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Use of CMC Na as Gelling Agent in Nanoemulgel Formulation of Methanol Extract of Sappan Wood (*Caesalpinia sappan* L)

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Abstract

Sappan wood (*Caesalpinia sappan* L.) contains brazilin including isoflavonoids which act as antimicrobials (against bacteria on skin) and antioxidants. Nano technology increases surface area, enhance stability, reduces skin irritation, protects from degradation, and has good drug delivery. Nano emulsion preparations can help permeability of the drug on the membrane surface. Nanoemulgel preparations can provide stability and good drug release compared to mixing drugs directly in a gel base. The purpose of this study were to determine the effect of different concentrations of CMC-Na gelling agents and which concentration of CMC-Na gelling agents that can produce the best nanoemulgel preparations. Nanoemulsion made from isopropyl myristate as oil phase, tween 80 as surfactant and polyethylenglycol as co-surfactant. Nanoemulsion formulations were tested by measurement of % transmittance by UV-Vis spectrophotometry at a wavelength 650nm. Nanoemulgel is made from nano emulsions used basis of CMC-Na at concentrations 3%, 4%, and 5%. The results showed percent transmittance was 71.86%. The results of physical quality and stability tests of the nanomulgel wood preparations showed an influence on the physical properties such as adhesion, dispersion and viscosity, the greater concentration of CMC-Na gelling agents in nanoemulgel preparations, the greater values adhesion and viscosity, while the spreadability was smaller. Concentration of CMC-Na gelling agent that produced the best of sappan wood nanoemulgel (Caesalpinia sappan L.) was 5% that stable by freeze-thaw testing.

Keywords: Nanoemulgel, Caesalpinia sappan L., Brazilin , CMC-Na

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1 Introduction

Sappan wood (*Caesalpinia sappan* L.) contains *brazilin* which is included in flavonoids as isoflavonoids that act as antimicrobials (against skin bacteria) and antioxidants [1]. Solubility of *brazilin* is difficult to dissolve in cold water, easily soluble in alcohol, ether and in the hydroxy alkaline solution[2]. Sappan wood can be used as an antioxidant, anti-inflammatory, antiacne, improving blood circulation, diarrhea and antiseptic drugs.

Emulsion preparation is one type of preparation that can help *permeability of the* drug on the membrane surface[3]. Gel system can help drug release and delivery of oil-based drugs and drugs that are difficult to dissolve[4]. Gel makes the preparation non-sticky or non-greasy, easily applied, and comfortable to use and increases patient compliance[5].

Nano technology with nanoscale size offers advantages more than conventional methods by increasing surface area, enhancing stability, reducing skin irritation, protecting from degradation, and having good drug delivery at intra-cell level[6]. Formulation nanoemulgel is done to maintain the stability of the compounds *brazilin* as antioxidants *antiacne*. Nanoemulgel is made from nanoemulsion mixed with *thickening agent* . Nanoemulgel is an emulsion preparation with a droplet size of 1-100 nm which is suspended in a hydrogel[7].

The formulation of sappan wood nanoemulgel with a concentration of 150 mg as an *anti-acne* therapy has been carried out using carbopol. In that study, there were no good nanoemulgel that produced because it had an unacceptable acidity by skin and poor physical stability[8].

CMC-Na is a polymer from nature and stable at pH 5-9, time required for CMC-Na to develop into a good gel structure is shorten[9]. The purpose of this study were to determine the effect of different concentrations of CMC-Na gelling agents and which concentration of CMC-Na gelling agents that can produce the best nanoemulgel preparations.

2 Materials and Methods

2.1 Sappan Wood extraction

The 500 grams powder of Sappan wood was macerated with 2,5L methanol (3x24 hours). Filtrate of maceration was filtered with flannel and filter paper. The methanol filtrate of sappan wood was collected in a container and the pulp were macerated again with 1.25 L of methanol (1x24 hours). F iltrate of second maceration was filtered and collected in a container, the pulp were macerated again with 1,25L methanol (1x24 hours). Filtrat of third maceration was filtered and collected in a container . The macerate was obtained and concentrated with a *rotary evaporator* at 50°C until obtained a concentrace extract.

2.2 TLC analysis

10 mg methanol extract of sappan wood (Caesalpinia sappan L.) was dissolved in 10 ml of ethanol p.a. TLC analysis used mobile phase chloroform: methanol (9: 1) in the chamber. Samples and raw quercetin were spotted on silica gel plates GF254. Elution distance was 8 cm and space of spot 1,5 cm. Detection with UV light 254. Brazilin compounds were indicated by the presence of blue phosphorescent that separate[10].

Table 1. Nanoemulgel Formula of Methanol Extract of Sappan wood

Material	Unit	Formula		
		I	II	III
Methanol extract of sappan wood	g	0,15	0,15	0,15
Isopropyl myristate	g	6	6	6
Tween 80	g	27,5	27,5	27,5
Polyethylene glycol (PEG) 400	g	27,5	27,5	27,5
CMC Na	g	3	4	5
Glycerin	g	10	10	10
Methylparaben	g	0,02	0,02	0,02
Distilled water ad	g	100	100	100

2.3 Formulation Nanoemultion of Sappan Wood Extract

150 mg of sappan wood extract dissolved in 9 ml of co-surfactant (PEG) by using a

magnetic stirrer with a speed of 800 rpm at 50°C until the whole extract dissolved . The oil phase was made by adding 6 ml of isopropyl myristate (HDI) to the extract solution with *magnetic stirrer* until it is homogeneous. The oil phase obtained was mixed with Tween 80 as much as 27,5 ml and 15,5 ml of co-surfactant (PEG)[8].

The mixture was stirred with a *magnetic stirrer* at a speed of 1000 rpm at 50 ° C until homogeneous. The mixture was mixed with 30 ml of distilled water drop by drop and stirred until a homogeneous mixture is obtained. The nanoemulsion formed was left alone for 24 hours until it was clear[11]. Measurement of % transmittance with UV-Vis spectrophotometry at a wavelength 650 nm. A blank solution used distilled water.

2.4 Formulation Nanoemulgel of Sappan Wood Extract (Caesalpinia sappan L.)

CMC-Na was developed in hot water for 15 minutes then crushed until homogeneous. CMC-Na was added with glycerin and methylparaben which had been dissolved in 3 ml of 96% ethanol. Nanoemulsion of Sappan Wood Extract was added to the mass of the gel that had formed and then stirred until homogeneous.

2.5 Physical Quality Test

2.5.1 Organoleptic Test

Examination of the shape, taste, smell and color was done by visual with took 0.25 grams to be touched, smelled, and seen in nature[12].

2.5.2 pH Measurement Test

pH of the stick was spreaded with 0.5 grams of the ointment preparation. Color changed that occur at the stick pH show the pH value of the ointment[13].

2.5.3 Homogeneity Test

Nanoemulgel preparations were taken 0.25 grams and then placed on a glass plate and then rubbed and felt to be seen and felt flat or not[13].

2.5.4 Viscosity Test

100 mL of nanoemulgel preparations were measured viscosity using a Rion VT 04-F viscomester in the appropriate rotor. The numbers that showed on the screen, after being stable and then read on the scale on the viscosimeter[14].

2.5.5 Spreadbility Test

0.5 gram of ointment was placed on a glass plate, another glass is placed on it and left for 1 minute. The diameter of preparations was calculated. After that, 50 grams, 100 grams and 150 grams of additional load were added in sequence and allowed to stand for 1 minute and then a constant diameter was calculated[15].

2.5.6 Adhesion Test

0.25 gram emulgel was smeared on top of the glass object. Then another glass object is placed on it. The glass object was installed on the test equipment and given a load of 0,5 kg for 5 minutes. Then released with a weight of 80 grams. The time is recorded until the two glass objects are released[16].

2.5.7 Protection Test

Wet the filter paper (10 x 10 cm) with phenolphthalein (PP) solution as an indicator, then dry. Smear the filter paper with 0,5 gram of preparation on one side of the surface as is usually the use of semisolid solid preparations. Other filter paper was wet with solid paraffin which was melted on the side of the paper. Stick it to the filter paper first, then drop the area with 1 drop of KOH. Observe the appearance of reddish stains on the wet paper with phenolphthalein solution. Record the time needed starting when the paper is dropped with KOH solution.

2.5.8 Stability Test

Stability test using the *freeze-thaw* method for 3 cycles. *Freeze-thaw is* carried out for 3 cycles with 1 cycle of 48 hours consisting of 24 hours at 4°C or 20°C and 24 hours at 40°C or 20°C[17]. Preparations were observed for physical stability, such as organoleptic, homogeneity, pH measurement, viscosity, spreadability, adhesion, and protection.

2.6 Data analysis

Result of physical test from the comparison of nanoemulgel formula of Sappan wood extract (*Caesalpinia sappan* L.) were analyzed using *one way anova* and stability test data were analyzed using *one sample t-test.*

3 Results and Discussion

3.1 Extraction of Sappan Wood (Caesalpinia Sappan L.)

Methanol can damage the cell walls of the sample so that polar and non-polar compounds can be dissolved in methanol[18]. Brazilian compounds are non-polar so they will be bound with solvents because methanol can dissolve polar and non-polar compounds.

The maceration method was chosen because it is effective for heat-resistant compounds (degraded by heat), the equipment used is relatively simple, inexpensive, and easily available[19]. The principle of the maceration method is soaking so that the liquid will penetrate the cell wall and enter the cell cavity containing the active compound and the active compound will dissolve.

The organoleptic product of the sappan extract is a thick, brown, odorless extract . The yield of the sappan wood extract was 5.94%. In a previous study yield of sappan methanol extract was 8.64%[10] and yield of methanol extract of sappan wood was 14.5%[20]. The yield obtained was smaller than the previous research, this was due to the lack of stirring frequency during the extraction process.

3.2 Analysis of Brazilin Compound with Thin Layer Chromatography (TLC)

TLC analysis of sappan wood extract used silica gel GF 254 as stationary phase and non-polar chloroform: metanol (9: 1) as mobile phase . The principle of thin layer chromatography (TLC) analysis is to separate the sample based on the polarity distribution between the sample and the solvent used.

The results of TLC are sappan wood extract and quercetin have Rf value 0.81 and hRf value 81 and blue spot. *Brazilian* compound is indicated by the presence of blue phosphorescent spot that separate[10].

The positive TLC results contain brazilin according to Isaiah's study, (2011) shown in Figure 1. (b) sample spots (S) more fluorescent blue. The bigger value of Rf the bigger distance of movement of these compounds on the TLC plate.

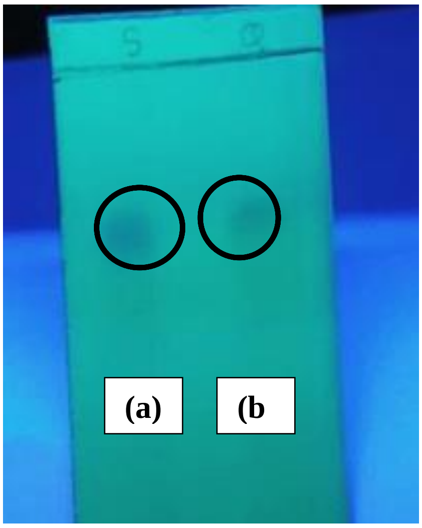

Figure 1. (a) Sample (b) Standard

3.3 Nanoemulsion of Methanol Extract of Sappan Wood

In the nanoemulsion formula there are oil phase, surfactant and co-surfactant. The oil phase uses 6% isopropyl myristate, the oil concentration is not too high so not cause nanoemulsion instability and difficulties when making nanoemulsion.

Tween 80 which is a non-ionic surfactant that is relatively non-toxic and does not irritate the skin compared to anionic surfactants or cationic surfactants[21]. The mechanism of action of surfactants is obtained because of the dual nature of the molecule. This dual property causes surfactants to be adsorbed at the interface to form a single layer where the hydrophilic group is in the water phase and the hydrocarbon chains in the oil phase. PEG 400 as a cosurfactant in formulas to assist surfactants in reducing interface stresses and increasing the solubilization of non-polar groups , providing clear and stable emulsions and no separation occurs[22].

Nanoemulsi percent transmittance measurement results of the seacng wood extract on a co-surfactant etanol 96% was 3,44%, a co-surfactant propilenglikol was 0,54%, a co-surfactant polyethylene glycol was 71,86%. The three co-surfactants still do not fulfill the transmittance range of 90% -100%. Polyethylene glycol co-surfactant is the best of the three co-surfactants because it has the highest percent transmittance was 71.86% and the visual mix shows clarity or transparency similar to aquades.

3.4 Nanoemulgel Formulation of Methanol Extract of Sappan Wood

Nanoemulgel is nanoemulsion that is mixed with *thickening agent*. In nanoemulgel sappan wood methanol extract using CMC-Na base.The mechanism of gel formation is the reaction of *entanglement* (extension of polymer chains), after CMC-Na is dispersed in water, CMC-Na polymer chains will elongate and form irregular polymer chains so that water will later be trapped in the polymer chains that are formed[9].

Glycerin is used as a humectant that can absorb water content so that it can maintain the water content of the preparation[9]. Methyl paraben or nipagin is used to inhibit the growth of fungi and preservatives with levels of 0.02 - 0.3%[9].

3.5 Physical Quality Test of Nanoemulgel of Sappan Wood Extract

3.5.1 Organoleptic Test of Nanoemulgel of Sappan Wood Extract

Checking the shape, taste, smell and color is done with the help of the senses. The organoleptic results in the form, odor and color of the three formulas have no difference at all. The three formulas are gel-shaped, with the characteristic odor of a weak, soft taste on the skin and pink. *The* CMC-Na gelling *agent had* no effect on the organoleptic test results of the nanoemulgel of methanol extract of sappan wood.

3.5.2 Homogeneity Test of Nanoemulgel of Sappan Wood Extract

The observation of the homogenity test showed that the three formulas had good homogenity, *gelling agent* CMC-Na no effect on the results of homogeneity tests of nanoemulge of methanol extract of sappan wood.

3.5.3 pH Measurement Test of Nanoemulgel of Sappan Wood Extract

pH test on all three formulas indicated that the third formula had same pH, it were 6. The third formula had pH that is acceptable for skin because of the pH which can be received by the skin is 5-10. The lower pH can irritate the skin and if the higher pH will make the skin dry. *The* CMC-Na *gelling agent* did not influence the nanoemulgel pH test results .

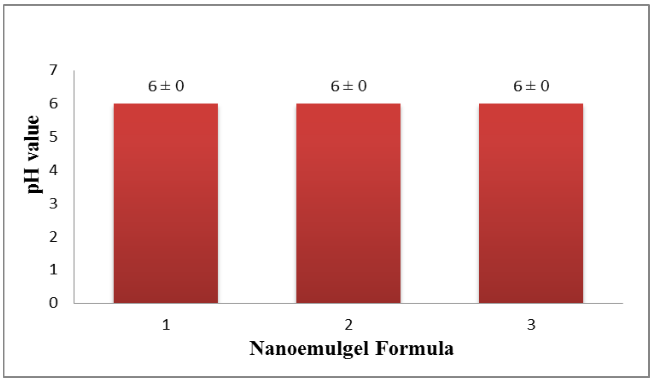

Figure 2. pH Test Results of Nanoemulgel of Sappan Wood Extract

3.5.4 Viscosity Test of Nanoemulgel of Sappan Wood Extract

Gel viscosity test aims to determined the thickness of nanoemulgel. Gel preparations that are too thick can inhibit the release of active substances.The addition of CMC-Na *gelling agent* can reduce the value of spreability and increase viscosity, because it can stretch interactions that occur between similar molecules[23]. Statistical test results showed significant results which is indicated by the *ANOVA* test value of 0,000 (<0.05). This showed that the CMC-Na concentration of each formula has a significant effect on the viscosity value.

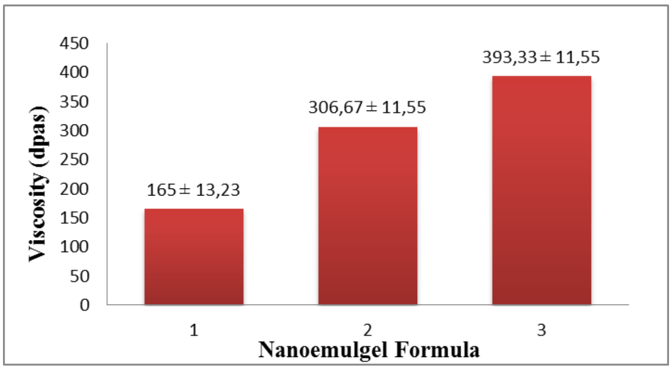

Figure 3 . Viscosity Test Results of Nanoemulgel of Sappan Wood Extract

The increase in the viscosity value is due to the nature of CMC-Na which absorbs water, the more water absorbed the Na^{+} will bind to OH thereby increasing the viscosity of the preparation and increasing the viscosity value.

3.5.5 Adhesion Test of Nanoemulgel of Sappan Wood Extract

The gel adherence test aims to determined the ability of the gel to adhere to the skin. The result of gel adhesion, the higher the concentration of CMC-Na, the higher the adhesion to the skin. The good adhesion of the gel is that the gel can adhere for more than 1 second[24]. Based on statistical analysis there was a significant difference in the stickiness of each formula which is indicated by the *ANOVA* test value of 0,000 (<0.05).

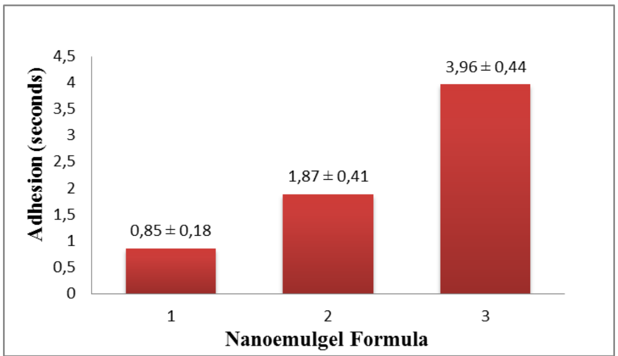

Figure 4 . Adhesion Test Results of Nanoemulgel of Sappan Wood Extract

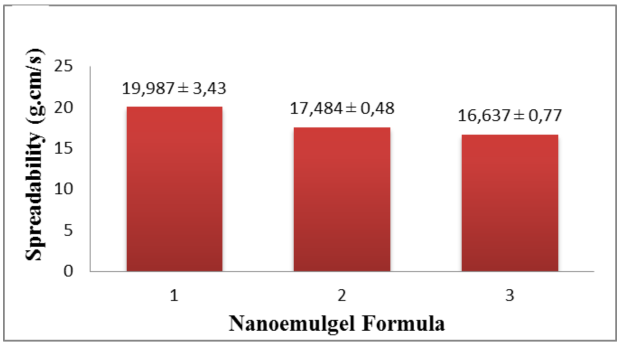

Figure 5. SpreadabilityTest Results of Nanoemulgel of Sappan Wood

The CMC-Na concentration in each formula gives a significant difference in the adhesion of each formula. Formula 3 has the highest viscosity value, preparation that stick around longer on the skin will give a longer therapeutic effect.

3.5.6 Spreadability Test of Nanoemulgel of Sappan Wood Extract

Spreadibility test aims to determined the gel's ability to spread when applied to the skin. The increasing concentration of CMC-Na (*gelling agent*) will increase the consistency of the preparation, so the spread of the three treatments decreases. This is due to the higher concentration of CMC-Na, the nanoemulgel is getting thicker (high viscosity), so that the gel is more difficult to spread.

Statistical test results from the spreadability test showed that the results were not significantly different from the dispersion value as indicated by the *ANOVA* test value of 0.196 (> 0.05), so that the concentration of CMC-Na as the *gelling agent* of the three formulas did not have a significant effect on the power value. spread.

3.5.7 Protection Test of Nanoemulgel of Sappan Wood Extract

This test was carried out to see the protective ability or protection of the nanoemulgel against strange influences from the external environment that can reduce the effectiveness of the emulgel[25].

If the nanoemulgel formula is less than 1 second, it can be concluded that the gel has poor protection. Formula 1 has the lowest protection and Formula 3 has the highest protection.

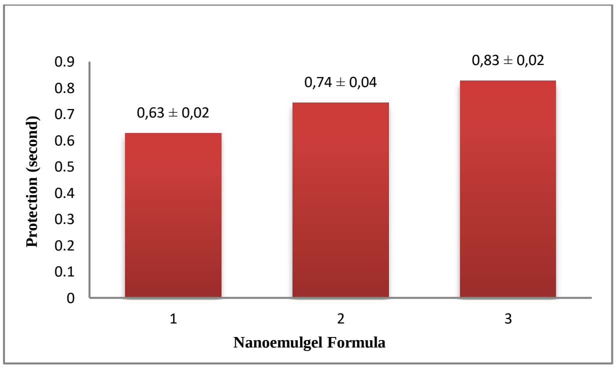

Figure 6 . Protection Test Results of Nanoemulgel of Sappan Wood Extract

Statistical test results from the protection test showed significant results which were indicated by the *ANOVA* test value of 0.001 (<0.05), so that the differences in CMC-Na concentrations in the three formulas gave different protection power from the nanoemulgel preparations.

3.6 Stability Test of Nanoemulgel of Sappan Wood Extract

The nanoemulgel physical stability test aims to determined the stability of the gel during storage. In all three formulas the organoleptic test results did not show changes in shape, taste, and odor, but there was a change in the color of the nanoemulgel preparations. Homogeneity test results and pH test results of the three nanoemulgel formulas did not change, namely remain homogeneous and pH 6, so that the three formulas were stable in organoleptic, homogeneity and pH tests of nanoemulgel preparations. In the viscosity test results, the results of the protection test and the results of the adhesion power test increased, but the spreadability test results decreased.

Table 2. Stability Test Results Nanoemulgel of Formulation 1 Sappan Wood Extract

Parameter	Evaluation test results before *freeze thaw*	Evaluation test results after *freeze thaw*	*P-value*	information
1. Organoleptic				
a. Form	Gel	Gel		
b. Color	Pink	Orange		
c. Rasa	Smooth	Smooth		
d. Smell	Typical	Typical		
2. Homogeneity	Homogeneous	Homogeneous		
3. pH test	6 ± 0	6 ± 0		
4. Viscosity Test (dpas)	165 ± 13.26	260 ± 17.32	0.015	Significantly different
5. Scattering Power Test (g.cm/s)	20 ± 3.43	19.62 ± 2.15	.001	Significantly different
6. Protection Power Test (seconds)	0.63 ± 0.02	0.79 ± 0.07	.001	Significantly different
7. Adhesion Test (seconds)	0.85 ± 0.18	0.9 1 ± 0.10	.001	Significantly different

Table 3 . Stability Test Results Nanoemulgel of Formulation 2 Sappan Wood Extract

Parameter	Evaluation test results before *freeze thaw*	Evaluation test results after *freeze thaw*	*P-value*	information
1. Organoleptic				
a. Form	Gel	Gel		
b. Color	Pink	Orange		
c. Rasa	Smooth	Smooth		
d. Smell	Typical	Typical		
2. Homogeneity	Homogeneous	Homogeneous		
3. pH test	6 ± 0	6 ± 0		
4. Viscosity Test (dpas)	306.67 ± 11.55	393 ± 11.55	0.016	Significantly different
5. Scattering Power Test (g.cm/s)	17.48 ± 0.48	16 , 45 ± 0.38	0,000	Significantly different
6. Protection Power Test (seconds)	0.74 ± 0.04	0.68 ± 0.06	0.002	Significantly different
7. Adhesion Test (seconds)	0.88 ± 0.41	1 , 87 ± 0.08	0,000	Significantly different

Table 4. Stability Test Results of Nanoemulgel of Formulation 3 Sappan Wood Extract

Parameter	Evaluation test results before *freeze thaw*	Evaluation test results after *freeze thaw*	*P-value*	information
1. Organoleptic				
a. Form	Gel	Gel		
b. Color	Pink	Orange		
c. Rasa	Smooth	Smooth		
d. Smell	Typical	Typical		
2. Homogeneity	Homogeneous	Homogeneous		
3. pH test	6 ± 0	6 ± 0		
4. Viscosity Test (dpas)	393.33 ± 11.55	400 ± 0	0.010	Significantly different
5. Scattering Power Test (g.cm/s)	16.64 ± 0.77	16.15 ± 0.81	.001	Significantly different
6. Protection Power Test (seconds)	0.83 ± 0.02	0.47 ± 0.04	0.002	Significantly different
7. Adhesion Test (seconds)	3.96 ± 0.44	4.28 ± 0.23	0,000	Significantly different

The results of the statistical formula 1 test showed a significant difference from the value of adhesion, the value of spreadability, the value of protection, and the value of viscosity indicated by the *P*-each *value is* 0.015, 0.001, 0.001, 0.001 (< 0.05). Preparations nanoemulgel in formula 1 was not stable in value of adhesion test, the value of spreadability test, the value of protection test, and the value of viscosity test.

The statistical test results of formula 3 showed that there are significant differences in the value of adhesion, spreability, protection, and viscosity value as indicated by P-values of 0.010 , 0.001, 0.002, 0.000 (<0, 05). Nanoemulgel preparations in formula 3 was unstable on the value of adhesion test , the value of spreadability test, the value of protection test, and the value of viscosity test because the CMC-Na base has a strong water holding capacity thereby increasing the viscosity test value[26].

Preparations nanoemulgel in the third formula has good stability on organoleptic test, the pH value and homogeneity, but the third formula is not stable in value of adhesion test, the value of spreadability test, the value of protection test, and the value of viscosity test for base CMC-Na has power strong water binding so that the base will absorb water and increase the value of the viscosity test[26].

4 Conclusions

Differences concentration of CMC-Na *gelling agents* in the nanoemulgel of sappan wood (*Caesalpinia sappan* L.) extract had an influence on the physical properties of adhesion, spreadability and viscosity. The best concentration of CMC-Na *gelling agent* that can produce the best nanoemulgel of sappan wood methanol extract (*Caesalpinia sappan* L.) is formula 3 with a concentration of 5%

5 Conflicts of Interest

The authors declare no conflict of interest.

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