

## **ANTIBACTERIAL EFFECTIVENESS (*Propionibacterium acnes*) OF KOMBUCHA FACE TONER FORMULA WITH GREEN TEA LEAF (*Camellia sinensis* L.)**

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### **ABSTRACT**

Acne is a process of chronic inflammation of the sebaceous glands in the skin caused by the bacteria *Propionibacterium acnes*. Approximately 80-100% incidence of acne occurs in adolescents. One solution is to use an antibacterial agent called catechins. It can be easily obtained from green tea, which has high catechin content. The amount of catechins used as antibacterial substances in green tea can also be increased by fermentation. In addition, lactic acid-fermented green tea can inhibit the growth of *P. acnes*. This study aimed to produce green tea kombucha face toner with anti-acne activity and good physical stability. This study included an experimental research. The research population was in the form of antibacterial compounds in face toner. The sample was green tea kombucha, which showed antibacterial activity at a concentration of 50% with an 18 mm Diameter of Inhibition Zone (DIZ). And the best formula is F1 with 0.84 mm DIZ, has a pH of 4 which is safe for the skin, clear solution, has clean power, it is also stable during storage at 30° and 40°C, but it has a rather disagreeable scent of toner.

**Keywords:** Acne, Antibacterial, Green Tea Leaves Kombucha, Face Toner

### **INTRODUCTION**

Acne is characterized by chronic inflammation of the sebaceous glands in the skin. In areas such as the face, neck, back, and shoulders, acne lesions usually develop because of the presence of sebaceous glands. Acne mostly from teens but also happens at all ages; it even becomes a psychological torture for one who has it. Approximately 80–100% of acne occurs in young adults, with age of 14-17 years old for women, and men between 16-19 years old (Ramdani, 2015) (Hanna, 2003).

For decades, active substances that have been used in anti-acne preparations include benzoyl peroxide, resorcinol/resorcinol monoacetate, sulfur, and isotretinoin. However, these active substances have side effects, such as bleaching hair, skin irritation, dry skin, and sensitive skin, have an odorous effect, and can cause systemic toxicity with the formation of reversible brown scales on the skin. Isotretinoin can even cause pregnancy disorders, which result in birth defects due to the disruption of organ or tissue development during pregnancy. Therefore it is necessary to develop potential alternative ingredients to be developed as anti-acne (Reveny, 2017), (Khanza, 2017).

Tea containing epigallocatechin, epigallocatechin-3-gallate, epicatechin-3-gallate, and epicatechin was found to be the most prominent, accounting for up to 30% of the total dry weight of fresh tea leaves. Various amounts of catechins are found in various types of local teas, such as white tea, green tea, oolong tea, and black tea. The highest catechins were found in white tea with a component of 13.22% (w/b) and green tea with 12.95% (w/b).

Therefore, in this study, green tea was used as the main raw material because its catechin content is not much different from that of white tea and has a more affordable purchase price. Catechins in tea have antibacterial activity and their levels increase when fermented (Jayabalan, 2014).

One of the fermented products is kombucha. It is a fermented tea with the addition of sugar and a symbiotic culture of bacteria and yeast (SCOBY). Kombucha broth has also been shown to exhibit antibiotic and antifungal activities. Research has shown that green tea kombucha has higher antimicrobial activity than black tea kombucha. At the time of fermentation, apart from lactic acid, acetic acid, other organic acids, and catechins, it was shown to contain a large amount of protein, which is known to specifically prevent various Gram-positive and Gram-negative microorganisms. The content of phenolic compounds in fermented tea (kombucha) increases due to an increasingly acidic environment and stimulation of enzymes by bacteria and yeast (Watawana, 2015), (Setyamidjaya, 200).

This study aimed to produce green tea kombucha face toner with anti-acne activity and good physical stability. Therefore, in this study, green tea leaves were fermented to obtain the maximum antibacterial activity from catechins. Natural ingredients, such as bacteria, when used in cosmetics, have low toxicity, high bioavailability, biodegradability, and zero waste; they are some of the advantages of using natural bacterial ingredients. Fermented products contain lactic acid, which is very good for the body and skin, one of which acts as an antibacterial agent on the skin by inhibiting the growth of acne-causing bacteria, *Propionibacterium acnes*, and plays a role in exfoliating dead skin (Anggraini, 2017); (Effendi, 2010).

A facial refresher (face toner) is the first step in any cosmetic treatment procedure. A soft and light formula on the skin and simple use are the advantages of using a toner that many toner users like the most, especially those with oily and acne-prone facial skin, mostly teenagers. Currently, the face toner is not only used as a facial skin refresher and temporarily closes skin pores, but also as an anti-acne on the skin.

## RESEARCH METHODS

This was an experimental study using a completely randomized design (CRD) with 12 treatments and 3 repetitions. The data obtained were the results of the analysis of lactic acid bacteria on green tea leaf kombucha and the results of the green tea leaf kombucha face toner inhibition test against *Propionibacterium acnes*. The population in this study was the antibacterial compound used in the face toner preparation formula. The sample in this study was green tea leaf kombucha, an anti-acne ingredient.

### Tools and materials

The tools used include; autoclave (Labtech), stir bar (Pyrex), spreader rod (Pyrex), beaker glass (Pyrex), petri dish (Pyrex), Erlenmeyer (Pyrex), incubator (Mettler), OSE wire (Usbeck Germany), caliper (MITUTOYO), magnetic stirrer (Vitalab), micropipette (Socorex), oven (Mettler), pipette (mico), universal pH paper (Sun-care), centrifugator (DM0412 DLAB), test tube (Pyrex), vertical glass tube (Pyrex), and analytical balance (Newtech).

Materials used include; distilled water (PT. Dipa Husada), *Camellia sinensis* L. green tea leaves (Tea Heaven), sugar (Rose Brand), bacterial isolates (*Acetobacter xylinum*) and yeast (*Saccharomyces sp.*), clindamycin 1% phosphate (Medi-Klin), 0.9% NaCl, nutrient broth (NB) (Merck), nutrient agar (NA) (Merck), and *Propionibacterium acnes*.

### Research Procedure

#### 1. The making of green tea kombucha and scoby (symbiotic culture of bacteria and yeast)

The green tea was weighed as much as 9% and then heated to 250 mL water until it reached a temperature of 78 °C, and green tea was added while stirring. The mixture was allowed to stand for 2 minutes then filtered and placed in a glass vessel. Granulated sugar (10 %) was added and homogenized with a stirrer bar until homogeneous. Allowed to

stand at 30-35°C then added culture of bacteria (*Acetobacter xylinum*) as much as 10%, and yeast (*Saccharomyces* sp.) as much as 10% grew well at 30 °C and incubated at 30-37°C for 48 hours (Sutarmi, 2005).

2. The making of a green tea kombucha solution

Green tea was weighed as much as 9%, and 1500 mL of water was heated to 78 °C, and green tea was added while stirring. The mixture was allowed to stand for 2 minutes then filtered and placed in a glass vessel. Then, 10% granulated sugar was added, and the mixture was homogenized. The samples were allowed to stand at room temperature 30-35°C then put in scoby tea mushrooms (symbiotic culture of bacteria and yeast). Add 15% kombucha starter culture, stir, cover with gauze, tie the lid with a rubber band and ferment for 14 days (Sutarmi, 2005).

3. The making of variations in the concentration of green tea leaf kombucha solution

The green tea leaf kombucha solution (50 mL) was added to 100 mL of distilled water at a concentration of 50%. Green tea leaf kombucha solution (25 mL) was added to 100 mL distilled water at a concentration of 25%. Green tea leaf kombucha solution (12.5 mL) was added to 100 mL distilled water at a concentration of 12.5%. Take 6.25 mL of green tea leaf kombucha solution and add distilled water to a final volume of 100 mL at a concentration of 6.25 %. Take 3.12 mL of green tea leaf kombucha solution and add distilled water to a final volume of 100 mL, at a concentration of 3.12%. Take 1.56 mL green tea leaf kombucha solution was added to 100 mL green tea concentration of 1.56 %. Take 0.78 mL of green tea leaf kombucha solution and add distilled water up to 100 mL for a concentration of 0.78 %.

4. Antibacterial activity test of green tea leaf kombucha solution

Nine treatments were performed, with three repetitions of each treatment. The five treatments consisted of various concentrations of fermented green tea kombucha and a negative control using a green tea solution before fermentation. Variations in concentrations of 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, and 0.78% were carried out using a green tea kombucha solution diluted using Aquadest (BPOM, 2014), (Effendi, 2014).

5. The minimum inhibition concentrations (MIC) test of green tea leaf kombucha solution

The solid dilution method was used to determine the MIC values. *Propionibacterium acnes* test bacteria were taken with an ose needle to make a bacterial suspension. Prepare 10 mL of NB media in a test tube and inoculate each 0.1 mL of bacterial suspension and 1 mL of green tea leaf kombucha and vortex until homogeneous for 24 hours and at 37°C and observe. Determined as MIC if there was still bacterial growth on NB media in the test tube with the smallest concentration. MBC was determined if there was no bacterial growth on NB medium with the smallest concentration.

Nutrient Agar medium (10 mL) was added to a sterile Petri dish and allowed to solidify as a basic ingredient. Five cylindrical cups were inserted septically into Petri dishes. A total of 15 mL of nutrient agar medium was mixed with 0.1 mL of *Propionibacterium acnes* bacterial suspension, then allowed to solidify. Then, five scavengers on the media were removed septically and 5 wells were formed. Then, 20 µL was dripped into the wells formed, each variant of green tea leaf kombucha concentration with the MIC value of the two types of *Propionibacterium acnes* bacteria. The Petri dishes were incubated at 35 °C for 24–48 hours in the treated petri dish. The clear area formed was measured with a caliper, and the Diameter of Inhibition Area (DDH) was measured in millimeters (mm) (BPOM, 2014) (Effendi, 2014).

6. Test the inhibitory power of kombucha face toner preparations from green tea leaves against *Propionibacterium acnes* bacteria).

A Completely Randomized Design (CRD) is the design used in this test. The variation in the green tea leaf kombucha face toner formula was obtained from the concentration determined based on the MIC in the inhibition test of the green tea leaf kombucha solution against *Propionibacterium acnes* bacteria.

NB media were prepared in test tubes, inoculated with 0.1 mL of bacterial suspension and 1 mL and kombucha green tea leaves and vortexed until homogeneous for 24 hours at 37°C and observed. Determined as MIC if there was still bacterial growth on NB media in the test tube with the smallest concentration. MBC was determined if there was no bacterial growth on NB medium with the smallest concentration.

Approximately 10 mL of nutrient agar medium was placed in a sterile petri dish and allowed to solidify (as a base material). Five cylindrical cups were inserted septically into Petri dishes. A total of 15 mL of nutrient agar medium was mixed with 0.1 mL of *Propionibacterium acnes* bacterial suspension and then allowed to solidify. Then, five scavengers on the media were removed septically and 5 wells were formed. Then, 1 mL of each variant F0 (negative control), F1, F2, F3, and F4 were used as positive controls (face toner with the active ingredient clindamycin phosphate 1%).

The Petri dishes were incubated at 35 °C for 24–48 hours in the treated petri dish. If a clear area is formed, bacterial inhibition occurs. The clear area was measured with a caliper, and the Diameter of Inhibition Zone (DIZ) was measured in millimeters (mm) (Nuralifah, 2018); (Fardin, 2016).

#### 7. Formulation of green tea kombucha face toner

Green tea leaf kombucha toner was formulated by adding the main ingredient to the concentration obtained from the antibacterial activity test of the green tea leaf kombucha solution with the best ability to inhibit acne-causing bacteria.

**Table I. Green Tea Leaf Kombucha Formula**

Materials	Variations Of Concentration Formula					Fungsions
	F0	F1	F2	F3	F4	
Green Tea Kombucha	-	X	(0.5)X	F1+F2	-	Antibacterial
Clindamisin fosfate	-	-	-	-	1	Positive control of antibacterial
Allantoin	0,5	0,5	0,5	0,5	0,5	<i>Shooting agent</i>
Gliseril	5	5	5	5	5	Humectan
Water	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100	Solvent

Information:

F0 : Is a negative control with a toner base composition

F1 : Is a face toner formula with an active ingredient concentration of X% KHM)

F2 : Is a face toner formula with a concentration of active ingredients (0.5 x X%)

F3 : Is a face toner formula with a concentration of active ingredients (F1 + F2)

F4: Is a face toner formula with a concentration of the active ingredient clindamycin phosphate 1%

## RESULTS AND DISCUSSION

### 1. Fermented Kombucha Green Tea Leaves

This study used the active ingredient, kombucha green tea leaves, which were fermented for 14 days, and the pH and Diameter of Inhibition Zone (DIZ) test. Subsequently, simple pasteurization was carried out at 45-50°C for 5 minutes. Simple pasteurization aims to kill *Acetobacter xylinum* and the yeast *Saccharomyces cerevisiae* present in green tea leaf kombucha after the desired secondary metabolites are produced as one of the target substances that act as antibacterial agents.



**Figure 1. Production of Green Tea Leaf Kombucha**

According to the journal (Wistiana, 2015) and (Indriyani, 2018) the amount of yeast and lactic acid bacteria increased on the 14th day, the total phenol content increased on the 14th day, and the total content of gluconic acid and other organic acids increased from day 14th day -6 and decreased on the 30th day. So it can be said that the effective duration of green tea leaf kombucha fermentation to obtain antibacterial activity like that causes acne like *Propionibacterium acnes* and *Staphylococcus aureus* is 14 days.

## 2. Green Tea Leaf Kombucha pH Test

Green tea leaf kombucha was measured for its pH value periodically on days 0th, 6th, and 14th day using a universal pH indicator to observe the increase in pH during the fermentation process.

**Table II. Green Tea Leaf Kombucha pH Results**

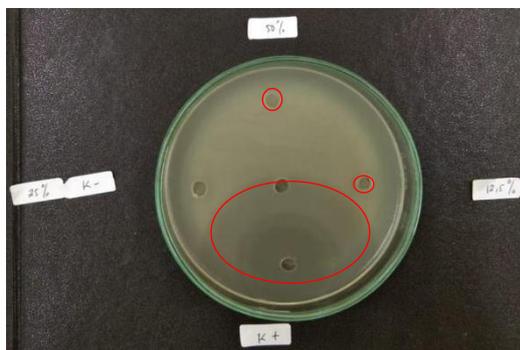
pH test day	pH value
Day-0	4
Day-6	3
Day14	3

These results indicate that pH decreases as fermentation progresses. This is due to the increased production of organic acids during the fermentation process, such that the pH of green tea leaf kombucha is lower than 4 on the pH test on day 0 and pH 3 on day 14.

## 3. Results of Determination of Minimum Inhibitory Concentration

MIC determination was carried out with 5 treatments with 2 repetitions for each treatment. The eight treatments consisted of various concentrations of fermented green tea kombucha, and a positive control of clindamycin solution. Minimum inhibitory concentration (MIC) was determined using the solid dilution method. Then, the test bacteria were inoculated and incubated at the optimum temperature in an anaerobic vessel. The distribution of active ingredient concentrations was determined at dilutions of  $10^{-2}$ ,  $10^{-4}$ , and  $10^{-5}$  for all concentration variants, and the positive control clindamycin was distributed at concentrations of 10-1 and 10-2.

The zero colonies that grew on the petri dish were determined as the Minimum Inhibitory Concentration (MIC) of green tea leaf kombucha in inhibiting the growth of *Propionibacterium acnes*.



**Figure 2.** Minimum Inhibitory Concentration Test of Green Tea Leaf Kombucha

**Table III.** MIC Value of Green Tea Leaf Kombucha

Green Tea Leaf Kombucha Concentration	Turbidity Value Before Incubation <i>Propionibacterium acnes</i>	Turbidity Value After Incubation <i>Propionibacterium acnes</i>
50	-	-
25	-	+
12,5	-	++
6,25	-	++
3,12	-	++
1,56	-	++
0,78	-	++
K+	-	-

Information:

- + (positive) = bacterial colony growing
- (negative) = bacterial colonies do not grow
- ++ = cloudy
- + = slightly cloudy
- = clear

These results indicate that green tea leaf kombucha can inhibit the growth of *Propionibacterium acnes* bacteria at the lowest concentration of 50%. This MIC value is the reference for the concentration of the main ingredient in green tea leaf kombucha anti-acne face toner. The turbidity test in this study did not use spectrophotometry because of limited laboratory equipment.

#### 4. Inhibitory Diameter Test Results

The diameter of the inhibition test was determined to determine the size of the inhibition zone of kombucha green tea leaves against *Propionibacterium acnes*.

**Table IV.** Test of the Diameter of Inhibitory Power of Green Tea Leaf Kombucha

Concentration (%)	Mean of Diameter of Inhibition Zone (DIZ) (mm) Green Tea Leaf Kombucha
50	18
25	0
12,5	0
6,25	0
3,12	0
1,56	0
0,78	0
Kontrol +	44,5
Kontrol -	0

Information:

Positive control (+) : Clindamycin

Negative control (-) : Green tea solution

The size includes the diameter of the hole

The results above show that the diameter of the inhibition formed was at a concentration of 50%, that is, with a zone of 18 mm. From this, it can be concluded that the Minimum Inhibitory Concentration (MIC) of kombucha green tea leaves is 50%, with an inhibition zone of 18 mm. The MIC value obtained was then entered into the formulation of the formula as the X% value.

##### 5. Results of Making Kombucha Green Tea Leaf Face Toner

The results of the green tea leaf kombucha toner formulation (Table I) by adding the main ingredient to the concentration obtained from the results of the antibacterial activity test of green tea leaf kombucha solution showed the best ability to inhibit acne-causing bacteria and slightly sour scent.



**Figure 3.** Results of Green Tea Leaf Kombucha Face Toner

##### 6. Face Toner Diameter of Inhibition Zone (DIZ) Result on Face Toner Formula

Based on the results of the green tea leaf kombucha face toner, a DIZ test was carried out, and the DIZ values were obtained for each formula.

**Table V. Diameter Inhibitory Power (DDH) on Face Toner Formula**

<b>Formula</b>	<b>Mean of DIZ (mm) <math>\pm</math> SD</b>
F0	0 $\pm$ 0
F1	2,2 $\pm$ 0
F2	0 $\pm$ 0
F3	2 mm $\pm$ 0
F4	19 mm $\pm$ 0

**Information:**

F0 : Negative control with the composition of the toner base

F1 : Formula face toner with active ingredient concentration X% (MIC)

F2 : Formula face toner with a concentration of active ingredients (0.5 x X%)

F3 : Formula face toner with a concentration of active ingredients (F1 + F2)

F4 : Positive control with the concentration of the active ingredient clindamycin phosphate 1%

SD : Standard Deviation

The size includes the diameter of the hole

Based on the results in the table above, it is shown that formulas F1, F2, and F3 have an average DIZ of 2.2 mm, 0, and 2 mm, respectively. Based on the composition of the formula, F3 contained more of the active ingredient kombucha green tea leaves than F1 and F2. Based on these findings. There may be an error (human error) in the manufacture of Formula F3 or during the pasteurization process, which exceeds the specified temperature, so that some of the active antibacterial substances in F3 are damaged.

**7. Evaluation of Face Toner Preparations (Alviniari, 2019) (Effendi, 2014)****a. Face Toner pH test**

Based on the results of the pH test on the green tea leaf kombucha face toner above, the pH of the green tea leaf kombucha face toner was obtained in the range of 4-5. Thus, it can be concluded that this preparation is safe for application to the skin.

**Table VI. Green Tea Leaf Kombucha Face Toner pH Test Results**

<b>Formula</b>	<b>pH mean <math>\pm</math> SD</b>
F0	5 $\pm$ 0
F1	4 $\pm$ 0
F2	4 $\pm$ 0,577
F3	4 $\pm$ 0
F4	5 $\pm$ 0

Organic acids are added during the fermentation process. This resulted in kombucha having increasingly acidic pH after fermentation.

### b. Face Toner Stability Test

Stability tests were carried out using the formulas F0 (without the addition of green tea leaf kombucha), F1 (green tea leaf kombucha concentration X% (MIC), F2 (0.5 x X%), F3 (F1 + F2), and F4 (positive control with concentration active ingredient clindamycin phosphate 1%). Stability tests were carried out with storage for 8 weeks and evaluated at 2nd, 4th, 6th and 8th week.

#### 1) Organoleptic Test

Organoleptic test results for kombucha face toner green tea leaves from week 0 to week 8

**Table VII. Organoleptic Test of Face Toner Storage Results**

Storage Temperature (°C)	Storage Time (weeks)	Organoleptic		
		Colour	Aroma	Textur
Room Temperature (30)	0	Yellow	Slightly sour smell	Liquid
	2			
	4	Yellow	Slightly sour smell	Liquid
	6	Yellow	Slightly sour smell	Liquid
	8	Yellow	Slightly sour smell	Liquid
High Temperature (40)	0	Yellow	Slightly sour smell	Liquid
	2		Slightly sour smell	
	4	Yellow	Slightly sour smell	Liquid
	6	Yellow	Slightly sour smell	Liquid
	8	Yellow	Slightly sour smell	Liquid

#### 2) The pH test

The pH stability test was carried out using a universal pH indicator at weeks 2, 4, 6, and 8 at 2 storage temperatures of 30 °C and 40 °C. The results show that green tea kombucha face toner is pH stable and safe for use on the skin.

**Table VIII. pH Stability Test Results**

Storage Temperature (°C)	Storage Time (weeks)	pH ± SD
30	0	4 ± 0
	2	
	4	4 ± 0
	6	4 ± 0
	8	4 ± 0

	0	4 ± 0
	2	
40	4	4 ± 0
	6	4 ± 0
	8	4 ± 0

## CONCLUSION

Based on the results of the research that has been done, it can be concluded that SCOBY (*symbiotic culture of bacteria and yeast*) can be made from the addition of the bacterial isolates *Acetobacter xylinum* and the yeast *Saccharomyces cereviceae*, and green tea leaf kombucha face toner has anti-acne activity and has good stability

## SUGGESTION

Green tea leaf kombucha must be purified by secondary metabolites that specifically act as antibacterial substances to obtain more effective antibacterial activity at lower concentrations. Therefore, it is necessary to perform qualitative and quantitative analyses of the organic acids produced during fermentation. Therefore, it is necessary to test the viscosity of green tea leaf kombucha face toner. In the preparation of face toner kombucha green tea leaves, it is necessary to add a fragrance that is safe and does not irritate the acne-prone skin.

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