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Growth Inhibitory Activities of Kalopanaxsaponins A and I against Human Pathogenic Fungi

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Antifungal activities of the compounds isolated from *Kalopanax pictus* against representative fungi of dermatomycosis were investigated using paper disc diffusion method. It was found that kalopanaxsaponins A and I were effective in inhibiting the growth of *Candida albicans* KCTC 1940 and *Cryptococcus neoformans* KCTC 7224 with minimum inhibitory concentration (MIC) of 25 μg/ml. It showed that antifungal activity of both compounds have strong selectivity against the fungi of dermatomycosis.

Key words : Kalopanaxsaponin, Antifungal activities, Minimum inhibitory concentration (MIC), Dermatomycosis

INTRODUCTION

The stem bark of *Kalopanax pictus* (Araliaceae) has been used as traditional crude drugs for the treatment of the feeble, neurotic pain, lumbago, various inflammation and especially for diabetesmellitus. A number of natural components have been isolated from this plant (Shao *et al.*, 1989a, b; Sano *et al.*, 1991) and further the effective constituents on liver damage have been reported (Lee *et al.*, 1995). Recently, we have reported the isolation of the active principle reducing hyperglycemia, hypercholesterolemia and hyperlipidemia in the streptozotocin-pretreated rat (Park *et al.*, 1998).

In the continuous biological studies on this plant materials, we investigated the antifungal effects of the compounds isolated from *K. pictus*. Because it is hard to develope antifungal drug compared with that of antibacterials, the developmental tempo on the former is slow. The reason is that many antifungals are toxic to human cell in addition to fungus cell which posed both eucaryotic cell structures. Practically, most synthetic antifungal drugs are showing the side effects such as the toxicities due to long-term administration against kidney and liver, headaches, skin hypersensitivity and endocrinal disorder (Yamaguchi, 1990; Yoo, 1997).

From these point of view, the studies on antifungal substances with little toxicities derived from natural products are progressive by many workers. Most fungal strains used in the reports for natural antifungal substances have been included plant pathogenic fungi, animal pathogenic fungi and rarely human skin fungi as the object of fungal strains. However, we examined the antifungal spectrum mainly aganist human pathogenic fungi such as three kinds of *dermatophytes*, the representative fungi strains of superficial dermatomycosis, which is classified by Ormby and Montgomery (1954) and clinically important, and two kinds of fungal strains exerting deep dermatomycosis, the representative fungal strains of candidiasis and one fungal strain belonging to *Penicillum* species.

The present paper describes the antifungal activity of the compounds isolated from *Kalopanax pictus* against human pathogenic fungi of dermatomycosis.

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MATERIALS AND METHODS

Materials

The experimental materials including liriodendrin, syringin, kalopanaxsaonin A, B, H and I, were isolated from *Kalopanax pictus* in our previous work (Park *et al.*, 1998), and were used. Structures of *Kalopanaxsaponin* A, B, H and I are shown in Fig. 1. As other materials, hederagenin was obtained from the isolation of total acid hydrolysate of the stem bark of *K. pictus* and ursolic acid (Δ^{12}-ursene-3β-ol-28-oic acid) is an authentic specimen. The fungal strains used in this experiment are as followings: Superficial dermatomycosis, *Tricophyton*

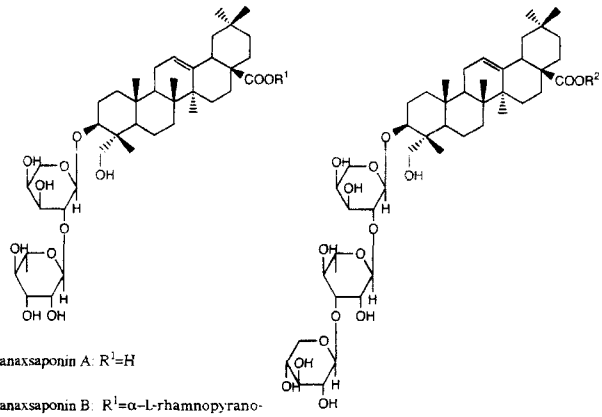

Fig. 1. Chemical structures of kalopanaxsaponins.

mentagrophytes KCTC 6077, *Microsporium gypseum* KCTC 1252 (from dermatophytosis of food), *Epidermophyton floccosum* KCTC 1246 (from dermatophytosis of hand); deep dermatomycosis, *Cryptococcus neoformans* KCTC 7224 (from clinical specimen, Minnesota), *Aspergillus niger* KCTC 1700; Candidiasis, *Candida albicans* KCTC 1940 (from man with bronchomycosis); *Penicillum avellaneum* 1253.

Antifungal activity test

Fungal strains used for the antifungal activity test are *C. albicans* as the representative fungi of candidiasis and *A. niger* as that of deep dermatomycosis. Test compounds were dissolved in dimethylsulfoxide (DMSO: 0.2%) and diluted with Saboraud dextrose broth to be prepared to 50, 100, 200, 400, 800 μg/ml solution in addition to 0.2% DMSO solution for blank test. Paper disc (Toyo Roshi Co. Ltd. Japan. 8 mm in diameter. 1.5 mm in thickness) was put on fungus-inoculated plate and 100 μl of each diluted test sample was loaded. After incubating each plate for 24~48 hrs at 28°C, the diameter of growth inhibitory zone was measured for antifungal activity.

Determination of minimum inhibitory concentration (MIC) of kalopanaxsaponins A and I

For determining MIC values, the spores of *C. albicans* and *C. neoformans* were collected by scratching or shaking the corresponding colony with loop in the medium added with Saboraud dextrose broth (pH 5.4). The microorganism solution was prepared for the concentration of the spore suspension to be T(%)=95 at 540 nm in UV spectrophotometer (Moore *et al.*, 1979). Other fungi solutions were prepared by the same way as that of *C. albicans* after they were incubated at 28°C for 7 days, and further spore suspension concentrations maintained to be T(%)=90 at 540 nm in UV spectrophotometer. The determination of MICs was conducted according to serial dilution method. 1 ml of Saboraud dextrose broth was disposed at thirteen test tube and followed by sterilized. 1 ml of test solution (400 μg/ml) was added in the first tube and serially diluted. And then 0.05 ml (spore number=10^5~10^6) of fungal solution was inoculated and incubated at 28°C for 7 days. MIC values were determined by judging visually the fungal growth in the series of test tubes.

RESULTS AND DISCUSSION

The research on natural antifungal substances has been continuously conducted for the search of new natural antifungal agents with little toxicities compared with commercial drugs. The naturally occurring antifungal substance effective to various fungal strains include terpenoids (iridoid, sesquiterpenoids, saponins and etc.), aliphatics (long chain alkanes and fatty acids), and aromatics (phenolics, flavonoids, stilbenes, bibenzyls, xanthones, benzoquinones and etc.) (Grayer *et al.*, 1994).

As the antifungal effects were shown in Table I, kalopanaxsaponin A and I were shown to be superior

Table I. Antifungal activities of the compounds isolated from *Kalopanax pictus*

Compounds	Diameter of inhibitory zone (mm)									
	C. albicans KCTC 1940					*A. niger* KCTC 1700				
	50	100	200	400	800 μg	50	100	200	400	800 μg
Liriodendrin	0	0	0	0	0	0	0	0	0	0
Syringin	0	0	0	0	0	0	0	0	0	0
Ursolic acid	0	0	0	0	0	0	0	0	0	0
Hederagenin	0	0	0	0	0	0	0	0	0	0
Kalopanaxaponin A	9.0	10.0	14.5	18.0	19.8	0	0	8.5	11.0	13.5
Kalopanaxaponin B	0	0	0	0	0	0	0	0	0	0
Kalopanaxaponin I	8.3	10.5	17.8	19.0	21.3	0	0	9.0	12.0	14.0
Kalopanaxaponin H	0	0	0	0	0	0	0	0	0	0

by the inhibitory zone from the 50 μg/ml to 200 μg/ml conc. against *A. niger*.

The Antifungal activity of Kalopanaxsaponin A and I against *C. albicans* were dose-dependent manner (Fig. 2). However, liriodendrin, syringin, ursolic acid and hederagenin were inactive. The growth of *C. albicans* and *A. niger* were not inhibited by any concentrations of these compounds.

Among seven compounds used in this test, only kalopanaxsaponin A and I have significant antifungal activities. Triterpenoid saponins, rather than those genins, have been reported to show often antifungal activities. Camellidin I and II which have been isolated from *Camellina japonicus* were reported to have antifungal effects against *Psetalotia longiseta* of plant pathogenic fungi (Ishidate *et al.*, 1953; Nishino *et al.*, 1986). It has been also demonstrated that 3-*O*-glucosides of hederagenin, bayogenin and medicagenic acid of the root of *Dolichos killimanscharicus* (Marston *et al.*, 1988) and sakurasosaponin of the leaves of *Rapanea melanophloes* (Kazuhiro *et al.*, 1993) exhibited antifungal effects. However, these effects are biological results against only plant pathogenic fungi. In addition, avenacins (Maizel *et al.*, 1964; Crombie *et al.*, 1984a, b; Grayer *et al.*, 1994) have been reported that the MICs are over the range of 25~50 μg/ml (Maizel *et al.*, 1964) against six kinds of plant pathogenic fungi and animal pathogenic fungi (25 μg/ml against *Tricophyton interdigitale*; 25 μg/ml against *Saccharomyces pastorianus*; 50 μg/ml against *Candida albicans, Collectotrichum pisi, Endocanidiophora fagacearum* and *Pythium irregulare*). As examined in the literatures, the antifungal report on naturally occurring compounds against human pathogenic fungi was hardly found. Moreover, antifungal principles of *Kalopanax pictus* have never been elucidated.

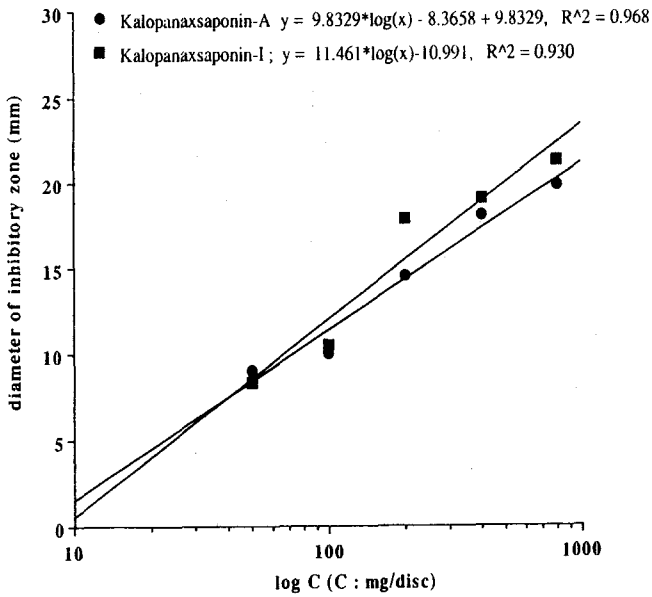

Fig. 2. Inhibitory zones vs. added amounts of kalopanaxsaponins A and I against *C. albicans* KCTC 1940.

The antifungal effects of kalopanaxsaponin A and I against the fungal strain occurring human dermatomycosis were illustrated in Table II. The MICs of kalopanaxsaponin A and I were 25 μg/ml to *C. albicans* KCTC 1940 and *C. neoformans* KCTC 7224. This value was estimated to be markedly low when compared with those of other natural antifungal agents, while it is remarkably high compared with those of clotrimazole (positive control) of synthetic antifungal agents. The MIC on *A. niger* occurring human deep dermatomycosis was observed at 200 μg/ml conc.. Antifungal effects on other strains were not observed (>200 μg/ml). This finding suggests that these two saponin compounds were selective to very limited fungal strains.

Although kalopanaxsaponin B and H are also hederagenin glycosides, they showed no activity against the fungi used in this experiment. This finding can be speculated that 28-*O*-glycoside instead of 17-free carboxyl removes the activity, maybe rather than the polarity due to increment of sugar moiety. It can be reminded that very little difference between the effects of kalopanaxsaponin A and I was found though numbers of sugar moiety were different each other. Marston *et al.* (1988) have reported the antifungal activity of hederagenin 3-*O*-glucoside against *Cladosporum cucumerium* of plant pathogenic fungi, while hederagenin and ursolic acid tested exhibited no activity. As examined in the present test results and literature data, it was

Table II. Minimum inhibitory concentration (MIC) of kalopanaxaponins A, B, H and I against various fungi (μg/ml)

Antifungal agents	KCTC[a] 6077	KCTC[b] 1252	KCTC[c] 1246	KCTC[d] 7224	KCTC[e] 1700	KCTC[f] 1940	KCTC[g] 1253
Kalopanaxaponin A	>200	>200	>200	25.0	200	25.0	>200
Kalopanaxaponin B	>200	>200	>200	>200	>200	>200	>200
Kalopanaxaponin I	>200	>200	>200	25.0	>200	25.0	>200
Kalopanaxaponin H	>200	>200	>200	>200	>200	>200	>200
Clotrimazole*	1.22	2.44	0.61	0.61	1.22	4.88	0.61

*Positive control

[a]*T. mentagrophytes* KCTC 6077, [b]*M. gypseum* KCTC 1252, [c]*E. flocossum* KCTC 1246, [d]*C. neoformans* KCTC 7224, [e]*A. niger* KCTC 1700, [f]*C. albicans* KCTC 1940, [g]*P. avellaneum* KCTC 1253

suggested that glycoside linkage to 3-OH of hederagenin played an important role in the exhibition of the antifungal activity.

It is our interests that kalopanaxsaponin A and I exhibited strong and specific antifungal activities to *C. albicans* and *C. neoformans*. On consideration of relatively low toxicity of natural compounds compared to synthetic compounds, these two saponins can be suggested as model compounds effective to candidiasis and the infection of *C. neoformans* of deep dermatomycosis.

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