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Chemo-Divergence in Essential Oil Composition among Thirty One Core Collections of *Ocimum sanctum* L. Grown under Sub-Tropical Region of Jammu, India

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ABSTRACT

Evaluation of thirty one core collections of *Ocimum sanctum* L. synonyms *O. tenuiflorum* L. collected from different ecological regions representing contrasting environment of India was carried out. All the collections were grown under sub-tropical region of Jammu, India. Study revealed wide range of variability in quantitive and qualitative attributes of oil. Essential oil content ranged between 0.16% ± 0.01% - 0.55% ± 0.08% showing the presence of fifteen constituents. Methyl eugenol (1.54% - 93.16%) and Eugenol (0.06% - 70.41%), were the major constituent. The other major constituent of the oil was *β*-Caryophyllene (4.60% - 33.77%) which was detected in almost all the collections. Borneol, Copane, *α* Caryophyllene were other constituents detected in almost all the accessions. *α* selinene was detected in traces in only three accessions (OS-01, OS-03, OS-50) and *β*-selinene was detected in four accessions (OS-01, OS-03, OS-50, OS-72. Accession OS-70 collected from Patna, showed distinct chemical profile having *β*-Elemene (32.81%), *β*-Caryophyllene (16.37%), Germacrene-D (18.05%), *β*-Ocimene (17.69%) and Copane (5.738%). Being distinct in oil profiling, Patna collection was designated as distinct chemotype. Collections OS-50 from Gwalior from Central India and OS-59 from Rajkot Western India have been identified as methyl eugenol (93.16%) and eugenol (70.41%) rich genotypes. The data collected provided useful information with respect to composition of essential oil among core collection evaluated representing various agro-climatic zones.

Keywords: *β*-Caryophyllene; Chemotype; Eugenol; Germacrene-D; Methyl Eugenol; *β*-Ocimene; Variability

1. Introduction

Genus *Ocimum* belonging to family Lamiaceae, consists of about 160 species [1] represents versatile group of aromatics. It is well discussed in Ayurveda as healing system. *Ocimum sanctum* (Tulsi) leaves have potent medicinal properties, acting as an expectorant and antiseptic, in addition to having use as insect-repellent [2]. Leaves are diaphoretic, anti-periodic; they are also used in bronchitis, gastric and hepatic disorders. Decoction of leaves is recommended for cough, malaise and in colds. It is a good mosquito repellant as well. Oil extracted from flowers is used in skin diseases and ring worm infection. Various studies have been performed with *Ocimum sanctum* for its antibacterial, antioxidant, antiulceric, antimalarial, antidiabetic, anti-inflammatory, antilipidemic, anticancer and immunomodulatory properties [3]. The oils from the leaves of *O. basilicum* and *O. sanc*tum contain phenols as aldehydes [4].

The therapeutic value of this is ascribed to its essential oils contents [5,6]. The perfume, pharmacy and food industries use aromatic essential oil extracted from the leaves and flowers of basil in variety of products [6]. The European genotype or a sweet basil is considered to have the highest quality aroma, containing linalool and methyl chavicol as major constituents [7]. Seasonal variation in the content of essential oil and its major constituents eugenol, methyl eugenol, and caryophyllene, in leaves of *O. sanctum* has been reported [8]. Eugenol-rich (54% of essential oil) genotype of sacred basil *O. sanctum* has been identified [9]. Use of sensitive analytical tools, GC-MS of the essential oil of *O. sanctum* leaves allowed the separation of 46 compounds of which 31 were completely or partially identified [10].

The therapeutic potential is distributed variably in off-types of *Ocimum sanctum* L. of different geographical and agro-climatic zones which implies that there is morpho-chemical and molecular diversity in it [11,12]. The natural variability needs to be studied and tapped for

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characterization of elite genotypes having desired combination of characters. This is important from commercial and pharmaceutical point of view to identify distinct biomolecule rich cultivar for bioprospection. The present proposed study is aimed to evaluate chemo-variation among thirty one core collections representing diverse agroclimatic regions of India. This will provide breeders an ample scope to undertake screening and selection of chemotypes and identify newer chemotypes. Based on the recorded distinct chemo profiling "elite" strains could be characterized for exploitation commercially for bioprospection of their value added products.

2. Material and Methods

2.1. Plant Material

Thirty one collections of *Ocimum sanctum* L. were subjected to evaluate oil profiling. Among these two accessions OS-06 and OS-07, GAU-2 (Shyam Tulsi) and IC 75730 (Puja Tulsi) were procured from NBPGR Delhi and remaining twenty nine core collections accessions were collected from different ecological regions of India ranging from sea level to 1220 masl. The details of various eco-regions where from these core collections were resourced are given in **Table 1**.

Table 1. Resource Locations of various core collections of *Ocimum sanctum* grown under Jammu conditions.

Acc. No	Location	Agro-climatic region	Annual rain fall (mm)	Latitude (North)	Longitude (East)	Altitude (masl)
OS-01	Jammu*	Subtropical	1100	32˚43'	74˚54'	400
OS-03	Old Delhi*	Subtropical	715	28˚61'	77˚23'	213
OS-06	NBPGR New Delhi*	Subtropical	715	28˚35'.	77˚12'	239
OS-07	NBPGRI New Delhi*	Subtropical	715	28˚35'.	77˚12'	239
OS-09	*Joginder nagar**	Subtropical	1092	31˚72'	76˚92'	1220
OS-10	Amritsar*	Subtropica	681	31˚63'	74˚87'	234
OS-48	Mathura*	Subtropical	593	27˚28' .	77˚41'	174
OS-49	Rasulpur*	Subtropical	600	28˚40'	76˚59'	187
OS-50	Gwalior**	Subtropical	700	26˚22'	78˚18'	197
OS-52	Baruasagar**	Subtropical to Semi-arid	876	25˚21'	78˚45'	210
OS-53	Jhansi**	Subtropical to Semi-arid	900	25˚43'	78˚58'	284
OS-56	Trivandrum*****	Tropical	1835	8˚29'	76˚57'	64
OS-57	*Kancheepuram****	Tropical	1213	11˚12'	78˚80'	58.5
OS-58	Junagarh***	Hot summers	1100	21˚51'	70˚46'	315
OS-59	Rajkot***	Moderate	276	23˚08'	71˚40'	*134*
OS-61	Thoriyali***	Semi-arid	500	22˚3'	70˚78'	134
OS-62	Surendra Nager***	Semi-arid	500	22˚43'	71˚38'	74
OS-63	Pune*****	Tropical wet and dry	722	18˚31'	73˚55'	560
OS-64	Kalawad***	Semi-arid	214	22˚13'	70˚23'	85
OS-65	Hyderabad*****	Tropical wet ,dry to semi-arid	1,019	17˚27'	78˚28'	536
OS-66	Dholka***	Moderate	400	22˚70'	72˚46'	16
OS-67	*Porbandar****	Moderate	81	21˚63'	69˚6'	14
OS-68	Ayodhaya*	Subtropical	896	26˚48'	82˚12'	667
OS-69	Saurashtra***	Arid	686.04	20˚40'	68˚60'	5
OS-70	Patna****	Tropical	100	25˚	85˚	53
OS-72	Channai*****	Tropical wet-and-dry	1,400	13˚04'	80˚17'	6
OS-73	*Madurai*****	Tropical dry and hot	721	9˚55'	78˚7'	135.
OS-76	Kolkata****	Tropical wet-and-dry	1583	22˚32'	88˚20'	12
OS-77	Bangalore*****	Tropical savanna	870	12˚97'	77˚56'	920
OS-79	*Sagar***	Subtropical	1000	24˚72'	80˚18'	452
OS-80	Bhubaneshwar****	Tropical monsoon	1200	17˚49'	81˚27'	45

Northern India*, Central India**, Western India***, Eastern India****, Southern India*****.

Seeds of all the core collections were subjected to seed viability test by triphenyl tetrazolium chloride (TTC) solution before sowing for raising nursery in well prepared pots in the second week of April. Six week old seedlings of all the accessions were planted in Farm Yard Manure (FYM) treated experimental plots during 2009 and 2011 at Experimental Farm of the Institute (32˚44'N Latitude and 74˚55'E Longitude and 400 masl) with a temperature ranging from 5˚C - 45˚C and total rain fall 500 mm with plant distance of 50 cms. The soil of experimental plots was sandy loam with pH 6.8, organic carbon 0.27%, available nitrogen, phosphorous and potash (200 kg/ha, 14 kg/ha and 136 kg/ha) respectively.

2.2. Essential Oil Extraction

Freshly harvested foliage (100gm) was used for extraction of essential oil by hydro-distillation method using Clevenger-type apparatus at 60˚C for 3 h. The oil was further analysed by the combination of GC and GC-MS. Essential oil content (percentage) was determined on air dried weight basis as an average of three samples. Mean (X): The mean value of the character was worked out by dividing the totals by corresponding number of observations.

Mean: Sum of X values/N (Number of values). Range: Lowest and highest values of each of character were recorded. Standard error: Standard error of difference of two means was calculated with the help of error mean square from the analysis of variance.

GC was performed on Perkin Elmer (USA) make gas chromatograph model auto system XL, equipped with FID and fused silica capillary column (30 m × 0.32 mm ID, 0.25 µm film thickness) coated with dimethyl polysiloxane (RTX-1). Restek (Bellefonte, PA, USA).

***GC analysis*:** Column oven temperature was programmed from 50˚C - 240˚C at 5˚C per min. Injector and detector temperatures were optimized at 250˚ and 270˚C respectively. Nitrogen gas at a flow rate of 1mL/min was used as the mobile phase. Injector split ratio was 1:80.

***Gas chromatograph-mass spectrometer*:** GC-MS analysis was carried out on a Varian GC-MS 4000 fitted with a Varian Factor Four VF-5 ms fused silica capillary column (30 m × 0.25 mm id, film thickness 0.25 um). Temperature programming of oven was from 50˚C to 240˚C at 5˚C/min rising rate. Helium was used as carrier gas at flow rate of 1 ml/min. Mass spectra were recorded over 50 - 300 amu range at 1 scan per sec with E.I. at 70 eV.

Identification of peaks was carried out by comparing their retention times with authentic samples injected under similar chromatographic conditions. Comparison of Kovat indices with literature values was carried out. The mass spectra were compared with those reported in the NIST and WILEY computer libraries and those published in literature [13] The relative amount (%) of individual components of the oil is expressed as percent peak area relative to total peak area from the GC/FID analyses of the whole extracts.

3. Results and Discussion

The chemical constituents of oil obtained from various samples were examined by the combination of GC, GC-MS and Kovat indices and data recorded is presented in **Table 2**.

The essential oil percentage of various accessions analyzed ranged between 0.16% ± 0.01% - 0.55% ± 0.08% with a mean value of 0.35%, which is comparable to earlier published reports [14-20]. The analysis of the oil showed the presence of fifteen constituents in 99.36% of the total volatile oil. Content of each constituent was compared among various collections. Analysis revealed methyl eugenol content between 1.54% - 93.16%, with a mean value of 73.89%. Among the various collections methyl eugenol content ranged between 90.50% - 93.16% in OS-59, OS-61, OS-62 and OS-65. These collections were designated as methyl eugenol rich clones. In accession OS-48, OS-49, OS-52, OS-53, OS-64, OS-67, OS-73, OS-76 and OS-79, methyl eugenol content re- corded as 80.15% - 88.19%. The oil profiling of OS-01, OS-03, OS-09, OS-10, OS-50, OS-68 and OS-69 show that these collections are rich in eugenol constituent and eugonal content ranged between 0.06% - 70.41% with a mean value of 30.37%. Identification of methyl eugenol rich collection which showed traces of eugenol content or absence possibly might be due to methylation of eugenol [21]. The occurrence eugenol is considered as of great ecological significance, it is documented to be an inhibitor of herbivory [22-24] as well as a good nematocide [25,26] fungicide [27,28] and bacteriocide [29]. Contrary to this, methyleugenol is an important insect pollinator attractant in many flowers, for pollinating moths and beetles in particular, and is a female phero- mone mimic for several fruit flies [30].

β-Caryophyllene was identified as major constituent which was detected in almost all the analysed collections. Its content ranged between 4.60% - 33.77%, with highest value (33.77%) recorded in accession OS-01 collected from Jammu. The presence of *α*-Caryophyllene in high proportion (19.42%) was only detected in (OS-01) whereas *α*-Caryophyllene Borneol, Copane were detected in almost all the accessions. ***α*-**selinene was present in OS-01, OS-03 and OS-50 in traces and *β*-selinene was detected in four accessions (viz. OS-01, OS-03, OS-50 and OS-72). Δ Cadinene, Elemol, Linalool and Caryophyllen oxide were present in traces in some accessions. The collection OS-70 from Patna, Bihar showed *β*-Ele-

Table 2. Essential oil array of various core collections of *Ocimum sanctum* L: Relative percentage of various components.

Acc No	*β*-Ocimene	Linalool	Borneol	Euqenol	Copane	*β*-Elemene	Methyl Eugenol	*β*-Caryo-phyllene	*α*-Caryo phyllene	Germa crene-D	*β* selinene	*α* Selinene	Delta Cadinene	Elemol	Caryo-phyllene oxide
OS-01	3.91		0.63	26.77	1.83	2.01		33.77	19.42	6.52	0.57	0.52			0.39
OS-03			0.50	64.22		10.20		21.00	2.05		0.15	0.28	0.10	0.1	0.15
OS-06		0.097	1.75	0.84	1.16	0.52	79.55	10.62	1.05	3.25			0.27	0.01	0.05
OS-07		0.063	1.25		1.2	0.39	78.74	12.19	1.22	3.83			0.30		
OS-09	0.73	0.867	3.43	44.95	3.85	1.31		30.03	3.15	7.34			1.06	0.25	1.27
OS-10	0.70	0.911	3.49	45.48	3.82	1.30		29.56	3.17	7.28			1.05	0.22	1.3
OS-48			0.14	0.60			87.65	9.47	0.89	0.19			0.03		0.06
OS-49			0.34			0.59	88.19	8.66	0.83	0.37			0.03	0.02	0.05
OS-50				70.41		12.92		12.34	1.34		0.17	0.22		0.18	0.31
OS-52	2.07		0.19	0.14	0.89	3.24	82.80	7.05	0.66	1.76			0.21	0.08	0.09
OS-53			1.01		0.45	2.43	85.80	6.46	0.64	1.19			0.11		
OS-56				20.97			25.16	11.8		15.45					
OS-57		0.178	1.64		2.82	0.77	78.49	8.09	0.81	5.36			0.72		0.18
OS-58	0.33	0.078	2.14	0.06	1.02	2.57	79.94	8.87	0.86	2.91			0.24	0.08	0.02
OS-59			0.15			0.62	93.16	4.60	0.44	0.25					0.05
OS-61						0.77	90.66	7.04	0.66						
OS-62			0.29		0.11	2.04	90.50	5.48	0.51	0.36					
OS-63				66.98			1.54	24.18	2.56	2.52			0.3		0.22
OS-64	0.40		0.35	0.22	0.35	3.78	82.49	9.69	0.88	0.70			0.12	0.03	0.11
OS-65				0.15		1.17	90.92	5.58	0.88						0.44
OS-66			0.75		1.35		77.15	14.39	1.32	3.31			0.30		
OS-67					0.20		86.12	10.83	1.00	0.51			0.07		
OS-68	6.58			22.58	3.44	19.75		27.20							
OS-69				54.38				30.35	1.60	2.79			0.46		
OS-70	17.67				5.73	32.81		16.37	0.84	18.05			1.92		
OS-72			1.06		4.50	0.45	74.55	9.10	0.93		6.68		0.76		0.25
OS-73			1.18		0.56	0.76	87.39	6.63	0.66	1.31					
OS-76				3.55			80.15								
OS-77				59.64			6.38	28.24							2.89
OS-79				64.65			80.55	15.65							1.02
OS-80	1.85		0.32		1.23	5.24	71.53	13.08	1.25	2.37				0.29	0.02
Mean	3.81 ± 1.00	0.36 ± 0.07	1.08 ± 0.18	30.37 ± 5.04	1.92 ± 0.30	4.80 ± 1.41	73.89 ± 4.63	14.61 ± 1.60	1.91 ± 0.65	3.98 ± 0.84	1.90 ± 0.57	0.34 ± 0.02	0.45 ± 0.08	0.14 ± 0.01	3.41 ± 2.29

mene (32.81%), *β*-Caryophyllene (16.37 %) ,Germacrene-D (18.05%), *β*-Ocimene (17.69%) and Copane (5.738%), as major constituents. The details of the oil profiling showing composition of three distinct core collections OS-50, OS-59, OS-70 are illustrated in **Figure 1**. The present results showed significant variability in the oil composition among thirty one core collections. Earlier report support the present observations [31]. A significant variation in chemical composition of the oil among the 270 accessions was recorded. Major constituents reported are eucalyptol, eugenol, and methyl chavicol. Composition of volatile oil of hybrids of *Ocimum* species for the purpose of studying the genetics of the chemical constituents showed that changes result in the new possible chemical formations through remote hybridization of various types [32].

The present data clearly indicates divergence with respect to essential oil composition in holy basil collected

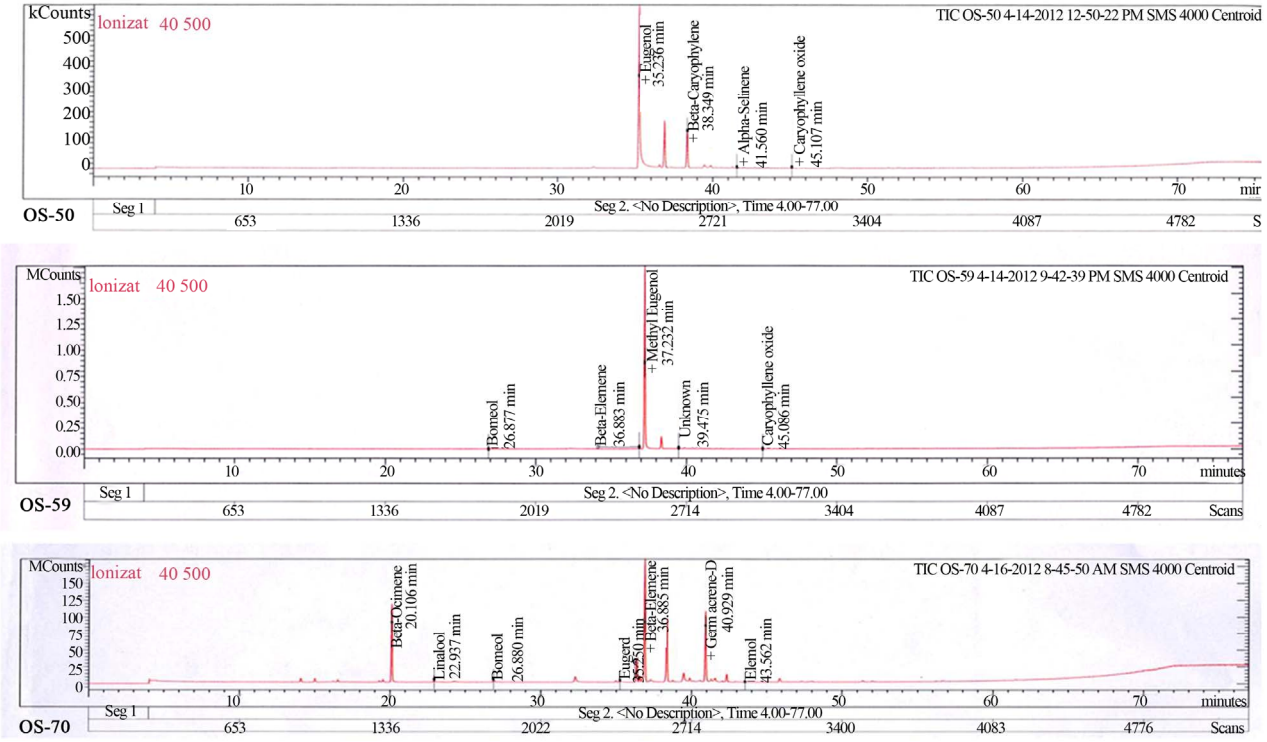

Figure 1. Legend GC/MS Chromatogram of selected core collections (a)-OS-50 Euqenol RT-35.236 min, *β*-Caryo-phyllene RT-38.349 min., *α* Selinene RT-41.560 min and Caryo-phyllene oxide RT-45.160, (b)-OS-59 Borneol RT-26.877min. Methyl Eugenol RT-37.232 min. Caryo-phyllene oxide RT-45.086) and (c)-OS-70 *β*- Ocimene RT-20.106min. B-Elemene RT-36.885 min. Germa crene-D RT-40.929 *β*-Caryo-phyllene RT- 38.358 min. using analytical conditions as detailed in Materials and Methods.

from different eco-zones representing quite contrasting environments. Diversity among collected accessions which depicts divergence in chemical spectrum of essential oil could possibly be attributed due to soil type, pH, extractable nutrients, temperature in various ecozones located in different parts of India. Secondly, relatively less conservative nature of the synthesis or accumulation of these constituents at harvest could possibly be another reason for variation in composition of oil. The collected data further validate and supports the earlier findings where the role of environmental conditions on basil productivity, oil content, and composition [16,33] have been documented. The present study and the earlier reports clearly point towards importance of germplasm evaluation in *Ocimum sanctum*, which needs to be carried out to identify high yielding chemotype rich in molecules of therapeutic importance. This will further help to exploit particular genotype for commercial purpose. The study lead to identify OS-50 and OS-59 collected from Gwalior from central India and Rajkot from western India, respectively as methyl eugenol (93.16%) and eugenol (70.41%) rich cultivars. On the basis of data OS-70 collected from Patna (Bihar) was quite distinct which suggest that this collection could be characterized as distinct chemotype having *β*-Elemene (32.81%), *β*-Caryophyllene (16.37%), Germacrene-D (18.05%) and *β*-Ocimene (17.69%) as major constituents among the thirty one evaluated core collections.

4. Conclusion

The present investigation has broaden knowledge base with respect to existing diversity with respect to essential oil array in thirty one core collections of *Ocimum sanctum* from different eco-geographical conditions having contrasting environment. This has helped to identify elite clone rich in particular components which could be exploited further for bioprospection of biomolecules.

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