ANTIMICROBIAL ACTIVITY OF MORINGA LEAF (*Moringa oleifera*) ETHANOL EXTRACT AND BACTERIA AGAINST *Staphylococcus aureus* AND THE FUNGI *Candida albicans*

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Abstract

Moringa plants are known in various parts of the world as vegetables that are rich in nutrients and have various properties, one of which is as an antimicrobial. This research was conducted to test the antimicrobial inhibition of Moringa leaf decoction and ethanol extract against the growth of Staphylococcus aureus and Candida albicans bacteria. The antimicrobial test in this study used the Disc diffusion method using various concentrations, including 25%, 50%, 75% and 100%. There were two test samples, Moringa leaf decoction and ethanol extract using the maceration method with 96% ethanol sailor. In addition to the test group, there was also a control group, namely a positive control using chloramphenicol and ketoconazole antibiotics, and a negative control using aquadest as a decoction solvent, and DMSO as an extract solvent. The results showed that Moringa leaf decoction and Moringa leaf ethanol extract could be used as antibacterial. The inhibition zones produced by Staphylococcus aureus at concentrations of 25%, 50% and 75% had a weak zone of inhibition for both decoction and ethanol extract of Moringa leaves. While the concentration of 100% stew and ethanol extract of Moringa leaves can inhibit by 7.25 mm and 8.5 mm which are included in the medium category. Moringa leaf extract can provide an inhibitory zone against Candida albicans. At a concentration of 25% it has an average inhibition zone of 1.75, at a concentration of 50% it has an average inhibition zone of 3, at a concentration of 75% has an average inhibition zone of 3.75. , at a concentration of 100% has an average inhibition zone of 4.5. So it can be concluded that the ethanol extract of Moringa leaves can inhibit the activity of the Candida albicans fungi with a weak category.

Keywords: *Moringa oleifera*, antimicrobial, *Candida albicans*, *Staphylococcus aureus*

Introduction

Infections caused by the fungus *Candida albicans* are commonly called Candidiasis. Based on available data, the prevalence of candida in adults is 3 -48% while in children 45-65%. Cutaneous candidiasis in Indonesia ranks third in the incidence of dermatophytosis, but in several cities, namely Makassar, Medan, and Denpasar, it ranks first in the incidence of dermatophytosis. The use of antifungal drugs without inappropriate indications can cause resistance to antifungals. With the prevalence of fungal infections and the limited number of

therapeutic options available, antifungal resistance may become a serious problem in the future . g.

Staphylococcus aureus is a bacterium gra m positively shaped round. According to Kotler & Sordillo, (2010) *Staphylococcus aureus* can cause diarrhea. Argudin *et al.* , (2010) also added that Staphylococcus enterotoxin is the main cause of food poisoning followed by diarrhea. In 2018 there were 10 outbreaks spread across 8 provinces, including West Java, Bali, West Nusa Tenggara, East Nusa Tenggara, West Kalimantan, Central Sulawesi, Maluku, and Papua. With diarrhea mortality rate during an outbreak of 4.76%. East Java is one of the provinces in Indonesia with a percentage of 76% around 151,878 cases.

One of the plants around the community that can be used as an antibacterial drug is Moringa oleifera or often called Moringa. According to Krisnadi's research, (2015) said that Moringa leaves contain active compounds of saponins, flavonoids, alkaloids, and tannins. Where these compounds can be used as antibacterials by destroying bacterial cell membranes. Antibacterial testing of Moringa leaf extract against Sthapylococcus aureus and Escherichia coli bacteria conducted by Dima et al., (2016) used the agar diffusion metgod by means of wells. Using maceration extract of Moringa oleifera leaves at a concentration of 80% included in the very strong category with an inhibition zone diameter of 22,66 mm.

Previous research has been done by (Budi et al., 2012) showed that 70% ethanol extract of Moringa leaves have a ktivitas anti fungi in tunju right with kemamp oth reduce furfur Malasseiza colony growth at concentrations of 18% ethanol extract .4 In Journal of Natural Sciences Research states that the presence of tannins can inhibit the formation of fungal cell walls, causing the death of organisms . This study aims to determine the inhibition of the decoction and ethanol extract of Moringa (Moringa oleifera) leaves against the growth of the fungus *Candida albicans* and the bacterium Staphylococcus aureus that causes diarrhea using the disk diffusion method (Kirby & Bauer test).

Materials and Methods

The tools used in this study were erlenmeyer, measuring cup, beaker glass, test tube, petri dish, oven, blender, sieve, glass jar, rotary evaporator, glass bottle, spirtus/bunsen lamp, au toclaf, incubator , spectrophotometer, dropper, analytical balance, loop needle, tweezers, thermometer, spoon, caliper and stirrer.

The materials used in this study were Moringa leaf (*Moringa oleifera* L.), *Staphylococcus aureus* test bacteria , *Candida albicans* , PDA (*Potato Dextrose Agar*) , sterile distilled water, 96% ethanol, *Nutrient Broth* (NB) media, filter paper , label paper, *Mueller Hinton Agar* (MHA), *M annitol Salt Agar* (MSA) , *Sulfide Indole Motility* (SIM) , Simmon Citrate, *Tri Sugar Iron Agar* (TSIA), *Methyl red-Voges Proskaue* (MR-VP), barium ($BaCl_2$), sulfuric acid (H_2SO_4), DMSO (*Dimethyl Sulfoxide*) and disc paper.

Sample Preparation

Moringa leaf amples that have been collected are then carried out wet sorting , then washed with running water. Samples that have been cleaned of impurities are then drained and separated between leaves and twigs . Then proceed with the p engeringan using the oven. The dried sample was mashed with a blender, then the resulting powder was sieved with 60 mesh, until a fine and homogeneous powder was obtained.

Extract Making

Extracts in this study used two extraction methods, namely maceration and decoction. Moringa leaf extract was obtained by maceration method by means of 200 grams of Moringa leaf simplicia powder soaked in 1500 ml of 96% ethanol solution . The sample was covered with aluminum foil and left for 5 days while stirring occasionally. After 5 days filtered using filter paper. The filtrate obtained was then thickened using a rotary evaporator, in order to obtain a thick extract of Moringa leaves . [6]

Preparation of extract stew is done by 20 grams of chopped bulbs Moringa leaves and add 20 ml of sterile distilled water in a beaker glas s , then heated to boiling for 15 minutes. Furthermore, the stew of Moringa leaves in a hot state is filtered using a glass funnel lined with filter paper.

Phytochemical Test

The alkaloid test was carried out by adding 1 ml of 2 N sulfuric acid to the sample and shaking it until 2 layers were formed. Add two drops of Dragrndrof's reagent in a test tube, and two drops of Wagner's reagent in a test tube. A positive result is indicated by the

formation of a brown precipitate. The flavonoid test is carried out by adding concentrated HCL to the sample and heating it on a water bath, then observing the color changes that occur. The saponin test was carried out by adding 5 ml of water to the sample , after which it was shaken vigorously for 10 minutes and then left for 10 minutes. Foam formed and persisted for more than 10 minutes indicated the presence of saponins . The tannin test was carried out by adding 5 drops of 10% NaCl and filtered. The filtrate was added and 10% glatin was added. After that, no changes occurred. Positive results with the formation of a white precipitate . Steroid test conducted by the sample is added by way of added 2 ml of H_2SO_4 concentrated. The presence of a red ring indicates an unsaturated steroid. Phenol test is done by way of adding $FeCl_3$ 1% to occur perubaha n colors.

Preparation of Test Solution

Created l olutions test concentration of 100%, 75%, 50% and 25% respectively of ethanol extract of Moringa leaves and decoction of the leaves of Moringa. By way of extracting the maceration or re busan each as much as 5 ml, 3.75 ml, 3.33 m l, and 2.5 ml , then each dissolved into 1 ml of solvent each (stew dissolved into distilled water, extract dissolved in DMSO).

Antifungal Test

The fungal suspension was swab evenly on the surface of the PDA media, then left for 5 minutes, the disc paper was dripped as much as 30-50 l with maceration extract and Moringa leaf decoction which had been made in concentrations of 100%, 75%, 50%, 25% and in control (positive & negative). Then using sterile tweezers. Then the disc paper is let on the surface of the PDA media and pressed a little so that it sticks. PDA media were incubated at 37 $^{\circ}$C for 24 hours

Antibacterial Test

Media *Mueller Hinton order* (MHA) each 10 ml which has dist erilkan poured on a sterile petri dish and ditung gu until solidified , the rest of the media MHA present in the test tube was added with 100 bacterial suspension test were homogenized with a vortex. Then it was poured into a petri dish containing MHA and then leveled by forming a figure eight . Paper discs that have been soaked for 15 minutes in maceration extract

and Moringa leaf decoction that have been made in various concentrations and in controls (positive and negative), then using sterile tweezers, the disc paper is placed in a sterile petri dish for 1 minute until no dripping liquid. Then the disc paper m is placed on the surface of the MHA media and pressed a little so that it sticks. The MHA medium was incubated at 37 ^{0}C for 24 hours.

Data analysis

The data obtained were carried out with One Way *Analysis Of Variant* (ANOVA) statistical test . Furthermore, a *post hoc* test was carried out if the *One Way Anova* test was meaningful. If the data distribution is not normal, then a non - parametric statistical test, namely the Kruskall-Wallis , is used to see the difference in the mean of each concentration . If there is a difference between concentrations, then proceed with the Mann-Whitney test. To determine the difference between the stew and the ethanol extract of Moringa leaves, a different test was carried out using an independent T-test.

Results and Discussion

In the phytochemical test results of the decoction and ethanol extract of Moringa leaves , it was found that there was no difference between the decoction and the ethanolic extract of Moringa leaves that were positive for alkaloids when added Dragendorph's reagent, indicated by the formation of a reddish-brown precipitate. Contains flavonoid compounds, characterized by changes in color to dark red and orange after being given treatment. Contains tannins, which are characterized by the formation of a white precipitate. Contains saponins, with the formation of a foam that can last more than 10 minutes. Contains stero id, indicated by a red ring. Contains phenol which is characterized by a change in color to greenish black.

Table 1. Phytochemical Test Results of Moringa Leaf Extract and Decoction

No	Test	Observation	Results	
			Stew	**Extract**
1.	Alkaloids filtrate + Dragendorf reagent	A brown precipitate is formed	+	+
2.	Flavonoids B + concentrated HCL filtrate C + HCL filtrate + Mg powder	Dark red Orange	+ +	+ +
3.	Saponins Extract + water → shake vigorously	Foam more than 10 minutes	+	+
4.	Tannins filtrate + gelatin salt	There is a white precipitate	+	+
5.	Steroids The filtrate B + acetic anhydride + H_2SO_4 concentrated C + H_2SO_4 filtrate	There is a change in color to yellow There mer ring ah	+ +	+ +
6.	Phenol Filtrate + $FeCl_3$	The color changes to greenish black	+	+

From the observation, it was found that the boiled sample of Moringa leaves and ethanol extract of Moringa leaves can be used as an antibacterial , this can be seen from the decoction and ethanolic extract of Moringa leaves that have an inhibitory zone against the bacterium *Sta phylococcus aureus* . Moringa leaf decoction at a concentration of 25% to 75% has an average inhibition zone diameter of 3.25 mm, 4.25 mm, 5.25 mm which is included in the category of weak inhibition zone. While at a concentration of 100% the diameter of the inhibition zone is 7.25 mm which is included in the category of medium inhibition zone . Inhibition zone testing on ethanol extract with a concentration of 25% to 75% has an average inhibition zone of 4.25 mm, 5.25 mm, 5.75 mm, which is included in the category of weak inhibition zone . Meanwhile, there was an extract with a concentration of 100% of the resulting inhibition zone of 8.5 mm which was included in the category of medium inhibition zone.

Table 2. Average Inhibitory Zone of Staphylococcus aureus to Moringa Leaf Ethanol Decoction and Extract

No	Concentration	Stew		Extract	
		Mean ± SD	**Category**	Mean ± SD	**Category**
1.	K+	11,75 ± 0,957[a]	Strong	10,75 ± 0,500[a]	Strong
2.	K-	0 ± 0[b]	-	0 ± 0[b]	-
3.	25%	3,25 ± 0,500[c]	Weak	4,25 ± 0,500[c]	Weak
4.	50%	4,25 ± 0,500[d]	Weak	5,25 ± 0,500[d]	Weak
5.	75%	5,25 ± 0,500[e]	Weak	5,75 ± 0,500[d]	Weak
6.	100%	7,25 ± 1,258[f]	Currently	8,5 ± 0,577[e]	Currently

From the inhibition zone test, Moringa leaf decoction samples could not inhibit the growth of the fungus *Candida albicans*. While the ethanol extract of Moringa leaves at a concentration of 25% produces an average inhibition zone of 1.75 mm, at a concentration of 50% produces an average inhibition zone of 3 mm, at a concentration of 75% produces an average inhibition zone of 3. 75 mm , at a concentration of 100% produces an average zone of inhibition of 4.5 mm.

Table 3. Average Inhibition Zone of Candida albicans to Moringa Leaf Ethanol Decoction and Extract

No	**Concentration**	**stew**	**Category**	**Ekstrak ± SD**	**Category**
1	CONTROL (+)	30,5	Very strong / Very strong	28,75 ± 0,957[A]	Very strong / Very strong
2	CONTROL (-)	-	-	1 ± 1,154 [B]	- - / Weak
3	25%	-	-	1,75 ± 0,500 [B]	- - / Weak
4	50%	-	-	3 ± 0,500 [C]	- - / Weak
5	75%	-	-	3,75 ± 0,500 [D]	- - / Weak
6	100%	-	-	4,5 ± 0,577 [D]	- - / Weak

In data processing, the diameter of the inhibition zone of Moringa leaf decoction and ethanol extract on the growth of *Staphylococcus aureus* bacteria has an abnormal data distribution value with a p value of 0.05. Followed by an alternative test, namely the Kruskal-Wallis analysis, from the results of the analysis it is known that the probability value is 0.00 for decoction and 0.001 for extract. This shows that there is a significant or significant difference. S elanjutnya to determine the significance (p 0.05) between groups, followed by Mann-Whitney test. In the Mann-Whitney test, the inhibitory zone of the ethanol extract of Moringa leaves, there was no significant difference at a concentration of 50% with a concentration of 75%.

On this research has been done, diketah ui that stew and ethanol extract of Moringa leaves have inhibitory to bacteria *Staphylococcus aureus* from konsenetrasi lowest to highest concentrations . The inhibition zones produced in boiled samples and ethanol extracts of Moringa leaves with high concentrations showed the widest inhibition zones for bacterial growth, this was influenced because the levels of active compounds contained in high concentrations were more than low concentrations . The wider the inhibition area formed around the paper disc, the greater the antibacterial power contained in the Moringa leaves.

The use of positive control in this study using chloramphenicol because it is included in the broad-spectrum antibiotics that can inhibit gram-positive and gram-negative growth. The negative control used in this study was *Dimethyl Sulfoxide* (DMSO) which was used as a solvent for the ethanol extract of Moringa leaves and sterile distilled water which was used as a solvent for decoction of Moringa leaves. *Dimethyl l Sulfoxide* (DMSO) is widely used as a solvent for extracts in various studies related to the antimicrobial test of plant extracts. (Natheer *et al.* , 2012) stated that the substance used as a negative control was the solvent used as a diluent for the extract. The purpose is as a comparison that the solvent used as a diluent does not affect the results of the antibacterial test. [9] The result of the inhibition zone of the negative control against the test bacteria was 0 mm. Ha l This shows that the use of the solvent DMSO and sterile distilled water did not affect the results of antibacterial tests.

The results obtained in the phytochemical test showed no difference between the extract and decoction of Moringa leaves. Phytochemical tests were carried out on ethanol extract and Moringa leaf decoction containing alkaloids, flavonoids, saponins, tannins, steroids, and phenols. According to (Retnowati, Bialangi and Posagi, 2011) these secondary metabolites can be used as antibacterial, this is due to the inhibition of these

bacteria due to the reaction of a chemical compound contained in Moringa leaves as antibacterial. F lav onoid have work as an antibacterial mechanism by inhibiting the function of the cell membrane with the car a permebealitas disrupt cell membranes and inhibit binding of enzymes such as ATP ase and phospholipase. [10]According to (Ibrahim and Kuncoro, 2012) alkaloid ju ga alleged can be used as an antibacterial by interfering components of the peptidoglycan in bacterial cell i , sehing ga composition of the cell wall are not fully formed and cause cell death.

In addition, the saponins found in Moringa leaves can also act as an antibacterial which causes leakage of proteins and enzymes from the cells. Saponins can sebag ai anti b akteri for surface active substances like detergents, saponins consequently will menurunk an surface tension of the bacterial cell wall and membrane permebialitas destroy resulting in the increase in permeability or cell leak and cause right Senya wa intracellular going out. [12]

According to (Amalia Sari and Nursanty, 2017) the mechanism of action as an antibacterial tannins could be expected to mengkerutkan the cell wall or cell membrane permeability thereby disrupting the cell itself, due to disruption of the permeability, the cell can not perform life activities so pertumbuha nnya terha mbat or even death. [13]The mechanism of action of steroids in Moringa leaves are associated with membrane lipids and sensitivity to the compound en steroids which cause leaks in liposomes.

The ethanol extract of Moringa leaves has a higher antibacterial activity when compared to Moringa leaf decoction with water as a solvent. This is because there are differences in the extraction method and the use of solvents. The use of solvents in the extraction will affect the chemical compounds that will be attracted, so that it will affect the secondary metabolite activity of the Moringa leaf plant. This causes the inhibition zone that is formed to be larger in the ethanolic extract of Moringa leaves because the use of polar ethanol solvents in Moringa leaf extract can attract most of the active compounds found in polar Moringa leaves. [13]This is reinforced by the research of Sulastri, (2015) which states that ethanol has the property of dissolving almost all substances, both polar, semipolar, and nonpolar and can optimally attract the life of flavonoids.

Antifungal activity of the ethanol extract of Moringa leaves can inhibit the growth *of Candid a albicans.* E kstrak ethanol Moringa leaves have antifungal activity against *Candida albicans* marked with terben their shapes inhibition zone at a concentration of 25%, 50%, 75% and 100%. From these results it is known that the higher the concentration of Moringa leaf ethanol extract used against *Candida albicans* , the higher the inhibition zone produced in the form of diameter. M ccording to studies (Siddique, *et.*

Al . 2016), which states that the increase in inhibitory zone along with an increase KONS entrasi Rand abkan because of the increased content of antifungal extracts increase the antifungal activity due to the active substance content of dissolved increases at higher concentrations in extracts tested

There was no inhibition zone in Moringa leaf decoction compared to Moringa leaf ethanol extract, due to differences in extraction methods and solvents used. The use of solvents in the extraction will affect the chemical compounds that will be attracted. The use of the boiling method with a temperature that is too high can result in the decomposition or destruction of the secondary metabolite content in Moringa leaves. This is supported by research conducted by (Puspitasari, 2018) which states that the content of secondary metabolites of simplicia that has been boiled will be damaged when heated at high temperatures.

In addition to the effect of the use of solvents and extraction methods, not the formation of inhibitory zone on the stew also can be influenced by the content of secondary metabolites contained in the Stew , allegedly because of the amount of compound m etabolit sekun der mentioned are not adequate to inhibit the growth of *C. albicans* . Phytochemical screening carried out in this study can only prove the presence of a secondary metabolite compound qualitatively. In addition, there has been no p en elitian out the amount of at least one compound metabol it secondary to inhibit *C. albicans* .

In the inhibition zone, the extra shelf ethanol produced against the fungus *Candida albicans* with the *disc diffusion* method produces a relatively small inhibition zone which is included in the weak category. However, the pitting method that has been carried out in the preliminary test provides a better inhibition zone and is included in the strong category. This proves that the choice of method is very influential on the inhibition process . This is in accordance with research conducted by (Prayoga, 2013) The use of the pitting method is considered to be more effective than the *disc diffusion* method by producing a larger inhibition zone due to the method of extracting the well directly entering into each hole, the inhibiting effect becomes stronger. [12] Whereas in the *disc diffusion* method , *the* discs are immersed in the sample and placed on top of the media.

In the decoction extraction method according to (Margaretta *et al.* , 2011) heating during the extraction process can cause damage to plant secondary metabolites . Bioactive components such as flavonoids, tannins, and phenols can be damaged at temperatures above 50 $^{\circ}$C because they can undergo structural changes and produce low secondary metabolites. [17] This is reinforced by a previous study conducted by (Verawati *et al.,* 2016) the maceration

method of dried leaves on piladang leaf samples had the highest total phenol content compared to the boiled method, with levels of 356.7619 mg/g in the maceration method. , and 69.3957 mg/g. [18] So that the difference in temperature and the extraction method used greatly affects the inhibition zone produced by the decoction and extract.

The weakness in this study is the inhibition zone produced is very small. When compared with previous research that has been carried out by (Dima, Fatimawali and Lolo, 2016) using the well method regarding the activity test of Moringa leaf extract against *Escherichia coli* and *Staphyloc occus aureus bacteria* using the well method at a concentration of 80% which has an inhibition zone of 20, 50 mm which is included in the strong category of *Staphylococcus aureus.* [19] The difference in the resulting inhibition zone is due to the different methods in the test.Research Sri *et al.,* (2017) and Haryati *et al.,* (2017) men unjukkan research results that the method of pitting nicer and more spacious than the inhibitory zone disk method. These results are also supported by the results of the study (Nurhayati *et al.,* 2020) which says that the activity generated pad a method su mura n is higher than the activity on the method of discs. This is because the well extract method can be directly inserted into each hole so that the effect to inhibit bacteria is stronger. Whereas in the *Dis- C diffusion* method , the disk cramp must be immersed in a drip plate containing ethanol extract of Moringa leaves and Moringa leaf decoction, then the disc is placed on top of the agar medium. According to (Prayoga, 2013) using the well method can produce a large diameter of the inhibition zone, because in the well method an osmolarity process occurs from a higher concentration of extract. Osmolarity occurs more thoroughly and is more homogeneous and the concentration of the resulting extract is higher and stronger to inhibit bacterial growth. [20] In addition, isolates are active not only on the top surface of the agar nutrient but also down to the bottom. [21] While the *Disc diffusion* method used antimicrobial isolates had activity only on the upper surface of the nutrient agar.

From the statistical analysis that has been carried out, the results showed that the decoction and ethanol extract of Moringa leaves on the growth of *Staphylococcus aureus* bacteria had abnormal data distribution (P 0.05) . From the results of the Mann-Whitney test, there was an insignificant or significant difference in the 50 % concentration with the 75% concentration in the ethanol extract of Moringa leaves. The result was not significant difference because the inhibition zones formed at concentrations of 50% and 75% did not differ much . U ji difference conducted to determine the activity of decoction and extract of Moringa leaves using the test T-test. From these results obtained a value of 0.663 which

means there is no significant difference between the stew and ethanol extract of Moringa leaves, or it can be said that the decoction and ethanol extract of Moringa leaves have the same activity . This is because the inhibition zones produced by decoction and ethanol extract of Moringa leaves are not much different.

Conclusion

Decoction and ethanol extract of Moringa leaves can memb erikan activity of antibacterial against *Staphylococcus aureus* at all concentrations with sig. 0.000 and 0.001 which were indicated by the formation of a clear zone around the paper disc. At a concentration of 25% , 50% and 75% had a weak zone of inhibition, while at a concentration of 100% had an average area of 7.2 5 mm and 8.5 mm which was included in the medium category . Analysis of the different test data showed that there was no significant difference between the stew and ethanol extract of Moringa leaves against *Staphylococcus aureus* bacteria , or it could be said that the decoction and ethanol extract of Moringa leaves had the same activity, with a value of 0.663. The ethanol extract of Moringa leaves has antifungal activity with a weak category , while the decoction of Moringa leaves does not have antifungal activity on the type of fungus *C andida albicans* using the *disc diffusion* method .

Acknowledgements

I would like to thank STIKES Anwar Medika Hospital and the entire community for their support in completing this research. I also thank the students who have collaborated in this research h.

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