Addis Ababa University
College of Natural and Computational Sciences
Department of Chemistry

MSc Thesis

INVESTIGATION OF CHEMICAL CONSTITUENTS AND ANTI-OXIDANT ACTIVITIES OF THE ESSENTIAL OILS OF *LIPPIA ADOENSIS* AND *OCIMUM SANCTUM* L.

By: Yemisrach Fikadu

Advisor: Professor B.S. Chandravanshi and Dr. Estifanos Ele

July, 2020

Addis Ababa, Ethiopia

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DECLARATION

I, the undersigned, declare that this Thesis entitled "Investigation of chemical constituents and anti-oxidant activities of the essential oils of *Lippia Adoensis* and *Ocimum Sanctum* L." is my original work and has not been presented for any degree in any other university before and all sources of materials used for this thesis have been appropriately acknowledged.

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List of Abbreviations

DPPH	1,1-Diphenyl-2-picryl-hydrazyl
EtOAc	Ethyl acetate
GC-MS	Gas chromatography - mass spectrometry
MAE	Microwave assisted extraction
MeOH	Methanol
MSD	Mass selective detector
NIST	National institute of standard and technology
RI	Retention index
R	Retention time
SD	Standard deviation
SFE	Supercritical fluid extraction
UV-Vis	Ultra-Violet Visible

ABSTRACT

In this study essential oils of *Lippia adoensis* leaf, *Ocimum sanctum* L. leaf and stem (together without separation) and the mixture of the two plants from Bishoftu and Debre Berhan sample area was analyzed using Gas chromatography mass spectrometry (GC-MS) and its antioxidant activities were assessed using UV-Vis spectroscopy. *Ocimum sanctum* L. from Bishoftu sample area showed 12 compounds. The detected major compounds were β-bisabolene (31.38%), 4-[(1E)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methyl-Cyclohexen (25.56%), eucalyptol (17.12%). The essential oil from stem and leaf of *Ocimum sanctum* L. from Debre Berhan sample area showed 20 compounds. The major compounds were β-bisabolene (24.45%), 4-[(1E)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methylcyclohexen (19.61%) and eucalypto (13.42%). *Lippia adoensis* from Bishoftu sample area showed 15 compounds with major components as linalool (66.60%) and caryophyllene (4.28%) and *Lippia adoensis* from Debre Berhan sample area showed 12 compounds with major component as linalool (86.11%). The essential oil of the mixture of *Lippia adoensis and Ocimum sanctum* L. from Bishoftu sample area showed 18 compounds with major components as linalool (62.54%) and from Debre Berhan sample area showed 21 compounds major components as linalool (48.47%) and octadecane (5.47%). The antioxidant activities were assessed using UV-Visible spectroscopy. The radical scavenging activity of the essential oils of *Lippia adoensis* and *Ocimum sanctum* L. was assessed using DPPH. The antioxidant activities of the sample were determined by comparing DPPH inhibition and standard ascorbic acid DPPH inhibition. The measured average antioxidant activities for essential oil of *Ocimum sanctum* L. from Bishoftu and Debre Berhan exhibited DPPH radical scavenging activities of 96.48% and 96.17% at 100µg/mL, respectively. *Lippia adoensis* from Bishoftu and Debre Berhan sample area exhibited DPPH radical scavenging activities of 92.58% and 93.37% at 100 µg/mL, respectively and the essential oil of mixture of two plants (*Lippia adoensis* and *ocimum sanctum*) from Bishoftu and Debre Berhan sample area exhibited DPPH radical scavenging activities of 95.25% and 96.42% at 100µg/mL, respectively, which is comparable to that of ascorbic acid standard that exhibited a 98.08 % at the same concentration.

Key words: Essential oils, Antioxidant activity, DPPH assay *Ocimum sanctum* L. and *Lippia adoensis*.

1. Introduction

Natural product is a substance produced by a living organism that is found in nature. It can be prepared by total synthesis. One of the natural products are spices. A spice is a seed, fruit, root, bark, or other plant substance foremost used for flavoring, coloring, or preserving food (Shelef, 1984). Spices are different from other plants, which are the leaves, flowers, or stems of plants used for flavoring. Many spices have antimicrobial and antioxidant properties (Shelef, 1984). Ethiopia has a very rich diversity of land, soil and climate, especially the highlands, that experience large amounts of rainfall (1000-2000 mm/year) and have cool temperatures are known to sustain a natural vegetation of Afromontane forests, while at lower elevations giving way to broadleaf forests and wooded grasslands. In the high peaks, arid xerophytic vegetation flourish, flat valleys, drier rocky/sandy areas and steep slopes producing marked changes within short distances in rainfall, temperature, and soil types with a culmination of diverse vegetation (Friis et al., 1982).

In Ethiopia between 6000 - 7000 species of higher plants are estimated (Berhan, 1991), and from this species 10-12% of these are endemic (Zuberi et al., 2014). And also Ethiopia has many languages, cultures and beliefs that have in turn contributed to the high diversity of traditional knowledge and practice of the people, which, among others include the use of medicinal plants. More than 95% of traditional preparations in the country are of plant origin (Demissew, 1989). The common medicinal plants sold in markets are uses include treating skin infections, vermifuge, pain relief and fumigation In Ethiopia; use of traditional medicinal plants is widely practiced. The traditional medicine use in Ethiopia having a large spread could be attributed to cultural acceptability, physical accessibility efficacy against certain type of disease and economic afford ability as compared to modern medicine (Abebe et al., 2001). The Ethiopian flora is 6,000 species of vascular plants are estimated which about 10% are endemic to be believes (Hedberg et al., 2003).

1.1. *Lippia Adoensis*

The genus *Lippia*, in the member of Verbenaceae family distributed in tropical Africa and America has 200 species (Demissew and Puff, Beitr, 1989). There are five *Lippia* species in Ethiopia specifically, *L. adoensis*, *L. javanica*, *L. grandifolia*, *L. adoensisis* and *L. carviodora* (Demissew and Puff, Beitr, 1989). In the Africa the leaves of some *Lippia* species are used as tea or spice (Uphof, 1968) and also South America and Central (Compadre et al., 1987). Others are used as to treatment that cold and fever, for abdominal problems (Rovesti, 1972). Some species have antifertility properties which are credited (Awas, and Demissew, 2009), while others in perfumery are important (Compadre et al., 1987).

L. adoensis to the Afromontane region is endemic in Ethiopia. It is found in forest margins and disturbed areas at altitude of 1900-2650 m in the regions namely, Wellega, Shoa, Gondar, Wello, Gojam, Tigtay, Harerge, Arsi, Sidamo, Gmamo Gofa, Keffa and Eritrea administrative (Chogo and Crank, 1982). The wild *L. adoensis* locally known as *Kesse* is used for ceramic utensils and washing wooden to significant spicy and fresh a pleasant smell. And also there are many medicinal applications. To prevent flu, indigestion and headache are taken boiled flowers and leaves (Asfaw, 1992).

Cultivated *L. adoensis* found in the gardens of the Oromo ethnic groups in Shoa Administrative Region and Gurage is locally known as *Kosseret.* The leaves of *Kosseret* are used for flavouring purposes and when compared to wild *L. adoensis* they are claimed to have higher flavour. Cultivated *L. adoensis* dried leaves are important ingredients in the preparation of spiced butter giving it a characteristic flavour and sweet pleasant spicy. In the preparation of Ethiopian dishes, like *Kitfo, Doro Wat*, Spiced butter is an important cooking fat. And also there are several spices are used in making flavoured (spiced) butter. In Ethiopian some of spices are cardamom, basil, fenugreek, black cumin, turmeric, white cumin, garlic and rue. But individuals have certain personalized ways of most communities; mixture spices with butter generally have more or less identical recipes for preparing spiced butter. The Gurages are generally regarded as the makers of the most popular *Kitfo* a dish prepared from minced, raw meat, fresh, lean, raw meat served in just melted spiced butter. Unique flavour imparted to it mainly from *Kosseret* used in the preparation of spiced butter is characterized of Gurage *Kitfo* (Helen, 1980). The picture of *L. adoensis* plant with leaf and flower is shown in Figure 1.

Figure 1: *Lippia adoensis* plant with leaf and flower.

1.2. Ocimum sanctum L.

The genus *Ocimum* L. (basil) is a member of the Labiatae family. *Ocimum* is an important medicinal and economic plant (Paton, et al, 1999). *Ocimum basilicum* L. is upright herbaceous annual aromatic, and medicinal, spice plants that belong to the Lamiaceae family (Helen, 1980). The name Basil is derived from the Greek word *basileus* which means "king" (Blank et al., 2004) and Ocimum species, "*Ocimum sanctum* L." is the native to Asia and popularly known as "Tulsi" and also important medicinal plant which is worshiped by many Indians and sacred. It is also called as Queen of herbs, Tulsi (Hindi), Holy Basil, Sacred Basil, etc. The word 'Holy Basil' itself is a confusing term used for the plants of the family Lamiaceae (Kumar et al, 2015). More than 150 species are consists in basil distributed in the tropics and subtropics of the world. The most commonly cultivated species in the world are *O. gratissimum*, *O. basilicum, O. xcitriodoru*, *O. minimo* L. and *O. tenuiflorum* L, *O. americanum* L. Basil is an aromatic herb that is used widely to flavour and add a distinctive aroma to food. A spice leaves can be used for fresh or dried. Essential oils extracted from flowers and fresh leaves can be used as additives in food, cosmetics and pharmaceuticals (Javanmardi, et al, 2002).

The plant has proven its effectiveness in contrast cold, fever, indigestion, respiratory syndrome, diarrhea, headache, bronchitis, hysteria and many more and cough (Pushpangadan and Bradu, 1995).

In Ethiopia, there are different ethnic group has different name for sweet basil namely, locally called as "Besso bila" in Amharic, "duguno" in Afan Oromo, "Gimenja" in Hadiya, "seseg" in Tigrigna, "qantalama" in sidamigna, "Kepowa" in Wolayita (Gebrehiwot, et al., 2015). Ethiopia is a mother of wide agro ecology as many authors mentioned which is makes the country suitable for cultivation of many aromatic and medicinal plants (Zuberi et al., 2014). This creates sufficient conditions for different cultivars of sweet basil to be cultivated widely throughout the country. The picture of *Ocimum sanctum* L. plant with leaf and flower is shown in Figure 2.

Figure 2: *Ocimum sanctum* L. plant with leaf and flower

Many study is on the composition of essential oils of different spices of *Ocimum sanctum* L. from different countries have been reported. Charls and Simon (1990) have studied essential oil composition and content of basil (*Ocmum* spiciec) from West Lafayette American. Dean et al., (1990) have reported essential oil profiles of several temperate and tropical aromatic plants: their antimicrobial and antioxidant activities.in international symposium on Medicinal and Aromatic

Plants from Scotland United Kingdom. Javanmardi et al., (2002) have studied Chemical characterization of basil (*Ocimum basilicum L*) found in local accessions and used in traditional medicines in Iran from Iran. Lee et al., (2005) have reported identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (Thymus vulgaris L.) and their antioxidant properties. Pandalia and Verma (2011) have determined comparative volatile oil composition of four *Ocimum* species from northern India. Hahn and Burkett (2013) have reported optimizing eugenol extraction conditions from fresh and dried samples of holy basil (*Ocimum sanctum*) from India. Yaldiz et al., (2015) have studied herb yield and chemical composition of basil (*Ocimum basilicum* L.) essential oil in relation to the different harvest period and cultivation conditions from Turkey. Gebrehiwot et al., (2015) have reported Chemical composition and antimicrobial activities of leaves of sweet basil (*Ocimum basilicum* L.) herb from Adama, Ethiopia. Beatovic et al., (2015) have studied chemical composition, antioxidant and antimicrobial activities of the essential oils of twelve *Ocimum basilicum* L. Cultivars grown in Serbia records of Natural Products from Surbia. Asfaw (1992) have reported chemical investigation on the essential oils of Endemic wild and cultivated *Lippia adoensis* Addis Ababa, Ethiopia. Terblanché and Kornelius (1996) have reported essential oil constituents of the genus *Lippia* (Verbenaceae) from South Africa. Adelani et al., (2016) have reported chemical composition and bioactivity of *Lippia adoensis* Hochst ex. Walp (Verneneaceae) leaf essential oil against c*allosobruchus maculatus* Fabrics from Ogbomoso, Nigeria.

However, there is no report in the literature on the chemical composition and antioxidant of essential oils of *Ocmum sanctum* L. and *Lippia adoensis* from Ethiopia. Therefore, it is worthwhile to investigate the chemical composition and antioxidant activities of essential oils *Ocmum sanctum* L. and *Lippia adoensis* from two different places in Ethiopia.

1.3. Objectives

1.3.1. General objective

The general objective of this study was to investigate the chemical components and antioxidant properties of essential oils *Ocimum sanctum* L and *Lippia adoensis* species.

1.3.2. Specific objectives of the study

- To extract of essential oil of *Ocimum sanctum* L. and *Lippia adoensis* species arial parts using hydro-distillation.
- To characterization of extracted oils of *Ocimum sanctum* L. and *Lippia adoensis* species essential oil components by gas chromatography coupled with mass spectrometry.
- To evaluate of antioxidant activities of the essential oils of *Ocimum sanctum* L. and *Lippia adoensis*.

1.4. Statement of the problem

In Ethiopia traditional healers may not understand the scientific explanation of their medicines, but they knew from their day to day activities, cultures, trends of elders about the healing potential of medicinal plants. Since there is a better understanding today how human beings interact with nature, thus scientists are in a good position to investigate the healing power of medicinal plants and the chemistry of their chemical constituents. Medicinal plants mainly contain mixture of different chemical compounds that may act individually, additively or in synergy to improve health. Therefore the rationale of this research work is to look for optimum technique for the production of essential oils from plant materials and to isolate compounds from essential oil solution of leaf and stem (without separation) part of *Ocimum sanctum* L. and *Lippia adoensis* leaf.

1.5. Significance of the study

This study provide scientific information concerning essential oil as traditional medicine and it also led to the identification of potential bioactive compounds from essential oils extracted from medicinal plants which can act as biological antioxidant activities.

2. Literature Review

2.1. Medicinal plants

Medicinal plants are currently in considerable significance view due to their special attributes as a large source of healing power phytochemicals that may lead to the development of new drugs. Most of the phytochemicals from plant sources such as phenolic and flavonoids have been reported to have positive impact on health and cancer prevention (Azwandia, 2015). High content ofphenolic and flavonoids in medicinal plants have been associated with their antioxidant activities that play a role in the prevention of the development of age-related disease particularly cause by oxidative stress. With regards to the beneficial phytochemicals in medicinal plants towards natural products in pharmaceuticals and cosmetic industry, the researches on medicinal plants particularly were as important as the research on conventional drugs (Amberber, 2011).

2.2. Phytochemicals of medicinal plants

Table 1: Some of the medicinal plants used for different purposes in Ethiopia (Megersa, et al., 2013).

No.	Scientific name	Local Amharic name	Family	Plant part, preparation and application	Disease treated
1	*Croton macrostachyus* Del.	Besana	Euphorbiaceae	Exudates put on the cut skin to stop bleeding, bark of croton put on fire and the smoke used as to protect mosquito bite, Juvenile leaves smashed and rubbed on affected part. dried root powdered and given to dog with 'injera' which suffered by rabies	Diarrhea, skin cut, mosquito repellant, ring worm rabies
2	*Carissa spinarum* L.	Agam	Apocynaceae	Fresh bark chewed early before having breakfast the bark	Stomachache,

				chewed or hold in teeth for 5-10 min	toothache,
3	*Olea europaea* L.*subsp. cuspidata (Wall. ex G. Don) Cif*	Weyra	Oleaceae	Fresh root chewed,	Stomach ache
4	*Zingiber officinale Roscoe*	Zenjeble	Zingebraceae	Chewed and swallowed	Tonsillitis
5	*Coffea arabica* L.	Buna	Rubiaceae	The dried coffee bean roasted and powdered then given to the patient by mixing with honey.	Diarrhea
6	*Citrus limon* (L.) Burn.f.	Lomi	Rutaceae	Squiz the fruit and massage on bleeding gum, crush the fruit and apply its content on skin burn.	Gum bleeding, skinburn
7	*Cordia africana* Lam	Wanza	Boraginaceae	Leaves of Cordia africana, leaves of Acanthus polystachius crushed together with Feces of goat then put on fire the ash mixed with butter and creamed on affected part.	Spider poison
8	*Lippia adoensis* Hochst. ex Wal	Kossert	Verbenaceae	Fresh leaves chewed	Burn on chest
9	*Catha edulis* (Vahl)	Chat	Celastraceae	Fresh leaves crushed and boiled in water with leaves of *Ruta chalepensis*L., fresh leaves	Cough

	Forssk ex Endl			of *Periploca linearifolia* Quart. –Dill. & A. Rich. and fresh leaves of *Englerina woodfordioides* Gilbert then sugar added while it is boiling, put off from the fire and make to cool finally a cup of tea will be taken for four days.	
10	*Echinops hispidus* Fresen	Kebrch o	Asteraceae	Dried bark put on fire and the smoke inhaled	Tonsil, evil eye
11	*Ricinus communis* L.	Gulo	Euphorbia ceae	Fresh leaves crushed and mixed with water and taken one cup of tea for 3 consecutive days.	Rabies
12	*Phytolacca dodecandra L' Herit.*	Endode	Phytolacca ceae	Few root powdered and mixed with water and drunk for two days, root of *Phytolacca dodecandra*, juvenile leaves of *Momordica foetida*leaves of *Justicia schimperiana*and juvenile leaves of *Croton macrostachyus* powdered together and very few given with tea before having breakfast for three days. One cup of tea is given for man whereas half cup of tea for children	Liver disease Gonorrhea
13	*Guizotia abyssinica*(L.f.) Cass	Nug	Asteraceae	Seed roasted powdered and the *Decoction drun*, seed powdered and rubbed on *Madaa gatiitii* of oxen	Swelling

2.3. Extraction methods

Extraction is the separation of medicinally active portions of plant using selective solvents through standard procedures (Azwandia, 2015). The purpose of all extraction is to separate the soluble plant metabolites, leaving behind the insoluble cellular marc (residue). The initial crude extracts using these methods contain complex mixture of many plant metabolites, such as alkaloids, glycosides, phenolic, terpenoids and flavonoids. Some of the initially obtained extracts may be ready for use as medicinal agents in the form of tinctures and fluid extracts but some need further processing. Several of the commonly used extraction methods are discussed below.

2.3.1. Hydro-distillation

In order to isolate essential oils by hydro-distillation, the aromatic plant material is packed in a still and a sufficient quantity of water is added and brought to a boil; alternatively, live steam is injected into the plant charge. Due to the influence of hot water and steam, the essential oil is free from the oil glands in the plant tissue. The vapor mixture of water and oil is condensed by indirect cooling with water. From the condenser, distillate flows into a separator, where oil separates automatically from the distillate water.

2.3.2. Supercritical fluid extraction (SFE)

Supercritical fluid (SF) or also called as dense-gas is a substance that shares the physical properties of both gas and liquid at its critical point. Factors such as temperature and pressure are the determinants that push a substance into its critical region. SF behaves more like a gas but have the solvating characteristic of a liquid. An example of SF is CO_2 that become SF at above 31.1 °C and 7380 kPa. Interest in supercritical CO_2 (SC-CO_2) extraction due to excellent solvent for nonpolar analytic and CO_2 is readily available at low cost and has low toxicity. Even though SC-CO_2 has poor solubility for polar compounds, modification such as adding small amount of ethanol and methanol enable it to extract polar compounds. SC-CO_2 also produces analyzes at concentrate form as CO_2 vaporizes at ambient temperature. SC-solvents strength can be easily altered by changing the temperature, pressure or by adding modifiers that lead to reduce extraction time. Optimization of SC-CO_2 on *Wadelia calendulacea* achieved its optimum yield at 25 MPa, 25 ºC temperature, 10% modifier concentration and 90 min extraction time (Falcão et al., 2017). A major disadvantage of this method is the initial cost of the equipment is very high (Naudeet al., 1998).

2.3.3. Microwave assisted extraction (MAE)

MAE utilizes microwave energy to facilitate partition of analytes from the sample matrix into the solvent (Trusheva et al., 2007). Microwave radiation interacts with dipoles of polar and polarizable materials causes heating near the surface of the materials and heat is transferred by conduction. Dipole rotation of the molecules induced by microwave electromagnetic radiation disrupts hydrogen bonding; enhancing the migration of dissolved ions and promotes solvent penetration into the matrix (Kaufmann and Christen, 2002). In non-polar solvents, poor heating occurs as the energy is transferred by dielectric absorption only (Taamalliet al., 2012).

2.3.4. Solvent extraction

The principle of solid-liquid extraction is that when a solid material comes in contact with a solvent, the soluble components in the solid material move to the solvent. Thus, solvent extraction of plant material results in the mass transfer of soluble active principle (medicinal ingredient) to the solvent, and this takes place in a concentration gradient. The rate of mass transfer decreases as the concentration of active principle in the solvent increases, until equilibrium is reached, i.e. the concentrations of active principle in the solid material and the solvent are the same. Thereafter, there will no longer be a mass transfer of the active principle from plant material to the solvent.

2.3.5. Soxhlet extraction

The Soxhlet extraction method integrates the advantages of the reflux extraction and percolation, which utilizes the principle of reflux and siphoning to continuously extract the herb with fresh solvent. The Soxhlet extraction is an automatic continuous extraction method with high extraction efficiency that requires less time and solvent consumption than maceration or percolation. The high temperature and long extraction time in the Soxhlet extraction will increase the possibilities of thermal degradation.

2.3.6. Cold pressing method

The term cold pressed theoretically means that the oil is expeller-pressed at low temperatures and pressure. Cold pressed method is one of the best methods to extract essential oils. This process is used for most carrier oils and many essential oils. This process ensures that the resulting oil is 100% pure and retains all the properties of the plant. It is a method of mechanical extraction where heat is reduced and minimized throughout the batching of the raw material. The cold pressed method is also known as scarification method. Cold pressed method is mainly used for extracting essential oils from plants, flower, seeds, lemon, tangerine oils. In this process, the outer layer of the plants contains the oil are removed by scrubbing. Then the whole plant is pressed to squeeze the material from the pulp and to release the essential oil from the pouches. The essential oil rises to the surface of the material and is separated from the material by centrifugation.

2.4. Chemistry of *Ocimum sanctum* L. plant

Ocimum sanctum L. plants are of essential oil with ant oxidative and antimicrobial activities and flavoring agent and essential oil with ant oxidative and antimicrobial activities (Javanmardi et al., 2003). Packaging and storage practices integrated with the blanching process are used to retain its antioxidant potential and to make the green color more potent. However existing practices are uninterested to define the quality of the processed *Ocimum* mixed products. Concentrations in *Ocimum* have total phenolic and phenolic subfamilies are important parameters to monitor the post-harvest quality loss in terms of its antioxidant activities. Extraction of *Ocimum sanctum* L. is prescribed for the management of skin disease, bronchitis, diarrhea, dysentery, malaria, arthritis, and insect bites. It also considered being a helpful for adapting to stress, balancing different processes in the body (Pattanayak et al., 2010).

Flavour and pleasant smell composition of *Ocimum* are complex due to the presence of phytochemicals; eugenol, camphor, eugenic acid, β-elemene, limonene, rosmarinic acid, camphene, ocimene, linalool, caryophyllene, methyl carvicol, urosolic acid, apigenin, cirsimaritin, isothymusin, isothymonin, orientin, rutin, caffeic vicenin, and rosmarinic acid (Pandalia and Verma, 2011). Volatile compounds are mainly responsible for its characteristic aroma while non-volatiles phenolic for example caffeic acid, rutin, and rosmarinic acid for pharmacological actions (Nupur et al., 2018). *Ocimum sanctum* L. has been recognized as a rich source of aromatic essential oil used mainly in pharmaceutical, cosmetic as well as food and flavouring industries (Charles and Simon, 1990).

Ocimum sanctum L. is one of the most widely grown plants for healing power used (Vani et al., 2009). There are three distinct varieties of holy basil exist: Krishna, which is the stronger flavored red-purple variety, Rama, which is the most commonly found white-green variety and Vana, which grows wild in the forests of South Asia and Malaysia (Mondal et al., 2009). Holy basil has been used for thousands of years. The plant is used as a treatment for a variety of conditions including the common cold, headaches, heart disease, inflammation, malaria, various forms of poisoning, stomach disorders, as well as spiritual and flavoring purposes (Pandya et al., 2011). Its essential oil and extracts have been indicated to have antioxidant, anticancer and anti-inflammatory, antimicrobial activities (Arranz et al., 2015). One of the compositions the essential oil of holy basil is eugenol, which is thought to be responsible for such properties (Hahnand Burkett, 2013).

A literature search for the phytochemicals constituents of *Ocimum sanctum* L. have been reported anti-inflammatory activity in rats (Godhwani et al., 1987) however, anti-inflammatory activity was found to be less active than aspirin (Singh et a1., 1996), found that the volatile oil of O. *sanctum* could leukotriene induced and inhibit arachidonic inflammation. Widely studied has been studied about biological activity of herbal plant essential oils (Boligon et al., 2013). Basil essential oils have antioxidant and antimicrobial properties of was reported (Lee et al., 2005). To consider composition of the oils is biological activities of great importance. When reported by (Beatovic´et al., 2015), the antioxidant activities of the oils was correlated with the compounds possessing a phenolic ring with an OH group (for example eugenol) and major proportion of volatile compounds. It is the essential oil with ant oxidative and antimicrobial activities and source of flavoring agent (Javanmardi, 2003). It has been reported that *Ocimum basilicum* L. (sweet basil) is the most economically important species (Maki and Kintzios, 2008). According to some sources of shrubs and plants from the sub-tropical regions and tropical of Asia are more than 150 species. The *Ocimum* species is *O. gratissimum*, *Ocimum tenuiflorum*, *Ocimum* *basilicum, Ocimum viride* and *Ocimum sanctum* in Europe, India, and Africa, (Pattanayak et al., 2010).

2.4.1. Composition of holy basil (*Ocimum sanctum* L.) plant

Ocimum sanctum L. plant has been used under cultivation for centuries in India for multipurpose. Major compound of leaves and in florescence contain 0.5–2% essential oil with eugenol (Yaldiz et al., 2015). One of the active constituents largely present in leaves and inflorescence of the herb is eugenol which reduces blood glucose levels in type-2 diabetics (Pushpangadan and Bradu , 1995). In a short duration economically sacred basil has huge potential for large scale cultivation increase medicinal and aromatic crop and it suitably fits in to the existing cropping pattern (Ajjan et al., 2009).

2.4.2. Phenolic compounds in the *Ocimum sanctum* L. leaf and their antioxidants capacity

The present in *Ocimum sanctum* L. plant phenolic compounds are namely eugenol (31.9–50.4%), 1,8-cineole (12.6–16.5%), estragole (9.7–12.9%), chavicol (0.7–2.0%), methyleugenol (0.2–0.3%) has been reported (Deans et al., 1990). The oil of *Ocimum sanctum* L. and eugenol have seen efficacy as aflatoxin production fungi toxicant inhibiting fungal. Hence, due to aflatoxin production these products may prove useful in controlling quantitative losses of food commodities due to fungal infestation as well as qualitative losses. In addition, eugenol has been also reported as an effective antioxidant compound (Kaur, Perkins, 1991) and thus can enhance the shelf life of food products by oxidation of unsaturated lipids and controlling free radical scavenging. Due to presence of some phenolic compounds in the essential oil the research were reported the reduction in fungal growth. It is observation from the study that eugenol (61.30%), plays key role in its antifungal activities is major component of *O. sanctum* essential oil (Pushpangadan and Bradu, 1995).

2.5. Chemistry of *Lippia adoensis* plant

Approximately 200 *Lippia* species have been identified (Terblanché and Kornelius, 1996) and are located around the Earth some of which have been reported to be insecticidal against mosquito larvae and insects of stored products (Carvalho et al., 2003). *Lippia adoensis* is that found around the world tropical shrub. Culturally, leaves of *L. adoensis* can be food additive and cooked as plants. The antioxidant activities and pharmaceutical of the species have been reported (Demissew et al., 1989; Asres and Bukar, 2002). It has been reported that due to soil nutrients available and geographical locations to the flora chemical composition of the essential oils from the same botanical species differ (Jemaa, 2012). And also designed to determine the chemical compounds in Nigeria-grown *Lippia adoensis* essential oil using gas chromatography-mass spectrometry (GC-MS) method and to evaluate the fumigant toxicity has been reported.

Many studies have shown the effectiveness of herbal in the control of many storage insects. Some plants families known to have insecticidal potentials include Annonaceae, Meleaceae, Piperaceae Asteraceae, Verbenaceae and Zingeberaceae. In essential oil the predominant compounds identified like eucalyptol, *α*-terpineol and *γ*-terpinene have been identified in different essential oil having pesticidal properties (Owolabi *et al*., 2014; Zoghbi *et al*., 2014). *Lippia* species have also been found to have larvicidal, antiseptic and antifungal activity (Abegaz et al. 1998).

2.5.1. Phenolic compounds in *Lippia adoensis* leaf and their antioxidants capacity

Linalool is one of the composition of the essential oils dominated were observed to be simple mixtures. This monoterpene alcohol comprised 73.19-82.75% and 68.06-76.29% of the oil from the leaves and flowers, respectively. When the leaves are crushed is attributable to linalool the sweet odor discharged. The second major component was found to be germacrene D, a sesquiterpene hydrocarbon (6.74-9.48% and 5.01-10.86% in flower in leaves). 6-caryophyllerie, Ocimene, fl-myrcene, 01-, 6-cadinene and α-farnesene were identified by GC-MS (Asfaw, 1992).

Comprises about 250 herbaceous species of shrubs and is widely distributed all over South America and Central, as well as tropical Africa (Terblanché and Kornelius, 1996). The species are distributed in the arid regions of the southwestern in the deciduous tropical forests of Central America, in the tropical savannas and United States of America of Brazil, which are the places with high indexes of endemic (Salimena, 2002). Among the prominent examples we can highlight the *L. origanoides*, which is recognized in the Mexican Pharmacopeia as a substitute for common ‘oregano’ (*Origanum vulgare*) and popularly known as ‘oregano’ in Mexico. It is, therefore, mostly used as in the preparation of several dishes and a condiment in the kitchen (Oliveira et al., 2006). The dried and milled leaves of some *Lippia adoensis* or fruits and the flowers of this genus have been used as a substitute for *Thymus vulgaris* in spice mixtures for meats and pizzas (Lorenzi and Matos, 2002; Santoro et al., 2007).

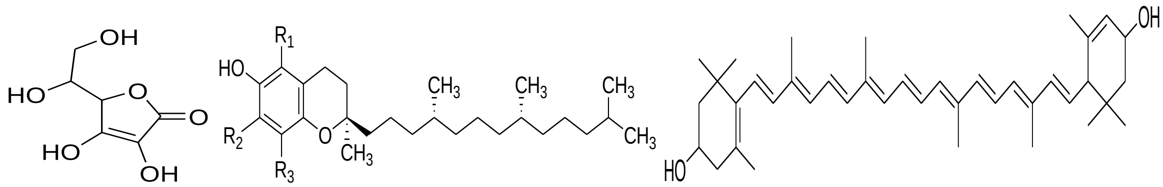
2.6. Antioxidant activity

Antioxidants are substances that their negative effects and neutralize free radicals. They act at different stages like interception, prevention and repair and by different mechanisms reducing agents by quenching singlet oxygen, donating hydrogen, trapping free radicals and acting as chelators (Devasagayam et al., 2004). Antioxidant containing foods used to help the human body to reduce singlet oxidative damage (Halliwell & Gutteride, 2007). There are two basic groups of antioxidants namely natural and synthetic ones. However, mainly the reason of use of these synthetic antioxidants they are suspected to be carcinogenic therefore has been restricted in some countries (Bartosz, 1997). Many researchers have focused on natural antioxidants and pure natural compounds and in the plant kingdom numerous crude extracts have been reported to have antioxidant properties. Therefore, utilization of more effective antioxidants of natural origin and the development are desired.

The DPPH analyze are free radical scavenging activity of the MeOH extract, EtOAc and isolated compounds were measured by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) method (Egwaikhide and Gimba, 2007). Usually performed as when the DPPH radical scavenging activity measure electron denoting capacity of antioxidant (Baumann et al., 1997). Considered to be DPPH is a model of a stable lipophilic radical. A chain reaction of lipophilic radicals is initiated by lipid auto-oxidation. Antioxidants react with DPPH reducing the number of DPPH free radicals to the number of their available hydroxyl groups. Therefore, the absorption at 517 nm is proportional to the amount of residual DPPH. It is visually noticeable as a discoloration from purple to yellow (Nishibe et al., 1995) and Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The high DPPH radical scavenging activity of these cultivars suggests their use in diseases arising from free radical attack.

% of radical scavenging activity = ($A_{standard} - A_{analyte}/A_{standard}$) × 100

The most common antioxidants extracts or exists in plants are vitamin (C, E and A), Carotenoids, and phenolic compounds (Santos-Sánchez et al., 2019) (Figure 3).



Vitamin C (ascorbic acid) Vitamin E (tocopherols) Vitamin A (Carotenoid) (Lutein)

Figure 3: Structures of vitamins C, E and A.

3. Materials and methods

3.1. Equipment, apparatus and chemicals

The equipment used to conduct this study was hydro-distillation set up, locally manufactured electrical stove, different size of Erlenmeyer flask, oven, What-man number 1 filter paper, digital balance, UV-Visible spectrometer (model: PerkinElmer UV Win Lab 6.0.3.0730/1.61.00 Lambda 900), gas chromatography-mass spectrometer (GC-MS) (Model: GC-7820A, Agilent Technologies; Detector-5977EMSD, USA), column: DB-1701, (30 m × 0.250 mm), and 0.25 micrometer particle size), measuring cylinder, beakers, heating gun, glass tube, stopper and test tubes.

The chemicals, reagents, and solvents used were hexane, pentane, methanol, ethyl acetate, ascorbic acid (standard) and DPPH used in this research.

3.2. Sample collection

About 0.5 kg of each of *Lippia adoensis* leaf from Bishoftu and Debre Berhan and *Ocimum sanctum* L. stem and leaf (together without separation) form Bishoftu and Debre Berhan were collected from street vendors. The mixtures of the two plants (250 g each) from Bishoftu and Debre Berhan were also prepared and used for essential oil extraction. Thus a total of six samples were analyzed.

3.3. Procedure

Lippia adoensis leaf and *Ocimum sanctum* L. steam and leaf (together without separation) were collected from Bishoftu and Debre Berhan, Ethiopia. The leaves were after cutting in to pieces, 0.5 kg of the sample was put into 5 L capacity round bottom flask containing 2 L of distilled water. Extraction of essential oils was done by Clevenger apparatus using hydro-distillation method. The essential oil was collected in air tight glass sample bottle and stored in a refrigerator at 4 C^0until analyses.

Figure 4: Hydro-distillation Clevenger apparatus set up used in essential oil extraction

3.3.1. Procedure of hydro-distillation of *Lippia adoensis* leaf and *Ocimum sanctum* L. steam and leaf (together without separation) oils

Lippia adoensis and *Ocimum sanctum* L. were prepared at room temperature by cutting the leaves and stem parts into pieces. Then the plant parts (500g for each plan) were placed in a round bottomed flask filled with placed in a round bottomed flask filled with 2L of distilled water. The Clevenger apparatus was attached to the round bottomed flask on one side and attached to a condenser on the other side. The plant material was heated using a heating mantel to boil the water. The process was kept for 3 hr. till the level of the extracted oil remained constant. Then the oil was separated from aqueous layer, measured and placed in a refrigerator for GC-MS and antioxidant analysis.

3.3.2. Procedure of sample preparation for GC-MS analysis

For GC-MS analysis of essential oil the standard stock solution was prepared. The stock solution of 200µg/mL prepared by taking 5µLof each sample and dissolved with pentane in 25mL volumetric flask. Then 200 µg/mL concentration of stock solution was diluted to 20µg/mL and transferred to a vial and subjected to analysis by using GC-MS.

3.3.2.1.GC-MS characterization of essential oil from *Lippia adoensis* and *Ocimum sanctum* L.

The essential oil obtained from *Lippia adoensis* leaf and *Ocimum sanctum* L. together with stem without separation were analyzed by GC system coupled with Agilent technology 5977E MSD system which was equipped with auto sampler. The chromatographic separation was done in DB-1701 column, (14%-cyanopropyl-phenyl)-methylpolysiloxane, which was 30 m in length and 0.25 µm in thickness at a pressure of 8 psi and 0.97989 mL/min flow rate. Ultra high pure (99.999%) helium gas, as the carrier gas, was used at constant flow mode. An Agilent 7820A auto sampler was used to inject 1µL of the sample with a split less injection mode into the inlet heated to 275 ºC with total run time of 29.33 min. Oven temperature was programmed with the initial column temperature of 60 ºC and hold-time 2 min. The column temperature was increased at a rate of 10 ºC/min until the temperature reached 200 ºC and then heated again at the rate of 3 ºC/min until the temperature reached to 240 ºC. No mass spectra were collected during the first 4 min of the solvent delay. The transfer line and the ion source temperature were 280 ºC and 230 ºC, respectively. The detector voltage was 1600 V and the electron energy was 70 eV. Mass spectral data were collected from 40–650 *m/z*. The name and structure of peaks were determined through NIST 2014 library search and retention index (RI) calculation.

3.3.3. Procedure of DPPH radical scavenging assay of *Lippia adoensis* leaf and *Ocimum sanctum* L. and their mixture essential oil

The radical scavenging activity of the essential oils of *Lippia adoensis* and *Ocimum sanctum* L. and the mixtures of the two plants was assessed using DPPH according to the procedure described by (Bhuiyan, et al., 2009).The essential oil extract was first dissolved in ethyl acetate to afford 1 mg/mL solution. It was then serially diluted in ethyl acetate to give concentrations of 500, 250, 125 and 62.5µg/mL. To 1 mL of each concentration, 4 mL of 0.004 % DPPH solution in methanol was added to make 100, 50, 25 and 12.5 µg/mL solutions. The mixtures were left to stand in the dark for 30 min and then the absorbance at 517 nm was recorded for each concentration using UV-Vis spectrophotometer. The percentage DPPH inhibition was calculated using the formula.

$$\% \text{ DPPH Inhibition} = \frac{A_{control} - A_{extract}}{A_{control}} \times 100$$

Where $A_{control}$ is the absorbance of DPPH solution and $A_{extract}$ is the absorbance of the test sample plus DPPH. All measurements were performed in triplicates. The same procedure was used to determine the radical scavenging activity of ascorbic acid standards.

4. Results and discussion

4.1. GC/MS analysis of essential oil extract from *Lippia adoensis* and *Ocimum sanctum* L. and their mixture

Lippia adoensis leaf and *Ocimum sanctum* L. stem and leaf together without separation essential oil extract was collected by hydro-distillation of 500 g of the plant materials and the mixture of the two plants each 250 g 1µL of 20µg/mL samples was analyzed by GC-MS.

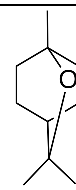
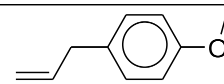
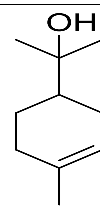
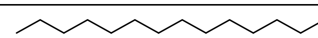
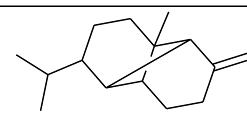
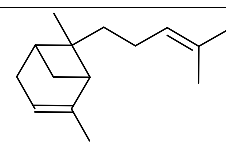
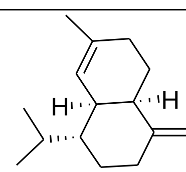
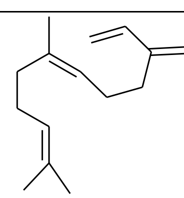
Retention index (RI) of the essential oil components of *Lippia adoensis* and *Ocimum sanctum* L.were also calculated by injecting a mixture of *n*-alkanes with the same experimental condition as that of the sample analysis and using the van den Dool and Kratz relationship.

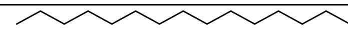
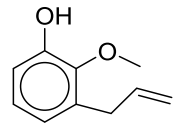
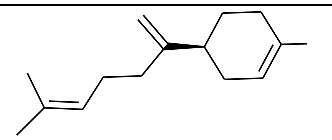
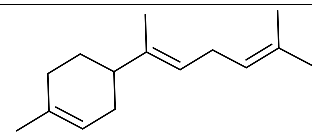
$$RI = \frac{100n + 100\,(Rt_{(unknown)} - Rt_{(n)})}{Rt_{(n+1)} - Rt_{(n)}}$$

Where RI is retention index of the analyte, n is the number of carbon atoms in the smaller *n*-alkane, Rt (unknown) is the retention time of the analyte, Rt(n) and Rt(n+1) are the retention times of the reference *n*-alkanes eluting immediately before and after the analyte, respectively.

Different numbers of compounds in the essential oils extracted were identified by GC/MS. The different components were characterized by matching their mass spectra with those of reference compounds recorded in NIST 2014 mass spectral library and confirmed by the retention index (RI) obtained from a series of *n*-alkanes. The essential oils constituents of *Lippia adoensis* and *Ocimum sanctum* L. and their mixture are summarized in Tables 2-7. The chromatograms of the essential oils of *Lippia adoensis* and *Ocimum sanctum* L. and their mixture are shown in Figures 5-10.

Table 2: GC-MS analysis of essential oil of *Ocimum sanctum* L. Bishoftu sample

Peak No.	R_t	RI	% of compound	Compound name	Structure
1	7.062	1032	17.1	Eucalyptol	
2	10.209	1289	7.48	Estragole	
3	10.358	1189	1.04	α - Terpineol	
4	11.638	1393	0.80	Tetradecane	
5	12.458	1456	1.33	β - Ylangene	
6	12.534	1462	2.32	trans-α-Bergamotene	
7	12.625	1469	2.92	ɤ - Muurolene	
8	12.868	1444	0.70	(Z) - β -Famesene	

9	12.917	1492	1.47	Pentadecane	
10	13.128	1509	5.64	2-methoxy-3-(2-propenyl)-Phenol	
11	13.528	1542	31.38	β-Bisabolene	
12	13.915	1574	25.56	4-[(1E)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methylcyclohexene	

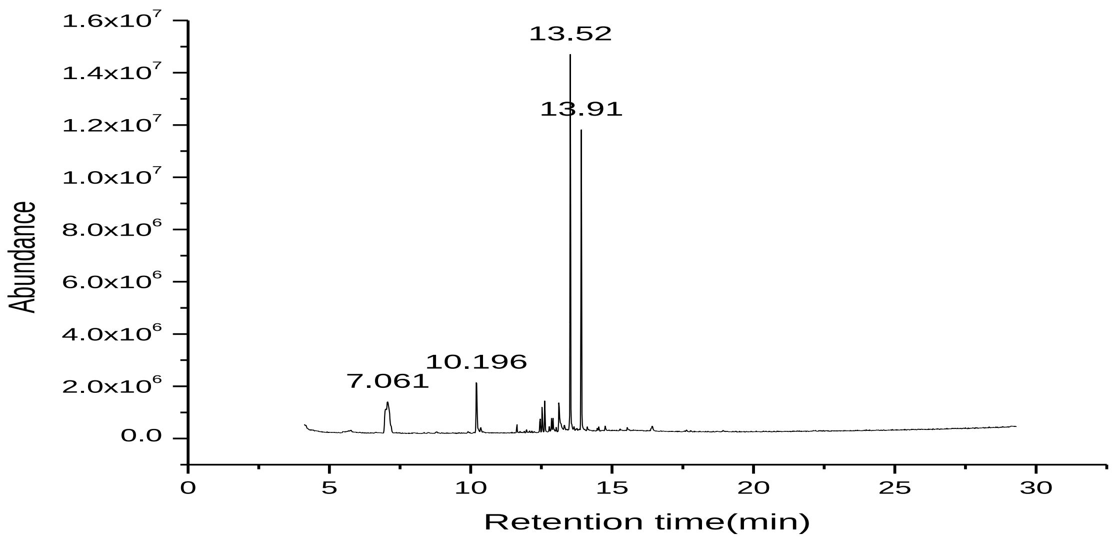
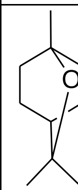
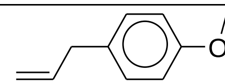
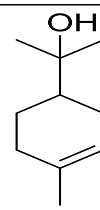
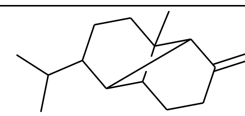
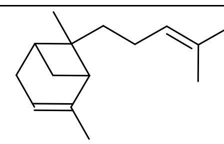
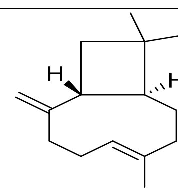
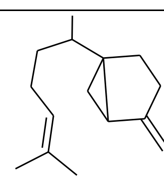
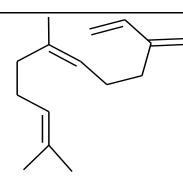
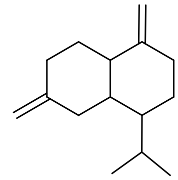
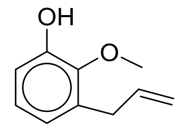
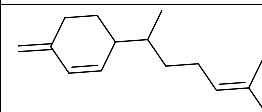
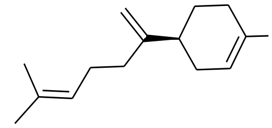
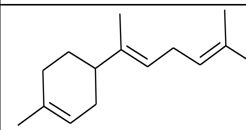
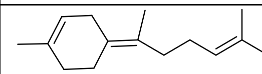
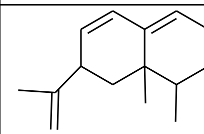
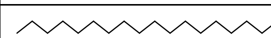
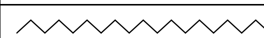
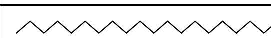
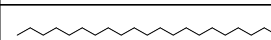


Figure 5: GC-MS chromatogram of essential oil of *Ocimum sanctum* L. from Bishoftu

Table 3: GC-MS analysis of essential oil of *Ocimum sanctum* L. Debre Berhan sample

Peak No	R_t	RI	% of compound	Compound name	Structure
1	7.066	1077	13.4	Eucalyptol	
2	10.212	1290	6.07	Estragole	
3	10.360	1189	0.75	α - Terpineol	
4	12.464	1457	1.07	β-Ylangene	
5	12.541	1462	1.76	trans-α-Bergamotene	
6	12.632	1470	2.20	Caryophyllene	
7	12.791	1482	0.40	Sesquisabinene	
8	12.874	1489	1.25	(Z)-β-Famesene	

9	13.033	1446	0.28	ε - Muurolene	
10	13.1315	1509	5.7934	2-methoxy-3-(2-propenyl)-Phenol	
11	13.323	1464	0.83	β- Sesquiphllandrene	
12	13.533	1542	24.45	β-Bisabolene	
13	13.9201	1574	19.61	4-[(1E)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methylcyclohexen	
14	14.764	1647	0.55	(E)-ɤ-Bisabolene	
15	15.284	1692	0.52	Heptadecane	
16	15.548	1716	0.56	(1s,7s,8aR)-1,8a-dimethyl-7-(prop-1-en-2-yl)-1,2,3,7,8,8a-hexahydronaphthalene	
17	16.386	1792	6.11	Octadecane	
18	17.577	1889	1.22	Nonadecane	
19	18.925	1992	8.89	Eicosane	
20	22.162	2193	3.53	Heneicosane	

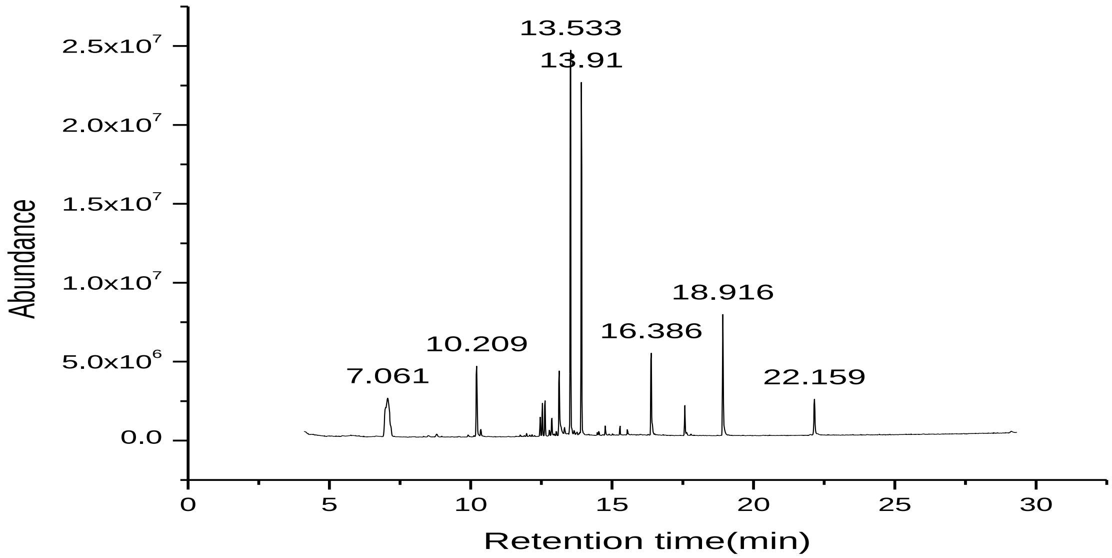
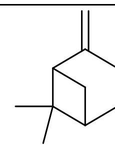
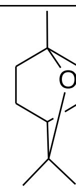
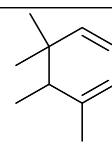
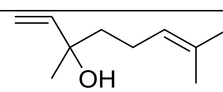
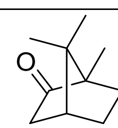
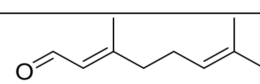


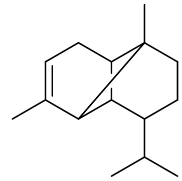
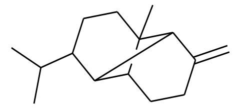
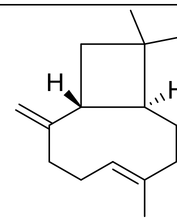
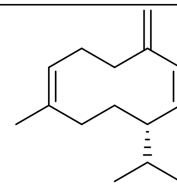
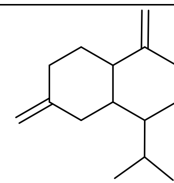
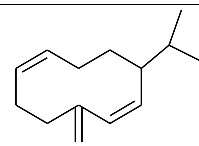
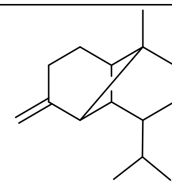
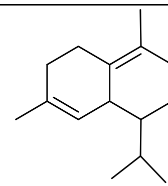
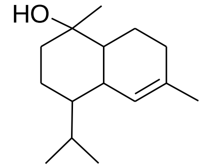
Figure 6: GC-MS Chromatogram of essential oil of *Ocimum sanctum* L. from Debre Berhan sample

The essential oil of *Ocimum sanctum* L. from Bishoftu sample area showed 12 compounds with major components as β-bisabolene (31.38%), 4-[(1E)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methylcyclohexen (25.56%), eucalyptol (17.12%) and minor components as estragole (7.48%), 2-methoxy-3-(2-propenyl)-phenol (5.64%), ɤ - muurolene (2.92%) and trans-α-bergamotene (2.32%), pentadecane (1.47%), β -ylangene (1.33%), α - terpineo (1.04%) and two very minor compounds that were indicated in Table 2 with concentrations of less than 1%. The essential oil of *Ocimum sanctum* L. from Debre Berhan sample area showed 20 compounds with major components as β-bisabolene (24.45%), 4-[(1E)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methylcyclohexen (19.61%) and eucalypto (13.42%) and minor components as eicosane (8.89%), octadecane (6.11%), estragole (6.07%), 2-methoxy-3-(2-propenyl)-phenol (5.79%), Heneicosane (3.53), caryophyllene (2.20%), trans-α-bergamotene (1.76%), (Z)-β-famesene (1.25%), nonadecane (1.22%),β-ylangene (1.07%) and additional minor compounds that are indicated in Table 3.

When essential oils of *Ocimum sanctum* L. from the two sampling areas were compared, it was found that their metabolite profiles are almost similar with minor differences. In the case of both plants species, there are three major compounds which are common to both. This cannot be a surprise as plants from identical species show similar metabolite profiles. These compounds can be considered as marker compounds that can differentiate *Ocimum sanctum* from other species. However, in this study it has been observed that some compounds exist only in one species which can be considered as geographical location based markers. *Ocimum sanctum* L. from Bishoftu sample showed ɤ - muurolene as the only compound different from sample collected from Debre Berhan. In contrary, plant sample obtained from Debre Berhan showed few more compounds that are absent in Bishoftu sample such as caryophyllene, sesquisabinene, ε – muurolene, (1s,7s,8aR)-1,8a-dimethyl-7-(prop-1-en-2-yl)-1,2,3,7,8,8a-hexahydronaphthalene, octadecane, nonadecane, eicosane and heneicosane. This could be due to differences in geographical locations and their climatic conditions.

Table 4: GC-MS analysis of essential oil of *Lippia adoensis* Bishoftu sample

Peak No.	R_t	RI	% of compound	Compound name	Structure
1	6.190	1020	1.92	β-Pinene	
2	7.104	1080	2.66	Eucalyptol	
3	8.538	1174	0.41	α-Pyronene	
4	8.801	1191	66.60	Linalool	
5	9.84	1263	0.40	(-) Camphore	
6	11.353	1240	0.92	β-Citral	

7	11.740	1402	1.38	α-Copaene	
8	12.467	1456	2.08	β-Ylangene	
9	12.635	1469	4.28	Caryophyllene	
10	12.860	1431	0.72	Isogermacrene D	
11	13.037	1446	0.71	ε - Muurolene	
12	13.507	1540	1.56	Germacrene D	
13	13.672	1598	0.59	β - Copaene	
14	13.768	1524	1.10	δ-cadioene	
15	16.292	1642	0.64	Tau (η)-Muuralol	

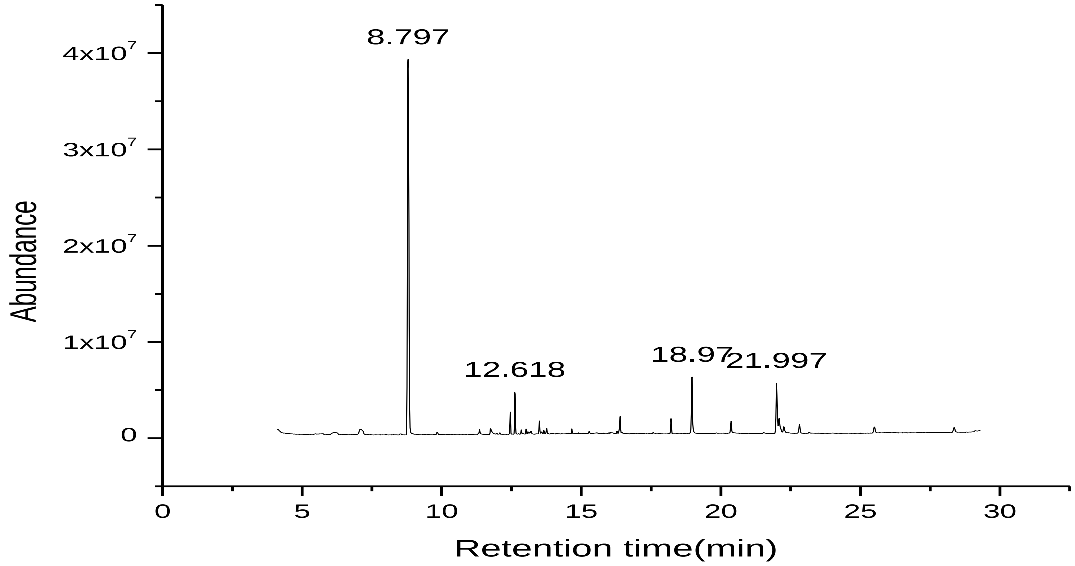
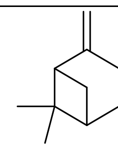
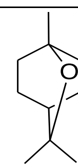
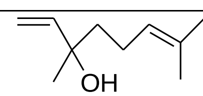
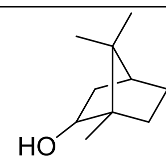
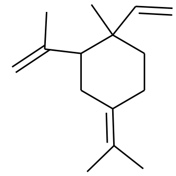
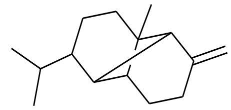
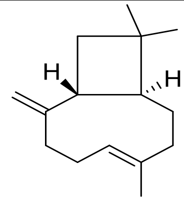
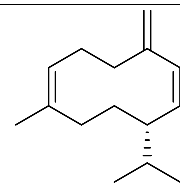
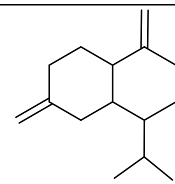
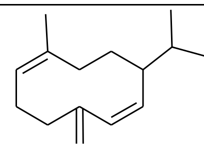
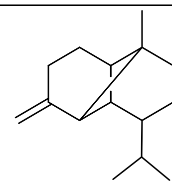
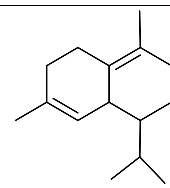


Figure 7: GC-MS chromatogram of essential oil of *Lippia adoensis* Bishoftu sample

Table 5: GC-MS analysis of essential oil of *Lippia adoensis* Debre Berhan sample

Peak No.	RT	RI	% of compound	Compound name	structures
1	6.153	1020	2.57	β-Pinene	
2	7.079	1080	2.75	Eucalyptol	
3	8.795	1191	86.11	Linalool	
4	10.191	1167	1.13	Endo-Borneol	

5	11.311	1344	0.37	Elemene Isomer	
6	12.459	1456	1.44	β-ylangene	
7	12.626	1457	1.95	Caryophyllene	
8	12.856	1487	0.44	Isogermacrene D	
9	13.029	1500	0.44	ε-Muurolene	
10	13.499	1540	1.07	Germacrene D	
11	13.666	1598	0.45	β - Copaene	
12	13.763	1524	0.60	δ-cadinene	

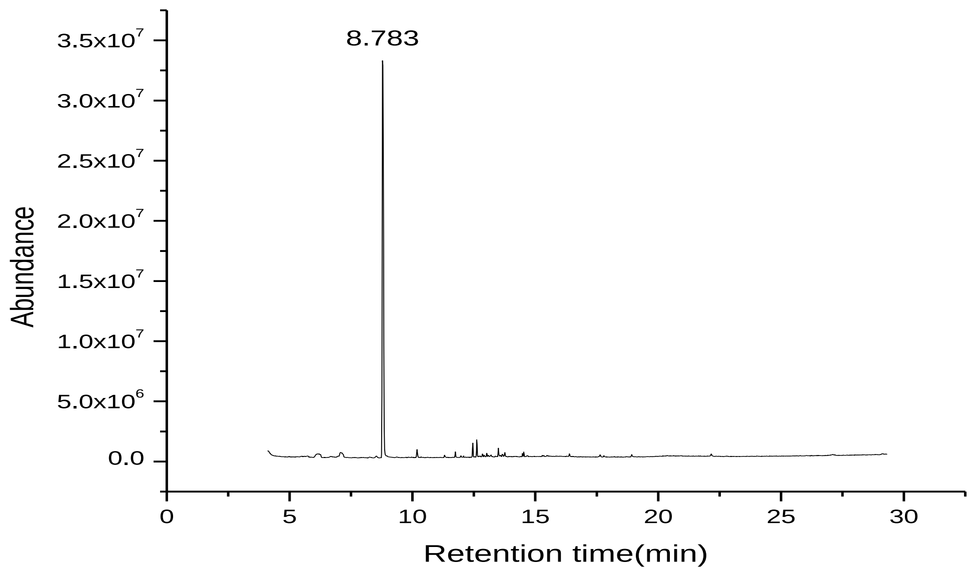


Figure 8: GC-MS chromatogram of essential oil of *Lippia adoensis* Debre Berhan sample

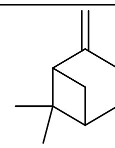
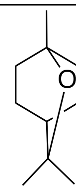
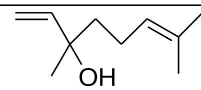
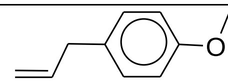
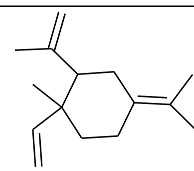
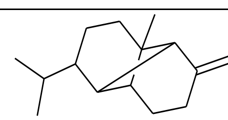
The essential oil leaf of *Lippia adoensis* from Bishoftu sample area showed 15 compounds with major components as linalool (66.60%), caryophyllene (4.28%) and minor components as eucalyptol (2.66%), β.-ylangene (2.08%), β-pinene (1.92%), germacrene D (1.56%), α-copaene (1.38%), δ-cadioene (1.10%) and very minor compounds listd in Table 4. The essential oil leaf of *Lippia adoensis* from Debre Berhan sample area showed 12 compounds with major component as linalool (86.11%) and minor components as eucalyptol (2.75%), β-pinene (2.57%), caryophyllene (1.95%), β-ylangene (1.44%), endo-Borneol (1.13%), germacrene D (1.07%) the remaining compounds there in the Table 5.

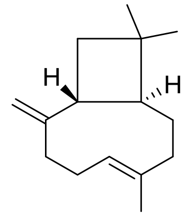
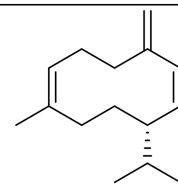
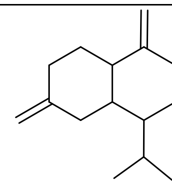
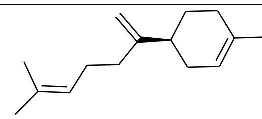
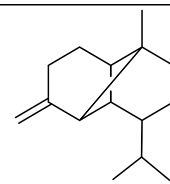
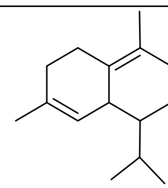
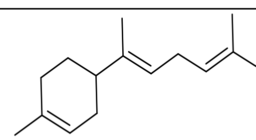
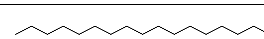
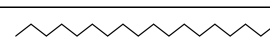
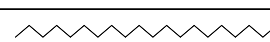
The two samples share 6 compounds in common including the major component, linalool. The compounds detected are in different concentrations as expected which could be due to variation in geographical location and other factors. The most amazing incident was that both samples showed metabolites which are unique to them. This shows how nature dictates what should be produced at particular time and specific environment so that the plant species would remain fit.

Another interesting finding was that all the compounds produced or detected in the essential oils were biogenetically produced through a single biosynthetic pathway which is terpenoid biosynthetic pathway. The metabolites are classified as monoterpenes with carbon number 10 and sesquiterpenes with carbon numbers 15. Of the compounds detected, only few of them contain a hetroatom, oxygen, in the form of epoxide, hydroxyl or carbonyl form. For the *Lippia adoensis* species one can consider linalool as a marker compound to identify the essential oils of the species from others.

When the metabolic profiles of the two plant species (*Lippia adoensis and Ocimum sanctum)* were compared, they only share one compound (caryophyllene) in common. The remaining compounds are different in structure.

Table 6: GC-MS analysis of essential oil of the mixture of two plants (*Lippia adoensis* and *Ocimum sanctum* L.) Bishoftu sample

Peak No.	R_t	RI	% of compound	Compound name	Structure
1	6.147	1020	1.79	β-Pinene	
2	7.06	1080	4.02	Eucalyptol	
3	8.793	1191	62.54	Linalool	
4	10.197	1288	1.37	Estragole	
5	11.306	1369	0.26	Elemen	
6	12.455	1456	1.34	β-Ylangene	

7	12.622	1469	2.23	Caryophyllene	
8	12.853	1487	0.41	Isogermacrene D	
9	13.026	1500	0.31	ε - Muurolene	
10	13.526	1542	3.05	β-Bisabolene	
11	13.661	1553	0.42	β - Copaene	
12	13.760	1524	0.56	δ-cadinene	
13	13.917	1574	1.12	4-[(1E)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methylcyclohexene	
14	15.279	1709	0.37	Heptadecane	
15	16.380	1792	5.12	Octadecane	
16	17.572	1889	1.10	Nonadecane	
17	18.920	1992	7.14	Eicosane	
18	22.157	2193	2.32	Heneicosane	

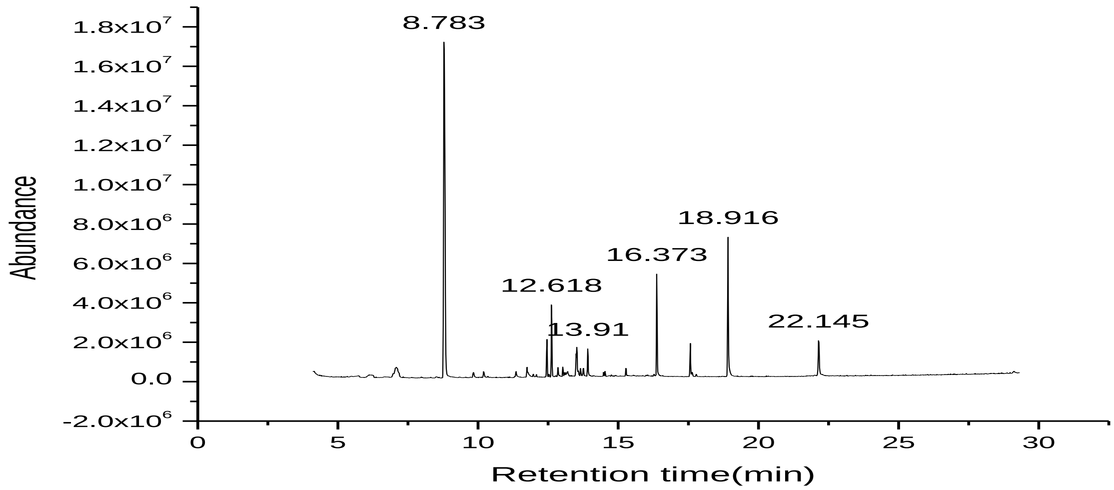
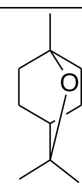
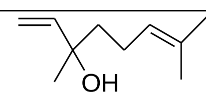
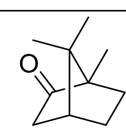
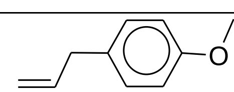
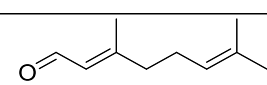
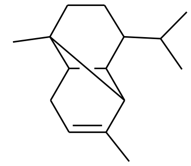
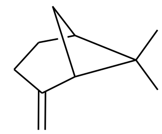
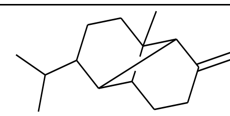
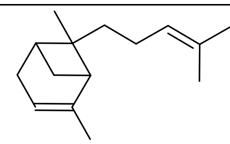
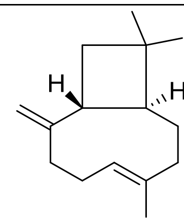
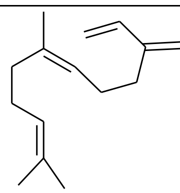
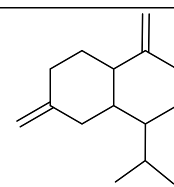
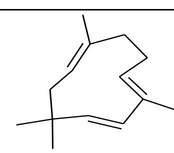
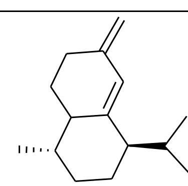
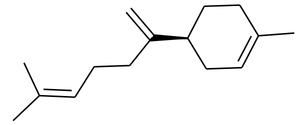
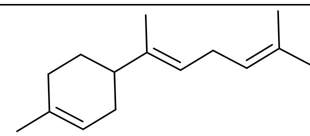
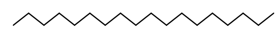
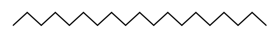


Figure 9: GC-MS chromatogram of essential oil of the mixture of two plants (*Lippia adoensis* and *Ocimum sanctum* L.) from Bishoftu sample

Table 7: GC-MS analysis of essential oil of the mixture of two plants (*Lippia adoensis* and *Ocimum sanctum* L.) Debre Berhan sample

Peak No.	R_t	RI	% of compound	Compound name	Structure
1	7.069	1080	4.58	Eucalyptol	
2	8.793	1191	48.47	Linalool	
3	9.838	1263	0.78	(-)-Camphore	
4	10.205	1288.7	0.76	Estragole	
5	11.357	1372	1.01	β-Citral	

6	11.751	1401	1.94	α-Copaene	
7	11.974	1418.5	0.26	β -Bourbonene	
8	12.458	1456	2.73	β - Ylangene	
9	12.533	1461.9	0.23	trans-α-Bergamotene	
10	12.626	1469	4.71	Caryophyllene	
11	12.857	1444	1.01	- β -Famesene	
12	13.028	1500	0.74	ε - Muurolene	
13	13.127	1508.6	0.64	Humulene	
14	13.195	1514	0.71	Bicyclosesquiphellandrene	

15	13.525	1541.8	4.57	β-Bisabolene	
16	13.916	1574	2.27	4-[(1E)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methylcyclohexene	
17	15.280	1709	0.54	Heptadecane	
18	16.379	1792	5.47	Octadecane	
19	17.573	1889	1.88	Nonadecane	
20	18.925	1992	9.42	Eicosane	
21	22.162	2193	3.71	Heneicosane	

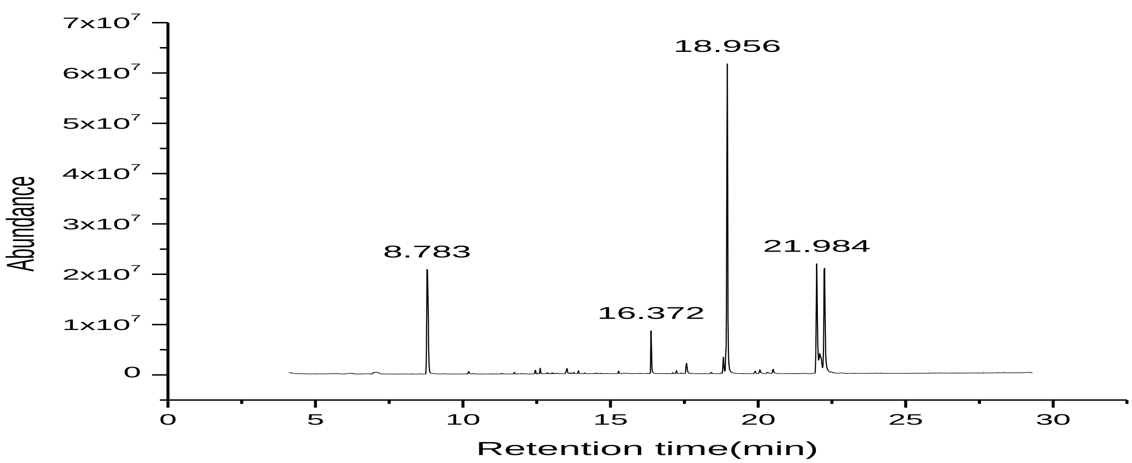


Figure 10: GC-MS chromatogram of essential oil of the mixture of two plants (*Lippia adoensis* and *Ocimum sanctum* L.) from Debre Brhan sample

The mixture of essential oil of *Lippia adoensis* leaf and stem and leaf (together without separation) of *Ocimum sanctum* L. from Debre Berhan sample area showed 22 compounds with major components as linalool (48.47%) and eicosane (9.42%), octadecane (5.47%) and minor components as caryophyllene (4.71%), eucalyptol (4.58%), β-bisabolene (4.57%), Heneicosane3.71%),β-ylangene (2.73%), 4-[(1E)-1,5-dimethyl-1-hexadien-1-yl]-1-methylcyclohexene (2.27%), α-copaene (1.94%),- β -famesene(1.01%), β-citra (1.01%)the remaining compounds there in the Table 7. The mixture of essential oil from leaf of *Lippia adoensis* and stem and leaf (together without separation) of *Ocimum sanctum* L. from Bishoftu sample area showed 19 compounds with major component as linalool (62.54%) and minor component α-eicosane (7.14%), octadecane (5.12%), eucalyptol (4.02%), β-bisabolene (3.05%), heneicosane (2.32%), caryophyllene (2.23%), β-pinene (1.79%), estragole (1.37%), β-ylangene (1.34%), 4-[(1E)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methylcyclohexene(1.12%) and nonadecane (1.10 %) and the remaining compounds there in the Table 6.

In order to investigate what can be happen when two different plant species (*Lippia adoensis* and *Ocimum sanctum*) are mixed and distilled, equal amount of the plants material from both species were mixed and hydrodistilled. The metabolite profiles of the mixtures showed metabolites those detected in individual species. The interesting part was that the co-distillation of the plant materials didn't affect the metabolite profile as such however, concentrations of some of the metabolites changed in significant amount. One of the incidents that caught our attention was the dramatically change in concentration of β-bisabolene. The measured concentration in both cases (mixtures of Debre Berhan and Bishoftu) was about 10 times less than the amount measured in individual species. This is likely due to chemical processes/reactions that have occurred during the distillation that transformed part of the β-bisabolene into another different compound.

In any case, mixing of the species to cook foods has its own advantage to get as many as possible compounds in food that may contribute towards flavor of the cook and also health benefit.The results of present study revealed that the compositions of the essential oils extracted from the mixture of two plants are different from that of the individual plant. This is because some of the compounds are present in the essential oils of the two plants and hence their percentages are increased while the percentages of those compounds which are present as minor components in the individual plants are decreased. Furthermore the total numbers of compounds in the essential oils of the mixture of the two plants are larger than that of individual plants. Therefore using the mixture of the two plants for spicing is preferable than the individual plant.

4.2. Antioxidant activity

4.2.1. Calibration curve data of ascorbic acid

The calibration curve data of ascorbic acid are given in Table 10. The calibration curve of ascorbic acid is shown in Figure 11. The relationship between concentration (µg/mL) of ascorbic acid and its average absorbance determined at 220 nm was used for the standard calibration curve. The regression equation obtained for ascorbic acid was y = 0.0028x + 0.0099 (R^2 = 0.9947).

Table 8: Calibration curve data of ascorbic acid **and % DPPH inhibition**

Concentration (µg/mL)	Absorbance of ascorbic acid at 220 nm	Absorbance at 517 nm	% DPPH Inhibition
100	2.07925	0.02550 ± 0.00029	98.1 ± 0.0253
50	1.6174	0.03860 ± 0.00185	97.1± 0.134
25	0.9308	0.06595 ± 0.00161	95.1 ± 0.120
12.5	0.4020	0.1128 ± 0.00186	91.5 ± 0.143
6.25	0.05534	0.1827 ± 0.00035	86.3 ± 0.0254

The results are reported as mean ± SD of three replicates.

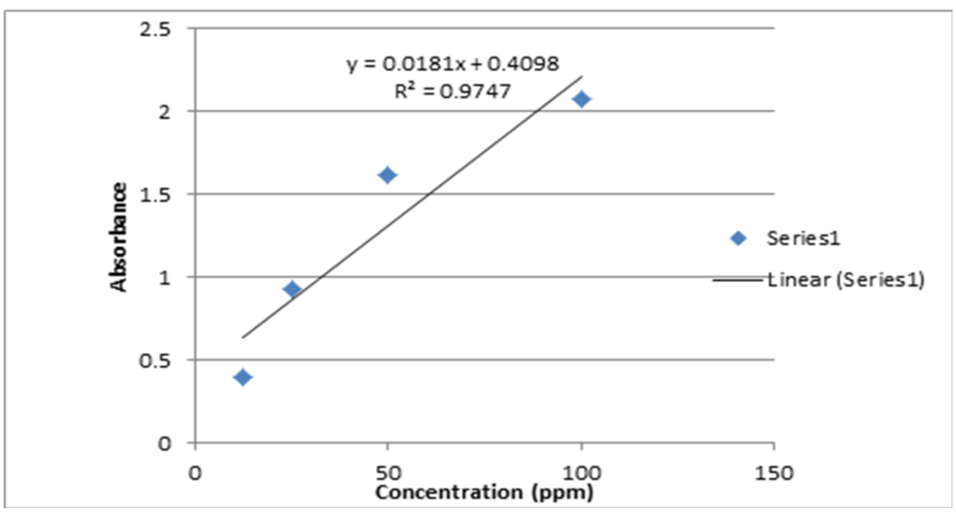


Figure 11: Calibration curve of ascorbic acid at 220 nm.

Table 9 shows the results obtained for antioxidant activity of the essential oil extract of *Ocimum sanctum* L. and ascorbic acid standards. The ethyl acetate solution of the essential oil *Ocimum sanctum* L. from the two sample area was able to reduce the stable DPPH radical blue to the yellow-colored 2,2-diphenyl-1-picrylhydrazyl indicating its potential as a radical scavenger. The essential oil of *Ocimum sanctum* L. from Bishoftu exhibited DPPH radical scavenging activities of 96.48% at 100 µg/mL while the essential oil of *Ocimum sanctum* L. from Debre Berhan sample area exhibited DPPH radical scavenging activities of 96.17% at 100 µg/mL. At the same concentration standard ascorbic acid scavenged the DPPH radical scavenging activities by 98.08 %.

Table 9: Radical scavenging activities of the essential oil of *Ocimum sanctum* L. and ascorbic acid standards determined using DPPH assay

Sample area	Concentration (µg/mL)	Absorbance of sample at 517 nm	Mean absorbance of sample at 517 nm	% DPPH inhibition EtOAc solution of *Ocimum sanctum* L. essential oil	% DPPH inhibition of ascorbic acid standards
Bishoftu *ocimum sanctum L.*	100	0.04776	0.047 ± 0.0018	96.48 ± 0.135	98.08 ± 0.0253
		0.04407			
		0.04810			
	50	0.04568	0.0502 ± 0.0044	96.24 ± 0.326	97.10 ± 0.134
		0.05724			
		0.04764			
	25	0.05121	0.0509± 0.00032	96.18 ± 0.024	95.05 ± 0.120
		0.05043			
		0.05121			
	12.5	0.05227	0.0573 ± 0.0040	95.69 ± 0.295	91.53 ± 0.143
		0.06335			
		0.05648			
Debre Berhan *ocimum sanctum*	100	0.05572	0.0509 ± 0.0045	96.17 ± 0.339	98.08 ± 0.0253
		0.05357			
		0.04361			
	50	0.05519	0.0553± 0.00022	95.84 ± 0.0147	97.10 ± 0.134

L.		0.05572			
		0.05519			
	25	0.05907	0.0599 ± 0.0012	95.50 ± 0.0878	95.05 ± 0.120
		0.05882			
		0.06190			
	12.5	0.2040	0.212 ± 0.0088	84.15 ± 0.657	91.53 ± 0.143
		0.2040			
		0.2255			

Table 12 shows the results obtained for antioxidant activity of the essential oil extract of *Lippia adoensis* and ascorbic acid standards.

The ethyl acetate soluble fraction of the leaves *Lippia adoensis* from the two sample area was able to reduce the stable DPPH radical blue to the yellow-colored 2,2-diphenyl-1-picrylhydrazyl indicating its potential as a radical scavenger. The essential oil of leaves *Lippia adoensis* from Bishoftu exhibited DPPH radical scavenging activities of 92.58 % at 100 µg/mL while the essential oil of *Lippia adoensis* from Debre Berhan sample area exhibited DPPH radical scavenging activities of 93.37% at 100 µg/mL which is comparable to that of ascorbic acid standard. At the same concentration standard ascorbic acid scavenged the DPPH radical scavenging activities by 98.08 %.

Table 10: Radical scavenging activities of the essential oil of *Lippia adoensis* and ascorbic acid standards determined using DPPH assay

Sample area	Concentration (µg/mL)	Absorbance of sample at 517 nm	Mean absorbance of sample at 517 nm	% DPPH inhibition EtOAc solution of *Lippia adoensis* essntial oil	% DPPH inhibition of ascorbic acid standards
Lippia adoensis	100	0.04557	0.0496 ± 0.005	92.58 ± 0.772	98.08 ± 0.0253
		0.05052			
		0.09022			
	50	0.06355	0.167 ± 0.005	75.04 ± 0.685	97.10 ± 0.134

Bishoftu		0.06448			
		0.06559			
	25	0.2513	0.367 ± 0.033	45.07 ± 4.95	95.05 ± 0.120
		0.2067			
		0.2067			
	12.5	0.3694	0.439 ± 0.0215	34.31 ± 3.221	91.53 ± 0.143
		0.3797			
		0.3694			
Lippia adoensis Debre Berhan	100	0.04557	0.333 ± 0.118	93.37 ± 1.84	98.08 ± 0.0253
		0.05052			
		0.09022			
	50	0.06355	0.0645 ± 0.0007	93.12 ± 0.0742	97.10 ± 0.134
		0.06448			
		0.06558			
	25	0.2513	0.222 ± 0.018	76.37 ± 1.943	95.05 ± 0.120
		0.2067			
		0.2067			
	12.5	0.3694	0.373 ± 0.004	60.24 ± 0.449	91.53 ± 0.143
		0.3797			
		0.3694			

Table 13: shows the results obtained for antioxidant activity of the essential oil extract of the mixture of two plants (*Lippia adoensis* and *Ocimum sanctum*) and ascorbic acid standards. The ethyl acetate solution of the essential oil of the leaves mixture of two plants (*Lippia adoensis* and *ocimum sanctum*) from the two sample area was able to reduce the stable DPPH radical blue to the yellow-colored 2,2-diphenyl-1-picrylhydrazyl indicating its potential as a radical scavenger. The essential oil of leaves mixture of two plants (*Lippia adoensis* and *Ocimum sanctum*) from Bishoftu exhibited DPPH radical scavenging activities of 95.25% at 100 µg/mL while the essential oil of mixture of two plants (*Lippia adoensis* and *Ocimum sanctum*) from Debre Berhan sample area exhibited DPPH radical scavenging activities of 96.42% at 100 µg/mL. At the same concentration standard ascorbic acid scavenged the DPPH radical by 98.08 %.

The results of present study revealed that the essential oils of *Ocimum sanctum* L. have higher DPPH radical scavenging activities than that of *Lippia adoensis* and the mixture of the two plants.

Table 11: Radical scavenging activities of the essential oil of the mixture of two plants (*Lippia adoensis* and *Ocimum sanctum* L.) and ascorbic acid standards determined using DPPH assay

Sample area	Concentration (µg/mL)	Absorbance of Sample at 517 nm	EtOAc extract of mixture of two plants. (*Lippia adoensis* & O*cimum sanctum* L.)	%DPPH inhibition EtOAc solution of (*Lippia adoensis* &*Ocimum sanctum* L.) essential oil	%DPPH inhibition of ascorbic acid standards
(*Lippia adoensis* and *ocimum sanctum*) Bishoftu	100	0.03268	0.0317 ± 0.0065	95.25 ± 0.973	98.08 ± 0.0253
		0.04038			
		0.02206			
	50	0.02710	0.0348 ± 0.0112	94.93 ± 1.760	97.10 ± 0.134
		0.02423			
		0.05311			
	25	0.07734	0.0548 ± 0.0138	91.78 ± 2.060	95.05 ± 0.120
		0.04447			
		0.04280			
	12.5	0.1928	0.187 ± 0.0190	72.11 ± 2.812	91.53 ± 0.143
		0.1581			
		0.2109			
(*Lippia adoensis* and O*cimum sanctum* L.) Debre Berhan	100	0.02423	0.0240 ± 0.00132	96.42 ± 0.199	98.08 ± 0.0253
		0.02577			
		0.02206			
	50	0.04954	0.0526 ± 0.00233	92.17 ± 0.349	97.10 ± 0.134
		0.05609			
		0.05217			
	25	0.07906	0.078 ± 0.00066	88.40 ± 0.097	95.05 ± 0.120
		0.07735			
		0.07754			
	12.5	0.2599	0.189 ± 0.057	66.86 ± 3.376	91.53 ± 0.143
		0.2042			
		0.1021			

The results of present study revealed that the essential oils of *Ocimum sanctum* L. have higher DPPH radical scavenging activities than that of *Lippia adoensis* and the mixture of the two plants.

4.2.2. UV-Spectra of 6 samples of ethyl acetate solution of the essential oils solution

The UV-Vis spectra of ethyl acetate solution of the essential oils of *Ocimum sanctum* L. and *Lippia adoensis* and their mixtures are shown in Figures 12-14.

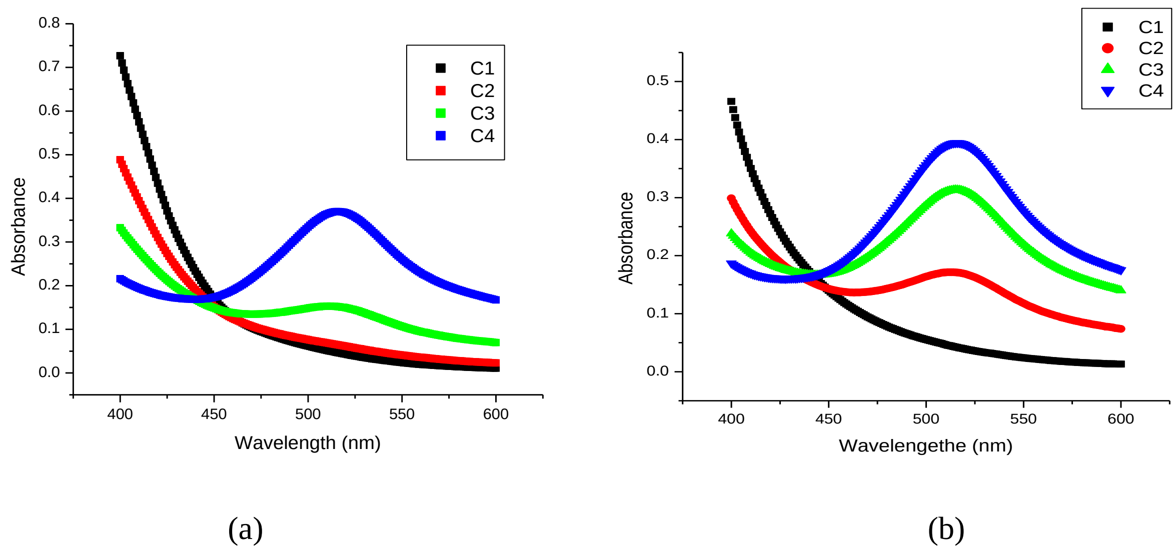
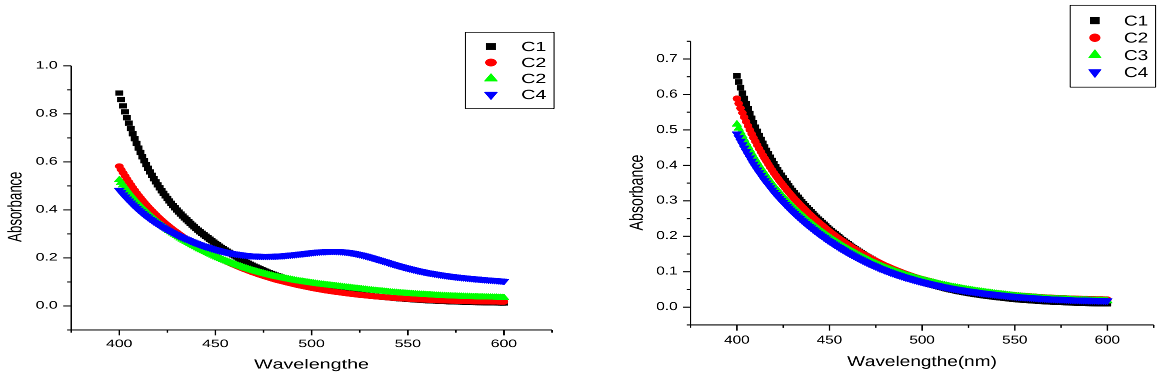


Figure 12: Absorbance of *Lippia adoensis* extracted (a) From Debre Brhan sample (b) From Bishoftu sample (C1 = 100 µg/mL, C2 = 50 µg/mL, C3= 25 µg/mL, C4= 12.5 µg/mL)

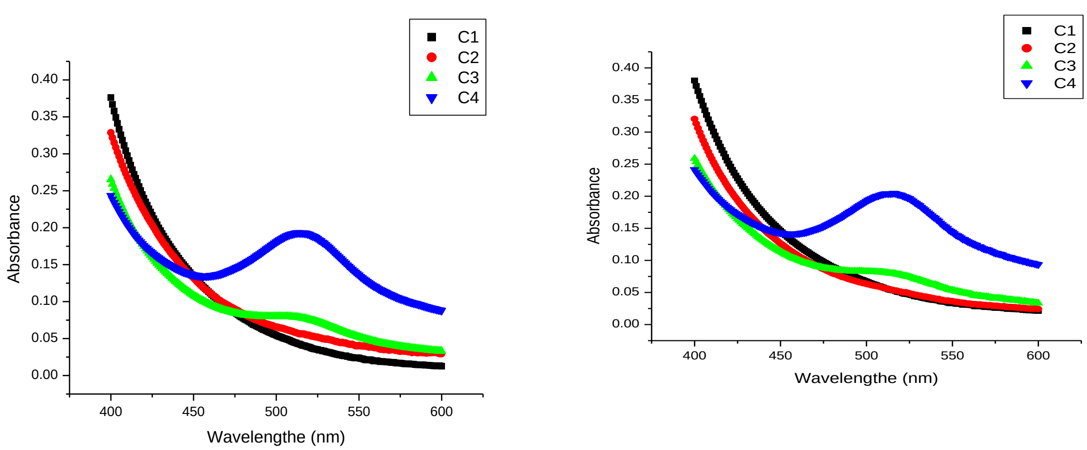
As indicated in Figure 11: UV–visible spectroscopy analysis of essential oils extract *Lippia adoensis* leaves, ethyl acetate solution of the essential oil from Bishoftu sample area has a higher absorbance than ethyl acetate solution of the essential oil of *Lippia adoensis* leaf from Debre Berhan *Lippia adoensis*. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity and this indicates the antioxidant capacity of essential oils from Bishoftu ethyl acetate solution of the essential oil of *Lippia adoensis*have lower free radical scavenging or antioxidant activity than Debre Brhan *Lippia adoensis*essential oilextracted. But all extracted shows almost similar decreasing trend in reducing power with the decreasing concentration of the samples.



(a) (b)

Figure 13: Absorbance of *Ocimum sanctum* extracted (a) From Debr Brhan (b) From Bishoftu(C1 = 100 µg/mL, C2 = 50 µg/mL, C3= 25 µg/mL, C4= 12.5 µg/mL)

As indicated in Figure 12: UV–visible spectroscopy analysis of essential oils extract *Ocimum sanctum* L. leaf and stem, from Debre Berhan sample area has a higher absorbance than Bishoftu *Ocimum sanctum*. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity and this indicates the antioxidant capacity of essential oils from Debre Berhan *Ocimum sanctum* L. have lower free radical scavenging or antioxidant activity than Bishoftu *Ocimum sanctum.* From Bishoftu sample area essential oil showed almost no peak on the UV–visible spectroscopy. This indicates higher reducing power or antioxidant activity.



(a) (b)

Figure 14: Absorbance of mixture of *Ocimum sanctum* and *Lippia adoensis* (a) From Bishoftu (b) From Debr Brhan (C1 = 100 µg/mL, C2 = 50 µg/mL, C3= 25 µg/mL, C4= 12.5 µg/mL)

As indicated in Figure 13: UV–visible spectroscopy analysis of essential oils extracted from mixture of *Lippia adoensis* leaves and *Ocimum sanctum* L. leaf and stem from Debre Berhan sample area has a higher absorbance than mixtures of mixture of *Ocimum sanctum* L. and *Lippia adoensis* from Bishoftu. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity and this indicates the antioxidant capacity of essential oils from Debre Berhan mixture of *Ocimum sanctum* L. and *Lippia adoensis* have lower free radical scavenging or antioxidant activity than Bishoftu mixtures. But all the essential oils showed almost similar decreasing trend in reducing power with the decreasing concentration of the samples.

5. **Conclusion**

In this work, essential oil of *Ocimum sanctum* L. stem and leaf (together without separation) and *Lippia adoensis* leaf from Bishoftu and Debre Berhan and their two mixtures were extracted by hydro-distillation and analyzed by GC-MS. The different components were characterized by matching their mass spectra with those of reference compounds recorded in NIST 2014 mass spectral library and confirmed by the retention index (RI) obtained by analyzing a mixture of *n*-alkanes using GC-MS under the same experimental condition as that of the sample analysis and using the van den Dool and Kratz relationship.

The antioxidant activity of the ethyl acetate extract of the essential oils from *Ocimum sanctum* L. stem and leaf (together without separation) and *Lippia adoensis* leaf and the mixture of the two plants were evaluated. Ethyl acetate solution of the essential oil of *Ocimum sanctum* from Debr Brhan and Bishoftu respectively 96.48% and 96.17% DPPH inhibition at 100µg/mL which are both comparable to that of ascorbic acid standard that exhibited 98.08% at 100µg/mL. Phenolic compounds are the major contributors to the antioxidant activity of *Lippia adoensis* leave and *Ocimum sanctum* stem and leaf (together without separation).

Generally the essential oils of *Ocimum sanctum* L. have highest DPPH radical scavenging activities than that of *Lippia adoensis* and the mixture of the two plants because of ethyl acetate extracted *Ocimum sanctum* from Bishoftu sample area has a lowest absorbance than *Ocimum sanctum* L. ethyl acetate solution of the essential oil of *Ocimum sanctum* L. from Debre Berhan *Ocimum sanctum* L. and also *Lippia adoensis* and the mixture of the two plants.

6. References

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