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Antibacterial Activity Test of Ethanol Extract of Bitter Gourd Leaves (Momordica Charantia L) Against Acne-Causing Bacteria (Staphylococcus Epidermidis)

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Abstract

Bitter melon leaves contain flavonoids, saponins, tannins and terpenoids, which have antibacterial potential. The aim of this research was to determine the antibacterial activity of an ethanol extract of bitter melon leaves. This antibacterial activity test was carried out using the disc diffusion method. The concentrations of bitter melon leaf ethanol extract used were 50%, 60%, and 70%. Clindamycin was used as a positive control and distilled water as a negative control. The data was obtained by using the one-way Anova statistical test. The test for antibacterial activity showed that the inhibitory zone was 9.07 mm wide at a 50% extract concentration, 10.72 mm wide at a 60% concentration, and 12.08 mm wide at a 70% concentration. There was a significant difference between the positive control with extract concentrations of 50%, 60%, and 70%, which was indicated by the Sig value. 0.005. The ethanol extract of bitter melon has an inhibitory effect on the growth of Staphylococcus epidermidis bacteria.

Keywords: Antibacterial, Bitter Melon Leaves, Staphylococcus Epidermidis

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Introduction

Acne is a skin illness that is caused by persistent inflammation and has an intricate etiology that involves numerous components (Mias et al., 2023). Acne typically manifests itself throughout the teenage years (Tuğrul et al., 2023). Genetics, hormonal activity during the menstrual cycle, stress, hyperactive sebaceous gland activity, cleanliness, food (Mohammed et al., 2023), and the use of cosmetics are all factors that might cause acne to appear (Ago Harlim et al., 2024). A blockage of the pores in the skin is the root cause of acne. This obstruction causes oil secretion to become impeded, which then causes the oil to become enlarged and dry, resulting in acne. The production of metabolites by Staphylococcus epidermidis, which can react with sebum and so increase the inflammatory process, is one of the ways in which this bacterium contributes to the development of cancer (Azzimonti et al., 2023). Skin clinics typically utilize antibiotics as a treatment for acne since these medications have the ability to reduce inflammation and eliminate bacteria (Tan et al., 2024). Problems that emerge as a result of continuing to use antibiotics for an extended period of time will lead to resistance, which is

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why other alternatives are being sought out that can minimize undesired side effects.

The fruit of the bitter melon plant, in particular, has been utilized extensively as a means of therapeutic treatment (Kumaree & Prasansuklab, 2023). There are a number of conditions that can be effectively treated with bitter melon, including fever, worm infections, coughing, sores, boils, constipation, malaria, and syphilis(Chaudhury, 2023). Canker sores, fever, coughing, malaria, dysentery, and rheumatism are all conditions that can be effectively treated with the leaves of bitter melon, which are another portion of the citrus fruit (Mittal et al., 2023). The leaves of bitter melon contain a variety of compounds, including alkaloids, flavonoids, saponins, and tannins. In addition to its antibacterial effects, this chemical (Andrade et al., 2023).

Based on the description above, this research aims to determine the anti-acid activity of bitter melon leaf ethanol extract against Staphylococcus epidermidis.

Research Method

Experimental research in a laboratory is the type of research being conducted. In the meantime, the research design that was utilized was a randomized design (CRD), which combined three repeats and five treatments. The bacteriology laboratory of the pharmacy faculty of Bhakti Wiyata Health Sciences Institute in Kediri was the location where this investigation was carried out. An Erlenmeyer, an analytical balance, a measuring cup, an oven, a porcelain cup, a tube rack, a test tube, a petri dish, a spirit lamp, a water bath, an autoclave, an incubator, a chamber, F254 silica gel, UV 254 light and 366 nm, tube wire, a sterile swab, and a caliper are some of the instruments that are utilized. Bitter melon leaves, the antibiotic clindamycin as a positive control, Aquades as a negative control, 70% ethanol as a solvent, MHA media, Nutrient Agar, concentrated hydrochloric acid, magnesium powder, FeCl, anhydrous acetic acid, sulfuric acid, glacial acetic acid, butanol, paper disks, and pathogenic bacteria (Staphylococcus epidermidis) are some of the materials that are utilized in this study.

Research Procedures Making Simplicial

After the bitter melon leaves have been collected, they are first sorted wet in order to separate dirt and foreign materials from the simplicial material. Later, they are washed with running water in order to remove dirt that is attached to the simplicial material. Following this, a chopping process is carried out in order to facilitate the drying process. Finally, the leaves are dried by airing them out in the open air. After blending the dry sample until it was completely smooth, the findings were placed in a glass container that was sealed.

Extraction

Maceration is the method that is utilized in the extraction process. After weighing and placing 150 grams of bitter melon leaf simplicial in a glass container (Jar), 1.5 liters of ethanol with a 70% concentration was added, and the mixture was macerated for three times twenty-four hours. After that, the extract that was produced is filtered in order to separate the filtrate from the residue. After macerating the residue that was obtained with 750 milliliters of ethanol at a concentration of 70 percent for two consecutive twenty-four hours, the filtrate and the residue were separated. A water bath was used to ensure that the obtained filtrate was concentrated to the point where a thick extract was discovered. The amount of yield is determined.

Phytochemical Screening

Phytochemical screening is carried out to determine the presence of flavonoids, saponins, tannins, terpenoids.

1) Analysis of flavonoid compounds

Identification of flavonoids was carried out based on the method used by Harith (2018) with modifications

2) Analysis of tannin compounds

Tannin identification was carried out based on the method used by Azizah (2018) with modifications

3) Analysis of saponin compounds

Saponin identification was carried out based on the method used by Harith (2018) with modifications

4) Analysis of terpenoid compounds

The identification of flavonoids was carried out using a method that was modified from the one that (Gamay et al., 2024) utilized, which was thin layer chromatography (TLC) analysis. A sample of bitter melon leaf extract was used for the TLC analysis, which utilized a stationary phase consisting of silica gel GF245 and a mobile phase consisting of n-butanol, acetic acid, and water in the ratio of 4:1:5. Ammonia vapor was used to treat the results of the TLC plate, and the results were observed using a UV lamp with wavelengths of 254 nm and 366 nm.

Antibacterial Activity Test

A technique known as modified Disc (Agar Diffusion) is utilized for the purpose of conducting the antibacterial test (Salam et al., 2023). To begin, non-glassware is sterilized in an autoclave located at 121 degrees Celsius for fifteen minutes, while glassware is sterilized in

an oven located at 160-170 degrees Celsius for two hours. The Bunsen fire is used to completely consume needles.

Creation of Slanted Media

Using a pipette, transfer twenty milliliters of liquid agar nutrient media into a test tube. After sterilizing the tube in an autoclave at 121 degrees Celsius for fifteen minutes, tilt the tube at an angle of thirty to forty degrees Celsius and allow it to remain at room temperature until it hardens. Finally, place the tube in the refrigerator.

Basic Media Creation

The media was prepared by first dissolving 3.8 grams of Mueller Hinton Agar (MHA) with 100 milliliters of distilled water, then homogenizing it with a magnetic stirrer, heating it over a water bath until it boiled and had a clear yellow color, and finally sterilizing the still liquid media with an autoclave at 121 degrees Celsius for fifteen minutes. In a sterile petri dish, the MHA media solution is poured, and then the patient is allowed to wait until the solution solidifies.

Bacterial Inoculation

Following the removal of bacteria using a sterile needle loop, the bacteria were scraped onto slanted agar media and then placed in an incubator at 37 degrees Celsius for a period of twenty-four hours. Establishing bacterial suspensions for testing. A wire mesh is used to collect bacteria, which are subsequently streaked onto nutritional agar media. The microorganisms are then cultured in an incubator at 37 degrees Celsius for 18 to 24 hours.

Preparation of Mc Standard Solution Farland

Using a sterile tube, the bacteria that had been inoculated were removed, and then they were suspended in a test tube that contained 10 milliliters of 0.9% sodium chloride until the turbidity of the test tube was equal to that of the standard Mc solution.

Antibacterial Testing Using the Disc Diffusion Method

Based on the findings of study conducted by (Haritha et al., 2023), antibacterial testing was performed using the disc diffusion method.

Processing and analysis of data

Following the Anova test, which was used to examine differences in the mean (average) diameter of the inhibition zone in the treatment groups, the Post Hoc test (multiple comparison test) was used to determine which data were different using the Tukey test. The Anova test was used to process and analyze the data.

Results and Discussion

As a consequence of the identification process, it was determined that Momordica charantia L. would be the plant that would be utilized in the antibacterial activity test. In order to identify the features of simplicial and bitter melon leaf extract, the organoleptic properties of bitter melon leaves were carried out through visual observation. The bitter melon leaf simplicial is a powder that is pale green in color and has a distinct odor. On the other hand, the bitter melon leaf extract is a thick substance that is blackish green in color and has a distinct odor. There was a yield of 12.75% and the amount of extract that was collected was 19.13 grams. Because ethanol content can have an effect on antibacterial activity and lead to false positives, the ethanol-free test was carried out to demonstrate that bitter melon leaf extract does not include any ethanol. This was done in order to demonstrate that the extract does not contain any ethanol. Based on the findings of the esterification test, it was determined that the extracted substance did not smell like ester anymore, which indicated that it had lost its ethanol content. Flavonoids, tannins, saponins, and terpenoids are all found in the ethanol extract of bitter melon leaves, according to the findings of a phytochemical examination. All of the results are presented in table 1.

Table 1. Phytochemical Screening Results for Active Compounds of Bitter Gourd Leaf Extract

Chemical Content	Reactor	Results (+) Literature	Extract Test Results	Information
Flavonoids	Sample+aqu ades + powder Mg+HCL concentrated	Color orange on sample	Sample colored orange	Positive contain flavonoids
Tannin	Sample+aqu ades+FeCl	Color Bluish black	Sample colored black	Positive contain tannin
Saponins	Sample+aqu nice	Foam Formed	Forms foam that lasts 3 minutes	Positive contain saponin
Terpenoids	Sample+aqu ades+ as. Acetate anhydrous+as. Concentrated sulfate	Formed purple blue red green	Formed purple red	Positive contain terpenoids

Following the application of ammonia vapor, the results of the TLC analysis revealed that the sample of bitter melon leaf extract included flavonoid chemicals, which were characterized by the appearance of yellow spots. By observing the sample spots with the UV

254 lamp, it was discovered that they were a dark green color and that they illuminated purple when exposed to the UV 366 (Dereje et al., 2023). For the purpose of this TLC investigation, the mobile phase consisted of n-butanol, acetic acid, and water in the proportions of 4:1:5, while the stationary phase was a GF254 silica gel paper plate. In Figure 1, you can see the results of the TLC study that was performed.

The position at which the compound stops

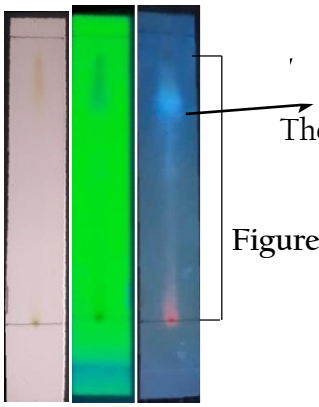

Figure 1. Detection of flavonoid compounds in bitter melon leaf extract

Information:

1. Visual appearance of the TLC plate after being treated with ammonia vapour
2. Appearance of the TLC plate under 254 nm UV light
3. Appearance of the TLC Plate under 366 nm UV light

The flavonoid compounds in the analytic medium undergo a reaction that causes them to create hydrogen bonds and adhere to the TLC plate. This reaction takes place throughout the process of compound separation. When the mobile phase starts to elute the TLC plate, it will dissolve the flavonoid compounds that have been identified on the baseline. This will occur when the mobile phase begins to elute the plate. The mobile phase moves over the chromatographic plate, which causes the compounds to move as well. The solubility of the compound in the solvent should be taken into consideration when determining the speed at which the chemical moves on the plate. The presence of a stain on the Rf indicates that absorption has ceased when the solvent travels without the chemical.

Staphylococcus epidermidis, which is one of the most frequent bacteria that causes acne after Propionibacterium acne, was employed in the antibacterial activity test. The disc diffusion method was utilized to conduct the test. This approach is typically selected by the majority of academics in Indonesia due to the fact that it is regarded as being more practical.

Test samples consisted of ethanol extracts of bitter melon leaves with concentrations of fifty percent, sixty percent, and seventy percent. Clindamycin was employed as the standard for the positive control, whereas distilled water was used for the negative control. Using the

disc diffusion method, investigations into the antibacterial activity of bitter melon leaf extract revealed that the extract has antibacterial activity against Staphylococcus epidermidis. However, the findings of these investigations varied depending on the quantity of the extract. It was shown that the 70% concentration of bitter melon leaf ethanol extract had the maximum inhibitory power in stopping the growth of Staphylococcus epidermidis bacteria, which measured 12.08 mm. This was the case among the three concentrations of the extract. Antibiotics belonging to the lincosamide class, such as clindamycin, exhibit a high level of action against a wide variety of facultative anaerobic bacteria. The mechanism of action of clindamycin involves the inhibition of protein synthesis in bacterial ribosomes, which in turn disrupts the process of peptide chain building in bacteria. The gram-positive bacteria known as Staphylococcus epidermidis is also susceptible to the antibiotic known as clindamycin. A concentration of 50% of bitter melon leaf ethanol extract demonstrated an inhibitory power of 9.07 millimeters, a concentration of 60% demonstrated an inhibitory power of 10.72 millimeters, a concentration of 70% demonstrated an inhibitory power of 12.08 millimeters, and the positive control demonstrated an inhibitory power of 23.29 millimeters. As a result of the antibacterial test, it was discovered that the diameter of the inhibition zone of the ethanol extract of bitter melon leaves was not comparable to that of the positive control. The results of the observation showed that the antibacterial effect increased with increasing concentration of the test extract. This indicates that additional research needs to be conducted with higher doses in order to determine the magnitude of the dose of bitter melon leaf ethanol extract that is capable of producing an antibacterial effect that is comparable to that of the positive control.

Based on the inhibition zone criteria according to (Yani, 2020), the antibacterial power of the ethanol extract of bitter melon leaves with a concentration of 50% (9.07 mm) has a bacterial inhibitory power which is categorized as medium (Liu et al., 2024), then the ethanol extract of bitter melon leaves with a concentration of 60% (10.72 mm) and a concentration of 70% (12.08 mm) has a bacterial inhibitory strength that is categorized as strong (Sheikhalipour et al., 2023). The inhibitory power of bitter melon leaf extract was demonstrated in earlier studies carried out by Pasometimes (2020) using 96% ethanol. The concentrations of 5000 ppm (16.99 mm), 10,000 ppm (18.78 mm), and 15,000 ppm (21.81 mm) were found to be effective in inhibiting the activity of the extract. Previous research carried out by Undap (2017) demonstrated that the 10% (0.2 cm2), 15% (0.3 cm2), 20% (0.4 cm2), and 25% (0.5 cm2) concentrations of the ethanol extract of bitter melon leaves exhibited antibacterial activity against Staphylococcus aureus bacteria.

Flavonoids, tannins, saponins, and terpenoids are some of the active compounds that are thought to be responsible for the antibacterial activity of bitter melon leaf extract (de Brito et al., 2023). Flavonoids are also thought to be present in the extract. Flavonoids are compounds that, due to the high level of polarity that they possess, have a tendency to possess antibacterial activity (Nguyen et al., 2023). Flavonoid compounds, which have a high polarity, are able to easily penetrate the cell walls of Staphylococcus epidermidis bacteria because of their high magnetic properties (Kiarashi et al., 2024). After successfully penetrating the cell wall, the compound will cause damage to the permeability of the cytoplasmic membrane. This will make it difficult for the bacteria to take in the nutrients they require to remain alive. Additionally, the proteins that comprise the cell will be released on their own due to the fact that the permeability of the cytoplasm has been damaged (Trybus et al., 2023), which will ultimately result in the death of the bacterial cell. Tannin is a polyphenolic compound that has a high molecular weight and is composed of hydroxy and carboxyl groups (Zhang et al., 2023). Its mechanism of action as an antibacterial agent involves a reaction with cell membranes, the inactivation of enzymes, and the inactivation of the function of genetic material (Ramos-Zúñiga et al., 2023). Tannin accomplishes this by inhibiting the enzymes reverse transcriptase and DNA topoisomerase, which prevents the formation of bacterial cells. In the year 2021, Adiningsih et al. In addition to having an ester structure, saponin is a compound that possesses chelating properties. For the purpose of reducing the amount of water that is present in bacterial cells, the ester form is composed of two parts that each possess distinct polar properties. Bitter melon leaf extract has been shown to be an effective anti-acne agent, according to research (Silvy, 2012). Its mechanism of action is as a chelator of metals in cosmetics that can cause acne, and it also has antibacterial activity against Staphylococcus epidermidis bacteria. Both of these properties have been demonstrated to be effective in treating acne. Terpenoids are hydrocarbon compounds that have the ability to inhibit the growth of bacteria by destroying the cell walls of such organisms. According to (Prasad et al., 2023), the formation of polymer bonds between terpenoids and porins leads to the damage of proteins, which in turn reduces the permeability of the cell wall. This, in turn, causes bacterial cells to lack nutrition, which can either inhibit bacterial growth or cause dead bacteria to die.

The results of the antibacterial activity test can be seen in Figure 2.

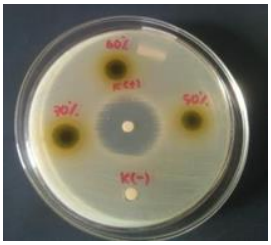

Figure 2. Results of the antibacterial activity test of ethanol extract of bitter melon leaves using the disc diffusion method

In the treatment groups, the results of the One Way Anova test revealed that there were significant differences in the inhibition zones, which were indicated by the Sig value. These differences were found to exist within the treatment groups. The standard deviation (α) is less than 0.05. This demonstrates that the differences that occur are the result of different treatments, as indicated by the fact that the value between groups is higher than the value within groups, as shown by the sum of squares column (El Mannoubi, 2023). The significance value obtained from the Post Hoc test indicates that there is a noteworthy distinction in the antibacterial activity between the positive control group and the purified extract group when the concentrations were 50%, 60%, and 70% (Ayachi et al., 2023). This is demonstrated by a sig value of 0.000, which is less than the threshold of 0.05. The results of this study indicate that the purified extract concentration group and the positive control group exhibited distinct effects in terms of inhibiting the growth of bacteria. It was determined through the post hoc analysis that the antibacterial activity of the ethanol extract of bitter melon leaves, which was present in three different concentrations, did not have a comparable level of activity to that of the positive control. After conducting post hoc tests, it was observed that there were significant differences in the diameter of the inhibition zone for purified extracts with concentrations of 50%, 60%, and 70% respectively. The sig values for these tests were 0.000 0.05.

Conclusion

A 50% concentration of the ethanol extract of bitter melon leaves has an inhibitory power of 9.07 millimeters, a 60% concentration has an inhibitory power of 10.72 millimeters, and a 70% concentration has an inhibitory power of 12.08 millimeters. The diameter of the inhibition zone is measured against the Staphylococcus epidermidis bacteria. Both the diameter of the positive control inhibition zone and the concentration of the extract have sig values of 0.000, which indicates that there is a significant difference in the antibacterial activity between the groups that were brought together for comparison.

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