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Development of nanoemulgel formula with purified *Anredera cordifolia* (Ten.) Steenis leaves extract for anti-inflammatory activity

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ABSTRACT: This study evaluates the potential of a nanoemulgel formulation of purified extract from *Anredera cordifolia* (Ten.) Steenis leaves, which is rich in flavonoids, as a substance that reduces inflammation. The aim of this study is to evaluate the effects of concentrations of Tween 80 and PEG 400 on the physicochemical characteristics and anti-inflammatory activities of nanoemulgel. Four nanoemulsion formulas (formula 1, formula A, formula B, and formula AB), which vary in the concentrations of Tween 80 and PEG 400, were tested for their characteristics and have met all the requirements for nanoemulsion characteristics by assessing percent transmittance, particle size, zeta potential and PDI value. Formula AB indicates the smallest particle size (84.233 ± 3.513 nm) and the highest transmittance percentage (99.367 ± 0.306%). The interaction of Tween 80 and PEG 400 affects the pH response, spreading ability, adhesion, and viscosity. Formula 1, formula A, formula B, and formula AB can be accepted or are within the optimal area. The anti-inflammatory test using the carrageenan model showed that formula A and formula AB had the best anti-inflammatory activity compared to the other formulas, approaching the activity of the positive control (betamethasone cream). Based on the assessment of edema volume, the anti-inflammatory activity was assessed, and formulae A and AB demonstrated a considerable inhibition of inflammation at the 4th, 5th, and 6th hour. The study's findings suggest that the purified extract from *Anredera cordifolia* (Ten.) Steenis leaf nanoemulgel has promising anti-inflammatory properties and is useful for topical application.

KEYWORDS: *Anredera cordifolia*; flavonoids; purified extracts; nanoemulgel; anti-inflammatory.

1. INTRODUCTION

The binahong plant (*Anredera cordifolia* (Ten.) Steenis), particularly its leaves, has been utilized empirically to treat various types of illnesses. As a traditional remedy, *Anredera cordifolia* (Ten.) Steenis is used to treat gout, diabetes, hyperglycemia, obesity, pain relief, and kidney failure, and to promote wound healing [1,2]. The dried *Anredera cordifolia* (Ten.) Steenis leaf has an effective antibacterial and antioxidant effect. [3,4]. Additionally, it is said that the stem leaves of *Anredera cordifolia* (Ten.) Steenis have anti-inflammatory properties.

Steroids and non-steroidal anti-inflammatory medicines, or NSAIDs, are frequently used to treat inflammation because they lessen discomfort and swelling. However, long-term use of NSAIDs can be harmful to the body, especially the digestive tract. The use of steroids can also lead to a weakened immune system, moon face, osteoporosis, and even hypertension. Therefore, traditional medicine can be used to reduce the side effects of chemical drugs. One of them is the extract of *Anredera cordifolia* (Ten.) Steenis leaves, which is considered to have relatively minor side effects [5].

Secondary metabolite substances including flavonoids, alkaloids, steroids, phenolic acids, saponins, tannins, and triterpenoids are found in the leaves of *Anredera cordifolia* (Ten.) Steenis [1]. The flavonoid compounds found in the extract of *Anredera cordifolia* (Ten.) Steenis include vitexin, isovitexin, morin (3,5,7, 2′,4′-pentahydroxyflavone), myricetin, apigenin, rutin, and quercetin [6-9]. It is well known that flavonoids block inflammatory mediators like NO, TNF-α, IL-1β, IL-6, and COX-2. Therefore, flavonoid compounds from the leaves of *Anredera cordifolia* (Ten.) Steenis contribute to anti-inflammatory activity. Research on the

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anti-inflammatory activity of chlorophyll has been conducted and has proven capable of inhibiting inflammatory mediators, specifically in the expression of the TNF-α gene [10]. By preventing the synthesis of pro-inflammatory mediators like NO (nitric oxide) and iNOS (inducible nitric oxide synthase), resin is also known to have anti-inflammatory properties [11]. This means that in order to get rid of undesirable substances, the 96% ethanol extract of *Anredera cordifolia* (Ten.) Steenis leaves needs to be purified. The purification of the 96% ethanol extract of *Anredera cordifolia* (Ten.) Steenis leaves uses n-hexane as a solvent, which is expected to attract interfering non-polar compounds.

The purified extract of (*Anredera cordifolia* (Ten.) Steenis) leaves containing flavonoid compounds has potential anti-inflammatory activity, making it suitable for development into a topical formulation. The formulation is to be developed from the purified extract of *Anredera cordifolia* (Ten.) Steenis is a nanoemulgel. Flavonoid compounds have low solubility in water, so a nanoemulgel formulation is needed to enhance the active substances in the purified extract of *Anredera cordifolia* (Ten.) Steenis, with the hope of improving bioavailability and therapeutic effects [12].

The formulation of the nanoemulgel consists of creating a nanoemulsion with an oil phase of VCO (Virgin Coconut Oil), an aqueous phase of distilled water, Tween 80 as the surfactant, and PEG 400 as the co-surfactant. The gel base used in making nanoemulgel preparations is Carbopol 940 [13]. Tween 80, a non-ionic hydrophilic surfactant, and PEG, a hydrophilic compound, have good solubility in water. Both of these compounds are non-toxic and do not irritate the skin. This is in accordance with the preparation of nanoemulgel formulations, where the gel base is made using water as a solvent [14]. The interfacial tension between the water and oil phases is lessened by Tween 80. PEG 400 will lessen interfacial tension by improving the oil phase's penetration into the surfactant monomer's hydrophobic regions. Higher concentrations of Tween 80 and PEG 400 will produce nanoemulsions with a high percentage of transmittance, which affects the clarity of the nanoemulsion and results in smaller particle sizes [15]. The hope is to produce an optimal nanoemulgel formulation of the purified extract of *Anredera cordifolia* (Ten.) Steenis leaves.

2. RESULTS

2.1 Purified Extract of *Anredera cordifolia* (Ten.) Steenis Leaves

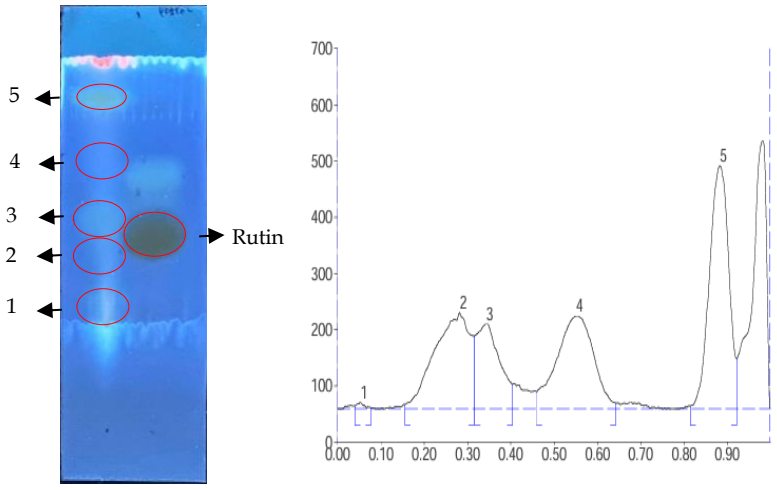
The extraction of *Anredera cordifolia* (Ten.) Steenis leaves using the ultrasonic method produces a thick, dark green extract with a distinctive aroma, yielding 15.17%, exceeding the Indonesian Herbal Pharmacopoeia standard of 11.91%. The ultrasonic method is more efficient compared to conventional maceration, which yields 12.32% [16]. A 96% ethanol solvent and an optimal temperature of 50°C are ideal for extraction because flavonoids easily dissolve in semi-polar solvents and are not heat-resistant [17]. Purification with n-hexane removes unwanted non-polar compounds, resulting in an extract yield of 34.25%.

2.2 Standardization of The Purification Extract of *Anredera cordifolia* (Ten.) Steenis Leaves

The determination of the moisture content, ash content, acid-insoluble ash content, and flavonoid content is part of the standardization process for the purified extract of *Anredera cordifolia* (Ten.) Steenis leaves. Making ensuring the extract is devoid of impurities and contains active components is the aim. To evaluate the effectiveness of the purification method in extracting flavonoids, the flavonoid content results in the purified extract were compared with the 96% ethanol extract. The results of standardizing the purified extract are displayed in Table 1, along with the amount of flavonoids in the 96% ethanol extract. Figure 1 shows the spots from the TLC plate's elution under UV light at 366 nm following exposure to ammonia vapor as well as the densitogram. According to Table 2 for the Rf data, it shows that at spot number 5, there is blue-green fluorescence. This blue-green spot is suspected to be a compound from the flavonoid group [18].

Table 1. Results of The Standardization

Parameter	Result	Category
Moisture Content	6.927 ± 2.435	<8.9%
Total Ash Content	4,625 ± 1.873	<7.2%
Acid-Insoluble Ash Content	0.038 ± 0.017	<1.8%
Flavonoid Content in Purified Extract	5.263 ± 0.344	-
Flavonoid Content in 96% Ethanol extract	2.390 ± 0.197	-



a. **b.**

Figure 1. Spot Appearance (Elution Result) (a) and Densitogram Result (b)

Table 2. Results of Color Observation of Spots and Rf Values in Chromatography Profile Determination

Spot	Rf Value	UV 366 nm Visual Observation
1	0.05	Blue
2	0.28	Light Blue
3	0.34	Light Blue
4	0.55	Light Blue
5	**0.88**	**Blue-Green**

2.3 Observation of Nanoemulsion Characteristics

The observation of percent transmittance, particle size, PDI value, and zeta potential value serves as parameters in determining the characteristics of the nanoemulsion formulation containing purified extract of *Anredera cordifolia* (Ten.) Steenis leaves. The differences in the characteristics of the nanoemulsions are evident from the four formulas with variations in the concentrations of Tween 80 and PEG 400 as determining factors for a good formula. Table 3 shows the values of each characteristic parameter of the tested nanoemulsion preparation.

Table 3. Results of The Characteristics of Nanoemulsions From The Four Formulas

Characteristics	Formula			
	1	A	B	AB
Transmittance Percentage (%)	96.300 ± 0.361	94.667 ± 0.569	98.667 ± 0.404	99.367 ± 0.306
Particle Size (nm)	119.700 ± 15.440	137.467 ± 13.396	95.267 ± 10.304	84.233 ± 3.513
PDI Value	0.632 ± 0.044	0.755 ± 0.021	0.240 ± 0.037	0.646 ± 0.056
Zeta Potential Value (mV)	-21.533 ± 1.305	-17.433 ± 0.907	-11.833 ± 0.757	-20.867 ± 0.306

2.4 Evaluation of Models for Optimization Process

Model analysis is necessary to initiate the optimization process. The best formula is found by utilizing observations of pH, spreadability, adhesion, and viscosity. Table 4 displays the outcomes of the model analysis for each of the four responses.

Table 4. Statistical Analysis Result

Response	pH		Spreadibility (cm)		Adhesion (second)		Viscosity (cPs)	
	[a]P value	[b]F value	[a]P value	[b]F value	[a]P value	[b]F value	[a]P value	[b]F value
Model	0.0004	21.30	0.0017	13.58	<0.0001	704.23	<0.0001	66.57
Tween 80	0.4511	0.6275	0.0194	8.50	<0.0001	246.29	<0.0001	160.17
PEG 400	<0.0001	61.70	0.0015	22.20	0.0003	37.75	0.0003	37.50
Tween 80*PEG 400	0.2452	1.57	0.0133	10.03	<0.0001	1828.65	0.1909	2.04

[a]P value is determine the significance values, [b]F value in two-way ANOVA indicates variation that significant

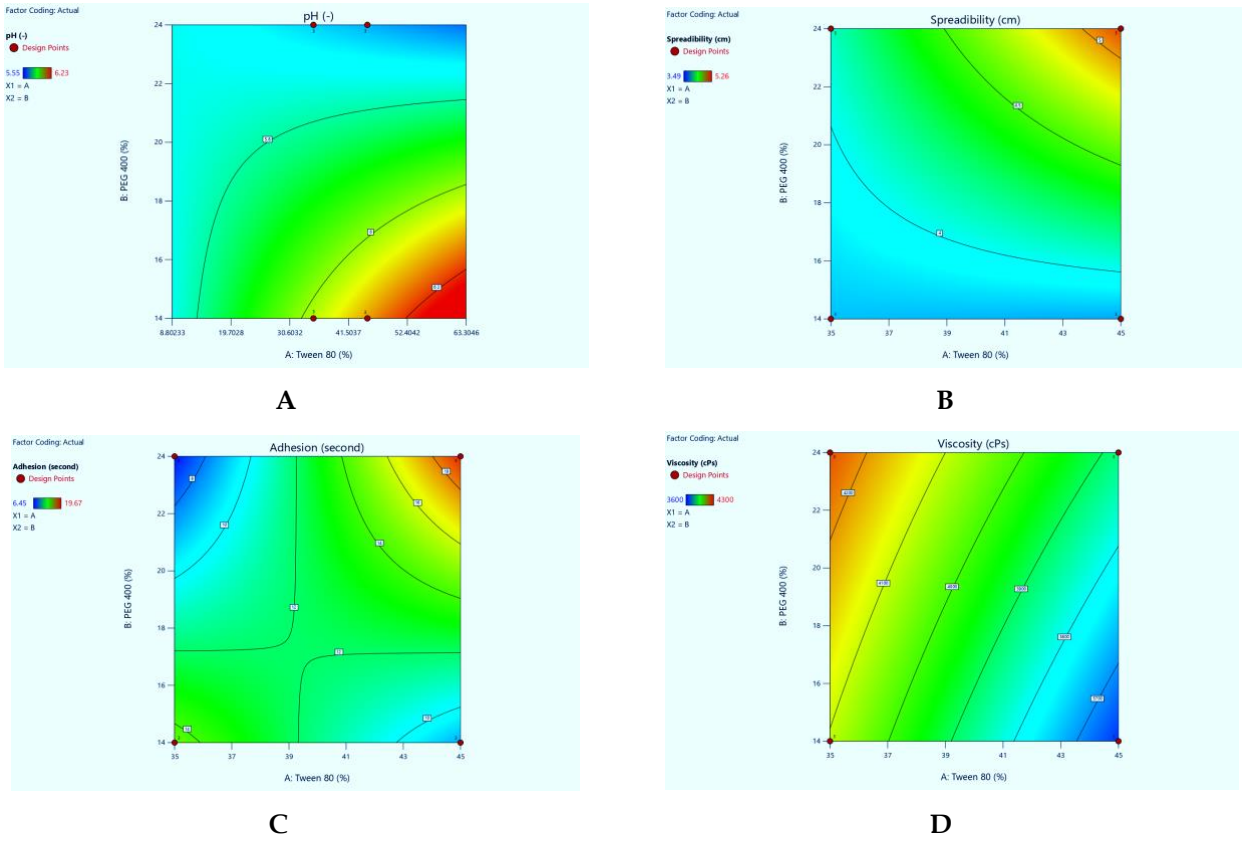


Figure 2. Contour Plot Response of pH (A), Spreadibility (B), Adhesion (C), and Viscosity (D)

2.5 Evaluation of Responses for the Optimization Process

The responses (pH, spreadability, adhesion, and viscosity) of the four formulations (formula 1, formula A, formula B, and formula AB) are shown in Figure 3 as being in the yellow area, suggesting that all four formulas are either within the ideal range or are acceptable. The optimal levels that fall within the prediction points for Tween 80 are 40 %w/w, and for PEG 400, they are 19 %w/w.

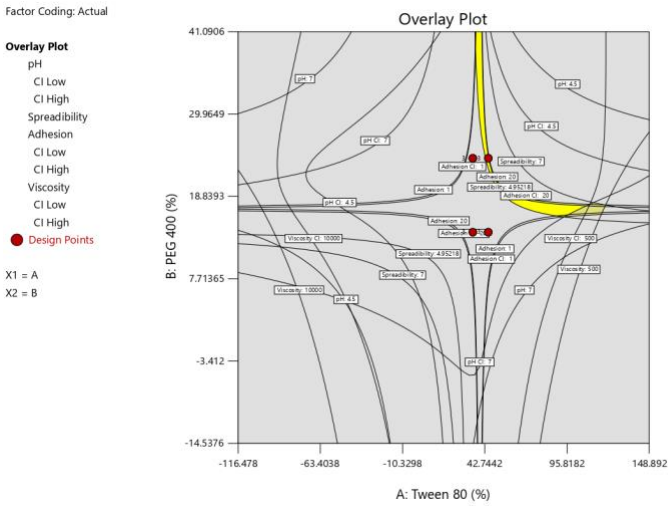


Figure 3. Overlay Plot for Response pH, Spreadibility, Adhesion, and Viscosity

2.6 Results of The Evaluation Test of The Nanoemulgel Formulations

The organoleptic characteristics, homogeneity, spreadibility, adhesion, pH, and viscosity test results of the four nanoemulgel formulas are shown in Table 5 and exhibit a significant degree of resemblance, falling within the expected range. However, there is a single power test of three formulas that are not within the required range.

Table 5. Result of The Evaluation test of The Nanoemulgel Formulations

Formula	Organoleptic	Homogeneity	pH	Spreadibility (cm)	Adhesion (second)	Viscosity (cPs)
1	Light yellow, Transparent, Odorless	Homogeneous, There are No Coarse Grains	6.02 ± 0.07	3.82 ± 0.29	14.52 ± 0.37	4093 ± 83
A	Light yellow, transparent, odorless.	Homogeneous, There are No Coarse Grains	6.13 ± 0.12	3.78 ± 0.19	8.69 ± 0.45	3633 ± 42
B	Light yellow, transparent, odorless.	Homogeneous, There are No Coarse Grains	5.69 ± 0.04	4.09 ± 0.47	6.64 ± 0.23	4247 ± 50.33
AB	Light yellow, transparent, odorless.	Homogeneous, There are No Coarse Grains	5.67 ± 0.11	5.14 ± 0.12	19.23 ± 0.41	3880 ± 40

2.7 Anti-inflammatory Activity of Nanoemulgel Formulations

The inflammation test calculates the amount of edema that carrageenan causes in lab rats. Using a plethysmometer, measurements were made every hour for six hours. Volume edema is inversely correlated with the inhibition percentage that each recipe produces in relation to the control group. At hour 0, the inhibition percentage is still high because the carrageenan has just been injected. However, at hours 1, 2, and 3, there was a decrease in the percentage of inflammation, indicating the release of inflammatory mediators. The subsequent increase in edema volume at hours 4, 5, and 6 indicates the anti-inflammatory activity of each formula. The data on the inhibition of inflammation in each test group can be seen in Figure 4.

The average percentage data of inflammation in the six test groups showed a normal and homogeneous distribution at the 1st and 3rd hours ($p>0.05$), which meets the criteria for ANOVA testing. On the other hand, the data were homogenous and not regularly distributed for the 0th, 2nd, 4th, and 6th hours. Thus, the differences between groups were assessed using the Kruskal-Wallis test, and the differences between each treatment group were assessed using the Mann-Whitney test.

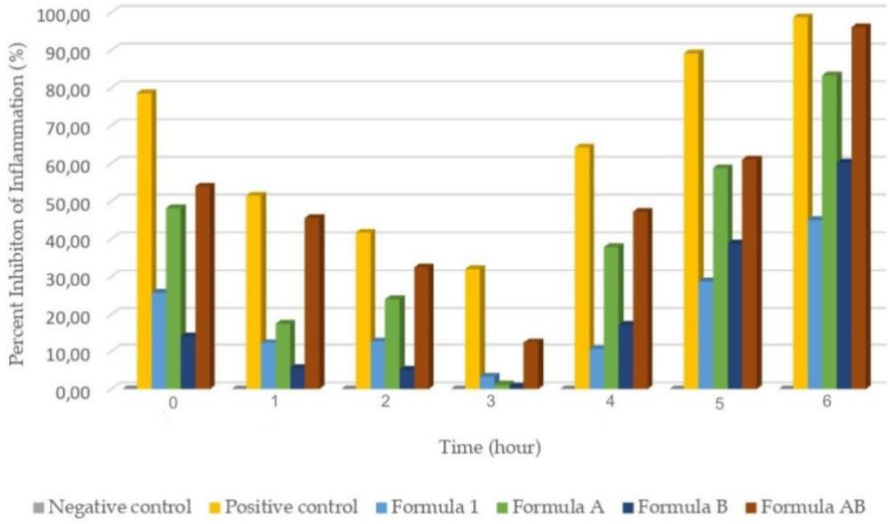


Figure 4. Percentage of Inhibition of Inflammation for Each Test Group

3. DISCUSSION

The preparation of *Anredera cordifolia* (Ten.) Steenis leaf extract using the ultrasonic method and 96% ethanol as a solvent. 96% ethanol is used as a solvent because it is the solvent employed in the extraction of flavonoids from the extract of *Anredera cordifolia* (Ten.) Steenis leaves [19]. The extraction of *Anredera cordifolia* (Ten.) Steenis leaves using the ultrasonic method is far superior in terms of extraction duration compared to the conventional maceration method [20]. The ultrasonic method employs ultrasonic vibrations greater than 20kHz on the surface of the powder. The yield obtained in this study is 15.17%, with theextract weightt being 60.685 g from 400 grams of *Anredera cordifolia* (Ten.) Steenis leaf powder.

Separating undesirable substances from the 96% ethanol extract of *Anredera cordifolia* (Ten.) Steenis leaves can be used to purify the extract. N-hexane is used as a solvent in the purification of the 96% ethanol extract of *Anredera cordifolia* (Ten.) Steenis leaves, and it is anticipated that this will draw non-polar molecules that are disruptive. The purification process will yield the desired compound with a higher concentration [21]. The yield obtained from the purified extract is 34.25%, with the weight of the extract obtained being 20.784 g. The purpose of determining the yield is to measure the concentration of chemical content dissolved in the solvent. The higher the yield percentage, the more chemical content there is in the sample.

The Indonesian Herbal Pharmacopoeia's criteria were met by the evaluated parameters, such as moisture content, total ash content, acid-insoluble ash content, and flavonoid content, according to the findings of standardizing the purified extract of *Anredera cordifolia* (Ten.) Steenis leaves. The moisture content, as indicated in Table 1 at 6.927 ± 2.435% w/w, is critical to the extract's stability [22]. The total ash content, which measures the quantity of inorganic material remaining after burning, is 4.625 ± 1.873% w/w. This includes both physiological and non-physiological ash, which comes from the plant tissue itself and external sources like sand, gravel, or dirt that sticks to the plant's surface. With an acid-insoluble ash level of 2.390 ± 0.197% w/w, the soil is contaminated with silica, which may irritate skin [23]. All these results suggest that the purified extract meets the established quality criteria.

The total flavonoid content of the purified extract of *Anredera cordifolia* (Ten.) Steenis leaves obtained are higher at 5.263 ± 0.344% w/w compared to the total flavonoid content of the 96% ethanol extract of *Anredera cordifolia* (Ten.) Steenis leaves, which is only 2.390 ± 0.197% w/w. This is because, during the purification process, flavonoid components may become more concentrated as non-flavonoid compounds or other impurities are removed. This will increase the flavonoid ratio in the purified extract [24]. Determining the chromatographic profile of the purified extract of *Anredera cordifolia* (Ten.) Steenis leaves are a crucial step in standardization to ensure the quality and quantity of flavonoids in the extract. This chromatography profile is analyzed through the appearance of the elution spots and the densitogram. Based on Figure 1, the elution under UV light at 366 nm shows a blue-green spot in the purified extract and the rutin standard, indicating the possible presence of flavonoid compounds [18]. However, in the routine standard, there is more than one spot, indicating the presence of contaminants that could affect the elution process. On the

other hand, the densitogram in image 1 shows the purified *Anredera cordifolia* extract (Ten.) Steenis leaves contain five spots with Rf values listed in Table 2.

The initial stage in the preparation of nanoemulgel involves first creating the gel base and the nanoemulsion. Nanoemulsions, which have particle sizes ranging from 20 to 200 nm, require high energy in their production process. Ultrasonic techniques are used to effectively reduce the size of nanoemulsion particles [25,26]. Nanoemulsions of purified extracts from *Anredera cordifolia* (Ten.) Steenis leaves were created in four formulas that varied based on the concentration of Tween 80 (A) and PEG 400. (B). The characteristics of the nanoemulsions described in Table 3 show differences caused by this variation in concentration. The transmittance percentage is an indicator of the success of nanoemulsion formation; if the transmittance percentage is over 99%, the nanoemulsion is considered clear or transparent [27]. With a transmittance percentage of 99.367 ± 0.306%, Formula AB shows that particle size reduction is successful. Particle size decreases with increasing transmission percentage. The particle sizes for formula 1, formula A, formula B, and formula AB are 119.700 ± 15.440 nm, 137.467 ± 13.396 nm, 95.267 ± 10.304 nm, and 84.233 ± 3.513 nm, respectively, with formula AB yielding the smallest particle size. Smaller particle sizes allow for higher penetration of active substances, enabling more active ingredients to reach the area of inflammation [28]. The polydispersity index value for formula 1, formula A, formula B, and formula AB ranges from 0 to 1. The values of the polydispersity index for the four formulas are 0.632 ± 0.044, 0.755 ± 0.021, 0.240 ± 0.037, and 0.646 ± 0.056. A monodisperse system is represented by a value of 0, and polydisperse particles are represented by a value of 1 [29]. The polydispersity index data show that the generated nanoemulsion has a reasonably homogeneous particle size, falling between 0.240 to 0.755 [30]. The zeta potential values of the nanoemulsions from the four formulas were found to be -21.533 ± 1.305 mV, -17.433 ± 0.907 mV, -11.833 ± 0.757 mV, and -20.867 ± 0.306 mV, respectively. A zeta potential of ±30mV is believed to be sufficient to ensure the physical stability of nanoemulsions [27].

The initial stage in the optimization process of nanoemulgel formulations involves evaluating the model to determine the parameters that will be used as benchmarks in the response analysis, to achieve an optimal formula. Based on Table 4, all parameters, including pH, spreadability, adhesion, and viscosity have met the criteria for a good model. A p-value indicating significance of less than 0.05 shows that factors such as Tween 80 (A), PEG 400 (B), and the interaction between Tween 80 and PEG 400 (AB) have a significant effect on the observed response. The factor Tween 80 (A) significantly influences the parameters of spreadability, adhesion, and viscosity, but does not affect the pH parameter. The factor Tween 80 (A) has a significant influence on adhesion with an F value of 246.29. The PEG 400 (B) factor has a significant influence on the pH parameters, spreadability, adhesion, and viscosity. The highest F value for the pH parameter is 61.70, which means that PEG 400 significantly affects the pH response.Meanwhile, the interaction between Tween 80 and PEG 400 (AB) only affects the spreadability and adhesion, with adhesion being the best response, showing an F value of 1828.65. The contour plot appearance is shown in Figure 2 for pH response, spreadability, adhesiveness, and viscosity in relation to the factors of Tween 80 and PEG 400. The generated plot indicates that only the red area has the Tween 80 and PEG 400 concentrations that are ideal for the dispersion response [30,31]. Based on the response analysis, a prediction point was obtained for the Tween 80 factor at a level of 40 %w/w and the PEG 400 factor at a level of 19 %w/w. The overlay plot can be seen in Figure 3, which shows that all responses (pH, spreadability, adhesion, and viscosity) are in the yellow area. This indicates that the Tween 80 and PEG 400 factors provide optimal responses in all four formulas.

Organoleptic testing to visually assess the physical characteristics of a formulation, with all four formulas exhibiting a light yellow color, transparency, and no odor. In the homogeneity test, it was also found that none of the four formulas contained coarse particles. The preparation must be homogeneous, indicating that the ingredients in the formulation are mixed together [25]. Based on the pH testing, all formulas fall within the range of 4.5-7.0, which is the pH range of human skin. It should be noted that Tween 80 has a pH range of 6.0-8.0, while PEG 400 has a pH range of 4.0-7.5 [31]. Formulas B and AB have pH values below 6.0, specifically 5.69 ± 0.04 and 5.67 ± 0.11, respectively. This is due to the higher content of PEG 400 in formulas B and AB compared to formulas 1 and A. The spreadability of the nanoemulgel preparation is crucial to ensure even distribution on the skin. The greater the spreadability of the preparation, the better it is, as a larger membrane area for application will enhance drug diffusion [32]. Good spreadability for nanoemulgel preparations is in the range of 5-7 cm. Among the four formulas, only formula AB meets the spreadability requirement at 5.14 ± 0.12 cm. The results for the other three formulas, namely formula 1, formula A, and formula AB, are not satisfactory as they fall within the range of 3.82-4.09 cm. The nanoemulgel formulation's adhesion time measures how long it takes for the formulation to stick to skin.

Longer durations indicate better adhesion times, thus it is anticipated that the formulation's skin contact time will likewise be extended [33]. A good adhesion test yields findings that are longer than 1 second. All four formulas have met the range, with formula AB showing the highest adhesion time of 19.23 ± 0.41 seconds and formula B showing the lowest adhesion time of 6.64 ± 0.23 seconds. Viscosity is an essential factor in producing nanoemulgel for effective application on the skin. The viscosity test of the purified extract nanoemulgel of *Anredera cordifolia* (Ten.) Steenis was conducted using a Brookfield viscometer. Finding the substance's viscosity value is the aim of this test. The thickness of that substance increases with a higher viscosity value [25,30]. The viscosity values of the four formulas are good as they fall within the range of 500-10,000 cPs.

Anti-inflammatory testing using a carrageenan model by observing the volume of edema in laboratory rats. Observations were conducted for 6 hours, from hour 0 to hour 6, using a plethysmometer. The percentage of inhibition in each formula relative to the control group is negatively correlated with the measured volume of edema. The negative control used was a nanoemulgel without extract, while the positive control was betamethasone cream. Betamethasone is used as a positive control because it has a mechanism for suppressing various pro-inflammatory mediators and cytokines. TNF-a, IL-6, and IL-8 are among the cytokines that betamethasone can suppress and cause inflammation [34].

The average percentage of inflammation data in the six test groups, including the negative control, positive control, formula 1, formula A, formula B, and formula AB, is normally distributed and homogeneous ($p < 0.05$) only at the 1st hour and the 3rd hour, allowing for the continuation of the ANOVA test. All test groups at hours 0, 2, 4, 5, and 6 were followed by the Kruskal-Wallis test to determine differences and the Mann-Whitney test to identify differences between groups. The percentage results of inflammation that did not differ significantly ($p > 0.05$) at hours 0, 1, 2, and 3 of the testing period in the test groups (formula 1, formula A, formula B, formula AB) compared to the negative control group indicate the release of inflammatory mediators following a 1% carrageenan injection. This occurs due to several mediators involved in inflammation, namely histamine, serotonin, and bradykinin, which are first detected in the early phase of inflammation about 1 hour after carrageenan induction. After the initial phase, a delayed phase process occurs (after 1 hour from the initial phase) involving neutrophil infiltration, which also contributes to the inflammatory response by producing pro-inflammatory cytokine mediators like IL-β and TNF-α. Carrageenan will cause the production and release of Nitric Oxide (NO) at the inflammation site for up to 3 hours [35,36].

Based on image 4, at the 4th, 5th, and 6th hours, both formula 1, formula A, formula B, and formula AB show an increase in the percentage of inflammation inhibition, indicating anti-inflammatory activity in all four formulas. Formula A and formula AB, as seen in the graph of inflammation inhibition percentage, show results that are most comparable to the percentage of inflammation inhibition of the positive control. Therefore, among the four formulas, formula A and formula AB are the best in anti-inflammatory activity. When looking at the concentration levels of Tween 80 and PEG 400 factors in formula A and formula AB, it can be concluded that the Tween 80 factor at the highest level (45 %w/w) has the greatest influence on the anti-inflammatory activity process. However, the anti-inflammatory activity testing was not conducted at the prediction point of Tween 80 concentration level (40 %w/w) and PEG 400 concentration level (19 %w/w). The flavonoid content in the purified extract of *Anredera cordifolia* (Ten.) Steenis leaves are thought believed to actively reduce inflammation by preventing the release of inflammatory mediators such as COX-2, NO, TNF-α, IL-1β, and IL-6 [37].

4. CONCLUSION

The standardization parameters indicate that the purified extract of *Anredera cordifolia* (Ten.) Steenis meets the Indonesian Herbal Pharmacopoeia's criteria and is suitable for development into a nanoemulgel preparation. The characteristics of the nanoemulsions in Formula 1, Formula A, Formula B, and Formula AB meet the necessary criteria, suggesting that these nanoemulsions are stable. The interaction between Tween 80 and PEG 400 in Formulas 1, A, and AB affects the pH, spreadability, adhesion, and viscosity. The optimal concentration for Tween 80 is predicted to be 40 %w/w, and for PEG 400, it is 19 %w/w. Anti-inflammatory activity tests show that Formulas 1, A, B, and AB all demonstrate increased inhibition of inflammation at the 4th, 5th, and 6th hours. Among these, Formulas A and AB exhibit inhibition percentages closest to that of the positive control, betamethasone, indicating that Formulas A and AB have the highest anti-inflammatory

activity. This is comparable to the factor of Tween 80 at the highest concentration level of 45 , which has an impact on anti-inflammatory activity.

5. MATERIALS AND METHODS

5.1 Materials

The plant material used is powdered leaves of *Anredera cordifolia* (Ten.) Steenis, obtained from the Cihargem area in Bandung, Indonesia. The determination of the plant *Anredera cordifolia* (Ten.) Steenis conducted at the Medicinal Plant Garden Laboratory, Faculty of Pharmacy, Sanata Dharma University, proves that the plant used in this research is indeed the binahong plant with the species *Anredera cordifolia* (Ten.) Steenis and the family Basellaceae, and it is also known by another name, madeira vin. Other materials include 96% ethanol, n-hexane, Tween 80, and PEG 400, which were acquired from Bratachem, Indonesia. Virgin coconut oil (VCO), carbopol 940, triethanolamine, 0.9% NaCl, carrageenan, and aquades purchased from CV. Aloin Labora, Indonesia. The other materials used are rutin, 37% HCl, aluminum chloride, and sodium acetate.

5.2 Methods

5.2.1 Determination of Anredera cordifolia (Ten.) Steenis and Ethical Clearance

The determination process of the plant *Anredera cordifolia* (Ten.) Steenis took place at the Medicinal Plant Garden Laboratory, Faculty of Pharmacy, Sanata Dharma University, resulting in a validation letter of determination no: 03/LKTO/Far-USD/XII/2023. The ethical clearance process was conducted at the Animal Ethics Committee, Faculty of Veterinary Medicine, Udayana University, resulting in the animal ethics approval certificate number: B/74/UN14.2.9/PT.01.04/2024.

5.2.2 Preparation of Anredera cordifolia (Ten.) Steenis Leaf Extract

The preparation of *Anredera cordifolia* (Ten.) Steenis leaf extract using the ultrasonic method. A total of 100 g of *Anredera cordifolia* (Ten.) Steenis leaf simplicia powder was weighed and then soaked with 1 L of 96% ethanol solvent (1:10) in an extraction container. The extraction vessel is placed in a water cleaning bath with a frequency of 42 kHz, and the process is carried out for 30 minutes. The extract was filtered to separate the filtrate from the residue. Resonance of sonication is repeated in the same way for a total of 2 times. The sonication extract was evaporated using a rotary evaporator at a temperature of 50°C to obtain a thick extract [38].

5.2.3 Preparation of Purified Extract from Anredera cordifolia (Ten.) Steenis Leaves

The liquid-liquid partition method is used to create a purified extract of *Anredera cordifolia* (Ten.) Steenis leaves. A thick extract of 0.5 grams of *Anredera cordifolia* (Ten.) Steenis leaves is added to 20 milliliters of 70% ethanol solvent. n-Hexane and distilled water are added to the separatory funnel in a 1:1 ratio, and then shaken for 5 minutes. After two phases have formed, the n-hexane phase is separated from the ethanol-water phase. This is done by adding n-hexane solvent in a 1:1 ratio to the n-hexane phase until it becomes clear. Next, a rotary evaporator is used to concentrate the ethanol-water phase at a temperature of 50°C until a thick extract is produced [39].

5.2.4 Standardization of Purified Extract of Anredera cordifolia (Ten.) Steenis Leaves

Determination of Moisture Content

Weigh 1 g of the purified extract that will be tested for moisture content using the gravimetric method. The moisture content requirement for the purified extract of *Anredera cordifolia* (Ten.) Steenis according to the Indonesian Herbal Pharmacopoeia is less than 8.9% [9].

Determination of Total Ash Content

2 grams of the sample are put into a ceramic crucible that has already been burned and dried. The Indonesian Herbal Pharmacopoeia stipulates that the total ash content of the purified extract of *Anredera cordifolia* (Ten.) Steenis cannot exceed 7.2% [9].

Determination of Acid-Insoluble Ash Content

Ash obtained by using 25 milliliters of diluted hydrochloric acid for five minutes in order to determine the total ash content. After passing through ash-free filter paper, the acid's insoluble portion is cleaned with hot water. Following that, the products were calcined at 600°C in a porcelain crucible until a consistent weight was reached. The Indonesian Herbal Pharmacopoeia stipulates that the purified extract of *Anredera cordifolia* (Ten.) Steenis leaves must have an acid-insoluble ash concentration of no more than 1.8% [9].

Determination of Flavonoid Chromatographic Profile

Preparation of the test solution of purified extract weighing 0.1 g and the standard solution of rutin weighing 40 mg is dissolved in 10 mL of ethanol P. Observations are made using TLC-densitometry by spotting the test solution and the rutin solution, each spotted with 10 µL on the TLC plate. Qualitative analysis was continued with the CAMAG TLC Scanner 4 densitometer [40].

Determination of Flavonoid Content

0.3 mL of the pure extract test solution was mixed with 1.5 mL of analytical grade ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M sodium acetate, and 2.8 mL of distilled water. Following mixing, the mixture is agitated and left to stand for 30 minutes at room temperature. measured absorption at a wavelength of 415 nm [40].

5.2.5 Preparation of Nanoemulsion Formula

The nanoemulsion formula can be determined using the factorial design method with 2 factors and 2 levels. The high level of surfactant concentration factor (Tween 80) is 45 %w/w, and the low level of surfactant concentration factor (Tween 80) is 35 %w/w. The high level of co-surfactant concentration factor (PEG 400) is 24 %w/w, and the low level of co-surfactant concentration factor (PEG 400) is 14 %w/w. Table 6 contains the design of the nanoemulsion formulation.

Table 6. Nanoemulsion Formula of Purified Extract from *Anredera cordifolia* (Ten.) Steenis Leaves

Material	Concentration (g)			
	Formula 1	Formula A	Formula B	Formula AB
Purified Extract	3	3	3	3
Tween 80	**35**	**45**	**35**	**45**
PEG 400	**14**	**14**	**24**	**24**
Virgin Coconut Oil	6	6	6	6
Methyl paraben	0.3	0.3	0.3	0.3
Propyl paraben	0.5	0.5	0.5	0.5
Aquadest	100	100	100	100

The first step is combining Tween 80 and PEG 400 in a beaker and homogenizing it for 10 minutes at 1000 rpm with a magnetic stirrer (mixture 1). Mixture 1 is given a dose of concentrated purified extract from *Anredera cordifolia* (Ten.) Steenis leaves, which is then homogenized for 10 minutes at 1000 rpm using a magnetic stirrer (mixture 2). Virgin coconut oil, the oil phase, is added to mixture 2 and homogenized for 10 minutes (mixture 3) with a magnetic stirrer running at 1250 rpm. After that, the mixture is sonicated at a frequency of 40 kHz using a Sonicator until a transparent nanoemulsion forms [41,42].

5.2.6 *Testing of Nanoemulsion Characteristics*

Determination of Polydispersity Index (PDI), Zeta Potential Value, and Particle Size

Determination of particle size using the Particle Size Analyzer SZ-100 with dynamic light scattering type. The particle size that can be classified as nanoemulsion ranges from 20-200 nm [35,43]. The polydispersity index values that fall within the monodispersity range are 0.05-0.7. The polydispersity index values that meet the polydispersity range are >0.7 [42]. A stable dispersion system is indicated by a zeta potential reading that is either less than -30 mV or more than +30 mV [42].

Determination of Transmittance Percentage Value

At 650 nm, the nanoemulsion sample was examined with a UV-Vis spectrophotometer. 90–100% is the range in which the predicted percent transmittance value falls [45].

5.2.7 Preparation of Nanoemulgel Formulation

The gel base used to create the nanoemulgel preparation is Carbopol 940. Carbopol 940 is added to distilled water that has been heated to 70°C, and the mixture is homogenized with a magnetic stirrer for 20 minutes at low speed. TEA is added to the mixture gradually until a homogeneous and clear gel base is formed. Next, the nanoemulsion is added to the gel base and sonicated with a Sonicator at a frequency of 40 kHz until a nanoemulgel is formed [46]. In Table 7, the amount of materials used for the preparation of nanoemulgel formulations is listed for each formula.

Table 7. Nanoemulgel Formula of Purified Extract from *Anredera cordifolia* (Ten.) Steenis Leaves

Material	Concentration (g)			
	Formula 1	Formula A	Formula B	Formula AB
Nanoemulsion	100	100	100	100
Carbopol 940	1.5	1.5	1.5	1.5
TEA	1	1	1	1
Aquadest	100	100	100	100

5.2.8 Evaluation of Nanoemulgel Preparation

Organoleptic Test

The organoleptic testing observed from the nanoemulgel preparation includes the texture, aroma, and color of the formulation [46].

Homogeneity Test

Applying a certain quantity of nanoemulgel preparation to a glass slide or other appropriate transparent medium is how homogenization testing is carried out. There shouldn't be any discernible coarse particles and the preparation should have a homogenous structure [46].

pH test

Utilizing a pH meter calibrated with buffer solutions of pH 4 and pH 7 to measure pH. After dissolving 0.5 g of nanoemulgel preparation in 5 mL of distilled water, the electrode is submerged in it, and the apparatus is kept in place until the pH value remains consistent. For skin application, the acceptable pH range for nanoemulgel formulations is 4.5 to 7 [46].

Spreadibility test

A transparent glass slide was placed on graph paper, and 0.5 g of the nanoemulgel preparation was spread out across it. A cover slip was placed over the transparent glass slide, and it was then exposed to weights weighing 50, 100, and 150 grams for one minute each. A nice semi-solid preparation can be spreadable between 5 and 7 cm [46].

Adhesion test

Two glass slides are covered with 0.25 g of the nanoemulgel preparation. The object glass was subjected to a 1 kilogram load for 5 minutes. The object glass was put on the testing apparatus after the load was taken out of it. The testing apparatus was loaded with 80 g, and the moment the sample was released from the object glass was noted. For semi-solid preparations, a good adhesion time is greater than 1 second [46].

Viscosity Test

Viscosity testing was conducted using a Brookfield viscometer with spindle number 5. The nanoemulgel sample was placed in a container, and the spindle speed was set to 50 rpm. The viscosity of a good nanoemulgel formulation ranges from 500 to 10,000 cPs [45].

5.2.9 Anti-inflammatory Activity Test

A total of 30 male Wistar strain white rats were after an 18 hour fast, drinking water was still provided. Measurement of the volume of the left hind foot of the mouse by inserting it into the plethysmometer up to the marked limit. The measurement results are recorded as the initial volume (Vo). The nanoemulgel preparation without extract, betamethasone cream, and the purified extract nanoemulgel preparations of formula 1, formula A, formula B, and formula AB were each applied at a dosage of 0.2 g to the left hind foot of the mice and left for 60 minutes. After 60 minutes, a 1% solution of carrageenan was injected subplantarly into the left foot of the mice at a volume of 0.1 mL. The volume of the left hind foot of the mice was measured up to the marked limit using a plethysmometer at hours 0, 1, 2, 3, 4, 5, and 6 after the administration of the 1% carrageenan solution. The measurement results were recorded as edema volume (Vt). In this test, what is observed is the change in the volume of the rats' foot edema per unit of time in response to the administration of the nanoemulgel preparation of purified extract from *Anredera cordifolia* (Ten.) Steenis leaves. The percentage of inflammation is computed using the subsequent formula:

$$\% \text{ Inflammation} = \mathbf{(Vt - Vo) / Vo \times 100\%}$$

Description:
Vt = volume of edema in the rat's foot over a unit of time
Vo = initial volume of the rat's foot

The percentage of inflammation inhibition is is computed using the subsequent formula:

$$\% \text{ Inhibition of Inflammation} = \mathbf{(a - b) / b \times 100\%}$$

Description:
a = average % inflammation in the negative control group
b = average % inflammation in the treatment and positive control groups.

5.2.10 Data Analysis

Data on the formulation of nanoemulgel using a factorial design was analyzed with a Two-Way ANOVA test using Design-Expert series 13®, with a significance threshold of $p<0.05$. The analysis of data for the anti-inflammatory activity test was conducted using a One-Way ANOVA test with SPSS 22 for Windows software to determine the differences among more than two sample groups, also with a significance threshold of $p<0.05$.

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