ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Antimicrobial Activities of Some Substances Used in Oral Cavity Spray

***Thawatchai Phaechamud[1, a], Juree Charoenteeraboon[2, b], Wanpen Saengthongpinit[3, 4, c], And Aruni Chuekaew[3, d]**

[1]Department of Pharmaceutical Technology, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom, Thailand 73000
[2]Department of Biopharmacy, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom, Thailand 73000
[3]Food Institute Project, Faculty of Science and Technology, Nakhon Pathom Rajabhat University, Nakhon Pathom, 73000 Thailand
[4]Food Science and Technology Program, Faculty of Science and Technology, Nakhon Pathom Rajabhat University, Nakhon Pathom, 73000 Thailand

ABSTRACT

Bad smell breath causes from the microbial accumulation in mouth which can be diminished by microbial reduction. The refresh and waking up feelings can generate from essential oils and the spicy taste of chili extract, respectively. The aim of this study was to investigate the antimicrobial activities of chili extract, essential oils and some additives in the oral cavity spray. The antimicrobial activities of the chili extract, capsaicin, peppermint oil, clove oil, cinnamon oil, citrofresh and frescolate against some microbes in oral cavity such as *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli* or *Candida albicans* were determined by agar cup diffusion method. Cinnamon oil exhibited the highest activity against *S. aureus* (MIC=1%v/v), *S. mutans* (MIC=1%v/v) and *C. albicans* (MIC=0.1%v/v). MIC of clove oil and peppermint oil against *S. aureus* were equally as 5%v/v, whereas clove oil (MIC=1%v/v) showed higher activity against *C. albicans* than peppermint oil (MIC>20%v/v). Both 1%w/v capsaicin solution and chili extract (containing 0.05%w/v capsaicin) did not inhibit *S. aureus* and *C. albicans*. The chili extract could inhibit the growth of *S. aureus, S. mutans and C. albicans* at concentration of 100%, 25% and 25%, respectively, whereas it could not inhibit the growth of *E. coli.* By comparison, frescolate could not inhibit the growth of *S. mutans* whereas citrofresh and cinnamon oil exhibited this activity. Therefore cinnamon oil and other selected substances exhibited the interesting antimicrobial activities against the microbes found in oral cavity.

Keywords: oral cavity spray, antimicrobial activities

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Cinnamon oil, which consists mainly of cinnamaldehyde has many functions including relieving fever, anticancer and antivirus properties. This oil also has strong antimicrobial action for variety of pathogenic microbes [1]. Cinnamon oil is the natural preservative and flavoring agent that is not toxic when it is added in food products. It can inhibit the growth of *Aspergillus flavus*, *Aspergillus parasiticus*, *Fusarium moniliforme* [2], *Lactobacillus sp.*, *Bacillus thermoacidurans, Salmonella sp., Corynebacterium michiganense, Pseudomonas striafaciens, Clostridium botulinum and Penicillium roqueforti* [3]. Cinnamon oil and thyme oils are active against *Salmonella enterica, Escherichia coli, Staphylococcus aureus* and *Listeria monocytogenes* [4]. Combination of cinnamon oil and thyme oil exhibited an additive effect against *Bacillus subtilis, Bacillus cereus* and *Staphylococcus aureus* and *E. coli* and *Salmonella typhimurium* and combination of cinnamon oil and clove oil displayed and additive effect against *B. subtilis, B. cereus, S. aureus,* and an indifferent effect against *E. coli* and *S. typhimurium* [5]. Therefore, cinnamon oil should be examined with respect to antimicrobial activity. Many volatile oils including peppermint oil demonstrated efficacy against hospital-acquired isolates and reference strains [6]. Clove oil and eugenol could be used as a natural antibacterial agent against cariogenic and periodontopathogenic bacteria [7].

The anti-pathogenic power of the citrofresh is due to a synergistic activity between the citrus fruit bioflavonoid complex and certain other naturally occurring organic acids. The active ingredients in citrofresh do not poison the bacteria however they act by destroying the cellular membrane of the micro-organisms. It can be used in a variety of oral care products. Frescolate is the menthyl lactate (5-methyl-2-(methylethyl) cyclohexyl alpha-hydroxypropanoate)**.** This compound has a mild cooling, fresh, minty properties which is used as cooling agent in the personal care products.

The objective of this study is to investigate the antimicrobial activity of chili extract, capsaicin, peppermint oil, clove oil, cinnamon oil, citrofresh and frescolate which can be used in the antimicrobial oral cavity spray.

MATERIALS AND METHODS

Materials

Capsaicin was purchased from Sigma and capsicum tincture (0.05% capsaicin in chili extract) code 81270 control No. R 18506, Bangkok Lab & Cosmetic Ltd., Rachaburi, Thailand was used as received. Tryptic Soy Agar (TSA) (lot No. 3056695, Difco, USA.), Tryptic Soy Broth (TSB) (lot 4259, Difco, USA.), Sabouraud Dextrose Agar (SDA) (lot 6166081, Difco-TM, Becton Dickinson and Company, USA) and Sabouraud Dextrose Broth (SDB) (lot 6345690, Difco-TM, Becton Dickinson and Company, USA) were used as received. Brain Heart Infusion agar and broth (BHI) were used for *Streptococcus mutans*. Clotrimazole and doxycycline hyclate were kindly supported from T Man Pharma Co. Ltd., Bangkok, Thailand. The 10 μg/ml ampicillin disk was purchased from Becton Dickinson and Company, France. Citrofresh and freshcolate were kindly supported from Bronson and Jacobs International Co.,

ISSN: 0975-8585

Ltd., Bangna, Bangkok, Thailand. Tween 80, dimethyl sulfoxide (DMSO), peppermint oil, cinnamon oil, clove oil and other chemicals were purchased from PC Drug Co., Ltd., Bangkok, Thailand.

Methods

For the antimicrobial activity testing the capsicin, capsicum extracts, selected oil and other substances were tested using the cup agar diffusion technique. The 10% DMSO containing 0.5%v/v tween 80 was used as the solvent for the test samples. Once an actively growing broth culture or suspension of microbes was obtained then the bacteria was inoculated and prepared by adjusting the turbidity of an actively growing broth culture in used broth to an optical density at 530 nm equivalent to $1x10^8$ cfu/ml. A sterile swab was dipped into the adjusted suspension before rotating and pressing on the inside wall of the tube. The inoculated microbe was spread on TSA for bacteria and SDA for fungi in three directions to ensure the complete spreading of the agar surface. Brain heart infusion agar and broth (BHI) were used for *Streptococcus mutans* and the aenarobic condition was applied using an anaerobic incubator (Hiramaya, Japan). Plates were opened for a few minutes to dry the spread culture. The sterile cyclinder cup was filled with the serial diluted tested sample of 150 µL/cup and placed for 30 min before incubated at 37 °C for 24 hrs. The antimicrobial activity was measured as the diameter (mm) of the clear zone of growth inhibition. The tests were carried out in triplicate and the mean clear zone ± S.D. was calculated. The sterile cyclinder cup filled with 10% DMSO containing 0.5%v/v tween 80 at the same amount of those test samples were employed as the negative control. The minimal inhibition concentrations (MICs) of the test samples against microbes were determined as the lowest concentration of sample which inhibited the microbial growth.

RESULTS AND DISCUSSION

Antimicrobial activity of (0.0625-1% w/v) capsaicin solutions and 0.00125-0.05% w/v of capsicum tincture was determined using agar cup diffusion method. There was no inhibition zone against *S. aureus* and *C. albicans* for these test samples (data not shown). Therefore the MIC of capsaicin and capsicum tincture should be higher than 1% and 0.05%w/v, respectively. Cinnamon oil exhibited the highest activity against *S. aureus* (MIC=1%v/v) (Table 1), *S. mutans* (MIC=1%v/v) (Table 2) and *C. albicans* (MIC=0.1%v/v) (Table 1). MIC of clove oil and peppermint oil against *S. aureus* were equally as 5%v/v, whereas clove oil (MIC=1%v/v) showed higher activity against *C. albicans* than peppermint oil (MIC>20%v/v) as presented in Table 3,4. The inhibition zone was larger than to measure the diameter for 0.5-4%v/v cinnamon oil and 20%v/v clove oil against *C. albicans*. Cinnamon oil exhibited the interesting powerful antimicrobial activity especially against *C. albicans* therefore it should be used in the developed oral cavity spray. However many users dislikes its smell and that the addition of other volatile oils or flavoring agents is necessary.

The MIC of capsicum tincture against *Staphylococcus aureus*, *Streptococcus mutans*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Candida albicans* were 100%, 25% and 25% w/v, respectively (Table 5). *Escherichia coli* could not be inhibit with these solutions. Therefore the capsaicin should not be the main antimicrobial in the developed oral cavity

ISSN: 0975-8585

spray. However the refresh and waking up feelings generating with the spicy taste of chili extract is interesting. By comparison the growth inhibition for *S. mutans* of cinnamon oil was higher than that of citrofresh whereas frescolate did not show the inhibition as presented in Table 6. Therefore frescolate which is menthyl lactate used as cooling agent did not inhibit these microbial growth however this result might be owing to its low solubility therefore its diffusion into the environmental agar was limited.

Table 1 Inhibition zone of cinnamon oil

Test sample	Inhibition zone (mm.)	
	S. aureus	*C. albicans*
4% v/v cinnamon oil	23.5 ± 2.12	ND
2% v/v cinnamon oil	22.5 ± 0.71	ND
1% v/v cinnamon oil	16 ± 1.41	ND
0.5% v/v cinnamon oil	-	ND
0.4% v/v cinnamon oil	-	26 ± 1.41
0.2% v/v cinnamon oil	-	21 ± 1.41
0.1% v/v cinnamon oil	-	12.5 ± 0.71
0.05% v/v cinnamon oil	-	-
0.025% v/v cinnamon oil	-	-
Solvent control	-	-
Doxycycline 2µg/mL	20 ± 0	-
Clotrimazole 40 µg/mL	-	21 ± 1.41

*(-) = no clear zone; N/A=not determined; ND = Inhibition zone larger than to measure

Table 2 Inhibition zone of cinnamon oil against *S. mutans*

Test sample	Inhibition zone ± SD (cm.)
5% v/v cinnamon oil	2.28±0.01
2% v/v cinnamon oil	1.70 ± 0.03
1% v/v cinnamon oil	1.07 ± 0.09
0.5% v/v cinnamon oil	-
0.2% v/v cinnamon oil	-
0.1% v/v cinnamon oil	-
0.05% v/v cinnamon oil	-
Negative control	-
Doxycycline 2 µg/ml	1.69 ± 0.02

*(-) = no clear zone

ISSN: 0975-8585

Table 3 Inhibition zone of clove oil

Test sample	Inhibition zone (mm.)	
	S. aureus	*C. albicans*
20% v/v clove oil	18.5 ± 2.12	ND
10% v/v clove oil	14.5 ± 3.54	
5% v/v clove oil	10.5 ± 0.71	18.5 ± 0.71
2.5% v/v clove oil	-	17.5 ± 0.71
1% v/v clove oil		9.5 ± 2.12
0.5% v/v clove oil	-	-
0.25% v/v clove oil	-	-
Solvent control	-	-
Doxycycline 2µg/mL	20 ± 0	-
Clotrimazole 40 µg/mL	-	20.5 ± 0.71

*(-) = no clear zone; N/A=not determined; ND = Inhibition zone larger than to measure

Table 4 Inhibition zone of peppermint oil

Test sample	Inhibition zone (mm.)	
	S. aureus	*C. albicans*
20% v/v peppermint oil	15.0 ± 0.82	-
10%v/v peppermint oil	10.3 ± 1.26	-
5%v/v peppermint oil	8.3 ± 1.26	-
2.5%v/v peppermint oil	-	-
Solvent control	-	-
Doxycycline 2 µg/mL	20.8 ± 0.96	-
Clotrimazole 40µ g/mL	-	20.5 ± 0.71

*(-) = no clear zone

Table 5 Inhibition zone of capsicum tincture

Capsicum tincture	Inhibition zone ± SD (cm.)			
	S. aureus	*S. mutans*	*C.albican*	*E.coli*
100%	0.93 ± 0.10	2.42 ± 0.10	2.54 ± 0.02	-
75%	-	2.18 ± 0.05	2.20 ± 0.12	-
50%	-	1.53 ± 0.04	1.36 ± 0.10	-
25%	-	1.45 ± 0.03	1.09 ± 0.12	-
10%	-	-	-	-
5%	-	-	-	-
1%	-	-	-	-
0.5%	-	-	-	-
Ampicillin 10 µg/ml	4.41 ± 0.05	5.57 ± 1.08	N/A	3.31 ± 0.05
Clotrimazole 10µg/ml	N/A	N/A	2.55 ± 0.13	N/A

*(-) no clear zone and N/A=not determined

ISSN: 0975-8585

Table 6 Inhibition zone of substances in oral cavity spray against *S. mutans*

Test samle	Inhibition zone (cm.)
Cinnamon oil*	2.47± 0.05
Citrofresh*	1.97± 0.08
Freshcolate*	0.00± 0.00
50 µg/ml Doxycycline**	4.70± 0.04

*not diluted; **positive control

CONCLUSION

The volatile oils including cinnamon oil, peppermint oil, clove oil, and citrofresh showed the antimicrobial activities with the different potency. These compounds exhibited the potential natural antimicrobial activity for addition in the oral cavity spray used for bad smell breath treatment. The refresh and waking up feelings generating from the used volatile oils and the spicy taste of chili extract can be obtained.

ACKNOWLEDGEMENTS

This research work was kindly supported by Government-Industries Cooperation Project, Division of Research & Evaluation, Commission on Higher Education, Ministry of Education, Thailand (Year 2010; Project: Oral Cavity Spray Containing Chili Extract and Essential Oils). We would like to thank Bangkok Lab & Cosmetic Ltd., Rachaburi, Thailand for his kind support the capsicum tincture. I would like to express my appreciation to Faculty of Pharmacy, Silpakorn University for the facility and support.

REFERENCES

[1] Wang G, Deng J, Ma Y, et al. JTCM 2012;32:19-24.
[2] Soliman KM, Badeaa RI. Food Chem Toxicol 2002;40:1669-1675
[3] Matan N, Rimkeeree H, Mawson AJ, et al. Int J Food Microbiol 2006;107:180–185
[4] Smith-Palmer A, Stewart J, Fyfe L. Lett Appl Microbiol 1998;26:118–122.
[5] Lu F, Ding Y, Ye X, et al. Agr Sci China 2011;10 :1482-7.
[6] Warnkee PH, Becker ST, Podschun R. J Cranio Maxill Surg 2009;37 :392-7.
[7] Moon S-E, Kim H-Y, Cha J-D. Arch Oral Biol 2011;56 :907-16.