SciMedCentral

Journal of Pharmacology & Clinical Toxicology

Short Communication

Antimicrobial Activity of Lippia Grata Schauer Essential Oil from Plants Submitted to Different Management Conditions-Short Comunication

Ana Valéria Vieira de Souza[1]*, Uiliane Soares dos Santos[2], Camila Hernandes[3], Flávio José Vieira de Oliveira[4], and Ana Maria Soares Pereira[3]

[1]*Embrapa Semiárido – Centro de Pesquisa Agropecuária do Trópico Semiárido (CPATSA), Brazil*

[2]*Universidade Estadual de Feira de Santana, Feira de Santana, Brazil.*

[3]*Universidade de Ribeirão Preto, Ribeirão Preto, Brazil*

[4]*Universidade Estadual da Bahia, Brazil*

***Corresponding author**

Ana Valéria Vieira de Souza, Embrapa Semiárido – Centro de Pesquisa Agropecuária do Trópico Semiárido (CPATSA), 56302-970, Petrolina, PE, Brazil, Email: ana.souza@embrapa.br

Submitted: 16 October 2018

Accepted: 24 October 2018

Published: 27 October 2018

ISSN: 2333-7079

Keywords

- Caatinga
- Verbenaceae
- Thymol
- Carvacrol
- Medicinal plant

Abstract

Lippia grata Schauer is a native Caating a species and presents high antimicrobial potential due to the presence of essential oils. Considering that different cultivation conditions can alter the content and chemical composition of essential oils with direct interference in antimicrobial activity, the objective of this work was to evaluate the antimicrobial activity of *Lippia grata* essential oil obtained from plants submitted to different management conditions in the field. The cultivation experiment was conducted with plants submitted to different doses of organic and mineral fertilization, in the presence and absence of irrigation and the essential oils were analyzed by CG-MS / CG-DIC. The antimicrobial assays were conducted against the strains *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger*. The major substances found in the essential oil were carvacrol, followed by thymol and the proportion of these compounds varied according to the type of fertilization and the presence or absence of irrigation. The essential oils extracted from plants of *L. grata* submitted to different cultivation conditions presented variation regarding the antimicrobial activity, being effective against all pathogens evaluated and superior to the commercial antibiotics used. Fertilization with 0.6 t a^{-1} of the mineral fertilizer and the intermediate dose of 40 t ha^{-1} of organic matter revealed excellent antimicrobial activity for the evaluated microorganisms.

INTRODUCTION

The genus *Lippia* (Verbenaceae) includes approximately 200 species of herbs, shrubs and small trees, most of which are distributed in the Neotropical region [1,2]. Brazil is the center of diversity of the genus, where 98 species with a high degree of endemism occur, more than half of which is located in the Serra do Espinhaço, located in the state of Minas Gerais [3]. In the northeastern region of Brazil, where the Caatinga biome is located, there is also a wide diversity of species of *Lippia*.*L. grata* is highlighted by the intense medicinal use advocated in ethnopharmacological surveys, which mention for it several popular denominations such as rosemary, bush rosemary, rosemary, and rosemary [4,5]. Pharmacological tests performed *in vitro* and *in vivo* with *L. grata* essential oil demonstrated cytotoxic activities on cancer cells [6,7], antimicrobial [8-10], as well as the use of the antimicrobial agent in the treatment of hypertension, inflammatory and analgesic [11-13].

It has recently been shown that thymol-rich *L. grata* essential oil is associated with commercial antibiotics and is effective in combating multi-drug resistant *S. aureus*, *Escherichia coli* and *P. aeruginosa* microorganisms [9]. In addition to the medicinal activity, the essential oil of this species has been used along with chitosan in the coating of fruits because it presents a preservative action, inhibiting the growth of pathogenic microorganisms that deteriorate foods [14,15]. Although this species has been the target in recent years of several pharmacological studies, which has become increasingly promising from a medicinal point of view, studies that correlate agronomic data with antimicrobial activity have not yet been developed. Thus, the objective of this work was to evaluate the antimicrobial activity of *Lippia grata* essential oil obtained from plants submitted to different field management conditions.

Fertile individuals were exsiccated and identified by Dra. Lúcia Helena Piedade Kiill da Embrapa Semiárido; a voucher specimen was deposited at the Trópico Semiárido Herbarium under number 7232.

The cultivation of *L. grata* was carried out at the Experimental Field of Bebedouro, belonging to Embrapa Semiárido, located in the municipality of Petrolina-PE, Brazil (376m altitude, 09 ° 23'35 ''S and 40 ° 30'27''W) and *L. grata* seedlings were produced from the rooting of cuttings collected in plants from a natural population found in the Caatinga area near the same institution. The cultivation experiment was conducted according to Souza et al. [16], where the treatments consisted of different doses of organic fertilization and mineral fertilization and presence and absence of and drip irrigation.

Essential oils extraction

The essential oils extraction procedure was conducted in triplicate. To this end, 100g of leaves was dried in an air-circulating oven at 40°C, for 48 h. The leaves were hydrodistilled in a Clevenger apparatus, at a 1:5 ratio (weight of plant material/ water volume), for 120 min. The hydrolate (water and essential oil) was dried with Na_2SO_4; the samples were stored in flasks sealed with Parafilm® and kept in a freezer until analysis.

Gas chromatographic analysis

The essential oils were qualitatively analyzed by gas chromatography/mass spectrometry (GCMS-QP2010 Ultra, Shimadzu Corporation, Kyoto, Japan) equipped with an AOC-20i (Shimadzu) automatic sampler. Separations were performed using Rtx®-5MS Restek fused silica capillary (5% -diphenyl-95% - dimethyl polysiloxane) of 30 mx 0.25 mm internal diameter (di), 0.25 mm thick film, in a constant stream of helium (99.999%) with rate of 1.2 ml min-1. An injection volume of 0.5 æl (5 mg ml $^{-1}$) was used, with a split ratio of 1:10. The temperature setting of the oven used was from 50°C (isotherm for 1.5 min), with an increase of 4°C/min, at 200°C, then at 10°C/min up to 250°C, ending with a 5 min isotherm at 250°C. CG-MS and CG-DIC data were simultaneously acquired using a detector separation system; the flow separation ratio was 4: 1 (MS: FID).

A restriction tube of 0.62 m x 0.15 mm d.i. (capillary column) was used to connect the splitter to the MS detector; a restriction tube of 0.74 m x 0.22 mm d.i. was used to connect the divider to the DIC detector. The temperature of the injector was 250ºC and the temperature of the source of ions was 200ºC. The ions were generated at 70 eV; at a scanning speed of 0.3 scans s-1 detected in the range of 40-350 Da. The DIC temperature was adjusted to 250°C, and the gas supplies for the DIC were synthetic air, hydrogen, helium at flow rates of 30, 300 and 30 ml min^{-1}, respectively. The quantification of each constituent was estimated by normalization of the peak area generated in the DIC - (%). Concentrations of the compounds were calculated from the areas of GC peaks and were arranged in order of GC elution. The compounds were identified by comparison with spectral data provided by the GC / MS system database (WILEY8, NIST107 and NIST21) and by using Kovats retention indexes [17].

The antimicrobial activity was determined by the microdilution test method using 96-well plates, following the guidelines of CLSI M7-A9 (bacteria), M27-A3 (yeast), and M38-A2 (filamentous fungus). The essential oils were assayed at a final concentration of 50 µL mL^{-1}, against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC 10231 e *Aspergillus niger* ATCC 16404. Readings were carried out after 20 (bacteria), 24 (*C. albicans*), and 48 h (*Aspergillus niger*) of incubation at 35°C. Amphotericin B (32 µg mL^{-1}). and gentamicin (64 µg mL^{-1}) (Sigma Aldrich®) were used as controls. The MIC (Minimum Inhibitory Concentration) was calculated as the lowest concentration of the essential oil that was able to avoid microorganism growth. The bacterial strains were cultured in BHI medium (Brain Infusion Heart-Himedia®), yeast in liquid Sabouraud Dextrose medium (Himedia®) and filamentous fungus in Sabouraud liquid Dextrose medium (Himedia®).

The obtained data were preliminarily analyzed in order to meet the basic hypotheses of ANOVA: additivity, independence, homoscedasticity and normality. The general model adopted for the test was:

$Yijk: \mu + \alpha i + \beta j + \gamma k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + \delta_{ijk} + e_{ijk}$, where:

μ: constant inherent in all installments;

αi: effect of organic fertilizer;

βj: effect of mineral fertilizer;

γk: effect of irrigation;

$\alpha\beta_{ij}$: interaction effect of organic fertilizer and mineral fertilizer;

$\beta\gamma_{jk}$: interaction effect of mineral fertilizer and irrigation

$\alpha\gamma_{ik}$: interaction effect of organic fertilizer and irrigation;

δ_{ijk}: interaction effect of organic fertilizer, mineral fertilizer and irrigation;

e_{ij}: experimental error associated to each installment.

Data were subjected to analysis of variance (5% significance) for Scott-Knott test and polynomial regression using Sisvar software [18].

Twenty-nine compounds were identified (97%) in the essential oils of *L. grata*, which consisted mainly of monoterpenes (87%). There was variation in the composition of the essential oil as a function of fertilization and irrigation, and this difference was more pronounced in relation to some minor compounds such as α-tujene, α-pinene, 1-octen-3-ol, Eb-ocimene, thymol acetate and aromadendrene, which were not quantified in all treatments. The major substances of the essential oil of *Lippia grata* were carvacrol, followed by thymol. Carvacrol content ranged from 79.0 to 71.5%, the lowest value obtained in plants fertilized with NPK, without organic matter and without irrigation. As for thymol, the variation was 14.3 to 4.9%, and the highest content was recorded in plants fertilized with NPK and that did not receive irrigation or organic fertilization [16].

When the plants were not irrigated and fertilized with NPK, a negative correlation between the amount of organic fertilizer and the content of thymol (r = - 0.86) and carvacrol (r = -0.80) was observed the higher the amount of organic matter the lower the content of these substances. In contrast, a positive correlation (r = 0.80) was observed between the amount of organic fertilizer and the thymol content in the plants that were irrigated and received NPK. The proportion of the major compounds in *L. grata* essential oil varied according to the type of fertilization and irrigation. The

Table 1: Minimum Inhibitory Concentration (MIC µL.mL^{-1}) of the essential oil of *Lippia grata* obtained plants cultivated with and without fertilization and irrigation, against *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* e *Aspergillus niger*..

Microorganism	Antibiótic	Organic fertilization (t ha^{-1})															
		0	20	40	60	0	20	40	60	0	20	40	60	0	20	40	60
		With Irrigation								Absent irrigation							
		NPK (15-9-20)				Absence NPK				NPK (15-9-20)				Absence NPK			
E. coli ATCC 25922	G 0.50a	0.62a	1.25b	0.62a	0.62a	0.62a	0.62a	1.25b	2.50c	2.50c	0.62a	0.62a	0.62a	0.62a	1.25b	1.25b	0.62a
S. aureus ATCC 6538	G 8.00c	0.62a	0.62a	0.62a	0.62a	0.62a	0.62a	0.62a	0.62a	0.62a	0.62a	1.25b	0.62a	0.62a	0.62a	0.62a	0.62a
C. albicans ATCC 102	A 4.00c	0.62a	0.62a	0.62a	0.62a	0.62a	0.62a	0.62a	1.25b	0.62a	0.62a	0.62a	0.62a	0.62a	0.62a	0.62a	0.62a
A. niger ATCC 16404	A 4.00b	0.62a	0.62a	0.62a	0.62a	0.62a	0.62a	0.62a	0.62a	0.62a	0.62a	0.62a	0.62a	0.62a	0.62a	0.62a	0.62a

G = gentamicina; A= anflericina B
MIC µL mL^{-1} - Minimum Inhibitory Concentration
Means followed by the same letter do not differ by the Scott-Knott p <0.05 tes

lowest proportion of carvacrol and thymol was 5:1, which was obtained in essential oil extracted from plants fertilized with NPK alone and without irrigation, while the highest proportion was 15: 1 obtained in plants that received only organic fertilization (20 t ha^{-1}) irrigated or not [16].

Determining the ratio of the essential oil compounds is important for establishing the standardization of the oil. The presentation of the results concerning the variation in the ratio between thymol and carvacrol by Souza et al. [16], is relevant because they are the main compounds with antimicrobial action, present in the oil of this species. Moreover, this variation in the proportion of the major compounds can determine the therapeutic effectiveness considering that some microorganisms are more susceptible to one compound than to another [19,20].

Irrigation and fertilization with NPK and organic matter interfered in the antimicrobial effect of *Lippia grata* essential oil. The oil activity on *E. coli* was the most negatively affected by cultural treatments, non-irrigated plants and only fertilized with organic matter (60 t ha^{-1}) or irrigated and fertilized with NPK only produced less effective essential oil with this microorganism (Table 1).

The results obtained in the antimicrobial assays showed that the action of the essential oil extracted from *L. grata* plants submitted to different management conditions were equal to or higher than the antibiotics used as references for all evaluated pathogens. For *E. coli*, 62% of the oils evaluated were as effective as gentamicin and for *S. aureus*, 94% were superior to the same antibiotic. As for yeast *C. albicans*, 94% of the evaluated treatments were effective to produce essential oil that presented MICs superior to anfterycin B and for fungus *A. niger* 100% of the oils were superior to the reference fungicide (Table 1).

This is the first time that the antimicrobial activity of *L. grata* oil against *A. niger* is being demonstrated and also the novel antibacterial activity of the essential oil of this species in the order of 0.62 µL mL^{-1}. Previously, Pessoa et al. [21], evaluated the bactericidal activity of essential oil of this species, which contained 54.4% carvacrol, 10.7% p-cymene and 1.8% thymol and found moderate activity for *E. coli* and *S. aureus*. The results obtained by Bitu et al. [22], showed that the minimum inhibitory concentration (MIC) ranged from 64-512 µL mL^{-1} to resistant strains of *E. coli* and S respectively. The oil extracted from fresh leaves was more active than that extracted from dry leaves.

The discrepant results reported on the antimicrobial activity of *L. grata* essential oil between the studies evaluated and those presented in the present study are mainly due to the studied chemotypes, in addition to the place of origin of the studied material, since there are significant variations regarding the content of the major components as well as the proportion between these substances. Another reason may be related to the wide variations in the presence and quantity of minority compounds that can act synergistically with the majority and therefore directly interfere in the antimicrobial activity.

Essential oils produced by plants grown under different irrigation and fertilization conditions presented different responses regarding the ability to inhibit the evaluated microorganisms. Plants of *Lippia grata* irrigated and fertilized with 0.6 t ha^{-1} of NPK (15-9-20), with the intermediate dose of 40 t ha^{-1} of organic matter, produced more biomass, higher essential oil content and excellent antimicrobial activity for the evaluated microorganisms.

ACKNOWLEDGMENT

The authors thank FACEPE, for granting the aid to research.

REFERENCES

1. Carvalho RRC, Laranjeira D, Carvalho Filho JLS, Souza PE, Blank AF, Alves PB, et al. *In vitro* activity of essential oils of Lippia sidoides e Lippia gracilis and their major chemical components against Thielaviopsis paradoxa, causal agent of stem bleeding in coconut palms. Química Nova. 2013; 241-244.

2. Atkins S. Verbenaceae, in: Kadereit, J.W. The Families and Genera of Flowering Plants Springer-Verlag, Heildelberg. 2004; 7: 449-468.

3. Salimena FRG. Kutschenco DC, Monteiro NP, Myssen C. Verbenaceae, in: Martinelli G, Moraes MA. (Eds.), Livro Vermelho da Flora do Brasil. Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Rio de

Janeiro. 2013; 1010-1016.

4. Lorenzi H, Matos FJA. Plantas Medicinais no Brasil: nativas e exóticas. Nova Odessa, Plantarum. 2012: 512.

5. Lorenzi H, Matos FJA. Plantas medicinais no Brasil: nativas e exóticas.2.ed. Nova Odessa: Plantarum. 2008: 544.

6. Melo JO, Fachin AL, Rizo WF, Jesus HCR, Arrigoni-Blank MF, Alves PB, et al. Cytotoxic effects of essential oils from three *Lippia grata Schauer* genotypes on HeLa, B16, and MCF-7 cells and normal human fibroblasts. Genetics and Molecular Research. 2014; 13: 2691-2697.

7. Ferraz RPC, Bomfim DS, Carvalho NC, Soares MBP, Da Silva TB, Machado WJ, et al. Cytotoxic effect of leaf essential oil of *Lippia grata* Schauer (Verbenaceae). Phytomedicine. 2013; 20: 615- 621.

8. Melo JO, Bitencourt TA, Fachin AL, Cruz EMO, Jesus HCR, Alves PB, et al. Antidermatophytic and antileishmanial activities of essential oils from *Lippia grata Schauer* genotypes. Acta Tropica. 2013; 128: 110-115.

9. Bitu VCN, Fecundo HDTF, Costa JGM, Coutinho HDM, Rodrigues FFG, Santana NM, et al. Chemical composition of the essential oil of *Lippia grata Schauer* leaves and its potential as modulator of bacterial resistance. Natural Product Res. 2014; 28: 399-402.

10. CRUZ EMO, COSTA-JUNIOR LM, PINTO JAO, SANTOS DA, ARAUJO SA. Acaricidal activity of *Lippia gracilis* essential oil and its major constituents on the tick Rhipicephalus (Boophilus) microplus. Vet Parasitol. 2013; 195: 198-202.

11. Riella KR, Marinho RR, Santos JS, Pereira-Filho RN, Cardoso JC, Albuquerque-Junior RLC, et al. Anti-inflammatory and cicatrizing activities of thymol, a monoterpene of the essential oil from *Lippia grata*, in rodents. J Ethnopharmacol. 2012; 143: 656-663.

12. Guilhon CC, Raymundo LJ, Alviano DS, Blank AF, Arrigoni-Blank MF, Matheus ME, et al. Characterisation of the anti-inflammatory and antinociceptive activities and the mechanism of the action of *Lippia grata* essential oil. J Ethnopharmacol. 2011; 135: 406-413.

13. Mendes SS, Bomfima RR, Jesus HCR, Alves PB, Blank AF, Estevam CS, et al. Evaluation of the analgesic and anti-inflammatory effects of the essential oil of *Lippia grata* leaves. J Ethnopharmacol. 2010; 129: 391-397.

14. Aquino AB, Blank AF, Santana LCLA. Impact of edible chitosan– cassava starch coatings enriched with *Lippia grata* Schauer genotype mixtures on the shelf life of guavas (*Psidium guajava* L.) during storage at room temperature. Food Chem. 2015; 171: 108-116.

15. Azevedo AN, Buarque PR, Cruz EMO, Blank AF, Alves PB, Nunes ML, et al. Response surface methodology for optimisation of edible chitosan coating formulations incorporating essential oil against several foodborne pathogenic bacteria. Food Control. 2014; 43: 1-9.

16. Souza AVV, Santos US, Corrêa RM, Souza DD, Oliveira FJV. Essential Oil Content and Chemical Composition of *Lippia gracilis* Schauer Cultived in the Sub-meddle São Francisco Valley. J Essential Oil Bearing Plants. 2017; 20: 983-994.

17. Adams R. Identification of essential oil components by gas chromatography / mass spectroscopy. Allured Publ. Corp., Carol Stream. 1995.

18. Ferreira DF. Sisvar: a computer statistical analysis system. Science and Agrotecnologia. 2011; 35: 1039-1042.

19. Vale-Silva L, Silva MJ, Oliveira D, Gonálves MJ, Cavaleiro C, Salgueiro L, Pinto E. Correlation of the chemical composition of essential oils from *Origanum vulgare* subsp. virens with their *in vitro* activity against pathogenic yeasts and filamentous fungi. J Medical Microbiol. 2012; 61: 252-260.

20. Bagamboula C F, Uyttendaele M, Debevere J. Inhibitory effect of and basil essential oils, carvacrol, thymol, estragole, linalool and p-cymene towards *Shigella sonnei* and *S. flexneri.* Food microbiol. 2004; 21: 33-42.

21. Pessoa ODL, Carvalho CBM, Silvestre JOVI, Lima MCL, Neto RM, Matos FJA, et al. Antibacterial activity of the essential oil from *Lippia aff. gracillis.* Fitoterapia. 2005; 76: 712-714.

22. BituV, Botelho MA, Costa JGM, Rodrigues FFG, Veras HNH, Martins KT, et al. Phythochemical screening and antimicrobial activity of essential oil from *Lippia gracillis.* Braz J Pharmacognosy. 2012; 22: 1: 69-75.

Cite this article

de Souza AVV, dos Santos US, Hernandes C, de Oliveira FJV, Soares Pereira AM (2018) Antimicrobial Activity of Lippia Grata Schauer Essential Oil from Plants Submitted to Different Management Conditions-Short Comunication. J Pharmacol Clin Toxicol 6(5):1121.