



Tyrosinase Inhibition from Green Tea (*Camellia sinensis* (L.) Kuntze) gel

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Abstract

Green tea (*Camellia sinensis* (L.) Kuntze) leaf has polyphenol substance that able to inhibit tyrosinase enzyme. Tyrosinase enzyme is one of the essential components that can be initiated melanin formation on the skin (melanogenesis). The natural inhibitory enzyme can be utilized in cosmetics and medicinal industries as depigmentation agent. Green tea leaf was formulated in the dosage form of a gel with carbomer 934 as a gelling agent. This study aimed to determine the optimum concentration of carbomer 934 on green tea leaf extract gel that has an inhibitory tyrosinase activity. This research used three carbomer 934 concentration, there was 0.5%, 0.75%, and 1% respectively. Which every formula was evaluated during six weeks involve organoleptic, homogeneity, viscosity, pH, centrifuge, and freeze-thaw test (during six cycles). The optimum formula was evaluated tyrosinase activity used a spectrophotometer-vis. The results showed that the great concentration of carbomer 934 was contained on the first formula, which has not phase separation and 49.62 ppm of IC₅₀ value. This research showed that green tea leaf extract gel with 0,5% carbomer 934 prevented tyrosinase activity.

Keywords: Carbomer 934, gel, green tea leaf extract, inhibitory of tyrosinase

Penghambatan Tirosinase dari Gel Daun Teh (*Camellia sinensis* (L.) Kuntze)

Abstrak

Daun teh hijau (*Camellia sinensis* (L.) Kuntze) memiliki kandungan senyawa polifenol yang dapat menghambat enzim tirosinase. Enzim tirosinase adalah salah satu komponen yang dapat menginisiasi pembentukan melanin kulit (melanogenesis). Inhibitor tirosinase dari sumber alam dapat dimanfaatkan sebagai agen depigmentasi pada kosmetik maupun obat. Daun teh hijau dibuat dalam bentuk sediaan gel dengan carbomer 934 sebagai gelling agent. Penelitian ini bertujuan untuk menentukan konsentrasi optimum dari carbomer 934 dan mengetahui aktivitas penghambatan gel terhadap enzim tirosinase. Ekstrak daun teh hijau dibuat dalam 3 formula dengan konsentrasi carbomer 934; 0,5% (Formula 1), 0,75% (Formula 2) dan 1% (Formula 3). Tiap formula dievaluasi selama 6 minggu meliputi organoleptik, homogenitas, viskositas, pH serta uji pemisahan fase, yaitu sentrifugasi dan freeze thaw (selama 6 siklus). Formula yang optimum diuji aktivitas penghambatan enzim tirosinase menggunakan spektrofotometer UV-Vis. Hasil penelitian menunjukkan bahwa konsentrasi optimum carbomer 934 pada formula 1, tidak mengalami pemisahan fase, serta memiliki nilai IC₅₀ sebesar 49,62 ppm. Penelitian ini menunjukkan carbomer 934 dengan konsentrasi 0,5% pada ekstrak daun teh hijau dapat menghambat aktivitas enzim tirosinase.

Kata Kunci: Ekstrak daun teh hijau, gel, inhibitor tirosinase, karbomer 934

1. Introduction

Hyperpigmentation is a disorder of facial skin pigments that is common because of an increase in the process of melanogenesis, including ultraviolet (UV) radiation, inflammation, hormones, and medications, which can cause darkening of the skin color.¹ Skin darkening is a condition in which the skin produces excessive melanin, the pigment which determines skin and hair color to a varying degree.² In addition, increasing melanin synthesis can cause local pigmentation or black spots on certain parts of the face.³ The synthesis of melanin involves tyrosinase as a critical enzyme that is catalyzing of tyrosine oxidation into dopaquinone.⁴ At a certain pH level, the process runs spontaneously, converting dopaquinone into dopa and dopachrome, which will further form melanin.⁵ Therefore, the process of melanogenesis can be inhibited. One of the procedures is the inhibition mechanism of tyrosinase enzymes on the skin either by using natural materials or synthetic materials.

One of the natural ingredients that have the efficacy of tyrosinase inhibitors is green tea leaves (*Camellia sinensis* L.). The methanol extract of 50% tea leaf has an inhibition activity of tyrosinase enzyme with an IC₅₀ value of 51,95 µg / ml.⁶ Fresh green tea leaves contain a precious component of polyphenol derivatives is around 10-30%, known as catechins.⁷ The main components of polyphenol from catechins derivatives can maximally inhibit the activity of tyrosinase that plays a role in the creation of skin pigmentation.⁸

Green tea leaf extract should be made in a particular physical form in a way to facilitate consumer's practicality as well as to extend the shelf life. The physical form widely used in the market is in the form of a cream. However, this form has a drawback of its instability, such as creaming or phase inversion. Referring to the relevant research, the best physical stability of a semisolid is in the form of gels, compared to other forms such as creams and ointments.⁹ Significantly too, gels have the advantage of having an

attractive physical appearance since it is clear and colorless,¹⁰ and it is also comfortable to use because it is not fatty, which causes excessive oil on the face. Thitimuta, et al. had determined the half-inhibition concentration (IC₅₀) of methanolic fresh tea leaves extract was 349 ± 9.00 µg/mL.¹¹

Carbomer or acrylic acid polymer is a gelling agent in a polymeric form that is derived from a synthetic material.¹² The excessed carbomer, compared to another gelling agent, has high stability and compatibility as well as low toxicity.¹³ There are several types of carbomers, such as carbomer 934, 940, 941, 971, and so on.¹² Carbomer 934 has good properties in the release of active substances compared with other gelling agents.¹³ Carbomer 934 can be used as a single-form gelling agent at a concentration of 0.5-2%.¹² The optimization of carbomer 934 concentration as a gelling agent in every gel preparation is pivotal to be done for the sake of identifying the optimum value of carbomer 934, which further generates a good gel physical stability parameters.

2. Methods

2.1. Instruments

The tools used are analytical scales (Ohaus), reflux apparatus, heating mantle, vacuum rotary evaporator (Eyela), oven (Mettler), pH meter (La Motte pH 5 Plus), viscometer using DVE-RVT type (Brookfield), centrifugator (K-Gemco), UV-Vis type UV-1601 spectrophotometer (Shimadzu), microscope (Yazumi), vortex mixer (K-Gemco), hotplate (Akebono), and glassware (Pyrex).

2.2. Materials

Dried green tea leaf powder obtained from the Indonesian Spice and Medicinal Crops Research Institute (ISMCR), 50% methanol, carbomer 934 (Shree Chemicals), methyl parabens, propyl parabens, propylglycol (Dow Chemical), triethanolamine, dimethylsulfoxide (Emsure), Folin Ciocalteu reagent (Sigma Aldrich), and aquadest. Materials to test the activity of tyrosinase inhibition are as a subject, mushroom tyrosinase (Sigma

Aldrich), levodopa as a substrate obtained from a grant from PT. Martina Berto, Tbk.

2.3. Procedure

2.3.1. Extraction of the Green Tea Leaves

A total of 20 grams of dried green tea leaves powder was extracted by reflux using 50% methanol solvent of 400 mL. The powder was extracted for 2 hours at the boiling temperature of the solvent, which is 78°C. The liquid extract was being concentrated by using a vacuum rotary evaporator device with a temperature of 55°C based on the concentrated temperature orientation. This process was run until a viscous extract could be poured and then finally weighed.¹⁴

2.3.2. Screening of Tanins Chemical

Component

Tanin on the extract was defined by qualitative analysis. The condensed extract was re-extracted with 80% ethanol by using an vertical cooler for 15 minutes, and then it was filtered. The obtained filtrate was evaporated over a water bath. On the remaining evaporation, hot and stirred distilled water was added. After the cold solution was centrifuged, the liquid on it was separated by decantation, and the obtained solution was used as the test solution. The test solution was added a 10% gelatin solution; as a result, a white precipitate will be developed.¹⁵

2.3.3. Formulation

Green tea gel was made in 3 formulas. The formula contained carbomer 934 by 0.5%, 0.75%, and 1%, respectively. Carbomer 934 was dispersed in 50% of distilled water until it was expanding.¹² After that, a pH check of Carbomer 934 was performed. The dispersion was then added with triethanolamine to increase the pH, homogeneous stirring was conducted to obtain a pH of 6 by using a colorimetric method (using a universal pH indicator), and finally, the gel base was formed. Methylparaben and propylparaben were dissolved by applying propyleneglycol.¹² Dimethylsulfoxide was dissolved in hot water until it was completely dissolved¹²; it was subsequently added to the methylparaben

and propylparaben solutions. As much as 1% of green tea leaf extract was dissolved in distilled water. The extract solution was mixed with a preservative solution and DMSO until it becomes homogeneous. The gel base was then added a bit by bit into the mixture until it becomes homogeneous. The remaining distilled water was added to the gel and stirred. The obtained gel was stored in a sealed container.

2.3.4. Evaluation of Physical Stability

Testing was done weekly for six weeks. Organoleptic examination of the gel observes a phase separation/syneresis (if any), color and clarity, and the odor it possesses. A homogeneity test of 0.5 g of the gel was placed between two glass objects, and then the presence of particles or inhomogeneity was observed under the light. The pH measurement of acidity (pH) was measured using a pH meter by dipping the electrode into a sample of green tea leaf extract gel. The pH value that appears on the tool screen was recorded. This measurement was made at room temperature.

The measurement of viscosity and flow properties was performed at speeds of 0.5, 1, 2, 4, 10, 20, and 30, then repeated at speeds of 20, 10, 4, 2, 1 and 0.5. Record the number indicating the value of viscosity and dial reading that appears on the screen.¹⁶

The phase separation test was carried out only once during the test. Centrifugation test of 20.0 grams of each sample was inserted into a centrifugation tube, centrifuged at 3750 rpm for 5 x 60 min, and then observed whether phase separation occurred.¹⁷ The phase separation cycle with the freeze-thaw method was carried out at six cycles for each formula. Each cycle was observed after 48 hours of storage at 4°C and 48 hours after at 45°C for 24 days.

2.3.5. Inhibition Activity Test of Tyrosinase

Green tea leaf extract gel was weighed and dissolved with methanol 50%. The sample solution was made by the concentration of 25, 50, 75, 100, 125, 150 ppm. This concentration was obtained from orientation. Tyrosinase

enzyme for inhibition activity test was purchased from Sigma-Aldrich and levodopa obtained from PT. Martina Berto.

Subsequently, read the absorption value (absorbance) by using UV-Vis spectrophotometer at the specified wavelength, which is 481.1 nm. The measured absorbance is the absorption of dopachrome formation.¹⁸ With the value of the obtained absorbance, calculate the percentage of the inhibition by using the formula of:

$$\% \text{ Inhibition} = \frac{(\text{Abs Normal Control} - \text{Abs Blank}) - \text{Abs Sample}}{(\text{Abs Normal Control} - \text{Abs Blank})} \times 100\%$$

The inhibition activity of the test sample was determined by the IC_{50} values calculated by using the linear regression equation.

2.3.6. Data Analysis

The absorbance value of the inhibition activity of tyrosinase was analyzed using a linear regression equation to determine the IC_{50} value.

3. Results

The obtained extract of green tea leaves had organoleptic characteristics that were viscous, blackish brown, aromatic smell of tea, and bitter taste. The yield of the extract was 53.76%. The obtained results of the organoleptic tests of each formula every week, from week 0 to week 6, had the same character that is the dark brown and obvious aromatic smell of tea. The shape of each

formula had a different shape of consistency. That was, the consistency of the form is slightly thick in formula 1, quite thick in the formula 2, and very viscous stable in the formula 3 until week 6. Having said that, the gel is organoleptically stable enough. The results of the homogeneity test were evenly colored, and no coarse or fine particles that do not coalesce in the gel. Accordingly, the gel had good homogeneity.

The pH test results from all the three formulas were in the range of 4.5-6.5 for six weeks period (Figure 1). There was a decreasing viscosity of the gel in the viscosity test (Figure 2). Statistical analysis shows that the viscosity was not affected by the time variable, but it was influenced by carbomer 934 concentration. The higher carbomer 934 concentration had greater viscosity with $p < 0.05$. In addition to the viscosity testing, the type of fluid properties was also examined. Formula 1 and 2 were discovered to had a plastic-type of flow properties and a quite stable thixotropy, starting from week 0 to week 6. Whereas, formula 3 had a viscoelastic type of flow properties. Centrifugation test was carried out at a speed of 3750 rpm for 5 x 60 minutes. The result of the centrifugation testing shows that the whole formula did not experience phase separation.

The results of freeze-thaw test show that the entire gel cycle did not experience any phase separation in formula 1. In contrast

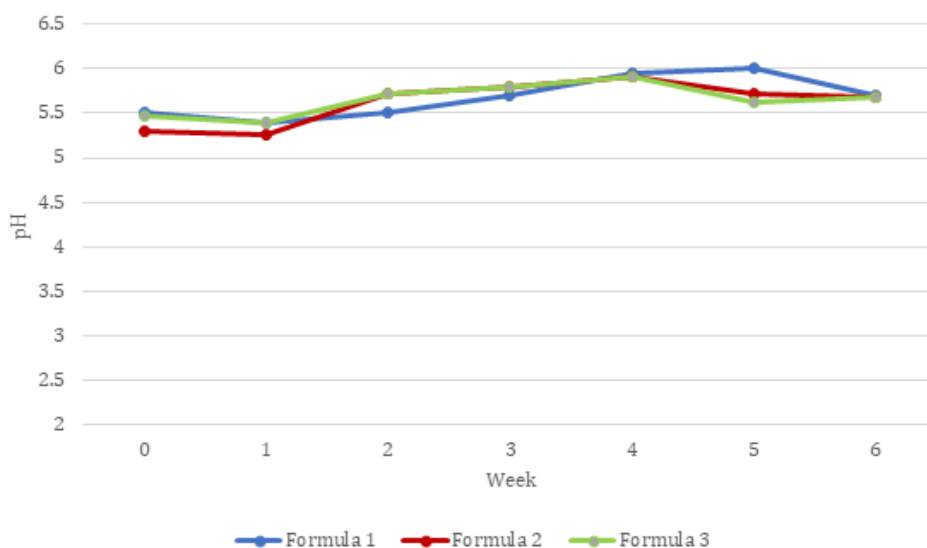


Figure 1. pH Testing Result

to formulas 2 and 3, which experience phase change or phase separation in the sixth cycle. Formula 1 was selected to proceed with the tyrosinase inhibition test based on the overall evaluation of its physical stability. The obtained value of IC_{50} from formula 1 was 49.62 ppm. The IC_{50} values indicated that the green tea leaf extract gel played a role as a tyrosinase inhibitor. These results indicated that it had greater effectiveness in inhibiting tyrosinase enzyme compared to the positive control of the extract. This occurs because the gel base could inhibit the works of the tyrosinase enzyme even though the percentage was low (See Figure 3).

4. Discussion

Referring to the literature; the obtained extract of green tea leaves had the same characteristics with Farmakope Herbal Indonesia, which are concentrated extract, blackish-brown, and bitter taste on the tongue.¹⁹ The yield was in line with the requirement of tea leaf extract yield that was not less than 7.8%.¹⁹ A visual test for homogeneity of the gel was in accordance with the literature. This assessment is useful to ensure no separation of phases, no syneresis (extrusion of water from a gel), and no foreign matter.²⁰

The pH values of all the formulations were in the range of 4.5-6.5, which is

considered acceptable to avoid the risk of irritation upon application to the skin. This result is in line with Anwar E (2012), the gel could be used without reducing the comfortable feeling during its application, and it elevates the absorption rate of the skin.²¹ The viscosity result showed that the higher carbomer obtained the greater viscosity. The high viscosity has a better ability to impart the consistency to semisolid preparations. Viscosity may impact skin retention of the dosage form and drug delivery or penetration via the skin.²⁰

Centrifugation and freeze-thaw test is useful to observe the phase separation of the gel. The centrifugation test did not predict the shelf life, but it only compared the stability of the three formulas. This result indicates the whole formula was stable enough by the centrifugal force. The freeze-thaw result indicates that formula 1 was the most stable formula for temperature, and it had a longer shelf life than any other formula. Some studies argued that the results of freeze-thaw testing for six cycles able to predict the physical stability of shelf life.²² Therefore, formula 1 can be concluded that the formula is the most stable and is predicted to has a longer shelf life than another formula. Moreover, phase separation of the gel product is one possible reason for a high variation of assay results from content uniformity test.²⁰

The IC_{50} formula 1 from this research

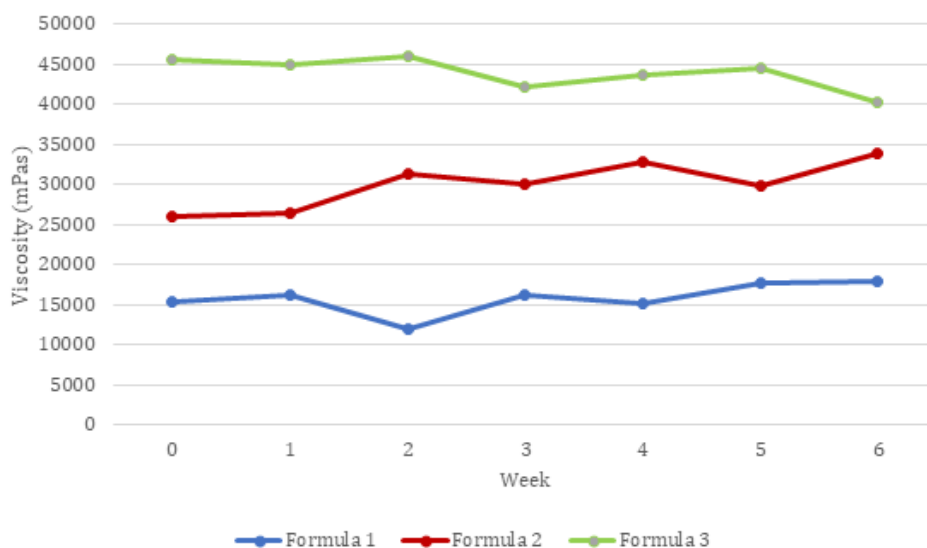


Figure 2. Viscosity Test

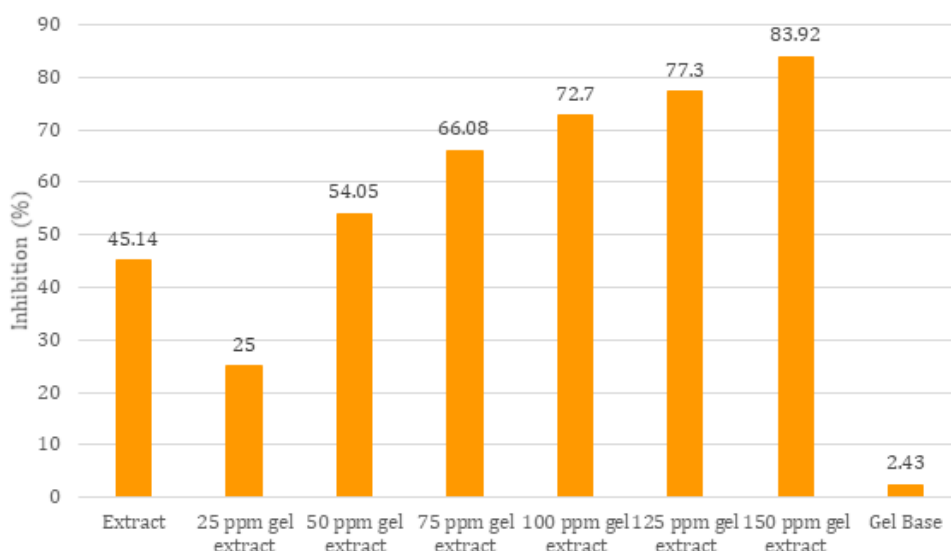


Figure 3. Tyrosinase inhibition value from variation extract concentration

was 49.62 ppm. The IC_{50} value of formula 1 is more effective inhibitory than IC_{50} extract tea leaves (349 ± 9.00 ppm), which is obtained in the previous study by Thitimuta S, et al.¹¹ Green tea is well known as a source of plant polyphenols which able to exhibit antimelanogenic effect as tyrosinase inhibitor.²³ The results show that the combination of green tea leaf extract with Carbomer 934 had an additive effect on the inhibitory ability of tyrosinase activity, which its reaction mechanism could not yet be explained.

5. Conclusion

Carbomer 934, as a gelling agent of green tea leaf gel, had good physical stability. Formula 1 had optimum inhibition of the mushroom tyrosinase enzyme, which is better than kojic acid ($IC_{50} = 49.62$ ppm and $IC_{50} = 61.38$ ppm, respectively). Therefore, green tea leaves gel has good potency to treat hyperpigmentation disorders because able to prevent melanogenesis by reducing the tyrosinase enzyme activity and can be a natural whitening cosmetic candidate. Carbomer 934, as a gelling agent of green tea leaf gel, had good physical stability. Formula 1 had optimum inhibition of the mushroom tyrosinase enzyme, which is better than kojic acid ($IC_{50} = 49.62$ ppm and $IC_{50} = 61.38$ ppm, respectively). Therefore,

green tea leaves gel has good potency to treat hyperpigmentation disorders because able to prevent melanogenesis by reducing the tyrosinase enzyme activity and can be a natural whitening cosmetic candidate.

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