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Antimicrobial activity of the essential oils of Brazilian species of the genus *Cunila* against foodborne pathogens and spoiling bacteria

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Abstract

The essential oils from aerial parts of six Brazilian species of the genus *Cunila* Mill. (Lamiaceae) currently used in beverages and food preparation, and in folk medicine, were obtained by steam distillation and analyzed by GC and GC/MS. The main components of the oils were: *Cunila galioides* citral (citral −77.9%), *C. galioides* menthene (mentha-*trans*-2,8-dienol −20.0%, limonene −13.6%, *trans*-ocimene −13.0%), *C. incisa* (1,8-cineole −42.9%, α-terpineol −14.0%), *C. spicata* (1,8-cineole −47.9%, α-terpineol −37.5%), *C. menthoides* (menthene −77.8%), *C. angustifolia* (sabinene −41.4%, γ-terpinene −11.4%), and *C. microcephala* (menthofuran −94.90%). These oils were screened for antibacterial activity against 15 bacterial species. The oil of *C. galioides* citral efficiently controlled the growth of *Bacillus* sp., *L. monocytogenes*, *S. aureus*, *A. hydrophila*, and *E. faecalis*, showing both contact and gaseous activity. Although less efficient, the other essential oils studied were effective against *Bacillus* species, *S. aureus*, and other specific bacteria. MIC and MCC values support their popular use, and indicate that they can be an efficient alternative for the control of foodborne and spoiling bacteria.

Keywords: *Cunila*; Essential oils; Antimicrobial activity

1. Introduction

Illness resulting from consumption of food contaminated with pathogenic bacteria and/or their toxins is a priority concern to public health. Data from industrialized and developing countries indicates that annually up to 10% or more of the population may have a foodborne disease. Indeed, the committee on Food Safety (FAO/WHO) concluded that illness due to contaminated food was perhaps the most widespread health problem in the world and an important cause of reduced economic productivity (Käferstein, Mortarjemi, & Bettcher, 1997). The factors contributing to the foodborne disease are changes in human demographics and behavior, technology and industry, international travel and commerce, microbial adaptation including antimicrobial resistance to antibiotics and other products (Altekruse, Cohen, & Swerdlow, 1997). In this context the identification and evaluation of natural products for the control of these pathogens, to assure consumers a safe, wholesome, and nutritious food supply, can be considered an important international challenge.

Antimicrobial compounds found in edible and medicinal plants include phenolic compounds and their subclasses, such as coumarins, flavonoids, and essential oils (Cowan, 1999; Janssen, Scheffer, & Baerheim Svendsen, 1987). Plant derived essential oils have been long used as flavoring agents in foods, beverages, confectionary products and toothpaste, among others. The versatile composition of plant essential oils and the large antimicrobial spectrum, associated with their low toxicity, make them potential natural agents for food preservation (Conner, 1993).

The genus *Cunila* (Lamiaceae) is formed by 22 species with two centers of distribution, one in Mexico with 10 species, and the other in the southern part of South America with 12 species. Species of the genus *Cunila* are used as spices, preparation of beverages, and as additives

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in mate tea. Other than as beverages, infusions of these species are all used in Brazilian popular medicine as stimulants, antispasmodics, emenagogues, antithermics, in the treatment of chronic cough and respiratory infections (Simões, Mentz, Irgang, Schenkel, & Stehmann, 1994). Due to their likeness to pennyroyal (*Mentha pulegium* L.), an European medicinal plant, several species of *Cunila* are popularly known as Poejo, in Brazil, or Poleo, in Uruguay and Argentina.

References to the oil composition of South American species of the genus *Cunila* reveals a large variation. *C. microcephala* and *C. fasciculata* oils have a large content of menthofuran (Bordignon, Schenkel, & Spitzer, 1997). The main constituents of *C. menthoides* are isomenthone, menthone and pulegone (Bordignon, Schenkel, & Spitzer, 1998), whereas those of *C. angustifolia* are sabinene, γ-terpinene and limonene (Bordignon, Schenkel, & Spitzer, 1999). Phytochemical study of the essential oil of different populations of *C. galioides*, revealed the existence of three chemotypes characterized by a large content of the two isomeric forms of citral, *trans*-β-ocimene, and menthenes, respectively (Echeverrigaray et al., 2003).

Considering the popular use of *Cunila* species, the purpose of the present study was to evaluate the effectiveness of the essential oils of six South-American species of the genus *Cunila* against common foodborne pathogens and spoilage bacteria.

2. Material and methods

2.1. Plant material

Cunila species were collected at the northeast mountains and central region of Rio Grande do Sul State, Brazil between October 2001 and March 2002, when the specimens were in the middle of their flowering period. A voucher specimen of each plant was deposited in the Herbarium of the University of Caxias do Sul, Brazil.

2.2. Preparation of essential oils and GC/MS analysis

Aerial parts of the plants were collected and dried in a chamber at 40 °C. The dried samples were subjected to hydrodistillation for 1 h using a Clevenger-type apparatus. The oil obtained was separated from water and dried over anhydrous Na_2SO_4 and stored at 4 °C. The identification of the chemical components of the oil samples was performed in a completed HP 6890 gas chromatograph using a mass selective detector HP 5973, equipped with Chemstation software and Wiley 275 spectra data. A HP-Innowax fused silica capillary column (30 m × 0.25 mm; 0.25 μm film thickness) was used. The chromatographic conditions were: column temperature 60 °C (8 min), 60–180 °C (3 °C/min), 180–230 °C (20 °C/min), 230 °C (20 min); interface 180 °C; split ratio 1:100; carrier gas, He (55.4 KPa); flow rate 1.0 ml/min; ionization energy 70 eV; mass range 40–350; volume injected 0.5 μl; solvent cut, 3.5 min.

GC analysis was performed on a HP 5973 gas chromatograph with FID detector using a HP-Innowax fused silica capillary column (30 m × 0.25 mm; 0.50 μm film thickness). The chromatographic conditions were: column temperature 40 °C (8 min), 40–180 °C (3 °C/min), 180–230 °C (20 °C/min), 230 °C (20 min); injector temperature 250 °C; split ratio 1:50; detector temperature 250 °C; carrier gas hydrogen (34 KPa); flow rate 1.0 ml/min; volume injected 0.2 μl.

2.3. Bacterial strains

Bacterial strains used in all antimicrobial assays were: *Aeromonas hydrophila* (ATCC 7966), *Aeromonas sobria* (ATCC 43979), *Bacillus cereus* (IBBac102), *Bacillus megatherium* (IBBac103), *Bacillus subtilis* (IBBac101), *Enterobacter cloacae* (IBEnt101), *Enterococcus faecalis* (ATCC 19433), *Escherichia coli* (ATCC 25922), *Listeria monocytogenes* (ATCC 7644), *Klebsiella oxytoca* (IBKle101), *Proteus mirabilis* (IBPro101), *Pseudomonas aeruginosa* (IBPse104), *Salmonella enterica* ser. Enteritidis (IBSal101), *Salmonella enterica* ser. Typhimurium (IBSal102), and *Staphylococcus aureus* (ATCC 6538). ATCC strains were obtained from the American Type Culture Colletion (Rockville, MD, USA). IB strains make part of the collection of the Institute of Biotechnology of the University of Caxias do Sul, and were isolated from food and clinical samples.

2.4. Antimicrobial assay using a disc diffusion method

Overnight LB cultures, adjusted to 10^8 CFU/ml were streaked on plates containing LB solid medium with 0.5% Tween-20. Filter paper discs (5 mm diameter, Whatman n°1) were impregnated with 10 μl of the essential oils, and placed on the center of the inoculated agar surfaces. After the plates were incubated at 37 °C for 24 h, the diameters of the distinctly clear zones were measured using a metric ruler. All tests were performed in triplicate and the antibacterial activity was expressed as the mean of inhibition diameters produced (in mm).

2.5. Minimum inhibitory concentration assay

Microdilution broth susceptibility assay was used (Koneman, Allen, Janda, Schreckenberger, & Winn, 1997). Stock solutions of essential oils were prepared in dimethylsulfoxide (DMSO). Serial dilutions of essential oils were prepared in sterile LB medium in 96-well microtiter plates. Freshly grown bacterial suspensions in LB were standardized to 10^8 CFU/ml, and added to the wells (10 μl). The last row containing only the serial dilution of essential oils without bacteria was used as the negative control. After incubation at 37 °C for 24 h the first well without turbidity was determined as the minimum inhibitory concentration (MIC).

2.6. Other antibacterial tests

The antibacterial activity of essential oils by gaseous contact was investigated using the microatmospheric technique described by Pellecuer, Allegrini, de Bouchberg, and Passet (1975). Briefly, after plating of the bacterial strains ($\sim 5 \times 10^8$ CFU) on LB medium, 5 mm Whatman n°1 discs soaked with 10 µl of the essential oils were located in the center of the cover of each Petri dish. Plates were incubated at 37 °C for 24 h, and the extension of the inhibition area determined.

Growth inhibition on different concentrations of the essential oils was assay by inoculating approximately 10^5 bacterial cells on 1 ml LB medium, adding the essential oil, and incubating the tubes at 37 °C for 24 h. Bacterial growth was evaluated by absorbance at 620 nm, and compared with the growth on the absence of the oil.

Cidal activity of the essential oils was evaluated transferring approximately 10^6 actively growing vegetative cells or spore suspension 96-well microtiterplate containing 200 µl LB medium and different concentrations of the essential oils (0–10 mg/ml) per well. After incubation at 37 °C for 6 h bacterial or spore suspensions were plated on LB agar plates, and incubated at 37 °C for 24 h. Colony forming units (CFU) were counted and the data expressed as percentage of the control (absence of essential oil). Minimal cidal concentration was defined as the minimal amount of essential oil (mg/ml) that result in no survivors.

Spore suspensions were obtained by growing *Bacillus* species during 48 h on LB broth, treated at 60 °C for 15 min, and concentrated by centrifugation.

3. Results

Twenty-nine phytochemicals were identified by GC/MS analysis as constituents of the essential oils of the *Cunila* species analyzed. These components and their retention indices are summarized in Table 1. As expected, chemical analysis revealed that there are qualitative and quantitative differences among the oils of *Cunila* species. The main constituent of the essential oils from the aerial parts of *C. galioides* chemotype citral were geranial (44.3%) and neral (33.7%), comprising a total of 77.9% of citral, where those of *C. galioides* chemotype menthene were mentha-*trans*-2,8-dienol (20.0%), limonene (13.6%) and *trans*-ocimene (13.0%). In contrast, *C. incisa* and *C. spicata* contained mainly 1,8-cineole and α-terpineol, in concentrations of 42.9% and 14.0%, and 47.9% and 37.5%, respectively. *C. menthoides* contained menthene (77.8%) and pulegone (14.4%). The highest content of a

Table 1
Main constituents (%) of the essential oils of *Cunila* species, as identified by GC/MS analysis

Phytochemicals[b]	RT[a] (min)	*C. galioides* citral	*C. galioides* menthene	*C. incisa*	*C. spicata*	*C. menthoides*	*C. angustifolia*	*C. microcephala*
α-Pinene	4.35	–	0.39	2.91	–	–	–	–
Thujone	4.52	–	–	1.23	–	–	–	–
Camphene	5.59	–	0.11	2.57	–	–	–	–
β-Pinene	7.21	–	0.39	1.14	–	0.87	–	–
Sabinene	8.07	–	0.28	6.74	5.97	0.90	41.4	–
Myrcene	10.84	–	0.43	1.28	–	–	–	–
α-Terpinene	11.16	0.64	–	0.87	–	–	–	–
Limonene	12.29	–	13.6	1.08	–	1.50	9.50	–
1,8-Cineole	12.47	–	0.32	42.9	47.9	0.40	4.70	2.38
γ-Terpinene	14.15	–	–	4.80	–	–	11.4	–
cis-Ocimene	14.67	–	2.22	–	–	–	–	–
trans-Ocimene	14.70	1.17	13.0	0.51	–	–	–	–
p-Cimene	16.50	–	–	4.41	–	–	–	–
δ-Carene	25.40	–	–	1.03	–	2.02	–	–
Menthofuran	25.95	–	–	–	–	–	–	94.9
Menthene	–	–	–	–	–	77.8	–	–
Linalool	29.47	1.41	6.77	2.52	–	–	0.60	–
β-Caryophyllene	30.26	1.84	5.08	–	4.01	0.88	–	2.73
Terpinene-4-ol	31.27	0.60	1.06	3.21	–	–	–	–
Mentha-*trans*-2,8-dienol	32.09	–	20.0	–	–	–	–	–
Pulegone	32.34	–	–	–	–	14.4	–	–
Neral	34.65	33.6	1.08	–	–	–	–	–
α-Terpineol	35.19	2.10	4.07	14.0	37.5	–	–	–
Germacrene	35.54	–	2.80	1.54	–	–	–	–
Geranial	35.92	44.3	1.86	–	–	–	–	–
2,3-Menthadiol	36.48	–	2.83	–	–	–	–	–
Geranil acetate	37.57	5.24	–	–	–	–	–	–
Aromadendrene	44.50	–	1.67	–	1.45	–	–	–
Elemol	44.77	–	–	–	3.13	–	–	–

[a] Retention time.
[b] Compounds present in trace amounts (<0.1%) were not registered.

Table 2
Zone of inhibition (mm) of *Cunila* essential oils against 15 bacterial strains

	C. galioides citral	*C. galioides* menthene	*C. incisa*	*C. spicata*	*C. menthoides*	*C. angustifolia*	*C. microcephala*
A. hydrophila	>50.0	6.0	6.0	8.0	6.0	8.0	6.0
A. sobria	>50.0	6.0	6.0	7.0	8.0	6.0	0.0
B. cereus	>50.0	>50.0	6.0	9.0	7.0	6.0	0.0
B. megatherium	>50.0	>50.0	7.0	>50.0	8.0	9.0	6.0
B. subtilis	>50.0	>50.0	8.0	13.0	7.0	5.0	6.0
E. cloacae	7.0	5.0	8.0	7.0	6.0	0.0	0.0
E. faecalis	>50.0	7.0	0.0	0.0	6.0	6.0	6.0
E. coli	9.0	6.0	5.0	5.0	>50.0	0.0	0.0
K. oxytoca	8.0	13.0	7.0	8.0	8.0	8.0	0.0
L. monocytogenes	>50.0	>50.0	6.0	6.0	7.0	7.0	0.0
P. mirabilis	5.0[a]	8.0	7.0	6.0	6.0	0.0	0.0
P. aeruginosa	0.0[b]	0.0	0.0	0.0	0.0	0.0	0.0
S. enteritidis	11.0	6.0	5.0	6.0	>50.0	0.0	0.0
S. typhimurium	14.0	6.0	5.0	6.0	>50.0	0.0	0.0
S. aureus	>50.0	12.0	5.0	7.0	5.0	0.0	0.0

[a] No growth under the disc.
[b] Bacterial growth under the disc.

single component was exhibited by *C. microcephala*, with 94.9% of menthofuran.

In a first set of experiments, antimicrobial activity was evaluated by the disc diffusion method. The results (Table 2) shows that from the seven essential oils studied, it was the essential oil of *C. galioides* chemotype citral which displayed the highest antibacterial activity, whereas those from *C. incisa*, *C. angustifolia*, and *C. microcephala* produced very small inhibition zones regardless of the test organism.

The high antimicrobial activity of *C. galioides* chemotype citral was confirmed by the microdilution broth assay (Table 3), exhibiting minimal inhibitory concentration values of 0.04 mg/ml against *B. cereus*, 0.31 mg/ml against *B. subtilis*, and 0.61 mg/ml against *B. megatherium* and *S. aureus*.

The essential oils of *C. incisa* and *C. spicata*, characterized by the presence of high concentrations of 1,8-cineole and α-terpineol, inhibited growth of *Bacillus*, *Aeromonas*, *L. monocytogenes*, and *S. aureus*.

Pseudomonas aeruginosa was not inhibited by the essential oils of *Cunila* species, and in general, Gram negative bacteria were less susceptible to the essential oils tested than the Gram positive species (Tables 2 and 3).

Evident antibacterial activity on the gaseous state was observed just for the essential oil of *C. galioides* chemotype citral on Gram positive bacteria (*B. cereus*, *B. megaterium*, *B. subtilis*, *L. monocytogenes, S. aureus*), and *Aeromonas* sp.

All the essential oils exhibited cidal activity on the bacteria they inhibited. However, the minimal cidal concentrations (MCC) were considerably higher than the MIC values. For the essential oil of *C. galioides* chemotype citral MCC values ranged between 2 mg/ml for *B. cereus* to 10 mg/ml for *A. hydrophila* and *S. enterica* ser. Typhimurium.

The effect of increasing concentrations of the essential oil of *C. galioides* chemotype citral was evaluated against *Bacillus* species and *S. aureus*. Fig. 1 shows that even low concentrations (0.1–0.6 mg/ml) of the essential oil of *C.*

Table 3
Antibacterial activity (MIC mg/ml) of the essential oils of *Cunila* species

	C. galioides citral	*C. galioides* menthene	*C. incisa*	*C. spicata*	*C. menthoides*	*C. angustifolia*	*C. microcephala*
A. hydrophila	2.50	5.00	5.00	5.00	>5.00	5.00	>5.00
A. sobria	5.00	>5.00	5.00	>5.00	5.00	2.50	>5.00
B. cereus	0.04	1.25	2.50	0.62	2.50	5.00	>5.00
B. megatherium	0.62	2.50	2.50	1.25	5.00	2.50	>5.00
B. subtilis	0.31	1.25	1.25	1.25	0.31	1.25	>5.00
E. cloacae	>5.00	>5.00	>5.00	>5.00	>5.00	>5.00	>5.00
E. faecalis	2.50	5.00	>5.00	5.00	>5.00	>5.00	2.50
E. coli	>5.00	>5.00	5.00	>5.00	>5.00	>5.00	>5.00
K. oxytoca	>5.00	>5.00	5.00	>5.00	>5.00	>5.00	>5.00
L. monocytogenes	1.25	>5.00	5.00	2.50	>5.00	>5.00	>5.00
P. mirabilis	5.00	5.00	>5.00	>5.00	>5.00	>5.00	>5.00
P. aeruginosa	>5.00	>5.00	>5.00	>5.00	>5.00	>5.00	>5.00
S. enteritidis	>5.00	>5.00	>5.00	>5.00	5.00	>5.00	>5.00
S. typhimurium	5.00	>5.00	>5.00	>5.00	>5.00	>5.00	>5.00
S. aureus	0.62	>5.00	5.00	2.50	5.00	2.50	>5.00

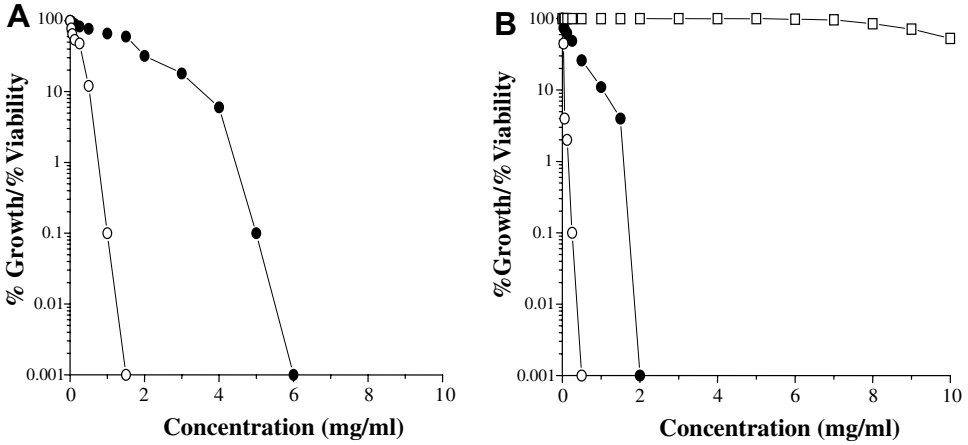

Fig. 1. Bacterial growth and cell viability on increasing concentrations of *C. galioides* chemotype citral essential oil. (A) *S. aureus* (–○–) growth and (–●–) viability. (B) *B. cereus* (–○–) growth, (–●–) viability of vegetative cells, and (–□–) viability of spores.

galioides chemotype citral are able to drastically reduce the growth of *S. aureus* and *B. cereus*, reducing vegetative cell viability. However, MCC was attained only at 6 mg/ml and 2 mg/ml for *S. aureus* and *B. cereus*, respectively. Spores of *B. cereus* resisted higher concentrations of the essential oil than did vegetative cells. Similar results were obtained with *B. subtilis* and *B. megatherium*, indicating that this is a general behavior of *Bacillus* spores.

4. Discussion

The essential oil compositions of the extracts used in the present study (Table 1) were similar to those previously reported for the *Cunila* species evaluated (Bordignon et al., 1997, 1998; Echeverrigaray et al., 2003). The essential oil of *C. angustifolia* used in our work had a high content of sabinene (41.4%) and γ-terpinene (11.4%), characterizing one of the chemotypes described by Bordignon et al. (1999).

As determined by the disk diffusion method (Table 2), most essential oils examined exhibited antimicrobial activity against one or more bacterial species. Due to the variation of diffusion and solubility properties of the different oils, the results obtained by the disk diffusion method are not directly comparable to those obtained with the microdilution broth assay (Kim, Marshall, & Wei, 1995). However, the oils that exhibited large inhibition zones for a given bacteria were confirmed as those with the lower MIC values (Tables 2 and 3).

The high antimicrobial activity of the oil of *C. galioides* chemotype citral (Tables 2 and 3) may be associated to its high content of geranial and neral, two isomers of citral, which have well documented antibacterial activity (Chalchat et al., 1997; Kim et al., 1995). An evaluation of the vapor activity of this oil confirms the antimicrobial activity of citral in the gaseous state (Inouye, Takizawa, & Yamaguchi, 2001).

The essential oils of *C. incisa* and *C. spicata*, characterized by the presence of high concentrations of 1,8-cineole and α-terpineol, compounds with well documented antimicrobial activity (Dorman & Dean, 2000; Magiatis, Skaltsounis, Chinou, & Haroutounian, 2002; Zaika, 1988), inhibited growth of *Bacillus*, *Aeromonas*, *L. monocytogenes*, and *S. aureus* (Table 2).

MIC values obtained with the essential oils, especially that of *C. galioides* chemotype citral, are comparable to those previously reported for very efficient antimicrobial plants (Cowan, 1999; Dorman & Dean, 2000; Hammer, Carson, & Riley, 1999; Sartoratto et al., 2004), indicating that the essential oil of this species, and of some other members of the genus *Cunila*, can be seen as potential antimicrobials for the control of some of the most important bacterial species present in food.

As previously observed in several plant extracts (Hammer et al., 1999), the essential oils of *Cunila* exhibited bacteristatic activity at low concentrations and bactericidal activity at higher concentrations (Fig. 1).

Spores of *Bacillus* species were resistant to the antimicrobial action of the essential oil of *C. galioides* chemotype citral. However, the growth and viability of their vegetative cells is drastically inhibited by low concentrations of this oil (Fig. 1). Similar results were obtained by Ultee, Kets, and Smid (1999) and Valero and Salmerón (2003) using carvacrol and the essential oils of several plants, respectively, indicating that this is a general effect. Even if not killing bacterial spores, the strong inhibition of bacterial growth and bactericidal effect on vegetative cells can guarantee the control of these species in food products (Valero & Salmerón, 2003).

In general, the antimicrobial activity of *Cunila* essential oils was more pronounced against Gram-positive than against Gram-negative bacteria, a fact previously observed with essential oils from other plant species (Nostro, Germano, D'Angelo, Marino, & Cannatelli, 2000; Ouattara, Simard, Holley, Piette, & Bégin, 1997). This general higher resistance among Gram-negative bacteria has been ascribed to the presence of their outer phospholipidic membrane, almost impermeable to lipophilic compounds (Nikaido & Vaara, 1985). The absence of this barrier in

Gram-positive bacterial allows the direct contact of the essential oil's hydrophobic constituents with the phospholipid bilayer of the cell membrane, where they bring about their effect, either causing an increase of ion permeability and leakage of vital intracellular constituents, or impairment of bacterial enzyme systems (Cowan, 1999; Wendakoon & Sakaguchi, 1995).

In summary, this study shows that the essential oils of the South American species of *Cunila*, especially those obtained from *C. galioides* chemotype citral, *C. incisa*, and *C. spicata*, currently used in folk medicine and food preservation, have remarkable antibacterial activities against foodborne and spoiling bacteria. However, if their oils or plant extracts are to be recommended for food preservation, issues of off-flavor, safety and toxicity should be addressed.

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References

Altekruse, S. F., Cohen, M. L., & Swerdlow, D. L. (1997). Emerging foodborne diseases. *Emerging Infectious Diseases, 3*, 285–293.

Bordignon, S. A., Schenkel, E. P., & Spitzer, V. (1997). The essential oil of *Cunila microcephala* and *Cunila fasciculate*. *Phytochemistry, 44*, 1283–1286.

Bordignon, S. A., Schenkel, E. P., & Spitzer, V. (1998). The essential oil of *Cunila menthoides* Benth (Lamiaceae). *Journal Essential Oil Research, 10*, 317–320.

Bordignon, S. A., Schenkel, E. P., & Spitzer, V. (1999). The essential oil of *Cunila angustifolia* (Lamiaceae). *Journal Essential Oil Research, 11*, 145–148.

Chalchat, J. C., Garry, R. P., Menut, C., Lamaty, G., Malhuret, R., & Chopineau, J. (1997). Correlation between chemical composition and antimicrobial activity. VI. Activity of some African essential oils. *Journal Essential Oil Research, 9*, 67–75.

Conner, D. E. (1993). Naturally occurring compounds. In P. M. Davidson & A. L. Branen (Eds.), *Antimicrobials in foods* (pp. 441–468). New York: Marcel Dekker.

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews, 12*, 564–582.

Dorman, H. J. D., & Dean, S. G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Microbiology, 88*, 308–316.

Echeverrigaray, S., Fracaro, F., Atti dos Santos, A. C., Paroul, N., Wasum, R., & Atti-Serafini, L. (2003). Essential oil composition of South Brazilian populations of *Cunila galioides* and its relation with the geographic distribution. *Biochemical Systematics and Ecology, 31*, 467–475.

Hammer, K. A., Carson, C. F., & Riley, T. V. (1999). Antimicrobial activity of essential oils and other plant extracts. *Journal of Applied Microbiology, 86*, 985–990.

Inouye, S., Takizawa, T., & Yamagushi, H. (2001). Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. *Journal of Antimicrobial Chemotherapy, 47*, 565–573.

Janssen, A. M., Scheffer, J. J. C., & Baerheim Svendsen, A. (1987). Antimicrobial activity of essential oils: a 1976–1986 literature review. Aspects of the test methods. *Planta Medica, 5*, 395–398.

Käferstein, F. K., Mortarjemi, Y., & Bettcher, D. W. (1997). Foodborne disease control: a transnational challenge. *Emerging Infectious Diseases, 3*, 503–510.

Kim, J., Marshall, M. R., & Wei, C. (1995). Antimicrobial activity of some essential oil components against five foodborne pathogens. *Journal of Agricultural and Food Chemistry, 43*, 2839–2845.

Koneman, E. W., Allen, S. D., Janda, W. M., Schreckenberger, P. C., & Winn, W. C. (1997). *Color atlas and textbook of diagnostic microbiology* (pp. 785–856). Philadelphia: Lippincott-Raven Publ.

Magiatis, P., Skaltsounis, A. L., Chinou, I., & Haroutounian, S. A. (2002). Chemical composition and in-vitro antimicrobial activity of the essential oils of three Greek Achillea species. *Zeitschrift für Naturforschung, 57*, 287–290.

Nikaido, H., & Vaara, M. (1985). Molecular basis of bacterial outer membrane permeability. *Microbiology Reviews, 49*, 1–32.

Nostro, A., Germano, M. P., D'Angelo, V., Marino, A., & Cannatelli, M. A. (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Letters in Applied Microbiology, 30*, 379–384.

Ouattara, B., Simard, R. E., Holley, R. A., Piette, G. J. P., & Bégin, A. (1997). Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. *International Journal of Food Microbiology, 37*, 155–162.

Pellecuer, J., Allegrini, J., de Bouchberg, S., & Passet, J. (1975). Place de l'essence de Satureia montana L. (Labiées) dans l'arsenal thérapeutique. *Plantes Médicinales et Phytothérapie, 9*, 99–104.

Sartoratto, A., Machado, A. L. M., Delarmelina, C., Figueira, G. M., Duarte, M. C. T., & Rehder, V. L. G. (2004). Compostion and antimicrobial activity of essential oils from aromatic plants used in Brazil. *Brazilian Journal of Microbiology, 35*, 275–280.

Simões, C. M., Mentz, L. A., Irgang, B. E., Schenkel, E. P., & Stehmann, J. R. (1994). *Plantas da medicina popular do Rio Grande do Sul* (4th ed.). Porto Alegre, Brazil: Editora UFRGS.

Ultee, A., Kets, E. P. W., & Smid, E. J. (1999). Mechanisms of action of carvacrol on the food-borne pathogen *Bacillus cereus*. *Applied and Environmental Microbiology, 65*, 4606–4610.

Valero, M., & Salmerón, M. C. (2003). Antibacterial activity of 11 essential oils against Bacillus cereus in tyndallized carrot broth. *International Journal of Food Microbiology, 85*, 73–81.

Wendakoon, C. N., & Sakaguchi, M. (1995). Inhibition of amino acid decaboxylase activity of *Enterobacter aerogenes* by active components of spices. *Journal of Food Protection, 58*, 280–283.

Zaika, L. L. (1988). Spices and herbs: their antimicrobial activity and its determination. *Journal of Food Safety, 9*, 97–118.