Brazilian Journal of Microbiology 45, 2, 699-705 (2014)
ISSN 1678-4405
Copyright © 2014, Sociedade Brasileira de Microbiologia
www.sbmicrobiologia.org.br

Research Paper

The antimicrobial efficacy of *Lippia alba* essential oil and its interaction with food ingredients

Terezinha Feitosa Machado[1], Nádia Accioly P. Nogueira[2],
Rita de Cássia Alves Pereira[1], Cívita Teixeira de Sousa[2],
Valéria Chaves Vasconcelos Batista[2]

[1]Embrapa, Fortaleza, CE, Brazil.
[2]Departamento de Análises Clínicas e Toxicológicas, Universidade Federal do Ceará, Fortaleza, CE, Brazil.

Submitted: November 24, 2011; Approved: September 9, 2013.

Abstract

The objective of this study was to evaluate the antimicrobial potential of *Lippia alba* essential oil (EOLa) and to investigate the effect of food ingredients on its efficacy. The antimicrobial potential of the oil was determined by the presence or absence of inhibition zones, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against Escherichia coli, *Listeria innocua*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella choleraesuis* and *Staphylococcus aureus*. The effect of food ingredients and the pH on the antimicrobial efficacy of oil was assessed by monitoring the maximum growth rate of *Listeria monocytogenes* in model media. The model media included potato starch (0, 1, 5 or 10%), beef extract (1, 5, 3, 6 or 12%), sunflower oil (0, 5 or 10%) and TSB broth at pH levels of 4, 5, 6 or 7. The EOLa showed efficacy at all concentrations (50%, 25%, 6.25%, 3%, 1.5%, 0.8%, 0.4% and 0.2%) evaluated, against all bacterial species, Gram-positive and Gram-negative. The antimicrobial efficacy of EO was found to be a function of ingredient manipulation. Proteins and lipids had a negative impact on the oil effectiveness, indicating the protective action of both on the microbial specie tested. On the contrary, at the highest concentration of starch (10%), the lower rate growth of *L. monocytogenes* was detected, therefore indicating a positive effect of carbohydrates on the oil effectivenes. Regarding the pH, the studies showed that the rate of microbial growth increased with increasing pH. It was concluded that the use of EOLa is more effective control pathogenic and spoilage bacteria when applied to starchy foods under an acidic pH.

Key words: essential oil, antimicrobial, food ingredient.

Introduction

Ensuring food safety while at the same time meeting the demands for nutritional conservation attributes and quality have driven the search for the development of alternative methods for food preservation. These increasing demands open new perspectives for the use of natural preservatives derived from plants, animals or microorganisms (Tiwari *et al.*, 2009). Addition of antimicrobial compounds to processed or non-processed foods, prolong their shelf life by reducing the microbial viability or growth rate (Beuchat and Golden, 1989).

Originally, the herbs and spices were used to change or improve the taste and flavor of food. However, products of secondary metabolism of plants, such as essential oils (EOs), naturally play an important role in the protection of plants against infectious agents (Bakkali *et al.*, 2008). Many of the constituents of these oils are known to delay or inhibit the growth of bacteria, fungi, viruses and insects (Burt and Reinders, 2003, Chorianopoulos *et al.*, 2008).

Lippia alba (Mill.) N. E. Brown (Verbenaceae) is a plant native from South America, popularly known as field lemon balm, bush lemon balm, Brazilian lemon balm, field

Send correspondence to T.F. Machado. Embrapa, Rua Dra. Sara Mesquita 2.270, 60511-110 Fortaleza, CE, Brazil. E-mail: terezinha.feitosa@embrapa.br.

rosemary, wild lemon balm and false Melissa among others (Biasi and, Costa, 2003). Leaves and roots of the plant are used in folk medicine in various forms. It is used for the treatment of gastric diseases, antipyretic diseases, as analgesic and as sedative (Barbosa Filho *et al.*, 2006; Julião *et al.*, 2003). Among the secondary metabolites reported for this species, the essential oil, which constituents include mono and sesquiterpenes, is highlighted (Pascual *et al.*, 2001). Ethanolic extracts of its leaves have been described to possess antimicrobial activity against *Staphylococcus aureus* (Sena Filho *et al.*, 2006) and different others microorganisms (Aguiar *et al.*, 2008), while the EO was characterized to with activity against *Bacillus subitilis, Enterococcus faecalis* (Alea *et al.*, 1997), *Candida albicans* (Duarte *et al.*, 2005), *C. parapsilosis*, *C. krusei*, *Aspergillus flavus* and *A. fumigatus* (Mesa-Arango *et al.*, 2009).

Because they are devoid of toxic actions, lemon balm herb infusions can be consumed in high doses (Matos, 1998) and its EO is promising for the control of pathogenic and spoilage food microorganisms. Thus, the objective of this study was to evaluate the efficacy *L. alba* essential oil against food spoilage and foodborne pathogenic bacteria and investigate the effect of food ingredients on its efficacy.

Materials and Methods

Plant material

Samples of leaves of *Lippia alba* (MILL.) N.E.Brown (*Verbenaceae*) were obtained from plant matrices from a medicinal garden belonging to Embrapa Tropical Agroindustry. Part of the raw material was dehydrated in a tray dryer with air circulation at temperatures of 30 °C for 24 h (Barbosa and Barbosa, 2006). A voucher specimen of the plant was deposited in Embrapa's Genetic Resources and Biotechnology Herbarium under the number CEN 73.791.

Essential oil extraction

The plant material, fresh and dried leaves, was submitted to hydro-distillation for 4 h using a Clevenger-type apparatus. The essential oil (EO) separated by centrifugation was subjected to drying with Na_2SO_4. The obtained oil was transferred to amber glass bottles with lids and stored at 2 °C until analyses (Craveiro *et al.*, 1976).

Gas chromatographic (GC) and gas chromatographic mass spectrometry (GC-MS) analysis

The percentage composition of essential oil was determined by GC-FID and the compounds were identified by GC-MS. GC analysis was carried out on a Shimadzu 2010 Gas Chromatograph equipped 2010 Gas Chromatograph equipped with an FID and 25 m x 0.25 mm x 0.25 μm WCOT column coated with diethylene glycol (AB-Innowax, 7031428, Japan). Both injector and detector (FID) temperatures were maintained at 260 °C. Helium was used as carrier gas at a flow rate of 3.0 mL/min at a column pressure of 152 kPa. Samples (0.2 μL) were injected into the column with a split ratio of 100:1. Component separation was achieved following a linear temperature program of 60-260 °C at 3 °C/min and then held at 260 °C for 10 min, with a total run time of 76 min. The percentage composition was calculated using peak normalization method assuming equal detector response. The samples were then analysed on same Shimadzu instrument fitted with the same column and following the same temperature program as above and the MS parameters used were: Ionisation Voltage (EI) 70 eV, peak width 2 s, mass range 40-700 m/z and detector voltage 1.5 V. Peak identification was carried out by comparison of the mass spectra with mass spectra available on database of NIST05, WILEY8 libraries and those of pure standards.

Microorganisms

The bacterial, pathogenic and spoilage food microorganisms employed in this study were *Escherichia coli* ATCC 10536, *Listeria innocua* ATCC 19115, *Listeria monocytogenes* ATCC 33090, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella choleraesuis* ATCC 10708 and *Staphylococcus aureus* ATCC 6538P. All cultures were maintained at -80 °C in brain and heart infused broth (BHI, Merk, Marcy l'Etoile, France) containing 10% glycerol. The cultures were prepared by subculturing 100 μL of each culture stock in 9 mL of BHI and incubating at 35 °C until they reached the exponential growth phase (12 h). Then, the obtained cultures were diluted in 0.85% NaCl solution until turbidity similar to 0.5 McFarland scale (Biomerieux Inc. Darmstadt, Germany), was obtained, corresponding to approximately 1.5×10^8 cfu/mL.

Antibacterial activity assay

The Agar diffusion test was performed as previously described (NCCLS, 2003a) with some modifications. 20 mL of Mueller-Hinton agar (Becton Dickinson, USA) were inoculated with 10^6 cfu/mL of one of the indicator strains and then poured onto a Petri dish and allowed to solidify. Wells of 5 mm diameter were aseptically perforated over the agar surface and 25 μL of serially EO dilutions in Tween 80 (Vetec, Duque de Caxias, Rio de Janeiro, Brazil) 1%, were added to the wells. The plates were kept at room temperature to allow dispersal and subsequently incubated under optimal conditions for growth of the target strains. The antimicrobial activity was visually evaluated as inhibition zones surrounding the wells. Sterile solutions of Tween 80 1% and amikacin (Sigma-Aldrich Co., St. Louis, MO, USA) 1.2 mg/mL were used as negative and positive controls, respectively.

Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC values were measured in sterile 96-well microtiter plates (NCCLS, 2003b) with modifications. Bacterial suspensions with cell density were adjusted to 10^8 cfu/mL, as previously described and were diluted in sterile BHI broth until 10^6 cfu/mL was reached. The wells were added with 80 μL of microbial suspensions, 100 μL of BHI broth (Merck) and 20 μL of binary dilutions of the EOLa from fresh and dry leaves (37.5 to 0.29 mg/mL and 42.74 to 0.33 mg/mL, respectively). In the control wells, 80 μL of microbial suspensions, 100 μL of BHI broth and 20 μL of sterile solution of amikacin 1.2 mg/mL or Tween 80 1% were added. After incubation (35 °C for 24 h) 10 μL of 3-(4,5-dimethylthiazyl)-2,5-diphenyltetrazolium bromide (MTT, Sigma Aldrich, St. Louis, MO, USA) (dissolved in phosphate buffered saline to a concentration 5 mg/mL) was pipetted into each well to stain the living cells. The MIC was defined as the lowest concentration of the test sample that resulted in a complete inhibition of microorganism growth.

The MBC was determined according to the method described by Baron *et al.* (1994). Aliquots of 50 μL were removed from the wells that showed no visible turbidity in the MIC determination test and plated on the surface of plate count agar (Merck). After incubation for 24 h at 35 °C, the colonies grown on the surface of the agar were counted. The concentration of EOLa able to inhibit the microbial growth to less than 0.1% of the initial inoculum was considered as the MBC.

Interactive effects of food ingredients and pH

The effect of food ingredients and pH on the antimicrobial efficacy of EOs was performed using a range of model media. *L. monocytogenes* ATCC 33090 was chosen as indicator strain (Gutierrez *et al.*, 2008b). MBC oil, previously established for the microbial species, was used (0.58 mg/mL). Model media were comprised as the following: (I) water soluble starch from potato (0, 1, 5 or 10%, Difco) in tripticase soy broth (TSB), (II) beef extract (1.5, 3, 6 or 12%, Difco) in distilled water and (III) sunflower oil (0, 5 or 10%) in TSB. Model media containing starch or beef extract were autoclaved prior to use. For the oil model media, the sunflower oil was autoclaved separately and then added to sterile TSB. Filter-sterilized Tween 80 (Merck) was added at 1% to facilitate mixing and to stabilize the emulsion. The pH of each model media was adjusted to 7.2. To determine the effect of pH on EO efficacy, TSB was adjusted to pH 4, 5, 6 or 7 with 1 N HCl solution.

The growth of *L. monocytogenes* in each model media containing EO was monitored using 96 well microplates. To the wells, 80 μL of each medium, 20 μL of EO and 100 μL of 10^6 cfu/mL of *Listeria* strain were added, which were assessed in micro plate spectrophotometer (Varian Cary, Cambridge, UK). Positive controls contained model media inoculated with the organism under investigation. Negative controls contained only EO and sterile model media. The survival curves of *L. monocytogenes* in model media were monitored at 490 nm over a 24 h period.

Statistical data analyses

Conventional statistical methods were used to calculate means and standard deviations of two simultaneous assays. To discover whether there were significant differences between the means, it was used Tukey test at $p < 0.05$ level.

Results

Chemical composition of *L.alba* essential oil

GC-MS analyses of the oil led to the identification of 36 different components, representing 98.30% of the total oil. The identified compounds are listed in Table 1. The oil showed a complex mixture of compounds which major constituents were e-citral (31.57%), neral (25.50%), d-limonemo (14.07%), germacrene D (5.47%), b-elemol (5.37%), g- terpinen (4.09%) and p-cymene (1.56%). According to Matos (1996), the majority presence of citral and limonene in oil, classifies it as the chemotype II.

Antibacterial activity assay

The L. alba EO showed antimicrobial activity at all concentrations tested with a broad spectrum of activity, inhibiting the growth of both Gram positive and Gram negative bacteria (Table 2). *S. aureus* was the species more sensitive to the oil. The fresh leaf oil was more efficient than the dry leaf oil against the bacterial tested, except for *L. innocua.*

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The values obtained for the MICs and MBCs of EOs from fresh and dried *L. alba* leaves presented in Table 3 confirm the antimicrobial potential of these substances on Gram positive bacteria. The antimicrobial potential was especially strong against *S. aureus*, which had its growth inhibited when exposed to 0.29 mg/mL of fresh leaves oil and made it impossible when exposed to 0.33 mg/mL of EOLa of dried leaves. These results corroborate those obtained by the diffusion method reported previously (Table 1).

Interactive effects of constituents of foods and pH

The maximum specific growth rate (μmax) of *L. monocytogenes* grown in different model media is indicated in Table 4. The antimicrobial effect of EOLa varied depending on the constituent manipulated. Proteins and lipids had a negative impact on the effectiveness of the oil.

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

Compound	IK[1]	Composition (%)
α - tujen	927	0.31
α - pineno	936	0.03
Sabinen	976	0.80
6-Metil-5-hepten-2-one	987	0.12
Mircen	990	0.45
a-Felandren	1010	0.04
4-Caren	1021	0.13
p-Cimen	1029	1.56
d-Limonen	1033	14.07
g-Terpinen	1061	4.09
4-Thujanol	1073	0.34
Linalol	1098	0.67
Citronellal	1151	0.07
Borneol	1174	0.10
Z-Geraniol	1226	1.15
Neral	1238	25.50
Carvone	1247	0.84
E-Geraniol	1250	0.77
E-Citral	1267	31.57
a-Copaene	1372	0.07
a-Bourbonene	1380	0.12
b-Cubebene	1384	0.39
a-Cedrene	1411	0.19
b-Cariofilene	1415	0.49
a-Humulen	1451	0.13
allo-Aromadendren	1455	0.19
Germacren D	1476	5.47
Biciclogermacren	1489	0.66
delta-Guaien	1499	0.12
gama-Cadinen	1508	0.50
b-Elemol	1543	5.37
Nerolidol	1557	0.20
Germacren-D-4-ol	1571	0.15
Guaiol	1590	0.56
a-Eudesmol	1649	0.57
Bulnesol	1666	0.63
Total identified		98.34

Kovats indexes calculated.

The higher concentration of these constituents, the larger were the averages of microbial growth, indicating protective action of these molecules on species tested. However, for all concentrations of proteins and lipids analyzed, rates of microbial growth were lower than for controls.

In the highest concentration of carbohydrate (10%) the lowest average growth of *L. monocytogenes* was detected, with significant differences ($p < 0.05$) on microbial growth of all tested concentrations of starch. When compared to controls, the positive effect of carbohydrate in antimicrobial efficacy of the oil was observed. *L. monocytogenes* growth in the presence of *L. alba* EO decreased with the decreasing pH of the medium.

Discussion

The traditional use of plants provides important information for the indication of the type of essential oil to be used for specific purposes in food and in the treatment of infectious diseases. Historically, it has been reported that the EOs have antimicrobial properties (Bajpai *et al.*, 2008; Burt and Reinders, 2003; Chorianopoulos *et al.*, 2008; Hoffman, 1987). However, because of its heterogeneous composition and the antimicrobial activity of many of its components, it seems unlikely that there is only one mechanism of action or that only one of its constituents is responsible for this action (Carson *et al.*, 2002). There is a consensus that the toxicity of essential oils to microorganisms is associated with the lipophilic character and the low molecular weight of their constituents. This allows the essential oil to rapidly cross the cellular membranes, causing changes in its structure and functions, with changes of permeability (Holley and Patel, 2005; Oussalah *et al.*, 2006).

Some studies have shown successful applications of EOs or their compounds, alone or in combinations with other methods of preservation, in the reduction or control of pathogenic and spoilage food microorganisms such as minimally processed vegetables (Gutierrez *et al.*, 2008b), fruits (Martínez-Romero *et al.*, 2007), fish (Mahmoud *et al.*, 2006), meat (Tassou *et al.*, 1995) and milk (Cava *et al.*, 2007).

In this study, the results of antimicrobial screening showed that the *L. alba* EO has potential antibacterial activity against *E. coli* ATCC 10536, *L. innocua* ATCC 19115, *L. monocytogenes* ATCC 33090, *P. aeruginosa* ATCC 9027, *S. choleraesuis* ATCC 10708 and *S. aureus* ATCC 6538P (Table 1). This can be due to the presence of E-citral, neral, d-limonene, germacrene D and g-terpinen, as well as Alpha-pinene, citronellal, a-felandren and a-copaene, major and minor components present in *L. alba* EO with a potential effect on pathogenic and spoilage food microorganisms (Burt and Reinders, 2003). These results are consistent with those cited by Tavares *et al.* (Tiwari *et al.*, 2009).

The MIC and MBC values found in this study (Table 2) were higher than those reported by Aquino *et al.* (2010), which quantified 0.39 μg/mL for *S. aureus* and 12.5 μg/mL for *E. coli.* However, they were similar to the values reported by Alea *et al.* (1997). These authors also reported the higher sensitivity of the Gram-positive strain *S. aureus* when compared to the tested Gram-negative strains, as well as a greater resistance of the *P. aeruginosa* and *Salmonella* sp strains.

Table 2 - Zone of Inhibition of the essential oil of fresh and dried *Lippia alba* leaves.

Microorganisms	EOLa fresh leaves		EOLaII dried leaves		Control (Amikacin)	
	IZ (mm)*	Conc %	IZ (mm)*	Conc %	IZ (mm)*	Conc mg/mL
S. auerus	15.5 ± 0.7	1.5	14.0 ± 1.4	1.5	35.0 ± 0.0	1.2
L.monocytogenes	9.0 ± 1.4	3	8.0 ± 0.0	3	35.0 ± 0.0	1.2
L. innocua	10.5 ± 0.7	3	13.0 ± 1.4	3	35.0 ± 0.0	1.2
E. coli	10.5 ± 0.7	25	9.0 ± 1.4	25	30.0 ± 0.0	1.2
P. aeruginosa	8.0 ± 0.0	25	7.0 ± 0.0	50	30.0 ± 0.0	1.2
S. choleraesuis	9.0 ± 0.0	50	9.0 ± 1.4	50	30.0 ± 0.0	1.2

Mean ± standard deviation (σ) of the halos of growth inhibition (mm) of the two tests. Well diameter: 5 mm. EOLa volume applied to each well: 25 μL. Negative control: Tween 80 1%.

Table 3 - Table 3. Inhibitory concentrations (CIM) and minimum bactericidal (CBM) of essential oils of fresh and dry *L. alba* leaves.

Microorganisms	EOLa of fresh leaves		EOLa of dries leaves	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
S. aureus	0.29	0.29	0.33	0.33
L. monocytogenes	0.58	0.58	1.33	1.33
L. innocua	0.58	1.17	1.33	1.33
E. coli	1.17	1.17	1.33	1.33
S. choleraesuis	9.37	9.37	5.34	5.34
P. aeruginosa	9.37	9.37	5.34	5.34

Controls: (+) amikacin 1.2 mg/mL; (-) Tween 80 1%

Table 4 - Specific maximum Growth of *L .monocytogenes* in models media containing 0.58 mg/mL of *L.alba* EO.

Model media	*Lippia alba* μmáx/24 h ($Abs_{490\ nm}$)	Control μmáx/24 h ($Abs_{490\ nm}$)
Beef extract (%)		
1.5	0.117 ± 0.006	0.472 ± 0.005
3.0	0.137 ± 0.003	0.442 ± 0.050
6.0	0.160 ± 0.003	0.479 ± 0.008
12.0	0.211 ± 0.005	0.515 ± 0.037
Starch media (%)		
0.0	0.113 ± 0.006	0.507 ± 0.066
1.0	0.147 ± 0.003	0.615 ± 0.042
5.0	0.167 ± 0.006	0.590 ± 0.158
10.0	0.140 ± 0.003	0.436 ± 0.025
Sunflower oil media (%)		
0.0	0.116 ± 0.005	0.620 ± 0.014
5.0	0.450 ± 0.028	0.678 ± 0.010
10.0	0.600 ± 0.019	0.705 ± 0.028
pH in TSB		
4	0.089 ± 0.002	0.251 ± 0.013
5	0.097 ± 0.004	0.452 ± 0.016
6	0.093 ± 0.006	0.519 ± 0.024
7	0.100 ± 0.005	0.588 ± 0.025

Means ± standard deviation (σ) values of Abs 490 nm.

Deans *et al.* (1995) observed that some essential oils seem to be more specific in their antimicrobial activity, exerting greater inhibitory activity against Gram-positive bacteria. The greatest resistance to EOs by Gram-negative bacteria seems to be associated to the structure of their cell wall lipopolysaccharide, which blocks the passage of hydrophobic oils. In this study, all Gram-negative strains tested showed a lower sensitivity to EOLa extracted from fresh leaves. However, when exposed to EO from dried leaves, the strain *E. coli* was as sensitive as the species of *Listeria*.

The presence of proteins and lipids determined a reduction in the efficacy of EOLa (Table 3). It is usually assumed that high levels of protein and fat in food protect in some way the bacteria from the EOs action (Tavares *et al.*, 2005). If the EO is dissolved in the lipid phase of the food, it will be relatively less available to act against the bacteria present in the aqueous phase. Gutierrez *et al.* (2008a) also reported the protective effect of lipids when the effect of EO of oregano and thyme was evaluated on *L. monocytogenes*. However, paradoxically, in the highest concentrations of protein, both EOs were more effective against the microbial species evaluated. Nevertheless, Dorman and Dean (2000) reported that the chemical structure of each component of an EO has direct influence on its antimicrobial activity, which possibly explains these results.

Unlike the protective effect of bacterial growth found for proteins and lipids, a high concentration of starch (10%)

determined a positive effect on the action of EOLa, reducing microbial growth. These results corroborate reports of Gutierrez *et al.* (2008a) and confirm the positive effect of carbohydrates on antimicrobial action of EOLa.

The growth rate of *L. monocytogenes* increased with the pH increasing. However, for all tested pHs microbial growth was lower than controls samples without EO.

According to Heinzmann and Barros (2007), one of the native plant species prominent to the development of new drugs is undoubtedly *L. alba*. Based on the results obtained in this study and others cited in the literature, it is possible to show that *L. alba* species is also promising for the development of a new preservative. Employing this species, several preclinical studies have been conducted, showing a number of activities related to its popular use. In parallel, a series of studies showed the effectiveness of essential oils and extracts to control the growth of microorganisms commonly involved in food spoilage and foodborne illnesses. However, despite the promising future, like other native species, *L. alba* requires a greater number of further studies, in order to generate a phytomedicine, herbal preservative or a scientifically proven product, validated to ensure its efficacy, safety and quality.

Conclusions

The antimicrobial efficacy of the *L. alba* EO was found to be a function of ingredient manipulation. The antimicrobial activity against *L. monocytogenes* was increased under higher starch concentrations and acidic pH conditions. High concentrations of protein and sunflower oil have decreased the effectiveness of EO.

References

Aguiar JS, Costa MCCD, Nascimento SC, Sena KXFR (2008) Atividade antimicrobiana de *Lippia alba* (Mill.) N. E. Brown (Verbenaceae). Rev Bras Farmacog 18:436-440.

Alea JA, Luis AG, Peréz AR, Jorge MR, Baluja R (1997) Composición y propiedades antibacterianas del aceite esencial de *Lippia alba* (Mill.) N. E. Br. Rev Cub Farmacol 30:29-35.

Aquino LCL, Santos GG, Trindade RC, Alves J, Santos PO, Alves BP, Blank AF, Carvalho LM (2010) Atividade antimicrobiana dos óleos essenciais de erva-cidreira e manjericão frente a bactérias de carnes bovinas. Alim Nutr 21:529-535.

Bajpai VK, Rahman A, Kang SC (2008) Chemical composition and inhibitory parameters of essential oil and extracts of Nandina domestica Thunb to control foodborne pathogenic and spoilage bacteria. Int J Food Microbiol 125:117-122.

Bakkali F, Averbeck, S, Averbeck D, Idaomar, M (2008) Biological effects of essential oils - A review. Food Chem Toxicol 46:446-475.

Barbosa FF, Barbosa LCA (2006) Influência da temperatura do ar de secagem sobre o teor e a composição química do óleo essencial de *Lippia alba* (Mill.) N, E. Brown. Quim Nova 29:1221-1225.

Barbosa Filho JM, Medeiros KCP, Diniz MFFM, Batista LM, Athayde Filho PF, Silva MS, Cunha EVL, Almeida JRGS, Quintana Júnior LJ (2006) Natural products inhibitors of the enzyme acetylcholinesterase. Braz J Pharmacog 16:258-285.

Baron EJ, Peterson LR, Finegold SM (1994) *Diagnostic Microbiology.* Mosby St. Louis, Missouri.

Beuchat LR, Golden DA (1989) Antimicrobials occurring naturally in foods. Food Technol 43:134-142.

Biasi LA, Costa G (2003) Propagação vegetativa de *Lippia alba*. Cien Rural 33:455-459.

Burt SA, Reinders RD (2003) Antibacterial activity of selected plant essential oils against Escherichia coli O157:H7. Lett Appl Microbiol 36:162-167.

Carson CF, Mee BJ, Riley TV (2002) Mechanism of action of Melaleuca alternifolia (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage and salt tolerance assays and electron microscopy. Antimicrob. Agents Chemother 46:1914-1920.

Cava R, Nowak E, Taboada A, Marin-Iniesta F (2007) Antimicrobial activity of clove and cinnamon essential oils against *Listeria monocytogenes* in pasteurized milk. J Food Prot 70:2757-2763.

Chorianopoulos NG, Giaouris ED, Skandamis PN, Haroutounian SA, Nychas GJE (2008) 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 acidbase sanitizers. J Appl Micrbiol 104:1586-1869.

Craveiro AA, Matos FJ, Alencar JWA (1976) simple and inexpensive steam generator for essential oils extraction. J Chem Educ 53:652.

Deans SG, Noble RC, Hiltunen R, Wuryani W, Penzes LG (1995) Antimicrobial and antioxidant properties of *Syzygium aromaticum* (L) Merr Perry: impact upon bacteria, fungi and fatty acid levels in ageing mice. Flavor Fragrance J 10:323-328.

Dorman HJD, Deans SG (2000) Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J Appl Microbiol 88:308-316.

Duarte MCT, Figueira GM, Sartoratto A, Rehder VLG, Delarmelina C (2005) Anti-*Candida* activity of Brazilian medicinal plants. J Ethnopharm 97:305-311.

Gutierrez J, Barry-Ryan C, Bourke P (2008a) The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredientes. Int J Food Microbiol 124:91-97.

Gutierrez J, Rodriguez G, Barry-Ryan C, Bourke P (2008b) Efficacy of plant essential oils against foodborne pathogens and spoilage bacteria associated with ready-to-eat vegetables: Antimicrobial and sensory screening. J Food Prot 71:1846-1854.

Heinzmann BM, Barros FMC (2007) Potencial das plantas nativas brasileiras para o desenvolvimento de fitomedicamentos tendo como exemplo l*ippia alba* (Mill.) N. E. Brown (Verbenaceae). Saúde 33:43-48.

Hoffman DL (1987) *The herb user's guide.* Thorsons Publishing Group. Wellingborough, UK.

Holley RA, Patel D (2005) Improvement in shelf-life and safety of perishable foods by plant essential oil and smoke antimicrobials. Food Microbiol 27:273-292.

Julião LS, Tavares ES, Lage CLS, Leitão SG (2003) Cromatografia em camada fina de extratos de três quimiotipos de

Lippia alba (Mill) N. E. Br. (erva cidreira). Rev Bras Farmacog 13:36-38.

Mahmoud BS, Yamazaki K, Miyashita K, Kawai Y, Shin IS, Suzuki T (2006) Preservative effect of combined treatment with electrolyzed NaCl solutions and essential oil compounds on carp fillets during convectional air-drying. Int J Food Microbiol 106:331-337.

Martínez-Romero D, Guillén F, Valverde JM, Bailén G, Zapata P, Serrano M, Castillo S, Valero D (2007) Influence of carvacrol on survival of Botrytis cinerea inoculated in table grapes. Int. J. Food Microbiol 115:144-148.

Matos FJA (1998) Farmácias Vivas. UFC, Ceará.

Mesa-Arango AC, Montiel-Ramos J, Zapata B, Durán C, Betancur-Galvis L, Stashenko E (2009) Citral and carvone chemotypes from the essential oils of Colombian Lippia alba (Mill.) N.E. Brown: composition, cytotoxicity and antifungal activity. Memórias do Instuto Oswaldo Cruz 104:878-884.

National Committee for Clinical Laboratory Standards-NCCLS (2003b) *Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically*, Approved Standard.

National Committee for Clinical Laboratory Standards-NCCLS (2003a) *Performance Standards for Antimicrobial Disk Susceptibility Tests,* Approved Standards.

Oussalah M, Caillet S, Lacroix M (2006) Mechanism of action of Spanish oregano, Chinese cinnamon, and savory essential oils against cell membranes and walls of *Escherichia coli* O157:H7 and *Listeria monocytogenes*. J. Food Prot 69:1046-1055.

Pascual ME, Slowing K, Carretero E, Mata DS, Villar A (2001) *Lippia*: tradicional uses, chemistry and pharmacology: a review. J. of Ethnopharmacol 76:201-14.

Sena Filho JG, Melo JGS, Saraiva AM, Gonçalves AM, Psiottano MNC, Xavier HS (2006) Antimicrobial activity and phytochemical prolife from the roots of *Lippia alba* (Mill.) N. E. Brown. Braz J Pharmacog 16:506-509.

Tassou C, Drosinos EH, Nychas GJE (1995) Effects of essential oil from mint (Mentha piperita) on Salmonella enteritidis and Listeria monocytogenes in model food systems at 4 °C and 10 °C. J Appl Bacteriol 78:593-600.

Tavares ES, Julião LS, Lopes D, Bizzo HR, Lage CLS, Leitão SG (2005) Análise do óleo essencial de folhas de três quimiotipos de *Lippia alba* (Mill.) N. E. Br. (Verbenaceae) cultivados em condições semelhantes. Rev Bras Farmacogn 5:1-5.

Tiwari BK, Valdramidis VP, O' Donnell CP, Muthukumarappan K, Bourke P, Cullen PJ (2009) Application of natural antimicrobials for food preservation. J Agric Food Chem 57:5987-6000.

All the content of the journal, except where otherwise noted, is licensed under a Creative Commons License CC BY-NC.