World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

FORMULATION AND PHYSICAL STABILITY STUDY OF NANOEMULSION GEL (NANOEMULGEL) CONTAINING BELIMBING WULUH (*AVERRHOA BILIMBI* L.) ETHANOLIC EXTRACT

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Article Received on 07/01/2021 **Article Revised on 27/01/2021** **Article Accepted on 17/02/2021**

ABSTRACT

Objective: Belimbing wuluh (*Averrhoa bilimbi* L.) is one of the plants that is potentially used as a source of antioxidants. Natural antioxidant contents such as vitamin C, carotenoids, flavonoids, tannins, and other polyphenol compounds are believed to prevent the photoaging process. The aim of this study is to formulate the ethanolic extract of Belimbing wuluh (*Averrhoa bilimbi* L.) into nanoemulsion gel dosage form and test its physical stability and antioxidant activity. **Methods:** Nanoemulgel was prepared by high pressure homogenizer method in various extract concentrations i.e. 1%, 2%, and 3%. The formulated nanoemulsion gel then tested for their physical stability using several methods, i.e. long-term stability tests, and cycling test.. In addition, an antioxidant capacity study was also carried out by using DPPH (2,2,-diphenyl-1-picryl hydrazil) damping method. **Results:** Nanoemulgel showed a stable physical appearance for 12 weeks at room temperature (25 ± 2^{o}C), high temperature (40 ± 2^{o}C), cycling test, and mechanical test. However, on a long-term physical stability test in low temperature (4 ± 2°C), all nanoemulgel undergone the Ostwald Ripening phenomena. The results of *in-vitro* antioxidant activity study showed poor IC_{50} value, i.e. 20520.09 µg/mL (F0); 18392.29 µg/mL (F1); 17868.80 µg/mL (F2); and 17287.625 µg/mL (F3), respectively. **Conclusion:** The formula is not an optimal formula to produce a nanoemulgel with good physical stability and antioxidant activity.

KEYWORDS: Antioxidant, *Averrhoa bilimbi L*, Formulation, Nanoemulsion Gel, Physical Stability.

INTRODUCTION

Skin is the largest organ of the human body that plays a role in the body's first-line defense mechanism against a variety of external disturbances, both physically, chemically, and biologically. One of physical disturbance that is often experienced by Indonesian people is UV exposure.

UV radiation is a very potential trigger in the formation of ROS (Reactive Oxygen Species) free radicals on the skin. Excessive ROS production in the epidermal layer will disrupt the balance of endogenous antioxidant systems produced by the body that trigger the occurrence of oxidative stress events that are suspected to be the main contributor of photoaging process.[1,2]

The use of antioxidants on the skin is considered to be able to inhibit the formation of ROS generated by sun exposure as an effective approach to prevent oxidative stress. Thus, the photoaging process caused by the presence of ROS can be inhibited.[3]

Belimbing Wuluh (*Averrhoa bilimbi L.*) is one of the plants that is potentially to be used as a source of antioxidants. Several studies have shown that fruits of these plants contain natural antioxidant compounds such as vitamin C, carotenoids, flavonoids, tannins and other polyphenolic compounds.[4] Therefore, the extract of this fruit that believed to prevent photoaging process will be formulated into nanoemulsion gel dosage form. Nanoemulsion is selected as the base of the bioactive extract due to the soft texture of semisolid on the skin. The present of small globules of nanoemulsion could modified the texture of the stratum corneum that can lead in enhancing of bioactive extract permeation into the skin

MATERIALS AND METHODS

Materials

Ethanolic Extract of Belimbing Wuluh (*Averrhoa bilimbi L.*) obtained from Balai Penelitian Tanaman Rempah and Obat (Bogor, Indonesia), Carbopol 940 (Lubrizol, Korea), Tween 80 (Brataco, Indonesia), Span 20 (Brataco, Indonesia), Propylenglicol (Brataco,

Indonesia), Isopropyl miristate (Palm-Oleo, Malaysia), Triethanolamine (Petronas Chemical), Metilparaben, Propylparaben, BHT, Na EDTA, and Aquademineralisata, Methanol, Acorbic acid, and DPPH (2,2,-diphenyl-1-picryl hydrazil).

Standardization of Belimbing Wuluh Ethanolic Extract

Standardization process of belimbing wuluh ethanolic extract consists of organoleptic examination, pH measurement, and antioxidant capacity study. Organoleptic study conducted on the physical appearance and odor of the ethanolic extract of belimbing wuluh; pH measurements of extract was conducted by dissolving 1 g of ethanolic extract condensed into 10 mL of aqua DM then measuring the pH using an INTSTRUMENT EUTECH 510 pH meter; and antioxidant capacity study performed by DPPH damping methods.

Formulation and Preparation of Nanoemulsion Gel

Nanoemulgel was made by using high energy methods namely High Pressure Homogenizer (HPH). The homogenization process was carried out at 500 bar pressure of 5 cycles. Nanoemulgel was made in 3 formulations with different ethanolic extract of belimbing wuluh ie 1%, 2%, and 3% in the same base composition.

A preliminary experiment was conducted to obtain a nanoemulsion with a clear, homogeneous, and stable physical appearance. The preliminary experiments were performed by varying the ratio of isopropyl myristate as an oil phase, Tween 80-Span 20 as a surfactant, and propylenglycol as a cosurfactant to form a ternary phase diagram using a water-titration.

The ratio of surfactant-cosurfactant mixture used was 1: 1. Aqueous titration method was used to prepare ternary phase diagrams by varying concentrations of oil (1:9 to 9:1) to a surfactants-cosurfactants mixture. The ternary phase diagram was made using the Chemix School version 3.60 software to obtain the nanoemulsion region. During the titration, samples were stirred to ensure homogeneity and visually monitored for ease of emulsification. The formulations that spontaneously formed emulsion without phase separation or creaming were considered as physically compatible and stable.

Characterization of Nanoemulgel Preparations

Organoleptic

In the organoleptic observations, nanoemulsion gel appearenace was observed whether it changes in color, odor, clarity, phase separation, and other changes that may occur during storage.

pH Measurement

The measurement of preparation pH was done by dissolving 1 g of the preparation into 10 mL aqua DM, then pH measurement was done by pH meter EUTECH INTSTRUMENT pH 510.

Measurement of Droplet Size Distribution and Potential Zeta Value

Measurements of droplet size distribution and zeta potential values were performed using Zetasizer Nano Series (Malvern, UK) with the help of Zetasizer ver 7.11 software. The sample solution was prepared by dissolving 1 g of the formula into 10 mL of aqua DM, then the solution was introduced into the cuvette as high as ± 1 cm from the bottom of the cuvette..

Transmission electron microscopy

Morphology of the optimized formulation was observed using transmission electron microscope (JEOL JEM-1010, Universitas Gadjah Mada, Indonesia). One drop of the diluted sample was dripped onto a carbon-coated copper grid and allowed to be absorbed, then stained with polar dye and allowed to dry at room temperature. After drying, globul of nanoemulsion gel morphology can be observed using TEM at room temperature (25 ± 2^oC).

Viscosity and Rheology

The Viscosity measurements were made using a Brookfield Viscometer at room temperature (25 ± 2^oC). On the viscosity measurement, one spindle number and one speed was selected. The experiment were conducted triplicate. As for rheological measurement, the spindle speed was set from low speed to high speed, then from high speed to low speed, gradually. In this measurement will be obtained dial reading number (dr).

Physical Stability Test

The physical stability tests were divided into two types of testing. First is the long-term stability test. This test was performed by storing the dosage at 4 ± 2°C, 25 ± 2^oC, and 40 ± 2^oC for 12 weeks. Organoleptic observations and pH measurements were performed every 2 weeks. In addition, the preparations also checked whether occur sineresis or another physical instability sign. This experiment was done triplicates.

The second test is a cycling test. Cycling test was performed as much as six cycles. Each cycle consists of storing the dosage form at cold temperature (4 ± 2°C) for 24 hours, then followed by storing the dosage form at hot temperature (40 ± 2 ° C) for the next 24 hours. In this test conducted organoleptic observation, as well as observed whether there is syneresis or another physical instability of the preparation. This experiment was done triplicate.

Antioxidant Activity Study with DPPH Damping Method (2,2, -dyphenyl-1-picryl hydrazil)

The preparation of the sample solution was carried out by dissolving 1 g of the preparation with methanol up to 50 mL to prepare a stock solution with a concentration of 20000 µg / mL. Then the stock solution was taken out by

using a pipette volume 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, and 6 mL and put it into 10 mL flask and the volumes was added up to 10 mL with methanol, obtaining 2000 µg / mL, 4000 µg / mL, 6000 µg / mL, 8000 µg / mL, 10000 µg / mL, and 12000 µg / mL concentration of sample solution.

DPPH solution was made by weighing 10.0 mg DPPH and then place into a 100 ml measuring flask, follow by addition of methanol to 100.0 ml to form a DPPH solution with a concentration of 100 ppm.

The solution which will be analyzed was prepared by taking 2 mL of each concentration and adding 1 mL of DPPH solution of 100 µg / mL concentration and 1 mL of methanol was then put into a coated reaction tube which covered with aluminum foil and then shaken. The spike solution was then incubated at 37 ° C for 30 minutes. The incubated test solution, was introduced into the cuvette for measured by using UV-Vis Spectrophotometry at maximum wavelength.

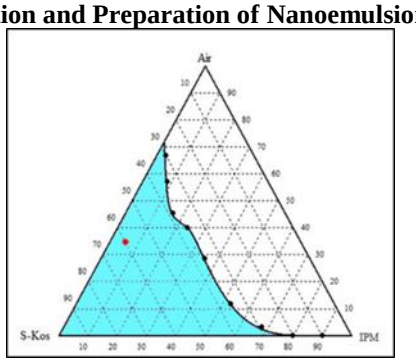
RESULT AND DISCUSSION

Standardization of Belimbing Wuluh Ethanolic Extract

Organoleptic observation of belimbing wuluh ethanolic extract was observed on physical appearance and the odor of belimbing wuluh ethanolic extract. Belimbing Wuluh ethanolic extract is brownish green (Pantone 132c) with typical sour smell of Belimbing Wuluh fruit. Belimbing Wuluh ethanol extract has pH 1.88. The low pH of etaholic extract is influenced by the content of organic acids and vitamin C contained in its fruits. One of the dominating acids is oxalic acid, usually ranging from 8.57 to 10.32 mg / g. Therefore, oxalic acid is suspected to be the cause of low pH of belimbing wuluh.[5] Changes in the composition of chemicals and organic acids during fruit ripening play a key role in chemical characteristics such as pH, total acidity, and sweetness.[6]

Figure 1: Organoleptic observation of belimbing wuluh ethanolic extract.

Formulation and Preparation of Nanoemulsion Gel



S-Kos = Surfactant-cosurfactant; IPM = isopropyl myristate.

Figure 2: Ternary phase diagram of isopropyl miristate, surfactant-cosurfactant, and water.

The blue area indicated by the pseudo-ternary phase diagram shows the area that can produce nanoemulsion. While the red dot indicated by the pseudo-ternary phase diagram showed a formula used to formulate nanoemulsion gel (nanoemulgel). The diagram shows that the red dot was still in the blue color area, so it can be assured that the oil, water, and surfactants-cosurfactant mixture used to formulate the nanoemulgel of belimbing wuluh ethanolic extract in recent study can produce nanoemulsion dosage form.

Evaluation of Nanoemulgel Dosage Form

Organoleptic

Nanoemulsion gel produced in recent research showed low viscocity, soft textured, spreadable, and comfortable enough to be applied to the skin even when slightly oily and sticky. The stickiness is due to the high concentration of Tween 80 added.

Blank formula (F0) and formula 1 (F1) have clear physical appearance, while formula 2 (F2) and formula 3 (F3) showed an opaque physical appearance. The difference in clarity that occurs may caused by differences in the size of globules that were formed. There was no phase separation occured in all formulas. This indicates that the concentration of surfactant (Tween 80 and Span 20) and cosurfactant (propylenglycol) used were in accordance with the HLB of the oil phase used.

Blank formula (F0) produced a pale greenish yellow preparation (Pantone 379c) with a Tween 80 smell. Formula 1 (F1) produced a brownish yellowish preparation (Pantone 7762c) with a distinctive smell of belimbing wuluh and Tween 80 blend. Formula 2 (F2) and Formula 3 (F3) produced a greenish-yellow preparation (Pantone 606c) with the same odor as formula 1 (F1). The different colors shown in the four preparations was due to the different concentration of the added extract of the belimbing wuluh The more extracts are added, the more tendencies for nanoemulgel to be greenish yellow.

Figure 3: Organoleptic observation of blank formula (F0), formula 1 (F1), formula 2 (F2), and formula 3 (F3).

pH Measurement

All nanoemulsion gel formulations showed varying pH. Different concentrations of belimbing wuluh ethanolic extract added to the four formulas will affect the pH of the resulting preparation. Therefore, ethanolic extract of belimbing wuluh itself has a very acidic pH that is 1.88 contributing to the decrease of pH. Formula 3 with 3% ethanolic extract had lower pH (4,20) compared to Formula 2 (5,41) and Formula 1 (7.04) containing 2% and 1% ethanolic extract, respectively. The blank formula (F0) that did not contain belimbing wuluh ethanolic extract tend to be alkaline with 7.44 pH.

Globul Size Distribution and Potential Zeta

Table 1: The result of the measurement of the distribuation of globul size and the zeta potential of the fourth gel nanoemulsion of the formula.

	F0	F1	F2	F3
Globul size (nm)	12,78	10,00	18,54	14,42
PDI	0,780	0,795	0,872	0,648
Zeta Potential (mV)	-28,3	-27,1	-16,3	-25,1

Measurement of globule size distributions and zeta potential in all formulas was performed using Malvern Zetasizer Nano Series. Based on Table 1, the nanoemulgel globule size of F0, F1, F2, F3 are 12.78 nm, 10 nm, 18,54 nm, and 14,42 nm respectively. All formula did not showed much variety in size due to the same concentration of oil added to all the formulation. Hadnadev, Dokic, Krstonosic, & Hadnadev (2013) suggests that the higher concentration of oil added to the o/w emulsion tend to increase the size of the globule formed.[7] The increased amount of oil added, the more internal phases would be dispersed.

Based on the theory expressed by Chellapa, Eid & Elmarzugi (2015), the globular diameters produced by the formula Formula (F0), Formula 1 (F1), Formula 2 (F2), and Formula 3 (F3) did not meet the size criteria of nanoemulsions (20 -200 nm).[8] All formulas produced a microemulsion droplet, i.e.emulsion system with globule size diameter of 1-100 nm, generally 10-50 nm.[9] The small diameter of the resulting droplet may be due to the high levels of surfactant-cosurfactant used in the formulation that caused the internal phase to be immobilized in the surfactant.[10]

From the data of Table 1 it can be seen that the polydispersity index (PdI) produced by the four formulas was very high. The Polydispersity Index (PdI) represents the value indicating the sample droplet size distribution.The Polydispersity index value that is greater than 0.7 indicates a broad an non-homogenoous droplet size distribution that was too broad and not homogenous. A good PdI value is less than 0.7 and the smaller the PdI value indicates the more homogeneous size of the particles produced by the sample. A formula which produces a PdI value above 0.7 can not use the Z-average value to express the average diameter of the globular size. The average value of the globule size that can be used for a formula with high PdI is Dmean-Volum value.[11] The uniformity of the sample droplet size in the present study could be due to the low pressure of high pressure homogenizer (HPH) used, or the lack of number of cycles carried out in the HPH process.

Besides the size of the globule, a zeta potential measurement was also performed. The zeta potential is an important parameters to measure the stability of the dispersion system and to find the possibility of flocculation or aggregation in the emulsion or suspension system.[11] The zeta potential regulates the repulsive degree between dispersed particles of equal charge and adjacent each other.[12] Particles having zeta potential values greater than + 30 mV and smaller than -30 mV indicate stable dispersion systems because they have surface charges that can prevent aggregation.[13] Based on the results (Table 1), the zeta potential value of the fourth formula was in the range of + 30 mV and -30 mV indicating the resulted nanoemulsion was not stable enough.

Transmission Electron Microscope

The morphology of nanoemulgel droplet was observed using Transmission Electron Microscope (TEM). In this study, morphological observations were performed only on formula 1 (F1), since formula 1 (F1) was considered to be the most optimal and stable formula compared to formula 2 (F2) and formula 3 (F3). The observations were performed with 150,000 times magnification. The result showed that the formula 1 (F1) had a round droplet. The nonhomogeneity of particle size belonging to formula 1 was also shown in Fig. 2 there was one large droplet with small droplets of different sizes around it. This was in line with the obtained PdI results, that was 0.6-0.8 which can be interpreted that the distribution of formulated nanoemulgel droplet size that was not homogeneous.

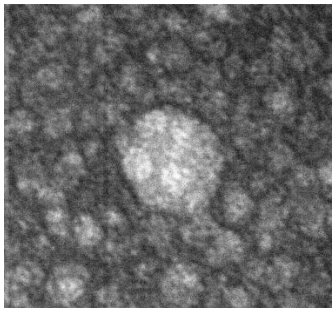


Figure 4: The morphology of nanoemulgel droplet observed by using Transmission Electron Microscope (TEM).

Viscocity and Rheology

The rheogram of nanemulsion gel formulas showed pseudoplastic flow properties. The nature of pseudoplastic flow was one type of non-Newton flow. The Increased rate of shear on products with pseudoplastic flow properties, resulting in the decreased of the viscosity. This type of Flow properties are shown in many pharmaceutical products such as emulsions, suspensions, liquid gels, creams, and so on.

The pseudoplastic flow curve starts from the origin (0,0) or near the origin with a low shear rate and no yield value as in the plastic stream. The curved portion in the rheogram shown by the materials with pseudoplastic flow properties, was due to the shearing action of the long-chain molecules such as Tween 80 and Span 20. The irregular molecules will arrange the long axis in the flow direction with increasing shear stress, increasing the shear rate at each subsequent shear stress.[14] The overlapping and decreasing curves that coincide indicate that if the shear stress get reduced, the system will immediately return to its original state. In its application, pseudoplastic flow showed that the preparation was a viscous preparation but quite easy to pour.

The viscosity value of a substance with pseudoplastic flow properties can not be determined with a single value, since there is no linear part of the curve.[14] The viscosity value of each formula at 5 rpm spindle 1 velocity was 700 cP (centipoise) for blank formula, 380 cP (centipoise) for formula 1, 200 cP (centipoise) for formula 2 and 180 cP (centipoise) for formula 3. The difference in viscosity value of the four nanoparticles gel preparations were interferred by the pH value of the preparation. The blank formula with 7.44 pH value (Alkaline) had a higher viscosity compared to formula 3 (F3) with 4.2 pH value (Acidic). Therefore, the Carbopol gel base added to the dosage will further expand or more viscous in a neutral pH atmosphere. The carboxyl group present in Carbopol, will ionize releasing protons at an alkaline pH, resulting in electrostatic repulsion from the negative charge which will cause the gel to swell.

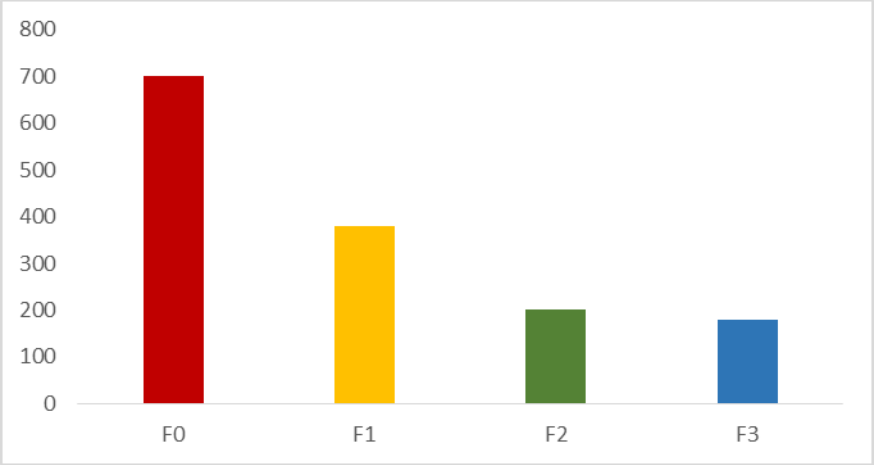


Figure 5: Viscosity measurement of blank formula (F0), formula 1 (F1), formula 2 (F2), and formula 3 (F3) at 0 week.

Physical Stability Test of Nanoemulsion Gel Form

Physical stability tests were performed to all formulas (F0, F1, F2, F3), aimed to determine the effect of several conditions on the physical stability of all four formulas and to ensure the quality, safety, and effectiveness of pharmaceutical dosage form during storage time are acceptable. There were several parameters observed in the physical stability test of the gel nanoemulsion preparation. The physical stability test was conducted using 3 methods, i.e. long term stability test, cycling test, and mechanical test.

After stored for 12 weeks (long-term stability test), the four nanoemulsion gel (F0, F1, F2, F3) dosage formulations at room temperature storage (25 ± 2°C) and high temperature (40 ± 2°C) showed stable physical appearance. There was no visible changes in color and odor and no phase separation or sineresis was found. Emulsion instability was shown in all four formulas stored at low temperature (4 ± 2°C). Visually, the four formulas stored at low temperatures were freezed and their colours turned milky white. However, the preparation were melted when placed at room temperature (25 ± 2°C). The turbidity level of all stored

four formulas at low temperatures were changed. At low temperature storage (4 ± 2°C), the four formulas became turbid in proportion to the length of storage. This phenomenon of instability is called the Ostwald Ripening phenomenon.[15] Ostwald Ripening is a phenomenon of emulsion instability that leads to gradual growth of large droplets at the expense of small droplets around it.[16]

The results of pH measurements of nanoemulgel stored in room temperature (25 ± 2°C) and low temperature (4 ± 2°C) for 12 weeks storage showed unsignificant variation in changes of pH, showing the four formulas were stable. The pH measurement of all four formulas stored inhigh temperature (40 ± 2°C) results were varied. The pH of the four formulas stored in high temperatures were decreased variably compared to formulas stored in room temperature and low temperature condition. This indicated the instability of the dosage at high temperature storage. The decrease in pH in all four formulas stored in high temperatures condition can be due to the oxidation events experienced by ascorbic acid and polyphenol compounds contained in the belimbing wuluh ethanolic extract. The release of H^+ ions from ascorbic acid and some polyphenol compounds due to the oxidation process, contributes to the decrease in the pH of the four preparations.

Observations of globular diameter were also performed at week 12 in order to see the stability of formulated nanoemulgel. The measurement results showed that the average globule diameter slightly increases. A significant increase of droplet diameter only occurs in blank formula (F0). The increase of the particle size may due to several factors, one of which was the potential zeta value possessed by the four formulas, so the interaction between formed globule may occur and led to the occurrence of flocculation events. The Ostwald Ripening event was highly possible to happen in all four formulas. Ostwald Ripening is a phenomenon of emulsion instability that leads to gradual growth of large droplets at the expense of small droplets around it.[16] But, to ascertain the occurrence of the Ostwald Ripening phenomenon further testing is required to ensure that the Ostwald Ripening phenomenon really occurs.

F0, F1, F2, F3 showed stable physical appearance after cycling test. No phase separation, crystal formation, or sineresis were found. This results indicated the amount of surfactants (Tween 80 and Span 20) added in the formula were able to stabilize the emulsion system, and Carbopol 40 used as a gelling agent, was able to retain water in its matrix so that the gel nanoemulsion before and after the cycling test remains stable.

Figure 6: After stored for 12 weeks, the four nanoemulsion gel (F0, F1, F2, F3) dosage formulations at room temperature storage (25 ± 2°C) and high temperature (40 ± 2°C) showed stable physical appearance, but instability was shown in all four formulas stored at low temperature (4 ± 2°C).

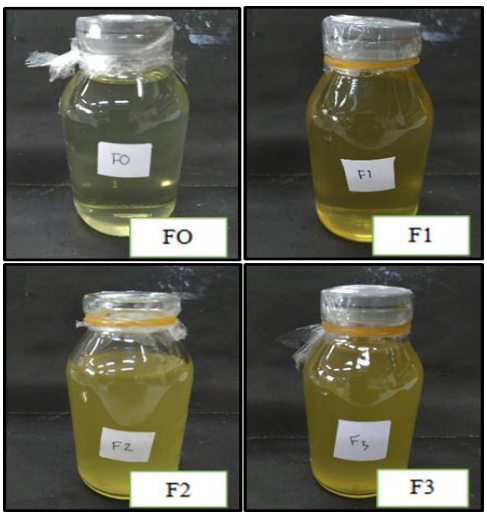


Figure 7: F0, F1, F2, F3 showed stable physical appearance after cycling test.

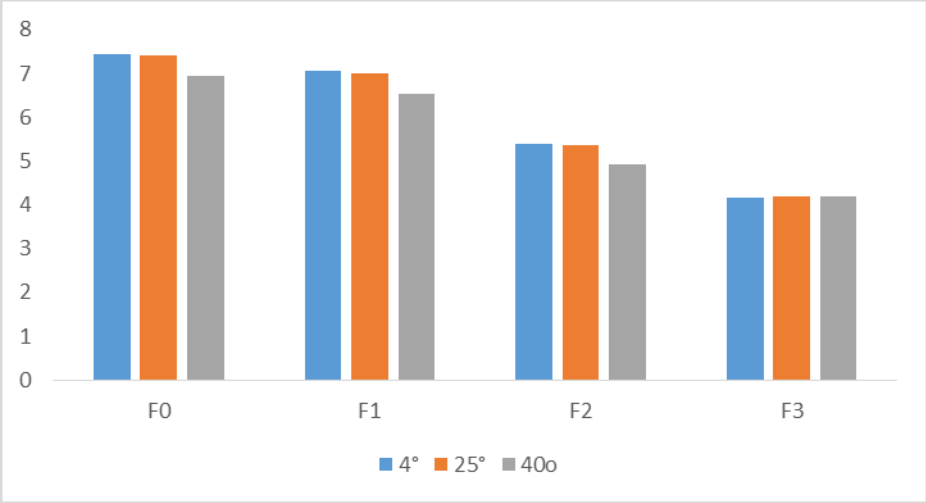


Figure 8: pH measurement of blank formula (F0), formula 1 (F1), formula 2 (F2), and formula 3 (F3) at low temperature (4 ± 2°C), room temperature (25 ± 2°C) and high temperature (40 ± 2°C) in 12 weeks.

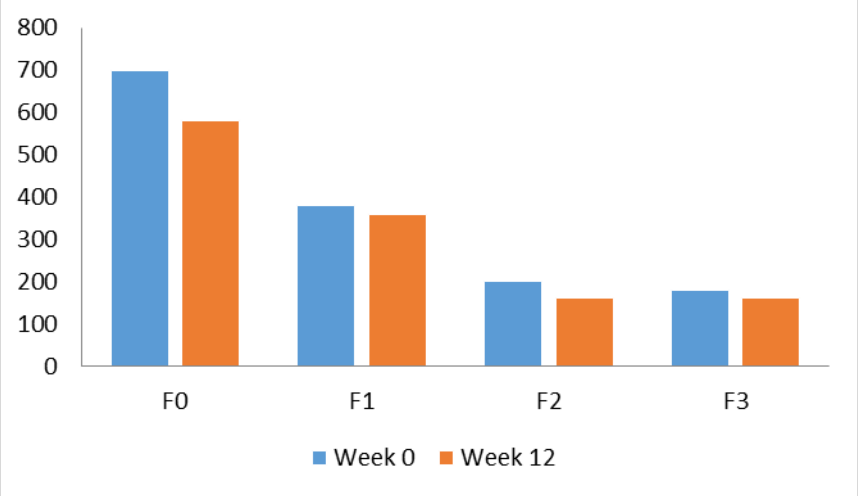


Figure 9: Viscosity measurement of blank formula (F0), formula 1 (F1), formula 2 (F2), and formula 3 (F3) at 0 week and 12 week.

Table 2: The result of the measurement of the distribuation of globul size and the zeta potential of the fourth gel nanoemulsion of the formula at 12 week.

	F0	F1	F2	F3
Globul size (nm)	152,17	11,83	18,24	14,9
PDI	0,701	0,720	0,843	0,667
Zeta Potential (mV)	-31,6	-33,5	-17,3	-26,6

Antioxidant Activity Test with DPPH Method (2,2, -dyphenyl-1-picryl hydrazil)

Measurement of antioxidant activity can be done in various ways, some of them by using lipid peroxidation method, tiobarbiturat, malonaldehid, β-carotene bleaching, DPPH, and thiocyanate. In this study, researchers chose to use a practical and sensitive method, the DPPH method. DPPH itself is a free radical compound or oxidizing agent that has unpaired electrons in its structure.

The working principle of this test is the presence of antioxidant compounds that will donate the H + ions on DPPH so that convert the free radical compounds DPPH which originally purple to non-radical compound DPP hydrazine with yellow color. The remaining DPPH is measured uptake at the specified maximum wavelength. The smaller the resulting absorption means the more DPPH compounds that can be neutralized by antioxidants. In this test, antioxidant activity was calculated by using the percentage of sample inhibition against DPPH, which was then plotted into the calibration curve. Antioxidant activity is expressed as IC50 value.[17]

Tabel 3: IC50 value of blank formula (F0), formula 1 (F1), formula 2 (F2), and formula 3 (F3).

Formulas	Antioxidant Capacity (IC_{50}) week 0	Antioxidant Capacity (IC_{50}) week 12
Extract	2500	-
F0 (blank formula)	20520,09	21257.5
F1	18392,29	21171,15
F2	17868,80	20784,065
F3	17287,625	18231

Based on the data from the table it can be seen that the formula 3 (F3) containing 3% ethanolic extract of belimbing wuluh had the smallest IC_{50} value compared to the formula 2 (F2), formula 1 (F1), and blank formula (F0) that containing 2%, 1%, and 0% ethanolic extract of belimbing wuluh, respectively. The value of IC_{50} is defined as the total antioxidant value needed to reduce free radical DPPH by 50% concentration. IC50 was calculated from all extracts based as the percentage of DPPH inhibited radicals.[18] The result showed that formula 3 (F3) had the greatest antioxidant activity compared to other formulas. This indicated that the more extracts of belimbing wuluh were added, the more antioxidant activity will increase.

The gel nanoemulsion preparations had lower antioxidant activity compared to pure ethanolic extracts and vitamin C standards. It may due to oxidation of antioxidant content during the formulation process, resulting in the decrease of antioxidant activity. As stated by Rahmawati and Bundjali (2012), vitamin C is highly susceptible to high temperatures (thermolabile), whereas the high pressure homogenizer carried out during the formulation heat that may damage the vitamin C content in the extract of starfruit.[19]

The antioxidant activity test at week 12 was performed to determine the tenacity of antioxidant activity showed in all four formulas during storage. Based on the test results, the four formulas showed the decrease of antioxidant activity during the storage, shown in the increasing IC_{50} value. The decrease in antioxidant activity during storage mau due to various factors such as storage temperature, humidity, light intensity, and others.

CONCLUSION

Nanoemulsion gel formulated with 5% isopropyl myristate as the oil phase, 30% Tween 80-Span 20 as surfactant, and 30% propylenglycol as cosurfactant showed a stable physical appearance for 12 weeks in room temperature (25 ± 2^{o}C), high temperature (40 ± 2^{o}C), cycling test, and mechanical test. However, in low temperatures of long-term physical stability (4 ± 2 ° C), the four formulas undergone instability phenomenon. The results of in vitro antioxidant activity study showed poor IC50 value, that was 20520.09 µg / mL (F0); 18392,29 µg / mL (F1); 17868.80 µg / mL (F2); And 17287,625 µg / mL (F3). Thus, the formulas are not optimal formulas for producing nanoemulsion gel with good physical stability and antioxidant activity.

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