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# Formulation And Activity Tests of Anti-Acne Toner 70% Ethanol Extract Black Garlic (*Allium sativum* L.)

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Abstract: Black Garlic (BG) is a fermented product of garlic heated at 60-70°C for 14 days. Black garlic has a black color and has an aroma and taste that is not too pungent like garlic. In Black Garlic S-allylcysteine helps the absorption of allicin making metabolism against bacterial infections easier. The purpose of this study was to determine the physical evaluation and antibacterial activity of 70% ethanol extract Black Garlic (EEBG) as well as the optimum concentration of Staphylococcus aureus, Staphylococcus epidermidis, and Propionibacterium acnes. The study used Single Black Garlic (Allium sativum L.) from Lumajang, East Java, fermented for 30 days at a temperature of 70°C and humidity of 90%. Single black garlic extract is formulated in 3 formulas, including formula 1 (0,5% extract), formula 2 (1% extract), formula 3 (1,5% extract), negatif control (base) and Wardah Acnederm Pure Refining Toner as a comparison. Toner were evaluated by organoleptic testing, pH, homogeneity, and antibacterial activity. The physical results of the toner preparation meet the physical quality requirements which include (organoleptic testing, pH, and homogeneity). The results of the antibacterial activity of toner preparations in formula 1 (0,5% extract), formula 2 (1% extract), formula 3 (1,5% extract), and Commercial toner for antibacterial. The conclusion of this study shows that EEBG has promising antibacterial effects and the evaluation of all instant granules has met the specified requirements.

Keywords: Antibacterial; Single black garlic; Toner

# Introduction

The skin is the outermost part of the body that borders the human environment. Skin is a comprehensive body structure that varies according to climate, age, gender, race, and body position. The skin has three main layers: the epidermis, dermis, and subcutaneous tissue. In addition, the skin has glands in the skin, and hair and nails have oil glands and sebaceous glands. The task of this gland is to moisturize the skin so that it is maintained and balanced, actively working and growing during puberty. This can cause skin diseases, one of which is acne vulgaris or acne(Sifatullah & Zulkarnain, 2021).

Acne arises due to many causative factors, including increased production of sebaceous glands,

colonization of acne-causing bacteria, androgen hormones that trigger increased sebum production, genetics, stress, cosmetics, and drugs(Dreno et al., 2015).

Bacteria that commonly infect acne are *Propionibacterium acnes, Staphylococcus aureus* and *Staphylococcus epidermidis*. These bacteria cause different effects, the bacteria Propionibacterium acnes produces lipase that breaks down, *Staphylococcus aureus* and *Staphylococcus epidermidis* infection causing irritation in the surrounding area will then swell, burst and then spread inflammation to the skin tissue(Kursia et al., 2016).

One of the treatments to make it easier to handle acne can be made in the form of preparations, one of which is face toner(Draelos, 2019). Topical treatment of acne with antibiotics and retinoids often leads to skin

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irritation and drug resistance. Antibiotic resistance reduces the effectiveness of treatment and can worsen acne(Draelos, 2019). Based on the side effects of synthetic drugs and antibiotics, people turn to herbal medicines such as black garlic (BG) as an alternative to acne treatment.

BG is the result of fermented garlic at controlled temperature and humidity for a certain period. The final product, called black garlic, changes its texture to become soft and has a sweet taste with a distinctive fruity aroma and taste(Wang et al., 2010). BG is reported as an antibacterial(Ngan et al., 2017), increase immunity(Wang et al., 2010), antioxidant (Jeong et al., 2009)(Col et 2016)(Lee et al., al., 2012), antidiabetic(Saravanan et al., 2009), anti-tumor(Jin-ichi Sasaki, Chao Lu et al., 2007), hepatoprotective(Shin et al., 2014)(Shintaro Kodai, Shigekazu Takemura, Shoji Kubo, 2015), anti-cancer(Xu et al., 2014), anti-cholesterol(Ha et al., 2015), and anti-inflammatory(Jeong et al., 2016).

In BG S-allylcysteine helps the absorption of allicin making metabolism against bacterial infections easier. The existence of antibacterial compounds that are higher than garlic is expected to be more effective in overcoming pathogenic bacteria that can cause diseases(Habeshian & Cohen, 2020). The latest research is that the 70% ethanol extract of a single black garlic is formulated in the form of a face toner, while other research is formulated in the form of nanoparticles or cream. Therefore, this research aims to to determine of the physical evaluation and antibacterial activity of 70% ethanol extract Black Garlic (EEBG) as well as the optimum concentration of Staphylococcus aureus, Staphylococcus epidermidis, and Propionibacterium acnes.

# Method

The Plant material used in this research was single garlic from Lumajang, East Java. All chemicals in this

 Table 1. Single Black Garlic extract Face Toner formula

study are analytical grade. Excipients in the toner formula are propylene glycol, Nipagin, Nipasol, 70% ethanol, glycerin and aquadest. Other ingredients are Muller Hinton Agar (MHA) media, 0.9% NaCl, Wardah Acnederm Pure Refining Toner (PT. Paragon Technology and Innovation, Tangerang), DMSO, Mc. Farland, and Propionibacterium acnes, Staphylococcus aureus and Staphylococcus epidermidis.

# Black Garlic Extraction

Garlic is weighed and placed in a climatic chamber with an optimal temperature of 70°C, and humidity of 90% for 30 days(Choi et al., 2014). The resulting Black Garlic is taken and aired according to the fermentation time. The extract was made using the maceration method with a ratio of single black garlic: 70% ethanol (1:2). The thick extract was frozen in a freeze dryer at -80ºC. The organoleptic profile, extract yield, ash content, water content, and residual solvent were analyzed.

Yield of extract compares the extracts obtained and the initial simplicias. Yield calculation:

Yield of extract = 
$$\frac{Condensed Extract Weight}{Initial Simplicia Weight} x 100\%$$
 (1)

# Phytochemical Screening

Phytochemical screening was conducted to observe secondary metabolite compounds in a single black garlic involving alkaloids, flavonoids, extract steroids/triterpenoids, saponins, and tannins.

Formulation of Toner using 70% Ethanol Extract of Single Black Garlic

The toner formulation used comes from research conducted by Marhaba et al., with slight modifications to the formula which can be seen in Table 1(Marhaba et al., 2021).

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Ingredients Name	F1	F2	F3	Negative control	Function		
Single black garlic	0.50	1	1.50	-	Active substances		
Nipagin	0.02	0.02	0.02	0.02	Preservatives		
Nipasol	0.02	0.02	0.02	0.02	Preservatives		
Glycerine	10	10	10	10	Humectants		
Propylene glycol	10	10	10	10	Humectants		
Aquadest add	100 ml	100 ml	100 ml	100 ml	Solvent		

A single black garlic extract preparation is made according to a predetermined concentration. Dissolve nipagin and nipasol with distilled water, stirring until homogeneous. Glycerine and propylene glycol are added to the preservative solution mixture. Dilute the black onion extract with distilled water, then take it according to the formula concentration. Add distilled

water up to 100 ml, and stir until homogeneous. Put into a container.

# Physical Evaluation of Toner

Organoleptic analysis of toner is carried out by examining the odor and color of the toner(Mulyani, 2022). The pH test was carried out using a pH meter, by

taking a 1 mL preparation and then adding it with 9 mL aquadest. The homogeneity test was carried out by taking 10ml of face toner formula preparation, then inserting the toner into the beaker glass and observing the arrangement of coarse particles or non-homogeneity in the toner preparation(Noor et al., 2023).

## Antibacterial Activity

A total of 15 ml of MHA media is put into a petri dish and left to solidify. Take a suspension of bacteria (Propionibacterium acnes, Staphylococcus aureus and Staphylococcus epidermidis) of 0.1 ml each and homogenize in each Petri dish. The MHA media that has been solidly perforated using a blue tip with a diameter of 6 mm, made as many as 5 wells (formula 1 (0,5% extract), formula 2 (1% extract), formula 3 (1,5% extract), negative control, positive control). MHA media that has undergone the treatment is incubated for 18-24 hours at a temperature of 37°C without being reversed.

The results of the antibacterial activity were observed visually by measuring the diameter of the inhibition zone around the well using a caliper with an accuracy of 0.05 mm.

## Data Analysis

Data analysis was performed using the SPSS version 25. antibacterial activity results using the One Way ANOVA. A p-value <0.05 was used to describe statistical significance.

# **Result and Discussion**

# Result of Single Black Garlic Fermentation

The result obtained is a single garlic simplicia that is fermented into a single black garlic obtained as much as 935 grams. The intensity of the color change in the garlic samples began to appear brown starting on the 17th day of the fermentation process with a temperature of 70°C and a humidity of 90%. Apart from that, changes in the taste of fermented garlic also occurred on the 17th day. The cause is the Maillard reaction, a non-enzymatic browning process between reducing sugars and primary amino acids during heating, producing a dark brown product known as melanoidin. The result of this reaction not only makes a changed color but also a milder aroma and sweeter taste (Wang et al., 2010).

The formation pathway of flavor compounds in the maillard reaction can be seen in Figure 1. Figure 1. shows the pathways involved in the formation of aroma and taste in Maillard's reaction. The pathway begins with a reaction between reducing sugars and amino acids which is then followed by cyclization, fragmentation, condensation and strecker degradation so that flavor compounds are formed. During high-temperature fermentation, some chemical compounds from fresh garlic are converted into Amadori/Heyns compounds, which are compounds formed in the maillard reaction(Kimura et al., 2017).



Figure 1. Flavor compound formation pathways in Maillard reactions(Kimura et al., 2017).

## Result of the Extraction Process and Its Characterization

Organoleptic extracts include a semisolid form, brown color; and the distinctive smell of a single Black Garlic Extract. The water content of an ethanol extract of a single black garlic was obtained at 1.58%, following quality standards, namely  $\leq 10\%$ , which indicates the amount of water in the material and maintains the durability of the extract during the storage period. In addition, the ash content of ethanol extract of a single black garlic obtained an ash content of 2.10%, this has met the standard is  $\leq$  16.6%, which reflects the mineral content of the extraction process (Kementrian Kesehatan, 2017). Finally, the residual solvent in the extract should not exceed 1%, and the residual solvent of ethanol extract of a single black garlic is 0.39%, meeting the requirements for use as a traditional medicinal ingredient.

## Result of the Phytochemical Screening

Phytochemical screening was carried out to determine the secondary metabolite compounds contained in a 70% ethanol extract Black Garlic qualitatively. The active compounds tested include alkaloids, flavonoids, saponins, tannins, and steroids/triterpenoids. The obtained secondary metabolite compounds are contained in Table 2.

**Table 2.** Single Black Garlic Phytochemical ScreeningTest Results

т (т.:	T ( D 1)
Types of Testing	l est Results
Alkaloids	-
Flavonoids	+
Saponins	+
Tannins	+
Steroids/Triterpenoids	-

Description:

(+): Secondary metabolites detected

(-) : Ssecondary metabolites not detected

# *Physical Evaluation Toner Preparation Organoleptic Test*

Commlo	Repli-			Result
Sample	cation	Color	Aroma	Shape
Formula 1	1	Brown	Typical black garlic	liquid
	2	Brown	Typical black garlic	liquid
	3	Brown	Typical black garlic	liquid
Formula 2	1	Brown	Typical black garlic	liquid
	2	Brown	Typical black garlic	liquid
	3	Brown	Typical black garlic	liquid
Formula 3	1	Brown	Typical black garlic	liquid
	2	Brown	Typical black garlic	liquid
	3	Brown	Typical black garlic	liquid
Negative	1	White	Typical black garlic	liquid
Control	2	White	Typical black garlic	liquid
	3	White	Typical black garlic	liquid

Description:

F1 = Single Black Garlic Extract Toner Preparation 0.5%

F2 = Single Black Garlic Extract Toner Preparation 1%

F3 = Single Black Garlic Extract Toner Preparation 1.5%

Result	of	Antibacterial A	Activity
Table	5.	Antibacterial	Activity

In the organoleptic test with three replications, the results were obtained by directly observing the instant granule preparation, including color, smell, and shape. The results were obtained from F1, F2, F3, and the negative control.

## Homogeneity Test

The results of the observation of the physical properties of the preparation showed that F1, F2, F3, and negative control showed the same results, namely homogeneity, and there was no arrangement of coarse particles in the face toner preparation. Based on the results of the evaluation above, the face toner preparations from 4 formulas have met the requirements of homogeneity in each formula.

## pH Test

pH testing is carried out using a pH meter until a constant pH is obtained. The results of the PH test evaluation can be seen in Table 4.

Formula	F	A		
ronnula	1	2	3	Average
F1	4,62	4,70	4,74	$4,68 \pm 0,11$
F2	4,45	4,53	4,57	$4,51 \pm 0,06$
F3	4,43	4,48	4,55	$4,\!48 \pm 0,\!06$
Negative control	6,02	6,02	6,04	$6,02 \pm 0,67$

Based on **Table 4** The results of the pH evaluation test of toner preparations According to the pH requirements, which are 4.5-6.5. pH testing using a pH meter.

	<u> </u>	Observation of bacterial growth			The average diameter	
Bacteria Test	eria Test Concentration inhibition zone (mm)		n zone (mm)	of the inhibition zone ±	Information	
		Ι	II	III	SD	
Staphyloccocus	F1	10.25	10.71	9.9	$10.29 \pm 0.33$	Strong
aureus	F2	11.37	11.12	12	$11.50 \pm 0.37$	Strong
	F3	11.8	11.77	13.27	$12.54 \pm 0.73$	Strong
	Negative	-	-	-	-	-
	control					
	Positive control	11.97	12.57	15.82	$13.45 \pm 1.69$	Strong
Staphyloccocus	F1	11.92	9.82	10.8	$10.85 \pm 0.86$	Strong
epidermidis	F2	14.52	11.95	13.5	$13.32 \pm 1.06$	Strong
	F3	14.75	12.9	15	$14.22 \pm 0.94$	Strong
	Negative	-	-	-	-	-
	control					
	Positive control	17.5	12.85	16.55	$15.63 \pm 2.01$	Strong
Propionibacterium	F1	8.4	4.97	7.05	$6.81 \pm 1.41$	Medium
acne	F2	11.92	10.8	11.42	$11.38 \pm 0.46$	Strong
	F3	12.22	11.1	11.72	$11.68 \pm 0.46$	Strong
	Negative	-	-	-	-	-
	control					
	Positive control	55.3	12.47	12.07	$26.61 \pm 20.29$	Very strong

The results of the antibacterial activity test of 70% ethanol extract black garlic in Table 5 show that it can inhibit Staphyloccocus aureus by forming a clear zone around the disc with a strong category. The clear zone formed is an inhibition zone for bacterial growth. So it can be concluded that 70% ethanol extract black garlic has the antibacterial activity of Staphyloccocus aureus. Furthermore, the results of testing the antibacterial activity of 70% ethanol extract black garlic in Table 8 can inhibit Staphyloccocus epidermidis bacteria by forming a clear zone around the disc with a strong category. The clear zone formed is an inhibition zone for bacterial growth. So it can be concluded that 70% ethanol extract black garlic has the antibacterial activity of Staphyloccocus epidermidis.

Furthermore, the results of testing the antibacterial activity of 70% ethanol extract black garlic in Table 5 can inhibit Propioni bacterium acne bacteria by forming a clear zone around the disc with a very strong category. The clear zone formed is an inhibition zone for bacterial growth. So it can be concluded that 70% ethanol extract black garlic has an antibacterial activity of Propioni bacterium acne, because 70% ethanol extract black garlic has secondary metabolite compounds of flavonoids, tannins and saponins. Black garlic contains flavonoid compounds and polyphenols. The main antibacterial component of garlic (*Allium sativum* L.) is the flavonoid component that is thought to be able to inhibit bacterial growth (Ngan et al., 2017).

#### Data Analysis Results

Based on the antibacterial activity test data of 70% ethanol extract black garlic obtained, it is statistically analyzed and processed using the IBM Statistical Product and Service Solution (SPSS) 27.0 application. Before conducting a one-way ANOVA test, a normality test and a homogeneity test are conducted first.

The results of the normality test (Shapiro-Wilk) show that the data is not normal. The Kruskal Wallis test was then carried out. The goal is to find out whether or not there is a difference between variables for data that does not meet the requirements of ANOVA testing. The results of the Kruskal Wallis test for antibacterial activity of Staphylococcus aureus, Staphylococcus epidermidis, and Propionibacterium acnes were respectively 0,028, 0,081, and 0,059. It shows that 70% ethanol extract black garlic of Staphylococcus aureus obtained a significance value of 0.028 or p<0.05 which means that there is a significant difference between the five treatment groups. So it is stated that it has antibacterial activity of Staphyloccocus aureus, the next step to carry out the mann-whitney test. The results of the Mann-Whitney test showed that 70% ethanol extract black garlic against Staphyloccocus aureus bacteria obtained negative control results compared to positive control obtained a significance value of 0.037 (<0.05) so that it can be stated that there is a significant difference which means that the positive control has Staphyloccocus aureus antibacterial activity. Furthermore, the negative control compared to the toner formula at concentrations of 0.5%, 1% and 1.5% obtained a significance value of 0.037 (<0.05) which can be stated that there is a significant difference which means that the toner formula has an antibacterial activity of Staphyloccocus aureus. 70% ethanol extract black garlic contains. The main antibacterial component of garlic (Allium sativum L.) is the flavonoid component that is thought to be able to inhibit bacterial growth. Comparable to the research of Manik *et al.* stated that the ethanol extract of kersen leaves against Staphyloccocus aureus contains flavonoid compounds that can inhibit Staphyloccocus aureus bacteria (Manik et al., 2014).

In 70% ethanol extract black garlic to measure the optimal concentration by comparing the positive control and 70% ethanol extract black garlic concentrations of 0.5% and 1%, a significance value of 0.050 (<0.05) was obtained, so it can be stated that there is a significant difference, which means that it has antibacterial activity against Staphyloccocus aureus bacteria. Meanwhile, 70% ethanol extract black garlic with a concentration of 1.5% obtained a significance value of 0.275 (>0.05) so it can be stated that there is no significant difference, which means that it has the same antibacterial activity as the positive control. While for *Staphylococcus epidermidis*, and Propionibacterium acnes was shown that the ethanol extract of 70% black garlic was obtained with a significance value of p>0.05 which means that there was no significant difference between the five treatment groups.

## Conclusion

Based on the research result the toner with 70% ethanol extract from a single Black garlic has been physically evaluated and shows results that meet cosmetic standards in terms of color, clarity, viscosity, and pH, so it has the potential to be further developed as a quality product. In addition, this face toner shows significant antibacterial activity against Propionibacterium acnes, Staphylococcus aureus, and Staphylococcus epidermidis.

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#### **Author Contributions**

A. A. S., contributed in conceptualizing the research idea, developing the product, S. M., contributed in analyzing data and writing the article. A. Y., contributed in writing, reviewing, and editing the article. A., contributed in collecting data.

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#### **Conflicts of Interest**

The authors declare that there is no conflict of interest regarding the publication of this article.

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