JPPIPA 9(2) (2023)

Jurnal Penelitian Pendidikan IPA

Journal of Research in Science Education

http://jppipa.unram.ac.id/index.php/jppipa/index

Antimicrobial Activity Test of Bitter Melon (*Momordica charantia* L.) Plant Extract Against *Staphylococcus epidermidis*, *Escherichia coli* Bacteria and *Candida albicans*

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Received: December 10, 2022
Revised: February 12, 2023
Accepted: February 25, 2023
Published: February 28, 223

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DOI: 10.29303/jppipa.v9i2.3699

Abstract: Bitter gourd (*Momordica charantia* L.) contains secondary metabolites of flavonoids, alkaloids, saponins, and steroids that act as antimicrobials. This study aimed to determine the antimicrobial activity of bitter melon (*Momordica charantia* L.) plant extract in N-hexane and ethanol solvents against *Staphylococcus epidermidis, Escherichia coli*, and *Candida albicans*. This research is an experimental laboratory study, namely the antimicrobial activity test of bitter melon plant extract on the growth of test bacteria using the agar diffusion method. The study's experimental design used a Completely Randomized Design with three repetitions at three concentration variations, namely 30%, 45%, and 60%. Then phytochemical screening was carried out to determine the active compounds in the bitter melon plant extract. Data on the inhibitory power of bitter melon plant extract were analyzed qualitatively, and the differences between concentrations were seen using the ANOVA (*Analysis of variance*) test. The results showed that bitter melon (*Momordica charantia* L.) plant extract in ethanol solvent showed activity against the growth of *Staphylococcus epidermidis, Escherichia coli* bacteria with a concentration of 60%, producing a powerful inhibition zone.

Keywords: Bitter melon (*Momordica charantia* L.); Antimicrobial activity; Secondary metabolites

Introduction

Learning natural sciences allows students Synthetic antibiotics are frequently used in the treatment of bacterial and fungal illnesses. The most widely used antifungal medications belong to the azole class and include fluconazole, imidazole (miconazole and ketoconazole), and polyenes (Neal, 2006). Meanwhile, amphenicol derivatives, aminoglycosides, and some penicillin derivatives, such as amoxicillin, are antibacterial drugs(Muntasir et al., 2022). However, it turns out that using synthetic antibiotics has negative effects, including irritations, allergies, and resistance to long-term use (Kementerian, 2011). The increasing resistance of bacteria makes researchers constantly look for new sources of antibiotics as safer alternative treatments. Sources of antimicrobials can be obtained from bioactive compounds in various plants, microbes, and marine organisms (Alfermann, 2000). One of the plants that have the potential to be developed into traditional medicine is bitter melon (*Momordica charantia* L.).

The bitter gourd or melon (*M. charantia* L.) is a Cucurbitaceae plant from tropical Asia (Maiti, 2012). Bitter melon plants include live annuals propagating or spreading with a buyer's tool as a channel. Characterized by a bitter taste, 5-ribbed stem with a length of 2-5 m, single-leaf, stemmed, alternately located, ovoid, the flowers are single yellow and have elongated rounded fruits with a nodule-nodule surface (Sambamurty, 2005).

Besides being able to process bitter gourd into various foods, only a few people know other benefits of other parts of the bitter melon plant, such as its leaves. Bitter melon leaves contain active plant substances such as flavonoids, phenols, and tannins (Azizah, 2018). The

How to Cite:
Rasmi, D.A.C., Zulkifli, L., & Ahadia, N. (2023). Antimicrobial Activity Test of Bitter Melon (Momordica charantia L.) Plant Extract Against Staphylococcus epidermidis, Escherichia coli Bacteria and Candida albicans. *Jurnal Penelitian Pendidikan IPA*, *9*(2), 454–458. https://doi.org/10.29303/jppipa.v9i2.3699

bitter melon's secondary metabolite composition, including alkaloids and flavonoids, can be an insecticide against *Aedes aegypti* mosquitoes (Dheasabel, 2018). The content of active compounds found in the bitter melon plant has a pharmacological effect on treating diseases such as diabetes mellitus, skin infection problems, vaginal inflammation, and hemorrhoids. In foreign countries such as Mexico, this plant treats dysentery, malaria, and puffiness (Kumar, 2010).

Research conducted by Riferty *et al*. (2018) found that the antibacterial activity test of the extract and fraction of bitter melon seeds (*Momordica charantia* L.) could inhibit the growth of *Propionibacterium acnes* bacteria with a MIC value at a 30% concentration of 8.9 mm. Meanwhile, Savira (2021), based on the results of her research, shows that bitter gourd leaf ethanol extract can inhibit the growth of *Streptococcus pyogenes* bacteria that cause laryngitis. Based on this description, the purpose of this study was to determine the effect of bitter melon plant extract on the growth of *Staphylococcus epidermidis, Escherichia coli,* and *Candida albicans* fungi as well as to establish the concentration of bitter melon plant extracts that were effective in preventing the development of these microorganisms.

Method

This type of research is experimental laboratory research using a Complete Randomized Design (Davis, 1971). The tools and materials used are research tools consisting of inoculation needles, ent needles, micropipettes, petridisks, autoclaves, bunsen lamps, hotplates, incubators, erlenmeyer (size 1L), magnetic stirrers, test tube beakers, ovens, laminar air flow, analytical balances, rotary evaporators, vortex mixers, blenders, and crossbars. Nutrient Agar, Muller Hintor Agar, Potato Dextrose Agar, solvent (ethanol and N-hexane), bitter melon plant (leaves and fruits), magnesium powder, concentrated HCl, dragendorf reagent, mayer reagent, HCl 2N, FeCl3 5%, chloroform, anhydrous acetate, H2SO4, *Staphylococcus epidermidis bacteria, Escherichia coli* and the fungus *Candida albicans.*

Data collection was carried out by measuring the diameter of the test microbial inhibition zone using the Kirby-Baurer well diffusion method. Two-way ANOVA was utilized to analyze the qualitative data, and continued with the BNT test was performed with a 5% significance level.

Research Procedure

1. Extract Creation

Samples of bitter melon leaves and fruits were sorted by wet and washed. Then drained and sliced into small pieces weighing 500 grams. Then it dries for 3–4 days. The dried leaves and fruit of bitter melon are crushed to form a powder. The simplicia in powder form is weighed as much as 100 grams and macerated in 500 ml of N-hexane until all parts are submerged for 48 hours with several stirrings. The results of the immersion were then filtered using sterile filter paper. The dregs formed in the first solvent were then macerated again using ethanol (70%) with the same treatment and volume in the first solvent. Thus, two kinds of bitter melon leaf and fruit extracts will be obtained: N-hexane and ethanol. Each extracted filtrate was then evaporated using a rotary evaporator to obtain a thick extract.

Phytochemical Test

a. Alkaloid Test

1 ml of the sample was put into a test tube, and 2-3 drops of Dragendorf's reagent were filled. The Dragendorf reagent will produce an orange-red precipitate if the reaction is successful.

b. Flavonoid Test

1 ml of the extract was added to 0.5 grams of magnesium powder and ten drops of concentrated HCI. If it reacts positively, it will produce an orange, pink, or red solution.

c. Saponin Test

A thick or concentrated extract of 5 ml is put into a test tube, added 10 ml of water is then, heated for 2-3 minutes and cooled. After cooling, it is shaken vigorously for 10 seconds. Saponins are shown by the formation of a stable 1-10 cm high foam for not less than 10 minutes. At the addition of 1 drop of HCl 2N, the froth will not disappear.

d. Tanin Test

A total of 1 ml extract was put into a test tube and added to a 5% FeCl3 solution. If it reacts positively, it will produce a strong purple or black color.

e. Steroid dan Triterpenoid Test

Steroid and triterpenoid tests were carried out by inserting 1 ml of extract into a test tube and adding 2 ml of chloroform and ten hatches of acetic anhydride. Furthermore, 3 drops of concentrated sulfuric acid were added through the wall of the test tube. A favorable response to steroids will cause a solution to turn blue, and a positive response to the presence of triterpenoids will cause a solution to turn red, orange, or purple.

Bioassay Test (Resistance)

The bacterial sensitivity test of the ethanol extract of the leaves and fruit of the bitter melon plant tested its activity against the growth of the test microbes on Muller Hinton Agar medium by the agar diffusion method using a well. 10 ml of MHA medium was added to the petridisk, and three wells with a 9 mm diameter

were created following solidification. The MHA-containing petridisk was then aseptically injected with bacteria using a sterilized cotton swab. The bitter melon's leaves and fruit extract were then tested in each wellbore in three different concentrations. After that, the antibacterial-infused medium was incubated for 24 hours at 37 °C.

The fungal sensitivity test was carried out by taking a suspension of the fungus *C. albicans* and inserting it into a petridisk, then pouring 10 ml of PDA media. The petridisk is then slowly shaken on the table's surface or *laminar airflow* so that the mold and media suspension is mixed evenly and allowed to stand until it solidifies. Then the manufacture of 3 well holes is carried out. In each good hole, extracts of leaves and fruits of the bitter gourd plant were injected in three concentration variations to be tested. The antibacterial injected media was then incubated for 24 hours at 37^{o}C.

Result and Discussion

The phytochemical test results of the leaves and fruits' bitter gourd plant extract (*Momordica charantia* L.) were positive for containing secondary metabolite compounds. Based on Table 1, phytochemical test results of the N-hexane extract of bitter melon leaves contain secondary metabolite compounds, namely alkaloids, and steroids. For N-hexane extract, the bitter gourd fruit only positively contains alkaloid compounds. Meanwhile, the results of the phytochemical test of extracted bitter gourd ethanol (Table 2) contain secondary metabolite compounds, namely alkaloids, flavonoids, saponins, and tannins. These phytochemical test results follow the results of research conducted by Azizah *et al*. (2018) that bitter gourd leaf ethanol extract contains alkaloids, flavonoids, saponins, and tannins.

Meanwhile, in the phytochemical test, the bitter melon extract was positive for containing alkaloid compounds, flavonoids, and saponins. Internal and external factors can influence the content of compounds of secondary metabolites of a plant. Internal factors can be genes, while external factors are light, temperature, humidity, pH, and where plants grow (Katuk, 2019).

Table 1. Phytochemical Test Results of N-hexane Extract of Bitter Melon Leaves and Fruits

Compound Group	Leaves Extract	Fruits Extract
Alkaloids	+	+
Flavonoids	-	-
Saponins	-	-
Tannins	-	-
Steroids/Triterpenoids	+	-

Description:
(+) : Contains class of compounds.
(-) : undetectable compound group.

The sensitivity test study showed that bitter melon plant extracts in concentrations of 30%, 45%, and 60% inhibited the growth of test microbes, namely *S. epidermidis*, *E. coli*, and *C. albicans fungi.* Extracts that can inhibit the growth of test microbes are ethanol extracts from leaves and bitter melon fruits. Whereas in the solvent N-hexane extract leaves and bitter gourd fruit, it cannot inhibit the growth of test microbes.

Table 2. Phytochemical Test Results of Ethanol Extract of Bitter Melon Leaves and Fruits

Compound Group	Leaves Extract	Fruits Extract
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	+
Tannins	-	-
Steroids/Triterpenoids	-	-

Description :
(+) : Contains class of compounds.
(-) : undetectable compound group.

The presence of antimicrobial activity from the extract of the bitter gourd plant on the leaves and fruits is characterized by an inhibitory zone of microbial growth test. The results of the inhibition zone measurements can be seen in Table 3.

Table 3. The activity of bitter melon leaf and fruit extracts in N-hexane and ethanol solvents against microbial test growth

Microbial Test	Types of extracts	Microbial Growth Inhibition Zone Diameter Test (mm)					
		N-hexane			Ethanol		
		30%	45%	60%	30%	45%	60%
Staphylococcus epidermidis	Leaf	0 (TA)	0 (TA)	0 (TA)	6.3 (S)	13.3 (K)	14 (K)
	Fruit	0 (TA)	0 (TA)	0 (TA)	3.8 (L)	6,7 (S)	7 (S)
Escherichia coli	Leaf	0 (TA)	0 (TA)	0 (TA)	16 (K)	17.5 (K)	26 (SK)
	Fruit	0 (TA)	0 (TA)	0 (TA)	3 (L)	4.7 (L)	5.3 (S)
Candida albicans	Leaf	0 (TA)	0 (TA)	0 (TA)	0 (TA)	0 (TA)	0 (TA)
	Fruit	0 (TA)	0 (TA)	0 (TA)	0 (TA)	0 (TA)	0 (TA)

Description: SK: Very Strong, K : Strong, S : Medium, L: Weak, TA: No activity

The activity of bitter melon leaf and fruit extracts in N-hexane solvents is known to be unable to inhibit the growth of test microbes; this can be seen from the absence of growth inhibition of test bacteria at all concentrations of the extracts tested. These results indicate that leaf and fruit extracts in N-hexane solvent do not have antimicrobial activity. Based on phytochemical test results, N-hexane extracts of bitter melon leaves and fruits contained alkaloid and steroid compounds. However, it did not show strong activity in inhibiting the growth of test microbes. Hamdani *et al*. (2016) explained that alkaloid compounds in a plant play an important role in antifungal and antibacterial activity. The possibility is that the concentration of active compounds in the extract of bitter melon leaves and fruits in the N-hexane solvent is insufficient to inhibit the growth of test microbes (Hamdani et al., 2016).

Based on (Table 3) ethanol extracts of bitter gourd leaves and fruits show varying abilities against microbial growth tests. Bitter melon extract in ethanol solvent concentrations of 30%, 45%, and 60% can inhibit the growth of *Staphylococcus epidermidis* bacteria classified as medium and strong. At the same time, the concentrations of 30%, 45%, and 60% are classified as strong and very strong against the growth of *Escherichia coli* bacteria. The bitter melon extract in ethanol solvents with a concentration of 30%, 45%, and 60% can inhibit the growth of *Staphylococcus epidermidis* and *Escherichia coli* bacteria belonging to the weak and moderate categories. Bitter melon leaves and fruits contain active compounds such as flavonoids, alkaloid saponins, and steroids that act as antibacterials.

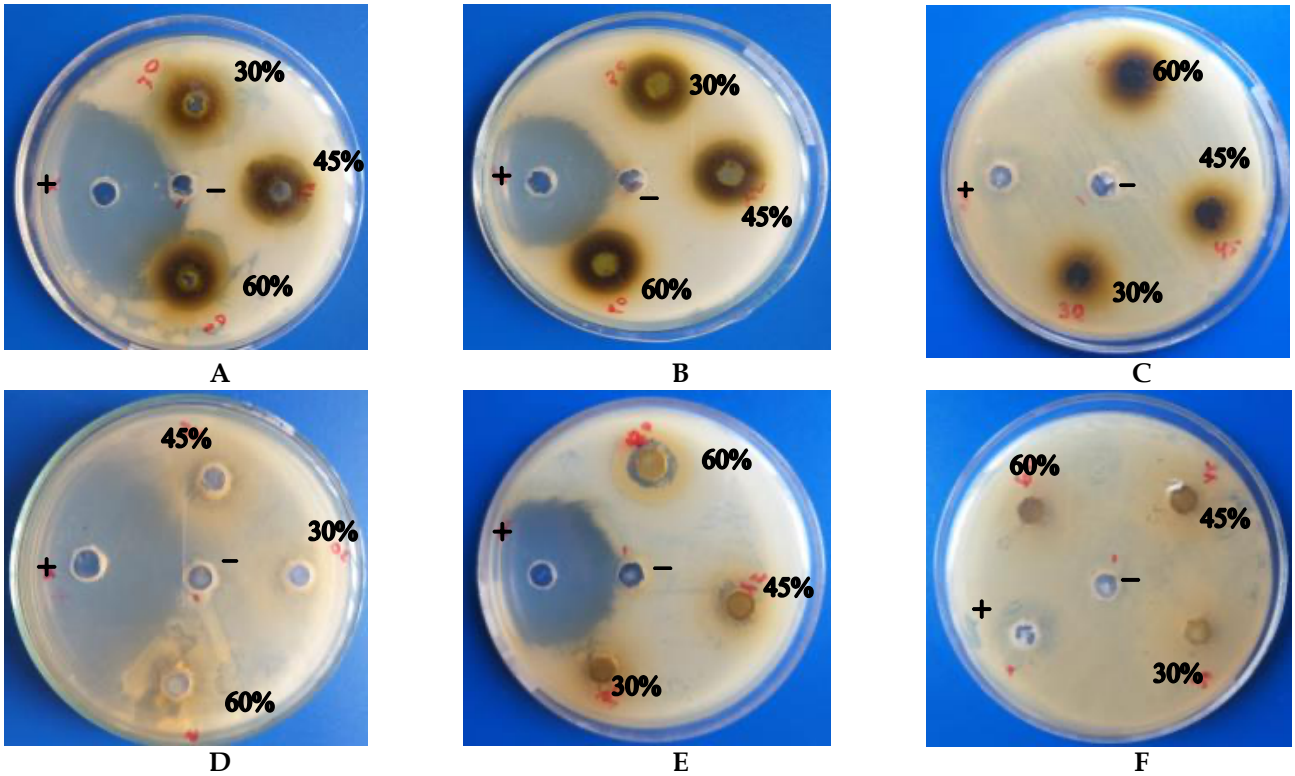

Figure 1. Test of antimicrobial activity of bitter melon plant extract (*M. Charantia* L.) against microbial growth. (A). Ethanol extract of bitter melon leaves against *S. epidermidis*, (B). Ethanol extract of bitter melon leaves against *E. coli* (C). Ethanol extract of bitter melon leaves against *C. albicans*, (D). Ethanol extract of bitter melon fruit against *S. epidermidis*, (E). Ethanol extract of bitter melon fruit against *E. coli*, (F). Ethanol extract of bitter melon fruit against C. *albicans*

According to Putri *et al*. (2019), the increase and decrease in the inhibitory zone are caused by the solubility properties of the active substance in the extract and the difference in diffusion velocity in the agar medium. Isramilda *et al*. (2020) also explained that the difference in the activity of the inhibitory power of srikaya leaf extract (*Annona squamosa* L.) against the growth of *Staphylococcus aureus* bacteria is due to differences in the number of active compounds contained in each concentration. The higher the concentration used, the higher the active compound.

The test results of the antimicrobial activity of bitter gourd leaf and fruit extracts showed that the solvent type factor and concentration variation obtained a P (sig) value of > 0,05. Based on the results of the analysis, it can be seen that H1 is rejected or H0 is

accepted at a test level of 5%. The solvent type and concentration variation factors did not significantly affect the growth of the microbial test inhibition. The results showed a P(sig) value of < 0.05 only found in the treatment of bitter melon leaf extract treatment for *Staphylococcus epidermidis* bacteria.

The solvent factor BNT test on bitter melon leaf and fruit extracts against the growth of *S. epidermidis* bacteria showed that the treatment with ethanol solvent was significantly different from the N-hexane solvent treatment. The analysis's results showed that ethanol solvents were more effective than N-hexane solvents at inhibiting the growth of test microorganisms in bitter melon leaf and fruit extracts.

Conclusion

Based on the results of the study, the following conclusions can be drawn: (1) Bitter melon plant extract (*Momordica charantia* L.) in N-hexane and ethanol solvents can inhibit the growth of *Staphylococcus epidermidis* and *Escherichia coli* bacteria. However, it cannot inhibit the growth of the *Candida albicans* fungus; (2) The ethanol extract of the bitter melon plant (*Momordica charantia* L.) parts of leaves and fruits can inhibit the growth of *Staphylococcus epidermidis* bacteria at a concentration of 60%, belonging to the category of very strong and very strong. While the *bacteria E. Coli belongs to the moderate category*.

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