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ACTIVITY OF *Heterothalamus psiadioides* Less (Asteraceae) ESSENTIAL OIL AGAINST *Listeria monocytogenes* STRAINS

ATIVIDADE DO ÓLEO ESSENCIAL DE *Heterothalamus psiadioides* Less. (Asteraceae) FRENTE A ISOLADOS DE *Listeria monocytogenes*

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ABSTRACT

Listeria monocytogenes is an important food-borne pathogen that may cause listeriosis, a rare but severe disease, with high fatality rates. Effective control of this pathogen leads to search for new approaches, including the use of natural products. *Heterothalamus psiadioides* Less. (Asteraceae) is a plant useful in South America folk medicine, to treat several disorders. This study aimed to analyze the antimicrobial activity of *H. psiadioides* essential oil against *L. monocytogenes*. Twenty-two *L. monocytogenes* (21 isolated from cheeses and one reference strain) were challenged against pure essential oil of *H. psiadioides* by agar disk diffusion assay. The effect of volatile constituents was also analyzed. The minimum inhibitory concentration (MIC) was determined at three temperatures (35 °C, 30 °C and 5 °C). The capacity of the essential oil to prevent cell adhesion was assessed using crystal violet assay. The essential oil showed activity against 20 *L. monocytogenes* strains, however, their volatile constituents did not show anti-*Listeria* effect. The MICs ranged from 32% (v/v) to 8% (v/v) among different temperatures. The isolates showed weakly adherents and the oil did not prevent cell attachment. In conclusion, the study demonstrated that the essential oil of *H. psiadioides* showed activity against different *L. monocytogenes* serovars. More researches are needed to clarify *L. monocytogenes* susceptibly to essential oils and action's mechanisms of *H. psiadioides* essential oil.

Keywords: *Listeria monocytogenes*; *Heterothalamus psiadioides*; essential oil; antimicrobial activity; biofilm formation.

RESUMO

Listeria monocytogenes é um relevante patógeno de origem alimentar que pode causar listeriose, uma doença rara, porém, severa e com altas taxas de mortalidade. O controle efetivo deste patógeno leva à busca de novas tecnologias, incluindo o uso de produtos naturais. *Heterothalamus psiadioides* Less. (Asteraceae) é uma planta usada na medicina popular da América do Sul para tratar diversas enfermidades. O objetivo deste estudo foi analisar a atividade do óleo essencial de *H. psiadioides* contra isolados de *L. monocytogenes*. Vinte e duas cepas de *L. monocytogenes* (21 isoladas de queijos e uma cepa referência) foram analisadas frente ao óleo essencial puro através do ensaio de disco difusão. O efeito dos constituintes voláteis também foi avaliado. A concentração inibitória mínima (CIM) foi determinada em três temperaturas (35 °C, 30 °C e 5 °C). A capacidade do óleo essencial em inibir a adesão celular foi analisada utilizando o ensaio de cristal violeta. O óleo essencial testado mostrou atividade contra 20 cepas de *L. monocytogenes*, no entanto, seus constituintes voláteis não demonstraram efeito anti-*Listeria*. As CIMs variaram de 32% (v/v) a 8% (v/v) entre diferentes temperaturas. As cepas de *L. monocytogenes* testadas mostraram-se fracas aderentes, e o óleo não foi capaz de impedir a fixação celular. Em conclusão, o estudo demonstrou que o óleo essencial de *H. psiadioides* apresentou atividade contra os diferentes sorovares de *L. monocytogenes*. Mais pesquisas são necessárias para elucidar a variabilidade de resposta de *L. monocytogenes* frente aos óleos essenciais assim como os mecanismos envolvidos com a atividade do óleo essencial de *H. psiadioides*.

Palavras-chaves: *Listeria monocytogenes*; *heterothalamus psiadioides*; óleo essencial; biofilme.

INTRODUCTION

Listeria is a Gram-positive, nonsporeforming, facultative anaerobic, flagellated and rod-shaped bacterium, commonly isolated from food samples (meats, meat products, milk and dairy products), soil, water, vegetation, and in the stool of healthy mammals, including humans (~5%) (1). The optimal growth temperature is between 30º and 37ºC, but growth occurs at 4ºC within a few days. *Listeria* spp. are facultative anaerobic, with growth between pH 6 and pH 9 and in medium supplemented with up to 10% (w/v) sodium chloride (1). The genus includes twelve different species (2), of which *Listeria monocytogenes* is considered the major human pathogen, causing listeriosis, a serious infection usually caused by eating food contaminated with this species (3).

Currently, 13 serovars (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e and 7) have been described for *Listeria monocytogenes*. Identification of a serotype has been based on diversity in somatic and flagellar antigens (3-5). Around 90% of strains isolated from patients with listeriosis belong to serovars 1/2a, 1/2b and 4b (6-8). According to Hofer et al. (2006) (9), the predominant serovars isolated from human clinical specimens in Brazil are 4b and 1/2a. The clinical syndromes associated with listeriosis include: central nervous system infections, such as meningitis and encephalitis, bacteremia, endocarditis, and stillbirths, fetal death or abortions in pregnant women.

The ubiquitous nature and growth characteristics, even at temperatures as low as 4°C, at extreme pHs, or in high saline concentrations, allows *L. monocytogenes* to survive in conditions normally used for food conservation and is the prerequisite for its relevance to their role as foodborne pathogens (10-12). Hence, disinfection approaches are required to ensure effective control of bacteria, improving food safety and reducing the risk of *L. monocytogenes* in food (13-15). Although the variety of disinfectants are approved for use by the National Regulatory Agencies, the demands for natural products, such as biocides, plant extracts and essential oils, have been increasingly reported (16,17).

A mixture of volatile compounds produced by plants, essential oils are characterized by a strong odor and formed by aromatic plants as secondary metabolites. Essential oils are synthesized by the plant as part of its defense system, and it provides an invaluable resource that has been used to find new drug molecules (18). A number of essential oils and several of their individual components exhibit antibacterial activity against foodborne pathogens *in vitro*, including *Listeria* sp. (16, 19). *Heterothalamus psiadioides* Less. (vernacular name: “alecrim-do-campo”, “vassoura” or “erva-formiga”) belongs to the Asteraceae family, grows as a shrub in the Southern Brazil, Argentina and Uruguay (20). In South America folk medicine, this species is used as an antipyretic, antidote against snake’s bite, anti-inflammatory, and against renal diseases (21, 22, 23). The major compound of the *Heterothalamus* genus is β-pinene (21, 24), which is one of the most widely distributed monoterpenes in plant species and major component of various essential oils (18). The interactions with other plants, including the cytotoxicity of the essential oil *of H. psiadioides*, were evaluated by Schmidt-Silva et al. (2012) (20); their results indicated that this essential oil could cause mitodepressive effects and chromosomal abnormalities in root meristems.

In Brazil, numerous studies have been conducted to evaluate the inhibitory activity of essential oils from the Asteraceae family against microorganisms (14, 25-29), but so far, there are few studies evaluating the activity of *Heterothalamus* sp. against microorganisms. So, the objective of this study was to evaluate the antimicrobial activity of essential oils from leaves of *H. psiadioides* against *L. monocytogenes.*

METODOLOGY

Essential oil of *H. psiadioides*

Leaves of *H. psiadioides* were collected in Porto Alegre (30° 3’S – 51° 7’W), Rio Grande do Sul State, Brazil, in May, 2012. The specimen is deposited in the ICN Herbarium of the Universidade Federal do Rio Grande do Sul (UFRGS) under the voucher number 175007. The essential oil (100% pure) was obtained from the Chemical Ecology and Chemotaxonomy Laboratory (UFRGS, Brazil). The chemical

characterization of the essential oil was performed by Lazarotto (2014) (30). The main compounds present are: β-pinene (44.65%), followed by limonene (6.50%), α-pinene (4.41%), trans-ocimene (2.30%) and myrcene (1.91%), with the monoterpenes representing 66.47% of the essential oil. The antimicrobial assays were conducted using pure essential oil or an aqueous solution prepared with a proportion of three parts of essential oil to one part of Dimethyl Sulfoxide (DMSO, Sigma-Aldrich) to yield a final concentration of 10% (v/v).

Bacterial strains, storage and inoculums preparation

A total of 22 *L. monocytogenes* strains (21 isolated from cheeses and one reference strain) were used in the present study (Table 1). The strains were provided by the Institute of Food Science and Technology of the Universidade Federal do Rio Grande do Sul (ICTA-UFRGS), National Laboratory of Farming and Animal Husbandry in Porto Alegre (LANAGRO/RS) and the Department of Microbiology of the Federal University of Rio Grande do Sul (ICBS-UFRGS). All strains were evaluated by the following phenotype tests: Gram stain, growth and morphologic characteristics on selective medium PALCAM Listeria Agar (BD Diagnostic Systems). The strains were stored at -20°C, and prior to assays, an aliquot of frozen cells was inoculated into Brain Heart Infusion Agar (BHIA, Oxoid) or Tryptone Soya Agar (TSA, HiMedia) and incubated at 35°C for 18 h.

Screening for antimicrobial activity by agar disk diffusion assay

The anti-listerial activity of the essential oil of *H. psiadioides* was investigated using the agar disk diffusion assay. At least three to five well-isolated colonies of the same morphological type were selected from BHIA or TSA. The colonies were transferred into a tube containing 4 to 5 ml of 0.9% sterile saline solution (w/v) until it matches the turbidity of a 0.5 McFarland standard (approximately $1x10^8$ CFU/mL). The inoculums were spread on the surface of Müeller-Hinton Agar (MHA, HiMedia). Sterile filter paper discs of 6 mm diameter were impregnated with 20 µL of pure essential oil and placed on the surface of the inoculated MHA. Sterile water was used as control. The plates were incubated at 35°C for 24 h and 48 h. Antibacterial activity was evaluated by measuring the diameter of the zones of inhibition, and the bacteria were considered sensible when the diameter was equal to, or greater than, 14 mm. Each assay was performed in duplicates on two separate experimental runs.

Antimicrobial activity by volatiles contact

The antimicrobial activity of the essential oil of *H. psiadioides* was also evaluated using gaseous contact. The assay was conducted based on Rodrigues et al. (2009) (31). Initially, Petri dishes containing MHA were inoculated, with suspension adjusted to the turbidity of a 0.5 McFarland standard of *L. monocytogenes* strains. One hundred microliters of pure essential oil or 10% aqueous solution (v/v) or sterile distilled water (control) were dropped onto a cotton ball attached to the upper part of the Petri dish, avoiding direct contact between bacteria and the essential oil. The plates were sealed and incubated at 35°C for 24 h. Antimicrobial activity was determined by their ability to suppress the growth of microorganisms in a closed system. Each assay was performed in duplicates on two separate experimental runs.

Determination of minimal inhibitory concentration (MIC) and bactericidal activity

The Minimum Inhibitory Concentration (MIC) was estimated by the microdilution method in BHI broth (BHI, Oxoid) using the protocol described by Jadhav et al. (2013) (14). Briefly, the essential oil of *H. psiadioides* was first diluted in DMSO, as described above. Serial dilutions of essential oil were carried out in BHI with concentrations ranging from 64% to 0.5% (v/v). Eight *L. monocytogenes* strains (ATCC 7644, LM 20, LM 32, LM 35, LM 43, LM 45, LM 46, LM 54)

were used in this assay. Ten microliters of the inoculum (10^6 UFC/mL) and 100 µL of essential oil solution were added to each well. The microplates were capped and incubated at three different temperatures: 35°C for 24 h; 30°C for 24 h; and 5°C for 5 days. The assays were performed in triplicates. Controls without the essential oil were prepared. The MIC was determined as the lowest concentration of the essential oil inhibiting visible bacterial growth.

In order to evaluate the bactericidal activity, aliquots from the wells displaying no growth were spread on BHIA agar and the plates were incubated for 24 h at 35°C.

Inhibition of initial cell attachment of *L. monocytogenes* by essential oil of *H. psiadioides*

The effect of the essential oil of *H. psiadioides* on biofilm formation ability of *L. monocytogenes* was assessed using the crystal violet assay (14, 32, 33). Dilutions of the essential oil of *H. psiadioides* equivalent to 0.5 x MIC and 1 x MIC were prepared using DMSO and TSB broth supplemented with 1% glucose (TSB-G). Individual wells of sterile 96-well flat-bottomed polystyrene microplates were filled with 150 µl of each solution and 50 µl of a 10^8 cfu/mL of bacterial suspension. Afterwards, the microplates were incubated for 48 h at 35°C. The optical density (O.D.) was measured at 492 nm (OD_{492}) in a spectrophotometer (Anthos 2010 Microplates Reader) (34). The O.D. cut-off (O.D.c) was defined as three standard deviations above the mean O.D. of the negative control. All strains were separated into categories using the O.D. measurement of bacterial films following Stepanovic et al. (2007) (33): O.D. ≤ O.D.c = non-adherent, O.D.c < O.D. ≤ (2x O.D.c) = weakly adherent, (2x O.D.c) < O.D. ≤ (4x O.D.c) = moderately adherent and (4x O.D.c) < O.D. = strongly adherent. Experiments were performed at least three times for each strain. The TSB-G used for dilutions of essential oils was used as negative control and *Staphylococcus epidermidis* American Type Culture Collection 35984 (ATCC) was used as positive control.

RESULTS

Antimicrobial activity of the essential oil of *H. psiadioides* against *L. monocytogenes*

The antimicrobial effects of the pure essential oil of *H. psiadioides* against different serovars of *L. monocytogenes* are presented in Table 1. The essential oil tested showed an inhibitory activity against 20 *L. monocytogenes* strains, with inhibition diameters ranging from 47.5 mm to 17.5 mm at 24 h of incubation. At 48 h of incubation, eleven strains were sensitive to the essential oil, with inhibition diameters ranging from 32.5 to 14.5 mm. Susceptibility to the essential oil was not correlated with different serovars. No antimicrobial activity of the volatile constituents of the essential oil of *H. psiadioides* was observed against *L.monocytogenes.*

Table 1. Antimicrobial activity of the pure essential oil of *H. psiadioides* against *L. monocytogenes* strains for 24 h and 48 h of incubation at 35 °C.

Strains	Serovar	Inhibition zone (mm) formed		Source
		24 h	48 h	
ATCC 7644	1/2c	42.5	20.0	Reference Strain
A5	1/2b	27.5	17.5	ICBS-UFRGS[a]
A11	1/2b	40.0	10.0	ICBS-UFRGS[a]
A15	n.d.	25.0	17.5	ICTA-UFRGS[c]
LM20	1/2b	10.0	8.0	ICBS-UFRGS[a]
LM30	n.d.	32.5	20.0	ICTA-UFRGS[c]
LM32	1/2b	27.5	15.0	LANAGRO[b]
LM34	1/2b	17.5	15.0	LANAGRO[b]
LM35	1/2b	47.5	27.5	LANAGRO[b]
LM36	1/2b	35.0	14.5	LANAGRO[b]
LM37	1/2b	34.0	11.0	LANAGRO[b]
LM38	1/2b	25.0	12.5	LANAGRO[b]
LM39	1/2b	35.0	10.0	LANAGRO[b]
LM40	4b	32.5	12.0	LANAGRO[b]
LM42	4b	20.0	13.5	LANAGRO[b]
LM43	4b	26.5	25.0	LANAGRO[b]
LM44	4b	10.0	10.0	LANAGRO[b]
LM45	1c	40.0	32.5	ICTA-UFRGS[c]
LM46	4c	32.5	10.0	ICTA-UFRGS[c]
LM47	4b	30.0	10.0	LANAGRO[b]
LM48	4b	42.5	10.0	LANAGRO[b]
LM54	1/2a	41.5	29.5	LANAGRO[b]

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[b] National Laboratory of Farming and Animal Husbandry, Brazilian Department of Agriculture;
[c] Institute of Food Science and Technology of Universidade Federal do Rio Grande do Sul;
n.d: not determined.

The MICs of the essential oil are presented in Table 2. The MIC observed at 35°C ranged from 16% (v/v) to 8% (v/v). At 5°C and 30°C, the MICs were equal to 32% (v/v) with the exception of isolate LM 35, which exhibited a MIC equal to 16% (v/v) at 30°C (Table 2).

Curiously, comparing the three temperatures tested, it was verified that under lower temperatures (30° C and 5° C)

the strains demonstrated less susceptibility to the essential oil of *H. psiadioides* than at 35° C, requiring higher concentrations of the oil to prevent its growth. As with the disk diffusion test, there were no differences between the serovars and MIC results.

Table 2. Minimal inhibitory concentration (MIC) of the essential oil of *H. psiadioides* against *L. monocytogenes* strains at different temperatures.

		Minimal inhibitory concentration at		
Strains	**Serovar**	**35°C**	**30°C**	**5°C**
ATCC 7644	1/2c	16%	32%	32%
LM 20	1/2b	16%	32%	32%
LM 32	1/2b	16%	32%	32%
LM 35	1/2b	16%	16%	32%
LM 43	4b	16%	32%	32%
LM 45	1c	16%	32%	32%
LM 46	4c	16%	32%	32%
LM 54	1/2a	8%	32%	32%

Considering the bactericidal activity, all *Listeria* were able to grow on BHIA at 35°C, indicating that the essential oil of *H. psiadioides* has bacteriostatic activity.

The effect of the essential oil on biofilm formation

The effect of the essential oil on initial cell attachment using concentrations equal to 0.5 x MIC and 1 x MIC is presented in Figure 1. The isolates tested showed weak adherence and the oil did not prevent cell attachment.

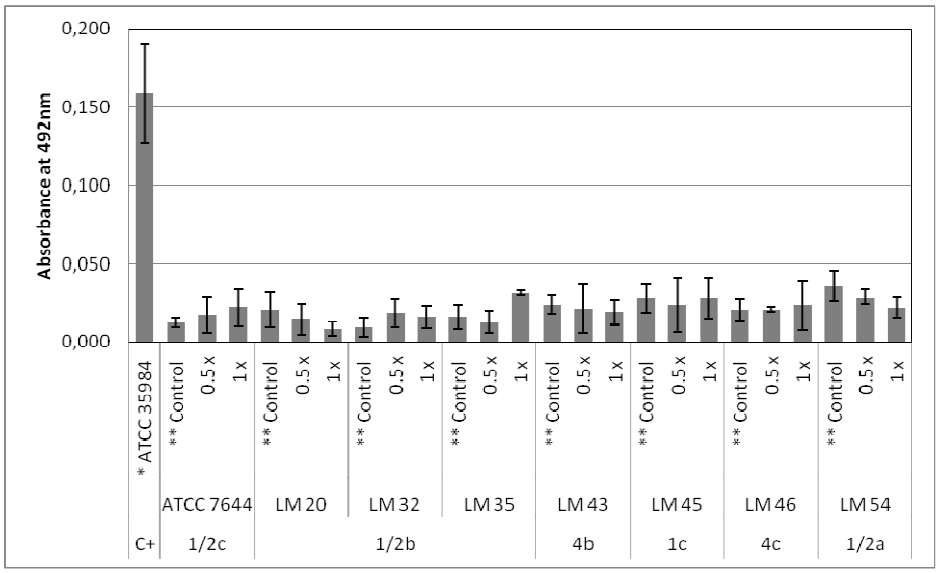

Figure 1. Effect of different concentrations (0.5x MIC and 1x MIC) of the essential oil of *H. psiadioides* on initial cell attachment against *L. monocytogenes* serovars incubated at 35 °C for 48 h.

* ATCC 35984 *Staphylococcus epidermidis* (positive control); ** Control: bacteria without essential oil.

DISCUSSION

The investigation for “green-products’’ derived from plants has been increasing. Consequently, plant extracts and their components are widely studied for their potential use in food as well as in medicine. The Asteraceae plant family, due to their secondary metabolites that act in chemical defense have been studied for their citotoxicity, allelopathy and antimicrobial characteristics, including antiviral, antibacterial and repellent use (20, 24, 25, 35). In the context of the antimicrobial characteristic, some researchers have been studying the anti-*Listeria* activity of essential oils from Asteraceae plants (14, 27, 28). Generally, such studies demonstrate similar results to those observed in the present study, in that we regard the magnitude of the inhibition zone as well as variability response among *L. monocytogenes* isolates in disk diffusion assay (14, 27, 29).

We could not observe a correlation between serovars and the sensibility to essential oils. Previous studies also revealed no difference or small differences in the sensitivities of the various serovars and species of *Listeria* to essential oils (37, 28, 36, 37). Therefore, it can be stated that, even within bacterial species, essential oil efficacy is dependent on the strain and in some cases on the strain's origin (29, 38). A study that screened the antibacterial activity of extracts from 13 Brazilian medicinal plants used to treat infectious diseases, reported antibacterial activity for five extracts from Asteraceae and, also pointed to a common compound responsible for this activity (39).

Although some authors claim that the antimicrobial effect of essential oils is due to an interaction between all the compounds present and not only to an individual component (17, 36), the biological properties can be a reflection of the major molecules present in the oils (18). The most representative molecules constituting the essential oil of *H. psiadioides* are monoterpenes. These components that have been found to interfere with the cell membrane functions in bacteria, crossing the cell membrane, penetrating into the interior of cells, interacting with intracellular sites and eventually causing cell death (14, 32). Monoterpene β-pinene is the major compound found in the essential oil here studied, and its bacteriostatic activity at low concentrations against *L. monocytogenes* strains has already been reported by Mourey & Cannilac (2002) (36). The essential oil of *H. psiadioides* also showed a bacteriostatic activity against *L. monocytogenes* strains, which would explain the results found in the bactericidal activity test and the decrease of inhibition zones in 48 h incubation.

The resistance of eight L. monocytogenes strains to the essential oil at lower temperatures (30°C and 5°C), when compared with MIC results at 35°C, were surprising for us. The hypothesis to explain the reported results is that some mechanism of *L. monocytogenes* to survival at low temperatures, such as changes in membrane composition and in gene expression, induction of proteins, stimulation of stress sigma factor (2) or flagella production below 30°C (40, 41), could be affecting, in some way, the essential oil’s mechanism of action, but further studies are needed to clarify these issues. The actual antibacterial activity of essential oils is not yet completely understood, it is most likely that there are several different kinds of damage and targets in the cell, including degradation of cell wall, damage to cytoplasmic membrane, damage to membrane proteins, leakage of cell contents, coagulation of cytoplasm and depletion of proton motive force (19, 42).

The disk diffusion assay and MIC determination are important methods to evaluate the antimicrobial activity, allowing direct contact between bacteria and compounds studied. Unlike these methods, in the gaseous contact assay, the contact between bacteria and volatile constituents occurs indirectly, which may have affected the results found here, where *L. monocytogenes* isolates were not sensitive, leading us to believe that the volatile compounds of the essential oil of *H. psiadioides*, by themselves, do not exhibit characteristics sufficient to prevent the growth of *Listeria*. The activity of the volatile fractions from *H. psiadioides* was not observed in the present study, contrasting with the cytotoxicity effect of essential oils of *H. psiadioides* and *H. alienus* for roots of lettuce and onion previously reported (20). This may be explained by prominent

differences between prokaryotes and eukaryotes characteristics, by their behavior towards the volatile constituents and by the characteristics of the method employed, which may not be the most appropriate to evaluate anti-*Listeria* activity of volatiles compounds.

Biofilm formation is an important problem; mainly for the food industry due to its resistance to a variety of approaches to eliminate or prevent its appearance, which can ultimately lead to food contamination and risks of outbreaks. The ability of *L. monocytogenes* to form biofilms is widely studied and it can vary among strains; however, the reasons for this variation remain unclear. There are attempts to find a relationship between biofilm formation with phylogenetic division, serotypes, persistent or sporadic strains and origin strains, but some disagreements still exist (12, 13, 15, 41). Djordjevic et al. (2002) (13) reported that strains from Division I (serotypes 4b and 1/2b) were significantly better at forming biofilm, than strains belonging to Division II (serotypes 1/2a and 1/2c). Nevertheless, Borucki et al. (2003) (12) found no significant statistical difference between strains, but it was observed that serovars 3a, 1/2c and 1/2a exhibited the highest average intensity values. The serovars 1/2c (15) and 1/2a (41), were also reported as more prolific biofilm formers. Although it is a small sample, our results agree with Borucki et al. (2003) (12), since there was no difference on biofilm formation between strains. The weak biofilm formation ability reported here, has also been observed in previous studies (15, 43). Though these results can be explained by the strains used, sample size and assay formats, the fact that *L. monocytogenes* do not form a classic biofilm, instead adhering to surfaces, is also a factor to be considered (12, 44).

The application of essential oils to prevent or eliminate *L. monocytogenes* biofilms has been researched under its importance for the food industry to control bacteria and demand for natural products with suitable properties. Most works evaluate the activity of essential oils, alone or in combinations, and their components in the *L. monocytogenes* preformed biofilm, usually varying the time of exposure to these agents (16, 17, 32). The results are quite variable, depending on plant species and compound characteristics tested. The prevention of cell attachment in *L. monocytogenes* strains, as performed in this study, has already been evaluated by Jadhav et al. (2012) (14) using yarrow essential oil (Asteraceae). Contrasting with previous works, the essential oil of *H. psiadioides* did not show preventive activity, because it was not able to modify the phenotype of biofilm formation among isolates. Although both plants belong to the same family, different results may be explained by the different composition of the essential oils.

CONCLUSION

The study demonstrated that the essential oil of *H. psiadioides* could be usefull against *L. monocytogenes.* The volatile constituents were not effective and the essential oil of *H. psiadioides* was unable to prevent cell adhesion. As in previous studies, the present issue reflects the complex variability of *L. monocytogenes* strains sensitivity to essential oils, demonstrating the need for further research in this area in view of the importance to control this pathogen and to reduce the risk of contamination.

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REFERENCES

(1) RAMASWAMY, V. et al. Listeria--review of epidemiology and pathogenesis. **Journal of Microbiology and Immunology Infection,** v.40, p. 4-13, 2007.

(2) List of Prokaryotic names with Standing in Nomenclature. LPSN. Available in:< http://www.bacterio.net/l/listeria.html>. Access:10 nov.2013.

(3) TORRES, K. et al. Patogenesis de *Listeria monocytogenes*, microorganismo zoonotico emergente. **MVZ-Córdoba,** v. 10, p. 511-543, 2005.

(4) ZHANG, Y. et al. Characterization of *Listeria monocytogenes* isolated from retail foods. **International Journal of Food Microbiology**, v.113. p. 47-53, 2007.

(5) LATHROP, A.A.; BANADA, P.P.; BHUNIA, A.K. Differential expression of InlB and Acta in Listeria monocytogenes in selective and nonselective enrichment broths. **Journal of Applied Microbiology**, v. 104, p.627–639, 2008.

(6) MCLAUCHLIN, J. et al. *Listeria monocytogenes* and listeriosis: a review of hazard characterization for use microbiological risk assessment of foods. **International Journal of Food Microbiology**, v.94, p.15-33, 2004.

(7) CHOU, C.H.; WANG, C. Genetic relatedness between *Listeria monocytogenes* isolates from seafood and humans using PFGE and REP-PCR. **International Journal of Food Microbiology**, v. 110, p.135-148, 2006.

(8) WANG, H.; LI Y.; SLAVIK, M. Rapid detection of *Listeria monocytogenes* using quantum dots and nanobeads-based optical biosensor. **Journal of Rapid Methods & Automation in Microbiology**, v. 15, p.67-76, 2007.

(9) HOFER, E.; DOS REIS, F.M.C.; HOFE, C.B. Sorovares de *Listeria monocytogenes* e espécies relacionadas, isoladas de material clínico humano. **Revista da Sociedade Brasileira de Medicina Tropical**, v. 39, p. 32-37, 2006.

(10) CHATURONGAKUL, S. et al. Modulation of stress and virulence in *Listeria monocytogenes*. **Trends Microbiology**, v.16, p.388- 396, 2008.

(11) UPADHYAY, A. et al. Antibiofilm effect of plant derived antimicrobials on *Listeria monocytogenes*. **Food Microbiology**, v. 36, p.79-89, 2013.

(12) BORUCKI, M.K. et al. Variation in biofilm formation among strains of *Listeria mocytogenes*. **Applied and Environmental Microbiology**, v. 69, p. 7336–7342, 2003.

(13) DJORDJEVIC, D.; WIEDMANN, M.; MCLANDSBOROUGH, L.A. Microtiter plate assay for assessment of *Listeria monocytogenes* biofilm formation. **Applied and Environmental Microbiology**, v. 68, p. 2950-2958, 2002.

(14) JADHAV, S. et al. Inhibitory activity of yarrow essential oil on Listeria planktonic cells and biofilms. **Food Control**, v. 29, p.125-130, 2013.

(15) HARVEY, J.; KEENAN, K.P.; GILMOUR, A. Assessing biofilm formation by *Listeria monocytogenes* strains. **Food Microbiology**, v. 24, p. 380–392, 2007.

(16) LEONARD, C.M. et al. Bioactivity of selected essential oils and some components on *Listeria monocytogenes* biofilms. **South African Journal of Botany**, v. 76, p. 676–680, 2010.

(17) SANDASI, M.; LEONARD C.M.; VILJOEN, A.M. The effect of five common essential oil components on *Listeria monocytogenes* biofilms. **Food Control**, v.19, p. 1070–1075, 2008.

(18) BAKKALI, F. et al. Biological effects of essential oils – A review. **Food and Chemical Toxicology,** v.46, p. 446–475, 2008.

(19) BURT, S. Essential oils: their antibacterial properties and potential applications in foods- a review. **International Journal of Food Microbiology**, v.94, p.223-253, 2004.

(20) SCHMIDT-SILVA, V. et al. Cytotoxicity of essential oils from two species of *Heterothalamus* (Asteraceae). **Australian Journal of Botany**, v.59, p.681-690, 2011.

(21) SUYENAGA, E.S. et al. Essential oil composition of *Heterothalamus psiadioides* Less. **Biochemical Systematics and Ecology**, v. 32, p. 83–86, 2004.

(22) BIAVATTI, M.W. et al. Etnhopharmacognostic survey on botanical compendia for potential cosmeceutic species from Atlantic Forest. **Revista Brasileira de Farmacognosia**, v.17, p.640-653, 2007.

(23) PALÁCIOS, S.M. et al. Screening of Argentinian plants for pesticide activity. **Fitoterapia**, v.78, p.580-584, 2007.
(24) DUSCHATZKY, C.B. et al. Evaluation of chemical and antiviral properties of essential oils from South American plants. **Antiviral Chemistry & Chemotherapy**, v. 16, p. 247–251, 2007.
(25) BASER, K.H.C. et al. The essential oil constituents and antimicrobial activity of *Anthemis aciphylla* BOISS. var. *discoidea* BOISS. **Chemical & Pharmaceutical Bulletin**, v.54, p.222-225, 2006.
(26) DIAS, J.F.G.; MIGUEL, O.G.; MIGUEL, M.D. Composition of essential oil and allelopathic activity of aromatic water of *Aster lanceolatus* Willd: (Asteraceae). **Brazilian Journal of Pharmaceutical Sciences**, v.45, p.497-474, 2009.
(27) LACIAR, A. et al. Antibacterial and antioxidant activities of the essential oil of *Artemisia echegarayi* Hieron. (Asteraceae). **Revista Argentina de Microbiología,** v.41, p. 226-231, 2009.
(28) FLORES, R.C. et al. Chemical composition of essential oil of *Baccharis salicifolia* (ruiz & pavon) PERS. and antibacterial activity. **Journal of the Chilean Chemical Society,** v.54, n. 4, p.475-476, 2009.
(29) VACA RUIZ, M.L. et al. Anti-listerial activity of plant essential oils from western region of Argentina. **Annals of Microbiology**, v. 56 , n. 4, p. 369-371, 2006.
(30) LAZAROTTO, D.C. **Fitotoxidez do óleo essencial de *Heterothalamus psidioides* Less. sobre *Arabidopsis thaliana* (L.) Heynh**. Dissertação de Mestrado – Programa de Pós-Graduação em Botânica, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brasil, 2014.
(31) RODRIGUES, F.F.; COSTA, J.G.; COUTINHO, H.D. Synergy effects of the antibiotics gentamicin and the essential oil of Croton zehntneri. **Phytomedicine,** v. 16, p. 1052-1055, 2009.
(32) de OLIVEIRA, M.M.M. et al. Control of planktonic and sessile bacterial cells by essential oils. **Food and Bioproducts Processing**, v. 90, p. 809–818, 2012.
(33) STEPANOVIC, S. et al. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. **APMIS**, v. 115, p. 891–899, 2007.
(34) VOGEL, L. et al. Biofilm production by Staphylococcus epidermidis isolates associated with catheter related bacteremia. **Diagnostic Microbiology and Infectious Disease**, v. 36 ,p.139–141, 2000.
(35) YANG, P.; MA, Y. Repellent effect of plant essential oils against *Aedes albopictus*. **Journal of Vector Ecology**, v.30, no.2, p.231-234, 2005.
(36) MOUREY, A.; CANILLAC, N. Anti-*Listeria monocytogenes* activity of essential oils components of conifers. **Food Control**, v. 13, p. 289–292, 2002.
(37) CANILLAC, N.; MOUREY, A. Antibacterial activity of the essential oil of *Picea excelsa* on *Listeria, Staphylococcus aureus* and coliform bacteria. **Food Microbiology**, v. 18, p. 261–268, 2001.
(38) SINGHA, A. et al. Efficacy of plant essential oils as antimicrobial agents against *Listeria monocytogenes* in hotdogs. **Lebensm.-Wiss. u.-Technol**, v. 36, p.787–794, 2003.
(39) HOLETZ, B.F. et al. Screening of Some Plants Used in the Brazilian Folk Medicine for the Treatment of Infectious Diseases. **Memórias do Instituto Oswaldo Cruz**, v. 97, p. 1027-1031, 2002.
(40) GRUNDLING, A. et al. *Listeria monocytogenes* regulates flagellar motility gene expression through MogR, a transcriptional repressor for virulence. **Proclamations of the National Academy of Sciences,** v.101, p.12318–12323, 2004.
(41) NILSSON, E.R.; ROSS, T.; BOWMAN, J.P. Variability in biofilm production by *Listeria monocytogenes* correlated to strain origin and growth conditions. **International Journal of Food Microbiology**, v. 150, p. 14–24, 2011.
(42) COX, S.D.et al. The mode of antimicrobial action of essential oil of *Melaleuca alternifolia* (tea tree oil). **Journal of Applied Microbiology**, v. 88, p. 170–175, 2000.
(43) ROCHO,S.R. **Avaliação da capacidade de formação de biofilme in vitro por *Listeria monocytogenes* isoladas de alimentos no RS**.Trabalho de Conclusão de Curso - Faculdade de Farmácia,Universidade Federal do Rio Grande do Sul, Porto Alegre, Brasil, 2009.

(44) KALMOKOFF, M. L. et al. Adsorption, attachment and biofilm formation among isolates of *Listeria monocytogenes* using model conditions. **Journal of Applied Microbiology**, v.91, p.725–734, 2001.

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