UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE FARMÁCIA

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

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Trabalho de Conclusão de Curso

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ACTIVITY OF ESSENTIAL OIL FROM *Heterothalamus psiadioides* Less (Asteraceae) 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 can cause listeriosis, a rare, but severe disease with high fatality rates. Effective control of this pathogen leads to search of new approaches, including the use of natural products. *Heterothalamus psiadioides* Less (Asteraceae) is a plant used in South America folk medicine to treat several infirmities. The aim of this study was analyze the activity of essential oil from *H. psiadioides* against *L. monocytogenes*. Twenty two strains of *L. monocytogenes* isolated from cheese were challenged in front to essential oil through 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). Biofilm formation among different strains serovars and capacity of the oil to prevent cell adhesion were assessed using crystal violet assay.The plant essential oil showed bacteriostatic action. However, their volatile constituents did not present anti-*Listeria* effect. The MICs ranged from 32% (v/v) to 8% (v/v) among temperatures and strains. The isolates tested showed weakly adherents and the oil did not prevent cell attachment. More researches are needed to clarify *L. monocytogenes* susceptibly to essentials oils and action's mechanisms of *H. psiadioides* essential oil.

KEYWORDS:

Listeria monocytogenes; *Heterothalamus psiadioides*; Essential oil; Biofilm

RESUMO

Listeria monocytogenes é um importante patógeno de origem alimentar que pode causar listeriose, uma doença rara, porém, severa 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* isoladas de queijos foram desafiadas frente ao óleo essencial 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 formação de biofilme entre diferentes sorotipos e a capacidade do óleo em evitar adesão celular foram analisadas utilizando o ensaio com cristal violeta. O óleo essencial *H. psiadioides* apresentou ação bacteriostática, entretanto, seus constituintes voláteis não demonstraram efeito anti-*Listeria*. As CIMs variaram de 32% (v/v) a 8% (v/v) entre temperaturas e isolados. As cepas de *L. monocytogenes* testadas mostraram-se fracas aderentes, e o óleo não foi capaz de impedir a fixação celular. Mais pesquisas são necessárias para elucidar a variabilidade de respostas 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 found in soil, water, vegetation, sewage and a wide variety of raw and processed foods, such as meats, meats products, milk and dairy products (1,2). *Listeria* species exhibit wide range of growth conditions, such as, refrigeration temperatures, acid pH and high salt concentrations (1). Currently, have been described 12 species of the *Listeria* genus (3), among them, the only species pathogenic to humans is *Listeria monocytogenes.* It is an important food-borne pathogen that can cause gastroenteritis in immune-competent people and/or listeriosis, a rare, but severe disease with high hospitalization and fatality rates, varying of 20 to 40% (1), primarily affecting elderly, pregnant, newborns and immune-compromised populations (2,4,5).

Listeria monocytogenes serotyping are differentiated according to the diversity in somatic and flagellar antigens. Nowadays, 13 serovars are described for this species: 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, 7 (6-8). Around 90% of strains isolated from patients with listeriosis belonging to serotypes 1/2a, 1/2b and 4b (1,4,9,10). According to Hofer et al. (2006) (11), the predominant serovars isolated from human clinical specimens in Brazil are 4b and 1/2a. The clinical syndromes associated with listeriosis can include central nervous system infections, such as meningitis and encephalitis, bacteremia, endocarditis and stillbirths, fetal death or abortions in pregnant women (1,5).

In the past decades, a several food-borne outbreaks caused by *L. monocytogene*s have been reported in Europe and in the United States. Since that, the interest for determine the risks associated with consumption of foods, virulence mechanisms of the bacteria, incidence of the disease and control strategies by food manufactures, government bodies, public health agencies and scientific branch have increased (1,12). Unlike of developed countries, in underdeveloped countries, including Brazil, the outbreaks and/or cases of listeriosis are underreported or under-diagnosed, although there are researches on the prevalence of *L. monocytogenes* strains isolated from patients (13). The ubiquitous nature and hardly growth characteristics, including tolerance to desiccation and ability to attach and form biofilm on a variety of equipment surfaces, allows *L. monocytogenes* to contaminate

and thrive in food-processing environment, resulting in great concern for food industry (1,14-17).

Biofilms are microbial communities that exist on abiotic and biotic surfaces and, once established, appear to present enhanced resistance to antimicrobial agents, sanitizers, disinfectants, desiccation and UV light (17-19). Hence, better disinfecting approaches are required to ensure effective control of bacterium, improving food safety and reducing the risk of listeriosis by ingestion of contaminated food (18,20,21). Although the variety of synthetic disinfectants approved for use by national regulatory agencies, there are concerns about the safety of these synthetic chemicals especially in the food processing industry, resulting in a growing demand for natural products, such as biocides, plant extracts and essential oils (12,22). A number of essential oils and several of their individual components exhibit antibacterial activity against food-borne pathogens *in vitro*, including the ability to control *Listeria* (12,23,24). It is a prospective area for research due to the rise of “green consumerism”, which stimulates the use and development of products derived from plants. Essentials oils are mixture of volatile compounds produced by plants, characterized by a strong odor, formed by aromatic plants as secondary metabolites. Known for their antiseptic, bactericidal, virucidal, fungicidal, others medical properties and their fragrance, they are used in preservation of foods and in medical products (25).

Heterothalamus psiadioides Less (vernacular name: “alecrim-do-campo”, “vassoura” or “erva-formiga”) belongs to the Asteraceae family and grows as a shrub in the Southern Brazil, Argentina and Uruguay (26). In South America folk medicine this species is used as an antipyretic, antidote of snake’s bite, anti-inflammatory (27), febrifuge and is potentially used for skin remedies (28) and against renal diseases (29). The major compound of genus *Heterothalamus* is β-pinene (27,30) which is one of the most widely distributed monoterpenes in plant species and major component of various essentials oils (25). The family Asteraceae present many constituents that have been studied for antimicrobial characteristics, cytotoxicity and allelopathy (31-33). The interactions with other plants, including the cytotoxicity of *H. psiadioides* essential oil, were evaluated by Schmidt-Silva et al. (2012) (26). Their results indicated that *H. psiadioides* essential oil can cause mitodepressive effects and chromosomal abnormalities in roots meristems.

Based on the cytotoxic effects of *H. psiadioides* essential oil already described in plants (26) and the antibacterial effect of plants belonging to Asteraceae family (20,31;34-36), this study aims to evaluate the action of essential oil from *H. psiadioides* against *L. monocytogenes,* since the control of this bacteria becomes required. To our knowledge, this is the first study to analyze the action of *H. psiadioides* against *L. monocytogenes* isolates.

METODOLOGY

Bacterial strains, storage and inoculums preparation

Twenty-two *L. monocytogenes* isolated from cheeses were provided by Institute of Food Science and Technology of Federal University of 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) were used in the present study. To confirm the purity of the cultures, the method of Gram Stain and verification of morphologic characteristics of colonies on selective medium PALCAM Listeria Agar (BD Diagnostic Systems) were made.

For standardization, the strains were inoculated in 3 mL of Tryptone Soya Broth (TSB, HiMedia) and incubated at speed of 80 RPM for 24h at 35°C .The aliquots of TSB culture were frozen at -20°C containing 50% (v/v) of glycerol.

Prior to each experiment, an aliquot of frozen cells was recovered onto Brain Heart Infusion (BHI, Oxoid) Agar or Tryptone Soya Agar (TSA, HiMedia) and incubated at 35 °C for 18h. For experimental procedures, a loopful of the BHI or TSA cultured of each isolate was dispersed in 0.9% saline solution (w/v) sterile until matched to the 0.5 McFarland turbidity standards (approximately $1x10^8$ CFU/mL).

Essential oil

The essential oil of *Heterothalamus psiadioides* was provided by Prof. Geraldo Luiz Gonçalves Soares of Institute of Bioscience of Federal University of Rio Grande do Sul (Porto Alegre - RS, Brazil). Specimens of *H.* psiadioides (175007) were deposited in a herbarium in Federal University of Rio Grande do Sul (ICN). The

assays were conducted using pure oil or a mixture with proportion of 3 parts of oil to 1 part of Dimethyl Sulfoxide (DMSO, Sigma-Aldrich).

Agar disk diffusion assay

The anti-listerial activity of *H. psiadioides* essential oil was investigated using the agar disk diffusion assay. The assay was carried out based on the recommendation of M2-A8/CLSI (2003) (37) with some modifications. The inoculums adjusted to a 0.5 MacFarland standard was uniformly spread on the surface of Müeller-Hinton Agar (MH, HiMedia) plates. Sterile filter papers discs of 6mm impregnated with 20 µL of essential oil were placed on the surface of the culture medium at the center of the dish. The plates were incubated at 35°C for 24h and 48h to observe whether the inhibition would remain the same. Before the times of incubations, the zones of inhibitions were measured (diameter in mm).

Volatile constituents assay

To analyze the activity of volatile constituents of essential oil from *H. psiadioides*, eight *L. monocytogenes* strains (ATCC 7644, LM20, LM 32, LM 35, LM 43, LM 45, LM 46, LM 54) were selected. The assay was conducted based on Schimidt-Silva et al. (2011) (28) with modifications. Initially, the inoculums was uniformly spread on surface of Müeller-Hinton agar plates. One hundred microlitres of pure oil (100%), 10% aqueous solution (v/v) or sterile distilled water (negative control) were dropped onto a swab attached to the cover of the plates avoiding direct contact with the bacteria. The plates were sealed and incubated at 35°C for 24h. The aqueous solution was prepared with essential oil, DMSO and sterile distilled water to yield concentration of 10% (v/v). After incubation time, the results were analyzed by visual inspection.

Determination of minimal inhibitory concentration (MIC) and bactericidal activity's assessment

Broth microdilution method in 96-well round-bottomed polystyrene microtiter plates was used to evaluate the MIC of *H. psiadioides* essential oil against eight *L. monocytogenes* strains (ATCC 7644, LM20, LM 32, LM 35, LM 43, LM 45, LM 46, LM

54). The assay was conducted as described by Jadhav et al. (2013) (20) with some modifications. Initially, a mixture of *H. psiadioides* essential oil and DMSO was made and added to the wells, containing the BHI medium. A serial dilution was performed to achieve a gradient from 64% to 0.5% (v/v) in a final volume of 100 µL. Ten microlitres of the inoculums were added to each well. The microplates were capped and incubated at 35°C and 30°C for 24h and at 5°C for 5 days. The assays were performed three times and controls negatives (broth medium) and positives (broth medium and inoculums) were presented on all tests. A microtiter plate containing only gradient of DMSO and inoculums was performed to exclude the eventual influence of this reagent on the MIC results. The minimal inhibitory concentration corresponds to the lowest concentration of *H. psiadioides* essential oil which inhibited visible growth of the bacteria.

For evaluate the bactericidal activity, 100 µL of the wells which there were no visible bacteria's growth were spread with Drigalski handle onto surface of BHI agar plates and incubated for 24h at 35°C.

Biofilm formation and activity of *H. psiadioides* essential oil on sessile cells

The effect of *H. psiadioides* essential oil on *L. monocytogenes* biofilm formation in flat-bottomed 96-well microplates was assessed using the modified crystal violet assay (38,39). The bacterial inoculums were prepared as previously described, replacing the 0.9% saline solution (w/v) for TSB broth containing 1% glucose (TSB-G). Solutions of *H. psiadioides* essential oil (equivalent to 0.5 x MIC and 1 x MIC) were prepared using TSB-G and DMSO. Fifty microlitres of each culture was pipetted into the wells with 150 µL of TSB-G, 0.5 x MIC or 1 x MIC to yield a final volume of 200 µL and incubated at 35°C for 48h. The cultures were added into the wells in quadruplicates. Each plate included 4 negative control wells comprising 200 µL of sterile TSB-G medium and 4 positive control wells comprising *Staphylococcus epidermidis* (ATCC 35984) inoculums which represents strongly formation biofilm. Elapsed incubation times, the content of each well was aspirated and washed three times with 200 µL of 0.9% saline solution (w/v) sterile. Then, the plates were shaken to remove the non-adherent bacteria and the sessile cells were fixed with 150 µL of methanol PA for 20 min. The sessile cells were stained with 150 µL of 0.5% crystal violet solution (w/v). After 10 min, the crystal violet solution was removed, the plates

were washed with distilled water and, after drying, 200 µl of 95% ethanol (v/v) was added to resolubilize the dye bound to adherent cells. The absorbance was read at 492nm (40) after 30 min on Enzyme-linked Immunosorbent Assay (ELISA) reader. To correct background staining and correct interpretation of results, the cut-off value to each plate was calculated conforming Stepanovic et al. (2007) (39). Then, this cut-off value was subtracted from the mean OD value obtained for each test strain included in the same plate and any positive value was considered biofilm formation. The classification based on Stepanovic et al. (2007) (39) also was used: non-adherent (OD final ≤ OD negative control), weakly adherent (OD negative control ≤ OD final ≤ 2 x OD negative control), moderately adherent (2 x OD negative control ≤ OD final ≤ 4 x OD negative control) and strongly adherent (4 x OD negative control ≤ OD final).

RESULTS

Essential oil activity against *L. monocytogenes*

The disk diffusion assay was used as screening test to observe the anti-*Listeria* activity of *H. psiadioides* essential oil against different strains. After 24h and 48h incubation with the oil at 35°C, the zones of inhibition's diameter were measured (Table 1). There was a decrease of inhibition zones between 24 and 48h incubation. While the inhibition halos for 48h incubation varied between 20 to 10 mm, the strains for 24h incubation presented larger inhibition halos, reaching values of 40, 30 and 20 mm of diameter. The isolates LM 20 (serovar 1/2b) and LM 44 (serovar 4b) showed the smallest inhibitions zones as many for 24h incubation as for 48h incubation. It was not possible to correlate serovars with different susceptibilities to essential oil, in view of variability of results found.

At volatile constituents activity assay, it was prospect the *anti-Listeria* activity of *H. psiadioides*. Different from that observed for plant roots meristems (26), wherein the contact with volatile caused mitodepressive effects and chromosomal abnormalities in onion and lettuce-root, the listerial growth on Müeller-Hinton agar was not altered. It was observed the same visual bacterial growth on negative control, 10% aqueous solution (v/v) and pure oil (100%) for the all isolates tested.

The MICs observed at 35°C were 16% (v/v) for isolates ATCC 7644, LM20, LM 32, LM 35, LM 43, LM 45, LM 46 and 8% (v/v) for isolate LM 54, whereas at 5°C and 30°C the eight strains tested showed MICs equal to 32% (v/v) with the exception of

isolate LM 35, which exhibited MIC equal to 16% (v/v) at 30°C. Curiously, comparing the three temperatures tested, it was verified that under lower temperatures (30°C and 5°C) the strains demonstrated being less susceptible to *H. psiadioides* essential oil than at 35°C, requiring higher concentrations of the oil for preventing its growth. As well as the disk diffusion test, there were no differences between the serotypes and MIC results. Regarding the evaluation of bactericidal activity, there was *Listeria* growth on all BHI agar plates when spread the content of MIC wells at 35°C, indicating that *H. psiadioides* essential oil do not have bactericidal activity at MIC.

Table 1. *Listeria monocytogenes* strains and diameter in millimeters (mm) of inhibition halos formed by essential oil from *H. psiadioides* in 24 and 48h incubation

***L. monocytogenes* Strains**	**Serovar**	**Diameter (mm)**		**Provider**
		24h	48h	
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]

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

The effect of the essential oil on biofilm formation

All the eight *L. monocytogenes* strains tested shown weak capacity to form biofilm in flat-bottomed 96-well microplates under experimental conditions independent of their serovars. The effect of the essential oil on initial cell attachment was investigated when adding the oil (at concentrations equal to 0.5 x MIC and MIC) at assay's start and comparing these results with those wells without the treatment (Figure 1). In some isolates there was an increase (ATCC 7644, LM 32, LM 35) and, in others, a decrease (LM 20, LM 54) of OD values when in contact with the *H. psiadioides* essential oil. However, the phenotype for biofilm remained the same between the strains with or without the essential oil.

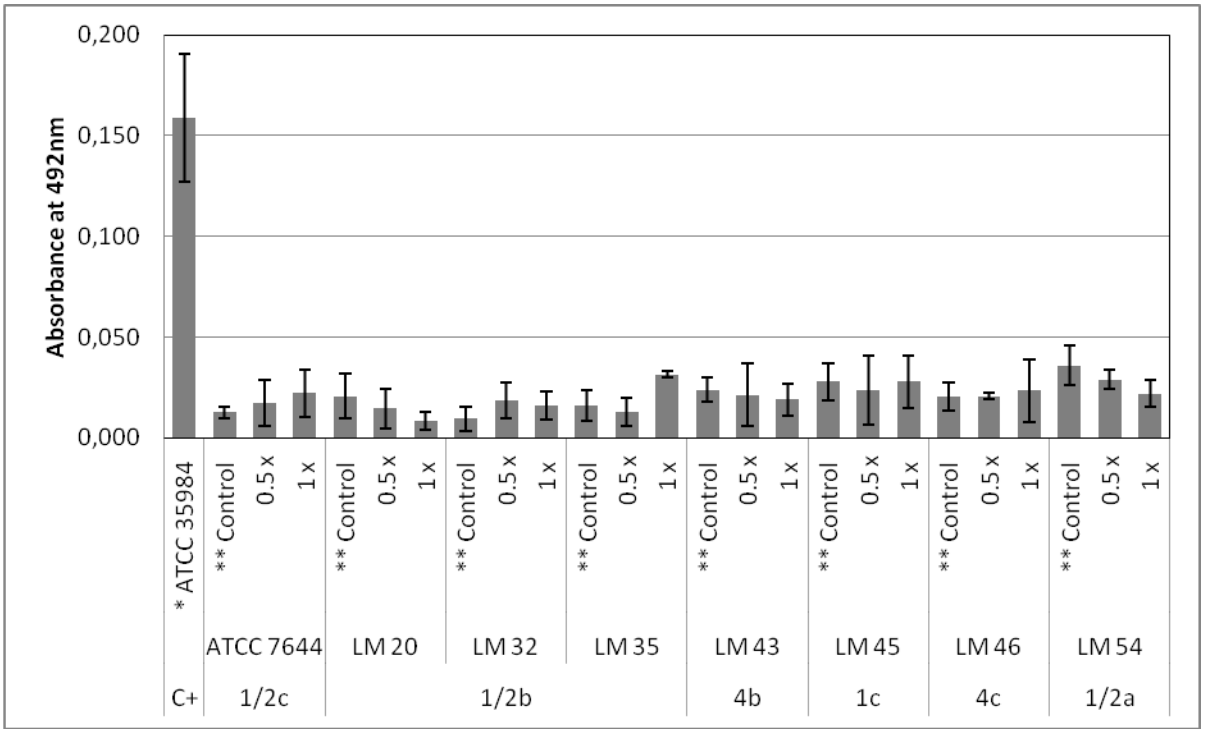

Figure 1. Effect of different concentrations (0.5 x MIC and 1x MIC) of essential oil from *H. psiadioides* on initial cell attachment in 6 *L. monocytogenes* serovars incubated at 35 ^{0}C for 48 h, determined by the crystal violet assay. * ATCC 35984 *Staphylococcus epidermidis* (strong biofilm former-positive control); ** Control: bacteria without essential oil.

DISCUSSION

The search for products derived from plants is crescent due to “green-consumism’’ and concerns about safety of synthetic products commonly used. Therefore, essential oils, plants extracts and their components are widely studied by their medical properties and potential for several industrial and healthy uses. Asteraceae plants’ family, due to their secondary metabolites that act in chemical defense, have been studied for their citotoxicity, allelopathy and antimicrobial characteristic, including antiviral, antibacterial and repellent use (26,30,31,41). In the context of antimicrobial characteristic, there are some researchers about anti-*Listeria* activity of essential oils derived from Asteraceae family’s plants (20,34-36). In general, such studies demonstrated similar results to observed in the present study, in regarding to the magnitude of the inhibition zone (20,34,36) as well as variability response among *L. monocytogenes* isolates in disk diffusion assay (34, 36).

In MIC determination, previous studies revealed no difference or small differences in the sensitivities of the various serovars and species of *Listeria* to essential oils (34,35,42,43), agreeing with our results. 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 origin (36,44). A study, which screened the antibacterial activity of extracts of 13 Brazilian medicinal plants used to treat infectious diseases, reported antibacterial activity of 5 extracts from Asteraceae family and also pointed to a common compound responsible for this activity (32).

Although some authors claim that the antimicrobial effect of essential oil is due to interaction between all the compounds present and not only due to an individual component (22,42,45), the biological properties can be a reflect of the majors molecules presents in oil (25,45). The most representative molecules constituting 90% of essential oil are monoterpenes (25), that have been found to interfere with the cell membrane functions in bacteria, crossing the cell membrane, penetrating into interior of the cells, interacting with intracellular sites and eventually causing cell death (20,38). The monoterpene β-pinene is the major compound of the genus *Heterothalamus* (27,30) and the bacteriostatic activity at low concentrations against *L. monocytogenes* strains have already been reported by Mourey & Cannilac (2002) (42). The *H. psiadioides* essential oil also showed a bacteriostatic activity against *L.*

monocytogenes strains, which would explain the results found at bactericidal activity test and the decrease of inhibition zones in 48h incubation.

The greater resistance of eight selected strains to *H. psiadioides* 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 *L.monocytogenes* mechanism of survival at low temperatures, such as changes in membrane composition, changes in gene expression, induction of proteins, stimulating of stress sigma factor (2) or the flagella production below 30°C (46,47) could be affecting in some way the essential oil's mechanism of action, but further study is needed to clarify these issues. The actual antibacterial action of essential oils is not yet completely understood, it is most likely that there are several 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 (23,48).

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, the contact between bacteria and volatile constituents occurs indirectly, which may be affected the results found at volatile form activity assessment, where *L. monocytogenes* isolates were not sensitive, leading us to believe that the volatile compounds of essential oil from *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 cytotoxicity effect of essential oils of *H. psiadioides* and *H. alienus* for roots of lettuce and onion previous reported (26). It may be explain by prominent differences between prokaryotes and eukaryotes characteristics, by their behavior towards the volatile constituents and by the own characteristics of the method employed, which may not be the most appropriate to evaluate the anti-*Listeria* activity of volatiles compounds.

Food-borne bacterial biofilm is an important concerned mainly for food industry due to its resistance to a variety approaches for eliminate or prevent its appearance, which can ultimately lead to food contamination and risks of outbreaks. Biofilm formation by *L.monocytogenes* is widely studied and it can vary among strains, however, the reasons for this variation remain unclear. There are attempts to link biofilm formation with phylogenetic division, serotypes, persistent or sporadic strains

and origin strains, but some disagreements exist yet (17,21,47,18). Djordjevic et al. (2002) (18) 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) (17) found no difference statically significant among strains, but it was observed that serovars 3a, 1/2c and 1/2a exhibited highest average intensity values. The serovars 1/2c (21) and 1/2a (47) also were reported as more prolific biofilm formers. Although it is a small sample, our results agree with Borucki et al. (2003) (17), once there was no difference on biofilm formation among strains. As well the variation among strains, the weak biofilm formation reported here also has been observed in previous studies (21,49). Though these results can be explained by the strains used, sample size and assay formats, the feature of *L. monocytogenes* do not form a classic biofilm but adheres to surfaces is a factor to be considered (17,50).

The application of essential oils to prevent or eliminate *L.monocytogenes* biofilm has been researched in view of its importance for the food industry to control of bacterium and demand for natural products with suitable properties. Most works evaluates 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 (12,22,38). The results are quite variable, depending on plant species and compounds characteristics tested. The prevention of cell attachment in *L. monocytogenes* strains, as performed in this study, have already been evaluated by Jadhav et al.(2012) (20), using yarrow essential oil (Asteraceae) at beginning assay. Disagreeing with previous work, the *H. psiadioides* essential oil did not show preventive active, 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 different formation of essential oils.

CONCLUSION

The *H. psiadioides* essential oil showed bacteriostatic activity against *L. monocytogenes* strains. At present study, it was observed that volatile constituents showed no effect against *L. monocytogenes* strains and the essential oil from *H. psiadioides* was unable to prevent cell adhesion. Also, it was verified that biofilm formation did not vary among the different serovars of *L. monocytogenes*, although the size sample tested. As previous studies, the present work reflects the complex variability of *L. monocytogenes* strains sensitivity to essential oils, demonstrating the need for further researches in this area in view of importance to control this pathogen and to reduce the risk of contamination.

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