CHEMICAL AND MOLECULAR CHARACTERIZATION OF FIFTEEN SPECIES FROM THE *LANTANA* (VERBENACEAE) GENUS

José Guedes de Sena Filho[1], Allívia Rouse Carregosa Rabbani[1], Tânia Regina dos Santos Silva[2], Ana Veruska Cruz da Silva[1], Igor Azevedo Souza[1], Maria José Bryanne Araujo Santos[1], Jemmyson Romário de Jesus[3], Paulo Cesar de Lima Nogueira[3], Jennifer M. Duringer[4]

[1]*Empresa Brasileira de Pesquisa Agropecuária - EMBRAPA, Coastal Tablelands, Av. Beira Mar, 3250, 49025-040, Aracaju, SE, Brazil.*

[2]*Herbário, Departamento de Ciências Biológicas, Universidade Estadual de Feira de Santana, BR 116, Norte, Km 03, 44031-460, Feira de Santana, BA, Brazil.*

[3]*LABORGANICS - Laboratório de Pesquisa em Química Orgânica de Sergipe Departamento de Química – CCET, Universidade Federal de Sergipe, Av. Marechal Rondon s/n - Jd. Rosa Elze, 49100-000 São Cristóvão, SE, Brazil.*

[4]*Department of Environmental & Molecular Toxicology, Oregon State University, 139 Oak Creek Building, Corvallis, OR 97331, USA.*

Corresponding author contact information

Name: José Guedes de Sena Filho

Phone: 055-79-4009-1387

Fax: 055-79-4009-1385

E-mail address: guedes@cpatc.embrapa.br

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 β-caryophyllene (19.0%) and α- caryophyllene (or humuleno, 33.0%) were the major components. *L. salzmannii* showed the presence of 58 compounds, the most abundant of which were β-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 (*L. radula, L. canescens, L. lucida, L. salzmannii and L. camara*), as well as 10 *Lantana* species published in the literature (*L. achyranthifolia, L. aculeata, L. balansae, L. hirta, L. involucrata, L. fucata, L. salviifolia, L. trifolia, L. velutina and L. xenica*) were performed. Species fell into three main groups. A cluster analysis of β-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 β-caryophyllene as a chemical marker for species belonging to the *Lantana* genus.

KEYWORDS: *Lantana*, *Lantana lucida*, *Lantana salzmannii*, β-caryophyllene, taxonomy, ISSR

Introduction

The *Lantana* genus consists of approximately 150 plant species, spanning from the tropics to the subtropics of the Americas, with a few members found in tropical Asia and Africa (Ghisalberti, E.L., 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 originates from their leaves (Ghisalberti, E.L., 2000; Walden et al., 2009). 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; Sena Filho, *et al.*, 2010).

Taxonomically, the *Lantana* genus is divided into four sections: Lantana, Callioreas, Rhytidocamara and Sarcolippia (Schauer 1847 and Briquet 1895, 1904). The divisions were based on floral and carpological characteristics, the best tools for classification available at the time. In general, this genus is very difficult to classify, due to the shape of its 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 (Rimpley & 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 β-caryophyllene was the major compound detected, together with phellandendrene, cubebene and elixene as minor components in an analysis of 15 species. (β-caryophyllene was not detected in the *Lippia* species evaluated; 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 individuals occupying different ecological niches (Santos *et al*. 2011, Costa *et al*. 2011, Silva *et al*. 2012). To optimally manage genetic resources for conservation and taxonomic categorization, an understanding of the genetic diversity within a species is indispensable (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 (*L. lucida* Schauer and *L. 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 (*L. achyranthifolia* (Hernandes, T *et al*., 2005), *L. aculeate* (Saxena & Sharma, 1999), *L. balansae* (De Viana et al, 1973), *L. camara* (Rana et al., 2005), *L. canescens* (Sena Filho et al., 2010), *L. hirta* (Walden et al., 2009), *L. involucrate* (Pino et al., 2006), *L. fucata* (de Oliveira et al., 2008), *L. radula* (Sena Filho et al., 2010), *L. salviifolia* (Ouamba et al, 2006), *L. trifolia* (Juliao et al, 2009), *L. velutina* (Walden et al, 2009) and *L. xenica* (Juliani et al., 2002)) using the Weighted Pair Grouping Method (WPGM). The third was to cluster the 15 *Lantana* species based on their ß-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 ß-caryophyllene results for chemical similarity, so that the taxonomy of this genus could be evaluated.

Methodology

Plant Material

Lantana salzmannii Schauer and *Lantana lucida* Schauer were collected in Itaporanga d'ajuda, State of Sergipe, Brazil in April of 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. *Lantana canescens* Kunth, *L. radula* Sw and *L. camara* L. were

collected in January of 2011 and identified by Prof. Dra Rita de Cassia Pereira. Voucher specimens were deposited at the Herbarium Dárdano deAndrade Lima (IPA), in the Instituto Pernambucano de Pesquisa Agropecuaria-Pernambuco State-Brazil under numbers 74048, 70004 and 86846, respectively.

Oil isolation procedure

The oil was obtained from 600 g of fresh leaves cut into pieces by hydrodistillation, for 4 hours, using a Clevenger-type apparatus (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. lucidas* was 0.6%.

Essential oil analysis

Oils from *L. lucida and L. salzmannii* were analyzed by GC/MS on a Shimadzu (Kyoto, Japan) model QP5050A gas chromatograph equipped with Shimadzu GC 10 software, using a fused silica capillary column (DB-5MS, 30 m X 0.25 mm i.d., 0.25µm d.f.) (J & W Scientific, Folson, CA, USA) and He as the carrier gas. The oven temperature program ramped from 40º-240ºC at 4ºC/min. Injector and detector temperatures were 250 ºC and 280 ºC, respectively. Samples (0.5µL) were injected using split injection with a ratio of 1:20. The MS was operated as follows: ionization energy of 70 eV; inter scan delay of 0.5 s; mass range from 40 to 350 amu. GC-FID analysis utilized a Perkin Elmer (Shelton, CT, USA) Clarus 500 gas chromatograph equipped with TC Navigator software, using an elite plot 5 capillary column (5% diphenyl- and 95% dimethyl polisiloxane, 30 m X 0.25 mm i.d., 0.25µm (Perkin Elmer)) 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.

Cluster analyses of essential oil chemical composition

For the first cluster analysis, we included chemical evaluation 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%; 2-5%, 5-10%, 10-15%, 15-20%, 20-25%, 25-30%, 30-35%, 35-40%, 40-45%, 45-50%, 50-55%, and 55-60%. We then performed a second cluster analysis using only β-caryophyllene content, with ranges of: 0-15%, 15-30% and 30-45%.

Based on the presence or absence of constituents in the oil of the species, a dissimilarity coefficient matrix (Jaccard, 1908) was calculated. 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/).

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 minutes for initial denaturation, followed by 45 cycles of denaturation at 94°C for 1 minute, 51.5°C for 45 seconds for primer annealing, 72°C for 2 minutes for extension, and finally one cycle of 72°C for 10 minutes 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 , USA), carried out at a constant voltage of 100 V for 90 minutes. The gel was then stained with ethidium bromide solution (5 mg/mL) for 15 minutes. 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).

Results and Discussion

Species of the *Lantana* genus were last subdivided over 100 years ago using only morphological characteristics (Schauer 1847 and Briquet 1895, 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 ß-caryophyllene as a chemical marker.

First, essential oil extracts from *Lantana 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 extract (Table 2). β-caryophyllene (19.0%) and α-humuleno (33.0%) were the major compounds; copaene (6.9%), biciclogermacrene (4.9%) and cubebene (4.3%) were minor compounds. In the *L. salzmannii* essential oil extract, 58 compounds were identified, representing 92.4% of all peaks. The most abundant compounds were β-caryophyllene (15.6%), selin-11-en-4-ol (11.2%) and *trans*-calameneno (6.6%) (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* extract from 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; Mendes et al., 2009; 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* (Figure 1A).

In order to support using ß-caryophyllene as a chemical marker for the *Lantana* genus, we performed a second clustering analysis grouping each species based on ß-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* (Figure 1B). The group *Rhytidocamara* was distinguished from the other species, as suggested by Briquet (1985, 1904) and Schauer (1847). When only the five species our group had collected were clustered for ß-caryophyllene content (Figure 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 ß-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 & 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 (Figure 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 (Figure 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, *Lantana 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; Julien Paolini, 2010) which must be taken into consideration when making associations between genes and component concentration. For example, gene expression of three monoterpene synthase genes (LaTPS12, LaTPS 23 and LaTPS 25) in *Lippia alba* (Verbenaceae) were recently evaluated and 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.

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.

Acknowledgments

The authors would like to thank Dr[a]. Rita de Cassia Perreira, and Dr[a] Olivia Cano for their great contribution in species identification.

References

Adams, R.P., Schwarzbach, A.E. and Pandey, R.N. 2003. The concordance of terpenoid, ISSR and RAPD markers, and ITS sequence data sets among genotypes: an example from Juniperus, *Biochem. Syst. Ecol*. ***31:*** 375–387.

Argyropoulou, C., Daferera D., Tarantilis P.A., Fassea C. and Polissiou M. 2007. Chemical compostition of the essential oil from the leaves of *Lippia citriodora* HBK (Verbenaceae) at two developmental stages, *Biochemical Systematics and Ecology* ***35***: 831-837.

Botelho, M.A., Nogueira N.A.P., Bastos G.M., Fonseca S.G.C., Lemos T.L.G., Matos F.J.A., Montenegro D., Heukelbach J., Rao V.S. and Brito G.A.C. 2007. Antimicrobial activity of the essential oil from *Lippia sidoides*, cavacrol and thymol against oral pathogens, *Brazilian Journal of Medical and Biological Research* ***40***: 349-356.

Briquet, I. 1895. Verbenaceae, in: A. Engler. & K. Prantl (eds.). Nat. Pflanzenfam. **4**(3A): 132-182.

Briquet, I., 1904. Verbenaceae, in: R. Chodat & E. Hassler (eds.), Plantae Hassleriane. Bull. Herb. Boissier ser. 2, **4**: 1062-1066.

Chowdhury, J.U., Nandi N.C. and Bhuiyan M.N.I. 2007. Chemical composition of leaf essential oil of *Lantana camara* L. from Bangladesh, *Bangladesh Journal of Botany* ***36***: 193-194.

Costa, S.M.O., Lemos T.L.G., Pessoa O.D.L., Pessoa C., Montenegro R.C. and Braz-Filho R. 2001. Chemical constituents from *Lippia sidoides* and cytotoxic activity, *Journal of Natural Products* ***64****:* 792-795.

Costa, T.S., Silva, A.V.C., Lédo, A.S., Santos, A.R.F. and Silva Junior, J.F. 2011. Diversidade genética de acessos do banco de germoplasma de mangaba em Sergipe, *Pesquisa Agropecuária Brasileira* ***46:*** 499-508.

Da Silva, N.A., da Siva J.K.R., Andrade E.H.A., Carreira L.M.M., Sousa P.J.C. and Maia J. G.S. 2009 Essential oil composition and Antioxidant capacity of *Lippia schomburgkiana, Natural Products Communications* ***4****:* 1281-1286.

Damasceno, E.I.T., Silva J.K.R., Andrade E.H.A., Sousa P.J.C. and Maia J. G.S. 2011. Antioxidant capacity and larvicidal activity of essential oil and extracts from *Lippia grandis*. Brazilian, *Journal of Pharmacognosy* ***21***: 78-85.

De Oliveira, J.S.C., Neves I.A., da Camara C.A.G. and Schwartz M.O.E. 2008. Essential oil composition of two *Lantana* species from mountain forests of Pernambuco (Northeast of Brazil), *Journal of Essential Oil Research* ***20***: 530-532.

De Viana, M.E.L., Talenti E.C.J. and Retamar J.Á. 1973. L´olio Essenziale di *Lantana balansae*, *Essenze Derivate Agrumari* ***43***: 299-306.

Doyle, J. 1991. DNA protocols for plants—CTAB total DNA isolation. In: Hewitt GM, Johnston A (eds) Molecular techniques in taxonomy, *Berlin Springer* 283–293.

Farajpour , M., Ebrahimi, M., Amiri, R., Noori, S.A.S., Sanjari, S. and Golzari, R. 2011. Study of genetic variation in yarrow using inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) markers, *African Journal of Biotechnology* ***10***(54): 11137-11141.

Fracaro, F., Zacaria, J. and Echeverrigaray, S. 2005. RAPD based genetic relationships between populations of three chemotypes of Cunila galioides Benth, *Biochem. Systematics Ecol*. ***33***(4): 409-417.

Hennebelle, T., Sahpaz S., Joseph H. and Bailleul F. 2008. Ethnopharmacology of *Lippia alba*, *Journal Ethnopharmacology* ***116***: 211-222.

Hernandes, T., Canales M., Avila J.G., Garcia A.M., Martinez A., Caballero J., Romo V.A. and Lira R. 2005. Composition and antibacterial activity of essential oil of *Lantana achyranthifolia* Desf (Verbenaceae), *Journal of Ethnopharmacology* ***96***: 551-554.

Jaccard, P. 1908. Nouvelles recherches sur la distribution florale, *Soc Vaud Sci Nat* ***44***: 223-270.

Jimenez-Arellanes, A., Meckes, M., Ramirez, R., Torres, J., Luna-Herrera, J. 2003. Activity against Multidrug-resistant *Mycobacterium tuberculosis* in Mexican Plants Used to Treat Respiratory Diseases, *J. Phytother. Res.* ***17***, 903–908.

Juliani, H.R., Biurrun, F., Koroch, A.R., Oliva, M.M., Demo, M.S., Trippi, V.S. and Zygadlo, J.A. 2002. Chemical constituients and antimicrobial activity of the essential oil of *Lantana xenica, Planta Medica* ***68*****:** 762-764.

Juliani,H.R., Simon J.E., Quansah C. and *et al.* 2008. Chemical diversity of *Lippia multiflora* essential oils from west Africa, *Journal of Essential Oil Research* ***20***: 49-55.

Juliao, L.S., Bizzo H.R., Souza A.M., Lourenço M.C., Silva P.E.A., Tavares E.S., Rastrelli L. and Leitão S.G. 2009. Essential oil from two *Lantana* species with antimycobacterial activity, *Natural Product Communications* ***4*****:** 1733-1736.

Kaneda, N., Lee Ik-Soo, Gupta M.P., Soejarto, D.D. and Kinghorn, A.D. 1992. (+)- 4 β-Hydroxyhernandulcin, a new sweet sesquiterpene from the leaves and flowers of *Lippia dulcis, Journal of Natural Products* ***55***: 1136-1141.

Khan, S.A., Hussain, D., Askari, E., Stewart, J.M.C.D., Malik, K.A. and Zafar Y. 2000. Molecular phylogeny of Gossypium species by DNA fingerprinting, *Theor Appl Genet* **101:** 931–938.

Maldonado, E., Díaz-Arumir H., Toscano R.A. and Martínez M. 2010. Lupane triterpenes with a δ- Lactone at ring E, from *Lippia Mexicana, Journal of Natural Products* ***73***: 1969-1972.

Manica-Cattani, M.F., Zacaria, J., Pauletti , G., Atti-Serafini , L. and Echeverrigaray, S. 2009. Genetic variation among South Brazilian accessions of Lippia alba Mill. (Verbenaceae) detected by ISSR and RAPD markers, *Braz. J. Biol.* ***69***(2): 375-380.

Marta, D., Mendes, A., Helena, T.A., Cristina, F.A., José, G., Barroso, A., Susana, S.F.B. and Luis, G.P. A Volatile and molecular characterization of two Portuguese endemic species: Angelica lignescens and Melanoselinum decipiens, *Biochemical Systematics and Ecology* ***37*** (2009): 98–105.

Martin, J.P. and Sanchez-Yelamo , M.D. 2000. Genetic relationships among species of the genus Diplotaxis (Brassicaceae) using inter-simple sequence repeat markers, *Theor Appl Genet* ***101*****:** 1234–1241.

Monteleone, I., Ferrazzini, D. and Belletti P. 2006. Effectiveness of neutral RAPD markers to detect genetic divergence between the subspecies *uncinata* and mugo of *Pinus mugo* Turra, *Silva Fenn* **40:** 391–406.

Muhayiamana, A., Chalchat J.A. and Garry R.P. 1998. Chemical composition of essential oils of *Lantana trifolia* from Rwanda, *Journal of Essential Oil Research* ***10***: 547-549.

Neves, L.A., de Oliveira J.C.S., da Camara C.A.G. and Schwartz M.O.E. 2008. Chemical composition of the leaf oil of *Lippia gracilis* Schauer from two localities of Pernambuco, *Journal of Essential Oil Research* ***20***: 157-160.

Nybom, H. and Weising, K. 2007. DNA profiling of plants. In: Kayser O, Quax WJ (eds) Medicinal plants biotechnology, from basic to industrial applications, *Wiley-VCH, Weinheim* 73–95.

Oliveira, D.R., Leitão G.G., Bizzo H.R., Lopes D., Alviano D.S., Alviano C.S. and Leitao S.G. 2007. Chemical and antimicrobial analyses of essential oil of *Lippia origanoides* HBK, *Food Chemistry* ***101***: 236-240.

Ortega-Nieblas, M.M., Robles-Burgueno, M.R., Acedo F.E., Gonzalez-Leon A., Morales-Trejo A., Vazquez-Moreno L. 2011. Chemical composition and antimicrobial activity of orégano (*Lippia palmeri* S. WATS) essential oil, *Revista Fitotecnia Mexicana* ***34***: 11-17.

Ouamba, J.M., Ouabonzi A., Ekouya A., Bessiere J.M., Menut C., Abena A.A. and Banzouzi J.M. 2006. Volatile constituents of the essential oil leaf of *Lantana salviifolia* Jacq.(Verbenaceae), *Flavour and Fragrance Journal* ***21***: 158-161.

Pandeló, D., Melo, T. D., Singulani, J. L., Guedes, F.A.F, Machado, M. A., Coelho, C. M; Viccini, L.F., Santos, M. O., Oil production at different stages of leaf development in *Lippia alba*, [online]. ahead of print [cited 2012-03-02], pp. 0-0 . Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0102-695X2012005000013&lng=en&nrm=iso>. Epub Jan 17, 2012. ISSN 0102-695X. http://dx.doi.org/10.1590/S0102-695X2012005000013.

Paolini,J., Barboni, T., Desjobert, J.M., Djabou, N., Muselli, A. and Costa, J. Chemical composition, intraspecies variation and seasonal variation in essential oils of Calendula arvensis L, *Biochem. Syst. Ecol*. ***38*** (2010) 865–874.

Pasakinskiene, I., C.M. Griffiths, A.J.E. Bettany, V. Paplauskiene and M.W. Humphreys. 2000. Anchored simple-sequence repeats as primers to generate species-specific DNA markers in Lolium and Festuca grasses, *Theor Appl Genet* ***100:*** 384–390.

Pascual, M.E., Slowing K., Carretero E., Mata D. S. and Villar A. 2001. Lippia: traditional uses, chemistry and pharmacology: a review, *Journal Ethnopharmacology* ***76****:* 201-214.

Pezhmanmehr, M., Hassani, M.S., Jahansooz, F., Najafi, A.A., Sefidkon, F., Mardi, M. and Pirseiedi, M. 2009.. Assessment of genetic diversity in some Iranian populations of Bunium persicum using RAPD and AFLP markers. Iran, *J. Biotechnol. **7***(2).

Pichersky, E. and Gershenzon, J. 2002. The formation and function of plant volatiles: perfumes for pollinator attraction and defense. Curr. Opin, *Plant Biol. **5***: 237–243.

Pino, J.A., Marbot R., Payo A., Chao D. and Herrera P. 2006. Aromatic plants from west of Cuba VII. Composition of the leaves oil of *Psidium wrightii* Krug et Urb., *Lantana involucrate* L., *Cinnamomum montanum* (Sw.) Berchtold et J.Persl. and *Caesalpinia violaceae* (Mill.) Standley, *Journal of Essential Oil Research* ***18***: 170-174.

Raina, S.N., Rani, V., Kojima, T., Ogihara, Y., Singh, K.P. and Devarumath, R.M. 2001. RAPD and ISSR fingerprints as useful genetic markers for analysis of genetic diversity, varietal identification, and phylogenetic relationships in peanut (*Arachis hypogaea*) cultivars and wild species, *Genome* **44:** 763–772.

Rana, V.S., Prasad D. and Blazquez M.A. 2005. Chemical composition of the leaf oil of *Lantana camara, Journal of Essential Oil Research* ***17***: 198-200.

Randrianalijaona, J.A., Ramanoelina P.A.R., Rasoarahona J.R.E. and Gaydou E.M. 2005. Seasonal and chemotype influences on the chemical composition of *Lantana camara* L. Essential oils from Madagascar, *Analytica Chimica Acta* ***545***: 46–52.

Reddy, M. P., Sarla, N. and Siddiq, E. A. 2002. Inter simple sequence repeat (ISSR) polymorphism and its application inplant breeding, *Euphytica* ***128:*** 9-17.

Rimpler, H. and Sauerbier, H. Iridoid glucosides as taxonomic markers in the genera Lantana, Lippia, Aloysia and Phyla, *Biochemical Systematics and Ecology* ***14*** **(**3): 307-310.

Santos, A.R.F, Ramos-Cabrer, A.M., Diaz-Hernandez, M. and Pereira-Lorenzo, S. 2011. Genetic variability and diversification process in local pear cultivars from northwestern Spain using microsatellites, *Tree Genetics and Genome* ***7:*** 1041-1056.

Saxena, V.K. and Sharma R.N. 1999. Antimicrobial activity of the essential oil of *Lantana aculeate, Fitoterapia* ***70***: 67-70.

Schauer, J. C. 1847. Verbenaceae, in: A. P. De Candolle (ed.). Prodr. 11: 522-700.

Sena Filho, J.G., Nimmo S.L., Xavier H.S., Barbosa-Filho J.M. and Cichewicz R.H. 2009. Phenylethanoid and lignan glycosides from polar extracts of *Lantana*, a genus of Verbenaceous plants widely used in traditional herbal therapies, *Journal of Natural Products* ***72***: 1344-1347.

Sena, J.G., Duringer, J.M., Uchoa, D.E.A., Xavier, H.S., Barbosa, J.M. and Braz, R. 2007. Distribution of iridoid glucosides in plants from the genus Lippia (Verbenaceae): An investigation of *Lippia alba* (Mill.) NE brown, *Natural Product Communications 2* ***7:*** 715-716.

Sena, J.G., Xavier, H.S., Barbosa Filho, J.G., Duringer, J.M. A. 2010 A Chemical Marker Proposal for the *Lantana* genus: Composition of the Essential Oils from the Leaves of *Lantana radula* and *L. canescens. Natural Products Communications* ***5:*** 635-640.

Senatore, F., Rigano D. 2001. Essential oil of two *Lippia* spp. (Verbenaceae) growing wild in Guatemala, *Flavour and Fragrance Journal* ***15***: 169-171.

Sharma, M., Sharma P.D. and Bansal M.P. 2008. Lantadenes and their esters as potential antitumor agents, *Journal of Natural Products* ***71***: 1222-1227.

Silva, A.V.C., Santos, A.R.F., Lédo, A. S., Feitosa, R.B., Almeida, C.S., Silva, G.M. and Rangel, M.,S.A. 2012. Moringa genetic diversity from germplasm bank using RAPD Markers, *Tropical and Subtropical Agroecosystems* ***15***: 31-39.

Silva, P.S., Viccini L.F., Singulani J.L., de Siqueira E.P., Zani C. L. and Alves T.M.A. 2010. Chemical composition of the essential oil and hexanic fraction of *Lippia* and *Lantana* species, *Brazilian Journal of Pharmacognosy* ***20***(6): 843-849.

Silva, T. R. S. 1999. Redelimitação e Revisão Taxonômica do gênero *Lantana* L. (Verbenaceae) no Brasil. Tese de doutorado. Universidade de São Paulo. São Paulo.

Sokal, R.R. and Michener, C.D. 1958. A statistical method for evaluating systematic relationships, *The Univ of Kansas Scient Bull* ***38:***1409–1438.

Suárez González, A.R., Martínez Nuñez, F.O., Castillo Villamizar, G.A. and Chacón S., M.I. 2007. Molecular characterization of aromatic species of the genus Lippia from the colombian neotropics, *Acta Hort.* ***756:*** 129-138.

Walden, A.B., Haber, W.A. and Setzer, W.N. 2009. Essential oil compositions of three *Lantana* species from Monteverde, Costa Rica, *Natural Product Communications* ***4:*** 105-108.

Wiejacha, K., Trzewik, A., Orlikowski, L.B., Szkuta, G. and Orlikowska, T. 2004. Genotypic differences between isolates of Phytophthora cinnamomi rands and P. citricola sawada obtained from twelve nursery plant species, *Journal of Plant Protection Research* ***44*** (3): 189-198.

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

Table 2. Volatile compounds identified in essential oil extracts from *Lantana salzmannii* and *Lantana lucida* leaves collected in the State of Sergipe, Brazil.

IRR exp.	IRR lit.	Compounds	*Lantana salzmannii*[a]	*Lantana lucida*[b]
924	924	α-tujene	0.12	-
930	932	α-pinene	0.05	-
970	969	sabinene	0.06	-
989	988	mircene	0.17	-
1005	1002	α-phelandrene	0.26	-
1006	1008	δ-3-carene	3.70	-
1015	1014	α -terpinene	0.16	-
1018	1020	p-cimene	0.45	-
1023	1022	o-cimene	1.26	-
1028	1024	limonene	2.84	-
1030	1026	1,8-cineol	0.32	-
1046	1044	(E)-β-ocimene	0.24	-
1057	1054	γ-terpinene	1.67	-
1080	1085	p-menta-2,4(8)-dieno	0.13	-
1084	1086	terpinolene	0.91	-
1099	1095	linalol	0.09	-
1179	1174	terpinen-4-ol	0.17	-
1335	1335	δ-elemene	0.56	-
1346	1345	α-cubebene	0.32	-
1370	1373	α-ylangene	0.10	-
1376	1374	α-copaene	3.18	6.91
1389	1389	β-elemene	2.37	4.43‡
1387	1389	β-cubebene		4.43‡
1406	1409	α-gurjenene	0.58	0.65
1421	**1417**	**β-caryophyllene**	**15.60**	**18.95**
1424	1434	γ-elemene	0.23	-
1430	1430	β-copaene	0.06	2.49
1435	1437	α-guaiene	0.17	-
1441	1442	6,9-guaiadiene	0.13	-
1449	1448	*cis*-muurula-3,5-diene	0.79	-

Table 2 (continued).

IRR exp.	IRR lit.	Compounds	*Lantana salzmannii*[a]	*Lantana lucida*[b]
1456	1452	α-humulene	3.24	33.02
1460	1458	allo-aromadendrene	1.17	0.88
1482	1478/1484	germacrene D	2.95	3.54
1483	1484	β-chamigrene	-	1.76
1490	1489	β-selinene	5.75	0.57
1491	1493	*Trans*-Muurola-4 (14), -diene	-	0.82
1495	1500	biciclogermacrene	-	4.87
1496	1498	αselinene	3.71	-
1497	1500	α-muurolene	-	2.79
1503	1505	(*E*,*E*)-α--farnesene	0.14	-
1507	1505	β-bisabolene	0.92	-
1513	1513	γ-cadinene	0.10	-
1518/1517	1522	δ-cadinene	1.58	11.33
1522	1521	*trans*-calameneno	6.60	-
1523	1528	*zonarene*	-	0.54
1533	1533	*trans*-cadina-1,4-diene	4.54	-
1541	1544	α-calacorene	0.21	-
1560	1561	(*E*)-nerolidol	1.97	-
1576	1577	espatulenol	0.31	-
1582	1582	caryophyllene oxide	1.97	-
1586	1586	gleenol	0.43	-
1595	1592	viridiflorol	0.26	-
1605	1602	ledol	0.88	-
1610	1608	epóxido de humuleno II	0.57	1.32
1628	1627	1-epi-cubenol	2.56	-
1638	1639	cariofila-4(12),8(13)-dien-5-(α/β)-ol	0.28	-
1642	1645	cubenol	1.04	-
1647	1644	α-muurulol	0.42	-
1655	1658	neo-intermedeol	1.40	-
1660	1658	selin-11-en-4-α-ol	11.24	-
1667	1655*	humulane-1,6-dien-3-ol	0.66	-
1671	1675	cadaleno	0.24	-
1679	1687	eudesma-4(15),7-dien-1β-ol	0.58	-

[a,b]Peaks listed in this table represent 92.4% and 94.9 %, respectively, of all peaks contained in the essential oil extract.
‡Peaks were grouped.

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

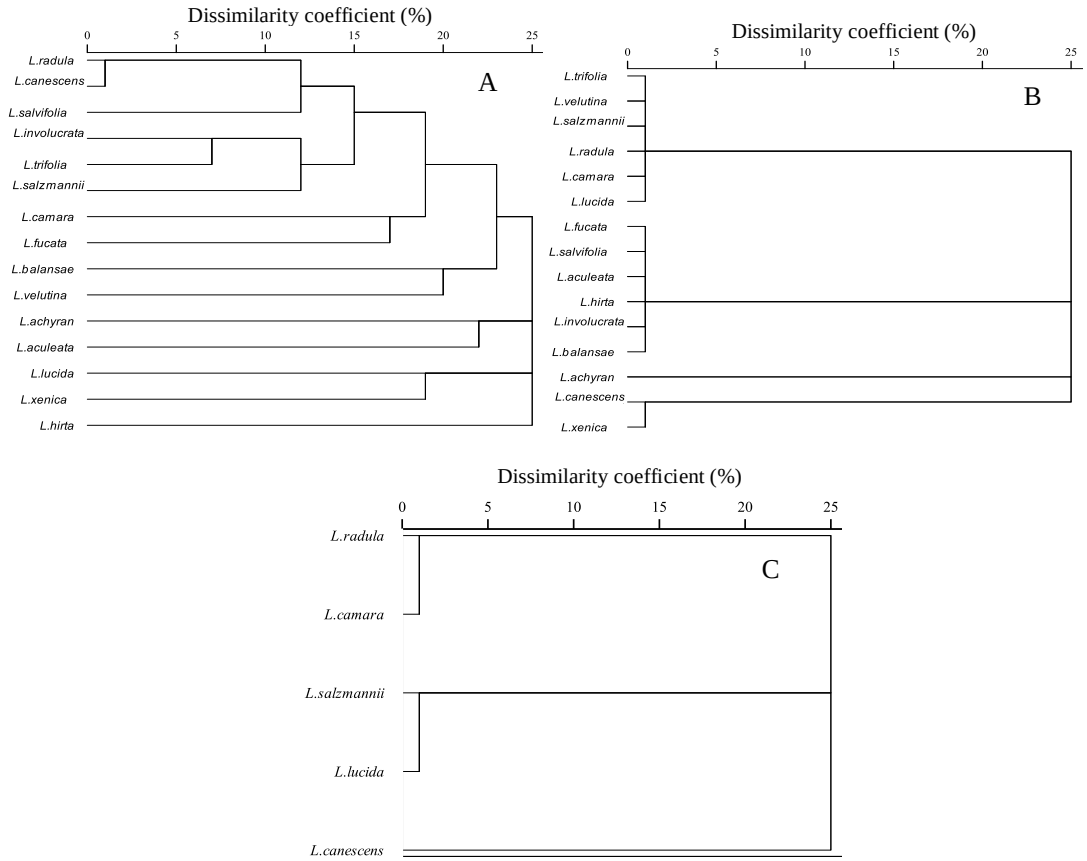

Figure 1. Dissimilarity phenograms derived from the: (A) chemical variation of 77 compounds observed in essential oil extracts from *Lantana* species; (B) amount of β-caryophyllene found in the 15 *Lantana* species used in this study; and (C) amount of β-caryophyllene evaluated in the five *Lantana*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

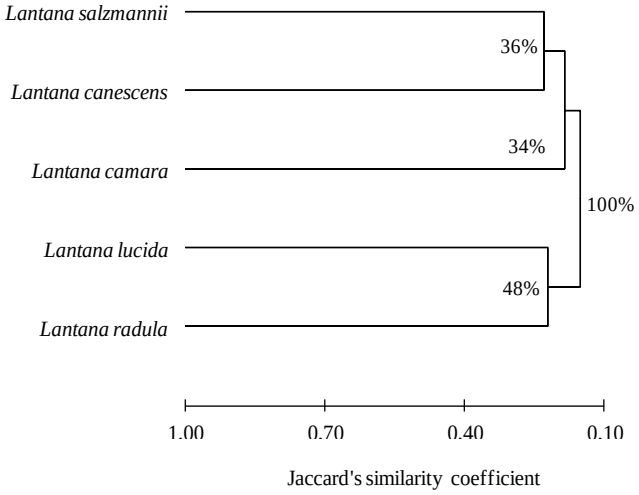

Figure 2. Dendrogram of genetic similarity from ISSR using the Jaccard coefficient and the Unweighted Pair Group with Arithmetic Mean method with bootstrap analysis for five species of *Lantana*.

Appendix 1. Volatile compounds identified in essential oil extracts from 13 *Lantana* species.

Volatile compounds	*L. achyranthifolia*[a]	*L. aculeata*[b]	*L. balansae*[c]	*L. camara*[d]	*L. canescens*[e]	*L. hirta*[f]	*L. involucrata*[g]	*L. fucata*[h]	*L.radula*[i]	*L. salviifolia*[j]	*L. trifolia*[k]	*L. velutina*[f]	*L. xenica*[l]
(E)-b-ocimene											3.0		9.2
(*E*)-Nerolidol							0.1	1.5					
(Z)-b-ocimene											15.1		
(Z,E)-α-fanesene											4.5		
1,8-cineol	5.03		24.4				0.3						
1-epi-cubenol													
1-*epi*-Cubenol													
Acetate de geranila			8.6										
Bicyclogermacrene								0.6			0.6	8.1	
Borneol			8.4										
Bornyl acetate		10.5											
Bulnesol							4.6						
Candina 4(5), 10(14) dieno	3.4												
Canphene		4.2			0.1								
Canphor	0.5		5.9										
Carvacrol	30.6						0.1						
Carveol			5.1										
Caryophyllene oxide				0.3			11.3	1		5.6	0.3		
Citral													
Davanone				7.3									
Dehidroaromadendreno					2.6								
Dipentene													
Elemol							9.7						
Elixene					8.6				10				
Eremofilene													
Eugenol		5.8					0.1				0.2		
Gemacrene A								4.5					
Gemacrene B								2.8					
Geraniol													
Germacrene D				10.9	0.2	24.5	3.7	11.6		1.7	20.3	37.5	6.6
Globulol													
Gossonorol													
Guaia 1(10), 11(12) dieno	2.8												
Humuleno epoxide II							3.9						
Isocaryophullene	10.7										2.8		
Khusimol								7.0					
Limonene			24.4	0.6			0.2				4.9	21.2	
Linalol			2.7				0.8			1.5	3.9		
Manoyl oxide								7.8					
Mentol													
Methyl chavicol		3.0											
Muurola-4,10(14)-dien-1-b-ol													

Appendix 1 (continued)

Volatile compounds	*L. achyranthifolia*[a]	*L. aculeata*[b]	*L. balansae*[c]	*L. camara*[d]	*L. canescens*[e]	*L. hirta*[f]	*L. involucrata*[g]	*L. fucata*[h]	*L.radula*[i]	*L. salviifolia*[j]	*L. trifolia*[k]	*L. velutina*[f]	*L. xenica*[l]
Myrcene		2.5								0.2			
Nerol		7.0											
Octen-3-ol						64.6				0.1	3.0		
p-cimene			4.5										
Sabinene							0.4						
Terpenyl acetate		4.2											
Trans isolongifolanone													
Trans-cadina-1,4-dieno													
Trans-calamenene													
Valencene								5.7					
y-curcumene				6.3									
y-terpinene					0.3		0.1					3.3	
y-candinene													13.3
α-bisabolol	11.2												
α-bornyl		7.2											
α-caryophyllene	3.78		11.5	11.5	2.6		4.9	2.3		0.5	4	2.8	
α-copaene				0.4			0.3		4.9	0.3	1.7		
α-eudesmol							24.9						
α-Muurolene					0.1		1.5		0.6	0.5	0.8		
α-phelandrene		18.9		0.6	0.3		0.1		1.2		0.1		
α-pinene		2.2	3.2				0.2				0.8		9.3
α-selinene									6.4				
α-terpineol		7.1					0.7				0.3		
β-bisabolene	5.7												
β-candinene	2.8	2.0							1.4				
β-cariophyllene		4.6	2.8	23.3	43.9	10.9	10.6	7.6	20.8	5.7	15.3	23.1	35.2
β-copaene										0.5		0.4	
β-cubebene					10.1				31	1			
β-elemene				2.8	0.3		1.2	0.6	0.9	0.5			
β-phelaphendrene					6.1				6.1		0.8		
β-pinene													
β-selinene													
δ-3-carene					0.3		0.3			0.2			
δ-cadinene				2.3	0.6		1.4				1.9		

a - Hernandes et al. (2005); b – Saxena & Sharma (1999); c – De Viana et al. (1973); d – Rana et al. (2003); e – Sena Filho et al (2010); f – Walden et al. (2009); g – Pino et al. (2006); h- de Oliveria et al. (2008); i – Sena Filho et al. (2010); j – Ouamba et al. (2005); k – Juliao et al. (2009); l – Juliani et al (2002).