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ORIGINAL RESEARCH

Application D-optimal method on the optimization of green tea gel (Camellia sinensis L.) formulation

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Abstract
Green tea leaves contain many polyphenols and antioxidants that can reduce cell damage and prevent premature ageing. Green tea extract is made in gel preparation to make it easier to use and increase convenience. HPMC base with propylene glycol additives can increase drug release and penetration into the skin. This study aims to optimize HPMC and propylene glycol levels in green tea extract gel formulations using the D-optimal method and calculate the total release of polyphenols using the Franz diffusion cell method. The extract is produced by infundation. The gel preparation was formulated by varying HPMC and propylene glycol levels in the manufacturing process. Each gel was evaluated for its physical properties, namely viscosity test, pH test, and dispersion test. The physical properties data obtained were then analyzed using Design-Expert software in the mixture design program to get the optimum formula. The optimum procedure brought was HPMC with a concentration of 6% and propylene glycol with a concentration of 17%, resulting in the best correlation between viscosity, dispersion, and pH, with a desirability value of 0.921. In the green tea gel preparation, the resulting concentration was 8.00 GAE/g sample, and the gel content was released at 360 min. was 3.30 GAE/g sample.

Keywords: D-Optional mixture design; green tea; HPMC; polyphenols; pharmacy care

Introduction

Skin is the main barrier against external antigens and other unwanted influences. Skin is susceptible to ageing caused by exposure to ultraviolet (UV) rays, pollution, and dust (Kim et al., 2018). One of the ways to prevent ageing is to use antioxidants to eliminate radicals. Green tea (Camellia sinensis) is a natural ingredient often used to avoid ageing. Green tea contains many polyphenols and antioxidants (Cavet et al., 2011) to reduce cell damage and slow ageing. The high content of polyphenols, especially the flavonoid group (Sudjatini, 2017), where the main flavonoid is catechins (about 20-30%) of the total dry weight of the leaves (Ardana et al., 2015). The antioxidant content of green tea is 100 times more effective than vitamin C and 25 times more effective than vitamin E as a skin freshener and regulator of free radical balance. The concentration of a good green tea extract used as an antioxidant is 1% (Purwanto & Zamzani, 2020).

The direct use of green tea as an antioxidant is considered less practical, so developing a more suitable dosage form is necessary. One is produced by making green tea extract as a gel. Gel preparations have advantages such as being easily distributed when applied to the skin, providing a cold sensation, and not causing scars on the skin (Afianti & Murrukmihadi, 2015). Good gel preparations can be obtained by formulating several types of gelling agents. Still, the most important thing is to pay attention to selecting gelling agents and other additives, such as propylene glycol. HPMC gel base is a gelling agent often used in cosmetic and medicinal preparations because it can produce a clear gel, is easily soluble in water, and has low toxicity. The research results (Madan & Singh, 2010) stated that the HPMC base has a good drug release rate and a wide distribution. In addition, there are additional ingredients in the form of propylene glycol, which according to research conducted by (Karande & Mitragotri, 2009) stated that propylene glycol could increase penetration into the skin by extracting fat and protein mechanisms, swelling in the stratum corneum, or expanding the partition coefficient and drug solubility in the skin formulation. Until now, there has been no research that optimizes the formula of green tea gel (Camellia sinensis) using the D-optimal mixture design method, where this method has advantages such as a smaller number of experiments, so the investigation becomes more efficient. In addition, it can analyze the relationship between factors and experimental responses simultaneously. Based on that point, the authors are interested in researching the optimization of the formulation of green tea (Camellia sinensis) gel preparations with the D-optimal method, as well as how the effect of variations in the concentration of HPMC and propylene glycol on the unconditional release of polyphenols in the preparation. The study aimed to evaluate the D-optimal method on the optimization of green tea gel for clinical use.

Method

Determination of polyphenolic compounds in the gel preparation was used UV-Vis Spectrophotometer (UV-Vis Spectrophotometer Shimadzu UV-1280 type) and studied polyphenols over a certain period using the Franz diffusion cell. Green tea (Camellia sinensis) is harvested from Baturiti Village, Tabanan, Bali. HPMC K100 is given from Colorcon Production, propylene glycol purchased from Bratachem, and all materials used are of pharmaceutical grade. The determination results show that the green tea plant used in the study can be ascertained to be a type of Camellia inensis. Prepared green tea leaves, then ground and mashed with a blender. According to the Indonesian Pharmacopeia, the powder obtained is then sieved with a mesh number 60 sieve to get a fine powder (Artanti et al., 2016). Green tea extract was obtained by the infundation method (Sugihartini et al., 2017), where the tea powder was weighed as much as 10 g, then added aquadest to 100 ml, and extracted by brewing at 95°C for 15 min (Fajar et al., 2018). Furthermore, the extract was fractionated with ethyl acetate two times. The fraction was thickened and evaporated to dryness to obtain a dry green tea extract (Sugihartini et al., 2017). The gel base was made using HPMC developed with distilled water and then allowed to stand for approximately 24 hours. Then mix PPG and PEG 400, stirring until homogeneous. Green tea extract, methylparaben, and propylparaben were added to a mixture of PEG and PPG and then stirred until homogeneous. The gel base is then put into the mix, then ground until homogeneous and a gel preparation is formed. Gel formulations were manufactured by the following table below **(Table 1)**.

Table 1. Formulation of Green Tea Leaf Gel (Camelia sinensis) using the D-optimal Method

Run No.	Green Tea Extract (%)	HPMC (%)	Propylene glycol (%)	Methyl Paraben (%)	Propyl Paraben (%)	Aquadest (%)
1	1.5	6.00	17.00	0.075	0.025	150
2	1.5	6.50	16.50	0.075	0.025	150
3	1.5	6.00	17.00	0.075	0.025	150
4	1.5	7.00	16.00	0.075	0.025	150
5	1.5	6.50	16.50	0.075	0.025	150
6	1.5	6.75	16.25	0.075	0.025	150
7	1.5	7.00	16.00	0.075	0.025	150
8	1.5	6.00	17.00	0.075	0.025	150
9	1.5	7.00	16.00	0.075	0.025	150
10	1.5	6.50	16.50	0.075	0.025	150
11	1.5	6.25	16.75	0.075	0.025	150

A total of 0.5 g of gel sample was placed on a round glass with a diameter of 15 cm. Another glass was placed on it and left for a minute. The diameter of the spread of the gel was measured. After that, 150 g of additional load was added and allowed to stand for a minute. Then, the constant diameter was measured (Artanti et al., 2016). The spread of 5-7 cm shows a semisolid, very comfortable consistency. Viscosity measurements were carried out with a Brookfield viscometer. As much as 15 mL of gel base was inserted into a cylindrical container, and then the viscosity was measured with a viscometer equipped with a spindle (Ardana et al., 2015). The spindle used is number 7, with a speed of 60 rotation per minute. The pH of the preparation was measured using a pH meter. The instrument was calibrated, washed with distilled water, and dried. Furthermore, the electrode is dipped into a gel base that has been dissolved in aquadest in a ratio of 1:10. The tool is allowed to stand until it shows a constant pH value (Ardana et al., 2015), and the pH value of the preparation. The gel base preparation's pH must follow the skin pH, namely 4.5-6.5. The optimal formula for green tea leaf gel (Camellia sinensis) was analyzed by a mathematical model approach (linear, quadratic, special cubic). The optimum procedure is determined based on the desirability value. The desirability value is a value that describes the correlation between observational data and model predictions.

The optimum formula was determined based on the physical properties of the gel, which consisted of a dispersion test of 5-7 cm (Garg et al., 2002), a pH test of 4.5-6.5, and a pH test of 4.5-6.5—desirability value of the model. The full release of polyphenolic compounds was tested with the Franz diffusion cell, with the dissolution medium used as a buffer solution of citrate pH four and the membrane used as filter paper. The temperature of the dissolution medium was 37°C, with a sampling volume of 2 ml and a sampling time of 6 hours. Furthermore, the determination of total polyphenol content was carried out by UV-Vis spectrophotometry. The sample weighed as much as 10 mg and dissolved in 10 mL of 96% ethanol. Then, 1 ml of the mixture was pipetted, and 0.4 mL of Folin-Ciocalteau reagent was added. Let stand for eight min.; add 4 mL of 7% Na2CO3 and distilled water up to 10 mL. Incubated for two hr. and measured the absorbance of the solution at maximum absorption of 730 nm by UV-VIS spectrophotometry. The results obtained are in the form of absorbance from the sample, and then the absorbance value of the model is plotted into a linear equation (Suhaenah, 2016).

Results

The results of the extraction of 160 g of green tea powder by the infundation method and fractionated with ethyl acetate as much as 13.60 g with a yield of 8.5%. The test was carried out by weighing 1 g of extract and then testing for the water content produced. Based on the resulting contour plot data, it is said that the higher the HPMC concentration and the lower the concentration of propylene glycol added to the formula, the higher the gel viscosity. The higher the viscosity produced, the more it will affect the spreadability of the gel preparation. Runs 1, 3, and 8 with an HPMC concentration of 6% resulted in the lowest average viscosity value, 44083.3 cps. While runs 4, 7, and 9 with an HPMC concentration of 7% produced the highest average viscosity value, 60900 cps.

Discussion

According to SNI 3945-2016 (Annuryanti et al., 2021), determining the water content meets the requirements of the maximum yield is 8%. The result of testing the water content of dry green tea extract is 7.97%, which is said to have met the requirements. HPMC has the advantage that it is non-toxic and non-irritating and has good viscosity stability when stored at room temperature and for a long time (Afianti & Murrukmihadi, 2015). Research conducted by (Karande & Mitragotri, 2009) stated that the additive in the form of propylene glycol in gel preparations could increase the penetration of the gel into the skin by the mechanism of fat and protein extraction, as well as swelling in the stratum corneum, thereby expanding the partition coefficient and drug solubility in the formulation. Using spindle no, the gel viscosity test was carried out with a Brookfield viscometer. Seven and stirred at 60 rpm—the viscosity test results after being processed by the Design Expert **(Figure 1)**. The greater the dispersion produced, the easier it is to be absorbed by the skin and provide maximum results (Maulina & Sugihartini, 2015; Sugihartini et al., 2017). Good dispersion results are between 3-5 cm (Afianti & Murrukmihadi, 2015). The dispersion results after being obtained by the Design Expert **(Figure 2)**.

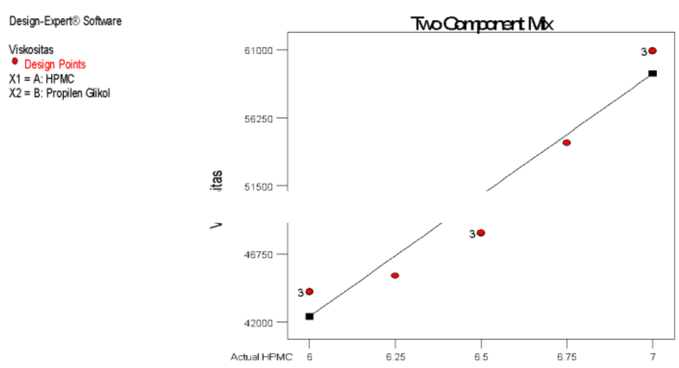

Figure 1. Correlation between the combination of HPMC and propylene glycol on viscosity value

Based on the resulting data, it is said that the higher the concentration of HPMC and the lower the concentration of propylene glycol added to the formula, the lower the dispersion power. The results of the dispersion obtained are 2.9-3.4, indicating that not all formulations meet the requirements of sound distribution. Formula 9 gives the smallest spreadability value, which is only 2.9 cm. This is because a higher concentration of HPMC can cause the viscosity value to increase and the spreadability value to decrease. pH testing is carried out to determine whether the preparations made are safe for use on the skin. The desired pH value is 4.5-6.5, which is the skin's pH range so it is not irritated due to different pH (Afianti & Murrukmihadi, 2015). The dispersion results after being obtained by the design expert **(Figure 3)**.

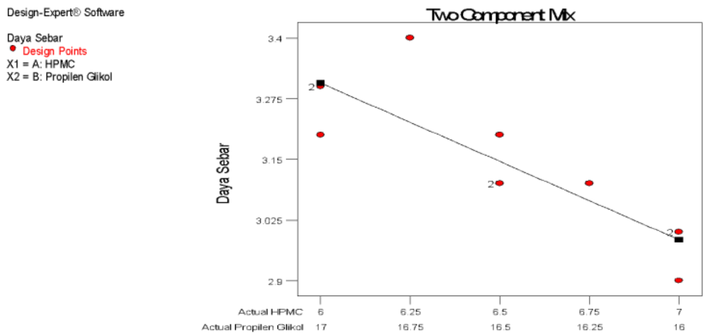

Figure 2. Correlation between the combination of HPMC and propylene glycol on spreadability value

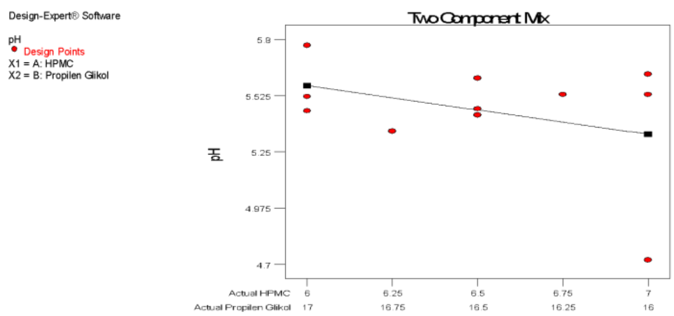

Figure 3. Correlation between the combination of HPMC and propylene glycol on pH value

Based on the resulting contour plot, data shows that all green tea gel extract formulas provide a pH value that is still in the normal pH range of the skin, which is 4.5-6.5, so it is safe and does not irritate the skin. The optimum formula is determined based on desirability **(Figure 4)**. The desirability value is a value that describes the closeness between the experimental results and predictions. After the data is processed using Design-Expert software, the resulting optimum formulation **(Table 2)**.

Table 2. Optimum Formulation of Green Tea Extract

Run	HPMC	PPG	Testing Result			Desirability
			Viscosity	Spreadability	pH	
1	6.00	17.00	42360.8	3.3	5.573	0.921

Based on the results of data processing, it was found that formula 1 has the highest desirability value, which is 0.921. In addition, the correlation value between viscosity, spreadability, and pH gave the best results. The greater the desirability value indicates the closeness of the results between the designs made and the results of the research that has been done. The resulting optimum formula will be further tested, namely, the release of polyphenol using a Franz diffusion cell. The optimum gel formula was repeated three times, and the result data were obtained **(Table 3)**. The resulting data shows that the value of each parameter generated by design experts is not significantly different from the research results. Furthermore, a Franz diffusion cell will test the replicated optimum formula for total polyphenols. The process of determining the absolute levels of polyphenols in the green tea gel sample begins with the preparation of a buffer solution of citrate pH 4.3 which is adjusted to the pH of the blood and followed by the manufacture of standard solutions of 1000 ppm gallic acid, 40 ppm common solutions, and serial solutions of 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm, and 60 ppm. The maximum wavelength resulting from the test is 763 nm. The standard curve for gallic acid is generated with a linear regression value of 0.9008 and shows good linearity. The sample concentration test was carried out by weighing 1 g of green tea gel sample and dissolved in 10 ml of citrate buffer solution pH 4.3, then centrifuging for 6 min at 1000 rpm. The filtrate was separated, then 1 ml was pipetted, put into a test tube, and citrate buffer pH 4.3 was added to the mark.

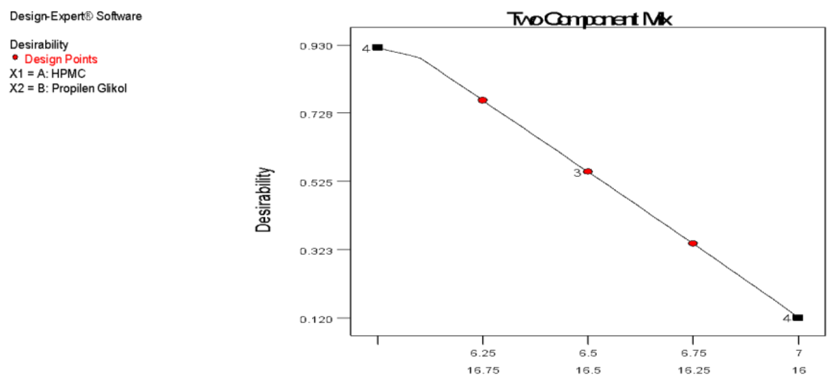

Figure 4. Optimum formulation of green tea extract

Table 3. Replication of Optimum Formulation of Green Tea Extract

Formulation	HPMC	PPG	Testing Result		
			Viscosity	pH	Spreadability
1	6.00	17.00	44120 cps	5.77	3.3 cm
2	6.00	17.00	44100 cps	5.52	3.2 cm
3	6.00	17.00	44090 cps	5.50	3.3 cm
		Average	44103.3 cps	5.596	3.267 cm

Then 0.5 ml of the solution was pipetted into a vortex tube, and 2.5 ml of Folin Ciocalteu, 10% reagent, was added. The answer was vortexed for 5 min, then allowed to stand for 5 min. Then, 2 ml of 7.5% Na2CO3 solution was added. The solution was incubated for 46 min at room temperature. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 763 nm. The total amount of polyphenols produced was 8.00 mg GAE/g sample.

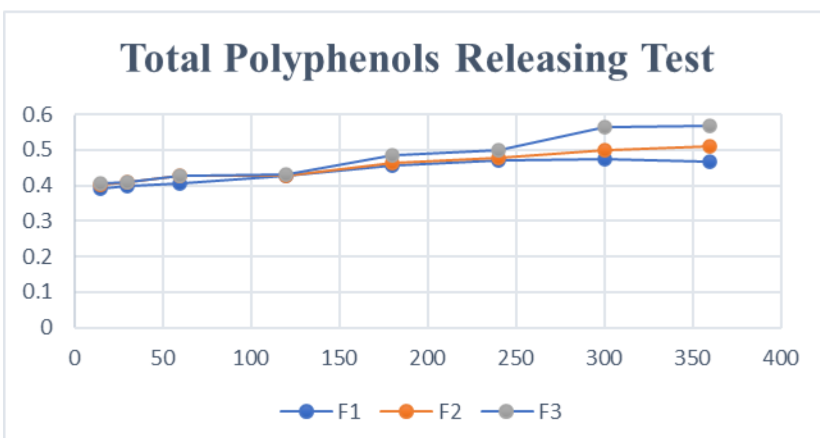

Figure 5. Absorbance value of optimum formulation

The total polyphenol release assay used the Franz diffusion cell method—testing using a filter paper membrane with a solvent in citrate buffer. The gel sample was weighed as much as 1 g and then put into the tool, and stirred with a magnetic stirrer at a speed of 200 rpm (Ayuningtias et al., 2017), which serves to accelerate the process of dissolving the penetrated substance and homogenize the concentration of the essence so that it is evenly distributed in the solution. The tool's temperature is maintained at 37±0.5oC, which describes the temperature of the human body. The sampling time was 15, 30, 60, 120, 180, 240, 300, and 360 min. (Putri et al., 2019). The volume of the sample pipetted was 4 ml, followed by an assay using UV-Vis spectrophotometry—the absorbance value of the gel preparation with the optimal formulation **(Figure 5).** The longer the penetration time, the higher the total released polyphenol content **(Table 4).** The total amount of polyphenol released at 360 min was 3.29 GAE/g sample.

This release test can be affected by several factors, such as the occurrence of pipetting errors and the presence of bubbles in the compartment, which causes inhibit the penetration.

Table 4. Calculation value of released green tea gel content

Time (minute)	Average Absorbance	Total Polyphenol Content Released (GAE/g)
15	0.3986	1.79
30	0.4061	1.89
60	0.4193	2.06
120	0.4283	2.18
180	0.4674	2.69
240	0.4827	2.89
300	0.5121	3.27
360	0.5138	3.29

Conclusion

Based on the research that has been done, it can be seen that the optimum formula of green tea gel is the use of HPMC and propylene glycol of 6% and 17%, and the resulting gel with the best viscosity, dispersibility, and pH, and has a desirability value of 0.921. In the green tea gel preparation, the concentration was 8.00 GAE/g sample, and the gel released at minute 360 was 3.29 GAE/g. Future studies is needed to evaluate the clinical impact of the finding.

Acknowledgement

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