Artículo original

Chemical composition and insecticidal action of essential oil from *Lantana montevidensis* (Spreng.) Briq. (Chumbinho) against *Nauphoeta cinerea*

Composición química y evaluación inseticida del aceite esencial de *Lantana montevidensis* (Spreng.) Briq. (Chumbinho) contra *Nauphoeta cinerea*

José Weverton Almeida Bezerra[1*] https://orcid.org/0000-0002-0966-9750
Maria Ivaneide Rocha[1] https://orcid.org/0000-0002-0312-9315
Allana Silva Rodrigues[1] https://orcid.org/0000-0003-2607-0632
Gledson Ferreira Macedo[1] https://orcid.org/0000-0002-2609-6894
Luana Vinuto Silva[1] https://orcid.org/0000-0001-9082-7471
Jeane Dantas Sousa[1] https://orcid.org/0000-0001-7965-8793
Viviane Bezerra da Silva[1] https://orcid.org/0000-0003-0581-2609
Débora de Menezes Dantas[1] https://orcid.org/0000-0002-2839-3550
Ma Aparecida Barbosa Ferreira Gonçalo[1] https://orcid.org/0000-0003-4974-2606
Rodolfo Sérgio de Oliveira[1] https://orcid.org/0000-0002-1716-6942
Luciano Temoteo dos Santos[1] https://orcid.org/0000-0002-9215-6832
Helder Cardoso Tavares[1] https://orcid.org/0000-0002-4379-9786
Samara Mendes de Sousa[1] https://orcid.org/0000-0002-5429-3844
Niwiarakelly da Silva Monte[1] https://orcid.org/0000-0001-6801-2591
Karina Vieralves Linhares[1] https://orcid.org/0000-0001-6567-3271

[1]Regional University of Cariri. Crato, CE, Brazil

[*]Corresponding author: weverton.almeida@urca.com

ABSTRACT

Introduction: Verbenaceae stand out among the botanical taxa containing species rich in essential oils. One species from this taxon, *Lantana montevidensis* (Spreng.) Briq., commonly known as trailing lantana, is characterized by containing monoterpenes and sesquiterpenes, essential oils with biological activity.

Objective: identify the chemical constituents of *L. montevidensis* essential oil and evaluate their insecticidal activity against *Nauphoeta cinerea*.

Method: *L. montevidensis* leaves were collected, dried and subjected to hydrodistillation. Chemical characterization was then performed by gas chromatography / mass spectrometry. For the biological assay against cockroaches, 20-day-old nymphs were selected and subjected to various concentrations (50 - 1 000 µg/ml of oil airborne), mortality being evaluated throughout 24 hours of exposure to the EO. Ethanol (C_2H_6O) was used as positive control.

Results: the study found that *L. montevidensis* oil has low insecticidal action, since mortality was only 10% in the 500 µg/ml group, whereas in the positive control group it was 95% at a 500 µg/ml concentration. Regarding the chemical composition of the oil, a total 22 constituents were found, two of which prevailed: β-caryophyllene (33.72%) and germacrene D (31.98%).

Conclusion: *L. montevidensis* displays insecticidal activity and contains sesquiterpene compounds.

Key words: terpenes; cockroaches; GC / MS; biopesticide

RESUMEN

Introducción: entre los taxones botánicos que presentan especies ricas en aceites esenciales, se destaca la Verbenaceae. Una especie de este taxón, *Lantana montevidensis* (Spreng.) Briq., conocida como *chumbinho*, es característica de presentar monoterpenos y sesquiterpenos, que juntos forman los aceites esenciales que presentan actividades biológicas.

Objetivo: identificar los constituyentes químicos del aceite esencial de *L. montevidensis* y evaluar su actividad insecticida contra *Nauphoeta cinerea*.

Metodología: las hojas de *L. montevidensis* fueron recolectadas, secas y sometidas a la hidrodestilación, posteriormente fue realizada su caracterización química por medio de la Cromatografía Gasosa acoplada a la Espectrometría de Masas. Para el ensayo biológico contra las cucarachas, fueron seleccionadas ninfas con 20 días de edad, las cuales fueron sometidas a diferentes concentraciones (50 - 1000 µg/mL de aceite por aire) siendo evaluada la mortalidad a lo largo de 24 horas de exposición del AE. Como control positivo se utilizó el etanol (C_2H_6O).

Resultados: el estudio apunta que el aceite de *L. montevidensis* presenta baja acción insecticida, ya que solo hubo un 10 % de mortalidad en el grupo de 500 µg/mL, mientras que en el control positivo hubo 95 % de mortalidad con la concentración de 500 µg/mL. En cuanto a la composición química del aceite, se encontraron un total de 22 constituyentes, que presentó dos constituyentes mayoritarios: el β-Cariofileno (33,72 %) y el Germacreno D (31,98 %).

Conclusión: el aceite esencial de *L. montevidensis* presenta acción insecticida y presenta compuestos sesquiterpénicos en su constitución.

Palabras clave: terpenos; cucarachas; GC/MS; biopesticida.

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Introduction

According to Brazilian legislation, agrochemicals are products resulting from physical, chemical or biological processes, which are used in agricultural production sectors. These products have as purpose the preservation of damages caused by pests to livestock.[1] However, they have negative effects on human health and the environment, since their formulas contain toxic compounds that can lead to mortality. And a current problem in our Brazilian territory is that in this the consumption of pesticides is the largest in the world.[2]

The pesticides are used in the fight against pests, which constitute the great majority of the atroopods. Among the pests are cited the Diptera and Blattodea orders, which have insects that bring economic losses in the event of the first order, is cited species *Ceratitis capitata* (Wiedemann) known as “mediterranean fly”. Already Blattodea, several species of cockroaches are known as being pests, like *Periplaneta Americana*.[3] Thus, over the years there has been an increase in the use of synthetic insecticides, which have caused the resistance of the target organisms and other consequences such as intoxication for non-target organisms.[4]

In this way, the demand for non-synthetic insecticides has increased and a positive fact is that in the evolutionary scale, the plants have acquired the possibility of producing several compounds that do not have a direct function in their growth and development, in which they are denominated as secondary compounds.[5] These compounds are linked to the protection of the plant, so that they can be used in the formulation of insecticides. These metabolites are divided into three chemically distinct groups terpenes, nitrogen compounds and phenolic compounds.[6]

Among the chemical groups mentioned, the most frequently studied insecticidal activity are terpenes, which in the case of monoterpenes and sesquiterpenes are volatile. The choice of these derivatives is due to two factors, the first is because the chemical constitution of the plants is variable, thus the pests would not present resistance in a long time, and the second is because the plants have low toxicity to humans, rapid degradation and reduced environmental impact.[7]

A plant rich in secondary components is the species *Lantana montevidensis* (Spreng.) Briq., commonly known as "chumbinho", is native both in Brazil and Uruguay, and in other parts of the world is considered an invasive species.[8] There are reports in the literature that the essential oil of *L. montevidensis* (EOLM) presents biological activities such as antibiotic modulator and antibacterial and antioxidant activity.[9,10] However, there are no records in the literature evidencing its insecticidal potential against organisms of the order Blattodea.

In recent years, considerable interest has been generated in the *Nauphoeta cinerea* known as cockroachlobster, a cockroach ovovivipara, as a potential alternative model for use in pharmacological research and basic toxicological studies. *Nauphoeta cinerea* mimics the behavioral and biochemical neuro changes observed in conventional animal models. These cockroaches are ideal experimental models because of their rapid reproduction cycle, small size and easy maintenance in the laboratory.[11]

Therefore, because of a great demand for alternative insecticides, this study aimed to evaluate the possible insecticidal effects of the essential oil of *L. montevidensis* leaves against the arthropod-model *N. cinerea* as well as to characterize this natural product phytochemically.

Methodology

Plant Material

The fresh leaves of *L. montevidensis* were collected in January 2015 in the Garden of Medicinal Plants of Regional University of Cariri - URCA in the city of Crato - Ceará - BR in time from 08:00 hrs, with latitude coordinates 7°22'S; -39°28' with an altitude of 490 m. The plant material was identified by Dr. Karina Vieralves Linhares, and deposited in the Herbarium Caririense Dárdano Andrade Lima - HCDAL of the Regional University of Cariri - URCA with #12,377.

Extraction of Essential Oil

The extraction of the essential oil of *L. montevidensis* (EOLM) was carried out in a hydrodistillation system as proposed by Matos,[12] with some modifications. For this purpose, after drying the leaves were ground to increase the contact surface and, consequently, to obtain a higher yield of OE. In the hydrodistillation system 150 g of the dried leaves were conditioned and heated to boiling for 2 hours. The oil was collected with a glass pipette and stored in a refrigerator at -10 °C until the completion of chemical and insecticide activity analysis.

Chemical Composition of Essential Oil

The essential oil after preparation was submitted to GC analysis in a Varian 3800 Gas Chromatograph equipped with a capillary fused silica column (25 m × 0.25 mm) coated with SE-54. The GC conditions used were: carrier gas He (1 mL/min); on column injector 200 °C; FID 250 °C; column temperature 60 °C to 325 °C at 4 °C/min. GC-MS analyses were performed on an HP 5973 - 6890 GC-MSD system operating in the EI mode at 70 eV, equipped with an HP-5 cross-linked capillary column (30 m × 0.25 mm). The temperature of the column and the injector were the same as those from GC. Identification of the constituents of *L. montevidensis* essential oil was based on retention index (RI), determined with reference of the homologous series of n-alkanes, C7-C30, under identical experimental conditions, comparing with the mass comparison of the mass spectra with those of NBS Library and those described by Adams.[13] The relative amounts of individual components were calculated based on the CG peak area (FID response).

Inventory and Creation of *Nauphoeta cinerea*

The cockroaches, *N. cinerea* were obtained from the Federal University of Santa Maria - UFSM provided by Professor Dr. João Batista Teixeira da Rocha. They were created and maintained at the Laboratory of Microscopy - LABOMIC, of the Regional University of Cariri - URCA, under temperature conditions of 25 ± 5 °C and relative humidity of 50%. The diet of adult cockroaches and nymphs consisted of dog food and water at will.

Insecticidal test against *Nauphoeta cinerea*

For the insecticidal assay the essential oil of *L. montevidensis* was contacted with filter paper and then attached to the 330 mL volume flask lid. Subsequently, 40 nymphs per group with 20 days of age were submitted to different concentrations of essential oil (50 - 1000 μg/mL oil per air) and mortality was evaluated over 24 hours of OE exposure. The assay was accompanied by a negative control and a positive control. In this case, for the positive control ethanol (C_2H_6O) was used, since it is toxic and volatile. The protocol followed the methodology of Bezerra *et al*.[(14)]

Statistical analysis

Statistical analyzes were performed using the software GraphPad Prism 6, using One-Way Variance Analysis (ANOVA), followed by the Tukey test at 95% reliability (p <0.0001).

Results

Chemical composition

The essential oil of *L. montevidensis* is constituted of mono and sesquiterpenes. The phytochemical analysis identified a total of 98.78% of the oil composition, so that 18 constituents were identified (Table 1). As a major constituent Germacrene D was identified with a total of 31.98% of the general constitution, followed by β-Caryophyllene (27.05%). As trace components, that is, less than 1% constitution, camphene (0.12%) were identified, sabinene (0.31%), camphor (0.49%), *t*-sabinene hydrate (0, 79%), β-Elemeno (0.82%), spathulenol (0.98%) so these types of constituents totaling 3.51%.

Table 1 - Chemical composition of *L. montevidensis* essential oil.

Constituents	**RI[a]**	**RI[b]**	**Chemical composition (%)**
Camphene	953	951	0,12
Sabinene	976	675	0,31
Terpinolene	1088	1079	2,01
Cis-Linalool Oxide	1074	1074	3,72
Linalool	1098	1199	1,11
Camphor	1143	1141	0,49
t-sabinene hydrate	1254	1257	0,79
α-Copaene	1376	1376	5,74
β-Elemene	1391	1389	0,82
β-Caryophyllene	1404	1401	27,05
(E)-Caryophyllene	1418	1423	2,39

Aromandendrene-allo	1461	1460	1,21
α-Humulene	1454	1451	5,11
Germacrene D	1480	1480	31,98
Bicyclogermacrene	1494	1497	6,04
α-Cadidene	1538	1538	3,84
Spathulenol	1576	1573	0,98
Caryophyllene Oxide	1581	1585	5,07
Total identified (%)	-	-	98.78

Relative proportion of essential oil constituents was expressed as a percentage.
[a]Relative Retention Index (Adams[11])
[b]Experimental Retention Index (based on the homologous series of *n*-alkane C_7-C_{30}).

Insecticide Activity of EOLM Against *Nauphoeta cinerea*

According to figure 1, it was demonstrated that the essential oil of *L. montevidensis* presented low insecticidal activity against *N. cinerea*, since at the concentration of 500 µg/mL there was only 10% mortality, while the positive control group had 100 % mortality from 50 µg/mL. The group of 1000 µg/mL caused a mortality of 25% in the nymphs.

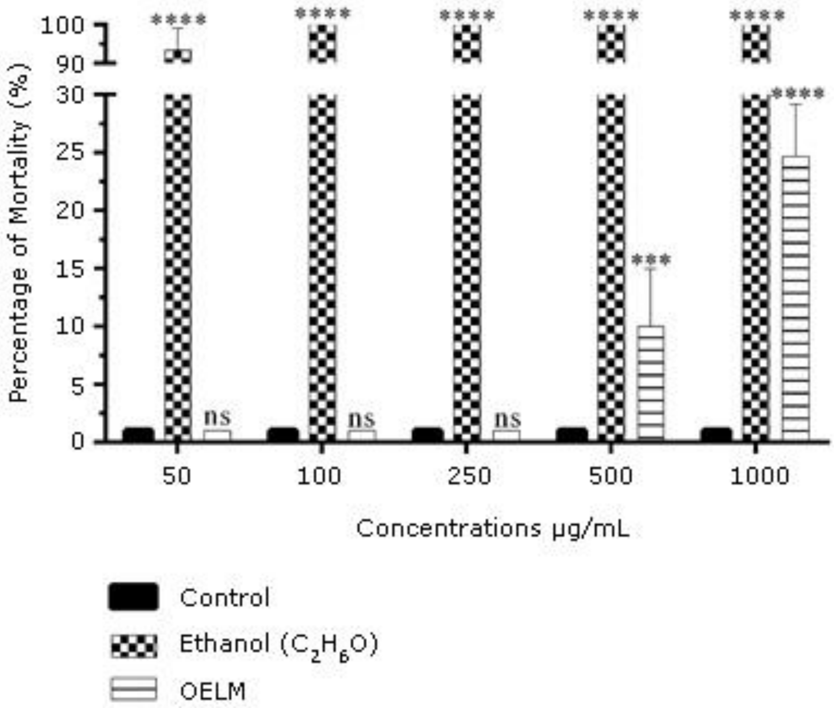

Fig. 1 - Insecticidal effect of the essential oil of *L. montevidensis* leaves (OELM), in front of the nymphs of *N. cinerea*. ns = no statistical significance by Anova One-way followed by Tukey *P*<0.05.

Discussion

Our phytochemical results corroborate with Sousa *et al*.[10] regarding the major compounds, however there are some differences in EO composition as for percentages. These variations in the amount of constituents may be due to differences in cultivation, collection periods, climatic stress, and especially the geographic origin of the plant.[15,16] An alternative to avoid a wide variation in the essential oil composition is that it should be extracted under the same conditions, resulting in a more constant composition.[17]

The essential oils are complex mixtures of monoterpenes and sesquiterpenes, which confer protection on plants, in some of these the composition may be heterogeneous, since the action of phyto-constituents may be joint.[18] Whereas in homogeneous oils, the action depends more on the quantity of that constituent. As with *Vanillosmopsis arborea* Barker (Asteraceae), which presents α-bisabolol as the major constituent with a presence percentage of 91.02% and a total of 4 constituents.[19]

Despite the low insecticidal action of *L. montevidensis* essential oil against the cockroach *N. cinerea*, this oil has an effective insecticidal action against the dipteran *D. melanogaster* This is justified because they are different organisms, however much they belong to the same order Hexápoda, the organization of these beings are different, while the cockroach is hemimetabola, the fly is holometabola, in this way there are biological differences.[20]

Although the insecticidal action of the oil has been low, this action can be attributed to the synergism of the chemical constituents present in the essential oil, since they presented moderate toxicity when tested alone and greater toxicity when combined with two or more constituents.[3] As reported by Di Pasqua *et al*.,[21] essential oils are typical lipophilic, and can cross the plasma membrane and affect its structures, such as polysaccharides, fatty acids and phospholipids, damaging the cytoplasmic components. Such damage can lead to the leakage of macromolecules and cell lysis, consequently, the death of biological organisms. This may explain the higher toxicity of the essential oils when compared to extracts.[22]

In this way, the essential oil of *Lantana montevidensis* presented a moderate insecticidal activity against the cockroach *Nauphoeta cinerea*, so that in high concentrations the oil is capable of inducing the mortality of the nymphs of the organism under study. The insecticidal action may be related to the phytochemicals of the oil, in this study germacrene D and β-Caryophylleno were identified as major. In this way, the isolation and tests evaluating the insecticidal action of these compounds must be realized.

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Conflicto de intereses

Los autores no refieren conflictos de interés

Authors contribution

José Weverton Almeida Bezerra: Planned the project and wrote the article.

Maria Ivaneide Rocha: Carried out phytochemical studies of essential oil.

Allana Silva Rodrigues: Helped to identify the species.

Gledson Ferreira Macedo: Carried out the bioassays with the cockroaches.

Luana Vinuto Silva: Carried out the bioassays with the cockroaches.

Jeane Dantas Sousa: Performed work orientation.

Viviane Bezerra da Silva: Extracted the essential oil of the species.

Débora de Menezes Dantas: Was responsible for all data collection and statistical analysis.

Ma Aparecida Barbosa Ferreira Gonçalo: Carried out phytochemical studies of essential oil.

Rodolfo Sérgio de Oliveira: Participated in article writing.

Luciano Temoteo dos Santos: Identified in the field the possible species with the presence of essential oils.

Helder Cardoso Tavares: Identified in the field the possible species with the presence of essential oils.

Samara Mendes de Sousa: Performed work orientation

Niwiarakelly da Silva Monte: Performed work orientation.

Karina Vieralves Linhares: Helped to identify the species.