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The Effect of Gelatin and Propylene Glycol on the Penetration Rate of Asiaticoside from Centella asiatica (L.) Urb Leaf Extract Gel

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Abstract: *Centella asiatica* (L.) Urb. is a plant known for its potential in scar treatment due to the presence of asiaticoside, a compound capable of promoting skin cell tissue repair. However, the large molecular size of asiaticoside hinders its penetration through the skin. This study aims to evaluate the effect of gelatin and propylene glycol on the transdermal penetration of asiaticoside from a gel formulation containing Centella asiatica leaf extract. Four different formulations were tested: a control (F1, without gelatin or propylene glycol); one with 15% propylene glycol (F2); one with 4% gelatin (F3); and one with 4% gelatin and 15% propylene glycol (F4). The physical characteristics of the formulations were assessed using several parameters: organoleptic properties, pH, homogeneity, centrifugation, spreadability, viscosity, and rheological behaviour. Penetration studies were performed using a Franz Diffusion Cell with a Whatman No. 1 membrane (pre-coated with Spangler solution) for 360 minutes. The penetration data were analyzed using Liquid Chromatography–Tandem Mass Spectrometry (LC–MS/MS) with a C18 BEH column (2.1 × 50 mm; 1.8 µm particle size). The results showed that each formulation exhibited distinct characteristics. The study findings indicated that both gelatin and propylene glycol, as well as their combination, significantly influenced the penetration of asiaticoside in the gel formulation. Propylene glycol, functioning as a penetration enhancer, was found to increase the penetration of asiaticoside in both gelatin-based and liquid formulations.

Keywords: Asiaticoside, Centella asiatica (L.), Franz Diffusion Cell, Gelatin, Penetration, Propylene Glycol.
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1. INTRODUCTION

Centella asiatica (L.) Urb, commonly known as pegagan in Indonesia, is a plant that grows and spreads widely in tropical regions. This plant has varying names and morphological forms across different regions in Indonesia and other countries. The differences in morphology and growing locations affect its bioactive compound content. Asiaticoside is one of the most abundant compounds in pegagan. Other compounds found in pegagan include tannins, vellarin, hydrocotylin, carotenoids, centeloside, mineral salts, and others (Sutardi, 2016). Asiaticoside is a triterpenoid glycoside classified under the alpha-amarin derivatives with sugar molecules (glucose and rhamnose). Asiaticoside has the ability to strengthen and enhance the repair of skin cells, making it widely used in topical products. However, due to its molecular weight of 911.1233 g/mol and the presence of the stratum corneum layer on the skin, the penetration of asiaticoside in topical applications is challenging (Li *et al*., 2020).

Topical administration is one route of drug delivery through the skin, with gel being one of its formulations. Gel is a semi-solid dosage form made from small inorganic particles or large organic molecules dispersed in a liquid (Danimayostu *et al*., 2017). Gel formulations are popular due to their appealing characteristics, including a cooling sensation, high adhesion, non-stickiness, ease of water removal, ease of application, and good absorption (Hastuty *et al*., 2018). A critical component in gel formulations is the gelling agent. Gelling agents are key components in the preparation of gels and are high molecular weight polymers that have multiple molecules cross-linked with the solvent molecules. The choice of gelling agent significantly affects the form and stability of the gel. Gelatin is one such gelling agent widely used in both food and non-food industries. Gelatin is a polypeptide compound obtained through partial acid or base hydrolysis of collagen derived from animal bones and skin (Suseno & Roswiem, 2018; Rauf *et al*., 2020). The presence of gelling agents can also aid in the delivery of drugs that are difficult to penetrate (Dahlizar *et al*., 2018).

Based on the data, asiaticoside's penetration through the stratum corneum is challenging due to its high molecular weight, necessitating the use of a penetration enhancer. Various penetration enhancers such as terpenes, alcohols, and surfactants are available (Dewi *et al*., 2019). Propylene glycol is one such enhancer classified as an alcohol. Propylene glycol can improve the solubility of drug compounds and disrupt the extracellular lipid components in the stratum corneum, thereby enhancing the diffusion process (Priani *et al*., 2013).

Given the background above, it is crucial to conduct research on the formulation of gel preparations containing asiaticoside from pegagan leaves, examining the impact of gelatin as a gelling agent and propylene glycol as a penetration enhancer. Penetration testing will be conducted using a Franz Diffusion Cell with a Whattman No. 1 membrane coated with Spangler liquid, which mimics skin conditions. The concentration of penetrated asiaticoside will be measured using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) due to its specific and sensitive ability to measure compounds based on molecular weight (Astuti *et al*., 2012).

This study addresses a relevant in the field of pharmaceutics and cosmeceuticals, specifically regarding gel formulations based on plant extracts to enhance the penetration and concentration of asiaticoside in the skin, due to its high molecular weight. The approach involves the combination of a chemical penetration enhancer (propylene glycol) and a gelling agent (gelatin) to facilitate dermal penetration, thereby improving bioavailability and ensuring therapeutic efficacy. This contributes significantly to the development of more effective topical delivery systems.

2. METHODS

Preparation of the formulations were carried using a digital analytical balance (GH 202, OGS, Japan), an overhead stirrer (IKA® RW 20 Digital), a hot plate (IKA® CMAG HS7), and an oven (Memmert Oven UN55, Germany). Characterization of the resulting formulations were performed using pH meter (Horiba F-52, Japan), viscometer (IKA® Rotavisc MV), the asiaticoside and *Centella asiatica* (L.) Urb Leaf extract gel was provided by the National Research and Innovation Agency, Serpong, Indonesia. Gelatin (Making Cosmetics, Snoqualmie United States), propylene glycol (Dow Chemical Pacific, Singapore). All others chemicals and solvents were of reagent grade and were obtained commercially, with majority from Wako Pure Chemical Industries, Ltd., Osaka, Japan.

This study refers to the formulation of gel preparations with four variations to evaluate the effects of using a gelling agent (gelatin) and propylene glycol on the penetration of asiaticoside from pegagan leaf extract gels (Budi & Rahmawati, 2019).

2.1 Gel Formulation

Table 1. Formulation of Pegagan Leaf Extract Gel

Material	Function	Formula (% w/v)			
		I	II	III	IV
Pegagan leaf extract	Active ingredient	5	5	5	5
Gelatin	Gelling agent	-	-	4	4
Propylene Glycol	Enhancer	-	15	-	15
DMDM Hydantoin	Preservative	0.6	0.6	0.6	0.6
Aquadest	Solvent	Ad 100	Ad 100	Ad 100	Ad 100

Information* :

- F1: No gelling agent and no enhancer
- F2: No gelling agent, with enhancer
- F3: No enhancer, with gelling agent
- F4: With gelling agent and enhance

2.2 Gel Preparation

Gelatin is dissolved in aquadest at a ratio of 1:20, as gelatin dissolves more readily in hot water. Pegagan leaf extract is dissolved in aquadest as needed and added to the gelatin solution. DMDM hydantoin, propylene glycol, and the remaining aquadest are then added to the gelatin solution. The components of the formulation are mixed using a magnetic stirrer at 30°C to enhance the homogeneity of the solution. The gel will form after being allowed to stand for a period of time (Rauf *et al*., 2020).

2.3 Evaluation of Gel preparations

a. Organoleptic Test

The gel is visually inspected for consistency, odor, and color. Typically, the gel should be clear and have a semi-solid consistency (Astuti *et al*., 2017).

b. pH Test

The pH of the gel is measured using a calibrated pH meter. One gram of gel is dissolved in 10 times its volume of aquadest. The gel is considered acceptable if its pH falls within the skin's pH range of 4.5 – 6.5 (Astuti et al., 2017).

c. Homogeneity Test

Homogeneity is assessed by observing the clarity of the gel or the absence of coarse particles. The gel is spread evenly on a glass plate and checked for the presence of coarse particles (Astuti *et al*., 2017).

d. Centrifugation

Centrifugation is performed to determine the stability of the gel. The gel is subjected to centrifugation for 30 minutes at 5000 rpm, and phase separation is observed (Bayti *et al*., 2021).

e. Spreadability Test

The spreadability of the gel is assessed by placing 1 gram of the pegagan leaf extract gel on a mica sheet, which is then placed on graph paper. Another mica sheet is placed on top, and the diameter of the spread gel is measured after applying a 50-gram weight for 1 minute. The weight is increased to 100 grams, 150 grams, and 200 grams, and the diameter of the spread gel is recorded. The test is conducted in triplicate (Senja & Renny, 2018).

f. Viscosity and Rheology Test

The viscosity and rheology of the gel are measured using two devices: the Brookfield UL Adapter viscometer for formulations F1 and F2, and the IKA Rotavis (me–vi) viscometer for formulations F3 and F4. For F1 and F2, approximately 16 ml of gel is placed in a sample tube up to the spindle mark. Measurements are taken at rotation speeds of 0.3, 0.6, 1.5, 3, 6, 12, 30, and 60 rpm. For F3 and F4, 100 grams of gel is placed in a beaker and measured using the IKA Rotavis (me–vi) viscometer with spindle 11 at appropriate speeds. A good viscosity range is 2000 – 4000 cps. Rheological properties are determined by varying the rotation speeds (Senja & Renny, 2018). Flow properties are assessed based on the shear rate (rpm) versus the force (dyne/cm²) (Ismayanti *et al*., 2021).

2.4 Penetration Test

The penetration of the gel is tested using a Franz Diffusion Cell with a 21 ml compartment filled with LCMS-grade aquadest, maintained at 37°C ± 1°C, and stirred at 300 rpm. Approximately 1 gram of gel is placed on a Whattman No. 1 membrane that has been soaked in Spangler solution for 15 minutes. The membrane is weighed and placed between the donor and receptor compartments. Samples are collected at 360 minutes (6 hours) using a syringe. The concentration of asiaticoside in the samples is measured using LC-MS/MS.

2.5 Determination of Asiaticoside Content Using LC-MS/MS

The asiaticoside content in the penetration samples is determined using LC-MS/MS with Multiple Reaction Monitoring (MRM) and an Acquity UPLC® C18 Waters BEH column (2.1 x 50 mm; 1.8 micrometers). The mobile phase consists of water, acetonitrile, and 0.1% formic acid. Analysis is conducted with a retention time of 17 minutes. Asiaticoside is identified based on its target molecular weight of m/z 976.4 ([M+NH4]+) as the parent ion and m/z 453.4 as the product ion (Gajbhiye *et al*., 2016).

3. RESULTS AND DISCUSSION

a. Organoleptic Test

The organoleptic test is conducted to assess the characteristics of the formulation, such as color, odor, and texture. According to Table 1, the observations show that all formulations have a deep green color and a distinctive aroma characteristic of pegagan leaf extract. The gel base exhibits a yellowish, transparent color due to the natural color of gelatin. The color and odor changes are attributed to the addition of pegagan leaf extract at a concentration of 5%. The concentration of the extract influences the intensity of the color; higher concentrations result in a more intense color (Prasongko *et al*., 2020).

Table 2. Organoleptic Properties of Pegagan Leaf Extract Gel

Formula	Color	Odor	Texture
F1	Deep green	Specific Pegagan Leaf Extract	Liquid
F2	Deep green	Specific Pegagan Leaf Extract	Liquid
F3	Deep green	Specific Pegagan Leaf Extract	Viscous
F4	Deep green	Specific Pegagan Leaf Extract	Viscous

Information * :

- F1: No gelling agent and no enhancer
- F2: No gelling agent, with enhancer
- F3: No enhancer, with gelling agent
- F4: With gelling agent and enhance

In addition to having the same color and odor, the formulations are distinguished by their texture, resulting in two types of formulations: F1 and F2 are liquid, while F3 and F4 are gels. This difference is due to the presence of the gelling agent, gelatin, in F3 and F4 at a concentration of 4%. Generally, gels form through the cross-linking of polymer chains, which leads to the formation of a helical structure due to hydrogen bonding, ionic interactions, and rigidity, creating a continuous three-dimensional mass (Nugrahaningsih *et al*., 2014). Gelatin gels form through the expansion of gelatin molecules during heating, which causes the breaking of gelatin molecular bonds and the binding of free liquid to form a gel (Agustini *et al.,* 2020).

b. PH Test

The pH test is conducted to determine the acidity level of a formulation. The acidity of the formulation is a crucial aspect of evaluation to ensure both safety and comfort during use (Agustiani et al., 2022). A gel with a pH that is too acidic can cause skin irritation, while a gel with a pH that is too alkaline can lead to skin dryness. Therefore, the formulation should have a pH range compliant with regulations, typically around pH 4.5 – 6.5 (Mursal *et al*., 2019).

Table 3. pH of Pegagan Leaf Extract Gel (n=3)

Formula	pH
F1	4.92 ± 0.010
F2	4.91± 0.012
F3	5.26 ± 0.006
F4	5.32 ± 0.036

Information* :

- F1: No gelling agent and no enhancer
- F2: No gelling agent, with enhancer
- F3: No enhancer, with gelling agent
- F4: With gelling agent and enhance

The pH of the formulations was measured by dissolving 1 gram of gel in 10 ml of solvent, then measuring with a calibrated pH meter. As shown in Table 2, the pH of F1 and F2 is approximately 4.9, while F3 and F4 have a pH around 5.3. The difference in pH is due to the inclusion of gelatin as a gelling agent. Gelatin has a pH range of 4.8 – 9.0, so its addition to the formulation increases the pH. The acidity or pH of gelatin is influenced by the solvent and the method used in its preparation (Elmitra, 2017).

c. Homogeneity Test

The homogeneity test is conducted by visually inspecting the formulation. The test involves placing the formulation between two glass plates. The purpose of this test is to ensure that all components are uniformly mixed in the gel formulation. Observations of the pegagan leaf extract gel show that there are almost no visible coarse particles, indicating good dispersion or dissolution of the extract. The even distribution of color also serves as an indicator of the formulation's homogeneity. These results suggest that the pegagan leaf extract gel meets the criteria for homogeneity (Astuti *et al*., 2017).

d. Viscosity and Rheology Test

Rheological testing determines the flow properties of a solution or semisolid by varying the rotational speed (rpm) to obtain different viscosity values. Flow properties are determined through the shear rate (rpm) versus shear stress (dyne/cm²) curve (Sulastri & Zamzam, 2018). F1 and F2 exhibit Newtonian flow properties (Figure 1), this is indicated by a linear curve relationship between shear stress and shear rate. Newtonian flow properties are characterized by a constant viscosity (Iswandana & Sihombing, 2017).

In contrast, F3 and F4 show thixotropic pseudoplastic flow properties. This is indicated by a decrease in viscosity (lower) at each shear rate from the descending curve compared to the ascending curve. This behavior may result from structural breakdown that does not quickly reform even when the applied pressure decreases or is removed (Indrawati & Sofia, 2018).

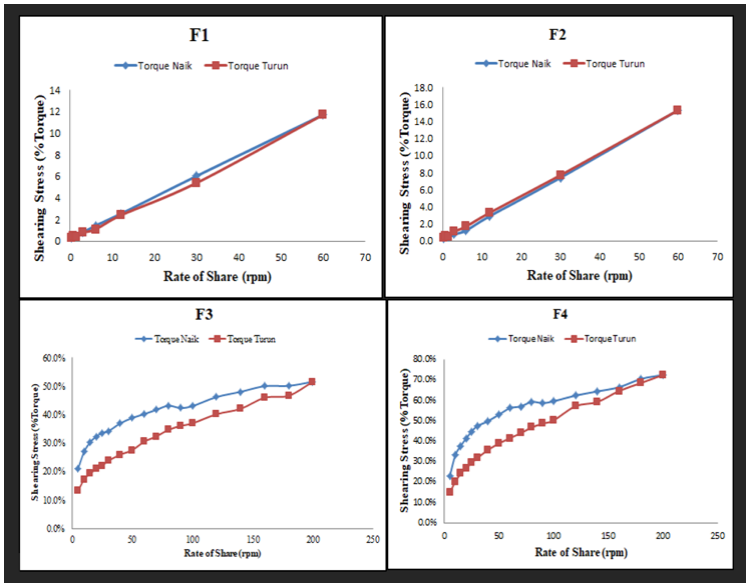

Figure 1. Rheology Curve of Pegagan Leaf Extract Gel
F1 (No *gelling agent* and *enhancer*), F2 (No *gelling agent*, with *enhancer*), F3 (No *enhancer*, with*gelling agent*), F4 (With *gelling agent* and enhancer)

The flow properties of the four gel formulations are influenced by their viscosity. Viscosity testing was conducted using a Brookfield UL Adapter viscometer for F1 and F2, and a Rotavis viscometer for F3 and F4. The formulations were placed in a container up to the spindle's immersion mark, and the rotational speed (rpm) of the spindle was adjusted.

Table 4. Viscosity of Pegagan Leaf Extract Gel

Formulation	Viscosity (cPs)
F1	6.7
F2	7.3
F3	4.307
F4	5.957

Information* :
- F1: No gelling agent and no enhancer
- F2: No gelling agent, with enhancer
- F3: No enhancer, with gelling agent
- F4: With gelling agent and enhance

Based on Table 3, the viscosity tests indicate that all four formulations have different viscosities due to the absence of a gelling agent (gelatin) in F1 and F2, resulting in lower viscosity and a colloidal form. In contrast, F3 and F4 contain gelatin, which leads to higher viscosity values. The standard viscosity range for gel formulations is between 6000 – 50000 cP (Hidayanti *et al*., 2015).

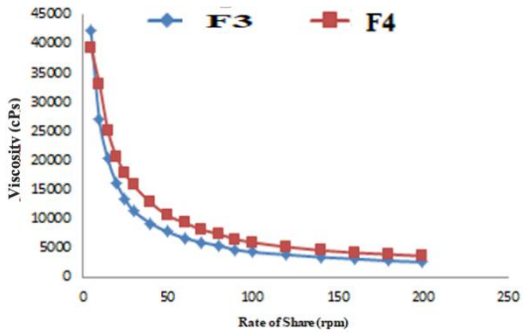

Figure 2. Viscosity Curve of Pegagan Leaf Extract Gel

The presence of gelatin as a gelling agent significantly affects the viscosity of the formulation. The concentration of gelatin used impacts the viscosity level; higher concentrations of gelatin result in increased gel viscosity (Sulastri & Chaerunisaa, 2016). The use of propylene glycol also influences the viscosity of the formulation. Gel formation occurs through the cross-linking of polymer chains, resulting in a helical structure due to hydrogen bonds, ionic interactions, and rigidity, thus forming a continuous three-dimensional mass. As shown in Figure 2, F4 exhibits higher viscosity than F3, which can be attributed to the presence of propylene glycol, leading to an increase in the viscosity of the formulation.

e. Centrifugation Test

The stability of a formulation during storage is a critical factor influencing its quality, necessitating centrifugation tests. The effect of gravitational force is used to evaluate the stability of the gel. The test involves observing phase separation in the formulation that has been centrifuged for 30 minutes at a rotational speed of 5000 rpm (Bayti *et al*., 2021).

Table 5. Centrifugation Test of Pegagan Leaf Extract Gel

Formula	pH
F1	Separation Occurs
F2	Separation Occurs
F3	No Separation Occurs
F4	No Separation Occurs

Information* :
- F1: No gelling agent and no enhancer
- F2: No gelling agent, with enhancer
- F3: No enhancer, with gelling agent
- F4: With gelling agent and enhance

Based on Table 4, the test results indicate that F1 and F2 showed phase separation, evidenced by the presence of sediment or brownish particles. This sediment is likely due to extracts that were not completely dissolved or well-dispersed in the gel formulations. F1 and F2, which have a liquid consistency, allowed undissolved particles to settle quickly. In contrast, F3 and F4 did not exhibit any sediment after testing, indicating better stability. The stability of F3 and F4 can be attributed to the presence of gelatin and propylene glycol, which enhance solubility and increase viscosity. This results in improved dispersion and a reduced rate of sedimentation (Bayti *et al*., 2021).

f. Spreadability Test

The spreadability test is a physical evaluation conducted to assess the ability of a formulation to spread by observing changes in the gel size before and after applying a predetermined weight. A good spreadability range for semisolid formulations is typically between 5 and 7 cm (Sulastri and Chaerunisaa, 2016).

Tabel 6. Spreadability of Pegagan Leaf Extract Gel (n=3)

Weight (gram)	Diameter (cm)	
	F3	F4
50	4.4 ± 0.4	3.7 ± 0.2
100	5.3 ± 0.1	4.6 ± 0.1
150	5.9 ± 0.2	5.4 ± 0.3
200	6.1 ± 0.2	5.6 ± 0.1

Information* :
- F3: No enhancer, with gelling agent
- F4: With gelling agent and enhance

Based on Table 5, F3 exhibits a larger spread diameter compared to F4. The difference in spread diameter between F3 and F4 is influenced by the viscosity of the formulation; higher viscosity results in a smaller spread diameter. The presence of propylene glycol in F4 increases its viscosity compared to F3, leading to a lower spreadability for F4. Conversely, F1 and F2 have lower viscosity, resulting in higher spreadability. The spreadability range can impact the release of the active ingredient and the comfort during application (Syam & Marini, 2020).

g. Penetration Test

In vitro penetration testing of gel formulations aims to determine the concentration of asiaticoside that penetrates through the skin over a specified period. The test is conducted using a Franz diffusion cell, which operates on the principle of placing a membrane between the donor and receptor compartments.

Typically of membranes used can be animal skin or synthetic. However, this test uses Whatman No. 1 membrane coated with Spangler's liquid. The use of a Spangler-coated membrane simulates a skin-like barrier, although it is not as complex. Initially, the weight of the Whattman No. 1 membrane is measured before and after soaking in Spangler's liquid to determine the percentage of absorbed liquid. The membrane's uniformity is considered acceptable if the absorbed Spangler's liquid percentage falls within the range of 102.19% to 131.22%. Based on Table 6, the weight of the membrane after soaking meets the required standards (Astuti *et al*., 2012).

Table 7. Weight Uniformity of Whatman No.1 Membrane

Formula	Weight Membran Soaking (gram)		Spangler's Liquid Entrapped (% w/v)
	Before	After	
F1	0.0464	0.0964	107
F2	0.0468	0.0975	108
F3	0.0472	0.0980	107
F4	0.0474	0.0986	108

Information*:
- F1: No gelling agent and no enhancer
- F2: No gelling agent, with enhancer
- F3: No enhancer, with gelling agent
- F4: With gelling agent and enhance

Penetration testing was conducted using 1 gram of gel formulation applied to the donor compartment. Approximately 21 ml of LC-MS/MS grade water was used as the receptor fluid in the receptor compartment. LC-MS/MS grade water was selected for its high quality, which meets the required standards based on physical and chemical parameters, such as Total Dissolved Solids (TDS), pH, and electrical conductivity (Ikhwanudin *et al*., 2020).

The test was performed at a temperature of 37°C ± 1°C, with a magnetic stirrer set at 300 rpm. The temperature was controlled to match normal human body conditions, as temperature can affect the penetration of substances (Sugiyati *et al*., 2015). The magnetic stirrer ensures uniform distribution of the penetrated substance within the compartment (Halim et al., 2013). Samples of 2 ml were collected at the 360 minute using a syringe, then transferred to a vial for concentration analysis using LC-MS/MS.

Based on the penetration test results, F1 and F2 exhibit higher penetration levels compared to F3 and F4. This is due to the lower viscosity of F1 and F2, which allows the active ingredient to more easily permeate the membrane. Conversely, F3 and F4 have higher viscosities due to the presence of gelatin, which forms a more viscous gel structure. In drug delivery systems, gel functions as a membrane that regulates the gradual release of active ingredients (Budiatin *et al*., 2014), and the viscosity of the formulation, influenced by the concentration of the gelling agent, affects the penetration level (Azmi *et al*., 2022). The higher viscosity in F3 and F4 inhibits the penetration of the active ingredient but provides a better gel consistency (Sulastri & Chaerunisaa, 2016).

Additionally, if we compare the liquid (F1 and F2) and gel formulations (F3 and F4) we can observed that F2 and F4 demonstrates greater penetration compared to F1 and F3 due to the presence of propylene glycol as an enhancer. Propylene glycol increases penetration by disrupting the intracellular lipid matrix, thereby facilitating the active ingredient's passage through the stratum corneum layer (Mulyana *et al*., 2016; Qisti *et al*., 2018).

4. CONCLUSION

Based on the conducted research, it can be concluded that the use of gelatin and propylene glycol in the formulation of Centella asiatica (L.) Urb extract gel affects the characteristics of the gel, including organoleptic properties, pH, homogeneity, spreadability, viscosity, and rheology. Gelatin has a more significant impact, leading to notable differences between formulas, particularly between those with gelatin and those without. The use of gelatin and propylene glycol influences the penetration of Centella asiatica (L.) Urb extract gel. Specifically, propylene glycol enhances penetration due to its role as a penetration enhancer.

5. CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation**.**

6. ACKNOWLEDGMENTS

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