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Research article

Inhibitory power test of moringa oleifera lamk against *Actinobacillus Actinomycetemcomitans* and its toxicity test to *SGOT SGPT* in rat

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Abstract: This study was to test the effectiveness of Moringa seed oil extract in inhibiting the growth of *Actinobacillus Actinomycetemcomitans* bacteria and to test the toxicity of Moringa seed oil extract. This type of research is experimental laboratory with *post test only control group design*. Pure Moringa leaf seed oil concentrations of 25%, 50% and 75% were used as experimental materials and the bacteria *Actinobacillus Actinomycetemcomitans* as test samples. The inhibition test used the Kirby-Bauer method while the SGOPT and SPGT examinations were used to measure the level of toxicity. The largest inhibition zone was found at a concentration of 75% at 4.73 and the smallest at a concentration of 25% at 2.23, while for positive control the inhibition zone was 16.44 and negative control was 0.10. Toxicity test shows that the safe dose that can be used is no more than 1250mg/kg, while toxicity occurs at a dose of 2500-5000 mg/kg. The results of statistical tests also showed a significant difference between the control and test groups of 2500 and 5000mg/kg. Conclusion : Moringa fruit seed oil (Moringa Olefiera L) has the greatest inhibitory power ata concentration of 75% with a dose of not more than 1250 mg/kg.

Keywords: *Moringa (Moringa Olefiera L). Germ inhibitory test. Toxicity test.*

INTRODUCTION

Indonesia as a tropical country has biodiversity and abundant natural resources, one ofwhich is the Moringa plant (Moringa oleifera L). The benefits of this plant have beenrecognized both nationally and internationally,[1,2] In Indonesia, the Moringa plant is used for food, medicine, cosmetic ingredients and traditional cultural rituals.[1] Almost all parts of the plant can be utilized, including the seeds of the Moringa fruit. [2,3] Moringa seeds contain high vegetable oil and have many health

benefits. Moringa seed oil contains natural compounds that have potential as antibacterials, generally containing flavonoids, tannins, steroids, polyphenols, terpenoids, alkaloids, and saponins.[4,5] In addition, Moringa is also a good source of natural antioxidants because it contains various types of antioxidant compounds such as ascorbic acid,flavonoids, phenolics and carotenoids. High concentrations of ascorbic acid, estrogen and β- sitosterol, iron, calcium, phosphorus, copper, vitamins A, B and C, α-tocopherol, riboflavin, nicotinic, folic acid, pyridoxine, β-carotene, protein, and especially amino acids essentials suchas methionine, cystine, tryptophan and lysine are present in the leaves and pods which make them an almost ideal dietary supplement. [6.7]

The most common oral infection is periodontitis. The 2018 Basic Health Research (Riskesdas) shows the prevalence of periodontitis in Indonesia is 74.1%. Globally, periodontitis as an infectious disease ranks 6th with a prevalence of 11.2% affecting nearly 743million people. *Actinobacillus Actinomycescomitans* is one of the bacteria found as the cause of periodontitis. The type is anaerobic in the form of gram-negative rods that play an importantrole in the development of periodontal disease such as the formation of periodontal pockets, destruction of periodontal fibers and alveolar bone.[8.9] Treatment of periodontal tissue infectionsusually uses synthetic antibiotics. Treatment of infection with synthetic antibiotics can bring its own problems, namely the side effects with antibiotics. In addition, the resistance of bacteria to antibiotics is increasingly worrying after the emergence of bacterial strains that are resistantto some commonly used antibiotics. Attempts to find other alternatives in the treatment of infections is to use traditional medicine.

The development of a new drug, the conditions that must be met to be able to increase to the next stage of use are safe and non-toxic. For this purpose, it is necessary to examine the toxicitytest, both acute and subchronic toxicity. Toxicity test methods can be divided into two types, namely, toxicity tests designed to evaluate all the general effects of a compound, and specific toxicity in detail.[10,11]

Toxicity test on white rats is one of the efforts to determine the toxicity and side effectson humans. Commonly performed toxicity tests are orally and dermally. The acute oral toxicitytest aims to determine the toxicity of a chemical when it enters the digestive tract directly, while the acute dermal toxicity test aims to

determine the toxicity of a chemical through contactwith the skin. Oral and dermal testing is done because poisoning often occurs through food anddrink as well as skin contact.[12]

The toxic effect of a drug is often seen in the liver because the liver plays an importantrole in detoxifying compounds that enter the body. Hepatotoxicity can occur due to the accumulation of xenobiotics in the liver which are excreted through bile so that the histopathological parameters of the liver and the levels of SGOT and SGPT enzymes in the testanimals are also seen. An increase in the activity of the SGOT and SGPT enzymes is a strongand sensitive indicator of abnormalities in liver cells.[13] Examination of *Glutamic Oxaloacetic Transaminase* (SGOT) or *Aspartarte aminotransferase* (AST) and *Serum Glutamic Piruvic Transaminase* (SGPT) or *Alanine aminotransferase* (ALT) is also aims to determine the inflammation that occurs in the body and is usually an indication of a disorder (inflammation)in the liver.[14]

This study aims to analyze what is the best concentration of Moringa seed oil that can inhibit the growth of *Actinobacillus Actinomycescomitans* bacteria and see the maximum dosethat can be used in the safe zone.

METHOD

This research is a laboratory experimental study with a *post test only control design*. The inhibition test was a diffusion test using the Kirby-Bauer method, while SGOPT and SPGTwere used to measure the level of toxicity.

1. Resistance Test
   a. Tools and Materials

      The tools used are caliper, ose, analytical balance, autoclave, filter paper, tweezers,measuring cup, dropper, measuring pipette, rack and test tube, petridish, erlenmayer, aluminum foil, incubator, bunsen and vortex, paper disc,swabs. The ingredients are Moringa seed oil, *Actinobacillus Actinomycetemcomitans* bacteria, Muller Hiton Agar (MHA) media, 95% ethanol, 0.9% NaCl, DMSO (Dymethyl Sulfoxide), spritus. Tools and materials are sterilized using an autoclave and 96% ethanol.[15]

   b. Preparation of culture media and test media for Muller Hiton Agar (MHA)

media

A total of 38 grams of MHA was dissolved in 1 L of distilled water then heated and stirred using a stirrer until homogeneous. The media was sterilized using an autoclave at 121°C, 1.5atm pressure and for 15 minutes. After sterilization, 15 ml is put into a petri dish which will be used as the medium in the test.

c. Rejuvenation of the bacteria *Actinobacillus Actinomycetemcomitans*

The *Actinobacillus Actinomycetemcomitans* bacteria were propagated by taking 1 Ose,scratched into solid media so that it tilted in a zigzag manner and the media tube was covered with cotton after that it was incubated at 37°C for 24 hours.[17]

d. Making positive control and negative control

The positive control used the antibiotic clindamycin which was dripped on the paperdisc. The negative control used is DMSO *(Dymethyl Sulfoxide)* which is the solvent in this test. The number of treatments that will be tried is three, namely treatments at concentrations of 25%, 50% and 75%, with the Federer formula, it is known that 8 repetitions are carried out, so that a total of 24 treatments are obtained.[18]

e. Bacterial Suspension Manufacturing

The suspension was prepared by inoculating 1-2 oses of the bacteria culture from Muller Hinton Agar (MHA) media into a tube containing 0.9% NaCl. Homogenize witha vortex, then equalize the turbidity with 0.5 Mc Farland solution ($1.5x10^8$ CFU/ml).[19]

f. Inhibition zone test

Actinobacillus Actinomycetemcomitans bacteria were cultured for 1x24 hours at 37°C in a petri dish and then treated with filter paper. The measurement of the inhibition zoneof the Moringa seed oil extract was carried out in a petri dish. [20] The test method used was the Kirby-Bauer method, namely the diffusion method with paper discs. Bacterialcultures were poured aseptically as much as 3 ml of sterile petri dishes containing MHAagar. Paper discs were put in Moringa seed oil with different concentrations, namely 25%, 50% and 75% and also in negative control (DMSO) and positive control (clindamycin

antibiotic) for approximately one minute. Paper discs that had been soaked in Moringa seed oil were placed aseptically on the surface of the MHA medium that had been inoculated with bacteria with sterile tweezers, then incubated at 37ºC for1X24 hours. After incubation, the clear zone around the paper disc was observed and the diameter was measured. The test of the inhibitory power of Moringa seed oil to thebacteria *Actinobacillus Actinomycetemcomitans* was carried out 9 times for eachconcentration tested.[21]

2. Toxicity test
   a. Tools and Materials[22]

      The tools used are analytical balance, cotton swab, Erlenmeyer glass, tweezers, stirringrod, 3cc syringe, capillary tube, mask, handscoon, vaculab tube, tip, evendor tube, andcentrifuge. The materials needed were 20 white rats consisting of 10 males and 10 females adapted for 7 days in a lab environment, Moringa seed oil, alcohol, corn oil, technical ether.

   b. Initial blood draw[23,24]

      Each white rat was taken blood through the tail and eyes, first the white rat was put intoa jar that had been given ether on cotton after the white rat fainted. Blood collection was done using a 3cc syringe. blood is put into a vaculab tube.

   c. Early white mouse blood and plasma separation[23]

      The blood was put into a centrifuge and rotated for 20 minutes at a speed of 2500 rpm.After that, the blood was taken with a micropipette whose tip had been attached and theblood plasma was transferred to the Evendor tube. The separated plasma will be storedin a refrigerator at 4°C for further processing.

   d. Making trial extract

      Preparation of stock solution with Moringa seed oil and corn oil solvent in a ratio of 1:150%, namely 20 ml of Moringa seed oil plus 20 ml of corn oil, as for the calculation ofthe dose are:

      1. Moringa oil 625 mg/kg, stock solution 2.5 ml added corn oil made up to 20 ml
      2. Moringa oil 1250 mg/kg, 5 ml stock solution added with corn oil made up to 20 ml

3. Moringa oil 2500 mg/kg, 10 ml stock solution added with corn oil made up to 20 ml
4. Moringa oil 5000 mg/kg, taken from stock solution without adding corn oil

e. Administration of extract to test animals [25]

In this study, 20 mice were divided into 5 treatment groups, each consisting of 4 whiterats consisting of 2 males and 2 females. Dosage in white rats was determined from thescales of the white rats and administered orally. The five treatment groups are:

1. Group I: healthy control, only given standard food and drink
2. Group II: given Moringa seed oil at a dose of 625mg/kg
3. Group III: given Moringa seed oil at a dose of 1250mg/kg
4. Group IV: given Moringa seed oil at a dose of 2500mg/kg
5. Group V: given Moringa seed oil at a dose of 5000 mg/kg

After that the mice were observed for seven days with standard food and drink.

f. Final blood draw (after seven days of observation)[3]

Based on the results of the research on the inhibitory power of Moringa seed oil, the measurement of the average inhibition value of the bacteria *Actinobacillus Actinomycetemcomitans* showed that a concentration of 25% had the smallest inhibitionzone of the three concentrations, namely 2.23±1.25, followed by a concentration of 50%, which was 3.71±1.78 and for a concentration of 75% it had the zone of inhibitionis 4.73±2.31. The greatest zone of inhibition occurred in the positive control group, namely 16.44±4.09 and for the negative control 0.10±0.05 to *Actinobacillus Actinomycetemcomitans* bacteria.

Shaphiro-Wilk statistical test was conducted to determine the value of normality and obtained p value > 0.05, which means the data isnormally distributed. The test was followed by a parametric test, namely One-way Anova. Based on the One-way Anova statistical test, a significance value of 0.000 ($p<0.05$) was obtained, which means that there was a significant effect of the mean diameter of the inhibition zone of various concentrations of Moringa seed oil on the inhibition of the bacteria *Actinobacillus*

Actinomycescomitans. To find out in detail which treatment group had a significant antimicrobial effect, a Post Hoc LSD test wascarried out. The results of the Post Hoc LSD test showed a significance ($p < 0.05$) between the treatment groups, this can be seen from the difference in the mean diameterof the inhibition zone formed.

g. Final blood plasma collection.[23]

After taking the blood, the blood is then put into a centrifuge and rotated for 20 minutesat a speed of 2500 rpm. After that, the blood was taken with a micropipette with a tip attached and the blood plasma was transferred to the Evendor tube.

h. Blood plasma examination to see the value of SGOT and SGPT

Blood samples were sent to the Hasanuddin University clinical pharmacy laboratory liver function tests, namely *Serum Glutamic Oxaloacetic Transaminase* (SGOT) and *Serum Glutamic Piruvic Transaminase* (SGPT).[26] Increased levels of SGOT and SGPTwill occur if there is an intracellular release of enzymes into the blood caused by necrosis of liver cells or acute damage, or in other words, levels of SGOT and SGPTwill increase in the blood when there is damage to liver cells.[27]

RESULTS

Examination of the inhibitory power of Moringa seed oil on the growth of *Actinobacillus Actinomycetemcomitans* bacteria at concentrations of 25%, 50% and 75% the results can be seen in Figure 1 and Table 1.

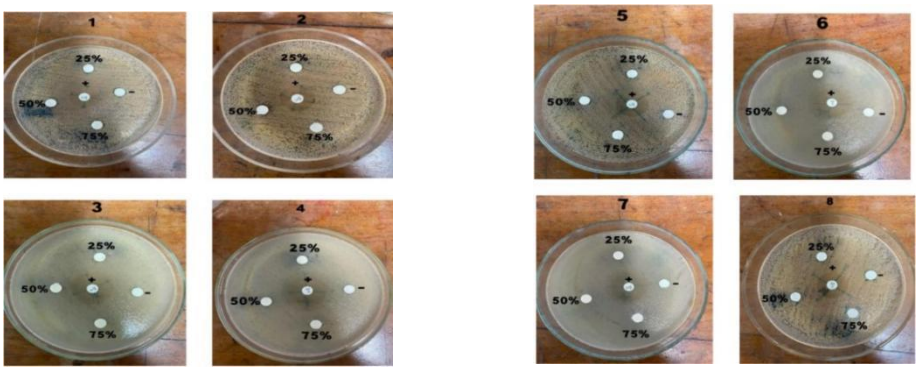

Figure 1. Formation of the inhibition zone on MHA that has been incubated for 24 hours

The results of the measurement of the average zone of inhibition in the study were obtainedwith the following data:

Table 1. The results of the measurement of the average value of the zone of inhibition of *Actinobacillus bacteria*

Group	N	Inhibition Zone
		Mean ± SD
Moringa Seed Oil 25%	8	2.23 ± 1.25
Moringa Seed Oil 50%	8	3.71 ± 1.78
Moringa Seed Oil 75%	8	4.73 ± 2.31
Positive Control	8	16.44 ± .09
Negative Control	8	0.10 ± 0.15

From Table 1 it can be seen that Moringa seed oil with a concentration of 25% has the smallest inhibition zone at 2.23 and the largest at 75% concentration at 4.73. The zone of inhibition in the positive control group was 16.44 and the negative control group was 0.10.

Table 2. Statistical test results of the zone of inhibition of *Actinobacillus Actinomycetemcomitans*

Consentration	N	*Actinobacillus Actinomycetemcomitans*	
		*Normalitytest**	*Comparisontest***
Moringa Seed Oil 25%	8	0.903*	
Moringa Seed Oil 50%	8	0.082*	
Moringa Seed Oil 75%	8	0.245*	0,0
Positive Control	8	0.191*	00
Negative Control	8	0.197*	

**Shapiro Wilk test>0.05; data distribution normal*

***One-Way Anova: p<0.05; significant*

The Shaphiro-Wilk statistical test was carried out to determine the value of normality and obtained a p value > 0.05, which means the data is normally

distributed so that the test iscontinued with the parametric test, namely Oneway Anova (table 2). Based on the One- way Anova statistical test, a significance value of 0.000 ($p<0.05$) was obtained, which meansthat there was a significant difference between the treatment groups. Furthermore, a further difference test (post hoc) was carried out to determine the difference between the 2 variables (Table 3).

Tabel 3. Post hoc statistical test results for *Actinobacillus Actinomycetemcomitans* inhibition zone bacteria.

Handling groups (i)	Proportion (j)	*Bacterial Inhibition Zone* Mean difference (i-j)	*p-value**
	50% Consentration	-1.47500	0.212
25% Consentration	75% Consentration	-2.49375*	0.039*
	Positive control	-14.20625	0.000*
	Negative control	2.13125	0.075
	75% Consentration	-1.01875	0.386
50% Consentration	Positive control	-12.73125	0.000*
	Negative control	3.60625	0.004*
75% Consentration	Positive control	-11.71250	0.000*
	Negative control	4.62500	0.000*
Positive Control	Negative control	16.33750	0.000*

**LSD: p<0.05; significant*

The results of the further difference test using the LSD test with a significance of $p<0.05$ showed a 25% concentration of Moringa seed oil compared to a 50% concentration and the negative control had a $p>0.05$, which means there was no significant difference or had the same effect. Meanwhile, the concentration of 25% compared to the concentration of 75% and the positive control had a p value of <0.05, which means that there is a significant difference or has a different effect. Likewise, the difference in the inhibition zone at a concentration of 50% compared to a concentration of 75% had a p value> 0.05, which meansthat there was no significant difference or had the same effect between these concentrations.

However, when compared with positive and negative controls, it has a p value of <0.05, whichmeans that there is a significant difference or has a different effect. The use of safe doses of Moringa seed oil at several doses was measured on the liver function of experimental animals by measuring SGOT and SGPT. The results can be seen in table 4.

Table 4. The difference in the mean value of SGOT and SGPT

Group	n	SGOT (average ± SD)	SGPT (average ± SD)
Healthy (control)	4	40,87 ± 5,28	35,84 ± 12,40
Moringa Seed Oil (625 mg/kg)	4	56,88 ± 11,09	60,48 ± 16,40
Moringa Seed Oil (1250 mg/kg)	4	63,33 ± 15,19	66,97 ± 15,11
Moringa Seed Oil (2500 mg/kg)	4	79,36 ± 7,44	102,76 ± 8,62
Moringa Seed Oil (5000 mg/kg)	4	88,58 ± 36,00	132,01 ± 38,49
P		0.020	0,000

The highest average SGOT value was found in the use of Moringa seed oil at the highestdose of 5000 mg/kg with a value of 88.58 and the lowest at the smallest dose of 625 mg/kg with a value of 56.88. The average value of SGPT also showed that the highest value was at the highest dose of 5000 mg/kg with a value of 132.01 and the lowest value of 35.84 at a doseof 625 mg/kg. The mean values of SGOT control were 40.87 and 75%d 35.84 for SGPT.

One-way ANOVA statistical test on the SGOT and SGPT values obtained a significance value of 0.020 and 0.000 respectively ($p<0.05$), which means that there is a significant difference between the treatment groups. Furthermore, a further difference test (post-hoc) with the LSD test was carried out to determine the difference between the 2 variables. Inhealthy controls, when compared with a dose of 625 mg/kg and a dose of 1250 mg/kg, the p value> 0.05, which means that there is no significant difference or has the same effect. Meanwhile, the healthy control when compared with a dose of 2500 mg/kg and a dose of 5000mg/kg had a p value <0.05, which means that there is a significant difference.

The results of the LSD further difference test on SGPT showed that healthy controls were compared with a dose of 625 mg/kg and a dose of 1250 mg/kg had a p value> 0.05, whichmeans that there was no significant difference or had the same effect. Meanwhile, the healthycontrol when compared with a dose of 2500 mg/kg

and a dose of 5000 mg/kg had a p value <0.05, which means that there is a significant difference or has a different effect.

Table 4 shows that the use of a dose of 625 mg/kg – 1250 mg/kg there is no differencein the value of SGOT and SGPT with healthy controls, so that the use of a dose of no more than 1250 mg/kg is still in the safe zone, while the use of a dose of 2500 mg/kg – 5000 mg/kgshowed a significant difference in results with healthy controls, so it can be said that liver damage or toxicity has occurred at that dose.

DISCUSSION

The many benefits of the Moringa tree either as a source of nutrition or as a medicinal ingredient have made the plant dubbed the Amazing Tree and The Miracle Tree.[4] Various studies have reported the benefits of Moringa as an antimicrobial as that Moringa starts from the leaves, seeds , flowers, roots and skin of Moringa are proven to contain antimicrobials. The content of flavonoids, saponins, triterpenoids and tannins in MO leaves was reported to also inhibit the growth of *Streptococcus mutans*, *Malassezia furfur*.[4] Moringa leaf extract is also reported to have antibacterial activity to *Staphylococcus aureus* and *Corynebacterium pseudotuberculosis*.[6]

The results of this study indicate that moringa seed oil with a concentration of 75% hasa greater inhibitory ability than the concentration of 50% and 25%. The higher the concentration of Moringa seed oil, the better the level of antibacterial effectiveness of the material. This proof is because the greater the concentration of Moringa seed oil, the more active substances it contains, so that the antibacterial activity is also higher1. The results of thisstudy are in accordance with what was reported by Agung Suprihadi that the antibacterial activity of 75% Moringa seed oil (Moringa Oleifera L) was able to inhibit the growth of Staphylococcus aureus and Escherichia coli bacteria better than the concentrations of 25% and50%.[1]

Toxicity test in this study used mice as experimental animals by using indicators of liver disorders through SGOT and SGPT examinations. The liver is one of the toxic target organs, after going through the gastroentistil system, it will be brought to the hepatic portal vein. Impaired liver function is a biochemical evaluation, including SGOT and SGPT enzymes.The results showed that a safe dose

that did not cause liver function impairment was a dose of625 mg/kg and 1250 mg/kg, while a dose of 2500 mg/kg and a dose of 5000 mg/kg showed impaired liver function as indicated by significantly different SGOT and SGPT values. with healthy control mice.[21]

Liver damage can be assessed by an increase in the activity of SGPT and SGOT enzymes in the blood. Increased levels of SGPT can reach 20-100times above the highest normal limit if there is necrosis of liver cells caused by drugs and toxic substances. The occurrence of damage to the liver is caused by a disturbance in the balance ofions, fluids or metabolic products such as free fat or the breakdown products of membrane phospholipids.[22]

The increased activity of SGOT and SGPT enzymes was caused by increased oxidativestress so that the production of oxidants and free radicals increased in number. Meanwhile, thenumber of cellular antioxidants remains so that they are unable to fight free radicals or oxidantattacks.[21] The membrane lipid peroxidation reaction by free radicals hasa direct impact on cell membrane damage, including changing fluidity, cross linking, membrane structure and function. If these conditions occur continuously and extreme, it can eventually cause cell death. [22]

CONCLUSION

Moringa fruit seed oil *(Moringa Olefiera L)* at a concentration of 75% has the most effective inhibitory power to the growth of *Actinobacillus Actinomycetemcomitans* bacteria. The recommended safe dose of Moringa leaf seed oil is no more than 1250 mg/kg. Further research is needed related to the inhibition test of Moringa oleifera seed oil (Moringa Oleifera Lamk) to the bacteria *Actinobacillus Actinomycetemcomitans* and the toxicity test of Moringa oleifera seed oil *(Moringa Oleifera Lamk)* as an anti-inflammatory drug.

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DATA AVAILABILITY

Data sets related to this article will be available upon request to the corresponding author.

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