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Antibacterial Activities Of Essential Oil From *Zingiber Cassumnar* Roxb. Against *Serratia marcescens,* Associated With Bacterial Keratitis

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Abstract

Introduction: *Serratia marcescens* (SM) is responsible for bacterial keratitis.The occurrence of antibiotics and disinfectants resistant - SM necessitate the discovery of new anti- SM agents from natural plants leads. **Methods:**The antibacterial activities of essential oils from 18 medicinal plants (*Zingiber cassumunar* Roxb.(A), *Zingiber officinale*(B), *Curcuma aromatica* Salisb. (C), *Chromolaena odoratum* (L.) (D), *Amomum biflorum* Jack (E), *Citrus maxima* (Burm.f).Merr.(F), *Syzygium aromaticum* (L.) Merr.& L.M.Perry. (G), *Citrus hystrix* DC. (H), *Kaempferia galanga* L. (I), *Alpinia galangal* (Linn.) Swartz (J), *Cocos nucifera* L.(K), *Curcuma xanthorrhiza* Roxb (L), *Mentha cordifolia* Opiz. (M), *Curcuma iners* Blume (N), *Eucalptus globulus* Labill. (O), *Litsea cubeba* Pers(P)., *Curcuma longa* L. (Q), and *Citrus reticulate* Blanco (R)), against 5 strains of SM, obtaining from Department of Medical Sciences Thailand Culture Collection (DMST); SM DMST4228, SM DMST 4424, SM DMST 5100, SM DMST 8845 and SM DMST 8846) were performed by using agar diffusion method (20 mg/disc). The minimum inhibitory concentrations (MICs) of the selected oil were determined by agar dilution method. The selected oil was investigated antibacterial potency comparable with 5 antibiotics (gentamicin:GEN, chloramphenicol:Cam, erythromycin:ERY, ampicillin:Amp, and tetracyclin:Tet) by using the agar diffusion method. **Results:** Among 18 essential oils, the antibacterial effect against the 5 strains of SM revealed that A (phlai oil) possessed significantly the strongest antibacterial efficacy with the range of its inhibition zones was 9.70-20.42 mm. The oil showed higher anti- SM activities against the 5 strains than AMP and ERY did. The best MIC value of A was 5 mg/mL. **Conclusion:**These findings indicate the possibility to use phlai oil for the topical treatment of bacterial keratitis against SM.

Keywords: S*. marcescens, Z.cassumunar* Roxb., agar diffusion, agar dillution, bacterial keratitis

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Introduction

Serratia marcescens (SM) is facultative aerobic, motile gram-negative rod within the family Enterobacteriaceae. This bacterium has a remarkably survival potential because of its low nutrient requirement, its antibiotics and disinfectants resistance, caused by variable cell wall permeability and the secretion to beta-lactamases (non-plasmid-mediated in SM) and its surfaces adherent ability. It is associated with contaminated contact lens solutions. It plays important role as one of the challenging organisms for testing contact lens solutions recommended by FDA guidelines (1989) (Parment P A, 1997). The previous studies reported the isolation of pure culture of it from contact lens solutions (particularly with chlorhexide) brought to ocular infections such as keratitis and conjunctivitis. An increase in the incidence antibiotics and disinfectants resistance of SM has brought about the necessity for developing new anti-SM agents. The finding out antibacterial efficacy against SM-caused bacterial keratitis, especially contact lens keratitis, from medicinal plants to use as an alternative agent has been accomplished. Therefore, the purposes of this study were to select, to investigate the strongest antibacterial efficacy against SM of essential oils from 18 medicinal plants, and to compare anti-SM activities of the selected oil with 5 antibiotics (Gentamicin(GEN), chloramphenicol(Cam), erythromycin(ERY), ampicillin(Amp), and tetracycline(Tet)). These antibiotics were commonly used for bacterial keratitis, which focused on broad-spectrum coverage against both gram-positive and gram-negative bacteria.

Materials and Methods

2.1 Preparation and isolation of extracts

The essential oils from 18 plants *(Zingiber cassumunar* Roxb. (A) ,*Zingiber officinale* (B), *Curcuma aromatica* Salisb. (C), *Chromolaena odoratum* (L.) (D), *Amomum Biflorum* Jack (E), *Citrus maxima* (Burm.f).Merr. (F), *Syzygium aromaticum* (L.) Merr. & L.M. Perry. (G), *Citrus hystrix* DC. (H), *Kaempferia galanga* L. (I), *Alpinia galangal* (Linn.) Swartz (J), *Cocos nucifera L*. (K), *Curcuma xanthorrhiza* Roxb (L), *Mentha cordifolia* Opiz. (M), *Cinnamomum iners* Blume (N), *Eucalyptus globulus Labill.* (O),*Litsea cubeba* Pers.(P), *Curcuma longa* L. (Q)*, and Citrus reticulata* Blanco (R)), were hydrodistilled by Clevenger-type apparatus for 6 h. The chemical constitutents of essential oils were examined by gas chromatography-mass spectrometry.

2.2 Antibacterial method

The antibacterial activities of the 18 essential oils against 5 strains of SM relevant to ocular infection , (1) SM DMST 4228, (2) SM DMST 4424, (3) SM DMST 5100, (4) SM DMST 8845 and (5) SM DMST 8846, obtaining from Department of Medical Sciences Thailand Culture Collection (DMST) were performed by agar diffusion and agar dilution method. The oil which possessed the strongest antibacterial effect would be selected to compare its potency with 5 antibiotics (GEN, Cam, ERY, Amp, and Tet) against the 5 strains of SM by agar diffusion method. The minimum inhibitory concentrations (MICs) of the selected oil from plant material were determined by agar dilution method.

2.2.1 Agar diffusion testing

According to the Clinical and Labo ratory Standards Institute (CLSI), M2-A9 (CLSI, 2006), the diameters of inhibition zones (mm.) of the 5 strains of SM were evaluated by agar diffusion method (20 mg/disc) using Mueller Hinton Agar. The plant material which possessed the strongest antibacterial effect would be selected to compare its potency with 5 antibiotics (GEN (10 µg/disc), Cam (30 µg/disc), ERY(15 µg/disc), Amp (10 µg /disc), and Tet (30 µg/disc)) against the 5 strains of SM by the same method. The antibacterial activity results were evaluated by measuring diameter of the inhibition zones of bacterial growth. All experiments were carried out in triplicate. One way's ANOVA was used for comparison among groups. tatistical significance was defined as $p < 0.05$.

2.2.2 Agar dilution testing

According to modified CLSI standards 2006 guidelines, M7-A7 (CLSI, 2006), the minimum inhibitory concentrations (MICs) of the selected plant material were determined by agar dilution method using Mueller Hinton Agar.

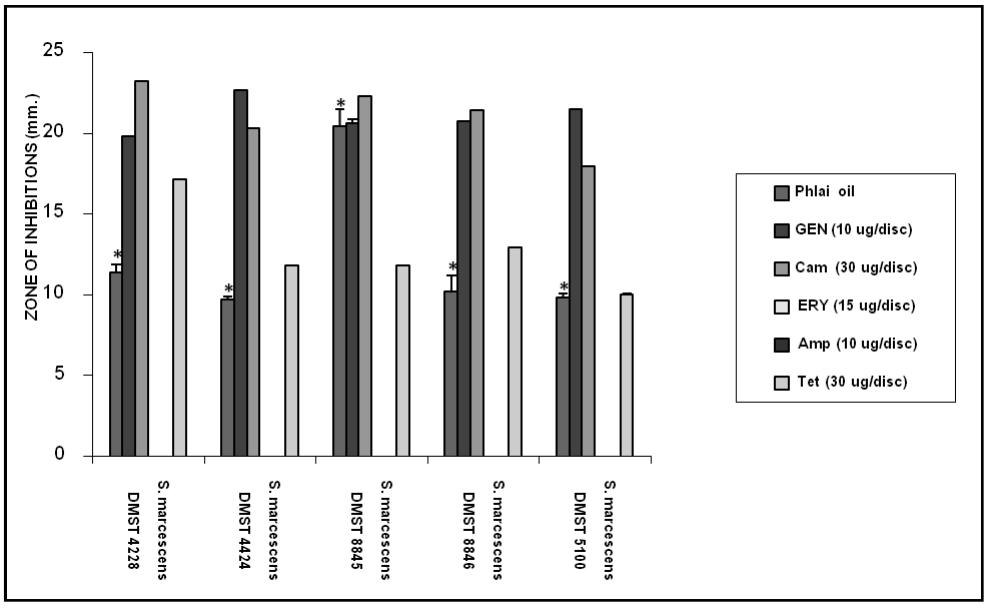
Results

Among the oils from 18 plants, the antimicrobial effects against all tested SM strains revealed that A (phlai oil) possessed the significantly strongest antibacterial effect with the range of its inhibition zones was 9.70-20.42 mm.($p< 0.05$). The antibacterial efficacy of essential oils of 10 plants (L, O, G, J, H, P, E, R, Q and B) demonstrated moderate activities with their inhibition zones ranging from 8.37-14.80, 8.27-14.44, 9.35-12.73, 8.76-12.50, 9.45-12.07,8.29-11.05, 8.07-10.46, 7.45-10.26, 7.77-9.94 and 7.67-9.75 mm.,respectively. The oil from 6 plants (F, M, K, C, I and D) showed weak antibacterial actions against the 5 strains of SM. They inhibited some of the tested strains. The ranges of their inhibition zone of F, M, K, C, I and D were 0.00-9.75, 0.00-8.64, 0.00-8.96, 0.00-8.22, 0.00-8.29, and 0.00-8.11 mm., respectively. N showed no antibacterial activities against all the tested SM strains. Therefore phlai oil, which contained mainly: sabinene (43 %), terpinene-4-ol (20 %), r-terpinene (8.5 %), α-terpinene (5.5 %) and α-pinene (2 %), was selected for the further study (Table 1). The comparison of antibacterial efficacy with 5 antibiotics (GEN, Cam, ERY, Amp and Tet) revealed that the oil exhibited higher anti- SM activities against the 5 strains than AMP and ERY did ($p < 0.05$). GEN and Cam showed higher antibacterial effect against the 5 stains of SM than the phlai oil did. The range of inhibition zones of GEN and Cam were 19.81-22.64 and 17.94-23.22 mm., respectively ($p < 0.05$). Phlai oil and Tet showed moderate antibacterial potencies against all tested strains with range of its inhibition zone were 9.82-20.42, 11.79-17.12 mm., respectively. Amp and ERY exhibited no antibacterial activities against all 5 strains of SM (Fig 1). There was no significant difference of anti-SM effect between phlai oil and 5 antibiotics to all 5 tested strains ($p < 0.05$). The MIC value of the oil against the 5 strains of SM using agar dilution method was 5 mg/mL.

Table 1 The comparison of inhibition zones of the oils from 18 medicinal plants (A to R) against the 5 strains of SM using agar diffusion method (20 mg/disc).

Oil Of Medicinal Plants	SM DMST 4228	SM DMST 4424	SM DMST 8845	SM DMST 8846	SM DMST 5100
A	11.39 ± 0.48	9.70 ± 0.19	20.42 ± 1.05	10.21 ± 1.00	9.82 ± 0.29
B	9.67	7.74 ± 0.01	9.75 ± 0.71	8.24 ± 0.27	7.67 ± 1.17
C	0	0	0	8.64	8.22
D	0	0	0	7.52 ± 0.05	8.11
E	10.46	8.17 ± 0.13	8.69	8.07	8.07
F	9.75	0	8.41 ± 0.32	7.93 ± 0.02	7.35 ± 0.30
G	12.73 ± 0.01	9.35	11.08	10.44 ± 0.01	10.08 ± 0.62
H	12.07 ± 0.01	9.83	11.15 ± 0.10	9.45	10.19 ± 0.25
I	0	0	0	8.29 ± 0.24	7.76
J	12.50 ± 0.46	9.04 ± 0.01	9.92	8.82 ± 0.08	8.76 ± 0.66
K	8.96 ± 0.28	7.73 ± 0.11	0	0	0.00
L	14.80 ± 2.08	10.07 ± 0.88	8.37	9.29 ± 0.39	9.02 ± 1.27
M	0	0	8.15 ± 0.69	8.64 ± 0.01	8.16
N	0	0	0	0	0
O	14.44	13.21 ± 0.49	13.79	8.65	8.27 ± 0.12
P	11.05 ± 0.42	8.29	9.32 ± 0.73	10.17 ± 0.50	10.80 ± 0.35
Q	9.94	7.87 ± 0.37	8.58 ± 0.16	8.06 ± 0.11	7.77 ± 0.61
R	10.26 ± 0.62	7.45 ± 0.01	7.94 ± 0.28	8.20 ± 0.37	8.25 ± 0.01

The result are presented as mean ± SD, n = 3, $p < 0.05$; analyzed by one way's ANOVA



The result are presented as mean ± SD, n = 3, * $p < 0.05$; analyzed by one way's ANOVA

Figure 1 The comparison of inhibition zones of the phlai oil (20 mg/disc) with 5 antibiotics against the 5 strains of SM using agar diffusion method.

Discussion

Among the tested extracts, phlai oil possessed the significantly strongest antibacterial effect against all the 5 strains of SM, associated with bacterial keratitis. *Z. cassumunar* Roxb. composed of abundant bioactive constituents such as monoterpenes hydrocarbons, sesquiterpene compound, sesquiterpene hydrocarbons, and sesquiterpene alcohols. Some previous studies revealed that sabinene and terpinene-4-ol in phlai oil had antibacterial activities (Wasuwat *et.al*., 1989; Giwanon *et al*.,2000.). The comparison of antibacterial effect of the oil with 5 antibiotics against the 5 strains of SM displayed that the oil was able to express anti- SM activities higher than 2 antibiotics, which had broad spectrum activity against gram-positve and gram-negative bacteria (Amp and ERY) did. Some previous studies reported that some gram-positive like *Staphy lococcus aureus* and gram-negative bacteria like *Pseudomonas aeruginosa* and SM were found in corneal infiltrates from contact lenses of patients (Zhou *et.al*., 2012). The result suggested that the essential oil from *Z. cassumunar* Roxb. may potentially to be an alternative anti-SM agent, and to be broad-spectrum antibacterial agent against gram-positve and gram-negative. Besides ocular infection, bacterial keratitis caused inflammation of the ocular surface or contact lens wear. Some previous studies reported the oil also had potent anti-inflammatory activity (Ozaki *et.al*., 1991; Jeenapongs *et.al*., 2003). Thus, the further stu

dies anti- inflammatory activity, antibacterial activities against other bacteria involved in microbial keratitis such as *P. aeruginosa* and *S. aureus* and toxicological effect of the oil should be conducted.

Conclusions

The oil from *Z. cassumunar* Roxb. provided antibacterial action against SM caused bacterial keratitis and also exhibited higher inhibitory effect against all 5 tests strains of SM than the 17 medicinal extracts and 2 antibiotics did. These findings indicate the possibility to develop the phlai oil as anti- SM agent from natural products.

Acknowledgements

We are grateful to Thailand Institute of Scientific and Technological Research (TISTR) for financial supporting.

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