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R. Giwanon.

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R. Giwanon[1], S. Rungsri[1], P. Limsiriwong[1], S. Nakakaew[1], and T. Srisom[1]

[1]*Pharmaceutical and Natural Product department (PNPD),Thailand Institute of Scientific and Technological Research(TISTR).35 Moo3Technopolis,PathumThani,12120.Thailand.*
E-mail address: rattanasiri@tistr.or.th

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INTRODUCTION

Rich sources of bioactive constituents (flavonoids, sesquiterpene lactones, essential oils, triterpene, diols, and triols) in Chrysanthemum family show pharmacological activities such as antimicrobial, anti-inflammatory, antioxidant, antimutagenic activities. Many previous studies reported the potential antimicrobial activity of *Chrysanthemum morifolium* Ramat and *C. indicum*. The increase in bacterial oral infections and the continuous epidemic of MRSA (Methicillin Resistant *Staphylococcus aureus*) relevant to nosocomial infection has brought about the necessity for developing new antibacterial agents. The finding out antibacterial efficacy against oral pathogens and MRSA from plants to use as an alternative medicine has been accomplished. Therefore, the purpose of this study was to investigate antibacterial potency from *C. morifolium* Ramat and from *C.indicum* against bacterial associated with oral infection .

MATERIALS AND METHODS

Preparation of extracts

2 parts (leaves and flowers) of *C.morifolium* Ramat and *C.indicum* collected from Chiang Mai province,Thailand were hydrodistilled and extracted to obtain oils and extracts. The essential oils from fresh flowers (A), fresh leaves (B) of *C.morifolium* Ramat. and from dried flowers of *C.indicum* (C) were hydrodistilled by clevanger-type apparatus for 6 h. The dried flowers (D), dried leaves (E) of *C.morifolium* Ramat., and dried flowers (F) of *C. indicum* were extracted with 95 % ethanol (v/v) 3 times for 48 hr. and were then evaporated. The yields of the 6 extracts (A, B, C, D , E and F) were 0.0038 % (v/v), 0.0204 %(v/v), 0.1853 %(v/v), 22.76 %(w/w), 15.42 %(w/w), and 20.38 % (w/w), respectively.

Antibacterial method

The antibacterial activities of 6 extracts against 5 bacterial strains relevent to oral infection (*S. aureus* subsp.aureus ATCC 43300 (MRSA), *S. aureus* subsp.aureus ATCC 29213 (Methicillin Sensitive *Staphylococcus aureus*, MSSA), *Streptococcus sanguinis* ATCC 10556, *Streptococcus pyogenes* ATCC 21059, and *Staphylococcus epidermidis* ATCC 12228) obtaining from ATCC Culture Collection, DMST Culture Collection (National Institute of Health; NIH) and TISTR culture collection were performed. Agar diffusion method was used in this study. All experiments were carried out in triplication. One way's ANOVA was used for comparison among groups. Statistical significance was defined as $p < 0.05$.

The Agar diffusion testing

The diameters of inhibition zones (mm) of the 5 oral pathogens were evaluated by agar diffusion method (16 mg/disc) following the Clinical and Laboratory Standards Institute (CLSI),2006. (previously National Committee for Clinical Laboratory Standards (NCCLs). 10 µg/disc of ampicillin (Amp) and 1µg/disc of oxacillin (OXA) were used as standard drugs. (CLSI Document M2-A8,2006).

RESULTS

Among tested extracts, the essential oils from both *C.morifolium* Ramat and *C.indicum* exhibited the higher antibacterial potency than the extract did. The range of inhibition zones of the oils against the 5 oral pathogens were 8.23-14.5, 9.32-14.07, and 0-12.85 mm., respectively, whereas the range of inhibition zones of the extracts (D and F) were 0-7.2 and 0-8.05 mm., respectively. E has no antibacterial effect against the tested strains (Fig 1). Among the oils from both selected plants, the antibacterial effect against all tested bacterial strains revealed that A possessed significantly the strongest antibacterial effect with the range of its inhibition zones was 8.23-14.5 mm.($p < 0.05$). The antibacterial efficacy of B demonstrated moderate antibacterial activity with its inhibition zones ranging from 9.32-14.07 mm. C inhibited the 4 oral pathogens (MRSA ATCC 4330, MSSA ATCC 29213, *S. sanguinis*, and

S. epidermidis) and the range of its inhibition zone was 8.47-12.85 mm. C had no antibacterial effect against *S.pyogenes* ATCC 21059. The oils from leaves and flowers part (A and B) of *C.morifolium* Ramat showed their antibacterial effect as well as 2 antibacterial drugs,Amp and OXA, against the 5 bacterial strains using the same method. The oil from flowers part of *C.indicum* exhibited only the 4 oral pathogens except *S.pyogenes* ATCC 21059 (Fig 2). Weak antibacterial action were found in the 2 ethanolic extracts (D and F) of flower part from the selected plants. D inhibited only MRSA ATCC 43330 and MSSA ATCC 29213 with range of its inhibition zone was 7-7.2 mm. and F had antibacterial activity against MSSA ATCC 29213 with its inhibition zones was 8.05 mm. E had no antibacterial action against the 5 pathogens. The extract from flowers part (D) of *C.morifolium* Ramat showed its antibacterial effect as well as 2 antibacterial drugs, Amp and OXA, against MRSA ATCC 43330 and MSSA ATCC 29213. The extract from the same part of *C.indicum* exhibited antibacterial effect only MSSA (Fig 3).

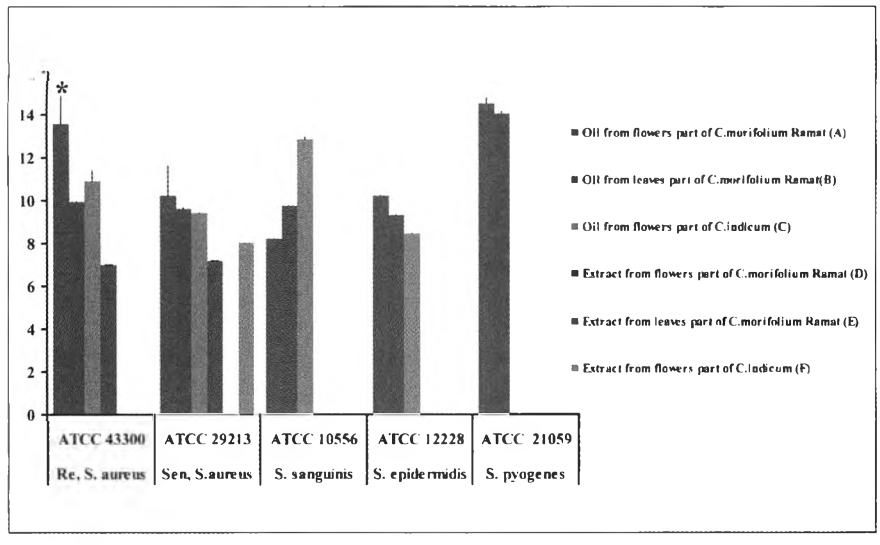

Fig 1 The comparison of inhibition zones of the oils and the extracts from *C.molifolium* Ramat and *C.indicum* against the 5 oral pathogens using agar diffusion method (16 mg/disc). * $p < 0.05$ level

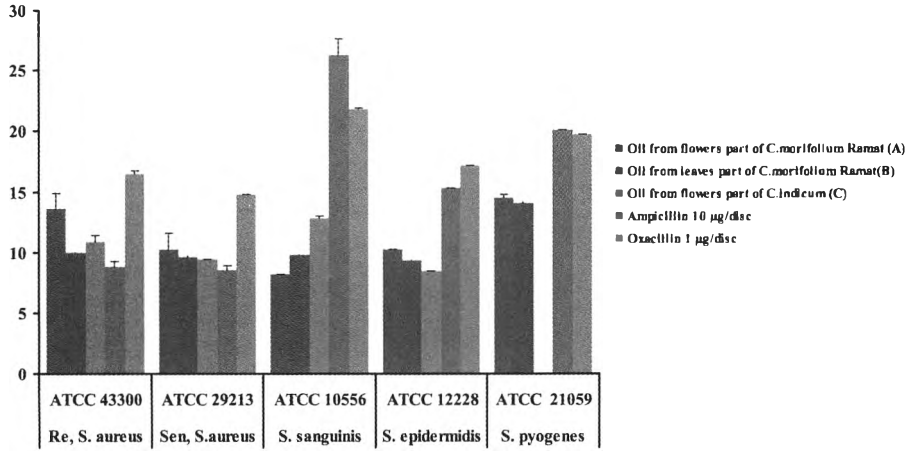

Fig 2 The inhibition zones of the oils from *C. molifolium* ramat and from *C. indicum* and 2 antibiotic drugs against the 5 oral pathogens using agar diffusion method (16 mg/disc)

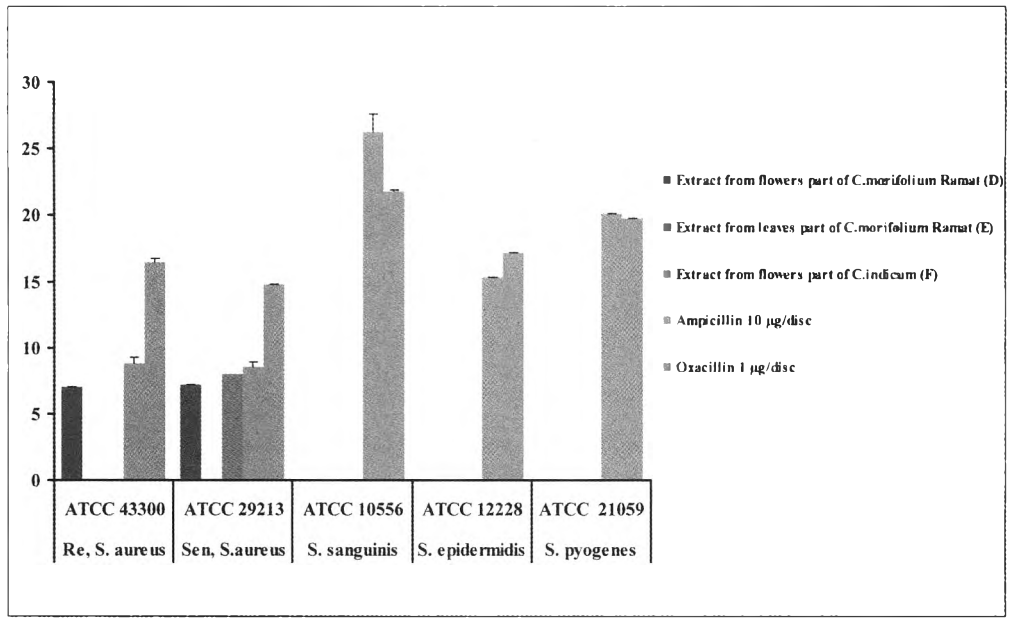

Fig 3 The inhibition zones of the extracts from *C. molifolium* Ramat, *C. indicum*, and 2 antibiotic drugs against 5 oral pathogens using agar diffusion method (16 mg/disc)

DISCUSSION

Among tested extracts, A possessed significantly strongest antibacterial effect against all the 5 bacteria associated with oral infection. C. *morifolium* Ramat composed of abundant bioactive constituents such as flavonoids, sesquiterpene lactones, essential oils, triterpene diols and triols. Some previous studies revealed antibacterial activities of flower extracts from C. *morifolium* Ramat against *S. aureus* and *S.epidermidis*. The comparison of antibacterial effect between the oil and antibiotics (Amp and OXA), against the 5 bacterial strains displayed that the oil was able to express antibacterial activity against oral pathogens as well as Amp and OXA did. The result suggested that the essential oil from flowers part of C. *morifolium* Ramat may potentially to be an alternative medicine for oral infection treatment. Thus, the further studies of the oil composition, the minimal inhibition concentrations (MIC), and the minimal bactericidal concentrations (MBC) should be conducted.

CONCLUSIONS

The oil from flowers part of *C.morifolium* Ramat provided antibacterial action against oral pathogens. The oil also exhibited an inhibitory effect against bacterial strains associated with oral infection as well as the 2 antibiotics did (ampicillin and oxacillin). These findings indicate the possibility to use the *C.morifolium* ramat oil for treating oral infection disease.

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