Screening of antibacterial and antifungal herbs used for treatment in traditional medicine

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ABSTRACT: Traditional medicine in Thailand usually uses many herbs to cure diseases. For a long time, many traditional medicinal doctors have used grinded herbs as drugs for the skin disease treatment. However, few herbs have been known so far. Therefore, it would be a great interest to widely screen more antibacterial and antifungal medicinal plants as a library to be used by traditional medicinal doctors. A total of 100 plants including known medicinal plants and interested plants were selected. The selected herbs were freshly grinded without addition of any solvents before subjecting to antibacterial and antifungal tests. The selected plants were screened for antibacterial and antifungal activities against pathogens causing dermatitis including *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. The result indicated that 5 selected medicinal plants, *Allium sativum* L., *Allium cepa* Linn, *Allium ascalonicum*, *Allium tuberosum* and *Capparis* sp. showed both antibacterial and antifungal activities. This finding is a pioneer demonstrating the use of grinded fresh fruits of *Capparis* sp. to inhibit some bacteria and fungi causing dermatitis. An addition of any solvents during leaf, bulb or fruit grinding was not required.

Keywords: antibacterial; antifungal; herps

Introduction

Medicinal plants are considered as a natural medicine for healing and preventing disease. Many parts of plant are used for medicinal purposes such as bulb, gel, leaves, roots, barks, fruit and peels. However, it lacks of proper scientific test. Plants that used to treat disease in traditional doctor should be confirmed by clinical demonstration (Cravotto et al., 2010). The use of plants to heal disease is found throughout human culture (Anne-Catherine, 2007).

Many Thai medicinal plants were used as medicine; for example, *Allium sativum* (garlic) was used to inhibit the infection of pathogens causing dermatitis such as *Candida albicans* (Low et al., 2008). Ethyl acetate fraction of *Allium cepa* showed inhibitory activities against *Bacillus subtilis*, *Bacillus cereus*, *Candida albicans*, *Escherichia coli, Kleibsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and *Erwinia carotovora* (Bakht et al., 2014). Chansukh et al. (2014) extracted nine traditional herb plants bearing quinonoids using petroleum ether and ethanol. The results indicated that antimicrobial potentials among selected Thai medicinal plants bearing quinonoid compounds can be used as crude

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drugs in traditional Thai medicine (Chansukh et al., 2014). From the previous study, the antimicrobial activity was done by extracting with various solvents before tested (Anywar et al., 2014; Bakht et al., 2013; Subramaniam et al., 2012). A few work supported the using fresh medicinal plants without any extraction was found. The aim of this study was to screen the antibacterial and antifungal herbs by determining their antimicrobial activity against pathogens causing dermatitis without any extraction to promote the using of fresh medicinal plant in Thailand. The pathogens used in this work were *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*.

Material and methods

Medicinal plants materials

Medicinal plants were divided into 2 groups, known medicinal plants and interested plants. Known medicinal plants were used follow by survey traditional doctor located in Roi Et and Buriram, Thailand. Interested plants were investigated and collected from Roi Et Rajabhat University forest. The leaves and fruit of each medicinal plant will be collected before used and were washed 3 times with sterilized distilled water.

Pathogens causing dermatitis

Three pathogens were used for antimicrobial activity included *Pseudomonas aeruginosa* TISTR 781, *Staphylococcus aureus* TISTR 1466 and *Candida albicans* TISTR 5779. All test pathogens used in this study were obtained from TISTR Culture Collection, Thailand Institute of Scientific and Technological Research. Each pathogen was maintained by subculturing and streaking onto appropriate medium periodically and kept at 4 °C before used.

Screening for antimicrobial activity

Single colony of each pathogen was transferred into suitable sterilized culture broth medium (NB for *P. aeruginosa* TISTR 781, *S. aureus* TISTR 1466 and YPD broth for *C. albicans* TISTR 5779) and incubated at 37 °C for 24 hrs. Each pathogen was adjusted to the cell density of a McFarland 0.5 using sterilized normal saline. One hundred microliters of each pathogen broth was spreaded onto agar medium. Antimicrobial activity was determined using modified agar disc diffusion method Drew et al. (1972). The cleaned freshly leaves or fruit of selected medicinal plants including known and interested plant were grinded using sterilized mortar. The grinded plants were transferred using aseptic technique with equal amount onto agar medium containing pathogens. Each plant was put 5 spots per plate. The antimicrobial activity plates were incubated at 37 °C for 24 hrs. The diameter of the inhibition zones around each of the grinded plant was taken as measure of the antibacterial activity.

Results and Discussion

Screening for antimicrobial activity

The present study investigated the antimicrobial (antibacterial and antifungal) activities of fresh form of known medicinal and interested plants located from Roi Et Rajabhat University forest. One hundred plants were used as substance to determine the antibacterial and antifungal activity against pathogens causing

dermatitis (Table 1). The result indicated that three of known medicinal plants, *Allium sativum* L., *Allium cepa* Linn, *Allium ascalonicum* and one of interested plant, *Capparis* sp. showed both antibacterial and antifungal activities. Bulb of known medicinal plants was used as antimicrobial substances. Fruit of *Capparis* sp. was used to determine the antimicrobial activity (Fig. 1.). This work focused on screening and using fresh plants to heal skin disease, so MIC MFC and MBC were not attended.

Table 1 One hundred plant samples which used as the sources of antibacterial and antifungal substances.

Plant species	Inhibition zone diameter (cm)*		
	C. albicans	P. aeruginosa	S. aureus
1. Bulb of *Allium sativum* L.	5.98	6.20	6.00
2. Bulb of *Alium cepa* Linn.	2.64	1.60	1.12
3. Bulb of *Allium ascalonicum*	1.41	1.10	1.00
4. Bulb of *Boesenbergia rotunda* (L.) Mansf	0	1.24	0
5. Leaf of *Limnophila geoffrayi* Bonati.	0	0.76	0
6. Leaf of *Fagraea fragrans* Roxb.	0	0.74	0
7. Leaf of *Hymenocardia wallichii.* Tul.	0	0.88	0
8. Leaf of *Psidium guajava* L.	0	0	0.76
9. Fruit of *Tamarindus indica* Linn.	0	1.68	1.82
10. Leaf of *Tagetes erecta* L.	0	0	0.78
11. Flower of *Fagraea fragrans* Roxb.	0	0	0.72
12. Fruit of *Oxyceros horridus* Lour.	0	0	0
13. Leaf of *Momordica charantia* L.	0	0	0.74
14. Bulb of *Zingiber officinale* Roscoe.	0	0	0.84
15. Fruit of *Areca catechu* Linn.	0	0.78	1.88
16. Leaf of *Azadirachta indica* A. Juss. var. *siamensis* Valeton.	0	0	0.96
17. Leaf of *Aganonerion polymorphum* Pierre ex Spire.	0	1.68	1.57
18. Leaf of *Heliotropium indicum* (Linn.) R.Br.	0	0.92	0
19. Leaf of *Garcinia cowa* Roxb. ex DC.	0	3.11	1.22
20. Leaf of *Allium tuberosum* Rottl. ex Spreng.	1.78	1.46	1.78
21. Flower of *Alpinia galanga* (L.) Willd.	0	0	+
22. Leaf of *Moringa oleifera* Lam.	0	0	+
23. Leaf of *Sesbania grandiflora* Desv.	0	0	0
24. Leaf of *Phyllanthus amarus* Schum & Thonn.	0	0	0
25. Flower of *Clitoria ternatea* L.	0	0	0
26. Leaf of *Clitoria ternatea* L.	0	0	0
27. Leaf of *Citrus hystrix* DC.	0	0	0
28. Leaf of *Cosmos sulphureus* Cav.	0	0	0
29. Flower of *Cosmos sulphureus* Cav.	0	0	0

30. Leaf of *Basella alba* L.	0	0	0
31. *Cuscuta chinensis* Lann.	0	0	0
32. Leaf of *Phyllanthus acidus* (L.) Skeels.	0	0	0
33. Leaf of *Cratoxylum formosum* (Jack) Dyer subsp.	0	0	0
34. Leaf of *Eurycoma longifolia* Jack.	0	0	0
35. Fruit of *Morinda citrifolia* L.	0	0	0
36. Leaf of *Morinda citrifolia* L.	0	0	0
37. Leaf of *Senna siamea* (Lam.)	0	0	0
38. Leaf of *Duranta drdcta* L.	0	0	0
39. Leaf of *Pandanus amaryllifolius* Roxb.	0	0	0
40. Leaf of *Piper betle* Linn.	0	0	0
41. Leaf of *Vernonia cinerea* Less.	0	0	0
42. Leaf of *Coccinia grandis* (L.) Voigt.	0	0	0
43. Leaf of *Ricinus communis* L.	0	0	0
44. Leaf of *Helichrysum bracteatum*	0	0	0
45. Leaf of *Ocimum basilicum* L.	0	0	0
46. Leaf of *Piper sarmentosum* Roxb.	0	0	0
47. Leaf of *Ocimum basilicum* L.f. var. *citratum* Back.	0	0	0
48. Leaf of *Mental cordifolia* Opiz.	0	0	0
49. Seed of *Citrus reticulata* Blanco.	0	0	0
50. Leaf of *Streblus asper* Lour.	0	0	0
51. Bulb of *Alpinia galanga* (L.) Willd.	0	0	0
52. Bulb of *Cymbopogon citratus* Stapf.	0	0	0
53. Flower of *Catharanthus roseus* (L.) G.Don.	0	0	0
54. Leaf of *Cassia alata* (L.) Roxb.	0	0	0
55. Leaf of *Peltophorum dasyrachis* (Miq.) Kurz.	0	0	0
56. Leaf of *Coriandrum sativum* L.	0	0	0
57. Leaf of *Syzygium gratum* (Wight) S.N. Mitra var. *gratum*	0	0	0
58. Leaf of *Mimosa pudica* L.	0	0	0
59. Leaf of *Zizyiphus mauritiana* Lamk.	0	0	0
60. Leaf of *Lasianthus cyanocarpus* Jack.	0	0	0
61. Leaf of *Capsicum frutescens* L.	0	0	0
62. Leaf of *Amaranthus lividus* L.	0	0	0
63. Leaf of *Ruellia tuberosa*	0	0	0
64. Leaf of *Melastoma malabathricum* L. subsp. malabathricum	0	0	0
65. Leaf of *Persicaria odorata*	0	0	0
66. Leaf of *Cleome gynandra* L.	0	0	0
67. Leaf of *Eryngium foetidum* L.	0	0	0
68. Leaf of *Tamarindus indica* L.	0	0	0
69. Leaf of *Averrhoa carambola* L.	0	0	0

70. Leaf of *Momordica cochinchinensis* (Lour.) Spreng.	0	0	0
71. Leaf of *Jatropha curcas* L.	0	0	0
72. Leaf of *Tradescantia spathacea* Stearn.	0	0	0
73. Leaf of *Morus alba* L.	0	0	0
74. Leaf of *Cyperus rotundus* Linn.	0	0	0
75. Flower of *Sesbania grandiflora* (L.) Desv.	0	0	0
76. Leaf of *Apium grsveolens* Linn.	0	0	0
77. Leaf of *Muehlenbeckia platyclada* (F.v.Muell.) Meissn	0	0	0
78. Bulb of *Stephania venosa* (BP.) Spreng.	0	0	ND
79. Leaf of *Annona Squamosa* Linn.	0	0	ND
80. Bulb of *Cymbopogon citrates* (DC. Ex Nees) Stapf.	0	0	ND
81. Blub of *Andrographis paniculata* (Burm.f.)	0	0	ND
82. Leaf of *Aloe vera* (Linn.) Burm. f.	0	0	ND
83. Bulb of *Curcuma longa* L.	0.86	0.89	ND
84. Leaf of *Tradescantia pallida* (Rose) D. Hunt.	0	0	ND
85. Leaf of *Gynura pseudochina* (L.) DC.	0	0.84	ND
86. Leaf of *Croton stellatopilosus* Ohba.	1.12	0	0
87. Leaf of *Alocasia cucullata* (Lour.)	0	0	0
88. Leaf of *Wrightia religiosa* Benth.	0	0	0
89. Leaf of *Euphorbia hirta*	0	0	0
90. Leaf of *Desmodium triflorum* (L.) DC.	0	0	0
91. Root of *Phyllanthus acidus* (L.) Skeels.	0.83	0	0
92. Root of *Azadirachta indica* A. Juss. var. *siamensis* Valeton	0	0	0
93. Leaf of *Achyranthes aspera* L.	0	0	0
94. Leaf of *Salacia chinensis* L.	0	0	0
95. Leaf of *Hoya* spp.	0	0	0
96. Fruit of *Capparis* spp.	2.40	2.62	2.51
97. Fruit of *Lagenaria siceraia* (Molina) Standley..	0	0	0
98. Leaf of *Ixora lobbii* Loud.	0	0	0
99. Leaf of *Kaempferia rotunda* L.	0	0	0
100. Leaf of *Ocha kirkii* Oliv.	0	0	0

*Average of inhibition zone diameter from 5 spots

ND; Not determine

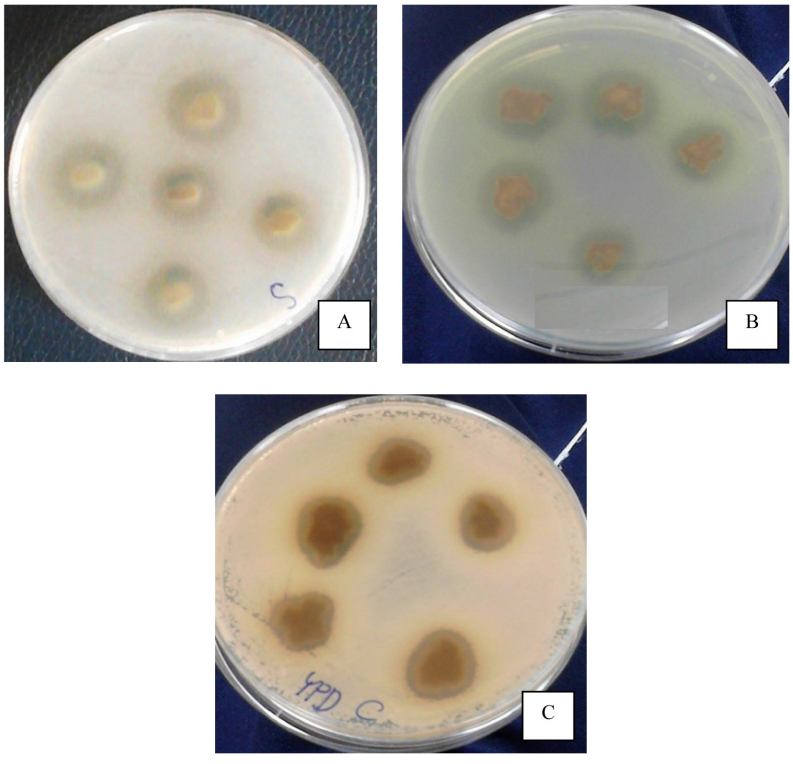

Figure 1 Antimicrobial activity of *Capparis* sp. fruit against *Staphylococcus aureus* TISTR 1466, *Pseudomonas aeruginosa* TISTR 781, and *Candida albicans* TISTR 5779.
(A) Inhibition zone of *Capparis* sp. fruit against *Staphylococcus aureus* TISTR 1466; (B) Inhibition zone of *Capparis* sp. fruit against *Pseudomonas aeruginosa* TISTR 781; (C) Inhibition zone of *Capparis* sp. fruit against *Candida albicans* TISTR 5779.

Many researches often use garlic as antimicrobial substance because garlic contains many compounds such as sulfur compounds like allicin, ajoene, allylmethyltrisulfide, diallyltrisulfide, diallyldisulphide andothers which present various biological properties like antimicrobial, anticancer, antioxidant, immunomodulatory, antiinflammatory, hypoglycemic, and cardiovascular effects (Viswanathan et al., 2014). *Allium cepa* and *Allium ascalonicum* also found in many studies that have antimicrobial activity (Hannan et al., 2010; Ramos et al., 2006; Dankert et al., 1979; Amin and Kapadnis, 2005). The ether extractof *Capparis erythrocarpos* showed antimicrobial activity against *C. albicans* and *S. aureus* with MIC value of 330 µg/ml and 400 µg/ml, respectively (Anywar et al., 2014). Root of *Capparis spinosa* was extracted with chloroform and showed bacteriostatic activity on the growth of *Deinococcus radiophirus* (Boga et al., 2011). The ethanol and dichloro-methane extract of aerial part of *Capparis deciduas* also presented antibacterial and antifungal against many pathogens (Keymanesh et al., 2009). The finding of this research is to promote the using fresh medicinal plant in traditional medicine to heal the skin disease in Thailand. Surprisingly, this study is an innovator demonstrating the use of grinded

fresh fruits of *Capparis* sp. to inhibit *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* causing dermatitis. An addition of any solvents during a grinding step was not required.

Conclusion

In this study, total of 100 plants including known medicinal plants and interested plants were selected. The selected plants were washed and grinded without addition of any solvents before subjecting to antibacterial and antifungal screening. The grinded plants were transferred using aseptic technique with equal amount onto agar medium containing pathogens causing dermatitis, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. The result indicated that 5 medicinal plants, *Allium sativum* L., *Allium cepa* Linn, *Allium ascalonicum*, *Allium tuberosum* and *Capparis* sp. showed both antibacterial and antifungal activities. This finding is a pioneer demonstrating the use of grinded fresh fruits of *Capparis* sp. to inhibit some bacteria and fungi causing dermatitis. An addition of any solvents during leaf, bulb or fruit grinding was not required.

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