ANALYSIS OF THE PHYTOCHEMICAL CONTENT OF GOTU KOLA LEAF EXTRACT (*Centella asiatica* L.) AND EFFICACY TEST AGAINST MICROBES *Pseudomonas solanacearum*

ABSTRACT

Pseudomonas solanacearum bacteria can cause wilt disease in tomatoes, chilies, tobacco, potatoes, and Solanaceae plants in general. The use of synthetic pesticides to treat wilt disease can cause negative impacts on plants, humans, and the environment, so it is necessary to find environmentally friendly pesticides. This research aims to determine the secondary metabolite compounds contained in the gotu kola leaf extract and to determine the antibacterial effect of gotu kola leaf extract against the bacteria *Pseudomonas solanacearum*. This research was carried out from January to March 2023 at the Wood Properties and Product Analysis Laboratory, Department of Agricultural Agrotechnology, Samarinda State Agricultural Polytechnic, Sungai Keledang, Samarinda Seberang District, Samarinda City, East Kalimantan. Research stages, namely: field observation of making Simplicia, making ethanol extract, analysis of the content of gotu kola leaf extract (phytochemical screening) consisting of alkaloids, flavonoids, tannins, saponins, carotenoids, and steroids as well as testing antimicrobial (anti-bacterial) activity. The results of the research show that there are secondary metabolite compounds in the crude ethanol extract of gotu kola leaves, namely Tannins, Flavonoids, Saponins, Alkaloids, and Steroids. The results of antibacterial activity tests on *Pseudomonas solanacearum* bacteria with concentrations of 2.5%, 5%, 10%, 15%, and 20% produced clear zone diameters with the following values: 10.3 mm, 12.3 mm, 10 mm, 11, 3 mm, and 10.7 mm, so it can be stated that the Minimum Inhibitory Concentration (MIC) is 5%.

INTRODUCTION

Pseudomonas solanacearum is a bacteria that causes wilt disease in tomatoes, chilies, tobacco, potatoes, and Solanaceae plants in general. This bacterium can attack its host at almost all phases of its development. This bacterial wilt disease is still a serious threat to farmers. The reason is that this bacterial attack causes wilting and even death of plants. Usually, this bacteria attacks many plants, including chilies, potatoes, melons, tomatoes, and various types of eggplant plants.

Bacterial attacks occur in the plant's xylem vessels, which will cause damage to this tissue and as a result, water transport to the leaves will be hampered. This obstruction of water flow will disrupt the photosynthesis process in the leaves so that no food is produced and cannot compensate for the evaporation that continues to occur. This disease has a big impact on plant growth so it is very detrimental to farmers. Eradication of this bacteria is usually with the bactericide agrimycin by pouring it into the soil around the plant (Samadi, 1996).

Synthetic pesticides are the most widely used means of controlling plant pests (OPT) by farmers in Indonesia (95.29%) because they are considered effective, easy to use, and economically profitable. The use of such synthetic pesticides can certainly pollute the environment and in turn, can leave pesticide residues on agricultural products. In the environment, pesticide residues can kill macro and micro-organisms and damage the natural balance. Meanwhile, in agricultural products, pesticide residues can harm human health, such as decreasing the immune system, disrupting kidney and liver function, encouraging cancer growth, and disrupting nerve function (http://cybex.pertanian.go.id/article/98725/dampak- synthetic-pesticides-and-how-to-reduce-their-residues/). The National Research and Innovation Agency (BRIN) warns that the use of synthetic pesticides can trigger various bad effects ranging from accumulation of residue in the body to resistance to plant pests. "Synthetic pesticides cause many ongoing problems both for farmers themselves and the environment (https://www.antaranews.com/berita/3814158/brin-ingangkat-dampak-buruk-pemakaian-pestisida-sintetis). As stated by Ibrahim and Sillehu (2022), The inappropriate use of pesticides endangers the health of farmers, consumers, and non-target microorganisms and has an impact on environmental pollution of both land and water up to 5 million cases of poisoning due to pesticides among workers in the agricultural sector, most of which occur in developing countries, one example of a developing country is Indonesia (Alfiansyah, 2024).

Persistent pesticides can remain in the environment for years, even decades so their impact on the environment and plants will accumulate in the environment and plants. Non-persistent pesticides can be

degraded in nature into other harmless compounds (detoxification occurs). This decomposition can take place chemically (photolysis) or biologically by plants or microorganisms. The effects of non-persistent pesticide residues can only last a few days to several months (Fitriadi & Putri, 2016) & (Amilia & Sunardi, 2016). The persistent nature of pesticides means that they experience long deposition in the soil causing soil degradation (Puspitasari & Khaeruddin, 2016).

Seeing the bad consequences caused by synthetic pesticides, it is necessary to find a solution, namely by using organic pesticides that are friendly to plants, the environment, and humans. Various types of plants can be used as ingredients in making organic pesticides.

Indonesia is one of the countries that has the largest medicinal plants in the world, namely around 80% of the world's herbs grow in Indonesia. Indonesia is known to have various plants that are useful as medicines (Muhlisah, 2007). One plant that is known to have medicinal properties is gotu kola (Centella asiatica). Gotu Kola is a wild plant that grows widely in plantations, fields, roadsides, and rice fields. This plant originates from tropical Asia, spread across Southeast Asia, including Indonesia, India, the People's Republic of China, Japan, and Australia, then spread to various other countries (Lestari, 2010). Gotu kola is a herbaceous plant; annual. This plant has no stem but has a short rhizome and various creeping stolons, about 10-80 cm long. The leaves of this plant are single, arranged in a research consisting of around 2-10 leaves. This plant sometimes looks a bit hairy, the leaf stalks are about 50 mm long. The leaves of this plant are kidney-shaped, wide, and round with a diameter of around 1-7 cm. The edges of the plant's leaves are serrated to jagged, especially towards the base of the leaf. This plant contains bioactive components, namely Asiaticoside, Aankunicide, Sotankunicide, Madekasoside, Brahmoside, Brahminoside, Brahmic acid, Madasiatic acid, Meso-inositol, Senteloside, Carotenoid, Hydrocotylin, Vellarin, Tannin and mineral salts such as Potassium, Sodium, Magnesium, Calcium. , and Iron (Wijayakusuma et al., 1994; Lasmadiwati et al., 2004), phosphorus, essential oils (1%), pectin (17.25%), amino acids and vitamin B (Santa and Bambang 1992), bitter Vellarine, and Tannic substances (Dalimartha 2006).

The phytochemical test is a preliminary test to determine the content of secondary metabolite compounds found in plants using certain reagents (Julianto, 2019). Research in Indonesia regarding the bioactive compound content of gotu kola plants has not yet been carried out much.

The research aimed to determine the metabolite compounds contained in gotu kola leaf extract, and to determine the antibacterial effect on *Pseudomonas solanacearum* bacteria.

2. RESEARCH METHODS

2.1. Time and Place

This research was carried out from January to March 2023 at the Wood Properties and Product Analysis Laboratory, Department of Agricultural Agrotechnology, Samarinda State Agricultural Polytechnic, Sungai Keledang, Samarinda Seberang District, Samarinda City, East Kalimantan.

2.2. Tools and materials

The tools used are cutting knives, drop pipettes, rotary evaporators, rotary vacuums, spoons, test tubes, autoclaves, magnetic stirrers, Petri dishes, aluminum foil, drop plates, measuring cups, test tubes, Erlenmayer, sample bottles, laminar airflow, ose needle, and blender.

The materials used are gotu kola leaves (Centella asiatica L.), cotton, Ethanol 95% and Ethanol 10% Nutrient agar, Acetone, Hydrochloric acid (HCl 2N), Dragendorff Reagent, Liebermann-Burchard Reagent, FeCl3 Reagent 1% (Iron(III) chloride), $CHCl_3$ (chloroform) reagent, NaCl (sodium chloride), bacteria Pseudomonas solanacearum, and Aquades.

2.3. Research Stages

The stages of research activities are:

(1) Field observations to determine the location for taking samples of gotu kola leaves.

(2) Sample preparation: gotu kola leaves were taken around the swamp land in the Kahoi Samarinda area. Gotu kola leaves are picked by hand, collected, and air-dried indoors, then 95 grams are taken and ground dry without adding water.

(3) Extraction of secondary metabolite compounds

A sample of crushed gotu kola leaves was weighed and weighed 40 grams, then macerated with 96% ethanol solvent and extracted for 2×24 hours until the extract solution was no longer colored; Then the solvent was filtered and evaporated using a rotary evaporator to obtain a crude ethanol extract;

(4) Phytochemical test

The ethanol extract obtained is then subjected to a phytochemical test to determine the types of secondary metabolite compounds contained in it, namely:

(a) Tannin test: the test is carried out by inserting 10 ml of the extract solution into a test tube and adding 1% (CH3COO)2Pb solution. Tannin is declared positive if a yellow precipitate forms in the reaction.

(b) Flavonoid test: the test is carried out by adding 1 ml of gotu kola leaf extract and adding a few drops of dilute sodium hydroxide (1% NaOH). The appearance of a clear yellow color in the extract solution and fading to colorless after adding dilute acid (HCl 1%) indicates the presence of flavonoids.

(c) Saponin test: the test is carried out by adding 1 ml of mushroom extract dissolved in Ethanol into a test tube and then adding 10 ml of hot water into the test tube. The solution is cooled and shaken for 10 seconds, and foam forms which persists for ± 10 seconds with a height of 1-10 cm and does not disappear if 1 drop of Hcl 2N is added, meaning it is positive for Saponin.

(d) Alkaloid test: This test is carried out by adding 1 ml of extract, adding 2 ml of KCl, then adding 1 ml of Dragendorff's solution. Changing the color of the solution to orange or red indicates that the extract contains alkaloids

(e) Carotenoid test: this test is carried out by adding 1 ml of extract, adding 2 ml of KCl, then adding 1 ml of Dragendorff's solution. Changing the color of the solution to orange or red indicates that the extract contains alkaloids

(f) Triterpenoid/Steroid test: in the Triterpenoid/Steroid test, if it is red or purple it means it is positive for Triterpenoids, if it is green and blue it means it is positive for Steroids. 1 ml of mushroom extract that has been dissolved in acetone in a test tube then add 10 drops of anhydride acetic acid and 2 drops of concentrated sulfuric acid sequentially. Next, the test tube is shaken and left for a few minutes. The reaction that occurs is followed by a color change. If it is red or purple it means it is positive for Triterpenoids, if it is green and blue it means it is positive for steroids.

(5) Sterilization of tools and materials

Petri dishes, test tubes, Erlenmayer, agar media (NA), nutrient broth media (NB), and all tools and materials except the crude ethanol extract that will be used, are sterilized in an autoclave for 1 hour at a temperature of 121◦ C and a pressure of 1 atm after washed clean, dried and wrapped in aluminum foil.

(6) Preparation of pure bacterial culture (*Pseudomonas solanacearum*):

(a) To make agar media, the first thing to do is weigh 4 grams of agar (nutrient), 2 grams of nutrient broth C6H12O6 glucose, and 200 ml H2O distilled water, then mix the ingredients that have been weighed and stir until evenly mixed, then cover. With aluminum foil, prepare test tubes, cotton, tube, multiculture, and Pseudomonas solanacearum bacteria.

(b) Before sterilizing the tube, it is closed first using cotton wool, and the test tube, tube, and 200 ml beaker are wrapped in aluminum foil, then sterilized using an autoclave for 1 hour, after being sterilized, leave it in the autoclave for 15 minutes.

(c) Next, put the sterilized materials and tools into the ESO laminar airflow apparatus, remove the aluminum foil fill the test tube with 10 ml of agar media, and wait 15 or 20 minutes until dry, then prepare the multi-culture tube, warm the multicultured tube, Wait until the tube is cold, warm the tip of the test tube and cotton wool then take the Pseudomonas solanacearum bacteria that are available for cultivation in the newly made media.

(7) Making positive controls (+) and negative controls (-):

(a) making a positive control (+) using 0.0250 mg chloramphenicol C11H12C12N2O5 dissolved in 10 ml acetone (C3H6O)

(b) Making a negative control (-) using acetone (C3H6O). The use of control (+) and control (-) in this study is as a comparison for antibacterial activity tests and the use of chloramphenicol is a standard comparison in antibacterial activity tests in the laboratory.

(8) Test antimicrobial activity by measuring the diameter of the clear zone.

2.4. Determination of MIC (Minimum Inhibitory Concentration)

Gotu kola leaf extract is made in several concentrations, namely 2.5%; 5%, 10%; 15%, and 20%. The solvent used is 10% acetone. Each concentration was weighed according to the concentration, then each concentration was given 10% Acetone solvent and shaken until the extract dissolved. Then the

antibacterial activity was tested and the lowest concentration of the extract that could still inhibit bacterial growth was the Minimum Inhibitory Concentration (MIC) value (Greenwood, 1945).

2.5. Antimicrobial Activity Assay

The antibacterial activity test was carried out aseptically using the well method. To test antimicrobial activity, 5 ml of nutrient broth (NB) media was poured into a petri dish and added with 15 ml of nutrient agar (NA) then left to solidify. After the agar solidifies, bacteria are spread that have been bred using sterilized cotton. Then the petri dish was incubated for 24 hours at a temperature of 35-37ºC. The clear area around the well indicates a positive test, namely the presence of antimicrobial activity. The diameter of the clear zone obtained was measured and compared with the standard compound chloramphenicol. In this study, the standard used was tetracycline (chloramphenicol + 10% acetone) and the extract in the antimicrobial activity test was crude ethanol extract.

2.6. Data Analysis Techniques

The data analysis technique used to test antimicrobial activity is by measuring the diameter of the clear zone around the resulting wellhole. Davis Stout quoted by Adriansyah (2005) states that the provisions for antibacterial strength are as follows: an obstacle area of more than 20 mm means very strong, more than an obstacle area of 10-20 mm (strong), an obstacle area of 5 mm (medium), and an obstacle area of less than 5 mm or means weak (Table 1).

Table 1. Bacterial Inhibition Classification

Diameter of inhibition area (DDH)	Growth inhibition response
>20 mm	Very strong
10-20 mm	Strong
5-9 mm	Currently
<5 mm	Weak

Source: (Adriansyah et al,.2005)

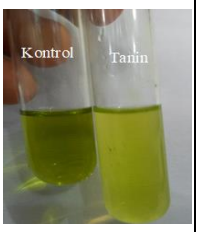
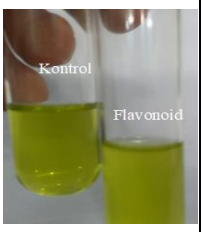
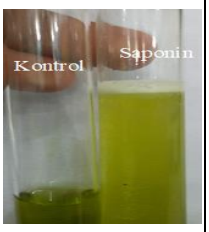
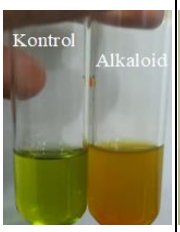
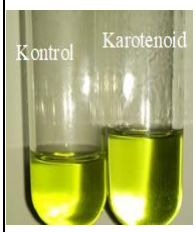
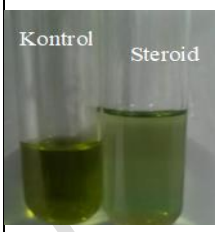
3. RESULTS AND DISCUSSION

3.1. Results of Phytochemical Screening Test for Gotu Kola Leaf Extract

Based on the results of the phytochemical test on gotu kola leaves, the results were obtained as presented in Table 2.

Table 2. Results of Phytochemical Screening of Crude Extract of Gotu Kola Leaves

Ethanol					
Tanin	Flavonoid	Saponin	Alkaloid	Carotenoid	Steroid

+	+	+	+	-	+
Description					
					
A yellow precipitate is formed.	A clear yellow color appears in the extract solution and fades.	Formation of persistent foam.	An orange color is formed.	No blue color forms on the surface	A green color is formed which indicates that it is positive for steroids.

Information :
+ = Contains secondary metabolite compounds
- = Does not contain secondary metabolite compounds

Based on the data in Table 2, it shows that in gotu kola leaf extract secondary metabolite compounds were found, namely tannins, flavonoids, saponins, alkaloids, and steroids, but did not contain carotenoids. Based on the data in Table 2, it shows that in gotu kola leaf extract secondary metabolite compounds were found, namely tannins, flavonoids, saponins, alkaloids, and steroids, but did not contain carotenoids. The results of another study reported by Hapsari et al (2017) stated that the ethanol extract of the gotu kola herb contains tannins, saponins, and steroids, but does not contain alkaloids and flavonoids. Furthermore, it was reported by Susetyarini and Nurrohman (2022) that gotu kola leaf extract contains flavonoids, alkaloids, tannins, and saponins as well as Vitamin C.

The tannin test showed positive results, namely that a yellow precipitate was formed. Tannin is a very complex component of organic substances, consisting of phenolic compounds that are difficult to separate and difficult to crystallize, precipitate proteins from solution, and combine with these proteins (Desmiaty et al., 2008).

The flavonoid test showed positive results. Flavonoids are polar compounds because they have several hydroxyl groups. Therefore, flavonoids generally dissolve in polar solvents such as ethanol. Ethanol functions as a flavonoid liberator from its salt form. The addition of concentrated hydrochloric acid functions to protonate flavonoids to form flavonoid salts. After adding magnesium powder, positive results were shown by changing the color of the solution to reddish black. The reddish-black color produced indicates the presence of flavonoids as a result of reduction by concentrated hydrochloric acid and magnesium (Harborne, 1987).

The Saponin test showed positive results, marked by the formation of persistent foam. Saponin is a glycoside form of sapogenin so it is polar. Saponin is a compound that is surface active and can cause foam if shaken in water (Kristanti et al., 2008). The appearance of foam in the saponin test indicates the presence of glycosides which can form foam in water which is hydrolyzed into glucose and other compounds (Marliana et al., 2005). The saponin compound will tend to be attracted by semi-polar solvents such as methanol.

The Alkaloid test shows positive results as indicated by the presence of an orange or red color. Alkaloids contain nitrogen atoms and are basic so to extract them requires the addition of sulfuric acid. The nitrogen atom which has a lone pair of electrons in the alkaloid replaces the iodine ion in the Mayer reagent, this results in the formation of a red precipitate when the Mayer reagent is added because the nitrogen in the alkaloid will react with the K+ metal ion from the Mayer reagent (Marliana et al., 2005). Alkaloids are generally in the form of crystals called alkaloid salts

The carotenoid test showed negative results because no blue color was formed on the surface. The characteristics of carotenoids are that they are sensitive to alkalis and very sensitive to air and light,

especially at high temperatures, and insoluble in water, glycerol, and propylene glycol (Kumalaningsih, 2007).

Steroids show positive results because a green color is formed. The compound analysis is based on the ability of the compound to form color with concentrated H_2SO_4 in anhydrous acetic acid solvent (Ciulei, 1984)

3.2. Antimicrobial Activity Test Results

To test antibacterial activity in this study, Pseudomonas solanacearum bacteria were used. The results of the antibacterial activity test of the crude ethanol extract of gotu kola leaves for 6 hours are presented in Table 3.

Table 3. Antibacterial Activity Test Results of Crude Ethanol Extract

Inhibition	Control +	Control -	Concentration of Extract Gotu kola leaves				
			2,5%	5%	10%	15%	20%
			Clear Zone Diameter (mm)				
Vertical	28 mm	0	10	12	10	11	11
Horizontal	23 mm	0	11	12	10	12	11
Oblique	27 mm	0	10	13	10	11	10
Amount	78 mm	0	31	37	30	34	32
Average	26 mm	0	10,3	12,3	10	11,3	10,7
Growth Inhibition Response			Strong	Strong	Strong	Strong	Strong

Source; Primary data is processed

In the antibacterial activity test, the data analysis technique used to test antimicrobial activity is by measuring the diameter of the clear zone produced. (Davis et al., 1971) stated that the provisions for antibacterial strength are as follows: a resistance area of 20 mm or more means very strong, a resistance area of 10-20 mm (strong), a resistance area of 5-10 mm (medium), and resistance area 5 mm or less means weak. So based on these provisions it can be seen that the crude extract of gotu kola leaves has antibacterial power which is included in the strong category at concentrations of 2.5%, 5%, 10%, 15%, and 20%.

Based on the data in Table 3, it shows that the control + concentration produces growth inhibition values ranging from 23 – 28 or with an average of 26 mm, while the control - concentration has an inhibition value of 0 mm. The results of the antibacterial activity test of the crude extract of gotu kola leaves on *Pseudomonas solanacearum* bacteria with a concentration of 2.5%; 5 %; 10 %; 15 and 20% produce clear zone diameters with the following values: 10.3 mm, 12.3 mm, 10 mm, 11.3 mm, and 10.7 mm, so it can be seen that the Minimum Inhibitory Concentration (MIC) value is equal to 2.5 %. The research results also showed that ethanol extract with a concentration of 5% showed more effective antibiotic activity compared to higher concentrations, namely 10%, 15%, and 20%. The research results reported by Ramdani (2022) show that the ethanol extract of gotu kola leaves can inhibit the growth of *Shigella dysenriae* bacteria at a concentration of 75% with an inhibitory power of 7.2 mm. which is classified as moderate inhibitory power. Furthermore, it was reported by Purba et al (2024) that a 100% concentration of ethanol extract from gotu kola leaves was able to show antimicrobial activity but could not inhibit the growth of *Streptococcus pneumoniae* bacteria.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1. Conclusion

Based on the research results and discussion, it can be concluded that:

1. The results of the phytochemical analysis show that there are secondary metabolite compounds in the crude ethanol extract of Gotu Kola leaves, namely Tannins, Flavonoids, Saponins, Alkaloids, and Steroids.

2. Based on the results of the antibacterial activity test, the crude extract of Gotu Kola leaves has been tested on *Pseudomonas solanacearum* bacteria with concentrations of 2.5%, 5%, 10%, 15%, and 20% producing clear zone diameters with the following values: 10.3 mm, 12.3 mm, 10 mm, 11.3 mm, and 10.7 mm. So it can be seen that the MIC (Minimum Inhibitory Concentration) is 5%.
3. Ethanol extract with a concentration of 5% shows more effective antibiotic activity compared to higher concentrations, namely 10%, 15%, and 20%.

4.2. Suggestions

Suggestions from this research are as follows:

1. In this research, further research needs to be done regarding the content of gotu kola leaves (*Centella asiatica* L.) using stem extract and then testing it against Pseudomonas solanacearum bacteria.
2. Gotu kola leaves should be thinly sliced before drying to facilitate the extract solution.
3. The stirring method used should use an automatic device and stir for a longer time so that the active substance can dissolve more optimally in the solvent used.
4. Make sure that in every extract-making or testing process, sterile equipment is used and the testing is carried out in Laminar Air Flow.
5. It is recommended that research be carried out by observing the phytochemical content to determine which is the most dominant among the phytochemical content of gotu kola leaves.
6. To increase the validity of the antimicrobial test results, an adjusted number of test Petri dishes should be made

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