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Chemical constituents and evaluation of cytotoxic and antifungal activity of *Lantana camara* essential oils

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Abstract: The essential oil (EO) of aerial parts of *Lantana camara* L., Verbenaceae, from Simões, Piaui, Northeast of Brazil, were obtained by hydrodistillation and analyzed by GC-FID and GC-MS techniques. In total, 68 compounds were identified. The most representative compounds of the oil were mono and sesquiterpenes. The main compounds found in the oil of the leaves in different months were β-caryophyllene (10.5%, in June of 2009), sabinene (7.98%, in September of 2008), limonene (7.68%, in September of 2008), spathulenol (11.64%, in September of 2008). The oil from stems of *L. camara* was characterized by a largest amount of sesquiterpenoids, with spatulenol (15.9%) and caryophyllene oxide (17.1% in June of 2009), as main components. β-Gurjunene (32.7%, in September of 2008) was the most prominent compound in the stems` oils, which was absent or at very low relative abundance in leaves. *L. camara* essential oils from leaves were cytotoxic to V79 mammalian cells and also to *Artemia salina*, showing 50% lethal concentration (LC50) values from 0.23 µg/mL. The in vitro data obtained in this study suggested that EO may also be effective treating yeast infection in patients infected with fluconazole and terbinafine resistant isolates, but its toxicity must be monitored carefully.

Article

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Introduction

The genus *Lantana*, Verbenaceae, comprises about 150 species. Species of the genus Lantana have been used in several applications in folk medicine due to its anti-rheumatic, carminative, antiseptic, antispasmodic, emetic, antifungal and antitumoral activities. *Lantana* sp. is also used against bronchopulmonary diseases (Ghisalberti, 2000; Lorenzi & Matos, 2002).

Many studies have reported that the genus *Lantana* displayed a variety of biological activities relating these activities to its chemical composition (Sousa et al., 2011; Sousa et al.,2010; Sousa et al., 2012; Costa et al., 2009; Costa et al., 2010; Sousa et al., 2009). Analysis of leaves stems and roots fixed extracts of genus *Lantana* indicated the presence of acid glycosides, alkaloids, flavonoids and triterpenes (Rwangabo et al., 1988; Herbert et al., 1991; Singh et al., 1991; Begum et al., 1995; O'Neill et al., 1998; Weir et al., 1998; Begum et al., 2000; Mello et al., 2005; Litaudon et al., 2009). These secondary metabolites show several biological activities: antibacterial (Costa et al., 2009; Jiménez-Arellanes et al., 2007; Pereira et al., 2008), fungicides (Rwangabo et al., 1988; Sousa et al., 2009), inflammatory (Julião et al., 2009), anti-nematodes (Begum et al., 2000), inhibitors of α-human thrombin (O'Neill et al., 1998; Weir et al., 1998), inhibitors of protein kinase C (Herbert et al., 1991) and inhibitors of protein Bcl-xL, a anti-apoptotic protein, of the family of Bcl-X (Hayashi et al., 2004; Litaudon et al., 2009).

In relation to chemical volatile constituents from genus *Lantana*, previously published studies showed a predominance of mono and sesquiterpenes (Sousa et al., 2010; Ngassoum et al., 1999; Silva et al., 1999; Misra & Laatsch, 2000; Alitonou et al., 2004; Hernández et al., 2005; Sonibare & Effiong, 2008). In this sense, we recently analyzed leaves of *Lantana* species by GC-MS

collected in Ceará, Brazil, and it showed that essential oils from these species have in its composition mainly sesquiterpenes and sesquiterpenoids (Costa et al., 2009; Sousa et al., 2009). In general, *Lantana* sp. has larvicidal potential (Costa et al., 2010) and considerable antibacterial activity, including antibacterial activity against *Sthaphylococcus aureus* (Sousa et al., 2010). Results of *L. camara*, for example, showed an effect against *Proteus vulgaris* and *Escherichia coli*. Notably, for this oil the evaluation of toxicity LC50 revealed results with minor values to limit pattern of 1000 µg/mL for *Artemia salina*. Moreover, the essential oil of *L. achyranthifolia* and *L. camara* showed antibacterial and antifungal activity against microorganisms that are actively working in respiratory and intestinal infections (Alitonou et al., 2004; Hernández et al., 2005; Sonibare & Effiong, 2008). Several species are considered toxic, but the most important toxic species is *L. camara*, to cause photosensitization in ruminants when they ingest large quantities of leaves (Bastianetto et al., 2005).

As can be seen in the literature, the presence of interesting compounds in species of the genus *Lantana* and their potential activity have motivated our phytochemical study with *L. camara* specie widely distributed in Simões, Piauí, Brazil. In this sense, the aim of the present study was to investigate the chemical composition of *L. camara* essential oil from leaves and stems in different periods. Furthermore, we also analyzed its biological properties by evaluation of antifungal activity and cytotoxic effects against larvae of *Artemia salina* (brine shrimp assay) and mammalian V79 cells (MTT).

Materials and Methods

Plant material

Leaves and stems of *Lantana camara* L., Verbenaceae, were collected in September of 2008 and June of 2009 in Simões, Piaui, Brazil. The voucher specimen was identified by Professor Jorge Yoshio Tamashiro (IQ-Unicamp), and deposited in the Herbarium Graziela Barroso of Federal University of Piaui, Brazil, under the registration number 27183.

Volatile constituent

Samples of fresh leaves and stems (about 300 g) of *L. camara* were subjected to hydrodistillation for 3 h in a Clevenger-type apparatus from which a yield of about 0.31% (leaves) and 0.17% (stems) of oil was obtained. The solvent used was peroxide free diethyl ether. The isolated essential oils were dried over anhydrous sodium sulfate and, after filtration, maintained under refrigeration before analysis.

Gas chromatography-mass spectrometry (CG-MS) and Gas chromatography (FID)

Gas chromatography (GC) analyses were performed on a Hewlett-Packard 5890 SERIES II equipped with a flame ionization detector (FID), column J & W Scientific DB-5 fused silica (30 m x 0.25 mm i.d. x 0.10 mm film thickness). Hydrogen was used as carrier gas at a flow rate of 1.0 mL/min; split mode (1:10). The injector and detector (FID) temperatures were maintained at 270 °C and 290 °C, respectively. The column temperature was maintained at 50 ºC for 5 min and then programmed to 180 ºC at a rate of 4 ºC/min, and finally increased to 260 °C at rate of 10 ºC/min for 10 min. Samples of 1 µL of essential oil diluted in 5% dichloromethane were injected and *n*-alkanes were used as reference points in the calculation of relative retention indexes. The percentage compositions were obtained from electronic integration measurements of peak area. The mass spectra were recorded on a Shimadzu GC-17A/MS QP5050A-GC-MS system (EI mode 70 eV, source temperature 270 °C, scanned mass ranged 43-350 amu). The operating conditions were as described above. The identity of each compound was determined by comparison of its retention index relative to C_8-C_{20} *n*-alkanes (Fluka Analytical, 1.0 mL Alkane Standard Solution), as well as of its spectra with the database library Wiley 229. The retention data (retention indexes) were compared to those of the literature (De Lima et al., 2009; Adams, 2007; Joulain & König, 1998).

Brine shrimp bioassay

The brine shrimp (*Artemia salina* Leach) lethality bioassay was carried out to investigate the toxicity of the essential oils of the leaves and stems. Brine shrimp eggs (Maramar) were hatched in artificial salt water (1.0 L of mineral water and 33 g of marine crude NaCl) and used after 24 h applying the modified literature method (Brasil et al., 2009; Parra et al., 2001). Experiments were conducted along with positive control and negative control and different concentrations (0.02; 0.04; 0.06; 0.12; 0.23; 0.25; 0.47; 0.50; 1.0; 5.0; 10.0; 25.0; 50.0 and 100 µg/mL to oil) in a set of three tubes per dose. The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes. Lethal concentration (LC50) values were obtained from the best-fit line plotting concentration versus percent lethality (Brasil et al., 2009; Meyer et al., 1982). The 50% lethal concentration (LC50) values of essential oil were determined by Probit analysis (SPSS 15.0).

V79 cell cytotoxic assay

Chinese hamster lung fibroblast cells (V79 cells) were cultured under standard conditions in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% heat-inactivated FBS (fetal bovine serum), 0.2 mg/mL L-glutamine, 100 IU/mL penicillin, and 100 µg/mL streptomycin. Cells were kept in tissue-culture flasks at 37 °C in a humidified atmosphere containing 5% CO_2 in air and were harvested by treatment with 0.15% trypsin-0.08% EDTA (ethylene diamine tetraacetic) in PBS (phosphate buffer solution). Cells (2x105 cells) were seeded in complete media and grown for one day prior to treatment with substances and before evaluation with MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay. The essential oils, dissolved in DMSO (dimethylsulfoxide), were added to FBS-free media to achieve the different designed concentrations (10, 25, 50 and 100 µg/mL), and the cells were treated for 3 h under standard conditions. The final DMSO concentration in the average never exceeded 0.2%, and the negative control was exposed to an equivalent concentration of solvent. 4-Nitroquinoline-N-oxide (4-NQO-0.2 µg/mL) was used as positive control. MTT reduction was performed according to Denizot & Lang (1986). Briefly after treatments, cells were washed once with PBS before the addition of 0.1 mL serum-free average containing yellow tetrazolium salt (MTT; 1 mg/mL) dye and incubated for 4 h at 37 °C. After incubation, the supernatant was removed and the purple formazan product was solubilized in 0.2 mL DMSO, stirred for 15 min, and its absorbance was measured at 570 nm. The absorbance of negative control (DMSO) cells was set as 100% viability and the values of treated cells were calculated as percentage of negative control (Péres et al., 2009).

Antifungal activity

The antifungal activity of the essential oil was investigated employing Disc Diffusion Method, according to CLSI (Clinical and Laboratory Standards) M44-A2 (2009). *Candida krusei* ATCC 6258, granted graciously by the Adolfo Lutz Institute of São Paulo and a sample of *Candida albicans* isolated from patients with onychomycosis from Teresina, Piauí. About 100 mg of the essential oil was dissolved in distilled water and acetone (20%) to a concentration of 100 mg/mL, 50 mg/mL, 25 mg/mL and 12.5 mg/mL. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth surrounding the oil. The complete antifungal analysis was carried out under strict aseptic conditions. The zones of inhibition were measured with antibiotic zone scale (Cecon-Brasil) in mm and the experiment was carried out in triplicates. The antifungal antibiotics (fluconazole, 25 µg; itraconazole, 10 µg; anfotericine B and terbinafine, 2 µg-Cecon-Brasil) and water/acetone 20% were used as positive and negative controls, respectively.

Results and Discussion

The identification of the essential oils' components was accomplished by comparison of their GC-MS retention indexes. Spectra were considered coincident if the similarity index was higher than 95%. To minimize the standard deviation arising from employing a sole substance as internal standard, we decided to employ a retention index, namely Kovat's Index obtained by the use of a mixture of the essential oil with eight *n*-alkanes as internal standards and using the equation of Van Den Dool & Dee Kratz (1963) and Kovat's Index experimental (KI_{exp}). This approach greatly improved the identification, especially for those compounds with very similar fragmentation patterns. We also used Kovat's indexes estimated by a computer program based on the least square linear regression that uses the retention times of a few known compounds in the chromatogram and compatibles Kovat's indexes from the literature (Alencar et al., 1990). The estimated Kovat's Index was done on the least square linear regression using six values of KI closer to values in the literature, two at each end and two intermediate, resulting in the following equation: KI=690.46404+0.0305 *Rt, where Rt represents the retention time of the sample constituent (R=0.99912; SD=14.2), (Figure 1).

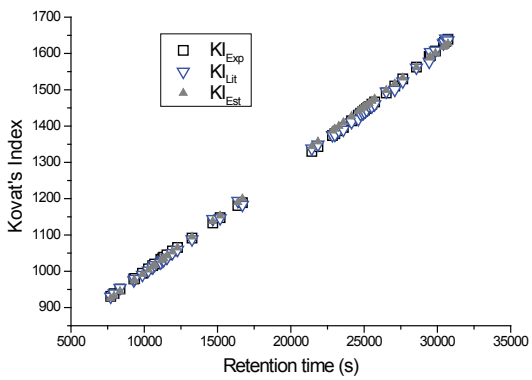

Figure 1. Comparison between values of Kovat's Index: KIexp (computed according to Van Den Dool & Dee Kratz (1963); KIest and KIlit*. KIa=Spectra were considered coincident if the similarity index was higher than 95%. We also used Kovat's indexes estimated by a computer program (Origin 5.0) based on the least square linear regression that uses the retention times of a few known compounds in the chromatogram and compatibles Kovat's indexes from literature Alencar et al. (1990). KI_{exp}=experimental Kovat's index (Van Den Dool & Dee Kratz (1963)).

The yields of essential oils obtained by hydrodistillation of the leaves and stems were 0.35% in June and 0.28% in September (w/w) and 0.21% in June and 0.13% in September (w/w), respectively. Table 1 gives the chemical composition and retention indexes of the compounds identified. In total 64 compounds were identified: accounting for 98.55% (in September of 2009) and 95.65% (in June of 2009) of leaves' oils; 98.78% (in June of 2009) and 99% (in September of 2008) of the stems' oil constituents, respectively. The most representative compounds of the oil were mono and sesquiterpenes. The oil from stems of *Lantana camara* L., Verbenaceae, was characterized by a large amount of sesquiterpenoids, with spatulenol (15.9%) and caryophyllene oxide (17.1% in June of 2009), as main components. β-Gurjunene (32.7%, in September of 2008) was the most prominent compound in the stems' oils, which was absent or at very low relative abundance in leaves. Unfortunately, there is no other study on stems' oil in the literature for comparison purposes. The main compounds found in the oil of the leaves in different months were β-caryophyllene (10.5%, in June of 2009), sabinene (7.98%, in September of 2008), limonene (7.68%, in September of 2008), spathulenol (11.64%, in September of 2008).

The results found show that there were significant differences between the essential oils yields obtained from different collection times (Sousa et al., 2009) as well as significant differences between the chemical composition in the leaves and stems. It can be observed that to obtain the highest essential oils yield, the collection must be carried out during the rainy season (when the yields are more significant).

In particular, the essential oil from leaves of *L. camara* showed a predominance of sesquiterpenes, similar to species found in other regions (Sousa et al., 2010; Costa et al., 2009; Silva et al., 1999; Randrianalijaona et al., 2005).

Statistical analysis demonstrated that oils showed qualitative and quantitative significant differences when a comparison is made across from the different months (Table 1). When comparing the results of our analyses with those in the literature we identify that our sample represents one new chemotype of *L. camara*. The very different compositions for *L. camara* are consistent with the wide diversity of chemical compositions observed in previous reports for these species (Da Silva et al., 1999).

Our findings indicate that the major compound of *L. camara* essential oils from leaves and stems are sesquiterpenes. We observed that the variability of the composition of essential oil content depends on season, climate and part of the plant studied. It should be noted that many sesquiterpenes identified in the essential oil extracted from leaves also occurred in the oil extracted from stems, independently of collection time. With concentration above 10%, the predominant compounds in the analysis described in the literature were: germacrene D, limonene, δ-curcumene, curcumene, davanone and 1,8-cineole (Ngassoum et al., 1999; Silva et al., 1999; Misra & Laatsch, 2000; Alitonou et al., 2004; Hernández et al., 2005; Sonibare & Effiong, 2008).

Table 1. Chemical composition of the essential oil, obtained by hydrodistillation, from leaves and stems of *L. camara.*

Components[a]	K.I.[b]	Area (%)			
		Leaves		Stems	
		June/2009	September/2008	June/2009	September /2008
α–thujene	931	0.99	----	----	----
α-pinene	938	3.06	----	----	----
camphene	951	0.31	1.47	----	----
β-pinene	974	----	3.68	----	----
sabinene	979	4.44	7.98	1.51	1.16
γ-pinene	980	0.25	----	----	----
myrcene	995	1.08	2.01	----	----
α-phellandrene	1007	0.76	3.17	----	----
δ-3-carene	1016	5.01	4.32	----	2.48
α-terpinene	1019	0.31	1.02	----	-
limonene	1024	----	7.68	1.51	2.22
β-ocimene	1032	----	2.38	----	----
p-cymene	1033	4.43	5.07	5.41	----
β-phellandrene	1037	4.82		----	----

Table 1. Chemical composition of the essential oil, obtained by hydrodistillation, from leaves and stems of *L. camara*. (cont.)

1,8-cineole	1039	1.00	2.67	1.31	1.47
cis-β-ocimene	1045	1.45	----	----	----
trans-β-ocimene	1056	2.20	----	----	----
γ-terpinene	1066	2.02	3.43	----	----
α-terpinolene	1091	0.87	1.36	----	----
linalool	1095	----	1.26	----	----
neo-allo-ocimene	1133	0.22	----	----	----
camphor	1148	0.08	0.75	----	----
terpinene-4-ol	1174	----	1.78	3.40	0.51
neo-dihydro carveol	1181	0.53	----	----	----
crytone	1189	0.14	----	----	----
δ-elemene	1330	0.28	----	----	----
bicicloelemene	1338	----	----	2.56	----
linalool propanoate	1339	----	----	1.69	----
α-terpinyl acetate	1344	3.65	----	----	----
α-ylangene	1374	0.20	----	----	----
α-copaene	1379	0.79	----	----	----
β-bourbonene	1387	0.44	----	3.63	----
β-elemene	1396	2.05	----	5.00	----
italicene	1404	----	----	----	1.05
α-cendrene	1414	0.53	----	----	----
iso-caryophyllene	1417	----	10.38	----	----
α-cis-bergamotene	1423	----	----	----	1.35
β-caryophyllene	1430	10.55	----	13.13	32.69
β-gurjunene	1435	0.56	----	0.47	----
γ-elemene	1440	1.50	----	0.47	----
α-guaiene	1444	0.37	----	----	----
guaiadiene <6,9>	1448	0.27	----	----	----
khusimene	1454	0.30	----	----	----
α-humulene	1460	1.99	1.45	2.23	3.30
allo-aromadendrene	1466	1.17	----	0.97	0.97
germacrene-D	1448	----	4.60	4.30	8.49
β-silinene	1487	----	----	1.05	----
γ-amorphene	1491	8.29	----	----	----
bicyclogermacrene	1510	10.62	11.69	12.96	13.93
δ-cadinene	1530	0.93	----	1.77	2.37
1,4-cadinadiene	1530	----	----	0.56	0.61
selina-3,5(11)-diene	1541	----	----	1.81	----
Junipene	1555	----	----	1.94	----
γ-gurjunene	1458	----	----	1.06	0.51
germacrene B	1562	2.73	0.97	-----	1.88
caryophyllene oxide	1581	----	5.57	17.10	6.63
spathulenol	1591	7.04	11.64	----	15.90
β-selinene	1592	----	1.16	----	----
khusimone	1594	1.23	----	----	----

Table 1. Chemical composition of the essential oil, obtained by hydrodistillation, from leaves and stems of *L. camara.* (cont.)

β-atlantol	1606	0.33	----	----	----
β-guaiene	1614	----	----	----	2.67
Ni	1623	----	1.31	----	----
1-epi-cubenol	1627	4.67	1.55	----	----
eemoligenol	1630	0.27	----	----	----
allo-aromadendrene epoxide	1633	0.24	----	0.91	----
β-acorenol	1639	0.68	----	----	----
Ni	1652	1.62	----	----	----
γ-cadinene	1665	----	----	7.90	----
Ni	1665	2.70	----	----	----
Ni	2120	----	----	3.59	0.21
neophytadiene	2223	----	----	0.59	----
Total (%)		99.97	99.86	98.78	99.21

[a]Compounds listed in order to their elution on the DB-5HT column. [b]Retention indexes on the DB-5HT column relative to C8-C_{20} *n*-alkanes. --- undetected. Ni: not identified.

The brine shrimp (*Artemia salina*) assay is a convenient preliminary toxicity test because brine shrimp is highly sensitive to a variety of chemical substances. Meyer et al., (1982) established a relationship between the level of toxicity and lethal concentration by extract of plants on larvae of *A. salina*, being toxic when those values are below 1000 mg/mL. Thus, we observed a high toxicity level of essential oils from leaves and stems of *L. camara* against *A. salina* with a lethal concentration LC50 0.234 µg/mL, with limits between 0.195 and 0.273 µg/mL (Figure 2) when compared to values for the controls beberine chloride (LC50 of 22.5 µg/mL) and strychnine sulfate (LC50 of 77.2 µg/mL) obtained by Meyer et al. (1982), confirming the earlier work which showed that *Lantana spp*. is highly toxic (Costa et al., 2009; Sonibare & Effiong, 2008).

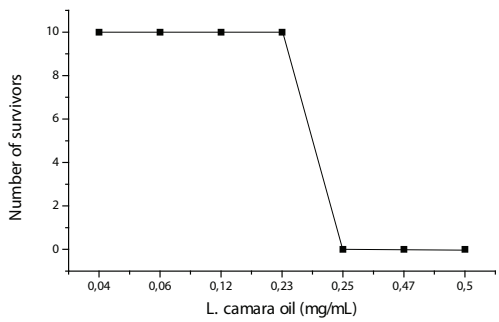

Figure 2. Cytotoxicity test with *A. salina* made with essential oil of *L. camara* in the range of concentrations between 0.02 a 0.5 mg/mL.

The cytotoxic effects of *L. camara* was evaluated in V79 mammalian cells. As can be seen in Figure 3, essential oil from *L. camara* leaves induces a significant decrease in cells survival at concentrations of 25 µg/mL and higher. The chemical composition analysis pointed as major compounds of the *L. camara* leaves essential oil the sesquiterpenes, bicyclogermacrene, (+) spathulenol, *trans*-caryophyllene and sabinene (Table 1). Generally, these major compounds determine the biological properties of the essential oils (Bakkali et al., 2008), nevertheless this effect can be influenced by the chemical composition and the synergism between the various components (Wright et al., 2007). Wright et al., (2007), working with essential oils from leaves of *Beilschmiedia* sp. nov., *Cinnamomum costaricanum*, *Ocotea Meziane* and *Ocotea* sp. nov. spatted that there was synergism between the major components and those minor one . Loizzo et al. (2008) evaluated various constituents of essential oils and found that α-pinene was inactive alone but had synergistic effect with other components of essential oil of *Cupressus sempervirens* spp. The cytotoxic effects of essential oil of *L. camara* can be related to the presence of terpenes (Bakkali et al., 2008).The terpenes as typical lipophiles can pass through the cytoplasmic membranes, disrupt the different layer of polysaccharides, fatty acids and phospholipids and permeabilize them, and the membrane damage can be related to essential oil cytotoxic effects (Bakkali et al., 2008). Moreover, the cytotoxic effects of these sesquiterpenes have been previously associated to normal and tumoral cells. Bicyclogermacrene has cytotoxic activity against HepG2 (hepatocellular liver carcinoma), MCF-7 (breast cancer), and PC-3 (prostate cancer) tumor cell lines, while spathulenol are toxic to both normal lung cells (MRC5) and gastric adenocarcinoma cells (AGS) (Boehme et al., 2008; Areche et al., 2009; Ashour et al.,

2009; Tundis et al., 2009). Effects of caryophyllene are controversial. Some studies indicate that caryophyllene, common in this species, does not exhibit any cytotoxic activity against human cells Prashar et al., (2006) and Legault et al., (2003), but other studies showed that β-caryophyllene is active against breast carcinoma cells lines BT-20 ATCC HTB 19, Kubo & Morimitsu (1995). According to Bakkali et al. (2008): α-terpinene, sabinene, limonene, cymene, β-caryophyllene have cytotoxic activities, dose dependent, when tested alone or added to the essential oil. But, the exact mechanism of these compounds is not known yet.

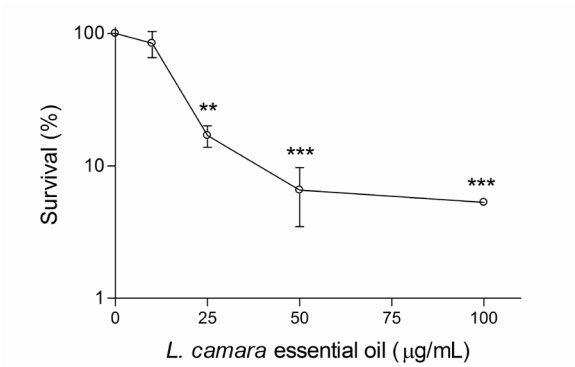

Figure 3. Cytotoxic effects of *L. camara* essential oil in V79 mammalian cells after 3 h exposure analyzed by MTT assay. Results are expressed as mean percentage in treated cells compared to control (solvent)±standard deviation of three independent experiments. Data significant in relation to control (solvent) at $^{**}p<0.01$ and $^{***}p<0.001$/one-way ANOVA–Dunet's multiple comparison test.

The relative efficacy of some commonly used antifungal antibiotics was compared with essential oil discs by employing the Filter paper Disc Diffusion Method. Table 2 summarizes the in vitro susceptibilities to the four antifungal agents (some of them used currently to treat candidiasis, Paiva et al., 2009) and for essential oil of *L. camara*. Resistance to fluconazole and terbinafine were mostly noted in *C. krusei*, but it was not observed in *C. albicans*, the most common pathogen among cutaneous candidiasis and of the oropharynx. *Candida krusei* is known to have intrinsic resistance, particularly to fluconazole and to azolic derivatives in general (Crocco et al., 2004; Rex et al., 2000). Cases of *Candida albicans* resistance to azolic derivatives in HIV-infected (human immunodeficiency virus) patients and in patients with invasive candidiasis were reported (Crocco et al., 2004). This essential oil remarkably inhibited the growth of the fungi tested: *C. krusei* ATCC 6258, and *C. albicans* isolated from patient with onychomycosis. However, its higher efficiency should be tested in larger concentration and also carefully check their toxicity.

Conclusions

In summary, when compared to literature data, our study evidenced some differences in the chromatographic profile as well as in quantitative composition of essential oil *Lantana camara* L., Verbenaceae. The cytotoxicity assay with *A. salina* was consistent with those obtained for V79 cells mammalian, and this oil remarkably inhibited the growth of tested fungi. Therefore, our data corroborate the toxicity of the essential oil of *L. camara*, as indicated in many references cited in this work; and because of its high toxicity all internal uses for humans must be excluded or done with great caution.

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Table 2. Antifungal activity of essential oil from leaves of *L. camara* in comparison with some antifungal antibiotics.

Essential oil inhibition (oil concentration 100 mg/mL, 50, 25 and 12.5 mg/mL)					Anfotericine	Fluconazole	Itraconazole	Terbinafine
Strains	Zone of inhibition in mm				Zone of inhibition in mm			
C. krusei	14	12	12	10	29	resistance	21	resistance
C. albicans	13	11	11	11	19	29	19	resistance

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