Songklanakarin J. Sci. Technol.
30 (4), 463-466, Jul. - Aug. 2008

Songklanakarin Journal of Science and Technology
http://www.sjst.psu.ac.th

Original Article

Antimicrobial activity of the ethanol extract and compounds from the rhizomes of *Kaempferia parviflora*

Sopa Kummee*, Supinya Tewtrakul and Sanan Subhadhirasakul

Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla, 90112 Thailand.

Received 28 March 2008; Accepted 11 July 2008

Abstract

The antimicrobial activity of the ethanol extract of *Kaempferia parviflora* was tested against human pathogens, including bacteria, yeast and dermatophyte fungi, using the agar disc diffusion. The ethanol extract exhibited strong anti-fungal activity against dermatophytes with clear zone values from 10.7-19.8 mm at concentration of 2 mg/disc. However, ethanol extract showed no activities against all bacteria and yeast tested. The ethanol extract and seven compounds of *K. parviflora* were further studied using agar dilution method against dermatophytes. It was found that the ethanol extract of *K. parviflora* exhibited strong anti-fungal activity against *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Microsporum gypseum* with MIC values of 62.5, 125 and 250 µg/ml, respectively. Only compound **6** (3, 5, 7-trimethoxyflavone) present in this extract showed appreciable anti-fungal activity with MIC values of 250 µg/ml against these three dermatophytes.

According to the obtained results, compound **6** could be responsible only in part of the antifungal effect of the EtOH extract. The antifungal activity of the EtOH extract of *K. parviflora* supports its traditional use for the treatment of dermatophyte infections

Keywords: antimicrobial activity, antibacterial activity, antifungal activity, *Kaempferia parviflora*

1. Introduction

Kaempferia parviflora belongs to the Zingiberaceae family and is locally known in Thai as Kra-chai-dam. The rhizome of this plant has been used for the treatment of allergy and gastrointestinal disorders as well as an aphrodisiac and for fungal infections (Pengcharoen, 2002). This plant has been denoted as Thai ginseng. The wine preparation of this plant is increasingly used in Thailand as a tonic and as an aphrodisiac. In Thai traditional medicine, the decoction of *K. parviflora* powder with ethanol has been reported to cure fungal infection, impotence, allergy, asthma, gout, diarrhea, dysentery, peptic ulcer and diabetes. The rhizome extract of this plant contains flavonoids such as 3,5,7,4´-tetramethoxyflavone, 5,7,4´-trimethoxyflavone and 5,7,3´,4´-tetramethoxyflavone (Yenjai *et al.*, 2004). The essential oil components from the rhizomes of *K. parviflora* are borneol (46.41%) and sylvestrene (25.30%) (Tanasiriwattana *et al.*, 1997)

K. parviflora has recently been reported to possess antimycobacterial, antiplasmodial, antifungal (Yenjai *et al.*, 2004), antibacterial (Tanasiriwattana *et al.*, 1997), antiviral (Phurimsak and Leardkamolkarn, 2005), antipeptic ulcer (Rujjanawate *et al.*, 2005) and antiallergic activities (Tewtrakul *et al.*, 2008). However, few studies have been conducted on the antimicrobial activity of *K. parviflora*.

The present study aimed to investigate the ethanol extract and compounds of *K. parviflora* for *in vitro* antimicrobial activity.

2. Materials and Methods

2.1 Plant materials

K. parviflora rhizomes were bought from a Thai

*Corresponding author.
Email address: sopa.k@psu.ac.th

traditional drug store in Songkhla province, Thailand. The voucher specimen is SKP 2061116. The plant material was identified by Assoc. Prof. Dr. Sanan Subhadhirasakul and a voucher specimen is kept at the herbarium of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla, Thailand.

2.2 Preparation of the plant extract and isolation

Two kilograms dried weight of *K. parviflora* were ground and macerated with ethanol at room temperature, 4 times (6 L x 4). The ethanol (EtOH) extract (267 g) was then concentrated and partitioned between water and hexane, and then the water layer was successively partitioned with chloroform. After that, the water layer was partitioned with ethyl acetate (EtOAc). Each fraction was evaporated to dryness in vacuo to give the following yields: hexane (14.1 g), chloroform (215.0 g), EtOAc (4.8 g) and water fractions (27.0 g), respectively. The hexane fraction (5.0 g) was chromatographed on silica gel column using hexane and EtOAc (95:5 to EtOAc 100%, 8,000 ml) to afford compound **1** (5-hydroxy, 3, 7-dimethoxyflavone, 370 mg), **2** (5-hydroxy, 7-methoxyflavone, 230 mg), **3** (5-hydroxy, 3, 7, 4'-trimethoxyflavone, 280 mg), **4** (5-hydroxy, 7, 4-dimethoxyflavone, 125 mg), **5** (5-hydroxy, 3, 7, 3', 4'-tetramethoxyflavone, 54 mg), **6** (3, 5, 7-trimethoxyflavone, 50 mg) and **7** (3, 5, 7, 4'-tetramethoxyflavone, 70 mg), respectively. The structures of **1**-**7** were elucidated by comparing the ^{1}H and ^{13}C-NMR spectral data with those reported in the literature (Agrawal, 1989; Harborne *et al.*, 1988; Jaipetch *et al.*, 1983).

2.3 Antimicrobial activity assay

1) Microorganisms

The tested organisms used in the study included three Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* TISTR 518, *Enterococcus faecalis* TISTR 459 and one Gram-negative bacterium: *Escherichia coli* ATCC 25922 which were kindly provided by the Department of Pathology, the Faculty of Medicine, Prince of Songkla University, Thailand, and the Thailand Institute of Scientific and Technological Research. Fungi used were one yeast, i.e. *Candida albicans* TISTR 5779, and three dermatophytes, i.e. *Trichophyton rubrum* SH-MU-2, *T. mentagrophytes* SH-MU-3 and *Microsporum gypseum* SH-MU-4, which were provided by the Thailand Institute of Scientific and Technological Research and Department of Microbiology, the Faculty of Sciences, Prince of Songkla University, Thailand.

2) Media

The medium used in the assay for the antibacterial test was Mueller Hinton agar (MHA, Merck, Germany) and that for the antifungal test was Sabouraud Dextrose agar (SDA, Merck, Germany).

3) Reference antibiotics

Tetracycline (30 µg/disc) (Oxoid, Oxoid Limited, England), norfloxacin (10 µg/disc, Oxoid, Oxoid Limited, England), tetracycline (Fluka, Fluka Chemie GmbH, Switzerland), amphotericin B (Sigma, Sigma chemical company, USA) and ketoconazole (Sigma, Sigma-Aldrich Chemie GmbH, Germany) were used as reference antibiotics.

4) Preliminary susceptibility test

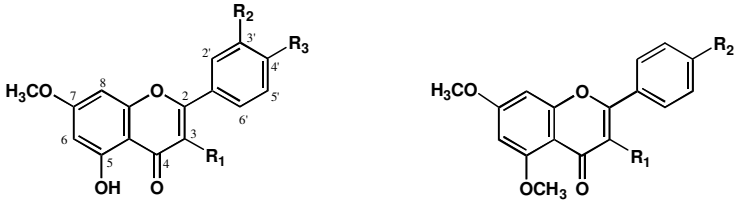
The disc diffusion method (Acar and Goldstein, 1996) was used to screen the antimicrobial activity. Sterile disks (6 mm) were impregnated with 10 µl of crude extract at concentration of 200 µg/ml. For bacteria, the microbes were placed on the surface of MHA, whereas SDA was used for fungi. The extract was tested in triplicate; control discs contained 10 µl of dimethyl sulfoxide (DMSO). The standard antibiotics consisted of tetracycline (30 µg/disc) for the Gram-positive bacteria, norfloxacin (10 µg/disc) for the Gram-negative bacteria, amphotericin B (10 µg/disc) for the yeast and ketoconazole (25 µg/disc) for the dermatophytes. The plates were incubated at 35°C for 18 h and 48 h for the bacteria and yeast, respectively. Plates with dermatophytes were incubated at 30°C for 4 days and the inhibition zone diameters were then measured.

5) Determination of minimum inhibitory concentration (MIC)

The MIC values were performed by the agar microdilution method according to reported procedures (Fenner *et al.*, 2005 and Navarro Garcia *et al.*, 2003) against dermatophytes. The dermatophytes were grown on SDA for 10 days. The inocula containing 10^3-10^4 cfu of each microorganism were spotted on agar supplemented with an extract or antibiotic at concentrations ranging from 15.62-2000 µg/ml for crude extracts, 1.9-250 µg/ml for pure compounds and 0.03-16 µg/ml for antibiotics. A number of wells were reserved in each plate for sterility control (no inoculum added), inoculum viability (no extract added) and the dimethyl sulfoxide control. Agar plates containing dermatophytes were incubated at 30°C for 7 days. The MIC was defined as the lowest concentration at which no visible growth was observed.

3. Results and Discussion

The hexane fraction was isolated to obtain seven methoxyflavone derivatives, whose structures are shown in Figure 1. *K. parviflora* ethanol extract was tested for antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria and fungi. The results revealed that the extract was active against three dermatophytes, i.e. *T.*



1 $R_1 = OCH_3, R_2, R_3 = H$
2 $R_1, R_2, R_3 = H$
3 $R_1 = OCH_3, R_2 = H, R_3 = OCH_3$
4 $R_1, R_2 = H, R_3 = OCH_3$
5 $R_1, R_2, R_3 = OCH_3$

6 $R_1 = OCH_3, R_2 = H$
7 $R_1, R_2 = OCH_3$

Figure 1. Structures of isolated compounds 1-7 from *Kaempferia parviflora* rhizomes

Table 1. Antimicrobial activity of *Kaempferia parviflora* ethanolic extract by the disc diffusion method

Microbes	Inhibition zone (mm)		
	EtOH extract[a]	Antibiotics	
Staphylococcus aureus ATCC 25923	-[b]	Tetracycline	26.5
Staphylococcus epidermidis TISTR 518	-	Tetracycline	34.6
Enterococcus faecalis TISTR 459	-	Tetracycline	29.4
Escherichia coli ATCC 25922	-	Norfloxacin	38.0
Candida albicans TISTR 5779	-	Amphotericin B	17.9
Trichophyton rubrum SH-MU-2	19.8	Ketoconazole	34.7
Trichophyton mentagrophytes SH-MU-3	13.7	Ketoconazole	33.8
Microsporum gypseum SH-MU-4	10.7	Ketoconazole	22.8

[a] Concentration of ethanol extracts (2 mg/disc)
[b] (-), no inhibition zone

Table 2. MIC values of *Kaempferia parviflora* ethanol extract and compounds 1-7 against dermatophytes by the agar dilution method

Compounds	MIC (μg/ml)		
	T. rubrum	*T. mentagrophytes*	*M. gypseum*
EtOH extract	62.5	125	250
1	> 250	> 250	> 250
2	> 250	> 250	> 250
3	> 250	> 250	> 250
4	> 250	> 250	> 250
5	> 250	> 250	> 250
6	250	250	250
7	> 250	> 250	> 250
Ketoconazole	0.5	8	16

rubrum, *T. mentagrophytes* and *M. gypseum* with MIC values of 62.5, 125 and 250 μg/ml, respectively, whereas it was inactive against *S. aureus*, *S. epidermidis*, *E. faecalis*, *E. coli* and *C. albicans* (Tables 1 and 2). 3,5,7,4′-Tetramethoxyflavone and 5,7,4′-trimethoxyflavone were reported to have mild antimycobacterial activity with the MIC values of 200 and 50 μg/ml, respectively (Yenjai *et al.*, 2004). The volatile oil from *K. parviflora* has been reported to show antibacterial activity against *S. aureus* and *Pseudomonas aeruginosa* (Tanasiriwattana *et al.*, 1997).

Among the isolated compounds, compound **6** (3, 5, 7-trimethoxyflavone) showed appreciable anti-fungal activity with MIC values of 250 μg/ml against the three dermatophytes mentioned above, whereas other compounds had no activity at the highest concentration tested (MIC > 250 μg/ml) (Table 2). According to the obtained results, compound **6** could be responsible only in part of the anti-fungal activity. The results indicate that the crude extract of *K. parviflora* showed higher antidermatophytic activity than the pure compounds. This behavior should be associated with a possible synergistic action of the compounds isolated from this plant. These findings support the traditional use of *K. parviflora* rhizomes for the treatment of dermatophyte infections.

Acknowledgements

The authors are grateful to the Thailand Research Fund (TRF) and the Commission on Higher Education (COE)

for financial support. We also thank the Faculty of Pharmaceutical Sciences for providing laboratory facilities.

References

Acar, J.F. and Goldstein, F.W. 1996. Disk susceptibility test. **In** Antibiotics in Laboratory Medicines, 4 th ed. pp. 1-51. Williams and Wilkins, Baltimore.

Agrawal, P.K. 1989. Carbon-13 NMR of flavonoids. Elsevier: Amsterdam.

Harborne, J.B., Agrawal, P.K. and Bansal, M.C. 1988. The Flavonoids. Chapman and Hall: London.

Fenner, R., Sortino, M., Kuze Rates, S.M., Dall´Agnol, R., Ferraz, A., Bernardi, A.P., Albring, A., Nör, C., von Poser, G., Schapoval, E. and Zacchino, S. 2005. Antifungal activity of some Brazilian *Hypericum* species. Phytomedicine., 12 : 236-240.

Jaipetch, T., Reutrakul, V., Tuntiwachwuttikul, P. and Santisuk, T. 1983. Flavonoids in the black rhizomes of *Boesenbergia pandurata*. Phytochemistry., 22 : 625-626.

Navarro Garcia, V.M., Gonzalez, A., Fuentes, M., Aviles, M., Rios, M.Y., Zepeda, G. and Rojas, M.G. 2003. Antifungal activities of nine traditional Mexican medicinal plants. Journal of Ethnopharmacology., 87 : 85-88.

Pengcharoen, O. 2002. Technology Chao Barn. Matichon Press, Bangkok, pp. 42-43.

Phurimsak, C. and Leardkamolkarn, V. 2005. Screening for antiviral effect of Thai herbs; *Kaempferia parviflora*, *Ellipeiopsis cherrevensis* and *Stemona tuberosa* against Dengue virus type-2. 31st Congress on Science and Technology of Thailand at Suranaree University of Technology, 18-20 October 2005.

Rujjanawate, C., Kanjanapothi, D., Amornlerdpison, D., Pojanagaroon, S. 2005. Anti-gastric ulcer effect of *Kaempferia parviflora*. Journal of Ethnopharmacology., 102 : 120-122.

Tanasiriwattana, N., Natakuatung, S. and Tanajaro, T. 1997. Chemical composition and antimicrobial activity of essential oil from *Kaempferia galanga*, *K. parviflora* and *K. angustifolia*. Senior proj. Chulalongkorn University.

Tewtrakul, S., Subhadhirasakul, S. and Kummee, S. 2008. Anti-allergic activity of compounds from *Kaempferia parviflora*. Journal of Ethnopharmacology., 116 : 191-193.

Yenjai, C., Prasanphen, K., Daodee, S., Wongpanich, V. and Kittakoop, P. 2004. Bioactive flavonoids from *Kaempferia parviflora*. Fitoterapia., 75 : 89-92.