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Research Article

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The media variance of production for anti microbe homogeny from the endophyte fungi of dahlia plant seed (*Dahlia variabilis*)

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ABSTRACT

Dahlia Plant Seed has been known as bio active homogeny content for anti microbe shown towards Escherichia coli aureus. This research is aimed to isolate the pure anti microbe from endophyte fungi fragmentation of Dahlia Plant Seed (Dahlia variabilis) and the structural determination of pure homogeny, as well as to conduct the pharmacological test found. In the first research step, it searched the optimum media as to product anti microbe from Endophyte fungi of Dahlia Plant Seed. This research used the fermentation media (Huang et al: 2007) varied the carbon source with peptone and ammonia nitrogen source. Screening was done with diffusion method in order to pathogenic microbe verily Escherchia coli, Staphylococus aureus, Aspergillus niger and Candida albicans. The extract concentration of impure endophyte fungi LBKRCC 41 for Fusarium sp 50 µl/disk showed that the small anti bacteria activity in the second media variance of Huang et al by watching in 5^{th} , 10^{th} , 15^{th} and 20^{th} days. Yet, the extract concentration of the same fungi for LBKURCC 43 (Sporothrix sp) was hard, and showed the big activity in the second day of in the same media variance. Media of Huang et al peptone variance gave the defend energy around 6 mm at E. coli and 8-9.5 mm at S. aureus. Media of Huang et al in ammonia variance gave the defend energy about 8-8,5mm at S. aureus. The hard Endophyte fungi of LBKURCC 43 Sporothrix sp 50 ul/disk gave the defend energy 20.65-23.3 mm at E. coli, 14.65-17.5 mm at S. aureus with peptone media variance of Huang et al (2007), for ammonia media variance of Huang et al (2007), it had the defend energy around 19.85-22.35mm at E .coli and 16.65-17.5 mm at S. aureus. This showed that the real difference and positive control applied. Yet, the both endophyte fungi did not show any activity to Aspergillus niger and Candida albicans. The second test result of metabolism with QC-MS was found out Xanthin and cathechin. The research result showed that the both endophyte fungi could be as the homogeny anti biotic source.

Keywords: Dahlia, Endophyte, Anti Microbe, Fermentation Media

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INTRODUCTION

Public in Indonesia suffer some diseases caused by several cases in which one of them is microbe, whether it is bacteria or fungi. Microbes such as *Escherichia coli, Staphylococcus aureus, Aspergillus niger and Candida albicans* can cause dangerous infection malady to mankind.

The usage of overdoses synthetic antibiotics could endanger for mankind health. An attempt to overcome the problem, namely by conducting a new anti biotic research from medic plant with doing the isolated endophyte microbe, especially endophyte fungi is mapped in plant tissue and could result the secondary metabolism concerning to nature [1].

In order to take directly bioactive homogeny from a plant was required bio mass so much or some of the plant itself. To make it efficiently, it was used a specific endophyte microbe sourced from plant mapping and expected to be able to result a number of bioactive homogeny to result antibiotic homogeny required without extracting from the plant itself. Every plant contains one or more from endophyte and has been found out the new anti bio active resulted from endophyte. It has been known that endophyte resulted bio active homogeny and almost 60% was anti biotic homogeny [2].

The endophyte microbe ability to produce secondary anti biotic homogeny which is suitable with the nature is a big chance and can be a priority to produce the secondary metabolic from endophyte microbes, isolated from the original plant which is more less than 300.000 from plant surrounding the earth. Each plant contains one or more Endophyte microbe which consists of bacteria and fungi and that through endophyte fungi where it could be produced metabolic homogeny fermentation in useful medic continually, reproduce ability in industrial scale for a short time, not underestimate ecological break [3].

Based on the previously research, it was known that hard extract of fermentation from endophyte fungi LBKURCC 41 and LBKURCC 43 verily *Fusarium sp* and *Sporothix sp* owned an activity of anti bacteria towards *Esherichia coli and Stophylococus aureus* [5]. Based on an optimum condition found above, it must be conducted the isolated pure homogeny of anti microbe and determine structural homogeny as to know about active genre, as well as toxicity seen a safety of pure homogeny.

The purpose of this research is to determine an optimum media to produce anti microbe homogeny from endophyte fungi LBKURRCC 41 and LBKURCC 43, so that it could be conducted the isolated homogeny of pure anti microbe from endophyte fungi fermentation of Dahlia plant seed (*Dahlia variabillis*) and also to determine the bioactive activity towards *Escherichia coli, Staphylococcus aureus, Aspergillus niger and Candida albicans.*

EXPERIMENTAL SECTION

The instruments used in this research was Spectrophotometer (Thermo genesi10SUV- VIS), Autocola1925x (Wiconsin Alumnium Foundry Co. Inc, Monitowoe), High Speed Micro Centrifuge Model CT 15RE, Vortex Mixer H- VM-300, Water Bath Grant SU28, Incubator Memmert, Rotary Shaker (Daihan Labtech Co. Ltd), oven, bowl, Petri ose needle, Bunsen, Gas Stove, Mirror Steer, Pincers, Filter Paper, Incubator, Foil Aluminium, Microscope, Cover Glass, Object Glass, Measure Glass, Reaction Tube, Volume Pipe, Erlenmeyer, Serong Range, Media Bottle, Incubator Shaker, Sentrifugasi, Analytical Scale, and Razor. The materials used were a media of Potato Dextrose Agar (PDA) in note : 1.10130.050, Sabouraud Dextrose Broth (SDB) in note 1.08339.0500, Sabouraud Dextrose Agar (SDA) Media of Nutrient Agar (NA) in note 1.05450.0500, Media of Nutrient Broth (NB) in note 1.05443.0500 (all media produced by Merck KGaA Germany); Natrium chloride (kel), calium Dehydrate Pospat (KH_2PO_4), Magnesium Sulfate hepta hydrate ($MgSO_4.{}_7H_20$), and sulfate iron of heptahydrate ($FeSO_4.{}_7H_2O$), Milipore Syrine filter 0.2 µm (Puradics TM 13 mm in note 6786-1302), Brake Paper 6 mm (macherey- nagel MN827ATD).

Preparations

Endophytic Fungi Fermentation

A number of 1 ml of inoculum of Endophytic fungi (5%) was inoculated into 200 ml of media (Huang *et al*, 2007), then it was incubated in 20 days in a hall of velocity 150 rpm. Every once in 5 days, it was conducted a test of anti microbe. The fermentation result of fungi culture was taken and centrifuged with velocity 5000 rpm in 20 minutes. To separate supernatant and cell biomass, fermented culture was filtered with using milliopore syringe filter 0.2 µm, so that it could be harder extract of endophyte fungi from Dahlia Plant. The hard extract of endophytic fungi was used to anti microbe test.

The Pathogenic Bacteria Reproduction

The test bacteria (*Escherichia coli and Staphylococcus aureus*) from landslide agar was inserted into medium of New Agar Nutrition aseptically and incubated in 24 hours. The growing colony was inoculated into Nutrition Broth medium and incubated in 24 hours at 37°C. After 24 hours, bacteria absorbed was measured. Bacteria was used to an anti bacteria test when OD reached 0.08-0.1 (as level as 10^7 CFU/mL). When OD is more than 0.1, it was liquidated by using NaCl 85%.

The Pathogenic Khamir Reproduction

The isolate of *Candica Albicans and Aspergillus niger* from miring agar was moved into a new PDA medium aseptically and incubated for 4 x 24 hours. It was inoculated again into Sabouroud Dextrose Broth (SDB) medium

and incubated for 48 hours. Khamir was ready to be used for anti khamir test when OD reached 0.08-0.1 (as level as 10^7 CFU/mL). When OD was higher than 0.1, it was conducted to be liquidated by using homogeny NaCl 0.85%.

Anti Bacteria Test

1 mL of pathogenic inoculum (OD 600 nm 0.1), as level as 10^7 CFU/mL [22] was inoculated into a reaction tube containing media of NA liquidate 15mL (50°C) and vortex, then this liquidated agar was poured into Petri bowl until solid. Each hard extract of endophytic sterile fungi 50 µL, dropped on sterile paper disc (6 mm) and then dried afterward. The positive control was Amoxsan 30 µg and negative control was the sterile fermentation medium. The paper disc was posted on NA medium containing the tested bacteria (*Escherichia coli* or *Staphylococcus aureus*) and the petridish was incubated in 37°. The inhibition zone around paper disc was measured post to be incubated in 24 hours.

Anti Khamir Test

1 mL of inoculums of pathogenic khamir (OD was 0.1 nm) as level as 10^7 CFU/mL was inoculated into the test tube containing 15 mL of liquidated PDA medium (50°C) and vortex, then poured into the petridish become solid. 50 µL sterilized endophytic isolate was dropped in the sterile paper disc (6 mm) and let dried. The positive control used was Ketoconazole (30 µg) and the negative control was sterilized fermentation medium. Paper disc was posted on PDA medium containing *Candida, Albicans,* and *Aspergilus*. The inhibition zone was measured after it was incubated in 2 x 24 hours.

Extract Endophyte Fungi Test of LBKUCC 41 and 43 with GC-MS.

Based on a test result of defend energy towards bacteria of *Escherichia coli, Staphylococcus aureus, Candida Albicans* and *Aspergilus niger* from petridish, endophyte fungi seemed had the biggest defend energy and conducted a secondary metabolism check resulted from endophyte fungi. The extract scale for fermentation fungi result was LBKURCC41 and 43 was 100 mg, stirred into 1 ml methanol, injected 1x ul into GC-MS by using Data Analysis of double range for Duncan test.

RESULTS AND DISCUSSION

The fermentation of fungi culture (LKBURCC 41 and LKBURCC 43) namely *Fusarium sp* and *Sporothix sp* were harvested in 5, 10, 15 and 20 days. It was harvested in the second session for both fungi (LBKURCC 41 and 43) verily Fusarium sp and Sporothix sp around 50 ml in action each other. The medium was Huang *et al* varied with sulfate ammonium, as a source of nitrate and Peptone, as carbon source, so that every endophyte fungi of LKBURCC 41 and 43 verily *Fusarium sp* and *Sporothix sp* has two kinds of fermentation medium. The results of inhibition zone towards microbes can be seen in the following table 1-7.

Table 1. Activity of endophyte fungi antimicrobe of LBKURCC 41 fermented at media Huang et al (2007) towards *Escherichia coli*

	Average of Inhibition Zone (mm)							
	Media Huang *et al* pepton variation				**Media Huang *et al* ammonia variation**			
Extract	Day 5	Day 10	Day 15	Day 20	Day 5	Day 10	Day 15	Day 20
Amoksan	20.5				20.5			
Control (-)	0				0			
LBKURCC 41	6	6	6	6	6	6	6	6

Table 2. Activity of Endophyte fungi antimicrobe of LBKURCC 41 fermentated at media Huang et al (2007) towards *Staphylococcus aureus*

	Average of Inhibition Zone (mm)							
	Media Huang *et al* pepton variation				**Media Huang *et al* ammonia variation**			
Extract	Day 5	Day 10	Day 15	Day 20	Day 5	Day 10	Day 15	Day 20
Amoksan	15.5				14			
Control (-)	0				0			
LBKURCC 41	8	8.5	9.5	9.5	8.5	8	8	8

Tabel 3. Activity of endophyte fungi antimicrobe of LBKURCC 41 fermented at media Huang et al (2007) towards *Aspergilus niger.L*

	Average of Inhibition Zone (mm)							
	Media Huang *et al* pepton variation				**Media Huang *et al* ammonia variation**			
Extract	Day 5	Day 10	Day 15	Day 20	Day 5	Day 10	Day 15	Day 20
Ketokonazol	14				14			
Control (-)	-				-			
LBKURCC 41	-	-	-	-	-	-	-	-

Table 4. Activity of endophyte fungi antimicrobe of LBKURCC 43 fermented at media Huang et al (2007) towards *Escherichia coli*

	Average of Inhibition Zone (mm)							
	Media Huang *et al* pepton variation				Media Huang *et al* ammonia variation			
Extract	Day 5	Day 10	Day 15	Day 20	Day 5	Day 10	Day 15	Day 20
Amoksan	10.85				10.6			
Control (-)	0				0			
LBKURCC 43	17.5	17.5	17	14.65	18.25	17.85	17	16.65

Table 5. Activity of endophyte fungi antimicrobe of LBKURCC 43 fermented at media Huang et al (2007) towards *Staphylococcus aureus*

	Average of Inhibition Zone (mm)							
	Media Huang *et al* pepton variation				Media Huang *et al* ammonia variation			
Extract	Day 5	Day 10	Day 15	Day 20	Day 5	Day 10	Day 15	Day 20
Amoksan	17				16			
Control (-)	0				0			
LBKURCC 43	23.3	20.9	20.9	20.65	22.35	22.1	21.3	19.85

Table 6. Activity of endophyte fungi antimicrobe of LBKURCC 43 fermented at media Huang et al (2007) towards A*spergilus niger.L*

	Average of Inhibition Zone (mm)							
	Media Huang *et al* pepton variation				Media Huang *et al* ammonia variation			
Extract	Day 5	Day 10	Day 15	Day 20	Day 5	Day 10	Day 15	Day 20
Ketokonazol	14.5				14			
Control (-)	0				0			
LBKURCC 43	-	-	-	-	-	-	-	-

Table 7. Activity of endophyte fungi antimicrobe of LBKURCC 43 fermented at media Huang et al (2007) towards *Candida albican*

	Average of Inhibition Zone (mm)							
	Media Huang *et al* pepton variation				Media Huang *et al* ammonia variation			
Extract	Day 5	Day 10	Day 15	Day 20	Day 5	Day 10	Day 15	Day 20
Ketokonazol	15.5				15			
Control (-)	0				0			
LBKURCC 43	-	-	-	-	-	-	-	-

Huang *et al* (2007) medium is a good medium for endophyte fungi fermentation where it was varied in carbon medium and nitrogen that will produce the bigger secondary metabolite. The screening activity result of hard extract of fermented endophyte fungi by using Huang *et al* medium from 5^{th} to 20^{th} in days can be seen at table of 1- 7 above. The hard extract of endophyte fungi *Fusarium sp* LBKURCC41 showed that activity of anti bacteria in Huang *et al* medium with medium of peptone varied in 5^{th} to 20^{th} days about *E.coli* bacteria gave the inhibition zone around 6 mm, to control the positive inhibition zone in 20.5 mm. Where as a result of inhibition zone towards bacteria of *E.coli* from endophyte fungi of Huang *et al* medium. Ammonia variance did not give the inhibition zone. Amoksan as positive control gave the inhibition zone 20.5 mm. For bacteria pathogenic of *S.aureus* had the inhibition zone with medium of Huang *et al* that varied peptone from 5^{th} to 20^{th} in days around 8-9.5 mm with positive control around 14 mm and amoksan as the positive control around 15.5 mm, for medium of Huang et al ammonia variance in 5^{th} until 20^{th} around 8-8.5 mm with positive control in 14 mm. The anti microbe activity of endophyte fungi LKBURCC 41 towards pathogenic fungi of *Aspergillus niger* did not have the inhibition zone, and neither did pathogenic fungi of *Candida albicans.* Ketonalzol, as postive control gave the inhibition zone 14 mm for *Aspergillus niger* and 15 mm for *Candida albicans.*

The hard extract of endophyte fungi *Sporothrix sp* LKBURCC 43 showed that anti bacteria activity towards pathogenic bacteria of *E.coli* around 20.65-23.3 mm from the 5^{th} until 20^{th} in days, for amoksan to give the inhibition zone in 17 mm with variance of peptone medium. The medium variance with ammonia gave the smaller inhibition zone from peptone medium variance verily 19.85,-22.35 mm and amoksan was 16 mm. For the inhibition zone towards *S. aureus* were 14.65,-17.5 from the 5^{th} to the 20^{th} in days. Inhibition zone of amoksan was 10.85 at peptone medium variance. The inhibition zones were 16.65-18,25 mm and 10,6 mm for variance of Huang *et al* medium and variance of ammonia amoksan from the 5^{th} to 20^{th} day respectively. The inhibition zone was towards *Aspergillus niger* pathogenic fungi and *Candida albicans* was not found in which ketonalzol around 14-15.5 mm.

Endophyte fungi which was inoculated at fermentation medium was about 5% from starter medium reproduced previously 24 hours. The measure of OD (optical density) was conducted by the time starter inoculated into fermentation, purposing a number of cells inoculated was the same as circulation for which OD was 660 nm in 0.1 as level as 107 CFU/mL [22]. Fermentation is one medium of anti microbe homogeny production from microorganism that was done by varying carbon and nitrogen sources from medium of Huang *et al* where sucrose

carbon source was changed with peptone and a source of extract nitrogen rag was changed with sulfate ammonium. This is aimed to see an influence of medium composition towards anti microbe homogeny production. When in fermentation process, the endophyte fungi would adopt to its nature, the growth of processed fermentation was begun from an adaptation step, exponential step, stationer step and last terminal step. At stationer step, nutrition medium was low so that endophyte fungi was in critics. In order to defend the life circulation of endophyte fungi homogeny, it would spread out secondary metabolism homogeny and become an emergency nutrition for a life [23]. Anti microbe homogeny from endophyte fungi was produced when to look at the stationer phase, because this phase, nutrition was low. A new anti microbe activity can be looked in 20 days. By the age of 20 days, nutrition medium was very enough, so that endophyte fungi did not produce any anti microbe homogeny.

An anti microbe test was done in every 5, 10, 15 and 20 days. It was conducted every day, yet the result would come in 5 days so that the test interval of anti microbe activity was in 5 days range. This is aimed to know about the optimal time for the highest anti microbe activity by signing the highest inhibition zone. A test of anti microbe activity was tested towards about four types of negative gram bacteria (*Escherichia coli*), positive gram bacteria (*Staphylococcus aureus*), pathogenic fungi of *Aspergillus niger* and *Candida albicans.* They are general pathogenic as disease effect to mankind [24]. A test of anti microbe extract activity of endophyte fungi of Dahlia plant was conducted by using paper disc diffusion method. The inhibition zone resulted was to compare with positive control, amoksan (50µg/b/v) for pathogenic bacteria and Ketokonazol (50µg/b/v) for pathogenic fungi. Negative control used a sterile medium to ensure anti microbe activity resulted was not from medium.

In Huang *et al* medium, variance with peptone and ammonia were used to replace nitrogen source. It consists of sucrose as carbon source changed by Na CMC, $NaNO_3$ as nitrogen source, KH_2PO_4 as phosfor the extract rag as nitrogen source was replaced by peptone and ammonia, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$ and KCl as sulfur and Mg^{+2},Fe_{+2} and K^+. The microbial activity by using variance of Huang medium et al can be observed in 5th day. The inhibition zone was higher than the 10th, 15th, and 20th days. This result shows that fungi LBKURCC 43 stepped the stationer phase. In this phase, nutrition in fermentation medium became low so that endophyte fungi would produce the secondary metabolite homogeny [23]. In the 20th day, the inhibition zone was so lower, caused by the dead phase so that a number of endophyte fungi producing anti microbe homogeny was low.

The extract activity of endophyte fungi was caused by NaCMC in medium of Huang *et al* source of complete carbon, as it had to be broken down. It was used by endophyte fungi. Sucrose would hydroleased first, and then glucose and fructose so that it could be used as source of nutrition of endophyte fungi. An activity of different microbe in every medium variance of Huang *et al* at endophyte fungi was caused by the difference of molecular structure for carbon source used in fermentation process. Glucose/dextrose as monosacharide is a homogeny directly used in metabolism, whereas NaCMC is disacharide stepping into hydrolease to be glucose and fructose where it is used for metabolism.

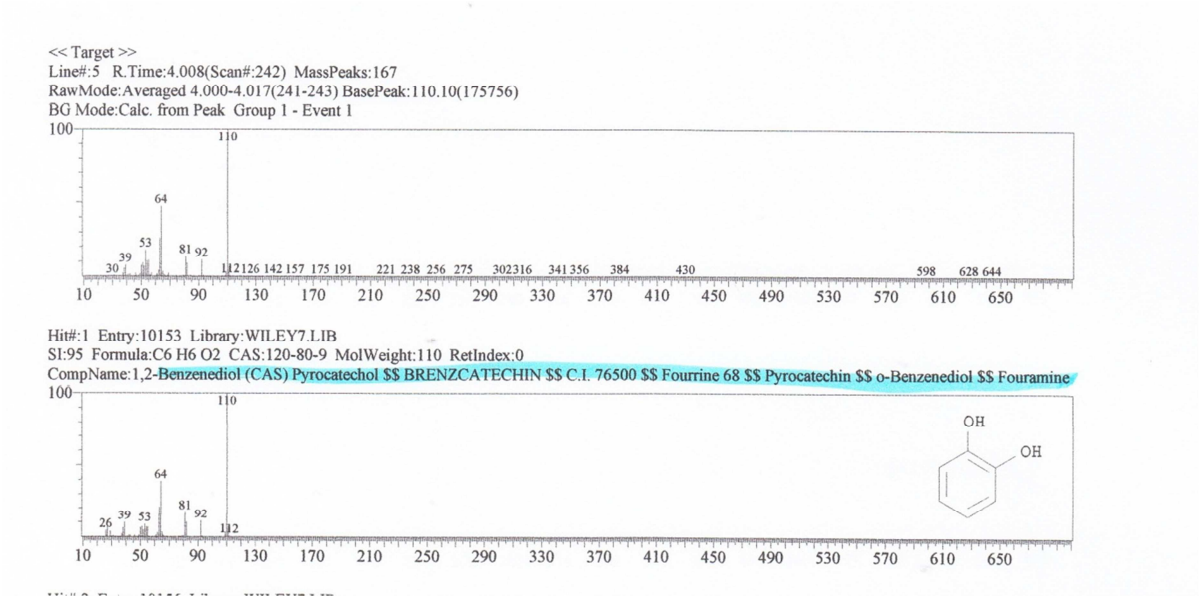

Figure 1. The chromatogram of endophyte fungi extract LKBURCC 43

A test result of homogeny contained in hard extract of endophyte fungi was conducted by using gas chromatography-mass spectrometer (GC-MS). The result from extract of endophyte fungi of LKBURCC 43 was xanthin and catechin homogeny (figure 1).

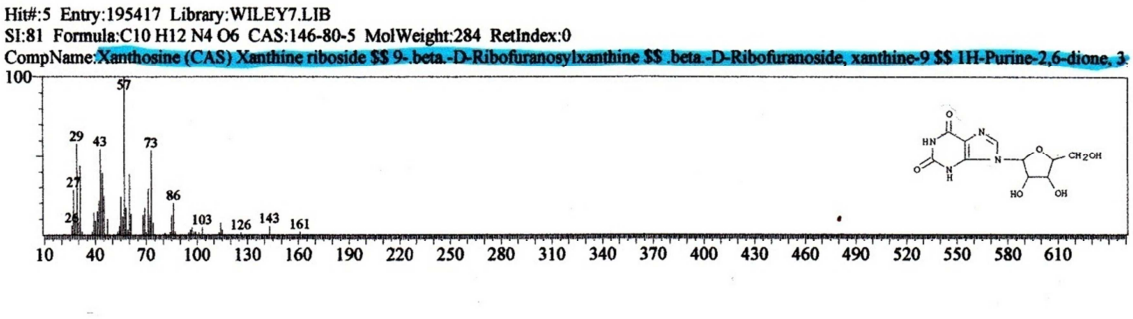

Figure 2. The mass spectrum of endophyte fungi extract LKBURCC 43

Cell membrane is functionalized as a selective support to homogeny concentration and defends anti homogeny concentration. The high concentration was based on active circulation through membrane. The focused concentration at cell surface would change the physical habits so that it could kill and defend cell [25]. Phenol, ammonium homogeny quarter would change mechanic of membrane permeability of bacteria cell so that it could break down the cell constituent in dead [26]. Advance test result by using Duncan's Multiple Range Test (DMRT) detected that inhibition zone of bacteria in extract of endophyte fungi LBKURCC 41 did not show the difference. In conclusion, extract of endophyte fungi LBKURCC 43 contained natural anti bacteria with the same inhibition zone in realistic LBKURCC 41 with $p < 0.05$.

CONCLUSION

Based on the result, it could be concluded that extract of endophyte fungi LBKURRC 43 gave the highest inhibition zone towards *Escherichia coli and Staphylococcus aureus*, which was 23.3 mm in the 5^{th} day with medium of Huang *et al* peptone variance. The optimum for medium of homogeny to produce anti microbe from endophyte fungi of Dahlia plant seed was Huang medium et al of peptone variance towards *Escherichia coli* in 5^{th} day. The test result of phytochemistry was by using gas chromatography (GC-MS) could be recognized a homogeny of Xanthin and Cathechin.

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