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Preparation and Characterization of Butterfly Pea (*Clitoria ternatea* L.) Nanoemulgel

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Abstract

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BACKGROUND: Butterfly Pea or Asian pigeonwing (*Clitoria ternatea* L.) has been widely used by people. This flower species contains flavonoid and phenolic with antioxidant activities. Further, the antioxidant in flowers can be processed into nanoemulgel facial makeup. The emulgel is expected to provide better drug delivery, thus maximizing the anti-aging effects.

AIM: The aim of this study was to identify the effect of the gel basis variation in the preparation and characterization of the nanoemulgel of Asian pigeonwing extract using the particle size analysis or PSA method.

METHODS: In this experimental laboratory research, the independent variable comprised the variants of gelling agent bases, namely, carbopol 940, hydroxy propyl methyl cellulose, and sodium carboxy methyl cellulose. Research tools involved magnetic stirrer, atomizer, spectrophotometer ultraviolet-visible, and particle size analyzer. The dependent variable consisted of the particle size, polydispersity index, and physical stability of nanoemulgel preparations. Instruments of the research encompassed three formula of emulgel, namely, F1 (using carbopol 940 0.2% base), F2 (using HPMC 0.2% base), and F3 (using Na CMC 0.2% base). All data were analyzed using the one-way ANOVA and Kruskal-Wallis test. The research was conducted from January to May 2021 at the Laboratory of Pharmacy Technology, Gorontalo Polytechnic of Health, the Natural Medicine Laboratory of the National Food and Drug Control Agency, and Ull Laboratory Yogyakarta.

RESULTS: The present work revealed that the F3 formula with Na CMC base is the best and most stable formula with the particle size measuring at 14.7 nm and polydispersity index (PDI) at 0.271.

CONCLUSION: The gel base variation of the preparation produces different physical quality of Asian pigeonwing nanoemulgel with the best characteristics in the formula based on Na CMC.

Introduction

Clitoria ternatea L. or Asian pigeonwing is a flower species with useful properties, such as antioxidant activities that capture free radical molecules caused by UV radiation. However, the antioxidant activities of the flower are rarely used in cosmetic industries. This notion underpins the rationale of the present work, that is, to explore the use of Asian pigeonwing as an antiaging product with its antioxidant agents, such as phenolic and flavonoid [1], [2], [3], [4], [5], [6].

The antioxidant activities in Asian pigeonwing can be processed into emulgel as an alternative preparation that provides better drug delivery systems. Emulgel is an emulsion made by mixing gelling agents into oil-in-water or water-in-oil emulsion; it has faster drug release systems than ointment and cream [7], [8]. Drug release systems on the skin are determined by the size of the particle, considering the skin membrane barrier. One of the factors that determine drug penetration into the skin is particle size. It is believed that size plays an important role in skin penetration [9].

The smaller the particle, the easier the drug penetrates the barrier and the better the effect of the drug, allowing better penetration to the skin by nanoemulgel [10].

Nanoemulgel is one of the topical preparations that have multiple release control systems, namely, hydrogel and nanoemulsion. Nanoemulsions are more stable than emulsions because nanoemulsions have a smaller droplet size of 20–500 nm and compared to conventional emulsions with a droplet size of 0.1–100 µm [11]. This preparation penetrates rapidly and produces active compounds faster due to its nano-size. Further, gelling agents can enhance the stability of nanoemulsion better by reducing the surface and interfacial tensions. It is of important paramount to ensure that the nanoemulgel carrier has met the particle size. Thus, characterization, that is, the measurement of particle and polydispersity index (PDI), was performed using a device called the particle size analyzer, or henceforth particle size analysis (PSA) [12].

The present work aims to prepare Asian pigeonwing nanoemulgel using variation gelling agent and identify the effect of them on the physical quality of the nanoemulgel.

Methods

Tools

Research tools involved magnetic stirrer, pH meter, ultrasonic cleaner, atomizer, spectrophotometer UV-Vis, and PSA.

Materials

Research materials involved Asian pigeonwings (*C. ternatea* L.) retrieved from Gorontalo city, carbopol 940, Na-CMC, VCO, Propylene glycol, Tween 80, DMDM hydantoin, butylated Hydroxytoluene, Aquadestilata, TEA, HCl, and magnesium powder.

Preparation of Asian pigeonwing extract infusion

As many as 100 mL of aquadest was added into 10 g of Asian pigeonwing simplicia, the formula was stirred until homogenous. The mix was boiled at 90°C for 15 min, stirred occasionally. Infused mix was filtered using filter paper and then squeezed.

Phytochemical screening of Asian pigeonwing flavonoid test

A total of 10 g of the sample was added into 100 mL of boiling water at 90°C; the mix was then boiled for 5 min before filtering. The filtrate was weighed at 5 mL, and then 0.05 mg of Mg powder and 1 mL of concentrated HCl were added, shaking it vigorously. Positive results were shown by the mixed color that changed to red, yellow, or orange [13].

Preparation of Asian pigeonwing flower powder extract

As many as, 2 L of the liquid extract of Asian pigeonwing was produced. A nozzle placed on the upper part was prepared to pass the liquid extract; the liquid was then sprayed with water. Furthermore, the liquid decomposed into powder granules. These granules were inserted into a drying chamber where hot air flows [14]. A total of 2 L of the liquid extract of Asian pigeonwing produced 250 g of the flower powder extract.

Preparation of Asian pigeonwing extract nanoemulgel

The formulation of Asian pigeonwing extract nanoemulgel comprised three different formulae using different gelling agents. Provided in the following Table 1 is the percentage of the materials in each formula.

Table 1: Asian pigeonwing extract nanoemulgel formula

Materials	F1 (%)	F2 (%)	F3 (%)
Water phase			
Butterfly pea extract	1	1	1
Propylene glycol	25	25	25
Tween 80	35	35	35
Dimethyloldimethyl hydantoin	0.5	0.5	0.5
Oil phase			
Virgin Coconut Oil	2	2	2
Butyl Hydroxy Toluene	0.1	0.1	0.1
Gel base			
Carbopol 940 and triethanol amine	0.2 and 0.8	-	-
Hydroxypropyl methylcellulose	-	0.2	-
Na carboxymethylcellulose	-	-	0.2
Aquadest	35.4 ml	36.2 ml	36.2 ml

Tween 80 and propylene glycol were mixed using a magnetic stirrer for 10 min at a speed of 500 rpm. Following the process was adding VCO and BHT; the mix was homogenized for 10 min. DMDM hydantoin and the flower extract, diluted using the aquadest for 10 min until homogenous, were then added. The nanoemulsion preparation was placed in an ultrasonic cleaner bath; the nanoemulsion was processed for 10 min to reduce the particle size.

The gel base was made by dispersing 0.2% carbopol 940 (F1), 0.2% HPMC (F2), and 0.2% Na CMC (F3) in aquadest in a separate medium until diluted. Na CMC was heated at 70° C until the gel mass was formed. TEA solution was added while stirring. The carbopol and TEA mix were homogenized using a magnetic stirrer at 350 rpm for 10 min at 70° C until the gel base was formed. The HPMC gel base was left in place for 24 h. Further, the gel base was added bit by bit into nanoemulsion and then mixed using a magnetic stirrer at 500 rpm for 10 min until nanoemulgel was formed.

Results

Water content of the simplisia and rendement results of Asian pigeonwing extract

Table 2 displays the calculation result of the water content of Asian pigeonwing simplisia.

Table 2: Water Content of simplisia and rendement results of Asian Pigeonwing extract

Tests	Initial weight	Recent weight	Yields (%)
Water content	664	272	40.96
Rendement	272	250	91.91

Flavonoid screening

Phytochemical screening was performed to identify the content of secondary metabolite of the flower sample using a specific reactant. The flavonoid test results of the flower powder extract that extract of the flower positive contain flavonoid. Sample 5 ml +Mg 0.0g mg +concentrated HCl 1 ml (shaken vigorously) yield a red one.

Physical stability

Transmittance percentage

The observation of the physical stability of nanoemulsion preparation was performed using a transmittance percentage test to examine the purity of the sample, as seen in Table 3.

Table 3: Transmittance percentage test results

Base	Replication 1 (%)	Replication 2 (%)	Replication 3 (%)	Average (%)
Nanoemulsion	99.96	99.90	99.85	99.90 ± 0.0449

pH and organoleptic test

The pH and organoleptic test results of the nanoemulgel of Asian pigeonwing extract are seen in Table 4.

Table 4: pH and organoleptic test results

Formula	pH	Color	Odor	Consistency	Homogeneity
F1	8.1 ± 0.0816	Clear Yellow	VCO-Like	Thick soft	Homogenous
F2	6.3 ± 0.0816	Clear Yellow	VCO-Like	Thick soft	Homogenous
F3	6.1 ± 8.8818	Clear Yellow	VCO-Like	Thick soft	Homogenous

Dispersibility test

Provided in Table 5 is the dispersity test results of the nanoemulgel of Asian pigeonwing extract.

Table 5: Dispersity test results

Formula	Weight (g)	Average (cm)
F1	0	4.4
	50 g	5.2
	100 g	5.2
Average (cm) F2	0	4.5
	50 g	5
	100 g	5
Average (cm) F3	0	4.8
	50 g	4
	100 g	4.7
Average (cm)		5.1
		4.6

Stability test

The results of the Asian pigeonwing nanoemulgel stability test showed that both F1, F2, and F3 are table.

Particle size test

Table 6 shows the results of the particle size test of the F3 formula (Na CMC gel base).

Table 6: Particle size test results

Formula	Particle Size (nm)			Average (nm)
	Replication 1	Replication 2	Replication 3	
F3	14.6	14.8	14.9	14.7 ± 0.1247

requirement, that is, ≤10. Higher water content (>10%) triggers the growth of microbes that are detrimental to the simplisia stability and thus affecting the storage resistance [15]. Table 2 displays the calculation result of the water content of Asian pigeonwing simplisia that meets the standard; the water content was 4%.

Rendement refers to the comparison between the dry weight of the sample with the weight of the material. The results of the rendement of Asian pigeonwing flower powder extract seen in Table 2 are classified standard. According to Farmakope Herbal Indonesia, an Indonesian reference book of pharmacy, the optimum rendement value of the extract should be less than 10% [16]. The condition is determined by the solvent type. Polar compounds will dissolve in polar solvents, *vice versa*. Aquadest was the solvent used in the extraction process since phenolic and flavonoids contained in Asian pigeonwing are water-soluble [17].

Red-colored solutions were formed as the results of phytochemical screening of the flavonoid test of the Asian pigeonwing. These results resonate with research by positive results were shown by [13] and [18], where the positive results are seen in the color of the mix that changed to red, yellow, or orange [13].

The transmittance percentage test results using the UV-Visible spectrophotometer at a wavelength of 650 nm reveal that the transmittance value was 99.90%. Such a high transmittance percentage indicates that the droplet size is getting smaller. If light passes through that emulsion system, the beams of light will be forwarded, and thus, the solution appears clear [19]. If the percentage of the transmittance of the sample is close to 100%, the sample has purity similar to the transmittance of aquadest at 100%. This indicates that the comparison of oil in the composition is smaller than the surfactant composition, and the co-surfactant has better purity [18], [20], [21].

The pH test on nanoemulgel preparation aims to identify and ensure that the pH is suitable for skin pH. The topical preparation should have a pH ranging from 4.5 to 6.5 to prevent skin irritation [22]. As seen in Table 4, F2 and F3 are the formulae of nanoemulgel preparation that fit with skin pH. F1 formula is excluded from the list due to the addition of triethanolamine (TEA) in the formulation process; the addition of TEA was to neutralize the acid trait of carbopol 940 to result in a clear solution; thus, the gel becomes transparent (Rowe, 2009). A high amount of TEA will bind more carboxylic groups, thus neutralizing the carboxylic groups and increasing the consistency of the gel. In addition, the turbidity of the gel is lowered [23].

The pH calculation using the ANOVA test reveals a significant result ($0.000 < 0.05$), confirming

Discussion

Water content is the parameter that is used to determine water residue after drying processes. The level of water content in the simplisia meets the quality

significant differences between pH values, and thus, the analysis proceeded to the *post hoc* test. According to the *post hoc* test, there is a significant difference between F1 and F2 and F1 and F3. However, there is no significant difference between F2 and F3.

The observation of the organoleptic test of the nanoemulgel of Asian pigeonwing extract does not indicate any changes while storing at room temperature; the color of each formula remains clear yellow. Similarly, the observation of the odor of the nanoemulgel of Asian pigeonwing extract does not indicate any changes while storing at room temperature. The preparation has a VCO-like odor as the VCO was used in the formulation process. Differences in consistency were also identified as each formula used a different gel base. The higher the concentration of the gelling agent, the more increased the consistency of the preparation. The soft consistency of the gel enables the gel to distribute evenly; also, the skin absorbs the gel quickly [24]. A homogeneity test was carried out to determine whether or not the mixing was evenly performed during the making of the preparation formula. It is also intended to identify the evenness of the nanoemulgel texture when applied to the skin. The observation reveals that the formulae have better homogeneity signified with all materials that are mixed evenly [25].

Good preparation is preferable if it can be dispersed evenly on the skin. Good dispersibility ranges between 5 and 7 cm [26]. Based on the results of the dispersibility test in the nanoemulgel of Asian pigeonwing extract, the replication was performed 3 times with different weights, that is, 0, 50 g, and 100 g. Table 5 reveals that the average results of F1 have met the requirement of good dispersibility. However, F2 and F3 did not meet good dispersibility requirements. The difference of gel bases can affect the decline in the dispersibility value. The higher the dispersibility index, the easier the medicine diffuses into the skin. Such a condition is because the wide dispersibility area provides a large membrane surface area that enables the medicine to diffuse into the skin, thus leading to a higher amount of substance penetrates and maximum efficacy [27].

The normality test reveals that the significance value is $0.00 < 0.05$, indicating that the data are not normally distributed. Following the normality test was the Kruskal–Wallis test since the data were not normally distributed ($0.556 > 0.05$). This can be concluded that there are no significant differences between the dispersibility value.

The stability test of the nanoemulgel of Asian pigeonwings extract was performed using the freeze-thaw method for three cycles. This method was carried out by observing the physical stability of the preparation while storing it at extreme temperature for three cycles. One cycle lasts 48 h: 24 h at temperature 4° C and 24 h at 40° C [25]. The physical stability test of the nanoemulgel of Asian pigeonwing extract was

central to determining nanoemulgel stability during the test. This was performed by examining the instability of emulsion, that is, creaming, sedimentation, flocculation, and coalescence [28]. According to stability test result, there is no emulsion instability in all formulae (F1, F2, and F3).

The selection of component and formula concentration used in making nanoemulgel must be considered. The oil phase in the nanoemulgel can affect the size of the globule and the stability of the nanoemulgel. Tiny globule causes a decline in the gravity force and Brownian motion prevents sedimentation or creaming. Furthermore, the globule prevents flocculation as a big surface area of the emulsion system can stimulate faster penetration of the active ingredients and provide esthetic value due to the transparent characteristic of nanoemulgel [20].

The particle size test was performed for the formula of the nanoemulgel of the Asian pigeonwing that had passed the evaluation phase with good stability of the preparation. As based on the transmittance percentage test, pH test, organoleptic test, stability test, and dispersibility test, the F3 formula has a transmittance percentage of 100%; moreover, the formula is clearer compared to F1 and F2. It is also stable at 4°C and 40°C and is suitable for the pH level of the skin.

The result of PSA revealed that the particle size is 14.7 nm. Considering this, F3 fits the characteristic of an acceptable nanoemulgel, where the nanoparticle size ranges from 10 to 200 nm [29]. The PDI value of F3 is measured at 0.271 (see Appendix 6). This value refers to the distribution of the particle size. If the PDI value is close to 0, the dispersity of the particle size is homogenous. If the PDI value is greater than 0.5, the heterogeneity is considered high. Samples with a PDI value greater than 0.7 have a very wide size distribution. This signifies that the size of the nanoemulgel of Asian pigeonwing has a homogenous particle size dispersion. PDI measures the homogeneity of nanoparticles, the smaller the PDI the more homogeneous nanoparticles. Nanoparticles with PDI smaller than 0.4 is considered acceptable for drug deliver. This is because differences among particle sizes are impactful on particle characterization [30].

Conclusion

The study confirms that the variation of gel bases in the preparation process of nanoemulgel contributes to the physical quality of the nanoemulgel of Asian pigeonwing extract. F3 formula with Na CMC 0.2% has clearer gel compared to F1 formula (carbopol 940) and F2 (HPMC); the preparation is deemed stable and suitable for skin pH. In addition, the formulation aspect also influences the characterization of the best formula, that is, F3 using the PSA method; the formula's

particle size was 14.7 nm, and the PDI value was measured at 0.271.

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