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Formulation and Evaluation of Platelet Rich Plasma (PRP) Lotion Preparations with Asiaticoside as an Antioxidant

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ABSTRACT

Currently, the use of PRP requires a person to visit a skin care clinic by doing a series of treatments directly. Therefore, in order to facilitate the use of PRP, asiaticoside lotion base products containing antioxidants can be used as a PRP product base. asiaticoside antioxidants serve to stabilize PRP when added to the base. This research uses Platelet-Rich Plasma (PRP) and Asiaticosida as active substances, Fancor® Uni-Embase as emollient and emulgator, DMDM hydantoin as preservative, and distilled water as carrier. The stages in this research include preparation of tools and materials, lotion base optimization, lotion preparation formulation, addition of active substances, lotion preparation evaluation, physical stability test, and antioxidant activity test. The results of the PRP lotion stability test with asiaticoside for 10 days showed significant results ($p<0.05$) which means that for 10 days there is a tendency to be unstable. Antioxidant activity test results obtained IC50 results on Asiaticosida of 57.63 µg/mL, on PRP of 196.1 µg/mL, on the base with the addition of Asiaticosida of 77.19 µg/mL, on F0 of 166.90 µg/mL, on F1 of 96.727 µg/mL, on F2 of 88.395 µg/mL, and on F3 of 82.017 µg/mL.

Key words: Lotion, PRP, asiaticoside, Antioxidant

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1. Introduction

At this time many people use various kinds of cosmetic preparations to care for the skin of both women and men. One of the cosmetic preparations for skin care is lotion. According to Indonesian Pharmacopeia III defines lotion as a liquid preparation used as an external drug in the form of a suspension or dispersion. Lotions can be solid in the form of a fine powder with a suitable suspending agent or an emulsion of the type seen in oil in water with a suitable surfactant (Ministry of Health RI, 1979).

Indonesia is a tropical country with various natural resources, including plants, some of which have been utilized both as cosmetics and as a means of preventing or treating diseases. One of the herbal plants, Centella asiatica, has a strong antioxidant effect. In Centella asiatica, there are many components of triterpenoid compounds and asiaticosides which are the main compounds that have antioxidant activity (Zainol et al in (Saputri & Damayanthi, 2015). Used as herbs, parts of Centella asiatica containing asiaticosides are found in stems (15.9%), leaves (82.6%), and roots (1.5%), so they are used as herbs (Zulkarnaen et al., 2015).

Currently, the use of PRP requires a person to visit a beauty clinic by doing a series of treatments directly. PRP treatment requires the use of needles to insert PRP into the skin. Therefore, in order to facilitate the use of PRP, researchers are interested in making a lotion base containing asiaticoside as an antioxidant that can be used as a PRP product base, it is hoped that this antioxidant can stabilize PRP when added to the preparation product. PRP (platelet-rich plasma) is a blood component of autologous biologically active products enriched with a number of growth factors, cytokines, and other plasma proteins (Lin et al., 2020). PRP contains a high concentration of platelets with a variety of bioactive proteins, suggesting that PRP can accelerate the process of tissue regeneration and healing (Lee et al., 2020). PRP has many platelets consisting of growth factors and other elements, which greatly help the regenerative healing process. PRP works by destroying platelet components that contain growth factors. PRP contains seven growth factors, including VEGF, EGF, FGF, IGF-1, PDGF, TGF β-1, and HGF (Taniguchi et al., 2019). PDGF plays a role in tissue remodeling and promotes the production of other growth factors. Growth factors in PRP can induce β-cell regeneration and increase β-cell mass by stimulating β-cell neogenesis and ductal cell differentiation into β-cells, as detected by increased c-peptide levels (Younis, 2019). PRP has experienced development and is increasingly advanced, many benefits of PRP include in terms of medical action, dermatology, and in terms of beauty. PRP can rejuvenate the skin and treat burns, chronic ulcers, diabetic ulcers, and hair loss (Satriyo et al, in Younis, 2019).

2. Method

This study is a laboratory experimental study conducted from March to June 2023. Platelet-Rich Plasma (PRP) obtained from volunteers and asiaticoside employed as active substance, Fancor® Uni-Embase used as emollient and emulgator, DMDM hydantoin used as preservative, and distilled water used as carrier. There are 4 formulas in this study, F0 with the addition of 2.5% PRP concentration without asiaticoside, F1 with the addition of 2.5% PRP concentration and 1% asiaticoside, F2 with the addition of 2.5% PRP concentration and 2% asiaticoside,

and F3 with the addition of 2.5% PRP concentration and 3% asiaticoside. Evaluation of lotion preparation includes organoleptic test, spreadability test, viscosity test, emulsion type test, homogeneity test, pH test, accelerated stability test, and antioxidant activity test (Uv-Vis spectrophotometry). The data obtained are presented in the form of tables and graphs and the evaluation results will be processed using IBM SPSS software.

3. Result

3.1 Optimization Results

The purpose of this optimization is to find out the right formula to determine the lotion base formula that meets the requirements of the literature.

Table 1. Lotion Base Formula

Material name	F1	F2	F3
Fancor® Uni-Embase	5	7.5	10
DMDM Hydantoin	5	5	5
Aquadest	Ad 100	Ad 100	Ad 100

Fancor® Uni-Embase was mixed with hot water at 70oC - 80oC and stirred until homogeneous, then waited until cool. After cooling, DMDM hydantoin was added. Then put into the lotion container.

After optimization, the best emulgator concentration was obtained to make lotion base, so PRP lotion with asiaticoside was formulated by making four different formulas, namely F0 with the addition of 2.5% PRP concentration without asiaticoside, F1 with the addition of 2.5% PRP concentration and 1% asiaticoside, F2 with the addition of 2.5% PRP concentration and 2% asiaticoside, and F3 with the addition of 2.5% PRP concentration and 3% asiaticoside.

Table 2: PRP lotion formula with asiaticoside

Composition (%)	F0	F1	F2	F3
PRP	2,5	2,5	2,5	2,5
Asiaticoside	-	1	2	3
Fancor® Uni-Embase	5	5	5	5
DMDM Hydantoin	0,6	0,6	0,6	0,6
Buffer pH 7	0,5	0,5	0,5	0,5
Triethanolamine (TEA) ad pH 7.4	qs	qs	qs	qs
Distilled water	ad 100	ad100	ad 100	ad100

Fancor® Uni-Embase was mixed with hot water and stirred until homogeneous, then waited to cool. After cooling, DMDM hydantoin, TEA, and Buffer were added. Then the pH was checked, after which asiaticoside and PRP were added and day 10.

3.2 Characterization

Lotion Base Reproducibility Test

Organoleptic Reproducibility Test

Table 3. Reproducibility Test on Organoleptic Characterization

Batch	Observation		
	Color	Smell	Shape
1	Yellowish white	Typical Asiaticoside	Somewhat viscous
2	Yellowish white	Typical Asiaticoside	Somewhat viscous
3	Yellowish white	Typical Asiaticoside	Somewhat viscous

Description:	1 = *Lotion* base formulation with FANCOR® UNI-EMBASE 5% and Asiaticoside 2%
	2 = *Lotion* base formulation with FANCOR® UNI-EMBASE 5% and Asiaticoside 2%
	3 = *Lotion* base formulation with FANCOR® UNI-EMBASE 5% and Asiaticoside 2%

Reproducibility Test for Homogeneity

Table 4. Reproducibility Test for Homogeneity

Batch	Homogeneity
1	Homogeneous
2	Homogeneous
3	Homogeneous

Description:	1 = *Lotion* base formulation with FANCOR® UNI-EMBASE 5% and Asiaticoside 2%
	2 = *Lotion* base formulation with FANCOR® UNI-EMBASE 5% and Asiaticoside 2%
	3 = *Lotion* base formulation with FANCOR® UNI-EMBASE 5% and Asiaticoside 2%

Reproducibility Test on Emulsion Type

Table 5. Reproducibility Test for Emulsion Type

Batch	Emulsion Type
1	M/A
2	M/A
3	M/A

Description:	1 = *Lotion* base formulation with FANCOR® UNI-EMBASE 5% and Asiaticoside 2%
	2 = *Lotion* base formulation with FANCOR® UNI-EMBASE 5% and Asiaticoside 2%
	3 = *Lotion* base formulation with FANCOR® UNI-EMBASE 5% and Asiaticoside 2%

Reproducibility Test Against pH

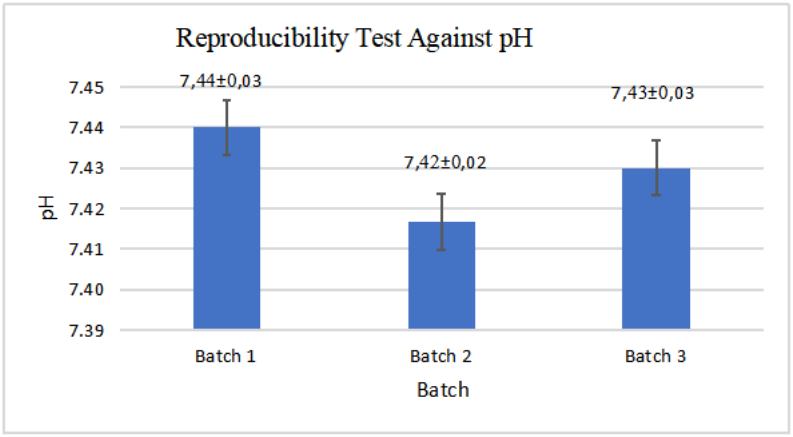


Image 1. Diagram of Reproducibility Test Results Against pH

In the pH test, the test was repeated three times, based on Figure 1, the average pH obtained in batch 1 was 7.44, batch 2 was 7.42, and batch 3 was 7.43. The value obtained is in accordance with the lotion pH requirements of 4.5-8 (SNI 16-4399-1996).

Reproducibility Test on Viscosity

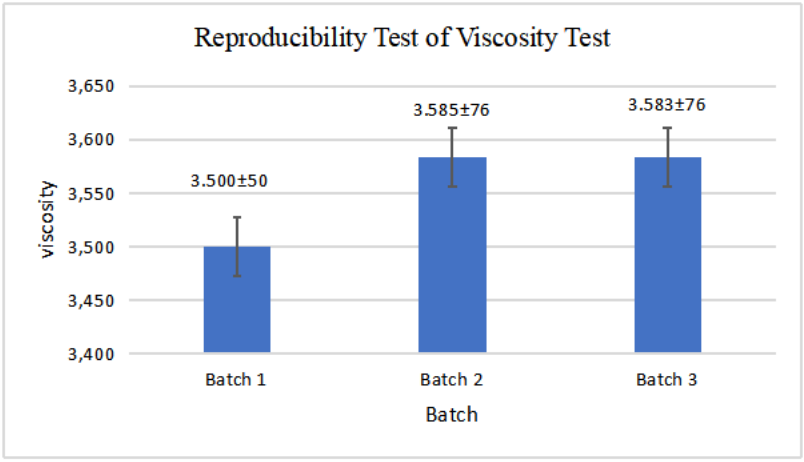


Image 2. Reproducibility Test on Viscosity

In viscosity testing using a Brookfield LV manual viscometer with a speed of 60 rpm and using spindle no 64. Viscosity testing was repeated three times, based on the diagram above, the average viscosity value obtained in batch 1 was 3,500, batch 2 was 3,583, and batch 3 was 3,583. The results obtained meet the lotion viscosity requirements of 2,000-50,000 cPs (SNI 16-4399-1996).

Reproducibility Test on Spreadability

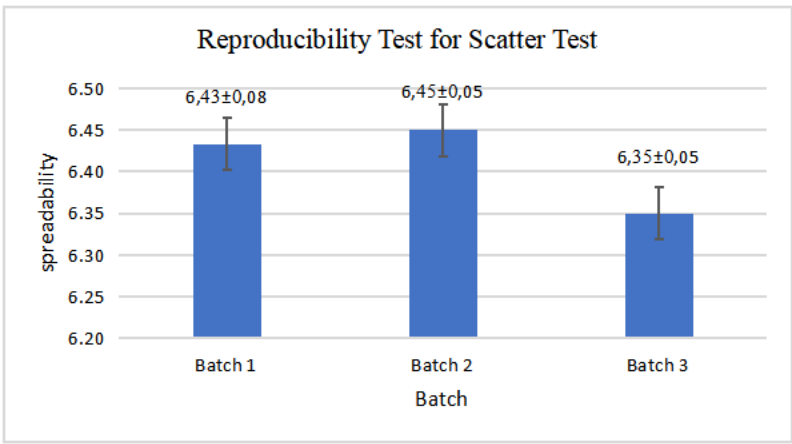


Figure 3: Reproducibility Test of Scatterability

In the spreadability test, the test was repeated three times, based on Figure 3 above, the average spreadability results obtained in batch 1 were 6.43, batch 2 was 6.45, and batch 3 was 6.35. The results obtained are in accordance with the lotion spreadability requirements of 5-7 cm (Garg, 2002).

Stability Test

Organoleptic Stability Test

Organoleptic test is done by observing changes using human senses including odor, consistency, and color (Mardikasari, et al., 2017).

Table 6: Stability test results on organoleptic.

Formula	Smell	Color	Shape
F0	No odor	White	Somewhat viscous
F1	Typical Asiaticoside	Yellowish white	Somewhat viscous
F2	Typical Asiaticoside	Yellowish white	Somewhat viscous
F3	Typical Asiaticoside	Yellowish white	Somewhat viscous

Stability Test for Homogeneity

Table 7. Results of Stability Test for Homogeneity

Day-	Formula			
	F0	F1	F2	F3
1	Homogeneous	Homogeneous	Homogeneous	Homogeneous
4	Homogeneous	Homogeneous	Homogeneous	Homogeneous
7	Homogeneous	Homogeneous	Homogeneous	Homogeneous
10	Homogeneous	Homogeneous	Homogeneous	Homogeneous

Description: F0 = Lotion Formulation with PRP 2.5%
F1 = Lotion Formulation with PRP 2.5% and 1% asiaticoside
F2 = Lotion Formulation with PRP 2.5% and asiaticoside 2%
F3 = Lotion formulation with PRP 2.5% and asiaticoside 3%

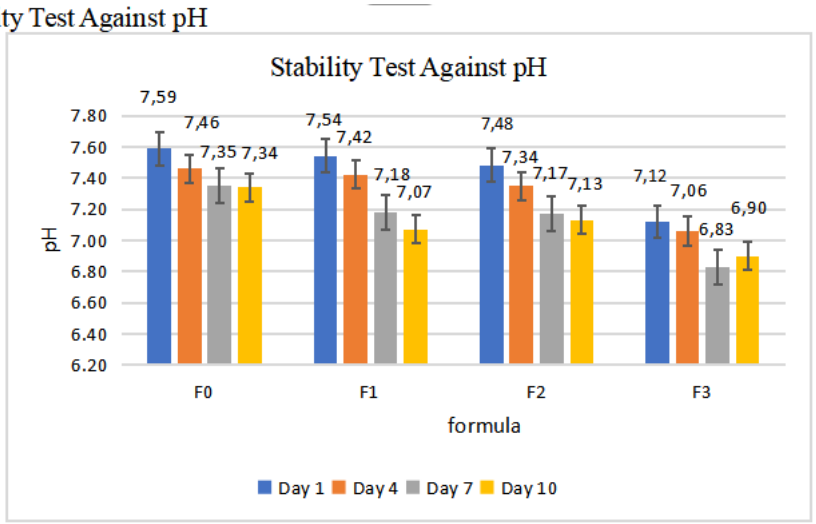


Image 4. Diagram of Stability Test Results Against pH

Description: F0 = Lotion Formulation with PRP 2.5%
F1 = Lotion Formulation with PRP 2.5% and asiaticoside 1%
F2 = Lotion Formulation with PRP 2.5% and asiaticoside 2%
F3 = Lotion formulation with 2.5% PRP and 3% asiaticoside

In the pH test, 3 repetitions were carried out to ensure accuracy. Based on Figure 4, the pH test for 10 days obtained results ranging from 6.83 to 7.59, in the four formulas the more days the average pH value drops but is still within the range of pH requirements. The value obtained is in accordance with the lotion pH requirements of 4.5-8 (SNI 16-4399-1996).

Stability Test on Viscosity

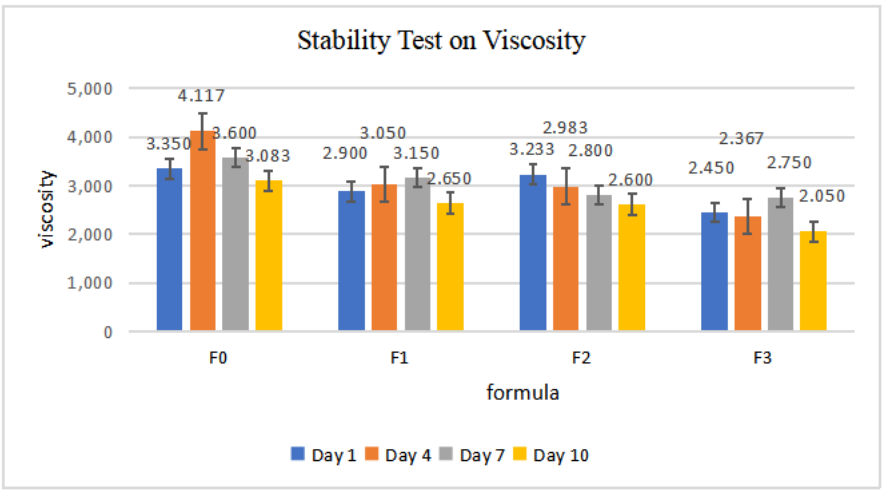


Image 5. Diagram of Stability Test Results on Viscosity

Description: F0 = Lotion Formulation with PRP 2.5%
F1 = Lotion Formulation with PRP 2.5% and 1% asiaticoside
F2 = Lotion Formulation with PRP 2.5% and asiaticoside 2%
F3 = Lotion formulation with PRP 2.5% and asiaticoside 3%

Viscosity testing was carried out 3 times to ensure the accuracy of the measurements. Based on Figure 5.10, the viscosity test for 10 days obtained viscosity results ranging from 2,050-4,117 cPs. During the 10 days there is an increase and decrease in viscosity value but the value obtained is still included in the lotion viscosity requirements of 2,000-50,000 cPs (SNI 16-4399-1996).

Stability Test on Spreadability

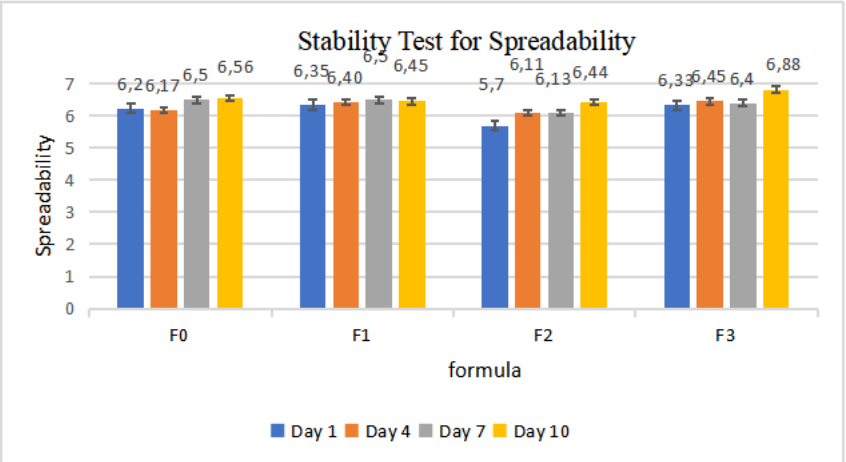


Figure 6: Diagram of Stability Test Results for Spreadability

Description: F0 = Lotion Formulation with PRP 2.5%
F1 = Lotion Formulation with PRP 2.5% and 1% asiaticoside
F2 = Lotion Formulation with PRP 2.5% and asiaticoside 2%
F3 = Lotion formulation with PRP 2.5% and asiaticoside 3%

The spreadability test was conducted 3 times to ensure the accuracy of the measurement. Based on Figure 6, the spreadability test for 10 days obtained results ranging from 5.7-6.82 cm, in the four formulas on average the longer stored, the more the spreadability area increases, but there is also a decrease in between storage times. However, the values obtained are still in accordance with the lotion spreadability requirements of 5-7 cm (Garg, 2002).

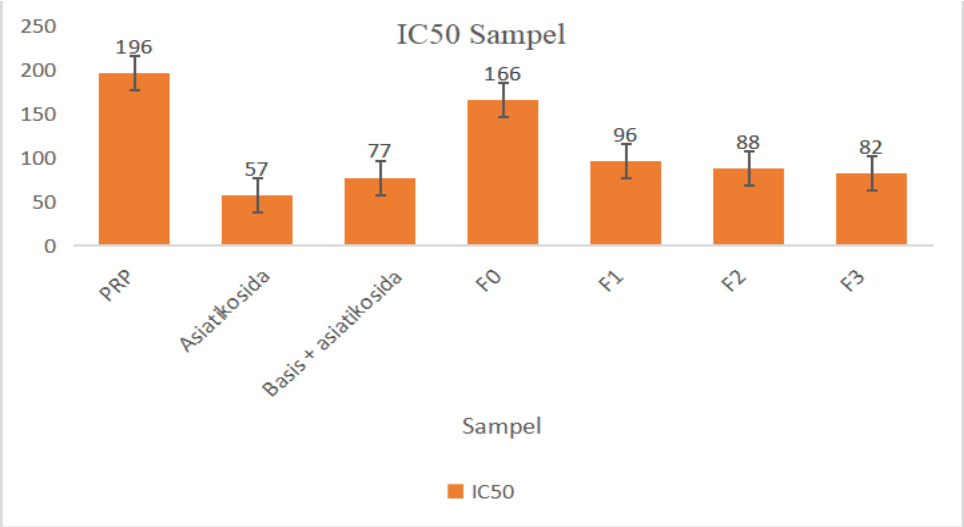
Stability Test on Emulsion Type

Table 8. Results of Stability Test on Emulsion Type

Day-	Formula			
	F0	F1	F2	F3
1	M/A	M/A	M/A	M/A
4	M/A	M/A	M/A	M/A
7	M/A	M/A	M/A	M/A
10	M/A	M/A	M/A	M/A

Description: F0 = Lotion Formulation with PRP 2.5%
F1 = Lotion Formulation with PRP 2.5% and 1% asiaticoside
F2 = Lotion Formulation with PRP 2.5% and asiaticoside 2%
F3 = Lotion formulation with PRP 2.5% and asiaticoside 3%

Antioxidant Activity Test



Description: F0 = Lotion Formulation with PRP 2.5%
F1 = Lotion Formulation with PRP 2.5% and 1% asiaticoside
F2 = Lotion Formulation with PRP 2.5% and asiaticoside 2%
F3 = Lotion formulation with PRP 2.5% and asiaticoside 3%

Based on Figure 7, the results of antioxidant activity test conducted on asiaticoside obtained IC50 value of 57.63 µg/mL, on PRP of 196.1 µg/mL, on the base with the addition of asiaticoside of 77.19 µg/mL, on F0 obtained a value of 166.90 µg/mL, on F1 obtained a value of 96.727 µg/mL, on F2 obtained IC50 value of 88.395 µg/mL, and on F3 obtained IC50 of 82.017 µg/mL. PRP has weak antioxidant, asiaticoside has strong antioxidant, base with added asiaticoside has strong antioxidant, F0 has weak antioxidant, F1 has strong antioxidant, F2 has strong antioxidant, and F3 has strong antioxidant.

4. Discussion

4.1. Reproducibility Test of Lotion Base

Reproducibility refers to the ability to reproduce the results of an analysis or experiment with the same procedures, data, and methods as originally used, thus obtaining maximum results. The reproducibility test used an optimized base, formula B1 with a concentration of 5% Fancor® Uni-Embase with the addition of 2% asiaticoside. In this test, there were 3 batches of repetition with manufacture on different days. Tests carried out include organoleptic test, pH test, homogeneity test, viscosity test, spreadability test, and emulsion type test.

4.2 Reproducibility Test on Organoleptic, Homogeneity, and Emulsion Type

The results of the organoleptic test were yellowish white in color, characteristic odor of asiaticoside, and slightly thick. While the homogeneity test is homogeneous and the emulsion type test is oil in water (M/A). In the three batches produced the same results of the

organoleptic test were yellowish white in color, characteristic odor of asiaticoside, and slightly thick. While the homogeneity test is homogeneous and the emulsion type test is oil in water (M/A). In the three batches produced the same results there were no changes, so it can be said that the organoleptic test, emulsion type test, and homogeneity test of lotion preparations made have good reproducibility.

4.3. Reproducibility Test for pH

In the pH test, the test was repeated three times, based on the diagram above, the average pH result obtained in batch 1 was 7.44, batch 2 was 7.42, and batch 3 was 7.43. When the pH test results were obtained, the data were statistically evaluated with SPSS to determine whether the formula repeated as many as three batches had differences between formulas. The pH data analyzed resulted in normal data ($p>0.05$) and homogeneous data of 0.653 ($p>0.05$). So that data processing is continued with the One Way Anova test, getting the result of 0.269 ($p>0.05$) which can be said that there is no significant difference in pH value. So it can be interpreted that the preparations made in terms of pH have good reproducibility.

4.4. Reproducibility Test on Viscosity

In the viscosity test, the test was repeated three times, based on the diagram above, the average viscosity value obtained in batch 1 was 3,500, batch 2 was 3,583, and batch 3 was 3,583. The viscosity test data obtained were then statistically analyzed using SPSS to determine whether there were differences in the formulas that were repeated as many as 3 batches. The viscosity data analyzed produced normal data, namely ($p>0.05$) and homogeneous data, namely 0.653 ($p>0.05$). So that data processing is continued with the One Way Anova test, getting the result of 0.302 ($p>0.05$) which can be said that there is no significant difference. So it can be interpreted that the preparations made have good reproducibility.

4.5. Reproducibility Test for Spreadability

In the spreadability test, the test was repeated three times, based on the diagram above, the average spreadability results obtained in batch 1 were 6.43, batch 2 was 6.45, and batch 3 was 6.35. Based on the results of the spreadability test obtained, it was then statistically analyzed using SPSS to determine whether there were differences in the formulas that were repeated as many as 3 batches. The analyzed spreadability data resulted in normal data, namely ($p>0.05$) and homogeneous data, namely ($p>0.05$). So that data processing is continued with the One Way Anova test, getting the result of 0.173 ($p>0.05$) which can be said that there is no significant difference. So it can be interpreted that the preparations made have good reproducibility.

4.6. Stability Test of PRP Lotion with Asiaticoside

At this stage, physical stability testing of PRP lotion preparations with asiaticoside was carried out at cold temperature storage (4oC) for 10 days, as for the day points tested, namely day 1, day 4, day 7, and day 10. This test was conducted on the four lotion formulations with the addition of 2.5% PRP

in each formula. There were three variations of asiaticoside concentration used, namely F1 by 1%, F2 by 2%, and F3 by 3%, while F0 was not given the addition of asiaticoside. Stability testing was conducted to determine the physical quality of lotion preparations using PRP with asiaticoside as antioxidant, including organoleptic test, viscosity test, homogeneity test, spreadability test, emulsion type test, and pH test.

4.7. Organoleptic Stability Test

Organoleptic observation of PRP lotion preparations with asiaticoside to determine the physical characteristics of the preparations made including odor, color, and shape (Mardikasari, et al., 2017). The results of organoleptic testing can be seen in table 5.9. The results of organoleptic testing of PRP lotion preparations with asiaticoside after storage for 10 days at 4oC include day 1, day 4, day 7, and day 10 have relatively the same organoleptic. In the four formulas made, the odor in F0 is odorless, while F1, F2, and F3 are typical of asiaticoside, this is because in F1, F2, and F3 there is the addition of asiaticoside. The shape produced from the four formulas is rather thick. Meanwhile, the color produced in F0 is white, this is because the lotion base is already white and there is no addition of asiaticoside to F0, this also indicates that PRP does not affect the color. In F1, F2, and F3 colors have a yellowish white color, this is due to the addition of asiaticoside to the lotion preparation. The observation results of the four formulas made did not show too significant changes in terms of changes in odor, color, and shape so that it can be said that the preparation is stable in terms of organoleptic.

4.8. Stability Test for Homogeneity

Homogeneity testing by checking the presence or absence of clumped granules in lotion preparations, the homogeneity test aims to determine the mixing of ingredients for lotion preparations. The homogeneity of the preparation can be influenced by the mixing procedure used during the preparation (Noer & Sundari, 2016). The homogeneity of the emulsion system can be influenced by the mixing method and equipment used during the emulsion manufacturing process (Noer and Sundari, 2016). After 10 days of testing at the points of day 1, day 4, day 7, and day 10 there was no significant change. PRP lotion preparations with asiaticoside showed homogeneous results characterized by the absence of clumped granules in the lotion.

preparation. In terms of the homogeneity test, it shows that the PRP lotion preparation with asiaticoside is not damaged because of the homogeneous preparation results.

4.9. Stability Test Against pH

The efficiency and stability of the drug, as well as its safety not to irritate the skin, are all verified through pH testing. pH in preparations is related to the effectiveness of preservatives, the stability of active substances, and the state of the skin (Fajriyah in Wulanawati et al., 2019).

The pH test for 10 days obtained results ranged from 6.83-7.59, in the four formulas the more days the average pH value decreased but was still within the pH requirement range.

The value obtained is in accordance with the lotion pH requirements of 4.5-8 (SNI 16-4399-1996). Changes in pH indicate a lack of stability of the preparation which can cause the preparation to be damaged during storage. Differences in the pH value of the preparation during storage can be caused by decomposing media such as storage temperature which can increase the acid or base content of a product (M.M et al., 2018). Changes in the pH of the preparation can also be caused by differences in the concentration of the active substance, with a higher concentration of extract, the pH of the preparation will decrease (Sugiharto & Dr. Cikra Ikhda Nur Hamida Safitri, 2020).

The data obtained were then processed using SPSS version 20, the initial stage is the requirements test including the normality test (Shapiro Wilk) and the homogeneity test (Levene's test). In the normality test and homogeneity test, the sig value was obtained. > 0.05 which means that the data is normally distributed and the data variance is homogeneous. So that data processing is continued with One Way Anova with the results obtained sig value. <0.05 which means that the average storage time of each formula is significantly different. So that data processing is continued using the Post Hoc Test. Post Hoc Test results in the pH F0 test day 1 with day 4, day 7, and day 10; day 4 with day 1, day 7, and day 10; day 7 with day 1 and day 4; day 10 with day-1 and day 4 there is a significant difference between storage times. In the pH test F1 day 1 with day 4, day 7, and day 10; day 4 with day 1, day 7, and day 10; day 7 with day 1, day 10 and day 4; day 10 with day- 1, day 7 and day 4 there is a significant difference between storage times. In F2 day 1 with day 4, day 7, and day 10; day 4 with day 1, day 7, and day 10; day 7 with day 1, day 10 and day 4; day 10 with day 1, day 7 and day 4 there is a significant difference between storage times. In the pH test of F3 day 1 with day 7 and day 10; day 4 with day 7 and day 10; day 7 with day 1, day 10 and day 4; day 10 with day 1, day 7 and day 4 there is a significant difference between storage times. So it can be interpreted that the four formulas are not very stable in cold temperature storage (4oC) for 10 days. This is reinforced by the instability of the pH value which is decreasing.

4.10. Stability Test for Spreadability

The purpose of the spreadability test is to evaluate the spreading properties of PRP lotion formulations containing asiaticosides when applied to the skin to ensure user comfort. The spreadability of the lotion preparation decreases when the same amount of pressure is applied in each test, and the relationship between spreadability and viscosity is inverse. The ability to spread is inversely correlated with the viscosity value where the low viscosity value increases the spreadability and vice versa, the high viscosity value decreases the spreadability (Arisanty, et al., 2020).

The spreadability test for 10 days obtained results ranging from 5.7-6.82 cm, in the four formulas on average the longer it is stored, the more the spreadability increases, but there is also a decrease between storage times. However, the value obtained is still in accordance with the lotion spreadability requirements of 5-7 cm (Garg, 2002). This is in accordance with the

lotion spreadability requirements of 5-7 cm (Garg, 2002). This is in line with the viscosity value that the longer stored the more liquid or down, so that the spreadability of the lotion is inversely proportional to the viscosity value (Oktaviasari & Zulkarnain, 2017). Some factors that affect changes in the spreadability value are temperature, storage, and excipients used. The longer the storage of the preparation, the lower the binding power of the thickener (Armadany et al., 2019). Changes in spreadability can also be influenced by less stable PRP.

The data obtained were then processed using SPSS version 20, the initial stage was the requirements test including the normality test (Shapiro Wilk) and the homogeneity test (Levene's test). In the normality test and homogeneity test, the sig value was obtained. > 0.05 which means that the data is normally distributed and the data variance is homogeneous. So that data processing is continued with One Way Anova with the results obtained sig value. <0.05 which means that the average storage time of each formula is significantly different. So that data processing is continued using the Post Hoc Test. Post Hoc Test results on the spreadability test F0 day 1 with day 7 and day 10; day 4 with day 10; day 7 with day 1; day 10 with day 1 and day 4 there are significant differences between storage times. In the F1 spreadability test day 1 with day 10; day 10 with day 1 there is a significant difference between storage times. In the spreadability test F2 day 1 with day 4, day 7, and day 10; day 4 with day 1; day 7 with day 1 and day 10; day 10 with day-1, day 7 and day 4 there is a significant difference between storage times. In the spreadability test F3 day 1 with day 7 and day 10; day 4 with day 7 and day 10; day 7 with day 1 and day 4; day 10 with day 1 and day 4 there was a significant difference between storage times. So it can be interpreted that the four formulas are not very stable in cold temperature storage (4^{o}C) for 10 days because in the One Way Anova test there are significant differences. This is reinforced by the instability of the spreadability area that changes during storage.

4.11. Viscosity Stability Test

The purpose of viscosity testing is to evaluate the viscosity of the lotion product made. Preparations with too high viscosity will be more difficult to extract from the container and more difficult to flow. The packaging process will also be affected by excessive viscosity because high viscosity produces significant resistance when putting the preparation into the container. In addition, too low viscosity is undesirable as it will cause dripping when applied (Martin, et al., 1993). The viscosity test used a Brookfield LV manual viscometer with a speed of 60 rpm and used spindle no 64.

The viscosity test for 10 days obtained viscosity results ranging from 2,050-4,117 cPs. During the 10 days there was an increase and decrease in viscosity values but the values obtained were still included in the lotion viscosity requirements of 2,000-50,000 cPs (SNI 16-4399-1996). Changes in viscosity can be caused by an increase or decrease in particle diameter size which causes the surface area to get smaller or larger (Mardikasari et al., 2020).

Changes in viscosity can also be influenced by PRP active ingredients that are less stable in the preparation.

The data obtained were then processed using SPSS version 20, the initial stage is the requirements test including the normality test (Shapiro Wilk) and the homogeneity test (Levene's test). In the normality test and homogeneity test, the sig value was obtained. > 0.05 which means that the data is normally distributed and the data variance is homogeneous. So that data processing is continued with One Way Anova with the results obtained sig value. <0.05 which means that the average storage time of each formula is significantly different. So that data processing is continued using the Post Hoc Test. The Post Hoc Test results in the F0 viscosity test day 1 with day 4, day 7, and day 10; day 4 with day 1, day 7, and day 10; day 7 with day 1, day 10 and day 4; day 10 with day-1, day 7 and day 0to-4 there is a significant difference between storage times. In the F1 viscosity test day 1 with day 7 and day 10; day 4 with day 10; day 7 with day 1 and day 10; day 10 with day- 1, day 7 and day 4 there is a significant difference between storage times. In the F2 viscosity test day 1 with day 7 and day 10; day 4 with day 10; day 7 with day 1; day 10 with day 1 and day 4 there is a significant difference between storage times. In the viscosity test of F3 day 1 with day 7 and day 10; day 4 with day 7 and day 10; day 7 with day 1, day 10 and day 4; day 10 with day-1, day 7 and day 4 there is a significant difference between storage times. So it can be interpreted that the four formulas are not very stable in cold temperature storage (4^{o}C) for 10 days because in the One Way Anova test there are significant differences.

4.12. Stability Test Against Emulsion Types

Emulsion type testing is carried out to determine whether the preparation made is an oil-in-water (O/W) or water-in-oil (O/W) emulsion type using the dilution method. The dilution method is carried out by diluting 1 gram of lotion preparation into 10 ml of distilled water (Megantara, et al., 2017).

The emulsion type test of the four formulas, namely the oil-in-water (O/W) emulsion type, is characterized by the mixing of the lotion preparation with water. The use of emulsifiers which tend to be more soluble in water causes this type of emulsion preparation, and can also be caused by water being used as a solvent (Rowe, et al., 2009). In the emulsion type test, the four formulas after being stored for 10 days at cold temperature (4oC) showed the same results from day 1, day 4, day 7, and day 10. This shows that the PRP lotion preparation with asiaticoside does not experience a change in emulsion type during storage time.

4.13. Antioxidant Activity Test

In this study, quantitative antioxidant activity was tested using the DPPH (1,1-diphenyl-2-picrylhydrazyl) technique against a base containing asiaticoside, PRP, asiaticoside, F1 (2.5% PRP and 1% asiaticoside), F2 (2, 5% PRP and 2% asiaticoside), and F3 (2.5% PRP and 3% asiaticoside). When performing antioxidant assays, the DPPH approach offers the advantage of being fast, easy, and requiring less sample and reagents.

The DPPH wavelength obtained in this test was λ 519 nm in methanol solvent in accordance with the literature which states that the maximum wavelength for DPPH is in the range 515-520 nm (Molyneux P, 2004). The next absorbance measurement will be carried out at this wavelength. Measurement of antioxidant activity in samples was carried out by reacting the sample and DPPH solution (1:1) in a dark place, covered with aluminum foil and tightly closed, to avoid the decomposition of the DPPH solution because it is easily oxidized. Vitamin C is used as a reference because it has electron donating groups on the C2 and C3 atoms. Vitamin C can absorb free radicals because of the presence of this group. The IC50 value for vitamin C is 8.147 g/mL, which shows that vitamin C is a strong antioxidant. Antioxidant activity was calculated by the IC50 value (Inhibition Concentration 50%) using the linear regression equation (y=bx+a).

The results of antioxidant activity tests carried out on asiaticoside obtained an IC50 value of 57.63 µg/mL, on PRP it was 196.1 µg/mL, on a basis with the addition of asiaticoside it was 77.19 µg/mL, on F0 a value of 166.90 was obtained. µg/mL, at F1 the value was 96.727 µg/mL, at F2 the IC50 value was 88.395 µg/mL, and at F3 the IC50 was 82.017 µg/mL. PRP has a weak antioxidant, asiaticoside has a strong antioxidant, base with added asiaticoside has a strong antioxidant, F0 has a weak antioxidant, F1 has a strong antioxidant, F2 has a strong antioxidant, and F3 has a strong antioxidant.

5. Conclusion

Based on the results of the research that has been carried out, it can be concluded that: Formula F0 with a concentration of 2.5% PRP, F1 with a concentration of 2.5% PRP and 1% asiaticoside, F2 with a concentration of 2.5% PRP and 2% asiaticoside, and F3 with a concentration of 2.5% PRP and 3 % asiaticoside can be formulated into lotion preparations. The four PRP lotion formulas with asiaticoside as an antioxidant during 10 days of storage at cold temperatures (4oC) tend to be less stable because there are significant differences in the spreadability test, viscosity test and pH test.

6. References

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