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ANTIBACTERIAL ACTIVITY OF EXTRACTS OF LEAVES OF LEILEM (*Clerodendrum minahassae* Teijsm. & Binn.) ON GROWTH GRAM-POSITIVE BACTERIA Staphylococcus aureus strains ATCC 25923

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Abstract

Leaf leilem (Clerodendrum minahassae Teism. & Binn.) commonly used by the Minahasa as food and traditional medicine for treating intestinal worms, abdominal pain, and pain in the chest. Gram-positive bacteria Staphylococcus aureus ATCC 25923 strain is a type of bacteria that is found in humans and can be the cause of the emergence of the disease. This research aims to know the types of solvents are most effective and how the antibacterial activity of extracts of leaves of leilem on the growth of the bacteria Staphylococcus aureus strains ATCC 25923. The solvent used is ethanol is polar, ethyl acetate which is semi polar, and n-heksan which is non polar. Preliminary research has been conducted on konsenterasi extract with 100 ppm 200 ppm 400 ppm and 800 ppm, the antibacterial activity of the most optimal at 800 ppm so the data will be used for the analysis in this study was konsenterasi 800 ppm. Deuteronomy was done by as much as 3 times. Positive control using antibiotics clindamicyn 400 ppm and negative controls using aquades and 10% DMSO. Isolation of bacteria using scratch in zig-zag and to test the antibacterial activity of using diffusion well in order. Results of the study showed that the percentage yield of solvent is ethanol that is 1,2329% in maceration 1:4. Drag the diameter of most bacteria are grown on ethyl acetate extract polar spring with a grade average of 12.6 mm. Based on the analysis of OneWay ANOVA showed different results for real then followed by Tukey test, to see that these three types of different extract significantly in inhibiting the growth of gram positive bacteria Staphylococcus aureus strains ATCC 25923. In conclusion, namely the first ethanol is a solvent that is most effective in dissolving the leaves leilem, both of the antibacterial activity in sequence from the most minor to the most massive is the n-heksan (non-polar), ethanol (polar), and ethyl acetate (semi polar). The third conclusion in the optimum konsenterasi 800 ppm, extract n-heksan n-heksan (non-polar), ethanol (polar), and ethyl acetate (semi polar) showed antibacterial activity significantly different.

Key words: ***Leilem Leaves, Staphylococcus aureus strain ATCC 25923, Antibacterial activity.***

INTRODUCTION

Biodiversity in Indonesia allows the existence of biological development potentialities in various fields, including in the field of industry. There are a wide variety of industrial products are produced from natural resources biodiversity, both used in the fulfillment of the

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needs of food or other products in meeting the needs of the community living day to day. The result of the processing of natural resources biodiversity that is not less important is in the field of pharmaceuticals, namely the production of medicines. Forest areas in Indonesia has vast areas of 120,35 million hectares and has approximately 80% of the total plant species are efficacious drugs (Heriyanto, 2006).

One of the plants that already have long been used in traditional medicine in Minahasa is a plant leilem (Clerodendrum minahassae Teijsm. & Binn.). The community of minahasa plant planting leilem usually this yard House and acreage estates. In addition to traditional medicinal plants, leilem is also utilized by communities in Minahasa as a vegetable that is usually cooked together with meat. This leilem plant included in the genus Clerodendrum and family Verbenaceae (Wiart, 2002). According to Burghardt (2006) Plant leilem (Clerodendrum minahassae Teijsm. & Binn.) shaped shrub and can grow into a tree with a height of 3–7 meters and a width of fork 4.6–9.1 meters. Leilem can also live on a neutral pH to acidic circumstances, have the tolerance to drought and can grow on the structure of soil clay and Sandy.

Plant parts from leilem who often used i.e. the leaves. In addition to groceries, leaves leilem is used also to treat various diseases. Diseases such as intestinal worms, mangi in infants (Adam, 2013). TBC (Sangi et al, 2008), and also pain in the chest and abdomen (Quattrocchi, 2012). One of the bacteria that can cause the emergence of a disease is the bacteria Staphylococcus aureus ATCC 25923 strains (ATCC American Type Culture Collection).According to Anonymous (2013) are saying that Staphylococcus aureus Infections became one of the causes of the occurrence of health problems including ulcers, acne. The enterotoxin produced can cause nausea and stomach pain.

One of the ways to prove the link between the drug plants with disease-causing bacteria is through a test of antibacterial activity with the addition of medicinal plants that have been dimaserasi so have shaped</http:> extracts.</http:> </http:> </http:> The lack of scientific data research results leilem plants as medicinal plants to treat diseases caused by microorganisms such as bacteria will make leilem only as traditional medicinal plants are not widely known. Because it is necessary holding of research on "antibacterial activity of Leilem leaf extract (*Clerodendrum minahassae* Teijsm. & Binn.) on the growth of Gram positive Bacteria Staphylococcus aureus strains ATCC 25923 ". The purpose of this research is to know the types of solvents are most effective in dissolving the leaves leilem and also find out the best antibacterial activity of extracts of leaves of leilem on the growth of the bacteria Staphylococcus aureus ATCC 25923 strains, as well as knowing the difference polar extracts activity, semi polar and non-polar leaves of leilem. The expected results of this research can be useful for the advancement of science, knowledge and technology, especially in the field of biology.

MATERIALS AND METHOD

2.1. Time and place of Research

This research was carried out for 3 months. Sampling done in Kelurahan leilem leaf Watulambot, Tondano Barat, Minahasa Regency with elevation 600 mdpl, average temperatures 18–26oC, humidity and an average of 70–95%. For the extraction of leaf leilem, bacterial inoculation, and antibacterial activity test was conducted in the laboratory of bio-Pharmacy and Molecular Biology Department of Biology Faculty of mathematics and Natural Sciences University of Manado.

2.2. Tools and materials Research

For the extraction of leilem leaves, the tool used is a rotary evaporator, sterile knife keeps, analytic scales, erlenmeyer flask, hanskun, filter paper, scissors, a measuring cup,

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spoon, glass jar, shaker incubator, spatula, bottle vial, aluminum foil, a mask, and a camera. To give the sign of every unit of the experiment used paper labels. the material used is leaves of leilem (Clerodendrum minahassae Teijsm. & Binn.) old fresh green to dark green, this is because the older leaves contain active compounds more compared with young leaves (Mann, 1987).

The solvent used was composed of organic solvent ethanol polar nature, ethyl acetate which is semi n-heksan polar and non polar. This refers to principles like dissolved like (Keenan, 1989) where the nature of the solvent will dissolve polar compounds which is polar, and vice versa in non polar solvents will dissolve non-polar compounds.

For bacterial isolation tool used is the glove, mask, sterile petri dish, erlenmeyer flask, sterile needle ose, volumetric pipette, bunsen, incubator, autoclaf, tweezers, cotton bud, hot plate, oven, analytic scales, sprayer, aluminum foil, cotton, spatula, test tubes, petri dish, electric stove hotplates, camera and label. The material used is medium Nutrient Both, Bacto Agar, Waterone, isolates the bacteria Staphylococcus aureus ATCC 25923 strains taken from Balai POM Manado, and alcohol.

To test the antibacterial activity, the tool used is a notebook, pipette tips, mikropipet, petri dish, mikropipet, slide, sterile test tubes, racks of test tubes, cotton bud, voteks, sprayer, sterile toothpick, bunsen, laminar air flow, filter paper, camera, paper label. The material used is leilem leaf extract, pure culture isolates of bacteria Staphylococcus aureus strains ATCC 25923, sterile aquades, DMSO (dimethyl sulfoksida), 70% alcohol, antibiotics checklists (Clindamicyn).

2.3. Design Research

The design used in this study was a randomized complete design method (RAL) with 3 factors on konsenterasi 800 ppm where preliminary research by testing the antibacterial activity of extract with konsenterasi 100 ppm 200 ppm 400 ppm and 800 ppm 800 ppm konsenterai, which has the power of drag optimum growing on the third type of extract. Each treatment was repeated as many as 3 times, so the total research unit the research unit is a 9. The factors in this study consists of the A Factor (extract with ethanol solvent), Factor B (solvent extract with ethyl acetate), Factor C (Extracts with solvents of n-heksan).

Prosedur Ekstraksi Daun Leilem

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RESULTS AND DISCUSSION

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Ethanol Extracts Of Leaves Of Leilem

The leaves of the leilem who have didestruksi dimaserasi when wet for 2 x 24 hours in a jar placed on Enivironmental Shaker Incubator ES 20/60 with a comparison of the 1:4 (leilem leaves as many as 100 grams and 400 mL ethanol) producing 300 mL filtrate solution extracts after filtered by qualitative filter paper 9 cm in diameter and 1 mm solution of filtering the blackish, dark green and very turbid. Screening process takes quite a long time about 12 hours, so there is a possibility the solvent evaporates. The solution out of the average filter paper 1 drop per 3 seconds.

After the filtrate is generated, the next step is to vaporize and memekatkan filtrate using a Rotary Evaporator Heidolph 562. Rotary process lasts for 4 hours with temperatures 44oC and rotation 60 rpm. The extract produced a brownish black, very thick, and has a smell like leaves leilem time dirajang, and no longer smells of ethanol. The extract of dirotari by using large-sized Rotary tube, after the completion of dirotari extract is transferred into small-sized vial bottle using a spatula. The number of ethanol extract of leilem leaves produced is as much as 1,2329 grams. Next a bottle vial containing the extract given label and placed in the refrigerator.

Ethyl Acetate Extracts Of Leaves Of Leilem

A comparison between the leaves of the leilem as much as 100 grams of organic solvent ethyl acetate and 400 mL of the extract solution generates a filtered after as many as 290 mL in the process of maceration for 2 x 24 hours. Filtering occurs during the 1 hour, aqueous extract with ethyl acetate faster screened compared to aqueous extracts of ethanol and n-heksan. The solution of the resulting extract is not clear, dark green and light green colored blackish on the surface.

The process of separation between leilem leaf extract and solvent ethyl acetate is done using a rotary evaporator with setting temperature 45, 2oC and rotation 60 rpm. The necessary duration of about 90 minutes. The resulting extract is as much as 0,7333 grams. The extract is concentrated is black, thick, and have the smell more fragrant than leilem extracts of ethanol and n-heksan. The extract is placed in a bottle a small vial, labelled, and stored in the refrigerator.

N-heksan extract of leaves of leilem

In the extraction process by using n-Heksan with the method of maceration by comparison are the same as on ethanol and ethyl acetate extract that is leilem leaves as many as 100 grams and n-Heksan as many as 400 mL, filtered extract solution generates after as many as 350 mL. This is due to the filtering process just a little over 3 hours due to the solution of the n-heksan extracts faster screened compared to ethanol. The solution of the resulting extract is yellow and pellucid.

On the process of evaporation and pemekatan by using a rotary evaporator, the temperature is set with 44, 5oC with rotation 60 rpm. The time required in the process of this separation is approximately 2 hours. Rotary process n-heksan leaf leilem this at first using the Rotary tube large and increasingly concentrated extracts, when transferred to a vial and bottle dirotari again using small-sized Rotary tube until all solvent is separated from the extract. It is intended to facilitate the transfer and uptake of the extract. Extract the resulting dark green yellowish, viscous oil, smelling just like the leaves of the leilem at the time of dirajang, and no longer smells of n-heksan. The resulting extracts after weighing 0,4146

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grams are weighed and placed in a small vial, bottle after being given a label placed on the extract in the refrigerator.

Percentage Yield And Extract

Determination of percentage yield performed as supporting data for the purpose of knowing the solvent that effectively produces the largest amount of extract yield. The abundance of leaves of leilem who have didestruksi before wet extracted is 100 grams, and the results obtained extracts different depending on the type of solvent. Leilem leaf extract with solvent ethanol produces extracts as much as 1,2329 grams. Leilem leaf extract with ethyl acetate solvents yielded as much as 0,7333 grams of extract. The solvent n-heksan produce leilem leaf extract as much as 0,4146 grams.

Tabel 1 : Data hasil analisis persentasi rendemen ekstrak daun leilem.

No	Jenis Pelarut / Polaritas	Berat Awal	Berat Ekstrak	% Rendemen
1.	**Etanol (Polar)**	100 gram	1,2329 gram	1,2329 %
2.	**Etil Asetat (Semi Polar)**	100 gram	0,7333 gram	0,7333 %
3.	**n – Heksan (Non – Polar)**	100 gram	0,4146 gram	0,4146 %

The results obtained in table 1 appear that solvent ethanol is polar compounds produce a percentage greater than the marinade with ethyl acetate and n-heksan. This indicates that this solvent is a solvent that is most effective in generating leilem leaf extract (Clerodendrum minahassae Teism. & Binn.).

Isolation Of Bacteria

Media used in this research is NA (Nutrient Agar). After the sterilization process media using Autoclaf Tommy SX 500 at 121o C and a pressure of 5 atm for about 2 hours then dihasilkanlah Nutrient Agar media obtained from the composition of the blend of NB (Nutrient broth) as much as 1.6 grams, BA (Bacto Agar) as much as 3.2 grams, and as many as 200 mL waterone. The resulting media are divided into two major groups, namely the media so tilted in test tubes that are used to culture bacteria and solid medium in the petri dish used for the treatment of leilem leaf extract. The resulting solid agar medium as much as 14 petri dish at a diameter of 8 cm petri dish where each petri dish containing 10 mL of medium. While the media so tilted is generated as many as 10 test tubes, each containing 5 mL, this is because if it is too thin or too thick then it will not be used for treatment.

The bacteria taken from balai POM Manado is as much as 3 bottles vial. Bacterial isolates showed an ivory white color with irregular-shaped colonies and scattered snow showers follow a pattern of inoculation on media so slanted. Isolates of bacteria Staphylococcus aureus ATCC 25923 strains stored in place of the incubation in the laboratory of the biology of UNIMA. Subculture is done every 3 x 24 hours as much as approximately 2 times i.e. prior to testing the antibacterial activity. Culture of bacteria to be used in the test was incubated for 2 x 24 hours. Before the bacteria cultures used in identification in accordance with the characteristics of the bacterium Staphylococcus aureus. The bacterial characteristics remain the same on 1 x and 2 x 24 hours only differ on where its density on incubation period of 1 x 24 hours solid colonies and 2 x 24 hours very dense colonies. This indicates that the incubation period is between 1 x and 2 x 24 hour life cycle of bacteria on the phase that is the exponential phase.

The Antibacterial Activity

Sterilization process is the first step and it is also very important in trials of antibacterial activity, this is to avoid the occurrence of contamination. Sterilization in the study carried out using autoclaf TOMY SX 500. All the tools and materials to be used sterilized beforehand.

The composition and the comparison test of the solution in this study is 8 mg extract plus 10 mL of solvent. The creation of the solution test done in a Laminar airflow UV Vis

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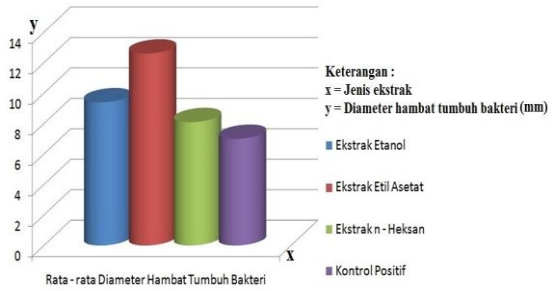
Biosan. Specific to the solution of the ethyl acetate extract test-and n-heksan done DMSO solvent, adding as much as 10%. Mixing solvent extract and use the vortices.

The well so that the formed round, totaling 4 on each petri dish except for the negative control of 6 well in order. This is because the most ideal composition for the number of wells in order to be on a petri dish is 4-6 wells in order in the observation zone nodes, the difference will be seen clearly. The diffusion of the well in order to be done in a Laminar airflow UV Vis Biosan. The creation of the well using a mikropipet pipette tip and sterile toothpick, each well contains both positive control treatment, negative control, and extract as much as 50 µl. Antibacterial activity can be seen from the clear zone formed around the well, the greater the diameter of the clear zone formed the greater activity of antibakterinya. Measurements of the clear zone around the well in order to be on treatment with ethanol extracts of leaves of leilem ethyl acetate extracts of leaves, leilem and n-heksan extract of leaves of leilem as well as a positive control with 3 times of Deuteronomy, when averaged, and made in the form of diagrams, then it will generate the following diagram:



daun leilem.

Figure 1 shows that the extract of leaves of leilem ethyl acetate soluble polar spring have antibacterial activity. After the ethyl acetate followed by ethanol extract which is polar, and then extract the n-heksan which is non-polar. One way ANOVA test results in table 2, obtained the data that generates p. value (0.00) < α (0.05) with F female (84,684) > F table (5.14). This means that H0 is rejected and the H1 is accepted, means at least one different average so that further tests done. Advanced test used is the test of Tukey, Tukey test results of every different type of extract treatment produce results the average difference.

Table 2: Results of one data processing way ANOVA for activity antibacterial leilem leaf extract on the growth of the bacteria gram positive Staphylococcus aureus strain ATCC 25923 with using SPSS 8

ANOVA

DHT

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	32.180	2	16.090	84.684	.000
Within Groups	1.140	6	.190		
Total	33.320	8			

Tukey test results table (table 3) of each different type of extract treatment produce results the average difference. From the table it can be seen that tukey each occupies 1 subset and no from the fourth subset that contains 2 or more the average difference in results. This means all the treatments in each factor has the result that shows the average difference is real. A subset of that range from very small results showed up to the most. The first subset contains on average for n-heksan, second subset contains the average for ethanol and the third subset contains the average for ethyl acetate.

Tabel 3 : Tabel Hasil Uji Tukey

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DHT				
Tukey HSD				
Perlakuan	N	Subset for alpha = 0.05		
		1	2	3
Ekstrak n – Heksan Daun Leilem 800 ppm	3	**8.1000**		
Ekstrak Etanol Daun Leilem 800 ppm	3		**9.4000**	
Ekstrak Etil Asetat Daun Leilem 800 ppm	3			**12.6000**
Sig.		**1.000**	**1.000**	**1.000**

Active compounds, both polar and non-polar leaves of leilem have antibacterial activity. The ethyl acetate extract notabennya is semi polar compounds, through the principle of like dissolved like active compounds can dissolve, the semi polar nature, but also the most active compounds that are polar and non-polar. That was the cause so that the antibacterial activity of extracts of ethyl acetate extract of greater than ethanol and n-heksan due to sinerginitas. This is in line with Poongothai and Rajan (2011) stating that the metabolite-secondary metabolites contained in plant cells have antibacterial activity with different makanisme working synergistically. In addition also there is sinergisitas between the active compounds contained in these extracts can cause occurrence, efficacy of herbal extracts used in treatment. Active compounds working synergistically menghasilakn better activity as well as lowering the potential toxicity of some single compound (Hernani, 2011).

The results of the analysis of plant phytochemicals leilem, showed that the plant contains leilem alkaloids and steroids (Sangi et al, 2008). According to Darsana dkk (2012) alkaloid compounds have antibacterial mechanism of action with the damaging component of bacterial Peptidoglycan constituent on the cell so that the cell wall layer is not completely formed. Further to steroids according to Ahmed (2007) mechanisms of steroid antibacterials i.e. steroids interact with membrane phospholipids in bacteria and make the cells become fragile.

CONCLUSION

From the results of research that has been carried out and the discussion, then the conclusions drawn are as follows:

1. Polar organic solvents of ethanol is the most effective solvent compared to solvent of n-heksan and ethyl acetate in dissolving the leaves leilem (Clerodendrum minahassae Teijsm. & Binn.).
2. The most excellent antibacterial activity in inhibiting the growth of gram positive bacteria Staphylococcus aureus strains ATCC 25923 is on treatment with ethyl acetate extract polar spring on konsenterasi 800 ppm.
3. Inhibitory activity of Gram-positive bacteria Staphylococcus aureus ATCC 25923 strain of ethanol extracts (Polar), ethyl acetate (semi polar), and n-heksan (non-polar) leaves of different leilem for real on konsenterasi 800 ppm.

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