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Antimicrobial Property of (+)-Lyoniresinol-3α-*O*-β-D-Glucopyranoside Isolated From the Root Bark of *Lycium chinense* Miller Against Human Pathogenic Microorganisms

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(+)-Lyoniresinol-3α-*O*-β-D-glucopyranoside (**1**) was isolated from an ethyl acetate extract of the root bark from *Lycium chinense* Miller, and its structure was determined using 1D and 2D NMR spectroscopy including DEPT, HMQC, and HMBC. (+)-Lyoniresinol-3α-*O*-β-D-glucopyranoside exhibited potent antimicrobial activity against antibiotic-resistant bacterial strains, methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from patients, and human pathogenic fungi without having any hemolytic effect on human erythrocytes. In particular, compound **1** induced the accumulation of intracellular trehalose on *C. albicans* as stress response to the drug, and disrupted the dimorphic transition that forms pseudo-hyphae caused by the pathogenesis. This indicates that (+)-lyoniresinol-3α-*O*-β-D-glucopyranoside has excellent potential as a lead compound for the development of antibiotic agents.

Key words: *Lycium chinense*, *Solanaceae*, (+)-Lyoniresinol-3α-*O*-β-D-glucopyranoside, Antimicrobial activity, MRSA, Stress response

INTRODUCTION

Lycii Radicis Cortex, which is the root bark of *Lycium chinense* Miller (solanaceae), has been used in traditional herbal medicine to treat fever and a lack of vigor. It has been reported to have hypotensive, hypoglycaemic, antipyretic and anti-stress ulcer activity in experimental animals (Funayama *et al.*, 1980; Morota *et al.*, 1987). A number of acyclic diterpene glycosides (Terauchi *et al.*, 1998), cyclic peptides (Yahara *et al.*, 1993), sesquiterpenes (Sannai *et al.*, 1982), spermine alkaloid (Funayama *et al.*, 1995), flavonoids (Terauchi *et al.*, 1997), and cerebrosides (Kim *et al.*, 1997) have been isolated from this plant. Previous work on the root bark of *L. chinense* confirmed the antioxidant (Han *et al.*, 2002) and antifungal activities (Lee *et al.*, 2004) of the phenolic compounds isolated from this plant. As part of an ongoing study on the root bark of this plant, also it was found that other compounds have a broad spectrum of antimicrobial activity on various human infectious pathogens. (+)-Lyoniresinol-3α-*O*-β-D-glucopyranoside (**1**) was isolated from this plant. Compound **1** displayed potent antimicrobial activity without any hemolytic activity against human erythrocytes, Moreover, this compound induced a stress response in *C. albicans* cells. This study reports the isolation and characterization of (+)-lyoniresinol-3α-*O*-β-D-glucopyranoside along with its antimicrobial activity.

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MATERIALS AND METHODS

General procedure

The melting point was obtained using a Fisher Scientific melting point apparatus and was uncorrected. The IR spectra were recorded on an IMS 85 (Bruker). The NMR spectra were recorded on a Varian Unity Inova 500 (500 MHz) spectrometer. The ^{1}H-^{1}H COSY, DEPT, HMQC, and HMBC NMR spectra were obtained with the usual pulse sequences. The HR-FABMS was determined on a JMS 700 (JEOL). Semi-preparative HPLC was performed on a Waters 600E (Delivery pump) with a UV Detector (Waters model 2487) using a μ Bondpak C_{18} (3.9 mm × 300 mm, Waters) column. The TLC and column chromatography were carried out on precoated Si Gel F_{254} plates (Merck,

art. 5715), RP-18 F_{254} plates (Merck, art. 15423), and Si gel 60 (Merck, 230-400 mesh).

Plant materials

The root bark from *L. chinense* (Solanaceae) was purchased from a local Korean herbal drug market in Gwangju, Korea, and was authenticated by the Department of Pharmacognosy, Chosun University. Voucher specimens were deposited in the Herbarium of the College of Pharmacy, Chosun University (893-16).

Extraction and isolation

The root bark (0.8 kg) of *L. chinense* was extracted with MeOH at room temperature, which afforded 137.3 g of residue. The methanol extract was suspended in water and partitioned sequentially with dichloromethane, ethyl acetate, and *n*-butanol. 3.0 g of the EtOAc fraction was subjected to column chromatography over a silica gel (300 g, 4.8 × 45 cm), and eluted with a CH_2Cl_2-MeOH-H_2O (8:1:0.1 → 6:1:0.1 → 4:1:0.1 → 2:1:0.1 → MeOH only) gradient system. The fractions were combined based on their TLC pattern to yield subfractions designated E1-E8. Subfraction E6 (223 mg) was further purified by column chromatography over a silica gel (100 g, 2.8 × 44.5 cm), and eluted with a $CHCl_3$-Me_2CO-MeOH-H_2O gradient system to afford eleven subfractions (E61-E611). Subfraction E69 (72 mg) was finally purified by semi-prep HPLC (Waters 600E), and eluted with an *i*-PrOH-MeOH-H_2O gradient system, which afforded compound **1** (7.40 mg).

(+)-Lyoniresinol-3α-*O*-β-D-glucopyranoside (1)

An amorphous powder, $[\alpha]_D^{24}$ +26.0° (*c* 0.5 in MeOH). UV (MeOH) λ_{max} nm (log ε): 276 (3.70); IR ν_{max} (KBr) cm^{-1}: 3400, 1570; FAB-MS *m/z* (rel. int.): 605.05 ($[M+Na]^+$, 86.73 %), 581.22 ($[M-H]^+$, 13.65 %); ^{1}H-NMR (CD_3OD, 500 MHz) δ : 1.71 (*m*, H-2), 2.09 (*m*, H-3), 2.59-2.74 (*m*, H-1), 3.25 (*m*, H-2α), 3.32 (*s*, -OCH_3), 3.30-3.84 (*m*, sugar H), 3.75 (*s*, -OCH_3), 3.76 (*m*, H-3α), 3.83 (*m*, H-2α), 3.86 (*s*, -OCH_3), 3.89 (*m*, H-3α), 4.28 (*d*, *J* = 8.0 Hz, anomeric H), 4.42 (*d*, *J* = 6.0 Hz, H-4), 6.43 (s, H-2',6'), 6.58 (s, H-8); ^{13}C-NMR (CD_3OD, 125 MHz) δ : 147.80 (*s*, C-3',5'), 147.45 (*s*, C-5), 146.40 (*s*, C-7), 138.17 (*s*, C-1'), 137.74 (*s*, C-6), 133.28 (*s*, C-4'), 128.99 (*s*, C-9), 125.26 (*s*, C-10), 106.64 (*d*, C-8), 105.71 (*d*, C-2',6'), 103.67 (*d*, C-1"), 77.06 (*d*, C-5"), 76.78 (*d*, C-3"), 74.00 (*d*, C-2"), 70.48 (*d*, C-4"), 70.23 (*t*, C-3α), 65.02 (*t*, C-2α), 61.65 (*t*, C-6"), 58.97 (*q*, OCH_3-5), 55.65 (*q*, OCH_3-3'/5'), 55.39 (*q*, OCH_3-7), 45.53 (*d*, C-3), 41.62 (*d*, C-4), 39.40 (*d*, C-2), 32.67 (*t*, C-1).

Bacterial and fungal strains

The methicillin-resistant *Staphylococcus aureus* (MRSA) strains used in this study were isolated from clinical human pathogens at the Kyungpook National University Hospital.

The *Saccharomyces cerevisiae* (KCTC 7296) and *Trichosporon beigelii* (KCTC 7707) were obtained from the Korean Collection for Type Cultures (KCTC) at the Korea Research Institute of Bioscience and Biotechnology (KRIBB) Taejon, Korea. The *Candida albicans* (TIMM 1768) was obtained from the center for Academic Societies, Osaka, Japan.

Antimicrobial activity

The bacterial cells grown in LB (Trypton 1%, Sodium chloride 1%, Yeast extract 0.5%) medium at 37°C, were seeded on 96-well microtiter plates at a density of 1×10^6 cells per well in 100 μL of LB medium. The serially diluted-sample solutions were added to each well. The cell suspension was incubated at 37°C for 6 h. After incubation, the optical density of each well was measured by a microtiter ELISA reader (Molecular Devices Emax, California, USA) at 620 nm. The antifungal activity was determined by growing the fungal strains in YPD medium (Dextrose 2%, Peptone 1%, Yeast extract 0.5%, pH 5.5) at 28°C. The fungal cells were seeded on 96-well microtiter plates in YPD medium at a density of 2×10^3 cells (100 μL per well). Ten μL of the serially-diluted sample solutions were added to each well, and the cell suspension was incubated for 16 h at 28°C. Five μL of a 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) solution [5 mg/mL MTT in phosphate-buffered saline (PBS), pH 7.4] was added to each well, and the plates were incubated for 3 h at 37°C. Thirty μL of 20% (w/v) SDS solution containing 0.02 M HCl was then added, and the plates were incubated at 37°C for 16 h to dissolve the formazan crystals that had formed (Lehrer *et. al.*, 1993). The turbidity of each well was measured at 580 nm using a microtiter ELISA reader (Molecular Devices Emax, California, U.S.A.).

Hemolytic activity

The hemolytic activity of compound **1** was determined by measuring the amount of hemoglobin released from the 8% suspensions of fresh human erythrocytes at 414 nm (Blondle and Houghten 1992). The human red blood cells were centrifuged and washed three times with phosphate-buffered saline (PBS: 35 mM phosphate buffer/0.15 M NaCl, pH 7.0). One hundred μL of the human red blood cells suspended 8% (v/v) in PBS were plated into 96-well plates, and 100 μL of the sample solution (from 50 to 3.125 μg/mL) was then added to each well. The plates were incubated for 1 h at 37°C and centrifuged at 150 × *g* for 5 min. One hundred μL of the aliquots were transferred to 96-well plates. Hemolysis was measured by its absorbance at 414 nm using an ELISA

plate reader (Molecular Devices Emax, Sunnyvale, CA, USA). Zero percent and 100% hemolysis was determined in PBS and 0.1% Triton X-100, respectively. The hemolysis percentage was calculated using the following equation:

$$\% \text{ hemolysis} = \frac{Abs_{414nm} \text{ in the sample solution} - Abs_{414nm} \text{ in PBS}}{Abs_{414nm} \text{ in 0.1\% Triton-X100} - Abs_{414nm} \text{ in PBS}} \times 100$$

Determination of intracellular trehalose

The *C. albicans* cell suspension containing compound **1** was incubated at 28°C for 1 h. The negative control was incubated without compound **1**, and the positive control was incubated with amphotericin B. The fungal cells were settled by centrifugation (12,000 rpm for 20 min), and only the cells were dried. 15 mg (dry weight) of the dried fungal cells were destroyed by boiling in a 0.025 mM potassium-phosphate buffer (pH 6.6) for Fifteen min. The crude neutral trehalose-containing fractions were then extracted by removing the cell debris. After then, the trehalose residue was digested with 0.05 unit of trehalase (sigma, T8778). After allowing the enzymatic reaction at 37°C to proceed for 30 min, the reaction suspension was mixed with H_2O, and a 16% DNS reagent (3, 5-Dinitrosalicylic acid 1%, NaOH 2%, Sodium potassium tartrate 20%) was then added (Sengupta *et al.*, 2000). For the reaction between glucose and the DNS reagent, the mixture was boiled for 5 min and then cooled. The color formation was measured at 525 nm.

Effect of (+)-lyoniresinol-3α-*O*-β-D-glucopyranoside on the dimorphic transition

C. albicans was maintained by periodic subculturing in a liquid YPD medium. Cultures of yeast cells (blastoconidia) were maintained in the liquid YPD medium at 37°C. Hyphal formation was induced by supplementing the cultures with 20% fetal bovine serum (Alvarez-Peral and Arguelles 2000). The dimorphic transition in *C. albicans* was investigated after incubating the cultures containing compound **1** at 37 °C for 48 h. The dimorphic transition to the hyphal forms was detected using phase contrast light microscopy (NIKON, ECLIPSE TE300, Japan).

RESULTS AND DISCUSSION

Structure elucidation

Compound **1**, an amorphous powder, $[\alpha]_D^{24}$ +26.0° (*c* 0.5 in MeOH), FAB-MS *m/z* 605.05 ([M+Na]$^+$), 581.22 ([M-H]$^+$) exhibited carbon signals that were assigned to the glucopyranosyl residue, and four aromatic methoxyl groups. Twelve carbon signals in the ^{13}C-NMR spectrum were assigned to two substituted benzene rings, and two carbinol carbons. In the ^{1}H-NMR spectrum, compound **1** displayed signals at δ 6.43 (*s*, H-2', 6'), 6.58 (*s*, H-8) 1.71 (*m*, H-2), 2.09 (*m*, H-3), 2.59-2.74 (*m*, H-1), 4.42 (*d*, *J* = 6.0 Hz, H-4). The above evidence indicated a 4-aryltetralin type lignan monoglucopyranoside for compound **1**. Based on the ^{1}H-, ^{13}C-NMR, DEPT, HMQC, and HMBC data, compound **1** was determined to be (+)-lyoniresinol-3α-*O*-β-D-glucopyranoside, which was previously isolated from *Stemmadenia minima* (Achenbach *et al.*, 1992).

Antimicrobial and hemolytic activities of (+)-lyoniresinol-3α-*O*-β-D-glucopyranoside

One of the most serious problems in hospitals nowadays is a secondary infection of antibiotics-resistant bacteria in the middle of treatment. The number of antibiotics-resistant bacterial strains has increased continuously as a result of the constant use of antibiotics to treat bacterial infections. This study tested the antibacterial effect of compound **1** against methicillin-resistant *Staphylococcus aureus* (MRSA) that was isolated clinically (Table I). The MRSA strains 1-4 showed antibiotic-resistance at high concentration of cefotaxime, 40 μg/mL. However, the MIC value of compound **1** was found to be 2.5-5 μg/mL. This result demonstrates that compound **1** has potent antibacterial activity against MRSA, which shows antibiotic-resistance to cefotaxime. Table II summarizes the antifungal activity of compound **1** highlighting the potent antifungal activity against the fungal strains tested in this study. The MIC values of compound **1** for *C. albicans, S. cerevisae* and *T. beigelii* were 5-10 μg/mL. Although the antifungal activity of compound **1** was less potent than that of amphotericin B currently used in clinical fungicidal agents, this result shows compound **1** has potent antifungal activity on human pathogenic fungi. The hemolysis percentage was measured against human erythrocytes at various concentrations of compound **1**, cefotaxime and amphotericin B used as the positive control (Table III). While amphotericin B displayed hemolytic activity against human red blood cells (RBCs) at all tested concentrations, compound **1** and

Table I. Antibacterial activity of (+)-lyoniresinol-3α-*O*-β-D-glucopyranoside (**1**) against methicillin-resistant *Staphylococcus aureus* (MRSA)

Samples	MIC (μg/mL)			
	MRSA 1	MRSA 2	MRSA 3	MRSA 4
Compound **1**	5	2.5-5	2.5	2.5
Cefotaxime	>40	>40	>40	>40

The bacterial strains were grown at 37°C in LB medium (Trypton 1%, Sodium chloride 1%, Yeast extract 0.5%). The bacterial cells were seeded at a density of 1×10^6 cells (100 μL per well) on the well of a 96-microtiter plate containing YPD media. The turbidity of each well was measured at 620 nm using a microtiter ELISA reader (Molecular Devices Emax, California, U.S.A.).

Table II. Antifungal activities of (+)-lyoniresinol-3α-*O*-β-D-glucopyranoside (**1**)

Samples	MIC (μg/mL)		
	C. albicans	*S. cerevisiae*	*T. beigelii*
Compound **1**	5	5-10	10
Amphotericin B	0.63	5	1.25

The fungal strains were grown at 28°C in YPD medium (Dextrose 2%, Peptone 1%, Yeast extract 0.5%, pH 5.5). The fungal cells were seeded at a density of 2×10^3 cells (100 μL per well) on the well of a 96-microtiter plate containing YPD medium. The antifungal activity of compound **1** was determined by medium-dilution method and a MTT assay.

Table III. Hemolytic activity of (+)-lyoniresinol-3α-*O*-β-D-glucopyranoside (**1**)

Samples	% Hemolysis (μg/mL)				
	50	25	12.5	6.25	3.125
Compound **1**	0	0	0	0	0
Cefotaxime	0	0	0	0	0
Amphotericin B	30	15	7	4	2

The hemolytic activity of the samples was evaluated by determining the amount of hemoglobin released from 8% suspensions of fresh human erythrocytes at 414 nm. The percentage hemolysis was calculated using the following equation: % hemolysis = [(Abs_{414nm} in the sample solution – Abs_{414nm} in PBS)/(Abs_{414nm} in 0.1% Triton-X 100 – Abs_{414nm} in PBS)] × 100.

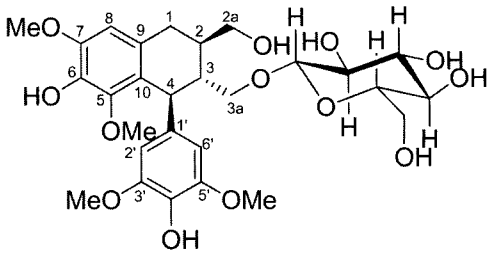

Fig. 1. Structure of (+)-lyoniresinol-3α-O-β-D-glucopyranoside (**1**) isolated from the root bark of *Lycium chinense*

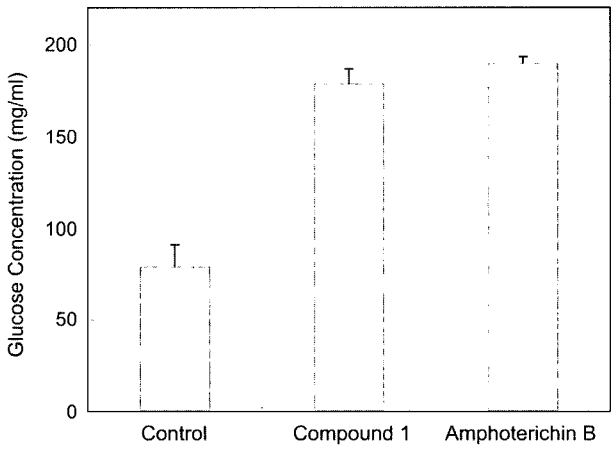

Fig. 2. Trehalose assay after treating *C. albicans* with compound **1** or amphotericin B. *C. albicans* cells treated with the sample or amphotericin B were incubated at 28°C for 1 h. The glucose concentration was determined using DNS method.

cefotaxime did not. This demonstrates the remarkable antimicrobial activity of compound **1** against various pathogenic microorganisms without hemolytic activity and its potential as a therapeutic agent showing a broad spectrum of antimicrobial activity.

Effect of (+)-lyoniresinol-3α-*O*-β-D-glucopyranoside as drug stress factor on *C. albicans*

In yeast, trehalose (α-D-glucopyranosyl-1, 1-α-D-glucopyranoside) accumulates by means of environmental stresses such as heat, desiccation, freezing and toxic agents (Benaroudj *et al.*, 2001). The level of glucose degradation by trehalase was estimated using the dinitrosalicylic acid (DNS) reagent in order to determine the amounts of intracellular trehalose accumulated by compound **1**. The glucose concentration in the *C. albicans* cells incubated with compound **1** was more than double that of the control cells incubated with amphotericin B (Fig. 2). The increased glucose concentration is believed to indicate an increase in intracellular trehalose by compound **1** as a toxic agent on *C. albicans* cells. This suggests that compound **1** affects the *C. albicans* cells as a drug stress factor inducing the accumulation of intracellular trehalose.

Effect of (+)-lyoniresinol-3α-*O*-β-D-glucopyranoside on morphological transition of *C. albicans*

The ability of some fungal strain(s) to undergo a morphological transition between unicellular forms and hyphae structures may be a simple model of cellular development. *C. albicans* is a prototypic dimorphic yeast. This diploid pathogen is becoming of increasing importance in human medicine. In *C. albicans*, dimorphism plays a key role in pathogenesis, with mycelial shapes being found during a host tissue invasion (Mclain *et al.*, 2000). In order to induce filamentation, the cultures were directly supplemented with serum. The effect of compound **1** on the dimorphic transition of *C. albicans* was investigated by examining cultures containing a MIC concentration of compound **1**, and incubating them with fetal bovine serum at 37°C for 48 h. Fig. 3 shows that compound **1** destroyed the hyphal forms at the MIC concentration, indicating that compound **1** can disrupt of the serum-induced filamentous form of *C. albicans*.

CONCLUSIONS

(+)-Lyoniresinol-3α-O-β-D-glucopyranoside (**1**) isolated

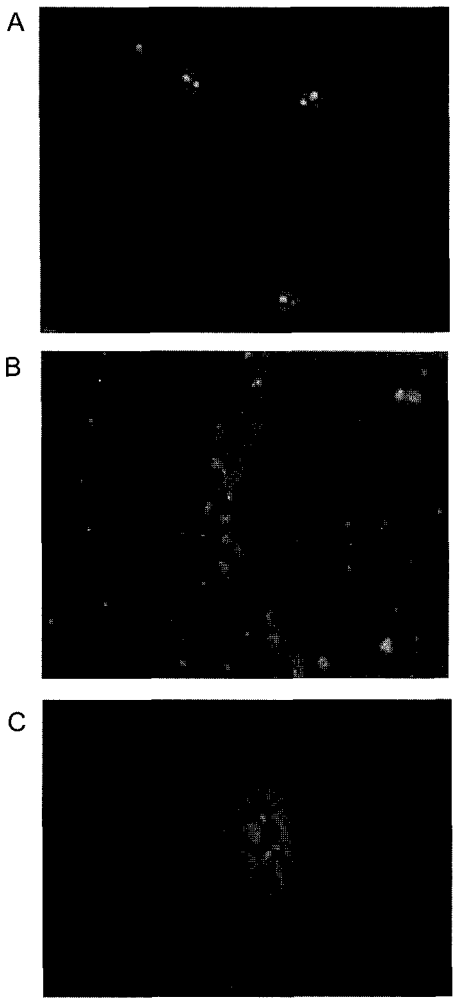

Fig. 3. The effect of (+)-lyoniresinol-3α-O-β-D-glucopyranoside (**1**) on the dimorphic transition in *C. albicans*. Each culture was incubated with the MIC concentration of the sample for 48 h in YPD medium with 20% FBS. (A) yeast control with no 20% FBS and sample, (B) with no treated sample, (C) treated with compound **1**

from the ethyl acetate soluble fraction of the root bark of *L. chinense* showed potent antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from patients and human pathogenic fungi without having any hemolytic effect on human erythrocytes. In addition, compound **1** was found to have potent and effective antifungal activity against *C. albicans*.

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REFERENCES

Achenbach, H., Löwel, M., Waibel, R., Gupta, M., and Solis, P., New lignan glucosides from *Stemmadenia minima*. *Planta Med.*, 58, 270-272 (1992).

Alvarez-Peral, F. J. and Arguelles, J.-C., Changes in external trehalase activity during human serum-induced dimorphic transition in *Candida albicans*. *Res. Microbiol.*, 151, 837-843 (2000).

Benaroudj, N., Lee, D. H., and Goldberg, A. L., Trehalose accumulation during cellular stress protects cells and cellular proteins from damage by oxygen redicals. *J. Biol. Chem.*, 276, 24261-24267 (2001).

Blondle, S. E. and Houghten, R. A., Design of model amphipathic peptides having potent antimicrobial activities. *Biochemistry*, 31, 12688-12694 (1992).

Funayama, S., Yoshida, K., Konno, H., and Hikkino, H., Structure of Kukoamine A, a hypotensive principle of *Lycium chinense* root bark. *Tetrahedron Lett.*, 21, 1355-1356 (1980).

Funayama, S., Zhang, G.-R., Nozoe, S., and Kukoamine, B., A spermine alkaloid from *Lycium chinense*. *Phytochemistry*, 38, 1529-1531 (1995).

Han, S.-H., Lee, H.-H., Lee, I.-S., Moon, Y.-H., and Woo, E.-R., A new phenolic amide from *Lycium chinense* Miller. *Arch. Pharm. Res.*, 25, 433-437 (2002).

Kim, S. Y., Choi, Y.-H., Huh, H., Kim, J., Kim, Y. C., and Lee, H. S., New antihepatotoxic cerebroside from *Lycium chinense* Fruits. *J. Nat. Prod.*, 60, 274-276 (1997).

Lehrer, R., Lichtenstein, A. K., and Ganz, T., Defensins: Antimicrobial and cytotoxic peptides of mammalian cells. *Annu. Rev. Immunol.*, 11, 105-128 (1993).

Lee, D. G., Park, Y., Kim, M.-R., Jung, H. J., Seu, Y. B., Hahm, K.-S., and Woo, E.-R., Antifungal effects of phenolic amides isolated from the root bark of *Lycium chinense*. *Biotechnol. Lett.*, 26, 1125-1130 (2004).

Mclain, N., Ascaniom, R., Baker, C., Strohaver, R. A., and Dolan, J. W., Undeclenic acid inhibits morphogenesis of *Candida albicans*. *Antimicrob. Agents Chemothr.*, 44, 2873-2875 (2000).

Morota, T., Sasaki, H., Chin, M., Sato, T., Katayama, N., Fukuyama, K., and Mitsuhashi, H., Studies on the crude drug containing the angiotensin I converting enzyme inhibitors (I) on the active principles of *Lycium chinense* Miller. *Shoyakugaku Zasshi*, 41, 169-173 (1987).

Sannai, A., Fujimori, T., and Kato, K., Isolation of (-)-1,2-dehydro-α-cyperone and solavetivone from *Lycium chinense*. *Phytochemistry*, 21, 2986-2987 (1982).

Sengupta, S., Jana, M. L., Sengupta, D., and Naskar, A. K., A note on the estimation of microbial glycosidase activities by dinitrosalicylic acid reagent. *Appl. Microbiol. Biotechnol.*, 53, 732-735 (2000).

Terauchi, M., Kanamori, H., Nobuso, M., Yahara, S., and Nohara, T., Detection and determination of antioxidative components in *Lycium chinense*. *Nat. Med.*, 51, 387-391

(1997).

Terauchi, M., Kanamori, H., Nobuso, M., Yahara, S., and Yamasaki, K., New acyclic diterpene glycoside, Lyciumoside IV-IX from *Lycium chinense* Mill. *Nat. Med.*, 52, 167-171 (1998).

Yahara, S., Shigeyama, C., Ura, T., Wakamatsu, K., Yasuhara, T., and Nohara, T., Cyclic peptides, acyclic diterpene glycoside and other compounds from *Lycium chinense* Mill. *Chem. Pharm. Bull.*, 41, 703-709 (1993).