TWEEN, SPAN AND SONICATION TIME OPTIMIZATION OF CANDLENUT SEED EXTRACT NANOEMULSION (ALEURITES MOLUCCANA (L.) WILLD) CENTRAL COMPOSITE DESIGN APPLICATION

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Abstract
Candlenut ethanol extract contains flavonoids and phenols that have benefits in the hair growth process. The formulation of the preparation into a nanoemulsion helps penetrate the stratum corneum of the scalp and keeps the flavonoids from being oxidized. Nanoemulsion is a dispersion of two liquids stabilized using a surfactant with a droplet size of 10 -100 nm. This research was conducted to obtain the optimal composition of tween 80, span 80 and sonication time. Data analysis usedstatistical analysis *two-way Analysis of Variance* (ANOVA) with 95% confidence level. This research is a quasi-experimental research using the optimization method of *Central Composite Design*. The factors used are tween 80, span 80 and sonication time. The nanoemulsion preparation was evaluated by conducting several physical properties tests such as organoleptic test, pH test, nanoemulsion type test, transmittance test, viscosity test, *Freeze-thaw cycle* and centrifugation test. From the statistical analysis of the *two - way Analysis of Variance* (ANOVA) the results that meet are the percent transmittance with a *p-value of* 0.040 (physical properties) and stability with a *p-value of* 0.016. The optimum points that meet the parameters of physical properties are F3, F4, F6, F7, F14 and F16. It is necessary to validate the recommended formula from theprogram *Minitab 17* and it is necessary to test the activity of nanoemulsion preparations.

Key words: [nanoemulsion, tween 80, span 8-, sonication, CCD]

INTRODUCTION

Hair is a crown for everyone because hair has a function other than to provide warmth, protection, but hair also provides beauty and supports appearance. Hair consists of roots and hair shafts. Hair is more responsive to environmental stimuli because the hair roots are supplied with blood through the nerves. Some disorders that can attack the hair such as *Cinities*, pearl disease and *alopecia. Cinities* is a term for white (gray) hair. This happens because hair pigment disappears, age factors, congenital and hereditary defects. *Alopecia* or baldness is caused by a disorder that causes hair to fall out continuously so that the head is bald (Sari and Wibowo, 2016). Candlenut contains active substances (flavonoids and phenols) which are efficacious to nourish hair and naturally blacken hair (Ulfa, 2003).

In Shoviantari's research (2019) entitled Hair Tonic Activity Test of Candlenut Oil Nanoemulsion, a hair growth test was conducted using rabbits as experimental animals. Observations were made for 21 days, the results showed that the part that was applied to the hazelnut oil nanoemulsion had the largest hair length compared to the part that was not applied to the nanoemulsion. Most drugs from natural substances that are synthesized directly have low solubility in water. Usually drugs that are poorly soluble in water have low absorption and consequently have low bioavailability. Therefore, a strategy is needed to overcome this problem. Oil-in-water (O/W) emulsions can be used as solutions. Emulsion delivery systems provide the advantage of protecting and releasing drugs that are poorly soluble in water (Macedo, 2014). It is hoped that the manufacture of oil-in-water emulsions will be more acceptable when used and will not make hair greasy (Shoviantari, 2019). This nanoemulsion is applied topically to the hair, in order to penetrate the stratum corneum on the scalp, then this compound is formulated into nanoemulsion preparations. The formulation into nanoemulsions can produce droplet sizes of 10-100 nm which can increase *active ingredient loading* and bioavailability. The small droplet size of the nanoemulsion makes it easier for the active substances to penetrate the stratum corneum on the scalp. Thermodynamically stable nanoemulsion stabilized by surfactants (Shoviantari, 2019).

Surfactants that are usually used in nanoemulsion preparations are nonionic *groups*. This group is recommended because it is more non-irritating.surfactants *Nonionic* also safer than other types of surfactants in terms of irritating the skin. The combination of lipophilic (span 80) and hydrophilic (tween 80) surfactants can produce an oil-in-water nanoemulsion system with the desired HLB system (Shoviantari *et.al,* 2019). The HLB balance is the concept that underlies the semi-empirical method in selecting the appropriate emulsifier or choosing a combination of emulsifiers in order to obtain a stable emulsion (Suhendra *et.al*, 2012). Emulsion stability is a separation process that lasts a long time so that the separation does not occur during the desired time interval. Suprobo and Rahmi (2015) mention that one of the internal factors that affect emulsion stability is particle size, the smaller the particle size, the higher the level of emulsion stability. The method used to reduce the particle size is the use of ultrasonic waves or using high energy (sonication) (Raharja and Damayanti, 2014).

In making O/W type nanoemulsion preparations, it is necessary to pay attention to the amount of tween 80 and span 80 used. Tween and span are used as surfactants that reduce the interfacial tension or surface tension that occurs between two different phases. Surfactants act as both emulsifier, wetting agent, foaming agent, and dispersant, which depends on the value of hydrophilic-lipophilic balance (HLB). The use of surfactants as surface tension lowering agents is based on the type of preparation to be made. The nanoemulsion to be made in the O/W type has an HLB value of more than 10 (> 10), while in the W/O type it is less than 10 (< 10). O/W nano preparations are expected to maintain the stability of flavonoids because flavonoids are coated by the oil phase of the preparation and the first to interact with foreign substances is the water phase which is the outermost part of the O/W nanoemulsion system. The use of a combination of surfactants with low and high HLB values leads to the formation of nanoemulsions with good stability when dissolved in water (Sadeq, 2020).

Based on the description above, nanoemulsion preparations are strongly influenced by the HLB of the surfactant and particle size. To obtain the desired HLB value, it is necessary to optimize the tween and span which are used as surfactants to obtain O/W type nanoemulsion preparations. Therefore, this research was conducted to obtain the optimal formula of tween 80, span 80 and sonication time of the nanoemulsion formulation of candlenut seed extract (*Aleurites moluccanus*) with the application of *Central Composite Design* (CCD). According to Riswanto (2019), CCD applications are generally used for several optimization purposes, such as formula optimization. The CCD application provides several formulas that can be used in optimizing tween 80, span 80 and sonication time so that the obtained formula is expected to make stable nanoemulsion preparations. The nanoemulsion formulation of candlenut seed extract will produce a nanoemulsion *spray* in the final form. In this formulation, preservatives are added which are used to keep the preparation from microbial contamination so that the preparation can be used in the long term.

METHODS MATERIAL

The materials used in this study were candlenut seed extract, VCO (*Virgin coconut oil*) oil (obtained from the FTSF Solid Laboratory of Sanata Dharma University), Tween 80, Span 80 (Brataco), 70% ethanol (obtained from the Pharmacognosy Phytochemical Laboratory of the University of Sanata Dharma), methyl paraben (Brataco), propylene glycol (Tekun Jaya), concentrated HCL, 10% NaOH, 1% $FeCl_3$ (obtained from the Pharmacognosy Phytochemical Laboratory of Sanata Dharma University) and Aquadest (obtained from the Organic Chemistry Laboratory of Sanata Dharma University) .

METHODS. This type of research is quasi-experimental using the *Central Composite Design* (CCD) method with a quasi-experimental design to obtain the optimal formula of surfactants tween 80 and span 80 and the sonication time in nanoemulsion formulation of candlenut seed extract (*Aleurites moluccana* L.Willd) with ethanol 70 %.

Sample 1

The candlenut seeds used are ready-to-use candlenut seeds purchased from the market. The contents are smoothed using a mortar and stamper. Then the powder was extracted using ethanol solvent by remaceration method. Take 4 kilograms of powder soaked in 6 liters of 70% ethanol for 3 days, stirred for 10 minutes every 2 hours then filtered. Filtering dregs soaked again with 70% ethanol as much as 4 liters left for 2 days then filtered. The results of the first and second filtrate are mixed and then evaporated using a *rotary evaporator* until a thick extract of candlenut seeds is obtained (Prasojo *et.al*, 2012).

Ingredients such as tween 80, span 80, candlenut seed extract, oil phase (VCO), methyl paraben and propylene glycol is put into a beaker glass then mixed using a *magnetic stirrer* for 10 minutes at a speed of 1000 rpm. *Aquadest* little by little and the stirring speed is increased to 1250 rpm for 10 minutes. Then homogenize all the mixed ingredients using ultraturax for 2 minutes with a scale of 2 and after that, sonication is carried out according to the specified time while stirring occasionally (Yuliani *et.al*,2016).The formula that produces nanoemulsion preparations that have good stability and physical properties will be put in a *spray***.**

Sampel 2

Factors used in the study were variations in concentration of tween 80, span 80 and sonication time. The leveling used is low level (-1), medium (0) and high level (+1) plus the *axial point* or *start point* (-α) and (+α). The number of factors used is three factors and the number of levels used is five levels, by replicating at the central point 4 times (in block 1 and block 2)

The optimization design is carried out by making variations on tween 80, span 80 and sonication time used as a factor. This experimental design uses the *Minitab 17* on *Central Composite Design* (Table1).

Table 1.. Experimental Design Optimization of tween factor 80, span 80 and sonication time using Minitab 17 (CCD)

StdOrder	RunOrder	PtType	Blocks	Tween 80 (b/b)	Span 80 (b/b)	Sonication (minute)
1	1	1	1	9	1	30
2	2	1	1	13	1	30
3	3	1	1	9	4	30
4	4	1	1	13	4	30
5	5	1	1	9	1	40
6	6	1	1	13	1	40
7	7	1	1	9	4	40
8	8	1	1	13	4	40
9	9	0	1	11	2,5	35
10	10	0	1	11	2,5	35
11	11	0	1	11	2,5	35
12	12	0	1	11	2,5	35
13	13	-1	2	7,734	2,5	35
14	14	-1	2	14,266	2,5	35
15	15	-1	2	11	0,0505	35
16	16	-1	2	11	4,9495	35
17	17	-1	2	11	2,5	26,833
18	18	-1	2	11	2,5	43,165
19	19	0	2	11	2,5	35
20	20	0	2	11	2,5	35
21	21	0	2	11	2,5	35
22	22	0	2	11	2,5	35

Qualitative test of flavonoid compounds was carried out with 10% NaOH reagent. The extract was taken 1 ml and then a few drops of 10% NaOh reagent were added. If there is a change in color to orange/orange, it indicates the presence of flavonoid content in the extract. The phenol compound test was carried out by adding 1% $FeCl3_{to}$ the ethanol extract of candlenut seeds. If there is a blacker color change than the color of the previous extract, it indicates a positive result (Ikalinus *et.al*, 2015).

Evaluation of Physical Properties and Stability of Nanoemulsions

a. Organoleptic Test. Organoleptic test is carried out by observing the shape, smell and color of the preparation visually (Shoviantari, 2019).
b. pH test. The pH test was carried out using a pH meter. The electrodes were calibrated using standard buffer solutions with pH 4 and 7 before use. Calibration is said to be complete if the

pH value shown on the screen matches the standard pH value and is stable. The pH measurement is carried out by dipping the electrode into the candlenut seed extract nanoemulsion, then the pH value will appear on the screen. This measurement was carried out at room temperature (Yuliani et.al, 2016).

c. Nanoemulsion type test. Testing the type of nanoemulsion of candlenut seed extract was carried out by the dilution method. The test was carried out by dissolving the sample preparation into the water phase and the oil phase with the ratio of each phase being 1:100. If the sample is completely soluble in aquadest, then the type of nanoemulsion is oil in water (O/W), whereas if the sample is soluble in oil, the type of nanoemulsion is water in oil (W/O) (Yuliani et.al, 2016).
d. Transmittance percent test. A sample of 1 ml of the preparation was taken and then dissolved in a 100 ml volumetric flask using aquadest. The percent transmittance of the solution was measured at a wavelength of 650 nm with a UV-Vis spectrophotometer. In this test, aquadest was also used as a blank (Yuliani et.al, 2016)
e. Viscosity Test. This measurement is carried out by taking 14 ml of the preparation sample and then putting it in a cup and installing it on the available solvent trap. This measurement uses a merlin VR viscometer whose speed is set to 200 rpm, three rotations for 30 seconds (Yuliani et.al, 2016).
f. Freeze – thaw cycle. Each nanoemulsion formula was stored at -10°C and 30°C/75% RH for 24 hours for 3 cycles. Nanoemulsions that have passed the freeze-thaw cycle were observed organoleptically, there was a phase separation of pH, percent transmittance, and viscosity (Yuliani et.al, 2016).
g. Centrifugation Test. The nanoemulsion was centrifuged using a centrifuge at a speed of 12000 rpm for 15 minutes. Then see whether there is a phase separation or not (Rachmawati *et.al*, 2015).

Statistical Data Analysis The data

Analysis technique used in this study was a two-way ANOVA (*two-way*) with a 95% confidence level. The *p-value* <0.05, then it indicates a significant difference between the physical properties and stability of the nanoemulsion preparation.ANOVA data analysis *Two-way* was performed on the *Central Composite Design* application *Minitab 17*. The data obtained are data from the evaluation of the physical properties and stability of nanoemulsion preparations.

RESULTS AND DISCUSSION

A. Extraction of Flavonoids from Candlenut Seed Oil

This study used flavonoids contained in candlenut seeds. The candlenut seeds used are candlenut seeds which are sold in the market at the booth, Depok, Sleman, Yogyakarta. Prior to extraction, the candlenut seeds were dried for approximately 5 days at a temperature of 50°C, then the candlenut was ground using a mortar and stamper after which it was sieved using a sieve no. 19. Then obtained as much as 4 kg of candlenut powder. Researchers did not pollinate because the crushed hazelnut is still a little wet. This is because candlenut contains oil and if it is powdered it will stick to the pollinator. After obtaining the candlenut powder, it was extracted by the maceration method. The maceration method was chosen because this method is easy and does not require heating, so it is unlikely that natural materials will be damaged or decomposed (Susanti and Bachmid, 2016). Maceration was carried out by weighing 200 g of candlenut powder and then adding 300 ml of 70% ethanol solvent into 500 ml Erlenmeyer. Ethanol 70% is used as a solvent because 70% ethanol is a universal solvent that can attract compounds that are soluble in non-polar or polar solvents with a polarity index of 5.2 (Padmasari et.al, 2013). To obtain a thick extract of ethanolic candlenut seeds, evaporation was carried out using a rotary evaporator. The thick extract of candlenut seeds was obtained as much as 1064.54 g. The yield was calculated from the number of candlenut extracts divided by the number of candlenut powder and obtained a yield of 26.669%. The ethanol extract of candlenut seeds is stored in a refrigerator in a closed and dark container.

$$\text{Yield calculation} : \frac{extract\ weight\ obtained}{hazelnut\ powder\ weight}\, x\ 100\%$$

$$= \frac{1064,54\ g}{3991,7}\ x\ 100\% = 26,669\%$$

B. Qualitative Test of Flavonoid and Phenol Compounds Qualitative

Test conducted with 10% NaOH obtained positive results, indicated that the color changed to orange/orange. The 10% NaOH reagent is an alkaline catalyst that causes the decomposition of flavonoid compounds into acetophenone molecules which are yellow to brown or orange in color. A positive result occurs with the reaction.

A positive phenolic result is positive if there is a change in color to darker than the previous extract color. Phytochemical test using $FeCl_3$ can show the presence of phenol groups. If there is a phenol group, it is also possible that there are tannins because tannins are polyphenolic compounds. The color change to darker/blackish occurs due to the formation of complex compounds between tannins and$_{et.al}$ FeCl3 (Ikalinus ,2015). Based on the results of the qualitative test, it was found that the ethanol extract of candlenut contains flavonoids and phenols.

C. Preparation of Nanoemulsion Preparation of Candlenut Seed Extract

Ingredients used are VCO, Tween 80, Span 80, methyl paraben, propylene glycol and thick extract of candlenut seeds. VCO is used as the oil phase in the manufacture of nanoemulsions. Based on Erawati's research (2017), it is stated that nanoemulsion preparations using VCO as the oil phase produce droplet sizes below 100 nm and VCO contains medium chain fatty acids so that they are easier to emulsify, are also safe for oral consumption and have good dissolving capacity (Sahumena *et al*. 2019). The combination of tween 80 and span 80 which is used as a surfactant in emulsion preparations can cover the oil phase. The presence of a polyoxyethylene chain from tween 80 and a span 80 ring into these two surfactants can be a steric barrier to the incorporation of droplets (Sahumena *et.al*, 2019).

Nanoemulsion preparations are used in the long term so that preservatives are needed so that the preparations are protected from contaminants. Methyl paraben functions as a preservative in nanoemulsion preparations because nanoemulsions contain high water content so that microbial contamination is easy. In addition to functioning as a humectant, propylene glycol in the preparation functions to prevent interactions between methyl paraben or other parabens with tween 80 (polysotbate 80). Rowe (2009) explained that 10% propylene glycol is able to potentiate or reduce the interaction between methyl paraben and nonionic surfactants such as tween 80. Candlenut seed extract is used as an active ingredient in preparations that can cause pharmacological effects, namely as hair growth. In the manufacture of nanoemulsions, it is necessary to pay attention to the mixing of ingredients. Where the nanoemulsion is made by entering the oil phase first, then followed by adding tween 80 and span 80, then adding propylene glycol, candlenut seed extract and methyl paraben into a beaker glass. The temperature used for sonication is 35°C.

D. Evaluation of Physical Properties and Stability of Nanoemulsion Preparations

Transmittance percentage is strongly influenced by the sonication time. Where the longer the sonication, the smaller the particle size will be obtained.Evaluation of the physical properties of nanoemulsions was carried out by organoleptic test where the preparation was expected to be clear and yellow in color. A clear preparation is an indicator that the preparation has a small droplet size. The pH measurement was carried out using a pH meter with aquadest for calibration. Based on SNI, the pH value for good hair tonic preparations is in the range of 3 - 7 so that the resulting preparation is not irritating (Shoviantari *et.al*,, 2019). The pH values obtained for each formula all met the desired pH value.

The transmittance percentage was seen using a UV-Vis spectrophotometer with a wavelength of 650 nm. A formula with a transmittance percentage of 90-100% shows that the formula has a clear and transparent visual appearance (Costa *et.al*, 2012). Several formulas have met good viscosity values, namely F3, F4, F6, F7, F14 and F16.

The viscosity value for nanoemulsion preparations is 1-100 cPa.s (Ayuningtias *et.al*, 2017). The viscosity of all formulas met the viscosity criteria of nanoemulsion preparations. The nanoemulsion type test was carried out to determine whether the preparations made were in accordance with the formulation desired by the researchers. Nanoemulsion preparations are formulated in the form of oil in water (O/W).

In table 2 the lowest percent transmittance value is in formula 1 (F1) with 76% transmittance percent and the highest is formula 7 (F7) with 98.9% transmittance percent with an average value of 86% transmittance and a standard deviation value (SD).) 7.68. The highest pH values obtained were 5.5 and 5.2 with an average pH value of 5.29 and a standard deviation of 0.07. The lowest viscosity of the preparation was in formula 5 (F5) with a viscosity value of 1.11 and the highest was 51.15 (F8) with an average value of 22.92 and a standard deviation of 17.01.

Table 2. Factors and levels in research

Factor	**Level**				
	-α (-1,633)	-1	0	+1	+α (1,633)
Tween 80	7,724g	9 g	11 g	13 g	14,266 g
Span 80	0,0505 g	1 g	2,5 g	4 g	4,9495 g
Sonication	26,833 minute	30 minute	35 minute	40 minute	43,165 minute

Note : High level= +1, Low level = -1, *center point* = 0, dan *axial point* = α

From the table above, it can be seen that several formulas already have good physical properties. Formulas that meet the parameters of good physical properties are F3, F4, F6, F7, F14 and F16.

Table 3 shows the results of the physical properties data after the *Freeze Thaw Cycle*. The value of percent transmittance and viscosity of each formula increased. The lowest percent transmittance value is in formula 1 which is 82.4% and the highest is in formula 8 with a percent transmittance value of 99.7% with an average value of 92 percent transmittance and a standard deviation value of 4.89. The highest viscosity of the preparation is in formula 15 which is 140 cPa.s and the lowest viscosity is 6.44 cPa.s with an average viscosity of 38.8 and a standard deviation of 32.06. The pH value obtained was not too different from the pH value in the physical properties test of the preparation. Where the highest pH is 5.5 and the lowest is 5.2 with an average pH value of 5.35 and a standard deviation of 0.13.

In Pratiwi's research (2018), it is stated that physical stability is an important parameter that must be fulfilled by the optimum formula. Because physical stability describes the durability of a preparation during storage or use of the preparation, where the preparation still has the same characteristics and properties as when it was first made. After testing the *Freeze Thaw cycle* with 3 cycles, the preparations were tested for organoleptic, pH, transmittance, viscosity and type of nanoemulsion. The data can be seen in table VII. No formula has good stability. In F7, F20 and F2 the percentage transmittance values obtained are not much different from the results of physical properties, namely 98.9%, 86.5% and 85.1%. At F7, F20, F21 there was an increase in viscosity with viscosity values of 22.69, 25.73 and 24.34 cPa.S, respectively. This increase in viscosity was due to the influence of the surfactant concentration used in the preparation which caused foculation between the dispersed particles, causing viscosity nanoemulsion preparations increased (Hakim *et.al*, 2018).

Table 3. Evaluation of the physical stability of the preparation

NO	**Formula**	**% Transmittance**	**pH**	**Viskosity (cPa.s)**	**Nanoemulsion type**
1	F1	82.4	5.3	6.44	O/W
2	F2	85.5	5.4	29.91	O/W
3	F3	98.1	5.4	16.34	O/W
4	F4	98.7	5.4	25.78	O/W
5	F5	88.4	5.2	85.76	O/W

6	F6	91.7	5.5	59.81	O/W
7	F7	98.9	5.2	22.69	O/W
8	F8	99.7	5.2	25.93	O/W
9	F9	92.3	5.2	28.63	O/W
10	F10	92.4	5.2	28.94	O/W
11	F11	95.1	5.2	20.13	O/W
12	F12	92.5	5.4	24.39	O/W
13	F13	87.1	5.5	15.72	O/W
14	F14	95.2	5.5	100.05	O/W
15	F15	88.1	5.5	140	O/W
16	F16	96.9	5.5	35.32	O/W
17	F17	90.5	5.5	57.64	O/W
18	F18	93.3	5.4	39.34	O/W
19	F19	92.7	5.2	20.17	O/W
20	F20	86.5	5.5	25.73	O/W
21	F21	85.1	5.3	20.46	O/W
22	F22	92.8	5.2	24.34	O/W
Quantity		92.0 ± 4.89	5.35 ± 0.13	38.8 ± 32.06	

Seen from all formulas there is an increase in transmittance percent. The increase in the transmittance percentage was caused by a change in the clarity of the nanoemulsion preparation because the particles in the preparation re-united to form a larger globule size so that when the percent transmittance measurement was measured only the solvent mixture was measured because the oil mixture had re-united to form a larger size than the other. the previous one (Aprilya, 2020). The pH value obtained increased and decreased in each formula. Changes in pH can be caused by environmental factors such as temperature and storage that is not good. Although there is a change in pH, the pH of the preparation is still included in the pH range for topical preparations (MM *et.al*, 2014). At the time of organoleptic testing, there was no visual change in the preparation even though there were changes in the percent transmittance, viscosity and pH. Researchers also conducted a centrifugation test to see if there was a separation in the preparation. The results obtained that there was no separation after the test was carried out.

E. Analysis of Physical Properties Data Using Minitab 17

After testing the physical properties of the nanoemulsion preparations, the data were analyzed using *Minitab 17*. The responses used were percent transmittance, pH and viscosity of the preparation.

1. Transmitter Response Regression, pH and Viscosity.

To see whether the difference in tween concentration, span and sonication time with CCD method affects the transmittance percentage, an analysis was carried out using *minitab 17*.

Transmittance regression equation (Figure 4)

transmittance = -12 + 4.8 tween + 39.6 span + 0.25 sonication+ 0.113 tween*tween
+ 0.575 span*span + 0.0375 sonication * sonication - 1.379 tween*span
- 0.079 tween* sonication - 0.675 span* sonication

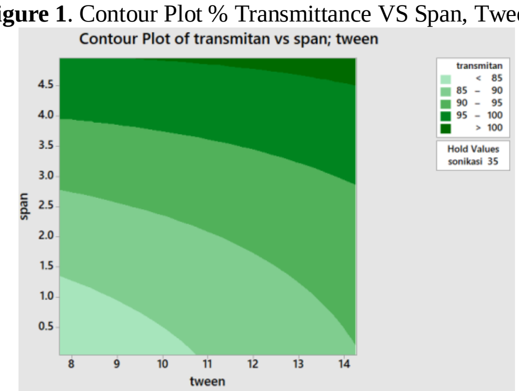
The results are declared significant if the response has a *p-value* <0.05. The *p-value* obtained by the researcher for the percent transmittance is 0.040 (< 0.05), which means that the research model does not significantly affect the transmittance percent. This is because the R-sq (R) in the model percent transmittance is 73.61% and the R-sq (Adj) value is 49.62%. The difference between the two R values in the model is 23.99%. The model is declared to have a significant influence if *the p-value* < 0.05, the value of R – sq is more than equal to 80% (≥ 80%) , the value of R – sq (Adj) is more than 80% (> 80%) with the difference between the two R values is 20% (Purba *et.al*, 2017). From the model above, it is stated that the *p-value* in the model is acceptable but for the value of R – sq, R – sq (Adj) and the difference in the value of R in the model is not acceptable. The results of the analysis of the values obtained indicate that the tween, span and sonication time factors can affect the percent transmittance seen from the *p-value*. Thus, the researcher can use the equations that have been obtained through the t-

wo away minitab 17. The obtained equation is used to predict the response when using level composition to factors such as tween, span and sonication time with optimal response results. *P-value* of pH and viscosity are 0.077 and 0.131. this model is not significant (> 0.05) so the researcher cannot use the equations that have been obtained from the ANOVA statistical test using *minitab 17*.

2. *Countour Plot dan Respon Surface Plot*

The results *of the contour plot* and *surface plot* obtained by data analysis using the CCD (*Central Composite Design*) method are as shown in Figures 1. In Figure 1 there are 5 colors that indicate the range of each percent transmittance. The first color is the transmittance area below 70%. The second color is the transmittance area with a range of 70% - 80%. The third color is the transmittance area with a range of 80% – 90%. The fourth color is the transmittance area with a range of 90% -100%. And the fifth color is the transmittance area with a range of more than 100 (> 100). The desired transmittance percentage in this research is 90%-100%. Where in this range is expected to produce a particle size of < 100 nm. Of the five colors displayed by minitab 17, the percent range of transmittance received is in the fourth color. Figure 8 shows the 3-dimensional structure of the *contour plot*.

Figure 1. Contour Plot % Transmittance VS Span, Tween



Program *minitab 17* provides predictive formulas that can be used to obtain preparations that meet the criteria. In Figure 9 above, it is shown that if you want to get a preparation with a pH value of 3-7, viscosity 1-100 cPa.S and a transmittance percentage of 90% - 100% it is recommended that the tween formula is 8.9122 g, span 4.9495 with sonication for 43.1650. The value of D (*Desirability*) displayed indicates the program's ability to produce the desired criteria. The range of D values is 0 – 1.0. if the value of D obtained is getting closer to 1.0, it shows the program's ability to produce the right results according to predictions. If using the composition as in the program, the formula made 0.9101 or 91.01% will meet the criteria.

F. Analysis of Stability Data Using Minitab 17

1. *Regression of Transmittance Response, pH and Viscosity*

Transmittance = 70.3 + 0.53 tween + 10.08 span - 0.44 sonication + 0.027 tween*tween
+ 0.274 span*span + 0.0156 sonication * sonication - 0.208 tween*span
+ 0.005 tween* sonication - 0.173 span* sonication

As previously explained, the response is said to be significant if *the p-value* of the model is less than 0.05 (< 0.05). The results obtained by researchers when conducting physical properties and stability tests were not too different. In this model, *the p-value* is 0.016 with an R-sq value of 78.48% and an R-sq (Adj) value of 58.91%. The *p-value* in this model is acceptable. Regression response of pH and Viscosity obtained *p-values* respectively are 0.133 and 0.072. The *p-value* of the pH and viscosity response is not acceptable, so the researcher cannot use the equation that has been obtained.

2. Countour Plot dan Respon Surface Plot

Stability of the preparation obtained results as shown in Figures 11 and 12. From *the contour plot* obtained 5 different colors. The first color area shows the percent transmittance area with a value below 85%. The second color area shows the percent transmittance with a range of 85% - 90%. The third color area shows the percent transmittance with a value of 90% - 95%. The fourth color area shows the transmittance percentage with a value of 95% - 100%, and for the fifth area shows the transmittance percentage with a value > 100%. Just as before, the percentage of transmittance that the researcher wants is in the range of 90% - 100%. The third and fourth color areas have met or are acceptable.

In this study, it was found that the ethanolic extract of the candlenut seeds contained flavonoids and phenols as indicated by a qualitative test tube by the researcher. This research did not get the optimum area but got the optimum point which was determined manually by the researcher. Determination of the optimum point is determined by looking at the criteria that meet the percent transmittance, pH, viscosity, type of nanoemulsion, and organolpetics from the physical properties of the preparation. The optimum point in this formula is at F3, F4, F6, F7, F14 and F16 because it meets the parameters of physical properties. The percent transmittance values are 97.5%, 96.3%, 97.5%, 98.9%, 92.4% and 96.1%. The respective viscosity values are 23.87, 23.42, 3.40, 2.34, 20.17 and 50.68. With pH 5.3, 5.2, 5.3, 5.2, 5.3 and 5.3. O/W nanoemulsion type and has a clear and yellow visual appearance.

CONCLUSION

The optimal composition of the formula that satisfies the stability parameters of the preparation was not obtained either from the viscosity value and the percentage of transmittance. There were 6 formulas that met the parameters of good physical properties seen from the percent transmittance, viscosity, pH, nanoemulsion type, and visual organoleptic test, namely F3, F4, F6, F7, F14 and F16.

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