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Inhibitory Power of Jatropha Curcas Leaf Extract on the Growth of Escherichia coli, Staphylococcus aureus, and Candida albicans Fungi

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ABSTRACT

This study aims to determine the effect of Jatropha curcas leaf extract on the inhibition of Escherichia coli, Staphylococcus aureus, and Candida albicans. This study was an experimental study which was arranged using a completely randomized design (CRD) consisting of 5 treatments (A0 = control (without Jatropha curcas leaf extract), A1 = 5% concentration (5 grams of Jatropha curcas leaf extract/95 ml aquadest), A2 = 10% concentration (10 grams of Jatropha leaf extract/90 ml of aquadest), A3 = 20% concentration (20 grams of Jatropha leaf extract/80 ml of aquadest), A4 = 30% concentration (30 grams of Jatropha leaf extract /70 ml of distilled water) With 3 replications with an incubation period of 24 hours and 48 hours.The parameters observed in this study were the number of clear zones (inhibitory zones) in Escherichia coli, Staphylococcus aureus, and Candida albicans, using a sliding bar. The results showed that the inhibition of Jatropha leaf extract was statistically very significant with a BNT value of a 0.01 = 0.15 and 0.13 and optimum inhibition at a concentration of 20% for Escherichia coli, for Staphylococcus aureus a BNT value of a 0.01 = 0.39 and 0.33 for optimum inhibition at a concentration of 20%, BNT a value of 0.01 = 0.52 and 0.29 for Candida albicans and optimum inhibition at a concentration of 30%.

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1. INTRODUCTION

Indonesia is a country that is rich in abundant natural resources, has a wide variety of useful plants, both in the health and industrial sectors. In the field of health, plants are widely used as basic ingredients in the process of making medicines. Meanwhile, in the field of industry, plants are used as alternative energy.

Indonesian people are used to using traditional medicines which generally come from plants to prevent disease or treat disease. The application of these drugs can be by drinking water extract from the plant or by placing finely ground simplicia on the affected area of the body. The lack of scientific information regarding the components contained in plants for traditional medicine has resulted in the economic value of these plants being very low. In addition, its use, which usually uses arbitrary doses, can cause unwanted effects. This situation prompted the writer to research plants that are well known by the community as traditional medicines.

One of the plants that people often use as a medicinal plant is Jatropha curcas (Jatropha curcas). Jatropha curcas alias jatropha is widely known by rural communities. This plant with a

Chinese name, Ma feng shu, is usually planted as a house fence, in gardens, or in tombs. In Sumatra, this plant is named Nawaih nawas, costal distance in Sulawesi, Lulu nau (Nusa Tenggara), and Muun mav (Maluku). According to the stories of many people, during the Japanese colonial era, people were forced to plant jatropha. The oil is extracted for use as ship fuel and weapons lubricants.

In Indonesia, this plant usually grows wild or is planted by the population as a hedge plant, because it is called Jatropha curcas. However, several groups of our society use this plant as a traditional medicine to cure various kinds of ailments: swelling from being hit, sprains, broken bones, bleeding wounds, itching, eczema, fungus between the toes. Besides that, it is also used to prevent colds for babies, treat leprosy, gonorrhea, rheumatism, worm medicine, and also to nourish hair.

Escherichia coli, or E. coli for short, is one of the main species of gram-negative bacteria. In general, the bacteria discovered by Theodor Escherich live in feces, and can cause health problems in humans, such as diarrhea, vomiting and other digestive problems.

Staphylococci are gram-positive, spherical cells usually arranged in grape-like irregular clusters. Staphylococcus grows rapidly on several types of media and actively metabolizes it. Staphylococcus aureus is coagulase positive, which distinguishes it from other species. Staphylococcus aureus is the main pathogen in humans. Nearly everyone has had some sort of S. aureus infection in their lifetime, from a severe food poisoning or minor skin infection, to an infection that can't be cured.

Candida albicans is one of the many types of yeast whose name is well known in the field of microbiology. Yeast itself is a single-celled microscopic fungus that reproduces vegetatively by forming a kind of bud (budding). Several types of yeast, including Candida albicans, have dimorphic properties. When in nature it will grow as a mycelium and when in the body it will grow as a yeast which reproduces by forming budding.

Candida albicans can naturally be found in the mucous membranes of the mouth and also in the vagina. Its existence in the human body is known as "normal flora". As normal vaginal flora, Candida albicans does not live alone but lives together with several other microorganisms.

From a search of several literatures and preliminary tests that have been carried out by the author, this research was conducted which aims to determine the inhibition of Jatropha curcas leaf extract on the growth of E. coli, S. aureus and C. albicans. The research results are expected to provide additional information about the concentration level of Jatropha leaves in inhibiting the growth of bacteria and fungi, so that this plant can be further used as medicine.

2. METHOD

2.1 Types of Research

This research is an experimental research by making variations on a single independent variable and then measuring its effect on the dependent variable.

2.2 Research Variable

The variables in this study consist of two variables. The independent variable is the concentration of Jatropha curcas extract, while the dependent variable is the growth of Escherichia coli, Staphylococcus aureus, and Candida albicans.

2.3 Research Design

This research was conducted using a completely randomized design (CRD) which was given concentrations of Jatropha curcas leaf extract with the following treatments: A0 = negative control (without Jatropha curcas leaf extract); A1 = concentration of Jatropha curcas leaf extract 5 %; A2 = concentration of 10% Jatropha curcas leaf extract; A3 = concentration of Jatropha curcas leaf extract 20 %; A4 = 30% concentration of Jatropha curcas leaf extract. The test bacteria used were Escherichia coli denoted B, Staphylococcus aureus denoted C, and Candida albicans fungus denoted D, so that the table obtained combinations of treatments A0B, A1B, A2B, A3B, A4B, A0C, A1C, A2C, A3C, A4C, A0D, A1D, A2D, A3D, and A4D.

2.4 Sampling Locations

The sample used in this study were fresh Jatropha curcas leaves whose leaves were not too young taken from BTN Samata Permai, Gowa Regency.

2.5 Time and Place of Research

This research was conducted in August 2009, at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Makassar State University, Makassar city.

2.6 Tools and Materials

The tools used in this study were as follows: blender, analytical balance, distillation apparatus, 100 ml measuring flask, test tube, measuring cup, Erlenmeyer tube, stir bar, wire loops, syringe, petri dish, diluent bottle, incubator, autoclave, spirit lamp, oven, funnel, tweezers, spreading rod, caliper and hole punch.

The materials used in this study were as follows: Jatropha curcas leaves, pure cultures of Escherichia coli, Staphylococcus aureus and Candida albicans, NB medium, NA medium, PDA, aquadest, cotton cover, aluminum foil, n-hexane, paper disk, whatman, spirit, HVS paper, and filter paper.

2.7 Research Procedure

The research started from the sterilization of tools and materials (sterilization using an oven; sterilization using an autoclave; sterilization using a Bunsen), making medium (making nutrient agar (NA) medium), making potato dextrose agar (PDA) medium), aquadest sterilization, making Jatropha curcas leaf extract (Jatropha curcas), Microbial Rejuvenation Test, Microbial Suspension Preparation, Inhibition Test and Observation and Data Processing.

2.8 Data analysis

The data that has been obtained is then analyzed by means of a Completely Randomized Design (CRD) at a confidence level of † = 0.01 and if the test has a significant effect, then the test is continued with the Least Significant Difference (LSD) test).

3. RESULTS AND DISCUSSION

3.1 Research result

The microorganisms used in this study were Escherichia coli which represented Gram negative, Staphylococcus aureus which represented Gram positive and Candida albicans which represented the fungus group. These three microorganisms were grown in NA and PDA medium which were then given Jatropha leaf extract with various concentrations. In this research, the agar diffusion method was carried out using paper disks. After incubation for 24 hours and 48 hours, the results showed that Jatropha curcas leaf extract could inhibit the growth of Escherichia coli, Staphylococcus aureus and Candida albicans by forming an inhibition zone around the paper disk. In the control observation (A0) there was no zone of inhibition of bacterial growth. In the treatment (A1, A2, A3, and A4) there is a zone of inhibition around the paper disk. The bacteria used represent Gram positive and Gram negative as well as microorganisms representing fungi aiming to determine the effect of antimicrobial substances contained in Jatropha curcas leaf extract which has a narrow or broad spectrum.

The presence of inhibition zones on both types of bacteria indicates that the antimicrobial substance of Jatropha leaf extract has a broad spectrum to inhibit microbial growth. According to Bibiana, antimicrobials are divided into 2 based on their spectrum in inhibiting microbes, namely broad and narrow spectrum.

3.1.1 Effect of Jatropha leaf extract on the growth of Escherichia coli

The average diameter of the inhibition zone of Jatropha leaf extract in the control group and the treatment group can be seen in Table 1. In the table it can be seen that the average diameter of the inhibition of E. coli growth in the observations after the 24-hour incubation period is greater than the incubation period 48 hours.

This shows that the active substance contained in Jatropha leaf extract began to decrease with increasing incubation period so that E. coli was active and grew again around the inhibition zone. Based on the results of measuring the average diameter of the inhibition zone for the control group (A0) and the treatment group (A1, A2, A3 and A4), the inhibition of bacterial growth during the 24-hour and 48-hour incubation period was greater at a concentration of 20% (A3) and began seen a decrease in growth inhibition at a concentration of 30% (A4)

Table 1. The average diameter of the inhibition zone (mm) for the growth of E. coli by several concentrations of Jatropha leaf extract was incubation period of 24 hours and 48 hours.

Treatment	Average clear zone diameter (mm)*	
	24 hour incubation	48 hour incubation
(A0) control	0.00a	0.00a

(A1) 5%	3.83c	3.48c
(A2) 10%	4.00d	3,65d
(A3) 20%	4.50e	4,15e
(A4) 30%	3,25b	2.90b
	BNT a 0.01 = 0.15	BNT a 0.01 = 0.13

Description : - * = including the diameter of the paper disc
- The numbers followed by the same letter are not statistically significantly different at the BNT test level of a 0.01.

Table 1 shows that the incubation period of 24 hours and 48 hours of Jatropha leaf extract at each concentration was significantly different, the 5% concentration was significantly different from the 30% concentration, as well as the 20% and 10% concentrations. This means that during the incubation period the chemical compounds are still active in inhibiting microbes. The highest inhibition at the 24 and 48 hour incubation period was at an extract concentration of 20%.

3.1.2 Effect of castor leaf extract on the growth of Staphylococcus aureus

The average diameter of the zone of inhibition of S. aureus growth by Jatropha leaf extract can be seen in Table 2. The results of statistical analysis using the F a test of 0.01 showed that the administration of Jatropha leaf extract had a very significant effect on the growth of S. aureus after the incubation period 24 hours and 48 hours.

Table 2 also shows that the average diameter of the inhibition zone for S. aureus by Jatropha leaf extract in the control and treatment groups was greater as the concentration of the extract increased, but decreased at a concentration of 30%.

Table 2. Average diameter of growth inhibition zones of S. aureus by various concentrations of Jatropha leaf extract after 24 and 48 hours of incubation.

Treatment	Average clear zone diameter (mm)*	
	24 hour incubation	48 hour incubation
(Ao) control	0.00a	0.00a
(A1) 5%	2,30b	1.98b
(A2) 10%	3.00c	2.65c
(A3) 20%	4.00d	3,65d
(A4) 30%	2.50b	2,15b
	BNT a 0.01 = 0.39	BNT a 0.01 = 0.33

Description : - * = including the diameter of the paper disc
- The numbers followed by the same letter are not statistically significantly different at the BNT test level of a 0.01.

Based on the statistical value using the BNT test a 0.01 showed that at 24 hours and 48 hours of incubation the average diameter of the inhibition zone of Jatropha leaf extract at a concentration of 5% was not significantly different from a concentration of 30%. During the incubation period the significantly different concentrations were 5% with 10% and 20%. Based on table 2, the highest inhibition zone was Jatropha leaf extract with a concentration of 20%.

3.1.3 Effect of castor leaf extract on the growth of Candida albicans

The average diameter of the zone of inhibition of C. albicans growth by Jatropha leaf extract can be seen in Table 3. The results of statistical analysis using the F a test of 0.01 showed that the administration of Jatropha leaf extract had a very significant effect on the growth of C. albicans after the incubation period 24 hours and 48 hours.

Based on the results of measuring the average diameter of the inhibition zone for the control group (A0) and the treatment group (A1, A2, A3 and A4), the inhibition of fungal growth during the 24-hour and 48-hour incubation period was greater as the concentration of Jatropha leaf extract increased.

Table 3. The average diameter of the growth inhibition zones of C. albicans by various concentrations of Jatropha leaf extract after 24 and 48 hours of incubation.

Treatment	Average clear zone diameter (mm)*	
	24 hour incubation	48 hour incubation
(A0) control	0.00a	0.00a
(A1) 5%	2.05b	1.83b
(A2) 10%	3,17c	2.83c
(A3) 20%	3,25c	3.00c
(A4) 30%	4,30d	3,92d
	BNT a 0.01 = 0.52	BNT a 0.01 = 0.29

Description : - * = including the diameter of the paper disc

- The numbers followed by the same letter are not statistically significantly different at the BNT test level of a 0.01.

Based on statistical values using the BNT test a 0.01 showed that at 24 hours and 48 hours incubation the average diameter of the inhibition zone of Jatropha leaf extract at a concentration of 5% was significantly different from a concentration of 10% as well as at a concentration of 20% and 30%, but not significantly different between concentrations of 10% and 20%. Based on table 3, the zone of inhibition of fungal growth during the 24-hour and 48-hour incubation period was greater as the concentration of Jatropha leaf extract increased. The highest inhibition zone was Jatropha leaf extract with a concentration of 30%.

3.2 Discussion

The results of observations and measurements of the average inhibition zone showed that Jatropha leaf extract at concentrations of 5%, 10%, 20%, and 30% could inhibit the growth of E. coli bacteria both incubation periods of 24 hours and 48 hours. The results of statistical analysis showed that during the 24-hour incubation period the 5% concentration was significantly different from the 10%, 20% and 30% concentrations. In the growth of these bacteria, concentrations were not found which were not significantly different, all concentrations were significantly different. This means that the active ingredient contained in Jatropha leaf extract inhibited the 24-hour incubation period, while the 5% concentration was still significantly different from the 10%, 20% and 30% concentrations during the 48-hour incubation period.

In the growth of these bacteria, concentrations were not found which were not significantly different, all concentrations were significantly different. Statistically, these data illustrate that the active ingredient contained in Jatropha leaf extract still inhibits the 48-hour incubation period even though it is bacteriostatic.

According to Jawetz (1992) 79, inhibited bacterial growth or bacterial death due to an antibacterial substance can be caused by inhibition of cell wall synthesis, inhibition of cell membrane function, inhibition of protein synthesis, or inhibition of nucleic acid synthesis.

The results of the average inhibition zone measurements showed that Jatropha leaf extract at concentrations of 5%, 10%, 20% and 30% could inhibit the growth of S. aureus bacteria both incubation periods of 24 hours and 48 hours. However, the results of statistical analysis showed that during the 24-hour incubation period, the Jatropha leaf extract concentration of 5% was not significantly different from the 30% concentration. . During this incubation period the concentrations were significantly different, namely 5% with 10% and 10% with 20% as well as 20% and 30%. whereas in the 48-hour incubation period the 5% concentration was not significantly different from the 30% concentration, this means that during the 24-hour incubation period the active chemical ingredients at these concentrations have the same effect in inhibiting the growth of S. aureus. During this incubation period the concentrations were significantly different, namely 5% with 10% and 10% with 20% as well as 20% and 30%. This shows that the concentration of 20% in the 48-hour incubation period still has an effect on inhibiting the growth of S. aureus.

The results of the average inhibition zone measurements showed that Jatropha leaf extract at concentrations of 5%, 10%, 20% and 30% could inhibit the growth of the fungus C. albicans in both 24 and 48 hour incubation periods. This shows that Jatropha curcas leaf extract at a concentration of 5% can inhibit the growth of C. albicans and further inhibit with increasing concentration of the extract. However, the results of statistical analysis showed that during the 24-hour incubation period, Jatropha leaf extract concentration of 10% was not significantly different from that of 20%. However, at a concentration of 5% it was significantly different from 10% as well as at concentrations of 20% and 30%. Whereas in 48 hours the 10% concentration was not significantly different from the 20% concentration, the 5% concentration was significantly different from 10%, 20% and 30%.

In the treatment group the highest concentration of Jatropha leaf extract inhibited the growth of C. albicans based on the analysis of BNT □ 0.01 was a concentration of 30% both at 24 hours and 48 hours of incubation. This is due to the higher concentration of Jatropha leaf extract, the more inhibitory substances (active ingredients) contained in the extract solution, but this only applies to the fungus group, while the bacteria group in the treatment group, the highest concentration of Jatropha leaf extract inhibits the growth of E. coli and S. aureus based on the analysis of the BNT test a 0.01 is a concentration of 20% both at 24 hours and 48 hours of incubation and begins to decrease at a concentration of 30%.

The ability of Jatropha leaf extract to inhibit the growth of S. aureus, E. coli and C. albicans is due to the fact that Jatropha leaves extract contains anti-bacterial substances. Jatropha leaf extract contains triacontranol, alpha-amirin, kaempesterol, beta-sitosterol, 7-keto-betasitosterol, stigmasterol, stigmas-5-en-3-beta-7-alfadiol, viteksin, isovitexin, and cyanide acid (HCN). In addition, it also contains saponins, flavonoids, tannins and polyphenolic compounds. 80 Some of these chemical compounds are thought to be inhibitors in inhibiting microbial growth. Flavonoid compounds are the largest group of phenolic compounds found in nature. These compounds are red, purple, and blue dyes. And as a yellow dye found in plants. Most of the natural flavonoid compounds are found in the form of glycosides. Glycosides are a combination of a sugar and an alcohol which are linked together through glycosidic bonds81. Flavonoids are known to be synthesized by plants in response to microbial infection so it is not surprising that they are effective in vitro against a number of microorganisms.

Polyphenols are compounds that come from nature and are found in plants. Its main use is as a powerful natural antioxidant. These antioxidant compounds eliminate free radicals, namely unstable molecules which are the main cause of aging and disease in humans and plants. These free radicals continuously attack the body and are metabolic products that produce oxidation processes. Oxidation can harm healthy cells in the body, and is one of the triggers for cancer, heart disease and stroke. Polyphenols can also reduce abnormal cells and inflammation, as well as restore these abnormal cells to be healthy.

Based on the results of the study, the average diameter of the inhibition zone for all treatment groups decreased after an incubation period of 48 hours. The ability of Jatropha leaf extract to inhibit the growth of S. aureus, E. coli and C. albicans can be classified as bacteriostatic compounds. Where the inhibition area (clear zone) that is formed again is slowly overgrown with bacteria. This is consistent with the statement that if the inhibition area remains clear for up to 48 hours, it indicates that the antibiotic or antimicrobial used is classified as bactericidal, while if during 24 hours of incubation the inhibition area is clear and then becomes cloudy after 48 hours incubation, it indicates that the antimicrobial used is bacteriostatic.

has decreased its working power, so that S. aureus, E. coli and C. albicans which are on the other side of the paper disk began to be able to adapt and neutralize the anti-bacterial substance, then bacteria began to grow around the clear area.

Materials used such as Jatropha leaf extract to interfere with the metabolism of microbial inhibition are referred to as antimicrobials, so they are known as antibacterial, antibacterial or fungicide and if they only inhibit growth then they are called bacteriostatic or fungistatic. A bactericidal is a substance which kills the vegetative forms of bacteria; Likewise, the terms fungicide, viricide and sporicide are defined respectively as agents that kill fungi, viruses and spores. While bacteriostatic is a condition that inhibits the growth of Staphylococcus aureus and Echerichia coli bacteria, likewise fungistatic describes the work of a material that stops the growth of the Candida albicans fungus.

4. CONCLUSION

Based on the results of research on the inhibition of Jatropha leaf extract on the growth of Escherichia coli, Staphylococcus aureus and Candida albicans, it can be concluded that: Jatropha leaves can inhibit the growth of Escherichia coli, Staphylococcus aureus and Candida albicans at concentrations of 5%, 10%, 20% and 30%. In the growth of E. coli and S. aureus began to experience a decrease in inhibition at a concentration of 30%. Whereas in the growth of C. albicans the ability of inhibition continues to increase along with increasing concentration. The concentration that provided the greatest inhibition of the growth of E. coli and S. aureus was 20%, while for C. albicans the greatest inhibition was 30%. On the growth of S. aureus at 5% concentration was not significantly different from 30% concentration and C. albicans growth at 10% concentration was not significantly different from 20% concentration. However, in contrast to E. coli and S. aureus, the concentrations of all E. coli growth were significantly different.

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It is recommended that further research be carried out by comparing commercial antibiotic drugs and further research on what chemical ingredients provide inhibition and are toxic to bacteria and the effects these substances cause if consumed by humans.

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