

The chemotypes of Ethiopian *Ocimum basilicum* L. (sweet basil) germplasms



Aynalem Gebre Gossa^{a,b}, Bizuayehu Tesfaye Asfaw^b, Magrate M. Kaigongi^{c,*}, Abiy Yenesew^d

^a Wondo Genet Agricultural Research Center, Ethiopian Institute of Agricultural Research (EIAR), P. O. Box 198, Shashemene, Ethiopia

^b School of Plant and Horticultural Science, Hawassa University, P. O. Box 05, Hawassa, Ethiopia

^c Kenya Forestry Research Institute, P.O. Box 20412-00200, Nairobi, Kenya

^d Department of Chemistry, University of Nairobi, PO Box 30197-00100, Nairobi, Kenya

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ABSTRACT

Sweet basil (*Ocimum basilicum* L.) exhibits significant chemical variability. This study characterized the chemical composition of 49 accessions of *O. basilicum* collected from different parts of Ethiopia using GC–MS. Results of this analysis revealed the presence of 46 compounds. Four compounds (eucalyptol, linalool, estragole, and eugenol) were found in all accessions, and seven compounds were found in large quantities of more than 10 %; methyl cinnamate (41.9 %), geraniol (27.9 %), linalool (25.41 %), eugenol (23.08 %), β -bisabolene (23.03 %), eucalyptol (17 %) and estragole (16.6 %). Cluster analysis grouped the 49 accessions into four chemotypes. Chemotype A consisting of eugenol/ estragole/ eucalyptol/ β -bisabolene, chemotype B (eucalyptol/estragole), chemotype C (linalool/geraniol) and chemotype D (methyl cinnamate/linalool). The results of this study indicated that the compounds found in Ethiopian *O. basilicum* accessions are highly diverse. Overall, the results of this study will provide scientific basis for future breeding programs of this crop.

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1. Introduction

Sweet basil (*Ocimum basilicum* L.) is an important herbaceous aromatic and medicinal plant that belongs to the Lamiaceae family. The genus *Ocimum*, comprises more than 150 species, is distributed all over the temperate and tropical regions of the world (Nassar et al., 2013). *O. basilicum* is native to tropical regions of Asia including Iran and India (Marwat et al., 2011). The species is commercially and extensively cultivated for essential oil production in many countries of the world (Wesolowska et al., 2012). *O. basilicum* is botanically described as a branched plant that grows between 0.3 and 1.3 m height, with light green silky leaves in opposite directions and containing many oily glands that store essentials oils. The basil flowers are colored from white to purple and arranged in a terminal spike (Sestili et al., 2018).

Ocimum species are characterized by a great chemical variability because of the abundant cross pollination, inter-specific hybridization, polyploidy (Raina and Misra, 2018). *O. basilicum* essential oil has several biological activities: anti-inflammatory and antioxidant (Akoto et al., 2020; Stanojevic et al., 2017) antimicrobial (Rubab et al., 2021), antiviral (Kubića et al., 2014), and anticancer (Perna et al., 2022) properties. In traditional medicine, they are used in the

treatment of malaria, colic vomiting, common cold, cough, headaches, diarrhea, inflammation, pain, and skin diseases (Purushothaman et al., 2018; Venâncio et al., 2011). Externally, they have been applied for the treatment of acne, loss of smell, insect stings, snake bites, and skin infections (Gebrehiwot et al., 2016). Due to their aroma, basils are widely utilized for flavor and fragrance in food, pharmaceutical, cosmetic, and aromatherapy industries (Egata, 2021; Hanif et al., 2019).

In Ethiopia, sweet basil has been cultivated and used for thousands of years as a culinary, spice, and medicinal herb and is locally called "Bessobila" (Abewoy, 2021; Egata, 2021; Tadesse et al., 2019; Tesfa et al., 2017). The tender stems, leaves, and flowers are dried, ground, and added to a different kind of locally prepared food and are also used for the treatment of malaria, headaches, and diarrhea (Demissew, 1993). It is a means of income generation for small-scale households by selling the aboveground part to local markets, with some of the harvest being exported to different countries (Tadesse et al., 2019).

The high levels of chemical variability *O. basilicum* is due to its wide distribution throughout the world, growing under different climatic conditions. Furthermore, the availability of various chemotypes and the production of new cultivars have resulted in a great variation in the essential oil composition and aroma among basil genotypes (Varga et al., 2017). Despite the growing domestic demand of essential oils in Ethiopia for aromatherapy, reports on the chemical

* Corresponding author.

E-mail address: mkaigongi@kefri.org (M.M. Kaigongi).

characterization of Ethiopian *O. basilicum* are meager. Morphological variability of 28 accessions of Ethiopian *O. basilicum* was studied and classified into six genotypes through morphological markers (Egata et al., 2017). The classification of genotypes by morphological features becomes difficult due to anthropogenic interference with selection, cultivation, and hybridization (Abdo, 2021). Further, chemical characterization can be used to separate the accessions based on the presence and concentrations of specific substances and to determine the intrinsic variability or variability among accessions of same species (Raina and Misra, 2018). Therefore, this study was aimed at investigating the contents and variability in the chemical composition of the essential oil of Ethiopian *O. basilicum* germplasm.

Studying the current status of chemotypes of Ethiopian *O. basilicum* and obtaining the chemical content will provide a guide for their management, assessment and identification of economically beneficial accessions. Chemotaxonomy and the comparative analysis of metabolic features have the potential to provide valuable information relating to ecology and evolution (Crockett and Robson, 2011) and was thus chosen in this study. To determine whether the studied *O. basilicum* accessions illuminate possible intraspecific relationships, metabolomics as a chemotaxonomic tool was selected because of its high resolution power in providing insights into plant diversification and evolution (Martucci et al., 2014).

2. Materials and methods

2.1. Plant materials and design of experiments

A total of 49 *O. basilicum* accessions were used in this study. These included 22 obtained from the Ethiopian Biodiversity Institute (EBI), 2 released varieties from Wondo Genet Agricultural Research Center, 23 accessions collected for this study from different parts of the country (SNNP, Oromia, and Harari), and 2 accessions obtained from abroad (one from Norway and one from Israel) (Table 1). The experimental fields were arranged in a 7 7 × 7 simple lattice design with two replications. Spacing of 1.5 m and 1 m was maintained between replications and plots, respectively. The experimental field consisted of 2.4 m × 3.6 m plots each containing 6 rows with 40 cm intra-row and 60 cm inter-row spacing. *O. basilicum* seeds were seeded in a 10 cm polyethylene bag in 2019 at Wondo Genet agricultural research center in a greenhouse. The potting medium was prepared by mixing a 3:2:1 ratio of topsoil, forest soil, and sand, respectively. Watering was done twice a week after all of the seedlings had emerged. After five weeks, the seedlings were transplanted to a lath house for hardening off for one week. Before transplanting the seedling to the main field, the experimental plot was prepared properly. Well- performing, vigorous and healthy-looking seedlings were transplanted to the main experimental field after 6 weeks. Each experimental plot contained a total of 36 plants. No fertilizer or chemical application was done during experimentation. All horticultural management was done as required.

2.2. Sample preparation and extraction of essential oil by hydro distillation

Composite samples of three-hundred-gram basil leaves and inflorescence were collected from each germplasm. Extraction was done by hydro distillation using Clevenger type apparatus for 3 h and the essential oil was collected after drying with anhydrous sulfate. The essential oil samples were stored in a refrigerator until GC–MS analysis.

2.3. GC–MS analysis of essential oil

GC–MS analysis was carried out in the Natural Product laboratory at Wondo Genet Agricultural Research centre using Abdo et al.

(2021) conditioning method for the genus *Ocimum*. The conditioning method used in the analysis of essential oil was Gas Chromatography–Mass Spectrometry (GC–MS) (Agilent model 7820 A) equipped with ALS. Solutions of essential oils (0.2 %) were prepared by dissolving 10 µL essential oil in 5 mL N-Hexane. The instrument was conditioned with a split/splitless injector mode, MS detector (5975), and HP-5 SM capillary column (0.25 mm i.d. × 30 m × 0.25 µm film thickness). The injector was operated on a split ratio of 1:10 with injection volumes of 1 µL and the injector temperature was set at 250 °C. The MSD interface temperature was set to 260 °C. Helium was used as carrier gas and controlled in constant flow mode at a linear velocity of 36.6 cm/sec. The oven temperature was programmed to start at 60 °C, which was held for 1 min and ramped at 5 °C/min to 80 °C with 3 min holding; the temperature was ramped at 4 °C/min to 180 °C and was held for 3 min. Finally, the temperature was raised from 25 °C/min to 300 °C for a duration of 6 min. The MSD was operated on scan mode in 40–500 m/z range, with ion source and transfer line temperatures held at 230 °C and 260 °C, respectively. The solvent delay time was 3.4 min and took 46.8 min for the total run (Lebanov et al., 2021).

2.4. Data analysis

Untargeted analysis of the data was performed using MarkerLynx (part of MassLynx 4.1, Waters, Milford, USA) to generate a matrix of accurate mass and retention time (AMRT) features uploaded to MetaboAnalyst, a free online software platform, to perform the chemometric analysis. Hierarchical cluster analysis (HCA) in form of a dendrogram was used to show the chemical relationship among the analyzed accessions. Interrelationships were revealed through HCA using Euclidean distance measure based on three components (MS data, relative abundances, and spatial distribution) for the test materials (Kaigongi and Lukhoba, 2021). The instrument variation was negligible and related samples clustered close to each other. Where samples from different sites appeared to cluster together, orthogonal partial least squares discriminant analysis (OPLS-DA) was performed to further separate them. Targetlynx, another application within Masslynx, was used to quantify the various tentatively identified metabolites in a relative fashion, against calibration curves of the standards (Kaigongi et al., 2020).

3. Results and discussion

3.1. Chemical composition of *O. basilicum* essential oils

The GC–MS analysis of essential oil of 49 *O. basilicum* accessions resulted in identification of 46 compounds, of which 16 are major essential oil compounds. These compounds were: β-pinene (0–1.50 %), eucalyptol (1,8-cineole) (2.05–17.00 %), β-ocimene (0.30–5.05 %), linalool (0.29–25.41 %), α -terpineol (0–1.79 %), estragole (0.46–16.6 %), nerol (0–4.40 %), geraniol (0–27.60 %), chavicol (0–7.00 %), citral (0–3.90 %), eugenol (0.62–23.08 %), methyl cinnamate (0–41.90 %), caryophyllene (0–2.51 %), humulene (0–2.51 %), β-bisabolene (0–23.03 %) and α-bisabolol (0–1.56 %). From the 16 major compounds, seven of them (Fig. 1) occur in more than 10 %. The 7 compounds were: methyl cinnamate (41.90 %), geraniol (27.90 %), linalool (25.41 %), eugenol (23.08 %), β-bisabolene (23.03 %) and eucalyptol (17.00 %), and estragole (16.60 %). The essential oil from the aerial parts of *O. basilicum* consists of a wide and varying array of chemical constituents, depending on variations in chemotypes, leaf and flower colors, aroma, and origin of the plants (Chalchat and Özcan, 2008; Varga et al., 2017). The findings from this study are in line with the report of Sajjadi (2006) who found linalool, methyl chavicol and caryophyllene among the main constituents in the oil of Iranian *O. basilicum*. Elsewhere, linalool, methyl cinnamate and methyl eugenol were recorded as the main constituents of oil

Table 1

Accession code, origin, source and coordinates for exact collection points as well as the country of origin of sweet Basil accessions used for the study.

No	Accession code	Region	Source	Latitude (°)	Longitude (°)	Altitude (m)	Country of origin
1	OB001	Tigray	EBI	14.092210N	38.633967E	2317	Ethiopia
2	OB002	Tigray	EBI	14.131408N	38.771450E	2017	Ethiopia
3	OB003	Tigray	EBI	14.165868N	38.900548E	2108	Ethiopia
4	OB004	Tigray	EBI	14.282391N	38.075276E	2172	Ethiopia
5	OB005	Tigray	EBI	14.276690N	39.462396E	2170	Ethiopia
6	OB006	Oromia	EBI	7.678924N	36.836128E	2198	Ethiopia
7	OB007	Oromia	EBI	7.673607N	36.831364E	2433	Ethiopia
8	OB008	Oromia	EBI	7.672788N	36.822438E	2336	Ethiopia
9	OB009	Oromia	EBI	7.675862N	36.830592E	1835	Ethiopia
10	OB010	Oromia	EBI	7.679945N	36.834111E	1611	Ethiopia
11	OB011	Oromia	EBI	7.679438N	36.834054E	1719	Ethiopia
12	OB012	Oromia	EBI	7.679751N	36.831301E	2107	Ethiopia
13	OB013	Amhara	EBI	10.684177N	37.345815E	1840	Ethiopia
14	OB014	Amhara	EBI	10.404703N	37.029965E	1840	Ethiopia
15	OB015	Amhara	EBI	10.618046N	37.422726E	1940	Ethiopia
16	OB016	Amhara	EBI	10.642327N	37.392513E	2570	Ethiopia
17	OB017	SWE	EBI	7.579963N	36.028147E	1944	Ethiopia
18	OB018	SWE	EBI	7.305727N	36.120158E	1791	Ethiopia
19	OB019	SWE	EBI	7.301587N	36.070719E	1378	Ethiopia
20	OB020	SWE	EBI	7.318825N	37.836348E	2532	Ethiopia
21	OB021	SWE	EBI	7.335819N	36.158994E	1768	Ethiopia
22	OB022	Sidama	EBI	6.799455N	38.435200E	1789	Ethiopia
23	OB023	Oromia	WGARC	8.982323N	37.867920E	1776	Ethiopia
24	OB024	Oromia	WGARC	8.979610N	37.875645E	1764	Ethiopia
25	OB025	Oromia	WGARC	8.976049N	37.881138E	1789	Ethiopia
26	OB026	Oromia	WGARC	8.705492N	37.888570E	1776	Ethiopia
27	OB027	Oromia	WGARC	8.436618N	37.885823E	1731	Ethiopia
28	OB028	Oromia	WGARC	9.033854N	38.355489E	1722	Ethiopia
29	OB029	Oromia	WGARC	9.066403N	38.580709E	1770	Ethiopia
30	OB030	Oromia	WGARC	8.976887N	37.652364E	2411	Ethiopia
31	OB031	Oromia	WGARC	8.995877N	38.124776E	2354	Ethiopia
32	OB032	SNNPR	WGARC	5.782638N	36.506218E	1733	Ethiopia
33	OB033	SNNPR	WGARC	6.091336N	36.462273E	1745	Ethiopia
34	OB034	SNNPR	WGARC	5.413618N	36.684746E	1432	Ethiopia
35	OB035	SNNPR	WGARC	6.109518N	37.759921E	1453	Ethiopia
36	OB036	SNNPR	WGARC	7.408383N	38.058499E	2132	Ethiopia
37	OB037	SNNPR	WGARC	7.309640N	38.120297E	2250	Ethiopia
38	OB038	SNNPR	WGARC	7.344373N	38.125104E	2050	Ethiopia
39	OB039	SNNPR	WGARC	7.272520N	38.067082E	1912	Ethiopia
40	OB040	SNNPR	WGARC	7.320455N	38.070350E	1961	Ethiopia
41	OB041	Oromia	WGARC	9.066403N	38.566976E	2031	Ethiopia
42	OB042	Harari	WGARC	9.322035N	42.114757E	1875	Ethiopia
43	OB043	SNNPR	WGARC	6.851478N	37.755629E	1785	Ethiopia
44	OB044	SNNPR	WGARC	6.848751N	37.759749E	1793	Ethiopia
45	OB045	Oromia	WGARC	8.971461N	37.542501E	2031	Ethiopia
46	OB046	Harari	WGARC	9.319817N	42.115138E	1965	Ethiopia
47	OB047	Norway	Norway	9.422338N	42.037339E	2034	Norway
48	OB048	Israel	Israel	13.782239N	39.515557E	2054	Israel
49	OB049	Harari	WGARC	13.954230N	39.574608E	2363	Ethiopia

OB- *Ocimum basilicum*; SNNPR- Southern Nations, Nationalities, and Peoples' Region; WGARC- Wondo Genet Agricultural Research Center; EBI- Ethiopian Biodiversity Institute; SWE- south west Ethiopia.

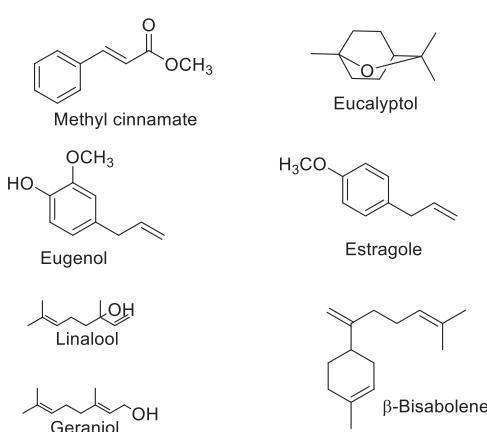


Fig. 1. Chemical structures of the 7 major compounds of *O. basilicum* essential oils.

samples collected from different geographical regions of this species ([Srivastava et al., 2018](#)), while [Varga et al. \(2017\)](#) recorded 1,8-cineole, linalool, linalool acetate, methyl chavicol, eugenol, trans-methyl cinnamate and trans- α -bergamotene from different cultivars of sweet basil. The major compounds recorded in this study are important for many kinds of biological activities, including anti-inflammatory, antibacterial, antiviral, antifungal, and muscle relaxant properties ([Avetisyan et al., 2017](#); [Carnesecchi et al., 2002](#); [Chen and Viljoen, 2010](#); [Koba et al., 2009](#); [Pavan et al., 2018](#)). In addition, these compounds have a wide application in the perfumery, cosmetics, and food flavoring industries ([Varga et al., 2017](#); [A. Wesolowska et al., 2012b](#)). Methyl cinnamate was the dominant constituent occurring up to 41.9 % (OB033) across accessions with an average of 3.24 % ([Table 2](#)). Three accessions, OB001, OB036, and OB042 recorded above 5 % methyl cinnamate content. Methyl cinnamate was not found in three of the accessions (OB046, OB013, and OB030). These results differ from values obtained in different studies elsewhere.

Table 2Major chemical composition of the essential oil of 49 *Ocimum basilicum* accessions.

Accessions	Chemical Composition (%)																
	CC1	CC2	CC3	CC4	CC5	CC6	CC7	CC8 RT	CC9	CC10	CC11	CC12	CC13	CC14	CC15	CC16	
	3.73	4.89	5.31	6.71	9.63	9.99	11.11	12.00	12.11	12.67	16.11	16.90	18.20	19.50	22.01	28.30	
351	OB038	0.90	10.89	2.23	0.46	1.18	11.60	0.28	—	0.95	—	15.12	0.92	2.46	2.20	17.73	1.20
	OB040	0.26	10.68	2.75	0.48	1.20	13.10	0.35	—	0.97	0.18	13.58	0.82	2.49	1.72	18.21	1.07
	OB032	1.48	12.17	5.05	1.92	1.27	10.90	0.44	—	1.23	0.44	16.49	5.86	1.93	1.72	12.23	0.76
	OB025	1.28	12.70	2.50	0.91	1.22	11.40	—	3.40	5.30	3.80	9.16	2.08	1.88	1.11	12.17	0.66
	OB021	0.65	7.35	2.47	1.69	0.81	8.95	1.13	—	1.33	1.41	5.49	4.28	2.01	1.06	21.16	1.56
	OB016	0.87	9.87	3.31	0.66	1.00	11.10	—	1.16	2.06	1.29	11.95	1.60	2.38	1.78	17.76	1.00
	OB027	0.82	10.22	1.96	0.72	1.23	11.10	—	—	0.72	—	17.37	1.78	2.28	2.03	17.56	0.96
	OB004	0.78	7.52	3.88	2.57	0.78	7.49	—	—	1.23	—	10.5	6.83	2.48	2.05	15.83	1.56
	OB017	0.54	7.89	2.98	0.95	0.84	10.30	—	—	0.56	0.11	18.8	2.64	2.03	1.59	18.24	1.35
	OB011	1.20	11.84	3.96	0.38	1.22	12.90	0.55	0.54	1.23	0.31	16.57	0.43	2.35	1.83	14.93	1.11
	OB005	0.29	5.74	2.69	1.00	0.74	7.27	—	0.21	0.70	0.26	23.08	2.19	1.97	1.48	18.68	1.25
	OB049	0.99	9.80	2.63	2.29	0.97	10.10	—	—	0.53	—	11.57	5.74	2.18	1.72	17.22	1.07
	OB045	1.13	13.56	2.21	0.38	0.43	12.00	—	2.68	2.67	1.74	5.16	0.56	2.44	1.73	17.18	1.16
	OB006	0.71	8.46	2.35	0.29	0.83	16.60	—	—	—	—	17.76	1.45	2.45	1.60	17.04	1.04
	OB029	0.89	10.21	2.37	4.43	1.10	9.55	—	5.27	—	—	11.29	0.59	2.10	1.89	15.68	1.00
	OB044	1.41	14.25	2.99	0.37	1.42	13.80	—	—	1.25	—	16.19	0.63	2.59	2.24	14.37	0.96
	OB037	0.75	11.04	1.68	2.39	0.41	13.80	—	—	1.19	—	12.52	5.02	2.39	2.23	15.01	1.24
	OB020	0.73	5.56	3.89	4.26	0.92	5.77	3.12	1.44	2.06	3.96	7.35	3.78	1.34	0.78	8.36	0.86
	OB024	0.65	6.50	4.37	0.41	0.92	10.20	—	—	1.07	0.14	18.67	1.21	1.94	1.25	11.81	0.76
	OB031	0.69	10.73	2.68	0.59	1.09	11.60	—	—	0.93	—	11.24	0.35	1.60	1.61	14.13	0.91
	OB008	0.34	7.50	1.02	0.63	1.21	11.60	—	—	1.08	—	16.42	—	2.08	1.78	17.77	—
	OB030	1.12	10.87	5.47	0.34	—	14.30	1.39	—	1.71	1.24	7.01	0.92	2.39	1.49	16.81	0.71
	OB015	0.51	4.10	0.57	10.59	—	13.90	0.33	—	2.01	1.40	0.62	1.09	0.43	0.44	3.22	0.27
	OB009	0.61	7.66	1.76	0.70	1.26	10.00	0.19	—	—	—	20.05	1.66	2.26	—	17.76	—
	OB003	1.05	9.45	2.91	0.86	1.15	10.30	0.23	—	0.88	—	12.79	0.49	2.45	1.96	15.94	1.07
	OB047	0.43	5.77	0.45	12.97	1.04	0.46	—	—	—	—	8.58	3.61	—	0.66	—	—
	OB010	0.39	8.28	1.28	0.30	1.01	13.30	—	—	—	0.86	10.09	0.55	2.31	1.89	12.59	1.15
	OB034	0.71	8.85	2.08	3.21	0.91	12.00	—	—	1.09	—	10.02	1.72	1.64	1.34	15.83	0.67
	OB048	0.32	3.77	0.31	25.41	0.81	0.78	—	1.08	—	—	8.20	4.42	—	0.82	—	—
	OB043	0.84	10.43	1.81	1.10	1.19	16.30	—	—	0.74	—	8.30	0.54	2.51	1.46	18.95	0.93
	OB035	0.96	12.17	3.28	1.50	1.20	13.80	—	—	0.85	—	11.96	0.72	1.78	2.28	15.88	0.91
	OB028	0.54	8.98	1.20	1.09	1.12	13.50	—	—	0.78	—	9.34	1.88	2.44	2.51	19.93	1.18
	OB022	0.77	12.19	1.44	0.67	1.20	12.00	0.58	—	1.16	0.47	9.93	1.48	1.83	1.94	9.44	1.24
	OB036	—	2.05	0.88	20.14	—	1.60	—	—	—	—	2.21	9.43	1.65	0.50	—	—
	OB012	0.67	7.87	2.88	2.42	0.89	9.21	—	—	0.66	—	17.02	0.67	1.75	1.39	17.03	0.80
	OB023	1.05	13.24	2.14	2.60	1.10	13.30	—	—	0.82	—	16.45	1.34	1.38	1.24	13.30	—
	OB018	0.90	9.97	4.52	1.15	0.89	12.90	4.39	—	4.30	3.73	5.18	0.51	2.07	2.08	12.30	1.06
	OB013	0.74	6.19	0.86	21.81	0.75	2.21	—	27.60	—	0.84	2.79	—	0.34	—	—	—
	OB007	0.77	10.10	1.68	0.57	0.95	16.30	—	—	0.75	—	11.67	0.63	2.45	1.66	19.99	1.14
	OB001	0.74	7.40	1.66	5.56	1.03	8.46	—	—	0.59	—	13.33	13.8	1.72	1.55	15.58	1.04
	OB014	0.93	12.79	1.81	1.64	1.57	12.50	—	—	0.85	—	11.8	0.68	1.83	2.22	18.82	—
	OB039	0.91	11.93	1.90	0.73	0.99	11.80	—	—	0.85	—	15.92	1.51	2.13	1.77	18.95	1.31
	OB002	0.72	10.17	1.92	0.69	1.09	11.60	—	—	0.91	—	14.99	1.35	2.15	1.82	20.88	1.25
	OB046	0.32	7.68	1.32	0.81	1.28	8.76	—	—	0.85	—	16.02	—	1.87	1.85	20.28	1.25
	OB041	0.42	9.06	1.22	0.81	1.21	13.10	—	—	0.91	—	6.83	0.86	1.96	1.85	23.03	1.17
	OB033	0.36	4.64	0.40	22.96	0.53	1.51	—	—	7.00	—	2.03	41.9	—	0.39	—	—
	OB026	1.42	17.00	1.19	2.63	1.79	12.10	3.57	—	3.61	3.16	3.55	7.11	1.23	0.88	14.26	—
	OB042	—	8.10	1.42	2.52	—	9.78	—	—	—	—	11.02	8.10	—	1.65	17.27	—
	Mean	0.74	9.27	2.45	3.61	0.95	10.60	0.34	0.91	1.23	0.44	11.54	3.24	1.80	1.53	14.4	0.80
	Range	0–1.50	2.05–17.00	0.3–5.00	0.29–25.41	0–1.79	0.46–16.60	0–4.40	0–27.60	0–7.00	0–3.90	0.62–23.08	0–41.90	0–2.50	0–2.50	0–23.03	0–1.6

CC= Chemical Composition, RT= Retention time, CC1 = β -Pinene, CC2= Eucalyptol, CC3= β -ocimene, CC4= Linalool, CC5= alpha-Terpineol, CC6= Estragole, CC7= Nerol, CC8= Geraniol, CC9= Chavicol, CC10= Citral, CC11= Eugenol, CC12= Methyl cinnamate, CC13= Caryophyllene, CC14= Humulene, CC15= β -bisabolene, CC16= α -bisabolol.

Taie et al. (2010), Wesolowska et al. (2012), Srivastava et al. (2018) and Abdo et al. (2021) reported that methyl cinnamate is one of the major components of *O. basilicum*. Geraniol was the second most abundant constituent, its content ranging from absent to 27.62 % with an average of 3.24 %. Three accessions recorded more than 2 % geraniol content, OB029 (5.27 %), OB025 (3.40 %), and OB045 (2.68 %), but it was absent in most of the accessions. These findings are in agreement with the reports of Al Abbasy et al. (2015), Nurzyńska-Wierdak et al. (2013) who reported that one of the major constituents of *O. basilicum* was geraniol (12.60 %).

Linalool was a dominant constituent ranging from 0.29 % (OB006) to (25.41 % (OB048) across accessions with an average of 3.61 %. Four accessions recorded above 20 %: OB048 (25.41 %), OB033 (22.96 %), OB013 (21.81 %), and OB036 (20.14 %) and also two accessions recorded above 10 %: OB047 (12.97 %) and OB015 (10.59 %). The findings are in line with Nurzyńska-Wierdak et al. (2013) who reported that the major constituent of *O. basilicum* was linalool (64.71 %). Similarly, Ismail (2006) reported that the major terpenes present in the sweet basil are linalool (44.18 %). Likewise, Antic et al. (2019) reported linalool as the dominant compound (40.97 %) in *O. basilicum*. Additionally, eugenol was present in all of the accessions analyzed with a range of 0.62 % to 23.08 % and an average of 11.54 %. One accession, OB009, recorded considerably higher eugenol content OB009 (23.08 %). Elsewhere, eugenol was reported as the major constituent of sweet basil (Antic et al., 2019; Sharopov et al., 2016; Srivastava et al., 2018).

β -bisabolene was also dominant constituent ranging from absent to 23.03 % (OB033) with an average of 14.40 %. β -bisabolene was the dominant compound in almost all the accessions analyzed except in five accessions (OB033, OB036, OB013, OB048, and OB047). This corroborates with the report of Abdo et al. (2021) who found that Bisabolene ranged from 45.79 % and 43.92 %. Eucalyptol was also one of the dominant constituents in this study ranging from 2.05 % (OB036) to OB026 (17.00 %) across accessions with an average of 9.27 %. Most of accessions recorded above 10.00 %. This is in agreement with findings of Milenković et al. (2019), who reported that the eucalyptol content of *O. basilicum* range from 8.70 % to 15.30 %.

O. basilicum essential oil has different biological activities based on various kinds of chemical compounds. Among the major chemical compounds geraniol is used for anti-inflammatory, antioxidant, neuroprotective, and anticancer effects (Carnesecchi et al., 2002; Chen and Viljoen, 2010; Pavan et al., 2018). In addition, geraniol is known to exhibit insecticidal and insect repellent properties and hence used as a natural pest control agent (Lapczynski et al., 2008; Pavan et al., 2018). Interestingly, geraniol has also been shown to sensitize tumor cells to commonly used chemotherapies and represents a promising cancer chemopreventive agent (Carnesecchi et al., 2002; Pavan et al., 2018). In addition, linalool exhibits various biological activities, antimicrobial, anti-inflammatory, anticancer, and anti-oxidant properties (Avetisyan et al., 2017). The high content of linalool, methyl cinnamate, eugenol, geraniol, and eucalyptol is highly valued in the cosmetics, perfume, flavor, and fragrance industries (Wesolowska et al., 2012). Therefore, the results of this study indicated that most of the major compounds crucial for different kinds of biological activities and industrial applications were found in Ethiopian basil accessions that can be exploited for future development of this plant.

3.2. Cluster analysis

The hierachal cluster analysis based on 16 major compounds led to segregation of samples into 4 clusters (Fig. 2). The number and name of accessions as well as their collection regions are presented in Table 3. Accessions collected from the same origin grouped in different cluster and accession from different origin fall in same cluster.

Cluster I: contain 38 accessions that cover 77.55 % of the accessions found in this group (Table 3). This group is characterized by a significant content of eugenol/estragole/ β -bisabolene of which represents chemotype A. One accession (OB005) contained considerably high content of eugenol (23.08 %). Similarly, another accession (OB006) contains high content of eugenol (17.76 %) and estragole (16.63 %). A high content of eucalyptol recorded from accession OB045 (14.24 %). In addition, 11 accessions from this chemotype recorded more than 15.00 %: eugenol content OB009 (20.05 %),

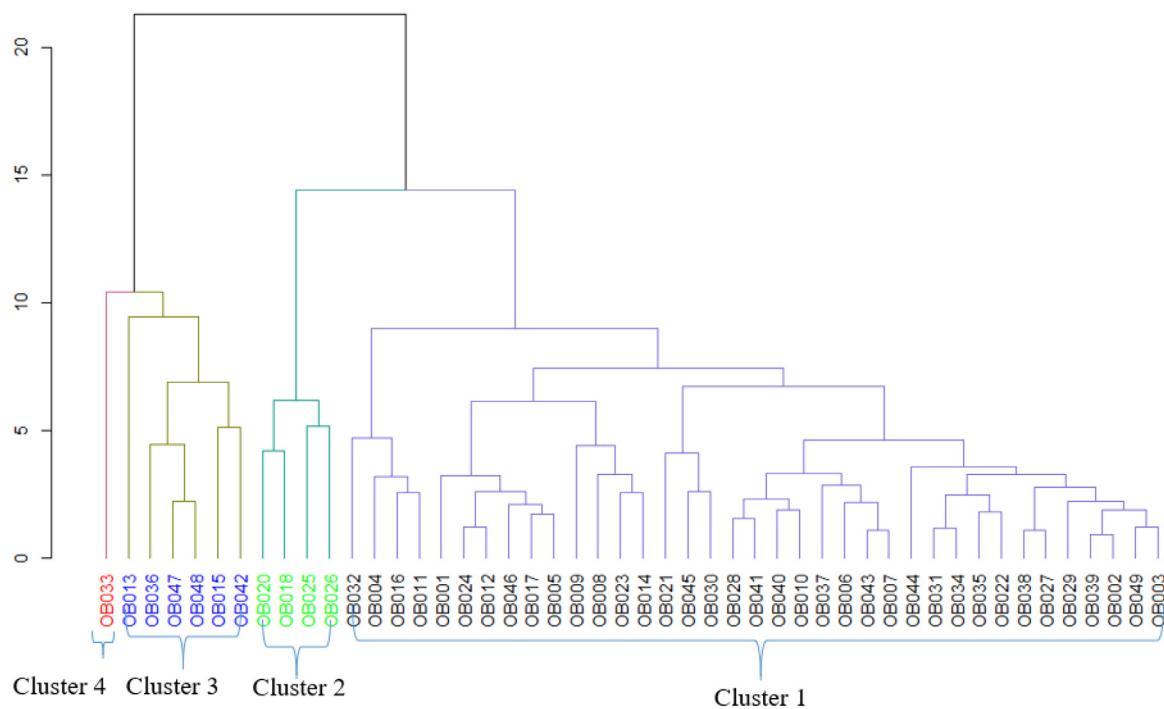


Fig. 2. Dendrogram showing clustering patterns for 49 accessions based on different chemical compounds of *Ocimum basilicum*.

Table 3Details of clusters of *O. basilicum* segregated from the 46 compounds.

Cluster	No of accessions	Accession in each cluster	Collection origin	Chemotype
I	38	OB032, OB004, OB016, OB011, OB01, OB024, OB012, OB046, OB017, OB005, OB009, OB008, OB023, OB014, OB021, OB045, OB030, OB028, OB041, OB040, OB010, OB037, OB006, OB043, OB007, OB044, OB031, OB034, OB035, OB022, OB038, OB027, OB029, OB039, OB002, OB049, OB003, OB020	Ethiopia	A
II	4	OB020, OB018, OB025, OB026	Ethiopia	B
III	6	OB013, OB036, OB047, OB048, OB015, OB042	Norway, Israel, Ethiopia	C
IV	1	OB033	Ethiopia	D

OB017 (18.8 %), OB024 (18.67 %), OBO16 (17.37 %), OB012 (17.02 %), OB011 (16.57 %), OB032 (16.49 %), OB023 (16.45 %), OB008 (16.42 %), OB046 (16.02 %), and OB038 (15.12). Moreover, two accessions in this group contained above 15 % estragole content: OB043 (16.03 %) and OB007 (16.03 %). Accession OB041 contained considerably high content of β -bisabolene (23.02 %). In addition, two accessions from this group recorded above 20 %: β -bisabolene OB002 (20.88 %) and OB046 (20.28 %).

Cluster II: This group contained four accessions that covered 8.16 % of the accessions (Table 3). This group is characterized by large content of eucalyptol/estragole which represents chemotype B. One accession (OB025) contained a considerably high content of eucalyptol (17 %) and estragole (12.06 %). From four accessions three of them recorded above 10 % estragole content: OB018 (12.96 %), OB025 (12.06 %) and OB026 (11.37 %).

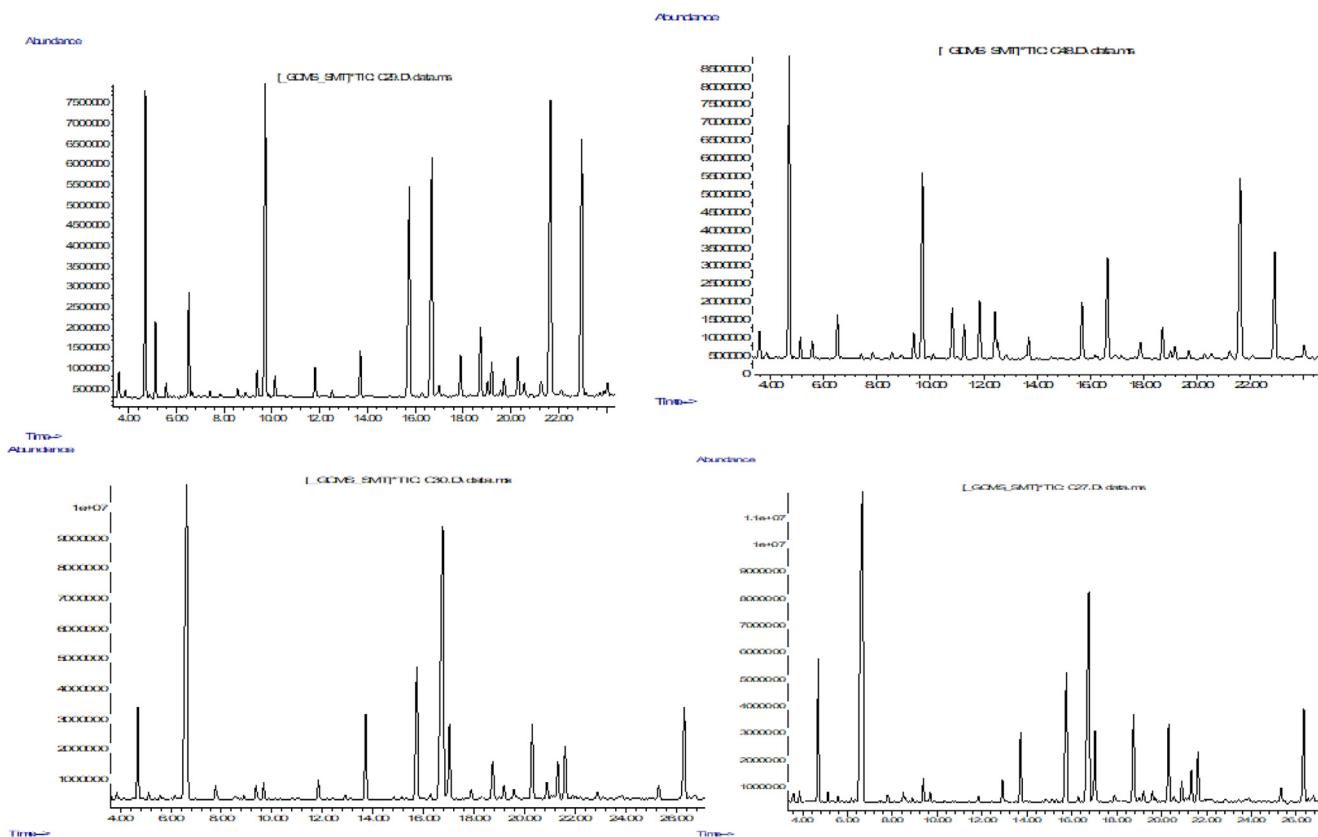
Cluster III: contain six accessions that represent 12.24 % of the accessions and is dominated by linalool /geraniol and representing chemotype C. From the six accessions, three accessions recorded above 20 %: OB048 (25.41 %), OB013 (21.81 %) and OB036 (20.14 %).

One accession (OB013) recorded a considerably high content of both geraniol (27.81 %) and linalool (21.81 %).

Cluster IV: contained one accessions that composed 2.04 % of the accessions dominated by methyl cinnamate/linalool representing chemotype D. Accession OB033 recorded a considerably high content of both methyl cinnamate (41.86 %) and linalool (22.96 %). This chemotype contains both the European chemotype which is characterized by high linalool content (Da Costa et al., 2015; Marotti et al., 1996; Sharopov et al., 2016; Srivastava et al., 2018; Varga et al., 2017), and the Tropical chemotype which is characterized by high methyl cinnamate (Abdo, 2021; Dris et al., 2017; Grayer et al., 1996; Srivastava et al., 2018; Telci et al., 2006; Wesolowska et al., 2012; Zheljazkov et al., 2008).

Chromatograms of the compounds representing the different clusters are shown in Fig. 3.

These findings are in line with the results of Marotti (Marotti et al., 1996) who reported three chemotypes for *O. basilicum*; linalool/methyl chavicol, and linalool/eugenol. Similarly, Telci et al. (2006) also reported seven chemotypes; 1) Linalool, 2) Methyl cinnamate, 3)

Fig. 3. GC-MS chromatogram of *O. basilicum* essential oils for each cluster.

Methyl cinnamate /linalool, 4) methyl eugenol, 5) Citral, 6) methyl chavicol (estragole), and 7) methyl chavicol/citral. Likewise, Bernhardt et al. (2014) found two chemotypes in *O. basilicum*; (I) Linalool-rich and (II) Linalool/methyl chavicol chemotype. In addition, Sharopov et al. (2016) characterized *O. basilicum* into six chemotypes; linalool (I), eugenol (II), estragole (III), methyl eugenol (IV), 1,8-cineole (V), and geraniol (VI). Elsewhere, Varga et al. (2017) reported five chemotypes; (A) High-linalool, (B) Linalool/trans- α -bergamotene, (C) Linalool/methyl chavicol, (D) Linalool/trans-methyl cinnamate and (E) High-methyl chavicol chemotype.

The present study demonstrated that the Ethiopian germplasms of *O. basilicum* varied significantly. This study pointed out intraspecific variations amongst germplasms from similar geographic origins while other samples exhibited chemotype similarity between germplasms from geographically different areas. The great intraspecific biochemical diversity found in this study was also observed in other taxa of the Lamiaceae family, specifically in species belonging to genera *Mentha* and *Thymus* (Tétényi, 1970). The great biochemical diversity among accessions of *O. basilicum* can be attributed to a long tradition of breeding for different purposes (Vieira and Simon, 2000). Hybridization between different accessions has generated compounds in the essential oil of hybrids that are not present in the essential oils of either parents (Costa et al., 2014). Due to a long breeding tradition (including interspecies crosses), the relationship between morphological diversity and chemical composition is complex. Because *O. basilicum* was and still is cultivated for different purposes, many hybrids exist as a result of combining different genotypes. In breeding programs aimed at obtaining ornamental cultivars, a selection of certain morphological features occurred. On the other hand, programs used for creating genotypes that exhibited

specific aromas used as spices or in pharmacology, selected accessions with certain biochemical profiles. As a result, we have a great number of cultivars that exhibit different morphological traits but show the same biochemical profile, and vice versa. Thus, biochemical traits, coupled with morphological analysis should be used for intra-specific characterization and germplasm management and determining the origin of cultivars (Varga et al., 2017). Morphological features of some of the accessions representing different chemotypes are showed in Fig. 4.

Discriminants identified based on the regions of sample collection were achieved through PCA loadings where 16 chemicals were identified (Fig. 5.). The discriminants were identified as outliers from the pool of chemicals in the PCA loadings (Máximo et al., 2006). Average concentrations of the chemical markers among accessions from different regions are shown in Fig. 6.

Extensive natural disparity in phytochemical profiles occurs between and within species of plants as an adaptation measure to different abiotic and biotic environments (Boccard and Rutledge, 2013). The most important players in the biosynthesis and accumulation of secondary metabolites include genetics, epigenetics, morphogenetic, ontogenic, and environmental factors (Kooke and Keurentjes, 2015). It can be argued that the metabolite production by the different populations of *O. basilicum* is possibly based on prevailing environmental conditions (Smirnoff and Stewart, 1985). This is probably influenced by epigenetic mechanisms which enhance phenotypic plasticity possibly as an adaptation to address the different levels of environmental stresses and in such cases, this can be transmitted successfully to the next offspring for numerous generations ultimately leading to transgenerational changes that evolve over time (Boccard and Rutledge, 2013).



Fig. 4. Pictorial representation of *O. basilicum* for each cluster, OB034 (cluster I), OB018 (cluster II), OB013 (cluster III), and OB033 (cluster IV).

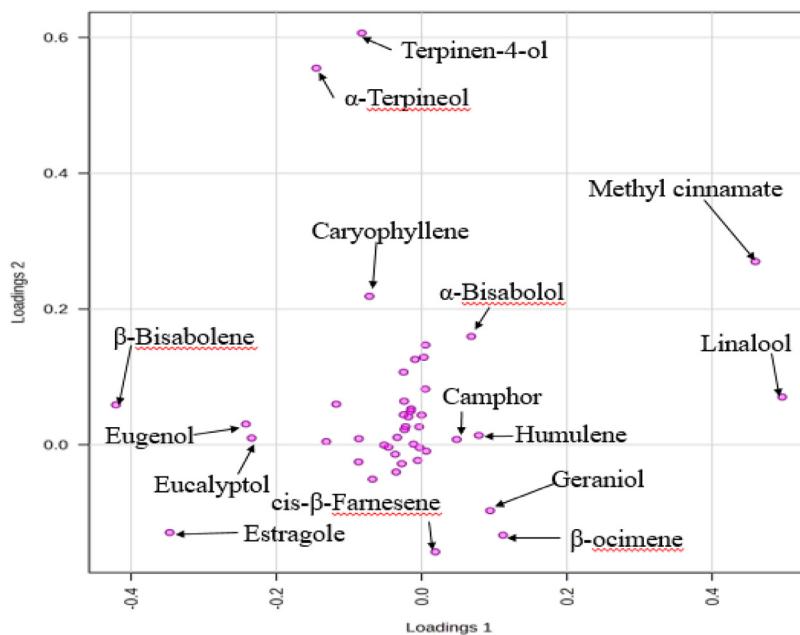


Fig. 5. PCA loadings plot of chemical markers of *O. basilicum* accessions based on regions of origin.

Previous investigations of *O. basilicum* chemotaxonomy differ in approach, by some degree, leading to different results. The type of material used for analysis plays an important role when conducting this type of research. Grayer et al. (1996) reported significant differences in the analysis of chemotypes when using fresh and freeze-dried material of the same plant. Environmental factors and plant growth stage should also be taken into consideration. In order to minimize the impact that pedo-climatic factors have on material, most researchers grow plants in a controlled environment, and collect material at the same growth stage (Vieira et al., 2001). The inclusion of different cultivars as well as the threshold and number of compounds included in PCA or CA affects the results

The major compounds found in our samples are synthesized via one of three biosynthetic pathways. Monoterpenes (1,8-cineole, linalool, linalool acetate) derive from the non-mevalonate biosynthetic pathway. Sesquiterpene trans- α -bergamotene derives from the mevalonate biosynthetic pathway, while phenylpropane derivatives (methyl chavicol, eugenol, trans-methyl cinnamate) are synthesized via the phenylpropene biosynthetic pathway (Iijima et al., 2004). Based on the major compounds present in the chemotype, the dominant biosynthetic pathway utilized by each chemotype can be determined. Combining this information with molecular data could assist in breeding distinct chemotypes with unique essential oil composition (Rastogi et al., 2014).

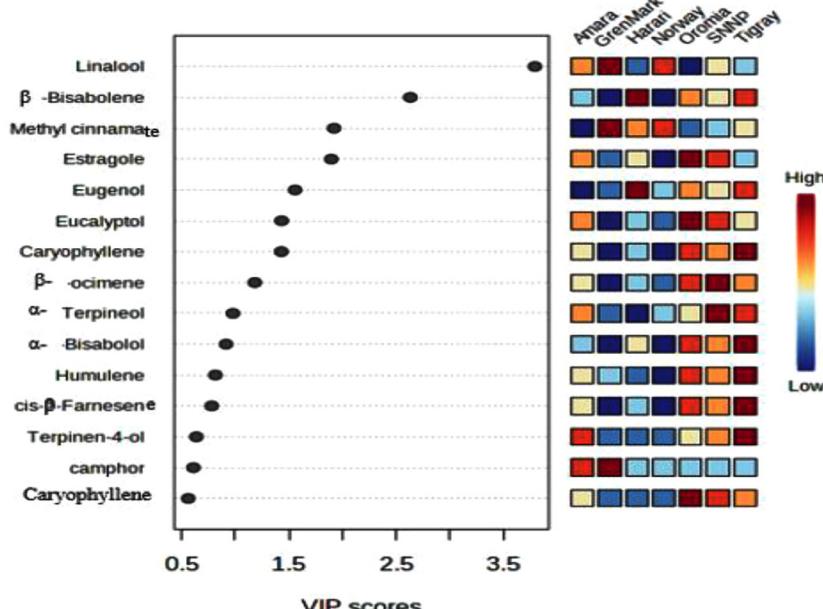


Fig. 6. Relative abundance of chemical discriminants in accessions from different regions. The dark red shows high concentration of the individual chemical in each region while the dark blue color implies low concentration.

4. Conclusion

Forty-nine *O. basilicum* accessions essential oil were analysed using GC-MS and a total of forty-six compound identified. The result indicated that four compounds (eucalyptol, linalool, estragole, and eugenol) were found in all accessions, and seven compounds were found in higher level of methyl cinnamate (0–41.90 %), geraniol (0–27.90 %), linalool (0.29–25.41 %), eugenol (0.62–23.08 %), β -bisabolene (0–23.03 %), eucalyptol (2.05–17.00 %) and estragole (0.46–16.60 %). One accession OB033 characterized by higher amount of both methyl cinnamate (41.86 %) and linalool (22.96 %). Accession OB013 characterized by higher content of geraniol (27.90 %) and linalool (21.81 %). Accession OB005 and OB006 characterized by higher content of eugenol (23.08 % and 17.76 %), respectively. One accession OB025 characterized by higher content of eucalyptol (17.00 %) and estragole (12.06 %). Accession OB041 characterized by higher content of β -bisabolene (23.02 %). The cluster analysis of the major compounds indicated that four chemotypes. Cluster I/Chemotype A: is characterized by a high content of; eugenol/estrone/ β -Bisabolene. Cluster II/Chemotype B characterized by a high content of; eucalyptol/estrone. Cluster III/Chemotype C; characterized by a high content of; linalool/geraniol and cluster IV/Chemotype D; characterized by a high content of; methyl cinnamate/linalool. The present study demonstrated that the Ethiopian germplasms of *O. basilicum* varied significantly. This study pointed out intraspecific variations amongst accessions from similar geographic origins while other samples exhibited chemotype similarity between accessions from geographically different areas. The observed high chemical constituent variabilities revealed the potential of Ethiopian basil germplasm for wider application. Due to the high content of linalool, methyl cinnamate, geraniol, estragole, eugenol and eucalyptol the analysed accessions (OB033, OB013, OB005, OB006, OB025, OB041) may be of interest in food, perfume, pharmaceutical, cosmetic, and aromatherapy industries.

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Declaration of Competing Interest

The authors have not declared any conflict of interests.

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