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IDENTIFICATION OF SECONDARY METABOLITES OF KIRINYUH LEAVES *(Chromolaena odorata* L.) AS ANTIBACTERIAL USING GC-MS

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ABSTRACT

This study aims to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against pathogenic bacteria and to determine secondary metabolites in kirinyuh leaves (Chromolaena odorata) using the GC-MS (Gas Chromatography-Mass Spectroscopy) method. The test bacteria used were Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa. The extraction method was carried out by maceration using 96% ethanol solvent. The crude ethanol extract of kirinyuh leaves in this study used concentrations of 30%, 40%, 50%, 60% and 70%. Using a UV-VIS spectrophotometer, the most effective concentration in inhibiting and killing the growth of Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa bacteria was a concentration of 70%, with an average OD (absorbance) value of -0.2029, -0.055, and -0.0523. Then secondary metabolite identification was carried out using GC-MS (Gas Chromatography-Mass Spectroscopy) ethanol-water (9:1). The test results showed that a concentration of 70% was the most effective in inhibiting and killing Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa bacteria. The content of secondary metabolites in the ethanol extract of kirinyuh leaves contains 33 secondary metabolite compounds, 6 of which are dominant compounds as antibacterials.

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Introduction

Indonesia is a country rich in biodiversity spread across various regions. This biodiversity can be used for treatment, namely as raw materials for making modern and traditional medicines. Indonesian people themselves have long known and used natural ingredients as medicine for various diseases. Many factors influence people to continue using natural ingredients as medicine, so that these natural ingredients continue to be developed for processing. Many types of Indonesian medicinal plants have been used as raw materials in making medicines, and these plants have even been clinically tested for phytochemical content, efficacy and safety of use.

Traditional medicine in Indonesia has been known to the public long before the existence of formal health services with modern medicines like today. Medicinal plants have relatively smaller side effects compared to chemical drugs. Traditional medicine is part of the community's cultural system which has enormous benefits in developing public health. WHO supports the back to nature movement by recommending the use of herbal medicines in maintaining public health, prevention and for treating various diseases.

Cases of infectious diseases in Indonesia include those caused by the bacteria Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa. Escherichia coli is a normal flora bacteria that can be found in the human intestine, is unique because it can cause primary infections such as diarrhea. Diarrhea is a condition where a person defecates with a softer or liquid consistency that occurs with a frequency of >3 times in 24 hours (Jap and Widodo, 2021). According to WHO and UNICEF, there are around 2 billion cases of diarrhea and 1.9 million toddlers die from diarrhea worldwide.

Every year. Based on Basic Health Research (2018), the prevalence of diarrhea for all age groups is 8% and the prevalence rate for toddlers is 12.3%, while in infants, the prevalence of diarrhea is 10.6%. Based on data from the 2020 Indonesian Health Profile, infectious diseases, especially diarrhea, are a contributor to death in children aged 29 days to 11 months.

*Staphylococcus aureus*can infect every tissue in the body and cause diseases with typical signs of inflammation, necrosis, and abscess formation. Staphylococcus aureus infection can come from direct contamination of wounds, such as post-operative infections (Laia et al., 2019). According to WHO (2014), around 6 million people suffer from chronic or acute wounds worldwide.

*Pseudomonas aeruginosa*types of opportunistic pathogenic bacteria that cause nosocomial infections that cause urinary tract infections (UTIs) (Soedarto, 2016). Based on WHO data, the number of urinary tract infection sufferers in the world reaches around 8.3 million people and is estimated to continue to increase to 9.7 million people. According to the Ministry of Health of the Republic of Indonesia (2016) the number of UTI sufferers in Indonesia reaches 90-100 cases per 100,000 population per year or around 180,000 new cases per year. Antibiotics are used as drugs to treat infectious diseases, their use must be rational, appropriate and safe. Irrational use of antibiotics refers to a situation where antibiotics are used without clear indications, the right dosage, and for a long period of time can have negative impacts, such as the occurrence of microorganism immunity to several antibiotics (resistance), increased side effects of drugs and even have an impact on death (Pratiwi, 2017)

Misuse and overuse of antibiotics are the main drivers of the development of drug-resistant pathogens. In this regard, the widespread use of antibiotics is one of the causes of resistance in microbes (WHO, 2023).

Indonesia as a tropical country has many types of plants used in pharmacological

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activities. Treatment using natural ingredients can be chosen to overcome various diseases. Traditional medicine is a material or concoction of ingredients in the form of plants, animal materials, mineral materials, liquid preparations (galenic) or a mixture of these materials that have been used for generations for treatment and can be applied according to the norms prevailing in society (BPOM RI, 2014). The development of the use of traditional medicine, especially from plants, to help improve public health has been quite widespread. One of the benefits of using medicine from these plants in humans is as an antibiotic.

One of the plants that can be used as an antibacterial drug is the kirinyuh leaf. Kirinyuh leaves (Chromolaena odorata) contain chemical compounds that have the potential to have antibacterial properties such as flavonoids, tannins, and saponins (Hidayatullah, 2018). According to Eriadi et al., (2016), the content of secondary metabolite compounds found in kirinyuh leaves is a group of alkaloids, flavonoids, steroids and saponins. Efforts to increase the use of kirinyuh leaves (Chromolaena odorata) as medicine, both traditional use, use of simple drugs, and phytopharmaceuticals. Gultom's research (2020) methanol extract of kirinyuh leaves contains secondary metabolite compounds that have antibacterial activity, namely the alkaloid, phenolic and flavonoid groups and have antibacterial activity against MDR bacteria.

Based on research by Munthe et al., (2016) it is known that kirinyuh leaf extract (Chromolaena odorata L) has antibacterial activity against Escherichia coli and Staphylococcus aureus. The Escherichia coli growth inhibition zone has an inhibitory power at a concentration of 25%, which is 1.9 cm, while the optimum inhibition zone on Staphylococcus aureus is obtained at a concentration of 20%, which is 1.2 cm. Rahayu's research (2017) found that ethanol extract of kirinyuh leaves with a concentration of 90% using the diffusion method has the potential to strongly inhibit the growth of Staphylococcus aureus with an inhibition zone diameter of 11.1 mm while the growth of Escherichia coli with an inhibition zone diameter of 7.93 mm. Research by Priono et al., (2016) on the effectiveness of kirinyuh leaves as an antibacterial with the diffusion method, has the best antibacterial activity on Escherichia coli with a concentration of 25% while Staphylococcus aureus with a concentration of 50%.

Based on the background above, this study will conduct a bioactivity test in the form of antibacterial properties of the kirinyuh plant extract (Chromolaena odorata) and identification of secondary metabolites using the GC-MS (Gas Chromatography-Mass Spectroscopy) method and which comes from the Berastagi area, Karo Regency, North Sumatra, samples were taken from the highlands because it is based on differences in the habitat of a plant that will affect the composition of the chemical compound content of a plant.

Materials and Methods

Place and Time of Research

This research was conducted at the Microbiology Laboratory of Medan State University and the Integrated Laboratory of the University of North Sumatra. The research period was from May 2024 to July 2024.

Population and Sample

The samples in this study were kirinyuh leaves (Chromolaena odorata) and 3 bacterial isolates, namely Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25924 and Pseudomonas aeruginosa ATCC 27853 obtained from the Microbiology Laboratory, Department of Clinical Pathology, University of North Sumatra Hospital, Medan.

Tools and materials

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The tools used in the study were maceration vessels, Erlenmeyer flasks, autoclaves, ovens, analytical scales, rotary evaporators, incubators, spectrophotometers, laminar air flow, filters, petri dishes, droppers, spatulas, spirit lamps, stirring rods, test tubes, blenders, hot plates, beaker glasses, tube racks, calipers, and a set of GC-MS (Gas Chromatography-Mass Spectrometry) tools.

The materials used in the study were kirinyuh leaf extract, Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa bacterial cultures, NA (Nutrient Agar) media, MHA (Mueller Hinton Agar) media, distilled water, 96% ethanol, filter paper, physiological NaCl.

Sample Extraction

The simplicia was weighed as much as 500 gr and put into an Erlenmeyer flask and soaked with 96% ethanol solvent until submerged. Soaking was done at room temperature and avoided from exposure to sunlight for 5 days. During the soaking period, the extract should be stirred every day to prevent sedimentation. After 5 days of soaking, then filter it using a funnel and filter paper. Repetition was carried out on the remaining simplicia from the filtering, repetitions can be done 2 times, where each time for 3 days of soaking. The extract was then rotated at a temperature of 40-50 ° C to separate the ethanol from the extract. The extract was evaporated using a hot plate so that the ethanol evaporated so that only the thick extract remained (Retnaningsih, 2016).

Making Bacterial Inoculum

Each of Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa bacteria were taken as much as 1 loop from the colony using a loop needle that had been sterilized over a Bunsen burner, then the bacterial sample was suspended in a tube containing 50 mL of 0.9% NaCl. The bacterial suspension was equated with a 0.5 Mc Farland standard tube, a solution with a Mc Farland turbidity standard of 0.5 is an equivalence of the concentration of microbes in a liquid medium with a density between 1.5 x 108 CFU/ml. The Mc Farland solution was made by mixing 0.05 ml of 1% BaCl2 solution with 9.95 ml of 1% H2SO4 then stored in a place protected from direct sunlight. Bacterial cultivation is done using the smear method, done by preparing a sterile cotton bud and dipping it into a standardized bacterial suspension using 0.9% NaCl then the sterile cotton bud is left for a while so that the liquid is absorbed into the cotton, after that the sterile cotton bud is squeezed by pressing it into the inside of the test tube while rotating it. After that the cotton bud is wiped on the surface of the NA media until the entire surface is tightly covered with a swab and is done 3 times. After bacterial cultivation is done, leave the NA media on the table for 5 minutes so that the bacterial suspension is absorbed into the media (Simanjuntak, 2008).

Minimum Inhibitory Concentration (MIC) Test of Kirinyuh (Chromolaena odorata) Leaf Extract Against Pathogens

The MIC study was conducted using the modified Kirby and Bauer liquid dilution method (Lennete et al., 1991) using Nutrient Broth (NB) liquid media and measured the absorbance with a UV-Vis spectrophotometer before and after incubation to see the growth of the test bacteria. A total of 4 ml of sterile NB media was put into each test tube, then 0.5 ml of extract was added with 5 concentration variations, namely 30%, 40%, 50%, 60%, 70%. Furthermore, 0.5 ml of bacterial suspension was added to this media at 10^6 CFU/ml which had been adjusted to the 0.5 Mc. Farland standard (Fatisa, 2013).

The test tubes were then measured for bacterial absorbance (Optical Density = OD) using a UV-Vis spectrophotometer (λ = 625 nm). Furthermore, the tubes were incubated for 18-24 hours at 37°C in an incubator. After incubation, the bacterial absorbance was measured again using a

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UV-Vis spectrophotometer (λ = 625 nm). MIC was determined by comparing the absorbance after the incubation treatment minus the absorbance before treatment. If the lowest concentration that inhibits bacterial growth, indicated by the absence of turbidity (bacterial OD is ≤ 0), then the Minimum Inhibitory Concentration (MIC) was obtained (Lennete et al., 1991).

Minimum Bacterial Concentration (MBC) Level Test of Kirinyuh (Chromolaena odorata) Leaf Extract Against Pathogens

To determine the MBC value, further tests were carried out, namely on all tubes used in the MIC that did not show any turbidity to bacteria, by taking 0.2 ml of the suspension that showed the MBC, then added to a test tube containing 5 ml of sterile NB media, the test tube was incubated for 12-18 hours at 37 ° C in an incubator, then the absorbance (OD) was measured using a UV-Vis spectrophotometer (λ = 625 nm) (Fatisa, 2013). If the measurement results show that the lowest concentration of the extract has an OD of 0 (no turbidity), then the Minimum Bactericidal Concentration (MBC) is obtained (Lennete et al., 1991).

Identification of secondary metabolites of kirinyuh leaves (Chromolaena odorata) using the GC-MS method

Kirinyuh leaf fractions were analyzed using Gas Chromatography-Mass Spectroscopy (GC-MS). Gas chromatography (GC) consists of a heated inlet, oven, and fused silica column (basically a circular glass tube) which takes into account the Sample Preparation, Evaporation, Separation, Detection phases. Mass Spectrometer (MS), MS will break down each separate compound originating from GC into ionized fragments. MS produces a graph called a mass spectrum. The sample was filtered using a filter membrane first, then the sample was injected as much as 20 µL with a C18 silica stationary phase and a mobile phase of an ethanol-water mixture (9:1) by gradient elution at a flow rate of 1.0 mL/min for 10 minutes with a wavelength of 366 nm. Then the device is operated to perform detection, the results of the detection of pure compounds are indicated by the formation of peaks with a certain retention time, the identity is given to the compound.

Data Analysis using Pub-Chem software

PubChem software can be accessed viahttp://pubchem.ncbi.nlm.nih.govStartin g from the search for the molecular formula, by searching for the formula will appear the search for identity by finding a certain chemical structure that is identical to the chemical structure. Next is the search for substructure and superstructure for example ethanol is a substructure of acetic acid, and acetic acid 30 is a superstructure of ethanol. The results of PubChem are: Substance Database will contain general information about chemical structures, synonyms, registrations, descriptions, websites, and related references connected to PubMed, 3D protein structures, and biological screening results.

Results and Discussion

Antibacterial Activity of Kirinyuh Leaf Extract (Chromolaena odorata)

Based on the results of the research that has been done that the extract of kirinyuh leaves (Chromolaena odorata) has antibacterial activity against Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa bacteria. Shows the antibacterial activity of kirinyuh leaves (Chromolaena odorata) against Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa bacteria with a concentration of 30% included in the weak category, and concentrations of 40%, 50%, 60% included in the moderate category. From the results obtained, a concentration of 70% is an effective concentration in inhibiting the growth of Escherichia coli, Staphylococcus

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aureus, and Pseudomonas aeruginosa bacteria.

Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) values

The determination of the minimum inhibitory concentration value was carried out using a UV-Vis spectrophotometer by measuring the turbidity value of each concentration treatment (absorbance value) after and before incubation in the test tube. It can be seen that the minimum inhibitory concentration in Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa bacteria is at a concentration of 70%. And at a concentration of 70% is the MIC value of the three bacteria, namely Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa. A negative ΔOD value (-) indicates a decrease in the absorbance value which means that there is a decrease in the number of bacteria after 24 hours of incubation, while a positive ΔOD value (+) indicates no decrease in the absorbance value meaning that there is still bacterial growth after 24 hours of incubation (Rori et al., 2018).

Based on the results of the research conducted, the MBC value of Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa bacteria at a concentration of 70%, which is an effective concentration to kill bacteria. Seen from the absorbance value after incubation, it decreased from the smallest to the largest concentration, indicating that bacterial growth was reduced, indicating that bacteria were killed.

The absorbance value cannot be used as a reference in determining the inhibitory activity of the extract against bacteria due to the varying levels of turbidity of the extract. The UV-Vis spectrophotometer also measures all levels of turbidity between the media, extracts, and bacterial suspensions, so it cannot determine whether the test bacteria can be killed or can still grow.

Results of secondary metabolite testing of kirinyuh leaf extract (Chromolaena odorata) using the GC-MS method

The results of secondary metabolite tests of kirinyuh leaves (Chromolaena odorata) using Gas Chromatography-Mass Spectroscopy (GC-MS) obtained 33 compounds in it. The results of ethanol solvent analysis showed 6 dominant secondary metabolite compounds based on their concentration. The identified metabolite compounds are: 1) 9-Octadecenoic acid, 2) 9-Octadecenoic acid 2,3-dihydroxypropyl ester, 3) AFLATOXI (SYNTHETIC), 4) Cyclopentaneundecanoic acid, methyl ester, 5) Acetic acid, anhydride, 6) Butane, 1-isocyano-. The six secondary metabolite compounds have high concentrations that are dominant over other compounds.

Kirinyuh contains many chemicals which are secondary metabolites such as terpenoids, limonoids, alkaloids, and flavonoids which are used by plants as a means of defense (Wijaya et al., 2018). The results of the identification of kirinyuh leaf extract using polar ethanol solvents that attract only polar analytes (Astarina et al., 2013).

Conclusion

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

Antibacterial Activity of Kirinyuh Leaves (Chromolaena odorata) against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa bacteria found that a concentration of 70% is an effective concentration in inhibiting bacterial growth.

Concentration of 70% ethanol extract of Kirunyuh leaves (Chromolaena odorata) becomes the Minimum Inhibitory Concentration (MIC) value and Minimum Bacterial Concentration (MBC) value against Escherichia coli, Stapylococcus aureus, and Pseudomonas aeruginosa bacteria.

The secondary metabolite content contained in the ethanol extract of kirinyuh

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leaves (Chromolaena odorata) using the GC-MS method contains 33 compounds.

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