Inhibitory Activity of Cinnamomum Zeylanicum and Eucalyptus Globulus Oils on Streptococcus Mutans, Staphylococcus Aureus, and Candida Species Isolated from Patients with Oral Infections

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KEY WORDS

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ABSTRACT

Statement of Problem: The Strep. mutans, Staph. aureus, Cand.albicans, and Cand. Glabrata are the most common cuases of oral infections and the use of chemical drugs usually leads to drug resistant microorganisms.
Purpose: The aim of this study was to investigate the antimicrobial activity of Cinnamon and Eucalyptus oils against this oral microorganism.
Materials and Methods: The oils were prepared by steam distillation and their inhibitory activity at different concentrations and the minimum inhibitory concentrations (MIC) were determined. Strep. mutans, multi drug resistant (MDR) Strep. mutans, Staph. aureus, methicilin resistant staph. areus, Candida. albicans, C.glabrata were used in this study. The data were analyzed using Chi square and Fisher statistical test.
Results: All of the bacterial and fungal isolates were sensitive to Cinnamon and Eucalyptus. Cinnamon oil showed strong promising inhibitory activity on all the S.mutans isolates at a concentration as low as 3.12%. Eucalyptus oil showed less inhibitory activity, as the least effective concentration of this oil was 25%. The MIC of Cinnamon and Eucalyptus oil ranged 12.8-51.2 and 64-256µg/ml, respectively.
Conclusion: Both Cinnamon and Eucalyptus oils exhibited antimicrobial activity but their effectiveness varied. Cinnamon oil showed stronger inhibitory activity as measured by MIC determination. S.mutans, the etiological agents of dental caries, were highly sensitive to Cinnamon oil and hence it may be used as an antiseptic in toothpaste, mouthwash or chewing gum for prevention of dental caries and other oral infections.

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Introduction

Infectious diseases represent an important cause of mortality among populations in developing countries. Although many new antimicrobial agents were produced in last decade, resistance to these drugs by the infectious agents has remarkably increased [1-3]. Moreover, the spread of drug resistant pathogens is one of the most serious threats to successful

treatment of microbial diseases. Due to the presence and increase of numerous drug resistant strains, there is an urgent need to develop novel antimicrobial agents; hence, much attention has been made on medicinal plants during the last decade [4-8]. Many hundred of plants worldwide are used in traditional medicine as a remedy for microbial infections. The oil extract of green tea (camellia sinensis) [9], garlic (Allium sativum) [4, 7], Cinnamon (Cinnamomum zeylanicum) [10], Eucalyptus globulus [11], Zataria multiflora [12], Artemizia feddei [13], and Rosmary (Rosmarious officinalis) [14] were the most extensively researched medicinal plants which have been reported to have cidal or inhibitory activity on various bacterial, fungal and viral agents.

Various microorganisms such as Streptococcus mutans, Staphylococcus species, Actinomyces, Bacteroides, fusiforms, candida species are present in the oral cavity of human beings which under special circumstances cause oral and systemic infections [9, 15-18]. Streptococcus mutans, the main causative agent for initiation and progression of dental caries, bacteremia and cardiovascular infections [15-16] have been reported to be highly sensitive to garlic oil while they were multiply-resistant to the routinely used antibiotics [4]. Other oral bacteria such as Prophyromonas gingivalis, Actinobacillus actionmycetecomitans, Fusobacterium nucleatum and Haemaphilus influenza were found to be sensitive to Cinnamon and Eucalyptus oils [10-11, 18-20].

According to many medical researches about antibacterial activity of the herbal drugs, in the present investigation we studied the possibility of the inhibitory activity of Cinnamon and Eucalyptus oils on S.mutans, S.aureus, Methicillin resistant staphylococcus aureus (MRSA), Candida albicans and Candida glabrata isolated from various oral infections of human subjects.

Materials and Methods

For this in vitro study, 188 patients with various oral infections such as dental caries, periodontal diseases, thrush, oral abscesses and oral lesions associated with artificial denture were included and sampled. S.mutans were isolated from the extracted rampant carious teeth. The extracted teeth were incubated in 10 ml Todd-Hewit broth (Merk- Germany) at 37°C in the presence of 5% CO_2 for 48 hours. A Mitis- Salivarious- Bacitracin- Agar (MSBA) medium was sub-cultured from Todd- Hewit broth and incubated at 37°C, 5% CO_2 for 72 hours. S.mutans were identified by biochemical tests as described elsewhere [4, 21]. Staphylococcus aureus were isolated from patients with oral abscesses, periodontal diseases, gingivitis or other related oral infections. The samples were inoculated in Mueller-Hinton broth and incubated at 37°C for 48 hours and S.aureus were then identified on the basis of colonial morphology and biochemical tests. Candida albicans and Candida glabrata were isolated and characterized from the oral samples of patients with thrush, oral abscesses and oral lesions associated with artificial denture. The samples were cultured on Cornmeal agar (CMA) containing Tween 80 and kept at room temperature for 3 weeks. Candida species were characterized by Germ tube test. All the isolates of S.mutans (n=82), S.aureus (n=57), C.albicans (n=30) and C.glabrata (n=19) were kept on culture media at 4°C until used. S.mutans ATCC 25175 and S.aureus ATCC 25923 were used as controls. Candida albicans (ATCC 90028) and C.glabrata (ATCC 90030) were also used as control.

Detection of Methicicillin resistant Staphylococcus aureus (MRSA)

All the S.aureus isolates were checked for sensitivity to methicillin. Detection of mecA gene or its product, penicillin Binding Protein (PBP2a), is considered as the gold standard for MRSA confirmation. However, the recent investigations suggest that disk diffusion using 30µg cefoxitin is also helpful and is superior to the most previously recom-

mended techniques [22-23]. Clinical Laboratory Standard Institute (CLSI) also has published zone diameter breakpoint guidelines for cefoxitin for demonstration of MRSA [24] .A total of 57 isolates of S.aureus were checked for methicillin resistance and Minimum Inhibitory Concentration (MIC) determination by using 30µg disk and E test strip of cefoxitin (AB Biodisk, Solna, Sweden). Briefly, cell suspension of each isolate of S.aureus was prepared and its turbidity was adjusted to No 0.5 Mac Farland. One tenth milliliter of this suspension was seeded on Mueller-Hinton BBL II agar (Becton-Dickinson, Heidelberg-Germany) and a 30 µg cefoxitin disk was placed on the agar and incubated at 36°C for 19 hours. The zones of inhibition were measured in mm by a caliper and then recorded. E test cefoxitin MIC testing was carried out using Mueller-Hinton BBL II agar, the plates were kept at 36°C for 19 and MICs were determined following the recommendations of the manufactures [22].

Antibiotic sensitivity by disk diffusion on S.mutans isolates

Antibiotic sensitivity profile of S.mutans (n=82) was determined according to the method of Bauer-Kirby [25]. Disks of penicillin, amoxicillin, tetracycline, imipenem, ceftriaxone, erythromycin clindamycin rifampin, vancomycin and teichoplanin were placed on a MSBA medium which was previously seeded with the test organism. Then the inhibition zones were measured after 72 hours of incubation at 37° C in the presence of 5% CO_2. Interpretation of resistance was based on Clinical Laboratory Standard Institute. Those isolates of S.mutans which were resistant to four or more of the abovementioned antibiotics were considered as Multi-Drug resistant (MDR) strains and were used to determine the antibacterial activity of Eucalyptus and Cinnamon oils. Broth microdilution susceptibility testing for estimation of MICs of amphotericin, flucytosine, fluconazole, itraconazole and voriconazole were carried out on all the candida isolates according to the recommendations of NCCLS as described in M27-A2.

Preparation of Eucalyptus globulus and Cinnamomum zeylanicum oils

Cinnamon and Eucalyptus oils were prepared by steam distillation for 3 hours according to Senhaji et.al [10]. The disteillation flask containing Cinnamon powder or Eucalyptus fresh leaves was connected to the steam generator by a glass tube and to a condenser to retrieve the oil. The recovered mixture was allowed to settle and the oil was withdrawn and kept at 4°C until used. Ten percent dimethyl sulfoxide (DMSO) with Tween 80 (0.5% V/V) was used as diluent for the antimicrobial activity testing.

Disk diffusion assay

The antimicrobial activities of Cinnamon and Eucalyptus oils were determined by disk diffusion test on solid media. MSBA, BAP, and CMA culture media were used for S.mutans, S.aureus, and Candida species, respectively. Cell suspensions of each isolate were prepared and adjusted to 108 Colony Forming Unit (CFU)/ml in sterile Mueller-Hinton broth and 0.1 ml of this suspension was seeded on appropriate culture media. A 6 mm in diameter sterile Wattman filter paper No 5 (Rund filter, Macherey-Nagel, D-5160 Doren Germany) was impregnated with 50µl of different concentrations (100, 50, 25, 12.5, 6.25 and 3.12%) of respective oils and placed on the abovementioned culture media. The plates were sealed with parafilm to avoid eventual evaporation of the test sample, kept at room temperature for 45 minutes to allow oil diffusion, and were then incubated at 37 or 30°C for 48-72 hours. The diameter of the zone of growth inhibition was measured and recorded. Sterile filter paper soaked in DMSO was used as control in each experiment. Inhibition zone of less than 7 mm was considered as negative.

MIC assay

The agar dilution method as recommended by National Committee for Clinical Laboratory Standards [26-27] with minor modifications was used. Final concentration of 0.5% (V/V) Tween 20 (Sigma) was incorporated into the agar medium to enhance the oil solubility. A series of two-fold dilution of each oil ranging from 0.2- 51.2µg/ml for Cinnamon and 16-256µg/ml for Eucalyptus oils were prepared in Mueller-Hinton agar at 48°C. The plates were allowed to stand at room temperature for 30 minutes and were then inoculated with 3µl aliquots of the cultures containing approximately 105 CFU/ml of each microorganism. The plates were incubated at 37 or 30°C for 24-72 hours. MICs were determined as the lowest concentration of the oil inhibiting the visible growth of each microorganism on the plates. Inhibition of bacterial and fungal growth on the plates containing test oil was judged by comparison with the growth in blank control plates. The data were analyzed using Chi-square and Fisher exact tests in SPSS version 11.5.

Results

In this study, 82 isolates of S.mutans, 57 of S.aureus, 30 of C.albicans, and 19 of C.glabrata were recovered from the oral cavity of patients with various oral infections. Out of 82 isolates of S.mutans, 20 (24.4%) were resistant to at least four antibiotics, so they were considered as Multi-Drug resistant-S.mutans (MDR- S. mutans). Table 1 presents the resistance rate of the S.mutans isolates. The highest resistance rate was observed for tetracycline while no resistance was detected to vancomycin and teichoplanin.

Table 2 shows the resistance patterns of 20 MDRS.mutans isolates. Five different patterns were identified: 17 isolates were resistant to 5 antibiotics (patterns No, 1, 2 and 3), 2 resistant to 4 (pattern No 4) and one isolate was resistant to 6 antibiotics (pattern No 5). According to the criteria suggested by CLSI [27], out of 57 isolates of S.aureus, 21 (36.8 %) were identified as MRSA.

Table 1 Resistance rate of 82 S.mutans isolates recovered from carious teeth to antibacterial agents by disk diffusion tests

Antibacterial agents	No of resistant isolates (%)
Tetracycline	20 (24.4)
Penicillin	18 (21.9)
Amoxicillin	15 (18.3)
Erythromycin	18 (21.9)
Rifampin	14 (17)
Clindamycin	12 (14.6)
Ceftriaxone	1 (1.2)
Imipenem	1 (1.2)
Vancomycin	0 (0)
Teichoplanin	0 (0)

Table 2 Resistance patterns of 20 MDR strains of S.mutans recovered from carious teeth to antibacterial agents by disk diffusion test

Pattern No.	Resistance pattern	No. of patterns
1	P, AMX, RIF, TET, E	8
2	P, AMX, TET, E, CLIN	5
3	P, E, CLIN, RIF, TET	4
4	AMX, CLIN, TET, RIF	2
5	P, E, IMIP, TET, CEF, CLIN	1
Total MDR strains		20

P-Penicillin, AMX- Amoxicillin, E- Erythromycin, RIF-Rifampin, TET- Tetracycline, CLIN-Clindamycin, CEF- Ceftriaxone, IMIP-Imipenem. MDR- multi drug resistant

Table 3 shows the results of cefoxitin sensitivity by MICE test and disk diffusion methods (Bauer-Kirby). The zone of growth inhibition around 30µg cefoxitin disk varied from 22-33 mm for non-MR-SA and 8-18 mm for MRSA. The MIC of Cefoxitin ranged 0.5- 4µg/ml and 6-24 µg/ml for

Table 3 Results of MIC-E test and disk diffusion technique, using cefoxitin strip and 30µg disk for identification of MRSA

Bacterial type	MIC range (µg/ml)	Range of inhibition zone (mm)
Non-MRSA (n=36)	0.5 – 4	22-23
MRSA (n=21)	6.0-24	8.0-18
MRSA ATCC 25923	12.0	16.0

MRSA- Methicillin Resistant Staphylococcus aureus. MRSA were characterized as described by Skov R et al [23] and CLSI [24].

Table 4 Antimicrobial activity of various concentrations of Cinnamon (C) and Eucalyptus (E) oils on microorganisms isolated from the oral cavity of patients with oral infections by disk diffusions tests

Con of Oils %		MDR S.mutans (n=20)	N.MDR S.mutans (n=62)	MRSA (n=21)	N.MRSA (n=36)	C.albicans (n=30)	C.glabrata (n=19)
100	C	62*	64	43	44	54	51
	E	41	38	29	38	48	45
50	C	51	52	28	28	27	27
	E	20	19	14	18	24	23
25	C	35	25	15	15	13	14
	E	9	8	0	8	12	12
12.5	C	21	15	9	8	8	8
	E	0	0	0	0	0	0
6.25	C	16	9	0	0	0	0
	E	0	0	0	0	0	0
3.12	C	9	8	0	0	0	0
	E	0	0	0	0	0	0

Abbreviations: MDR-multi drug resistant, MRSA-methicillin resistant S.aureus, * - figures are the mean diameter of inhibition zones in mm. 10% Dimethyl sulfoxide was used as control, which showed no zone of inhibition.

Non-MRS-A and MRSA, respectively ($p < 0.05$). All the isolates of C.albicans (n= 30) and C.glabrata (n=19) were sensitive to amphothericin B, flucytosine and voriconazole with MIC ranging 0.12-0.5µg/ml. However, a total of 4 (21%) of C.glabrata and 6 (20%) of C. albicans showed decreased susceptibility to itraconazole (MIC > 0.5µg/ml) and fluconazole (MIC > 16µg/ml). The inhibitory activity of Cinnamon and Eucalyptus oils on the aforementioned bacterial and fungal isolates from the oral cavity of patients with oral infections as measured by disk diffusion is summarized in Table 4. Cinnamon oil was found highly active on both MDR and Non-MDR-S.mutans at concentrations 3.12-100% by producing the zone of inhibitions ranging 8-62 mm in diameter. The least active concentration of this oil on other microorganisms tested was 12.5% producing inhibition zone of 8 mm in diameter. Eucalyptus oil at concentrations of 100, 50 and 25% was effective on MDR and Non-MDR-S.mutans producing zone of inhibition ranging 8-41 mm in diameter. The least active concentration of this oil on other microorganisms tested was 25% producing 8 mm inhibition zone. There was no inhibition of growth by 10% DMSO used as control. Table 5 is a summary of s the MIC of both oils. Cinnamon oil showed maximum activity with MIC values ranging from 12.8-51.2µg/ml, followed by Eucalyptus oil with MIC values of 64-> 256µg/ml against all the bacteria and unicellular fungi tested.

Discussion

One of the most important problems in the successful treatment of infectious diseases is the developent and spread of resistant pathogens to antimicrobial agents. Besides the side effects, high consumption of various antibiotics may lead to the development of supra-infection when antibiotics eradicate normal flora. Despite the advent and development

Table 5 Minimum inhibitory concentrations (MIC 90) of Cinnamon (C) and Eucalyptus (E) oils (µg/ml) against bacterial and fungal isolates of patients with oral infections.

Oils	MDR S.mutans	N. MDR S.mutans	MRSA	N.MRSA	C.albicans	C.glabrata
C	12.8	12.8	25.6	25.6	51.2	51.2
E	64	64	128	128	256	>256

Abbreviations: MDR-multi drug resistant, MRSA- methicillin resistant Staphylococcus aureus. N-none

of antimicrobial agents, bacterial and fungal infections are still a major health problem; therefore, there is an urgent need to develop new antimicrobial compounds, with higher activity, greater sensitivity, and less toxicity. The studies carried out on medicinal plants during the last decade indicate that essential oils or the extracts of some medicinal plants could be considered as a potential source of novel antimicrobial agents [4-14, 19-20]. In vitro studies in this investigation showed that both Cinnamon and Eucalyptus oils exhibited inhibitory activity on all the microorganisms tested but their effectiveness varied.

S.mutans were the most susceptible isolates to cin-8/7namon oil at a concentration as low as 3.12%, while Eucalyptus oil was not effective on S.mutans at this concentration (Table 4). MRSA, Non MRSA, C.albicans, and C.glabrata regardless of their sensitivity to antibiotics and antifungal agents were equally sensitive to Cinnamon oil and their growth was inhibited at 12.5% whereas Eucalyptus oil was effective at the concentration of 25%, indicating stronger activity of Cinnamon oil.

Quale JM et al. [28] have shown that consumption of commercially available Cinnamon bark could improve oral candidiasis of patients with HIV infections. Fluconazole resistant C.albicans (n=6, 20%) and C.glabrata (n=4, 21%) examined in our study were equally sensitive to Cinnamon oil with MIC= 51.2 µg/ml. Eucalyptus oil, though effective on these unicellular fungi, showed much higher MIC (256->256 µg/ml). Several studies have shown that Cinnamon oil had a strong and consistent inhibitory activity against various pathogenic microorganisms [29-30] while other investigators have reported the better antimicrobial activity of Eucalyptus oil [20, 31].

The data obtained in this study indicate the stronger activity of Cinnamon oil as it showed lower MICs on all the microorganisms tested (Table 5). The antimicrobial activity of Cinnamon oil is attributed to the presence of cinnamaldehyde which is the predominant active component found in Cinnamon oil [32]. Ooi LS et al [33] have found both cinnamon oil and cinnamaldehyde equally active on Gram positive and Gram negative bacteria (MIC= 75-600 µg/ml) and unicellular fungi (MIC=100-450 µg/ml). Cinnamaldehyde was also shown to inhibit the growth of antibiotics resistant and sensitive Helicobacter pylori [34] and hence Cinnamon bark taken orally may prevent gastric ulcer caused by these bacteria.

Cinnamon oil was not harmful when consumed in food products and may be used as an agent to inhibit the growth of bacteria, molds, and yeast cells in food preservation. However, there are occasional case reports of allergic contact dermatitis [35] and stomatitis [36] among the consumers of Cinnamon oil. Eucalyptus oil has been used in inhalation therapy to treat bronchitis and sinusitis. Inhalation of Eucalyptus vapor augments the output of respiretory tract fluid and maintain the ventilation and drainage of the sinuses. Eucalyptus oil is well documented as being extremely toxic if ingested particularly among children [37]. However, systemic Eucalyptus oil poisoning post-topical application has been reported [38].

Complex extract of the tea tree and Eucalyptus oils has been shown to have strong bactericidal activity against MRSA [39]. This complex has been administered percutaneously into bone, to treat an intractable MRSA infection of the lower tibia in an adult male patient and his symptoms resolved after 3 months [40]. In our study, both MRSA and non-MRSA were equally sensitive to Eucalypt-us oil with MIC=128µg/ml. However, considering the low inhibitory activity of this oil, as measured by MIC determination, its toxicity and side effects, its application as antiseptic is limited to topical use.

Conclusion

Cinnamon oil which exhibited stronger inhibitory

activity particularly on S.mutans, the etiological agent of dental caries, which is not toxic when ingested and has no harmful reactions and side effects, could be used as an antiseptic and may be incorporated into tooth paste, mouthwash and chewing gum for prevention of dental caries and other oral infections.

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References

[1] Sakoulas G, Moellering RC Jr. Increasing antibiotic resistance among methicillin-resistant Staphylococcus aureus strains. Clin Infect Dis 2008; 46 Suppl 5: 360-367.

[2] Kohanteb J, Dayaghi M, Motazedian M, Ghayumi MA. Comparison of biotyping and antibiotyping of Pseudomonas aeruginosa isolated from patients with burn wound infection and nosocomial pneumonia in Shiraz, Iran. Pak J Biol Sci 2007; 10: 1817-1822.

[3] Kohanteb J, Sadeghi E. Penicillin-resistant Streptococcus pneumoniae in Iran. Med Princ Pract 2007; 16: 29-33.

[4] Fani MM, Kohanteb J, Dayaghi M. Inhibitory activity of garlic (Allium sativum) extract on multidrug-resistant Streptococcus mutans. J Indi-an Soc Pedod Prev Dent 2007; 25: 164-168.

[5] Pesewu GA, Cutler RR, Humber DP. Antibacterial activity of plants used in traditional medicines of Ghana with particular reference to MRSA. J Ethnopharmacol 2008; 116: 102-111.

[6] Caelli M, Porteous J, Carson CF, Heller R, Riley TV. Tea tree oil as an alternative topical decolonization agent for methicillin-resistant Staphylococcus aureus. J Hosp Infect 2000; 46: 236-237.

[7] Martin KW, Ernst E. Herbal medicines for treatment of bacterial infections: a review of controll-ed clinical trials. J Antimicrob Chemother 2003; 51: 241-246.

[8] Aqil F, Khan MS, Owais M, Ahmad I. Effect of certain bioactive plant extracts on clinical isolates of beta-lactamase producing methicillin resistant Staphylococcus aureus. J Basic Microbiol 2005; 45: 106-114.

[9] Hirasawa M, Takada K, Makimura M, Otake S. Improvement of periodontal status by green tea catechin using a local delivery system: a clinical pilot study. J Periodontal Res 2002; 37: 433-438.

[10] Senhaji O, Faid M, Kalalou I. Inactivation of Escherichia coli O157:H7 by essential oil from Cinnamomum zeylanicum. Braz J Infect Dis 2007; 11: 234-236.

[11] Salari MH, Amine G, Shirazi MH, Hafezi R, Mohammadypour M. Antibacterial effects of Eucalyptus globulus leaf extract on pathogenic bacteria isolated from specimens of patients with respiratory tract disorders. Clin Microbiol Infect 2006; 12: 194-196.

[12] Khosravi AR, Eslami AR, Shokri H, Kashanian M. Zataria multiflora cream for the treatment of acute vaginal candidiasis. Int J Gynaecol Obstet 2008; 101: 201-202.

[13] Cha JD, Jung EK, Kil BS, Lee KY. Chemical composition and antibacterial activity of essential oil from Artemisia feddei. J Microbiol Biotechnol 2007; 17: 2061-2065.

[14] Luqman S, Dwivedi GR, Darokar MP, Kalra A, Khanuja SP. Potential of rosemary oil to be used in drug-resistant infections. Altern Ther Health Med 2007; 13: 54-59.

[15] Tomás I, Pereira F, Llucián R, Poveda R, Diz P, Bagán JV. Prevalence of bacteraemia following third molar surgery. Oral Dis 2008; 14: 89-94.

[16] Jones DJ, Munro CL. Oral care and the risk of bloodstream infections in mechanically ventilated adults: A review. Intensive Crit Care Nurs 2008; 24: 152-161.

[17] Shouval DS, Bilavsky E, Avitzur Y, Shapiro R,

Amir J. Staphylococcus aureus bacteremia complicating herpes simplex virus type 1 stomatitis: case report and review of the literature. J Periodontol 2008; 79: 376-378.

[18] Li L, Redding S, Dongari-Bagtzoglou A. Candida glabrata: an emerging oral opportunistic pathogen. J Dent Res 2007; 86: 204-215.

[19] Inouye S, Takizawa T, Yamaguchi H. Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. J Antimicrob Chemother 2001; 47: 565-573.

[20] Takarada K, Kimizuka R, Takahashi N, Honma K, Okuda K, Kato T. A comparison of the antibacterial efficacies of essential oils against oral pathogens. Oral Microbiol Immunol 2004; 19: 61-64.

[21] Beighton D, Russell RR, Whiley RA. A simple biochemical scheme for the differentiation of Streptococcus mutans and Streptococcus sobrinus. Caries Res 1991; 25: 174-178.

[22] Skov R, Smyth R, Clausen M, Larsen AR, Frimodt-Møller N, Olsson-Liljequist B, Kahlmeter G. Evaluation of a cefoxitin 30 microg disc on Iso-Sensitest agar for detection of methicillin-resistant Staphylococcus aureus. J Antimicrob Chemother 2003; 52: 204-207.

[23] Skov R, Smyth R, Larsen AR, Bolmstrôm A, Karlsson A, Mills K, et al. Phenotypic detection of methicillin resistance in Staphylococcus aureus by disk diffusion testing and Etest on Mueller-Hinton agar. J Clin Microbiol 2006; 44: 4395-4399.

[24] Clinical Laboratory Standard Institute 2005. Performance Standards for antimicrobial susceptibility testing 15th informational supplement M10-0-S15. Clinical and Laboratory Standard Institute. Wayne, PA.

[25] Forbes BA, Sahm DF, Weissfeld AS. Laboratory methods for detection of antibacterial resistance-in: Roche J, Parker SJ, Mc Adam L, Editors. Baily and Scott's diagnostic microbiology. 10th ed., Mosby Inc: St Louis; 1998. p. 250-272.

[26] Kohanteb J, Bazargani A, Saberi-Firoozi M, Mobasser A. Antimicrobial susceptibility testing of Helicobacter pylori to selected agents by agar dilution method in Shiraz-Iran. Indian J Med Microbiol 2007; 25: 374-377.

[27] Prudent D, Perineau F, Bessiere JM, Michel GM, Baccou JC. Analysis of the essential oil of wild oregano from Martinique (Coleus aromaticus Benth.): evaluation of its bacteriostatic and fungistatic properties. J Essen Oil Res 1995; 7: 165- 173.

[28] Quale JM, Landman D, Zaman MM, Burney S, Sathe SS. In vitro activity of Cinnamomum zeylanicum against azole resistant and sensitive Candida species and a pilot study of Cinnamon for oral candidiasis. Am J Chin Med 1996; 24: 103-109.

[29] Matan N, Rimkeeree H, Mawson AJ, Chompreeda P, Haruthaithanasan V, Parker M. Antimicrobial activity of Cinnamon and clove oils under modified atmosphere conditions. Int J Food Microbiol 2006; 107: 180-185.

[30] Aureli P, Constantini A, Zolea S. Antimicrobial activity of some plant essential oils against Listeria monocytogenes. J Food Prot 1992; 55: 344-348.

[31] Cimanga K, Kambu K, Tona L, Apers S, De Bruyne T, Hermans N, et al. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. J Ethnopharmacol 2002; 79: 213-220.

[32] Simić A, Soković MD, Ristić M, Grujić-Jovanović S, Vukojević J, Marin PD. The chemical composition of some Lauraceae essential oils and their antifungal activities. Phytother Res 2004; 18: 713-717.

[33] Ooi LS, Li Y, Kam SL, Wang H, Wong EY, Ooi VE. Antimicrobial activities of **C**innamon oil and cinnamaldehyde from the Chinese medicinal herb Cinnamomum cassia Blume. Am J Chin Med 2006; 34: 511-522.

[34] Ali SM, Khan AA, Ahmed I, Musaddiq M, Ahm-

ed KS, Polasa H, et al. Antimicrobial activities of Eugenol and Cinnamaldehyde against the human gastric pathogen Helicobacter pylori. Ann Clin Microbiol Antimicrob 2005; 4: 20.

[35] Campbell TM, Neems R, Moore J. Severe exacerbation of rosacea induced by Cinnamon supplements. J Drugs Dermatol 2008; 7: 586-587.

[36] Tremblay S, Avon SL. Contact allergy to Cinnamon: case report. J Can Dent Assoc 2008; 74: 445-461.

[37] Webb NJ, Pitt WR. Eucalyptus oil poisoning in childhood: 41 cases in south-east Queensland. J Paediatr Child Health 1993; 29: 368-371.

[38] Darben T, Cominos B, Lee CT. Topical Eucalyptus oil poisoning. Australas J Dermatol 1998; 39: 265-267.

[39] Anderson JN, Fennessy PA. Can tea tree (Melaleuca alternifolia) oil prevent MRSA? Med J Aust 2000; 173: 489.

[40] Sherry E, Boeck H, Warnke PH. Percutaneous treatment of chronic MRSA osteomyelitis with a novel plant-derived antiseptic. BMC Surg 2001; 1: 1.