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Chemical and molecular characterization of fifteen species from the *Lantana* (Verbenaceae) genus

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ABSTRACT

The essential oil from two *Lantana* species (*Lantana lucida* Schauer and *Lantana salzmannii* Schauer) were evaluated for their chemical composition by GC/MS. Results showed 17 predominant compounds for *L. lucida*, among which (*E*)-caryophyllene (19.0%) and α-humulene (33.0%) were the major components. *L. salzmannii* showed the presence of 58 compounds, the most abundant of which were (*E*)-caryophyllene (15.6%) and selin-11-en-4-ol (11.2%). Next, cluster analyses of the chemical composition of the volatile fraction of five Lantana species from our studies (*Lantana radula*, *Lantana canescens*, *L. lucida*, *L. salzmannii* and *Lantana camara*), as well as 10 *Lantana* species published in the literature (*Lantana achyranthifolia*, *Lantana aculeata*, *Lantana balansae*, *Lantana hirta*, *Lantana involucrata*, *Lantana fucata*, *Lantana salviifolia*, *Lantana trifolia*, *Lantana velutina* and *Lantana xenica*) were performed. Species fell into three main groups. A cluster analysis of (*E*)-caryophyllene content was also performed which resulted in the 15 *Lantana* species being segregated into four main groups. In addition, Inter-Simple Sequence Repeat (ISSR) was used to evaluate the genetic variation between five *Lantana* species collected from northeastern Brazil (*L. radula*, *L. canescens*, *L. lucida*, *L. salzmannii* and *L. camara*). Analysis showed a 36% similarity between, *L. salzmanii* and *L. canescens*, and a 48% similarity between *L. lucida* and *L. canescens*. Overall, results, indicate that it is possible to discriminate between groups of *Lantana* taxa based on both their chemical, composition and ISSR markers. In addition, this study provided further support for using (*E*)-caryophyllene as a chemical marker for species belonging to the *Lantana* genus.

1. Introduction

The *Lantana* genus consists of approximately 150 plant species, geographically spanning from the tropics to the subtropics of the Americas, with a few members found in tropical Asia and Africa (Ghisalberti, 2000). *Lantana* species are used in folk medicine for many diseases and for ornamentation in gardens (Chowdhury et al., 2007). They have a very pungent odor which

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originates from their leaves (Ghisalberti, 2000; Walden et al., 2009). To date, many studies have described the chemical composition and pharmacological activity of a variety of *Lantana* species (Jimenez-Arellanes et al., 2003; Julião et al., 2009; Sena Filho et al., 2009, 2010).

Taxonomically, the *Lantana* genus is divided into four sections: Lantana, Callioreas, Rhytidocamara and Sarcolippia (Schauer, 1847; Briquet, 1904). The divisions are based on floral and carpological characteristics, the best tools for classification available at the time they were made. In general, specimens from this genus are very difficult to classify, due to the shape of their inflorescence which changes with age and flower color. A taxonomic study of four genera from the Verbenaceae family (*Lippia*, *Lantana*, *Aloysia* and *Phyla*) proposed using iridoid glucosides as a taxonomic marker for this family (Rimpler and Sauerbier, 1986). This study contributed a great deal of information regarding the chemotaxonomy of Verbenaceae. Unfortunately, the presence and type of iridoid glucosides in plants from the morphologically similar *Lippia* and *Lantana* genera are virtually indistinguishable, and so are not very helpful in differentiating between them. Sena Filho et al. (2010) proposed a chemical marker for the *Lantana* genus, in which (*E*)-caryophyllene was the major compound detected, together with phellandendrene, cubebene and elixene as minor components in an analysis of 15 species. In the *Lippia* species evaluated, (*E*)-caryophyllene was not detected as the major compound; rather, it was suggested that species belonging to the *Lippia* genera would contain limonene, citral, carvacrol, β-myrcene, camphor and thymol as their main chemical markers.

Recently, in addition to the phenotypic characteristics of an individual plant species, genetic characteristics have been found to be useful and oftentimes necessary in distinguishing between plant species, cultivars and/or individuals occupying different ecological niches (Santos et al., 2011; Costa et al., 2011; Silva et al., 2012). In addition, an understanding of the genetic diversity within a species is indispensable to optimally manage genetic resources for conservation and taxonomic categorization, (Azizi et al., 2009). DNA analysis based on molecular markers such as Inter-Simple Sequence Repeat (ISSR) can be taxonomically useful in phylogenetic studies to distinguish between plant species and subspecies (Khan et al., 2000; Raina et al., 2001; Monteleone et al., 2006). These markers are not affected by environmental conditions, and have become increasingly important for surveying genetic diversity and for genotype identification of medicinal plants (Nybom and Weising, 2007).

Thus, the first aim of this study was to evaluate the essential oil of two endemic *Lantana* species from the salt marsh Atlantic forest landscape in Brazil (*Lantana lucida* Schauer and *Lantana salzmannii* Schauer). The second was to characterize the intraspecific variation of the essential oil composition in natural populations of those *Lantana* species, as well as 13 *Lantana* species published in the literature (*Lantana achyranthifolia* (Hernandes et al., 2005), *Lantana aculeate* (Saxena and Sharma, 1999), *Lantana balansae* (De Viana et al., 1973), *Lantana camara* (Rana et al., 2005), *Lantana canescens* (Sena Filho et al., 2010), *Lantana hirta* (Walden et al., 2009), *Lantana involucrate* (Pino et al., 2006), *Lantana fucata* (De Oliveira et al., 2008), *Lantana radula* (Sena Filho et al., 2010), *Lantana salviifolia* (Ouamba et al., 2006), *Lantana trifolia* (Juliao et al., 2009), *Lantana velutina* (Walden et al., 2009) and *Lantana xenica* (Juliani et al., 2002)) using the Weighted Pair Grouping Method (WPGM). The third was to cluster the 15 *Lantana* species based on their (*E*)-caryophyllene concentration. The last was to perform a cluster analysis using molecular characterization of five *Lantana* species (*L. radula*, *L. canescens*, *L. lucida*, *L. salzmannii* and *L. camara*) by ISSR–PCR and compare this to the (*E*)-caryophyllene results for chemical similarity, so that the taxonomy of this genus could be evaluated.

2. Methodology

2.1. Plant material

L. salzmannii Schauer and *L. lucida* Schauer were collected in Itaporanga d'ajuda, Sergipe, Brazil in April 2011. Voucher specimens (J. G. de Sena Filho) were deposited at the Herbarium of the Universidade Estadual de Feira de Santana (HUEFS), Bahia, Brazil under the numbers HUEFS 178287 and HUEFS 178288, respectively. *L. canescens* Kunth, *L. radula* Sw and *L. camara* L. were collected in January of 2011 and identified by Dr. Rita de Cassia Pereira. Voucher specimens were deposited at the Herbarium Dárdano de Andrade Lima (IPA), in the Instituto Pernambucano de Pesquisa Agropecuaria, Pernambuco, Brazil under numbers 74,048, 70,004 and 86,846, respectively.

2.2. Oil isolation procedure

The oil was obtained by hydrodistillation over 4 h using a Clevenger-type apparatus with 600 g of fresh leaves cut into pieces (Sena Filho et al., 2010). The oil was dried with anhydrous sodium sulphate and stored at −20 °C in a sealed amber bottle until chemical analysis was performed. The yield afforded from *L. salzmanni* was 0.8% and from *L. lucida* was 0.6%.

2.3. Essential oil analysis

Essential oil analyses of *L. lucida* and *L. salzmannii* were performed on a Shimadzu QP5050A GC/MS system equipped with an AOC-20i auto-injector. A J&W Scientific DB-5MS (coated with 5% phenyl–95% dimethylpolysiloxane) fused capillary column (30 m × 0.25 mm × 0.25 μm film thickness) was used as the stationary phase. Helium was used as the carrier gas, at a flow rate of 1.2 mL/min. The column temperature program was as follows: 40 °C for 4 min, raised to 220 °C at 4 °C/min, then heated to 280 °C at 20 °C/min. The injector and detector temperatures were 250 °C and 280 °C, respectively. Samples (0.5 μL in

CH_2Cl_2) were injected with a 1:20 split ratio. MS were taken at 70 eV with a scan interval of 0.5 s and fragments from 40 to 350 Da.

The retention indices were obtained by mixing the oil sample with a C_9–C_{18} linear hydrocarbon mixture (Van den Dool and Kratz, 1963). The volatile components were analyzed by GC/MS, and identification was made by comparing retention indices and mass spectra (Adams, 2007) with those in the literature, as well as by computerized matching of the acquired mass spectra with those stored in the NIST and Wiley mass spectral libraries and other published mass spectra.

GC analyses were carried out using a Perkin Elmer (Shelton, CT, USA) Clarus 500 gas chromatograph fitted with a flame ionization detector (FID) and TC Navigator software. Separation of the compounds was achieved with a Perkin Elmer Elite Plot 5 capillary column (5% diphenyl–95% dimethylpolysiloxane, 30 m × 0.25 mm i.d. X 0.25 μm film thickness) and N_2 as the carrier gas. The other parameters (oven temperature program, injector and detector temperature and amount of sample) were the same as those used for the GC/MS analysis described above. Peak areas and retention times were measured using an electronic integrator and TC Navigator software. The percentage composition of each component was determined by dividing the area of the component by the total area of all components isolated under these conditions, without an FID response factor correction.

2.4. Cluster analyses of essential oil chemical composition

For the first cluster analysis, we included chemical components of the essential oils from the two *Lantana* species evaluated in this study, as well as 13 species referenced in the literature (*L. achyranthifolia*, *L. aculeate*, *L. balansae*, *L. camara*, *L. canescens*, *L. hirta*, *L. involucrate*, *L. fucata*, *L. radula*, *L. salviifolia*, *L. trifolia*, *L. velutina* and *L. xenica*) that had at least a 2% or greater recovery of any compound (Appendix 1). Species were clustered using ranges of all volatile compounds reported in the literature: 0–2%; 2.01–5%, 5.01–10%, 10.01–15%, 15.01–20%, 20.01–25%, 25.01–30%, 30.01–35%, 35.01–40%, 40.01–45%, 45.01–50%, 50.01–55%, and 55.01–60%. Then, a second cluster analysis was performed using only (*E*)-caryophyllene content, with ranges of: 0–15%, 15.01–30% and 30.01–45%.

Based on the presence or absence of constituents in the oil of a species, a dissimilarity coefficient matrix was calculated (Jaccard, 1908). Clustering of the matrix for both analyses was carried out using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster algorithm (Sokal and Michener, 1958). Statistical analysis was performed using the PASW Statistics v. 18 software (http://www.ibm.com/us/en/).

2.5. Genetic analysis with ISSR markers

Five *Lantana* species (*L. radula*, *L. canescens*, *L. lucida*, *L. salzmannii*, and *L. camara*) were analyzed by ISSR. DNA was isolated from young leaves as previously described by Doyle (1991). Fourteen primers were used to screen for polymorphism (Table 1). The reaction volume was 20 μL, and consisted of 2 μL genomic DNA (40 ng), 1 μL of each primer (0.2 μM), and a mix composed of: 14.4 μL ultrapure sterile water, 2 μL 10X buffer (3 mM $MgCl_2$, 100 mM $MgSO_4$, 100 mM KCl, 80 mM $(NH_4)_2SO_4$, 100 mM Tris–HCl) (Neo Taq, Koma Biotech, Korea), 0.4 μL dNTP (10 mM) and 0.2 μL Taq polymerase (5 U/μL). PCR amplification was performed in a PTC-100 thermocycler (MJ Research, Inc., Watertown, MA, USA) using a cycle of 95 °C for 5 min for initial denaturation, followed by 45 cycles of denaturation at 94 °C for 1 min, 51.5 °C for 45 s for primer annealing, 72 °C for 2 min for extension, and finally one cycle of 72 °C for 10 min for final extension. For fragment visualization, a 2% agarose gel (1X TEB: 89 mM TRIS, 89 mM boric acid, 2.5 mM EDTA, pH 8.3) was used in a horizontal electrophoresis system (Sunrise, Gibco BRL,

Table 1
Primers and number of polymorphic fragments generated from five species of *Lantana*.

Primer[a]	Sequence 5′–3′	NPF[b]
ISSR 1	CACACACACACAGG	6
ISSR 2	CTCTCTCTCTCTCTCTAC	6
ISSR 4	CACACACACACAAC	3
ISSR 8	GAGAGAGAGAGAGG	6
ISSR 10	GAGAGAGAGAGACC	6
ISSR 12	CACCACCACGC	7
ISSR 13	GAGGAGGAGGC	6
ISSR 14	CTCCTCCTCGC	7
843	CTCTCTCTCTCTCTCTRA	5
807	AGAGAGAGAGAGAGAGT	10
810	GAGAGAGAGAGAGAGAT	8
823	TCTCTCTCTCTCTCTCC	5
835	AGAGAGAGAGAGAGAGYC	7
841	GAGAGAGAGAGAGAGAYC	5
845	CTCTCTCTCTCTCTCTRG	6

[a] Primers ISSR 1–14 were from Invitrogen (New York, NY USA); 843, 807, 810, 823, 835, 841 and 845 were from Integrated DNA Technologies (Coralville, IA, USA).
[b] NPF – Number of polymorphic fragments generated.

USA), carried out at a constant voltage of 100 V for 90 min. The gel was then stained with ethidium bromide solution (5 mg/mL) for 15 min. The ISSR fragment amplification products were visualized under ultraviolet light using a Gel Doc L-Pix image system (Loccus Biotecnologia, Brazil).

ISSR markers were scored for the presence (1) or absence (0) of a fragment, and a data matrix of I-scores were generated and similarity coefficients calculated using Jaccard's arithmetic complement index (Jaccard, 1908). The dendrogram was constructed using the UPGMA cluster algorithm (Sokal and Michener, 1958) to determine the robustness of the dendrogram; the data was bootstrapped with 10,000 replications using FreeTree software (http://web.natur.cuni.cz/flegr/programs/freetree.htm) and, for visualization of the cluster, we used XLSTAT software (www.xlstat.com).

3. Results and discussion

Species of the *Lantana* genus were last subdivided over 100 years ago using only morphological characteristics (Schauer, 1847; Briquet, 1904). With the advent of more advanced tools for both chemical and genomic analysis, our objective in this study was to refine the classification of species in this genus, especially in regards to testing the proposal of using (*E*)-caryophyllene as a chemical marker.

First, essential oils from *L. lucida* and *L. salzmannii* were evaluated for their chemical composition. Seventeen volatile compounds from *L. lucida* were identified which amounted to 94.9% of all peaks in the essential oil (Table 2). (*E*)-caryophyllene (19.0%), α-humulene (33.0%), δ-cadinene (11.3%), α-copaene (6.9%), bicyclogermacrene (4.9%) and β-cubebene (4.4%) were the major compounds detected. In the *L. salzmannii* essential oil, 58 compounds were identified, representing 92.9% of all peaks. The most abundant compounds were (*E*)-caryophyllene (15.6%), selin-11-en-4α-ol (11.2%), *trans*-calamenene (6.6%), β-selinene (5.8%) and *trans*-cadina-1,4-diene (4.5%) (Table 2). A complete listing of the volatile components of *L. lucida* and *L. salzmannii* essential oil extracts and their percentages are presented in Table 2. Notably, monoterpenes were only present in *L. salzmannii* (12.4%); none were detected in the *L. lucida* essential oil used in this study.

In general, terpenoids have been used effectively as chemotaxonomy markers; chemical variation in this group of compounds has been used to define intra- and inter-specific variability in a variety of plant species (Sena Filho et al., 2007; Adams et al., 2003). In this context, our research group performed a clustering analysis of 13 *Lantana* species from the literature (Appendix 1), as well as two *Lantana* species, endemic to Brazil, whose volatile components had not been previously characterized, for the variation of 77 mono- and sesquiterpenes in their essential oils. Plant species came from the following *Lantana* genus subdivisions: Lantana (*L. camara*, *L. lucida* and *L. aculeate*), Rhytidocamara (*L. achyranthifolia*) and Callioreas (*L. xenica*, *L. canescens*, *L. balansae*, *L. hirta*, *L. involucrata*, *L. fucata*, *L. salviifolia*, *L. salzmannii*, *L. radula*, *L. trifolia*, and *L. velutina*). The cluster analysis found three major groupings using a level of 23% dissimilarity (73% similarity): 1) *L. radula*, *L. canescens*, *L. salviifolia*, *L. involucrate*, *L. trifolia*, *L. salzmannii*, *L. camara*, *L. fucata*; 2) *L. balansae*, *L. velutina*; 3) *L. achyranthifolia*, *L. aculeate*; 4) *L. lucida*, *L. xenica*; and 5) *L. hirta* (Fig. 1A).

In order to support using (*E*)-caryophyllene as a chemical marker for the *Lantana* genus, we performed a second clustering analysis grouping each species based on (*E*)-caryophyllene content which resulted in four main groups: 1) *L. trifolia*, *L. velutina*, *L. salzmannii*, *L. radula*, *L. camara*, *L. lucida*; 2) *L. fucata*, *L. salvifolia*, *L. aculeata*, *L. hirta*, *L. involucrata*, *L. balansae*; 3) *L. achyranthifolia*; and 4). *L. canescens*, *L. xenica* (Fig. 1B). The group Rhytidocamara was distinguished from the other species, as suggested by Briquet (1904) and Schauer (1847). When only the five species our group had collected were clustered for (*E*)-caryophyllene content (Fig. 1C), a total of three groups were observed: 1) *L. radula*, *L. camara*; 2) *L. salzmannii*, *L. lucida* and 3) *L. canescens*. We believe that the isolation of *L. canescens* from the other *Lantana* species is due to the exceedingly high amount of (*E*)-caryophyllene it contains (43.9% versus 15.6–23.3% in the other four species).

In addition, the five *Lantana* species that we collected from northeastern Brazil (*L. radula*, *L. canescens*, *L. lucida*, *L. salzmannii* and *L. camara*) were subjected to a cluster analysis using molecular characterization by ISSR markers. ISSR is a powerful technique that has been used to resolve species/subspecies differences in a genus or to differentiate between genera in a family. For example, ISSR was used to distinguish between genera for *Lolium*, *Festuca* (Pasakinskiene et al., 2000) and *Diplotaxis* (Martin and Sanchez-Yelamo, 2000), as well as several aromatic and medicinal plant groupings (Farajpour et al., 2011; Pezhmanmehr et al., 2009; Manica-Cattani et al., 2009; Suárez González et al., 2007; Fracaro et al., 2005). In our study, ISSR fingerprints clearly distinguished all five tested species (Fig. 2). The 14 ISSR primers generated a total of 93 fragments, 100% of which were polymorphic. The primer with the highest number of fragments was 807 (10 fragments), while the lowest was ISSR 4 (3 fragments). Genotypes of the five species of *Lantana* selected for this study were clustered by UPGMA using the Jaccard coefficient (JC), estimated from the binary data (Fig. 2). The similarity mean was 0.17 JC (0.10–0.23 JC, Table 3). We observed a clear separation of two groups, with *L. camara* being the most isolated of the five species. Interestingly, *L. canescens* and *L. salzmannii* contain a large variety of mono- and sesquiterpenes; the ISSR results corroborate the cluster analysis performed on essential oil components which found a 36% similarity between the two species. Our results suggest that the genetically directed production of volatile compounds is correlated in the *Lantana* species we studied, and could be a possible alternative for grouping the species taxonomically.

Combining the chemical results with those using ISSR provided additional information on the similarity of the *Lantana* species evaluated in this study. We suggest that species in the same grouping may have similar routes of biosynthesizing secondary compounds, which result in activation of similar genes. However, the presence or absence of insect and other parasites and other environmental factors could affect the metabolic routes that are activated, (Pichersky and Gershenzon, 2002; Paolini et al., 2010) which must be taken into consideration when making associations between genes and

Table 2
Essential oil composition of *Lantana salzmannii* and *Lantana lucida* leaves collected in Sergipe, Brazil.

Compounds	RI[a]	RI[b]	% Peak area	
			L. salzmannii	*L. lucida*
α-thujene	924	924	0.1	–
α-pinene	930	932	0.1	–
sabinene	970	969	0.1	–
oct-1-en-3-ol	980	974	0.1	–
myrcene	989	988	0.2	–
α-phellandrene	1005	1002	0.3	–
δ-3-carene	1006	1008	3.7	–
α-terpinene	1015	1014	0.2	–
p-cymene	1018	1020	0.5	–
o-cymene	1023	1022	1.3	–
limonene	1028	1024	2.8	–
1,8-cineole	1030	1026	0.3	–
(*E*)-β-ocimene	1046	1044	0.2	–
γ-terpinene	1057	1054	1.7	–
p-mentha-2,4(8)-diene	1080	1085	0.1	–
terpinolene	1084	1086	0.9	–
linalool	1099	1095	0.1	–
terpinen-4-ol	1179	1174	0.2	–
δ-elemene	1335	1335	0.6	–
α-cubebene	1346	1345	0.3	–
α-ylangene	1370	1373	0.1	–
α-copaene	1376	1374	3.2	6.9
β-elemene	1389	1389	2.4	4.4‡
β-cubebene	1387	1389	–	‡
α-gurjunene	1406	1409	0.6	0.7
(*E*)-caryophyllene	**1421**	**1417**	**15.6**	**19.0**
γ-elemene	1424	1434	0.2	–
β-copaene	1430	1430	0.1	2.5
α-guaiene	1435	1437	0.2	–
guaia-6,9-diene	1441	1442	0.1	–
cis-muurola-3,5-diene	1449	1448	0.8	–
α-humulene	1456	1452	3.2	33.0
allo-aromadendrene	1460	1458	1.2	0.9
germacrene D	1482	1484	3.0	3.5
β-chamigrene	1483	1484	–	1.8
β-selinene	1490	1489	5.8	0.6
trans-muurola-4(14),5-diene	1492	1493	–	0.8
bicyclogermacrene	1495	1500	–	4.9
α-selinene	1496	1498	3.7	–
α-muurolene	1497	1500	–	2.8
(*E*,*E*)-α-farnesene	1503	1505	0.1	–
β-bisabolene	1507	1505	0.9	–
γ-cadinene	1513	1513	0.1	–
δ-cadinene	1518	1522	1.6	11.3
trans-calamenene	1522	1521	6.6	–
zonarene	1523	1528	–	0.5
trans-cadina-1,4-diene	1533	1533	4.5	–
α-calacorene	1541	1544	0.2	–
(*E*)-nerolidol	1560	1561	2.0	–
spathulenol	1576	1577	0.3	–
caryophyllene oxide	1582	1582	2.0	–
gleenol	1586	1586	0.4	–
viridiflorol	1595	1592	0.3	–
ledol	1605	1602	0.9	–
humulene epoxide II	1610	1608	0.6	1.3
epi-cuben-1-ol	1628	1627	2.6	–
caryophylla-4(12),8(13)-dien-5-(α/β)-ol	1638	1639	0.3	–
cubenol	1642	1645	1.0	–
α-muurolol	1647	1644	0.4	–
neo-intermedeol	1655	1658	1.4	–
selin-11-en-4α-ol	1660	1658	11.2	–
humulane-1,6-dien-3-ol	1667	1655	0.7	–
cadalene	1671	1675	0.2	–
eudesma-4(15),7-dien-1β-ol	1679	1687	0.6	–
Total peaks identified			92.9	94.9

Retention indices obtained from a DB-5 MS column and calculated according to [a] Van den Dool and Kratz (1963) or [b] Adams (2007). ‡Co-eluting peaks.

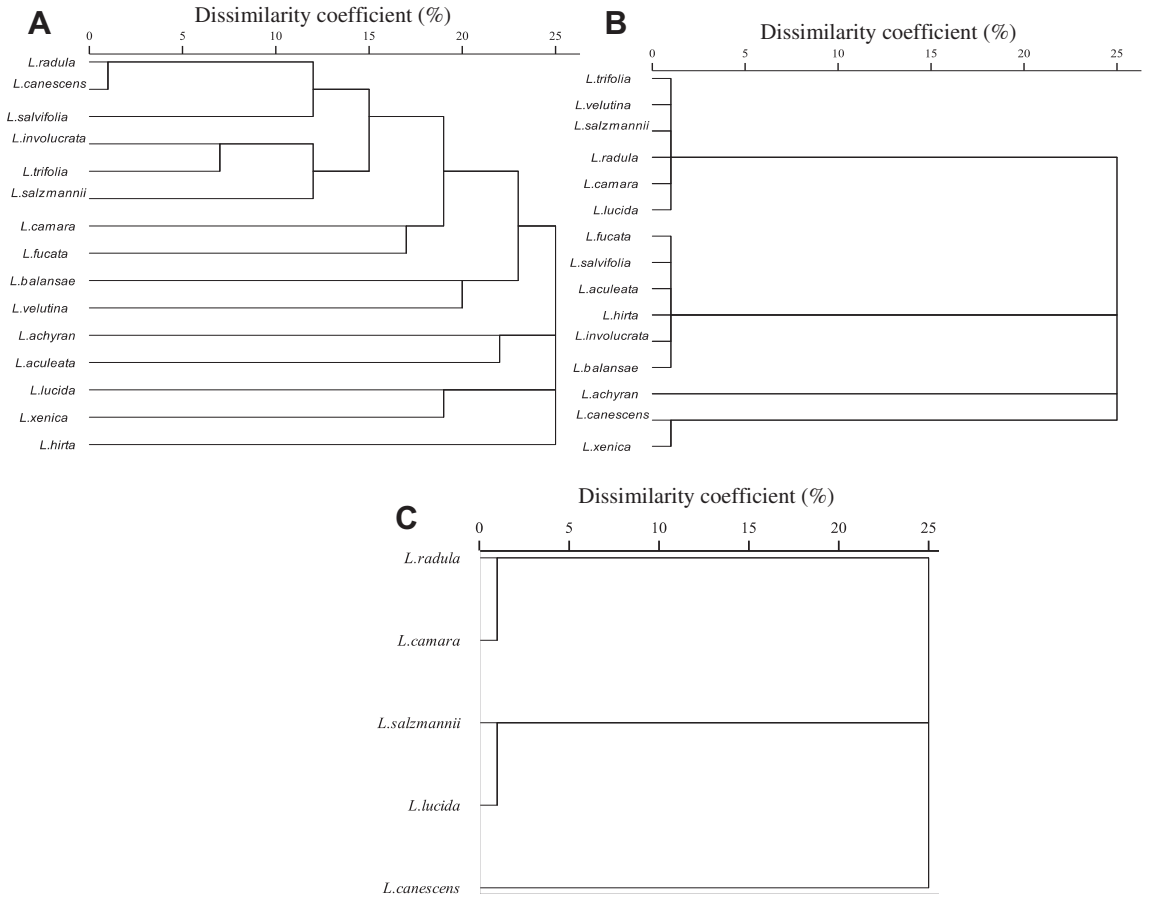

Fig. 1. Dissimilarity phenograms derived from the: (A) chemical variation of 77 compounds observed in essential oil extracts from *Lantana* species; (B) amount of (*E*)-caryophyllene found in the 15 *Lantana* species used in this study; and (C) amount of (*E*)-caryophyllene evaluated in the five *Lantana* species which have been collected by our group.

component concentration. For example, gene expression of three monoterpene synthase genes (LaTPS12, LaTPS 23 and LaTPS 25) in *Lippia alba* (Verbenaceae) were recently evaluated; results showed that the production of essential oils was higher in young versus older leaves (Pandelo et al., 2012). Thus, further research is necessary to more thoroughly evaluate the genetic and chemical correlations regarding essential oil production and its components in *Lantana* species.

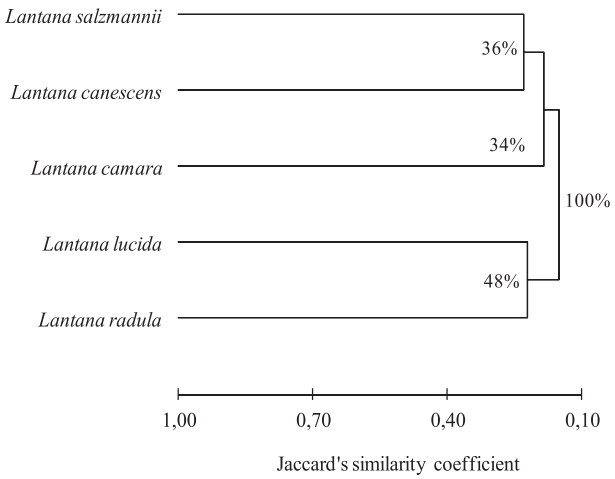

Fig. 2. Dendrogram of genetic similarity from ISSR using the Jaccard coefficient and the Unweighted Pair Group with Arithmetic Mean method with bootstrap analysis for five species of *Lantana*.

Table 3
Genetic similarity among five species of *Lantana* using the Jaccard coefficient.

	L. radula	*L. canescens*	*L. salzmannii*	*L. camara*	*L. lucida*
Lantana radula	1.00				
Lantana canescens	0.23	1.00			
Lantana salzmannii	0.19	0.23	1.00		
Lantana camara	0.11	0.16	0.21	1.00	
Lantana lucida	0.22	0.10	0.15	0.13	1.00

In summary, we characterized the essential oils from two Brazilian species of *Lantana* which had not been previously reported, as well as differentiated the chemical and genetic characteristics of 15 *Lantana* species through cluster analyses. The idea of combining chemical, genetic and morphological evaluations and synthesizing taxonomic relationships using currently available statistical software has the potential to greatly refine botanical taxonomy and aid in the most accurate identification/separation of species to date. The compilation of the three different analyses performed here provides an example of classifying plants using a multidisciplinary approach. This work will support future studies on the genetic and chemical evaluation of *Lantana* species and other genera belonging to the Verbenaceae family which should include a larger number of species so that a more complete study of the chemical, genetic and taxonomic diversity can be performed.

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bse.2012.07.024.

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