Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
16(4): 510-516, Out./Dez. 2006
Received 03/25/06. Accepted 08/30/06

Artigo

Effectiveness of *Lippia sidoides* Cham. (Verbenaceae) essential oil in inhibiting the growth of *Staphylococcus aureus* strains isolated from clinical material

Fillipe Pereira de Oliveira[1], Edeltrudes de Oliveira Lima[1]*, José Pinto de Siqueira Júnior[2], Evandro Leite de Souza[3], Bernadete Helena Cavalcante Santos[4], Humberto Medeiros Barreto[2]

[1]Laboratório de Micologia, Departamento de Ciências Farmacêuticas, Universidade Federal da Paraíba, 58051-970, João Pessoa, PB, Brasil,
[2]Departamento de Biologia Molecular, Centro de Ciências Exatas e da Natureza, Universidade Federal da Paraíba, 58051-970, João Pessoa, PB, Brasil,
[3]Departamento de Nutrição, Centro de Ciências da Saúde, Universidade Federal da Paraíba, 58051-970, João Pessoa, PB, Brasil,
[4]Laboratório de Bacteriologia Clínica, Departamento de Ciências Farmacêuticas, Universidade Federal da Paraíba, 58051-970, João Pessoa, PB, Brasil

RESUMO: "Atividade do óleo essencial de *Lippia sidoides* Cham. (Verbenaceae) na inibição do crescimento de *Staphylococcus aureus* isolados de material clínico". *Staphylococcus aureus* apresenta-se como um microrganismo patogênico clássico sendo comumente reconhecido como agente etiológico de infecções comunitárias e hospitalares. Considerando o conhecimento acerca das propriedades biológicas apresentadas por *Lippia sidoides* Cham. (Verbenaceae), este estudo teve como objetivo avaliar a efetividade de seu óleo essencial em inibir o crescimento de cepas de *S. aureus* com diferentes perfis de resistência antibiótica isoladas de material clínico. Os resultados mostraram destacável atividade anti-*S. aureus* do óleo essencial de *L. sidoides* notada por grandes halos de inibição do crescimento bacteriano (15-21 mm). A CIM encontrada foi de 0.4 µL/mL para todas as cepas ensaiada. A CIM apresentou destacável efetividade de inibição da viabilidade de *S. aureus* em caldo durante vinte e quatro horas de interação caracterizando um efeito bacteriostático. Estes resultados mostram a destacável atividade antiestafilocócica do óleo essencial de *L. sidoides*, bem como suportam a possibilidade de seu uso racional como agente antimicrobiano alternativo.

Unitermos: *Lippia sidoides*, *Staphylococcus aureus*, óleo essencial, atividade antimicrobiana.

ABSTRACT: *Staphylococcus aureus* is a classical pathogen microorganism and it is commonly recognized as etiological agent of many community and nosocomial infections. Regarding the knowledge about the biological properties of *Lippia sidoides* Cham. (Verbenaceae), this study aimed to evaluate the effectiveness of its essential oil in inhibiting the growth of *S. aureus* strains isolated from clinical materials and with different profiles of antibiotic resistance. The results showed prominent anti-*S. aureus* activity of *L. sidoides* essential oil noted by large growth inhibition zones (15-21 mm). MIC found was of 0.4 µL/mL for all assayed strains. MIC presented prominent effectiveness in inhibiting the viability of *S. aureus* in broth during twenty-four hours of exposure characterizing a bacteriostatic effect. These results show the promising anti-staphylococcal property of *L. sidoides* essential oil and support the possibility of its rational use as alternative antibacterial agent.

Keywords: *Lippia sidoides*, *Staphylococcus aureus*, essential oil, antimicrobial activity.

INTRODUCTION

Staphylococcus genus is widely spread in nature being part of the indigenous micoflora of skin and mucosa of animal and birds. Some *Staphylococcus* species are frequently recognized as etiological agents of many animal and human opportunistic infections (Nostro et al., 2004). *S. aureus, S. epidermidis, S. saprophyticus* and *S. haemolyticus* are the most important species as community and nosocomial human infection causing agents. In addition of causing different kinds of intoxications, *S. aureus* has been the most common etiological agent of festering infections which can attack different tissues and/or organs (e.g. furuncle, carbuncle, abscess, myocarditis, endocarditis, pneumonia, meningitis, bacterial arthritis) (Verhoff et al., 1999; Pereira et al., 2004). Capsule, peptdioglican, teicoic acids, A protein, adesins and synthesis of enzymes and extracelullar toxins are some virulence attributes present in/on *S. aureus* cell (Nostro et al., 2004).

* *E-mail: edelolima@yahoo.com.br, Tel. + 55-83-32167026, Fax+ 55-83- 32167094*

ISSN 0102-695X

All bacterial species are described as possessing some potential to develop strains with high resistance to various antibiotics, so that many cellular mechanisms involved in the rising of this biological phenomenon have been elucidated (Woolhouse, 2002; Wright, 2003). Among the bacterial genera able to develop changes in their sensitivity to antimicrobials, staphylococcal species have been recognized as having increasing and worrying antimicrobial resistance (Georgopapadakou, 2002; Nostro et al., 2004).

Over fifty years antibiotics have been applied in industry, agricultural, livestock and domestic environment for treating or inhibiting quickly the most infections. This use and, many times, misuse of antimicrobials in both human and animal medicine has been responsible for an unprecedent selection in the microbial evolution (White et al., 2002). Consequently, the society is facing the most serious dilemma of public health: the rising of infectious diseases which present etiological agents resistant to many and, some times, all clinically used antimicrobials (Souza et al., 2005a). For patients, the antimicrobial resistance increases the morbidity and mortality, while for healthcare institutions it means increasing costs (Dancer, 2001; Kuner; Oho, 2003).

Regarding the increasing clinical importance given to nosocomial and community bacterial infections and the progressive development of antimicrobial resistance, a great number of scientific researches emphasizing the antibacterial properties of plant products has been carried out (Hernández et al., 2003; Silva-Santos et al., 2004; Duarte et al. 2005; Gayoso et al., 2005; Michelin et al., 2005; Lima et al., 2006a; Lima et al., 2006b). Filtrates, infusions, macerated, juices, extracts and cataplasms from plants with medicinal properties have been applied in the treatment of various diseases since antiquity (Annuk et al., 1999; Hernández et al., 2003).

Lippia sidoides Cham. (Verbenaceae) is a plant native from Brazilian northeastern and it has showed valuable biological properties rising as potential source of biologically active compounds (Matos, 1994). *L. sidoides* leaves by-products have been applied in folk medicine for acne, wounds, skin and scalp infections treatment. Still, their infusion has been used for treating allergic rhinitis and vaginal, buccal and oropharyngeal infections (Sousa et al., 1991; Martins et al., 1994).

This study was carried out with the purpose of evaluating the effectiveness of *L. sidoides* essential oil in inhibiting the growth and survival of *S. aureus* strains isolated from clinical samples, which presented different profiles of resistance to various antibiotics conventionally applied in clinical therapy.

MATERIAL AND METHODS

Essential oil

Lippia sidoides Cham. (Verbenaceae) essential oil was supplied by Laboratory of Pharmaceutic Technology, Department of Pharmaceutical Sciences, Health Sciences Center, Federal University of Paraíba and its extraction was performed by hidrodistillation procedure (Craveiro et al., 1981; Matos, 1988).

Essential oil solutions used in antimicrobial assays were obtained according to the following procedure: 600 µL of the essential oil, 40 µL of Tween 80 and q.s.f. 5 mL of sterile water were added in sterile assay tube and shaken for 5 minutes using Vortex (Fanem), obtaining a solution with final essential oil concentration of 120 µL/mL. Following serial dilution technique, it was obtained solutions with concentrations of 60, 30, 15, 0.8, 0.4, 0.2, 0.1, 0.05, 0.025, 0.12 and 0.06 µL/mL (Souza et al., 2005b).

Microorganisms

S. aureus strains used as test microorganisms were isolated from clinical material (Table 1) by standard procedures (Murray, 1999; Konneman, 2001). The most strains presented resistance profile to some antibiotics applied in clinical therapy (Table 2). Antibiotic resistance study was carried out according to NCCLS (1999). Stock cultures were maintained on Muller-Hinton agar slants at 7 °C (± 1 °C). Overnight cultures inoculated in Muller-Hinton agar slants at 37 °C were used to prepare the bacterial inoculum used in the antimicrobial assays. The inoculum was of 10^6 colony forming units per mL (cfu/mL) prepared in sterile saline solution (0.85%) and standardized according to the turbidity of McFarland scale 0.5 tube and adjusted for presenting the wished microbial population (Fromtling et al., 1983).

Antimicrobial assays

Anti-staphylococcal effectiveness of *L. sidoides* essential oil was assessed in three steps: screening, determination of Minimum Inhibitory Concentration-MIC and MIC interference on the bacterial cell viability.

Solid medium diffusion technique using filter paper discs was used for screening the antibacterial activity. For this, 1 mL of the bacterium suspension (approximately 10^6 cfu/mL) was uniformly spread on sterile agar Muller-Hinton Petri dishes. After bacterial suspension absorption by agar, filter paper discs (Whartman n. 1, diameter 6mm) were soaked with 20 µL of the essential oil and placed on the inoculated agar (Nair et al., 2005; Sahin et al., 2004). The system was incubated at 37 °C for 24 hours. At the end of the incubation period, the bacterial growth inhibition zone diameter was measured using calipers and expressed in millimeters. It was considered as positive antibacterial activity when observed growth inhibition zone with diameter equal to or higher than 10 mm diameter (Lima et al., 1993).

Control of sensitivity of the assayed strains to

Table 1. Origin of *Staphylococcus aureus* strains used in the antimicrobial assays.

Strains	Origin
BH1	windpipe secretion
BH2	ocular secretion
BH5	nasal secretion
BH8	wound secretion
BH10	nasal secretion
BH13	ocular secretion
BH14	nasal secretion
BH15	abscess
BH17	vaginal secretion
BH18	artritis liquid
BH19	wound secretion
BH22	nasal secretion

Table 2. Resistance profile of *Staphylococcus aureus* strains to clinically used antibiotics.

Antibiotic	Strains											
	BH 1	BH 2	BH 5	BH 8	BH 10	BH 13	BH 14	BH 15	BH 17	BH 18	BH 19	BH 22
Ampicilin	R	R	S	R	R	R	R	R	R	R	R	R
Amoxicilin	R	S	S	S	S	S	S	S	-	-	S	S
Amoxicilin[a]	-	R	S	R	R	R	R	R	-	R	R	R
Azitromicin	-	R	S	-	R	R	S	S	-	R	R	S
Cefalotin	R	-	S	S	S	S	S	S	S	S	S	S
Cefaclor	-	S	S	S	-	S	S	S	S	R	S	-
Cefadroxil	R	S	S	S	S	S	S	S	S	S	S	S
Cefalexin	R	S	S	S	S	S	S	S	S	S	S	S
Clindamicin	R	S	S	S	S	S	S	S	-	-	S	S
Cleritromicin	-	R	S	-	R	R	S	S	-	-	R	S
Ciprofloxacin	R	-	-	S	S	-	-	-	-	-	-	-
Eritromicin	-	R	S	-	R	R	S	S	S	R	R	S
Gentamicin	R	S	S	S	S	S	S	S	-	S	S	S
Gatifloxacin	R	-	-	-	-	-	-	-	S	S	-	-
Oxacilin	R	S	S	S	S	S	S	S	S	S	S	S
Penicilin	R	R	S	R	R	R	R	R	-	-	R	R
Ampicilin-sulbactan	R	S	S	S	S	S	S	S	S	-	S	S
Tercoplamin	S	-	-	S	-	-	-	-	-	S	-	-
Vancomicin	S	-	-	S	-	-	-	-	-	S	-	-
Sulfametoxazole-Trimetropin	-	S	S	S	S	S	S	S	S	-	S	S

[a] clavulonic acid; R: resistant; S: sensitive; (-): no tested

Tween 80 was carried out by solid medium diffusion technique using filter paper discs (Bauer; Kirby, 1966). Also, the viability of the bacterial strains was assessed by verifying their capacity to grow in Muller-Hinton agar without addition of essential oil.

Microplate bioassay was used to determine the essential oil MIC. For this was used a microplate with 96 wells (flatted bottom) and cap. The 96-well microplates were prepared dispensing into each well 100 µL of doubled strength Brain Heart Infusion broth inoculated with 1mL of the bacterium inoculum prior the assay (1:9 v/v). A 100 µL of the essential oil solution with their respective concentration was transferred into seven consecutive wells. Final volume in each well was 200 µL. The solution having the highest concentration was added into the first well and the smallest concentration was added into the penultimate well. The last well contained 200 mL of Brain Heart Infusion broth inoculated with the bacterium inoculum was used as positive control (strain viability). The microplate was aseptically sealed, followed by mixing on plate shaker (300 rpm) for 30s, and incubated at 28 – 30 °C/48hs (Viljoen et al., 2003; Sahin et al., 2004). Antibacterial activity was detected using a colorimetric method by adding 200 µL of resauzurin staining (0.1%) aqueous solution in each well at the end of the incubation period. MIC was defined as the lowest

Table 3. Inhibitory activity of *L. sidoides* essential oil on *Staphylococcus aureus* strains isolated from clinical material.

Strains	Essential oil [a] (20µL/disc)	MIC (µL/mL)	Control	
			strain viability [b]	Tween 80[c]
BH1	18	0.4	+	+
BH2	18	0.4	+	+
BH5	18	0.4	+	+
BH8	15	0.4	+	+
BH10	18	0.4	+	+
BH13	15	0.4	+	+
BH14	18	0.4	+	+
BH15	15	0.4	+	+
BH17	18	0.4	+	+
BH18	16	0.4	+	+
BH19	21	0.4	+	+
BH22	18	0.4	+	+

[a] screening results showed in bacterial growth inhibition zones diameter (mm)
[b] microbial growth in agar without addition of essential oil
[c] absence of microbial growth inhibition by Tween 80

essential oil concentration able to inhibit the bacteria growth, as indicated by resauzurin staining (bacteria died cells are not able to change the staining color by visual observation – blue to red) (Palomino et al., 2002; Burt; Reinders, 2003).

Viable cells count method was used to carry out the study about the interference of the MIC essential oil on the bacterial cell viability. 5 mL of doubled strength Brain Heart Infusion broth was inoculated with 1 mL of the bacterium suspension. After that, 4 mL of the essential oil solution with concentration adjusted to provide an essential oil final concentration similar to the MIC previously determined, was added to the system and followed for shaking for 30s using Vortex. The system was incubated at 37 °C. At different time intervals (1, 2, 4, 8, 12 and 24 hs) of exposure, 1 mL of the suspension was serially diluted in PBS (10^{-1} - 10^{-5}) and inoculated in sterile Muller-Hinton agar Petri dishes for 24 hs at 37 °C (Arora; Kaur, 1999; Viljohen et al., 2003). In the control assay, the essential oil solution was replaced by sterile distilled water. After the incubation period, the number of colonies (cfu/mL) was counted and expressed in log of cfu/mL.

All antimicrobial assays were performed twice and the results were expressed as average of the two repetitions.

Statistical analysis

Statistical analysis was carried out by descriptive statistic study and determination of statistically significant difference (P<0.05) by Tukey test to the bacteria viability study. For this was used Sigma stat 2.03 computer program

RESULTS AND DISCUSSION

In the last years there has been a great scientific interest in chemical and pharmacological investigations regarding the biological properties of medicinal plants (Silva et al., 2003; Rocha et al., 2005; Barbosa-Filho et al., 2005; Barbosa-Filho et al., 2006a; Barbosa-Filho et al., 2006b; Barbosa-Filho et al., 2006c). It is known that medicinal plants have been source of many drugs applied in clinical procedures (e.g morphine, emetine, rutine). Essential oils are involved in many important actions related to the plant survival, playing prominent role in its defense against microorganisms (Cechinel-Filho et al., 1998; Siqui et al., 2000). The use of essential oils as antimicrobial agents presents two principal characteristics: i) their natural origin meaning more safety for users and environment; ii)there is low risk of rising microbial resistance to their action because essential oils are mixtures of several compounds that, apparently, presents different antimicrobial action making more difficult the microbial adaptability (Daferera et al., 2003).

Table 3 shows the inhibitory activity of *L. sidoides* essential oil on *S. aureus* strains isolated from clinical material. As can be seen, the essential oil at absolute concentration showed effectiveness in inhibiting all bacteria strains with inhibition zones diameters between 15-21 mm (average: 17.3; s.d.:1.8). Seven out from the twelve assayed strains showed inhibition zones with diameter of 18 mm. Smallest inhibition zones (15 mm) were found to *S. aureus* BH8, BH13 and BH22, while the largest one (21 mm) was found to *S. aureus* BH19. MIC value was 0.4 µL/mL for all assayed strains. This MIC value is smaller than other found in some studies emphasizing the anti-*S. aureus* properties of

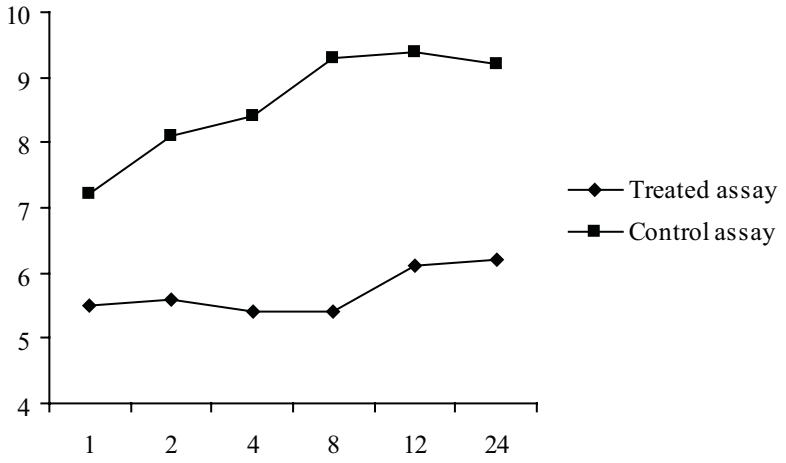

Figure 1. Interference of *Lippia sidoides* essential oil MIC (0.4 µL/mL) on *Staphylococcus aureus* BH1 cell viability.

medicinal plants (Carson et al., 1995; Caeli et al., 2000; McGaw et al., 2000; Hernández et al., 2003; Nostro et al., 2004). Antimicrobial effectiveness of *L. sidoides* essential oil on different pathogen microorganisms (e.g. *P. aeruginosa, B. subtilis, S. aureus, E. coli, Streptococcus* spp., *Trichosporon* spp.) also was reported by other researches (Lemos et al., 1990; Laxoste, 1994; Lima et al., 1994; Siani et al., 2000; Pontes, 2002; Costa et al., 2005; Oliveira et al, 2006). The essential oils of *Lippia sidoides* was active against larvae of *Aedes aegypti* and *Culex quinquefasciatus* (Costa et al., 2005).

Aligiannis et al. (2001) proposed a classification about the antimicrobial potential of plant products, based on MIC results: strong inhibitors – MIC up to 0.5 µL/mL; moderate inhibitors – MIC between 0.6 and 1.5 µL/mL; weak inhibitors – MIC above 1.6 µL/mL. Regarding the MIC values found for all assayed *S. aureus* strains, the classification criteria above cited confirms the strong anti-staphylococcal property of *L. sidoides* essential oil. Still, this intense anti-*S. aureus* activity becomes more important when regarded that the most assayed strains showed resistance to at least three clinically used antibiotics (Table 2).

Figure 1 shows the interference of *L. sidoides* essential oil MIC on the cell viability of *S. aureus* BH1. This strain was chosen for this assay because it presented high antibiotic resistance profile being resistant to twelve out from the fourteen tested antibiotics. MIC showed strong effectiveness in inhibiting the bacterial growth in broth with significant difference ($P<0.05$) with respect to the control assays. After twenty-four hours of exposure the control assay presented microbial count greater than 10^9cfu/mL. On the other hand, the treated assay showed count around 10^6cfu/mL (next to the initial inoculum). From two hours of exposure on the treated assay showed microbial count around 99.9% (1000 folds) smaller than the one found in the control assay.

Antimicrobial effect showed by *L. sidoides* essential oil on *S. aureus* can be recognized as bacteriostatic effect. Bacteriostatic property is characterized when some compound is able to cause the bacteria failure to grow in broth, but it is cultured when the broth is plated onto a suitable agar (Smith-Palmer et al, 1998; Burt, 2004). Thymol, carvacrol, p-pinene, α-pinene, mircene, 1,8 cineole, γ-terpinene, p-metadiene, methyl-thymol, methyl-carvacrol, linalool, 4-terpineole, γ-elemene are some compounds found in *L. sidoides* essential oil (Lemos et al., 1990; Matos; Oliveira, 1998; Leal et al., 2003). Thymol has been recognized as the active compound related to the antibacterial and antifungical property of *L. sidoides* essential oil (Matos; Oliveira, 1998). Thirteen compounds that don't belong the class of the essential oils, 3-O-acetyloleanolic acid, methyl 3,4-dihydroxy benzoate, lapachenol, tecomaquinone, tectoquinone, tectol, acetilated tectol, quercetin, luteolin, glucoluteolin, lippisidoquinone, taxifolin and isolariciresinol, were isolated from ethanol extract of *Lippia sidoides* (Costa et al., 2002).

The results obtained in this study showed the strong anti-staphylococcal property of *L. sidoides* essential oil noted by small MIC value and effectiveness in inhibiting the microbial growth in broth. The MIC value found for all assayed strains was prominently smaller than other MIC values found by many researches emphasizing the antimicrobial potential of medicinal plants on *S. aureus* strains. These data are promising and could encourage further researches on phytochemical, toxicological and pharmacological aspects of *L. sidoides* by-products in order to support their possible rational use in the antimicrobial therapy, particularly, in anti-*S. aureus* therapy.

REFERENCES

Aligiannis N, Kalpotzakis E, Mitaku S, Chinou IB 2001. Composition and antimicrobial activity of the essential oil of two *Origanum* species. *J Agric Food Chem 40*: 4168-4170

Annuk H, Hirmo S, Turi E, Mikelsaar M, Arak E, Wadstrom T 1999. Effect on cell surface hydrophobicity and susceptibility *Helicobacter pylori* to medicinal plant extracts *FEMS Micrbiol Lett 172*: 41-45.

Arora D, Kaur J 1999. Antimicrobial activity of spices. *Int J Antimicrob Agent 12*: 257-262.

Barbosa-Filho JM, Vasconcelos THC, Alencar AA, Batista LM, Oliveira RAG, Guedes DN, Falcão HS, Moura MD, Diniz MFFM, Modesto-Filho J 2005. Plants and their active constituents from South, Central, and North America with hypoglycemic activity. *Rev Bras Farmacogn 15*: 392-413.

Barbosa-Filho JM, Piuvezam MR, Moura MD, Silva MS, Lima KVB, Cunha EVL, Fechine IM, Takemura OS 2006a. Anti-inflammatory activity of alkaloids: A twenty-century review. *Rev Bras Farmacogn 16*: 109-139.

Barbosa-Filho JM, Medeiros KCP, Diniz MFFM, Batista LM, Athayde-Filho PF, Silva MS, Cunha EVL, Almeida JRGS, Quintans-Júnior LJ 2006b. Natural products inhibitors of the enzyme acetylcholinesterase. *Rev Bras Farmacogn 16*: 258-285.

Barbosa-Filho JM, Martins VKM, Rabelo LA, Moura MD, Silva MS, Cunha EVL, Souza MFV, Almeida RN, Medeiros IA 2006c. Natural products inhibitors of the angiotensin converting enzyme (ACE). A review between 1980-2000. *Rev Bras Farmacogn 16*: 421-446.

Bauer AW, Kirby WMM 1966. Antibiotic susceptibility testing by standardized single disk method. *Amer J Clin Pathol* 45: 493-496.

Burt S 2004. Essential oils: their antibacterial properties and potential applications in foods – a review. *Int J Food Microbiol* 94: 223-253.

Burt SA, Reinders RD 2003. Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. *Let Appl Microbiol 26*: 162-167.

Caelli M, Porteuns J, Carson CF, Heller R, Riley TV 2000. Tea tree as an alternative topical decolonisation agent for methicillin-resistant *Staphylococcus aureus*. *J Hosp Infect 46*: 236-237

Carson CF, Cookson BD, Farrey HD, Riley TV 1995. Susceptibility of methicillin resistant *Staphylococcus aureus* to the essential oil of *Malaleuca alternifolia*. *J Antimicrob Chemother 35*: 421-424.

Cechinel Filho V, Yunes RA 1998. Estratégias para obtenção de compostos farmacologicamente ativos a partir de plantas medicinais. Conceitos sobre modificação estrutural para otimização da atividade. *Quím Nova 21*: 99-105.

Costa SMO, Lemos TLG; Pessoa ODL, Assunção JCC, Braz-Filho R 2002. Constituintes químicos de *Lippia sidoides* (Cham.) Verbenaceae. *Rev Bras Farmacogn 12(Supl.)*: 66-67.

Costa JGM, Rodrigues FFG, Angélico EC, Silva MR, Mota ML, Santos NKA, Cardoso ALH, Lemos TLG 2005. Estudo químico-biológico dos óleos essenciais de *Hyptis martiusii*, *Lippia sidoides* e *Syzigium aromaticum* frente às larvas do *Aedes aegypti*. *Rev Bras Farmacogn 15*: 304-309.

Craveiro AA, Fernandes AG, Andrade CHS, Matos FJA, Alencar JW, Machado MIL 1981. *Óleos essenciais de plantas do Nordeste.* Fortaleza:Ed.UFC.

Daferera DJ, Ziogas BN, Polissiou MG 2003. The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis* subsp. *michiganensis. Crop Protection 22*: 39-44.

Dancer SJ 2001. The problem with cephalosporins. *J Antimicrob Chemother 48*: 463-478.

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

Fromtling RA, Pui-Yu H, Shadomy S 1983. *In vitro* inhibitory activities of 2 new orally absorbable imidazole, derivatives: BAYN 7133 and BAYI 913. *Sabouraudia 21:* 179-184.

Gayoso CW, Lima EO, Oliveira VT, Pereira FO, Souza EL, Lima IO, Navarro DF 2005. Sensitivity of fungi isolated from onychomycosis to *Eugenia cariophyllata essential oil* and eugenol *Fitoterapia 76*: 247-249.

Georgopapadakou NH 2002. Infectious disease 2001: drug resistance, new drugs. *Drug Resistance Updates*. *5:* 181-191.

Hernández T, Canales M, Avila JG, Duran A, Caballero J, Romo de Vivar A, Lira R 2003. Ethnobotany and antibacterial activity of some plants used in traditional medicine Zapolitán de las Salinas, Puebla (México). *J Ethnopharmacol 88*: 181-188.

Koneman EW 2001. *Diagnóstico Microbiológico - Texto e Atlas Colorido*. São Paulo: Medsi.

Kunner D, Oho AM 2003. Odesafio do controle da resistência aos antimicrobianos os hospitais. *Pharmacia Brasileira 36*: 90-92.

Laxoste E, Mandim D, Chaumont JP, Plumel MM, Craveiro AA, Matos FJA 1994. *XII Simpósio de Plantas Medicinais Brasileiras*. Fortaleza, Brasil.

Leal LKAM, Oliveira VM, Araruna SM, Miranda MCC, Oliveira FMA 2003. Análise de timol por CLAE na tintura de *Lippia sidoides* Cham. (alecrim-pimenta) produzida em diferentes estágios de desenvolvimento da planta. *Rev Bras Farmacogn 13(Supl.)*: 9-11.

Lemos TLG, Matos FJA, Alencar JW, Craveiro AA, McChesney JD. 1990. Antimicrobial activity of essential oil of Brazilian plants. *Phytother Res 4*: 82-84.

Lima EO, Gompertz OF, Giesbrecht AM, Paulo MQ 1993. In vitro antifungal activity of essential oil obatained from officinal plants against dermatophytes. *Mycoses 36*: 333-336.

Lima EO, Gomes STA, Oliveira NMC, Moraes VF, Paulo MQ, Carvalho ML, Araújo VC 1994. Atividade antifúngica de óleos essenciais sobre espécies de *Candida*. *XII Simpósio de Plantas Medicinais Brasileiras*. Fortaleza, Brasil.

Lima, IO, Oliveira RAG, Lima EO, Farias NMP, Souza EL 2006a. Atividade antifúngica de óleos essenciais sobre espécies de *Candida. Rev Bras Farmacogn 16*: 197-201.

Lima MRF, Ximenes CPA, Luna JS, Sant'Ana AEG 2006b. The antibiotic activity of some Brazilian medicinal plants. *Rev Bras Farmacogn 16*: 300-306.

Martins ER, Castro DM, Castellani DC 1994. *Plantas medicinais*. Viçosa: Ed. UFPR

Matos FJ 1988. *Introdução a fitoquímica experimental*. Fortaleza: Ed.UFC.

Matos FJ 1994. *Farmácias vivas*. Fortaleza: Ed.UFC.

Matos FJA, Oliveira F 1998. *Lippia sidoides* Cham. – Farmacognosia, química e farmacologia. *Rev Bras Farm 79*: 84-87.

McGaw LH, Jager AK, van Staden J 2000. Antbacterial, anthelmintic and antiamoebic activity of African medicinal plants. *J Ethnopharmacol 63*: 253-256.

Michelin DC, Moreschi PE, Lima AC, Nascimento GGF, Paganelli MO, Chaud MV 2005. Avaliação da atividade antimicrobiana de extratos vegetais. *Rev Bras Farmacogn 15*: 316-320.

Murray PR 1999. *Manual of Clinical Microbiology*. Washington: Ellen Jo Baron.

Nair MKN, Vasudevan P, Venkitanarayanan K 2005. Antibacterial effect of black seed oil on *Listeria monocytogenes*. *Food Control 16:* 395-398.

National Committee for Clinical Laboratory Standards – NCCLS 1999. *Performance standards of antimicrobial disk susceptibility test*. Atlanta, USA.

Nostro A, Blanco AR, Cannatelli MA, Enea V, Flamini G, Morelli I, Roccaro S, Alonzo V 2004. *FEMS Microbiol Lett 230*: 191-195.

Oliveira RAG, Lima EO, Vieira WL, Freire KRL, Trajano VN, Lima, IO, Souza EL, Toledo MS, Silva-Filho RN 2006. Estudo da interferência de óleos essenciais sobre a atividade de alguns antibióticos usados na clínica. *Rev Bras Farmacogn 16*: 77-82.

Palomino JC, Martin A, Camacho M, Guerra H, Swings J, Portaels F 2002. Resauzurin microtiter assay plate: simple and inexpensive method for detection of drug resistance in *Mycobacteriu*m tuberculosis. *Antimicrob Agent Chemother 46*: 2720-2722.

Pereira MSV, Siqueira Júnior JP, Takaki GMC 2004. Eliminação de resistência a drogas por fluoroquinolonas em *Staphylococcus aureus* de origem bovina*. Pesq Vet Bras 24*: 11-14.

Pontes ZBVS 2002. *Atividade antifúngica de produtos naturais e sintéticos sobre espécies de Trichosporon Behrend.* João Pessoa, 173p. Tese de Doutorado - Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba.

Rocha LG, Almeida JRGS, Macedo RO, Barbosa-Filho JM 2005. A review of natural products with antileishmanial activity. *Phytomedicine 12*: 514-535.

Sahin F, Gulluce M, Daferera D, Sokmen A, Sokmen M, Polissiou M, Agar G, Ozer H 2004. Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. v*ulgare* in the Eastern Anatolia region of Turkey. *Food Control 15*: 549-557.

Siani AC, Sampaio ALF, Sousa MG, Henriques MGMO, Ramos MFS 2000. Óleos essenciais. *Biotecnologia Ciência e Desenvolvimento 16*: 38-43.

Silva JS, Moura MD, Oliveira RAG, Diniz MFFM, Barbosa-Filho JM 2003. Natural products inhibitors of ovarian neoplasia. *Phytomedicine 10*: 221-232.

Silva-Santos A, Antunes AMS, Bizzo HR, D'Avila CA, Souza-Santos LC 2004. The application of essential oils and terpenics/terpenoids compounds in the fields of pharmaceutic and cosmetic through the knowledge registered in patents. *Rev Bras Farmacogn 14(Supl. 1)*: 48-50.

Siqui AC, Sampaio ALF, Sousa MC, Henriques MGMO, Ramos MFS 2000. Óleos essenciais – potencial anti-inflamatório. *Biotecnologia Ciência e Desenvolvimento 16*: 38-43.

Smith-Palmer A, Stewart J, Fyfe L 1998. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. *Lett Appl Microbiol 26:* 118-122.

Sousa MP, Matos MEO, Matos FJA 1991. *Constituintes ativos de plantas medicinais brasileira*s. Fortaleza: Ed.UFC.

Souza EL, Stamford TLM, Lima EO, Trajano VN 2005a. Emergencia e resistencia microbiana. *Laes & Haes 26*: 112-128.

Souza EL, Lima EO, Freire KRL, Sousa CP 2005b. Inhibitory action of some essential oils and phytochemicals on the growth of various moulds isolated from foods. *Braz Arch Biol Technol 48*: 245-250.

Verhoeff J, Beaujean D, Vlok H; Baars A, Meyler A; Van Der Werkwn C, Weersink A 1999. A dutch approach to methicillin-resistance *Staphylococcus aureus*. *Eur J Clin Microb Infect Dis 18*: 461-466.

Viljoen A, Van Vuuren S, Ernst E, Lepser M, Demirci B, Baser H, van Wyk BE 2003. *Osmitopsis astericoides* (Asteraceae) - the antimicrobial activity and essential oil composition of a Cape-Dutch remedy. *J Ethnopharmacol 88*: 137-143.

White DG, Zhao SZ, Simjee S, Wagner DD, McDermont PF 2002. Antimicrobial resistance of foodborne pathogens. *Microb Infect 4*: 405-41.

Woolhouse MEJ 2002. Population biology of emerging and re-emerging pathogens. *Trend Microbiol 10*: 3-7.

Wright GD 2003. Mechanisms of resistance to antibiotics. *Curr Opin Chem Microbiol 7*: 563-569.