

Optimization of Formulation and Antioxidant Activity Evaluation of Green Betel Leaf (*Piper betle* L.) Oil Toner

Optimasi Formula Dan Uji Aktivitas Antioksidan Toner Minyak Daun Sirih Hijau (Piper betle L.)

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ABSTRACT

Exposure to free radicals can damage the skin. Green betel leaf oil, which has antioxidant activity, can be formulated into a toner. This study aimed to determine the optimal toner formula and to evaluate its antioxidant activity. Optimization was performed using a factorial design with two factors: Tween 80 and PEG 400 concentrations. The optimum formula, determined with Design Expert 13, consisted of 10% Tween 80 and 5% PEG 400, with a predicted pH of 5.66 and 99% transmittance. Validation using a One-Sample T-Test showed no significant difference ($p > 0.05$) between the predicted and actual results. The optimized toner had a pH of 5.69, a transmittance of 99.2%, a particle size of 12.2 nm, and very strong antioxidant activity ($IC_{50} = 43.29 \mu\text{g/mL}$). In this research, an optimum toner formula was obtained which has good physical properties and very strong antioxidant activity.

Keyword: Toner, Green Betel Leaf Oil, Antioxidant, Optimization, Design Factorial

ABSTRAK

Paparan radikal bebas dapat menyebabkan kerusakan pada kulit. Minyak daun sirih hijau memiliki aktivitas antioksidan dan dapat diformulasikan menjadi sediaan toner namun pada formula sebelumnya dihasilkan toner yang keruh. Penelitian ini bertujuan untuk memperoleh formula optimum toner dan mengetahui aktivitas antioksidan. Metode pada optimasi menggunakan desain faktorial dengan dua faktor yaitu konsentrasi tween 80 dan PEG 400. Penentuan formula optimum toner menggunakan Design Expert 13. Formula optimum toner minyak daun sirih hijau menunjukkan hasil formula terbaik tween 80 (10%) dan PEG 400 (5%) dengan prediksi pH 5,66 dan persen transmitan 99%. Prediksi design dan hasil uji dibandingkan dengan One Sample T-Test diperoleh hasil yang tidak berbeda

signifikan dengan nilai signifikansi > 0,05. Formula optimum toner yang dihasilkan memiliki sifat fisik yang baik, ditunjukkan dengan nilai pH 5,69 yang sesuai dengan pH kulit dan persen transmittan 99,2%. Toner minyak daun sirih hijau menunjukkan aktivitas sebagai antioksidan yang sangat kuat dengan nilai IC₅₀ rerata sebesar 43,29 µg/mL, ukuran partikel 12,2 nm. Pada Penelitian ini diperoleh formula optimum toner yang memiliki sifat fisik yang baik dan aktivitas antioksidan sangat kuat.

Kata Kunci: Toner, Minyak Daun Sirih Hijau, Antioksidan, Optimasi, Desain Faktorial

LINTRODUCTION

Exposure to free radicals from air pollution, ultraviolet radiation, and unhealthy lifestyles is a major challenge to skin health. As the outermost layer of the body, the skin is vulnerable to damage caused by free radicals (Sayuti & Yenrina, 2015). Antioxidants can be found in various Indonesian plants, one of which is green betel leaf. Green betel leaf essential oil (*Piper betle* L.) contains sesquiterpene hydrocarbons (6.86–39.71%) and chavibetol ranging from 23.52% to 81.32%, which have antioxidant properties (Das et al., 2022). According to Christiani et al. (2023), green betel leaf essential oil has an IC₅₀ (Inhibition Concentration) value of 5.67 µg/mL, and research by My et al. (2020) reported an IC₅₀ value of 3.71 µg/mL.

A facial toner is a cosmetic preparation used to refresh the face and prepare the skin for the application of moisturizer (Khansa & Mardhiyah, 2017). Toners are generally clear to transparent solutions. For individuals with oily skin, toner can help reduce excessive sebum

secretion. In addition, toner can provide a cooling sensation to the skin. Toners are made using water as the base and other supporting ingredients, including active compounds, humectants, emollients, surfactants, preservatives, colorants, and fragrances (Draelos & Thaman, 2006). In toner formulations, ingredients containing antioxidants should be added to support the effectiveness of skin care. In a study by (Adityas et al., 2024), the use of tea tree oil as the active ingredient still resulted in a cloudy toner appearance.

This study aims to obtain an optimal toner formula with good physical properties and maximum antioxidant activity. The factorial design optimization method was used with variations in the concentrations of surfactant and cosurfactant, with pH and percent transmittance as the responses. Tween 80 was used as the surfactant, and PEG 400 was designated as the cosurfactant because it has short carbon chains that allow it to fill gaps between surfactants and reduce interfacial tension (Asri et al., 2017).

II. METHOD

The research employed an experimental approach using Design Expert version 13 (trial) with a factorial design method. In this study, two factors were optimized: the concentration of the surfactant Tween 80 (5–10%) and the cosurfactant PEG 400 (5–10%), each at two levels (high and low). The upper and lower boundaries were established based on previous research and subsequently input into the system design expert (Al Hasanah et al., 2023). The combination of the two factors and two levels (2^2) resulted in four formulas, with each formula prepared in triplicate. The formula obtained from the design can be seen in the following Table I.

Table I. Design of formulation optimization

Composition	Concentration (%) (w/v)			
	F1	F2	F3	F4
Green betel leaf oil	0.8	0.8	0.8	0.8
Tween 80	5	5	10	10
PEG-400	5	10	5	10
Propylene glykol	8	8	8	8
Aqua ad	100	100	100	100

The measured responses consisted of pH values and percent transmittance data.

A. Determination of the Optimum Formula

The optimum formula was determined based on the pH and percent

transmittance test results from the four formulas in Table 1. The formula selection was carried out using Design Expert software with a factorial design method to obtain a high desirability value. Desirability refers to how well a formulation meets the criteria for the final product, with an ideal value approaching (Al Hasanah et al., 2023).

B. Verification of the Optimum Formula

The optimum toner formula obtained from the factorial design was verified by reformulating it. Physical evaluations, including pH and percent transmittance, were then conducted. The results of the optimum formula were compared with the values predicted by the Design Expert version 13 (trial) software. The analysis was performed using a One Sample T-Test.

C. Preparation of Green Betel Leaf Oil Toner

The green betel leaf oil was weighed into a vial, after which Tween 80 was added and the mixture was stirred using a magnetic stirrer at 500 rpm for 10 minutes. PEG-400 was then added and stirred until homogeneous, followed by the addition of propylene glycol. Distilled water was gradually added while stirring for an additional 5 minutes.

D. Evaluation of the Toner Formulation

The toner formulation was evaluated through organoleptic testing to observe its physical characteristics, including appearance, color, and odor. The pH was measured using a pH meter (Indonesian Ministry of Health, 2020). Further evaluations included percent transmittance testing, particle size analysis (Maharini et al., 2020), and determination of the polydispersity index, where values approaching zero indicate a more uniform particle distribution (Danaei et al., 2018). Zeta potential measurement, stability testing.

E. Antioxidant Activity Test of Green Betel Leaf Oil Toner

The antioxidant activity of the toner was evaluated using the DPPH method with a 96-well microplate reader. A stock solution (1000 µg/mL) was prepared by dissolving 1.25 g of toner in methanol p.a. to 10 mL. This solution was further diluted to obtain a 100 µg/mL working solution. Serial two-fold dilutions were prepared in the microplate to obtain final concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 µg/mL. Methanol p.a. was used as the blank. Subsequently, 80 µL of DPPH solution (80 µg/mL) was added to each well. The mixture was incubated for 30 minutes at room temperature in the dark, and absorbance was measured using a

microplate reader. The percentage of inhibition and IC₅₀ values were then calculated (Agistia et al., 2024; Dehpour, 2009).

F. Data Analysis

The pH and percent transmittance data obtained from the optimum experimental formula and the predicted formula generated by Design Expert were analyzed using a One Sample T-Test. Meanwhile, the pH and percent transmittance data of the toner before and after the freeze-thaw stability test were analyzed using a Paired T-Test.

III. RESULTS AND DISCUSSION

In this study, the toner formulation from *P. betle* leaf oil was optimized using a combination of the surfactant Tween 80 and the co-surfactant PEG-400, employing a factorial design optimization method and evaluating antioxidant activity using the DPPH assay. Toners are generally clear solutions that serve several functions, including refreshing the skin, restoring its pH after cleansing, and providing hydration (Draelos & Thaman, 2006).

Green betel leaf is widely known in traditional medicine for its various therapeutic properties, such as antibacterial activity, wound-healing benefits, strengthening teeth, and antioxidant effects (Widiyastuti et al., 2020). The essential oil

of green betel leaf exhibits strong antioxidant activity, with an IC_{50} value of 5.67 $\mu\text{g/mL}$ reported by Christiani et al. (2023), and an IC_{50} value of into toner formulations serves as a supportive component that enhances skin care routines.

Green betel leaf oil is a clear, light-yellow liquid with a characteristic aroma of betel leaves. It is insoluble in water but highly soluble in 96% ethanol. The specific gravity of the oil is 0.964, its refractive index is 1.501, and its pH is 5.02. The oil contains sesquiterpene hydrocarbons (6.86–39.71%) belonging to the terpenoid group, chavibetol ranging from 23.52% to 81.32%, and eugenol.

The antioxidant activity of green betel leaf oil was evaluated using the DPPH method, which is widely used due to its simplicity, speed, sensitivity, and minimal sample requirement. The antioxidant activity test using DPPH in 80 $\mu\text{g/mL}$ methanol showed an inhibition percentage of 66.59% at 100 $\mu\text{g/mL}$. The percent inhibition data and the natural logarithm of the concentration were plotted to obtain a linear regression equation $y = 16.479x - 9.6688$ with an R^2 value of 0.962. This linear regression equation was used to calculate the IC_{50} value, which was determined to be 37.371 $\mu\text{g/mL}$. This result indicates that the antioxidant activity of green betel leaf oil is classified as very

strong, as it falls below 50 $\mu\text{g/mL}$. For comparison, vitamin C exhibited an IC_{50} value of 23.796 $\mu\text{g/mL}$, which also indicates very strong antioxidant activity ($IC_{50} < 50 \mu\text{g/mL}$).

The amount of oil used in the formulation was calculated based on the IC_{100} value, as the added oil was expected to inhibit 100% of free radicals. From the IC_{100} calculation of green betel leaf oil, an IC_{100} value of 776.708 $\mu\text{g/mL}$ was obtained. When converted into % (g/mL), this corresponds to 0.0776708 g/mL, which is approximately 0.08 g/mL. This value was then multiplied by a scaling factor of $10 \times IC_{100}$, resulting in a final concentration of 0.8% green betel leaf oil for the formulation.

The excipients used in the toner included Tween 80, PEG 400, propylene glycol, and distilled water. All excipients were evaluated prior to use to ensure compliance with the specifications stated in the Indonesian Pharmacopoeia, 6th Edition, and the Handbook of Pharmaceutical Excipients.

Tween 80 is a non-ionic surfactant that is considered safe due to its non-toxic and non-irritating characteristics. It is widely used in pharmaceutical formulations because of its favorable lipophilic–hydrophilic balance and its stability in weak acidic and basic solutions (Ramli et al., 2019). To enhance its surfactant activity,

Tween 80 is often combined with co-surfactants such as PEG 400. This combination can form a more compact film at the oil–water interface (Kale & Deore, 2017). PEG 400 itself is a short-chain glycol that is colorless, viscous, and odorless. In addition, PEG 400 functions as an emollient and enhances penetration in topical formulations (Ramli et al., 2019).

The optimization of the green betel leaf oil toner formulation was carried out using Design Expert version 13 (trial) through a factorial design method. The factorial design approach offers several advantages, including greater efficiency in terms of cost, time, and accuracy compared to the traditional trial-and-error method, which is typically more expensive, time-consuming, less efficient, and more prone to failure.

The optimization process was conducted by entering the variables to be optimized into Design Expert version 13 (trial). The factorial design applied in this study was a 2^2 design. The number “2” represents the levels—low and high—of the ingredient concentrations being optimized, while the second “2” refers to the factors, namely the variables examined in the formulation (Bolton & Bon, 2009). The concentration of surfactant applied in the formulation ranged from 5–10%, with 5% as the low level and 10% as the high level, while the concentration of co-

surfactant used was also within the range of 5–10%. The selection of surfactant and co-surfactant concentrations was based research (Al Hasanah et al., 2023; Rowe et al., 2009).

The data for each variable were entered into Design Expert version 13 (trial) using the concentration variations of each component (Tween 80 and PEG 400), as presented in Table I, which shows the optimization design of the toner formulation. Subsequently, the four formulas were prepared into facial toner preparations with three replications for each formula, and the pH and percent transmittance responses were evaluated.

pH measurement is an essential parameter in cosmetic product analysis. According to Tranggono & Latifah (2007), a good cosmetic product should have a pH close to that of the skin, ranging from 4.5 to 6.5. The evaluation results of the four optimized toner formulas showed that the pH values obtained met the acceptable pH range for skin. The pH data are presented in the Table II:

Table II. pH of the toner formulation

F	pH$\pm$ SD
F1	5.25 $\pm$ 0.050
F2	5.67 $\pm$ 0.086
F3	5.66 $\pm$ 0.067
F4	5.76 $\pm$ 0.109

According to Hilmarni et al., (2022), a pH value below 4.5 may cause

skin irritation, while a pH above 6.5 may lead to dryness and flakiness of the facial skin. Among the four formulas, F1 showed the lowest pH value, F2 and F3 exhibited similar pH values, and F4 had the highest pH value; however, all four formulas remained within the acceptable physiological pH range for the skin.

Percent transmittance was used as a response parameter to describe the clarity of the preparation. The closer the percent transmittance is to 100%, the better the toner's ability to transmit light, indicating good stability and homogeneity of the formulation. The evaluation results of the four optimized toner formulas showed percent transmittance values approaching 100%. The percent transmittance data are presented in Table III.

From Table III, it can be observed that F2 has the lowest percent transmittance, while F3 shows the highest. The pH and percent transmittance response data from the four toner formulas were

entered into Design Expert version 13 (trial) to obtain the optimum formula. The optimum formula was determined based on the desirability value (0–1), where values approaching 1 indicate a formulation that better meets the desired product criteria (Isnaeni et al., 2022).

Table III. Percent transmittance value

F	Mean $\pm$ SD
F1	97.6 $\pm$ 1.302
F2	96.5 $\pm$ 0.205
F3	99.0 $\pm$ 0.216
F4	98.9 $\pm$ 0.478

The selected optimum formula was Formula 3, as it had the highest desirability value, approaching 1, at 0.900. In the resulting contour plot of desirability, the orange color was observed to be approaching red. The contour plot for pH appeared green, whereas the contour plot for percent transmittance appeared orange. These results in Figure 1.

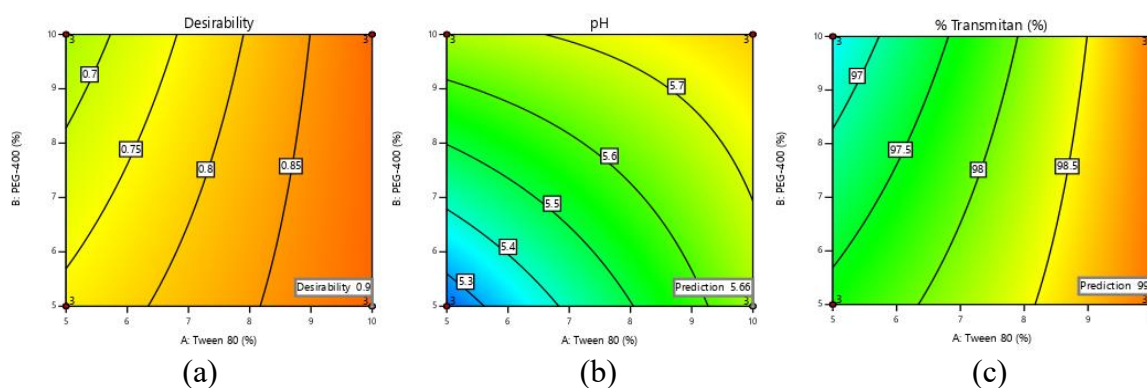


Figure 1. Contour plots of the optimum toner formula: (a) desirability contour plot, (b) pH contour plot, and (c) percent transmittance contour plot.

The contour plot serves as a graphical representation of the response, where areas with more intense red indicate higher response values, while areas shifting toward blue indicate lower response values. The predicted optimum formula generated

by Design Expert version 13 (trial) using the factorial design method consisted of 10% Tween 80 and 5% PEG 400. The interaction between Tween 80 and PEG 400 on the pH and percent transmittance responses can be seen in Figures 2.

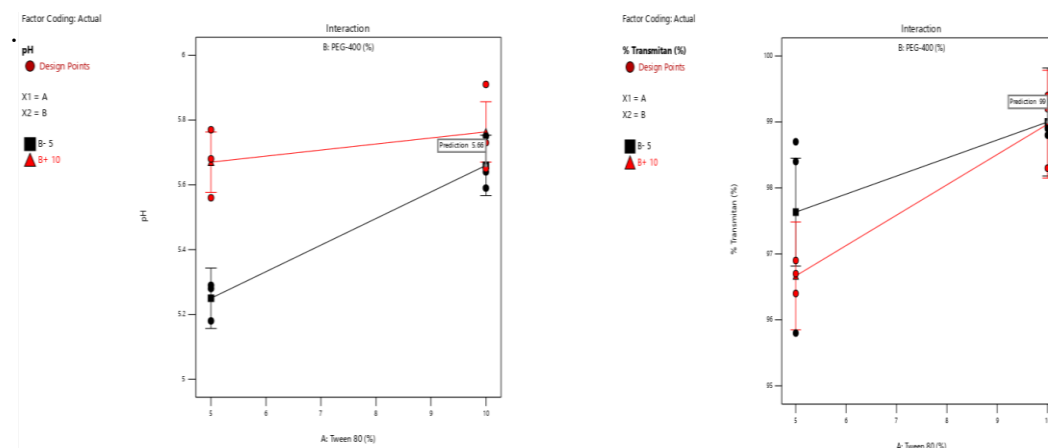


Figure 2. Interaction plot of Tween 80 and PEG 400 on (a) the pH and (b) the percent transmittance of the green betel leaf oil toner formulation

In the graphs, the X_1 axis represents the concentration of Tween 80, while the Y axis represents the response variables, namely pH and percent transmittance. The black line corresponds to the low-level concentration of PEG 400, whereas the red line represents the high-level concentration of PEG 400.

The analysis of the interaction plots indicates that using higher levels of Tween 80 and PEG 400 affects the pH response; the higher the concentration of both components, the higher the resulting pH, and vice versa. This is because the pH of Tween 80 ranges from 6.0 to 8.0, while PEG 400 has a pH range of 4.0–7.5 (Rowe et al., 2009). In the interaction plot for

percent transmittance, increasing the concentration of Tween 80 also leads to an increase in percent transmittance, whereas the use of a high level of PEG 400 results in a lower percent transmittance.

The verification results of the optimum formula were obtained through the point prediction feature in Design Expert version 13 (trial), which identified one optimal solution out of the four formulas, namely F3, with predicted values of pH 5.66 and percent transmittance of 99%. The optimum formula with a desirability value of 0.900 was then prepared again into a toner formulation with three replications.

The observed pH values of the three replications were 5.88, 5.62, and 5.56, with a mean $\pm$ SD of 5.69 ± 0.14 , which meets the acceptable skin pH range of 4.5–6.5. The observed percent transmittance values for the three replications were 99.3%, 99.1%, and 99.4%, with a mean $\pm$ SD of 99.2 ± 0.12 . Statistical analysis using a One Sample T-Test was conducted to determine whether there were significant differences between the experimental pH and percent transmittance values of the optimum formula compared to the predicted values from Design Expert version 13 (trial).

The One Sample T-Test results showed a Sig. (2-tailed) value of 0.811 for pH and 0.094 for percent transmittance. Based on these results, there were no significant differences between the experimental and predicted values, as indicated by Sig. (2-tailed) > 0.05 . These findings demonstrate that the data are valid, meaning the verification results are consistent with the predictions generated by Design Expert version 13 (trial). A test is considered acceptable when the Sig. (2-tailed) value is greater than 0.05; if the value is less than 0.05, it indicates a significant difference between the datasets.

The organoleptic evaluation optimum formula showed that the toner formulation had a pale yellow color, a liquid form, and a characteristic betel leaf aroma, while the base formulation appeared

clear, liquid, and odorless. The pale yellow color in the toner was attributed to the addition of green betel leaf oil, which has an orange to brownish hue at a concentration of 0.8%, as well as the use of Tween 80, which also has a yellowish color.

The optimum formula toner preparation was then subjected to a storage stability test using the freeze–thaw method for six cycles (24 days). Organoleptic evaluation and phase separation observations were carried out in each cycle, while the pH and percent transmittance were measured before and after the stability test. The results showed that the formulation remained stable, as indicated by the absence of any changes in the physical appearance of the toner before and after the freeze–thaw stability test, as well as the absence of phase separation among the components contained in the toner.

Based on the organoleptic evaluation prior to the stability test, the base appeared clear, liquid, and odorless, whereas the toner preparation exhibited a pale yellow color, a liquid consistency, and a characteristic betel leaf odor. Following the stability test, the organoleptic characteristics of both the base and the toner remained unchanged.

The percent transmittance test was conducted to assess the clarity of the preparation, where higher clarity is reflected by percent transmittance values

approaching 100%. The results showed that the percent transmittance of the toner base increased during the freeze–thaw stability test. In contrast, the percent transmittance of the toner preparation decreased slightly during the freeze–thaw cycles. Prior to the test, the percent transmittance values were 99.7, 99.8, and 99.6%, with a mean $\pm$ SD of $99.7\% \pm 0.08$. After the stability test, the values decreased to 99.1, 99.1, and 99.0%, with a mean $\pm$ SD of $99.0\% \pm 0.05$. The Wilcoxon test indicated that this decrease was not statistically significant, as shown by a Sig. (2-tailed) value of 0.109 ($p > 0.05$). The Wilcoxon test was used as an alternative because the percent transmittance data were not normally distributed ($p < 0.05$) based on the normality test.

pH evaluation was performed to determine the acidity or alkalinity of the preparation and to ensure that the product's pH remains within the physiological range of the skin Tranggono Sari, S. W. & Wilapangga, A. (2024). Formulasi dan Uji Stabilitas Sediaan Mikroemulsi Kalium Diklofenak Menggunakan Tween 80 : Span 80 Sebagai Surfaktan. *Jurnal Ilmiah Farmasi Akademi Farmasi*, 7(1), 118-126. <https://doi.org/10.53864/jifakfar.v7i1.179>

Latifah, 2007). The results showed that the pH of the toner base decreased during the freeze–thaw stability test. Prior

to the test, the base exhibited a pH of 5.40, which decreased to 5.22 after the test.

The pH values of the toner replicates before the stability test were 5.69, 5.78, and 5.57, with a mean $\pm$ SD of 5.68 ± 0.09 . After the stability test, the pH values were 4.90, 5.17, and 5.07, with a mean $\pm$ SD of 5.05 ± 0.11 . Statistical analysis using the Paired Sample T-test indicated a significant difference between pH values before and after the freeze–thaw stability test, with a Sig. (2-tailed) value of 0.017 ($p < 0.05$).

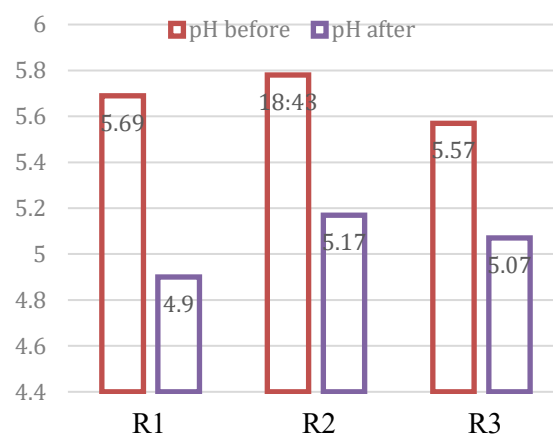


Figure 3. Stability test
Notes: R is replication

These findings demonstrate that the pH of the preparation decreased during the freeze–thaw cycles. In formulations containing Tween 80, this reduction in pH may be attributed to the degradation and oxidation of Tween 80, which can generate peroxides. As a nonionic surfactant, Tween 80 is susceptible to degradation at elevated temperatures, particularly under fluctuating thermal conditions such as those encountered during freeze–thaw cycles.

This degradation process produces acidic peroxide compounds, which contribute to the overall decrease in formulation pH (Mittag et al., 2022). pH measurements were conducted over an 8-week storage period at room temperature. The results showed that the pH decreased throughout the 8 weeks of storage. Based on the Paired T-Test, a significant difference was observed between the pH of the toner in week 1 and week 8, as indicated by a Sig. (2-tailed) value of 0.003 ($p < 0.05$).

This decrease may be attributed to storage conditions in which the temperature was not strictly controlled, as storage temperature can influence the pH stability of a formulation. Under such conditions, degradation of Tween 80 may occur. The degradation process produces peroxide compounds that are acidic in nature, thereby contributing to the reduction in the formulation's pH (Mittag et al., 2022).

The droplet size of the oil globules in the toner formulation was measured using a particle size analyzer. The measurements obtained from each replication were 12.2 nm, 12.2 nm, and 12.3 nm, with a mean $\pm$ SD of 12.2 ± 0.05 nm. These results indicate that the oil globule

size in the toner formulation falls within the nanometer range, specifically between 10–200 nm (Jaiswal et al., 2015).

Such small droplet sizes are essential in nanoemulsion formulations, as smaller particle sizes can enhance emulsion stability and improve the bioavailability of active ingredients. The roles of Tween 80 as a surfactant and PEG 400 as a co-surfactant contribute significantly to the resulting nanoemulsion particle size. The surfactant adsorbs onto the surface of the oil droplets and forms micelles, thereby reducing interfacial tension and promoting the formation of nanoemulsions with smaller particle sizes (Handa et al., 2021).

The polydispersity index (PDI) of the toner formulation was measured to evaluate the distribution of oil globule sizes within the emulsion. The measurements from each replication showed PDI values Table IV.

The PDI ranges from 0 to 1, where values closer to 0 indicate a monodisperse system, whereas values closer to 1 represent a polydisperse system. PDI reflects the uniformity of droplet size within nanoemulsions (Ermawati et al., 2023).

Table IV. Polydispersity index and zeta potential

Parameters	R1	R2	R3	Mean $\pm$ SD
PDI	0.120	0.175	0.222	0.172 ± 0.04
Zeta potential	-22.6 mV	-22.9 mV	-22.1 mV	-22.5 ± 0.33 mV

Zeta potential is an important indicator of nanoemulsion stability. According to Marzuki et al. (2019), zeta potential values exceeding +30 mV or below -30 mV indicate good emulsion stability because sufficiently strong electrical charges on the droplets prevent flocculation by promoting mutual repulsion. Although the zeta potential value of -22.5 mV does not reach the optimal stability threshold (± 30 mV), the existing negative charge can still contribute to emulsion stability. This value represents an acceptable level of initial stability, particularly when supported by an appropriate formulation approach.

The green betel leaf toner formulated using the optimum composition was subsequently evaluated for its antioxidant activity. Testing at a concentration of 100 $\mu\text{g/mL}$ showed an inhibition percentage of 62.91%, and the average IC_{50} value of the toner was 43.29 $\mu\text{g/mL}$, indicating that the toner possesses very strong antioxidant activity ($\text{IC}_{50} < 50$ $\mu\text{g/mL}$)

These findings demonstrate that the antioxidant activity of green betel leaf oil remains very strong even after being incorporated into the toner formulation.

Results of the One Sample T-Test showed that the IC_{50} value of the toner was not significantly different from that of the

pure green betel leaf oil, with a Sig. (2-tailed) value of 0.213 ($p > 0.05$).

According to Nirmalayanti (2021), screening various types of cosurfactants is essential to obtain nanoemulsions, utilizing phase diagrams to determine suitable component concentrations within the nanoemulsion formation region. Pseudoternary phase diagrams are used to identify areas where specific combinations of components can form nanoemulsions in an aqueous medium. The pseudoternary diagram for PEG 400 shows that this cosurfactant still possesses a nanoemulsion formation region. In contrast, the pseudoternary diagram using glycerin as a cosurfactant shows no nanoemulsion region. Therefore, PEG 400 is preferred as a cosurfactant due to its better miscibility with oil compared with glycerin (Priani et al., 2023).

IV. CONCLUSION

Green betel leaf oil, serving both as the active ingredient and the oil phase, was successfully formulated into a toner optimized using a factorial design approach. The optimum formula obtained was F3, consisting of 0.8% green betel leaf oil, 10% Tween 80, and 5% PEG-400. This formulation produced a clear appearance with a percent transmittance of 99.2% and an average pH of 5.69, and it remained stable without physical changes or phase

separation. The nanoemulsion exhibited an average particle size of 12.2 nm, a polydispersity index of 0.172, and a zeta potential of -22.5 mV. Additionally, the toner demonstrated very strong antioxidant activity with an IC_{50} value of $43.29 \mu\text{g/mL}$ ($IC_{50} < 50 \mu\text{g/mL}$).

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