

# The study of glabridin modified with phosphorylcholine groups and its efficacy research in the whitening essence

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## Results & Discussion:

### Introduction:

Glabridin is a natural compound extracted from licorice, which has many effects in cosmetics. Glabridin could inhibit the activities of tyrosinase, dopachrome tautomerase and DHICA oxidase in melanocytes [1]. Therefore, it has fast, efficient and safe whitening and freckle removing effect, and is also recognized as the most effective natural whitening ingredient in the world [2].

However, its application in cosmetics is limited due to its extremely insoluble in water. Therefore, it's necessary to modify the glabridin and improve its water-solubility and transdermal absorption property, which can be improved by modifying with phosphorylcholine-based compound. 2-Methylacryloxyethyl phosphorylcholine (MPC) is a hydrophilic group of phospholipids which constitutes the elementary unit of cell membrane [3]. Compound containing phosphorylcholine groups is commonly used in the biomedical field, such as organ transplantation, artificial blood vessels, hemodialysis and so on [4].

This study aims to introduce the glabridin modified with phosphorylcholine-based compound, which shows the transparent aqueous solution and nd also to evaluate the improvement effect of its inhibition of tyrosinase activity and of melanin production. The glabridin modified with phosphorylcholine-based compound was applied into a whitening essence, and then was clinically evaluated its whitening effect, thus implementing further application of glabridin in cosmetics.

### Materials & Methods:

**Materials**  
The compound contain phosphorylcholine groups was prepared by Shanghai Oli using green technology. Glabridin modified with phosphorylcholine-based compound which shows the transparent aqueous solution with 2% glabridin content and Glabridin-90 power (not modified) was also obtained by Shanghai Oli.

**Methods**  
Cell experiment was used to characterize the effect of glabridin aqueous solution on cell survival rate, and the differences in inhibition effects on tyrosinase activity and melanin production of glabridin before and after modification. The glabridin modified with phosphorylcholine-based compound is formulated into a whitening essence. The specific formula is also given to analyse the clinical whitening effect and visual sensory evaluation.

**Detection of B16 cell proliferation activity**  
According to the content of glabridin in glabridin-90 and glabridin aqueous solution, the final concentration of glabridin was formulated by PBS solution as follows: Glabridin aqueous solution: 0.000002%, 0.00002%, 0.0002%, 0.002%, 0.02%, 0.2%.

**Detection of tyrosinase activity inhibition**  
Sample preparation: glabridin-90 and glabridin aqueous solution were diluted 10 times with PBS solution, with reference to cell proliferation detection. The final concentration of glabridin was as follows:Glabridin-90: 0.000008%, 0.00008%, 0.0008%, Glabridin aqueous solution: 0.000002%, 0.00002%, 0.0002%.

**Detection of Melanin production inhibition**  
Sample preparation: glabridin-90 and glabridin aqueous solution were diluted 10 times with PBS solution, with reference to cell proliferation detection. The final concentration of glabridin was as follows: Glabridin-90: 0.000008%, 0.00008%, 0.0008%, Glabridin aqueous solution: 0.000002%, 0.00002%, 0.0002%.

**Clinical whitening efficacy evaluation**  
Before using whitening essence, the subjects at the age of 30 to 50 and meeting the criterion of only a small difference in the melanin index between the left and right sides of the face were required to clean their faces with the benchmark facial cleanser. They were then asked to wait 30 minutes after drying their faces. They were also given a test sample and asked them to use it on their faces every morning and evening.

**Visual sensory evaluation**  
The facial follow-up tests were performed at 7, 14, 28 and 56 days respectively after the use of the samples. The subjects' facial photos before and after using the whitening essence were compared.

### References:

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**Glabridin modified with phosphorylcholine-based compound**  
The compound contain phosphorylcholine groups can be utilised to encapsulate the active substance and realize the application of drugs. A transparent glabridin aqueous solution was obtained by modifying glabridin-90 with phosphoryl choline compounds. The Figure 1 shows the appearance of glabridin-90 (A) and the glabridin aqueous solution (B). No solubilizer was added to glabridin aqueous solution. The surface modification technology was used to dissolve glabridin in water. It is clear that the glabridin aqueous solution is colorless and transparent, indicating that glabridin is completely dissolved in water after being modified by phosphorylcholine compounds. It contributes to the application of glabridin in cosmetics [5].

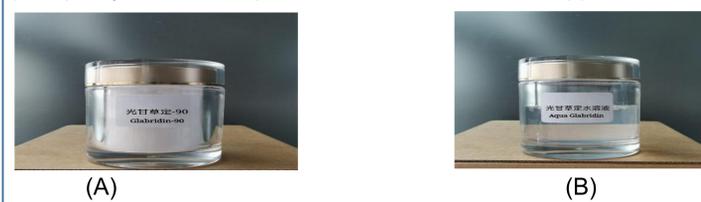


Figure 1 Appearance of glabridin-90 (A) and glabridin aqueous solution (B)

**Cell experiment**  
Cell experiment was used to characterize the effect of glabridin aqueous solution on cell survival rate, and the differences in inhibition effects on tyrosinase activity and melanin production of glabridin before and after modification.

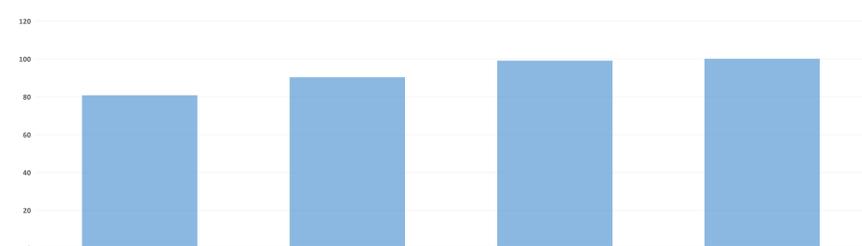


Figure 2 The effect of glabridin aqueous solution on the proliferation activity of B16 cells

**Clinical whitening efficacy evaluation**  
The glabridin modified with phosphorylcholine-based compound is formulated into a whitening essence. The test lotion was composed of 4% glabridin aqueous solution, together with other ingredients. It means the whitening essence contains 0.08% glabridin (w/w). The clinical whitening efficacy of glabridin was evaluated by instrument detection and visual sensory evaluation. During the evaluation of clinical whitening efficacy, the volunteers did not experience any discomfort such as pruritus, tingling, burning, and/or any clinical symptoms such as desquamation, erythema, papules, blisters, etc.

**Visual sensory evaluation**  
Figure 3 is the comparison of the subjects' facial photos before and after use. As you can see, after 56 days of using the whitening essence, the subjects' faces became brighter. The glossiness and delicacy were improved, which was consistent with the above clinical test data. Therefore, glabridin whitening essence indicates brilliant whitening effect.



Figure 3

## Conclusions:

Glabridin is completely water-soluble after being modified by phosphorylcholine compounds, which has a broad application prospect in the field of cosmetics. The cell experiments have also demonstrated that glabridin is a safe and effective natural whitening ingredient.

Compared with the unmodified glabridin, the glabridin modified by phosphorylcholine-based compound has better inhibitory effects on tyrosinase activity and melanin production, respectively.

During the evaluation of clinical whitening efficacy, after 56 days of using the whitening essence, the ITA° increased by 25.7%, the skin melanin and skin heme decreased by 32.5% and 20.5% respectively. The volunteers generally deem their skin more even, smoother and lighter than before. The glabridin whitening essence has a significant effect on brightening skin tone, eliminating inflammation and redness, and improving skin glossiness.