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Research Article

Two Anti-Glycation Agents from Flowers of *Epiphyllum Oxypetalum* (Dc.) Haw

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Abstract

Background: *Epiphyllum oxypetalum* (DC.) Haw. is a plant of great ornamental value. In recent years, it has been gradually used in food and cosmetics, especially in cosmetics.

Aims: Delve into the ingredients of *E. Oxypetalum* (DC.) Haw. to explore their beauty effects.

Methods: The ingredients of *E. Oxypetalum* (DC.) Haw. were isolated and purified by HLB resin column chromatography. The structure was identified by Nuclear Magnetic Resonance spectroscopy (NMR) and Mass Spectrometry (MS). The anti-glycation effect was evaluated by NƐ-Carboxymethyl-lysine (CML) immunofluorescence assays.

Results: Two flavonoids, isorhamnetin-3-O-robinobioside and isorhamnetin-3-O-rutinoside were isolated from flowers of *E. Oxypetalum* (DC.) Haw. 150 µM isorhamnetin-3-O-robinobioside was equivalent to positive control (PC, 0.75 mM aminoguanidine, AG), while 18.75 µM isorhamnetin-3-O-rutinoside was equivalent to PC and it was firstly isolated from *E. Oxypetalum* (DC.) Haw.

Conclusion: The results showed that two flavonoids had good anti-glycation effect as excellent anti-glycation agents.

Keywords: *Epiphyllum oxypetalum* (DC.) Haw.; Isorhamnetin-3-O-robinobioside; Isorhamnetin-3-O-rutinoside; Anti-glycation; Flavonoid

Introduction

E. Oxypetalum (DC.) Haw., as a plant of the genus Epiphyllum (the Cactus family), was used in traditional medicine, cosmetics, and food [1,2]. The flowers have good beauty effects, especially in moisturizing, whitening, improving ATP content and so on [3-5]. At present, there are few studies on the ingredients of *E. Oxypetalum* (DC.) Haw., especially the monomer compounds, which seriously hinder the in-depth study on the correlation between the ingredients and beauty effects.

In this paper, we isolated two flavonoids, isorhamnetin-3-O-robinobioside and isorhamnetin-3-O-rutinoside from flowers of *E. Oxypetalum* (DC.) Haw. At present, the anti-inflammatory and antioxidant effects of the two flavonoids have been reported, and we have also independently studied the effects, such as anti-inflammatory, anti-aging and anti-glycation [6-9]. Surprisingly, the anti-glycation effect of the two flavonoids was far greater than the anti-inflammatory and anti-aging effect.

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Materials and Methods

Materials

Flowers of *E. Oxypetalum* (DC.) Haw. were purchased from Fujian, China. HLB resin was provided by WePure Biotech Co., Ltd (Guangzhou, China). Silica gel (100-200 mesh) and all organic solvents were purchased from Shanghai Titan Scientific Co., Ltd. (Shanghai, China). Human dermal fibroblasts were purchased from Guangdong Biocell Biotechnology Co., Ltd. (Guangdong, China). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Triton X-100 were purchased from Sigma-Aldrich Chemical Co., Ltd. (Missouri, America). Dimethyl Sulfoxide (DMSO) was purchased from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Dulbecco's Modified Eagle's Medium (DMEM) and Phosphate-Buffered Saline (PBS) were purchased from Thermo Fisher Scientific (Massachusetts, America). AG and Methylglyoxal (MGO) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Paraformaldehyde (PFA) and mounting medium were purchased from Shanghai Biyuntian Biotechnology Co., Ltd. (Shanghai, China). Bovine Serum Albumin (BSA) was purchased from Yeasen Biotechnology (Shanghai) Co., Ltd. (Shanghai, China). Anti-CML antibody [CML26] (primary antibody) was purchased from Abcam (Cambridge, United Kingdom). Cy3 conjugated goat anti-mouse IgG (second antibody) was purchased from Wuhan Servicebio Technology Co., Ltd. (Wuhan, China).

Preparation of isorhamnetin-3-O-robinobioside and isorhamnetin-3-O-rutinoside

Flowers of *E. Oxypetalum* (DC.) Haw. were extracted with boiling water for 1 hour and then filtered. The filtrates were concentrated, then three-fold volume of 95% ethanol was added. Precipitate was removed, then ethanol was removed from the supernatant. The

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residue liquid was subjected to HLB resin column chromatography using 50% ethanol and 95% ethanol as eluent. The eluting section with 95% ethanol was concentrated to dry and was further separated by silica gel column chromatography. The elution system was ethyl acetate: ethanol: water = 85:10:5.

Compound characterization

1H-NMR and 13C-NMR were recorded using Bruker AVANCE III HD 600 MHz NMR spectrometer (Bruker, Germany) with DMSO-d6 (D = 99.9% + TMS = 0.03%) as the solvent. MS was obtained on Agilent 6120 Ion Trap LC/MS 500 analysis system (Agilent, USA).

Cell viability assays

The cell viability was tested by MTT assay. The fibroblasts were seeded into 96-well plates and cultured overnight (5%CO2, 37°C). According to the rate of plate laying is 80%, fibroblasts were treated. Zero group had no fibroblasts but only 100 µL DMEM. Solvent Control group (SC) had fibroblasts and 100 µL DMEM. Test groups were consisted of isorhamnetin-3-O-robinobioside (300, 150, 75, 37.5, 18.75, 9.38, 4.69, 2.34 µM) and isorhamnetin-3-O-rutinoside (300, 150, 75, 37.5, 18.75, 9.38, 4.69, 2.34 µM). After 24 hours of treatment (5%CO2, 37°C), the supernatant was removed and MTT was added to each well and incubated at 37°C in dark Place for 4 hours. The supernatant was then removed and 150 µL DMSO was added to each well. Absorbance (A) was read by enzyme-labeled instrument (MK3, Thermo Scientific Multiskan, America) at 490 nm. Cell viability (%) = (ATest - AZero)/(ASC - AZero) × 100%.

CML immunofluorescence assays

The fibroblasts were seeded into 24-well plates (15000 cells per well) and cultured for 24 hours (37°C, 5%CO2). Except the Blank Control group (BC), all the other groups were treated with 0.3 mM MGO to mimic glycation. At the same time, PC (0.75 mM AG), isorhamnetin-3-O-robinobioside (150, 75, 37.5, 18.75 µM) and isorhamnetin-3-O-rutinoside (150, 75, 37.5, 18.75 µM) were utilized to treat the cell models respectively. After 72 hours of incubation, the fibroblasts were washed with PBS and fixed with 4% PFA. Then fibroblasts were permeated with 0.1% Triton X-100 and blocked with 2.5% BSA for 1 hour. The fibroblasts were incubated with primary antibody (dilution 1:500) at 4°C for 10 hours. Staining was visualized using the secondary antibody (dilution 1:800) at room temperature for 1 hour. The stained slides were mounted with a mounting medium containing DAPI. Inverted Fluorescence images were captured by fluorescence microscope (Leica DMi8, Heidelberg, Germany), and Image J was used for quantitative analysis.

Statistical analysis

All data were expressed as the mean ± Standard Deviation (SD) and were analyzed by one-way analysis of variance (ANOVA) using Graph Pad Prism 9.5 (Graph Pad, USA). A P-value of <0.01 and <0.05 was considered as a statistically significant difference.

Results

Compound characterization

The final result is two yellow amorphous compounds. The low polar one was identified as isorhamnetin-3-O-robinobioside from the following spectral data (Figure 1). 6,10-11 1H-NMR(DMSO-d6, 600 MHz) δ: 12.61(1H, s, 5-OH), 10.87 (1H, s, 7-OH), 9.78 (1H, s, 4'-OH), 8.01 (1H, d, J = 2.1 Hz, H-2'), 7.50 (1H, dd, J = 2.1, 8.4 Hz, H-6'), 6.97 (1H, d, J = 8.5 Hz, H-5'), 6.43 (1H, d, J = 2.3 Hz, H-8), 6.26 (1H, d, J = 2.1 Hz, H-6), 5.46 (1H, d, J = 7.8 Hz, H-1''), 4.42 (1H, d, J = 1.0 Hz, H-1'''), 3.84 (3H, s, -OCH3), 1.06 (3H, d, J = 6 Hz, H-6'''); 13C-NMR (DMSO-d6, 150 MHz) δ :177.8 (C-4), 164.7 (C-7), 161.7 (C-5), 156.9 (C-2), 156.9 (C-9), 149.8 (C-3'), 147.4 (C-4'), 133.6 (C-3), 122.4 (C-6'), 121.5 (C-1'), 115.6 (C-5'), 113.9 (C-2'), 104.5 (C-10), 102.2 (C1''), 100.5 (C-1'''), 99.2 (C-6), 94.2 (C-8), 74.0 (C-5''), 73.4 (C-3''), 72.3 (C-4'''), 71.6 (C-2''), 71.1 (C-3'''), 70.9 (C-2'''), 68.7 (C-5'''), 68.4 (C-4''), 65.6 (C-6''), 56.4 (-OCH3), 18.3 (C-6'''); ESI-MS m/z 625 [M+H]+ 100%, m/z 647 [M+Na]+ 41%.

The high polar one was identified as isorhamnetin-3-O-rutinoside from the following spectral data (Figure 1). 12 1 H-NMR(DMSO-d6, 600 MHz) δ: 12.58 (1H, s, 5-OH), 7.86 (1H, d, J = 2.1 Hz, H-2'), 7.52 (1H, dd, J = 2.1, 8.4 Hz, H-6'), 6.91 (1H, d, J = 8.5 Hz, H-5'), 6.44 (1H, d, J = 2.3 Hz, H-8), 6.20 (1H, d, J = 2.1 Hz, H-6), 5.42 (1H, d, J = 7.8 Hz, H-1''), 5.13 (1H, d, J = 1.0 Hz, H-1'''), 3.84 (3H, s, -OCH3), 1.03 (3H, d, J = 6 Hz, H-6'''); 13C-NMR (DMSO-d6, 150 MHz) δ: 177.8 (C-4), 163.6 (C-7), 161.6 (C-5), 156.9 (C-2), 156.9 (C-9), 149.9 (C-3'), 147.3 (C-4'), 133.4 (C-3), 122.8 (C-6'), 121.6 (C-1'), 115.8 (C-5'), 113.7 (C-2'), 104.4 (C-10), 101.6 (C1''), 101.4 (C-1'''), 99.3 (C-6), 94.3 (C-8), 76.8 (C-3''), 76.4 (C-5''), 74.7 (C-2''), 72.2 (C-4'''), 71.0 (C-3'''), 70.8 (C-2'''), 70.5(C-4''), 68.8 (C-5'''), 67.3 (C-6''), 56.1 (- OCH3), 18.2 (C-6'''); ESI-MS m/z 625 [M+H]+ 100%, m/z 647 [M+Na]+ 46%.

Isorhamnetin-3-O-rutinoside was firstly isolated from *E. Oxypetalum* (DC.) Haw. It was epimer of isorhamnetin-3-O-robinobioside, a glucose unit replaced galactose unit in isorhamnetin-3-O-rutinoside, which was confirmed by comparison on TLC with authentic sample after hydrolysis.

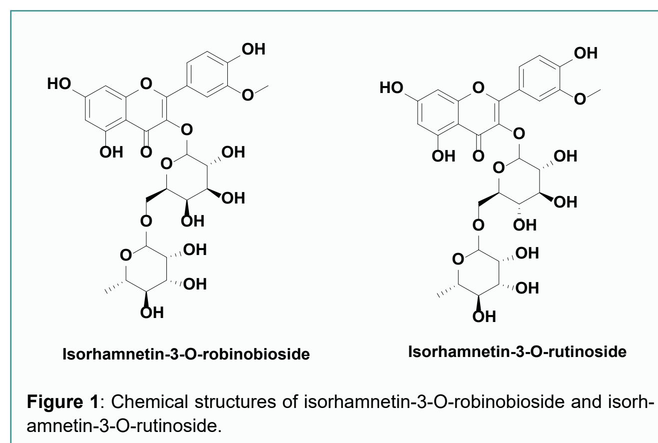

Figure 1: Chemical structures of isorhamnetin-3-O-robinobioside and isorhamnetin-3-O-rutinoside.

Cell viability

Neither of the two flavonoids show apparent human dermal fibroblast growth inhibition or cytotoxicity even at a high concentration of 300 µM (Figure 2). According to the data of cell viability, the concentration range with tolerant cytotoxicity of the two flavonoids could be anticipated to extend to far above 300 µM. Isorhamnetin-3-O-robinobioside and isorhamnetin-3-O-rutinoside would be considered safe materials if more cell and animal models were utilized to evaluated their toxicity.

CML immunofluorescence assays

Maillard reaction is a complex reaction between the amino group of a protein and the carbonyl group of a reducing sugar [13,14]. Modern scientists use Non-Enzymatic Glycosylation (NEG) to describe the Maillard reaction in living organisms [15]. AGEs, a complex group of isomeric compounds, is one of the end products of

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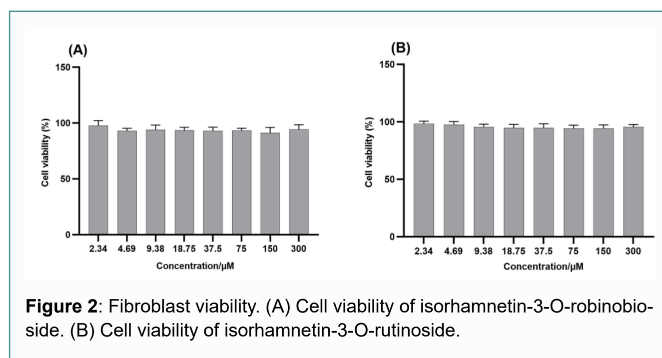

Figure 2: Fibroblast viability. (A) Cell viability of isorhamnetin-3-O-robinobioside. (B) Cell viability of isorhamnetin-3-O-rutinoside.

NEG and can cause a series of skin problems [16]. More than 20 kinds of AGEs have been discovered, the most representative is CML, it is also one of the main markers of glycosylation in human body.

Since CML doesn't carry fluorescence, we used immunofluorescence technology, so as to detect the content of CML. The stronger the fluorescence, the higher the CML content, the more serious the glycation. MGO is considered to be an important glycating agent and precursor of AGEs, mainly reacting with protein residues, leading to the formation of AGEs [17,18]. After 0.3 mM MGO treatment, the CML immunofluorescence expression was significantly enhanced. After treatment with 18.75, 37.5, 75, 150 μM isorhamnetin-3-O-robinobioside, the CML immunofluorescence expression was significantly reduced, especially at 150 μM, which was equivalent to AG. After treatment with 18.75, 37.5 and 75, 150 μM isorhamnetin-3-O-robinobioside, the expression was significantly reduced, all concentrations were equivalent to AG. AG is a typical synthetic inhibitor of AGEs, which acts by clearing dicarbonyl intermediates in the glycation reaction [11]. It shows both flavonoids can inhibit CML production very well (Figure 3).

Discussion

The glycation process in human tissues is a very long process, and excessive generation and accumulation of AGEs will cause irreversible damage to the body. Skin is the largest organ of the human body, and collagen and elastic fibers are the main supporting molecules of skin tissue structure [19]. The accumulation of AGEs, also CML, can reduce skin elasticity, increase skin wrinkles, stimulate inflammatory response, cause skin pigmentation and so on [20-22].

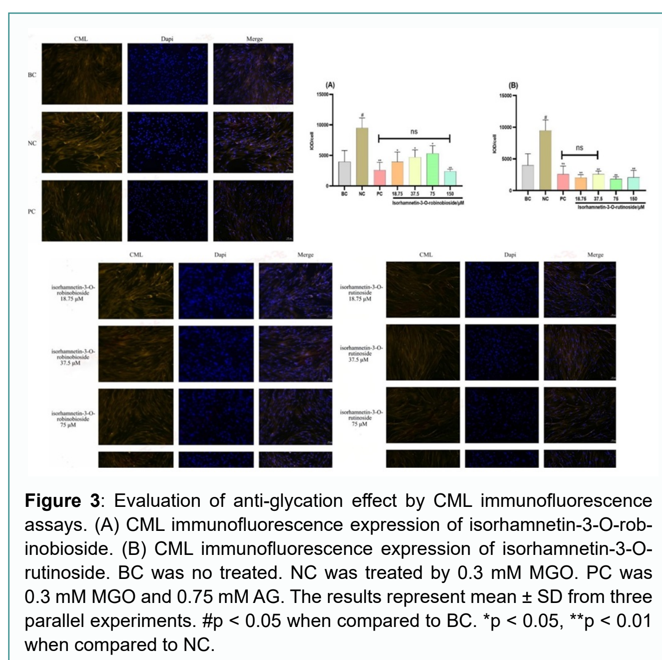

Figure 3: Evaluation of anti-glycation effect by CML immunofluorescence assays. (A) CML immunofluorescence expression of isorhamnetin-3-O-robinobioside. (B) CML immunofluorescence expression of isorhamnetin-3-O-rutinoside. BC was no treated. NC was treated by 0.3 mM MGO. PC was 0.3 mM MGO and 0.75 mM AG. The results represent mean ± SD from three parallel experiments. #p < 0.05 when compared to BC. *p < 0.05, **p < 0.01 when compared to NC.

Though isorhamnetin-3-O-rutinoside was epimer of isorhamnetin-3-O-robinobioside, which only one hydroxyl spatial orientation was different from the latter. However, in the concentration range of this experiment, the inhibition of CML of isorhamnetin-3-O-rutinoside is better than that of isorhamnetin-3-O-robinobioside on the whole. Unfortunately, these two flavonoids did not show good dose-concentration dependence within the concentration range of this experiment, and further exploration is needed in the future, but this does not affect our finding that they are excellent anti-glycation agents. Because when it is equivalent to AG, even the highest dosage of 150 μM is only 1/2 of 0.3 mM AG, not to mention the low concentration of isorhamnetin-3-O-rutinoside, the dosage is even lower. Of course, we can further study the anti-glycation effect of *E. Oxypetalum* (DC.) Haw., and use network pharmacology, molecular docking and other means to clarify the relationship between these two compounds and the anti-glycation effect of *E. Oxypetalum* (DC.) Haw. and the mechanism of their anti-glycation effect.

Conclusion

Today, "green" and "natural" are still the main theme of the cosmetics industry, and plant extracts are still highly to persue. With the progress and development of The Times, people are no longer satisfied with the beauty effect brought by plant extracts, but want to clearly play the mechanism behind the beauty effect. Therefore, the in-depth study of plant extract components is conducive to the in-depth exploration of its beauty mechanism, and also helps to find better beauty active substances.

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