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Isolation and Analysis of Chemical Components of Garlic (*Allium sativum L.*) Tuber Essential Oil As Well As Antibacterial and Antioxidant Activity Tests

Dimas A. Pratama[1],and Sabarmin Perangin-angin[2*]

[1,2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Jalan Bioteknologi No.1 Kampus USU Medan 20155, Indonesia

Abstract. The essential oil from garlic tubers (*Allium sativum L*) was isolated by hydrodistillation method using the Stahl apparatus. Garlic tubers were distilled for 4 to 5 hours to produce as much oil as 0.05% (w/ w). The chemical components of essential oil of garlic tubers were analyzed using GC-MS spectroscopy. The GC-MS results showed that there were 11 components with 5 main components, namely diallyl disulfide (44.98%), 1,3-diallyl tri sulfane (13.63%), allyl sulfide (13.06%), methyl allyl disulfide (11.87%), and methyl allyl trisulfide (4.84%). The antibacterial activity of essential oil of garlic tubers with diffusion method to use concentration variation of 5%, 10%, and 15% have strong antibacterial activity against Gram-positive bacteria like *Staphylococcus aureus* and have a medium to strong antibacterial activity against Gram-negative bacteria like *Escherichia coli*. The antioxidant activity of the essential oil of garlic tubers with DPPH (2,2-diphenyl-1-picrylhydrazil) showed an IC_{50} value was 22.863 mg/L and a very strong antioxidant group.

Keywords: Antibacterial, Antioxidant, Essential Oil, Garlic Tubers (*Allium sativum L*)

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1 Introduction

Essential oils or known as etheric oils or flying oils (essential oils, volatile) are produced by certain plants. Some plants as natural sources of essential oils are the Lauraceae, Myrtaceae, Rutaceae, Myristicaceae, and Labiatae families (Sudaryani & Sugiharti, 2005; Agusta, 2000). Essential oils are produced from certain plant tissue parts such as roots, stems, bark, leaves, flowers, fruit, or seeds. The oil is volatile at room temperature without decomposition, has a bitter taste, smells good according to the smell of the producing plant, and is generally soluble in organic solvents (Sudaryani & Sugiharti, 2005; Lutony & Rahmayati, 2002).

*Corresponding author at: Department of Chemistry, Faculty of Mathematics and Natural Sciences Universitas Sumatera Utara, Medan, Indonesia.

E-mail address: sabarminp@yahoo.com

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Garlic is an herbal plant that has been known to have been used as a cooking spice and as a medicine since 3000 BC by the Chinese and Central Asian tribes who used it to maintain health. Garlic is included in the Liliaceae family, which is a plant that contains 1% to 2% essential oil which has very varied chemical compounds. Garlic forms white tubers. A tuber consists of 8 to 20 cloves (scallions) and ribbon-shaped leaves 30 to 60 cm long (Hernawan & Setyawan, 2003; Lawrence & Lawrence, 2011).

Staphylococcus aureus is a Gram-positive, facultative aerobic and anaerobic bacterium that distinguishes it from other species. *Staphylococcus aureus* is the main pathogen for humans. Almost everyone will experience some type of *S. aureus* infection throughout their life, ranging from food poisoning or minor skin infections to severe life-threatening infections (Prihandani et al, 2015; Nasution, 2014).

Escherichia coli is a foodborne bacteria that can cause various gastrointestinal disorders. E.coli is a Gram-negative, facultatively anaerobic, and non-sporing bacterium that often causes lower gastrointestinal disorders in warm-blooded animals. E.coli causes disease because it produces a toxin called Shiga toxin (Prihandani et al, 2015).

Antioxidants are compounds that can counteract or reduce the effects of oxidants in the body. Such as free radicals that cause oxidation of fats in foods that cause damage to fats. Antioxidants work by donating electrons to free radical molecules because free radicals act as electron acceptors. Antioxidants are also able to neutralize free radicals by accepting electrons (Ramadhan, 2015; Rohman, 2016; Lawrence & Lawrence, 2011).

Amin et al, (2014) reported that there were 19 compounds found in garlic essential oil where diallyl disulfide was the compound with the highest number of 26.54%. Prihandani et al, (2015) reported that garlic extract has antibacterial activity against *Staphylococcus*, *Escherichia Salmonella typhimurium*, and *Pseudomonas aeruginosa* bacteria. The bioactive compound that has an important role was allicin, which is volatile with sulfur. Lawrence & Lawrence, (2011), (2011), the antioxidant activity of garlic essential oil that grew in North India, reported that garlic essential oil has potential as an antioxidant and the IC_{50} result from garlic essential oil was obtained at 0.5 mg/mL.

2 Materials and Methods

2.1 Equipments

In this study, the equipment used were water distillation apparatus, syringe, measuring flask, beaker glass, vial bottle, analytical balance, test tube, serology pipette, autoclave, Petri dish, tweezers, Erlenmeyer, ose needle, incubator, volume pipette, rubber ball, caliper, cotton, cling

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wrap, a set of ultraviolet-visibe (UV-Visible), gas chromatography-mass spectrometry (GC-MS) Spectrophotometer, napkins, aluminum foil, bunsen, disc paper, and vortex.

2.2 Materials

The materials used were local garlic (*Allium sativul L*), Na_2SO_4, NaCl, Ethanol, dimethyl sulfoxide (DMSO), nutrient agar (NA), Mueller Hinton Agar (MHA), DPPH, Ethanol *Stapyhlococcus aureus*, *Escherichia coli*, aquadest, and heating oil.

2.3 Isolation of Garlic (*Allium sativum L*) Tubers Essential Oil

A total of 300 g of garlic (*Allium sativum L*) tubers were cleaned from the skin, then sliced, and then put into 1000 mL of the bottom flask, then added ± 400 mL of aquadest, and connected to a Stahl apparatus equipped with an oil heater. Heated to boiling at 100 to 110ºC for ± 4-5 hours to produce distillate water with essential oils. The essential oil obtained was put into a vial, added NaCl to separate the oil, then the oil was separated from the water layer, then anhydrous Na_2SO_4 was added and allowed to stand for 24 hours and decanted. Next, the oil obtained was put into a vial, weighed, and then stored in the refrigerator. The chemical content was analyzed using GC-MS spectroscopy and continued with antibacterial and antioxidant activities tests.

2.4 Analysis of Garlic (*Allium sativum L*) Tubers Essential Oil with GC MS

The essential oils that have been obtained were analyzed for their chemical components using GC-MS spectroscopy.

2.5 Preparation of Nutrient Agar (NA) Media

A total of 7 g of NA was put into an Erlenmeyer and then dissolved in 250 mL of aquadest and heated until all dissolved and boiled.

2.6 Preparation of Oblique Agar Media and Bacterial Culture Stock

The sterile test tubes were put in 10 mL of NA media, then sterilized in an autoclave at 121ºC for 15 minutes. Let stand at room temperature until solidified in an inclined position to form an angle of 30 to 45º. The *Staphylococcus aureus* bacteria culture from the main strain was taken with a bent needle and then inoculated on the surface of the nutrient media so it was tilted by scratching, then incubated at 35ºC for 18 to 24 hours. The same treatment was performed on the *Escherichia coli* bacteria culture.

2.7 Preparation of Bacterial Suspension

A total of 10 mL of aquadest was put into a test tube and then sterilized in an autoclave at 121ºC for 15 minutes, then *Staphylococcus aureus* bacteria colonies were taken from the bacterial culture stock with a bent ose needle, then put into 10 mL of sterile aquadest, homogenized with a vortex, and then measured the absorbance of the blank was sterile distilled water with a

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wavelength of 600 nm. The same treatment was conducted on the Escherichia coli bacteria culture.

2.8 Preparation of Muller Hinton Media Agar (MHA)

A total of 9.5 g of MHA powder was put into an Erlenmeyer, then dissolved in 250 mL of aquadest and heated until all dissolved and boiled. Then sterilized in an autoclave at 121ºC for 15 minutes.

2.9 Preparation of Variations in Concentration of Garlic (Allium sativum L) Tubers Essential Oil

Essential oil was made in concentrations of 5%, 10% and 15% (v/v). Each essential oil was pipetted as much as 0.05 mL, 0.1 mL, and 0.15 mL and then put into a vial. Next, added 0.95 mL, 0.9 mL, and 0.85 mL of DMSO sequentially into the vial containing the essential oil, then homogenized using a vortex.

2.10 Antibacterial Activity Test of Garlic (*Allium sativum L*) Tubers Essential Oil

A total of 15 to 20 ml of sterile Mueller Hinton Agar (MHA) media were put into a sterile Petri dish, then allowed to solidify. A sterile cotton swab was taken, then dipped into a suspension of *Staphylococcus aureus* bacteria, and then scraped into MHA media which had solidified until evenly distributed. Then put the disc paper that has been soaked with chloramphenicol as a positive control, DMSO as a negative control, and essential oil of garlic (*Allium sativum L)* tubers with various concentrations into a petri dish containing *Staphylococcus aureus* bacteria, then incubated in an incubator at 35ºC. for 18 to 24 hours. Next, the diameter of the inhibition zone around the disc was measured. The same procedure Was done on *Escherichia coli* bacteria.

2.11 Preparation of 2.2- diphenyl-1-picrylhydrazyl (DPPH) Solution

In order to make 0.3 mM DPPH solution was prepared with as much as 11.83 mg DPPH powder put into 100 mL of the volumetric flask, then added ethanol to the marked line, then homogenized.

2.12 Preparation of Garlic (*Allium sativum L*) Tubers Essential Oil Variations

Garlic tuber essential oil was prepared 1000 ppm of mother liquor by dissolving 0.010 g of essential oil with ethanol in a 10 mL of volumetric flask. Then, 100 ppm of mother liquor was prepared by pipetting 1 mL of 1000 ppm of mother liquor into 10 mL of volumetric flask and diluted with ethanol to the marked line. The 100 ppm solution was diluted to make the 4, 6, 8, and 10 ppm concentration variations to the antioxidant activity test.

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2.13 Antioxidant Activity Test of Blank Solution

A total of 2.5 mL of ethanol was added as much as 1 mL of 0.3 mM DPPH solution in a test tube and left for 30 minutes in the dark room. Then, the absorbance was measured with a wavelength of 517 nm.

2.14 Sample Antioxidant Activity Test

A total of 2.5 mL of garlic tubers essential oil with a concentration of 4 ppm was put into a test tube and added 1 mL of 0.3 mM DPPH solution, homogenized, and left for 30 minutes. After that, the absorbance was measured with a maximum wavelength of 517 nm and carried out with the same treatment for a concentration of 6, 8, and 10 ppm.

3 RESULT AND DISCUSSION

3.1 Determination of Essential Oil Content

This process was repeated 7 times with the total number of samples of garlic tubers used being 2334 g with the amount of essential oil obtained was 1.0747 g.

$$\%\text{oil} = \frac{Weo(g)}{W1\,(g)} \text{x} 100\%$$

$$= \frac{1.0747}{2334} \text{ x } 100\% = 0.05\%$$

Description :

W_{eo} = weight of essential oil

$W_{1\,=}$ weight of garlic tuber

3.2 GC-MS Analysis

The essential oil obtained was analyzed by Gas Chromatography-Mass Spectroscopy (GC-MS). The GC chromatogram of the hydrodistillation garlic tubers essential oil obtained 11 peaks of the compounds presented in figure 1 and Table 1.

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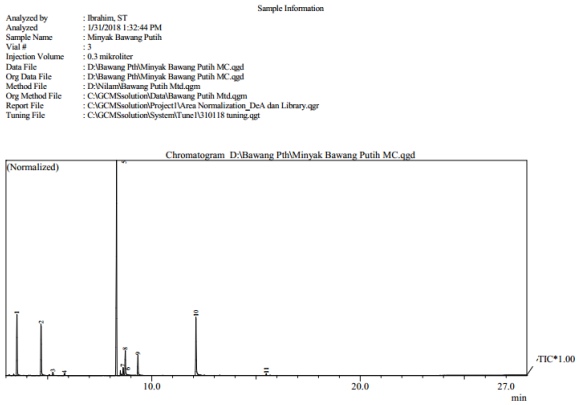

Figure 1. GC chromatogram of garlic tubers essential oil.

Tabel 1. The GC-MS results of garlic tubers (*Allium sativum L*) essential oil according to Library Wiley and Library Nist standards

No	Formula Molecule	Area (%)	Time Retention (Minute)	Massa Relative	Compound Allegedy
1	$C_6H_{10}S$	13.06	3.529	114	Allyl sulfide
2	C_4H_8S	11.87	4.686	120	Methyl allyl disulfide
3	$C_4H_8S_2$	0.72	5.255	120	Trans propenyl methyl disulfide
4	$C_2H_6S_3$	0.44	5.811	126	Dimethyl trisulfide
5	$C_6H_{10}S_2$	44.98	8.308	146	Diallyl disulfide
6	B_5H_9	1.18	8.492	113	Pentaborane
7	$C_6H_{10}S_2$	2.06	8.626	146	Diallyl disulfide
8	$C_6H_{10}S_2$	6.85	8.729	146	Diallyl disulfide
9	$C_4H_8S_3$	4.84	9.332	152	Methyl allyl trisulfide
10	$C_6H_{10}S_3$	13.63	12.120	178	1.3 Diallil tris sulfide
11	$C_6H_{10}S_2$	0.36		15.491	146 Diallyl disulfide

In this study, the chemical components of garlic tubers essential oil obtained were 11 components, namely allyl sulfide, methyl allyl disulfide, trans propenyl methyl disulfide, dimethyl trisulfide, diallyl disulfide, pentaborane, diallyl disulfide, diallyl disulfide, methyl allyl trisulfide, 1.3-diallyl trisulfide, 1.4-diallyl tetra sulfide, and diallyl disulfide were the most abundant components (44.98%).

3.3 Garlic Tubers Essential Oil Activity Test

The results of the antibacterial activity of the essential oil of garlic tubers with concentrations of 5%, 10%, and 15% (v/v) on *Staphylococcus aureus* and *Escherichia coli* bacteria were carried out by the agar diffusion method shown in Table 2;

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Tabel 2. The measurement result of the inhibitory zone of antibacterial activity of garlic tubers (*Allium sativum L*) essential oil

Bacteria	Clear Zone Diameter of Essential Oil (mm)				
	DMSO (K)	Chlorine amphere nikol	5%	10%	15%
Staphylococcus aureus	0	29.7	10.2	11	11.5
Escherichia coli	0	29	7	10.75	12.75

The formation of a clear area or zone around the disc paper indicated the inhibition of bacterial growth due to the influence of bioactive compounds found in the essential oil of garlic tubers. Salima (2015), this antibacterial activity was thought due to the content of allicin and ajoene, an organosulfur component possessed by garlic. Allicin was an active compound that acted as an antibacterial, by inhibiting the synthesis of RNA, DNA, and bacterial proteins, resulting in the death of bacteria.

Determination of the strength of antibacterial activity was grouped based on the area of the inhibition zone, i.e. a diameter of 20 mm or more was defined as very strong, a diameter of 10 to 20 mm was defined as strong, a diameter of 5 to 10 mm was moderate, and a diameter of 5 mm or less was weak (Komang et al, 2017).

3.4 Antioxidant Activity Test of Garlic Tubers (*Allium sativum L*) Essential Oil

The antioxidant activity test was performed using the method DPPH on essential oil of garlic tubers as a free radical to obtain the IC_{50} (Inhibitor Concentration) value observed using a UV-Visible spectrophotometer at a maximum wavelength of 517 nm.

Tabel 3. The antioxidant test of garlic tubers of essential oil

Sample (ppm)	Absorbance	%Damping
Blank	0.934	-
4	0.853	8.672
6	0.818	12.419
8	0.813	12.955
10	0.709	24.089

By using the least square equation (the equation that describes the relationship between the concentration of the test compound and the percentage of radicals scavenging), the IC_{50} value obtained was 22.863 mg/L. The bioactive compound in garlic that has an important role as an antioxidant was allicin (Sanah, 2016).

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Tabel 4. The level of the antioxidant power of the DPPH method

Sample (ppm)	Absorbance
Very Strong	< 50 g/ml
Strong	50-100 g/ml
Currently	101-150 g/ml
Weak	>150 g/ml

Based on Table 4 indicated that IC_{50} value obtained shows that the essential oil of garlic tuber (*Allium sativum L*) was a very strong antioxidant group. The reaction mechanism of between DPPH with allicin presented in figure 2, while radical antioxidant dimer reaction indicated in figure 3.

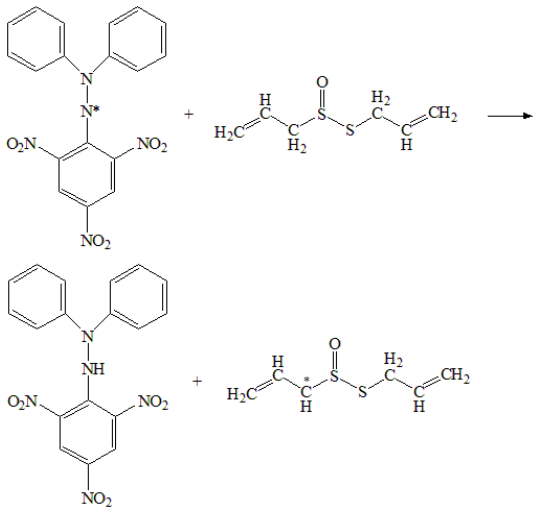

Figure 2. The reaction mechanism between DPPH with allicin

Antioxidants that become radicals then reacted with other molecules that were similar or the same (Molyneux, 2004).

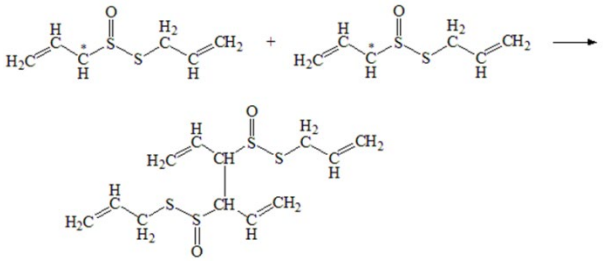

Figure 2. The dimer reaction of radical antioxidants.

4 Conclusion

Based on the data obtained in this study, it can be concluded that:

1. The main chemical components of garlic tubers (*Allium sativum L*) essential oil from hydrodistillation which was analyzed by GC-MS spectroscopy were 11 compounds. There were

5 components with large amounts, namely diallyl disulfide (44.98%), 1,3-diallyl trisulfide (13.63%), allyl sulfide (13.06%), methyl allyl disulfide (11.87%), and methyl allyl trisulfide (4.84%).

2. Garlic (Allium sativum L) essential oil has strong antibacterial activity on Gram-positive like *Staphylococcus aureus* and has moderate to strong antibacterial activity on Gram-negative like *Escherichia coli* bacteria.

3. Antioxidant activity of garlic tubers (*Allium sativum L*) essential oil by DPPH method (2,2-diphenyl-1-picrylhydrazil) obtained was an IC_{50} value at 22.863 mg/L and belongs to the group of very strong antioxidants.

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