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CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITIES OF THE ESSENTIAL OILS OF *LIPPIA ADOENSIS* HOCHST ex. WALP AND *OCIMUM SANCTUM* LINN

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ABSTRACT. Essential oils of *Lippia adoensis* Hochst ex. Walp leaf, *Ocimum sanctum* Linn leaf and stem and mixture of two plants from Bishoftu and Debre Berhan (Ethiopia) were analyzed using gas chromatography-mass spectrometry. The essential oil of *O. sanctum* from Bishoftu showed 12 compounds with major components being β-bisabolene (31.38%), 4-[(1Z)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methylcyclo-hexen (25.56%), eucalyptol (17.12%). While that from Debre Berhan contained 20 compounds with major components being β-bisabolene (24.45%), 4-[(1Z)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methylcyclohexen (19.61%) and eucalyptol (13.42%). The essential oil of the leaves of *Lippia adoensis* from Bishoftu showed 15 compounds with major components as linalool (66.60%), caryophyllene (4.28%) and that from Debre Berhan showed 12 compounds with linalool (86.11%) as major compound. The essential oil of the mixture of *L. adoensis* and *O. sanctum* from Bishoftu showed 18 compounds with major compound being linalool (62.54%) and that from Debre Berhan showed 21 compounds with major compounds being linalool (48.47%) and eicosane (9.42%), octadecane (5.47%). The essential oils of *O. sanctum* and *L. adoensis* and their mixture from the two places exhibited DPPH radical scavenging activity of 96.48% and 96.17%, 92.58% and 93.37%, and 95.25% and 96.42% at 100 µg/mL, respectively. The antioxidant activities of essential oils were comparable to that of ascorbic acid which exhibited 98.08% at the same concentration (100 µg/mL).

KEY WORDS: *Ocimum sanctum*, *Lippia adoensis*, Essential oil, Antioxidant activity, DPPH assay

INTRODUCTION

Natural product is a substance produced by a living organism that is found in nature; such substance include spices. A spice is a seed, fruit, root, bark, or other plant part widely used for flavoring, coloring, or preserving food [1]. Many spices have antimicrobial and antioxidant properties [1].

The genus *Lippia* is a member of Verbenaceae family having about 200 species, distributed in tropical Africa and America [2]. There are five *Lippia* species in Ethiopia [2]. In Africa the leaves of some *Lippia* species are used as tea or spice [3], and also in South America [4]. Some species have antifertility properties [5], while others used in perfumery are important [4]. The essential oil of leaves and flowers has been reported [6]. The antioxidant activities of some of the species have also been reported [2]. *Lippia* species have also been found to have larvicidal, antiseptic and antifungal activity [7].

Lippia adoensis is endemic in Ethiopia. It is found in forest margins and disturbed areas at altitude of 1900-2650 m in many regions [8]. The wild *L. adoensis* locally known as *Kesse* is used for washing ceramic utensils and wooden materials to signify spicy and pleasant smell. There are also many medicinal applications. To prevent flu, indigestion and headache, boiled flowers and leaves of *L. adoensis* are taken [9].

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 *L. adoensis* are

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used for flavoring purposes and when compared to wild *L. adoensis* they are claimed to have higher flavour. Dried leaves of cultivated *L. adoensis* are important ingredients in the preparation of spiced butter giving it a characteristic flavor and pleasant sweet aroma. In the preparation of Ethiopian dishes, like *Kitfo*, *Doro Wat*, spiced butter is an important cooking fat. Several other spices are also used in making flavoured (spiced) butter. Some of these spices are cardamom, basil, fenugreek, black cumin, turmeric, white cumin, garlic and rue. Unique flavour imparted to *Kitfo* is mainly from *L. adoensis* used in the preparation of spiced butter, which is characteristics of Gurage *Kitfo* [10].

The genus *Ocimum* (basil) is a member of the Labiatae family [11]. *Ocimum* is an important medicinal and economic plant [12, 13]. *Ocimum basilicum* L. is upright herbaceous annual aromatic and spice plants [10]. It is widely used to flavor and add a distinctive aroma to food. Essential oils extracted from flowers and fresh leaves can be used as additives in food, cosmetics and pharmaceuticals [11]. The plant has proven its effectiveness in curing cold, fever, indigestion, respiratory syndrome, diarrhea, headache, bronchitis, hysteria and cough [14].

Several studies have been reported on the composition of essential oils of different species of *Ocimum* from different countries [15, 16]. Charles and Simon [17] have studied essential oil composition and content of *Ocimum* species from West Lafayette, America. Dean *et al.* [18] have reported essential oil profiles of several temperate and tropical aromatic plants including *Ocimum* species and their antimicrobial and antioxidant activities. Javanmardi *et al.* [11] have studied chemical characterization of local accessions *O. basilicum* L. used in traditional medicines in Iran. Lee *et al.* [19] have reported identification of volatile components in *O. basilicum* and its antioxidant properties. Pandalia and Verma [20] have determined comparative volatile oil composition of four *Ocimum* species from northern India. Yaldiz *et al.* [21] have studied chemical composition of *O. basilicum* essential oil in relation to the different harvest period and cultivation conditions from Turkey. Gebrehiwot *et al.* [22] have reported chemical composition and antimicrobial activities of leaves of *O. basilicum* from Adama, Ethiopia. Beatovic *et al.* [23] have studied chemical composition, antioxidant and antimicrobial activities of the essential oils of twelve *O. basilicum* cultivars grown in Serbia.

O. sanctum essential oil is used as flavoring agent and possess antioxidative and antimicrobial activities [11]. Extraction of *O. sanctum* is prescribed for the management of skin disease, bronchitis, diarrhea, dysentery, malaria, arthritis, and insect bites. It is also considered to be helpful for adapting to stress, balancing different processes in the body [24].

Flavour and pleasant smell composition of *Ocimum* species are complex due to the presence of phytochemicals [20]. Volatile compounds are mainly responsible for its characteristic aroma while non-volatiles phenolic for example caffeic acid, rutin, and rosmarinic acid for pharmacological actions [25]. *O. sanctum* has been recognized as a rich source of aromatic essential oil used mainly in pharmaceutical, cosmetic as well as food and flavouring industries [17].

Abegaz *et al.* [9] have reported chemical composition of the essential oils of endemic wild and cultivated *L. adoensis* in Addis Ababa, Ethiopia. Terblanché and Kornelius [26] reported essential oil constituents of the genus *Lippia* (Verbenaceae) from South Africa. Babarinde *et al.* [27] have reported chemical composition and bioactivity of *L. adoensis* (Verneneaceae) leaf essential oil against *Callosobruchus maculatus* from Ogbomoso, Nigeria.

However, information on the chemical composition and antioxidant activities of essential oils of *O. sanctum* and *L. adoensis* from Ethiopia is scarce and limited. Furthermore, there is no study on the chemical composition and antioxidant activities of essential oils of the mixtures of the two plants. Therefore, it is worthwhile to investigate the chemical composition and antioxidant activities of essential oils of *O. sanctum* and *L. adoensis* and their mixtures from two different places in Ethiopia.

The objective of this study was to identify the chemical components and investigate the antioxidant properties of essential oils of *O. sanctum* and *L. adoensis* and their mixtures.

EXPERIMENTAL

Equipment and chemicals

The equipment used to conduct this study was hydro-distillation set up, locally manufactured electrical stove, digital balance, UV-Visible spectrometer (model: PerkinElmer UV Win Lab 6.0.3.0730/1.61.00 Lambda 900), gas chromatograph-mass spectrometer (GC-MS) (Model: GC-7820A, Agilent Technologies; Detector-5977EMSD, USA), column: DB-1701 (30 m × 0.250 mm, 0.25 µm particle size). The chemicals and solvents used were hexane, pentane, methanol, ethyl acetate, ascorbic acid and DPPH.

Sample collection

About 0.5 kg of each of *L. adoensis* leaf and *O. sanctum* stem and leaf (together without separation) from Bishoftu and Debre Berhan, Ethiopia 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 collected.

Procedure for hydrodistillation

The leaves of *L. adoensis* and stem and leaves of *O. sanctum* were cut in to pieces and 0.5 kg of the sample was put into a 5 L round bottom flask containing 2 L of distilled water. Extraction of essential oils was done by Clevenger apparatus using hydro-distillation method. The plant material was heated using a heating mantel to boil the water and the process was continued for 3 h till the level of the extracted oil remained constant. Then the essential oil was separated from aqueous layer, collected in air tight glass sample bottle, measured and placed in a refrigerator at 4 ºC until GC-MS and antioxidant analysis.

Sample preparation for GC-MS analysis

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

GC-MS characterization of essential oil

The essential oil obtained from *L. adoensis* leaf and *O. sanctum* leaf and 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 1 µL 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.

The essential oil extract of *L. adoensis* leaf and *O. sanctum* (stem and leaf together without separation) was collected by hydrodistillation of 500 g of the plant materials and the mixture of the two plants (each 250 g). A 1 µL of 20 µg/mL samples solution was analyzed by GC-MS.

Retention index (RI) of the essential oil components of *L. adoensis* and *O. sanctum* were 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 [28]:

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

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.

Procedure for DPPH radical scavenging assay

The radical scavenging activity of the essential oils of *L. adoensis* and *O. sanctum* and the mixtures of the two plants was assessed using DPPH according to the procedure described by Bhuiyan *et al.* [29]. The essential oil 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 62.5 µg/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.

Data analysis

Data analysis was done using statistical software (SPSS version 20) to analyze the differences of antioxidant activities between concentrations; and between sample and ascorbic acid at $p < 0.05$.

RESULTS AND DISCUSSION

GC/MS analysis of essential oil extract from L. adoensis and O. sanctum and their mixture

The representative chromatograms of the essential oils of *L. adoensis* and *O. sanctum* are shown in Figures 1 and 2.

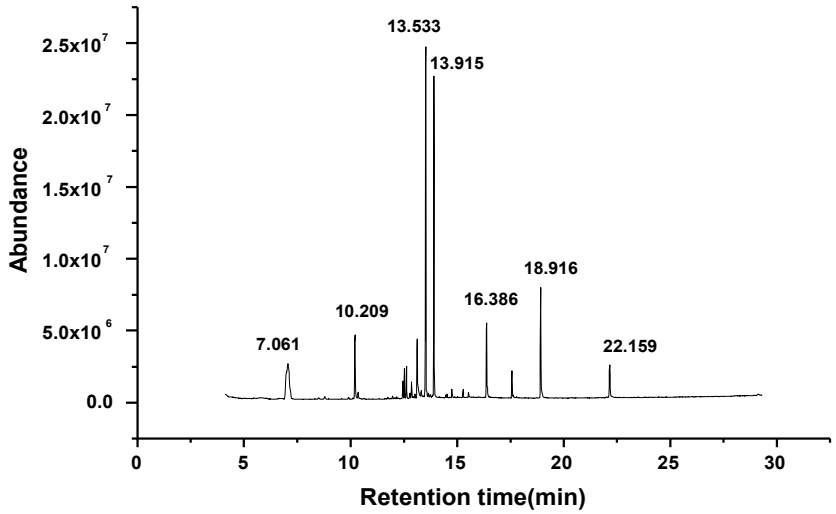

Figure 1. GC-MS chromatogram of essential oil of *Ocimum sanctum* L. from Debre Berhan.

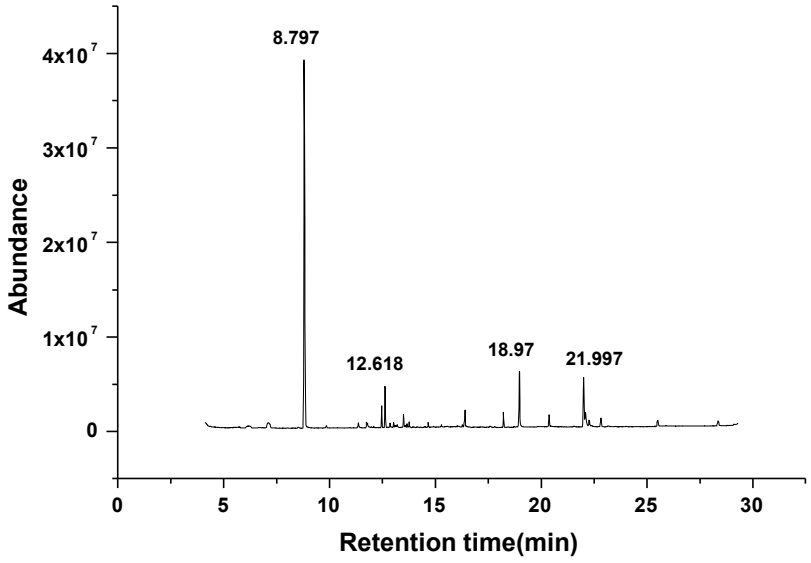

Figure 2. GC-MS chromatogram of essential oil of *Lippia adoensis* from Bishoftu.

The essential oil of *O. sanctum* from Bishoftu showed 12 compounds with major compounds being β-bisabolene (31.38%), 4-[(1Z)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methylcyclo-hexen (25.56%), eucalyptol (17.12%) and minor compounds being estragole (7.48%), 2-methoxy-3-(2-

propenyl)-phenol (5.64%), γ-muurolene (2.92%) and *trans*-α-bergamotene (2.32%), pentadecane (1.47%), β-ylangene (1.33%), α-terpineol (1.04%) and two very minor compounds that are indicated in Table 1 with concentrations of less than 1%. Twenty compounds were identified in the essential oil of *O. sanctum* from Debre Berhan with major compounds being β-bisabolene (24.45%), 4-[(1Z)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methylcyclohexen (19.61%) and eucalyptol (13.42%) and minor compounds being 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 as indicated in Table 2.

Table 1. Results of GC-MS analysis of essential oil of *Ocimum sanctum* L. from Bishoftu.

Peak No.	R_t	RI	% of compound	Compound name
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	γ-Muurolene
8	12.868	1444	0.70	(Z)-β-Farnesene
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	E-α-Bisabolene
12	13.915	1574	25.56	4-[(1Z)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methylcyclohexene

Table 2. Results of GC-MS analysis of essential oil of *Ocimum sanctum* L. from Debre Berhan.

Peak No.	R_t	RI	% of compound	Compound name
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-Farnesene
9	13.033	1446	0.28	ε-Muurolene
10	13.1315	1509	5.79	2-Methoxy-3-(2-propenyl)-phenol
11	13.323	1464	0.83	β-Sesquiphllandrene
12	13.533	1542	24.5	E-α-Bisabolene
13	13.9201	1574	19.6	4-[(1Z)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methylcyclohexen
14	14.764	1647	0.55	(E)-γ-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

When essential oils of *O. sanctum* from the two sampling areas were compared, it was found that their metabolite profiles are almost similar with minor differences. There are three major compounds which are common to *O. sanctum* samples from the two areas. 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 *O. 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. *O. sanctum* from Bishoftu showed γ-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 the sample from Bishoftu 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.

The essential oil of the leaves of *Lippia adoensis* from Bishoftu showed 15 compounds with major compounds as linalool (66.60%), caryophyllene (4.28%) and minor compounds 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 listed in Table 3. The essential oil of the leaves of *L. adoensis* from Debre Berhan showed 12 compounds with linalool (86.11%) as major compound and minor compounds as eucalyptol (2.75%), β-pinene (2.57%), caryophyllene (1.95%), β-ylangene (1.44%), endo-borneol (1.13%), germacrene D (1.07%) and some very minor compounds listed in Table 4.

Table 3. Results of GC-MS analysis of essential oil of *Lippia adoensis* from Bishoftu.

Peak No.	R_t	RI	% of compound	Compound name
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	66.6	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-cadinene
15	16.292	1642	0.64	Tau (η)-muuralol

The essential oil of *L. adoensis* from two sample sites shares 6 compounds in common including the major compound, linalool. The compounds detected are in different concentrations as expected which could be due to variation in geographical location and other factors. Variation in composition pattern of essential oils is attributable to changes in environmental factors and geographical location [30, 31]. Interestingly, 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 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 *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 4. Results of GC-MS analysis of essential oil of *Lippia adoensis* from Debre Berhan.

Peak No.	RT	RI	% of compound	Compound name
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

The essential oil of mixture of leaf of *L. adoensis* and stem and leaf *(*together without separation) of *O. sanctum* from Bishoftu showed 18 compounds with major compound as linalool (62.54%) and minor compounds 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-[(1Z)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methylcyclo-hexane (1.12%) and nonadecane (1.10%) and some other minor compounds listed in the Table 5. The essential oil of the mixture of *L. adoensis* leaf *and* stem and leaf *(*together without separation) of *O. sanctum* from Debre Berhan showed 21 compounds with major compounds as linalool (48.47%) and eicosane (9.42%), octadecane (5.47%) and minor compounds as caryophyllene (4.71%), eucalyptol (4.58%), β-bisabolene (4.57%), heneicosane (3.71%), β-ylangene (2.73%), 4-[(1Z)-1,5-dimethyl-1-hexadien-1-yl]-1-methylcyclohexene (2.27%), α-copaene (1.94%), β-famesene (1.01%), β-citra (1.01%) and some other minor compounds listed in Table 6.

In order to investigate what would happen, when two different plant species (*Lippia adoensis and Ocimum sanctum*) are mixed (equal amount of the plants material from both species) and hydrodistilled. The essential oil profiles of the mixtures showed metabolites those detected in individual plant species. The interesting part was that the co-distillation of the plant materials did not affect the qualitative metabolite profile in terms of the identified compound; however, concentrations of some of the metabolites changed in significant amount. There was an obvious change in the concentration of β-bisabolene. The measured concentration in both cases (mixtures of two plants from 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.

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; hence, their percentages 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.

Table 5. Results of GC-MS analysis of essential oil of the mixture of two plants (*Lippia adoensis* and *Ocimum sanctum*) from Bishoftu.

Peak No.	R_t	RI	% of compound	Compound name
1	6.147	1020	1.79	β-Pinene
2	7.06	1080	4.02	Eucalyptol
3	8.793	1191	62.5	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-[(1Z)-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

Table 6. Results of GC-MS analysis of essential oil of the mixture of two plants (*Lippia adoensis* and *Ocimum sanctum*) from Debre Berhan.

Peak No.	R_t	RI	% of compound	Compound name
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-Farnesene
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	E-α-Bisabolene
16	13.916	1574	2.27	4-[(1Z)-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

Comparison of chemical composition of essential oils of the present study with results reported in literature

Two studies were reported on the chemical composition of essential oils of leaves of *Lippia adoensis* from Nigeria. In one study, the major compound was found to be linaloon (81.30%) [6]. While in another study the major compounds were eucalyptol (28.36%), *α*-terpineol (25.99%) and *γ*-terpinene (15.24%) [27]. The major compounds in the essential oils of leaves of cultivated *L. adoensis* from four different places (Addis Ababa, Butajira, Sodo, Ghedo) in Ethiopia were linalool 73.19-82.75%, germacrene D: 6.74-9.48% [9] while there was a wide variation in the major compounds in the wild *L. adoensis* with limonene (3.44-32.30%), perillaldehyde (0.11-26.90%), piperitenone (0.15-44.48%), geranial (2.08-18.80%), ipsdienone (0.81-14.89%) [9]. In contrast to these, in the present study, the major compounds in essential oils of cultivated *L. adoensis* leaf from two places in Ethiopia were found to be linalool (66.60%) in the sample from Bishoftu and linalool (86.11%) in the sample from Debre Berhan. Variation in composition pattern of essential oils is attributable to changes in environmental factors and geographical location [30, 31].

Charles and Simon [17] investigated the composition of essential oil of basil (*Ocimum* spp.) from USA under different extraction conditions and reported the concentration of linalool, the major constituent, as 48.2% and 48.1% in oils obtained by hydro- and steam distillation, respectively, but 62.8% by organic solvent extraction. Similarly, methyl chavicol was 31.6% and 28.3% by hydro- and steam distillation, respectively, but only 16.2% by organic solvent extraction.

Lee *et al.* [19] studied the aroma of basil and reported that the major aroma compounds of basil were 3,7-dimethyl-1,6-octadien-3-ol (linalool; 3.94 mg/g), 1-methoxy-4-(2-propenyl) benzene (estragole; 2.03 mg/g), methyl cinnamate (1.28 mg/g), 4-allyl-2-methoxyphenol (eugenol; 0.896 mg/g), and 1,8-cineole (0.288 mg/g).

Pandalia and Verma have investigated the comparative volatile oil composition of four *Ocimum* species from Northern India. The essential oil compositions of cvs. 'Green' (CIM-Ayu) and 'Purple' (of *O. sanctum*) were almost the same, and both the cultivars were dominated by phenylpropanoids (68.1% and 73.5%) represented mainly by eugenol (67.4% and 72.8%, respectively). Sesquiterpene hydrocarbons (22.4% and 22.6%) constituted the second major class of compounds, mainly dominated by β-elemene (11.0% and 10.9%), β-caryophyllene (7.3% and 8.4%) and germacrene D (2.4% and 2.2%).

Yaldiz *et al.* [21] reported the chemical composition of basil (*Ocimum basilicum* L.) essential oil in relation to the different harvest period and cultivation conditions in Turkey. Methyl cinnamate (8.2-19.5 %) and linalool (6.9-42.7 %) were the major compounds in all harvests with respect to open-field conditions and kiwi plantations, respectively. Furthermore, eugenol (2.3-13.0 %), germacrene-D (1.5-15.9 %) and farnesene (0.2-21.1 %) were found to be high in green basil and the highest values were obtained from second harvest in open-field conditions. The percentage of 1.8 cineole in green basil plants growing under open-field conditions was 10.9 %.

Gebrehiwot *et al.* [22] have reported the chemical composition of essential oil extracted from the leaves of sweet basil (*O. basilicum*) from Haramaya, Ethiopia was found to have estragole (38.22%) as a major compound followed by 1-isopropyl-4-methylenecyclohex-1-ene (11.10%).

Beatovic *et al.* [23] have investigated the chemical composition of the essential oils of twelve *O. basilicum* cultivars grown in Serbia. The major compounds found were linalool (11.5-58.6%), α-*trans*-bergamotene (1.0-19.3%), β-bisabolene **(**0.6-23.8%), *trans*-methyl cinnamate (0.1-31.4%), eugenol (0.8-16.2%), neral (0.05-12.8%), methyl chavicol (estragole) (0.1-83.6%), 1,8-cineole (0.1-14.3%). In another study [16], the chemical composition of the essential oil of *O. sanctum* grown in Serbia were reported sesquiturpene hydrocarbonate β-cariophyllene is a predominant component in the essential oil with 63.80%. Ilić *et al.* [32] studied the chemical composition of the essential oils of three *O. basilicum* cultivars from Serbia. They reported the

major compounds as linalool (13.68-40.97%), eugenol (8.97-10.83%), α-bergamotene (1.95-9.25%) and epi-bicyclosesquiphellandrene (7.03-8.70%).

In the present study, the major compounds in the essential oils from the leaves of *O. santrum* from Bishoftu were E-α-bisabolene (31.38%) and 4-[(1Z)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methylcyclohexene (25.56%) and that from Debre Berhan E-α-bisabolene(24.45%) and 4-[(1Z)-1,5-dimethyl-1,4-hexadien-1-yl]-1-methylcyclohexen (19.61%). Variation in composition pattern of essential oils is attributable to changes in genetic and environmental factors and geographical location [30, 31].

Antioxidant activity

Table 7 shows the results obtained for antioxidant activity of the essential oil extract of *O. sanctum* and ascorbic acid standards. The ethyl acetate solution of the essential oil of *O. sanctum* from the two locations of plant collection 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 *O. sanctum* from Bishoftu exhibited DPPH radical scavenging activities of 96.48% at 100 µg/mL while the essential oil of *O. sanctum* 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%.

The DPPH radical scavenging activities of the essential oil of *O. sanctum* from Bishoftu showed no significant differences at $p < 0.05$ at all the four concentrations and also with that of ascorbic acid. However, there is a significant differences at $p < 0.05$ at the low concentration (12.5 µg/mL) between the DPPH radical scavenging activities of the essential oil from Debre Berhan and ascorbic acid.

Table 8 shows the results obtained for antioxidant activity of the essential oil extract of *L. adoensis* and ascorbic acid standards. The essential oil of *L. adoensis* from Bishoftu exhibited DPPH radical scavenging activities of 92.58% at 100 µg/mL while the essential oil of *L. adoensis* from Debre Berhan exhibited DPPH radical scavenging activities of 93.37% at 100 µg/mL which is comparable to that of ascorbic acid standard. The DPPH radical scavenging activities of the essential oil of *L. adoensis* from the two locations showed significant differences at $p < 0.05$ at all the four concentrations and also with that of ascorbic acid.

Table 7. Radical scavenging activities of the essential oil of *Ocimum sanctum* and ascorbic acid standards determined using DPPH assay.

Sample area	Concentration (µg/mL)	%DPPH inhibition of sample	%DPPH inhibition of ascorbic acid
Bishoftu	100	96.48 ± 0.135	98.08 ± 0.0253
	50	96.24 ± 0.326	97.10 ± 0.134
	25	96.18 ± 0.024	95.05 ± 0.120
	12.5	95.69 ± 0.295	91.53 ± 0.143
Debre Berhan	100	96.17 ± 0.339	98.08 ± 0.0253
	50	95.84 ± 0.0147	97.10 ± 0.134
	25	95.50 ± 0.0878	95.05 ± 0.120
	12.5	84.15 ± 0.657	91.53 ± 0.143

Table 8. Radical scavenging activities of the essential oil of *Lippia adoensis* and ascorbic acid standards determined using DPPH assay.

Sample area	Concentration (µg/mL)	%DPPH inhibition of sample	%DPPH inhibition of ascorbic acid
Bishoftu	100	92.58 ± 0.772	98.08 ± 0.0253
	50	75.04 ± 0.685	97.10 ± 0.134
	25	45.07 ± 4.95	95.05 ± 0.120
	12.5	34.31 ± 3.221	91.53 ± 0.143
Debre Berhan	100	93.37 ± 1.84	98.08 ± 0.0253
	50	93.12 ± 0.0742	97.10 ± 0.134
	25	76.37 ± 1.943	95.05 ± 0.120
	12.5	60.24 ± 0.449	91.53 ± 0.143

Table 9 shows the results obtained for antioxidant activity of the essential oil extract of the mixture of two plants (*L. adoensis* and *O. sanctum*) and ascorbic acid standards. The ethyl acetate solution of the essential oil of two plants (*L. adoensis* and *O. 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 (*L. adoensis* and *O. sanctum*) from Bishoftu exhibited DPPH radical scavenging activities of 95.25% at 100 µg/mL while the essential oil of mixture of two plants (*L. adoensis* and *O. 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%.

Table 9. Radical scavenging activities of the essential oil of the mixture of two plants (*L. adoensis* and *O. sanctum*) and ascorbic acid.

Sample area	Concentration (µg/mL)	%DPPH inhibition of sample	%DPPH inhibition of ascorbic acid
Bishoftu	100	95.25 ± 0.973	98.08 ± 0.0253
	50	94.93 ± 1.760	97.10 ± 0.134
	25	91.78 ± 2.060	95.05 ± 0.120
	12.5	72.11 ± 2.812	91.53 ± 0.143
Debre Berhan	100	96.42 ± 0.199	98.08 ± 0.0253
	50	92.17 ± 0.349	97.10 ± 0.134
	25	88.40 ± 0.097	95.05 ± 0.120
	12.5	66.86 ± 3.376	91.53 ± 0.143

The DPPH radical scavenging activities of the essential oils of the mixture of the two plants from the two locations showed significant differences at $p < 0.05$ at all the four concentrations and also with that of ascorbic acid.

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

CONCLUSION

In this work, essential oil of *Ocimum sanctum* stem and leaf (together without separation) and *Lippia adoensis* leaf from Bishoftu and Debre Berhan and their two mixtures were extracted by hydrodistillation and analyzed by GC-MS. 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 plants. Furthermore the total numbers of compounds in the essential oils of the mixture of the two plants are larger than that of individual plants. Thus mixing of the spices to

cook foods has its own advantage to get as many as possible compounds in food that may contribute towards flavor of the food and health benefit. Therefore using the mixture of the two plants for spicing is preferable than the individual plant. The antioxidant activity of the essential oils from *O. sanctum* stem and leaf (together without separation) and *L. adoensis* leaf and the mixture of the two plants were evaluated. The essential oils of *O. sanctum* have higher DPPH radical scavenging activities than that of *L. adoensis* and the mixture of the two plants. The DPPH radical scavenging activities of the essential oils are comparable to that of ascorbic acid standard at the same concentration.

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