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Research Article

The Effect of Glycerin and Squalane on Skin Moisture in Green Tea (*Camellia sinensis L.*) Nanostructrure Lipid Carrier Preparation

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ABSTRACT

Healthy skin requires optimal moisture levels to maintain its barrier function and prevent transepidermal water loss (TEWL). This study aims to analyze the effect of adding glycerin (humectant) and squalane (emollient) in Nanostructured Lipid Carrier (NLC) nano green tea extract (*Camellia sinensis* L.) preparations on skin moisture, as measured by TEWL parameters. This experimental study used three formulas: F1 (without moisturizer), F2 (with squalane), and F3 (with glycerin). Twelve healthy volunteers participated as test subjects. Physicochemical characteristics of the preparation, including organoleptic properties, pH, viscosity, particle size, PDI, and zeta potential, were evaluated. Additionally, antibacterial activity, entrapment efficiency, and TEWL values (before and after application) were measured. Data were analyzed using the Shapiro-Wilk normality test, homogeneity test, One-Way ANOVA, and Post-Hoc Tukey HSD tests. The results showed that use of a humectant (glycerin) and an emollient (*squalane*) in the NLC formulation exerts an influence on the reduction of *Transepidermal Water Loss* (TEWL) in the skin. Based on the *Post Hoc Tukey HSD* test, the F1 formulation without additional moisturizer provides the most significant TEWL reduction effect compared to the F2 (with *squalane*) and F3 (with glycerin) formulas ($p < 0.05$). Meanwhile, F2 (with *squalane*) only shows a significant advantage compared to F3 (with glycerin) after reaching the 45th minute. This indicates that the addition of *squalane* as an emollient is more effective in increasing hydration and repairing the skin barrier function compared to the use of glycerin in the NLC system.

INTRODUCTION

The skin is the largest organ in the body, with its main functions being to protect against external factors, control water loss, and maintain physiological balance. Skin health is greatly influenced by adequate moisture levels to maintain the skin barrier function. However, environmental exposure, aging,and the use of certain chemicals can cause a decrease in skin moisture, contributing to dehydration, premature aging, and epidermal dysfunction (Proksch et al., 2008) (Uehara et al., 2023). The majority ofthe skin barrier function is thought to be performed by the stratum corneum (SC). The stratum corneum's health is a good indicator of the skin barrier's performance. Transepidermal water loss (TEWL) is the process by which an organism loses water to the environment; the SC has a role in this regulation as well. It is well-established that transepidermal water loss (TEWL) and stratum corneum (SC) hydration exhibit a significant inverse correlation. Low SC hydration is commonly associated with high TEWL readings, which indicate poor skin barrier function. Hence, to keep dry skin looking

healthy,it is essential to maintain healthy skin by increasing its barrier function (Proksch et al., 2008).

One strategy for maintaining skin moisture is the use of moisturizers. Moisturizers are products designed to increase skin hydration (Butarbutar et al., 2020). Based on their mechanism of action, moisturizers can generally be divided into humectants and emollients (Partogi, 2011). Humectants are ingredients that work by drawing water from the deeper layers of the epidermis to the stratum corneum. Humectant moisturizers are also moisturizing products that prevent evaporation and thinning. Commonly used humectant moisturizers consist of several ingredients, one of which is glycerin, which can maintain skin moisture (Ld et al., 2018). As in the study by Setiani et al. (2024), this study found thatout of three formulations that used different percentages of glycerin 10%, 15%, and 20% F3 (20%) had the highest moisture content and met all evaluation criteria, making it the best formulation. The skin's moisture level can be increased and maintained by glycerin, hence a higher concentration of glycerin results in a higher moisture content. A humectant, glycerin draws water from the air and holds it in the skin, keeping the stratum corneum moist (Nur Faradila et al., 2022).

Emollients, meantime, are substances that improve skin texture by acting on intercorneocytes, which will desquamate (Nadeak et al., 2022). Squalane is one of the chemicals that is used rather often. Emollient squalane reduces Transepidermal Water Loss (TEWL) by smoothing and strengthening the skin barrier function by filling the spaces between corneocytes (Febriaty et al., 2020). By removing the hydrogen link, squalene is converted to squalane, a saturated version of the compound. When it comes to oxidative stability, squalane outshines squalene. Because of this, squalane's primary purpose is as a moisturiser (Sethi, 2016). According to Berfeld et al. (2019), squalane has been used in 294 cosmetic formulations with concentrations ranging from ~0.1% to >50%. This ingredient is used in various cleansing, moisturizing, and skin care products.

In the cosmetics industry, nanoparticle technology is increasingly being used to improve the effectiveness of moisturizers. One promising technology is the use of Nanostructured Lipid Carriers (NLC). Nanostructured Lipid Carriers (NLC) were developed to address several shortcomings of Solid Lipid Nanoparticles (SLN). NLC can increase the loading of active compounds and minimize damage to active compounds, as the active compounds are encapsulated in lipids. The NLC system, which consists of lipids, can inhibit water evaporation. In addition, NLC can increase skin hydration, which causes an increase in the gaps between corneocytes, making drug penetration into the skin easier. Furthermore, the small particle size can also reduce water evaporation from the skin (Aryani et al., 2021). The use of glycerin and squalane in the NLC system has the potential to increase the effectiveness of skin hydration,while optimizing the moisturizing function in suppressing TEWL (Butarbutar et al., 2020). Furthermore,green tea extract (Camellia sinensis L.) is one of several natural components that are finding a home in cosmetic recipes. One of the many polyphenols found in green tea is catechin, which can make up as much as 67% of the total. Epigallocatechin gallate is the primary catechin found in green tea. With an IC50 value of 6.435 ppm, en tea extract exhibited extremely potent antioxidant activity, as demonstratedby a study conducted by Priani et al. (2024). Catechins are polyphenolic chemicals and secondary metabolites that plants naturally make that are part of the flavonoid family. Oxidation, extreme heat, light, and acidic conditions are particularly harsh on catechins. And pure catechins are barely soluble incold water but dissolve in hot water, alcohol, and ethyl acetate with ease; they're also colourless. According to Aryani et al. (2021) and Rauf et al. (2015), catechins cannot be dissolved in chloroform, benzene, or ether. The low solubility of catechins is a major obstacle in their utilization, as it leads to inconsistent absorption; therefore, nanostructured lipid carrier (NLC) technology is employed to address this issue. A core matrix is created by combining specific solid and liquid lipids; surfactants are then used to stabilise the matrix, resulting in nano-structured lipid carriers (NLC). Liquid lipids have theability to decrease the regularity of the crystal lattice, which allows more room for the active ingredientsto be accommodated and ultimately increases the absorption effectiveness (Nurrohim et al., 2022). Hence, nanostructured lipid carriers (NLC) were employed in the creation of a topical delivery method for green tea extract. The purpose of this research is to examine the impact of glycerin and squalane in green tea extract nanocosmetics on TEWL-measured skin hydration. More effective skin moisturising formulations based on nanotechnology are anticipated to be developed as a result of this study.

METHODS

Equipment

Analytical balance (Ohaus PA312), oven (Memmert®), micropipette (Socorex®),hotplate stirrer (Thermo Scientific™ Cimarec+™), magnetic stirrer, spoon, iron spatula, tweezers, sonicator (Elma-Ultrasonic S10H), UV spectrophotometer-Vis (Shimadzu*), PSA cuvette, Tewameter TM 300 Centrifuges 5430R (eppendorf), Autoclave KT 40-D, Rotary Evaporator, Homogenizer, pH meter (Ohaus), Biosafety Cabinet (Esco LA2-3A1-E), Viscometer Rion DV-E, glassware (IWAKI and Pyrex) suchas beakers, measuring cups, measuring pipettes, measuring flasks, droppers, test tubes, stirring rods,and watch glasses.

Materials

Green tea leaves (Kulonprogo, Yogyakarta), glyceryl monostearate, isopropyl myristate, tween 80, demineralized water, glycerin, squalene, 96% ethanol, distilled water, Mayer's reagent, Dragendorf's reagent, nutrient agar (NA), 70% ethanol, aluminum foil, Staphylococcus aureus bacteria (Cv.Ardchem, Yogyakarta), and quercetin standard (SIGMA).

The equipment used in this study included Ultraviolet-Visible (UV–Vis) spectrophotometer (PerkinElmer Lambda 356) analytical balance (Ohaus), filter paper, hot plate (Stuart), stopwatch, pH meter (ATC), separating funnel (Pyrex), incubator (Memmert), 50 mesh sieve (Retsch), glass funnel and rotary evaporator (IKA® RV 10), water bath (Memmert), thin layer chromatography (TLC) plate, chamber, and UV lamp.

Extract Preparation

A total of 500 g of green tea leaf simplisia was extracted using the maceration method with 70% ethanol for three days while stirring occasionally. All maceration results were combined and then evaporated using a rotary evaporator (Nurrohim et al., 2022).

Phytochemical Screening

The following is the phytochemical screening procedure for green tea leaf extract, conducted with reference to the research by Pratama et al. (2024), Malik et al. (2014), Pananginan et al.(2020):

Alkaloid test

After three grammes of each extract were concentrated in a water bath, one millilitre of sodium hydrochloride solution was added. Two test tubes were used to separate the filtrate. Three drops of Dragendroff's reagent were applied to the first tube. To the second tube, three drops of Mayer's reagent were introduced. If a white precipitate forms in the Dragendorf tube and an orange precipitate in the Mayer tube, it means there is an alkaloid.

Flavonoid test

After drying out 1 gramme of each extract by evaporation, dissolve the leftovers in 1 to 2 millilitres of 96% ethanol. Then, combine 2 millilitres of 2N hydrochloric acid with half a gramme of zinc powder. After standing for one minute, add ten drops of concentrated hydrochloric acid. If within 2-5 minutes an intense red color occurs, indicating the presence of flavonoids.

Saponin test

After the distilled water has cooled, add 3 grammes of each extract to 10 millilitres, shake well for 10 seconds, and set aside. The creation of a stable foam that is 1-10 cm high and remains after adding 1 drop of 2n hydrochloric acid is indicative of a successful outcome, which must be maintained for at least 10 minutes.

Tannin test

In a 1 ml mixture of 10% iron (III) chloride, 1 gramme of each extract was introduced. If a dark blue, blackish blue, or greenish black color forms, this indicates the presence of a compound Tannin.

Preparation 0f Nanostructured Lipid Carrier (NLC)

The oil and water phases were dissolved at 65°C. After that, the water phase was poured into the lipid phase and stirred using a magnetic stirrer at 1500 rpm for 30 minutes. Then, after 10 minutes of homogenization, the NLC green tea leaf extract was subjected to 30 minutes of sonication. (Farlina et al., 2023).

Testing of physical

Organoleptic

Organoleptic testing was carried out by observing changes in shape, color and odor of the NLC preparation (Saputra et al., 2023).

Viscosity

The test was conducted by preparing a 100 mlL sample and placing it in a container/cup. The sample was examined using a Rion DV-E viscometer. Ensure that the sample is free of bubbles and evenly distributed in the cup. Then, turn on the device, use rotor no. 2, and observe the viscometer pointer pointing to the number on the viscosity scale, then record it (Rochman et al., 2022).

Table 1. Green Tea Extract NLC Formulation

Ingredient	F1	F2	F3
Green Tea leaf extract	1	1	1
Glyserin	-	-	5
Squalane	-	5	-
Glyseryl monostearate	4	4	4
Isopropyl miristate	6	6	6
Tween 80	4	4	4
Demineralized water	Ad 100	Ad 100	Ad 100

pH test

pH was measured with the use of a pH meter. After inserting the electrode into the sample, the reading of the pH meter was recorded. It was carried out three times in total (Rochman et al., 2022).

Particle Size Test, Polydispersity Index, And Zeta Potential

The assay will be held at the Islamic University of Indonesia, Yogyakarta. In order to determine how big the nano preparation's particles are, a Particle Size Analyser will employ the Dynamic Light Scattering (DLS) technique. In order to determine the particle size, mix 1 millilitre of NLC green tea extract with 10 millilitres of demineralised water and use the PSA (Nurrohim et al., 2022; Rohmah et al., 2019). The zeta potential value is evaluated using a zeta potential analyzer with the Dynamic Light Scattering (DLS) method employing photon correlation spectroscopy at a temperature of (25 °C) by diluting the sample 10 times with demineralized water and viewing it (Rohmah et al., 2019).

Entrapment Efficiency

The entrapment efficiency testing process was carried out by following the research reference conducted by Rosita et al. (2019) by diluting the 100 pm green tea extract stock solution made with concentrations of 4 ppm, 6 ppm, 8 ppm, 10 ppm, and 12 ppm with 0.4 mL, 0.6 mL, 0.8 mL, 1.0 mL, and
1.2 mL of 100 ppm quercetin intermediate solution, then placed in a 10 mL volumetric flask. Next, ethanol pa was added to each volumetric flask up to the mark. Absorbance was measured at the maximum wavelength obtained. After adding two millilitres of green tea extract to a 10-milliliter measuring flask, the mixture was topped off with ethanol and filtered. After that, the filtrate was spun in a centrifuge at 13,000 rpm for half an hour. A 10 mL measuring flask was filled to the mark with ethanol pa after 0.5 mL of supernatant was transferred to it.

The next step was to use a UV-Vis spectrophotometer to determine the absorbance at its peak wavelength. There were two separate analyses of the absorbance reading. The quercetin free from the NLC system in the centrifugation supernatant indicates the amount of free drug or active substance outside the delivery system. The entrapment efficiency was calculated using the entrapment efficiency percentage (EE%) formula. fter obtaining the concentration of green tea extract in the supernatant, the entrapment efficiency provided data on the percentage of active substances successfully absorbed by the nanoparticles (Jafar et al., 2019).

$$EE\% = \frac{Total\ added\ dru+ - Free\ dru+}{Total\ added\ dru+} x\ 100\%$$

Preparation of Test Media and Antibacterial Testing

The medium will be prepared using the well diffusion method by pouring 15 ml of NA solution into a Petri dish for the base medium and then allowing it to solidify. After solidification, a 7 mm well is placed at a distance so that the observation area does not overlap. Next, mix the bacterial suspension into the nutrient agar culture medium, and pour 15 ml of NA into the culture medium for the second layer. After the second layer solidifies, the well plate is removed aseptically from the Petri dish to form wells that will be used in the antibacterial test. The wells will be filled with 0.2 g and then dripped onto the well plate. Then incubate at

37°C for 24 hours. After incubation for 24 hours, observations are made (Pananginan et al., 2020).

Transepidermal Water Loss (TEWL)

Transepidermal Water Loss (TEWL) testing was conducted using 12 healthy volunteers (6 males and 6 females) aged between 18 and 25 years who had signed an informed consent form. The volunteers were then prepared by not using any skincare products for 24 hours and not showering for 4 hours before the experiment began. Before the measurement was taken, the volunteers' forearms were washed with warm water, dried carefully, and left for 30 minutes. They then entered the measurement room to allow their skin to adapt to the room environment (Husein et al., 2020). Transepidermal water loss (TEWL) was measured using a tewameter. The first stage involved measuring the TEWL value of the skin area on the upper arm to measure the water content in the skin before applying the nano preparation as a skin base. This TEWL value was the initial TEWL value (to) of the skin before treatment. After the initial TEWL measurement, the skin is reapplied with 0.5 grams of nano preparations F1, F2, and F3 at each point. TEWL measurements were taken every 15, 30, 45, and 60 minutes consecutively. The ability of the nano preparations to reduce skin TEWL was determined by the change in TEWL (Δ TEWL) pressed as a percentage, according to the following equation (Febriaty et al., 2020).

$$\Delta \text{TEWL} = \frac{(TEWL_n) - TEWL_0}{TEWL_0}$$

RESULTS AND DISCUSSION

The sample used in this study was green tea leaves (*Camellia sinensis L.*) obtained from Kulonprogo, Yogyakarta. In this study, two maceration processes were carried out. The results of extraction I yielded 115.69 grams of concentrated green tea extract with a yield of 23.13%. Furthermore, the second extraction yielded 94.49 grams of concentrated green tea extract with a yield of 18.89%. According to the Indonesian Herbal Pharmacopoeia, the extract yield is considered good if the yield value is not less than 10%, because the higher the yield, the higher the secondary metabolite compounds extracted.

To find out how many secondary metabolites were in the crude medicine and extract, phytochemical screening was done. The findings are displayed in Table 2. The presence of alkaloids, flavonoids, saponins, and tannins in green tea extract is illustrated in Table 2. The chemical substances in question possess antimicrobial properties.

Green tea extract served as the active ingredient in this study's Nanostructured Lipid Carrier (NLC), which was manufactured using a variety of materials including solid and liquid lipids, humectants, emollients, surfactants, and solvents. The aqueous phase of green tea extract NLC manufacture started with a 15-minute magnetic stir-frying of demineralised water and Tween 80 on a hotplate set at 65°C. Then, the lipid phase was prepared by mixing glyceryl monosterate into isopropyl myristate on a hotplate at 65°C in a magnetic stirrer until homegeneous. Once mixed, add the green tea extract and stir again with a magnetic stirrer until homogeneous. If the lipid phase is already homogeneous, continue by mixing the aqueous phase into the lipid phase, then stir with a magnetic stirrer by 30 minutes on a hotplate at 65°C. The next step is to place the preparation in a homogenizer for 10 minutes to produce smaller and more homogeneous lipid particles (Fachriani et al., 2023). Then, the NLC is sonicated for 30 minutes to help reduce the particle size by utilizing the ultrasonic waves generated in the device to break the particles into smaller sizes (Husni dan Kartika, 2017). The physical evaluation of the NLC preparation consists of organoleptic testing, pH testing, and viscosity testing.

Table 2. Phytochemical Screening Result

Test	Result	Information
Alkaloids	+	1. There are white deposits Pananginan et al.(2020) 2. There is an orange precipitate Pananginan et al. (2020)
Flavonoids	+	There is a dark red color Pananginan et al. (2020)
Tannin	+	Blue-black or greenish Pananginan et al. (2020)
Saponin	+	Foam formation Pananginan et al. (2020)

Note: if the sign is (+) then it means it is positive and if the sign is(-)it means it does.

Figure 1. NLC Preparation of Green Tea Extract

Table 3. pH Test dan Viscosity Test

Formula	pH ± SD	Viscosity ± SD
1	6,68 ± 0,02	40 ± 0.0
2	6,89 ± 0,03	60 ± 0.0
3	7,12 ± 0,01	40 ± 0.0

Preparation F1 (without added moisturizer) yielded a brownish-green hue, a unique scent, and a smooth consistency.as shown in Figure 1, according to organoleptic test observations. A pale brownish- green colour, a distinct scent, and a liquid texture were the results of Preparation F2 (with the addition of squalane). The third preparation, which included glycerin, turned out to be a liquid with a brownish- green hue, a distinct smell, and a smooth consistency.

Based on Table 3 it can be seen that in the pH test of the NLC preparation, all formulas F1 (without added moisturizer), F2 (with added squalane), and F3 (with added glycerin) have met the desired requirements based on the normal skin pH range, which is 4.5-7.0. The next test was the viscosity test, which was conducted to measure the thickness or flow resistance of the preparation. Viscosity is an important parameter in pharmacy because it can affect the stability, absorption, and application of the product on the skin (Saputra et al., 2023).

Viscosity testing was performed using a Rion viscometer. Rotor number 3 was used, which was installed on the Rion viscometer and locked in a counterclockwise direction. A 100 ml sample of NLC was placed in a cup, ensuring that it was free of bubbles and evenly distributed on the surface of the cup. The rotor was then placed in the center of the cup containing the NLC sample, and the device was turned on. The rotor rotated and the viscosity indicator needle moved to the right in dPas (decipascal-second) units (Martin et al., 2011). The viscosity test was conducted on day 3, yielding the results listed in Table 3. Based on Table 3, the difference in viscosity among the NLC formulas is due to the physical and chemical properties of the moisturizers used. Squalane increases viscosity due to its interaction with the lipid phase, while glycerin does not have the same effect because it interacts more with the aqueous phase.

This explains why F2 has a higher viscosity than F1 and F3 (Nur Faradila et al., 2022), This was followed by particle size testing. The results of particle size testing of the three formulas, as listed in Table 3, produced particle sizes of <1000 mm, indicating that the particle sizes of the three formulas fall within the particle size range of the LC system, which is 10-1000 nm. The degree of uniformity in a system can be described by its polydispersity index (PDI) value; in a monodisperse system, a smaller PDI value indicates a more uniform distribution of particles. If the polydispersity index value is less than 1, it means that the samples are polydisperse, and if it is close to 0, it means that the samples are monodisperse.

Every one of the three sample formulas exhibits monodispersity, according to the table's polydispersity index size test findings. The NLC monodisperse system is very homogeneous and uniform because its particle sizes are all quite close together (16). To keep the dispersion system stable and avoid aggregation, a high zeta

potential value suggests that there is a strong repulsive force between the particles. Based on the zeta potential measurements of NLC preparations F1, F2, and F3, they meet the requirements in the category above 30 mV (Febriani et al., 2022). Preparation F1 without moisturizer, NLC particles are only affected by the charge from the lipids and surfactants used. This results in a high zeta potential value (-51.6 mV), indicating excellent electrokinetic stability. In F2, with the addition of squalane, a non-polar emollient that can affect the charge distribution on the particle surface.

The addition of squalane tends to reduce the negative charge on the particles, causing the zeta potential value to decrease to -34.1 mV. The addition of glycerin can affect the electrical double layer around the particles, causing the zeta potential value to decrease slightly to -35.1 mV. The difference in zeta potential values between F1, F2, and F3 is due to the effect of moisturizers on the charge distribution on the particle surface. F1 has the best electrokinetic stability (-51.6 mV) because there are no moisturizers affecting the particle charge. F2 (with squalane) and F3 (with glycerin) have lower zeta potential values (-34.1 mV and -35.1 mV), but are still within a good stability range. emollients such as squalane and glycerin can affect the electrical double layer around the particles, thereby decreasing the zeta potential value (Wahyuni et al., 2022). The zeta potential values of the NLC preparations (F1, F2, and F3) ranged from -34.1 mV to -51.6 mV. According to literature, a colloidal dispersion system is considered stable and less prone to aggregation if it possesses an absolute zeta potential value above ±30 mV (Puthukulangara et al., 2025; Marín et al., 2017).

Entrapment efficiency testing was conducted to determine the amount of active substance absorbed in the NLC system. Absorption efficiency testing was performed using a UV-Vis spectrophotometer. The working method of UV-Vis spectrophotometry is based on light absorption at a light has a wavelength between 200-400 nm, while visible light has a wavelength of 400-750 nm (Iskandar, 2017) (Listiyana et al., 2020).

Entrapment efficiency testing was conducted at a wavelength of 374 nm. Absorption efficiency (EE) is the percentage of active ingredients trapped in lipid particles. Lipophilic preparations usually have an EE value between 90-98%, while hydrophilic preparations have an EE value of 30-50% (Ciecila et al., 2025). In this study, the absorption efficiency produced by the three formulas (F1, F2, and F3) did not meet the requirements for lipophilic extract materials because they did not meet the NLC topical preparation range of 90-98%. This could be due to the addition of a higher ratio of liquid lipids to the solid lipid matrix, which could disrupt the regularity of the crystal lattice. This disruption creates an imperfect structure (Rochman et al., 2022).

Table 4. Particle Size, PDI, and Zeta Potential

Formula	Particle Size ± SD (nm)	PDI ± SD	Zeta Potential ± SD (mV)
1	137,7 ± 0,854	0,269 ± 0,064	-51,6 ± 0,265
2	173,8 ± 0,781	0,125 ± 0,019	-34,1 ± 1,323
3	156,1 ± 1,873	0,453 ± 0,026	-35,1 ± 3,381

Table 5. Absorption Effciency

Formula	Formula 1	Formula 2	Formula 3
Replication1	48,51	49,76	47,47
Replication i 2	40,83	51,42	46,02
$\bar{x}$ ± SD	44,67 ± 5,431	50,59 ± 1,174	46,75 ± 1,025

Table 6. Inhibitory Zone Diameter of Green Tea Extract

Green Tea Extract	R1	R2
	4 mm	3,5 mm
	2,5 mm	3 mm
	3 mm	3,5 mm
$\bar{x}$ ± SD	3,17 ± 0,76	3,33 ± 0,29

Based on Table 5, the addition of glycerin to F2 significantly increased the EE value from 44.67% to 50.59% and also reduced the standard deviation (SD). The effect of squalene as a hydrophilic material increased the ability of the matrix to trap or stabilize the active ingredient (green tea extract) within it. Then, in F3, which also had glycerin added, the EE value increased from 44.67% to 46.75%, but the increase was smaller than that of squalane in F2. The EE value shows that formula variations have an effect. F2 (with squalane) produced the highest absorption efficiency value of 50.59 ± 1.174. This increase indicates that squalane (F2) is the most effective additive for improving the ability of NLC to trap green tea extract.

Antibacterial testing of green tea extract at a concentration of 1% in the three formulas (F1, F2, and F3). Antibacterial effectiveness can be observed from the formation of an inhibition zone measured around the well. The results obtained in the antibacterial test can be seen in Table 6.

An inhibitory zone was observed in the green tea extract test, as shown in Table 6, but it was deemed weak. An inhibition zone of 20 mm or more is deemed very strong, an inhibition zone of 11-20 mm is deemed strong, an inhibition zone of 6-10 mm is deemed moderate, and an inhibition zone of 5 mm or less is deemed weak, accordance with Davis and Stout (2009). The amount of green tea extract used can affect this. A stronger inhibitory effect on bacterial growth may be observed at higher concentrations.

If the extract concentration is too low, its antibacterial activity is not sufficient to produce a large inhibition zone, indicating that the higher the concentration of the extract used, the larger the diameter of the resulting inhibition zone. Based on these criteria, the inhibitory power of green tea extract against Staphylococcus aureus bacteria is classified as weak, because the results obtained are in the range of <5 mm. Thus, it is known that the concentration used is a concentration that is less effective in inhibiting *Staphylococcus aureus* bacteria. Transepidermal Water Loss (TEWL) testing was conducted after obtaining ethical clearance from the Ethics Committee of the Faculty of Medicine, Duta Wacana Christian University, with Number: 1756/C.16FK/2025. The confidentiality of the research subjects' data was guaranteed, and before the experiment was conducted, the subjects were given informed consent and asked to sign for legal approval. Transepidermal Water Loss (TEWL) is a method in dermatology that studies the skin barrier in vivo. In this study, three formulas were used, namely F1 (without added moisturizer), F2 (with added squalane), and F3 (with added glycerin).

The next step was to run a normality test was conducted to determine whether the residual values in the data follow a normal distribution. Based on the normality test criteria, data are considered normally distributed if the significance value (sig) is greater than 0.05. Conversely, if the significance value is less than 0.05, the data are considered not normally distributed [49]. Based on Table 7 of the *Shapiro–Wilk* normality test results across all measurement times (15, 30, 40, and 60), the *p-value* obtained was greater than 0.05 in all formula groups. Thus, it can be concluded that the TEWL reduction data in this study are normally distributed, so that further statistical analysis can be performed using a parametric test, namely *One-Way ANOVA*, to determine the differences in TEWL reduction between formulas. Meanwhile, the TEWL variable after measurement that is not normal is more appropriately analyzed using a non-parametric test such as *Kruskal–Wallis*. Table 7 shows that the TEWL variable has a significance level of 0.173 for the Kolmogorov-Smirnov test and a value of 0.404 for the Shapiro-Wilk test, both of which are higher than the 0.05 threshold. This points to a regularly distributed distribution for the TEWL data prior to measurement. The data following measurement for the TEWL variable does not follow a normal distribution, as indicated by the Shapiro- Wilk value of 0.001 and the Kolmogorov-Smirnov significance value of 0.047. On the other hand, the TEWL decrease (%) variable had significant values greater than 0.05 for both the Kolmogorov-Smirnov and Shapiro-Wilk tests, with values of 0.200 and 0.438, respectively. Statistics on the percentage decline in TEWL follow a normal distribution.

Table 7. TEWL Measurement Result

Subject	Formula	Time	TEWL_Before (g/h.m2)	TEWL_After (g/h.m2)	% TEWL decrease
1	1	1	19.22	8.97	53.33%
1	1	2	19.22	10.08	47.55%
1	1	3	19.22	9.36	51.30%
1	1	4	19.22	7.55	60.72%
2	1	1	19.43	9.05	53.42%
2	1	2	19.43	7.45	61.66%
2	1	3	19.43	7.96	59.03%
2	1	4	19.43	8.01	58.78%
3	1	1	14.92	10.65	28.62%
3	1	2	14.92	7.53	49.53%
3	1	3	14.92	5.79	61.19%
3	1	4	14.92	5.42	63.67%
4	1	1	15.78	8.97	43.16%
4	1	2	15.78	7.73	51.01%
4	1	3	15.78	7.03	55.45%
4	1	4	15.78	6.87	56.46%
5	2	1	12.62	11.72	7.13%
5	2	2	12.62	9.12	27.73%
5	2	3	12.62	8.43	33.20%
5	2	4	12.62	6.74	46.59%
6	2	1	11.39	8.07	29.15%
6	2	2	11.39	7.85	31.08%
6	2	3	11.39	6.95	38.98%
6	2	4	11.39	5.74	49.60%
7	2	1	11.46	8.94	21.99%
7	2	2	11.46	6.13	31.08%
7	2	3	11.46	6.11	38.98%
7	2	4	11.46	5.96	47.99%
8	2	1	11.51	8.62	25.11%
8	2	2	11.51	7.76	32.58%
8	2	3	11.51	7.47	35.10%
8	2	4	11.51	6.70	41.79%
9	2	1	10.05	7.34	26.97%
9	3	2	10.05	7.25	27.86%
9	3	3	10.05	7.06	29.75%
9	3	4	10.05	6.93	31.04%
10	3	1	17.6	15.86	9.89%
10	3	2	17.6	15.64	11.14%
10	3	3	17.6	14.58	17.16%
10	3	4	17.6	14.31	18.69%
11	3	1	8.33	7.69	7.68%
11	3	2	8.33	7.64	8.28%
11	3	3	8.33	7.42	10.92%
11	3	4	8.33	6.07	27.13%
12	3	1	12.62	11.72	7.13%
12	3	2	12.62	9.12	27.73%
12	3	3	12.62	9.43	25.28%
12	3	4	12.62	8.74	30.74%

Table 8. *One Way* ANOVA Test

		Sum of Squares	df	Mean Square	F	Sig.
15	Between Groups	2179.376	2	1089.688	10.263	.005
	Within Groups	955.576	9	106.175		
	Total	3134.951	11			
30	Between Groups	2272.704	2	1136.352	15.589	.001
	Within Groups	656.052	9	72.895		
	Total	2928.756	11			
40	Between Groups	2587.157	2	1293.578	31.118	.000
	Within Groups	374.132	9	41.570		
	Total	2961.288	11			
60	Between Groups	2204.431	2	1102.216	61.486	.000
	Within Groups	161.337	9	17.926		
	Total	2365.768	11			

Table 9. *Post Hoc Test*

Dependent Variable	(I) Formula	(J) Formula	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
15	F1	F2	23.78750*	7.28612	.024	3.4446	44.1304
		F3	31.71500*	7.28612	.005	11.3721	52.0579
	F2	F1	-23.78750*	7.28612	.024	-44.1304	-3.4446
		F3	7.92750	7.28612	.544	-12.4154	28.2704
	F3	F1	-31.71500*	7.28612	.005	-52.0579	-11.3721
		F2	-7.92750	7.28612	.544	-28.2704	12.4154
30	F1	F2	17.96250*	6.03716	.038	1.1067	34.8183
		F3	33.68500*	6.03716	.001	16.8292	50.5408
	F2	F1	-17.96250*	6.03716	.038	-34.8183	-1.1067
		F3	15.72250	6.03716	.067	-1.1333	32.5783
	F3	F1	-33.68500*	6.03716	.001	-50.5408	-16.8292
		F2	-15.72250	6.03716	.067	-32.5783	1.1333
45	F1	F2	18.25250*	4.55907	.008	5.5236	30.9814
		F3	35.96500*	4.55907	.000	23.2361	48.6939
	F2	F1	-18.25250*	4.55907	.008	-30.9814	-5.5236
		F3	17.71250*	4.55907	.009	4.9836	30.4414
	F3	F1	-35.96500*	4.55907	.000	-48.6939	-23.2361
		F2	-17.71250*	4.55907	.009	-30.4414	-4.9836
60	F1	F2	13.41500*	2.99386	.004	5.0561	21.7739
		F3	33.00750*	2.99386	.000	24.6486	41.3664
	F2	F1	-13.41500*	2.99386	.004	-21.7739	-5.0561
		F3	19.59250*	2.99386	.000	11.2336	27.9514
	F3	F1	-33.00750*	2.99386	.000	-41.3664	-24.6486
		F2	-19.59250*	2.99386	.000	-27.9514	-11.2336

*. The mean difference is significant at the 0.05 level.

Based on Table 8 of the *one-way ANOVA* test, it is known that the TEWL data *One-Way ANOVA* test was conducted to determine whether there are differences in TEWL reduction between formulas (F1, F2, and F3) at each observation time. The testing criterion is that if the *p-value* < 0.05, then

there is a significant difference between the formula groups.

From Table 8 on the *One-Way ANOVA* test results, a *p-value* < 0.05 was obtained across all observation times (15, 30, 45, and 60). Thus, it can be concluded that there are significant differences in TEWL reduction between formulas (F1, F2, and F3) at each measurement time. To determine which formulas have significant differences, further analysis needs to be conducted using a follow-up test (*post hoc test*) using the Tukey test.

Next, the data were analyzed using a *Post Hoc Test*. The *Post Hoc* test was performed using the *Tukey HSD Test* method. The *Tukey HSD* test was conducted to determine which formula pairs have significant differences in TEWL reduction. The testing criterion is that if the *p-value* < 0.05, then there is a significant difference between the formula pairs. Based on the *Tukey HSD* test results, at the 15th-minute and 30th-minute measurements, significant differences occurred between F1 and F2 as well as F1 and F3, whereas F2 and F3 did not differ significantly. Meanwhile, at the 40th-minute and 60th-minute measurements, all formula pairs showed significant differences in TEWL reduction.

CONCLUSIONS

Based on the results of the research and data analysis that have been conducted, it can be concluded that: The use of a humectant (glycerin) and an emollient (*squalane*) in the NLC formulation exerts an influence on the reduction of *Transepidermal Water Loss* (TEWL) in the skin. Based on the *Post Hoc Tukey HSD* test, the F1 formulation without additional moisturizer provides the most significant TEWL reduction effect compared to the F2 (with *squalane*) and F3 (with glycerin) formulas (p < 0.05). Meanwhile, F2 (with *squalane*) only shows a significant advantage compared to F3 (with glycerin) after reaching the 45th minute. This indicates that the addition of *squalane* as an emollient is more effective in increasing hydration and repairing the skin barrier function compared to the use of glycerin in the NLC system.

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CONFLICT OF INTEREST

All authors have stated that they have no financial or other competing interests in this research. The study's design, data collection, analysis, and publication of the results were unaffected by any of the declared financial or material supports or sources of financing. The writers are entirely responsible for the reliability and validity of the findings since they had complete access to all data.

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