Antifungal Activity against Food-Borne Fungi of *Aspidistra elatior* Blume

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An antifungal compound was isolated from *Aspidistra elatior* Blume. The methanol extract showed antifungal activity against *Saccharomyces cerevisiae, Hansenula anomala, Mucor mucedo,* and *Candida albicans.* The powdered methanol extract was dissolved in water and partitioned with hexane, ethyl acetate, *n*-butanol, and then water. Only the *n*-butanol fraction exhibited antifungal activity. The antifungal compound in the *n*-butanol fraction was finally isolated by silica gel chromatography and HPLC. Analysis by fast-atom bombardment mass spectroscopy and Fourier-transform nuclear magnetic resonance spectroscopy revealed that the structure of the antifungal compound is aspidistrin (diosgenin 3-*O*-β-lycotetraoside). The minimum inhibitory concentration of aspidistrin was 2.5 μg/mL against *S. cerevisiae*, 10 μg/mL against *H. anomala*, 10 μg/mL against *M. mucedo*, and 50 μg/mL against *C. albicans*. This is the first report of antifungal activity against food-borne fungi of aspidistrin.

Keywords: *Aspidistra elatior Blume; aspidistrin; antifungal activity*

INTRODUCTION

Aspidistra elatior Blume (Japanese name, Haran), an evergreen plant, has been used in folk medicine as a diuretic, an expectorant, a tonic, and so on. Pharmaceutical interest in *A. elatior* Blume promoted research on its pharmaceutically functional compounds, and several kinds of compounds have been isolated. For example, several steroid compounds from *A. elatior* Blume have been reported (Mori and Kawasaki, 1973; Hirai *et al.*, 1982; Konishi *et al.*, 1984). It is well-known that Haran has been used as a serving material instead of a dish and also for decoration of Japanese food like Sushi (sliced raw fish with rice) and Sashimi (sliced raw fish) in Japan. We postulated that there is some relationship between the traditional usage of Haran and the preservative effects of its constituents for food. This idea attracted us to verify the active constituents of Haran. Here, we describe the isolation, identification, and antifungal activity of an antifungal compound from *A. elatior* Blume.

MATERIALS AND METHODS

Materials. The air-dried *A. elatior* Blume was collected in Yokkaichi, Mie, Japan. Nutrient broth and malt extract medium were obtained from Nissui Pharmacy Ltd. (Japan).

Extraction and Isolation of Compound 1. The chipped, air-dried *A. elatior* Blume (562 g) samples were extracted with methanol (2 L, three times), and the methanol solution was evaporated under reduced pressure at 45 °C. The antimicrobial activity of the methanol extract was tested against several microorganisms by the paper disk method (Table 1). The methanol extract (80.9 g) was dissolved in 1 L of water and then partitioned in turn with 1 L of hexane, ethylacetate, *n*-butanol, and water. The fractions were screened for their antifungal activities, with *Saccharomyces cerevisiae* IFO 0203 as the bioindicator [paper disk (PD) method, 10 mg/disk]. The *n*-butanol fraction (13.7 g) showed antifungal activity. A 6.0-g portion of the *n*-butanol fraction was chromatographed on an SiO_2 column (50 × 600 mm, BW-820MH, Fuji Silysia Chemical Ltd., Japan), with CH_3Cl:MeOH:H_2O (30:10:1, v/v) as the solvent. The eluates were divided into five fractions: A (130 mg), B (1460 mg), C (410 mg), D (940 mg), and E (1350 mg). Fraction D showed antifungal activity against *S. cerevisiae*. The active compound (**1**) was finally purified from fraction D by HPLC on an SiO_2 column (8.0 × 250 mm; Develosil 60-5; CH_3Cl:MeOH, 5:1, v/v; Nomura Chemical Company, Ltd., Japan).

Table 1. Antimicrobial Activity of *A. elatior* Blume

microorganism	GIZ (mm)[a] (methanol extract)	MIC (μg/mL)[b] (aspidistrin)
S. cerevisiae IFO 0203	16	2.5
H. anomala IFO 0136	15	10
C. albicans IFO 1061	9	50
Rhodotorula rubra IFO 0001	—	>100
M. mucedo IFO 7684	11	10
Aspergillus niger ATCC 3275	—	100
Rhizopus chinencis IFO 4745	—	>100
Penicillium chrysogenum IFO 4626	—	>100
Staphylococcus aureus IFO 12732	—	>100
Bacillus subtillis IFO 3007	—	>100
Escherichia coli IFO 3547	—	>100
Pseudomonas aeruginosa IFO 3080	—	>100

[a] GIZ, growth inhibition zone obtained by the PD method (disk diameter = 8 mm). [b] MIC, minimum inhibitory concentration.

Structural Properties of 1. The melting point was determined on a Yanagimoto micro melting point apparatus (hot-stage type, MS-S3). The optical rotation was measured with a digital polarimeter (model DIP-370; cell length, 1 cm; JASCO Company, Ltd., Tokyo). The carbon-13 and distortionless enhancement by polarization transfer (DEPT)-mode NMR spectra were measured with a JEOL GSX-400 spectrometer at 100.6 MHz, and chemical shifts are reported on a δ (ppm) scale, with tetramethylsilane (TMS) as the internal standard. The high-resolution, fast-atom bombardment mass spectroscopy (HR-FAB-MS) spectrum was measured with a JEOL JMS-DX 303.

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Table 2. ^{13}C NMR Chemical Shifts of 1

	carbon no.	compound **1** (ppm)	reported value[a] (ppm)
aglycon	1	37.5 (t)	37.6
	2	30.1 (t)	30.2
	3	78.2 (d)	78.5
	4	39.2 (t)	39.4
	5	141.0 (s)	141.2
	6	121.6 (d)	121.6
	7	32.3 (t)	32.4
	8	31.8 (d)	32.0
	9	50.3 (d)	50.5
	10	37.0 (s)	37.2
	11	21.1 (t)	21.2
	12	39.9 (t)	40.0
	13	40.4 (s)	40.6
	14	56.6 (d)	56.8
	15	32.2 (t)	32.3
	16	81.4 (d)	81.2
	17	63.0 (d)	63.1
	18	16.4 (q)	16.4
	19	19.4 (q)	19.5
	20	42.0 (d)	42.1
	21	15.0 (q)	15.0
	22	109.3 (s)	109.3
	23	31.6 (t)	31.8
	24	29.3 (t)	29.4
	25	30.6 (d)	30.7
	26	66.9 (t)	67.0
	27	17.3 (q)	17.3
galactose	1	102.7 (d)	102.8
	2	73.2 (d)	73.1
	3	75.3 (d)	75.3
	4	79.9 (d)	79.7
	5	76.2 (d)	76.0
	6	60.5 (t)	60.7
glucose (inner)	1	105.0 (d)	104.9
	2	81.1 (d)	81.2
	3	86.8 (d)	87.2
	4	70.7 (d)	70.7
	5	77.7 (d)	77.9
	6	62.5 (t)	62.7
glucose	1	105.2 (d)	104.9
	2	75.1 (d)	75.1
	3	78.8 (d)	78.6
	4	70.5 (d)	70.5
	5	77.6 (d)	77.5
	6	62.9 (t)	63.1
xylose	1	104.9 (d)	104.7
	2	75.6 (d)	75.6
	3	78.7 (d)	78.6
	4	71.0 (d)	71.3
	5	67.4 (t)	67.2

[a] Hirai *et al.*, 1982.

Bioassays. Bacteria were precultured overnight in nutrient broth (Nissui Pharmacy Ltd., Japan) at 37 °C, and fungi were precultured in a malt extract medium (Nissui Pharmacy Ltd., Japan) at 25 °C. Two types of assays were employed; they are, the PD method and measurements of the minimum inhibitory concentration (MIC; Serit *et al.*, 1991). For the PD method, plates were prepared by mixing 0.5 mL of the preculture with 10 mL of the agar medium. The 100 μL containing 10 mg of methanol extract was impregnated into a PD (Toyo Roshi Company, Ltd., Japan) of 8 mm diameter and 1.5 mm thickness. After air-drying, the PD was placed on the agar plate and incubated for 3 days (for fungi) or 1 day (for bacteria) before measuring the diameter of the growth inhibition zone. For the MIC measurements, plates were prepared by mixing the agar medium with a sample solution of each concentration. A loophole of the preculture was smeared on the agar plate and, after incubating, the microbial growth was visually observed.

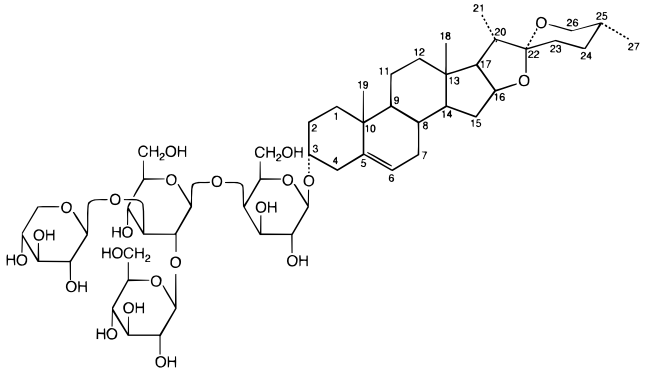

Figure 1. Structure of the antifungal compound aspidistrin.

RESULTS AND DISCUSSION

Purification of 1. The methanol extract of *A. elatior* Blume was assayed against several microorganism species by the PD method. A growth inhibition zone around the PD of the extract against four species of fungi was observed. The antifungal activity was in the following order: *S. cerevisiae*, *Hansenula anomala*, *Mucor mucedo*, and *Candida albicans*. In contrast, antibacterial activity was not observed at a similar dose (Table 1). The methanol extract was subsequently partitioned with solvents. The active compound (**1**) was purified by HPLC on an SiO_2 column from the *n*-butanol fraction (see Materials and Methods). We obtained 416.6 mg of the active compound (**1**) from the methanol extract of *A. elatior* Blume.

Structure of 1. The physical properties of **1** are as follows: a white powder, mp 265−267 °C (dec) and $[\alpha]_D$ −68° (*c* 1.08, pyridine). A molecular formula of $C_{50}H_{80}O_{22}$ (calcd as 1032.8954) was indicated by HR-FAB-MS. The FAB-MS spectrum showed a molecular peak at *m*/*z* (%) 1033 ($M + H^+$, 7%) and fragment ion peaks at *m*/*z* (%) 901 (2), 871 (2), 739 (3), 577 (26), and 397 (100). These peaks indicated the presence of one pentose and three hexoses in **1**. The chemical shifts of ^{13}C NMR and DEPT-mode NMR spectra in pyridine-d_5 are shown in Table 2. The NMR spectra showed the presence of 50 carbon signals, four anomeric carbon signals (δ 102.7, 104.9, 105.0, and 105.2), one set of double-bond carbon signals (δ 121.6 and 141.0), and four methyl carbon signals (δ 15.0, 16.4, 17.3, and 19.4; Table 2). The physical properties, the ion peaks of FAB-MS, and the chemical shifts of ^{13}C NMR (Hirai *et al.*, 1982) are consistent with those of aspidistrin. Therefore, **1** was proposed as aspidistrin (diosgenin 3-*O*-β-lycotetraoside; Figure 1).

Minimum Inhibitory Concentration of Isolated Aspidistrin. The MICs of aspidistrin against *S. cerevisiae* IFO 0203, *H. anomala* IFO 0136, *M. mucedo* IFO 7684, and *C. albicans* IFO 1061 were 2.5, 10, 10, and 50 μg/mL of medium, respectively (Table 1). Aspidistrin showed strong activity against the fungi that was similar to that of the methanol extract of *A. elatior* Blume. Therefore, it could be generally concluded that the major antifungal activity of *A. elatior* Blume can be ascribed to aspidistrin, which account for 0.17% of the air-dried *A. elatior* Blume (by weight).

Conclusion. The results of this study clearly indicate that the active antifungal compound in *A. elatior* Blume is aspidistrin. Aspidistrin has antifungal activity against three yeasts and one mold, but does not exhibit antibacterial activity, as shown in Table 1. Other authors also reported antifungal activity of aspidistrin against *Piricularia oryzae* (Imai *et al.*, 1967) and four

species of plant pathogens (Takano and Hasegawa, 1990). These results indicate that aspidistrin might be specifically an antifungal rather than an antibacterial compound.

This is the first report of the antifungal activity against food-borne fungi of aspidistrin. The antifungal properties of aspidistrin might make this compound useful for the control of fermentation by yeasts or for the therapy of candidosis (Garciahermoso *et al.*, 1995; Johnson *et al.*, 1995; Vancutsem, 1995). Our results suggest that the use of Haran might not only be for decoration of food but also for its antifungal properties.

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