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

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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
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 *H and 13C*-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, 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 micro-dilution 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 10^3-10^5 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.*
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.

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References


