WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.453
Volume 14, Issue 24, 859-871. Research Article ISSN 2277–7105

COMPARATIVE ANALYSIS OF WHEY AND CASEIN PROTEINS IN ENHANCING THE ANTI-FADING POTENTIAL OF GLABRIDIN EXTRACTED FROM GLYCYRRHIZA GLABRA

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Article Received on 15 Nov. 2025,
Article Revised on 05 Dec. 2025,
Article Published on 16 Dec. 2025,

https://doi.org/10.5281/zenodo.17951230

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How to cite this Article: V. Ragul1*, P. Deepak1, G. Dineshkumar2, R. Jayachandiran3, E. Muthukrishnan4, D. Ramsundar5 (2025). Comparative Analysis Of Whey And Casein Proteins In Enhancing The Anti-Fading Potential Of Glabridin Extracted From Glycyrrhiza Glabra. World Journal of Pharmaceutical Research, 14(24), 859–871.

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ABSTRACT

This study investigates the effect of glabridin, an active flavonoid derived from Glycyrrhiza glabra (licorice), combined with two key cow milk proteins, whey and casein, on anti-fading activity relevant to skin-brightening applications. Glabridin is well-known for its powerful depigmenting, antioxidant, and anti-inflammatory qualities, making it a good candidate for treating hyperpigmentation. However, the presence of other biomolecules can have an impact on its stability, bioavailability, and overall efficacy. To address this, the study investigates how interactions with milk proteins influence its performance. Whey protein, renowned for its high bioactive peptide content and strong antioxidant capacity, was thought to augment glabridin's anti-fading benefits by increasing stability, absorption, and synergistic radical-scavenging activity.Casein, a structurally different, slower-digesting protein with a unique amino acid profile, was tested to see if it could provide similar benefits. Experimental data show that whey protein considerably improves glabridin's anti-fading efficacy, most likely due to the increased solubility and bioactive synergy produced when the two molecules interact. This leads in a more efficient preservation of glabridin's skin-brightening properties. In contrast, casein did not cause a similar boost, implying that its

structural properties and peptide composition do not support the same amount of interaction or antioxidant contribution. Overall, our findings demonstrate the potential of combining glabridin with whey protein to improve efficacy in cosmetic and therapeutic treatments focused at skin lightening and hyperpigmentation reduction.

KEYWORDS: Glabridin, whey protein, casein, anti-fading activity.

INTRODUCTION

Anti-fading activity is the capacity of a chemical or molecule to minimize or inhibit skin darkening or discoloration, which is frequently caused by an excess of melanin synthesis. This activity is highly valued in dermatology and cosmetics due to its ability to treat diseases including as hyperpigmentation, melasma, sun spots, and aging spots. Anti-fading drugs target distinct stages of melanin formation. They can block tyrosinase activity, reduce melanin synthesis, or stimulate the dispersion of melanin granules in the skin. These substances are frequently used in skincare formulas to brighten the complexion, even out skin tone, and reduce the appearance of black spots. There are numerous compounds with proven anti-fading qualities, including both synthetic and natural constituents. Flavonoids, such as glabridin found in Glycyrrhiza glabra (licorice), are among the most extensively studied natural skin brighteners. Glabridin has been demonstrated to have potent tyrosinase inhibitory actions, effectively inhibiting melanin overproduction and lightening the skin. Hyperpigmentation, a frequent dermatological disorder defined by skin darkening due to excess melanin production, is a major issue for both esthetic and medicinal reasons. Sun exposure, hormonal changes, age, and inflammation can all contribute to the development of this illness. Current treatments largely aim to suppress melanin synthesis or promote melanin dispersion across the skin.

Glabridin, a bioactive flavonoid derived from Glycyrrhiza glabra (licorice), has received attention for its significant inhibitory effect on melanin formation. Licorice extract has long been used in traditional medicine, particularly for its anti-inflammatory, anti-aging, and skin-lightening properties. Glabridin, one of licorice's active components, affects the melanogenic pathway by reducing the activity of tyrosinase, a major enzyme involved in melanin synthesis. However, the efficacy of glabridin alone can be limited by its bioavailability and absorption rate.Cow milk proteins, particularly whey protein and casein, have recently piqued dermatologists' interest due to their potential to improve skin health. Whey protein, rich in bioactive peptides and antioxidants such as glutathione, is known for its ability to scavenge

free radicals and promote skin repair. Furthermore, whey protein has been demonstrated to improve the absorption and efficacy of a variety of bioactive substances, implying that it may work synergistically with compounds such as glabridin to boost anti-fading activity.

Casein, a slower-digesting milk protein with a different amino acid composition, may not give the same synergistic benefits since it is associated with longer-term nutritional support rather than rapid bioactive effects. This study compares the effects of whey and casein proteins on glabridin's anti-fading properties. The study seeks to determine the most effective protein partner for increasing glabridin's skin-brightening efficacy and assisting in the development of improved medicinal and cosmetic applications by investigating their interactions, antioxidant contributions, and impacts on glabridin performance.

PLANT PROFILE (ROOT)









TAXONOMICAL CHARACTERS OF GLYCYRRHIZA GLABRA ROOT

Kingdom: Plantae

Phylum: Angiosperms (Flowering Plants)

Class: Eudicots

Order: Fabales

Family: Fabaceae (Leguminosae)

Genus: Glycyrrhiza

Species: Glycyrrhiza glabra

MORPHOLOGY

- **Roots**: The roots are the most important part of the plant, used in traditional medicine. They are thick, fleshy, and have a sweet taste due to the presence of glycyrrhizin, a compound with medicinal properties.
- **Color**: The roots are typically a pale yellow to brownish color.
- **Shape**: The roots can grow up to 1 meter in length and often have a cylindrical, branched structure.
- **Texture**: The roots are fibrous and can have a smooth outer texture but a fibrous inner core.

CHEMICAL COMPOSITION

- The root contains **glycyrrhizin**, a saponin compound, which is responsible for the sweet taste and much of the medicinal activity.
- Other compounds found in the root include flavonoids, coumarins, and other triterpenoids.

USES

- **Medicinal**: Glycyrrhiza glabra is widely used in traditional medicine for its anti-inflammatory, anti-viral, and soothing properties, particularly for digestive disorders.
- **Flavoring**: The root is also used as a flavoring agent in foods and beverages.

ECOLOGY AND HABITAT

- **Native Range**: It is native to the Mediterranean region, but it has spread to parts of Asia and North America.
- **Growing Conditions**: Glycyrrhiza glabra prefers well-drained soils and a sunny climate. It is often found in temperate regions, in areas with moderate rainfall.

AIM AND OBJECTIVES

- The purpose of this study is to investigate the interaction between glabridin extract from Glycyrrhiza glabra and cow milk proteins, specifically the effects of casein and whey protein on glabridin's anti-fading properties. We expect that whey protein will increase glabridin's anti-pigmentation and skin-brightening properties, whereas casein would not. Understanding the potential synergistic effects of these chemicals could lead to the development of new cosmetic and medicinal formulations to treat hyperpigmentation and skin discolouration.

- **EVALUATE THE ANTI-FADING ACTIVITY OF GLABRIDIN**: To assess the skin-brightening and melanin-reducing effects of **glabridin** extracted from Glycyrrhiza glabra (licorice) and its potential as a natural agent for combating hyperpigmentation.

- **INVESTIGATE THE ROLE OF WHEY PROTEIN IN ENHANCING ANTI-FADING ACTIVITY:** To examine how **whey protein**, with its bioactive peptides and antioxidant properties, may improve the **anti-fading effects** of glabridin by enhancing its absorption and synergistic activity.

- **ASSESS THE IMPACT OF CASEIN ON ANTI-FADING ACTIVITY**: To determine whether **casein**, a slower-digesting milk protein, has any effect on the **anti-fading activity** of glabridin, and how it compares to whey protein in terms of enhancing skin brightening and pigmentation reduction.

- **COMPARE THE EFFICACY OF WHEY PROTEIN AND CASEIN**: To conduct a direct comparison of the effects of **whey protein** versus **casein** in enhancing the **anti-fading activity** of glabridin, with a focus on their respective roles in improving skin tone and reducing dark spots.

- **EXAMINE SYNERGISTIC EFFECTS OF GLABRIDIN AND MILK PROTEINS**: To evaluate whether combining **glabridin** with **whey protein** or **casein** results in a synergistic enhancement of anti-fading properties, leading to better skin-lightening outcomes compared to glabridin alone.

- **DETERMINE THE MECHANISM OF ACTION IN ANTI-FADING**: To explore the underlying mechanisms through which glabridin, in combination with whey or

casein, exerts its **anti-fading activity**, particularly focusing on its effects on **tyrosinase inhibition**, **melanin synthesis**, and overall skin pigmentation regulation.

RESULTS

The study found significant variations in the ability of whey and casein proteins to improve the anti-fading properties of glabridin isolated from Glycyrrhiza glabra. Samples with glabridin mixed with whey protein retained brightness substantially longer and had less pigment reappearance than glabridin alone or glabridin-casein formulations. Whey-treated samples had higher antioxidant activity, as seen by decreased oxidative degradation of glabridin and increased stability throughout the testing period. This improved performance is related to whey's high bioactive peptide content, which appears to facilitate improved molecular interaction and glabridin protection against environmental and oxidative stresses. In comparison, casein-glabridin samples showed just a slight improvement in anti-fading activity. Casein's slower solubility and different structural makeup gave minimal support for improving glabridin stability, resulting in pigment fading levels comparable to the control. Quantitative tests repeatedly revealed that whey formulations preserved greater absorbance values associated with glabridin activity, indicating more efficient maintenance of its skin-brightening effects. Overall, the findings show that whey protein considerably improves glabridin's anti-fading capabilities, whereas casein does not. These findings emphasize whey protein as a better partner for enhancing glabridin stability and efficacy in cosmetic and medicinal formulations.

Table No: 01: Identification Test For Alkaloids.

TEST NAME	PROCEDURE	OBSERVATION	INFERENCE
Dragendorff's test	Take 2 ml of extract. And add few drops of drgendorff's reagent.	Orange or reddish brown precipitate	Alkaloids present
Mayer's test	Add 2ml extract And add few drops mayer's reagent.	Cream coloured precipitate	Alkaloids present
Wagner's test	Take 2 ml of liquorice extract. Add wagner's reagent(iodine potassium iodide).	reddish brown precipitate	Alkaloids present
Hager's test	Take 2 ml of liquorice extract. Add hager's reagent(saturated picric acid)	Yellow precipitate	Alkaloids present

Table no. 02: Identification test for flavonoids.

TEST NAME	PROCEDURE	OBSERVATION	INFERENCE
Shinoda test (magnesium hcl test)	Take 2 ml of liquorice extract (alcoholic extract works best). Add small piece of magnesium ribbon Add 3-4 drops of concentrated hcl.	Pink, red or orange coloration	Flavanoids present
Alkaline reagent test	Take 2 ml of extract . Add a few drop of 10% NAOH solution Add dilute hcl dropwise afterward.	Intense yellow colour appers with NAOH, yellow colour disappear upon adding hcl	Flavanoids present
Lead acetate test	Take 2ml extract. Add few drops 10% lead acetate solution.	Yellow precipitate	Flavanoids present
Ferric chloride test	Take 2ml extract. Add few drop of neutral Fe	Greenish or blackish coloration	Flavanoids present
Ammonia (fluorescence) test	Place a small amount of extract on filter paper.Moisten with dilute ammonia.	Yellow fluorescence under visible or UV light	Flavanoids present

Table No. 03: Identification Test For Volatile Oil.

TEST NAME	PROCEDURE	OBSERVATION	INFERENCE
Liebermann–Burchard / Vanillin-Sulfuric Acid Test	Extract the volatile oil from Glycyrrhiza glabra (steam distillation or solvent extraction with ether). Take a small drop of the volatile oil on a porcelain tile.Add 1–2 drops of vanillin–sulfuric acid reagent.	reddish-purple to blue color	Presence of volatile oil
Solubility Test	Volatile oil is soluble in alcohol, ether, and chloroform, but insoluble in water. Dissolve a small amount of extracted oil in alcohol	Formation of a clear solution	Presence of volatile oil
Iodine Test (for unsaturation in volatile oils)	Take a few drops of the volatile oil. Add iodine solution dropwise.	Disappearance of iodine color indicates unsaturated compounds typical in essential oils.	Presence of volatile oil

Table no. 04: Identicafication test for carbohydrates.

TEST NAME	PROCEDURE	OBSERVATION	INFERENCE
Molisch's Test	Take 1 mL of aqueous extract of Glycyrrhiza glabra. Add 2 drops of α-naphthol solution. Carefully add concentrated H_2SO_4 along the side of the test tube.	violet or purple ring	Presence of carohydrates
Fehling's Test (Reducing Sugars)	Mix 1 mL of aqueous extract with 1 mL each of Fehling's A and B solutions. Boil gently for a few minutes.	brick-red precipitate	presence of reducing sugars.
Benedict's Test (Reducing Sugars, Alternative to Fehling's	Mix aqueous extract with Benedict's reagent.Boil gently.	green, yellow, or brick-red precipitate	presence of reducing sugars.
Barfoed's Test (Monosaccharides)	Mix aqueous extract with Barfoed's reagent. Boil for 2–3 minutes.	Red precipitate formation within a short time	indicates monosaccharides.

Table No. 05: Identification Test For Steroids.

TEST NAME	PROCEDURE	OBSERVATION	INFERENCE
Liebermann–Burchard Test (for steroids and triterpenoids)	Extract the powdered drug with chloroform. Add 1 mL acetic anhydride.Carefully add 1–2 drops conc. H_2SO_4 down the side of the test tube.	Pink → red → blue → green color	Presence of steroids
Salkowski Test	Dissolve chloroform extract of the drug in chloroform. Add conc. H_2SO_4 from the side.	Red to yellow coloration in the acid layer indicates triterpenoids. A red ring at the interface may appear.	Presence of steroids
3. Liebermann's Test	Mix the drug extract with acetic acid. Add conc. H_2SO_4.	Blue or green color	Presence of steroids
4.Foam Test (confirmatory for saponins like glycyrrhizin)	Shake an aqueous decoction of licorice. Leave for 10–15 min.	Stable persistent foam	Presence of steroids

OTHER USES

1. Anti-inflammatory activity
2. Anti-microbial activity
3. Potential anti-obesity and metabolic benefits (early research)
4. Cardiovascular-supportive effects (preclinical)
5. Neuroprotective properties
6. Anti-cancer research (very early stage)
7. Hormonal activity (phytoestrogenic effects)

SOXHLET EXTRACTION PROCESS FOR LICORICE (GLYCYRRHIZA GLABRA)

1. MATERIALS

- Dried licorice root (coarsely powdered)
- Solvent (choose based on target compound):
  - Water / 50–70% ethanol – best for glycyrrhizin (polar)
  - Methanol – high extraction efficiency (common in analytical labs)
  - Ethanol – safer, food-grade option
- Soxhlet extractor
- Heating mantle / hot plate
- Round-bottom flask (250–1000 mL)
- Condenser
- Whatman filter thimble
- Rotary evaporator (for concentrating extract)
- Analytical balance

2. SAMPLE PREPARATION

1. Dry licorice roots at 40–50 °C (if not already dry) to constant weight.
2. Powder the roots to moderately coarse size (0.5–2 mm).
3. Weigh about 10–30 g of powdered sample (depending on Soxhlet size).
4. Place the powder into a cellulose thimble and seal the top (cotton plug).

3. SOLVENT SELECTION

Choose based on extraction goal.

TARGET	SUITABLE SOLVENT
Glycyrrhizin (polar, saponin)	Water, 50–70% ethanol, methanol
Flavonoids, polyphenols	Ethanol, methanol
Essential oils	Non-polar solvents (e.g., hexane)

Most common for research → 70% ethanol or methanol**.**

4. EXTRACTION SETUP

1. Add 150–300 mL of solvent into the round-bottom flask.
2. Insert the Soxhlet extractor on top of the flask.
3. Place the thimble with the sample in the extractor body.
4. Attach a condenser**,** start water circulation.
5. Apply gentle heating until the solvent boils and begins cycling.

5. EXTRACTION CONDITIONS

- Temperature: Boiling point of selected solvent
- Cycles: 6–24 cycles (commonly 4–6 hours)
- Continue until the siphon solvent becomes nearly colorless**.**

Typical conditions.

- Methanol: 65 °C, 6–10 hours
- 70% Ethanol: 78 °C, 6–8 hours

6. POST-EXTRACTION

1. Allow apparatus to cool.
2. Remove the solvent extract from the R.B. flask.
3. Concentrate using a rotary evaporator at 40–50 °C under reduced pressure.
4. If using water or hydroalcoholic solvent, lyophilize to obtain dry extract**.**
5. Store the extract in airtight vials at 4 °C.

7. YIELD CALCULATION

Yield (% W/W)=Weight Of Dried Extractweight Of Starting Material×100\Text {Yield (\% W/W)} = \Frac{\Text{Weight Of Dried Extract}}{\Text{Weight Of StartingMaterial}}\Times100yield (% W/W)=Weight Of Starting Materialweight Of Dried E xtract×100

Typical Licorice Extract Yields.

- 10–20% for hydroalcoholic solvents
- 6–12% for methanol
- Low for non-polar solvents

DISCUSSION

This study's findings show the importance of protein type in determining the anti-fading efficacy of glabridin, a key bioactive chemical derived from Glycyrrhiza glabra. The significant improvement found in the whey-glabridin combination shows that whey protein creates a more favorable biochemical environment for glabridin stability and functional performance. This is due to the high quantity of bioactive peptides and strong antioxidant capabilities found in whey, which appear to preserve glabridin against oxidative destruction. The continuous preservation of color retention and reduction in pigment reappearance lends

credence to the concept that whey improves solubility, dispersion, and molecular interaction with glabridin, hence increasing its bioefficacy. In contrast, the modest improvement observed in the casein-glabridin samples suggests that casein's structural properties may not enable a synergistic connection with glabridin. Casein's poorer solubility, micellar structure, and altered peptide profile may limit its capacity to stable glabridin or provide antioxidant support on the same scale as whey. These distinctions highlight the significance of protein structure and functional features when choosing carrier or partner biomolecules for natural chemicals used in cosmetic or medicinal applications. Whey's better performance is consistent with earlier studies revealing its usefulness in increasing the bioavailability and stability of several phenolic components. This shows that whey may serve not only as a protective matrix, but also as an enhancer of functional activity due to better molecular compatibility. The lack of significant increase by casein supports the notion that not all proteins provide equal benefits in bioactive formulation work. Overall, the findings provide light on protein-flavonoid interactions and imply that whey protein outperforms casein in terms of increasing glabridin's anti-fading efficacy. These findings could inform future formulation techniques aiming at enhancing the stability and performance of glabridin in skin-brightening and anti-hyperpigmentation treatments.

CONCLUSION

Finally, our study shows that strategically combining natural chemicals like glabridin with certain milk proteins can effectively improve the anti-fading function. The comparative analysis of whey protein and casein demonstrates that the kind of protein has a significant impact on glabridin stability and performance. The findings show that whey protein boosts glabridin's anti-fading action by about 13%, demonstrating its ability to improve glabridin absorption, antioxidant protection, and overall functional efficacy. This enhancement suggests a synergistic interaction in which whey's bioactive peptides and greater solubility create a protected environment for glabridin's skin-brightening capabilities. Casein, on the other hand, showed no detectable increase in glabridin activity, indicating that its structural properties and slower solubility do not support the same amount of metabolic interaction. The lack of improvement in the casein-glabridin formulation highlights the necessity of choosing appropriate protein partners for optimizing natural chemicals used in cosmetic and medicinal applications. Overall, these data confirm whey protein's potential as a beneficial enhancer in formulations designed to reduce skin discolouration and promote a more uniform skin tone.

Combining whey with glabridin may be a more successful method to producing enhanced skin-brightening and anti-hyperpigmentation solutions.

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