Research Article

Antiaging of Cucurbitane Glycosides from Fruits of Momordica charantia L.

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Methanol extracts of Momordica charantia L. fruits are extensively studied for their antiaging activities. A new cucurbitane-type triterpenoid (1) and nine other known compounds (2–10) were isolated, and their structures were determined according to their spectroscopic characteristics and chemical derivatization. Biological evaluation was performed on a K6001 yeast bioassay system. The results indicated that all the compounds extended the replicative lifespan of K6001 yeast significantly. Compound 9 was used to investigate the mechanism involved in the increasing of the lifespan. The results indicated that this compound significantly increases the survival rate of yeast under oxidative stress and decreases ROS level. Further study on gene expression analysis showed that compound 9 could reduce the levels of UTH1 and SKN7 and increase SOD1 and SOD2 gene expression. In addition, it could not extend the lifespan of the yeast mutants of Uth1, Skn7, Sod1, and Sod2. These results demonstrate that compound 9 exerts antiaging effects via antioxidative stress and regulation of UTH1, SKN7, SOD1, and SOD2 yeast gene expression.

1. Introduction

Fruits of Momordica charantia L. are edible healthy vegetable in Asia and commonly known as bitter melon or bitter gourd because of their bitter taste. Given their nutritional potential, they are used as traditional Chinese herbal medicine to treat several ailments, such as diabetes, constipation, abdominal pain, kidney stones, piles, pneumonia, and improve appetite [1–5]. M. charantia contains biologically active phytochemicals, such as polysaccharides, proteins, flavonoids, glycosides, saponins, steroids, alkaloids, essential oils, and triterpenes [5–10]. Many of these phytochemicals exhibit antitumor, anti-inflammatory, immunomodulation, and antidiabetic activities and the ability to reduce oxidative stress [5].

Aging is a dominating risk factor for age-related diseases, including cancer, metabolic disease, cardiovascular disease, and neurodegenerative illnesses [11]. As the aging population is increasing dramatically throughout the world, aging has drawn great attention because of huge expenses for medical care and serious consequences of the related diseases. Interventions that delay aging were found to have a greater effect on the quality of life compared with disease-specific approaches [12]. In our previous studies [13–17], a yeast mutant K6001 was employed in the bioassay system, and ganodermasides A–D, phloridzin, nolinospirose F, and parishin with significant antiaging potential from natural sources were obtained.

Basing on the K6001 bioassay system, we isolated one novel cucurbitane glycoside (1) and nine known cucurbitane-type triterpenoids (2–10) from the fruits of M. charantia L. (Figure 1). Essential studies on the action mechanism suggested that these cucurbitane glycosides could improve the antioxidative properties of yeasts. The yeast genes of youth 1 (UTH1), skinhead-7 (SKN7), and superoxide dismutase (SOD) may also be involved in the action.

2. Material and Methods

2.1. General. The chemical reagents used were of HPLC grade and purchased from TEDIA (Rhode Island, USA). The others were of analytical grade and obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).
The preparative HPLC system was equipped with two ELITE P-230 pumps and an UV detector. Optical rotations were determined on a JASCO P-1030 digital polarimeter. High-resolution ESI-TOF-MS analyses were performed on an Agilent Technologies 6224A Accurate-Mass TOF LC/MS system (Santa Clara, CA, USA). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AV III-500 spectrometer (Bruker, Billerica, MA, USA). Column chromatography was performed over the silica gel (200–300 mesh, Yantai Chemical Industry Research Institute, Yantai, China) or reversed phase C18 (Octadecylsilyl, ODS) silica gel (Cosmosil 75C18-OPN, Nacalai Tesque, Japan).

2.2. Plant Material and Yeast Strains. Fruits of *M. charantia* were purchased from Liangzhu market of Hangzhou, Zhejiang Province, China, 2011. The identity of this plant was confirmed by an associate professor Liurong Chen, and a voucher specimen (number 20110712) was preserved in Zhejiang University Institute of Materia Medica. The yeast strains BY4741 and mutants of *uth1*, *skn7*, *sod1*, and *sod2* with K6001 background are from Prof. Matsuura in Chiba University, and K6001 strain with W303 background is from Prof. Breitenbach in Salzburg University.

2.3. Extraction and Isolation. About 1.5 kg (dry weight) of the material was smashed and extracted with methanol (MeOH) for 3 days with shaking at room temperature. The extract was filtered and concentrated to obtain a crude extract (224 g), which was partitioned with ethyl acetate (EtOAc) and water. The active EtOAc layer (30 g) was subjected to a silica gel open column with *n*-hexane/acetone (99 : 1, 98 : 2, 95 : 5, 90 : 10, 80 : 20, 70 : 30, 60 : 40, 50 : 50, and 0 : 100) and acetone/MeOH (50 : 50 and 0 : 100). The EtOAc layer yielded nine fractions after the combination based on TLC analysis. The eighth active fraction of 4.0 g (13.9 g in total) was subsequently separated by ODS open column with MeOH/H2O (60 : 40, 70 : 30, 75 : 25, 80 : 20, 90 : 10, and 100 : 0), and 11 samples were obtained (fr.1–fr.11). The four active samples of fr.6–fr.9 were further separated as follows:

Fr.6 (600 mg) was introduced to an ODS open column with MeOH/H2O (70 : 30, 73 : 27, 75 : 25, 77 : 23, 80 : 20, 83 : 17, 85 : 15, and 100 : 0) to yield nine samples (fr.6-1–fr.6-9). Fr.6-6 (72 mg) was further separated by a silica gel open column chromatography with dichloromethane (CH2Cl2)/MeOH (100 : 0, 99 : 1, 98 : 2, 97.5 : 2.5, 95 : 5, 90 : 10, and 0 : 100), and the sixth active fraction (11.6 mg) was purified by HPLC [C30-UG-5 (φ 10 × 250 mm, Nomura Chemical), mobile phase: acetonitrile (MeCN)/H2O (90 : 10), flow rate: 3 mL/min, and detector: 210 nm] and yielded compound 1 (4.6 mg, *t*R = 33.5 min) and compound 2 (1.0 mg, *t*R = 30.7 min).

Fr.7 (277 mg) was separated in a silica gel open column with chloroform (CHCl3)/MeOH (100 : 0, 98 : 2, 95 : 5, 90 : 10, and 0 : 100) and yielded compound 3 (10.6 mg, *t*R = 15.9 min), compound 4 (1.8 mg, *t*R = 17.1 min), and compound 5 (1.9 mg, *t*R = 18.7 min).

Fr.8 (340 mg) was introduced to a silica open column with *n*-hexane/CHCl3 (50 : 50, 30 : 70, and 0 : 100) and CHCl3/MeOH (97 : 3, 95 : 5, 90 : 10, and 0 : 100) to give compound 6 (1.8 mg, *t*R = 13.9 min) and compound 7 (1.6 mg, *t*R = 17.1 min).
[CAPCELL PAKC$_{18}$] Shiseido (q10×250 mm), mobile phase: MeCN/H$_2$O (63:37), flow rate: 3 mL/min, and detector: 210 nm) and yielded compound 6 (10.2 mg, $t_R = 22.0$ min), compound 7 (4.9 mg, $t_R = 22.9$ min), and compound 8 (1.3 mg, $t_R = 32.0$ min).

Fr.9 (600 mg) was separated by silica gel open column with CHCl$_3$/MeOH (100:0, 100:1, 100:2, 100:3, 100:5, 90:10, and 0:100), and fr.9-1–fr.9-8 were obtained. Then, fr.9-4 (332 mg) was further separated by ODS open column with MeOH/H$_2$O (90:10, 95:5, and 100:0), and the third fraction (42 mg) was purified by HPLC [CAPCELL PAKC$_{18}$] Shiseido (q10×250 mm), mobile phase: MeCN/H$_2$O (60:40), flow rate: 3 mL/min, and detector: 210 nm) and yielded compound 9 (5.0 mg, $t_R = 28.7$ min) and compound 10 (8.0 mg, $t_R = 38.3$ min).

2.3.1. Compound 1. White solid; [a]$_D^{20}$-78.6 (c 0.2, CH$_3$OH); high-resolution ESI-TOF-MS m/z 653.4029, calculated for C$_{37}$H$_{58}$O$_8$Na$^+$ 653.4024; data of $^1$H NMR and $^{13}$C NMR are shown in Table 1.

2.3.2. Charantoside IV (2). Colorless solid; [a]$_D^{28}$-143.9 (c 0.16, CH$_3$OH) MS m/z 623 (M + Na)$^+$, C$_{38}$H$_{60}$O$_{9}$Na; $^{13}$C NMR (125 MHz, pyridine-d$_5$): $\delta = 15.4, 19.3, 19.3, 20.6, 21.5, 24.2, 26.0, 28.0, 28.7, 31.4, 33.8, 37.2, 39.4, 40.5, 45.7, 45.9, 49.3, 51.0, 52.7, 63.7, 69.7, 72.9, 73.5, 76.6, 80.5, 85.5, 86.3, 104.3, 115.1, 120.3, 130.4, 134.6, 135.1, and 142.9. The structure was identified based on comparison of MS, $^1$H, and $^{13}$C NMR data with literature [18].

2.3.3. Monnordicoside F$_2$ (3). White solid; [a]$_D^{20}$-101.0 (c 0.94, CHCl$_3$:CH$_3$OH = 1:1); MS m/z 641 (M + Na)$^+$, C$_{39}$H$_{60}$O$_{9}$Na; $^{13}$C NMR (125 MHz, pyridine-d$_5$): $\delta = 15.4, 19.3, 19.3, 20.6, 21.5, 24.3, 26.0, 28.0, 28.6, 31.3, 31.3, 31.4, 33.8, 37.0, 39.4, 39.9, 40.5, 45.7, 45.8, 49.3, 50.6, 52.7, 63.7, 69.7, 70.1, 72.9, 73.5, 76.6, 80.5, 85.5, 86.3, 104.2, 124.6, 130.4, 134.6, and 142.1. The structure was identified based on comparison of MS, $^1$H, and $^{13}$C NMR data with literature [19].

2.3.4. Goyaglycoside-b (4). White solid; [a]$_D^{20}$-100.7 (c 0.2, CH$_3$OH); MS m/z 671 (M + Na)$^+$, C$_{39}$H$_{60}$O$_{9}$Na; $^{13}$C NMR (125 MHz, pyridine-d$_5$): $\delta = 15.3, 19.1, 19.3, 20.4, 21.7, 23.7, 25.3, 27.8, 28.6, 31.3, 31.3, 31.3, 34.3, 37.0, 39.5, 40.0, 42.0, 42.6, 45.7, 48.6, 48.7, 50.8, 58.1, 63.7, 69.7, 70.1, 72.2, 74.2, 77.0, 83.9, 86.0, 102.8, 112.8, 124.7, 132.0, 133.6, and 142.1. The structure was identified based on comparison of MS, $^1$H, and $^{13}$C NMR data with literature [4].

2.3.5. Karaviloside III (5). White solid; [a]$_D^{28}$+70.9 (c 0.1, CH$_3$OH); MS m/z 657 (M + Na)$^+$, C$_{37}$H$_{62}$O$_{9}$Na; $^{13}$C NMR (125 MHz, pyridine-d$_5$): $\delta = 16.0, 18.5, 19.4, 23.1, 26.3, 28.3, 29.3, 29.4, 29.6, 30.8, 31.3, 31.3, 33.2, 34.8, 35.4, 37.1, 39.8, 40.0, 42.4, 46.7, 48.7, 49.3, 50.6, 56.6, 63.8, 69.7, 70.1, 72.5, 73.9, 76.1, 78.0, 88.3, 105.4, 119.5, 124.2, 142.2, and 148.4. The structure was identified based on comparison of MS, $^1$H, and $^{13}$C NMR data with literature [20].
2.3.6. Charantoside VI (6). White solid; [α]_{D}^{20} -72.7 (c 0.48, CH$_3$OH); MS m/z 655 (M + Na)$^+$, C$_{45}$H$_{60}$O$_{18}$Na; $^{13}$C NMR (125 MHz, pyridine-d$_5$); δ = 15.0, 18.8, 19.3, 20.3, 20.6, 21.5, 24.2, 26.0, 26.3, 28.0, 29.1, 31.5, 33.8, 34.2, 39.4, 40.5, 43.4, 45.7, 45.9, 49.3, 51.7, 52.7, 55.7, 63.7, 69.7, 72.9, 73.5, 76.6, 78.0, 80.5, 86.3, 102.7, 132.0, 133.5, and 135.5. The structure was identified based on comparison of MS, $^{1}$H, and $^{13}$C NMR data with literature [18].

2.3.7. Charantagenin E (7). Colorless solid; [α]_{D}^{28} -104.3 (c 0.11, CH$_3$OH); MS m/z 685 (M + Na)$^+$, C$_{38}$H$_{62}$O$_{9}$Na; $^{13}$C NMR (125 MHz, pyridine-d$_5$); δ = 15.2, 18.8, 19.1, 20.4, 20.4, 21.7, 23.7, 25.3, 26.2, 27.8, 29.0, 31.4, 34.2, 34.2, 39.5, 42.0, 42.6, 43.4, 45.7, 48.5, 48.7, 51.8, 55.7, 58.0, 63.7, 69.7, 72.2, 74.2, 76.9, 77.0, 83.9, 86.0, 102.8, 112.8, 127.8, 132.0, 133.5, and 135.5. The structure was identified according to the comparison among MS, $^{1}$H, and $^{13}$C NMR data in literature [21].

2.3.8. Charantoside II (8). White solid; [α]_{D}^{20} -67.1 (c 0.2, CH$_3$OH); MS m/z 685 (M + Na)$^+$, C$_{38}$H$_{62}$O$_{9}$Na; $^{13}$C NMR (125 MHz, pyridine-d$_5$); δ = 15.2, 18.5, 19.1, 19.3, 20.4, 21.6, 23.7, 25.2, 26.2, 27.7, 28.8, 31.4, 33.2, 34.2, 39.5, 42.0, 42.6, 43.7, 45.9, 48.5, 48.7, 51.7, 56.0, 58.0, 63.6, 69.6, 72.2, 74.1, 75.2, 76.9, 84.0, 85.9, 102.9, 112.8, 127.8, 132.0, 133.5, and 134.9. The structure was identified based on comparison of MS, $^{1}$H, and $^{13}$C NMR data with literature [18].

2.3.9. Momordicoside G (9). White solid; [α]_{D}^{28} -90.2 (c 1.0, CH$_3$OH); MS m/z 655 (M + Na)$^+$, C$_{38}$H$_{60}$O$_{18}$Na; $^{13}$C NMR (125 MHz, pyridine-d$_5$); δ = 15.5, 19.3, 20.6, 21.5, 24.3, 26.0, 26.5, 26.9, 28.0, 28.6, 31.5, 33.8, 36.7, 39.4, 40.1, 40.5, 45.7, 45.9, 49.3, 50.6, 50.7, 52.7, 63.7, 65.3, 67.9, 72.9, 73.5, 75.3, 76.6, 80.6, 85.5, 86.3, 104.2, 128.8, 130.4, 134.6, and 138.1. The structure was identified based on comparison of MS, $^{1}$H, and $^{13}$C NMR data with literature [19].

2.3.10. Gayaglycoside-d (10). White solid; [α]_{D}^{28} -124.9 (c 0.1, CH$_3$OH); MS m/z 685 (M + Na)$^+$, C$_{38}$H$_{62}$O$_{9}$Na; $^{13}$C NMR (125 MHz, pyridine-d$_5$); δ = 15.3, 19.1, 19.3, 20.4, 21.6, 23.7, 25.3, 26.4, 26.9, 27.8, 28.6, 31.3, 34.3, 36.8, 39.5, 40.1, 40.5, 42.0, 42.6, 45.7, 48.5, 48.7, 50.5, 50.8, 58.1, 63.7, 69.6, 72.2, 74.1, 75.3, 77.0, 83.9, 86.0, 102.8, 112.8, 128.9, 132.0, 133.6, and 138.0. The structure was identified based on comparison of MS, $^{1}$H, and $^{13}$C NMR data with literature [19].

2.4. Acid Hydrolysis and Sugar Analysis of Compound 1. The absolute configuration of sugar moity of compound 1 was determined according to a previously reported method [22, 23]. Briefly, compound 1 (0.5 mg) in anhydrous 2.0M HCl in MeOH (1 mL) was heated at 80°C with reflux for 4h. The reaction solution was evaporated and partitioned between chloroform and water. The residue of aqueous layer was heated with 0.5 mg L-cysteine methyl ester in pyridine (200 μL) at 60°C for 1h; then, o-tolyl isothiocyanate dissolved in 100 μL pyridine (7 mg/mL) was added to the reaction mixture and further reacted at 60°C for 1h. After that, the reaction mixture was dried and analyzed by LC/HRESIMS with the following conditions: Agilent Extend C18 column (3.5 μm, 3.0×100 mm); DAD detection, 210 nm; t = 0 min.
with PBS, and their DCF fluorescence was measured by a fluorescent plate reader at excitation and emission wavelengths of 488 and 525 nm, respectively.

2.8. Real-Time Quantitative PCR Analysis. BY4741 yeast cells were cultured in glucose medium following the addition of 0, 1, 3 μM compound 9. RNA was extracted from yeast cells in the exponential phase through the hot-phenol method. Reverse transcription was performed using a cloned AMV first-strand cDNA synthesis kit (Invitrogen, California, USA) with oligo (dT) adaptor primers and 5 μg of yeast total RNA. Real-time PCR was performed using the CFX96-Touch (Bio-Rad, Hercules, USA) and SYBR Premix EX Taq™ (TakaRa, Otsu, Japan). Thermal cycling parameters for UTH1 and SKN7: 40 cycles, 94°C for 15 s, 55.4°C for 15 s, and 68°C for 20 s; for SOD1 and SOD2: 40 cycles, 94°C for 15 s, 60°C for 25 s, and 72°C for 10 s. Primers used were as follows: for UTH1, sense 5′-CGC CTC TTC TCT TT-3′ and antisense 5′-ACC ATC GGA AGG TTG TTC AG-3′; for SKN7, sense 5′-AGT TGT CAG CGA CGG TCT TT-3′ and antisense 5′-GCT GTG GCA CCA TCT AGG TT-3′; for SOD1 sense 5′-CAC CAT TTT CGT CCG CCT TT-3′ and antisense 5′-TGG TGT CTC TGC TGG TC-3′; for SOD2, sense 5′-CTC CGG TCA AAT CAA CGA AT-3′ and antisense 5′-CTG TGG CCA GAA GAT CTG AG-3′; for TUB1, sense 5′-CCA AGG GCT ATT TAC GTG GA-3′ and antisense 5′-GGT GTA ATG GCC TCT TGC AT-3′. The amount of UTH1, SKN7, SOD1, and SOD2 was normalized to that of TUB1.

2.9. Statistical Analysis. One-way analysis of variance was performed using GraphPad Prism biostatistics software (San Diego, CA, USA) to analyze the data. Significant differences were compared by two-tailed multiple t-tests with Student–Newman–Keuls test. Data were expressed as means ± SEM of triplicate experiments. A P < 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Structure Elucidation of Compound 1. Compound 1 has the molecular formula C37H58O8 as determined by HR-ESIMS measurement. The 1H NMR data showed six methyl groups at δH 0.83 (3H, s), 0.90 (3H, s), 0.91 (3H, s), 0.95 (3H, d, J = 5.6 Hz), 1.47 (3H, s), and 1.92 (3H, s), along with six olefinic protons at δH 9.46 (1H, s), 5.10 (1H, s), 5.63 (1H, dd, J = 3.6, 9.7 Hz), 5.76 (1H, m), 6.18 (1H, dd, J = 2.10, 9.7 Hz), and 6.32 (1H, d, J = 15.3 Hz). Several multiple peaks at δH 3.94–4.76 and the signal of an anomeric proton [δH 5.52 (1H, d, J = 7.8 Hz)] indicated the existence of a sugar moiety. The 13C NMR data revealed the presence of 37 carbon signals. With the combined signals of 13C NMR and DEPT, the 37 carbon signals were attributed to six olefinic carbons (δC 115.1, 130.4, 132.0, 133.6, 135.1, and 143.0), one anomeric carbon (δC 102.8), one oxygenated quaternary carbon (δC 86.0), six oxymethines (δC 69.7, 72.2, 74.2, 77.0, 83.9, and 112.8), one oxymethylene (δC 63.7), one methoxy group (δC 58.1), four quaternary sp3 carbons (δC 39.5, 45.8, 48.6, and 48.7), four methines (δC 37.3, 42.1, 42.7, and 51.2), seven methylenes (δC 19.2, 23.7, 27.8, 28.7, 31.3, 34.3, and 40.5), and six methyl groups (δC 15.3, 19.3, 19.4, 20.4, 21.7, and 25.3). Detailed analysis of the 1H-1H COSY spectra led to the determination of the partial structures depicted by the bonds (Figure 2, in bold bonds). In the HMBC spectrum, these partial structures were connected to yield the following gross structures: H-3 to C-5; H-6 to C-5; H-8 to C-9; H-10 to C-9; CH3-18 to C-12, C-13, C-14, and C-17; H-19 to C-9, C-10, C-11, and −OCH3; CH2-28 to C-3, C-4, and C-29; CH2-29 to C-4, C-5, and C-28; CH2-30 to C-8, C-13, C-14, and C-15; H-23 to C-25; H-24 to C-25, C-26, and C-27; H-26 to C-24; CH3-27 to C-24; and −OCH3 to C-19. The signals of H-3 to C-1′ of allose and anomeric proton H-1′ of allose to C-3 in the HMBC indicated the location of the sugar moiety (Figure 2). The β anomeric configuration of allose was determined from its coupling constant J (7.8 Hz) of anomeric protons (δH 5.52). The absolute configuration of the sugar moiety was further confirmed by the degradation of compound 1 and through the comparison of the retention time of its aldose thiocarbamate derivative (tR = 9.287 min) with those of the following aldose thiocarbamate standards: L-cysteine-D-allose (tR = 7.460 min), D-cysteine-D-allose (tR = 9.127 min), and D-cysteine-D-allulose (tR = 7.460 min).

The relative stereochemistry of compound 1 was deduced by nuclear overhauser enhancement spectroscopy (NOESY) analysis. As shown in Figure 2, the broad singlet signal of H-3 appeared at δH 3.73 thereby suggested the α configuration of this proton. The NOESY correlations of...
The yeast generations of K6001 were as follows: control (7.70 ± 0.48); Res at 10 μM (10.20 ± 0.42***); compound 1 at 1 μM (9.20 ± 0.49*) and at 3 μM (9.25 ± 0.42*); compound 2 at 1 μM (9.03 ± 0.43*) and at 3 μM (9.00 ± 0.42*); compound 3 at 1 μM (11.03 ± 0.53***) and at 3 μM (9.68 ± 0.55**); compound 4 at 1 μM (11.08 ± 0.50***) and at 3 μM (10.25 ± 0.56**); compound 5 at 1 μM (10.40 ± 0.45***) and at 3 μM (10.70 ± 0.45**); compound 6 at 1 μM (9.68 ± 0.57**) and at 3 μM (10.10 ± 0.46**); compound 7 at 1 μM (9.68 ± 0.55**) and at 3 μM (9.75 ± 0.47**); compound 8 at 1 μM (9.63 ± 0.52**) and at 3 μM (9.73 ± 0.55**); compound 9 at 1 μM (9.75 ± 0.47**) and at 3 μM (10.13 ± 0.41**); and compound 10 at 1 μM (9.55 ± 0.42**) and at 3 μM (10.08 ± 0.39***) (*P < 0.05, **P < 0.01, and ***P < 0.001, compared with the control).

Figure 3: Effect of compounds 1–10 on the replicative lifespan of K6001 yeast strain. The average lifespan of K6001 was as follows: control (7.70 ± 0.48); Res at 10 μM (10.20 ± 0.42***); compound 1 at 1 μM (9.20 ± 0.49*) and at 3 μM (9.25 ± 0.42*); compound 2 at 1 μM (9.03 ± 0.43*) and at 3 μM (9.00 ± 0.42*); compound 3 at 1 μM (11.03 ± 0.53***) and at 3 μM (9.68 ± 0.55**); compound 4 at 1 μM (11.08 ± 0.50***) and at 3 μM (10.25 ± 0.56**); compound 5 at 1 μM (10.40 ± 0.45***) and at 3 μM (10.70 ± 0.45**); compound 6 at 1 μM (9.68 ± 0.57**) and at 3 μM (10.10 ± 0.46**); compound 7 at 1 μM (9.68 ± 0.55**) and at 3 μM (9.75 ± 0.47**); compound 8 at 1 μM (9.63 ± 0.52**) and at 3 μM (9.73 ± 0.55**); compound 9 at 1 μM (9.75 ± 0.47**) and at 3 μM (10.13 ± 0.41**); and compound 10 at 1 μM (9.55 ± 0.42**) and at 3 μM (10.08 ± 0.39***) (*P < 0.05, **P < 0.01, and ***P < 0.001, compared with the control).

Figure 4: Effect of compound 9 on the antioxidative ability of yeast cells and ROS level of yeast. (a) BY4741 yeast cells in the control group and compound 9-treated groups were dropped in the same YPGlucose agar plate mixed with 9 mM H₂O₂. After four days, the growth of yeast cells in different groups was photographed. (b) Effect of compound 9 on the survival rates of yeast under oxidative stress condition. Control, 48.31 ± 4.94; Res, 72.74 ± 6.19*; compound 9 at 1 μM, 71.21 ± 2.75* and at 3 μM, 68.73 ± 5.89*. The experiment was conducted at least thrice. Vertical bars represent the mean ± SEM of three assays (*P < 0.05). (c) The change of ROS level of yeast after administration compound 9 at 1, 3 μM. Control, 2.77 ± 0.15**; Res, 1.79 ± 0.15**; compound 9 at 1 μM, 1.98 ± 0.08**, and at 3 μM, 1.73 ± 0.22*. Vertical bars represent the mean ± SEM of 6 repeats (*P < 0.05 and **P < 0.01, compared with the control).
major cross-peaks of H-3α/Me-28, Me-28/H-10, H-10/Me-30, and Me-30/H-17 indicated the α-orientation of these protons. The correlations between H-1β/H-19, H-19/H-8, H-8/Me-18, and Me-18/H-20 suggested the β-orientation of these groups. The double bond at C-23 and C-24 was elucidated by COSY correlations, whereas the transgeometry was determined from the coupling constant, $J_{23-24} = 15.3$ Hz.

The above evidence suggested that compound 1 was structurally similar to (19R,23E)-5β, 19-epoxy-19-methoxycucurbita-6, 23-25-trien-3β-ol 3-O-β-D-allopyranoside (Figure 1).

3.2. Identification of the Known Compounds. Compounds 2–10 (Figure 1) were identified by comparing their spectroscopic data with those in literature.

3.3. Antiaging Activity in K6001 Yeast Strain. All isolated cucurbitane triterpenoids (1–10) were tested for antiaging activity through the K6001 bioassay method at different optimum concentrations. All the compounds at 1 and 3 μM extended the replicative lifespan of K6001 significantly (Figure 3), demonstrating that the cucurbitane triterpenoids isolated from M. charantia L. fruits have antiaging effect in yeast.

3.4. Compound 9 Improves the Oxidative Resistance and Decreases ROS Production of Yeast. Studies on mechanism of action were conducted with compound 9 because of its abundance and good activity. Oxidative stress is one of the primary causes of aging, as indicated in various model organisms [24]. Therefore, the effect of compound 9 on the oxidative resistance of yeast was first tested. The growth of yeast cells was inhibited at 9 mM H$_2$O$_2$, whereas incubation with compound 9 at 1 or 3 μM remitted the inhibition (Figure 4(a)). The effect was further confirmed in another assay. As shown in Figure 4(b), the survival rate of the control group was 48.31% ± 4.94%, whereas that in the experimental groups increased to 71.21% ± 2.75% (compound 9 at 1 μM, $P < 0.05$) and 68.73% ± 5.89% (compound 9 at 3 μM, $P < 0.05$). The experiments indicated that compound 9 enhances the oxidative resistance of yeast cells. Furthermore, we detected the ROS level of yeast after administration compound 9 at 1 and 3 μM. As we expected, the ROS level of yeast in the resveratrol and compound 9 groups were significantly decreased compared with the control.
group (Figure 4(c), \( P < 0.01 \), \( P < 0.01 \), and \( P < 0.05 \), respectively. These results suggested that compound 9 extended the replicative lifespan via inhibition of oxidative stress.

3.5. Compound 9 Extends Yeast Lifespan via Modification of \( UTH1 \), \( SKN7 \), \( SOD1 \), and \( SOD2 \) Gene Expression. It is well known that antioxidative stress is one of mechanisms of action for antiaging. \( UTH1 \) gene essentially takes part in oxidative stress regulation, and deletion of \( UTH1 \) gene will lead to extend the replicative lifespan of yeast [25]. \( SKN7 \) is upstream gene and is a stress response transcription factor in \( Saccharomyces cerevisiae \) [26]. Superoxide dismutases (SOD) are major ROS scavenging enzymes and can convert superoxide anion to hydrogen peroxide [24]. Real-time PCR analysis was performed to examine the molecular mechanism of compound 9-mediated lifespan extension. The significant gene expression reduction or increase of \( UTH1 \), \( SKN7 \), \( SOD1 \), and \( SOD2 \) was observed in the compound 9 treatment groups (Figure 5). These results suggested that compound 9 produced antiaging effect via regulation \( UTH1 \), \( SKN7 \), \( SOD1 \), and \( SOD2 \) yeast gene expression.

3.6. Antiaging Effects of Compound 9 Diminished in \( UTH1 \), \( SKN7 \), \( SOD1 \), and \( SOD2 \) Mutations with \( K6001 \) Background. To investigate the role of these genes in the antiaging activity of compound 9, we used the mutants of \( UTH1 \), \( SKN7 \), \( SOD1 \), and \( SOD2 \) as shown in Figure 6, compound 9 at 3 \( \mu M \) did not affect the replicative lifespan of \( UTH1 \) (Figure 6(a)) or \( SKN7 \) mutants (Figure 6(b)), neither of \( SOD1 \) (Figure 6(c)) or \( SOD2 \) mutants (Figure 6(d)). These results were further indicated that these four genes were involved in the mechanism of action of compound 9.

4. Conclusions

A novel cucurbitane-type triterpenoid and nine known compounds were isolated and identified from the fruits of \( M. charantia \). All the compounds showed antiaging effect in
yeast. The antiaging activities of these cucurbitane-type triterpenoids depended on their antioxidative ability and the regulation of the UTH1, SKN7, SOD1, and SOD2 yeast genes. Apart from being one of the most well-known vegetables and frequently used as a traditional medicine because of its health benefits, *M. charantia* has potential as an antiaging functional food.

**Conflicts of Interest**

The authors declare no financial or commercial conflict of interest.

**Authors’ Contributions**

Xueli Cao and Yujuan Sun contributed equally to the article.

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