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Comparison of *Jatropha curcas* (Linn) Leaf Extract and *Nystatin* Effectivity on *Candida albicans* Growth Inhibition

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ABSTRACT

Fungal infection is a health problem that is faced around the world and arose in developing countries. Oral candidiasis is a fungal infection that affects oral mucosa. Lesion of oral candidiasis is caused by *Candida albicans,* which is considered as an opportunistic infection in humans. Antifungal therapy is necessary to inhibit the growth of these fungi. Traditional medicine becomes the alternative therapy to face this. One of Traditional therapy is *Jatropha curcas* (Linn) leaf. *Jatropha curcas* (Linn) leaf contains natural substances like flavonoid, tannin, and saponin that function as Antifungal. To measure the effectivity comparison between 50% concentration of Jatropha curcas (Linn) leaf extract with 100.000 IU of nystatin to the growth of *Candida C. albicans.* The research method is a laboratory experiment. The design of this study was posttest only control group design with agar / Kirby Bauer diffusion method. In this research, nine repetitions were carried out on three inhibitory treatment groups using 50% concentration of Jatropha curcas (Linn) leaf extract, 100.000 IU nystatin, and control (sterile aquades). The instrument in this research is a caliper with a millimeters (mm) unit. Shapiro wilk normality test showed p>0.05 to all groups. It shows that all groups were normally distributed. So, it fulfills the parametric test requirement, namely one-way ANOVA. This One-Way ANOVA parametric test indicated whether or not there is differentiation among the individuals that given different treatments. From the test, we can conclude that there is a different inhibitory test between 50% concentration of *Jatropha curcas* (Linn) leaf extract with nystatin to the growth of C. *albicans*. Nystatin is more effective rather than Jatropha curcas (Linn) leaf extract. But *Jatropha curcas* (Linn) leaf extract has the potential to inhibit the growth of *C. albicans* so that it can be taken into consideration as an alternative drug for the growth of the *Candida C. albicans.*

Keywords: *Jatropha curcas* (Linn) leaf extract, nystatin, the inhibition of the fungi, *Candida albicans*

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INTRODUCTION

Indonesia is a country that has a biodiversity that ranks second largest in the world after Brazil. Most of the diversity is potentially a medicinal plant. One of these commonly used plants as a medicine by the community is the distance of the fence (*Jatropha curcas L.*). Jatropha is a biodiesel plant that can grow in several tropical areas, such as in Indonesia. Some parts of this plant can be utilized as a traditional medicinal plant.[1]

Jatropha plant is one of the natural ingredients that is often used as a medicinal ingredient because it has many benefits to treat a variety of diseases, both stem, leaf, fruit, and its thrill. The leaves of the Jatropha plant are an easy solution because these plants are found in the yard. The leaves of Jatropha plant use communities to treat acute pulpitis, canker sores, and oral candidiasis caused by Candida fungi.[2]

Oral candidiasis is one of the fungal infections concerning oral mucosa. These lesions are caused by the fungus *Candida C. albicans*, which causes opportunistic infections in humans. Candidiasis in systemic diseases caused an increase in the mortality rate of about 71-79%.[3] Therefore, it is essential to know the treatment of oral Candidiasis by inhibiting the fungus C. albicans.

The active compound leaves Jatropha that can inhibit the growth of C. C. are flavonoids, saponins, and tannins. The mechanism of action of flavonoids and tannins is utilizing protein denaturation, disrupting the lipid layer, and resulting in deterioration of cell walls. At the same time, saponin compounds can lower the surface tension resulting in a rise in permeability or Cell leaks and causes intracellular compounds to come out.[4] These active substances could be extracted from the leaf of the fence distance.[5]

The method above is supported by some previous research. Parka Agnita et al., comparing the power of extract and the decoction of leaf-spacing (*Jatropha curcas L.*)
to the growth of *C. albicans* obtained the results that the leaf extract distance Fence more effectively inhibit than decoction of Jatropha.[1] Sukmawati et al., reporting the antimicrobial effect of the Fence range extract (Jatropha curcas L) in inhibiting growth *C. albicans*. She reported results that the Minimum Inhibitory Concentration (MIC) achieved at a 25% concentration with a 12.08 mm barrier zone and a Minimum Fungicidal Concentration (MFC) at a 75% concentration with a 16.72 mm barrier zone against the fungus *C. albicans*.[4]

In addition to using natural materials such as distance plants, not a few people also use chemicals for treatment. One chemical drug that is often used to treat the fungus *C. albicans* is Nystatin. Nystatin is an antifungal drug used to treat fungal infections of *b* on the oral cavity, throat, intestines, and vagina. Nystatin has antifungal activity via the binding of sterols (especially ergosterol) in the fungi's cell membrane. The result of this bonding makes the membrane unable to function again as a selective barrier, and potassium and other cell components will be lost.[6] Nystatin's side effects are nausea, vomiting, and diarrhea, which may irritate the mucosa when administered at high doses. Another side effect of Nystatin will harm health when consumed continuously.[7]

This study then aimed to compare the effectiveness of leaf extract fence (Jatropha curcas L) and Nystatin toward inhibiting the growth of the C. *albicans*.

METHOD

The test materials used in this study are handscoen, masks, fungus *C. albicans*, cotton, alcohol, leaf fence distance (Jatropha curcas Linn), Nystatin, aquades sterile, ethanol 96%, blank disks (paper discs), aluminum foil and the media Sabouraud Dextrose Agar (SDA).

This research is an experimental research laboratory with the draft posttest only control group. This study was conducted from May to June 2019. It was conducted at the Pharmaceutical Biology Laboratory of the Department of Pharmacy UIN Alauddin Makassar for the manufacture of extracts and laboratory microbiology of the Faculty of Medicine Hasanuddin University to test the mushroom C*. albicans.*

The samples used in the study consisted of 3 treatment groups, namely leaf extract of Jatropha curcas Linn, a concentration of 50% as a treatment group of 1, Nystatin as a treatment group 2, and aquadest steril as a negative control. The power test method uses the diffusion method of the discs. Each treatment group was repeated nine times.

Jatropha curcas L commonly used as a plant fence. Prepared leaf fence (Jatropha curcas Linn) is still young with the criterion: it has a length of 10-15 cm, a width of 5-10 cm, located 3-4 leaves from the tip of the chain. The leaf retrieval was performed in the morning between 07.00-11.00 am. This time is considered a peak active metabolism and will increase a chance to get large enough substance. Subsequently, the dry sorting performed to separate the damaged leaves before drying for three days.

Jatropha leaf extract using maceration method implemented maceration methods, and performed in pharmacy biology of the Department of Pharmacy UIN Alauddin Makassar. The freshly made Jatropha curcas Linn (400 grams) is dried. The dry fence leaves were ground to powder and obtained simplisia as much as 210 grams. Then it is macerated by using the simplisia of leaf fence distance into the jars and dissolved using ethanol solvent 96% for three days with a ratio of 1:10. After that, it was filtered using a filter paper to separate the pulp with a solvent containing the active compounds. Active compounds separation was carried out using a rotary evaporator so that the active compounds were obtained in the form of concentrated extracts. Jatropha leaf extract was deposited into a sterile glass sealed bottle and stored in the refrigerator.

After 100% extract obtained 100%, 50% dilution was gained by adding sterile aquades. The suspension of *C. albicans* measured its absorption by the standard Mc Farland 0.5 using Densichek. Media Saburoud Dextrose Agar (SDA) warm poured on the petri dish then inoculated. *C. albicans* and awaited to become dense. The barrier of the fence (Jatropha curcas Linn) on the growth of the fungus *C. albicans* was done by the method of diffusion discs. The initial stage is sterile cotton dipped into the test mushroom suspension. Then, it rotated several times and pressed the tube over the liquid to remove excessive inoculum. The entire media surface is to be inoculated with the test mushroom by repeating the cotton-filled mushroom suspension by streaking on the whole surface to Bolden with the zig-zag movement until the media is closed.

Pre-soaked disc-paper in the sample solution of 50% leaf extract, Nystatin 100,000 IU, and sterile aquades as much as 1 ml for 15 minutes were placed on the surface of the media to which it has been inoculated. The test fungus uses sterile tweezers. After that, the new individual discs were placed on the media. The Media has contained a test fungus then incubated at 370C for 2x24 hours. The Data obtained processed and analyzed statistically.

RESULTS

Our results showed that on a concentration of 50%, a visible clear area zone (dark to slight green) that is not overgrown by *C. albicans*, while in Nystatin is visible in the area of a clear zone in the vicinity of the eastern pit that is not Overgrown by *C. albicans.* Sterile Aquades (K-) there is no barrier zone. The observed barrier zone observation Data can be seen in table 1.

Furthermore, the data obtained by statistical analysis test conducted to know the significance of the influence of the resistance between nystatin and 50% fence leaf extract against *C. albicans*, before statistically tested, test the normality of data to know the distribution of normal data or not. The test data used is a Shapiro test – Wilk obtained p nystatin value = 0.373 and p value of leaf extract concentration fence range 50% = 0.722 means normal distribution data (p>0.05). It shows that all distributed groups are normal. Thus a qualified parametric test is One Way Anova.

The parametric test of One Way Anova indicates the absence of difference between the individual treatment of one with other treatment individuals. The Data obtained from the statistical test of one way ANOVA aims to determine the difference in the barrier zone of each type of intervention. Because the value p = 0.000 < 0.05 then Ho is rejected. This means there is a meaningful difference in each type of intervention of nystatin concentration and leaf extract of 50%. It can be concluded that at least 1 group of hating power treatments affect the growth of *C. albicans* fungus. Besides, the value of P-value obtained is smaller than 0.05 so that the resistance test affecting the growth of *C. albicans.*

DISCUSSION

The main subjects in this study were the fence distance leaves (*Jatropha curcas* Linn) and Nystatin. The leaves are one part of the plant that many contain compounds of secondary metabolites which are active compounds. Research conducted by Sharma et al suggests that the ethanol extract from *Jatropha curcas L*inn contains alkaloid substances, saponin, tannins, terpenoids, steroids, glycosides, phenol compounds, and flavonoids. The research conducted by Nwokocha et al, shows that among the four species *Jatropha (J. Curcas, J. Podagrica, J. Multifida, and J. Gossypifolia*), *Jatropha Curcas* which have the highest content of tannins and saponins. The concentration of tannins on the leaf observed was 7.43% for *J. Curcas*, 6.79% for *J. Podagrica*, 5.16% for *J. Dentaria* and 5.14% for *J. Gossypifolia*. Meanwhile, saponins concentration on the leaves of the four species *Jatropha is J. Curcas* (4.89%) *J. Gossypifolia* (4.15%), *J. Dentaria* (3.15%), and *J. Podagrica* (3.15%).[8]

Chemical compounds in *Jatropha curcas* Linn are antimicrobial, flavonoids, tannins and saponins. According to Vijayalakshmi, the flavonoids compounds and tannins have been shown to inhibit fungal growth.[4] substances Most major that can inhibit the growth of *C. albicans* is tannins. Tannin is an organic compound

consisting of a mixture of complex polyphenols compounds, constructed from C, H, O. Tannin elements can function to damage the main components of the cell wall compilers consisting of chitin, adhesion and lipids because they have antiseptic properties, Bacteriostatic and fungistatic.[9] The antifungal mechanisms belonging to tannins are the ability to inhibit the synthesis of the khitin used for the formation of cell walls in fungi and damage the cell membranes so that mushroom growth is hindered.[6]

Saponin is as a polar-shaped surfactant will lower the surface tension of the sterol membrane from the cell wall of *C. albicans*, resulting in impaired membrane permeability resulting in the inclusion of materials or substances required can be interrupted. The antifungal properties of saponins stem from the formation of a compound bond of polar saponins with lipoprotein and a group bond of non polar saponins with plasma membrane fat of mushroom cells. The bond causes the fat to rupture and a filling and disruption of the permeability of fungal cell membranes. It causes the exact cells of *C. albicans* and finally the death of mushroom cells.[6]

The mechanism of action of flavonoids in inhibiting the growth of fungi is to form complex compounds with extracellular proteins. Flavonoids are easily dissolved substances that can damage the cell membranes of the fungi and are followed by the discharge of intracellular compounds and work by means of protein denaturation, disrupting the lipid layer, and resulting in damage to the cell walls. It can occur because the flavonoids are lipophilic so that it will bind to phospholipid-phospholipids on the cell membrane of fungi and interfere with cell permeability.[1]

Prajitno explains that phenol compounds and flavonoids are one of the antimicrobial that works by interfering with the function of cytoplasmic membranes. Volk & Wheeler explains the damage to the membrane allowing important inorganic ions, nucleotides, coenzymes and amino acids to seep out the cells. In addition, it can prevent the inclusion of important materials into cells because the cytoplasmic membrane also controls the active carriage into the cell. Thus resulting in cell death or cell inability to grow. Prajitno, explains that H + ions of the phenol compounds and their derivatives (flavonoids, tannins) will attack polar groups (phosphate clusters) so that the phospholipids molecules on the microbial cell walls will break down into glycerol, carboxylic acids and phosphoric acid. In such circumstances, the phospholipid is incapable of maintaining the form of the cytoplasmic membrane, consequently the cytoplasmic membrane will leak and the microbes will experience a growth obstacle of even death.[10]

In addition to working by elaborating phospholipids on cell membranes, phenols also work by denatured protein. The protein denaturation process is a reactive carbonyl group that reacts with the amino group of proteins. So that the proteins are denatured, which means that there is a change in the composition of the polypeptide chain that causes the protein to clot so that the solubility becomes low. In such circumstances the proteins do not work anymore and When such conditions continue to cause death in bacteria and fungi.[10]

The assay activity of *Jatropha curcas L*inn to the fungus growth of *C. albicans* shows different results against each given treatment. This suggests that nystatin and the concentration of extracts indicate the presence of different antifungal activity. Equivalent to some previous research, in research conducted by Sukmawati et al, obtained the average concentration of the barrier zone formed at each concentration is 12.08 mm for a concentration of 25%; 14.98 mm for a concentration of 50%; 16.72 mm for concentrations of 75% and 18.45 mm for the concentration of 100% with the Sumuran method. All extracts are tested able to inhibit the growth of the fungus *C. albicans* indicated by the formation of a subterrent diameter around the Sumuran.[4]

Different from the research conducted. At *Jatropha curcas L*inn concentrations 50% of the concentration was obtained on average the result of the hate zone of about 8.4 mm with a disc diffusion method 9 times the repetition. Many factors affect the size difference of the barriers that are obtained. In accordance with previous research by Soemarno, stated that there are several factors affecting the broad size of the inhibitory zone i.e. (1) Suspension turbidity in accordance with McFarland (2) incubation temperature, to obtain optimal growth, Incubation is done at 370C, because sometimes there is a fungus that is less fertile growth, (3) Incubation time 2x24 hours (4) in order to-agar, thickness to be around 4 mm, if less than the thickness then the diffusion of the drug will be faster, and if More than that thickness, then the diffusion of the drug will be slower, and (5) the distance between the holes is recommended at least 15 mm, to avoid the occurrence of overlapping zone of obstacles.[4]

In addition, the selection of the Extract method also greatly affects the area of the barrier zone. Some commonly used methods are the pitting method and the diffusion method of the discs. The pitting method is to make holes in the media so that they have been inoculated using a special tool to punch them. The number and layout of the holes is adjusted to the research purpose, then the holes are injected with the extracts to be tested. After incubation, mushroom growth is observed to see there is no area of obstacles around the hole. Different with the method of diffusion discs. Diffusion discs IE using disc paper that has been soaked extract is placed on the surface of the media so that it has inoculated test mushrooms using sterile tweezers. Of these two methods there is a discrepancy in the formed zone where the pitting method results in a barrier zone diameter greater than that of the disc diffusion method. Based on the research of Susanna DKK, the more extracts are incorporated, the more the compounds of secondary compounds are contained in them, thus being able to inhibit the growth of fungi characterized by the formation of the inhibitory zone diameter. According to the research of the Novel Kojong et al, the thing that occurs in the pitting method is the osmolarity process of the concentration of extracts from the leaf jatropha so that osmolarity occurs More thorough and more homogeneous as well as the concentration of higher-produced extracts and stronger to inhibit the fungus *C. albicans* in addition, according to the Goddess et al, the Rise and fall of the inhibited zone can also be caused by the nature of Solubility of active substances on the extract and the speed difference of diffusion in the media agar.[11,12]

In this study, Nystatin was chosen as the subject of research because Nystatin is a major drug group in the fight against *C. albicans.* Nystatin has antifungi activity by means of binding sterols (especially ergosterol) in the cell

membrane of fungi. The result of this bonding makes the membrane unable to function again as a selective barrier, and potassium and other cell components will be lost. The main action of Nystatin is against *Candida* spp.[6]

The concentration treatment of *Jatropha curcas L*inn and Nystatin in research shows that there is a difference in the inhibitory zone, meaning that Nystatin is more effective than the *Jatropha curcas L*inn extract in inhibiting the growth of the fungus *Candida* C. albicans. In the research done obtained results at a concentration of 50% inhibition of *Jatropha curcas L*inn extract of 8.44 mm Hence the response of the growth barrier is moderate, while in nystatin 100,000 IU amounted to 14.7 mm the response of the growth is strong. The research is according to the opinions of Ardiansyah, et al. Which states that there are several classifications of antimicrobial forces, namely (1) the area of 20 mm or more, means very strong, (2) the area of 10-20 mm, which means strong, (3) the area of 5-10 mm (4) of the region is a 5 mm barrier, weak. 4 Explanation above shows that the results of this research can prove the hypothesis that has been taken is not in accordance with the results of the study, because the results obtained that nystatin more effective than with *Jatropha curcas L*inn extract, but extracts *Jatropha curcas L*inn has a resistance to the growth of fungus *C. albicans*, so it is expected that it can be used as an alternative remedy for the growth of fungus*. C. albicans*.

CONCLUSION

Hedge leaf extract (*Jatropha curcas L*inn) has potential inhibition of *C. albicans* growth suggesting its capability to use alternative medicine consideration for the growth of the fungus *C. albicans*.

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Table 1. The results of a barrier zone diameter measurement

No.	Treatment	Number of replication (9X)	Mean (mm)
1	Nystatin	132.4 mm	14.7
2	50% *Jatropha curcas* (Linn) leaf extract.	78.9 mm	8.7
3	Negative control	52.92 mm	5.88