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Optimization of HIB Value Combination of Tween 60 and Span 80 on Cream Formulation of Ethanol Extract of Green Tea Leaves (*Camellia Sinensis* L.)

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ABSTRACT

Green tea (*Camellia sinensis* L.) is known to have ability to protect skin against free radicals. This is supported by polyphenol compound catechin. This research aims to determine the optimum Hydrophilic-Lipophilic Balance (HLB) value of Tween 60 and Span 80 compositions on the optimum cream formula of ethanol extract of green tea leaves. Tea leaves are extracted by macerating using 70% ethanol. Catechin in extract is known from Thin Layer Chromatography (TLC) test with silica gel 60 F_{254} as stationary phase and ethyl acetate:aquadest:formic acid (18:1:1 v/v) as mobile phase. Antioxidant activity is determined by 2,2-Diphenyl-1-picryhydrazyl (DPPH) method and value of Inhibition Concentration 50% (IC_{50}) is then calculated. Formula optimization using Design Expert® version 7.1.5 (DX 7) software, Simplex Lattice Design (SLD) method with two components Tween 60 and Span 80. Cream is characterized according to physical properties organoleptic, homogeneity, viscosity, pH, spreadability, adhesiveness, and cream type. The optimum formula obtained is then tested for physical stability for 4 weeks at room temperature (28±2°C) and data are statistically analyzed using one-way ANOVA. The extract contains catechin proved with Retention factor (Rf) value 0.8 and has antioxidant activity with IC_{50} value 56.35 ppm. 6.4% Tween 60 and 3.6% Span 80 result an optimum HLB value 11.1. It has viscosity 2897.50±35.94 mPa.s, spreadability 18.44±0.06 cm^2, adhesiveness 0.85±0.05 seconds, and pH 4.530±0.002. Statistical test shows that the cream is significantly altered at pH, but does not significantly change in viscosity, spreadability, and adhesiveness after being stored for 4 weeks.

Key words: optimization; HLB; Tween 60; Span 80

INTRODUCTION

Cosmetics are a necessity that absolutely need to be fulfilled nowadays. Utilization of natural or herbal ingredients as active ingredients in cosmetic preparations is now getting more common. The possibility of negative reactions on skin due to mixtures of chemical compounds causes consumers to switch to herbal cosmetic products (Singh *et al.,* 2011).

One of the plants widely known by the public is tea. Various studies show that green tea is beneficial for preventing cancer, osteoporosis, cardiovascular, Parkinson's disease and Alzheimer's, as well as lowering blood pressure. Meanwhile, for beauty care, green tea is used to slim down, prevent early aging, eliminate bad breath and get rid of acne (Soraya, 2007).

Green tea has antioxidant properties. These properties lie in the presence of polyphenols in the form of catechins. Antioxidants of green tea could ward off free radicals and stop the proliferation of cancer cells. Free radicals could damage DNA, which could result in faster aging process (Chacko *et al.,* 2010).

In this study, ethanol extracts of green tea leaves are formulated into cream. Creams are semisolid preparations, in the form of emulsions that contain no less than 60% of water and are intended for external use (Ministry of Health of the Republic of Indonesia, 1979). Creams contain emulsions between oil phase and water phase and are stabilized by an emulsifier. One type of emulsifier is non-ionic surfactants. Non-ionics surfactants are non-electrolytes and therefore, they are not sensitive to the changes in pH of medium. They are more neutral because they are not ionized as is the case with ionic surfactants (Mahato, 2007). Therefore, these surfactants are more stable in both acidic and alkaline conditions.

The non-ionic surfactants used is a combination of Tween 60 and Span 80. Tween 60 is a water-soluble emulsifier that has a HLB value of 14.9 and thus, it could form O/W emulsions. Meanwhile, lipophilicity characteristics of Span 80 are more dominant, and this surfactant has a HLB value of 4.3 so that it forms W/O emulsions (Rowe *et al.,* 2009). HLB value difference between these two surfactants could result in differences in

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physical characteristics and physical stabilityof a cream. The selected cream produced from the combination of these two surfactants is the O/W-type so that the HLB value is in the range of 8-18 (Allen *et al.,* 2004).

Based on those reasons, this research aims to optimize the HLB value in cream formulation of green tea leaves ethanol extract. The method used is Simplex Lattice Design with two components, namely Tween 60 and Span 80.

METHODOLOGY

Tools and Ingredients

Pollinator, kitchen pot, stainless steel frying pan, spatula, bleached calico fabrics, aluminum foil, extracts bottles, electric stove (Robusta®), silica gel 60 F_{254}, capillary pipe, TLC chamber, weighing bottles, oven, desiccator without desiccant, analytical balance (Adventurer™), UV-Vis spectrophotometer, cuvette, micropipette (Gilson Pipetman, Germany), porcelain cups, water bath (Memmert®), ultra turrax T25 (Janke & Kunkel Ika®-Labortechnik), cream pots, glasswares (PYREX-Germany), Viscotester Brookfield, pH-meter (HANNA™), laboratory glass plates (used for homogeneity test), spreadability test apparatus (Pharmaceutical Technology Laboratory, Faculty of Pharmacy UGM), adhesive testing equipment (Pharmaceutical Technology Laboratory, Faculty of Pharmacy UGM), object glasses, and stopwatch (Alba Digital Stopwatch).

Green tea leaves obtained from Desa Nglinggo, Pagerharjo, Samigaluh, Kulon Progo, DI Yogyakarta, ethanol 70% (pharmaceutical grade), ethanol (pro analysis), DPPH (2,2-Diphenyl-1-picrylhydrazyl) radicals, pure vitamin C, ethyl acetate (pro analysis), formic acid (pro analysis), ammonia, $FeCl_3$ solution. The materials used for pharmaceutical grade formulation are Tween 60, Span 80, liquid paraffin, white vaseline, cetyl alcohol, methylparaben, and distilled water.

Work Procedures

Preparation of Dried Green Tea Leaves

Green tea leaves were cut after they were cleaned from dirt with running water. After that, the leaves were heated in an oven at 55°C for 2-3 days. Dried green tea leaves were then pollinated using a grinder. Finally, dried green tea powder was stored in a dark bottle.

Preparation of Green Tea Leaves Ethanol Extract

Extraction was carried out by maceration using a 70% ethanol solvent. One part of dried green tea powder was put into a maceration apparatus and then it was added with 10 parts of ethanol. The mixture was soaked in ethanol 70% for the first 6 hours while stirred occasionally, and then it was left to sit for 18 hours. Macerates were separated by filtration. This process was repeated at least twice with the same type and amount of solvent. All macerates were collected to be evaporated until thick extracts were obtained (Ministry of Health of the Republic of Indonesia, 2008). The obtained thick extracts were stored in a brown bottle. Yield of extracts is expressed according to equation (1):

$$\text{Yield}(\%\text{w/w}) = \frac{\text{thick extract weight}}{\text{simplicia powder weight}}\ x\ 100\%$$

Evaluation of Extract Quality

Organoleptic Test

Observation on the color, smell, and texture of the extracts was conducted.

Loss on Drying Determination

Weighing bottle with constant weight were prepared (heated at 105°C for 30 minutes). Two grams of thick extracts were put into weighing bottles until it became a layer of 5-10 mm. The bottle was opened and dried in an oven at 105°C for 5 hours. After the bottle was removed from the oven and was left to sit for 15 minutes, it was weighed again. The opened weighing bottle and the extracts were heated again for 30 minutes and weighed afterward (Ministry of Health of the Republic of Indonesia, 2008).

This process was repeated until constant weight was obtained and then the loss on drying was calculated. Loss on drying is expressed in equation (2):

$$\text{Loss on Drying}=\frac{\text{initial weight (g)}-\text{final weight (g)}}{\text{initial weight (g)}}x100\%$$

Thin Layer Chromatography Test

One gram of green tea leaf ethanol extract was diluted in 10 mL of ethanol p.a. and later sonificated for 10 minutes. The elution length is 8 cm. The mobile phase used was acetate:distilled water:formic acid (18:1:1 v/v), while the stationary phase used was silica gel 60 F_{254} that had been activated by heating in an oven at 30-40ºC for 10 minutes.

After elution, the spots were evaporated with ammonia and observed with UV_{254} light, a pale blue color indicates the presence of catechins. Spots visualization with $FeCl_3$ reagent (Robinson, 1995 in Rustanti *et al.,* 2013). Rf value of catechin in the sample was then compared to the value in literature, which is 0.88 (Amarowicz, 2005 in Rustanti *et al.,* 2013). Rf value is expressed

Table I. Formula of Green Tea Leaves Ethanol Extract Cream

Materials (gram)	*Run*							
	R1	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**
Green tea extracts	1	1	1	1	1	1	1	1
Tween 60	0	5	2.5	10	0	10	7.5	5
Span 80	10	5	7.5	0	10	0	2.5	5
Liquid paraffin	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
White vaseline	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5
Cetyl alcohol	2	2	2	2	2	2	2	2
Methylparaben	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Aquadest	51.85	51.85	51.85	51.85	51.85	51.85	51.85	51.85

Table II. HLB value of Green Tea Leaves Ethanol Extract Cream

Run	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**
HLB	4.3	9.6	6.9	14.9	4.3	14.9	12.2	9.6

according to equation (3):

$$Rf = \frac{\text{length traveled by compound from original point}}{\text{length traveled by solvent from original point}}$$

Antioxidant Activity Test (DPPH method)
Solutions Test Preparation

Preparation of 0.4 mM DPPH solution, 15.7 mg of DPPH radical powder was put into a 100.0 mL volumetric flask and was added with ethanol p.a until the volume was exactly 100.0 mL.

Preparation of green tea leaves extract solution, a standard extract solution of 1000 ppm concentration was prepared, and then concentrations of 20, 40, 60, 80, and 100 ppm were prepared with a volume of 5.0 mL each.

Preparation of vitamin C solution, a 1000 ppm standard solution of vitamin C was prepared, and then concentrations of 10, 15, 20, 25, and 30 ppm were made with a volume of 5.0 mL each.

Determination of λ maximum for DPPH, 1.0 mL solution of 0.4 mM DPPH was added with ethanol p.a. until the volume was exactly 5.0 mL. After that, the mixture was vortexed and absorbance scanning was conducted using spectrophotometer at 30 minutes operating time with a wavelength range of 450-550 nm in order to obtain a description of its maximum absorbance.

Absorbance measurement of test compounds, a total of 500.0 µL sample solutions (green tea extracts and vitamin C solutions) were respectively added with 1.0 mL solution of 0.4 mM DPPH and ethanol p.a. until the volume was exactly 5.0 mL. The mixture was vortexed and left to sit for 30 minutes. Then, its absorbance was read at λ maximum. The blank used was ethanol p.a. This test was conducted with 2 replications. Antioxidant activity (AA) is measured through equation (4):

$$AA\ (\%) = \frac{\text{Abs blank} - \text{Abs sample}}{\text{Abs blank}} x100\%$$

Formulation and Evaluation of Physical Properties of Cream

The formula used refers to *Dermatological Preparations of The Tropics* (1991), with modifications to the addition of active and inactive substances. Formula optimization using SLD with two components by DX 7 software produced eight runs (Table I). The HLB values obtained (Table II).

All ingredients were weighed and grouped into oil or water phase. The oil phase consists of liquid paraffin, white vaseline, cetyl alcohol, and Span 80 heated over a water bath to 70°C. The water phase consists of distilled water, Tween 60, and methylparaben also heated to 70°C. The oil phase was homogenized using ultra turrax. The water phase was then gradually added to the oil phase while both of them were still hot. The mixture was continuously stirred until it thickened and resembled a cream, and after the cream was formed it was left heated to 60°C. Extracts that had been dissolved in distilled water were added into the cream and they were stirred until homogeneous. After that, the cream was put into a tightly closed container (pot).

The physical properties of the cream, which include organoleptic; homogeneity; viscosity; spreadability; adhesiveness; pH; and cream type, were then tested.

Optimal Formula Determination

Optimal formula determination was conducted using SLD method in DX 7 software. The goal and importance of each quantified parameters of the physical properties of the cream i.e.

Table III. Data of Viscosity, Spreadability, Adhesiveness, and pH of Eight Run Creams

Run	Viscosity (mPa.s)	Spreadability (cm^2)	Adhesiveness (seconds)	pH
R1	6077.5	12.00	0.95	5.015
R2	2062.5	18.51	0.91	5.063
R3	3452.2	13.12	0.85	4.935
R4	1582.5	24.28	0.83	4.145
R5	6312.5	13.15	0.91	4.549
R6	1757.5	18.40	0.95	4.419
R7	2697.5	18.07	0.85	4.651
R8	3215	19.89	0.68	4.721

spreadability, adhesiveness, viscosity, and pH were determined. Based on the lower and upper limit (Table IV), the obtained optimal formula has a higher concentration of Tween 60 compared to that of Span 80. The mixed HLB value of concentration ratio between Tween 60 and Span 80 was then calculated using equation (5):

Tween 60 A = A % x HLB A
Span 80 B = B % x HLB B
Mixed HLB = A + B

Optimal Formula Verification

The production of optimal cream formula using the method and evaluated the physical properties of the same cream as in the eight run optimization. The test results are then compared with the predictive value of DX 7 software SLD method using statistical analysis of one sample t-test with a 95% confidence level. The optimal formula verification is used to prove the validity of the SLD method by predicting the optimal formula.

Physical Optimal Formula Stability Test

The optimal cream formula obtained was then tested for physical stability at room temperature (28±2ºC) for 4 weeks to see if the optimal cream formula still had the same physical properties during storage. Tests conducted include organoleptic, homogeneity, spreadability, adhesiveness, viscosity, pH, and cream type test. Tests are conducted every week for 4 weeks of storage.

Method of Analysis

Data is analyzed using IBM SPSS Statistics Version 24 software using the *Shapiro-Wilk* statistic test to see whether the data obtained is normally distributed or not. Data is normally distributed if it has a significance value > 0.05. If the data are normally distributed, the data is analyzed by one sample t-test, whereas if the data is not normally distributed, the data is tested by *Mann-Whitney.* The confidence level used is 95%.

To see the difference response of the physical properties (viscosity, spreadability, adhesiveness, and pH) of optimal cream formula, the data for 4 weeks of storage are tested for normality using *Shapiro-Wilk.* Data that are normally distributed are then analyzed by one-way ANOVA and data that are not normally distributed are analyzed by *Kruskal-Wallis*. Organoleptic, homogeneity, and cream type test are analyzed descriptively.

RESULTS AND DISCUSSION

The results of the determination showed that the plants tested were tea (*Camellia sinensis L.*) based on testing in the Dept. Pharmacy Biology, Faculty of Pharmacy, UGM. The extract produced is in the form of thick dark brown extract, smelled of tea, and thick texture. Maceration of tea powder with a weight of 1000 grams produces a thick extract of 240.4 grams, so that the yield of extract produced is 24.04%. Determination of the drying shrinkage extract was 17.33% which showed that the number of compounds lost or evaporated during the drying process was 17.33%. Qualitative test extracts are conducted by KLT by looking at the value of Retention factor (*Rf*). The results of the chromatogram indicate the separation into three colored bands under UV_{254}, which is at *Rf* 0.6, 0.8, and 0.9. After being sprayed with $FeCl_3$ reactor, a blackish blue ribbon appears with *Rf* 0.8. The *Rf* is close to the *Rf* of the EGCG compound in the literature, which is 0.88 (Amarowicz, 2005 in Rustanti *et al.,* 2013). This shows that the extract contains catechin compounds.

The measurement of antioxidant extract activity was initiated by scanning the maximum wavelength (λ). The maximum wavelength obtained is 518 nm with an absorbance value of 0.814. Positive control used is vitamin C. The measurement of antioxidant activity is indicated by IC_{50} value, which is the concentration of antioxidant compounds that gives % inhibition of 50%. Measurements is done three times, and the average IC_{50} is obtained for extract was 56.35 ppm, whereas the average IC_{50} for vit. C of 18.53 ppm.

Table IV. Data Determination of Optimal Cream Formula

Criteria	*Goal*	*Lower Limit*	*Upper Limit*	*Importance*	DX 7 Software Prediction Value
Spreadability	*Maximize*	13.5	18	(++++)	18.50
Adhesiveness	*In range*	0.8	0.95	(+++)	0.81
pH	*Target to* = 4,7	4.5	5.063	(+++)	4.780
Viscosity	*Target to* = 2180	2000	4000	(++++)	2180

Table V. Comparison of the Prediction Value and the Actual Value of the Optimal Formula

Response	Prediction Value	Actual Value	Significance	Interpretation
Viscosity	2170	2897.50	0.000	Significantly different
Spreadability	18.50	18.44	0.150	Not significantly different
Adhesiveness	0.81	0.85	0.157	Not significantly different
pH	4.780	4.530	0.000	Significantly different

This means vit. C is more effective as an antioxidant than extract.

Cream optimization with the DX 7 software SLD method produce eight run. The test is conducted by replication three times for each run. Eight run creams show brownish white, smell of tea, soft texture, and visually homogeneous. Data of viscosity, spreadability, adhesiveness, and pH (Table III). The increase in the composition of Tween 60 will affect to the spreadability and adhesiveness, while the increase in the composition of Span 80 has an effect on viscosity and pH. The presence of Tween 60 which is hydrophilic is binding stronger in the water phase so that it can reduce the consistency of the M/A type cream, and resulting in a high spreadability. Whereas, Span 80 is a lipophilic surfactant and is able to bind stronger to the oil phase, so it will provide high viscosity. The cream type assessment is done by the dilution method. The cream is diluted with aquadest, set as positive or M/A type when dispersed evenly in the aquadest. Six run showed the M/A type cream, while R1 and R5 showed negative test results. The larger composition of Span 80 will produce A/M type cream because Span 80 is a lipophilic surfactant.

From the seven physical properties of creams, which are used for the determination of optimal formula are spreadability, adhesiveness, viscosity, and pH. These four responses are then set to their goal and importance. The optimal formula obtained has a ratio of concentration of Tween 60 greater than Span 80 by considering the lower limit and upper limit of the four responses (Table IV). Prediction value of spreadability in DX 7 software SLD method exceeds the upper limit, which is 18.50. This can be caused by the degree of importance is quite high, namely (++++). Based on the analysis of DX 7 software SLD method, obtained the optimal formula at concentration of Tween 60 6.4% and Span 80 3.6% with a value of desirability of 0.934. Desirability is the desired value and has a range of 0-1, so if desirability is close to 1, the recommended formula is more in accordance with the desired criteria. Comparison of concentrations of 6.4% Tween 60 and 3.6% Span 80 gives mixed HLB values of 11.1 which are calculated by equation (5). Data determination of the optimal formula (Table IV).

Data of viscosity, spreadability, adhesiveness, and pH response are tested for normality using *Shapiro-Wilk* with a confidence level of 95%. The results show data of viscosity, spreadability, adhesiveness, and pH normally distributed. Statistical analysis is continued with one sample t-test to see the difference between the actual values with SLD predictions. The results of the analysis (Table V). The test results are not significantly different if the significance value was > 0.05. Spreadability and adhesiveness responses show results that are not significantly different, whereas viscosity and pH responses show significantly different results. The optimal cream formula was then tested for physical stability at room temperature (28±2°C) for 4 weeks including organoleptic, homogeneity, viscosity, spreadability, adhesiveness, pH, and cream type test. Data of viscosity, spreadability, adhesiveness, and pH during storage (Table VI).

During 4 weeks of storage, the cream does not undergo significant changes, remains brownish white, smells of tea, soft texture, and visually homogeneous. Data of viscosity, spreadability, adhesiveness, and pH were normally distributed according to the *Shapiro-Wilk* test with a significance value of 0.054; 0.680; 0.080; and

Table VI. Data of Viscosity, Spreadability, Adhesiveness, and pH of the Optimal Cream Formula during Storage

Week-	Viscosity (mPa.s)	Spreadability (cm^2)	Adhesiveness (seconds)	pH
0	2897,5 ± 35,94	18,44 ± 0,06	0,85 ± 0,05	4.529 ± 0.002
1	2885,0 ± 26,46	18,25 ± 0,08	0,88 ± 0,03	4.538 ± 0.004
2	2867,5 ± 17,08	18,33 ± 0,13	0,86 ± 0,03	4.531 ± 0.006
3	2887,5 ± 17,08	18,25 ± 0,09	0,86 ± 0,06	4.532 ± 0.003
4	2907,5 ± 32,02	18,39 ± 0,05	0,96 ± 0,08	4.539 ± 0.003

0.961. Subsequent tests are parametric with one-way ANOVA. One-way ANOVA test gives significance of 0.335; 0.034; 0.069; and 0.005 for viscosity, spreadability, adhesiveness, and pH. Viscosity and adhesiveness show a significance value of > 0.05, so there is no significant difference, while for spreadability and pH the significance value is < 0.05, which means that there is a significant difference for 4 weeks of storage. Therefore, a post-hoc test was conducted in the form of Tukey HSD to see on what week there were significant differences. The results show that the significance value of spreadability is 0.062. This shows that there is no significant difference in the cream that is stored from week 0 to week 4. Unlike with pH, there are significant differences when it is stored in weeks 0-1 and weeks to 0-4. Significant values obtained were 0.026 and 0.022, respectively. The optimal cream formula for M/A type after being tested by the dilution method and did not experience cream-type reversal (inversion) for 4 weeks of storage.

CONCLUSION

From the research above, it can be concluded that the ethanol extract of green tea leaf contains catechin compounds with an *Rf* value of 0.8 and are antioxidant with an IC_{50} value of 56.35 ppm. The optimal cream formula according to the DX 7 software analysis SLD generate a combination of 6.4% Tween 60 and 3.6% Span 80. The actual value of spreadability and adhesiveness response did not significantly different from the predictive value of the DX 7 software SLD method, but it is significantly different on the viscosity and pH response. After testing its physical stability for 4 weeks at room temperature (28±2°C), organoleptic, homogeneity, adhesiveness, spreadability, viscosity, and cream type did not experience significant changes, but pH underwent significant changes.

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