



DESIGN AND ANALYSIS OF LUTEOLIN-BASED ANTI-OXIDANT GEL FOR SKIN CARE

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ABSTRACT

This study presents the Design and Analysis of a Luteolin-based antioxidant gel for skin care. The hydrogel was prepared using a simple, eco-friendly method, making it a promising candidate for cosmetic applications. The various parameters including pH, viscosity, spreadability, stability, and anti-oxidant activity were evaluated to ensure the efficacy and usability of the hydrogel. The result indicates that the gel exhibits significant anti-oxidant and anti-aging properties, making it a valuable ingredient in skincare formulations. Physical and chemical compatibility studies were conducted to confirm the compatibility of the drug with the selected excipients. Luteolin, the major ingredient is a natural flavonoid, found in plants, vegetables, and fruits. It possesses anti-oxidative, anti-aging, and anti-inflammatory properties. Recent scientific literature has reported the cardiac protective effects of Luteolin in vivo and in vitro.

This study focuses on luteolin's antioxidant activity and clearly illustrates the anti-aging effect brought about by luteolin through its anti-oxidative mechanism.

A topical gel formulation always has shown advantages over other formulations by offering better drug delivery and as it is less greasy and can be quickly removed from the skin when compared to cream, ointment, and paste. Gel formulation has improved application properties and consist.

INTRODUCTION

Nowadays, skin damage and skin-related issues are increasing tremendously. Among them, skin aging has been a major concern worldwide. Aging is a phenomenon that is characterized by folds, ridges, and creases in skin that occurs due to loss of body mass, poor hydration, disintegration of dermis, and epidermis junction. A reduction in the number of fibroblasts that synthesize collagen and vessels that supply the skin leads to an increase in the laxity and hence forms wrinkles.

Ultraviolet radiation from the sun leads to various immediate and long-term deleterious effects, including acute erythema (sunburn), degradation of collagen and elastin, and wrinkled appearance of the skin (Photo aging). Antioxidants are skin protectants from damage and aging by neutralizing free radicals. It also prevents wrinkles, inflammation, and sun damage. People can consume through diet or by the application of skin care products topically. Antioxidant defense mechanisms play a crucial role in protecting the skin from oxidative damage. The number of antioxidants in the epidermis is greater than in the dermis. Antioxidants are available in synthetic and natural substances.

Advantages of gel formulation:

- Gels are easy to prepare when compared to other formulations.
- Gel is an elegant non-greasy formulation
- It has better adherence properties when applied to the site
- Gels are biocompatible and ecofriendly
- They possess better flexibility
- It is easy to modify

Disadvantages of gel formulation:

- The gelators may irritate.
- Solvent loss from formulation dries the gel
- Expensive

MATERIALS AND METHODS

Luteolin belongs to a group of substances called Flavanoids. It is a flavone with a yellow crystalline appearance. Luteolin is a substance found in several plant species, including those used in traditional medicine to treat many diseases. It is widely distributed in the plant kingdom and has been studied extensively for its pharmacological properties, such as anti-inflammatory, antioxidant, and neuroprotective.

METHODOLOGY**A. Collection of Luteolin Powder**

Luteolin powder was obtained from HUBEI HEILONGJIANG BIOTECHNOLOGY Co., Ltd China, which had passed the test according to the standards and was certified by Ging Wang, Xiang Huang, and Xiu Zhang.

B. Drug Characterization studies**a. Melting Point**

A small quantity of luteolin is added into a capillary tube. Melting point of the drug was determined by using Melting point apparatus.

b. Solubility studies

The solubility nature of luteolin was checked by dissolving 1mg of drug in various solvents like methanol, ethanol, acetone, propylene glycol, chloroform, benzene and distilled water to analyze the solubility.

c. Fourier Transform Infrared Spectroscopy

Infrared spectroscopy was used to detect the functional groups of bio-molecules present in drug and drug excipients. The sample was mixed with potassium bromide salt using a mortar and pestle and compressed into a thin pellet and the sample was put into IR spectrometer. The results were recorded on an FTIR spectrometer between the range $4000-400\text{cm}^{-1}$. The wavelength of light absorbed is characteristic of the chemical bonds.

C. Formulation of Luteolin antioxidant gel

A weighed quantity of the polymer Carbopol 934 was kept in distilled water for half an hour to swell. The drug luteolin is weighed accordingly and dissolved in propylene glycol and ensure that it is fully solubilized. This step may require gentle heating below 40°C . Overheating should be prevented.

Once dissolved add luteolin solution to the hydrated gel base while stirring continuously with a glass rod to uniformly distribute. Add preservative phenoxy ethanol as required and almond oil for fragrance. Make up the volume to 50 ml using distilled water and add triethanolamine (drop-wise) to adjust the pH of the gel to 4.7-5.7. Multiple trials were conducted by varying the concentration of carbapol, stirring time, and stirring speed to obtain a stable and elegant gel.

Table no 1: Formulation of Anti-oxidant gel containing Luteolin

SL.NO	INGREDIENTS	WORKING FORMULA
1	LUTEOLIN	1g
2	PROPYLENE GLYCOL	4.5g
3	CARBOMER	1.75g
4	GLYCERIN	3g
5	TRIETHANOLAMINE	As required
6	PHENOXYETHANOL	0.4g
7	ALMOND OIL	As required
8	DISTILLED WATER	Q.S

EVALUATION STUDIES

a. Organoleptic properties

Organoleptic properties such as color, odor, clarity, and homogeneity of the gel were observed by visual inspection.

b. Determination of pH

The pH meter was calibrated using a standard buffer solution. About 1g of gel was weighed and dissolved in 100 ml of distilled water and its pH was measured.

c. Viscosity

The viscosity of gel was measured using a Brookfield viscometer where 100ml was analyzed using spindle no. 64 at 100 rpm

d. Spreadability

The spreadability of the formulation was determined by measuring the spreading diameter of one gram of sample between two horizontal glass plates(10cm,20cm)after one minute. Spreadability denotes the extent of the area to which the formulation spreads on application to the skin. The bioavailability and efficiency of a formulation also depend on its spreading value. The spreading was expressed in terms of time in seconds taken by two slides to slip off from the gel placed between the slides under a certain load.

Two glass slides of standard dimensions were taken. For this purpose, the gel was applied in between two glass slides and they were pressed together to obtain a film of uniform thickness by placing 100gm weight for 5 minutes.

The spreadability (S) can be calculated using the formula

$$S = M.L / T$$

e. Drug content

Accurately weighed 1g of the gel and transferred it to the 100 ml of volumetric flask containing 20 ml of phosphate buffer pH 5.5. The volumetric flask was shaken for 30 minutes and the volume was made up to 100ml with phosphate buffer pH 5.5 solution. Sample absorbance is determined by a UV spectrophotometer at 271nm.

f. Evaluation of antioxidant activity (DPPH ASSAY METHOD)

- i. Preparation of DPPH solution
0.1ml of DPPH is dissolved in 100ml of methanol.
- ii. Preparation of sample solution
Dilute the gel sample in different concentrations such as 0.5mg/ml, 1mg/ml, and 2mg/ml, and make it into 100ml with methanol.
- iii. Preparation of standard solution
Dilute the ascorbic acid in different concentrations such as 0.5mg/ml, 1mg/ml, and 2mg/ml, and makeup into 100ml with methanol.
- iv. Test procedure
Add the 2ml DPPH solution into 3 test tubes and add 1ml of sample solution from each concentration into 3 test tubes. Incubate at room temperature for 30 minutes in the dark.
In the same way, 2ml DPPH solution is added to the 3 test tubes, and 1 ascorbic acid 1ml from different concentrations into each test tube. Incubate for 30 minutes.
- v. Measuring absorbance
After 30 minutes, use a spectrophotometer to measure the absorbance of solution at 517nm
- vi. Calculating antioxidant activity

$$\text{Percentage radical scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

g. Stability study at room temperature

Optimized formulation kept in a tightly closed air-tight container and stored at room temperature for three months and observed for any visible changes in color, odor, and physical state.

RESULTS**a. Melting point**

The melting point of luteolin drug was determined by melting point apparatus and was found to be 330°C.

b. Solubility studies

The solubility studies of Luteolin in various solvents were determined

Table no 2: solubility studies of Luteolin

SOLVENTS	LUTEOLIN
Methanol	Soluble
Ethanol	Soluble
Chloroform	Soluble
Propylene Glycol	Soluble
Acetone	Soluble
Benzene	Insoluble
Distilled Water	Insoluble

c. FTIR studies of drug, drug & excipient

The Fourier Transform Infrared Spectroscopy (FTIR) of the drug Luteolin and drug along with the excipient was performed.

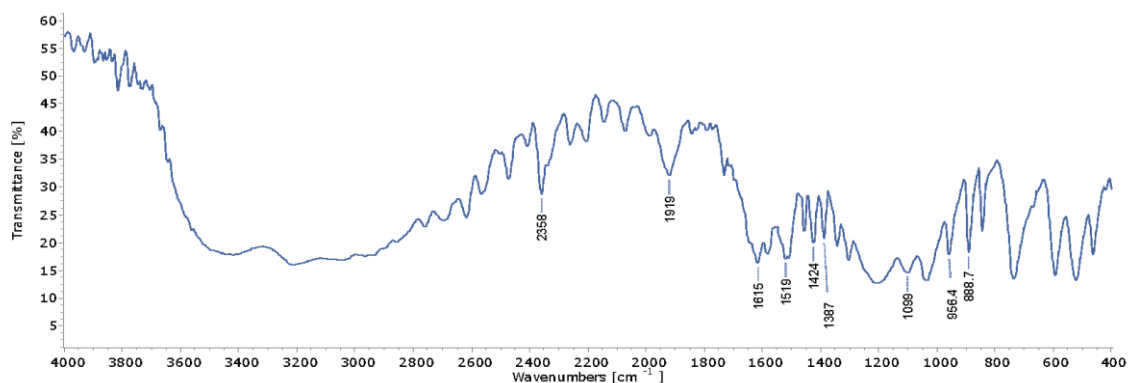
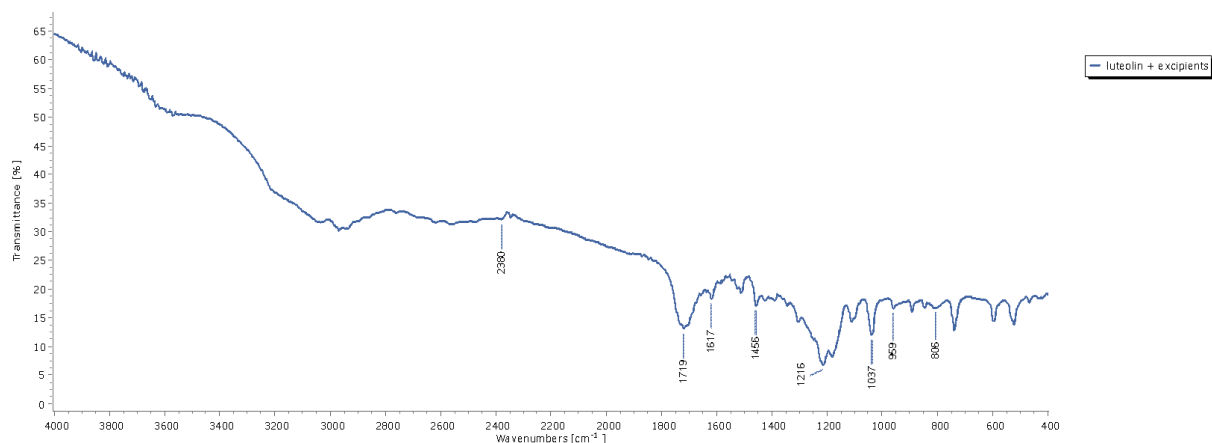
FTIR of the drug Luteolin**FTIR of drug and excipient****d. Formulated gel**

Figure No 1. Formulated Luteolin gel

e. Organoleptic properties**Table no 3: Organoleptic characteristics**

COLOR	Pale yellow
ODOUR	Pleasant fragrance
TEXTURE	Smooth
APPEARANCE	Translucent gel

f. Determination of pH**Table no 4: pH of anti-oxidant gel**

Formulation	pH
FORMULATED GEL	5.31

g. Viscosity**Table no 5: Viscosity of anti-oxidant gel**

Formulation	Viscosity
FORMULATED GEL	4698 Cp

h. Spreadability**Table no 6: Spreadability of anti-oxidant gel**

Formulation	weight used (gm)	length (cm)	time taken (sec)	spreadability
FORMULATED GEL	100	7.5	60	12.5g.cm/s

i. Drug content**Table no 7: Drug content of anti-oxidant gel**

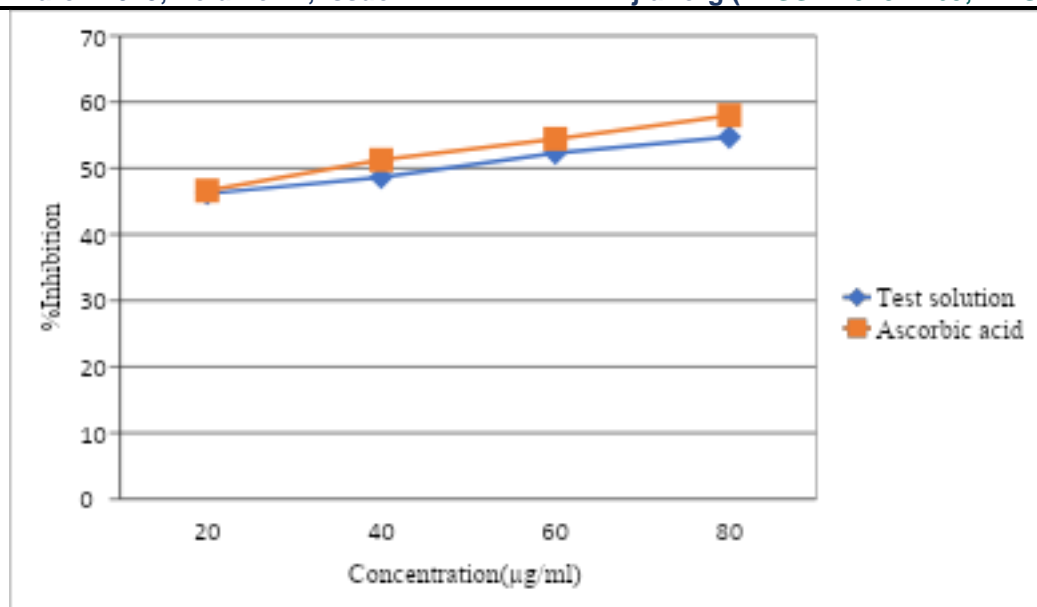
Formulation	Drug Content
FORMULATED GEL	81% w/w

j. Anti- oxidant Activity (DPPH ASSAY METHOD)

The test solution percentage inhibition was calculated using the formula and the graph was plotted by concentration ($\mu\text{g/ml}$) vs percentage inhibition. The slope obtained with equation $y=mx+c$ was used to calculate the IC_{50} value $x=(y-c)/m$. The percentage inhibition in different concentrations along with IC_{50} value was obtained.

Table no 8: Anti- oxidant Activity (DPPH ASSAY METHOD)

SL.NO	SOLUTIONS	% INHIBITION(DPPH-SCAVENGING)				$\text{IC}_{50}(\mu\text{g/ml})$
		20 $\mu\text{g/ml}$	40 $\mu\text{g/ml}$	60 $\mu\text{g/ml}$	80 $\mu\text{g/ml}$	
1	Test solution	46.15	48.63	52.3	54.67	47.3
2	Std.drug (Ascorbic acid)	46.62	51.24	54.43	57.98	36.08



k. Stability study at room temperature

The optimized formulation was kept in an air-tight container and stored at room temperature for three months. No visible changes in color, odor, or physical state were observed.

CONCLUSION

Over the last decade, researchers and scientists have become more interested in pharmaceutical semisolid dosage forms, particularly gels. Skin is a key site for systemic and local drug administration. Gels are defined as semi-rigid systems in which an interlacing three-dimensional network of particles or solvated macromolecules of the dispersed phase restricts the movement of the dispersing medium. Photoaging is a term used for the characteristic skin changes induced by chronic UVA and UVB exposure. Antioxidants help to protect the skin from damage and aging by neutralizing free radicals. They also prevent wrinkles, inflammation, and sun damage. Antioxidant defense mechanisms play a crucial role in protecting the skin from oxidative damage. The present study aims at formulating an antioxidant gel and evaluating various parameters. The luteolin-infused antioxidant gel shows a yellow color and a translucent appearance with a pleasant odor. It shows acidic pH, optimum spreadability, and proper viscosity. Antioxidant gel preparation contains luteolin as the active ingredient and shows a promising antioxidant effect thereby providing an anti-aging effect to the skin. Luteolin can be incorporated in pharmaceutical gel for skin care intended to premature aging and cure various skin problems.

Thus, we conclude this study by assuring the importance of luteolin in skin care and protection by incorporating it into pharmaceutical hydrogel formulation which was found to be stable and compatible for topical application.

REFERENCES

1. Gendrisch F, Esser PR, Schempp CM, Wölfl U. Luteolin as a modulator of skin aging and inflammation. *Phytochem Rev.* 2020;19(3): 571-583. doi:10.1007/s11101-020-09616-w
2. Mohammed AA, Ibrahim AA. Pathological roles of reactive oxygen species and their defense mechanism. *Saudi Pharm J.* 2004;12:1-18.
3. Seelinger G, Merfort I, Schempp CM. Antioxidant, antiinflammatory and anti-allergic activities of luteolin. *Planta Med.* 2008;74:1667-1677.
4. Sastri TK, Sharma S, Patel A, et al. Novel gels: An emerging approach for delivering therapeutic molecules and recent trends. *Gels (Basel).* 2022;8(5):316. doi:10.3390/gels8050316.
5. Waugh A, Grant A. Ross & Wilson Anatomy and Physiology in Health and Illness. 12th ed. Edinburgh: Elsevier; 2018.

6. Loyd VA, et al. Ansel's pharmaceutical dosage forms and drug delivery systems .9th ed. Philadelphia: Lippincott Williams& Wilkins;2011.
7. Kim JK, Kang KA, RYU YS, Piao MJ Han X, Oh MC ,et al. Introduction of endoplasmic reticulum stress via reactive oxygen species mediated by Luteolin in melanoma cells. Anticancer Res .2016;36:2281-2289.
8. Chinmoy Bhuyan*, Dipanakar Saha, Bipul Rabha, Topical gels as drug delivery system: A Review, Journal of Pharmaceutical Research International, Volume-33(47A):344-357.
9. Sunitha Dontha. A REVIEW ON ANTIOXIDANT METHODS. Asian J Pharm Clin Res [Internet]. 2016 Oct. 1 [cited 2024 Nov. 19];9(8):14-32
10. Ganceviciene R, Liakou AI, Theodoridis A, Makrantonaki E, Zouboulis CC. Skin anti-aging strategies. Dermatoendocrinol. 2012 Jul 1;4(3):308-19. doi: 10.4161/derm.22804. PMID: 23467476; PMCID: PMC3583892.