Bioscience
Volume 5 Number 1, 2021, pp. 44-56
ISSN: Print 1412-9760 – Online 2541-5948
DOI: 10.24036/0202151110068-0-00

BIOSCIENCE
http://ejournal.unp.ac.id/index.php/bioscience

Anti-Microbial Activities of Shallots (*Allium cepa* L.) Extract and Garlic (*Allium sativum* L.) Extract on the Growth of Peat Soil Bacteria

Chaidir Adam [1]*

Biology Education Program, Faculty of Teacher Training and Education, University of Palangka Raya, Palangka Raya[1*]

Email: chaidir.adam03@gmail.com

Abstract. This study aims to examine the antimicrobial activity of shallots (*Allium cepa* L.) and garlic (*Allium sativum* L.) extracts on the growth of peat soil bacteria. This type of research is an experimental study using a Completely Randomized Design with four levels of treatment. Test for bacterial activity using the agar diffusion method with the paper disc technique. Data were analyzed statistically using One Way ANOVA and the Games-Howell post-hoc test at the 5% significance level. The results showed that shallots (*Allium cepa* L.) and garlic (*Allium sativum* L.) extracts had antimicrobial activity against the growth of tested bacteria as indicated by the presence of a clear zone as an indicator of inhibition of bacterial growth. Garlic has better antimicrobial activity seen from the diameter of the clear zone that appears in the garlic extract treatment ranging from 8-13 mm, whereas in the treatment of onions it ranges from 3–5 mm.

Key Words: antimicrobial, *Allium cepa, Allium sativum,* medicinal plants

1. Introduction

Prevention or treatment of diseases caused by pathogenic bacteria usually uses compounds that have antimicrobial activity. Antimicrobial compounds are compounds that can inhibit or interfere with microbial growth and metabolism (Cowan, 1999); (Sartelli et al., 2016); (Asif, 2017). Terms such as anti-bacterial or anti-fungal are used for antimicrobial compounds that inhibit specific groups of microbes, namely anti-bacteria for bacteria and anti-fungal for fungi. Two mechanisms are primarily due to the antibacterial behavior of an agent, which involves chemically interfering with the synthesis or function of essential components of bacteria and/or circumventing traditional antibacterial resistance mechanisms (Amerikova et al., 2019; Gonelimali et al., 2018; Khameneh et al., 2019). There are many targets for antibacterial agents, including bacterial protein biosynthesis; bacterial cell-wall biosynthesis; degradation of bacterial cell membranes;

replication and repair of bacterial DNA; and inhibition of a metabolic pathway (Khameneh et al., 2019).

Shallot (*Allium cepa* L.) and garlic (*Allium sativum* L.), based on several research results, have antimicrobial activity (Benkeblia, 2004); (Azu & Onyeagba, 2006). Shallot extract (*Allium cepa* L.) has anti-bacterial activity against *Escherichia coli, Salmonella typhi*, and *Bacillus subtilis* which infect the gastrointestinal tract (Azu & Onyeagba, 2006). The use of garlic as an antimicrobial (especially anti-bacterial) has long been used to fight bacterial infections, including inhibiting *Aerobacter, Aeromonas, Bacillus, Citrella, Citrobacter, Clostridium, Enterobacter, Escherichia, Klebsiella, Lactobacillus, Leuconostoc, Micrococcus, Mycobacterium, Proteus, Providencia, Pseudomonas, Salmonella, Serratia, Shigella, Staphylococcus, Streptococcus* and *Vibrio* (Bhandari, 2012).

Several studies have examined the diversity and composition of soil microbial populations, including peat soils (Mandic-Mulec et al., 2014). Peat soils are rich in bacterial communities that help fertilize the soil as a denitrifier that is involved in denitrification and nitrogen fixer (Kusai & Ayob, 2020). Pathogenic bacteria are also found in the peat soils, such as *Bacillus anthracis* that is known as a pathogen that infects humans, wildlife, and livestock (Irenge & Gala, 2012; Wall et al., 2015). This study aims to examine the antimicrobial activity of shallot extract (*Allium cepa* L.) and garlic extract (*Allium sativum* L.) on the growth of peat soil bacteria.

2. Materials and Methods

2.1. Materials

The tested bacteria in this experiment were bacteria isolated from peat soil on Jl. Mahir Mahar, Palangka Raya, Central Kalimantan. Soil samples were diluted by serial dilution method and then planted on NA (Nutrient Agar) media as culture stock. The instruments and materials used in this study are presented in Table 1 and Table 2.

Table 1. List of Research Instruments

No.	Instrument(s)	amount
1	Petri Dish	8 pc(s)
2	Pipette	2 pc(s)
3	*Aluminum Foil*	Sufficiently
4	*Autoclave*	1 unit
5	Analytical Balance	1 unit
6	*Hot Plate*	1 unit
7	Test Tube	2 pc(s)
8.	Inoculation Loop	1 pc(s)
9.	Bunsen	1 pc(s)

Table 2. List of Research Materials

No.	Material(s)	amount
1	Shallots (*Allium cepa* L.)	500 g
2	Garlic (*Allium sativum* L.)	500 g
3	Aquadest	Sufficiently
4	Nutrient Agar (NA) Media	14 g
5	Amoxicillin	Sufficiently
6	Alcohol 70%	Sufficiently
7	Paper	Sufficiently
8	Latex Gloves	1 pair
9	Cotton	Sufficiently

2.2. Methods

This study used a completely randomized design with 4 levels of treatment and 4 replications (Table 3). Consisting of 4 groups, namely two control groups (positive control and negative control) and two treatment groups.

Table 3. Research Design

T	R			
	1	2	3	4
C-	$(C-)_1$	$(C-)_2$	$(C-)_3$	$(C-)_4$
C+	$(C+)_1$	$(C+)_2$	$(C+)_3$	$(C+)_4$
T1	$(T1)_1$	$(T1)_2$	$(T1)_3$	$(T1)_4$
T2	$(T2)_1$	$(T2)_2$	$(T2)_3$	$(T2)_4$

Annotation:

C- : Negative control, without shallots (*Allium cepa* L.) and garlic (*Allium sativum* L.) extracts
C+ : Positive control, treatment using generic drug: Amoxicillin
T1 : Treatment 1, Shallots (*Allium cepa* L.) Extract
T2 : Treatment 2, Garlic (*Allium sativum* L.) Extract

2.2.1. Procedure for Making Shallots (*Allium cepa* L.) and Garlic (*Allium sativum* L.) Extract

a. Peeled 500 grams each of shallots (*Allium cepa* L.) and garlic (*Allium sativum* L.) then dried in the sun to dry.
b. Dried shallots (*Allium cepa* L.) and garlic (*Allium sativum* L.) are then mashed by pounding.
c. Soaking the refined shallots (*Allium cepa* L.) and garlic (*Allium sativum* L.) in distilled water for 24 hours.

d. After 24 hours, the soaking was filtered to obtain the extract of shallots (*Allium cepa* L.) and garlic (*Allium sativum* L.) with water solvent.

2.2.2. Sterilization

a. Beaker Glass, Erlenmeyer Flask, Test Tubes, Pipettes, and Petri Dishes are washed with soap until clean then dry.
b. Wrapped in paper based on the type of glassware, before being put into the Autoclave.
c. Sterilize the wrapped glassware with an autoclave at 121^0C (15 psi) for 15 minutes.
d. Wait until the pressure reaches 0 psi on the Geared Gauge Autoclave, then open the cover, remove the sterilized glassware.

2.2.3. Procedure for Making Nutrient Agar (NA) Media

a. Weigh 14 grams of Nutrient Agar (NA) using a digital Ohaus Balance.
b. Dissolve using 500 ml of distilled water in the Erlenmeyer Flask.
c. Place the Erlenmeyer flask containing 14 g / 500 ml NA solution on top of the Magnetic Heated Stirrer.
d. After the 14 g / 500 ml NA solution has turned clear (clear), turn off the magnetic heated stirrer.
e. Coat the top of the Erlenmeyer flask using aluminum foil, then sterilize the clear NA media using an Autoclave at 1210C (15 psi) for 15 minutes.
f. Wait until the pressure is 0 psi on the Geared Gauge Autoclave, then open the cover, remove the NA media that has been sterilized.
g. After the sterilized NA medium is not too hot, then pour it into 8 (eight) Petri dishes.

2.2.4. Procedure for Making Physiological Saline Solution

a. Weigh 0.85 grams of NaCl using a digital Ohaus Balance and measure 100 ml of distilled water.
b. Put the weighed salt into a sterile Erlenmeyer flask and add 50 ml of distilled water, then mix until homogeneous.
c. Once homogeneous, add another 50 ml of water (total 100 ml) and cover with a cotton ball and aluminum foil.
d. Sterilize using an autoclave at 121^0C (15 psi) for 15 minutes.
e. Wait until the pressure is 0 psi on the Geared Gauge Autoclave, then open the cover, remove the physiological saline solution of 0.85 g / 100 ml that has been sterilized.

2.2.5. Procedure for Making Bacterial Suspension

a. Prepare a BaCl2 + H2SO4 (MacFarland) comparison solution.
b. Dilute the bacterial colony sample from the culture stock using inoculation loop into a test tube containing 10 ml of physiological saline solution.
c. The turbidity of the suspension was adjusted according to the MacFarland solution.

2.2.6. Treatment Procedures

a. Prepare treatment solutions: (1) negative control (distilled water); (2) positive control (Amoxicillin); (3) treatment 1 (shallot extract); and (4) treatment 2 (garlic extract).
b. Soak the paper disc (cut using a paper punch) in each solution.
c. Cover the solution with aluminum foil.

Fig. 1. Preparation of the Treatment Solutions

d. Streak the bacterial sample on NA media
e. Take and place the soaked paper disc from each treatment solution on a petri dish. (4 pieces/treatment solution, one petri dish for one treatment)
f. Label each petri dish with C- (Negative Control), C+ (Positive Control), T1 (Treatment 1: Shallot Extract), and T2 (Treatment 2: Garlic Extract).

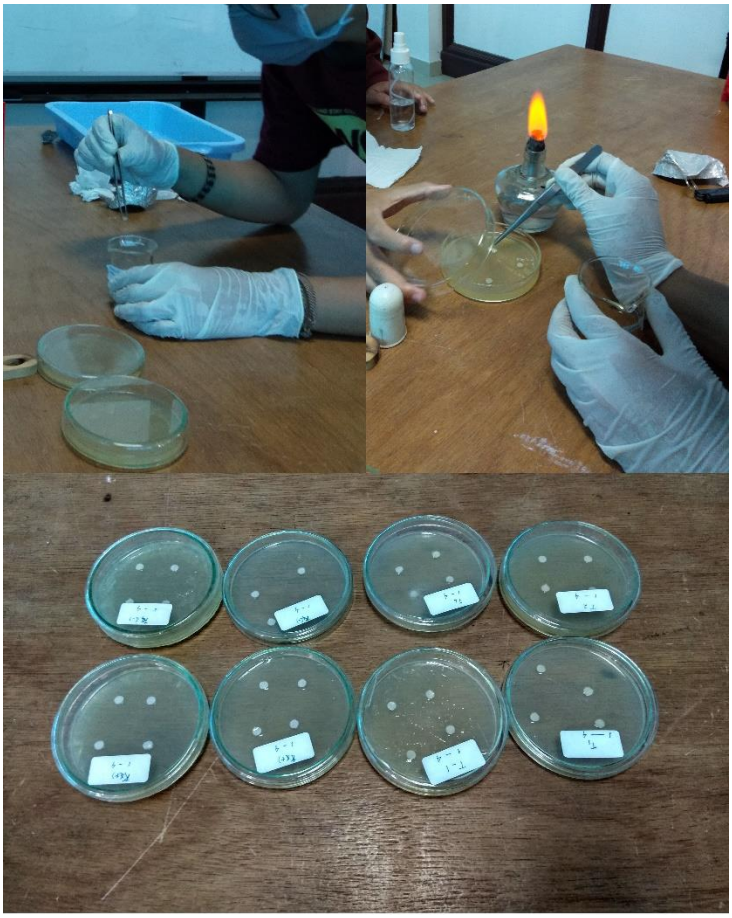

Fig. 2. Treatment Procedures

2.2.7. Anti-Microbial Activity Test

The anti-microbial activity was observed from the results of measurements of the clear zone/inhibition zone diameter at 24 hours and 48 hours after bacterial inoculation and treatment.

2.2.8. Data Analysis

The data obtained from each treatment level were analyzed using the One Way ANOVA formula at the 5% significance level. The data will be analyzed using the SPSS 23 program. The hypothesis in this study is as follows:

H_0 : Shallot extract (*Allium cepa* L.) and garlic extract (*Allium sativum* L.) do not have an antimicrobial activity that inhibits bacterial growth.

H_1 : Shallot extract (*Allium cepa* L.) and garlic extract (*Allium sativum* L.) have an antimicrobial activity that inhibits bacterial growth.

If the p-value <0.05, then H_0 is rejected. This shows that there is a significant difference between treatments and to find out which treatment is the best, it is necessary to carry out post-hoc tests, namely the Bonferroni test (if the variance is homogeneous) and the Games-Howell test (if the variance is not homogeneous).

3. Result and Discussion

3.1. Results

The research data is in the form of measurements of the clear zone diameter which is an indicator of the presence of antimicrobial activity against bacteria. Measurements were carried out twice, precisely at 24 hours (24h) and 48 hours (48h) after the inoculation of the bacterial sample and the treatment. The data on the results of clear zone measurements at 24h and 48h are presented in Table 4, Table 5, and Figure 1.

Table 4. Clear Zone Diameter Measurement Data at 24h (mm)

T	R				Mean (mm)
	1	2	3	4	
K-	0,0	0,0	0,0	0,0	0,0
K+	14,0	13,0	13,0	19,0	14,8
T1	4,0	4,0	5,0	3,0	4,0
T2	11,0	9,0	11,0	7,0	9,5

Table 5. Clear Zone Diameter Measurement Data at 48h (mm)

T	R				Mean (mm)
	1	2	3	4	
K-	0,0	0,0	0,0	0,0	0,0
K+	14,0	13,0	14,0	20,0	15,3
T1	5,0	4,0	5,0	4,0	4,5
T2	13,0	10,0	11,0	8,0	10,5

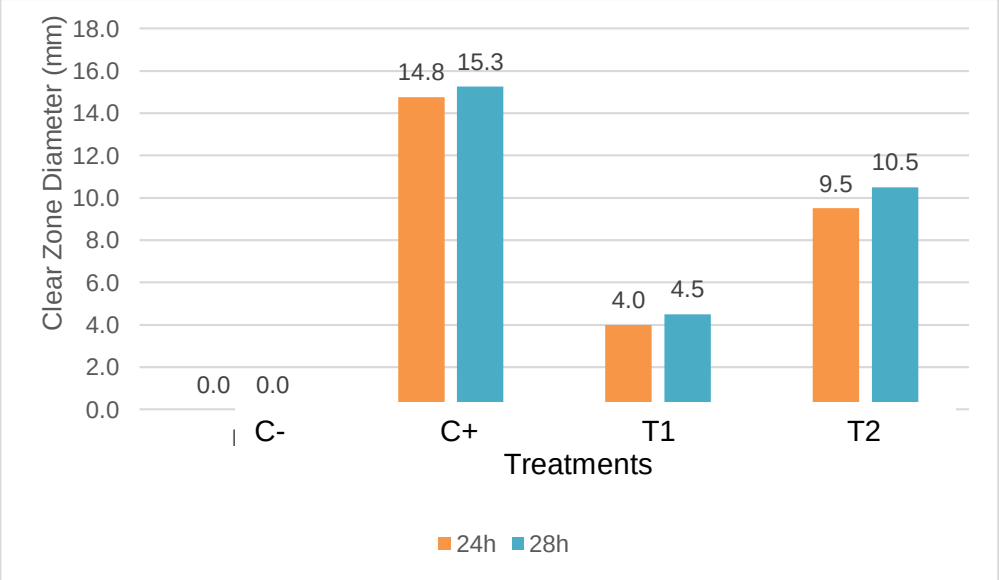

Fig. 1. Comparison diagram of clear zone mean diameter at 24h dan 48h

The results of the One Way ANOVA analysis on the 24h and 48h data using the SPSS program in Table 6 show that there is a significant difference (reject H0) at the 5%

significance level as indicated by the F value (24h = 90.563; and 48h = 81.760) and the p-value (0.000 <0.050).

Table 6. Results of One Way Anova Analysis on 24h and 48h Data Using SPSS 23

		Sum of Squares	df	Mean Square	F	Sig.
Clear Zone Diameter 24h	Between Groups	8.547	3	2.849	**90.563**	**.000**
	Within Groups	.377	12	.031		
	Total	8.924	15			
Clear Zone Diameter 48h	Between Groups	9.147	3	3.049	**81.760**	**.000**
	Within Groups	.448	12	.037		
	Total	9.594	15			

Post-hoc tests were carried out to determine which treatment was the most ideal between T1 (onion extract) and T2 (garlic extract). The results of the homogeneity test showed that the p-value (sig.) Was 0.024 (24h) and 0.026 (48h) less than 0.05, which means that the data variance was not homogeneous. Then a further Games-Howell test was carried out using the SPSS 23 program with the detailed results presented in Table 7 and Table 8 which show that between Positive Control (C+) and Treatment 2 (T2) were not significantly different. This means that the inhibition (antimicrobial activity) of garlic extract (Allium sativum L.) is better than that of shallot extract (Allium cepa L.). The length of the clear zone diameter between treatments is presented in Figure 3.

Table 7. Results of Games-Howell Analysis on the 24h Data Using SPSS 23

No.	Treatments		Mean Difference (I-J)	Keterangan
	I	J		
1	C-	C+	-1,9750*	Significant
		T1	-,9000*	Significant
		T2	-1,4500*	Significant
2	C+	C-	1,9750*	Significant
		T1	1,0750*	Significant
		T2	**.5250**	**Not Significant**
3	T1	C-	.9000*	Significant
		C+	-1,0750*	Significant
		T2	-.5500*	Not Significant
4	T2	C-	1,4500*	Significant
		C+	**-.5250**	**Not Significant**
		T1	.5500*	Significant

Table 8. Results of Games-Howell Analysis on the 48h Data Using SPSS 23

No.	Treatments		Mean Difference (I-J)	Keterangan
	I	J		
1	C-	C+	-2,0250*	Significant
		T1	-.9500*	Significant
		T2	-1,5500*	Significant
2	C+	C-	2,0250*	Significant
		T1	1,0750*	Significant
		T2	**.4750**	**Not Significant**
3	T1	C-	.9500*	Significant
		C+	-1,0750*	Significant
		T2	-.6000*	Significant
4	T2	C-	1,5500*	Significant
		C+	**-.4750**	**Not Significant**
		T1	.6000*	Significant

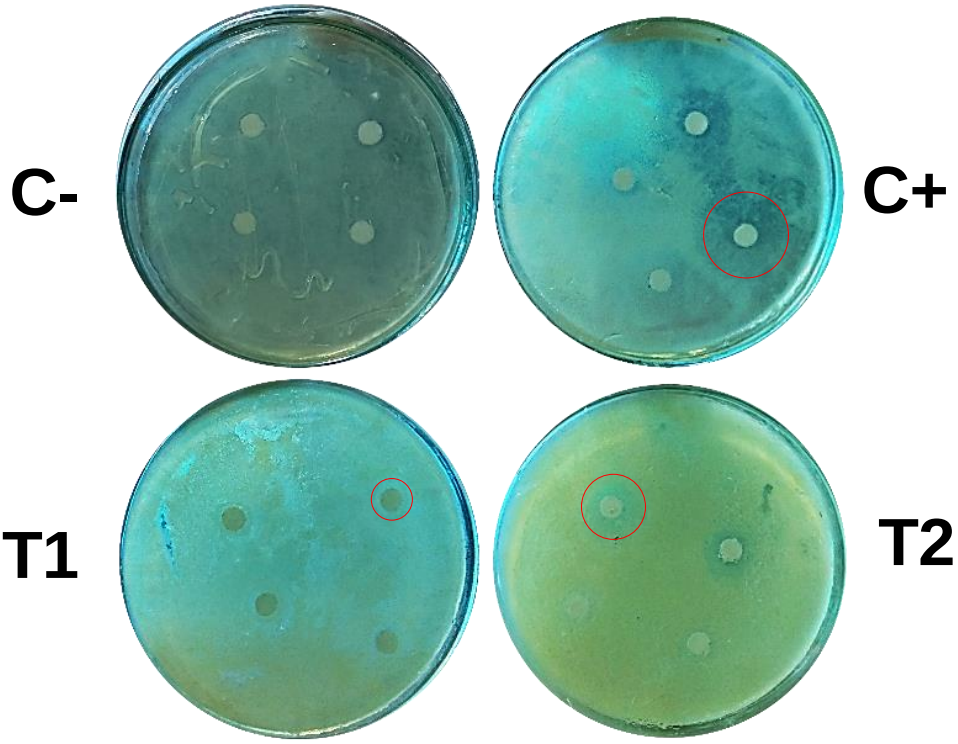

Fig. 3. Comparison of the Diameter Length of the Clear Zone in Each Treatment

3.2. Discussion

Extracts of shallots (*Allium cepa* L.) and garlic (*Allium sativum* L.) have long been and are widely used as antimicrobials to inhibit the growth of bacteria, fungi, and other parasitic microorganisms. This study tested the antimicrobial activity of shallot (*Allium cepa* L.) and garlic (*Allium sativum* L.) extracts against tested bacteria. Bacterial

samples were obtained from peat soil and grown on NA media. The results showed that both extracts had antimicrobial activity against the growth of the tested bacterial colonies. This is indicated by the clear zone on the treated media and the results of the one-way ANOVA analysis (p-value 0.000 <0.050). Research by (Yousufi, 2012) dan (Zine & Zine, 2015) shows that onion extract and garlic extract have antimicrobial activity inhibiting the growth of *E. coli, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi, Aerobacter aerogenes,* and *Proteus vulgaricus.*

Shallots (*Allium cepa* L.) contain many phytochemical compounds, most of which are hydrocarbons and their derivatives (Griffiths et al., 2002). Quercetin and allicin are the main components of shallots that act as antimicrobials, especially antibacterials (Shrestha et al., 2016), quercetin is bound to the bacterial enzyme DNA gyrase and allicin inhibits enzymes that contain thiol (Ankri & Mirelman, 1999). DNA gyrase is an essential bacterial enzyme that catalyzes ATP-dependent negative super-coiling of double-stranded closed-circular DNA, which is included in the topoisomerase enzyme group (Reece & Maxwell, 1991)(Papillon et al., 2013). Gyrase has a different affinity for various molecules, making it an ideal antibiotic target (Engle et al., 1982). Shallots also contain flavonoids and polyphenols which based on several research results show that these compounds have antibacterial activity (Hendrich, 2006)(Ani et al., 2006). Garlic (*Allium sativum*) also contains many phytochemical compounds that have pharmaceutical effects, including allicin which is the most potent compound as an antibacterial agent (Shrestha et al., 2016).

The results of post hoc analysis using Games-Howell showed that garlic extract (*Allium sativum* L.) was not significantly different from the positive control (C+; Amoxicillin). This means that the inhibitory power of garlic extract against bacterial growth is almost the same as that of commercial antibiotics (Amoxicillin). The diameter of the clear zone (inhibition zone) of garlic extract ranged from 8-13 mm, larger than the diameter of the clear zone of shallot extract which ranged from 3-5 mm at the same concentration.

Shallot extract (*Allium cepa* L.) actively inhibits gram-positive bacteria but is weak against gram-negative bacteria (Kirilov et al., 2014). This suggests that the tested bacterial strains obtained from peat soil are thought to be gram-negative bacteria so that the inhibition (antimicrobial activity) of shallots decreases. (Shrestha et al., 2016) through research that has been conducted, concluded that the mixture of garlic extract is more potential as an antibacterial than red onion extract, even the mixture of garlic and onion (1:1) still shows better antimicrobial activity.

4. Conclusion

The conclusions of this study are: (1) shallot extract (*Allium cepa* L.) and garlic extract (*Allium sativum* L.) have antimicrobial activity against the growth of tested bacteria which is indicated by the presence of a clear zone as an indicator of growth inhibition. bacteria; and (2) Garlic has better antimicrobial activity seen from the diameter of the clear zone that appears in the garlic extract treatment ranging from 8 - 13 mm, while in the treatment of onions it ranges from 3 - 5 mm.

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