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Fungicidal effect of isoquercitrin via inducing membrane disturbance

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ABSTRACT

Isoquercitrin is a flavonoid isolated from *Aster yomena*, which has been used as a traditional medicinal herb. In the present study, we investigated the antifungal activity and the underlying mechanism of isoquercitrin. Isoquercitrin had a potent effect in the susceptibility test against pathogenic fungi and almost no hemolysis. Propidium iodide and potassium release assays were conducted in *Candida albicans*, and these studies confirmed that isoquercitrin induced membrane damage, thereby, increasing permeability. Membrane potential was analyzed using 3,3′-dipropylthiacarbocyanine iodide [$DiSC_3(5)$], and the transition of membrane potential was indicated by an increased fluorescence intensity. To further analyze these results using model membranes, giant unilamellar vesicles and large unilamellar vesicles that encapsulated calcein were prepared and the detection of calcein leakage from liposomes indicated that membrane was disturbed. We further verified membrane disturbance by observing the disordered status of the lipid bilayer with 1,6-diphenyl-1,3,5-hexatriene fluorescence. Moreover, changes in size and granularity of the cell were revealed in flow cytometric analysis. All these results suggested the membrane disturbance and the degree of disturbance was estimated to be within a range of 2.3 nm to 3.3 nm by fluorescein isothiocyanate–dextran analysis. Taken together, isoquercitrin exerts its fungicidal effect by disturbing the membrane of cells.

1. Introduction

The medicinal properties of numerous herbal plants are widely known and have been used for many years to treat a variety of conditions [1]. They can exert physiological actions on the human body, producing therapeutic effects such as anti-hepatotoxic and anti-cancer activity [2]. In addition, many of the anti-microbial compounds that have been isolated from medicinal herbs have low toxicity and elicit minimal side effects [3]. For this reason, there has been increased interest in the medicinal properties of various herbal plants. Specifically, most plants produce organic biomolecules in response to infections or stress caused by microorganisms to protect themselves as chemical barriers [4].

Phytochemicals are small, organic, naturally occurring biomolecules that are secondary metabolites from plants [5]. Phytochemicals may be effective at combating or preventing diseases due to their antioxidant effects. The major phytochemicals are flavonoids, phenolic compounds, and tannins [6]. Flavonoids, which are phenolic structures that contain one carbonyl group, have been shown to inhibit multiple viruses [7,8]. Phenolic compounds are the simplest bioactive phytochemicals consisting of a single substituted phenolic ring. They possess several biological properties, including anti-microbial, anti-viral, and antioxidant activity. Tannins are polymeric phenolic substances that have anti-infective actions [8].

Aster yomena is a perennial herb that grows mainly in South Korea. It is used as a traditional medicine in the treatment of inflammation, colds, and asthma [9]. Isoquercitrin, a dietary flavonoid, is found in medicinal and dietary plants, including herbs, flowers, and vegetables [10]. It has been shown that isoquercitrin has a wide range of therapeutic properties, including anti-inflammatory, antioxidant, and anti-allergic activities [11]. Recently, other effects of isoquercitrin have been reported. Isoquercitrin can potentially be used to prevent lipid metabolism disorder through activation of the adenosine monophosphate-activated protein kinase [10]. Furthermore, anti-asthmatic activity and the possible use as a diuretic have been demonstrated [12,13]. However, to the best of our knowledge, there has yet been no study demonstrating the effect of isoquercitrin against fungi. Here, we report the antifungal activity of isoquercitrin isolated from *A. yomena* against various fungal strains and demonstrate its mode of action.

Abbreviations: CH_2Cl_2, dichloromethane; EtOAc, ethyl acetate; MIC, minimum inhibitory concentration; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide; $DiSC_3(5)$, 3,3′-dipropylthiacarbocyanine; SD, standard deviation; GUV, giant unilamellar vesicle; ITO, indium tin oxide; LUV, large unilamellar vesicle; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; DPH, 1,6-diphenyl-1,3,5-hexatriene; FITC, fluorescein isothiocyanate; FD, FITC-labeled dextran

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2. Materials and methods

2.1. Isolation of isoquercitrin

The aerial parts of *A. yomena* Makino (Asteraceae) were collected and air-dried. Next, 1.9 kg of these aerial parts was extracted three times with methanol (MeOH) under reflux, and 120.1 g of residue was produced. The MeOH extract was suspended in water, which was then partitioned sequentially with equal volumes of dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), and n-butanol (BuOH). Each fraction was evaporated in vacuo to yield CH_2Cl_2 (23.6 g), EtOAc (15.2 g), and n-BuOH (48.8 g) residues and water (48.2 g) extract. Column chromatography (CC) was used, and the EtOAc fraction (10.0 g) was chromatographed over a silica gel column using a gradient solvent system of $CHCl_3$:MeOH (5:1 → 1:3) to give five subfractions (E1–E5). The E2 (3.7 g) subfraction was subjected to MCI® Gel CC eluted with a gradient solvent system of MeOH:H_2O (1:1 → 1:0) to yield six subfractions (E21–E26). The E21 (640.1 mg) subfraction was subjected to RP-18 CC eluted with a gradient solvent system of MeOH:H_2O (1:3 → 1:0) to yield nine subfractions (E211–E219). The E218 (60.3 mg) subfraction was purified by silica gel CC ($CHCl_3$:MeOH = 5:1) to produce isoquercitrin (20.2 mg). The physicochemical and spectral data of isoquercitrin, including ^{1}H NMR, ^{13}C NMR, and HSQC, were identical to those reported in the literature (Fig. 1) [14,15].

Yellow powder; $[\alpha]_D^{20}$ − 85° (MeOH, *c* 0.06); EI-MS *m/z*: 464 [M^+]; ^{1}H NMR (300 MHz, CD_3OD) δ: 7.71 (1H, d, *J* = 2.0 Hz, H-2′), 7.57 (1H, dd, *J* = 8.4, 2.0 Hz, H-6′), 6.86 (1H, d, *J* = 8.4 Hz, H-5′), 6.37 (1H, d, *J* = 1.8 Hz, H-8), 6.19 (1H, d, *J* = 1.8 Hz, H-6), 5.24 (1H, d, *J* = 8.0 Hz, H-1″), 3.70–3.29 (6H, m, H-2″–6″). ^{13}C NMR (75 MHz, CD_3OD) δ: 179.6 (s, C-4), 166.1 (s, C-7), 163.1 (s, C-5), 159.1 (s, C-2), 158.6 (s, C-9), 150.0 (s, C-4′), 146.0 (d, C-3′), 135.8 (s, C-3), 123.3 (d, C-6′), 123.2 (s, C-1′), 117.7 (d, C-2′), 116.1 (d, C-5′), 105.8 (d, C-1″), 104.5 (s, C-10), 100.0 (d, C-6), 94.9 (d, C-8), 78.5 (d, C-3″), 78.2 (d, C-5″), 75.9 (d, C-2″), 71.3 (d, C-4″), 62.7 (t, C-6″).

2.2. Antifungal susceptibility test and hemolytic activity assay

Candida albicans (ATCC 90028) and *Candida parapsilosis* (ATCC 22019) were obtained from the American Type Culture Collection (ATCC) (Manassas, VA). *Malassezia furfur* (KCTC 7744), *Trichophyton rubrum* (KCTC 6345), and *Trichosporon beigelii* (KCTC 7707) were obtained from the Korean Collection for Type Cultures (KCTC). The *M. furfur* was cultured at 32 °C in a modified YM broth (Difco) containing 1% olive oil, and the fungal strains were cultured in YPD broth (Difco) with aeration at 28 °C. Fungal cells (2×10^6/mL) were inoculated into YPD or YM broth with 1% olive oil and then dispensed into microtiter plates at a volume of 0.1 mL/well. The minimum inhibitory concentration (MIC) was determined using a standard microdilution method and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay [16,17]. Following overnight incubation, the growth was measured using a microtiter ELISA Reader (Molecular Devices Emax, CA) by monitoring the absorption at 580 nm. The MIC values were determined by three independent assays.

The hemolytic activity of the compounds was assessed by determining the release of hemoglobin from an 8% suspension of human erythrocytes at 414 nm with an ELISA reader. The percentage of hemolysis was calculated using the following equation: hemolysis (%) = 100 [(Abs_{414nm} in the compound solution − Abs_{414nm}) / (Abs_{414nm} in 0.1% Triton X-100 − Abs_{414nm})] [18].

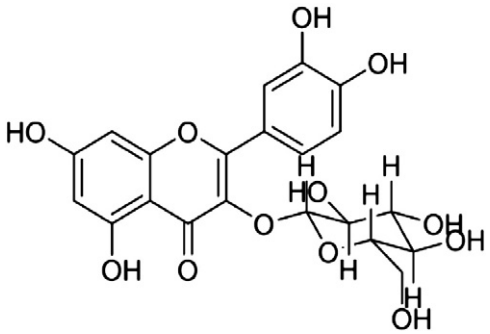

Fig. 1. Structure of isoquercitrin.

2.3. Propidium iodide influx

C. albicans cells (2×10^6/mL), centrifuged at 8000 rpm for 5 min, were suspended in phosphate buffered saline (PBS) and treated with the compounds at a concentration of 2.5 μg/mL. After incubation for 4 h at 28 °C, cells were harvested by centrifugation and suspended in PBS. The cells were then treated with 9 μM propidium iodide and incubated for 5 min at room temperature. The cells were analyzed using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA) [19].

2.4. Potassium release

Potassium release was assessed to examine the change in ion concentration resulting from treatment with the compounds. *C. albicans* cells (OD = 1.0) were resuspended in PBS and treated with the compounds. After incubation for 5-minute intervals at 28 °C, the cells were centrifuged at 13,000 rpm for 10 min. The supernatant was measured using an ion-selective electrode (ISE) meter (Orion Star A214, Thermo Scientific, Singapore). The cells were sonicated to determine 100% potassium release. The percentage of potassium release caused by the compounds was calculated as follows: potassium release (%) = 100 × ($[K^+] - [K^+]_0$) / ($[K^+]_t - [K^+]_0$), where $[K^+]$ represents the potassium release achieved after addition of the compounds and $[K^+]_0$ and $[K^+]_t$ represent the potassium release without the compounds and with sonication, respectively [20].

2.5. Change in membrane electrical potential

To detect the change in the membrane electrical potential, a membrane potential sensitive probe, 3,3′-dipropylthiacarbocyanine iodide [$DiSC_3(5)$] was used. *C. albicans* cells (2×10^6/mL) were centrifuged at 10,000 rpm for 5 min and washed with Ca^{2+} and Mg^{2+}-free PBS. Changes in the fluorescence, caused by the collapse of the membrane potential by 2.5 μg/mL of the compounds, were continuously monitored using a spectrofluorometer (Shimadzu RF-5301PC; Shimadzu, Japan) at an excitation wavelength of 622 nm and an emission wavelength of 670 nm. To ensure reproducibility, the measurement was repeated two times under each condition [21].

2.6. Preparation of giant unilamellar vesicles

Giant unilamellar vesicles (GUVs) were prepared using indium tin oxide (ITO)-coated glass. Lipids, composed of phosphatidylcholine (PC)/phosphatidylethanolamine (PE)/phosphatidylinositol (PI)/ergosterol (5:4:1:2, w/w/w/w), were prepared at a concentration of 3.75 mg/mL in chloroform. The lipid solutions were coated onto an ITO-coated glass for 5 min in a spin coater (Spin Coater, ACE-1020 Series) and then evaporated under vacuum for 2 h. The lipid-coated and uncoated glass were separated by a distance of 2 mm with a thin Teflon spacer. The chamber was filled with 10 mM HEPES buffer (pH 7.2) through a hole in the silicon spacer. The application of a 1.7-V (peak-to-peak) and 10-Hz sine wave was immediately applied to the ITO electrodes for 2 h at room temperature. GUVs from the ITO glass were then detached with the following conditions: 4-V (peak-to-peak) and 10-Hz sine wave for 10 min. The GUVs were treated with the compounds and changes in the GUVs were observed with an inverted microscope (Nikon Eclipse Ti-S, Japan) [22].

Table 1
The antifungal activity of isoquercitrin and amphotericin B.

Fungal strains	MIC (μg/mL)	
	Isoquercitrin	Amphotericin B
C. albicans ATCC 90028	2.5	1.3
C. parapsilosis ATCC 22019	2.5	1.3
M. furfur KCTC 7744	5.0	2.5
T. rubrum KCTC 6345	2.5	2.5
T. beigelii KCTC 7707	5.0	1.3

2.7. Calcein leakage

Large unilamellar vesicles (LUVs), PC/PE/PI/ergosterol (5:4:1:2, w/w/w/w), and encapsulating calcein were prepared by vortexing the dried lipids in a dye buffer solution (70 mM calcein, 10 mM Tris, 150 mM NaCl, and 0.1 mM EDTA [pH 7.4]). The suspension was freeze-thawed in liquid nitrogen for 11 cycles and extruded through polycarbonate filters (two stacked 200-nm pore size filters) with a LiposoFast extruder (Avestin Inc., Ottawa, Canada). Untrapped calcein was removed via gel filtration on a Sephadex G-50 column. The leakage of calcein from the LUVs was monitored by measuring the fluorescence intensity at an excitation wavelength of 490 nm and an emission wavelength of 520 nm with a spectrofluorometer (Shimadzu RF-5301PC, Shimadzu, Japan). To determine 100% dye release, 30 μL of 1% Triton X-100 was added to the vesicles. The percentage of dye leakage caused by the compounds was calculated as follows: dye leakage (%) = $100 \times (F - F_0) / (F_t - F_0)$, where F represents the fluorescence intensity achieved after addition of the compounds and F_0 and F_t represent the fluorescence intensities without the compounds and with Triton X-100, respectively [23].

2.8. Fluorescence intensity of the plasma membrane

Fluorescence emitted from the plasma membrane of *C. albicans* cells labeled with 1,6-diphenyl-1,3,5-hexatriene (DPH) (Molecular probes, Eugene, Oregon) was used to monitor changes in membrane dynamics. *C. albicans* cells (2×10^6/mL) treated with compounds were incubated for 1 h at 28 °C and fixed with 0.37% formaldehyde. After washing with cold PBS, the cells were frozen with liquid nitrogen and then thawed with warm PBS twice. The suspensions were incubated with 0.6 mM DPH for 45 min at 28 °C and washed with PBS three times. PBS was added to the supernatant and the fluorescence intensity of DPH was measured using a spectrofluorometer (Shimadzu RF-5301PC, Shimadzu, Japan) at 350 nm excitation and 425 nm emission wavelengths [24].

2.9. Morphological change

C. albicans cells (2×10^6/mL), centrifuged at 8000 rpm for 5 min, were suspended in PBS and treated with 2.5 μg/mL of the compounds. After incubation for 4 h at 28 °C, the cells were harvested by centrifugation at 12,000 rpm for 5 min and suspended in PBS. The morphological changes were then analyzed. Non-stained living cells were evaluated for each sample by determining their position on a forward scatter (FS) versus side scatter (SS) contour plot [19].

2.10. Leakage of fluorescein isothiocyanate-labeled dextrans

FITC-labeled dextran (FD) 10 and FD20 (molecular weight = 10 and 20 kDa) were used to evaluate the extent of membrane damage induced by the compounds. Lipids [PC/PE/PI/ergosterol (5:4:1:2, w/w/w/w)] were prepared, and the chloroform was removed with an evaporator for 30 min. To encapsulate the FD in the liposomes, the dried lipids were vortexed with 2.4 mg/mL of FD in a dye buffer solution (10 mM Tris, 150 mM NaCl, and 0.1 mM EDTA [pH 7.4]). The method for preparing liposomes containing FD was the same as that for preparing liposomes containing calcein. The leakage of FD from the liposomes was monitored by measuring the fluorescence intensity at an excitation wavelength of 494 nm and an emission wavelength of 520 nm using a spectrofluorometer (Shimadzu RF-5301PC, Shimadzu, Japan). The maximum fluorescence intensity was determined by adding 30 μL of 1% Triton X-100 to a liposome suspension. The percentage of FD leakage was calculated in the same manner as the percentage of calcein leakage [25].

Table 2
Hemolytic activity of compounds against human erythrocyte cells.

Compounds	Hemolysis (%)						
	80.0 μg/mL	40.0 μg/mL	20.0 μg/mL	10.0 μg/mL	5.0 μg/mL	2.5 μg/mL	1.3 μg/mL
Isoquercitrin	16.78	0	0	0	0	0	0
Amphotericin B	100	46.65	16.05	0	0	0	0

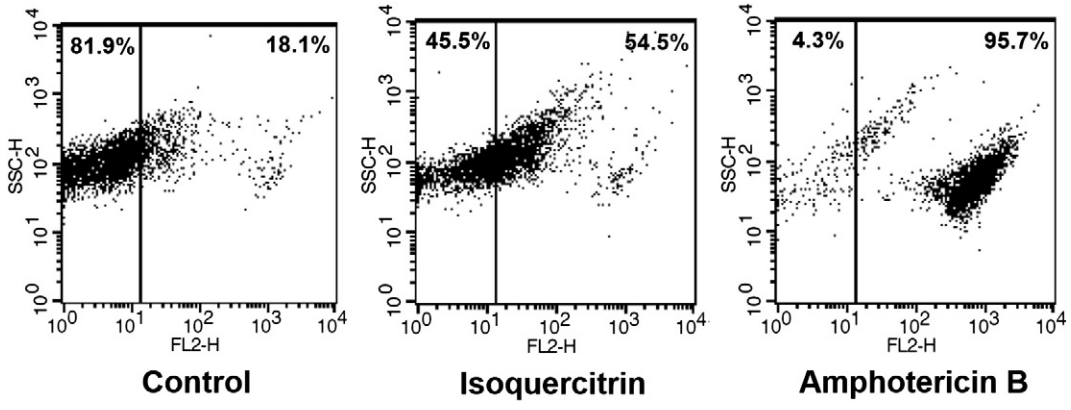

Fig. 2. Analysis of membrane permeabilization by propidium iodide staining in *C. albicans*. The percentages indicate the population of cells stained with propidium iodide.

3. Results and discussion

3.1. Antifungal and hemolytic activities

Isoquercitrin is a phytochemical, specifically a flavonoid, isolated from *A. yomena*. The antifungal activity of isoquercitrin has not previously been described in detail so we examined its effect and mode of action. The effect of isoquercitrin on various pathogenic fungal strains was determined using the susceptibility test. Amphotericin B, an antifungal agent used to treat serious fungal infections, was used as a positive control [26,27]. The results showed that isoquercitrin was effective as an antifungal treatment with MIC values of 2.5–5.0 μg/mL, but the effect was less than that of amphotericin B, which had MIC values of 1.3–2.5 μg/mL (Table. 1). These ranges were sufficient to eliminate the pathogenic fungi. Furthermore, to evaluate the presence of hemolytic activity, human erythrocytes were treated with various concentrations of isoquercitrin, ranging from 1.3 μg/mL to 80.0 μg/mL (Table. 2). Isoquercitrin did not induce hemolytic activity at concentrations less than 80.0 μg/mL, whereas amphotericin B induced hemolytic activity at 20.0 μg/mL. Considering all of these results, we confirmed that isoquercitrin had a potent antifungal effect with almost no hemolysis.

3.2. Effect of membrane disruption

Phytochemicals are generally known to cause membrane disturbance resulting in the loss of membrane integrity [28]. We therefore investigated whether isoquercitrin targets the cell membrane. First, we analyzed membrane damage by monitoring the influx of propidium iodide. Propidium iodide, a DNA-staining fluorescent probe, is impermeable to the cell membrane and only penetrates the cell if there are severe lesions on the membrane, which results in an increase in fluorescence intensity [29,30]. Increased fluorescence intensity was shown in cells treated with compounds (Fig. 2). The percentage of propidium iodide influx following exposure to isoquercitrin and amphotericin B was 54.5% and 95.7%, respectively, whereas that of the control was 18.1%. These influxes of PI, impermeable molecule, indicated that isoquercitrin did induced membrane lesions, decreasing membrane integrity and increasing membrane permeability.

To confirm membrane permeabilization in respect of the efflux of molecule, a potassium ion release assay was performed. The concentration of potassium ions within the cell is generally high and it must remain as such when there are no changes in the environment for homeostasis to be maintained [20,31]. When normal maintenance of the cell is compromised due to changes such as membrane damage and permeabilization, potassium ions are released from the cell. After treatment with compound, the potassium ion concentration outside of the cell was increased (Fig. 3). Cells treated with isoquercitrin and amphotericin B for 20 min showed a 37.0% and 80.7% release of potassium ions, respectively, as compared to untreated cells, which released only 10.0% of potassium ions. We therefore confirmed that isoquercitrin affects fungal cells by damaging the cell membrane, causing membrane permeabilization, and altering the potassium ion concentration inside and outside the cells [32,33].

The inner membrane maintains negative polarization through maintenance actions such as potassium influx and potassium ion which are essential for the maintenance of the membrane potential. As shown with the potassium ion release assay, potassium ions are released from the cell when the membrane is disturbed, and this may contribute to the change in membrane potential [34]. We then performed one additional experiment with $DiSC_3(5)$ to detect the change in membrane potential. $DiSC_3(5)$ is a voltage-sensitive fluorescent probe used for examining membrane depolarization [35]. It accumulates in the cell membrane, where it self-quenches its own fluorescence, depending on the membrane potential [36]. After $DiSC_3(5)$ (at 50 s) was added to a *C. albicans* cell suspension, cells were treated with isoquercitrin or amphotericin B (at 200 s) (Fig. 4). The fluorescence intensity was shown to increase as soon as the cells were treated, whereas the intensity of untreated cells remained at baseline level. These results showed that the cell membrane was disturbed and that membrane potential dissipated with the transition of ions.

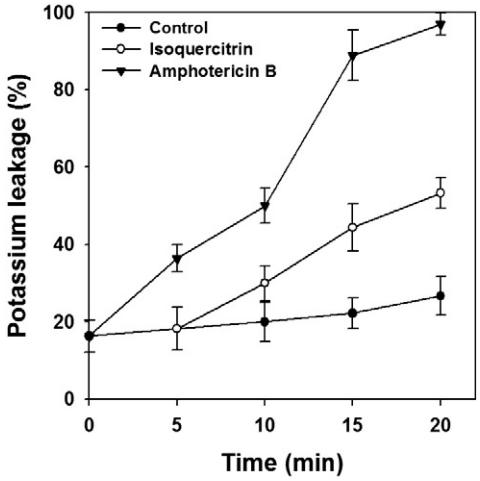

Fig. 3. The percentage of potassium released from *C. albicans* induced by the compounds was measured every 5 min. Results are based on three independent experiments, performed in triplicate. The error bars represent the standard deviation (SD).

3.3. Action related to membrane disturbance

We prepared artificial cells, namely GUVs and LUVs, to study the mode of action of isoquercitrin on the membrane [22,23]. These liposomes, which encapsulate calcein, have been useful for model membrane experiments. Membrane disturbance can be observed optically using inverted fluorescence microscopy to monitor changes in fluorescence and the shape of the GUVs [37]. GUVs, mimicking a fungal membrane, have been used to examine the physical and biological properties of membranes, including changes in shape, interactions of macromolecules, and membrane fusion [38]. When treated with isoquercitrin or amphotericin B, the diameter of the GUVs diminished and fluorescence intensity faded due to the leakage of calcein leakage over time (Fig. 5A). By contrast, the fluorescence intensity and size of control GUVs were maintained. With these results, we confirmed that isoquercitrin induced membrane disturbance [39].

Subsequently, calcein leakage from LUVs was investigated. If the liposome membrane is disturbed, calcein, an impermeable molecule, can leak out of the membrane [40,41]. Isoquercitrin or amphotericin B

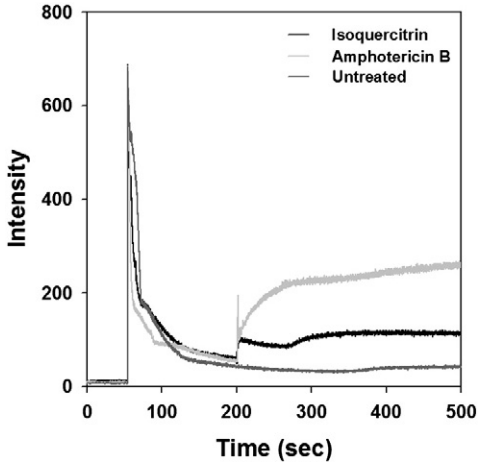

Fig. 4. Depolarization of membrane potential was detected by using $DiSC_3(5)$. $DiSC_3(5)$ was added at t = 50 s. The compounds were added at t = 200 s.

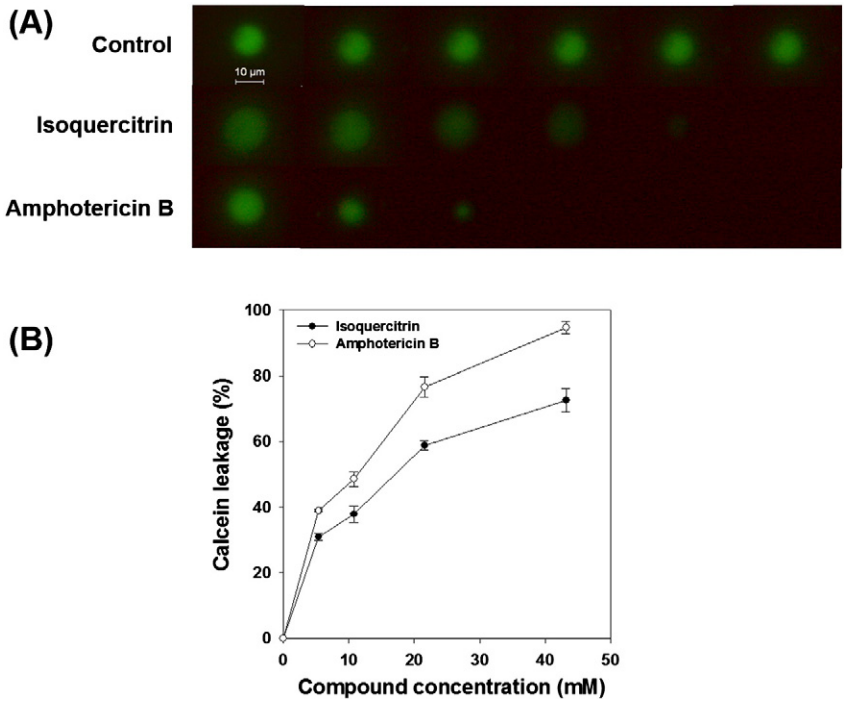

Fig. 5. (A) The response of GUVs, which comprised phosphatidylcholine (PC)/phosphatidylethanolamine (PE)/phosphatidylinositol (PI)/ergosterol (5:4:1:2, w/w/w/w), in the presence of the compounds at 0, 10, 20, 30, 40, and 50 s. The scale bar represents 10 μm. (B) Calcein leakage from LUVs [PC/PE/PI/ergosterol(5:4:1:2, w/w/w/w)] induced by the compounds (5.4, 10.8, 21.6, and 43.2 μM).

was applied in a dose-dependent manner (MIC × 1, ×2, ×4, ×8), and calcein leakage gradually increased dependent on the concentration of the compounds (Fig. 5B). Treatment with isoquercitrin or amphotericin B induced the calcein leakage from the LUVs, respectively. Furthermore, calcein leakage percentages increased in a dose-dependent manner. These results suggest that isoquercitrin disturbs the membrane and that efficiency of membrane disturbance is concentration-dependent [42].

To further verify the existence of membrane disturbance induced by isoquercitrin, we performed an in vivo test. Changes such as lipid bilayer

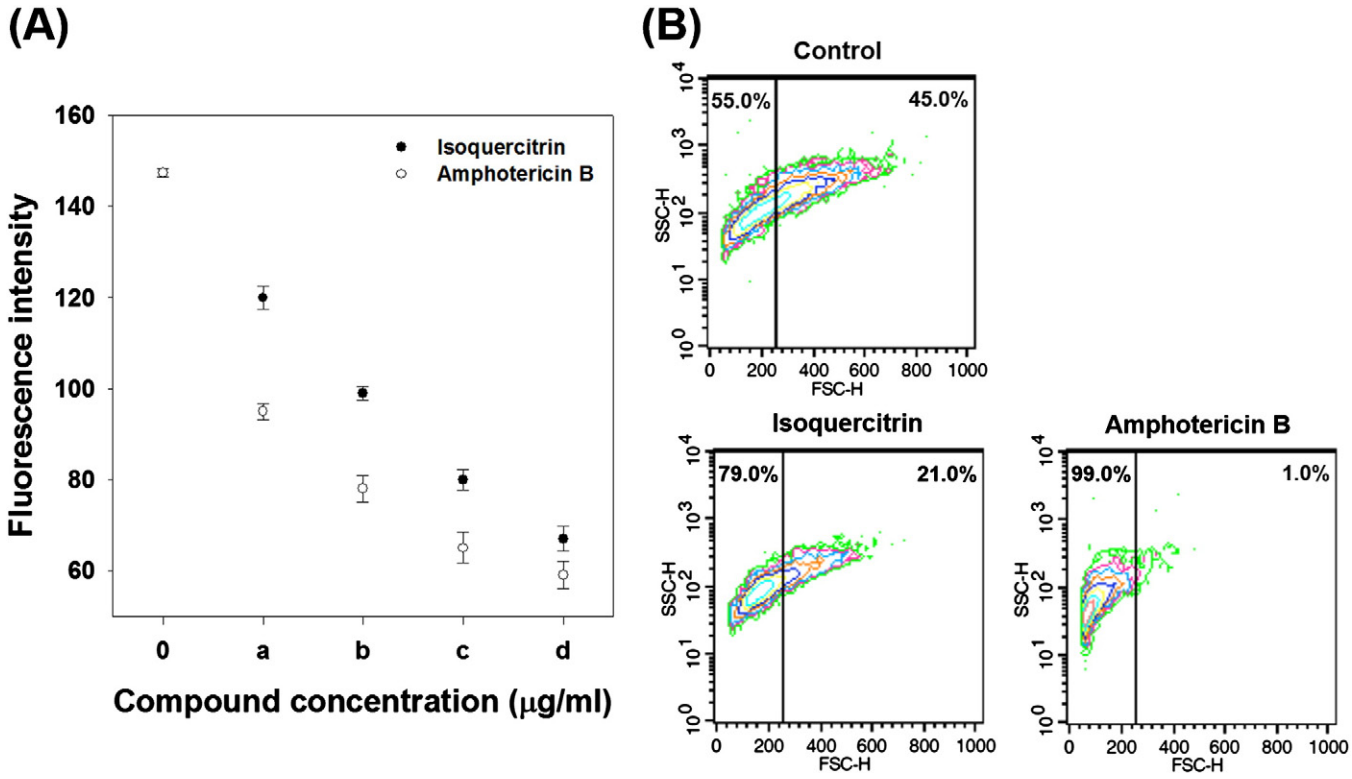

Fig. 6. (A) DPH fluorescence intensity of *C. albicans* cell treated with a, 2.5; b, 5.0; c, 10.0; and d, 20.0 μg/mL of compounds (as indicated). The error bars represent the SD values for three independent experiments, performed in triplicate. (B) Flow cytometric analysis treated with compounds. FSC is an indicator of cell size, and SSC (log) is an indicator of cell granularity.

perturbation and membrane loss are associated with membrane disruption and pore formation [43,44]. For this reason, we performed a DPH assay in *C. albicans*. DPH, a membrane fluorescent probe, is hydrophobic and associates with the lipophilic tails of phospholipids in the cytoplasmic membrane but does not disturb its structure [45,46]. Disorder in membrane lipids is inevitable when membrane is perturbed or pores form and then DPH becomes detached from the membrane under these conditions. The concentration of isoquercitrin and amphotericin B used for treatment was doubled (MIC × 1, ×2, ×4, ×8) and the fluorescence intensity of cell treated with compound was detected. As the concentration of compounds increased, the membrane became more unstable, DPH detached, and fluorescence intensity thereby decreased (Fig. 6A). Consequently, we conclude that isoquercitrin induced perturbation of membrane lipid bilayer and disorder in membrane.

When there are pores, which form unexpectedly, or other damage to the membrane such as membrane disturbance, the ions can move freely across the membrane and the driving force for the movement of the water is largely removed. Therefore, we examined morphological changes such as cell shrinkage (Fig. 6B). FSC (x-axis) is an indicator of cell size, and SSC (y-axis) is an indicator of cell granularity [47]. When cells were exposed to isoquercitrin or amphotericin B, a significant decrease in cell size and extensive shrinkage were observed. Decreased FSC values following treatment with the compounds were 24.0% (isoquercitrin) and 44.0% (amphotericin B) of the total cell population compared to baseline (established in control conditions). These shrinkages demonstrated that, membrane permeability increased and cell shrinkage occurred along with the loss of ions and osmotic pressure.

3.4. Degree of membrane disturbance

An FD leakage assay was conducted with molecules different in molecular weight and radius. FDs, dextrans linked with fluorescein isothiocyanate, have been widely used in diverse contexts such as degree of permeability and pore size [48,49]. To evaluate the degree of disturbance induced by the compounds, the leakage of FD10 (Stokes–Einstein radius = 2.3 nm) and FD20 (Stokes–Einstein radius = 3.3 nm) from liposomes was measured (Fig. 7) [50]. Treatment with isoquercitrin or amphotericin B resulted in the release of 26.7% and 53.6% of FD10 from the liposomes, respectively. However, treatment with either compound did not result in the release of FD20 from the liposomes. Based on these results, the degree of disturbance was assumed to be between 2.3 and 3.3 nm. These size ranges indicate that intracellular components can move inside and outside of the cell freely. Considering all results, we conclude that isoquercitrin induces membrane disturbance in fungal cells. Due to these unexpected exchanges, cell homeostasis will not be consistently maintained; hence, membrane permeabilization and osmosis are induced, resulting in cell death [51].

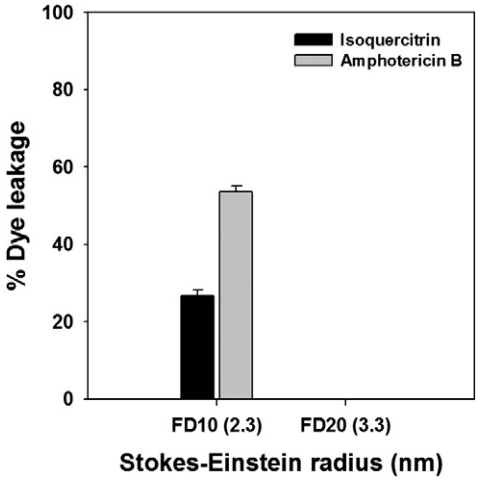

Fig. 7. Percentage of FITC-dextrans (FDs), leaking from liposomes, induced by treatment with isoquercitrin and amphotericin B.

Isoquercitrin acts as a potent antifungal agent with almost no hemolysis in humans. Based on several membrane experiments, isoquercitrin targets the cell membrane and causes membrane permeabilization and depolarization. Additionally, we demonstrate that perturbation of the membrane lipid bilayer and cell shrinkage (caused by osmotic pressure) which occurred with the addition of isoquercitrin, lead to cell death. Hence, isoquercitrin is suitable for clinical applications requiring a potent and stable effect on the human body.

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