Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(6): 201-208
Received: 16-09-2019
Accepted: 20-10-2019

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Chemical compositions of fresh volatiles aromas of some Kenyan *Ocimum* species

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Abstract
Plants of genus *Ocimum* have unique aromas hence the likelihood of chemical profiles of their fresh volatiles being different. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of head space volatiles of fresh aerial parts of *O. kenyense*, *O. kilimandscharicum* and *O. lamiifolium* species led to identification of four major classes of chemical constituents namely; monoterpenoids (15.16-56.01%), benzenoids (16.68-43.41%), sesquiterpenoids (3.35-22.77%) and non-terpenoids (7.62-15.62%). Fresh aromas of *O. kenyense* species from Laikipia and Nyeri Counties contained major constituents such as β-pinene (13.0-13.3%), *p*-ethylacetophenone (9.65-11.3%), *p*-methoxyacetophenone (8.74-11.6%), eucalyptol (6.10-8.80%) and ethyl isovalerate (7.62-7.80%). Limonene (9.94%) and camphor (19.4%); and eucalyptol (19.93%) and linalool (19.2%) were identified as major constituents of *O. kilimandscharicum* species from Kirinyaga and Nyeri Counties, respectively. α-Phellandrene (11.92%), limonene (7.65%) and (*E*)-β-ocimene (7.04%) were identified as major constituents of *O. lamiifolium* species (Nyandarua County) while α-phellandrene (18.69%) and β-sabinene (15.73%) were identified as major compounds of *O. lamiifolium* species (Nakuru County).

Keywords: *Ocimum*, Fresh, Volatile, Aroma

Introduction
The *Ocimum* species are annual and perennial herbs and shrubs of *Lamiaceae* family indigenous to tropical America, Asia and Africa [1, 2]. It is estimated that genus *Ocimum* consists of approximately 200 species with numerous varieties [3]. Out of these, 14 *Ocimum* species such as *O. kenyense*, *O. kilimandscharicum*, *O. lamiifolium*, *O. masaiense*, *O. suave*, *O. forskolei*, *O. fischeri*, *O. spicatum*, *O. cufudontii*, *O. spectabile*, *O. angustifolium*, *O. filamentosum*, *O. turkanaense* and *O. mearnsii* have been identified in Kenya [4]. Individual species within genus *Ocimum* show significant variations in the aroma, morphology and chemical compositions [5].

Genus *Ocimum* plants are cultivated for their valuable essential oil which is widely used in aromatherapy treatment, perfumery of toiletry products, dental products, pharmaceutical products, cosmetics and food flavours [6-9]. A recent study reported traditional use of *Ocimum basilicum* natural aroma as stress and depression relieving aromatherapeutic agent, body and clothing perfume, food flavouring and preservative agent in Ethiopia [10].

Chemical analysis of *Ocimum canum* species, locally known as Otimajo in Northern Cameroon led to identification of linalool (44.9%) and geraniol (38.2%) as the major chemical components responsible for its distinctive floral-fruity aroma. Otimajo is a well known spice and is commonly used to flavor fish soup by local communities in Cameroon [11]. GC-MS analysis of head space sampled volatiles of fresh basil leaves (*Ocimum basilicum*) flavored yogurt led to identification of linalool and eucalyptol as its major flavour constituents [12]. The study established that unique aroma added by both fresh and dried basil to food makes it a good alternative natural flavouring agent for yogurt and other dairy products [13].

Headspace sampled mosquito repellent odour of Eritrean *O. forskolei* species was analyzed by Dekker and co-workers using GC-MS. (*E*)-β-ocimene, 4-hexenyl acetate, 3-hexenol, 1-octen-3-ol, linalool, α-copaene, α-humulene, methyl salicylate, methyl cinnamate and (*E*)-β-caryophyllene were identified as key chemical constituents of *O. forskolei* species' odour. Gas Chromatography-Electro-Antennographic Detector (GC-EAD) analysis singled out methyl salicylate and methyl cinnamate as novel mosquito repellent compounds of *O. forskolei* species odour [14]. In Ethiopia, analysis of fresh leaf head space of *O. suave* species, led to identification of (*E*)-β-ocimene as an effective volatile mosquito repellent [15]. Head space sampling technique is preferred for ecological studies since it gives a more realistic picture of volatile profile of plant emissions detected by insects [16].

Volatile phytochemical constituents of *Ocimum* species have attracted a great deal of interest from researchers due to their potential as natural perfumes, flavors, aromatherapeutics and repellents. Several studies have been conducted on chemistry species in Kenya [17-19]. However, prior to this work, there has been no report on chemical investigation of fresh aroma volatiles' compositions of Kenyan *Ocimum* species. This study presents the first report on chemistry of aroma volatiles of fresh aerial parts of *O. kenyense, O. kilimandscharicum* and *O. lamiifolium* species growing in various agro ecological zones in Kenya.

2. Materials and Methods

2.1 Plant collection and identification

Fresh leaf and floral plant materials of each of the selected *Ocimum* species were collected in May 2014 from natural populations in Laikipia, Nyeri, Nyandarua, Nakuru and Kirinyaga Counties in Kenya. Botanical identification of the plant materials was carried out at the Herbarium of Department of Botany, University of Nairobi, Kenya where voucher specimens AN/001/2014, AN/002/2014 and AN/003/2014 were deposited.

Fresh aerial parts of each selected *Ocimum* species were picked from two different agro ecological areas. *Ocimum kenyense* species was collected from Nyeri and Laikipia Counties while *Ocimum* kilimandscharicum species was collected from Nyeri and Kirinyaga Counties. *Ocimum lamiifolium* species was collected from Nyandarua and Nakuru Counties. Freshly plucked plant materials were wrapped in moist tissues and transported to laboratory for volatiles' collection at the Centre for African Medicinal and Nutritional Flora and Fauna (CAMNFF) in Masinde Muliro University of Science and Technology in Kakamega County, Kenya.

2.2 Air entrainment of selected *Ocimum* species' volatiles

Samples of fresh aerial parts of each selected *Ocimum* species weighing 10 g were separately placed into glass containers with volume of 1 liter and sealed with Teflon tape. Collection of volatile components was conducted on different Soxhlet cleaned adsorbent glass tube filters using air entrainment kit for 20 minutes [14, 20]. The glass filters contained 40 mg of porapak Q polymer on which volatile constituents of *Ocimum* species aerial parts' aromas adsorbed. Clean air was pulled for 20 minutes through an empty glass (1 liter) fitted with a glass tube filter to provide a control sample. After collection of volatiles, the tubes were heat sealed in glass tubes and refrigerated until needed for analysis. The volatiles were eluted from the porapak Q filters with GC grade dichloromethane where 100 µl samples were collected and used for chemical analyses.

2.3 Chemical analyses

Qualitative and quantitative analyses of the volatiles aromas was performed using gas chromatograph fitted with flame ionization detector (GC-FID) and gas chromatograph coupled with mass spectrometer (GC-MS). The GC-FID analysis was performed on GC HP 5890 II chromatograph equipped with split less injector attached to DB-5 MS column (25 m × 0.32 mm × 0.52 mm film thickness) and fitted to FID. Helium Carrier gas was used at flow rate of 1 ml/ min and split ration of 1:30. The injector temperature and detector temperature were set at 250 ºC and 270 ºC, respectively while column temperature was linearly programmed from 40 to 240 ºC (at the rate of 5 ºC per minute). Similar analytical conditions were employed for GC-MS analysis where HP GC 1800 II equipped with DB-5 MS column (30 m x 0.25 mm, 0.25 mm film thickness) was used. Transfer line was heated at 270 ºC. Mass spectra were acquired on E1 mode (70 eV) in m/z range of 0-400 a.m.u with a scan time of 1.5 seconds.

2.4 Chemical identification of fresh volatiles of selected *Ocimum* species

Identification of volatile chemical constituents was conducted by matching mass spectra and retention indices were compared with those of authentic standards in addition to NIST library, Kovat index databases [21] and literature [22-24].

3. Results and Discussion

3.1 Chemical composition of fresh volatiles of selected *Ocimum* species

GC-MS analyses of head space volatiles of selected *Ocimum* species lead to identification of a total of thirty, twenty-nine and sixteen chemical constituents in fresh volatiles of Kenyan *O. kenyense*, *O. kilimandscharicum* and *O. lamiifolium* species.

3.1.1 Chemical composition of *Ocimum kenyense* species fresh volatiles

A total of nineteen chemical constituents were identified in fresh volatiles of *O. kenyense* species from both Laikipia and Nyeri Counties (Table 1).

Table 1: Chemical constituents of *O. kenyense* species fresh volatiles

GC Peak	RI	Identity of the compound	Amount in %	
			Laikipia	Nyeri
1	849	Ethyl isovalerate	7.80	7.62
2	974	β-pinene	13.00	13.30
3	1026	Eucalyptol	8.80	6.10
4	1028	*p*-cymene	5.83	5.09
5	1030	*m*-cymene	5.38	5.00
6	1055	1,3-Diethyl benzene	2.19	2.31
7	1163	4-ethylbenzaldehyde	2.05	2.10
8	1174	2-ethylbenzaldehyde	0.82	0.76
9	1195	Estragole	1.15	1.05
10	1273	*p*-ethylacetophenone	9.65	11.31
11	1290	*p*-methoxyacetophenone	8.74	11.68
12	1417	(*E*)-β-Caryophyllene	1.20	1.26
13	1435	1,2-Diacetylbenzene	1.99	2.10
14	1451	1,4-Diacetylbenzene	1.55	2.09
15	1452	α-Humuulene	-	4.93
16	1505	β-Bisabolene	2.25	6.10
Monoterpenoids			21.80	19.40
Sesquiterpenoids			3.45	12.29
Benzenoids			39.35	43.41
Non-Terpenoid			7.80	7.62
% Identified chemical constituents			72.40	82.72

Fifteen compounds were identified in fresh volatiles of aerial parts of *O. kenyense* species from Laikipia County (OKE-LKP). The fresh volatiles were characterized by seven major compounds namely ethyl isovalerate (peak 1) (7.80%), β-pinene (peak 2) (13.0%), eucalyptol (peak 3) (8.80%), o-cymene (peak 4) (5.83%), *m*-cymene (peak 5) (5.38%), *p*-ethylacetophenone (peak 10) (9.65%) and *p*-methoxyacetophenone (peak 11) (8.74%). Minor chemical compounds such as β-bisabolene (peak 16) (2.25%) and 1, 3 diethylbenzene (peak 6) (2.19%) among others, were also present in fresh volatiles of OKE-LKP (Figure 1).

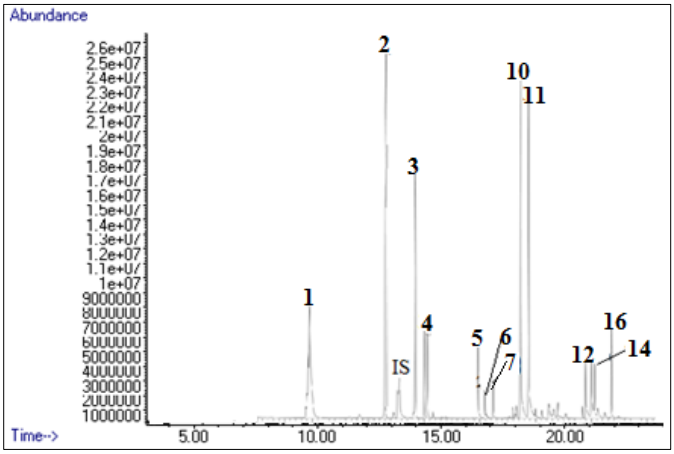

Fig 1: A representative total ion chromatogram of fresh volatiles of *O. kenyense* (Laikipia)

Sixteen chemical compounds were reported in fresh volatiles of aerial parts of *O. kenyense* species from Nyeri County (OKE-NYR). The fresh volatiles were characterized by eight major compounds namely ethyl isovalerate (Peak 1) (7.62%), β-pinene (peak 2) (13.34%), eucalyptol (peak 3) (6.10%), *o*-cymene (peak 4) (5.09%), *m*-cymene (peak 5) (5.00%), *p*-ethylacetophenone (Peak 10) (11.31%), *p*-methoxyacetophenone (peak 11) (11.68%) and β-bisabolene (peak 16) (6.10%). Minor compounds such as α-humulene (peak 15) (4.93%) and 1,3-diethylbenzene (peak 6) (2.31%) among others, were also present in OKE-NYR fresh volatiles (Figure 2).

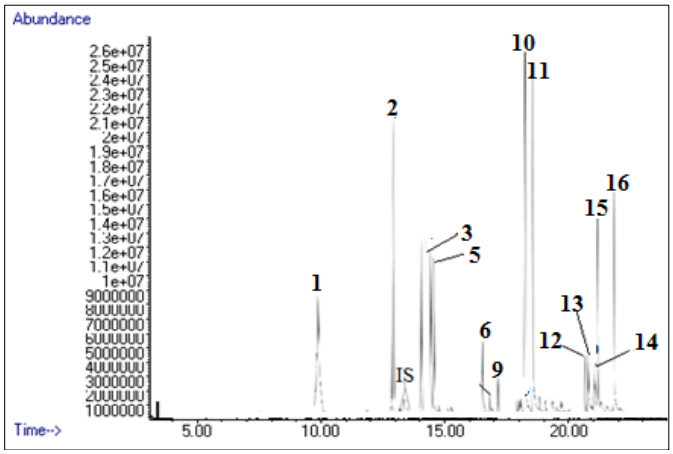

Fig 2: Total ion chromatogram of fresh volatiles of *O. kenyense* (Nyeri)

3.1.2 Chemical composition of *Ocimum kilimandscharicum* species fresh volatiles

A total of thirty chemical constituents were identified in fresh volatiles of *O. kilimandscharicum* from both Kirinyaga and Nyeri Counties (Table 2).

Table 2: Chemical constituents of *O. kilimandscharicum* species fresh volatiles

GC Peak	RI	Identity of the compound	Amount in %	
			Kirinyaga	Nyeri
1	932	α-pinene	2.08	1.73
2	946	Camphene	6.11	0.63
3	974	β-pinene	2.03	4.73
4	1022	*o*-cymene	5.30	5.80
5	1024	Limonene	9.94	-
6	1026	Eucalyptol	5.19	19.93
7	1040	1,4-Diethylbenzene	-	1.50
8	1044	(*E*)-β-ocimene	3.63	-
9	1055	1,3-Diethyl benzene	1.18	1.06
10	1074	Sabinene hydrate	0.81	1.01
11	1083	Fenchone	-	1.43
12	1093	6-Camphenone	3.46	-
13	1095	Linalool	-	19.20
14	1141	Camphor	19.40	1.42
15	1163	4-ethylbenzaldehyde	0.87	0.83
16	1168	Endo-borneol	0.53	-
17	1195	Estragole	-	3.02
18	1249	Geraniol	3.68	-
19	1273	*p*-ethylacetophenone	3.85	3.95
20	1290	*p*-methoxyacetophenone	3.88	3.84
21	1304	Lavandulyl acetate	1.08	-
22	1350	Eugenol	-	3.85
23	1351	α-Cubebene	-	1.00
24	1409	α-Gurjunene	-	0.53
25	1417	(*E*)-β-Caryophyllene	4.11	3.37
26	1435	1,2-Diacetylbenzene	1.60	1.86
27	1454	(*E*)-β-Farnesene	2.37	2.33
28	1472	γ-Muurolene	0.92	0.81
29	1484	Germacrene-D	1.35	2.61
30	1503	Germacrene-A	0.67	0.65
Monoterpenoids			56.01	49.07
Sesquiterpenoids			12.44	11.78
Benzenoids			16.68	25.71
% Identified chemical constituents			85.13	86.56

Twenty-three compounds were reported in fresh volatiles of *O. kilimandscharicum* species from Kirinyaga County (OKI-KRN). The fresh volatiles were characterized by five major compounds namely camphene (peak 2) (6.11%), *p*-cymene (peak 4) (5.30%), limonene (peak 5) (9.94%), eucalyptol (peak 6) (5.19%) and camphor (peak 14) (19.47%). Minor compounds such as (*E*)-β-cymene (peak 8) (3.63%), 6-camphenone (peak 12) (3.46%), geraniol (peak 18) (3.68%), *p*-ethylacetophenone (peak 19) (3.85%), *p*-methoxyacetophenone (peak 20) (3.88%) and (*E*)-β-caryophyllene (peak 25) (4.11%) among others, were also present in fresh volatiles of OKI-KRN species (Figure 3).

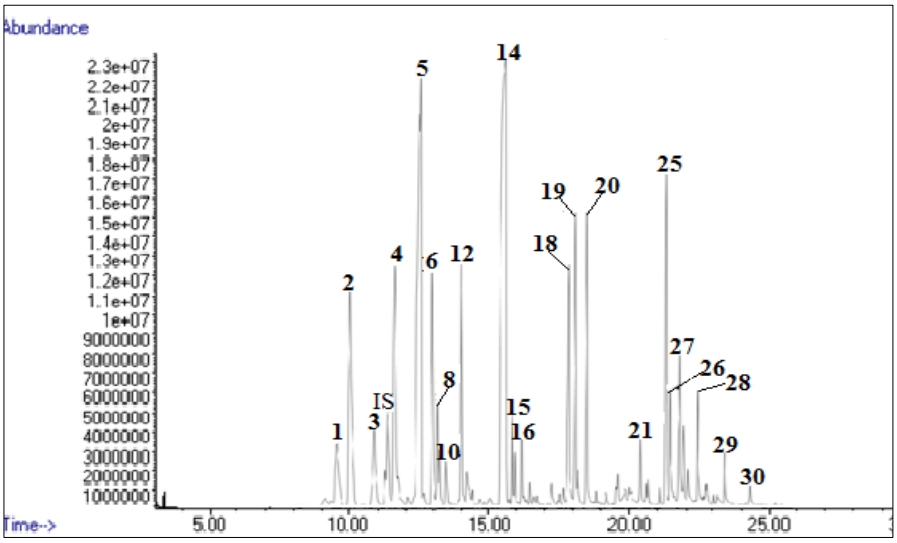

Fig 3: Total ion chromatogram of fresh volatiles of *O. kilimandscharicum* (Kirinyaga)

Twenty-four chemical constituents were identified in fresh volatiles of aerial parts of *O. kilimandscharicum* species from Nyeri County (OKI-NYR). Three major chemical constituents namely *p*-cymene (peak 4) (5.80%), eucalyptol (peak 6) (19.93%) and linalool (peak 13) (19.2%) characterized the fresh volatiles' aroma. Minor chemical constituents such as estragole (peak 17) (3.02%), *p*-ethylacetophenone (peak 19) (3.95%), *p*-methoxyacetophenone (peak 22) (3.85%), (*E*)-β-caryophyllene (peak 25) (3.37%), (*E*)-β-farnesene (peak 27) (2.38%) and germacrene-D (peak 29) (2.61%) among others, were also present in fresh volatiles of OKI-NYR species (Figure 4).

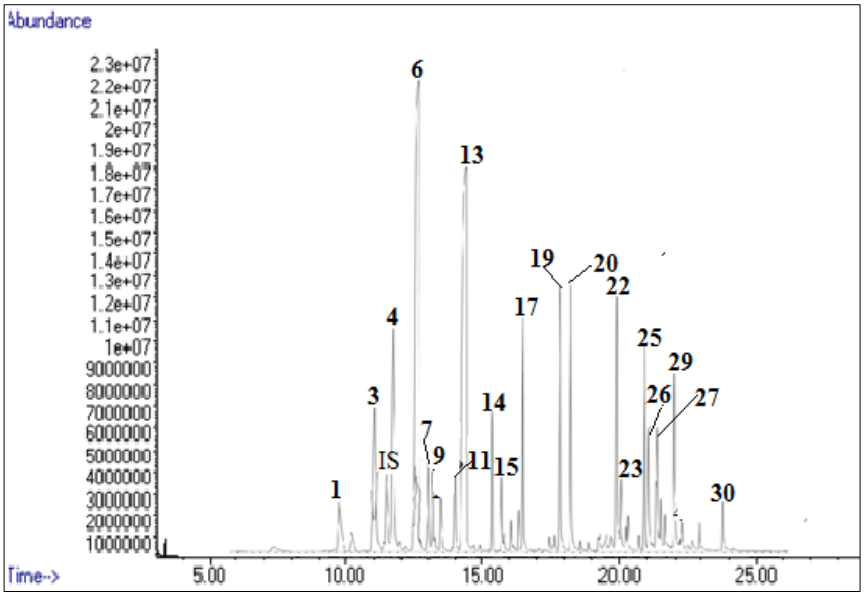

Fig 4: Total ion chromatogram of fresh volatiles of *O. kilimandscharicum* (Nyeri)

3.1.3 Chemical composition of *Ocimum lamiifolium* species fresh volatiles

A total of twenty-nine chemical constituents were identified in fresh volatiles of *O. lamiifolium* species from both Nyandarua and Nakuru Counties (Table 3).

Table 3: Chemical constituents of *O. lamiifolium* species' fresh volatiles

GC Peak	RI	Identity of the compound	Amount in %	
			Nyandarua	Nakuru
1	932	α-pinene	1.88	1.29
2	974	β-pinene	-	1.06
3	976	β-Sabinene	-	15.73
4	980	1-Octen-3-ol	2.88	4.64
5	995	3-Octanal	-	4.41
6	1002	α-phellandrene	18.69	11.92
7	1008	δ-3-carene	1.16	0.89
8	1022	*o*-cymene	2.71	1.48
9	1024	Limonene	7.65	-
10	1044	(*E*)-β-ocimene	7.04	-
11	1055	1,3-Diethyl benzene	1.68	1.87
12	1074	Sabinene hydrate	-	4.69
13	1097	Nonan-2-ol	-	2.82
14	1106	1,2-Diethyl benzene	-	2.00
15	1110	Octen-3-yl acetate	3.05	3.75
16	1163	4-ethylbenzaldehyde	1.23	1.61
17	1174	2-ethylbenzaldehyde	-	0.55
18	1273	*p*-ethylacetophenone	4.73	6.61
19	1290	*p*-methoxyacetophenone	4.76	6.59
20	1370	Carvacrol acetate	2.80	-
21	1409	α-Gurjunene	1.77	-
22	1417	(*E*)-β-Caryophyllene	0.72	0.88
23	1484	Germacrene-D	-	1.47
24	1492	(*Z*)-β-Guaiene	1.20	-
25	1494	Bicyclogermacrene	2.70	-
26	1524	Δ-Cadinene	0.58	-
27	1574	Germacrene-D-4-ol	0.55	0.66
28	1435	1,2-Diacetylbenzene	1.11	2.23
29	1451	1,4-Diacetylbenzene	1.33	1.85
Monoterpenoids			36.42	15.16
Sesquiterpenoids			7.52	22.77
Benzenoids			17.54	24.79
Non-terpenoids			8.73	15.62
% Identified chemical constituents			70.21	78.34

Twenty-two chemical compounds were identified in fresh volatiles of aerial parts of *O. lamiifolium* species from Nyandarua County (OLA-NYD). The fresh volatiles were characterized by three major compounds namely α-phellandrene (peak 6) (18.69%), limonene (peak 9) (7.65%) and (*E*)-β-ocimene (peak 10) (7.04%). On the other hand, minor compounds such as octen-3-yl acetate (peak 15) (3.05%), 1-octen-3-ol (peak 4) (2.88%), *p*-ethylacetophenone (peak 18) (4.73%) and *p*-methoxyacetophenone (peak 19) (4.76%) and bicyclogermacrene (peak 25) (2.70%), among others were identified in fresh volatiles' aroma of *O. lamiifolium* species from Nyandarua County (OLA-NYD) (Figure 5).

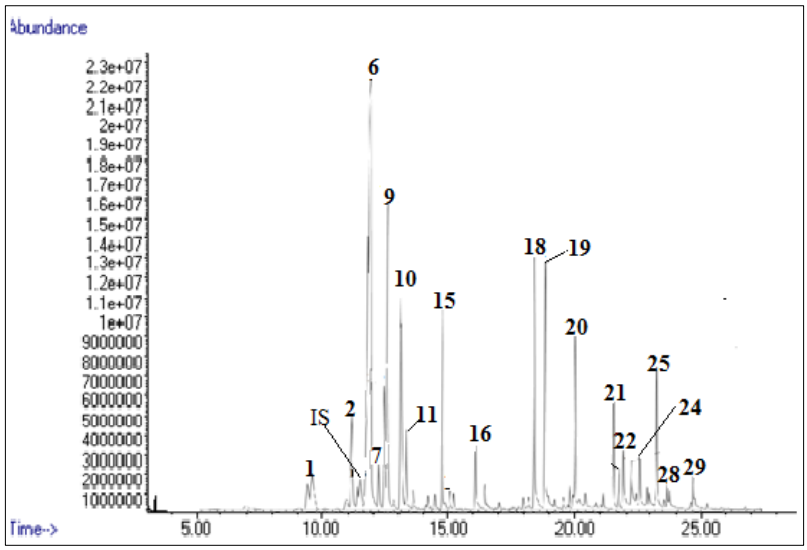

Fig 5: Total ion chromatogram of fresh volatiles of *O. lamiifolium* (Nyandarua)

Twenty-three chemical constituents were identified in fresh volatiles of aerial parts of *Ocimum lamiifolium* from Nakuru County (OLA-NKU). Five major constituents namely β-sabinene (peak 3) (15.73%), α-phellandrene (peak 6) (11.92%), sabinene hydrate (peak 12) (4.69%), *p*-ethylacetophenone (peak 18) (6.61%) and *p*-methoxyacetophenone (peak 19) (6.59%) characterized the fresh volatiles' aroma. On the other hand, minor constituents such as octen-3-yl acetate (peak 15) (3.75%), 3-octanol (peak 7) (4.41%), nonan-2-ol (peak 13) (2.82%) and 1, 2 diacetylbenzene (peak 28) (2.23%) among others, were identified in OLA-NKU species fresh volatiles' aroma (Figure 6).

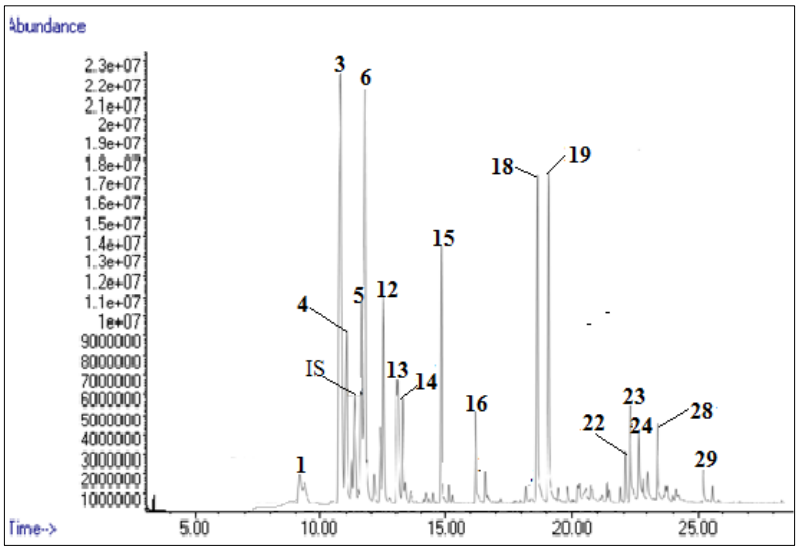

Fig 6: Total ion chromatogram of fresh volatiles of *O. lamiifolium* (Nakuru)

Fresh volatiles' aromas of *O. kenyense* species were dominated by benzenoid phytochemical constituents (39.35-43.41%) while *O. kilimandscharicum* species' aroma was dominated by monoterpenoid constituents (49.07-56.01%). In *O. lamiifolium* species, dominant classes of fresh volatiles' phytochemical constituents varied with agro ecological zones of origin. Fresh volatiles aroma of *O. lamiifolium* species from Nakuru County was dominated by benzenoid (24.79%) and sesquiterpenoid (22.77%) constituents while its counterpart from Nyandarua County was dominated by monoterpenoid constituents (36.42%).

In this study, chemical constituents such as 1,3-diethylbenzene (1.06-2.31%), 4-ethylbenzaldehyde (0.87-2.10%), *p*-ethylacetophenone (3.85-11.31%), *p*-methoxyacetophenone (3.84-11.68%), (*E*)-β-caryophyllene (0.72-4.11%) and 1,2-diacetylbenzene (1.11-2.10%) were identified in fresh volatiles of all investigated *Ocimum* species. However, there were notable variations in volatile constituents of *O. kenyense*, *O. kilimandscharicum* and *O. lamiifolium* species' fresh volatiles' aromas based on species and agro ecological zones of origin. β-Pinene, *p*-ethylacetophenone and *p*-methoxyacetophenone were identified as major constituents of fresh volatiles' aroma of *O. kenyense* species from both Laikipia and Nyeri Counties. However major constituents of fresh volatiles' aroma of *O. kilimandscharicum* and *O. lamiifolium* species varied with agro ecological zones of origin.

Camphor and limonene were identified as major constituents of fresh volatiles' aroma of *O. kilimandscharicum* species from Kirinyaga County while linalool and eucalyptol were identified as major constituents of fresh volatiles' aroma of its counterpart from Nyeri County. α-Phellandrene, limonene and (*E*)-β-ocimene were identified as major constituents of fresh volatiles' aroma of *O. lamiifolium* species from Nyandarua County while α-phellandrene and β-sabinene were identified as major constituents of fresh volatiles' aroma of its counterpart from Nakuru County.

Previous studies on head space volatiles of *O. basilicum* species from China and Czech Republic revealed notable variations in their respective chemical profiles. Analysis of head space volatiles of floral parts of *O. basilicum* species from China led to identification of α-bergamotene (57.1%), cedrene (16.4%), eugenol (13.1%) and δ-cadinol (11.8%) as its major chemical constituents [25]. In a separate study, GC-MS analysis of head space-solid phase micro extraction (HS-SPME) volatiles of five different cultivars of *O. basilicum* species grown under ecological and conventional cultivation in Czech Republic led to identification of four major constituents. Linalool (16-32%), eucalytol (3-20%), eugenol (9-22%) and α-bergamotene (1-20%) were identified in all investigated cultivars of *O. basilicum* species from Czech Republic [7].

Findings of this study concur with those of previous studies that have demonstrated variations in volatile constituents of the same *Ocimum* species based on agro ecological areas of origin due to associated edaphic and climatic factors [26]. Factors such as solar irradiation [13, 27], temperature [28], rainfall [29] and soil type [30-32] among others, have been reported to contribute significantly to observed variations in quality and quantity of volatile emissions in *Ocimum* species.

4. Conclusion

This study reports chemical compositions of fresh volatiles' aromas of Kenyan *O. kenyense*, *O. kilimandscharicum* and *O. lamiifolium* species for the first time. Distinct variations in chemical profiles of fresh volatiles' aromas of the three investigated *Ocimum* species were observed in this study. Therefore, the unique fresh volatiles' aroma of each investigated Kenyan *Ocimum* species could be attributed to distinct variations in their respective chemical profiles. The natural aroma of *Ocimum* species is largely influenced by its most dominant constituent(s) thus plants growing in certain ecological zones in Kenya could be potential sources of

aromatherapeutics, perfumes, food flavors and insect repellents.

5. Acknowledgment
We thank International Foundation of Science (IFS) for awarding research grant [F 5773-1] and National Research Fund (NRF), Kenya for awarding research grant NACOSTI/RCD/ST&I/6TH CALL PhD/265 research grant.

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