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Antioxidant Activity Test Of Kepel Leaf Extract Gel Preparation (Stelechocarpus Burahol) With Variations Of Carbopol 940 Concentration Using The Dpph Method

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Abstract

Kepel leaves (Stelechocarpus burahol) have the potential as a source of natural antioxidants. This study aims to determine the effect of variations in Carbopol 940 concentration on the physical quality, stability, and antioxidant activity of kepel leaf extract gel. The extract was obtained through maceration using 96% ethanol and formulated into a gel with variations in Carbopol 940 concentrations of 1%, 1.5%, and 2%. Evaluations included organoleptic, homogeneity, pH, spreadability, adhesiveness, viscosity, physical stability using a cycling test, and antioxidant activity using the DPPH method. Data were analyzed using SPSS. The results of the kepel leaf extract study showed very strong antioxidant activity with an IC50 value of 10.14 ± 0.89 ppm. All gel formulas met the physical quality requirements of the preparation. Increasing the concentration of Carbopol 940 decreased the pH value and spreadability, but increased the viscosity. The formula containing 1.5% Carbopol 940 showed the best physical characteristics and stability. The antioxidant activity of the gel preparation showed IC50 values of 226.61 ± 7.68 ppm (F1); 230.96 ± 3.11 ppm (FII); and 241.56 ± 4.66 ppm (FIII), respectively, where the entire formula was classified as a very weak antioxidant.

Keywords: Antioxidants, Carbopol 940, Kepel Leaves, Gel

INTRODUCTION

Oxidative stress is a condition that occurs when the formation of free radicals exceeds the capacity of the body's antioxidant defense system. The accumulation of free radicals can trigger damage to biomolecules, such as lipids, proteins, and DNA, thus contributing to premature aging and the development of various degenerative diseases (Liochev, 2013; Liguori et al., 2018). Therefore, the use of antioxidant compounds is an important approach to inhibiting oxidation reactions and reducing the negative impacts of free radicals. The growing public interest in natural ingredients has also encouraged the use of natural antioxidants as a safer alternative to synthetic antioxidants (Adhyanti & Darsini, 2022).

Kepel leaves (*Stelechocarpus burahol*) are a plant with the potential to be developed as a source of natural antioxidants because they contain various secondary metabolites, especially phenolic and flavonoid compounds that can donate hydrogen atoms or electrons to neutralize free radicals (Herlina et al., 2018). Several studies have reported that ethanol extract of kepel leaves has very strong antioxidant activity based on the DPPH method with an IC50 value that is categorized as very strong (Rosalina, 2014; Isferina, 2022). These results indicate that kepel leaves have the potential to be further developed as an active ingredient in topical preparations.

Gel preparations are chosen as delivery systems because they are easy to apply, provide a comfortable sensation on the skin, are non-sticky, and are suitable for the delivery of hydrophilic active compounds (Yanhendri & Yenny, 2012). The characteristics of the gel are greatly influenced by the type and concentration of the gelling agent. Carbopol 940 is a widely used gelling agent because it is able to produce gels with high viscosity, good stability, and physical characteristics suitable for topical preparations (Hidayanti et al., 2015). Changes in the concentration of Carbopol 940 can affect the pH, viscosity, spreadability, adhesion, and stability of the preparation, thus potentially affecting the release of active substances.

Many studies have been conducted on the antioxidant activity of kepel leaf extract, including its development in the form of a cream preparation. However, research on the effect of varying Carbopol 940 concentrations on the physical quality, stability, and antioxidant activity of kepel leaf extract gel

is still limited. Therefore, this study aims to evaluate the effect of varying Carbopol 940 concentrations on the physical quality, stability, and antioxidant activity of kepel leaf extract gel (*Stelechocarpus burahol*) using the DPPH method and determine the formula that provides the best preparation characteristics.

RESEARCH METHODS

Tool

The equipment used includes a UV-Vis spectrophotometer (Shimadzu UV-1800), rotary evaporator (IKA RV-10), viscotester VT-04 (Rion Co., Ltd.), pH meter, analytical balance, oven, water bath, blender, 60 mesh sieve, and standard laboratory glassware.

Material

The research materials consisted of kepel leaves (*Stelechocarpus burahol*) obtained from Denggung, Sleman, Yogyakarta Special Region, 96% ethanol, Carbopol 940, propylene glycol, methyl paraben, triethanolamine (TEA), DPPH, quercetin, methanol pa, distilled water, phytochemical screening reagents, and commercial gel containing Calendula officinalis as a positive control.

Sample preparation and extract preparation

Plant identity was confirmed through morphological identification at the Pharmaceutical Biology Laboratory of Gadjah Mada University. Kepel leaves were sorted, washed, dried at 40°C, then ground and sieved using a 60-mesh sieve to obtain a simple powder. Drying loss was determined gravimetrically in accordance with the provisions of the Indonesian Herbal Pharmacopoeia.

Extraction was carried out using a maceration method using 96% ethanol at a 1:8 ratio of the drug to the solvent for three days with periodic stirring. The filtrate was then concentrated using a rotary evaporator at 50°C and then evaporated using a water bath until a thick extract was obtained.

Testing the characteristics of powder and extract

Characterization of the extract included organoleptic observations, determination of water content using the azeotropic distillation method, and phytochemical screening for alkaloids, flavonoids, tannins, saponins, steroids, and triterpenoids according to the Harborne method.

Gel formulation

Kepel leaf extract was formulated into a gel preparation with an extract concentration of 5% using Carbopol 940 as a gelling agent at concentrations of 1% (F1), 1.5% (F2), and 2% (F3). The gel base without extract was used as a negative control, while a commercial gel containing Calendula officinalis was used as a positive control. The composition of each formula is presented in Table 1.

Table 1. Formula of ethanol extract gel of kepel leaves (*Stelechocarpus burahol*)

Material	Formula (grams)					
	F1	F2	F3	K1	K2	K3
Kepel leaf extract (active ingredient)	10	10	10	-	-	-
Carbopol 940 (gelling agent)	2	3	4	2	3	4
Propylene glycol (humectant)	30	30	30	30	30	30
Methylparaben (preservative)	0.4	0.4	0.4	0.4	0.4	0.4
TEA (alkalizing agent)	1	1	1	1	1	1
Aquadest (solvent)	156.6	155.6	154.6	166.6	165.6	16
Total	200	200	200	200	200	2

Making gel

Carbopol 940 was dispersed in distilled water until it swelled, then neutralized using TEA. Kepel leaf extract and methyl paraben were dissolved in propylene glycol, then gradually mixed into the gel base while stirring until homogeneous. All formulas were made using the same procedure according to varying Carbopol 940 concentrations.

Evaluation of supplies

Physical quality testing included organoleptic, homogeneity, pH, viscosity, spreadability, and adhesiveness. Physical stability was evaluated using a cycling test method for six cycles at 4°C and 40°C alternately with a storage time of 24 hours in each condition. The antioxidant activity of the extract and gel preparation was determined using the DPPH method at a wavelength of 517 nm, then the IC_{50} value was calculated based on a linear regression equation.

Data analysis

Data were analyzed using SPSS. Data distribution was tested with Shapiro–Wilk, while homogeneity of variance was tested using Levene. Data that met the assumptions of normality and homogeneity were analyzed using One-Way ANOVA followed by the Tukey HSD test. Data that were normally distributed but not homogeneous were analyzed using Welch ANOVA followed by the Games–Howell test. Parameter changes before and after the cycling test were analyzed using a paired sample t-test at a 95% confidence level ($p<0.05$).

RESULTS AND DISCUSSION

Plant Identification Results

Identification results indicate that the sample used is a kepel leaf (*Stelechocarpus burahol* (Blume) Hook.f. & Thomson) from the Annonaceae family. The identification stage is an important initial step to ensure the authenticity of the raw material, ensuring that the research uses the correct species. Accurate plant identity influences the secondary metabolite content obtained and supports the validity of the research results.

Sample Preparation Results

The sample preparation process yielded 1.70 kg of dried herbal medicine from an initial fresh leaf weight of 4.31 kg, while the pollination process yielded 1.663 kg of herbal medicine powder. The weight loss was primarily due to reduced water content during the drying process. Water reduction is necessary to inhibit enzyme activity and microorganism growth, thus ensuring greater storage stability and maintaining the quality of active compounds prior to extraction.

Results of testing the characteristics of powder and extract

The average drying loss of the medicinal plant, 8.60%, meets the Indonesian Herbal Pharmacopoeia's requirements of no more than 10%. This value indicates that the medicinal plant has a relatively low water and volatile compound content. A water content that meets the requirements is an indicator of the quality of medicinal plants because it can increase material stability, reduce the risk of microbial growth, and minimize the possibility of active compound degradation during storage.

Extraction using the maceration method using 96% ethanol produced a yield of 7.916%, thus meeting the requirements of the Indonesian Herbal Pharmacopoeia, which stipulates a yield of kepel leaf extract of at least 5.5%. The use of 96% ethanol was chosen because it is able to dissolve various secondary metabolites, especially phenolic and flavonoid compounds, which are known to act as antioxidants.

Organoleptic observations showed that the extract was thick, dark green to black, and had a distinctive aroma of kepel leaves. Determination of the water content yielded an average value of 8.63%, still below the maximum limit of the Indonesian Herbal Pharmacopoeia (17.8%). This value indicates that the extract has good stability and is relatively resistant to microbial growth during storage.

Phytochemical screening showed that the ethanol extract of kepel leaves contained alkaloids, flavonoids, tannins, steroids, and saponins, while no triterpenoids were detected. The presence of phenolic compounds, particularly flavonoids, supports the extract's potential as an antioxidant source because this class of compounds is known to donate hydrogen atoms or electrons to stabilize free radicals. These results also align with research by Elfasyari (2019) which reported a similar secondary metabolite profile in the ethanol extract of kepel leaves.

Results of Physical Quality Testing of Gel Preparations

Physical quality evaluation was conducted to determine the effect of variations in Carbopol 940 concentration on the characteristics of the gel preparation. Parameters observed included organoleptic properties, homogeneity, pH, spreadability, adhesiveness, viscosity, and physical stability. The results of the organoleptic and homogeneity tests are presented in Table 2.

Table 2. Results of organoleptic and homogeneity tests of kepel leaf extract gel

Formula	Organoleptic			
	Form	Color	Aroma	Homogeneity
F1	Semi-solid	Dark green	Special extract	Homogeneous
F2	Semi-solid	Dark green	Special extract	Homogeneous
F3	Semi-solid	Dark green	Special extract	Homogeneous
K1	Semi-solid	Clear white	Odorless	Homogeneous
K2	Semi-solid	Clear white	Odorless	Homogeneous
K3	Semi-solid	Clear white	Odorless	Homogeneous

Organoleptic Test Results

All formulas produced semi-solid preparations with a consistency suitable for a gel preparation. The formula containing the extract had a dark green color and a distinctive aroma of kepel leaves, while the gel base without the extract was clear and odorless. The differences in color and aroma were influenced by the presence of the extract, while variations in the concentration of Carbopol 940 did not cause changes in organoleptic characteristics. This indicates that increasing the concentration of the gelling agent within the range used has a greater effect on rheological properties than on the physical appearance of the preparation.

Homogeneity Test Results

All formulas showed homogeneous results without any coarse grains or phase separation. Good homogeneity indicates that all components, including the kepel leaf extract, are evenly distributed within the gel base. Uniform distribution of active ingredients is essential to ensure consistent dosage with each use and to support the quality and effectiveness of topical preparations.

The results of pH, spreadability, adhesion and viscosity tests can be seen in table 3.

Table 3. Results of pH, spreadability, adhesion and viscosity tests

Formula	pH	Spread power (cm)	Adhesion (seconds)	Viscosity (cps)
F1	4.96 ± 0.09	5.74 ± 0.32	3.80 ± 0.68	5,320 ± 200
F2	4.66 ± 0.01	5.11 ± 0.04	4.26 ± 1.76	15,360 ± 222.71
F3	4.64 ± 0.03	4.87 ± 0.15	4.01 ± 0.50	27,800 ± 1,257.29
K1	5.91 ± 0.02	4.71 ± 0.17	14.14 ± 3.98	31,280 ± 1,162.07
K2	5.00 ± 0.02	4.06 ± 0.04	8.42 ± 3.68	34,173.33 ± 847.89
K3	4.82 ± 0.02	4.11 ± 0.11	14.58 ± 4.15	38,400 ± 1,058.30

Results of pH Test of Kepel Leaf Extract Gel Preparation

The pH value of all formulas is in the range of 4.64–4.96, thus meeting the physiological pH range of the skin and potentially minimizing the risk of irritation when applied topically. Increasing the concentration of Carbopol 940 causes a tendency to decrease the pH value. This condition is related to the nature of Carbopol as a polyacrylic acid polymer containing carboxylic groups. Although it has been neutralized using triethanolamine (TEA), increasing the amount of Carbopol can increase the contribution of acid groups in the gel system, resulting in a lower pH of the preparation. This finding is in line with the research of Hidayanti et al. (2015) who reported that Carbopol concentration affects the pH characteristics of gel preparations.

Statistical analysis using One-Way ANOVA followed by Tukey's HSD test showed that variations in Carbopol 940 concentration significantly affected pH ($p<0.05$). Thus, changes in gelling agent concentration are a factor determining the acidity characteristics of gel preparations.

Results of Spreadability Test of Kepel Leaf Extract Gel Preparation

Spreadability is a parameter that describes the gel's ability to spread across the skin surface, thus affecting ease of application and distribution of the active ingredient. Testing was conducted using graduated loads, while statistical analysis was based on measurements with a maximum load of 250 grams because this reflects the gel's spreadability under the greatest pressure.

Formula 1 and Formula 2 had spreadability within the range that meets the requirements of gel preparations, while Formula 3 showed slightly lower values. The decrease in spreadability with increasing Carbopol concentration indicates that the formation of a denser polymer network causes viscosity to increase, thus reducing the gel's ability to flow and spread. This inverse relationship between viscosity and spreadability is a general characteristic of Carbopol-based gel preparations.

The results of the One-Way ANOVA analysis showed that variations in Carbopol 940 concentration significantly affected the spreadability of the preparation ($p<0.05$). This finding indicates that the selection of the gelling agent concentration needs to be considered to achieve a balance between viscosity and ease of application to the skin.

Results of the Adhesive Power Test of Kepel Leaf Extract Gel Preparation

Adhesion testing is performed to determine the preparation's ability to maintain contact with the skin surface. The longer the contact time, the greater the opportunity for the active ingredient to diffuse, thus increasing the effectiveness of the topical preparation.

All formulas demonstrated adhesion times of more than one second, thus meeting gel quality requirements. The formula containing the extract had lower adhesion than the negative control. This is thought to be due to the extract affecting the Carbopol network structure, thus reducing the gel's adhesion.

Although the adhesion value tended to increase with increasing Carbopol concentration, statistical analysis showed that the difference was not significant ($p>0.05$). These results indicate that the Carbopol 940 concentration range of 1–2% did not significantly change the adhesion ability of the preparation.

Viscosity Test Results of Kepel Leaf Extract Gel Preparation

Viscosity is an important parameter that affects physical stability, ease of use, and release of active ingredients from gel preparations. All formulas had viscosities within the gel dosage form requirements, namely 2,000–50,000 cPs, thus still meeting physical quality standards.

Increasing the concentration of Carbopol 940 resulted in a gradual increase in viscosity. This indicates that the higher the Carbopol concentration, the more three-dimensional network is formed, thus increasing the system's ability to withstand flow. Conversely, the formula containing the extract showed lower viscosity compared to the negative control. The presence of the extract is thought to disrupt the interactions between the Carbopol polymer chains, resulting in less than optimal gel structure formation.

Statistical analysis using One-Way ANOVA followed by Tukey HSD test showed that variations in Carbopol 940 concentration significantly affected the viscosity of the preparation ($p<0.05$). Based on these results, it can be concluded that Carbopol concentration is the main factor determining gel viscosity, which will then affect other physical characteristics of the preparation, especially spreadability.

Physical Stability Test

Physical stability is an important parameter in gel preparation development because it determines the formulation's ability to maintain its characteristics during storage. In this study, stability was evaluated using a cycling test, which involves alternating storage at low (4°C) and high (40°C) temperatures for six cycles to simulate temperature changes that may occur during product distribution and storage. The results of the stability test are shown in Table 4.

Table 4. Results of the gel stability test of kepel leaf extract

Testing	Formula	Before the cycling test	After the cycling test	p-value results
pH	F1	4.96 ± 0.09	5.36 ± 0.01	0.005
	F2	4.66 ± 0.01	5.13 ± 0.01	0.001
	F3	4.64 ± 0.03	5.11 ± 0.01	0.030
	K1	5.91 ± 0.02	6.15 ± 0.07	0.039
	K2	5.01 ± 0.02	5.42 ± 0.02	0.002
	K3	4.82 ± 0.02	5.20 ± 0.01	0.001
Spread power	F1	5.74 ± 0.32	6.30 ± 0.05	0.002
	F2	5.11 ± 0.04	5.07 ± 0.26	0.775
	F3	4.87 ± 0.15	4.40 ± 0.10	0.078
	K1	4.71 ± 0.17	5.09 ± 0.32	0.268
	K2	4.06 ± 0.04	4.66 ± 0.40	0.029
	K3	4.11 ± 0.11	4.19 ± 0.07	0.184
Adhesive power	F1	3.80 ± 0.68	2.68 ± 0.60	0.020
	F2	4.26 ± 1.76	2.26 ± 0.29	0.192
	F3	4.01 ± 0.50	2.55 ± 0.48	0.013
	K1	14.14 ± 3.98	4.98 ± 2.18	0.118
	K2	8.42 ± 3.68	6.50 ± 2.42	0.632
	K3	14.58 ± 4.15	15.23 ± 7.77	0.818
Viscosity	F1	5,320 ± 200	4,813.3 ± 122.2	0.047
	F2	15,360 ± 222.71	13,893.3 ± 1,086.1	0.112
	F3	27,800 ± 1257.3	24,746.7 ± 2,223.3	0.051
	K1	30,946.7 ± 1328.1	29,400 ± 1,143.33	0.139
	K2	34,173.3 ± 847.9	32,773.3 ± 515.9	0.071
	K3	38,400 ± 1,058.30	37,560 ± 634.98	0.430

*differences are considered significant if $p<0.05$

All formulas maintained their shape, color, aroma, and homogeneity after the cycling test. No phase separation or organoleptic changes were observed, indicating that the gel system maintained its integrity despite temperature fluctuations. These results indicate that Carbopol 940 is capable of forming a sufficiently stable gel network to maintain the dispersion of its components during storage. Similar findings were also reported for cucumber extract gel and butterfly pea flower extract gel formulations, which maintained their organoleptic characteristics after stability testing with varying Carbopol 940 concentrations.

pH testing showed statistically significant changes in all formulas after cycling ($p<0.05$). However, all pH values remained within the physiological skin pH range, thus the formulations were considered suitable for topical use. These changes are thought to be due to changes in the Carbopol polymer structure during repeated heating and cooling, which affects the degree of ionization of the carboxylate groups in the gel system.

In the spreadability parameters, FII, FIII, K1, and K3 did not experience significant changes ($p>0.05$), while F1 and K2 showed significant changes. The decrease in spreadability stability is thought to be related to changes in the gel network structure during temperature cycling, thus affecting the gel's ability to flow when subjected to pressure. In contrast, Formula 2 was able to maintain a relatively constant spreadability, indicating a good balance between the viscosity and elasticity of the gel system.

The adhesion test results showed that Formula 2 remained stable after cycling, while Formula 1 and Formula 3 experienced significant changes. This indicates that a Carbopol 940 concentration of 1.5% produces a gel structure that is better able to maintain adhesive properties compared to lower or higher concentrations. Too low a polymer concentration results in a less strong gel network, while too high a concentration has the potential to form a stiffer structure that is more susceptible to changes during storage.

In viscosity testing, only F1 experienced a significant change ($p<0.05$), while FII, FIII, and all controls remained stable. These results indicate that increasing the Carbopol concentration produces a denser polymer network, thus better maintaining viscosity during storage. Several studies of Carbopol-based gel formulations have also reported that increasing the Carbopol concentration improves rheological stability and maintains the physical characteristics of the preparation during storage.

Overall, FII demonstrated the best physical stability, maintaining most of the quality parameters after cycling. These results indicate that the use of Carbopol 940 at a concentration of 1.5% resulted in the best physical quality, including viscosity, spreadability, adhesion, and gel system stability, compared to other formulas.

Antioxidant Activity Test

Antioxidant activity testing was performed using the DPPH method to evaluate the ability of extracts and gel preparations to scavenge free radicals. This method is widely used because it is simple, sensitive, and able to describe a compound's ability to donate hydrogen atoms or electrons to DPPH radicals. The results of the antioxidant activity test can be seen in Table 5.

Table 5. Results of antioxidant test of kepel leaf extract and gel

Formula	IC50 ± SD	Information
Quercetin (comparator)	11.75±0.25	Very strong
Kepel leaf extract	10.14 ± 0.89	Very strong
F1	226.61 ± 7.68	Very weak
F2	230.96 ± 3.11	Very weak
F3	241.56 ± 4.66	Very weak
K2 (-)	4377.9 ± 376.03	Not active
K (+)	131.49 ± 5.5	Currently

Ethanol extract of kepel leaves showed very strong antioxidant activity with an IC_{50} value of 10.14 ± 0.89 ppm. This activity is thought to be related to the flavonoid and phenolic compounds identified in phytochemical screening. These compounds are known to have the ability to donate protons or electrons, thereby stopping oxidation chain reactions and stabilizing free radicals (Blois, 1958; Rice et al., 1996).

After being formulated into a gel preparation, all formulas experienced an increase in IC_{50} values so that their antioxidant activity decreased to a very weak category. This decrease in activity does not necessarily indicate a loss of active compounds, but rather reflects a reduction in the number of compounds that can diffuse into the test medium during the analysis time. The Carbopol matrix forms a three-dimensional network that can slow the release of active compounds so that contact between the antioxidant compounds and DPPH radicals is more limited. A similar phenomenon has also been reported in various Carbopol-based antioxidant gel formulations, where the antioxidant activity of the preparation is lower than that of the extract even though the extract has high activity (Rowe et al., 2009).

In addition to the influence of the gel matrix, the formulation process is also suspected to contribute to the decreased antioxidant activity. The heating stage during gel preparation has the potential to cause the degradation of some thermolabile phenolic and flavonoid compounds, thus reducing their free radical scavenging capacity. Nevertheless, all formulas still demonstrated superior antioxidant activity compared to the negative control, thus concluding that kepel leaf extract continues to contribute to the antioxidant activity of the formulation (Taylor & Aulthon, 2022).

The results of statistical analysis showed that variations in Carbopol 940 concentrations of 1%, 1.5%, and 2% did not significantly affect antioxidant activity ($p>0.05$). Although there was a tendency for the IC_{50} value to increase with increasing Carbopol concentration, the difference was not statistically significant. This indicates that in the concentration range used, Carbopol 940 had a greater effect on the physical characteristics of the preparation than on its antioxidant activity. Therefore,

Formula 2 was chosen as the best formula because it was able to provide a balance between physical quality, stability, and antioxidant activity.

CONCLUSION

Based on the results of the evaluation of physical quality, stability test, and antioxidant activity, Formula II containing 1.5% Carbopol 940 showed the best physical characteristics and stability compared to the other formulas. Variations in Carbopol 940 concentration significantly affected the pH, spreadability, and viscosity of the preparation ($p<0.05$). Although the antioxidant activity of the gel preparation was lower than that of the extract, all formulas still showed free radical scavenging activity.

This study is limited by the fact that antioxidant activity was only evaluated using the DPPH method, which does not fully describe the antioxidant activity mechanism. Furthermore, stability testing was conducted using a cycling test, a long-term stability test, and an in vitro release test. Therefore, further research is needed to evaluate the active ingredient release profile and long-term storage stability.

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