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Antifungal Activity of Phenanthrene Derivatives from Aerial Bulbils of *Dioscorea batatas* Decne

Eun-Joo Kum, Sang-Jo Park[1], Bong-Ho Lee[1], Jong-Sik Kim[2], Kun Ho Son and Ho-Yong Sohn*

Department of Food and Nutrition, Andong National University, Andong 760-749, Korea,
[1]Experiment Station of Bioresources, Gyeongbuk Agricultural Technology Administration Andong 760-749, Korea
[2]Department of Biological Science, Andong National University, Andong 760-749, Korea

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Plants of the genus *Dioscorea* have long been used as oriental folk medicine, and *Dioscorea batatas* Decne has been cultivated for healthy food in Korea. Although the bulbils were produced 2,000 ton annually, there are few reports for bioactive compounds in bulbils. In this study, three phenanthrenes and two phenanthraquinones were isolated from the aerial bulbils of *D. batatas* Decne, and their structures were elucidated. Among them, compound **2** (6-hydroxy-2,7-dimethoxy-1,4-phenanthraquinone) has not been reported previously. Evaluation of antimicrobial activities based on disk-diffusion assay, MIC and MFC showed the compound **12** (6,7-dihydroxy-2,4-dimethoxyphenanthrene) has strong antimicrobial activity with 25 μg/ml of MIC and MFC against *Candida albicans*. Our results suggested that compound **12** has a potent antifungal activity, and the antimicrobial activity and its spectrum are modulated by hydroxylation and methoxylation of phenanthrene ring moiety of the compound.

Key words – 6,7-Dihydroxy-2,4-dimethoxyphenanthrene, antifungal activity, *Dioscorea batatas* Decne, fungicidal activity

Plants of the genus *Dioscorea* have long been used as folk medicine to treat intestinal spasm, asthma, rheumatoid arthritis, bronchitis, poor appetite, frequent and uncontrollable urination and other diseases[3,10]. Recently, it was demonstrated that the leaves of *D. preussii* have a strong antileishmanial activity[11], and dioscin, prosapogenin A of dioscin and beta-sitosterol, from the *Dioscorea* have strong antifungal activity[1,12,15,16].

Among the *Dioscorea* genus which has over 600 species, *D. batatas* Decne has been cultivated in Korea, and recognized as a healthy food and digestive. In Korea, the annual production of yam exceeds 4,311 ton and the consumption has been increased during last decades[13]. Although the amount of bulbils of yam annually produced is estimated to 2,000 ton, only limited amount of bulbils is used as a seed for next year, and the majority of bulbils is discarded. In Andong area, a major producing district in Korea, the aerial bulbils of this edible yam have been used as seeds and digestive healthy food like a yam tubers. The taste of boiled bulbils is similar to that of boiled potato or sweet-potato.

*Corresponding author
Tel : +82-54-820-5491, Fax : +82-54-823-1625
E-mail : hysohn@andong.ac.kr

Recent studies revealed that the root and rhizome of *D. batatas* Decne contains various bioactive substances, such as steroidal saponin[2], beta-sitosterol, antifungal bibenzyls, antifungal phenanthrenes[4,19], and immune cell stimulating mucopolysaccharide[3]. However, the studies on the bioactive compounds from the aerial bulbils still remained rudimentary. Simply, *in vitro* micropropagation by direct induction and determination of diosgenin content in bulbils were reported[14,17].

The antimicrobial and spasmolytic activities were observed with different phenanthrene derivatives. For example, 3,7-dihydroxy-2,4-dimethoxyphenanthrene and 3,7-dihydroxy-2,4,8-trimethoxyphenanthrene from the orchid *Scaphyglottis livida* [5], 1,5,7-trimethoxy-9,10-dihydrophenanthrene-2,6-diol and 1,5,7-trimethoxyphenanthrene-2,6-diol from *Nidema boothii*[7], 2,5-dihydroxy-3,4-dimethoxyphenanthrene, and 9,10-dihydro-2,5-dihydroxy-3,4-dimethoxyphenanthrene from *Maxillaria densa*[6], and 2,5-dihydroxy-4,9,10-trimethoxyphenanthrene, 2,5-dihydroxy-4-methoxyphenanthrene and 2,5,9-trihydroxy-4-methoxy-9,10-dihydrophenanthrene from *Dendrobium plicatile*[8]. In addition, similar antifungal phenanthrenes were reported in yam tuber[4,19].

In this study, we isolated different phenanthrenes and phenanthraquinones from the aerial bulbils of *D. batatas*

Decne, and evaluated antimicrobial activities of isolated phenanthrene derivatives. Our results suggest that 6,7-dihydroxy-2,4-dimethoxyphenanthrene possess a potential as an antifungal agent, and the antimicrobial activity and its spectrum may be modulated by hydroxylation and methoxylation of phenanthrene ring moiety of the compound.

Materials and Methods

Plant material, extraction and isolation

The aerial bulbils of yam (*D. batatas* Decne) were harvested from farm of the Gyeongbuk Agricultural Technology Administration, Kyungpook, Korea in November, 2002. A voucher specimen (A-0360) was deposited at the Department of Food and Nutrition, Andong National University, Korea. The aerial bulbils of yam were chopped, dried at 60 °C, ground to powder, and stored at 4°C before use. The powder (65 kg) was extracted with refluxing methanol for 3 days. After evaporation of methanol in vacuo, the residue (1,030 g) was suspended in water and then extracted with CH_2Cl_2. The CH_2Cl_2-soluble fraction (198 g) was subjected to silica gel (7734, Merck Co., USA) column chromatography eluted with hexane with ethylacetate (gradient 0 to 70%) to give seven fractions designated as D1 to D7. The fraction D5 was chromatographed over silica gel with hexane:ethylacetate (98:2~9:1) to give ten subfractions. The seventh subfraction was chromatographed over silica gel with CH_2Cl_2 to give compound **1** and **10.**

The fraction D6 was subjected to silica gel column chromatography eluted with hexane:ethylacetate (98:2~6:4) to give twelve subfractions. The ninth subfraction was re-chromatographed over silica gel with CH_2Cl_2:methanol (1000:0~1000:8) to give compound **2** and **11.** The fraction D7 was subjected to silica gel column chromatography eluted with CH_2Cl_2:methanol (100:0~95:5) to give four subfractions. The first subfraction was chromatographed over silica gel with hexane:ethylacetate (8:2) to give compound **12.**

Identification of compound 1, 2, 10, 11 and 12.

Melting points (MP) were taken on a Fisher apparatus without correction. Optical rotations were measured on a Rudolph autopol III. Infra Red (IR) spectra were recorded in KBr on a Bruker FT-IR spectrophotometer, and UV spectra were recorded on Hitachi U-3010 spectrophotometer. Nuclear Magnetic Resonance (NMR) spectra were obtained on a Varian Unity INOVA500 (500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR) spectrometer using tetramethylsilane as an internal standard at room temperature. Electrical ionization mass spectra and fast-atom bombardment mass spectra were obtained on a JEOL JMS-700 MSTATION. Silica gel and TLC plate used were Kieselgel 60 (70-230 mesh, Merck) and Kieselgel 60 F_{254} (precoated silicagel, Merck), respectiviely.

Antibacterial activity assay

Bacterial strains used were *Proteus vulgaris* KCTC 2433, *Salmonella typhimurium* KCTC 1926, *Escherichia coli* O157 ATCC 43895, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus mutans* JC-2, and *Staphylococcus aureus* KCTC 1916. The antibacterial activity was evaluated by disk-diffusion assay as determined by growth inhibition zone [18,20]. The bacteria to be tested were grown in nutrient broth (Difco Co. USA) at 37°C for 24 h, and collected by centrifugation. After spreading of each microorganism ($2x10^6$ cells) on nutrient agar (Difco Co., USA), disk of Whatman No.2 filter paper (diameter of 6 mm) containing the isolated compound (70 μg) was applied, and the growth inhibition was measured after 24 hr. Compounds **1, 2, 10, 11,** and **12** were dissolved in dimethylsulfoxide, respectively. Dimethylsulfoxide (0.5%) was used as a solvent control, and ampicillin (1 μg/disk) was used as a positive control. All data were presented as the mean values of triplicates for each microorganism.

Antifungal activity assay

Fungal strains used were *Candida albicans* ATCC 10231 as a representative fungus of Candidiosis, *Botrytis cinerea* KACC 40574 as a plant pathogen and *Saccharomyces cerevisiae* IFO 0233 as a control strain. The antifungal activity was evaluated by disk-diffusion assay, and determination of minimum inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) using Sabouraud dextrose broth (Difco Co., USA), as previously reported[18,20]. For MIC determination, the fungi were grown in Sabouraud dextrose broth at 30°C for 24 h, and then 0.1 ml of cell culture was inoculated with 0.9 ml of fresh medium with different concentrations of phenanthrenes and phenanthraquinones (0, 1.5, 3.1, 6.2, 12.5, 25, 50, 70, and 100 μg/ml), which were dissolved in dimethylsulfoxide. The MIC was defined as the lowest concentration able to inhibit any visible microorganism growth, and was determined by measuring cell growth OD after 48 h. The

MFC was determined by culturing 0.1 ml of the vortexed broth from all tubes in the MIC assay on Sabouraud dextrose agar plates at 30°C for 48 h. The MFC was defined as the lowest concentration which growth of fungal colony was completely inhibited. Dimethylsulfoxide (0.5%) was used as a solvent control, and miconazole and amphotericin B (1.0, 1.5, and 2.0 μg/ml) were used as positive controls. All data were presented as the mean values of triplicates for each microorganism.

Results and Discussion

From the bulbils of *D. batatas* Decne, three phenanthrenes and two phenanthraquinones were identified by comparison of their physico-chemical properties and spectral data with those reported in the literature[4,9,19].

Compound **1** showed following characteristics and identified as a 6-hydroxy-2,4,6-trimethoxyphenanthrene (batatasin I) [4,19]. MP 142~143 ℃; UV λ_{max} (CH_3OH) nm : 220, (sh)237, (sh)250, 259, 282(sh), 293(sh), 306(sh), 328, 344, 360; FAB-MS *m/z* 284 $[M]^+$; IR (KBr) ν_{max} cm^{-1} : 3494 (O-H), 1624, 1483, 880, 852, 825, 816, 679, 619 (aromatic ring), 1611, 1578, 1527, 1502, 1462, 1437, 1394, 1367, 1336, 1302, 1285, 1267, 1207, 1193, 1163, 1156, 1140, 1088, 1061, 1033, 956, 940; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ : 3.89 (3H, *s*, 2-OCH_3), 3.91 (3H, *s*, 7-OCH_3), 4.05 (3H, *s*, 4-OCH_3), 6.78 (1H, *d*, *J*=2.5 Hz, H-3), 7.00 (1H, *d*, *J*=2.5 Hz, H-1), 7.36 (1H, *s*, H-8), 7.51 (1H, *d*, *J*=9.0 Hz, H-10), 7.66 (1H, *d*, *J*=9.0 Hz, H-9), 8.90 (1H, *s*, H-5), 9.33 (*s*, 6-OH); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ : 158.8 (C-4), 157.1 (C-2), 146.8 (C-7), 146.3 (C-6), 134.2 (C-10a), 127.5 (C-9), 125.9 (C-8a), 124.7 (C-4b), 123.8 (C-10), 114.4 (C-4a), 112.4 (C-5), 108.7 (C-8), 101.3 (C-1), 98.7 (C-3), 55.7 (4-O$\underline{C}H_3$), 55.3 (7-O$\underline{C}H_3$), 55.2 (2-O$\underline{C}H_3$).

Compound **2** showed following characteristics and identified as a 6-hydroxy-2,7-dimethoxy-1,4-phenanthraquinone, which has not been reported previously. MP 250~253 ℃; IR (KBr) ν_{max} cm^{-1} : 3440 (O-H), 1634, 1487 (1,4-pheananthrquinone C=O), 1612, 1583, 1568, 1535, 1436, 1398, 1366, 1270, 1236, 1165, 1132, 1072, 1024, 939, 886, 850, 798, 750; EI-MS *m/z* 284 $[M]^+$, 269, 255, 226, 213, 185, 170, 142; HREI-MS *m/z* 284.0688 (calculated for $C_{16}H_{15}O_5$, 284.0683); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ : 3.85 (3H, *s*, 2-OCH_3), 3.95 (3H, *s*, 7-OCH_3) 6.25 (1H, *s*, H-3), 7.43 (1H, *s*, H-8), 7.85 (1H, *d*, *J*=8.5 Hz, H-10), 8.09 (1H, *d*, *J*=8.5 Hz, H-9), 8.96 (1H, *s*, H-5), 10.29 (6-OH); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ : 188.92 (C-4), 181.08 (C-1), 158.79 (C-2), 151.69 (C-6), 151.46 (C-7), 134.15 (C-8a), 132.98 (C-9), 129.77 (C-10a), 126.44 (C-4b), 124.77 (C-4a), 119.85 (C-10), 111.95 (C-3), 109.91 (C-5), 107.98 (C-8), 56.87 (2-O$\underline{C}H_3$), 56.12 (7-O$\underline{C}H_3$)

Compound **10** showed following characteristics and identified as a 7-hydroxy-2,4,6-trimethoxyphenanthrene[4]. MP 174 ℃; UV λ_{max} (CH_3OH) nm : (sh)237, (sh)253, 260, 282(sh), 293(sh), 305(sh), 329, 345, 362; IR (KBr) ν_{max} cm^{-1} : 3434 (O-H), 1612, 1486, 870, 837, 805 (aromatic nucleus), 1450, 1442, 1417, 1280, 1215, 1165, 1140, 1088, 1058, 1022, 935; FAB-MS *m/z* 284 $[M]^+$; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ : 3.89 (3H, *s*, 4-OCH_3), 3.95 (3H, *s*, 6-OCH_3), 4.08 (3H, *s*, 2-OCH_3), 6.80 (1H, *d*, *J*=3.0 Hz, H-3), 7.01 (1H, *d*, *J*=2.5 Hz, H-1), 7.22 (1H, *d*, *J*=2.0 Hz, H-8), 7.54 (1H, *d*, *J*=9.0 Hz, H-10), 7.58 (1H, *d*, *J*=8.5 Hz, H-9), 8.98 (1H, *s*, H-5), 9.35 (1H, *s*, 7-OH); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ : 158.57 (C-2), 156.92 (C-4), 147.87 (C-6), 145.29 (C-7), 133.92 (C-10a), 126.96 (C-9), 126.88 (C-8a), 124.63 (C-10), 123.55 (C-4b), 111.89 (C-8), 114.76 (C-4a), 108.75 (C-5), 101.45 (C-1), 98.98 (C-3), 55.93 (2-OCH_3), 55.24 (6-OCH_3), 55.16 (4-OCH_3).

Compound **11** showed following characteristics and identified as a 7-hydroxy-2,6-dimethoxy-1,4-phenanthraquinone (dioscoreanone)[9]. MP 250~253 ℃; IR (KBr) ν_{max} cm^{-1} : 3436 (O-H), 1635, 1482 (1,4-phenanthraquinone C=O), 1583, 1418, 1340, 1277, 1233, 1213, 1167, 1135, 1070, 1027, 947, 868, 790; FAB-MS *m/z* 285 $[M+H]^+$; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ : 3.86 (3H, *s*, 2-OCH_3), 3.96 (3H, *s*, 6-OCH_3), 6.23 (1H, *s*, H-3), 7.26 (1H, *s*, H-8), 7.87 (1H, *d*, *J*=8.5 Hz, H-10), 8.03 (1H, *d*, *J*=9.0 Hz, H-9), 9.0 (1H, *s*, H-5), 10.3 (*s*, 7-OH); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ : 188.5 (C-4), 180.3 (C-1), 158.2 (C-2), 152.4 (C-6), 149.5 (C-7), 134.3 (C-8a), 131.6 (C-9), 128.6 (C-10a), 124.73 (C-4b), 124.69 (C-4a), 120.0 (C-10), 111.2 (C-3), 110.2 (C-8), 106.0 (C-5), 56.3 (2-OCH_3), 55.4 (6-OCH_3).

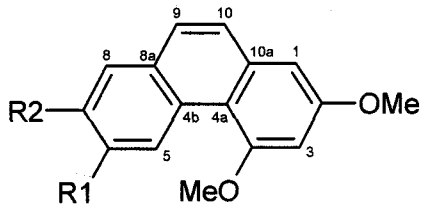
Compound **12** showed following characteristics and identified as a 6,7-dihydroxy-2,4-dimethoxyphenanthrene[19]. MP 206 ℃; UV λ_{max} (CH_3OH) nm: 220, (sh)253, 260, 283(sh), 293(sh), 306(sh), 330, 345, 363; IR (KBr) ν_{max} cm^{-1} : 3383 (O-H), 1616, 1587, 1548, 1504, 1486, 866, 839, 814, 673 (aromatic nucleus), 1466, 1446, 1423, 1396, 1273, 1211, 1200, 1169, 1130, 1087, 1065, 935; EI-MS *m/z* (rel. int.) 270 (100) $[M]^+$, 255 (18), 237 (10), 227 (16), 212 (16), 184 (11), 135 (10); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ : 3.88 (3H, *s*, 2-OCH_3), 4.04 (3H, *s*, 4-OCH_3), 6.76 (1H, *d*, *J*=2.5 Hz, H-3), 6.97 (1H, *d*, *J*=3.0 Hz, H-1), 7.17 (1H, *s*, H-8), 7.45 (1H, *d*, *J*=9.0 Hz, H-10), 7.53 (1H, *d*, *J*=9.0 Hz, H-9), 8.86 (1H, *br s*, H-5), 9.30

(1H, *d*, *J*=6.5 Hz, 7-OH), 9.33 (1H, *d*, *J*=7.0 Hz, 6-OH); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ : 158.59 (C-4), 156.73 (C-2), 145.76 (C-6), 144.54 (C-7), 133.85 (C-10a), 127.10 (C-9), 126.12 (C-8a), 123.79 (C-4b), 123.60 (C-10), 114.47 (C-4a), 112.41 (C-5), 111.86 (C-8), 101.27 (C-1), 98.57 (C-3), 55.61 (4-OCH_3), 55.05 (2-OCH_3). The structures of compound **1**, **2**, **10**, **11**, and **12** were shown in Fig. 1.

Antimicrobial activity of isolated compounds was evaluated against various pathogenic, food spoilage and plant pathogenic microorganisms based on disk-diffusion assay (Table 1). Ampicillin showed high activity against gram positive-, and gram negative- bacteria, while miconazole was active against fungi. Compound **12** (6,7- dihydroxy-2,4-dimethoxyphenanthrene) showed strong and broad-spectrum antimicrobial activity, but compound **1** (batatasin 1) is slightly active against only fungi. Compound **2**, **10**, and **11** did not show antibacterial and antifungal activity. Compound **11** has minor antibacterial activity against *S. epidermidis*. Compound **12** was more effective against *S. cerevisiae* or *C. albicans* than *B. cinerea*.

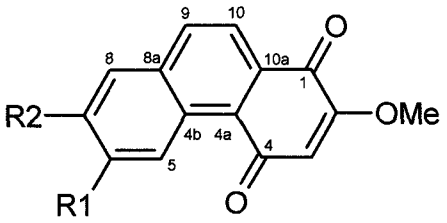
The MICs of compound **12** against different bacteria and fungi were determined (Table 2). Miconazole and ampho

(a)



1: R1=OH, R2=OMe (6-hydroxy-2,4,6-trimethoxyphenanthrene)
10: R1=OMe, R2=OH (7-hydroxy-2,4,6-trimethoxyphenanthrene)
12: R1, R2=OH (6,7-dihydroxy-2,4-dimethoxyphenanthrene)

(b)



2: R1=OH, R2=OMe (6-hydroxy-2,7-dimethoxy-1,4-phenanthraquinone)
11: R1=OMe, R2=OH (7-hydroxy-2,6-dimethoxy-1,4-phenanthraquinone)

Fig. 1. Structures of the (a) phenanthrenes (compound **1**, **10** and **12**) and (b) phenanthraquinones (compound **2** and **11**) from the aerial bulbils of *D. batatas* Decne.

Table 1. Evaluation of antimicrobial activities of phenanthrenes and phenanthraquinones from the bulbils of *D. batatas* Decne against different bacteria and fungi*

Compound	Diameter of growth inhibition zone (mm)								
	Fungi			Gram negative bacteria			Gram positive bacteria		
	CA[1]	BC	SC	PV	ST	EC	SE	SM	SA
Ampicillin	-[2]	-	-	28	20	13	13	15	30
Miconazole	17	12	12	-	-	-	-	-	-
1	9	8	10	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	9	-	-
12	18	8	19	25	20	9	13	15	25

*70 μg of samples (compounds **1**, **2**, **10**, **11**, and **12**), and 1 μg of ampicillin or 1 μg of miconazole were used per disk, respectively.
[1]CA: *Candida albicans*, BC: *Botrytis cinerea*, SC: *Saccharomyces cerevisiae*, PV: *Proteus vulgaris*, ST: *Salmonella typhimurium*, EC: *Escherichia coli* O157, SE: *Staphylococcus epidermidis*, SM: *Streptococcus mutans*, SA: *Staphylococcus aureus*. [2]- : No inhibition.

Table 2. MICs of compound **12** (6,7-dihydroxy-2,4-dimethoxyphenanthrene) against different bacteria and fungi

Compound	Minimal Inhibitory Concentration (μg/ml)[1]								
	Fungi			Gram negative bacteria			Gram positive bacteria		
	CA[2]	BC	SC	PV	ST	EC	SE	SM	SA
Ampicillin	-[3]	-	-	1.25	1.25	1.25	2.5	5.0	0.63
Miconazole	1.5	2.0	1.5	-	-	-	-	-	-
Amphotericin B	1.5	2.0	1.5	-	-	-	-	-	-
12	25	125	25	50	50	> 100	100	> 100	50

[1]MIC: Minimal Inhibitory concentration (μg/ml),
[2]CA: the abbreviations of bacteria and fungi were the same as those of Table. 1.
[3]- : No inhibition.

tericin B at the concentration of 2.0 μg/ml completely inhibited fungal growth and prevented fungal colony formation. Ampicillin showed strong activity against different bacteria. Compound **12** showed strong antifungal activity with MIC value of 25 μg/ml against *C. albicans* and *S. cerevisiae*, while MIC value against *B. cinerea* was 125 μg/ml. Against *P. vulgaris*, *S. typhimurium* and *Staphylococcus aureus*, compound **12** showed activities with MIC value of 50 μg/ml. These results were consistent with the previous report[19], although only growth inhibition was reported. Our results indicated that the antimicrobial activity of phenanthrene is modulated by hydroxylation or methoxylation.

MFC values of compound **12** against *C. albicans* and *S. cerevisiae* were 25~50 μg/ml, and the values were similar to MIC, suggested that the cell growth inhibition is mediated by fungicidal action. The effect of compound 12 on the growth of the fungi was investigated (Fig. 2). After treatment of compound 12 to culture (10^6 cell/ml), the viable cell was counted using Sabouraud dextrose agar plates every 3 h. The suppressive effects of compound **12** were evident and showed concentration dependent manner. After treatment of compound **12** at 25 μg/ml concentration, the cell viability was rapidly decreased showing first-order kinetics. These results indicate that compound **12** has a prominent fungicidal activity, and suggest that 6,7-dihydroxy-2,4-dimethoxyphenanthrene in the bulbils of yam is responsible for defense mechanism against microbial infection. Further researches on the mode of action of 6,7-dihydroxy-2,4-dimethoxyphenanthrene, and modification of the structure for activity increase are necessary.

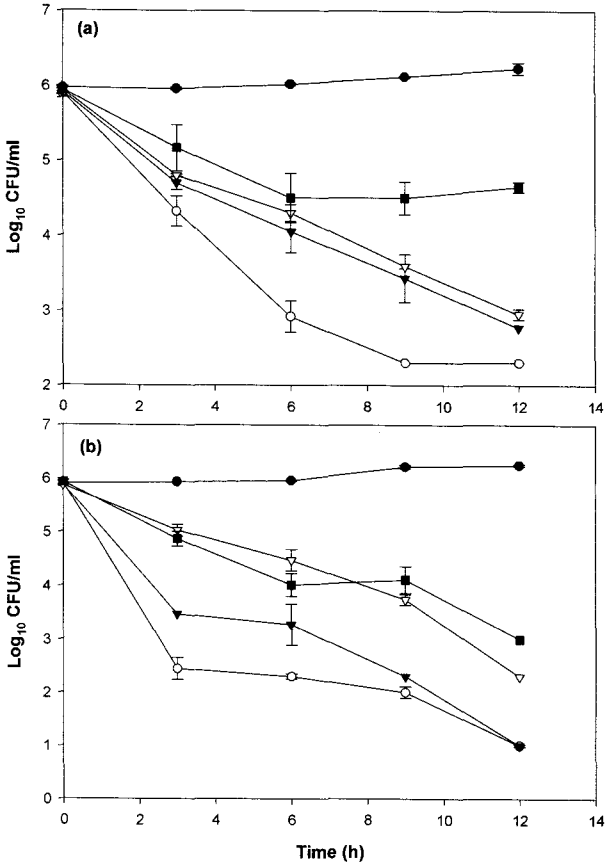


Fig. 2. The suppressive effect of compound **12** (6,7-dihydroxy-2,4-dimethoxyphenanthrene) on the growth of (a) *C. albicans* and (b) *S. cerevisiae*. Symbols: •, no treatment; ▽, 25 μg/ml; ▼, 50 μg/ml; ○, 100 μg/ml of compound **12** and ■, 1 μg/ml of miconazole.

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References

1. Aderiye, B. I., S. K. Ogundana, S. A. Adesanya, and M. F. Roberts. 1996. Antifungal properties of yam (*Dioscorea alata*) peel extract. *Folic Microbiol.* **41**, 407-412.
2. Cai, J., M. Liu, A. Wang, and Y. Ju. 2002. Apoptosis induced by dioscin in Hela cells. *Biol. Pharm. Bull.* **25**, 193-196.
3. Choi, E. M., S. J. Koo, and J. K. Hwang. 2004. Immune cell stimulating activity of mucopolysaccharide isolated from yam (*Dioscorea batatas*). *J. Ethnopharmacol.* **102**, 1851-190
4. Coxon, D. T., S. K. Ogundana, and C. Dennis. 1982. Antifungal phenanthrenes in yam tubers. *Phtochemistry* **21**, 1389-1392.
5. Estrada, S., A. Rojas, Y. Mathison, A. Israel, and R. Mata. 1999. Nitric oxide/cGMP mediates the spasmolytic action of 3,4'-dihydroxy-5,5'-dimethoxybibenzyl from *Scaphyglottis livida*. *Planta Med.* **65**, 109-114.
6. Estrada, S., R. A. Toscano, and R. Mata. 1999. New phenanthrene derivatives from *Maxillaria densa* (1). *J. Nat. Prod.* **62**, 1175-1178.
7. Hernandez-Romero, Y., J. I. Rojas, R. Castillo, A. Rojas, and R. Mata. 2004. Spasmolytic effects, mode of action, and structure-activity relationships of stilbenoids from *Nidema boothii*. *J. Nat. Prod.* **67**, 160-167.
8. Honda, C., and M. Yamaki. 2000. Phenanthrenes from *Dendrobium plicatile*. *Phytochemistry* **53**, 987-990.
9. Itharat, A., A. Plubrukarn, P. Kongsaeree, T. Bui, N. Keawpradub, and P. J. Houghton. 2003. Dioscorealides and Dioscoreanone, novel cytotoxic naphthofuranoxepins, and 1,4-phenanthraquinone from *Dioscorea membranacea* Pierre. *Org. Lett.* **5**, 2879-2882.
10. Kwon, C.-S., H. Y. Sohn, S. H. Kim, J. H. Kim, K. H. Son,

J. S. Lee, J. K. Lim, and J.-S. Kim. 2003. Anti-obesity effect of *Dioscorea nipponica* Makino with lipase-inhibitory activity in rodents. *Biosci. Biotechnol. Biochem.* **67**, 1451-1456.
11. Lamidi, M., C. DiGiorgio, F. Delmas, A. Favel, Mve-Mba C. Eyele, M. L. Rondi, E. Ollivier, L. Nze-Ekekang, and G. Balansard. 2005. *In vitro* cytotoxic, antileishmanial and antifungal activities of ethnopharmacologically selected Gabonese plants. *J. Ethnopharmacol.* **102**, 1851-190
12. Li, K., Y. Tang, J. P. Fawcett, J. Gu, and D. Zhong. 2005. Characterization of the pharmacokinetics of dioscin in rat. *Steroids* **70**, 525-530.
13. Ministry of Agriculture and Foresty. Korea. 2004. *Statistical Data.*
14. Narula, A., S. Kumar, K. C. Bansal, P. S. Srivastava. 2003. In vitro micropropagation, differentiation of aerial bulbils and tubers and diosgenin content in *Dioscorea bulbifera. Plant Med.* **69**, 778-779.
15. Sautour, M., A. C. Mitaine-Offer, T. Miyamoto, A. Dongmo, and M. A. Lacaille-Dubois. 2004. A new steroidal saponin from *Dioscorea cayenensis. Chem. Pharm. Bull.* **52**, 1353-1355.
16. Sautour, M., A. C. Mitaine-Offer, T. Miyamoto, H. Wagner, and M. A. Lacaille-Dubois. 2004. A new phenanthrene glycoside and other constituents from *Dioscorea opposita. Chem. Pharm. Bull.* **52**, 1235-1237.
17. Seong, N.-S. C.-H. Park, and S.-T. Lee. 1995. Micropropagation by adventitious bud formation and direct bulblet induction of *Discorea batatas. Kor. J. Breed* **27**, 56-57.
18. Sohn, H.-Y., K. H. Son, C.-S. Kwon, G.-S. Kwon and S. S. Kang. 2004. Antimicrobial and cytotoxic activity of 18 prenylated flavonoids isolated from medicinal plants: *Morus alba* L., *Morus mongolica* Schneider, *Broussnetia papyrifera* (L.) Vent, *Sophora flavescens* Ait and *Echinosophora koreensis* Nakai. *Phytomedicine* **11**, 666-672.
19. Takasugi, M., S. Kawashima, K. Monde, N. Katsui, T. Masamune, and A. Shirata. 1987. Antifungal compounds from *Dioscorea batatas* inoculated with *Pseudomonas cichorii. Phytochemistry* **26**, 371-375.
20. Yin, M.-C. and S.-M. Tsao. 1999. Inhibitory effect of seven *Allium* plants upon three *Aspergillus* species. *Int. J. Food Microbiol.* **49**, 49-56.

초록 : 재배마 *(Dioscorea batatas Decne)*의 주아로부터 분리된 phenanthrene 유도체의 항진균 활성

금은주 · 박상조[1] · 이봉호[1] · 김종식[2] · 손건호 · 손호용*
(안동대학교 식품영양학과, [1]안동대학교 생명과학과, [2]경북농업기술원 생물자원연구소)

마속 식물들은 오래 전부터 다양한 민간처방에 사용되어 왔으며, 재배마의 근경은 다양한 생리활성물질들이 보고되어, 건강식품으로 인식되고 있다. 재배마의 주아는 매년 2,000톤 이상 생산되고 있으나, 다음해의 종자로 일부 이용될 뿐 거의 폐기되고 있으며, 생리활성에 대한 보고도 거의 없는 실정이다. 본 연구에서는 마의 주아로부터 3종의 phenanthrene 및 2종의 phenanthraquinone를 분리하였으며, 이들의 구조를 규명하였다. 이들 중 6-hydroxy-2,7-dimethoxy-1,4-phenanthraquinone은 처음으로 분리되었다. 분리된 5종을 대상으로 항균활성을 평가한 결과, 6,7-dihydroxy-2,4-dimethoxyphenanthrene은 *Candida albicans*에 대해 25 μg/ml의 MIC와 MFC를 나타내어, 강력한 항균활성으로 실제적 사용 가능성을 나타내었다. 본 연구결과는 phenanthrene 링 구조의 -OH 및 $-OCH_3$ 수식이 항균활성 및 항균 스펙트럼 결정의 중요한 인자임을 제시하였다.