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 lots of polyphenols and antioxidants that can reduce cell damage and prevent premature aging. 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 increase drug penetration into the skin. This study aims to optimize the levels of HPMC and propylene glycol 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 obtain the optimum formula. The optimum formula obtained 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-Optimal mixture design; green tea; HPMC; polyphenols

Introduction

Skin is the main barrier against external antigens and other unwanted influences. Skin is susceptible to aging caused by exposure to ultraviolet (UV) rays, pollution, and dust (Kim et al., 2018). One of the ways to prevent aging is to use antioxidants to eliminate radicals. Natural ingredients that are often used for preventing aging are green tea (Camellia sinensis). Green tea contains many polyphenols and antioxidants (Cavet et al., 2011) so that it can reduce cell damage and slow down the aging process (Syah, 2006). 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). According to research conducted by (Soraya, 2007), 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 it is necessary to develop a more suitable dosage form. One of them is developed by making green tea extract in the form of 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 (Pramuji Afianti & Murrukmihadi, 2015). Good gel preparations can be obtained by formulating several types of gelling agents, but the most important thing is to pay attention to the selection of gelling agents and other additives such as propylene glycol. HPMC gel base is one of the gelling agents that are often used in cosmetic and medicinal preparations because it can produce a clear gel, is easily soluble in water, and has low toxicity. The results of the research by (Madan & Singh, 2010) stated that the HPMC base has a good drug release rate, and has 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 can increase penetration into the skin by extracting fat and protein mechanisms, swelling in the stratum corneum, or increasing the partition coefficient and drug solubility in the skin. 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 experiment 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 total release of polyphenols in the preparation.

**Method**

**Design**

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.

**Materials**

Green tea (Camellia sinensis) is harvested from Baturiti Village, Tabanan, Bali. HPMC K100 is given from PT. Colorcon, propylene glycol purchased from Bratachem, and all materials used are of pharmaceutical grade.

**Plant Determination**

The results of the determination show that the green tea plant used in the study can be ascertained to be a type of Camellia sinensis.

**Green Tea Powder Preparation**

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 obtain a fine powder (Artanti et al., 2016).

**Extraction of Green Tea Leaf Powder (Camelia sinensis)**

Green tea extract was obtained by the infusion method (Sugihartini et al., 2017), where the tea powder was weighed as much as 10 g, then added aquadest to 100 ml, extracted by brewing at 95°C for 15 min. (Fajar et al., 2018). Furthermore, the extract was fractionated with ethyl acetate 2 times. The fraction was then thickened and evaporated to dryness to obtain a dry green tea extract (Sugihartini et al., 2017).

**Gel Formulation**

Gel base was made by using HPMC developed with distilled water, then allowed to stand for approximately 24 hr. Then mixed PPG and PEG 400, stirred until homogeneous. Green tea extract, methylparaben, and propylparaben were added to a mixture of PEG and PPG, then stirred until homogeneous. The gel base is then put into the mixture, then ground until homogeneous and a gel preparation is formed. Gel formulations were manufactured by the following table below (Table 1).

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

**Spreadability Test**

0.5 g of the 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, added 150 g of additional load and allowed to stand for a minute, and then the constant diameter was measured (Artanti et al., 2016). The spread of 5-7 cm shows a semisolid consistency which is very comfortable.
Viscosity Test

Viscosity measurements were carried out with a Brookfield viscometer as much as 15 mL of gel base was inserted into a cylindrical container, 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 rpm.

pH Test

Measurement of the pH of the preparation was carried out using a pH meter. The instrument was first calibrated, then washed with distilled water, then 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 is the pH value of the preparation (Rawlins, 2003). The pH of the gel base preparation must be following the skin pH, namely 4.5-6.5 (Latifah & Iswari, 2013).

Data Analysis

The optimal formula for green tea leaf gel (Camellia sinensis) was analyzed by a mathematical model approach (linear, quadratic, special cubic). The optimum formula is determined based on the desirability value. The desirability value is a value that describes the correlation between observational data and model predictions.

Formula Optimization

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 (Tranggono & Latifah, 2007), and a pH test of 4.5-6.5 (Tranggono & Latifah, 2007). desirability value of the model.

Total Polyphenol Release Study

The test for the total release of polyphenolic compounds was carried out with the Franz diffusion cell with the dissolution medium used in the form of a buffer solution of citrate pH 4, and the membrane used in the form of 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 was weighed as much as 10 mg, then 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 8 min., add 4 mL of 7% Na2CO3 and add distilled water up to 10 mL. Incubated for 2 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, then the absorbance value of the sample 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, 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, which was 44083.3 cps. While runs 4, 7, and 9 with HPMC concentration of 7% produced the highest average viscosity value, which was 60900 cps.

Discussion

According to SNI 3945-2016 (Annuryanti et al., 2021), the determination of the water content meets the requirements if the maximum yield is 8%. The result of testing the water content of dry green tea extract is 7.97%, and this result 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 can 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 increasing the partition coefficient and drug solubility in the formulation. The gel viscosity test was carried out with Brookfield viskosimeter using spindle no. 7 and stirred at 60 rpm. The results of the viscosity test 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 results of dispersion after being obtained by the Design Expert (Figure 2).
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, and these results indicate that not all formulations meet the requirements of good dispersion. Formula 9 gives the smallest spreadability value, which is only 2.9 cm. This is because the higher the 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, where this is the pH range of the skin so it is not irritated due to different pH (Afianti & Murrukmihiadi, 2015). The results of dispersion after being obtained by the Design Expert (Figure 3). 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 the desirability value (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>
<thead>
<tr>
<th>Run</th>
<th>HPMC</th>
<th>PPG</th>
<th>Viscosity</th>
<th>Spreadability</th>
<th>pH</th>
<th>Desirability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.00</td>
<td>17.00</td>
<td>42360.8</td>
<td>3.3</td>
<td>5.573</td>
<td>0.921</td>
</tr>
</tbody>
</table>

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 3 times, and the result data were obtained (Table 3).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>HPMC</th>
<th>PPG</th>
<th>Viscosity</th>
<th>pH</th>
<th>Spreadability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.00</td>
<td>17.00</td>
<td>44120 cps</td>
<td>5.77</td>
<td>3.3 cm</td>
</tr>
<tr>
<td>2</td>
<td>6.00</td>
<td>17.00</td>
<td>44100 cps</td>
<td>5.52</td>
<td>3.2 cm</td>
</tr>
<tr>
<td>3</td>
<td>6.00</td>
<td>17.00</td>
<td>44090 cps</td>
<td>5.50</td>
<td>3.3 cm</td>
</tr>
<tr>
<td>Average</td>
<td>6.00</td>
<td>17.00</td>
<td>44103.3 cps</td>
<td>5.596</td>
<td>3.267 cm</td>
</tr>
</tbody>
</table>

Based on the resulting data, it shows that the value of each parameter generated by design experts is not significantly different from the research results. Furthermore, the replicated optimum formula will be tested for total polyphenols using a Franz diffusion cell.

The process of determining the total 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. Followed by the manufacture of standard solutions of 1000 ppm gallic acid, 40 ppm standard 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 is said to show 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 centrifuged for 6 min. at 1000 rpm. The filtrate was separated, then 1 ml was pipetted, put into a test tube, then added citrate buffer pH 4.3 to the mark. 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 solution 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.

The total polyphenol release assay was carried out using the Franz diffusion cell method. Testing using filter paper membrane with a solvent in the form of 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 substance so that it is evenly distributed in the
solution. The temperature of the tool is maintained at 37±0.5°C which describes the temperature of the human body. The sampling time used was the 15, 30, 60, 120, 180, 240, 300, and 360 min. (Putri et al., 2019). The volume of the sample that was 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).

**Figure 5.** Absorbance Value of Optimum Formulation

![Total Polyphenols Releasing Test](image)

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

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 inhibited the penetration.

**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 sample.

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**Conflict of interest**
The author claim that there are no competing conflicts of interest.

**References**


