Alim. Nutr., Araraquara
v. 22, n. 3, p. 339-343, jul./set. 2011

ISSN 0103-4235
ISSN 2179-4448 on line

ESSENTIAL OILS OF *CYMBOPOGON* SP. IN THE CONTROL OF FOODBORNE PATHOGENIC BACTERIA*

Danilo Florisvaldo BRUGNERA**
Maíra Maciel Mattos de OLIVEIRA***
Roberta Hilsdorf PICCOLI****

■ABSTRACT: In this study, the agar well diffusion technique was used to determine the antibacterial activity of *Cymbopogon nardus* (citronella) and *Cymbopogon citratus* (lemongrass) essential oils, which were applied at different concentrations. The bacterial species used were the foodborne pathogens *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.* Both essential oils presented antibacterial activity in most concentrations tested. The Minimum Inhibitory Concentrations (MICs) founded were: 7.81µL/mL (*S. aureus*) and 3.90µL/mL (*E. coli* and *P. aeruginosa*), for *C. nardus* essential oil; and 3.90µL/mL (*S. aureus, E. coli* and *P. aeruginosa*), for *C. citratus* essential oil. The essential oils used were shown as promising natural antibacterials for pathogenic bacteria control in the food industry.

■KEYWORDS: Bacterial pathogens; food microbiology; natural antibacterials; agar well diffusion technique.

INTRODUCTION

Currently, the microbiological contamination of foods constitutes one of the biggest dangers from a public health point of view. The presence of microorganisms in foodstuffs can be related, mainly, to contaminated raw material, flaws in the sanitization procedures, use of water with poor microbiological quality and food handlers with inappropriate hygienic practices. Once present in food, some bacteria, called pathogenic, can cause food-transmitted diseases. Among the species frequently involved in outbreaks are *Staphylococcus aureus*, pathogenic *Escherichia coli* [11,15,24,30] and *Pseudomonas aeruginosa*, that can be mentioned as one of the prevalent species in waterborne diseases. [3,10,13,31]

In order to ensure safe food, the search for new substances capable to control bacterial growth has become a new research area. According to Dimitrijević et al.[12] the increased demand of consumers for additive-free, fresher, more natural tasting foods and with a smaller impact on the environment, while maintaining the microbiological safety, provokes many researchers to investigate the antimicrobial effects of natural compounds. The use of essential oils has shown to be a promising alternative for the antimicrobials commonly used in the food industry. Recent research points to a possible use of the essential oils as natural preservatives in foods [1,5,27] and as sanitizer solutions in the control of bacterial biofilms formed on industrial surfaces. [9,22]

The use of essential oils as antimicrobial agents in the food industry should be a result of previous laboratory studies. Several methodologies are available for the *in vitro* evaluation of the essential oils antimicrobial activity and one of the most used is the agar diffusion assay. [23,26,28] One of the advantages of this technique is that it allows to estimate the degree of microbial growth inhibition measuring the diameter of the inhibition zone formed. According to Kalemba & Kunicka, [17] the effectiveness of a specific essential oil is represented by the size of the inhibition zone formed on the agar surface, around the filter paper disk or the cavity.

The genus *Cymbopogon* belongs to the family Poaceae and possesses more than 100 species in tropical countries, including Brazil. Of those species, approximately 56 are aromatic. A few of them should be given special attention for their wide use in folk medicine and high content of essential oils with quite varied purposes, such as therapeutic and cosmetic. [19] Within this genus are *Cymbopogon citratus* (D.C.) Stapf. (lemongrass), native to India, known for producing an essential oil rich in citral, [20] and *Cymbopogon nardus* (L.) Rendle (citronella), native to Ceylon, known for the repellent power of its essential oil rich in citronellal. [6] The use of essential oils from species of the genus *Cymbopogon*, such as *C. citratus* and *C. nardus*, for the control of foodborne pathogenic bacteria is an interesting alternative, since these plants have a high essential oil yield, [23] are widely distributed in Brazilian territory, in addition to being easy to cultivate. [3,19]

Considering the importance of the study of new natural substances capable of controlling the presence of pathogenic bacteria in food industry, the agar well diffusion technique was used to determine the antibacterial activity of different concentrations of *C. nardus* and *C. citratus* essential oils against *S. aureus*, *E. coli* and *P. aeruginosa.*

* Research carried out with financial support from the Research Support Foundation of the State of Minas Gerais (FAPEMIG).
** Doctoral student in Food Science – Department of Food Science – UFLA – 37200-000 – Lavras – MG – Brazil. E-mail: danilobrugnera@hotmail.com.
*** Postdoctoral student in Agricultural Microbiology – Department of Biology – UFLA – 37200-000 – Lavras – MG – Brazil.
**** Associate Professor – Department of Food Science – UFLA – 37200-000 – Lavras – MG – Brazil.

MATERIAL AND METHODS

Fresh leaves of *C. nardus* and *C. citratus* (2000g) were collected from Medicinal Plant Nursery of the Federal University of Lavras in Minas Gerais, Brazil. Essential oils were extracted by hydrodistillation using a modified Clevenger apparatus. Fresh *C. citratus* and *C. nardus* leaves were chopped and placed with water in a 4L round-bottom flask. The flask was coupled to the modified Clevenger apparatus and the extraction was performed for 2.5 hours with the temperature maintained at approximately 100°C. The hydrolate obtained was centrifuged (321.8 x *g*) for 5 minutes, with the essential oil being removed with a Pasteur pipette and stored at refrigeration temperature in glass flasks wrapped in aluminum foil. [16,23]

The strains used were *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 25853. During the experiment, they were maintained in freezing medium (15mL glycerol, 0.5g bacteriological peptone, 0.3g of yeast extract and 0.5g NaCl, per 100mL of distilled water, with the final pH adjusted to 7.2–7.4). For reactivation, an aliquot of the stock culture was transferred to test tubes containing Trypic Soy Broth (TSB), which were incubated at 37°C for 24 hours. Later, the strains were streaked in Petri dishes containing Trypic Soy Agar (TSA), which were incubated at 37°C for 24 hours. For inocula standardization, the McFarland scale was used. Of the colonies formed on the TSA surface, some were removed and transferred to test tubes containing saline solution (NaCl 0.85% w/v). The suspensions were standardized using tube 5, corresponding to approximately 1.5 x 10^9 CFU/mL.

The antibacterial activity of the essential oils was assessed by the agar well diffusion technique, using the methodology described by Oliveira et al., [23] with some modifications. Müeller Hinton agar with 0.5% v/v of Tween 80 was used as culture medium. For the preparation of the essential oil deposition wells, an initial layer of culture medium was deposited in Petri dishes (100x20mm), on which, after solidification, glass beads were deposited. The overlay culture medium, containing 10^8CFU/mL of the bacterial inoculum, was deposited on the glass beads on the surface of the initial layer. The amount of overlay culture medium used was 15mL, 14mL being Müeller Hinton agar and 1mL of saline solution 0.85% w/v containing the bacterial inoculum (1.5x10^9CFU/mL). The addition and subsequent homogenization of the saline solution containing the bacterial inoculum to the agar was conducted when this was at a temperature of approximately 45°C. After the solidification of the overlay, the glass beads were removed with the aid of sterile tweezers, creating the wells. In each Petri dish, 5 wells were created. Soon afterwards, 10µL of different concentrations (0; 3.90; 7.80; 15.60; 31.20; 62.50; 125.00; 250.00 and 500.00), expressed in µL/mL, of the essential oils of C. *citratus* and *C. nardus,* were transferred to the wells. The concentrations were obtained after dilution of the essential oils in ethanol. The concentration of 0µL/mL, contained only ethanol, was used as positive control for the bacterial growth. The Petri dishes were incubated at 37ºC for 24 hours and after this period the diameters of the inhibition zones formed were measured, subtracting 3mm regarding the diameter of the well. Three repetitions were done. The Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration of essential oil that resulted in inhibition of the bacterial growth.

RESULTS AND DISCUSSION

The essential oils of *C. nardus* and *C. citratus* presented antibacterial activity at most of the used concentrations. However, different behaviors were noticed according to the bacterium and the essential oil used. Regarding the *C. nardus* essential oil, *E. coli* and *P. aeruginosa* were more sensitive, presenting a MIC of 3.90µL/mL. For *S. aureus* the MIC of *C. nardus* was 7.81µL/mL. For the essential oil of *C. citratus*, *E. coli*, *S. aureus* and *P. aeruginosa* presenting a MIC of 3.90µL/mL. In general, it can be said that starting from the minimum concentrations necessary for an inhibitory effect, the zone diameters increased with the increase of the concentrations (Table 1).

In the large majority of cases, Gram-positive bacteria are more sensitive to essential oils than Gram-negative, because the outer membrane of Gram-negatives, rich in lipopolysaccharides, difficult the penetration of antimicrobial agents. [7] However, according to the results obtained in this study for the essential oil of *C. nardus* (Table 1), only the Gram-negative bacteria (*E. coli* and *P. aeruginosa)* were sensitive to the lowest concentration used (3.90µL/mL). Similar result was founded by Bussata et al.,[5] that evaluating the antimicrobial activity of oregano essential oil, obtained a MIC of 0.690mg/mL for *Streptococcus mutans*, value superior to that of all of the appraised Gram-negative bacteria, such as *E. coli, Aeromonas* sp. and *Salmonella choleraensius*, that presented MIC of 0.460mg/mL. According to Koyama et al., [18] many of the essential oil compounds have the ability to break or to penetrate the lipidic structure present in the outer membrane of Gram-negative bacteria.

P. aeruginosa is one of the most resistant bacteria to essential oils. [4,29] However, in this study, *P. aeruginosa* showed to be very sensitive to the essential oils used, especially *C. citratus* at the highest concentrations (62.50 to 500.00µL/mL) (Table 1). Similar result was observed by Getahun et al., [14] who verified that the *Mentha spicata* essential oil exhibited a wide spectrum of antibacterial activity against all of the tested strains, including multiresistant strains of *P. aeruginosa.*

Differences in the antibacterial activity among essential oils of different plant species, as observed in this study for *C. nardus* and *C. citratus* (Table 1), happen due to ecological and growth factors and are related to the concentration and nature of essential oils constituents, as well as to possible synergistic interactions. [8] Oladimeji et al. [21] mention that the antimicrobial activity of the essential

Table 1 – Antibacterial activity of the essential oils from *Cymbopogon nardus* and *Cymbopogon citratus*, using agar well diffusion method, expressed by diameter (mm) of inhibition zone.

C. nardus	**Diameter (mm)**		
(µL/mL)	***Escherichia coli***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***
0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
3.90	2.88 ± 0.24	0.00 ± 0.00	1.98 ± 0.65
7.81	3.61 ± 0.46	1.23 ± 0.38	1.75 ± 0.18
15.62	2.96 ± 0.86	1.80 ± 0.16	1.88 ± 0.26
31.25	3.42 ± 0.35	2.48 ± 0.48	1.81 ± 0.80
62.50	3.80 ± 0.92	4.83 ± 0.62	2.80 ± 0.97
125.00	5.08 ± 1.78	5.00 ± 0.74	2.98 ± 0.59
250.00	5.23 ± 0.33	6.51 ± 1.65	4.21 ± 0.29
500.00	8.03 ± 1.92	5.37 ± 1.16	6.30 ± 1.07
C. citratus	**Diameter (mm)**		
(µL/mL)	***Escherichia coli***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***
0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
3.90	3.27 ± 0.16	2.17 ± 0.47	2.30 ± 0.21
7.81	4.67 ± 1.31	2.58 ± 0.31	2.98 ± 0.46
15.62	4.50 ± 0.94	3.92 ± 0.42	4.28 ± 0.16
31.25	3.95 ± 1.04	3.73 ± 0.10	4.75 ± 1.59
62.50	5.05 ± 0.74	5.83 ± 0.12	12.08 ± 2.66
125.00	6.68 ± 0.37	8.30 ± 0.47	14.33 ± 4.29
250.00	6.48 ± 1.15	8.58 ± 0.59	15.92 ± 2.55
500.00	8.05 ± 0.80	9.12 ± 1.52	20.83 ± 3.30

The results are expressed as the average of three repetitions ± the standard deviation.

oils is strongly connected to their chemical composition. The chemical composition of the essential oils that were used in this study was previously determined by our research group [22,23] and monoterpenes was related as the majority constituents. For the essential oil of *C. citratus* there was a prevalence of geranial (42.91%) and neral (30.90%), compounds that isomerically form the citral. For *C. nardus*, mainly, citronellal (34.60%), geraniol (23.17%) and citronellol (12.09%) were found.

According to Sikkema et al., [25] the action mechanism of monoterpenes involves mainly toxic effects on the structure and function of the cell membrane. As a result of their lipophilic character, the monoterpenes will preferably dislocate from the aqueous phase towards the membrane structures. Bakkali et al. [2] mention that the accumulation of the essential oil constituents in the lipid double layer of the cytoplasmic membrane will confer a characteristic of permeability. In bacteria, cytoplasmic membrane permeabilization is associated to dissipation of the proton motive force, regarding reduction of the ATP pool, internal pH and electric potential, and loss of ions, such as those of potassium and phosphate.

CONCLUSION

The essential oils used in this study were shown as promising natural antibacterials for the control of pathogenic bacteria. This fact is of great importance for the food industries, where the development of new control tools related to the production of safe foods is strictly necessary to avoid the occurrence of foodborne diseases. In this context, it is emphasized that this work should serve as an incentive for subsequent research related to the use of these essential oils as natural preservatives in foods or in sanitizers solutions for surfaces that come in contact with foodstuffs. Studies with practical applications of these essential oils are scarce in the literature, mainly those related to the essential oil of *C. nardus*, which demonstrates that this is a new line of research to be continued.

BRUGNERA, D. F.; OLIVEIRA, M. M. M.; PICCOLI, R. H. Óleos essenciais de *Cymbopogon* sp. no controle de bactérias patogênicas veiculadas por alimentos. **Alim. Nutr**., Araraquara, v. 22, n. 3, p. 339-343, jul./set. 2011.

■RESUMO: Neste estudo, a técnica de difusão em cavidade feita em ágar foi utilizada para determinar a atividade antibacteriana dos óleos essenciais de *Cymbopogon nardus* e *Cymbopogon citratus*, os quais foram testados em diferentes concentrações. As espécies bacterianas utilizadas foram os patógenos alimentares *Staphylococcus aureus*, *Escherichia coli* e *Pseudomonas aeruginosa*. Ambos os óleos essenciais apresentaram atividade antibacteriana na maioria das concentrações. As Concentrações Mínimas Inibitórias (CMIs) encontradas foram: 7,81µL/mL (*S. aureus*) e 3,90µL/mL (*E. coli* e *P. aeruginosa*), para o óleo essencial de *C. nardus*; e 3,90µL/mL (*S. aureus, E. coli* e *P.*

aeruginosa), para o óleo essencial de *C. citratus*. Os óleos essenciais utilizados neste estudo mostraram-se como antibacterianos naturais promissores para o controle de bactérias patogênicas nas indústrias de alimentos.

■PALAVRAS-CHAVE: Patógenos bacterianos; microbiologia de Alimentos; antibacterianos naturais; técnica de difusão em cavidade feita em ágar.

REFERENCES

1. AQUINO, L. C. L. et al. Atividade antimicrobiana dos óleos essenciais de erva-cidreira e manjericão frente a bactérias de carnes bovinas. **Alim. Nutr.**, v. 21, p. 529-535, 2010.
2. BAKKALI, F. et al. Biological effects of essential oils: a review. **Food Chem. Toxicol.**, v. 46, p. 446-475, 2008.
3. BARWICK, R.S. et al. Surveillance for waterborne-disease outbreaks - United States, 1997-1998. **MMWR CDC Surveill Summ.**, v. 49, p. 1-21, 2000.
4. BOUHDID, S. et al. Antibacterial and antioxidant activities of *Origanum compactum* essential oil. **Afr. J. Biotechnol.**, v. 7, p. 1563-1570, 2008.
5. BUSSATA, C. et al. Evaluation of *Origanum vulgare* essential oil as antimicrobial agent in sausage. **Braz. J. Microbiol.**, v. 38, p. 610-616, 2007.
6. CASTRO, L.O.; RAMOS, R.L.D. **Principais gramíneas produtoras de óleos essenciais:** *Cymbopogon citratus* (DC) Stapf., capim-cidró, *Cymbopogon martinii* (Rox.) J.F. Watson, palma-rosa, *Cymbopogon nardus* (L.) Rendle, citronela, *Elyonurus candidus* (Trin.) Hack. , capim-limão, *Vetiveria zizanioides* (L.) Nash, vetiver. Porto Alegre: FEPAGRO, 2003. 28p.
7. CEYLAN, E.; FUNG, D. Y. C. Antimicrobial activity of spices. **J. Rapid Methods Autom. Microbiol.**, v. 12, p. 1-55, 2004.
8. CHANG, S. T.; CHEN, P. F.; CHANG, S. C. Antibacterial activity of leaf essential oils and their constituents from *Cinnamomum osmophloeum*. **J. Ethnopharmacol.**, v. 77, p. 123-127, 2001.
9. CHORIANOPOULOS, N. G. et al. Disinfectant test against monoculture and mixed-culture biofilms composed of technological, spoilage and pathogenic bacteria: bactericidal effect of essential oil and hydrosol of *Satureja thymbra* and comparison with standard acid–base sanitizers. **J. Appl. Microbiol.**, v. 104, p. 1586-1589, 2008.
10. CRAUN, G. F.; CALDERON, R. L.; CRAUN, M. F. Outbreaks associated with recreational water in the United States. **Int. J. Environ. Health Res.**, v. 15, p. 243-262, 2005.
11. DENNY, J.; BHAT, M.; ECKMANN, K. Outbreak of *Escherichia coli* O157:H7 Associated with Raw Milk Consumption in the Pacific Northwest. **Foodborne Pathog. Dis.**, v. 5, p. 321-328, 2008.
12. DIMITRIJEVIĆ, S. I. et al. A study of the synergistic antilisterial effects of a sub-lethal dose of lactic-acid and essential oils from *Thymus vulgaris* L., *Rosmarinus officinalis* L. and *Origanum vulgare* L. **Food Chem.**, v. 104, p. 774-782, 2007.
13. ECKMANNS, T. et al. An outbreak of hospital-acquired *Pseudomonas aeruginosa* infection caused by contaminated bottled water in intensive care units. **Clin. Microbiol. Infect.**, v. 14, p. 454-458, 2008.
14. GETAHUN, Z.; ASRES, K.; MAZUMDER. A. Essential oil composition, antibacterial and antioxidant activities of four *Mentha* species growing in Ethiopia. **Ethiopian Pharm. J.**, v. 25, p. 91-102, 2007.
15. GRANT, J. et al. Spinach-associated *Escherichia coli* O157:H7 Outbreak, Utah and New Mexico, 2006. **Emerg Infect Dis.**, v. 14, p. 1633-1636, 2008.
16. GUIMARÃES, L. G. L. et al. Influência da luz e da temperatura sobre a oxidação do óleo essencial de capim-limão (*Cymbopogon citratus* (D.C.) Stapf). **Quím. Nova**, v. 31, p. 1476-1480, 2008.
17. KALEMBA, D.; KUNICKA, A. Antibacterial and antifungal properties of essential oils. **Curr. Med. Chem.**, v. 10, p. 813-829, 2003.
18. KOYAMA, S. et al. A new substance (yoshiksol) with an interesting antibiotic mechanism from wood oil of Japanese traditional tree (kisohinoki), *Chamaecyparis obtuse*. **Gen. Pharmacol.**, v. 28, p. 797-804, 1997.
19. LORENZI, H.; MATOS, F. J. A. **Plantas medicinais no Brasil:** nativas e exóticas cultivadas. Nova Odessa: Plantarum, 2002. 512p.
20. MING, L. C. et al. Yield of essential oil and citral content in different parts of lemongrass leaves (*Cymbopogon citratus* (DC.) Stapf) - Poaceae. **Acta Hort.**, v. 426, p. 555-559, 1996.
21. OLADIMEJI, F.A. et al. Effect of autoxidation on the composition and antimicrobial activity of essential oil of *Lippia multiflora*. **Pharmacol. Lett.**, v. 11, p. 64-67, 2001.
22. OLIVEIRA, M. M. M. et al. Disinfectant action of *Cymbopogon* sp. essential oils in different phases of biofilm formation by *Listeria monocytogenes* on stainless steel surface. **Food Control**, v. 21, p. 549-553, 2010.
23. OLIVEIRA, M. M. M. et al. Rendimento, composição química e atividade antilisterial de óleos essenciais de espécies de *Cymbopogon*. **Rev. Bras. Pl. Med.**, v. 13, p. 8-16, 2011.
24. SCHMID, D. et al. Outbreak of staphylococcal food intoxication after consumption of pasteurized milk products Austria. **Wien Klin Worhenschr**, v. 121, p. 125-131, 2009.

25. SIKKEMA, J.; DE BONT, J. A. M.; POOLMAN, B. Interactions of cyclic hydrocarbons with biological membranes. **J. Biol. Chem.**, v. 269, p. 8022–8028, 1994.

26. SILVA, A. B. et al. Antibacterial activity, chemical composition, and cytotoxicity of leaf's essential oil from brazilian pepper tree (*Schinus terebinthifolius,* RADDI). **Braz. J. Microbiol.**, v. 41, p. 158-163, 2010.

27. SOLOMAKOS, N. et al. The antimicrobial effect of thyme essential oil, nisin and their combination against *Escherichia coli* O157:H7 in minced beef during refrigerated storage. **Meat Sci.**, v. 80, p. 159-166, 2008.

28. SOUZA, E. L.; STAMFORD, T. L. M.; LIMA, E. O. Sensitivity of spoiling and pathogen food-related bacteria to *Origanum vulgare* L. (Lamiaceae) essential oil. **Braz. J. Microbiol.**, v. 37, p. 527-532, 2006.

29. TADTONG, S. et al. Antimicrobial activities of essential oil from *Etlingera punicea* rhizome. **J. Health Res.**, v. 23, p. 77-79, 2009.

30. WOOLAWAY, M. C. et al. International outbreak of staphylococcal food poisoning caused by contaminated lasagne. **J. Hyg.**, v. 96, p. 67-73, 1986.

31. YODER, J. S. et al. Surveillance for waterborne-disease outbreaks associated with recreational water United States, 2001-2002. **MMWR Surveill Summ.**, v. 53, p. 1-22, 2004.

Recebido em: 02/06/2011

Aprovado em: 30/09/2011