Abstract Book

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The Effect of *Cera alba*Concentration Variations on The Physical Stability of Black Cumin (*Nigella sativa*L.) Oil Balm Stick

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ABSTRACT

Black cumin oil from black cumin seeds contained thymoquinone (TQ) and known to have anti-inflammatory activity. Topical anti-inflammatory drugs can be made in the form of balm. In this study, black cumin seed oil was formulated into a stick balm in order to facilitate its use.Method in this study,black cumin oil was formulated in the form of a balm stick using *cera alba*, *adeps lanae,*and VCO as a base. Balm stick was made into 5 formulas with varying concentrations of *cera alba*(30%, 32.5%, 35%, 37.5% and 40%). The evaluation of the balm stick was carried out on organoleptic, homogeneity, melting point, hardness and yield value, followed by 8 weeks physical stability evaluation at room temperature. The results obtained were analyzed using Two Way ANOVA and Kruskal Wallis statistical analysis. Physical stability test results indicated an increase in melting point in the balm stick on a weekly basis (50.17^{o}C, 51.33^{o}C, 52.83^{o}C, 55.33^{o}C and 57.17^{o}C respectively), while the results of the hardness test and yield value in stick balm were 102.89dyne/cm^{2},113.54dyne/cm^{2},125.93dyne/cm^{2},137.79dyne/cm^{2}and148.68dyne/cm^{2}respectively, which were also showed an increase in value. In spite of increased on melting point, hardness test and yield value on physical stability tests, this result still met the requirements. Based on the 8 weeks physical stability evaluation at room temperature, it can be concluded that**i**ncreased concentration of *cera alba*in the balm stick formulation affected the melting point, hardness test and yield value of black cumin oil balm stick, however, the results were still in the range of requirements.

Keywords: Black cumin oil, cera alba, balm stick, physical stability.

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The Effect of *Cera alba* Concentration Variations on The Physical Stability of Black Cumin (*Nigella sativa* L.) Oil Balm Stick

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ABSTRACT

Aim: Black cumin oil from black cumin seeds contained thymoquinone (TQ) and known to have anti-inflammatory activity. Topical anti-inflammatory drugs can be made in the form of balm. In this study, black cumin seed oil was formulated into a stick balm in order to facilitate its use.

Methods: In this study, black cumin oil was formulated in the form of a balm stick using *cera alba*, *adeps lanae*, and VCO as a base. Balm stick was made into 5 formulas with varying concentrations of *cera alba* (30%, 32.5%, 35%, 37.5% and 40%). The evaluation of the balm stick was carried out on organoleptic, homogeneity, melting point, hardness and yield value, followed by 8 weeks physical stability evaluation at room temperature. The results obtained were analyzed using Two Way ANOVA and Kruskal Wallis statistical analysis.

Results: Physical stability test results indicated an increase in melting point in the balm stick on a weekly basis (50.17ºC, 51.33ºC, 52.83ºC, 55.33ºC and 57.17ºC respectively), while the results of the hardness test and yield value in stick balm were 102.89dyne/cm^2, 113.54dyne/cm^2, 125.93dyne/cm^2, 137.79dyne/cm^2 and 148.68dyne/cm^2 respectively, which were also showed an increase in value. In spite of increased on melting point, hardness test and yield value on physical stability tests, this result still met the requirements.

Conclusion: Based on the 8 weeks physical stability evaluation at room temperature, it can be concluded that **i**ncreased concentration of *cera alba* in the balm stick formulation affected the melting point, hardness test and yield value of black cumin oil balm stick, however, the results were still in the range of requirements

Keywords: Black cumin oil, cera alba, balm stick, physical stability.

INTRODUCTION

Black seed (*Nigella sativa* L.) or better known as habbatussauda is a plant that widely used as a traditional treatment for a number of diseases and cooking spices. The pharmacological effects of Black seed include anti-inflammatory, analgesic, antioxidant, antibacterial, antidiabetic, anticancer and to enhance the immune system. [1] One component of black seed which can be used as an active ingredient in the preparation is in the form of oil. Black seed oil is a type of volatile oil that has a distinctive aroma and can provide a warm feeling that is able to provide a relaxation

effect when inhaled and used. [2] One of the chemical compounds contained in black seed oil is Thymoquinon.

Thymoquinon has a pharmacological effect as anti-inflammatory.[3] The growing development of science in pharmaceutical technology, now anti-inflammatory preparations can be made in the form of balsam preparations. Balsam is a product similar to ointment, used as an external medicine that serves to relieve or eliminate pain. [2] Balsam is usually used by applying it directly with the hand so that it can cause stickiness and discomfort. Balsam now has a new dosage form in the form of a stick so that its use as an anti-inflammatory preparation is more practical and easy. Balsam stick preparations consist of several main base components namely oil, fat and wax.

Oil has a function as an emollient, fat can function as a binder between the water phase and the oil phase and malam has a function as a giver of shape and keeps the preparation solid when in a hot temperature.[4] Malams that can be used as a base for balsam sticks are cera alba (beeswax), carnauba wax, paraffin wax and candelilla wax. In each material has different physical-chemical properties because between one ingredient and the other has different components of constituent compounds. Cera alba is a natural malam obtained from the *Apis mellifera* L. nest. *Cera alba* as wax has the ability to oil binding, maintain the consistency of the preparation and increase the melting point.[5] Physical stability test is a test conducted to determine the stability level of a preparation in a certain time to maintain the initial physical properties of a preparation. Observation of physical stability includes organoleptic, homogeneity, melting point and hardness test.[4]

Several studies have been carried out on the use of cera alba in a formula. The basis of *cera alba* as a malam greatly influences the physical properties and stability of semisolid and topical solid preparations, especially the preparation of balsam stick. Alima (2015) said that increasing the concentration of cera alba can improve physical properties in the form of hardness in the balsam stick preparation and the concentration of cera alba which is good for use in balsam stick is 30%.[6] Chairina (2014) said that the dosage formula using malam cera alba is a more physically stable preparation compared to the formula that uses *carnauba wax* malam. [7]

The difference in the concentration of cera alba will affect the hardness and consistency which will ultimately affect the quality and physical stability of the balsam stick preparations. In making this balsam, it refers to previous research conducted by Alima (2015) which obtained the results of methyl salicylate balsam stick with good melting and hardness using 30% cera alba. This

research will be made of balsam stick using an active ingredient of black seed (*Nigella sativa* L.) by distinguishing the concentration of cera alba. The active ingredient used is in the form of black seed oil obtained by buying it from PT. Lantabura International. Cera alba as malam (*wax*) was used with 5 different concentrations, namely concentrations of 30, 32.5, 35, 37.5 and 40%. The difference in the concentration of cera alba is used to see the effect caused on the physical stability of the balsam stick. Observations were carried out starting from week 0 to week 8 in storage conditions at room temperature (± 25°C). Observations made were in the form of organoleptic, homogeneity, melting point, hardness and yield value observation.

MATERIAL AND METHODS

Materials

The ingradients used in this study were acetic anhydrous acid, concentrated sulfuric acid (H_2SO_4), black seed oil purchased from PT. Lantabura International, adeps lanae, cera alba, cetyl alcohol and *Butyl Hydroxytoluene* (BHT) were obtained from PT. Brataco, virgin coconut oil (VCO) was obtained from CV. Herba Bagoes.

Methods

Identification test of active substances and excipients

a. Black seed oil (*Thymoquinon*)

Organoleptic test was done by obeserving at the shape, color and odor.

Qualitative test was done by taking enough black seed oil into a test tube, then add with anhydrous acetic acid (AAA) and concentrated sulfuric acid (H_2SO_4) in a ratio of 3:1, the positive results of the terpene will produce a brownish red color.

b. Cera alba

Organoleptic test was done by placing the material in a container with a white background then the shape, color and odor were observed.

This melting point test used method IV. The melting point test was carried out by melting the compound at the lowest possible temperature, inserting it into the capillary tube where both ends were open to a depth of 10 mm. Then the capillary tube cooled at 10°C or more for 2 hours. Capillary pipes containing substances were inserted into the melting point device, adjust the

temperature rise from 1ºC/minute. Afterward the fusion observed when the initial substance melted until it dissolved completely. The results of the observation were recorded. [8]

c. Adeps lanae

Organoleptic test was done by placing the material in a container with a white background then the shape, color and odor were observed.

This melting point test used method IV. The melting point test was carried out by melting the compound at the lowest possible temperature, inserting it into the capillary tube where both ends were open to a depth of 10 mm. Then the capillary tube cooled at 10°C or more for 2 hours. Capillary pipes containing substances were inserted into the melting point device, adjust the temperature rise from 1ºC/minute. Afterward the fusion observed when the initial substance melted until it dissolved completely. The results of the observation were recorded. [8]

d. Cetyl Alcohol

Organoleptic test was done by placing BHT on a white background container then the shape, color and odor were observed.

The BHT melting point test was carried out by method I. BHT was taken sufficiently then crushed into a very fine powder, then put into a capillary tube where one end was closed, the capillary pipe was compressed by tapping on a solid surface. The temperature was set to 30ºC below the BHT melting temperature. Then the capillary pipe was inserted into the melting point, set the temperature rise from 1ºC/minute. Afterward the fusion observed when the initial substance melted until it dissolved completely. The results of the observation were recorded. [8]

e. Butyl Hydroxytoluene (BHT)

Organoleptic test was done by placing BHT on a white background container then the shape, color and odor were observed.

The BHT melting point test was carried out by method I. BHT was taken sufficiently then crushed into a very fine powder, then put into a capillary tube where one end was closed, the capillary pipe was compressed by tapping on a solid surface. The temperature is set to 30ºC below the BHT melting temperature. Then the capillary pipe is inserted into the melting point, set the temperature rise from 1ºC/minute. Afterward the fusion observed when the initial substance melted until it dissolved completely. The results of the observation were recorded. [8]

f. *Virgin Coconut Oil* (VCO)

Organoleptic test was done by placing the material in a container with a white background then the shape, color and odor were observed.

Density test using method I. Density test was carried out with a clean and dry picnometer. The pycnometer was weighed and the weight was recorded as W_1. Water boiled then cooled to 25ºC, the water was put into the picnometer and then weighed and recorded as W_2. The water in the picnometer was removed and then dried. The oil is put into the picnometer and then the picnometer was weighed with a temperature of ± 25ºC, recorded the weight as W_3. Then calculate the density of oil weight with the following formula.[8]

$$\rho = \frac{w_3 - w_1}{w_2 - w_1} \quad \text{..... (1)}$$

Formula Preparations

In this study 5 formulas were made to determine the effect of different concentrations of cera alba on the physical stability of balsam stick preparations with active ingredients of black seed oil. The full formula can be seen in table 1.

Table 1. Formula of Balsam *Stick* Preparation

No	Material	Function	Jumlah				
			F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)
1.	Black seed oil	Active substance	5	5	5	5	5
2.	*Cera Alba*	Hardener	30	32,5	35	37,5	40
3.	*Adeps lanae*	Binder	10	10	10	10	10
4.	Cetyl alcohol	Plasticizer	10	10	10	10	10
5.	Butyl Hydroxytoluene	Antioxidant	0.1	0.1	0.1	0.1	0.1
6.	VCO	Emollient	ad 100	ad 100	ad 100	ad 100	ad 100

Balsam *stick* Preparation

All materials used were weighed. Butyl hydroxitoluene (BHT) was dissolved with VCO (Mass 1). Cera alba was melted in a steam cup above the water tank at a temperature maintained at ± 65oC (Mass 2). Once the alcohol was added to the melted cera alba, it was stired until melt and

homogeneous. Adeps lanae was put into the molten mixture, stirring until it melted and was homogeneous. The steam plate was lowered from the water bath, added butyl hydroxitoluene which had been dissolved in VCO, and stirred rapidly until it was homogeneous (Mass 3). Afterward, black seed oil was put into a mixture of mass 3, stirred until homogeneous and poured into mold. Furthermore, evaluation of the preparation included organoleptic, homogeneity, melting point, hardness and yield value.[9]

Evaluation and Physical Stability Test of Balsam *stick*

a. Organoleptic Test

Organoleptic testing was done by observing the shape, color and odor of the preparations that have been made. [10] Tests on physical stability of the preparation were carried out for 8 weeks which was observed every week from week 0 to week 8 with storage at room temperature.

b. Homegeneity Test

The homogeneity test was done by cutting the middle part of the balsam preparation transversely, then placing it between two pieces of object glass with a black background and visually observed. Homogeneity was indicated by the absence of coarse grains. [11] Tests on physical stability of the preparation were carried out for 8 weeks which was observed every week from week 0 to week 8 with storage at room temperature.

c. Melting Point Test

Melting point test was carried out using a capillary pipe with an internal diameter of 1.1-1.2 mm and a column height of 75 mm. Balsam sticks were inserted into the capillary pipe to a depth of approximately 10 mm. Then the capillary pipe was placed into a digital melting point device with the appropriate position and set with a temperature rise of 1ºC. The initial temperature fusing until it melts perfectly was recorded. [4] Tests on physical stability of the preparation were carried out for 8 weeks which was observed every week from week 0 to week 8 with storage at room temperature.

d. Hardness Test

The hardness test of the preparation was carried out using a *penetrometer*. The balsam stick was placed in the middle just below the *penetrometer* needle, then the start button was pressed. The *penetrometer* needle would move down through the preparation of the balsam stick for five seconds. The scale lever was pressed and would show the penetrating depth of the *penetrometer*

needle. The translucent depth was shown in units of 10^{-1} mm. The bigger translucent depth penetrometer needle indicated the preparation of balsam stick was getting softer. The test was carried out three times in each formula and then the average translucent depth was calculated. This test was carried out for 8 weeks which was observed every week from week 0 to week 8 with storage at room temperature. [4] In regard to the translucent needle depth results, the *yield value* can be calculated using the formula. The *yield value* can be used as a scattering power data. Tests on physical stability of the preparation were carried out for 8 weeks which was observed every week from week 0 to week 8 with storage at room temperature.

Analysis

Based on the tested data on melting and hardness, black seed stick (*Nigella sativa* L.) balsam was prepared using statistical analysis, first tested for normality and then homogeneity. The results of the data from the physical stability test in the form of melting point and hardness were statistically analyzed using a two-way ANOVA test (95% confidence level) to determine the differences in each formula and then continued with the Tukey HSD test to determine the significant differences of each formula and storage time.[12] In regard of the yield value, a Kruskal-Wallis non-parametric analysis was conducted and a significance value was observed whether there were significant differences.

RESULTS

Material Identification

Material identification was done before making preparations. All ingredients were identified to ensure that the characteristics and purity of the materials used in the study. The identification results can be seen in Table 2.

Evaluation and Physical Stability of Balsam *Stick*

Organoleptic Test of Balsam *Stick.*

The results of organoleptic test observations on the four balsam stick formulas can be seen in Table 3. The results of organoleptic test of the balsam stick preparation on the five formulas produced the same shape, color, odor and texture, namely rod-shaped, pale yellow, odoring a little typical of black seed with non-greasy texture. Physical stability tests were carried out by storing

balsam stick sticks at room temperature (± 25°C) with a storage period of 8 weeks. Observations were conducted on physical changes in the form of color, odor, crystal formation and the presence of liquid oil (*sweating*) on the surface of the balsam stick. The results of stability evaluation showed no change in orgnoleptis black seed oil balsam *stick.* The observations result can be seen in Tables 4.5 and 6.

Table 2. Material Identification Test Results

Material	Identification Test	Identification Result	Standard
Black seed oil	Organoleptic	Brown liquid, typical odors	The oily liquid is brown chesnut and has a typical odor [15]
	Black seed oil + 3 drops AAA + 1 drops H_2SO_4	Brownish red	Brownish red (Terpenoid)[16]
Cera Alba	Organoleptic	Flat, weak odors, yellowish white	Solid, yellowish-white color with a characteristic weak odor [8]
	Melting point	58-62ºC	62-65ºC[8]
Adeps Lanae	Organoleptic	Fat, yellow or pale yellow, typical odors	The mass is like fat, sticky, yellow and has a typical odor.[8]
	Melting point	30-38ºC	38-44ºC[8]
Cetyl alcohol	Organoleptic	Solid granule, white, weak characteristic odor	Granule or white cube, white, weak characteristic odor [8]
	Melting point	45-48ºC	45-50ºC[8]
Butyl Hydroxytoluene	Organoleptic	Solid crystal form, clear, odorless	Solid, white color and a characteristic weak odor [8]
	Solubility	Easy to dissolve in VCO with a ratio of 1:15	Insoluble in water and propylene glycol, easily soluble in ethanol, chloroform and ether [8]
Virgine Coconut Oil	Organoleptic	The form of liquid, colorless, weak odor.	Clear liquid, pale yellow, weak odor [17]
	Density	0.9204 g/ml	0.940 g/ml – 0.950 g/ml[17]

Homogeneity Test of Balsam *Stick*

The homogeneity test results on the balsam stick preparation showed that the five balsam stick formulas showed homogeneous results, no lumps, granules or rough points appeared in the preparation from the mixture of the materials used. Physical stability evaluation testing was carried out for 8 weeks, starting at week 0 to week 8 which was stored at room temperature. The homogeneity test of the balsam stick preparation in each formula showed the same results (homogeneous).

Table 3. Organoleptic Test Results of Balsam *Stick* Preparation.

Formula	Shape	Color	Odor	Texture
Formula I	Stick	Pale yellow	slightly smelling black seed	Not greasy
Formula II	Stick	Pale yellow	slightly smelling black seed	Not greasy
Formula III	Stick	Pale yellow	slightly smelling black seed	Not greasy
Formula IV	Stick	Pale yellow	slightly smelling black seed	Not greasy
Formula V	Stick	Pale yellow	slightly smelling black seed	Not greasy

Table 4. Physical Stability Test Results at Room Temperature Week 0 - 2

F	Week														
	0					1					2				
	W	B	KM	SW	K	W	B	KM	SW	K	W	B	KM	SW	K
I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
III	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 5. Physical Stability Test Results at Room Temperature Week 3 - 5

F	Week														
	3					4					5				
	W	B	KM	SW	K	W	B	KM	SW	K	W	B	KM	SW	K
I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
III	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 6. Physical Stability Test Results at Room Temperature Week 6 – 8

F	Week														
	6					7					8				
	W	B	KM	SW	K	W	B	KM	SW	K	W	B	KM	SW	K
I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
III	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Note:

F	= Formula	KM	= Surface crystal
W	= Color	SW	= *Sweating*
B	= Odor	K	= Consistency
(+)	= Perumaterial occured	(-)	= Perumaterial did not occur

Melting Point Test of Balsam *Stick*

The first evaluation results at the melting point were calculated as the melting point results at week 0. The melting point results obtained on formula 1 was 50.17^{o}C, formula 2 was 51.33 ^{o}C, formula 3 was 52.83^{o}C, formula 4 was 55.33^{o}C and formula 5 was 57.17^{o}C. This showed that all balsam stick formulas met the requirements of the melting point, which ranges from 50-70°C. These results can be seen in Figure 1. In the evaluation results showed an increase in melting point of the formula because the higher concentration of cera alba could increase the melting point.

These results can be seen in Figure 2. The results of statistical analysis of the melting point between the formulas and the time indicated that there were significant differences in each formula on each week. This was indicated by the significance value 0.000 <0.05.

Hardness Test and *yield value* of Balsam *Stick*

Evaluation results of balsam stick hardness from formula 1 to formula 5 were 4.17 mm, 3.97 mm, 3.77 mm, 3.6 mm, 3.47 mm. These results can be seen in Figure 3. In physical stability testing the hardness increased which marked by a decrease in the number of the penetrometer needle scale, which means the preparation of the balsam stick was getting harder. These results can be seen in Figure 4. Based on the evaluation results on the calculation of the yield value, it can be seen that from one to the fifth formula produces yield value that meets the requirements, which was 102.89 dyne/cm^2, 113.54 dyne/cm^2 , 125.93 dyne/cm^2, 137.79 dyne/cm^2 and 148.68 dyne/cm^2. Which means that in formulas 1 to 5 had good dispersion. These results can be seen in Figure 5. In the physical stability test, the calculation of yield value showed the increasing results in each formula on each week. This can be seen in Figure 6. The results of the statistical analysis of the hardness between the formulas and the time showed that there are significant differences in each formula on each week. This is indicated by the significance value 0.000 <0.05.

DISCUSSION

Organoleptic Test of Balsam *Stick*

The organoleptic test results from 5 formulas showed the same organoleptic characteristik. This proves that the difference in the concentration of cera alba did not affect the shape, color, odor and texture of the balsam stick preparation. The formation of surface crystals on the balsam stick was also not formed, this wass because during the process of making and forming the balsam stick was carried out at temperatures above the critical temperature ie the range at 12-16^{o}C (Novotna). In the physical stability test, the aroma from the balsam stick for 8 weeks found did not change. This can happen because the oil component of VCO or oil in the active substance, black seed oil did not experience oxidation reactions during the 8-week storage. The oxidation reaction would occur if there was a damage to the fat which results in a rancid odor in the preparation of the balsam stick. [13]

In addition, the physical appearance of the balsam *sticks* also did not change. This was due to the absence of the formation of malam crystals on the surface of the balsam *stick* preparation and no removal of oil liquid (sweating) on the surface of the balsam stick preparation.

Homogeneity Test of Balsam *Stick*

The homogeneous results from balsam stick was due to wax base, fat and oil formulated perfectly. In the physical stability test, the homogeneity of the balsam *stick* did not change. This showed that there was no effect of increasing the concentration of cera alba on the homogeneity of the balsam *stick* preparation.

Melting Point Test of Balsam *Stick*

The melting point of all balsam stick formulas for 8 weeks met the requirements. The evaluation results of the balsam *stick* melting point showed that the difference in the concentration of cera alba in each formula produced different melting points. The higher the concentration of cera alba, the greater the yield of the melting point. This was because the higher the concentration of cera alba, the more carbon bonds contained in the hydrocarbon bonds in cera alba, so that the energy needed to break the carbon bonds will be even greater. One carbon bond can be broken down by an energy of 57 kcal.

Hardness Test and *yield value* of Balsam *Stick*

The results of balsam stick hardness were seen from the translucent depth of the needle in the preparation of the balsam stick. The higher of the number on the penetrometer scale showed the deeper the needle penetrated to the balsam stick, which means the number of needle depths was getting larger and the resulting balsam stick preparations were getting softer. The preparation of balsam stick was said to be soft if it has a translucent depth of 9-10.5 mm with a load of 50 g. [4] The *penetrometer* (Koehler) in the laboratory had a 102.5 g load, so the result of the balsam stick hardness test wass determined by the *yield value*. The *yield value* showed the pressure needed to apply the preparation in order to spread. A good *yield value* ranges from 100-1000 dyne/cm^2. [14]

In the physical stability testing, the hardness increased which marked by a decrease in the number of the penetrometer needle scale, which means the preparation of the balsam stick was getting harder. Increased hardness of the fomulas occured due to the increased concentration of cera alba in each formula, this could occured because the fatty acids contained the malam will be bonded to one another. This was what made the strength of fatty acids to be able to increase the hardness of balsam sticks.[13] Similarly, the results of the calculation of the *yield value* obtained.

The physical stability testing from the calculation of *yield values* showed increasing results in each formula. The results of the calculation of the *yield value* were inversely proportional to the translucent needle depth results in the preparation.

CONCLUSION

Based on the results of the study it can be concluded that differences in the concentration of cera alba F1 (30%), F2 (32.5%), F3 (35%), F4 (37.5%), F5 (40%) to physical stability showed an increase melting point and *yield value*. The results of the hardness showed a decrease in the number on the penetrometer scale which means that the preparation of balsam stick was getting harder.

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