrane proteins by penetrating in to the phospholipids layer of the bacterial cell wall, binding to proteins and blocking their normal functions. Because of their lipophilic nature, essential oils and their compound scan influence the percentage of unsaturated fatty acids and their structure. However, because of the variety of molecules present in plant extracts, their antimicrobial activity cannot be accredited to a single mechanism but to a number of diverse mechanisms at various sites of the bacterial cell outer and inner components, affecting the functions of cell membrane, cytoplasm, enzymes, proteins,fatty acids, ions, and metabolites (Sakkas and Papadopoulou, 2017).Therefore, considering the currenturgent need for new antimicrobials, it is imperative for the plant essential oils and their constituents to be further investigated with regard to their potential as antimicro bialagents.

5. SUMMARY, CONCLUSION AND RECOMMENDATION

5.1. Summary

Basil (*Ocimum basilicum* L.) also known as sweet and garden basil is the most common species in Lamiaceae family (mint family). Microbiological hazards that brings about the foodborne diseases being increasingly important public health problems. The emergence of increased multidrug resistant bacteria has initiated development of new antimicrobial agents.Essential oils from aromatic plants) and herbal extracts have attracted scientific interest due to their potential use as antimicrobial and/or antioxidant compounds in food.Basil (*Ocimum basilicum* L.) is an aromatic plant used extensively to add aroma and flavor in food. Traditionally, basil has been used as a medicinal plant.

In Ethiopia, the tender stems, leaves, and flowers are dried, ground, and added to sauces either alone or mixed with other spices to provide a fine flavour to stews. It is an important ingredient in berbere and shiro powders and the preparation of clarified (spiced) butter. The dried leaves can be used 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 kinds of stew. On account of such justification the present study was aimed to examine physicochemical properties and biological activities of oil extracted from basil leaves and flowers.

The oil extraction was done in Soxhelt apparatus using hexane as a solvent. Then, physicochemical properties of the oil extract were assessed based on oil content, specific gravity, acid value, percent free fatty acid and peroxide value. The antioxidant activities were investigated based on free radical scavenging activities of DPPH and hydrogen peroxide. The antimicrobial experiment was arranged as 2x1x5 [2 source extracts (oil extract from leaves and flowers of sweet basil at three concentration levels), 1 solvent system i.e. hexane, 5 test organisms (2 bacteria: *Escherichia coli* and *Staphylococcus aureus;* three fungi *(Aspergillus versicolor, A. Niger, Candida albicans*)] completely randomized factorial design in two replications. The antimicrobial activities using disc diffusion method and broth dilution method. In addition, the least concentration of extract that show antimicrobial activity was

selected for further determining the minimum inhibitory concentration (MIC): minimum bactericidal (MBC) and minimum fungicidal concentrations (MFC).

Physicochemical properties of *Ocimum basilicum*(L)leaf and infloresence presented significance differences between leaf and infloresence oil extracts were obtained for oil yield, specific gravity, acid value (ACV), and free fatty acid values. Significantly higher oil yield was observed for leaf oil (62.50%) than fruit oil (48.50%).

The antioxidant activities of *Ocimum basilicum* (L) leaf and flower oil extracts recorded significantly higher antioxidant activities with respect to DPPH(86.45%)for leaf oil extracts. However, significantly higher ascorbic acid content (13.42%) and hydrogen peroxide scavenging activity (5.50) were observed for *O.basilicum* flower oil extract.

The antimicrobial activity of the oil extracts was evaluated against two bacteria: *E. coli* (gram negative), *S. aureus* (gram positive), and three fungal pathogens including *Aspergillus versicolor, A. niger*and *Candida albicans* by the disc diffusion assay. All test microbes were susceptible to all tested oil extracts, although the degree of inhibition varied among the oil extracts.

The mean zone of inhibition at highest concentration (3µl/ml) against bacterial test pathogens ranged from 12.50±0.50 mm to 15.47±0.50mm, while 11.97±0.45mm to 15.90±0.85mm against fungal test pathogens. The strongest antibacterial activity with maximum zone of inhibition (15.47mm) at highest concentration (3µl/ml) of the oil was recorded for *O. basilicum* (L.) infloresence oil extract against *S. aureus* while the weakest antibacterial activity (12.50mm) was observed for leaf oil against *E. coli* indicating that *S. aureus* was more susceptible than *E. coli*. Hence flower oil has exhibited more antibacterial potential than leaf oil in sweet basil (*O. basilicum* L.). On the other hand, the strongest antifungal activity with maximum zone of inhibition (15.90mm) was recorded for leaf oil against *C. albicans* as the weakest antifungal activity with minimum zone of inhibition (11.97mm) was recorded for infloresence oil against *A. niger* indicating that leaf oil extract was more effective antifungal potential than leaf oil extract in *O. basilicum* (L.).

The strength of the antimicrobial activities of *O. basilicum* (L.) leaf and infloresence oil extracts was assessed by MIC, MBC and MFC. The strongest bactericidal activity with MIC

(0.09µl/ml) and corresponding MBC (0.19 µl/ml) was exhibited by the infloresence oil extract against *S. aureus* while the weakest bactericidal activity with MIC (0.38 µl/ml, the largest value) and MBC (0.75 µl/ml) was recorded for leaf oil extract against *E. coli* indicating that *S.aureus* is more susceptible to the oil extract than *E. coli. Thus,* flower oil extract possesses stronger antibacterial potential than leaf oil extract in sweet basil (*O. basilicum* L.).

Ocimumbasilicum (L.) leaf oil extract has demonstrated strongest antifungal activity with MIC (0.125µl/ml, the least value) and MFC (0.09µl/ml) against *C. albicans* where as the weakest antifungal activity with MIC (0.63µl/ml) and MFC (1.25µl/ml) was obtained for the flower oil extract against *A.niger*showing that*C. albicans* was the most susceptible to the oil extract, while*A. niger*was the most resistant to the oil extract, and the leaf oil was more effective antifungal potential than the inflorescence oil in *O. basilicum*(L.).

5.2. Conclusion

The higher DPPH value of leaf oil extract indicates higher antioxidant activities and the presence of higher essential omega-3 fatty acids in *O. basilicum*(L.) leaf oil. The antioxidant activities of leaf oil was found to be significantly higher than infloresence oil extract in *O. basilicum* indicating that leaf oil might possess better biological activities, oil quality and pharmacological applications.The strongest antibacterial activity with maximum zone of inhibition was recorded for *O. basilicum* (L.) infloresence oil extract against *S. aureus* while the weakest antibacterial activity was observed for leaf oil against *E. coli* indicating that *S. aureus* was more susceptible than *E. coli*. Hence infloresence oil has exhibited more antibacterial potential than leaf oil in sweet basil (*O. basilicum* L.). The strongest antifungal activity with maximum zone of inhibition was recorded for leaf oil against *C. albicans*asthe weakest antifungal activity with minimum zone of inhibition was recorded for infloresence oil against *A. niger*indicating that leaf oil extract was more effective antifungal potential than leaf oil extract in *O. basilicum* (L.).The results obtained indicate that these essential oils have antioxidant, antibacterial and antifungal activity and can be used as natural antioxidant and antimicrobial agents in medicine, food industry and cosmetics.

5.3. Recommendation

- The major components of basil oil vary extensively, depending on genetic factors, geographical origins, nutritional status, the extracted plant parts (stem, leaf, and infloresence), and the extraction methods and solvent used. Thus further studies are required to obtain more reliable results using more efficient conduct more efficient oil extraction methods.
- Further studies are also needed to assess the effect of genotypes and locations on biological activities of the oil extracts.
- The antimicrobial activities of sweet basil oils and other extracts obtained from different *Ocimum spp* need to be studied;
- The antimicrobial potential of the oil extracts need to be optimized with various types of Gram-positive and Gram-negative bacteria.
- Toxicity of the extracted oils must be evaluated using model test with model animal (most often with rats) .
- Studies also need to be conducted to correlate the antimicrobial activities with the essential oil chemistry.

6. REFERENCES

Ajayi, I. A., Oderinde, R. A., Kajogbola, D. O. and Uponi, J. I. 2006. Oil Content and Fatty Acid Composition of Some Underutilized Legumes from Nigeria. *Food Chem.* 99:115–120.

Alemu A., Garedew W., Gebre A. 2018. Essential oil yield and yield components of basil (*Ocimumbasilicum*L.) as affected by genotype and intrarow spacing at Jimma, SW Ethiopia. *ActaAgrobot.* 71(3):1743.

ALfawaz, M. A. 2004. Chemical Composition and Oil Characteristics of Pumpkin (*Cucurbita Maxima*) Seed Kernels. Res. Bult., No. (129), *Food Sci. Agric. Res.* Center, King Saud Univ. 5–18.

Alves-Silva J. M., Dias dos Santos S. M. , Pintado M. E. , Pérez-Álvarez J. A., Fernández-López J. *et al.* 2013. Chemical composition and *in vitro* antimicrobial, antifungal and antioxidant properties of essential oils obtained from some herbs widely used in Portugal. *Food Control* 32: 371-378.

AOAC(1990). *Official Methods of Analysis*, 15th ed. Association of Official Chemists' Washington DC.

AOAC, (2000). Official Methods of Analysis of the AOAC International (2000). 7th Edition, Volume II. Gaithersburg, Maryland, USA.

Baba G., Lawal AO, Shariff HB. 2012. Mosquito repellent activity and phytochemical characterization of essential oils from *Strigahermonthica, Hyptisspicigera*and *Ocimumbasilicum*Leaf Extracts. *Br J Pharm Toxicol,* 3:43-8.

Banchio, E., Bogino, P. C., Zygadlo, J., & Giordano, W. 2008. Plant growth promoting rhizobacteria improve growth and essential oil yield in *Origanummajorana*L. *Biochemical Systematics and Ecology*, 36: 766-771.

Botsoglou, N. A., Christaki, E., Florou-Paneri, P., Giannenas, I., Papageorgiou, G., Spais, A. B. 2004. The effect of a mixture of herbal essential oils or _- tocopheryl acetate on performance parameters and oxidation of body lipid in broilers, *S. Afr. J. Anim. Sci.*, 34: 52–61.

Botsoglou, N. A., Florou-Paneri, P., Christaki, E., Fletouris, D. J., and Spais, A. B. 2003. Inhibition of lipid oxidation in long-term frozen stored chicken meat by dietary

oregano essential oil and á-tocopheryl acetate supplementation, *Food Res. Int.,* 36: 207–213.

Bozin, B.; Mimica-Dukic, N.; Samojlik, I.; Goran, A.; Igic, R. 2008. Phenolics as antioxidants in garlic (*Allium sativum* L., Alliaceae). *Food Chem*. 111: 925–929.

Bozkurt, M., Kucukyilmaz, K., Pamukcu, M., Cabuk, M., Alcicek, A., and Catli, A. U. 2012. Long-term effects of dietary supplementation with an essential oil mixture on the growth and laying performance of two layer strains, *Ital. J. Anim. Sci.,* 11: 23–28.

Brenes, A. and Roura, E. 2010. Essential oils in poultry nutrition: main effects and modes of action, *Anim. Feed. Sci. Technol.,* 158: 1–14.

Buchbauer G. 2012. A review on recent research results (2008-2010) on essential oils as antimicrobials and antifungals. A review. *Flavour Frag J* 2012;27:13-39.

Charles, D. J.; Simon, J. E. 1990. Comparison of extraction methods for the rapid determination of essential oil content and composition of basil (*Ocimum*spp.). *J. of the American Society for Horticultural Science,* 115(3): 458-462.

Chokechaijaroeparn O, Bunyaprahatsava N, Kongchensin S. 1994. Mosquito repellent activities of Ocimum volatile oils. *Phytomed,* 1:135.

Clinical and Laboratory Standards Institute (CLSI) (2015). Document M45. Methods for Antimicrobial Dilution and Disk Susceptibility of Infrequently Isolated or Fastidious Bacteria; Approved Guideline. Third Edition. CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA.

Clinical and Laboratory Standards Institute (CLSI, 2012). Performance standards for antimicrobial disk susceptibility tests; approved standards – Eleventh Edition. CLSI document M02-A11. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, PA, 19087 USA, 2012.

Copetta, Andrea; Lingua, G; Berta, G; *et al.* 2006. "Effects of three AM fungi on growth, distribution of glandular hairs, and essential oil production in Ocimumbasilicum L. var. Genovese". *Mycorrhiza*. 16(7): 485–494.

Cozzi, R., Ricordy, R., Aglitti, T., Gatta, V., Petricone, P. and De-Salvia, R. (1997), "Ascorbic acid and beta-carotene as modulators of oxidative damage", *Carcinogenesis,* 18: 223-228.

Deshpande R. S., Tipnis H. P. 1997. Insecticidal activity of *Ocimumbasilicum*L. *Pesticides* 11:1–12.

Dorman, D., Dastmalchi, K., Oinonen, P., Darwis, Y., Laakso, I. and Hiltunen, R. (2008), "Chemical composition and in vitro antioxidant activity of lemon balm extract", *Lebensmittl-wissenschaft und-technologie,* 41: 391-400.

El-Ghorab, A.H., Shaaban, H.A., El-Massry, K.F. and Shibamoto, T. (2008), "Chemical composition of volatile extract and biological activities of volatile and less-volatile extracts if juniper berry (Juniperusdrupacea L.) fruit", *J. Agric. Food Chem.,* 56:
5021-5025.

El-Ghorab, A.H., Shibamoto, T. and Ozcan, M.M. (2007), "Chemical composition and antioxidantactivities of buds and leaves of capers (Capparis ovata Desf. Var. canescens) cultivated in Turkey", *J. Essent. Oil Res*.,19: 72-77.

Eriotou E., Anastasiadou K., Nikolopoulos D., Koulougliotis D. (2015). Antimicrobial and Free Radical Scavenging Activities of Basil (*Ocimumbasilicum*)Essential Oil Isolated from Five Plant Varieties Growing in Greece. *J. Nutr. Food Sci.* 5: 367.

Fernandes, P. C.; Fcanali, R. Teixeira, J. P. F.; Furlani, P. R.;Marques, M. O. M. 2004. Cultivo de manjericãoemhidroponiaemdiferentessubstratos sob ambienteprotegido. *Horticultura Brasileira*,,22(2): 260-264.

Freire-Moran L, Aronsson B, Manz C, Gyssens IC, So AD, *et al.* 2011. Critical shortage of new antibiotics in development against multidrug-resistant bacteria - Time to react is now. *Drug Resist Update* 14: 118-124.

Gill, B. S.; Randhawa, G. S. 2000. Effect of different row and plant spacings on yield and quality of French basil oil. *Journal of Research Punjab Agricultural University*, 36(3-4): 191-193.

Grayer RJ, Kite GC, Goldstone FJ, Bryan SE, Paton A, Putievsky E. 1996. Infraspecific taxonomy and essential oil chemotypes in sweet basil, *Ocimumbasilicum*. *Phytochemistry* 43:1033-9.

Grayer, R. J., Kite, G. C., Goldstone, J., Bryan, S. E., Paton, A., &Putievsky, E. 1996. Intraspecific taxonomy and essential oil chemotypes in sweet basil, *Ocimum basilicum*. *Phytochemistry, 43*, 1033-1039.

Gutierrez J., Barry-Ryan C., Bourke P. 2009. Antimicrobial activity of plant essential oils using food model media: Efficacy, synergistic potential and interactions with food components. *Food Microbiol* 26: 142-150.

Hosseini, S. A., Naseri, M., Zarai, A., Lotfollahian, H., Riyazi, S. R., and Meimandipour, A. 2013a. Effect of lemon oil on gastrointestinal tract, blood parameter and immune responses in broilers, *Ann. Biol. Res.*, 4, 47–51.

Javanmardi J., Khalighi A., Kashi A., Bais H. P, Vivanco J. M. 2002. Chemical characterization of basil (*Ocimumbasilicum*L.) found in local accessions and used in traditional medicines in Iran. *J Agric Food Chem*, 50:5878-83.

Khatri, M., Nasir, M. K. A., Saleem, R., & Noor, F. 1995. Evaluation of Pakistani sweet basil oil for commercial exploitation. *Pakistan Journal of Scientific and Industrial Research*, 38: 281-282.

Kwee E. M., Niemeyer E. D. 2011. Variations in phenolic composition and antioxidant properties among 15 basil (*Ocimumbasilicum*L.) cultivars. *Food Chem* 128: 1044-1050.

Labrador V., Freire P. F., Martín J. P., Hazen M. J. 2007. Cytotoxicity of butylated hydroxyanisole in Vero cells. *Cell BiolToxicol* 23: 189-199.

Lachowicz K. J, Jones G. P, Briggs D. R, Bienvenu FE, Palmer M. V, Ting S. S. T. *et al*. 1996. Characteristics of essential oil from basil (*Ocimumbasilicum*l.) Grown in australia. *J Agric Food Chem* 44:877–81.

Loizzo, M. R., Bonesi, M., Menichini, F., Tenuta, M. C., Leporini, M., Tundis, R., 2016: Antioxidant and carbohydrate-hydrolysing enzymes potential of *Sechiumedule*(Jacq.) Swartz (Cucurbitaceae) peel, leaves and pulp fresh and processed. *Plant Food Hum. Nutr.* 71: 381-387.

Maia, N.B.; Bovi, O. A.; Perecin, M. B.; Marques, M. O. M.; Granja, N. P.; Trujillo, A. L. R. 1996. New crops with potential to produce essential oil with high linalool content helping preserve rosewood – an endangered Amazon species. *Acta Horticulturae,* Wageningen, .39-43.

Manosroi A, Dhumtanom P, Manosroi J. 2006. Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines. *Cancer Lett*, 235:114-20.

Markovic V. V. and Bastic L. V. 1975.Characteristics of Pumpkin Seed Oil. *J. Am.Oil Chem. Soc.* 53: 42–44.

Marotti M, Piccaglia R, Giovanelli E. 1996. Differences in essential oilcomposition of basil (*Ocimumbasilicum*L.) Italian cultivars related tomorphological characteristics. *J Agric Food Chem,* 44:926–9.

Marotti, M., Piccaglia, R., Giovanelli, E. 1996. Differences in essential oil composition of Basil (*Ocimumbasilicum*L.) italian cultivars related to morfological characteristics. *J. of Agricultural Food Chemistry* , 44(12): 926- 3929.

May A., Bovi O. A., Maia N. B., Barata L. E. S., Souza R. C. Z; De Souza E. M. R, De Moraes A. R. A; Pinheiro M. Q. 2008. Basil Plants Growth and Essential Oil Yield in a Production System With Successive Cuts.*Bragantia, Campinas,* 67(2): 385-389.

Morshed, H., Islam, M. S., Parvin, S., Ahmed, M. U., Islam, M. S., Mostofa, A G. M. and Bin Sayeed, M. S. 2012. Antimicrobial and Cytotoxic Activity of the Methanol Extract of *Paederiafoetida*Linn. (Rubiaceae*). Journal of Applied Pharmaceutical Science* 02 (01); 77-80.

Mousavi S.M., Bagheri G., Saeidi S. 2015 Antibacterial Activities of the Hydroalcoholic Extract of *Portulaca*o*leracea*Leaves and Seeds in Sistan Region, Southeastern Iran. *IntJ Infect.* 2(2):e23214.

Mrkovacki, N., Đalovic, I., Josic, D., Bjelic, D., &Brdar-Jokanovic, M. 2016. The effect of PGPR strains on microbial abundance in maize rhizosphere in field conditions. *Ratarstvoipovrtarstvo, 53*, 15-19.

Muni, R.; Dasha, R.; Naqvi, A. A.; Sushil, K. 2002. Effect of plant density and harvesting time on the yield and the quality of essential oil in *Ocimum*species. *Journal of Medicinal and Aromatic Plant Sciences,* 24 (2): 393-396.

Nadeem M., Faqir Muhammad Anjum F. M., Khan M. I., and Tehseen S., El-Ghorab A., Sultan J. I. 2013. Nutritional and medicinal aspects of coriander (*Coriandrumsativum* L.) A review. *British Food Journal*, 115(5): 743-755.

Newell D. G., Koopmans M., Verhoef L, Duizer E, Aidara-Kane A, et al. 2010. Food-borne diseases-The challenges of 20 years ago still persist while new ones continue to emerge. *Int J Food Microbiol* 139: S3-S15.

Nguyen P. M., Kwee E. M., Niemeyer E. D. 2010. Potassium rate alters the antioxidant capacity and phenolic concentration of basil (*Ocimumbasilicum*L.) leaves. *Food Chem* 123: 1235-1241.

Nichols D. S. and Sanderson K. 2003. The Nomenclature, Structure, and Properties of Food Lipids. In *Chemical and Functional Properties of Food Lipids* (Z.E. Sikorski, and A. Kolakowska, eds.) PP. 29–59, CRC Press.

Nielsen, S.S. (2002): *Introduction to the ChemicalAnalysis of Foods. Pp* 183-204.

Okoye F. B. C, Obonga W. O., Onyegbule F. A., Ndu, O. O., Ihekwereme C. P. 2014. Chemical composition and anti-inflammatory activity of essential oils from the leaves of *Ocimumbasilicum* L. and *Ocimumgratissimum* L. (Lamiaceae). *Int J Pharm Sci Res* 5:2174-80.

Oliveiraa JS, Livia A Porto, Charles S Estevam, Rosana S Siqueira, Pericles B Alves, Edenilson S Niculau, Arie F Blank, *et al*. 2009. Phytochemical screening and anticonvulsant property of *Ocimumbasilicum*leaf essential oil. *BolLatinoam Caribe Plant Med Aromat* 8:195-202.

Opalachenoiva G, Obreshkova D. 2003. Comparatve studies on the activity of basil an essential oil from *Ocimumbasilicum*L.-against multidrug resistant clinical isolates of the genra staphylococcus, enterococcus and pseudomonas. *J Microbial Methods* 54:105, 10.

Pushpangadan, P., and Bradu, B. L. 1995. Basil. In: K. L. Chadha, & R. Gupta (Eds.),.*Advances in horticulture. Medicinal and aromatic plants, 11* (pp. 627-657). New Delhi: Malhotra Publishing House.

Rattanachaikunsopon P, Phumkhachorn P. 2010. Antimicrobialactivity of basil (Ocimum *basilicum*) oil against *Salmonella enteritidis* in vitro and in food. *Biosci. Biotechnol. Biochem*.74: 1200-1204.

Runyoro, D., Ngassapa, O., Vagionas, K., Aligiannis, N., Graikou, K., &Chinou, I. 2010. Chemical composition and antimicrobial activity essential oils of four *Ocimum*species growing in Tanzania. *Food Chemistry*, *119*, 311-316.

Saikia N., Nath S. C. 2003. Evaluation of essential oil characters of sweet basil (*Ocimum basilicum*Linn.) growing at assam valley condition of Northeast India. Proceedings of

the national symposium on “Biopropecting of Commercially Important Plants”, Jorhat, India; 2003. p. 73-8.

Sajjadi, S. E. 2006. Analysis of the essential oils of two cultivated basil (*Ocimumbasilicum* L.) from Iran, Daru, 14: 128–130.

Sakkas H, Gousia P, Economou V, Sakkas V, Petsios S,Papadopoulou C. 2016. In vitro antimicrobial activity of fiveessential oils on multidrug resistant gram-negative clinicalisolates. *J. Intercult. Ethnopharmacol.* 5: 212-218.

Sakkas H. and Papadopoulou, C. 2017. Antimicrobial Activity of Basil, Oregano, and Thyme Essential Oils. *J. Microbiol. Biotechnol.*, 27(3): 429–438.

Sethi S, Om Prakash, Chandra M, Punetha H, Pant A. K. 2013. Antifungal activity of essential oils of some Ocimum species collected from different locations of Uttarakhand. *Indian J Nat Prod Sci*, 4:392-7.

Shan B., Cai Y. Z., Brooks J. D., Corke H. 2007. The *in vitro* antimicrobial activity of dietary spice and medicinal herb extracts. *Int J Food Microbiol* 117: 112-119.

Silva MGV, Vieira IGP, Mendes FNP, Albuquerque IL, Santos R. N, Silva F. O., *et al*. 2008. Variation of ursolic acid content in eight *Ocimum*species from northeastern brazil. *Molecules* 13:2482-7.

Simon, J. E., Quinn, J., Murray, R. G. 1990. Basil: a source of essential oils. In: Janick, J.; Simon, J. E. (Eds.). Advances in new crops. Portland: Timber Press, pp. 484-489.

Tajkarimi M. M., Ibrahim S. A., Cliver D. O. 2010. Antimicrobial herb and spice compounds in food. *Food Control* 21: 1199-1218.

Telci I, Bayram E, Yılmaz G, Avc B. 2006. Variability in essential oil composition of turkish basils (*Ocimumbasilicum*L.). *BiochemSystEcol*;34:489-97.

Umerie, S. C., Anaso, H. U.; Anyasoro, L. J. C. 1998. Inseticidal potentials of *Ocimum basilicum*leaf extracts. *Bioresource Technology*, 64(3): 237-239.

Vahdatpour, T., Nikpiran, H., Babazadeh, D., Vahdatpour, S., and Jafargholipour, M. A. 2011. Effects of Protexin®, Fermacto® and combination of them on blood enzymes and performance of Japanese quails (*Coturnix Japonica*), *Ann. Biol. Res*., 2: 283-291.

Villalobos-Pascual MJ, Acosta-Ballesta MC. 2003. Chemicalvariation in an *Ocimum basilicum*gerplasm collection andactivity of the essential oils on *Callosobruchus maculatus*.*Biochem. Syst. Ecol.* 31: 673-679.

Vina A, Murillo E. 2003. Essential oil composition from twelve varieties of basil (*Ocimum* spp) grown in Colombia. *J BrazChem Soc.,* 14:744-9.

Wellenreiter, R. H., Mowrey, D. H., Stobbs, L. A., and d'Assonville, J. A. 2000: Effects of avilamycin on performance of broiler chickens, *Vet. Ther.,* 1, 118–124.

Wierdak R. N., Borowski B., Dzida K., Zawiślak G., Kowalski R. 2013. Essential oil composition of sweet basil cultivars as affected by nitrogen and potassium fertilization. *Turk J.Agric.*,37:427-36.

Yakhkeshi, S., Rahimi, S., and GharibNaseri, K. 2011. The effects of comparison of herbal extracts, antibiotic, probiotic and organic acid on serum lipids, immune response, GIT microbial population, intestinal morphology and performance of broilers, *J. Med. Plants*., 10: 80–95.

Yayasinghe C, Gotoh N, Aoki T, Wada S.2003. Phenolics composition and antioxidant activity of sweet basil (*Ocimumbasilicum*L.). *J Agric Food Chem,* 51:4442-9.

Zheljazkov V. D., Cantrell C. L., Tekwani B., Khan S. I. 2008. Content, composition, and bioactivity of the essential oils of three basil genotypes as a function of harvesting. *J Agr Food Chem* 56: 380-385.

7. APPENDICES

Appendix Table 1. Raw data for physicochemical properties and antioxidant activities

Source of oil extract	Rep	Oil yield	Spgr	ACV	FFA	PV	DPPH-SA	PVsc	AA
Infloresence	1	47	0.67	4.21	2.12	2.00	1.90	4.60	11.84
Infloresence	2	50	0.71	4.49	2.26	2.60	2.00	6.40	15.00
Leaf	1	65	0.93	2.81	1.41	3.00	86.4	2.40	7.52
Leaf	2	60	0.86	3.37	1.69	2.80	86.5	2.30	9.04

Spgr: specific gravity; ACV: acid value; FFA: free fatty acid value; PV: peroxide value; DPPH: 2, 2- diphenyl-1-picrylhydrazyl; HPSA peroxide scavenging activity; AA: ascorbic acid.

Appendix Table 2. Raw data for antibacterial activity based on diameter of zone of inhibition

Test pathogen	Source of Oil extract	Rep	Concentration of the oil extract			Ampicillin (1µl/ml)
			1µl/ml	2µl/ml	3µl/ml	
E. coli	Infloresence	1	9	12	14.2	19
E. coli	Infloresence	2	10	12.6	15	18.5
E. coli	Infloresence	3	9.4	11.5	14.5	19.1
E. coli	Leaf	1	0	8	13	19.4
E. coli	Leaf	2	0	9.6	12	18.5
E. coli	Leaf	3	0	8.7	12.5	19.2
S. aureus	Infloresence	1	10	13	15	19.1
S. aureus	Infloresence	2	11	12.5	15.4	18.6
S. aureus	Infloresence	3	10.7	12.3	16	19
S. aureus	Leaf	1	10	11	14	18.4
S. aureus	Leaf	2	9.3	10.5	14.5	18
S. aureus	Leaf	3	9.7	10.8	15	18.8

Appendix Table 3. Raw data for antifungal activity

Test pathogen	Source ofoil extract	Rep	Concentration of the oil extract			Ketokonazole (1µl/ml)
			1µl/ml	2µl/ml	3µl/ml	
A. versicolor	Infloresence	1	9	12	14	17.5
A. versicolor	Infloresence	2	9.7	11.5	14.5	18.5
A. versicolor	Infloresence	3	9.5	11.6	13.6	18
A. versicolor	Leaf	1	10.1	12.5	15.5	18
A. versicolor	Leaf	2	10.5	12	15.2	17.5
A. versicolor	Leaf	3	9.8	11.5	16	16.5
A. niger	Infloresence	1	6	10	12	18
A. niger	Infloresence	2	7	11	11.5	17.5
A. niger	Infloresence	3	6.8	11.5	12.4	18
A. niger	Leaf	1	8	11	13	17
A. niger	Leaf	2	8.8	11.8	13.5	17.5
A. niger	Leaf	3	9	12	14	17.5
C. albicans	Infloresence	1	10.2	11.5	12	17.5
C. albicans	Infloresence	2	9.5	12.1	12.5	18
C. albicans	Infloresence	3	10.4	12.4	13	17
C. albicans	Leaf	1	10.5	12	15	17.5
C. albicans	Leaf	2	11	13	16	17.5
C. albicans	Leaf	3	10.8	13.5	16.7	18

Appendix Figure 1.Essential Oil extraction and concentration of crude extract in water bath.

Appendix Figure 2. Some activities during determination of physicochemical properties

Appendix Figure 3. Activity during antimicrobial test

Appendix Figure 4. Data collection on antimicrobial activities