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Chemical composition, antimicrobial and anti-acetylcholinesterase activities of essential oil from *Lantana camara* (Verbenaceae) flowers

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***Lantana camara* L. is considered a weed, but is used in agriculture and traditional medicine due to its different pharmacological effects. The objective of this study was to determine the chemical composition and the bioactivity of essential oil from *L. camara* flowers collected in Boa Vista, Roraima, Brazil. Essential oil was obtained using a Clevenger type apparatus. The oil obtained was analyzed by gas chromatography coupled to flame ionization detector (GC-FID) and gas chromatography coupled to mass spectrometer detector (GC-MS) and its bioactivity was assayed against fungi and bacteria, as well as for acetylcholinesterase inhibition. The chemical composition of essential oil presented major compounds contrasting with the literature, while its bactericidal and fungicidal activity was excellent, reaching over than 90% inhibition against yeast. A potent anti-acetylcholinesterase activity, superior than 77% inhibition, was also observed.**

Keywords: *Lantana camara*, flowers essential oil, antiacetylcholinesterase, antimicrobial

INTRODUCTION

Lantana camara L. (Verbenaceae) is a shrub with distribution in tropical, subtropical and temperate regions, being considered a weed difficult to control. However, *L camara* has ornamental uses, and is reported to improve soil quality for agriculture as well as possessing insecticide, antifungal, and herbicide activities. In folk

medicine, this species is known by its sudorific and antipyretic activities, action on bronco-lung problems, rheumatism and against scabies (Patel, 2011; Passos et al., 2009; Lorenzi, 2008; Kohli et al., 2006; Lorenzi, 2002). The essential oil of its flowers and leaves possess a variety of chemical compounds with leishmanicidal, antimicrobial, anticancer, antiulcer, anti-inflammatory activities, among others. However, in high doses, these species can be toxic to some animals (Oyourou et al., 2013; Machado et al., 2012; Montanari et al., 2011; Sousa et al., 2011; Costa et al., 2009; Sharma and Kumar, 2009).

The objectives of this work was to analyze the chemical constitution of essential oil obtained from dried flowers of *L. camara* collected in Boa Vista, Roraima, and to evaluate its bioactivities on the acetylcolinesterase inhibition, and on the pathogenic microorganisms *Escherichia coli*, *Salmonella typhimurium* (Gram-negative bacteria), *Staphylococcus aureus* and *Streptococcus sanguinis* (Gram-positive bacteria), an yeast (*Candida albicans*) and the filamentous fungi *Aspergillusflavus* and *Fusariumproliferatum*.

MATERIALS AND METHODS

Plant material and essential oil extraction

The flowers of *L. camara* were collected in the Cauamé Campus of Federal University of Roraima (UFRR) in Boa Vista, Roraima, Brazil. The plant material was identified by José Ferreira Ramos (Instituto Nacional de Pesqusias da Amazonia, INPA), and a voucher specimen (268126) was deposited at the INPA Herbarium.

The flowers were dried at room temperature and 100 g of the sample were used to obtain the essential oil by hydrodistillation using a Clevenger type apparatus. The essential oil was dried over anhydrous sodium sulphate and stored at -20°C before analysis (Rubiolo et al., 2010; Sefidkon, 2002).

GC/FID analysis

The essential oil was analyzed on a HP 7820A Gas Chromatograph (GC) equipped with a flame ionization detector (FID) using a capillary column (HP5 30 m × 0.32 mm × 0.25 microns, Agilent). Column temperature: 70°C (0 min) at 3°C min^{-1} up to 240°C. Gun: 250°C Split (1:30). FID Detector: 260°C. Carrier gas: hydrogen at 3 ml min^{-1}. Vol injection: 1 µl. Essential oil was diluted at 1% in chloroform. Data acquisition software used was Compact EZChrom Elite (Agilent). The quantitative analysis was accomplished using standard areas from the chromatograms obtained by GC-FID.

Gas chromatography/mass spectrometry analysis

A GCMS-QP2010 ULTRA (Shimadzu) was used. Column: Rxi-1MS 30 m × 0.25 mm × 0.25 microns (Restek). Column Temp: 70°C (2 min), 5°C min^{-1} to 250°C. Injector: 250°C Split (1:20), GC-MS interface at 250°C. MS detector (electron impact at 70 eV) temperature was 250°C. Carrier gas: helium at 1.5 ml min^{-1}. Vol injection: 1 µl. Essential oil was diluted at 0.1% in chloroform.

Data acquisition software used was GC-MS Solution (Shimadzu) together with NIST11 library. Identification of peaks was made by comparison of the mass spectra obtained by GC-MS spectra with the NIST11 library and also by comparing the Kovats indices calculated by GC-FID and literature data.

Antibacterial and yeast assay

E. coli (ATCC 25922), *S. tiphymurium* (ATCC 14028), *S. aureus* (ATCC 25923) and *S. sanguinis* (ATCC 49456) bacteria and *C. albicans* (ATCC 18804) yeast were used in the assay. Concentrations assayed were 500, 250, 125, 62.5, 31.25, 15.6, and 3.9 µg ml^{-1} (Zacchino and Gupta, 2007). Samples were weighed and dissolved in DMSO to 50 mg ml^{-1}. 40 µl of this solution was added to a flask containing 960 µl of BHI (Brain Heart Infusion) broth (working solution). A pre-inoculum was prepared in which the bacteria and the yeast, stored under refrigeration, were transferred with a platinum loop to test tubes containing 3 ml of freshly made BHI broth. The tubes were incubated at 37°C for 18 h. Then, the pre-inoculum (500 µl) was transferred to tubes containing 4.5 ml of sterile distilled water. The tubes were homogenized and the concentration adjusted to 0.5 of McFarland turbidity standard (10^8 CFU ml^{-1}), thereby obtaining the inocula used in the bioassays.

Assays were performed in 96-microwell plates in duplicate. 100 µL of BHI broth was added to each well. In the first well, 100 µl of working solution were also added. The solution was homogenized and 100 µl transferred to the next well and so on until the last well, from where 100 µl was discarded. Then, 100 µl of microorganism inocula were added to wells. Eight different concentrations of each sample were tested. A positive control devoid of the working solution allowed us to examine microorganism growth. A negative control, which lacked the inoculum permitted us to discount the colour coming from the working solution. A control plate containing 100 µl of BHI culture medium and 100 µl of sterile distilled water were added to the experiment as a control of BHI broth sterility.

Another control was also prepared, containing the standard antibiotics Ampicillin (antibacterial), miconazole and nystatin (antifungals) to observe the activity of these antibiotics over the microorganisms. Microorganism growth was measured in ELISA plate reader (492 nm) immediately after ending the experiment (0 h). They were incubated at 37°C and read again after 24 h of experiments, ending the test. Results were calculated as percentual inhibition using the formula:

$$\% \text{ inhibition} = 100 - AC - AC \times 100AH - AM$$

AC = absorbance of the sample; AC = absorbance of control sample; AH = absorbance of microorganisms in the control control and AM = absorbance of culture medium control.

Filamentous fungi assay

Filamentous fungi used in this test were A. flavus (CCT 4952) and

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F. proliferatum (CML 3287). DMSO was used for sample preparation and the concentration of sample in the assay was 250 mg mL^{-1}. Sabouraud broth was used for fungal growth. A spore suspension at a concentration of 5×10^{-5} spores ml^{-1} was used after spores counting on a Neubauer chamber. The sample incubation time was 48 h after which absorbance was read at 490 nm on a microtitre plate reader. Data were processed using the Outlier method, Grubbs test with 95% significance level. The percentage of inhibition was calculated by using the formula:

$$\% \text{ inhibition} = 100 - AC - AC \times 100AH - AM$$

AC = absorbance of the sample; AC = absorbance of control sample; AH = absorbance of microorganisms in the control control and AM = absorbance of culture medium control.

Acetylcholinesterase (AChE) inhibition assay

Aliquots of a working solution (25 µl) (sample in DMSO 10 mg ml^{-1}) were added to microplate wells, positive and negative controls were also prepared. To the first five wells of a column (positive control), 25 µl of an eserine solution prepared at 10 mg ml^{-1} (in Tris/HCl at pH 8.0) was added. Then, 25 µl of acetylthiocholine iodide (ATChI, Sigma A5751); the reaction mixture, 125 µl of 5',5-dithio-bis (2-nitrobenzoate) (DTNB, Sigma D8130) and 50 µl of Tris/HCl (pH 8) containing 0.1% (m/v) bovine serum albumin were added to each well. Absorbance was measured at 405 nm every 1 min for 8 times. Then 25 µl (0.226 U ml^{-1}) of Electric eel AChE (type VI-S) provided by Sigma (C3389-500UN) in Tris/HCl were added to each well. Absorbance was measured at 405 nm by 10 times (Frank and Gupta, 2005; Ellman et al., 1961). Percentual inhibition was calculated using the formula:

$$\% \text{ inhibition} = 100 - \frac{(PCA)}{(PSA)} \times 100$$

Where PCA = (absorbance of the sample with enzyme - absorbance of the sample without enzyme); PSA = (absorbance of negative control with enzyme - absorbance of negative control without enzyme).

RESULTS AND DISCUSSION

The essential oil from flowers of *L. camara* provided 0.30% yield (Table 1), a yield lower than that obtained for an Iranian sample, 0.63% (Sefidkon, 2002), but was higher than that obtained for an India species, 0.08% (Khan et al., 2002). GC-FID and GC-MS analyses of the essential oil of *L. camara* showed presence of 20 compounds, accounting for 82% of the total components (Figure 1, Table 1). The principal compounds and their respective concentrations in the essential oil of *L. camara* dried flowers were germacrene D (23.7%), germacrene B, (13.2%), β-caryophyllene (9.5%), longicyclene (8.4%), followed by β-sesquiphellandrene (4.9%), EE-farnesene (4.9%) and γ-muurolene (3.7%), also varied.

The concentrations of compounds detected in the essential oil of *L. camara* flowers showed to be influenced by the collection spot. For instance, in the oil obtained from a *L. camara* voucher collected in India (Khan et al., 2002), the major compounds identified were β-elemene (14.5%), germacrene D (10.6%), α-copaene (10.7%), α-cadinene (7.2%), β-caryophyllene (7.0%) and γ-elemene (6.8%); on the other hand, the essential oil obtained from *L. camara* flowers grown in Iran showed sabinene (16.5%), β-caryophyllene (14.0%), 1,8-cineole (10.0%), bicyclogermacrene (8.1%) and α- humulene (6.0%) as major compounds (Sefidkon, 2002). El Baroty et al. (2014) report the essential oil chemical composition of L. camara flowers obtained in Cairo, Egypt, where the majority chemicals differ from those presented in Table 1 among other differences. The yield differences, variations in chemical composition and their respective yields can be result of a number of biotic and abiotic factors (Figueiredo et al., 2008). The variations in the chemical composition can lead to different bioactive effects against diseases and microorganisms responsible for causing various pathologies. *L. camara* essential oil was assayed for acetylcholinesterase inhibition and, as the result, a good inhibition was detected reaching 77.15% of inhibition.

Neurodegenerative syndromes like Alzheimer's disease have been causing great concern worldwide. This disease causes difficulties in language, memory, emotional behaviour, personality and cognitive abilities (Singh et al., 2013). The World Health Organization (WHO), presents alarming data for Alzheimer's disease. Since it was estimated in 2010 about 35.6 million people were already suffering with this illness, with projections that this value would be tripled by 2050, with approximately 115.4 million people directly affected by Alzheimer's disease (WHO, 2012). Plants components have been extensively screened for their potential for acetylcholinesterase inhibition, since many plants can be used for the treatment of neurodegenerative diseases (Mukherjee et al., 2007). According to the classification for acetylcholinesterase reducing potential of crude extracts, weak inhibitors present inhibitory value below 30%; moderate inhibitors present 30 to 50% inhibition, and potent inhibitors show over 50% of inhibition enzyme (Vinutha et al., 2007).

Considering this, essential oil of *L. camara* dried flowers stands out as a potent inhibitor of AChE enzyme. This inhibitory potential may have been highlighted by the synergism of the several chemical constituents present. It has been found that the inhibitory potential can be associated to synergistic compounds. As an example, interactions between 1,8-cineole/α-pinene and 1,8-cineole/caryophyllene oxide are reported to improve reduction of acetylcholinesterase activity; the same effect can be also caused by miscellaneous compounds (Singh et al., 2013; Savalev et al., 2003). Therapies involving essential oils can be accomplished in several ways, the

Table 1. Percentage composition of the *L. camara* flowers essential oil.

RI*	RI (Lit)**	Compound	Percentage
941	939	α-Pinene	-
978	981	Sabinene	0.2
983	972	β-Pinene	-
1009	1004	δ-3-Carene	0.5
1032	1030	1,8-Cineole	-
1108	1100	Linalool	2.7
1315	1340	δ-Elemene	0.4
1323	1328	α-Cubebene	0.8
1362	1373	Longicyclene	8.4
1378	1385	β-Cubebene	2.3
1381	1393	β-Elemene	2.3
1407	1425	β-Caryophyllene	9.5
1418	1436	δ-Muurolene	3.7
1443	1467	Humulene	2.8
1475	1487	Germacrene D	23.7
1491	1517	Bicyclogermacrene	0.7
1497	*1500*	*E,E*-Farnesene	4.9
1522	1560	β-Sesquiphellandrene	4.9
1554	1562	Germacrene B	13.2
1581	1573	Caryophyllene oxide	0.9
Others			18.1
Total			100.0
Extraction yield (%)			0.30

*RI: Retention index calculated by GC using an *n*-alkane series under the same conditions as for samples; **RI (Lit): Retention index (Literature); Flavornet by Terry Acree and Heinrich Arn (2004).

Table 2. Inhibition of gram (+) and gram (-) bacteria by the essential oil from *L. camara* flowers.

Bacteria	500 µg ml^{-1} (%)	250 µg ml^{-1} (%)	125 µg ml^{-1} (%)	31.25 µg ml^{-1} (%)	3.91 µg ml^{-1} (%)
E. coli	42	35	23	17	19
S. typhimurium	81	52	48	51	44
S. aureus	89	56	44	17	-
S. sanguinis	92	7	6	-	-

- : No known inhibition.

most common being aromatherapy. It was possible to observe improvement in the cognitive function of patients with Alzheimer's through aromatherapy (Savalev et al., 2003). Essential oils act in the central nervous system with excellent results in improving the living conditions and treatment of several diseases, especially neurodegenerative diseases like Alzheimer's and Parkinson (Dobetsberger and Buchbauer, 2011; Jimbo et al., 2009).

Filamentous fungi *A. flavus* and *F. proliferatum* were inhibited by *L. camera* essential oil (44.52 and 28.97%, respectively). These microorganisms affect humans and crops causing a lot of damage, especially economic losses in various parts of the world (Mulè et al., 2004; Howard, 2002). Gram (+) and gram (-) bacteria (Table 2) and the yeast were inhibited in the assay showing activity

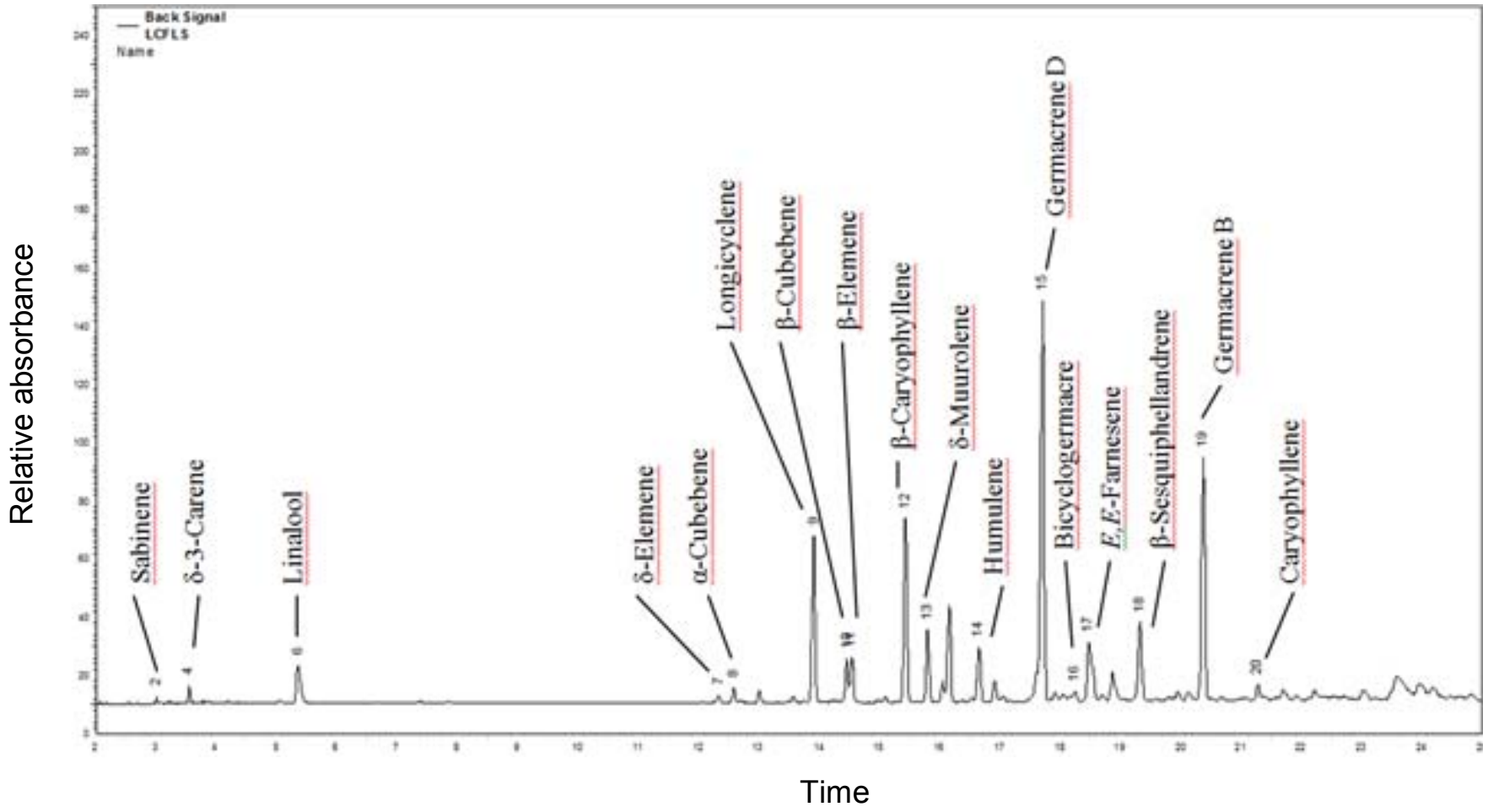

Figure 1. Chromatogram of the essential oil from *L. camara* flowers.

for the essential oil from L. camara flowers. The values ranged from 3.91 µg ml^{-1} to 500 µg ml^{-1}, potentially some satisfactory results in this bioassay. Ampicillin was used as a control, a standard clinical use of antibiotics, was very efficient in the concentrations shown. Growth of the pathogenic yeast, C. albicans, was inhibited more than 90% at all concentrations. It is noteworthy that there was 95% of *C. albicans*

inhibition at 15.6 µg/ml, while the positive controls, miconazole and nystatin where less active at the same concentration (92 and 91%, respectively).

Pathogenicity of this yeast is usually associated to low immunity in decurrence of aging, infection or therapies, which can lead to the development of candidiasis. This infection affects the skin, oral cavity, esophagus, gastrointestinal tract, vagina and the human vascular system (Calderone and Fonzi, 2001). The above results reveal the potential of this essential oil against pathogenic microorganisms, broadening the biological importance of the species, already known for its actions as antipyretic, antimutagenic, and insecticide, among others (Naz and Bano, 2013; Seth et al., 2012; Zandi-Sohani et al., 2012; Kurade et al., 2010; Sharma and Kumar, 2009; Sonibare and Effiong, 2008; Verma and Verma, 2006; Barre et al., 2005; Deena and Thoppil, 2000; Siddiqui et al., 1995).

CONCLUSION

Despite *L. camara* is considered a weed, it has several biological benefits, being a promising source of a natural and powerful oil that acts as an AChE inhibitor. This essential oil may be a possibility for the development of new medicines to treat neurodegenerative diseases. Besides, it has efficacy against pathogenic microorganisms that affect humans, therefore it is used as an alternative antibiotic is also suggested.

Abbreviations

AChE, Acetycholinesterase; **GC-FID,** gas chromatography using flame ionization detector; **AD,** Alzheimer's disease.

Conflicts of interest

Authors have none to declare.

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