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Effect of Ethanol Solution Concentration in the Extraction Process of *Centella asiatica* L. Bioactive Components Using Microwave-Assisted Extraction (MAE) Method

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Abstract. The Pegagan plant has a scientific name in the form of *Centella asiatica* L. which is included in the Centella genus, Apiaceae family, and kingdom Plantae. Pegagan (*Centella asiatica* L.) has distinctive bioactive components, namely triterpene ester glycoside compounds in the form of asiaticoside and madecassoside, and triterpene group compounds in the form of Asiatic acid and madecassic acid. In this study, the extraction of bioactive components from pegagan was carried out using the Microwave-Assisted Extraction (MAE) method and will study the effect of ethanol solvent concentration in the extraction of bioactive compounds. Analysis of the extracted bioactive content was carried out by analyzing the total phenol content using the Folin-Ciocalteau reagent and ANOVA analysis. The results obtained from the study were in the form of total phenol content as an indication of the presence of bioactive compounds, namely at operating conditions of 450 watts of power, 50% ethanol concentration with a radiation time of 15 minutes which resulted in a total phenol content of 21.9244 mg AGE/g sample. In the ANOVA analysis with ethanol solvent, variables that gave a significant response to the total phenol content were microwave power, radiation time, and ethanol concentration with an R-square value of 95.31%. The effect of ethanol concentration on the total phenol content produced, namely the concentration of pure ethanol solvent will produce extracts with the smallest total phenol content, the effect of extraction time on total phenol content, namely the longer extraction time will increase the total phenol content. Maximum total phenol content using ethanol solvent that is, at operating conditions of 450 watts of power, 10% ethanol concentration with a radiation time of 15 minutes which resulted in a total phenol content of 520 mg AGE/g sample.

Keywords: *Microwave-assisted extraction (MAE), pegagan, ethanol, ANOVA, Folin-Ciocalteu.*

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1. Introduction

Pegagan (*Centella asiatica* L.) is a plant that has been used as a traditional medicine in the form of fresh, dry, and powdered ingredients. Pegagan is a wild plant originating from tropical Asia and spreading throughout Southeast Asia, including Indonesia, India, the Republic of China, Japan, and Australia. Pegagan (*Centella asiatica* L.) contains bioactive substances such as asiaticoside and madecassoside, triterpene ester glycosides, and triterpene group compounds such as Asiatic acid and made basic acid (Sutardi, 2016). Extracts for the extraction of bioactive compounds can be carried out using conventional extraction methods such as maceration, and non-conventional extractions which include Soxhlet extraction, enzyme-assisted extraction, subcritical water, ultrasound, supercritical fluid extraction (SFE), and Microwave-assisted Extraction (MAE). The microwave-assisted extraction method is an extraction method using microwave radiation to heat the solvent used. Microwave-assisted extraction is a method of extracting materials and liquids that uses microwave radiation in the heating process. Microwave-assisted extraction (MAE) is an extraction technique to extract bioactive compounds from plants with solvents that are safe for humans (Rinawati *et al*., 2020).

The pegagan plant has been used for hundreds of years, especially in the dermatology and cosmetology industries. The use of pagan plant extracts can be used for wound healing, skincare that is getting dull and reduces signs of premature aging accelerates the growth of collagen in the skin so that it can repair and regenerate skin when it is damaged by acne(Budi & Rahmawati, 2019). Ethnopharmacology is a branch of medical science that studies the use of plants as drugs or components in traditional medicine by people from generation to generation. The components of the pegagan plant (*Centella asiatica* L.) which are useful for traditional medicine according to research (Dharmono, 2007) include all parts of the plant from roots, stems, leaves, flowers, and fruit.

According to ethnoeconomic studies, pegagan has no significant economic value because it is not traded. This is because this plant is quite common and is only classified as a wild plant. However, the pegagan plant still has important economic value for the Indonesian people because of the availability of the pegagan plant as a medicine for diseases such as coughs and wounds. If people suffer from this disease, they do not have to spend a lot of money to get treatment. Gotu kola is used by the community as a source of animal feed other than as herbal plants. In terms of ecology, pegagan is a component that has a vital function in the ecosystem where this plant grows as one of the producers of oxygen needed by living things (Dharmono, 2007).

This research focuses on the extraction of bioactive components from pegagan (*Centella asiatica* L.) using the Microwave-Assisted Extraction (MAE) method to determine the effect of the concentration of ethanol solvent in the extraction process. This study aims to determine the effect of ethanol concentration on the extraction process using the Microwave-assisted Extraction (MAE) technique. The research findings are intended to provide insight into the relevance of the solvents used in the extraction process and allow for the development of extraction methods that produce the highest-yielding bioactive compounds while maintaining optimal operating conditions.

2. Materials and Methods

2.1 Materials

Pegagan leaf powder (*Centella asiatica* L.) with a size of 40 mesh, Na_2CO_3 (Sodium Carbonate), 96% Technical Ethanol, Folin-Ciocalteu Reagent, Gallic Acid, Aquades.

2.2 Methods

The research procedure was carried out in 3 stages, including (1) sample preparation, (2) extraction using microwaves assisted extraction (MAE), and (3) analysis of research results. The drying process is carried out under the sun for 20 hours by using a dry indicator in the form of easily breaking dry pegagan leaves. Then weigh the raw materials are mashed with a particle size of 40 mesh with a mass of 1 gram. The extraction process was carried out with several variables including a solvent concentration of 10%, 50%, and 90%, 150 watts of microwave power, 300 watts, and 450 watts, and extraction time of 5 minutes, 10 minutes, 15 minutes. The results of the extraction process were stored in a vial measuring 8 ml at a temperature of 40ºC. Then, the extraction results were analyzed using total phenol analysis.

2.2.1 Preparation of Gallic Acid Solution

Weigh 0.01 grams of gallic acid, then add 1 ml of ethanol and add distilled water until the volume becomes 100 ml.

2.2.2 Determination of Maximum Length

Take 1 ml of gallic acid mother liquor with a concentration of 100 ppm, put it in a test tube, and add 1 ml of Folin reagent, shake the two liquid mixtures until they are homogeneous, and let stand at room temperature for 4-8 minutes. Add 4 mL of 10% Na_2CO_3 solution into a test tube, shake until homogeneous, and let stand for 15 minutes. After that, a Vis-spectrophotometer with a wavelength range of 700-800 nm was used to examine the solution.

2.2.3 Create a Gallic Acid Calibration Curve

100 ppm gallic acid mother liquor, taken 1, 3, 5, and 7 ml. Then the solution was diluted using distilled water to a final volume of 10 ml, resulting in a solution with a concentration of 10, 30, 50, and 70 ppm. Then 0.2 ml of each solution was taken and put into a test tube and added 1 ml of Folin-Ciocalteu reagent was then shaken until the mixture of the two solutions is homogeneous, then allowed to stand at room temperature for 8 minutes. Then 3 ml of 10% Na_2CO_3 was added, shaken until homogeneous, and allowed to stand for 30 minutes at room temperature. The maximum wavelength absorption has been determined. Then it is used to create a calibration curve using the regression equation y = ax+b.

2.2.4 Determination of Total Phenol Content

Take 0.1 ml of extract, add 9.9 ml of distilled water (dilution 100 times), and add 1 ml of Folin-Ciocalteu reagent, then shake until the solution is homogeneous and let stand for 8 minutes. Then, 3 mL of 10% Na_2CO_3 was added to the mixture, shaken until the solution was homogeneous, then allowed to stand for 1 hour at room temperature. Measure the absorption using a vis-spectrophotometer using the maximum wavelength. The content analysis repeats 3 times so that the phenol content obtained was as mg gallic acid equivalent/gram of fresh sample.

3. Result and Discussions

3.1 Result

The research was carried out from November 2020 until December 2020 at the Basic Chemistry Laboratory and Bioprocess Laboratory, Engineering/Chemical Engineering Study Program, Department of Mechanical Engineering, Faculty of Technic, University of Jember. This study used the extracted pegagan plant to extract bioactive compounds or components using the microwave-assisted extraction method. The results of the measurement of the total phenol content of the pegagan extract can be seen in Table 1.

Table 1. Total Phenol Content of Pegagan

No.	Power (Watt)	Solvent Contentration (%)	Time (minute)	Average Absorbance	Total Phenol (mg AGE/g sample)
1.	150	10	5	0.4475	298.8461538
2.	150	10	10	0.5450	423.8461538
3.	150	10	15	0.5235	396.2820513
4.	150	50	5	0.5710	457.1794872
5.	150	50	10	0.5980	491.7948718
6.	150	50	15	0.5900	481.5384615
7.	150	90	5	0.4570	311.025641

No.	Power (Watt)	Solvent Contentration (%)	Time (minute)	Average Absorbance	Total Phenol (mg AGE/g sample)
8.	150	90	10	0.5075	375.7692308
9.	150	90	15	0.5210	393.0769231
10.	300	10	5	0.5545	436.025641
11.	300	10	10	0.5625	446.2820513
12.	300	10	15	0.5845	474.4871795
13.	300	50	5	0.6055	501.4102564
14.	300	50	10	0.5975	491.1538462
15.	300	50	15	0.6140	512.3076923
16.	300	90	5	0.5015	368.0769231
17.	300	90	10	0.5180	389.2307692
18.	300	90	15	0.5675	452.6923077
19.	450	10	5	0.5530	434.1025641
20.	450	10	10	0.5800	468.7179487
21.	450	10	15	0.6200	520
22.	450	50	5	0.6055	501.4102564
23.	450	50	10	0.5920	484.1025641
24.	450	50	15	0.5540	435.3846154
25.	450	90	5	0.5220	394.3589744
26.	450	90	10	0.5775	465.5128205
27.	450	90	15	0.5935	486.025641

3.2 Discussions

3.2.1 Maximum Wavelength Measurement Result

The maximum wavelength measurement was carried out using a vis-spectrophotometer. The purpose of determining the maximum wavelength is to determine the area of absorption in the mother liquor that has the highest absorbance. The steps taken in determining the wavelength are inserting the distilled water into a vis spectrophotometer using a cuvette to ensure the cleanliness of the equipment to be used. The next step is to set the wavelength range used around 400-800 nm. This is by following the research conducted by (Sukmawati *et al.*, 2018), with a maximum wavelength in the range of 400-800 nm for analysis using a Vis Spectrophotometer and states that the 400-800 nm range obtains the ideal wavelength results in determining the presence of bioactive compounds in herbal medicinal plants. The next step is to determine the sample content by inserting a cuvette containing a sample solution into the Vis Spectrophotometer, and the wavelength with the highest absorbance is 765 nm.

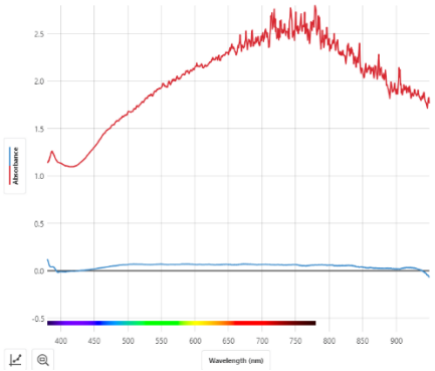

Figure 1. Maximum Wavelength Measurement

3.2.2 Gallic Acid Standard Curve

Preparation of a standard curve for gallic acid with several concentrations of gallic acid, namely 10, 30, 50, and 70 ppm. The result of measuring the maximum wavelength is 765 nm. Measurement of the absorbance of standard solutions of gallic acid from several concentrations was measured based on the maximum wavelength obtained. Based on the absorbance measurements made, a calibration curve was obtained which stated the relationship between gallic acid concentration and absorbance expressed by a linear line.

The requirements for the accepted analytical method for the correlation coefficient (r) obtained from the range 0.996 ± 1 are used to determine the total phenolic content of the pegagan extract (Tahir *et al*., 2017). Based on this, the linear regression equation y = 0.0078x + 0.2144 with a correlation coefficient of R^2 is 0.9949. The correlation coefficient results obtained are by following the acceptance requirements, namely 0.996 so that it can be used to determine the total phenol content.

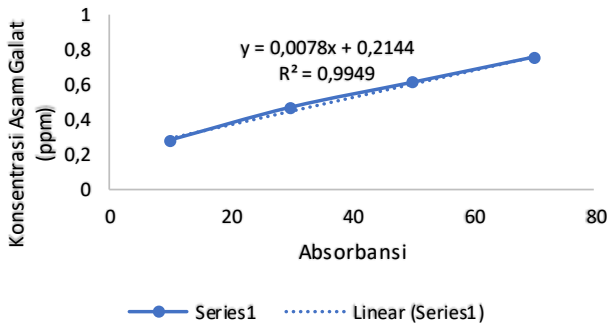

Figure 2. Gallic Acid Standart Curve

3.2.3 Effect of Variables (Power, Solvent Concentration, and Extraction Time) on Total Phenol Content

Based on the results of the study, the effect of the variables on the total phenol content can be seen in figures 3, 4, and 5. Based on Figure 3, it can be seen that at 5 minutes of extraction time with 10% and 50% ethanol concentrations the total phenol content increased, while at 90% ethanol concentration the total phenol content decreased significantly. At 150 watts the highest concentration of total phenol content was at a concentration of 50% for 10 minutes resulting in a total phenol content of 481.5384615 mg AGE/g sample, while the lowest total phenol content was at a concentration of 10% with an extraction time of 5 minutes of 298.8461538 mg AGE/g sample. In general, the concentration of ethanol solvent and extraction time affect the total phenol content, with the result that at 150 watts the total phenol content increased and decreased from the solvent concentration variable at the time of extraction.

In Figure 4 it can be seen that the graph of the total phenol content increased and decreased from the solvent concentration variable at several extraction times with a power of 300 watts. In the extraction time variable of 5 minutes, the ethanol concentration of 10% and 50% of the total phenol content increased, while the ethanol concentration of 90% of the total phenol content decreased significantly. At 300 watts the highest concentration of total phenol content was at a solvent concentration of 50% with an extraction time of 15 minutes which resulted in a total phenol content of 512.3076923 mg AGE/g sample, while the lowest total phenol content was at a concentration of 90% with an extraction time of 5 minutes of 368.0769231 mg AGE/g sample.

Based on Figure 5, it is known that the graph has a trend of increasing and decreasing the total phenol content of the variable concentration of ethanol solvent at several extraction times with a power of 450 watts. At the 5-minute extraction time variable, the ethanol concentration of 10% and 50% of the total phenol content increased, while at the 90% ethanol concentration, the total phenol content decreased significantly. At 450 watts the highest concentration of total phenol content was at 10% ethanol concentration with an extraction time of 15 minutes which resulted in a total phenol content of 520 mg AGE/g sample, while the lowest total phenol content was at a concentration of 90% with an extraction time of 5 minutes of 394.3589744 mg AGE/g sample.

The extraction process runs optimally when the polarity of the solvent is compatible with the substance to be dissolved. The yield of the extract produced during the extraction process will be higher if the polarity of the solvent is compatible with the substance. The effect

of ethanol concentration on the total phenol content produced, namely the concentration of pure ethanol solvent will produce extracts with the smallest total phenol content, this is because polyphenol extraction is very dependent on the polarity of the solvent, and using pure solvents is not effective for the separation of polyphenols whose ingredients come from plants. This is following the research conducted by (Kristanti *et al*., 2019)), which states that the increase in the concentration of ethanol solvent is inversely proportional to the total phenol content obtained. The ethanol solvent in the extraction process acts as a solute with the plant matrix. This shows that the combination of water and ethanol solvents works well for extracting bioactive chemicals from plant sources (Gunathilake *et al*., 2019). The total phenol content in this study increased when a solvent with a concentration of 10% and 50% was used, but the total phenol content decreased when a solvent with a concentration of 90% ethanol was used. The thing that needs to be known during the extraction process is that the higher the concentration of ethanol used, the higher the yield of the extract. Increasing the solvent concentration will affect the polarity of the solvent so that it can increase the solvent's ability to extract fewer polar compounds. Problems that arise when extracting a less polar solvent can cause cell walls that have the same properties to be degraded automatically so that the content of bioactive compounds from herbal plants is easier to extract. However, the use of fewer polar solvents causes a reduction in the polar compounds that can be extracted. This is following the research, so based on the results obtained, the highest yield was obtained at the time of extraction using ethanol with a concentration of 10%.

At 150 W and 300 W microwave power for 5 minutes to 10 minutes, the effect of extraction time on total phenol content resulted in findings with a tendency to increase total phenol content. According to (Kristanti *et al*., 2019), the yield of bioactive components extracted within 5 to 10 minutes experienced a significant increase while the yield obtained for more than 10 minutes was not significantly different and tended to remain constant. Based on this statement, it can be seen that the extraction time has a significant impact on the yield, and the extraction time that is too long or too short can change the physical and chemical characteristics of the extracted material. When the extraction period is too short, the solubility of phenolic compounds is less than optimal, and the material is not extracted completely, but when the extraction time is too long the phenolic compounds will be destroyed automatically.

At 450 W microwave power for 15 minutes, the effect of extraction time on the total phenol content increased. The addition of time in the extraction process can increase the penetration of the solvent into the substance, making it easier for the solvent to extract

compounds from the material. The increase in total phenol content was caused by the sample interacting directly with the solvent for a longer period so that the amount of material extracted was greater until the optimal time limit was reached. However, after the optimal duration was reached, the addition of extraction time did not affect the amount of phenolic bioactive compounds extracted. According to (Kristanti *et al*., 2019) in his research on Supercritical Fluid Extraction and Maceration Technology on Zingiber: Antioxidant Activity and Phytochemical Content, he knows the fact that the longer the extraction time, the higher the yield of the extract because solvents can penetrate the material and break down plant cell walls during the process. extraction process and causes the amount of extracted compounds to increase. This is in line with the findings of this study, which determined that 15 minutes was the best time to produce the highest total phenol content yield.

Microwave power ranges from 150 watts to 450 watts, which has enhanced the effect of total phenol concentration. With increasing microwave power, the content of bioactive compounds also increases. According to a study conducted by (Yingngam et al., 2020)when the microwave power was increased from 300 to 600 W, the content of bioactive compounds increased. This is because the increase in microwave power increases the solubility and diffusion of bioactive components out of the plant matrix, and increases the pressure in the plant matrix. However, if the microwave power is greater than 700 watts, the concentration of bioactive compounds will decrease because the power used is too high, causing the material to degrade automatically.

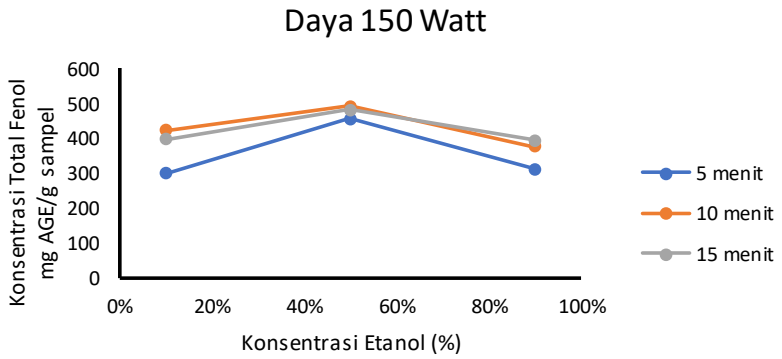

Figure 3. Effect of Variables on Total Phenol Concentration at Power 150 Watt

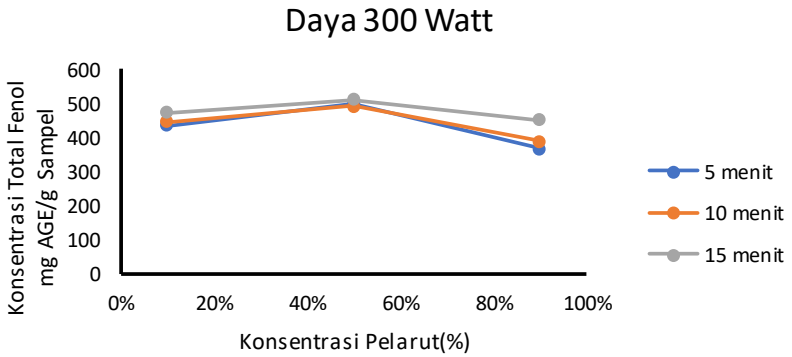

Figure 4. Effect of Variables on Total Phenol Concentration at Power 300 Watt

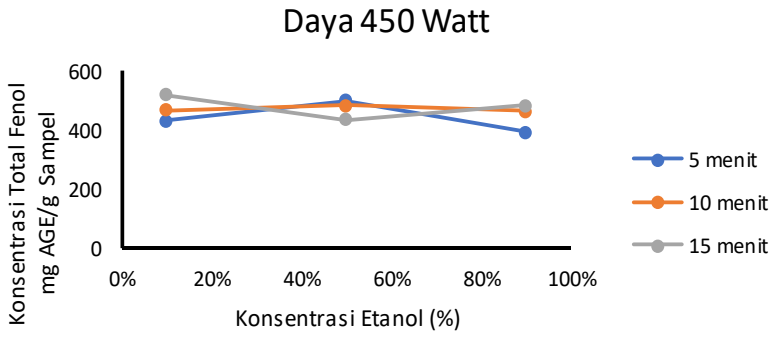

Figure 5. Effect of Variables on Total Phenol Concentration at Power 450 Watt

3.2.4 Analyze with ANOVA

The ANOVA technique was used for statistical analysis in this study. The purpose of the ANOVA analysis was to determine whether the factors used in the pegagan extraction affected the final product, and the variables were considered significant if the p-value of the

ANOVA analysis technique was less than 0.05 (5 percent). The data obtained were used to test the normal distribution as the first stage in the analysis procedure. Before running the ANOVA test, one of the assumptions is that the results of the probability plot on the ANOVA must be normally distributed. The results of the probability test carried out using the Kolmogorov-Smirnov technique can be seen in Figure 4.12 with a p-value of 0.05 and a plot that follows a diagonal line, which shows that the analysis using the Two-way ANOVA general linear model is acceptable.

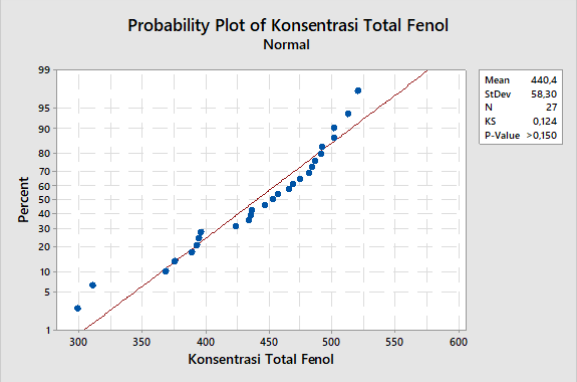

Figure 6. Graph of Normality Test for Total Phenol Content

After the data were normally distributed, ANOVA analysis was performed. The results of the ANOVA analysis are as follows:

Table 2. ANOVA Analysis Results

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Power	2	19386	9693.2	18.72	0.001
Concentration	2	29544	14772.1	28.52	0.000
Time	2	12103	6051.4	11.68	0.004
Time*Concentration	4	10845	2711.3	5.24	0.023
Power*Time	4	3923	980.8	1.89	0.025
Concentration*Time	4	8434	2108.5	4.07	0.043
Error	8	4143	517.9		
Total	26	88379			

The P-value is said to be significant or significant if the P-value <0.05. Based on Table 2, the results of the ANOVA analysis showed that the microwave power, extraction time, and ethanol concentration had a significant effect on the total phenol content with a p-value <0.05. The conclusion obtained in the research model of pegagan plant extract (*Centella asiatica* L.) is that the variables used are variables that have a significant or significant effect on the extract's total phenol content.

Based on the ANOVA analysis carried out, the R square value of 95.31% showed that the model was by following the research results. The value of R square is stated according to the model if the percentage generated is more than 75% (Yingngam *et al.,* 2020), so it can be concluded that the equations used to predict the influential variables are appropriate and can be used to predict the actual results of the study.

4. Conclusion

The effect of ethanol solvent concentration in the process of extracting the bioactive components of pegagan is the higher the concentration of ethanol solvent used does not increase the yield of the resulting extract, based on research that has been carried out the optimum concentration in research is 10%, the maximum total phenol content using ethanol solvent is at conditions operating power of 450 watts, 10% ethanol concentration with a radiation time of 15 minutes which resulted in a total phenol content of 520 mg AGE/g sample. In the ANOVA analysis with ethanol solvent, power, extraction time, and solvent concentration variables gave a significant response to the total phenol content, that is with an R square value of 95.31%.

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