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MORPHO-CHEMICAL DESCRIPTION AND ANTIMICROBIAL ACTIVITY OF DIFFERENT *OCIMUM* SPECIES

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Abstract: Basil is a popular medicinal and culinary herb, and its essential oils have been used extensively for many years in food products, perfumery, dental and oral products. Basil essential oils and their principal constituents were found to exhibit antimicrobial activity against a wide range of Gram-negative and Gram-positive bacteria, yeast, and mould. The essential oils obtained from aerial parts of three different species of *Ocimum* comprising twenty one germplasm lines were investigated for their essential oil composition and antimicrobial activity during 2010. Essential oils from seventeen germplasm lines in *Ocimum basilicum* and two each in *Ocimum tenuiflorum* and *Ocimum gratissimum* were investigated for anti-microbial activity against four bacterial strains (*Staphylococcus aureus*, *Bacillus* sps., *Escherichia coli* and *Pseudomonas aeruginosa*). The morpho-chemotypes exhibited wide variability for morphological and chemical traits. Anti-bacterial activity was found to be high for *Staphylococcus aureus*, moderate for *Escherichia coli*, low for *Bacillus* and *Pseudomonas aeruginosa* was highly resistant. The essential oils of Pale Green-Broad Leaves (*O. basilicum*) and CIM Ayu (*O. gratissimum*) exhibited significant antibacterial activity against both *S. aureus* and *E. coli* signifying them promising for anti-bacterial activity. No relationship was observed between chemotype specificity and anti-bacterial activity, indicating that apart from major components of essential oil, minor components and other factors may be responsible for anti-microbial activities.

Key words: *Ocimum basilicum*, *Ocimum gratissimum*, *Ocimum tenuiflorum*, Methyl chavicol, Eugenol, Linalool, Antimicrobial activity

Introduction

There is a significant growth in the world trade for essential oils (growing approximately at 11% per year [BIZZO & al. 2009]). The exports and internal consumption of essential oils have shown a significant increase in India also in the recent past. In India essential oils are produced from many species and those belonging to the genus *Ocimum* also contribute modestly to the total exports.

The genus *Ocimum* L. (Lamiaceae) consisting of several species is very well distributed in tropical and subtropical Africa, Asia and South America [GUPTA, 1994]. They occur in various parts of tropical Asia, America and sub tropical regions of the world and found to grow from sea-level to an altitude of about 1800 m. The genus is represented by nine species in India. Among the various *Ocimum* species, Sweet basil (*O. basilicum* L.) is commercially and extensively cultivated for essential oil production in India and other countries. In India Sweet basil is cultivated in various States viz. West Bengal, Maharastra, Uttar Pradesh, Madhya Pradesh, Bihar, Jammu, Assam etc.

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The oil of sweet basil is used extensively in condimentary products, cosmetics, and toiletry, perfumery and confectionery industries. The oil has moderate export value and the oil is reported to have bactericidal, insecticidal and also medicinal properties. The oil also contains protein, carbohydrates and relatively high concentrations of vitamins A and C.

O. gratissimum L. occurs throughout India and is also cultivated for it's essential oil. Several studies reported the chemical composition and antimicrobial activity of the essential oil of *O. gratissimum* L. [CHARLES & SIMON, 1990; CASTEELS & al. 1993; BASSOLE & al. 2005; ANANDA & al. 2010].

Ocimum essential oils are composed of aroma compounds such as eugenol, methyl eugenol, citral, linalool, geraniol and thymol which are required as raw materials for the pharmaceutical, cosmetics and food industries [GUPTA, 1994; BIZZO & al. 2009]. Many of these essential oils are used in folk medicine [GRAYER & al. 1996], exhibit other biological activities such as antimicrobial [PRASAD & al. 1986; NAKAMURA & al. 1999; BASSOLE & al. 2005], insecticide [DE PAULA & al. 2003; PAULA & al. 2004], antioxidant [GANIYU, 2008] and analgesic activities [FRANCA & al. 2008].

Even though numbers of reports on anti-microbial activities of *Ocimum* essential oil are available, studies across different *Ocimum* species are negligible. So the present investigation on the morphological features, chemical composition and antimicrobial activities of the volatile oils produced by twenty one chemotypes belonging to three different species, i.e. *Ocimum tenuiflorum*, *Ocimum basilicum* and *Ocimum gratissimum* was carried out.

Material and methods

Experimental site and design of the experiment

The present study was undertaken to evaluate the variability of twenty one chemotypes of *Ocimum* belonging to three different species for their suitability for commercial cultivation in the Deccan region of India during 2009-2010. The experiment was laid out at the research farm of Central Institute of Medicinal and Aromatic Plants (CIMAP), Research Centre, Boduppal, Hyderabad, Andhra Pradesh, India.

The experimental site is located at an altitude of 542 m above mean sea level with a geographical bearing of 78°8' longitude and 17°32' latitude. Semi-arid tropical climate zone of Hyderabad has an average rainfall of 800 mm per year. The soil of the experimental field is a red sandy loam (alficusto chrept) with pH 8.1 (1.25 soils to solution ratio), EC – 1.25 ds/m, organic C – 0.3%, total N – 0.03%, available P – 10 ug/g soil and exchangeable K – 128 ug/g soil.

Morphological variability

The seeds were sown during May-June, 2009. Three weeks old seedlings were transplanted in the main field with a row to row distance of 60 cm and plant to plant distance of 45 cm. There were twenty one plots replicated three times in a completely randomized design. Each treatment was accorded 3x4 m plots. In all there were 63 experimental plots. Morphological observations were recorded at the time of harvesting on ten randomly selected plots in each treatment.

Essential oil extraction

The aerial parts of *O. basilicum*, *O. tenuiflorum* and *O. gratissimum* were collected in flowering stage from experimental plots during 2010 – 2011 for the extraction of

essential oils. For the extraction of essential oils, freshly collected herbage of three *Ocimum* species were subjected to hydro-distillation using a Clevenger-type apparatus for 3.5 h.

The essential oils collected were dried over anhydrous sodium sulphate and stored at 4 °C until the analysis was carried out.

Antimicrobial cultures

Pure cultures of the strains obtained from National Collection of Industrial Microorganisms (NCIM), NCL, Pune, India were used in the experiment. Cultures are maintained on Nutrient Agar (Hi-Media, India) slopes at 4 °C and sub cultured before use.

The Gram-positive and Gram-negative bacterial strains used for the investigation are listed below:

a. *Staphylococcus aureus* – Gram-positive
b. *Bacillus* sps – Gram-positive
c. *Escherichia coli* – Gram-negative
d. *Pseudomonas aeruginosa* – Gram-negative

Determination of antibacterial activity

In vitro antibacterial activity of essential oils of twenty one *Ocimum* chemotypes was studied against four microbial cultures using Agar Diffusion Well Method.

Muller-Hinton medium was used for test assay. Essential oils were diluted to give 50 µg, 100 µg, 200 µg, 400 µg and 800 µg per 100 micro liters concentration. The overnight broth of inoculum was seeded on agar plates (1.5*108 CFU/ml). Eight mm diameter wells were prepared in seeded agar plates and test compound was introduced in each well. The solvent used for preparing essential oil solution was absolute alcohol. Solvent control well was run for every assay. All the plates were incubated at 37 °C for 24 hrs. The antimicrobial spectrum of the essential oils were determined in terms of zone sizes around each well i.e. diameter of inhibition zones. The experiment is replicated thrice following a completely randomized design.

GC analysis

GC analysis was carried out using Varian CP-3800 with Galaxie chromatography data system fitted with flame ionization detector (FID) and an electronic integrator. Separation of the compounds was achieved employing a Varian CP-Sil 5CB capillary column (ID: 50 m X 0.25 mm; film thickness 0.25 µm) with 5% dimethyl polysiloxane. Nitrogen was the carrier gas at 0.5 ml/min constant flow rate. The column temperature program was: 120 °C (2 min) to 240 °C (6 min) at 8 °C/min ramp rate. The injector and detector temperature were 250 °C and 300 °C respectively. Samples (0.2 µL) were injected with a 20:80:20 split ratio. Retention indices were generated with a standard solution of *n*-alkanes (C_6-C_{19}). Peak areas and retention times were measured by an electronic integrator. The relative amounts of individual compounds were computed from GC peak areas without FID response factor correction.

Results and discussion

Morphological description

Description about the morphological characters of the three *Ocimum* spp. are presented in Tab. 1. Among the three species, *O. gratissimum* was tall and *O. basilicum* showed wide variability for plant height ranging from short (44.00 cm) to medium plant height (125.00 cm). Canopy spread of tall *O. gratissimum* lines was relatively less than *O. basilicum* and *O. tenuiflorum* and both species recorded high number of leaves/plant. *O.*

gratissimum with less number of leaves/plant had large leaf area with lengthier and broader leaves. *O. basilicum* showed wide range for number of inflorescences/plant. *O. tenuiflorum* lines recorded higher number of inflorescences/plant. However, *O. tenuiflorum* with higher inflorescence number showed moderate number of whorls/ inflorescence (17) whereas *O. gratissimum* exhibited higher number of whorls/inflorescence (27-28). *O. basilicum* exhibiting wide variation for whorls/inflorescence.

O. gratissimum had lengthy peduncles, while the petiole lengths were high in *O. basilicum* and *O. tenuiflorum*. The flowers were relatively large in *O. basilicum* with large size bracts, sepals and petals. The stem colour was green or greenish purple in *O. basilicum*, whereas greenish purple in *O. tenuiflorum* and *O. gratissimum*. Flower colour of *O. basilicum* was white (some germplasm lines beared whitish purple flowers). *O. tenuiflorum* had purple/purplish white flowers, while the flower colour of *O. gratissimum* was purplish white. *O. basilicum* exhibited a range of colours for stamen colour ***i.e.*** white or creamy white or whitish purple. Stamen colours of *O. tenuiflorum* and *O. gratissimum* were similar to flower colour. Seeds were black in *O. basilicum* and brown in remaining two species.

Chemical description

The compositions of the essential oils of *O. basilicum* (seventeen chemotypes), *O. tenuiflorum* (two chemotypes) and *O. gratissimum* (two chemotypes) are presented in table (Tab. 2). In total twenty nine compounds were identified in the oils representing 78.0 - 99.6% of the oil. Methyl chavicol (5.2 - 87.0%), eugenol (0.0 - 50.0%), linalool (1.4 - 69.0%), limonene (0.1 - 10.3%), β-carophyllene (0.1 - 5.8%) and methyl cinnamate (only one chemotype - 61.0%) were the major constituents in *O. basilicum* oils.

Eugenol (0.9 - 84.0%), methyl eugenol (0.0 - 73.0%) and β-carophyllene (5.5 - 6.9%) were the major constituents in *O. tenuiflorum* oils. In *O. gratissimum* oils, eugenol and limonene + 1,8 cineole were found to be the major constituents. These eight major compounds were considered for a cluster analysis to identify possible chemotypes. The resulting dendrogram (Fig. 1) showed the existence of 3 main clusters and 3 single germplasm line clusters.

The first group was formed by *O. basilicum* germplasm lines 1, 3, 14, 10, 13 and 17, which could be identified as "methyl chavicol" chemotypes. Their oils, having very little variation in quantitative composition, showed high percentage of methyl chavicol (73.0 - 87.0%) with small quantities of linalool and eugenol. The second major cluster consisted of *O. basilicum* germplasm lines 2, 12, 4, 5, 7, 6 and 9, which comprised of three sub clusters (2 & 12; 4, 5 & 7 and 6 & 9) all having methyl chavicol as one of the major component along with linalool. The essential oil of lines 2 and 12 belonging to sub cluster-1 comprised of methyl chavicol (42.0 - 62.0%) as the major component along with linalool (18.0 - 24.0%) and line-2 had 25.0% eugenol in addition. Equal quantities of methyl chavicol (34.0 - 39.0%) and linalool (39.0 - 43.0%) were characteristic in the essential oils of germplasm lines 4, 5 and 7 belonging to second sub-cluster. On contrary, germplasm lines 6 and 9 in the remaining third sub-cluster possessed high amount of methyl chavicol (48.0 - 52.0%) in comparison to linalool (27.0 - 29.0%).

Third major cluster comprised of all the three species having eugenol or methyl eugenol as the major constituent. Germplasm line 11 belonging to *O. basilicum* consisted of equal quantities of eugenol (33.4%) and linalool (29.8%), while *O. gratissimum* lines were rich in eugenol (62.0 - 74.8%). Germplasm line 20 belonging to *O. tenuiflorum* had the highest eugenol (84.0 %) content and germplasm line 21 belonging to the same species was

rich in methyl eugenol (73.0%). *O. basilicum* germplasm lines 15, 8 and 16 in single genotype clusters were rich in specific component i.e. eugenol (15.0%), linalool (69.0%) and methyl cinnamate (61.0%) respectively.

Antimicrobial activity

Among the four bacterial strains tested, anti-bacterial activity was found to be high for *Staphylococcus aureus*, moderate for *Escherichia coli*, low for *Bacillus. Pseudomonas aeruginosa* was highly tolerant. Leading nosocomial agent *Staphylococcus aureus* a catalase positive, coagulase positive strain and gram positive cocci was found to be most sensitive to all the *Ocimum* oils tested. *O. basilicum* germplasm lines with wide differences for morphology and essential oil composition were highly variable for anti-bacterial activity also against *S. aureus* (Tab. 3).

Eugenol rich Exotic line and methyl chavicol rich Pale Green-Broad Leaves germplasm line showed profound anti- *S. aureus* activity (Fig. 2) with average inhibition zone of 45.6 mm and 41.2 mm, respectively. Moderate effects were exhibited by OB Tall, Globe P-15, Globe General, Citral-II and Dark Green-Broad Leaves. Anti- *S. aureus* effects were low for Linalool-II, MC-1, Linalool-III, Vikarsudha, MCN-9 and Kushmohak, while the inhibitory zones were very low for essential oils from MCN-10, MCN Narrow Leaves and Mulagu-II.

Eugenol rich essential oils obtained from *O. gratissimum* OG Original and *O. tenuiflorum* CIM Ayu exhibited very high inhibitory activity against *S. aureus* with average inhibitory zones of 46.4 mm and 34.6 mm respectively. Methyl eugenol rich *O. tenuiflorum* CIM Kanchan showed low antibacterial activity against *S. aureus*.

The essential oil of MCN-9 majoring in methyl chavicol (42.0%) along with eugenol (25.0%) and linalool (18.0%), which showed low anti- *S. aureus* activity exhibited relatively high anti- *E. coli* activity with average inhibition zone of 20.8 mm ranging 15.0 mm - 23.0 mm from 50-800 µg concentration (Tab. 4; Fig. 3). Relatively moderate resistance against *E. coli* were shown by essential oils from Citral-II (17.2 mm), Globe P-15 (16.8 mm), Pale Green-Broad Leaves (15.4 mm), Linalool-II (15.2 mm) and CIM Ayu (15.2 mm). The essential oils of Pale Green-Broad Leaves (*O. basilicum*) and CIM–Ayu (*O. gratissimum*) were more active against both *S. aureus* and *E. coli* signifying them promising for anti-bacterial activity.

Pseudomonas aeruginosa a highly adaptive aerobic Gram negative bacterium and a leading nosocomial pathogen, was highly resistant to the essential oils belonging to all the three species. In the present study, enhanced production of pigmentation i.e diffusible blue green pigment phytocyanin was noticed in the presence of *Ocimum* oils. The observation that production of diffusible pigment phytocyanin, which is highly enhanced in presence of *Ocimum* essential oils may be an indication that this pigment acts as a protective barrier for *Pseudomonas* against antimicrobial substances. The difference in the antimicrobial activity of Gram positive and Gram negative bacteria can be attributed to the difference in their cell wall composition. Gram negative bacteria are resistant due to presence of outer membrane acting as a barrier to many environmental or plant natural substances. The resistance exhibited by *Pseudomonas* can be attributed to the small pore size of its cell wall due to the outer membrane complexity [LOPEZ & al. 2005].

Conclusions

The different chemotypes of all the three *Ocimum* species exhibited variability for morphological characters, chemical composition and anti-bacterial activity. No relationship was observed between chemotype specificity and anti-bacterial activity, indicating that apart from major components of essential oil, minor components and other factors may be responsible for anti-microbial activities.

Tab. 1. Morphological characterization of different *Ocimum* species i.e. *O. basilicum* (17 genotypes), *O. tenuiflorum* (2 genotypes), *O. gratissimum* (2 genotypes)

S. No	Morphological trait	*Ocimum basilicum*	*Ocimum tenuiflorum*	*Ocimum gratissimum*
1	Plant height (cm)	44 - 125	95-120	96-150
2	Canopy spread (cm)	37 - 94	60-92.3	64-76
3	Leaves/ plant	350-4032	2210-2850	989-1200
4	Leaf length (cm)	3.2-11.6	5.4-5.6	10.4-15.7
5	Leaf width (cm)	1.4-5.1	2.9-3	6.4-6.7
6	Inflorescence/ plant	15-294	289-295	70-276
7	No. of Whorls/ Inflorescence	12 - 27	17	27-28
8	Petiole length (cm)	0.3-2.6	2.1-2.5	4-4.6
9	Peduncle length (cm)	13-33.5	14.2-30.2	15-17.8
10	Bract length (cm)	0.6-1.4	0.4	0.6-0.8
11	Bract width (cm)	0.3-0.6	0.3	0.2-0.3
12	Sepal length (cm)	0.4-0.9	0.4-0.5	0.4-0.5
13	Sepal width (cm)	0.1-0.3	0.1-0.2	0.1-0.3
14	Petal length (cm)	0.6-1.1	0.4-0.5	0.4-0.6
15	Petal width (cm)	0.2-0.4	0.2-0.3	0.2-0.3
16	Stem colour	Green / Greenish Purple	Greenish Purple	Greenish Purple
17	Flower color	White/Whitish Purple	Purple/Purplish white	Purplish white
18	Stamen color	White/Creamy white/Whitish Purple	Purple/Purplish white	Purplish white
19	Seed colour	Black	Brown	Brown

Tab. 2. Percentage composition of the essential oils of twenty one chemotypes belonging to three *Ocimum* species

Constituents	*Ocimum basilicum* Germplasm Lines										
	MC - 1	**MCN - 9**	**Broad dark green**	**Globe General**	**Linalool II**	**Mulugu II**	**Kushmohak**	**Citral II**	**Linalool III**	**Globe P-15**	**Exotic**
terpinen-4-ol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Methyl chavicol	73.0	42.0	87.0	34.0	39.0	48.0	38.4	5.2	52.0	75.5	0.2
(Z)methyl cinnamate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Eugenol	0.8	25.0	2.3	6.9	tr	0.1	3.3	nd	0.1	0.3	33.4
Methyl cinnamate	nd	nd	nd	nd	tr	nd	nd	nd	nd	nd	nd
geranyl acetate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
methyl eugenol	0.6	1.6	0.6	1.5	0.1	0.4	2.7	nd	0.4	0.7	0.1
β-elemene	tr	nd	nd	nd	tr	tr	1.0	nd	tr	0.3	1.0
β-caryophyllene	0.6	0.5	0.2	1.6	0.8	1.1	1.4	1.7	0.8	0.6	5.7
α- humulene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
germacrene D	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
caryophyllene oxide	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B-eudesmol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
α –thujene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
α-pinene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Campene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Sabinene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Myrcene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
limonene+1,8 cineole	5.3	1.3	3.0	2.7	1.7	6.3	1.4	4.7	2.7	2.7	10.3
(Z)-β-ocimene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
γ- terpinene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
terpinolene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Linalool	6.9	18.0	1.4	39.0	43.0	29.0	40.6	69.0	11.9	11.9	29.8
Camphor	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Borneol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Citronellol	nd	nd	nd	nd	nd	nd	0.7	0.7	0.7	nd	nd
Citral-I	nd	nd	nd	nd	nd	nd	tr	2.5	1.5	nd	nd
Geraniol	nd	nd	nd	nd	nd	nd	1.5	0.2	0.7	nd	nd
Citral-II	nd	nd	nd	nd	nd	nd	tr	3.5	2.3	nd	nd
Total	87.2	88.4	94.5	85.7	84.6	84.9	91.0	87.5	73.1	92.0	80.6

Contd., **Tab. 2**

Constituents	*Ocimum basilicum* Germplasm Lines						*Ocimum gratissimum* Germplasm Lines		*Ocimum tenuiflorum* Germplasm Lines	
	CIM Saumya	**Vikarsudha**	**Broad Pale Green**	**MCN 10**	**MC Narrow**	**OB Tall**	**OG Variant**	**OG Original**	**CIM -AYU**	**CIM-kanchan**
terpinen-4-ol	nd	nd	nd	nd	tr	nd	tr	0.6	tr	tr
Methyl chavicol	62.0	87.0	87.0	6.6	6.0	40.0	0.4	0.1	tr	tr
(Z)methyl cinnamate	tr	tr	nd	nd	8.0	nd	tr	tr	tr	tr
Eugenol	1.3	tr	2.3	50.0	0.3	0.1	74.8	62.0	84.0	0.9
Methyl cinnamate	tr	tr	nd	nd	61.0	nd	tr	tr	tr	tr
geranyl acetate	tr	tr	nd	nd	tr	nd	tr	0.1	tr	tr
methyl eugenol	tr	1.1	0.6	1.3	0.3	0.5	tr	tr	tr	73.0
β-elemene	0.5	tr	tr	tr	0.9	tr	tr	0.5	7.5	0.5
β-caryophyllene	0.2	0.1	0.2	0.8	0.5	2.0	1.5	1.8	6.9	5.5
α- humulene	tr	0.1	nd	nd	0.3	nd	tr	0.2	tr	0.4
germacrene D	0.9	tr	nd	nd	0.6	nd	tr	tr	tr	tr
caryophyllene oxide	tr	0.2	nd	nd	tr	nd	tr	tr	tr	1.5
B-eudesmol	1.5	1.3	nd	nd	0.2	nd	tr	tr	tr	0.1
α –thujene	tr	tr	nd	nd	tr	nd	tr	0.1	tr	tr
α-pinene	0.1	0.4	nd	nd	0.1	nd	tr	tr	0.9	0.1
Campene	tr	tr	nd	nd	tr	nd	tr	0.1	0.1	tr
Sabinene	tr	tr	nd	nd	tr	nd	tr	0.2	tr	tr
Myrcene	0.4	0.1	nd	nd	0.3	nd	tr	0.2	tr	0.1
limonene+1,8 cineole	1.5	0.1	3.0	1.3	0.9	5.0	10.8	7.7	0.1	0.1
(Z)-β-ocimene	1.5	0.3	nd	nd	1.2	nd	tr	0.4	tr	0.1
γ- terpinene	tr	tr	nd	nd	tr	nd	tr	0.1	tr	0.1
terpinolene	tr	0.1	nd	nd	tr	nd	tr	tr	tr	tr
Linalool	24.0	1.8	1.4	18.0	16.0	32.0	tr	0.3	0.1	tr
Camphor	tr	tr	nd	nd	0.2	nd	tr	18.0	tr	tr
Borneol	0.6	0.1	nd	nd	tr	nd	tr	0.3	tr	tr
Citronellol	nd	nd	nd	nd	nd	nd	tr	tr	tr	tr
Citral-I	nd	nd	nd	nd	nd	nd	tr	tr	tr	tr
Geraniol	nd	nd	nd	nd	nd	nd	tr	tr	tr	tr
Citral-II	nd	nd	nd	nd	nd	nd	tr	tr	tr	tr
Total	94.5	92.7	94.5	78.0	96.8	79.6	87.5	92.7	99.6	82.4

nd: not detected tr: traces

Tab. 3. Antimicrobial activity of pure essential oils obtained from twenty one chemotypes belonging to three sp. of *Ocimum* tested at different concentrations on *Staphylococcus aureus*

S. No	Chemotype	Inhibition zone in mm (millimeters)						
		CONCENTRATION (µg)						
		50	100	200	400	800	Range	Average
1	MC - 1	8	8	17	20	22	8-22	15
2	MCN - 9	11	19	18	16	30	11-30	18.8
3	Broad Dark Green	11	20	28	34	48	11-48	28.2
4	Globe General	17	20	24	34	40	17-40	27
5	Linalool II	14	12	16	10	17	10-17	13.8
6	Mulugu II	2	4	10	10	20	2-20	9.2
7	Kushmohak	12	10	11	26	38	10-38	19.4
8	Citral II	20	16	30	27	48	16-48	28.2
9	Linalool III	8	8	20	12	40	8-40	17.6
10	Globe P-15	18	20	24	30	38	18-38	26
11	OB Tall	20	14	21	23	25	14-25	20.6
12	Exotic	38	42	50	50	48	38-50	45.6
13	CIM Saumya	18	18	20	34	40	18-40	26
14	Vikarsudha	4	20	20	20	30	4-30	18.8
15	Broad Pale Green	40	40	38	40	48	38-48	41.2
16	MCN 10	1	1	1	10	11	1-11	4.8
17	MCN Narrow	1	1	6	10	12	1-12	6
18	OG Variant	16	17	20	22	42	17-42	23.4
19	OG Original	30	50	50	50	52	30-52	46.4
20	CIM AYU	38	17	42	46	30	17-46	34.6
21	CIM Kanchan	10	12	8	14	14	8-14	11.6

Tab. 4. Antimicrobial activity of pure essential oils obtained from twenty one chemotypes belonging to three sp. of *Ocimum* tested at different concentrations on *Escherichia coli*

S. No	Chemotype	Inhibition zone in mm (millimeters)						
		Concentration (µg)						
		50	100	200	400	800	Range	Average
1	MC - 1	5	10	10	15	10	5 - 15	10
2	MCN - 9	15	18	28	20	23	15 - 28	20.8
3	Broad Dark Green	16	8	13	8	16	8 - 16	12.2
4	Globe General	10	12	14	16	17	10 - 17	13.8
5	Linalool II	11	16	13	17	19	11 - 19	15.2
6	Mulugu II	11	8	12	12	20	8 - 20	12.6
7	Kushmohak	2	12	11	10	14	2 - 14	9.8
8	Citral II	20	10	20	14	22	10 - 22	17.2
9	Linalool III	6	12	8	10	20	6 - 20	11.2
10	Globe P-15	6	16	10	24	28	6 - 28	16.8
11	OB Tall	4	10	8	12	28	4 - 28	12.4
12	Exotic	4	11	10	12	16	4 - 16	10.6
13	CIM Saumya	10	1	12	6	26	1 - 26	11
14	Vikarsudha	1	2	4	2	8	1 - 8	3.4
15	Broad Pale Green	8	15	12	12	30	8 - 30	15.4
16	MCN 10	6	7	8	12	12	6 - 12	9
17	MCN Narrow	1	1	1	10	11	1 - 11	4.8
18	OG Variant	2	6	6	10	12	2 - 12	7.2
19	OG Original	4	4	8	8	18	4 - 18	8.4
20	CIM AYU	8	12	12	16	28	8 - 28	15.2
21	CIM Kanchan	11	10	14	8	6	6 - 11	9.8

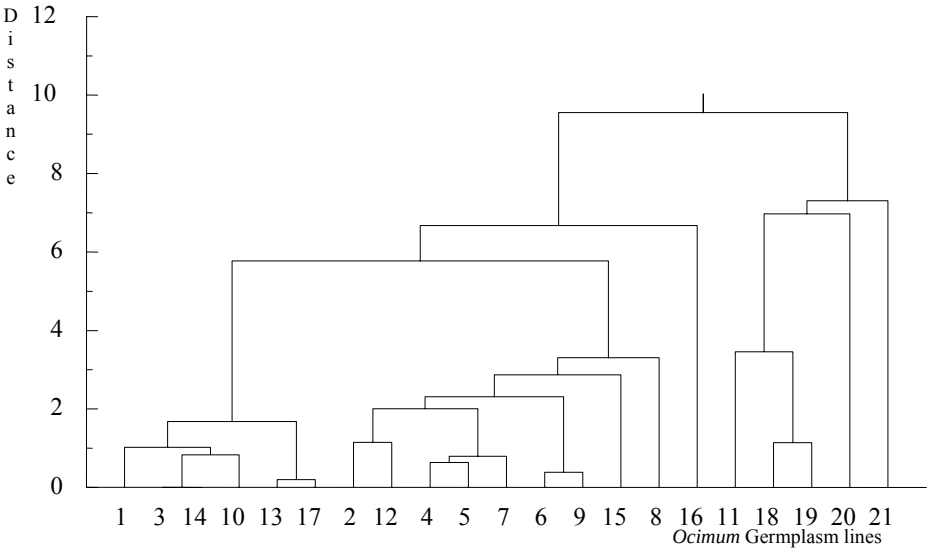

Fig. 1. Cluster analysis of twenty one chemotypes belonging to three species of *Ocimum* based on eight major essential oil components

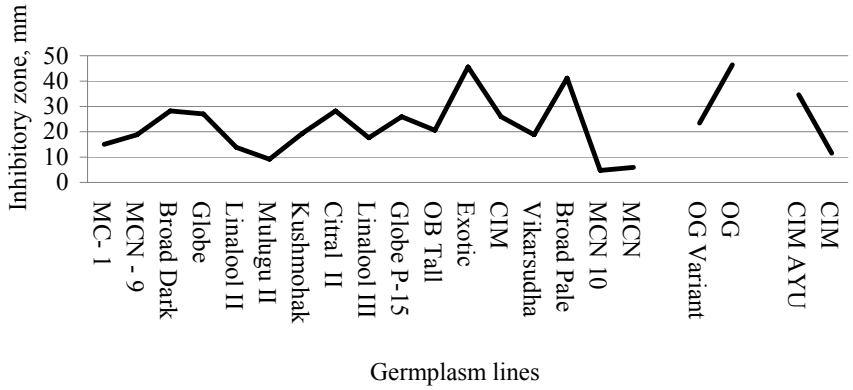

Fig. 2. Antimicrobial activity of pure essential oils obtained from twenty one chemotypes belonging to three species of *Ocimum* tested at different concentrations on *Staphylococcus aureus*

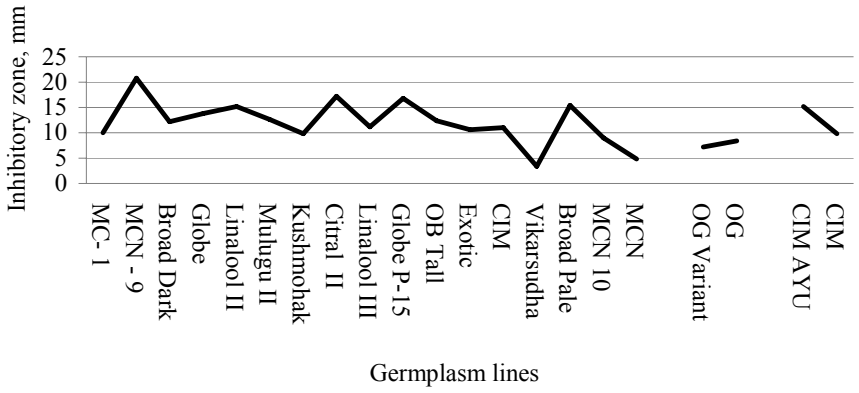

Fig. 3. Antimicrobial activity of pure essential oils obtained from twenty one chemotypes belonging to three species of *Ocimum* tested at different concentrations on *Escherichia coli*

References

1. ANANDA A. K., MOHAN M., HAIDER S. Z. & SHARMA A. 2010. Essential oil composition and antimicrobial activity of three *Ocimum* species from Uttarakhand (India). *International Journal of Essential Oil Therapeutics*. **4**: 111-114.
2. BASSOLE I. H. N., NEBIE R., SAVADOGO A., OUATTARA C. T., BARRO N. & TRAORE S. A. 2005. Composition and antimicrobial activities of the leaf and flower essential oils of *Lippia chevalieri* and *Ocimum canum* from Burkina Faso. *Afric. J. Biotech*. **4**: 1156-1160.
3. BIZZO H. R., HOVELL A. M. C. & REZENDE C. M.2009. Óleos essenciais no Brasil: Aspectos gerais, desenvolvimento e perspectivas. *Quim Nova*. **32**: 588-594.
4. CASTEELS P., AMPE C., JACOBS F. & TEMPST P. 1993. Functional and chemical characterizations of Hymenoptaecin, an antibacterial polypeptide that is infection-inducible in the Honeybee (*Apis mellifera*). *J. Biol. Chem.* **268**: 7044-7054.
5. CHARLES D. J. & SIMON J. E. 1990. Comparison of extraction methods for the rapid determination of essential oil content and composition of basil. *J. Amer. Soc. Hort. Sci.* **115**: 458-462.
6. DE PAULA J. P., CARNEIRO M. R.G. & PAUMGARTTEN F. J. R. 2003. Chemical composition, toxicity and mosquitorepellency of *Ocimum selloi* oil. *J. Ethnopharmac*. **88**: 253-260.
7. FONSECA M. C. M., BARBOSA L. C. A., NASCIMENTO E. A. & CASALI V. W. D. 2006. Essential oil from the leaves and flowers for *Porophyllum ruderale* (Jacq.) Cassini (Asteraceae). *J. Essent. Oil Res.* **18**: 345-347.
8. FRANCA C. S., MENEZES F. S., COSTA L. C. B., NICULAU E. S., ALVES P. B., PINTO J. E. B. & MARÇAL R. M. 2008. Analgesic and antidiarrheal properties of *Ocimum selloi* essential oil in mice. *Fitoterapia*. **79**: 569-573.
9. GANIYU O. 2008. Antioxidative potential of *Ocimum gratissimum* and *Ocimum canum* leaf polyphenols andprotective effects on some pro-oxidants induced lipidperoxidation in rat brain: an in vitro study. *Amer. J. Food Technol.* **3**: 325-334.
10. GUPTA R. 1994. Basil (*Ocimum* spp.). *G-15 Gene Banks for Medicinal and Aromatic Plants Newsletter*. **1**: 1-3.
11. LOPEZ P., SANCHEZ C., BATLLE R. & NERIN C. 2005. Solid- and vapor-phase antimicrobial activities of six essential oils: Susceptibility of selected foodborne bacterial and fungal strains. *Journal of Agricultural and Food Chemistry*. **53**: 6939-6946.

12. MOGHADDAM A. M. D., SHAYEGH J., MIKAILI P. & SHARAF J. D. 2011. Antimicrobial activity of essential oil extract of *Ocimum basilicum* L. leaves on a variety of pathogenic bacteria. *African Journal of Traditional, Complementary and Alternative Medicines*. **5**: 187-193.
13. NAKAMURA C. V., UEDA-NAKAMURA T., BANDO E., MELO AFN, CORTEZ DAG & DIAS FILHO BP. 1999. Antibacterial activity of *Ocimum grantissimum* L. essential oil. *Mem Inst Oswaldo Cruz*. **94**: 675-678.
14. PAULA J. P., FARAGO P. V., CHECCHIA LEM, HIROSE K. M. & RIBAS J. L. C. 2004. Atividade repelente do óleo essencial de *Ocimum selloi* Benth. (variedade eugenol) contra o *Anopheles braziliensis* Chagas. *Acta Farm Bonaerense*. **23**: 376-378.
15. PRASAD G., KUMAR A., SINGH A. K., BHATTACHARYA A. K., SINGH K. & SHARMA V. D. 1986. Antimicrobial activity of essential oils of some *Ocimum* species and clove oil. *Fitoterapia*. **57**: 429-432.