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Effect of Collection Time on Essential Oil Composition of *Lantana camara* Linn (Verbenaceae) Growing in Brazil Northeastern

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Abstract: To verify the composition variation of its essential oil along 24 hours, leaves of *Lantana camara* Linn. of medicinal plant collection of the Department of Chemical Biology of URCA-Crato–CE, were collected every three hours during one day (of the 07:00 to 19:00 h) in April, 2008. The leaves had its oil extracted by hydro-distillation in Clevenger apparatus during three hours with two repetitions. The chemical composition analysis of the extracted essential oils was carried out in gas chromatography/mass spectroscopy. The phytochemical profile of the essential oils presented significant difference in function of the harvest. The seven most abundant were: germacrene D (24.50-6.15%), biciclogermacrene (33.32-14.27%), spathulenol (25.04-1.06%), eremophilene (20.64-1.93%), valecene (33.70-0.84%), viridiflorene (19.46%) and 1,10-di-epi-cubenol (27.93-21.32). The different result found here indicates the existence of different chemotypes of *L. camara.*

Keywords: Keywords: *Lantana camara* Linn*;* essential oil; chemical variability; chemical composition**.**

1. Introduction

The Verbenaceae family comprises about 100 genera and 2000 species distributed in tropics and subtropics, mainly in temperate zone of southern hemisphere [1]. *Lantana* is a genus of about 150 species that are very popular as popular ornamental garden plant [2]. *Lantana* species are known to are virtually immune to herbivory owing to the presence of a wide array of phytochemicals of diverse groups [3]. The plants have been used in many parts of the world to treat a wide variety of disorders as antirheumatic, stimulant, sudoriparous, to treat bronchitis and asthma and in biologic control [4,2].

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Lantana camara Linn, common in America and Africa, is a shrub cultivated world-wide as an ornamental plant, but now, *L.* camara is considered as agressive and it has been regarded as one of the 10 most noxious weeds in the world. Its leaves have been used in folk medicine to treat fever, influenza, astham, bronquitis and many other deseases [2].

L. camara leaves are aromatic and their essential oil is reported to be inseticidal, acting as bees, mosquitoes and flies repellent. Besides this, the essential oil possess antibacterial and antifungical properties [5]. Seeds essential oil is antifungical and antiseptic and the flowers essential oil promotes the *Aedes* mosquitoes oviposition [4,6].

Many aromatic and/or medicinal plants species have been the target of studies that relate the time influence on essential oil chemical composition. This occurs because the time of material collect is a relevant aspect to be taken into account when dealing with essential oil production.

Previous reports showed chemical composition variation in *L. camara* leaves essential oils from different origins [8-11]. Different compositions were observed in samples obtained from distinct regions of Brazil [12]. The sazonal variation of leaves and flowers essential oils from *L. camara* collected in Madagascar was studied by Randrianalijaona et al., (2005) [13]. Despite this, until this moment, there is no literature record regarding the daytime chemical variation, and these justify this work.

In the present study, we were interested to evaluate whether the chemical composition of *L. camara* essential oil from Cariri (Ceará, Brazil Northeastern) would vary according to the daytime collection.

2. Materials and Methods

2.1. Plant Material

Leaves of *Lantana camara* Linn were collected in April, 2008, from the Small Aromatic and Medicinal Plants Garden of the Natural Products Research Laboratory (LPPN) at University Regional do Cariri (URCA), Crato, Ceara state, Brazil. A total of 15 samples (200g/each) were collected 7:00 a.m.; 10:00 a.m.; 1:00 p.m.; 4:00 p.m. e 7:00 p.m. A voucher specimen (#1662) was deposited in the "Herbário Caririense Dárdaro de Andrade Lima" of Regional University of Cariri, Crato.

2.2. Essential Oil Extraction

Samples of fresh leaves (200 g) were submitted to hydrodistillation process for two hours, in a Clevenger-type apparatus [14]. The essential oils collected were subsequently dried by anhydrous sodium sulfate (Na_2SO_4), and kept refrigerated at < 4 ºC until be analyzed.

2.3. Essential Oil Analysis

Oil analysis was performed using a Shimadzu GC-17 A/ MS QP5050A (GC/MS system): DB-5HT capillary column (30 m x 0.251 mm, 0.1 µm film thickness); helium carrier gas at 1.7 mL/min; injector temperature 270 ºC; detector temperature 290º C; column temperature 60º C (2 min) – 180º C (1 min) at 4º C/min, then 180º – 260º C at 10º C/min (10 min). Scanning speed was 0.5 scan/sec from m/z 40 to 450. Split ratio (1:30). Injected volume: 1 µL of 5 mg/mL solution ethyl acetate. Solvent cut time = 3 min. The mass spectrometer operated using 70 eV of ionization energy. Identification of individual components was based on their mass spectral fragmentation based on two computer library MS searches (Wiley 229), retention indices, and comparison with published data [15,16].

2.3. Statistical analysis

The yields obtained were submitted the statistical analysis using Analysis of Variance (ANOVA) followed by the Newman-Keuls Multiple Comparison Test. The results with $p < 0.05$ were considered to be significant.

3. Results and Discussion

The results found here show that there was significantly differences ($p< 0.05$) between the essential oils yields obtained from different collect times (Figure 1). The less yield (0.01%) was recorded at 7:00 a.m., and the higher one (0.09%) was obtained at 7:00 p.m. It can be perceived that to obtain the highest essential oils yield, the collect must be realized after 1:00 p.m., when the yields are more significatives. In many other reports about *Lantana* genus it was observed aerial part essential oil yield variation (0.01-0.4%) [17-19].

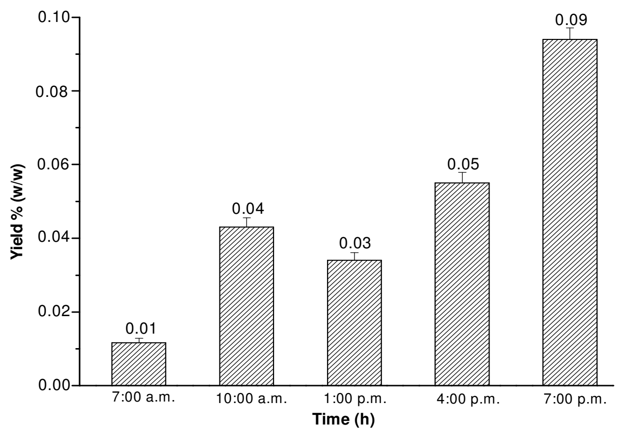

Figure 1: Average yield of essential oil of *Lantana camara* Linn at different times of collection.
*The values of the average yields are statistically significant with each ($p < 0.05$ – ANOVA followed by the Newman-Keuls Multiple Comparison Test).

In Table 1, the essential oil chemical composition can be found. Qualitative and quantitative differences can be observed, when a comparison is made between the different daytime collection (Figure 2). The GC/MS analysis identified 17 compounds (94.82%) at 7:00 a.m., 14 (89.06%) at 10:00 a.m., 13 (93.63%) at 1:00 p.m., 16 (97.96%) at 4:00 p.m. And 12 (97.11%) at 7:00 p.m.. The sesquiterpenes were predominant as shown by others authors [12,13].

Table 1. Chemical composition and percentages of essential oil components of *L. camara* Linn obtained at five different times of day.

Components	IR[a] sam.	IR[b] lit.	Composition (%)				
			7:00 a.m.	**10:00 a.m.**	**1:00 p.m.**	**4:00 p.m.**	**7:00 p.m.**
Δ-elemene	1330	1330[26]	-	-	-	0.32	-
α-copaene	1366	1376[17]	0.22	0.49	0.60	1.19	0.31
β-elemene	1385	1393[17]	1.22	1.19	1.31	2.06	1.48
Z-caryophyllene	1409	1405[28]	-	4.13	-	-	-
β-caryophyllene	1414	1418[17]	-	-	-	5.78	-
trans-caryophyllene	1412	1422[27]	-	-	23.79	-	0.33
E-caryophyllene	1422	1424[28]	11.32	-	-	-	-
β-gurjunene	1428	1430[17]	-	-	0.23	0.38	-
Aromadendrene	1441	1434[29]	4.81	3.53	-	-	-
α-guaiene	1445	1443[12]	0.21	-	-	-	-
α-humulene	1448	1451[29]	0.37	-	4.56	-	-
Alloaromadendrene	1449	1459[12]	-	-	-	1.15	0.21
γ-gurjunene	1472	1470[26]	9.80	3.45	-	-	-
Germacrene D	1473	1480[17]	21.30	22.90	15.10	24.50	6.15
Eremophilene	1480	1486[28]	5.64	1.93	3.27	11.15	20.64
Aristolochene	1485	-	-	1.18	-	-	-
Biciclogermacrene	1490	1484[17]	-	-	20.74	33.32	14.27
Viridiflorene	1498	1499[28]	-	-	19.46	-	19.46
Valecene	1500	1496[30]	33.70	0.84	-	-	-
Germacrene A	1508	1505[12]	-	-	-	-	1.05
γ-cadinene	1514	1513[17]	0.66	-	1.07	-	0.82
Δ-cadinene	1516	1514[29]	-	-	-	1.54	-
Germacrene B	1543	1547[26]	0.36	1.17	1.05	1.45	3.55
γ-elemene	1546	1549[10]	-	-	-	5.57	-
Spathulenol	1566	1576[17]	1.93	25.04	1.97	1.06	-
Caryophyllene oxide	1571	1577[26]	0.32	1.25	-	-	-
Viridiflorol	1577	1587[12]	0.24	0.64	-	5.32	-
1,10-di-epi-cubenol	1609	1617[26]	-	21.32	-	-	27.93
α-cadinol	1628	1652[17]	-	-	0.48	-	-
Torreyol	1636	1630[29]	-	-	-	0.37	-
Camphor	1140	1140[17]	0.49	-	-	-	-
E-phytol	2100	2113[25]	2.23	-	-	2.80	1.12
Total identified			94.82	89.06	93.63	97.96	97.11

[a]. relative retention indices experimental: n-alkanes were used as reference points in the calculation of relative retention indices.
[b]. relative retention indices (literature values)

A strong daytime variation of the main constituents was found (Table 1). The constituents that appeared in majority were: germacrene D (24.50-6.15%), biciclogermacrene (33.32-14.27%), spathulenol (25.04-1.06%), eremophilene (20.64-1.93%), valecene (33.70-0.84%), viridiflorene (19.46%) e 1,10-di-epi-cubenol (27.93-21.32), Figure 2.

Valencene, identified as the main constituent at 7:00 a.m (33.7%), was found at the concentration of 0.84% at 10:00 p.m. The presence of this compound was not detected at the other collection times. *Υ*-gurjunene was present at 7:00 a.m. (9.8%) and 10:00 a.m. (3.34%). 1,10-di-epi-cubenol was recorded at 10:00 a.m. (21.32%) and it was the main constituent at 7:00 p.m. (27.93%). Sphatulenol was found at 7:00 a.m, 10:00 a.m. (main constituent, 25.04%), 1:00 p.m and 4:00 p.m.

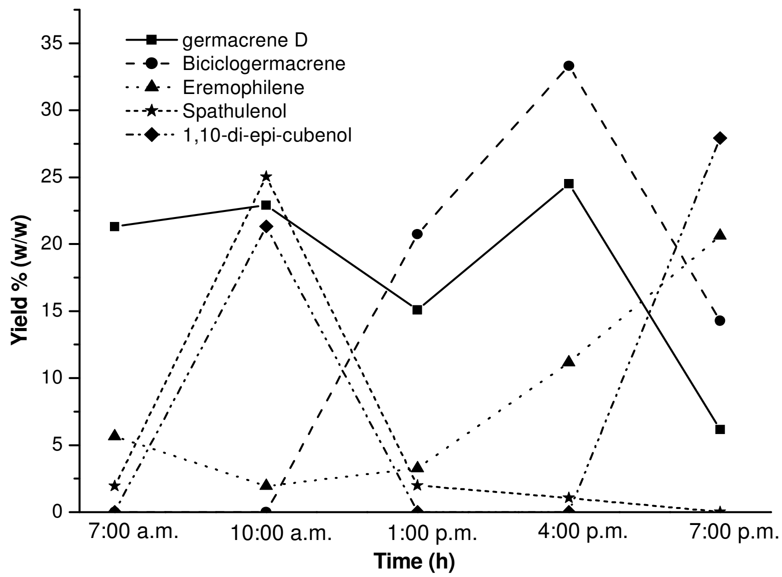

Figure 2. Variation in major constituents of essential oil of *Lantana camara* Linn collected at different times.

Martins and Santos (1995) [20] related that, according to the plant active substance, there are daytimes that the constituent concentration is less or higher. This standard is important to correlate with the biological activities, incluinding antibacterial, inseticidal and antifungical [21]. Simões and Spitzer (2001) [22] hightlight that ambiental influence (temperature, relative humidity, sun exposition and wind) are fundamental factors that are able to cause variations.

Caryophyllene isomers (*Z*, *E* e β caryophyllene) were present between the main constituents of essential oil at different daytime. According to Chowdhury et al., (2007) [23] this constituents are commom in aerial parts essential oils from *Lantana* genus.

Germarece D was present as one of the main constituents at all collection times. The highest levels were at 7:00 a.m. (21.3%), 10:00 a.m. (22.9%) and 4:00 p.m. (24.5%). A decreased was observed at 1:00 p.m. (15.1%) and 7:00 a.m. (6.15%), Figure 2. Bicyclogermacrene was not found at morning, but it was the main constituent at 4:00 p.m. (33.32%), Table 1. Silva et al, (1999) [12] verified high levels of these sesquiterpenes in *Lantana camara* essential oils obtained from North of Brazil.

Eremophilene was identified in all analyzed samples, achieving the highest levels at 1:00 p.m. (11.15%) and 7:00 p.m. (20.5%). Viridiflorene had a similar behavior (19.46% at 1:00 and 7:00 p.m). It´s important to note that these constituents were not identified in previous reports.

As reported by others authors [12,13,17,24] viridiflorol (5.32-0.24%), germacrene A (1.05%) germacrene B (3.55-0.36%), *E*-phytol (2.23-1.12%), α-copaene (1.19-0.22%), β-elemene (2.06-1.22%), Υ-cadinene (1.07-0.66%) and β-gurjunene (0.38-0.23%) were in minority.

Comparing the results found here with previous reports, it can be verified a strong indication that the chemical variation may be related to the existence of chemiotypes of *Lantana camara.*

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