

Formulation of Butterfly Pea Flower (*Clitoria ternatea*) Kombucha Gel and Burn Wound Healing Test on White Rabbits

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Abstract. Burns are a condition in which skin tissue is lost or damaged due to contact with a high-risk heat source, requiring immediate medical attention and proper treatment. Fermented butterfly pea flowers using Symbiotic Cultures of Bacteria and Yeasts (SCOBY) produce kombucha that can increase total flavonoids, anti-inflammatory potential, and protection against oxidative damage. Previous studies showed that 2% butterfly pea flower SCOBY kombucha could optimally heal burns. This study aimed to determine the effect of butterfly pea flower kombucha concentration on the physical characteristics of the gel and its burn healing ability in male white rabbits, as well as to determine the best concentration. The research design used was a post-test only control group design. Dried butterfly pea flowers were fermented using SCOBY for 14 days, and the resulting kombucha was then subjected to phytochemical screening. Next, the kombucha was made into gels with concentrations of 10%, 15%, and 20% and tested for burn healing using white rabbits. Second-degree burns were created using the Akhoondinasab method, and gel application was done twice daily for 14 days, followed by observation of burn area reduction and healing percentage. The test results showed that butterfly pea kombucha contains flavonoids, tannins, alkaloids, and triterpenoid steroids. Statistical tests using two-way ANOVA showed that kombucha concentration affects the physical characteristics of the gel and burn healing ability in rabbits. A 20% kombucha concentration produced gel with the best physical characteristics and was able to heal burns by day 11, comparable to the positive control (*Bioplacenton gel*).

1 Introduction

Burns are a condition of skin damage due to tissue loss due to contact with heat sources such as fire, flames, exposure to hot water, contact with hot objects, electric shock, chemicals, and solar radiation. According to the World Health Organization burns are a global public health problem, causing around 180,000 deaths annually [1]. Burns carry a high risk of infection, dehydration, and other serious complications so they require immediate medical attention and can be harmful to the body if not treated properly. The

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main cause of complications and death in burn patients is infection [2]. Therefore, burns face complex problems that require special attention. Wound healing is the process of continuously restoring damaged skin tissue to the body. The wound healing process occurs through four stages: hemostasis, inflammation, proliferation, and maturation [3]. When a wound occurs, inflammation usually occurs as a sign of the body's defense against the infection process. Anti-inflammatory drugs have a therapeutic effect that can inhibit and reduce inflammation. Inflammation can be caused by physical injury, bacterial infection, heat, antibody-antigen interactions, and other factors. The mechanism of action of anti-inflammatory drugs is by inhibiting the release of prostaglandin hormones into damaged tissues [4].

Butterfly pea flower (*Clitoria ternatea*) is a plant rich in anthocyanins, flavonoids, and potent polyphenols [5]. Flavonoid compounds have potential due to their anti-inflammatory and antibacteria properties [6]. In a previous study, topical application of 10% butterfly pea flower extract gel can heal burns by significantly affecting STAT3 expression in Wistar rats suffering from thermal burns [7]. Furthermore, butterfly pea flowers were fermented using Symbiotic Culture of Bacteria and Yeast (SCOBY) to produce kombucha which causes the bioconversion of these compounds into bioactive molecules with higher stability and bioavailability, and increases total flavonoids thereby strengthening the anti-inflammatory potential and protection against oxidative damage [8]. The organic acids in kombucha can stabilize flavonoids, increasing their bioavailability. The benefits of the kombucha fermentation process include serving as a source of antibacterial, anti-inflammatory, and antioxidant properties, as well as playing a vital role in improving intestinal microflora and anticancer properties [9]. A 2% kombucha sample showed potential anti-inflammatory activity with an IC₅₀ value of 140.22 ppm. Kombucha from butterfly pea flowers (*Clitoria ternatea L.*) has a high flavonoid content, which is known to have antioxidant and anti-inflammatory effects [10]. Based on the description above, research was conducted to determine the effect of butterfly pea flower kombucha gel as a burn wound healer.

2 Method

This research uses the true experimental method (**Fig. 1**). Some of the tools used in this study are. Analytical balance (Santorius 2402), pH meter, glassware (Iwaki Pyrex), water bath, test tube, mirror tube, dropping plate, chamber, Silica Gel GF 254 plate, 254 nm UV lamp, rabbit cage, adhesiveness tester, spreadability tester, Brookfield viscometer, mortar, stamper, probe, climatic chamber.

The materials used in this study were SCOBY or initial kombucha culture, dried butterfly pea flowers, butterfly pea flower pollen, male white rabbits, bioplacenton gel, Carbopol, TEA, propylene glycol, glycerin, nipagin, nipasol, and distilled water. Materials for the color reaction test: K₃[Fe (CN)₆], FeCl₃, Mg powder, HCl (p), amyl alcohol, 1% FeCl₃, 2N HCl, Dragendorff's reagent, Mayer's reagent, anhydrous acetic acid, concentrated H₂SO₄. Materials for TLC: a. Eluents: butanol: glacial acetic acid: water (4:1:5), butanol: glacial acetic acid: water (4:1:5). Alkaloids: methanol-NH₄OH(p) (200:3). b. Spotted Appearance, ammonia (P) vapor, iron (III) chloride and Dragendorff.

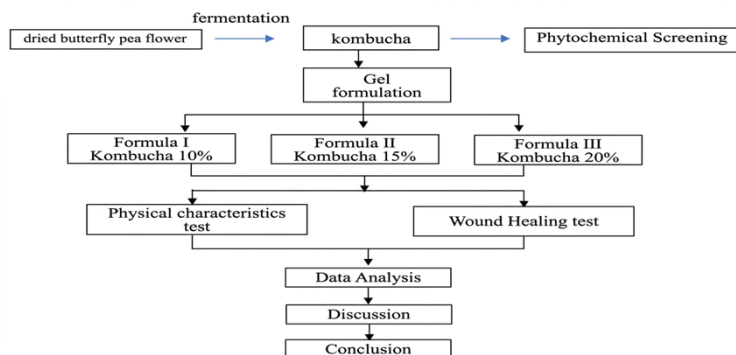


Fig. 1. Flow chart of the research

The research design uses an experimental research method with a post-test only controlled group design. The object used in this study was the effect of butterfly pea flower kombucha on skin burns in rabbits injured/ traumatized by a hot iron with the following stages:

2.1 Preparation of Kombucha

Dried butterfly pea flowers, whole and finely ground, are packaged in a 1-gram tea bag and brewed with 250 ml of water at 75°C for 9 minutes. The resulting butterfly pea tea is stored in a sterilized glass jar with the addition of 40% sugar. The butterfly pea tea is left to reach room temperature for 2 hours. The starter and SCOBY are added to the glass jar after the tea reaches room temperature ($\pm 28-30^{\circ}\text{C}$). Cover with cloth and store in a place away from direct sunlight for 10-14 days [11].

2.2 Phytochemical screening

Phytochemical screening is a cost-effective lab method to detect and identify major groups of natural compounds (phytochemicals) like alkaloids, tannins, flavonoids, saponins, and phenols in kombucha, revealing potential medicinal properties. This screening uses chemical reaction and TLC methods.

2.3 Formulation butterfly pea flower kombucha gel

Table 1. Composition of kombucha gel

Ingredient	Formula			Function of Ingredient
	I	II	III	
Kombucha	10%	15%	20%	Active ingredient
Carbopol 940	2%	2%	2%	Gelling agent
Triethanolamine (TEA)	2%	2%	2%	Alkalizing agent
Propylene glycol	10%	10%	10%	Co-solvent
Nipagin	0.1%	0.1%	0.1%	Preservative
Glycerin	5%	5%	5%	Humectant
Essence rosae	Qs	Qs	Qs	Perfume
Distilled water ad	100	100	100	Solvent

The research variables are as in this research, butterfly pea flower kombucha is made in gel form with concentrations of 10%, 15% and 10% with the composition as show in **Table 1**.

Carbopol 940 was developed in 10 parts of hot distilled water in a mortar, TEA was added and homogenized. Propylene glycol, which had previously been dissolved in hot distilled water at 90°C, was added and stirred until homogeneous. Butterfly pea flower kombucha was mixed with glycerin, added to the base, and the remaining water was added to the base and stirred until homogeneous.

2.4 Physical characteristics test of kombucha gel

- a. Test organoleptic
Observations of the preparation included the aroma, color, and texture of each gel formulation, which were observed three times.
- b. Homogeneity
The preparation was tested using two slides, with the sample placed on one slide and spread evenly. A good preparation should be homogeneous and free of clumped particles.
- c. Spreadability test
This test was conducted by weighing 0.5 grams of gel, placing it in a round glass, and placing the other slide on top of it, and leaving it for 1 minute. Afterward, 150 grams of weight was added, left for 1 minute, and the constant diameter was measured.
- d. Adhesion test
The adhesion test was conducted by weighing 0.5 grams of gel, placing it in the center of a glass slide, covering it with another glass slide and applying a 1 kg load for 5 minutes. The glass slide was then mounted on a tester with an 80 grams load, and the time required for the two glass slides to detach was measured.
- e. Viscosity
The viscosity test was conducted using a Brookfield viscometer by immersing the viscometer spindle in 100 grams of the sample placed in a beaker at an appropriate speed. The viscosity of the sample was measured on the scale inside the instrument after stability was achieved. To assess the stability of the sample, this viscosity test was performed three times over a period of one month.
- f. Acidity testing (pH test)
The pH test was conducted using universal pH paper dipped into the diluted gel sample. The color change was compared with a universal pH standard. Gel preparations generally have a pH between 4 and 6.
- g. Physical stability testing
The physical stability of the preparation was tested using a cycling test. The butterfly pea flower kombucha gel preparation was stored at 4 °C ± 2 °C for 24 hours (this treatment represents one cycle). The experiment was repeated six times. The physical condition and pH of the preparation were compared before and after the test.
- h. Preparation and treatment of experimental animals: White rabbits
This was a true experimental study with a post-test only control group design. The inclusion criteria were male Wistar rabbits, normal body weight (150-220 grams), 8-

12 weeks old before adaptation for 7 days, and visual observations showed healthy, active, and no anatomical abnormalities.

2.5 Wound healing test

This was a true experimental study with a post-test only control group design. Inclusion criteria were male Wistar rabbits with normal body weight (150-220 grams), 8-12 weeks of age, and a 7-day adaptation period. Visually, the rabbits appeared healthy, actively moving, and showed no anatomical abnormalities. Exclusion criteria included weight loss >10% (less than 150 grams), diarrhea during the study period, behavioral changes (refusal to eat, lethargy), or death during the study. After 24 hours (day 8), the rabbits were shaved in five sections, and their backs were smeared with 10%, 15%, and 20% kombucha gel from butterfly pea flowers, as well as gel base (control -) and Bioplacenton gel (control +). The wound healing process was observed from day 1 to day 14.

2.6 Data analysis

All results of the gel's physical characteristics and burn healing calculations and percentages were recorded and processed using statistical tests in Microsoft Excel and Statistical Product and Service Solutions (SPSS). This study conducted statistical analysis to test comparative hypotheses for numerical measurement scales using more than two unpaired data sets. If the data distribution was normal according to the Shapiro-Wilk normality test, a one-way ANOVA test was used. If the data distribution was not normal, the Kruskal-Wallis test was used. The data obtained in the study were then tested using a two-way ANOVA test using SPSS version 23.

3 Results and discussion

The results of making butterfly pea flower kombucha can be seen in **Fig. 2**. The kombucha is bluish-purple in color, with a watery consistency and a pH of 2.05. The resulting kombucha was separated from the SCOBY and phytochemical screening was carried out to determine the compounds contained in it. The test results showed that butterfly pea flower kombucha contains flavonoids, tannins, alkaloids, and steroid triterpenoids. The flavonoid content is thought to play a role as an antioxidant, antibacterial, and anti-inflammatory agent, which is very important in wound healing [6]. The kombucha gel were prepared in 3 formulas, as shown in **Table 1**. All the gel were blue, clear and transparent (**Fig. 2**). Visual organoleptic testing of the gel preparations revealed a blue color in all three gels, with the higher kombucha concentration resulting in a stronger/deeper blue color. This is due to the presence of anthocyanins, whose color is influenced by pH; the more acidic the solution, the stronger the color.



Fig. 2. Butterfly pea flower kombucha

Kombucha with enrichment of *Clitoria ternatea* kombucha. It shows that the more kombucha added, the more intense its color formed. Gel with the addition of *Clitoria ternatea* kombucha (w/w) is shown in **Fig. 3**. It can be seen that the more kombucha added, the more purple the color becomes. The pH of butterfly pea flower kombucha after 14 days of fermentation reaches around 2. This acidity is influenced by the activity of probiotics that convert sugars into organic acids, while also stabilizing the anthocyanins (blue pigments) of the butterfly pea flower. The fermentation duration has a significant effect on lowering the pH value of kombucha over time. The longer the fermentation (typically 7-14 days), the higher the activity of microorganisms (yeast and acetic acid bacteria) in converting sugars into organic acids (acetic acid & gluconic acid) [12]. Results of the test characteristics of butterfly pea flower kombucha gel is shown in **Table 2**.

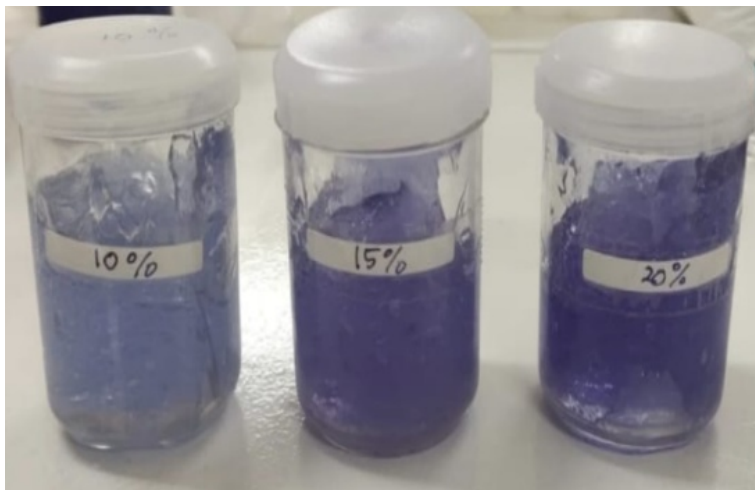


Fig. 3. Butterfly pea flower kombucha gel (10%, 15%, 20%)

Table 2. Results of the test characteristics of butterfly pea flower kombucha gel

Evaluation	Concentration of butterfly pea flower kombucha in the gel		
	10%	15%	20%
Organoleptic			
Form	Gel	Gel	Gel
Color	Blue +	Blue ++	Blue +++
Odor	Rose	Rose	Rose
Homogeneity	Homogeneous	Homogeneous	Homogeneous
pH	5.465±0.167	5.318±0.100	5.218±0.064
Spread ability (cm)	3.60±0.286	3.90±0.037	4.05±0.206
Adhesion Test (sec)	1.21±0.074	1.17±0.041	1.12±0.047
Viscosity (cP)	31365±1595.01	30730±830.60	28623±1473
Cycling test (3 cycles)	Stable	stable	Stable

The homogeneity test for the gel preparations showed that all three formulations were homogeneous because the particles were evenly distributed. Increasing the kombucha concentration in the gel did not affect the homogeneity.

The pH test results showed that all three formulas met the skin pH requirements of 4.5-6.5, making them safe to use. Increasing the concentration of butterfly pea flower kombucha would lower the pH of the preparation. This occurs because the pH of kombucha is very acidic, namely 2.05. The pH test data obtained were analyzed using statistics, obtained normally distributed data with a significance value ($p>0.05$) and the data obtained were homogeneous with a significance value ($p<0.05$). Continued with the parametric test, a significance value ($p<0.05$) was obtained, which means there was a difference between groups.

Spread ability testing to determine the ability of the gel to spread on the skin. The wider the distribution diameter, the greater the contact area of the active ingredient with the skin. Based on **Table 2**, it shows that the higher the concentration of kombucha in the gel, the higher the spread ability because kombucha is a thin liquid, so increasing the concentration of kombucha will reduce the viscosity of the gel and the spread ability will be greater. The spread ability test data obtained were analyzed using statistics, obtained normally distributed data with a significance value ($p>0.05$) and the data obtained were homogeneous with a significance value ($p<0.05$). Continued with the parametric test, a significance value ($p<0.05$) was obtained, which means there was a difference between groups.

Adhesion testing aims to determine the gel's ability to adhere. High adhesion of the gel results in a longer adherence time to the skin, thus prolonging penetration time, allowing for greater absorption of the active ingredient and maximizing the burn healing effect. Based on **Table 2**, it shows that the higher the concentration of kombucha in the gel, the lower the adhesion. The adhesion test data obtained were analyzed using statistics, obtained normally distributed data with a significance value ($p>0.05$) and the data obtained were homogeneous with a significance value ($p<0.05$). Continued with the parametric test, a significance value ($p<0.05$) was obtained, which means there was a difference between groups.

The viscosity test results showed a decrease in viscosity with increasing kombucha concentration. This occurs because the resulting butterfly pea flower kombucha is

thinner than the gel base. Based on **Table 2**, it shows that the higher the concentration of kombucha in the gel, the lower the viscosity. The viscosity test data obtained were analyzed using statistics, obtained normally distributed data with a significance value ($p > 0.05$) and the data obtained were homogeneous with a significance value ($p < 0.05$). Continued with the parametric test, a significance value ($p < 0.05$) was obtained, which means there was a difference between groups.

To ensure stability, a cycling test was carried out with 3 cycles and then physical characteristics tests such as pH, spreadability, adhesion and viscosity. The conclusion was that the butterfly pea flower kombucha gel was stable, as the results of these physical characteristics did not differ statistically significantly.

Table 3. Burn wound diameter

Treatment	Replication	Burn wound diameter (mm)		
		day-1	day-6	day-11
Control -	1	20	19	17
	2	20	19	15
	3	20	18	16
	4	20	18	16
Control +	1	20	11	0
	2	20	10	1
	3	20	11	0
	4	20	10	0
Gel kombucha 10%	1	20	13	5
	2	20	14	4
	3	20	14	4
	4	20	13	5
Gel kombucha 15%	1	20	13	3
	2	20	12	3
	3	20	14	2
	4	20	14	3
Gel kombucha 20%	1	20	11	0
	2	20	11	0
	3	20	11	1
	4	20	10	0

The purpose of a burn healing test is usually to create a standard burn wound on test animals (mice or rabbits), apply the preparation, and then observe parameters such as the percentage of healing, the rate of wound closure, and histopathological changes over a specified period. This is compared with a negative control (ointment base) and a positive control (a standard medication such as Bioplacenton).

This study used four male rabbits weighing 2.5-3 kg. Each rabbit was given five second-degree burns. Kombucha gel of 10%, 15%, and 20%, along with a control (bioplacenton gel) and a control (gel base) applied twice daily for 14 days until all burns healed. The diameter of the burns was recorded from day one until healing; the healing process took 14 days. The wound diameters were measured on days 1, 6, and 11, and the average healing percentage was calculated. The burn wound diameter results are shown in **Table 3**.

Butterfly pea flower kombucha tea gel has high potential to accelerate burn healing due to its high content of active compounds such as flavonoids, tannins, alkaloids, and

steroid triterpene. This combination promotes skin regeneration, with studies showing optimal healing of burns occurring by day 11. Data on burn wound healing diameter followed by the calculation of the average % burn wound healing as shown in **Table 4**.

Table 4. Average wound healing (%)

Evaluation	Average % burn wound healing		
	day-1	day-6	day-11
control negative	0	7.5	14.38
control positive	0	47.5	98.75
Gel 10%	0	32.5	77.50
Gel 15%	0	33.75	86.25
Gel 20%	0	46.25	98.75

The average wound healing percentage data on days 1, 6, and 11 were analyzed using statistics, obtained normally distributed data with a significance value ($p > 0.05$) and non-homogeneous with a significance value ($p > 0.05$). Continued with a non-parametric test, obtained a significant value ($p < 0.05$), which means there is a difference between groups. This means that there is an influence of the concentration of butterfly pea flower kombucha in the gel on the average percentage of wound healing. This is shown more clearly in the graph of average wound healing in **Fig. 4**.

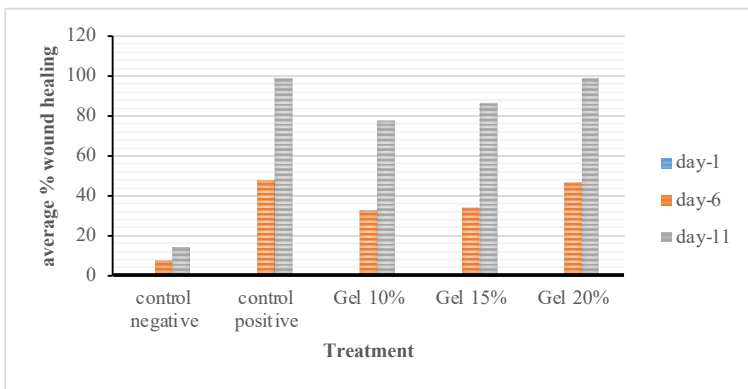


Fig. 4. Average % wound healing

Fig. 4 shows that the concentration of butterfly pea flower kombucha in the gel affects the average percentage of wound healing and the speed of healing. Statistics show that increasing the concentration of kombucha significantly increases the average wound healing rate. This is because the higher the concentration of kombucha in the gel, the greater the amount of anti-inflammatory flavonoids, which can accelerate wound healing. The gel containing 20% kombucha has the same wound healing ability as the positive control, namely Bioplacenton gel, which is widely known as a potential burn healing drug.

4 Conclusion

The study demonstrates that incorporating telang flower (*Clitoria ternatea*) kombucha into a gel formulation provides significant therapeutic activity for the healing of second-degree burns. It was observed that increasing the concentration of butterfly pea flower

kombucha directly influences both the physical properties of the gel and its overall efficacy in treating burn wounds in rabbit models. Notably, the formulation containing a 20% concentration of kombucha yielded the most optimal physical characteristics and achieved complete wound healing by the 11th day, a result equivalent to the positive control (Bioplacenton gel).

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