

## Variation in the Hexanic Extract Composition of *Lippia graveolens* in an Arid Zone from Mexico: Environmental Influence or True Chemotypes?

Tzasna Hernández<sup>a,\*</sup>, Margarita Canales<sup>a</sup>, Angel Duran<sup>a</sup>, Ana María García<sup>a</sup>, José Guillermo Avila<sup>a</sup>, Luís Hernández-Portilla<sup>b</sup>, Martha Alvarado<sup>a</sup>, Marisol Romero<sup>a</sup>, Bárbara Terán<sup>a</sup>, Patricia Dávila<sup>c</sup> and Rafael Lira<sup>c</sup>

<sup>a</sup>Laboratorio de Fitoquímica, UBIPRO, Facultad de Estudios Superiores-Iztacala, Universidad Nacional Autónoma de México, Tlalnepantla 54090, Edo. de Mexico, Mexico

<sup>b</sup>Laboratorio de Fisiología Vegetal UBIPRO, Facultad de Estudios Superiores-Iztacala, Universidad Nacional Autónoma de México, Tlalnepantla 54090, Edo. de Mexico, Mexico

<sup>c</sup>Laboratorio de Recursos Naturales, UBIPRO, Facultad de Estudios Superiores-Iztacala, Universidad Nacional Autónoma de México, Tlalnepantla 54090, Edo. de Mexico, Mexico

**Abstract:** The hexanic extract composition of two populations of *Lippia graveolens* growing in an arid zone in the Tehuacán-Cuicatlán Valley (Zapotitlán Salinas, Puebla), Central Mexico, in different seasons of the year, and in contrasting conditions of ecological disturbance has been studied by GC and GC-MS. Regarding to the composition of these extracts, qualitative differences among the two populations were found. Thus, 9 compounds were identified from the extracts of the Zone A (the less disturbed) and 10 from the ones of Zone B (the most disturbed). Thymol was detected as the major compound in most of the individuals sampled, while at the season level, September was the more productive month for zone B (higher content of identified compounds) and the only one that presents antibacterial activity. *Lippia graveolens* displays quantitative and qualitative variations both within and between natural plant populations.

**Key Words:** Arid zones, *Lippia graveolens*, Thymol, Zapotitlán Salinas.

### INTRODUCTION

In a recent study on the phytochemical basis of the traditional knowledge on the plants used for the treatment of gastrointestinal infectious diseases in traditional medicine from Zapotitlán Salinas, *Lippia graveolens* was one of the most important and effective plants for those purposes [1]. In addition, the study also identified that some of the most important compounds responsible for that effectiveness were thymol and carvacrol. It is widely known, however, that the production of this kind of secondary metabolites could be determined by climatic factors, season of the year and/or the development stage of the plant [2-5].

Considering the above mentioned, the objective of this work was to determine, in different seasons of the year, and for contrasting conditions of ecological disturbance, the antibacterial activity and the existence of inter and intra population variation in the composition of the hexanic extracts of *Lippia graveolens* populations growing in an arid zone in the Tehuacán-Cuicatlán Valley in Central Mexico. The final goal was to determine if this variation could potentially be recognized as chemotypes deserving attention for their conservation in a seed bank. For the purposes of this paper, a chemotype (sometimes called chemovar) is a chemically

distinct entity in a plant or microorganism, with differences in the composition of the secondary metabolites [4, 6].

### STUDY SITE

Zapotitlan Salinas is a small village located within the limits of the Tehuacán Cuicatlán Biosphere Reserve in central Mexico. Zapotitlán Salinas is located in the south-eastern part of the Valley of Tehuacán-Cuicatlán, in the State of Puebla (Fig. 1). The climate is arid with a mean annual rainfall of 542.5mm and annual temperature of 19.8°C [7]. According to the classification proposed by Rzedowski (1978) [8], there are three general vegetation types in the zone, namely, arid tropical shrubland, thorn forest and tropical deciduous forest, with thorn-scrub as the predominant vegetation. Mediterranean type vegetation commonly called "mexical" or chaparral is also present, covering hills up to 2000 m [9]. The Chocho-Popoloca is the dominant ethnic group in the region [1, 10, 11].

### EXPERIMENTAL

#### Field Work

#### Plant Material

Between May 2005 and March 2006 the samples were collected in the Terrazas Aluviales, in Zapotitlán Salinas, Puebla (central Mexico) in two places under clearly contrasting disturbed conditions (named Zones A and B). Zone A, known as Las Granjas, corresponds to the less disturbed condition (continuous cover of vegetation, soil primary structure with a well defined superficial horizons), whereas

\*Address correspondence to this author at the Tzasna Hernández. Laboratorio de Fitoquímica, UBIPRO, Facultad de Estudios Superiores-Iztacala, Universidad Nacional Autónoma de México, Tlalnepantla 54090, Edo. de Mexico, México; Tel: + 52-5-623-11-36; Fax: + 52-5-623-12-25; E-mail: tzasna@servidor.unam.mx



an analysis of variance (multifactor ANOVA) of the average values of the compounds obtained was done. p-values of 0.001 or less were considered statistically significant. This analysis was performed by using the Statistics software version 6.0.

## RESULTS AND DISCUSSION

Hexanic extracts were prepared from the samples obtained at the different collection sites and seasons. Regarding to the composition of these extracts, qualitative differences among the two populations were found. Thus, 9

compounds were identified from the extracts of the Zone A and 10 from the ones of Zone B. The compounds identified were: 3-Carene,  $\alpha$ -Pinene,  $\rho$ -Cymene, Carvacrol, Thymol, Isocaryophyllene ( $\gamma$ -caryophyllene),  $\alpha$ -Humulene, Guaia-1(5), 7(11)-diene, Thymol methyl ether in both zones and  $\alpha$ -Thujene was present only in zone B (Tables 1 and 2) which suggest the possibility that more secondary metabolites are produced in disturbed zones [15].

The hexane extracts were characterized by high percentages of monoterpenes, especially oxygenated ones (Thymol methyl ether, Carvacrol, Thymol) as well as alkyl-

**Table 1. Statistics Parameters of *Lippia graveolens* Hexane Extracts from Zone A (Las Granjas)**

C	RT	December 2005			March 2006		
		Mean (%)	Min	Max	Mean (%)	Min	Max
$\alpha$ -Pinene	4.73	0.11 $\pm$ 0.16	0	0.32	0	0	0
$\rho$ -Cymene	5.27	3.77 $\pm$ 2.84	0.51	12.57	4.00 $\pm$ 3.48	0	13.20
3-Carene	5.90	0.60 $\pm$ 0.53	0.09	1.98	0	0	0
Thymol methyl ether	9.02	0	0	0	0.33 $\pm$ 0.58	0	1.83
Carvacrol	10.10	0.03 $\pm$ 0.88	0	0.52	0.88 $\pm$ 3.11	0	13.53
Thymol	10.31	21.60 $\pm$ 17.11	2.82	80.29	11.11 $\pm$ 11.23	0	35.97
Isocaryophyllene	12.37	0.41 $\pm$ 0.32	0	0.64	0.10 $\pm$ 0.28	0	1.11
$\alpha$ -Humulene	12.82	0.16 $\pm$ 0.16	0	0.69	0.33 $\pm$ 1.46	0	6.51
Guaia-1(5), 7(11)-diene	12.93	0.008 $\pm$ 0.03	0	0.15	0	0	0

C, Compound; RT, Retention Time, n=20 individuals.

**Table 2. Statistics Parameters of *Lippia graveolens* Hexane Extracts from Zone B (Jardín Botánico)**

C	RT	May 2005			September 2005			December 2005			March 2006		
		Mean (%)	Min	Max	Mean (%)	Min	Max	Mean (%)	Min	Max	Mean (%)	Min	Max
$\alpha$ -Thujene	3.86	0.002 $\pm$ 0.21	0	0.04	0.54 $\pm$ 0.97	0	4.02	0.003 $\pm$ 0.01	0	0.06	0	0	0
$\alpha$ -Pinene	4.73	0.06 $\pm$ 0.10	0	0.29	0.81 $\pm$ 1.02	0	4.75	0.09 $\pm$ 0.13	0	0.58	0.02 $\pm$ 0.11	0	0.52
$\rho$ -Cymene	5.27	1.60 $\pm$ 1.42	0	4.05	5.75 $\pm$ 3.20	0	10.15	2.63 $\pm$ 1.58	0	6.55	3.65 $\pm$ 3.91	0	17.67
3-Carene	5.90	0.49 $\pm$ 0.64	0	2.26	2.58 $\pm$ 1.95	0	5.51	0.43 $\pm$ 0.33	0	1.34	0.07 $\pm$ 0.17	0	0.55
Thymol methyl ether	9.02	1.31 $\pm$ 1.66	0	5.89	2.02 $\pm$ 2.28	0	7.32	0.28 $\pm$ 0.57	0	1.92	0.30 $\pm$ 0.43	0	0.43
Carvacrol	10.10	0.41 $\pm$ 0.68	0	2.02	1.00 $\pm$ 1.94	0	6.50	0.27 $\pm$ 0.55	0	2.01	1.41 $\pm$ 3.17	0	12.96
Thymol	10.31	17.95 $\pm$ 8.50	0.60	36.58	32.59 $\pm$ 19.49	0	68.66	15.82 $\pm$ 9.70	0	39.42	8.33 $\pm$ 7.97	0	30.06
Isocaryophyllene	12.37	0.59 $\pm$ 0.72	0	2.55	1.76 $\pm$ 1.30	0	4.51	0.39 $\pm$ 0.30	0	1.32	0.18 $\pm$ 0.29	0	1.12
$\alpha$ -Humulene	12.82	0	0	0	0.03 $\pm$ 0.12	0	0.53	0.08 $\pm$ 0.12	0	0.21	0.08 $\pm$ 0.14	0	0.51
Guaia-1(5), 7(11)-diene	12.93	0.20 $\pm$ 0.38	0	1.28	0.92 $\pm$ 0.84	0	1.97	0.07 $\pm$ 0.13	0	0.49	0	0	0

C, Compound; RT, Retention Time, n=21 individuals.

monoterpenes ( $\alpha$ -Thujene,  $\alpha$ -Pinene,  $\rho$ -Cymene, 3-Carene) and some sesquiterpenes (Isocaryophyllene,  $\alpha$ -Humulene, Guaia-1(5), 7(11)-diene). In all sites and seasons, the oxygenated monoterpenes were present in higher percentages than monoterpenes hydrocarbons. Thymol was detected as the major compound in most of the individuals sampled, while at the season level, September was the more productive month.

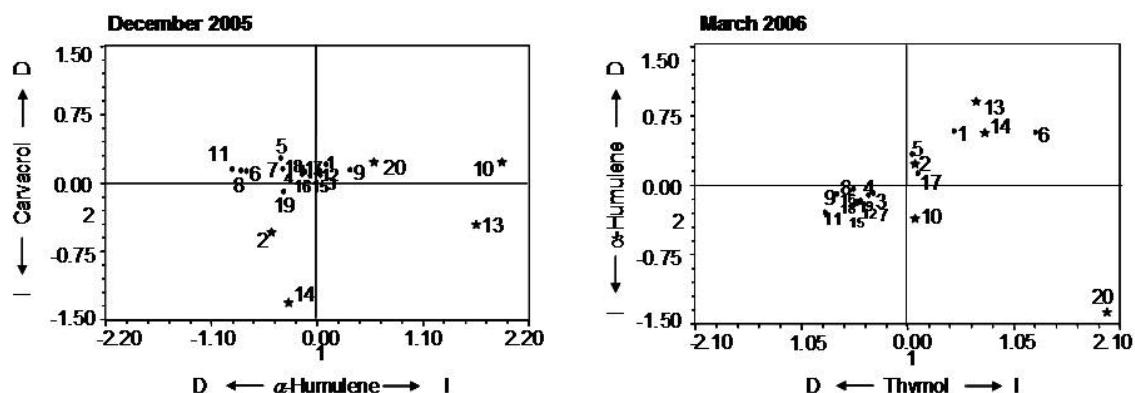
The results of the multifactor ANOVA analysis revealed significant differences in the concentration and composition of the identified substances for the different sampling seasons within each zone (seasonal variation). In the only case where a comparison among the two populations was possible (December 2005/March 2006 samples), the results revealed significant differences within and among populations in the concentration and composition of most of the identified compounds (inter-population variation) (Table 3).

Multivariate analysis revealed intrapopulation variation in the two sites, which seems to be associated to the season, as well as to the quantitative differences in concentration of the identified metabolites (Tables 1 and 2; Figs. 2 and 3). As a consequence, it is difficult to identify individuals that could be recognized as outliers (and possible chemotypes carriers) respect to the rest of the population. The most common condition is that, in both sites and for the different seasons, almost any individual be found in different parts of the plot, or be included in the group with more individuals. Nevertheless, some exceptions to this pattern were documented for the two zones. Thus, for the Zone A, individuals 2, 10, 13, 14 and 20, were systematically placed as outliers in the two sampling seasons (Fig. 2). In the case of the zone B, individuals 5 and 8, were always placed as outliers in the plot (Fig. 3), while individuals 2, 10 and 12 were also outliers at least in three of the seasons. Table 4 summarizes these results. These individuals should be more carefully

**Table 3. Results of the Multifactor ANOVA Analysis (F and P Values)**

		Identified Compounds									
		$\alpha$ -Thujene	$\alpha$ -Pinene	$\rho$ -Cymene	3-Carene	Thymol methyl ether	Carvacrol	Thymol	Isocaryophyllene	$\alpha$ -Humulene	Guaia-1(5), 7(11)-diene;
A and B zone	F	0.95	0.06	1.19	0.52	1.43	0.61	2.64	0.20	1.0	4.78*
	P	0.3324	0.8139	0.2770	0.4736	0.2361	0.4384	0.1083	0.6557	0.3216	0.0318*
Dec 2005 and march 2006	F	0.95	12.15*	0.85	45.60*	2.95	4.03*	11.61*	15.54*	0.27	7.24*
	P	0.3324	0.0081*	0.3583	0.001*	0.0901	0.0480*	0.0010*	0.0002*	0.6080	0.0087*
Zone/season collection	F	0.95	0.54	0.33	3.12	2.25	0.08	0.32	0.60	0.25	4.78*
	P	0.3324	0.4637	0.5636	0.0809	0.1373	0.7769	0.5721	0.4400	0.6183	0.0318*
Zone A	F	-	9.43*	0.05	26.26*	6.37*	1.50	5.25*	10.76*	0.25	1.00
	P	-	0.0039*	0.8168	0.001*	0.0159*	0.2278	0.0276*	0.0022*	0.6206	0.3236
Zone B	F	6.33*	10.87*	8.79*	24.53*	6.89*	1.14	13.50*	16.87*	3.07*	17.00*
	P	0.0007*	0.001*	0.001*	0.001*	0.0004*	0.3369	0.001*	0.001*	0.0327*	0.001*

\*indicate significant differences; F, value reported in statistics tables; P, calculated probability.



**Fig. (2).** Groups/Outliers identified in Zone A. I, increase; D, decrease; •, group; ★, outliers.

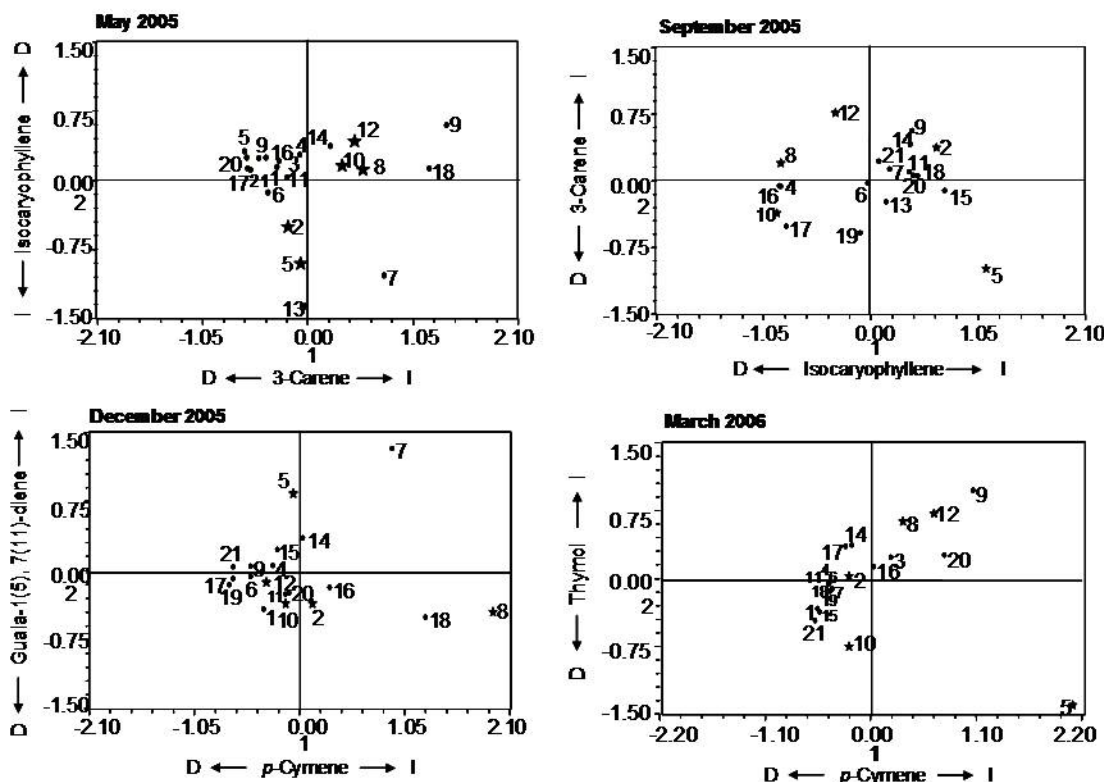


Fig. (3). Groups/Outliers identified in Zone B. I, increase; D, decrease; •, group; ★, outliers.

Table 4. Summary of Individuals Consistently Found as Outliers in Zones A and B

Zone A		Zone B			
Dec 2005	March 2006	May 2005	Sept 2005	Dec 2005	March 2006
2		2	2	2	2
5	5	5	5	10	10
8	8	8	8	13	13
10	10		10	14	14
12	12		12	20	20

Each column represent the outliers (number of individual studied) of each season/zone.

studied in order to determinate if they can be recognized as true chemotypes.

Antibacterial activity was only found for the September samples from Zone B, which coincides with the season of major concentration of secondary metabolites ( $\alpha$ -Pinene,  $p$ -Cymene, Thymol) (Table 5) which are already known to exhibit antibacterial activity [16-19], and the season when people collects the plant [1, 11]. It is important to stand out that although in most of the samples thymol was present, antibacterial activity was only found for the September samples, this is due probably to antagonistic effects between some components that affect the antimicrobial activity for the hexanic extracts [20].

In conclusion, *Lippia graveolens* displays quantitative and qualitative variations within and between natural plant populations, which are expressed in response to the contrasting conditions of the two sites where they live. That is why the rare individuals (outliers in the analysis) are interesting for further study, since their chemical features could have a genetic basis more than an environmental one.

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**Table 5. Antibacterial Activity of the Hexanic Extracts of *Lippia graveolens* Collected in Zone B (September 2005)**

Individual Number	Inhibition Zone (mm)	
	<i>S1</i>	<i>Vch No01</i>
Positive Control	32.00 ± 0.50	35.00 ± 0.50
1	nd	nd
2	9.33 ± 1.53	8.00 ± 0.58
3	nd	nd
4	12.33 ± 1.53	11.00 ± 0.00
5	7.67 ± 0.58	7.00 ± 0.00
6	11.67 ± 0.58	16.33 ± 0.58
7	9.67 ± 0.58	8.67 ± 0.58
8	9.00 ± 0.00	9.00 ± 0.00
9	10.67 ± 0.58	13.33 ± 1.15
10	10.33 ± 0.58	11.67 ± 1.53
11	7.00 ± 0.00	6.33 ± 0.58
12	7.67 ± 0.58	7.00 ± 0.00
13	6.33 ± 0.58	6.67 ± 0.58
14	8.00 ± 0.00	7.33 ± 0.58
15	10.00 ± 0.00	12.00 ± 0.00
16	30.00 ± 0.00	30.00 ± 0.00
17	11.67 ± 1.53	10.33 ± 1.15
18	30.00 ± 0.00	30.00 ± 0.00
19	18.33 ± 5.03	12.67 ± 1.15
20	10.33 ± 0.58	9.33 ± 0.58
21	11.33 ± 0.58	16.00 ± 0.00

Positive control: Chloramphenicol (25 µg), *S1. Sarcina lutea*; *V.ch. No-01. Vibrio cholerae*; nd, not determined.

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