

## Evaluation of Butterfly Pea Flowers for Antioxidant Activity and its Potential as Antioxidant Soap

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**Abstract:** *Clitoria ternatea*, often known as butterfly pea, is a flowering vine plant in the Fabaceae family with oval and acute leaves. Due to free radicals, many severe diseases were occurred such as cancer, diabetes, and cardiovascular and neurological illnesses. Aside from that, if the cellular antioxidant defense mechanism is depleted, free radicals can easily spread. Increasing the antioxidant defense layer in the human body system will eventually aid in lowering free radicals from harming cellular components. Extraction is crucial to obtain its bioactive components which contribute to its antioxidant properties, in which in this study, the extract of *C. ternatea* was received through the process removal of solvent using rotary evaporator. Therefore, this study was performed to evaluate the antioxidant compounds found in *C. ternatea* extract obtained. DPPH assay was conducted in this study as a form of analysis of antioxidant compound in the *Clitoria ternatea* extract, in which *Clitoria ternatea* extract as the sample and ascorbic acid is used as the standard. The absorbance values were measured using UV-Vis spectrophotometer at 517 nm with five different concentrations measured. Though DPPH assay was fully performed, the results obtained from the assay did not fully match with other studies. The comparison of DPPH assay performed during this study was compared to Varsha Jadhav study of antioxidant compound of *Clitoria ternatea*. However, one of the reasons why there were errors in this study might be the extraction method applied for this butterfly pea flowers. Other than that, this study also conducted survey based on the potential antioxidant soap made from *Clitoria ternatea* extract. Once the soap was fully made and packaged, the soaps were distributed among 30 respondents and the respondents were asked to answer a form to study their preferences in soap and feedbacks after using this potential antioxidant soap from *C. ternatea*. In this survey, it is shown that respondents are mostly likely interested in this butterfly pea flowers soap, however, if there were any follow-up questions based on their usage of the respondents for a period of time, it will sum up a significant data of this soap. Lastly, the estimation of cost analysis based on this soap shown that this soap has the potential of commercialized in the market as it can obtain high amount of profit per product sold.

**Keywords:** *Clitoria ternatea*, Antioxidant Compounds, Antioxidant Soap

## 1. Introduction

The focus of this research is on how to analyze the *Clitoria ternatea* flowers extract in terms of antioxidant components and activity as well as to experiment the potential of producing antioxidant soap using *C. ternatea* flowers [1]. Extensive experimental and clinical research has been conducted on the antioxidant activity of edible plants, which includes free radical scavenging, restricting the beginning or propagation of chain reactions, and minimizing cellular molecular [2].

The flowers of *Clitoria ternatea* contain a variety of phytochemical compounds, including flavonoids, and the predominant colorant components are anthocyanins. The blue tint of *Clitoria ternatea* flowers is frequently used as a natural source of food coloring in the preparation of a variety of cuisines. The *Clitoria ternatea* flowers extract will be extracted to characterize its antioxidant components and activities for further processing.

To obtain antimicrobial and antioxidant activity, most commercial soaps use synthetic chemicals such as triclosan and paraben for antimicrobial action, and butylated hydroxyanisole and butylated hydroxytoluene for antioxidant and preservative effects. In 2016, the United States Food and Drug Administration (FDA) has banned 19 antiseptic active ingredients for antiseptic soap products that are used in household setting, including triclosan, as there are potential risks and not identified as GRAS / GRAE (Generally Regarded as Safe / Effective) [3]. Therefore, replacing synthetic with natural antioxidants will have beneficial health effects [4,5]. In healthy human cells, electron pairing with biological macromolecules such as proteins, lipids, and DNA can stabilize free radicals [6]. Aside from that, if the cellular antioxidant defense mechanism is depleted, free radicals can easily spread [7].

Recently, there are strong consumer demand and interest in natural and safe cosmetics and skincare, including antiseptic soaps that are plant-based, biodegradable and free of artificial colorings or preservatives. Increasing the antioxidant defense layer in the human body system will eventually aid in lowering free radicals from harming cellular components.

### 1.1 Objectives

The objective for this study is to perform antioxidant assays of *Clitoria ternatea* extract using 2,2-Diphenyl-1-Picryl-Hydrazyl-Hydrate (DPPH) assay, to produce *Clitoria ternatea* soap using the extract and to survey the perception of *Clitoria ternatea* extracts as soaps by sample distribution and surveys to 30 respondents.

### 1.2 *Clitoria ternatea* and the benefits

*Clitoria ternatea* is commonly known as butterfly pea flowers or *bunga telang* as in Malaysia. Butterfly pea flowers has small size for the diameter of the flowers which are at 2 to 4 cm length. It has diverse shades of blue with a yellow or pure white coloration with a big standard petal. Since it is a vine or a creeper plant, it has a spindly stem. Butterfly pea flowers are also very sensitive to temperature and difference of pH. Hence, the making of food or beverages from butterfly pea flowers can be crucial in the preparation. Although it is very sensitive in temperature and pH changes, it is excellent in growing in either tropical monsoon country or even at a drought area for a long period of time. Different countries had their own naming of *Clitoria ternatea*, such as in Sudan, it is named as Kordofan pea, Cunha in Brazil and Pokindang in the Philippines [8].

Aside from that, *Clitoria ternatea* is known in a naturally self-pollinated flowers, however, due to the determination of genotype segregation, cross-pollination of *Clitoria ternatea* can occur [9]. *Clitoria ternatea* is also a self-seeding plant and can distribute a large number of seeds without any human intervention, when its dry pod crack. Butterfly pea has been found in many tropical and subtropical regions, where it has become naturalized [9]. Due to the attractiveness of the butterfly pea has, it is vulnerable to continuous heavy grazing by animals.

Butterfly pea flower has widely been used as a natural colorant in food. As for example in Kelantan, they used butterfly pea flowers to dye the color of the rice for their well-known dish known as *nasi kerabu*. Butterfly pea flower is also found in many shampoos, conditioners, and other hair care products. *Clitoria ternatea* helps moisturize hair follicles, promotes hair growth, reduces hair fall, and slows greying. Based on the previous studies that have been published on the medicinal side effects of *Clitoria ternatea*, it includes the effect on antipyretic, analgesic, and anti-inflammatory [10]. Butterfly pea's antioxidants can help relax stomach muscles, which aids digestion in human's digestive system. According to several published studies on rats and other animals, the administration of butterfly pea flowers extract daily can increase the levels of acetylcholine in their brain.

In this study, the evaluation of butterfly pea flower was needed to measure the content of antioxidant activity found and with the evaluation, the process of making soap was planned. The antioxidant soap was decided to be made due to the low production in the market, as often sold in the market is antibacterial soap. Based on the research, DPPH assay was performed as a test to measure the amount of antioxidant found in this flower.

### 1.3 Antioxidant Soap

There are a variety of processing methods that can be used in soap production, including kettle boiling, hydrolysis/neutralization, continuous or cold saponification with various fats and feedstocks, all of which result in final products with properties that are more suitable for use in personal hygiene. The quality of the final product was decided by the disagreeable qualities of the finished products, such as objectionable odors, high levels of unreacted caustic soda, and saponified lipids, restricting its usage in personal hygiene.

One of the oldest chemical industries, the soap industry produces cleaning chemicals for the personal skincare, restaurant, food, and laundry industries [11]. Heat, light, and heavy metals, notably copper or iron, promote auto-oxidation, resulting in product discoloration and the creation of undesirable breakdown products with an unpleasant odor. During the soap-making process, saponification is described as the process of combining triglycerides with a strong base to generate fatty acid metal salts [12]. The fatty acid components of toilet soaps and cosmetics are such that they are easily auto oxidized when exposed to oxygen in the air. Based on this study, the soap that was decided to be made is an antioxidant soap made from butterfly pea flower, in which with the content of antioxidant found in this flower would aid in removing free radicals out of one's body.

## 2. Materials and Methods

### 2.1 Materials

Materials that are needed to for the preparation of *Clitoria ternatea* soap is *C. ternatea* extract, soap base containing palm oil, extra virgin olive oil and coconut oil. These oils have different function during the production of soaps. For DPPH assay, the chemicals and standard that are used during the experimental procedure are DPPH powder, methanol, and ascorbic acid standard. UV-Vis spectrophotometer is used to measure the absorbance value.

### 2.2 Methods

#### 2.2.1 Preparation of *C. ternatea* soap

The butterfly pea flower extract was obtained through the process of removing solvent in the rotary evaporator which in this case, the solvent was acetic acid and ethanol with concentration of 100 mg/mL. *Clitoria ternatea* extract (100 mg/ml), soap base and water are the formulation's active components that was used in this soap making process. The formulas for the production process of 30 exact butterfly pea flower soaps with weight is 0.01 gram and size measurement are 3.5 cm x 2 cm x 1 cm each were shown in Table 1 below. Figure 1 shows the picture of the butterfly pea flower soap. For a 1kg of soap base,

300mL of water should be added to the mixture. In this study, 152 g of soap base was used and 46mL of water is added to the mixture. Only 0.01 gram of butterfly pea flower extract was needed for this process as the extract itself are already concentrated and it required to be diluted with water added in the formula. The mixture was melted in the 700 W microwave for 3 minutes. After that, the soap was poured into silicone mold and left to cool at room temperature before placing it into chiller for about 3 to 4 hours to set.



**Figure 1: Butterfly pea flower soap**

**Table 1: The amount needed for preparation of *Clitoria ternatea* soaps**

No	Soap base (g)	Water (mL)	Butterfly Pea Flower extract (g)
1	152	46	0.01

### 2.2.2 *C. ternatea* Soap Evaluation Survey

The butterfly pea flowers soap was distributed beforehand to the users to enable the users to use and evaluate the butterfly pea flowers soap. Then, the survey was given to the user to complete. There are 14 questions that required to be answered and divided into 3 sections which are Section A, Section B (I) and Section B (II).

Section A is to analyze the demographics of the respondents, in which the questions is regarding age and gender of the respondents. Section B (I) is about preferences in daily use of soap as a personal hygiene product. Since COVID-19 still ongoing, people take greater care in personal hygiene to reduce and prevent infections. The use of soap with antiseptic, antibacterial and antioxidant properties are in high demand in the community. This will ensure that not only them are safe from COVID-19 but also their families. This section is to identify what kind of preferences are needed in a soap based on customer's or user's point of view. Last section is Section B (II) in which to gather feedbacks on butterfly pea flower soap after the respondents have the experience to use the soap given to them. In this section, there were satisfaction poll question in which to answer based on fragrance, colours, lather, packaging and shape of the soap. The poll was created on a three-point scales; 1 for Very Satisfied, 2 for Neutral and 3 was for Very Dissatisfied.

### 2.2.3 Analysis of 2,2-Diphenyl-1-Picryl-Hydrazyl-Hydrate (DPPH) scavenging activity.

The antioxidant activity of butterfly pea extracts was measured using UV-Vis spectrophotometry. Firstly, 1mM of DPPH solution was prepared by mixing 0.012 g of DPPH powder to 30mL of pure methanol. The DPPH methanolic solution was then wrapped in an aluminum foil after the mixture fully combined and then stored at the chiller before immediate use.

The ascorbic acid stock solution was prepared at 1 mg/mL concentration. The ascorbic acid stock solution was then diluted using methanol to 5 different concentrations which are 0.05 mg/mL, 0.1

mg/mL, 0.15 mg/mL, 0.20 mg/mL and 0.25 mg/mL. Five different concentration of the butterfly pea flower extract was prepared; which are 10 mg/mL, 15 mg/mL, 20 mg/mL, 25 mg/mL and 30 mg/mL.

Next, the DPPH methanolic solution was added into the test tube respectively to the total volume of 3mL. As for ascorbic acid, 2.9 mL of DPPH methanolic solution was added to 0.1 mL of sample, while 2.5 mL of DPPH methanolic solution was added to 0.5 mL of *C. ternatea* extract. The samples were then mixed using vortex mixer for few seconds before being wrapped with aluminum foil and kept in the dark at room temperature for 30 minutes.

The absorbance of each sample was measure using UV-Vis spectrophotometer at 517 nm. Methanol alone was utilized as a negative control, whereas ascorbic acid (standard) was employed as a positive control. The absorbance of the control and samples is measured, and the percentage DPPH scavenging activity will be determined as:

$$DPPH^{\circ} \text{ scavenging activity (\%)} = 1 - \left[ \frac{(A_{\text{sample}} - A_{\text{blank}})}{(A_{\text{control}} - A_{\text{blank}})} \right] \times 100 \quad Eq. 1$$

Each assay was performed in triplicate.

### 3. Results and Discussion

#### 3.1 The Production of Butterfly Pea Flower Soap

The recipe of the butterfly pea flower soap was 152 gram of soap base, 46 mL of water and 0.01 gram of butterfly pea flower extract without any fragrance added. Based on the recipe, 30 bars of butterfly pea flower soaps can be produced. When mixed with water, lather from the soap was observed. The packaging for this butterfly pea flower soap is crucial as it is small in shape and it requires a small pack for the user's satisfaction in carrying it freely. The packaging was a zipper bag in XS size with specifications of 50 mm x 90 mm x 0.06 mm and the material is PE. Once the soap was ready, the survey will be distributed with the soap. The various of ages and respondents were required to answer a survey, from below 17 years old, 18 till 30 years old and 31 years old above.

#### 3.2 Analysis of Butterfly Pea Flower Soap Based on Survey Distribution

This analysis is to gather the user's perception regarding the butterfly pea flowers soap distribution. Based on the surveys, during the satisfaction poll questions, briefly the respondents were very satisfied with the fragrance (46.7 %), colour (46.7 %), lather (50.0 %), packaging (43.3 %) and shape (36.7 %) of this soap. The questions regarding the effect of using this butterfly pea flowers soap also was asked, in which the respondents answered 'Yes' is 28.6 % and 'Maybe' is 46.4 %. In this survey, it is shown that respondents are mostly likely interested in this butterfly pea flowers soap, however, if there were any follow-up questions based on their usage for a week or two, it will sum up a significant data on how this butterfly pea flower soap acts as a potential antioxidant soap.

#### 3.3 Determination of Antioxidant Compounds through DPPH Free Radical Scavenging Activity

The antioxidant potential of extracts from butterfly pea flowers sample was assessed using a DPPH radical scavenging test. After the DPPH radical was successfully scavenged, it will change the original colour which is purple to yellow. After that, the absorbance will be seen decreasing at 517nm. Based on the Table 3.1 and Table 3.2, it shows the values of absorbance obtained from UV-Vis spectrophotometer of the sample extract of butterfly pea flower and the ascorbic acid. The values of these absorbance were taken in a triplicate value to calculate the average absorbance of each sample.

**Table 2: The value of different concentration for the sample extract of butterfly pea flower vs absorbance**

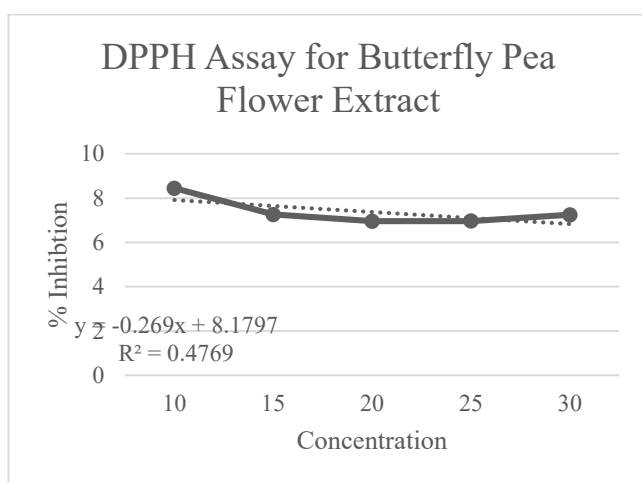
Concentration (mg/mL)	Absorbance (Ab)	Average absorbance (Ab)
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Methanol	0	0	0	0
Methanol + DPPH	-9.9999	-9.9999	-9.9999	-9.9999
10	0.757	0.74	0.736	0.7443333
15	0.633	0.623	0.621	0.6256667
20	0.597	0.595	0.594	0.5953333
25	0.597	0.597	0.596	0.5966667
30	0.625	0.624	0.624	0.6243333

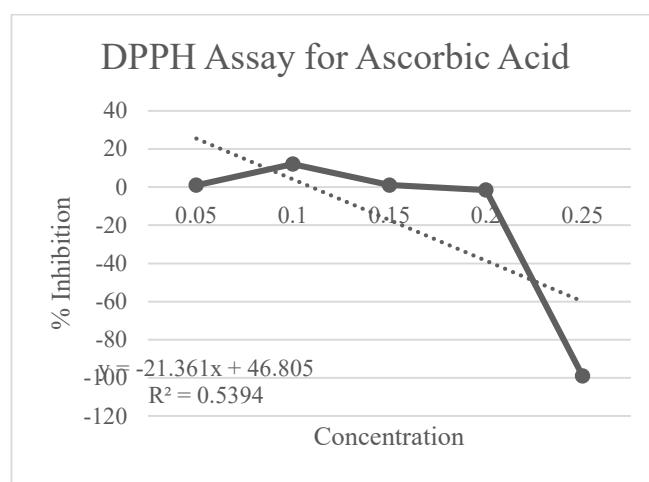
**Table 3: The value of different concentration for the sample of ascorbic acid**

Concentration (mg/mL)	Absorbance (Ab)			Average absorbance (Ab)
Methanol	0	0	0	0
Methanol + DPPH	-9.9999	-9.9999	-9.9999	-9.9999
0.05	0	0	0	0
0.1	1.107	1.109	1.107	1.1076667
0.15	0.007	0.006	0.007	0.0066667
0.2	-0.253	-0.253	-0.253	-0.253
0.25	-9.9999	-9.9999	-9.9999	-9.9999

Based on Figure 2 and 3, the comparison between DPPH assay for butterfly pea flower extract and ascorbic acid shows a very different trendline. However, graph of % inhibition for butterfly pea flower extract still showing a decreasing value of % inhibition. Trendline for DPPH assay for ascorbic acid shows a proper drop from the lowest concentration to the highest concentration.



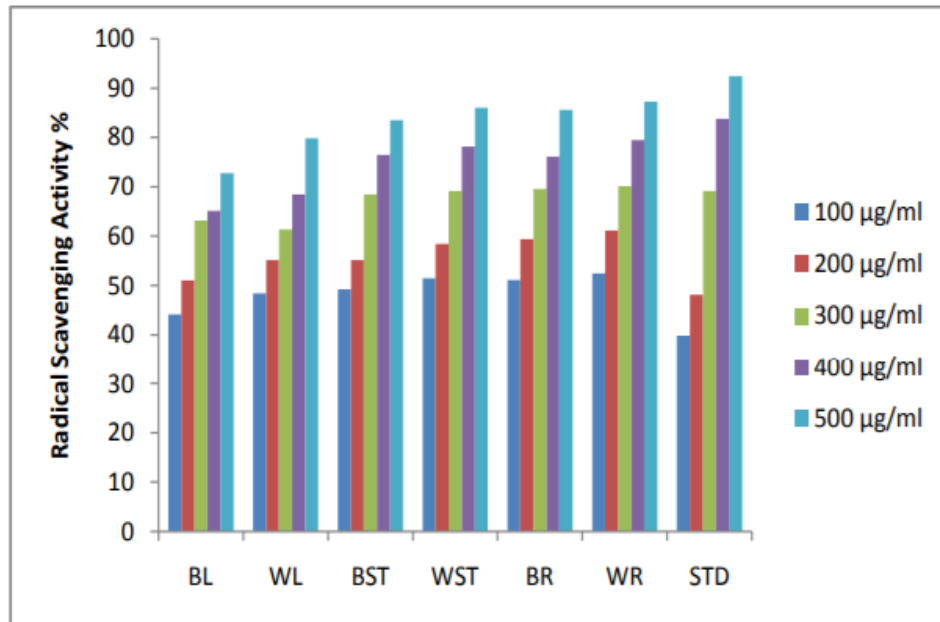
**Figure 2: Graph of DPPH Assay of Butterfly Pea Flower Extract**



**Figure 3: Graph of DPPH Assay of Ascorbic Acid**

However, as to refer in another study from Varsha Jadhav (2013) in Figure 4, it shows that the trendline supposed to be increasing between concentration and % of inhibition.

This error may occur due to the contamination during the process of dilution of concentration between sample extract of butterfly pea flowers and ascorbic acid. Moreover, based on calculation in the journal from Varsha Jadhav (2013), it shows the formula of % inhibition is as followed:



**Figure 4: DPPH radical scavenging activity method. (BL= Blue variety leaves, BST= Blue variety stem, BR= Blue variety root, WL= White variety leaves, WST= White variety stem, WR= White variety root, STD= Ascorbic Acid) [13]**

### 3.4 Estimation of Cost Analysis Based on The Production of Butterfly Pea Flower Soap

The butterfly pea flowers soap has the potential of getting commercialized in the market as an antioxidant herbal soap as it contains the antioxidant compound based in the findings of analysis in DPPH assay. Based in Table 4, it shows the estimation of the product costing of butterfly pea flower soap.

**Table 4: The estimation of the product costing of butterfly pea flower soap**

<b>Variable costs</b>			
<b>Item</b>	<b>Cost</b>	<b>Unit</b>	<b>Amount per unit</b>
Butterfly pea flower extract	RM0.08	1 kg of soap	RM0.08
Soap base	RM0.05	1 kg of soap	RM0.05
Silicon mould	RM0.01	1 kg of soap	RM0.01
Packaging	RM0.15	1 kg of soap	RM0.15
Transportation	RM0.20	1 kg of soap	RM0.20
Electrical power	RM0.50	1 kg of soap	RM0.50
<b>Variable costs per unit:</b>			<b>RM0.99</b>

<b>Fixed costs</b>			
<b>Item</b>	<b>Cost</b>	<b>Period of time</b>	<b>Annual total</b>
Marketing ads		Annual	RM300
Management and administration		Annual	RM1,000
Business registration, fixed tax		Annual	RM500

<b>Items you would need to depreciate</b>	<b>Cost</b>	<b>Useful life in years*</b>	<b>Annual depreciation*</b>
Bottling machine	RM600	10	RM60

<b>Loan you need to amortize</b>	<b>Loan amount</b>	<b>Interest</b>	<b>Payback period in years</b>	<b>Number of payment in a year</b>	<b>Annual amount due</b>
Loan @ 7% interest	RM5,000	7%	3	100	RM1,848.37

<b>Items you would need to amortize</b>	<b>Cost</b>	<b>Useful life in years*</b>	<b>Annual depreciation*</b>
Computers	RM2,000	6	333.3333333
	0		0

**Total fixed costs: RM4,041.70**

Based on the estimation of cost analysis, if the product was sold for 2000 units, then per unit fixed cost is RM 2.02. Cost per unit is calculated by this formula;



$$(\text{Total unit sold} / \text{Total fixed cost}) + \text{Variable cost per unit}$$

If the product is produced reaching 5,700 units, the cost per unit (which included variable and fixed cost) is RM 1.70. If the selling price per unit was set to RM 9.90, thus the profit obtained will be RM 8.91 per unit of soap. Then, if the selling reached 1000 units, the profit is RM 4, 868.00, which will cover the product costing. Based on this estimation cost analysis, this butterfly pea flower soap has the potential to be commercialized in the herbal soap for medicinal benefits.

#### 4. Conclusion

This study is focused on the evaluation of antioxidant activity found in *C. ternatea* and its potential to be commercialized as an antioxidant herbal soap. Though the DPPH antioxidant assay was handled according to published standard protocol, it still did not achieve the same result as other published studies. There is a need to confirm the antioxidant properties from *C. ternatea* using other alternative antioxidant assays such as Ferric Reducing Antioxidant Power (FRAP), Hydroxyl Radical Scavenging Activity (HRSA), Superoxide Radical Scavenging Activity (SRSA), or Total Phenolic Content (TPC) assays. Besides, improvement in extraction method also can enhance the result obtained for the antioxidant compounds testing. Alternative extraction methods can be useful to see whether extraction methods could affect the antioxidant properties of *C. ternatea* extract. Additionally, this study also investigated the potential of producing antioxidant soap from the butterfly pea flower extract. In this survey, it is shown that respondents are mostly likely interested in this butterfly pea flowers soap, however, if there were any follow-up questions based on their usage for a set amount of time, it will sum up a significant data on how this butterfly pea flower soap acts as a potential antioxidant soap. Lastly, based on the estimation of cost analysis for this butterfly pea flower soap, it shown that this soap has the potential for being herbal soap that can be commercialized in the market, with RM 8.91 per unit of product sold. This will be possible if this soap was further analyzed for toxicity and allergen assessment and gain a higher significant data to provide testimonials from the users.

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