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ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF VARIOUS BREAD FRUIT LEAVES (ARTOCARPUS ALTILIS (PARKINSON) FOSBERG)

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ABSTRACT: Breadfruit (Artocarpus altilis (Parkinson) Fosberg) empirically has been used in certain communities in Indonesia as a traditional medicine. Biosynthesis process can change colour leaves of breadfruit from green leaves (GL), yellow leaves (YL), fallen yellow (FY), fallen dry (FD), and the process fermentation of green leaves become fermented green (FG). The purpose of this study was to determine whether the methanol extract of breadfruit leaves variations have activity to Escherichia coli, Staphylococcus epidermidis, Propionibacterium acnes and Candida albicans and determination of Minimum Inhibitory Concentration (MIC). Simplicia was processing maseration used methanol, phytochemical screening on the fifth methanol extracts, botanicals characterization, activity coli, test against to Escherichia Staphylococcus epidermidis, *Propionibacterium acnes*, and activity test against to *Candida albicans*. Active extracts determined Minimum Inhibitory Content (MIC) and TLC. MIC used agar diffusion method. The test results showed antibacterial and antifungal activity variations of fermented green gives effective results than other breadfruit leaf variations. MIC values for the bacterium Escherichia coli, Staphylococcus epidermidis, Propionibacterium acnes, and the Fungi Candida albicans in a row by fermentated green extract 20mg/mL; 47,5mg/mL; 15mg/mL; and 475mg/mL. Test results on a TLC bioautography ethyl acetate fraction with the developer n-Hexane: Ethyl Acetate (7:3) provide clear zone at Rf 0.08; 0.13; 0.23; 0.28; 0.32; 0.38; 0.42; 0.50; 0.55; 0.60; and 0.63.

INTRODUCTION: Indonesia has a high biodiversity so as to produce new compounds that have medicinal properties. Breadfruit (*Artocarpus altilis* (Parkinson) Fosberg) is a plant that is easy to obtain and empirically been used by the people of Indonesia for traditional medicine. Almost all parts of this plant has been used as medicine (leaves, fruit, root bark and sap).

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The root bark breadfruit efficacious as antiplatelet ¹, activity as antifungal and antioxidant ², the resin efficacious as a cure diarrhea and dysentery ³ the leaves are useful as antihypertensive, antidiabetic, antioxidant, and anticancer. ^{3, 4, 5, 6} Secondary metabolites were isolated from *Artocarpus* genus consisting of terpenoids, flavonoids, stilbenoid, arilbenzofuran, and neolignan. A group of flavonoids are compounds most commonly found of *Artocarpus*.⁷

Biosynthesis process can cause discoloration of the breadfruit leaves of fresh green leaves (GL), yellow leaves (YL), fallen yellow (FL), fallen dry (FD), and the fermentation process fresh green leaves become fermented green leaves (FG). Additional processing of fresh green leaves become green leaf fermentation been with this process which was originally expected to be a leaf of fresh green leaves, will turn in to yellow leaves, because the fermentation process can accelerate the aging of leaves. Leaf fermentation is done by stacking leaves during 5 days after the process of picking and washing.³

Based on research conducted by ⁸ comparing the levels of total flavonoids of the fifth methanol extract of leaves of breadfruit as correlation with antioxidant activity with results breadfruit leaves dried had higher levels of total flavonoids supreme and there is no direct correlation between the levels of total flavonoids of antioxidant activity of the fifth extract methanol breadfruit leaves.

Based on the above background, it conducted research on activity assay variation breadfruit leaf consisting of green leaf, yellow leaf, fallen yellow leaf, fallen dry leaf and fermented green leaf against *Staphylococcus epidermidis, Eschericia coli,* and *Propionibacterium acnes* and the Fungi *Candida albicans.*

2. Methodology:

2.1 Tools: The tools used in this study, among others, rotary evaporator (IKA®), water bath (Nuohai), autoclave, Laminar Air Flow (LAF), perforator size 6 mm, incubator (Jenaco®), flask, beaker, cup Petri, rod stirrer, pipette volume, micro pipette (Socorex), erlenmeyer, cuvette, measuring cups, analytical balance (Henherr®) as well as tools used in the process of screening and characterization of such porcelain cup, oven (Memmert), furnace (Branstead Thermolyne), desiccator, wood clamps, tube racks, drip plate, spatula, burning spirits.

2.2 Material: The plant material used in this study is the variations breadfruit leaves. Other ingredients needed are distilled water, methanol, ethyl acetate, n-hexane, screening reagents, culture media: Nutrient Agar and Potato Dextrose Agar. The bacteria used are *Propioni bacterium acnes*, *Staphylococcus epidermidis*, *Escherichia coli*, and the Fungi *Candida albicans*. Positive control used clindamycin, chloramphenicol, and Nystatin.

2.3 Preparation of Material: Material in the form of variations of breadfruit leaves freshly collected and cleaned with water. Then select, chopped and dried by heating in the sun. For green breadfruit leaf fermentation. Fresh green breadfruit leaves are cleaned with water and then dried. Leaves stacked \pm 30 leaves during 5 days (the upper leaves are not used) and then dried by lining up the leaves.

2.4 Characterization and Screening Crude Extract **Phytochemicals:** Phytochemical of screening performed on variations of the fresh green leaves of breadfruit, green fermentation, stuck yellow, yellow fall and tumble drier to determine the content of secondary metabolites. In general, compounds tested include testing of alkaloids, flavonoids. tannins, phenolics, triterpenoids, steroids, quinones, monoterpenes, saponins. Simplicia sesquiterpene. and characterization includes determination of the ash content, content of ethanol soluble extract, and levels of water soluble extract.

2.5 Extraction: Powder simplicia of variation of of breadfruit, fresh green leaves green fermentation, stuck yellow, yellow dry fall and fall respectively in maceration using methanol for 3 days, then each filtered using filter paper. Each filtrate of each variation simplisia leaves separated from the solvent using a rotary evaporator at a temperature of 45°C, in order to obtain a thick extract variation of fresh green leaves of breadfruit, fermented green, yellow leaves, fallen yellow and fallen dry.

2.6 Testing Antibacterial Activity: Antibacterial against Propionibacterium activity acnes. Staphylococcus epidermidis and Escherichia coli were tested using agar diffusion method. In the media Nutrient Agar (NA) of 15 mL put 0.1 mL bacterial suspension measured transmittannya, then homogenized and left to solidify. After it was made 6 holes using the perforator diameter of 6 mm. Every hole is filled sample extract that has been diluted with a solvent 5% Tween 80 at a concentration of 10%, 5%, 2.5%, 1.25% 50 mL using a micropipette. The test is performed triplo, then incubated at 37°C for 18-24 hours. After that the inhibition area diameter is measured using a caliper.

2.7 Testing Antifungal Activity: Antifungal activity against Candida albicans Fungi were tested using agar diffusion method. In the media Dexto Potato Agar (PDA) included as many as 15 mL of bacterial suspension 0.1mL measured transmittannya, then homogenized and left to solidify. After it was made 6 holes using the perforator diameter of 6 mm. Every hole is filled sample extract that has been diluted with a solvent 5% Tween 80 at a concentration of 100%, 80%, 50%, 30% 50 mL using a micropipette. The test is performed triplo, then incubated at 25°C for 24 hours. After that the inhibition area diameter is measured using a caliper.

2.8 Fractionation Breadfruit Leaf Extract Variation Methanol: Variations methanol extract of leaves of breadfruit most effectively provide antibacterial and antifungal activities fractionated using Liquid-Liquid Extraction method (ECC) using a solvent mixture of n-hexane: Ethyl Acetate and thickened the fraction.

2.9 Thin Layer Chromatography (TLC): Each sample was spotted on silica gel plates. Developers used is a ratio of solvent n-hexane: ethyl acetate. After the sample spotted on the plates silica gel chromatography inserted into the chamber and then the chamber was closed chromatography. After that, awaited and observed to mobile phase and rise to the surface of the plate boundary. Spotting the rise observed by spraying the TLC plates using chromogenic reagent and observed under ultra violet light with a wavelength of 254 and 366 nm, and its Rf is calculated by measuring the distance soluble compounds.

2.10 Determination of Minimum Inhibitory concentration (MIC):

2.10.1 Bacteria: MIC most active extracts were tested using the agar diffusion method. In the media Nutrient Agar (NA) of 15 mL put 0.1 mL bacterial suspension measured transmittannya, then homogenized and left to solidify. After it was made 6 holes using the perforator diameter of 6 mm. Every hole is filled sample extract that has been diluted with a solvent 5% Tween 80 at a concentration of derivative of the concentration of extract that still provide antibacterial activity of 50 mL using a micropipette. The test is performed triplo, then incubated at 37° C for 18-24 hours.

After that the inhibition area diameter is measured using a caliper.

2.10.2 Fungi: MIC most active extracts were tested using the agar diffusion method. In the media Dexto Potato Agar (PDA) included as many as 15 mL of 0.1 mL bacterial suspension measured the transmittance and then homogenized and left to solidify. After it was made 6 holes using the perforator diameter of 6 mm. Every hole is filled sample extract that has been diluted with a solvent 5% Tween 80 at a concentration of extract that still delivers the antifungal activity of 50 mL using a micropipette. The test is performed triplo, then incubated at 25°C for 24 hours. After that the inhibition area diameter is measured using a caliper.

2.11 Bioautography: TLC plates are placed on the surface of the media Nutrient Agar (NA) for bacterial and surface-handed Potato Agar (PDA) for fungi that have been inoculated with the microorganisms analyzed.

2.12 Analysis of Data: Data obtained on the test antibacterial and antifungal activities, analysis using Analysis of Variance (ANOVA) followed by Tukey's test using SPSS version 16.

RESULT AND DISCUSSION:

3.1 Determination Plants: Determination plants in Herbarium conducted in the School of Biological Sciences and Technology (SITH) Bandung Institute of Technology. Results of determination stating that the plants were checked properly is *Artocarpus altilis* (Parkinson) Fosberg.⁸

3.2 Extraction Results: Breadfruit leaf extract variation is obtained by applying a cold way is by The solvent used is methanol maceration. redestilation. Maceration been selected for secondary metabolites cannot stand the heat then selected by maceration. Selection of methanol as a solvent is the universal solvent because methanol is expected the contents in a breadfruit leaf extract variation in such compounds are non-polar, semipolar and polar. To obtain an extract viscous done evaporation, temperature used 45°C. This is done to keep the compounds that are not heat resistant not damaged by heating.

| TABLE | 1: YIELD | RESULTS | BREADFRUIT | LEAF |
|--------|----------|------------|---------------|------|
| VARIAT | ION METH | ANOL EXT | RACT | |
| No | Breadfr | uit Leaves | Extract Yield | (%) |

| No | Breadfruit Leaves | Extract Yield | (%) |
|----|----------------------|---------------|-----|
| 1. | Green Leaves (GL) | 8.29 | |
| 2. | Yellow Leaves (YL) | 5.21 | |
| 3. | Fallen Yellow (FY) | 10.39 | |
| 4. | Fallen Dry (FD) | 31.08 | |
| 5. | Fermented Green (FG) | 7.19 | |

From the results of the collection and processing of breadfruit leaves, extracts the highest yield obtained from the leaves of breadfruit fallen dry (FD) amounted to 31.08% and the lowest yield of vellow leaves of breadfruit (YL) of extract is 5.21%. These results indicate that the component compounds in the leaves of breadfruit fallen dry (FD) extracted more with methanol compared with other breadfruit leaves. Breadfruit leaves fallen dry which is expected to have a low water content, has a low weight as well, when compared with other types of leaves, so that the specific gravity of this low will be more likely to cause the extracted compounds. Methanol is a universal solvent, which means that methanol will dissolve the compounds that are non polar, semi-polar and polar.

The ResultofPhytochemicalScreeningBreadfruitLeafExtractVariation:Phytochemicalscreening or commonly called thephytochemicalscreening is an early stage toidentify the content of secondary metabolites foundin plants.

 TABLE 2: RESULTS PHYTOCHEMICAL SCREENING OF

 BREADFRUIT LEAF VARIATION EXTRACT

| Metabolite | GL | FG | YL | FY | FD |
|----------------|----|----|----|----|----|
| Alkaloids | - | - | - | - | - |
| Flavonoids | + | + | + | + | + |
| Tannins | + | + | + | + | + |
| Penolics | + | + | + | + | + |
| Monoterpen and | + | + | + | + | + |
| sesquiterpen | | | | | |
| Steroid | + | + | + | + | + |
| Triterpenoids | - | - | - | - | - |
| Quinone | + | + | + | + | + |
| Saponin | - | - | - | - | - |

Description: GL = Green Leaves Breadfruit,GF = GreenFermentation Breadfruit Leaves, YL = Yellow Leaves Breadfruit, FY = Fallen Yellow Breadfruit Leaves, FD =Fallen Dry Breadfruit Leaves, (+) = Identified, (-) = Not identified

The results obtained from the screening phytochemical extracts is the fifth positive breadfruit leaf methanol extract containing flavonoids, tannins, phenolics, monoterpenes, sesquiterpene, a steroid, and quinones.

3.4 Results Characterization of Crude Leaves Breadfruit: Characterization simplicia find out the quality requirements simplisia to be processed into ingredients. Determination of ash content is done to describe the internal and external mineral content originating from the beginning to the process of formation of the extract and determine the metal content contained in crude drugs. Assay of the water soluble extract and extract content of ethanol soluble compounds aimed to determine substances dissolved in water or dissolved in ethanol.

| TABLE | 3: | VARIATION | CHARACTERIZATION |
|---------|-----|-------------|------------------|
| RESULTS | BRE | ADFRUIT LEA | VES |

| | - | | |
|------------|-----------|--------------|-------------|
| Breadfruit | Total Ash | Water | Ethanol |
| leaves | Content | Soluble | Soluble |
| | (%) | Material (%) | Extract (%) |
| GL | 13.50 | 64.23 | 11.91 |
| YL | 26.00 | 32.94 | 8.92 |
| FY | 27.36 | 35.94 | 12.44 |
| FD | 21.50 | 18.34 | 59.34 |
| FG | 20.50 | 21.91 | 4.95 |

Results of the characterization of crude drugs, determination shown by the largest ash content breadfruit leaves fallen yellow by 27.36% and ash content of the smallest indicated by the fresh green leaves of breadfruit of 13.50%. Determination of ash content gives an overview and inorganic mineral element content contained in crude drugs. Sari assay aims to provide an initial overview of the amount of the compound content. Results of the assay of the largest ethanol soluble extract shown by breadfruit leaves fallen dry by 59.34% and the content of ethanol soluble extract the smallest indicated by the green breadfruit leaf fermentation 4.95%.

The content in the leaves of breadfruit fallen dry is widely thought to have compounds that are semi to non-polar. Results of the assay of the largest watersoluble extract is shown by the fresh green leaves of breadfruit by 64.23% while the content of water soluble extract the smallest indicated by breadfruit fallen dry by 18.34%. The content of the fresh green leaves of breadfruit is widely thought to have polar compounds which have a polarity such as water. Testing Results Antibacterial Activity of Variation Breadfruit Leaf against Bacteria Escherichia coli, Staphylococcus epididermis, and Propionibacterium acnes: The test results of antibacterial activity with some variation of the concentration used is 10mg / mL; 25mg / mL; 5mg / mL; 100mg / mL and 150mg / mL can be seen in **Table 4.**

| TABLE 4: | TESTING | RESULTS | ANTIBACTERIAL | ACTIVITY | AGAINST | STAPHYLOCOCCUS | EPIDERMIDIS, |
|-----------------|-----------|----------|-----------------|----------|---------|-----------------------|--------------|
| ESCHERIC | HIA COLI, | AND PROP | IONIBACTERIUM A | CNES | | | |

| Bacteria | Extract | Extract The Average Diameter of the Inhibitor | | | | |
|----------------------------|-----------|---|------------------|------------------|-----------------|------------------|
| | | The Concentration (mg/ml) | | | | |
| | | 10 | 25 | 50 | 100 | 150 |
| Staphylococcus epidermidis | GL | 6 | 6 | 8.83±0.49 | 11.77±0.32 | 12.0±0.6 |
| | GF | 6 | 6 | 10.77 ± 0.21 | 13.23±0.3 | 12.97±0.06 |
| | YL | 6 | 6 | 6 | 6 | 6 |
| | FY | 6 | 6 | 10.77 ± 0.21 | 12.27±0.23 | 12.33±0.1 |
| | FD | 6 | 6 | 6 | 6 | 6 |
| | Control + | 14.8 ± 0.06 | 14.8 ± 0.06 | 14.9 ± 0.17 | 14.8 ± 0.17 | 14.9±0.1 |
| | Control - | 6 | 6 | 6 | 6 | 6 |
| Propionibacterium acnes | GL | 6 | 12.4±0.17 | 12.1±0.2 | 11.57±0.06 | 13.33±0.23 |
| | GF | 6 | 13.7±0.2 | 12.7±0.1 | 13.0±0.17 | 13.53±0.06 |
| | YL | 6 | 10.97 ± 0.06 | 11.87 ± 0.06 | 12.1±0.06 | 13.1±0.1 |
| | FY | 6 | 14.20 ± 0.17 | 10.13 ± 0.06 | 12.13±0.06 | 10.97 ± 0.11 |
| | FD | 6 | 12.10±0.3 | 12.10±0.1 | 12.03±011 | 12.8 ± 0.11 |
| | Control + | 14.7±0.17 | 14.8 ± 0.06 | 14.8 ± 0.17 | 15.0 ± 0.1 | 14.87 ± 0.06 |
| | Control - | 6 | 6 | 6 | 6 | 6 |
| Eschericia coli | GL | 6 | 11.8 ± 0.1 | 11.4 ± 0.2 | 12.0±0.15 | 13.7±0.17 |
| | GF | 6 | 11.7±0.17 | 11.53±0.3 | 14.3±0.29 | 14.8 ± 0.1 |
| | YL | 6 | 10.53 ± 0.06 | 10.37 ± 0.06 | 12.0±0.06 | 11.87 ± 0.06 |
| | FY | 6 | 11.57 ± 0.06 | 10.87 ± 0.4 | 11.77±0.15 | 13.77±0.35 |
| | FD | 6 | 11.8 ± 0.1 | 10.37±0.06 | 11.83±0.15 | 14.9±0.1 |
| | Control + | 10.5±0.17 | 9.8±0.32 | 10.1±0.17 | 9.7±0.3 | 9.6±0.17 |
| | Control - | 6 | 6 | 6 | 6 | 6 |

Description: Perforator diameter = 6 mm, Solvent used = 5% Tween 80, GL = Green Leaves, GF = Green Fermented, YL = Yellow Leaves, FY = Fallen Yellow, FD = Fallen Dry

The solvent used to dissolve the extract is 5% tween 80, because the use of DMSO, dissolved extract cannot be perfect and there is still a precipitate. It can be seen from the data table above, most of the breadfruit leaf extract of green fermentation can inhibit the bacteria Escherichia coli. Staphyolococcus epidermidis and Propionibacterium acnes which is indicated by giving a clear zone most large compared to extract variation breadfruit leaves the other, because of secondary metabolites that produce green fermentation has antibacterial and antifungal activity of the highest compared with the fresh green, yellow stuck, yellow fall, and fall is dry, then the variations of green breadfruit leaf

fermentation at a later stage will be carried out fractions. To test *Staphyolococcus epidermidis bacteria, Propionibacterium acnes* and using a positive control dose of clindamycin with $50\mu g / mL$ while the test bacteria *Escherichia coli* chloramphenicol using a positive control at a dose of 1 mg / mL.

3.6 Test Results Antifungal Activity Variations breadfruit leaf Fungi *Candida albicans*: The test results antifungal activity with some variation of the concentration used is 1000mg / ml; 800mg / ml; 500mg / ml; and 300mg / ml can be seen in **Table 5.**

| Fungal | Extract | The Average Diameter of the Inhibitory (mm) | | | | | |
|-----------------|-----------|---|---------------------------|------------|-----------|--|--|
| | | | The Concentration (mg/ml) | | | | |
| | | 1000 | 800 | 500 | 300 | | |
| Candida albican | GL | 6 | 6 | 6 | 6 | | |
| | GF | 13.97±0.15 | 13.4±0.3 | 13.33±0.06 | 6 | | |
| | YL | 6 | 6 | 6 | 6 | | |
| | FY | 6 | 6 | 6 | 6 | | |
| | FD | 6 | 6 | 6 | 6 | | |
| | Control + | 23.2±0.11 | 23.0±0.3 | 23.2±0.06 | 23.4±0.17 | | |
| | Control - | 6 | 6 | 6 | 6 | | |

TABLE 5: RESULTS OF TESTING ANTIFUNGAL ACTIVITY

Description: Perforator diameter = 6 mm. Solvent used = 5% Tween 80. GL = Green Leaves, GF = Green Fermented, YL = Yellow Leaves, FY = Fallen Yellow, FD = Fallen Dry

The solvent used to dissolve the extract is 5% tween 80, because the use of DMSO, dissolved extract cannot be perfect and there is still a precipitate. It can be seen from the table above only green breadfruit leaf extract fermentation that gives the test fungal activity against *Candida albicans*, the green breadfruit leaf extract fermentation has been done fraction. Positive control used is Nystatin with a dose of 1 mg / ml.

of Fractionation **Breadfruit** 3.7 Results Fermentation Green Leaves: Green breadfruit leaf extract fermentation fractionation using a solvent n-hexane, ethyl acetate, and methanol, which can dissolving compounds are secondary metabolites based on the nature of polarity. Nhexane can attract Metaboli secondary compounds that are non-polar. Ethyl acetate can be interesting compounds are secondary metabolites that are semi-polar. Methanol can be interesting compounds are polar. So that the compounds are interested secondary metabolite has been based on polarity. The liquid fraction obtained is then thickened.

TABLE 6: RESULTS FRACTIONATION BREADFRUITFERMENTATION GREEN LEAVES

| No. | Fraction | Extract Weight Condensed | % Yield |
|-----|---------------|-----------------------------|---------|
| 1. | N-hexane | 2,89 gram | 4,51 |
| 2. | Ethyl acetate | 7,43 gram | 11,61 |
| 3. | Methanol | 1,04 gram | 1,62 |

3.8 Results of Screening Fractionation Bread fruit Green Fermentation Leaf: Phytochemical screening purposes is to determine secondary metabolites that terrdapat fraction fermented breadfruit leaf green.

| TABLE | 7: | RESULTS | OF | SCF | REENING |
|---------|-------|-----------|------|------|---------|
| FRACTIO | NATIC | ON BREADF | RUIT | LEAF | GREEN |
| FERMENT | ΓΑΤΙΟ | N | | | |

| | Fraction n- Hexane | Fraction Ethyl Acetate | Fraction Methanol |
|---------------|-----------------------|------------------------------|----------------------|
| Alkaloids | - | - | - |
| Flavonoids | + | + | + |
| Tannins | - | - | + |
| Penolics | - | + | + |
| Monoterpen | | | |
| and | + | + | + |
| sesquiterpen | | | |
| Steroid | + | + | + |
| Triterpenoids | - | - | - |
| Quinone | + | + | + |
| Saponin | - | - | - |

Results of screening fraction green breadfruit leaf fermentation is not different from the results of screening fermented breadfruit leaf extract green, this means that no content is lost during the fractionation process.

3.9 Determination of Minimum Inhibitory Concentration Results (MIC) of the methanol breadfruit extract of leaves of Green Against Fermentation Bacteria: Testing Minimum Inhibitory Concentration (MIC) is intended to determine the minimum concentration required to inhibit the growth of bacteria. MIC Determination is done by using the agar diffusion method. The result leaves methanol extract of fermented green breadfruit has a MIC value of 15 mg / mL of the bacteria Propionibacterium acne, 47.5mg / mL against Staphylococcus epidermidis, and 20mg / mL of the bacterium Escherichia coli.

TABLE 8: RESULTS DETERMINATION OF MINIMUMINHIBITORY CONCENTRATION METHANOL EXTRACTSBREADFRUIT GREEN FERMENTATION LEAF BACTERIAAGAINST PROPIONIBACTERIUM ACNE

| (| Concentration (mg / mL) | Diameter(mm) | Control (-) |
|---|-------------------------|--------------|-------------|
| | 22.5 | 11.9 | 6 |
| | 20 | 11.0 | 6 |
| | 17.5 | 10.8 | 6 |
| | 15 | 9.9 | 6 |
| | 12.5 | 6 | 6 |

TABLE 9: DETERMINATION OF MINIMUM INHIBITORYCONCENTRATIONMETHANOLEXTRACTSBREADFRUITLEAFGREENFERMENTATIONAGAINSTSTAPHYLOCOCCUSEPIDERMIDIS

| Concentration (mg / mL) | Diameter (mm) | Control (-) |
|-------------------------|---------------|-------------|
| 47.5 | 8.9 | 6 |
| 45 | 6 | 6 |
| 42.5 | 6 | 6 |
| 40 | 6 | 6 |
| 37.5 | 6 | 6 |
| 3.5 | 6 | 6 |
| 32.5 | 6 | 6 |
| 30 | 6 | 6 |
| 27.5 | 6 | 6 |
| 25 | 6 | 6 |

TABLE 10: RESULTS OF DETERMINATION OF MINIMUM **INHIBITORY** CONCENTRATION **METHANOL** EXTRACTS BREADFRUIT GREEN FERMENTATION LEAF AGAINST BACTERIA ESCHERICHIA COLI Concentration (mg / ml) Diameter (mm) Control ()

| Concentration (ing / ini) | Diameter (mm) | |
|---------------------------|---------------|---|
| 22.5 | 11.1 | 6 |
| 20 | 10.7 | 6 |
| 17.5 | 6 | 6 |
| 15 | 6 | 6 |

3.10 Results of Determination Minimum Inhibitory Concentration (MIC) of the methanol extract breadfruit Green Fermentation leaves Against Fungi *Candida albicans:* In testing the breadfruit leaf extract variation only green fermented breadfruit leaves only provide activity against *Candida albicans* Fungi. The result leaves methanol extract of fermented green breadfruit has a MIC value with the concentration of 475mg / ml against the Fungi *Candida albicans*.

TABLE 11: RESULTS OF DETERMINATION MINIMUMINHIBITORY CONCENTRATION METHANOL EXTRACTSBREADFRUIT GREEN FERMENTATION LEAF AGAINSTCANDIDA ALBICAN

| Concentration (mg / mL) | Diameter (mm) | Control (-) | | | |
|-------------------------|---------------|-------------|--|--|--|
| 475 | 10.9 | 6 | | | |
| 450 | 6 | 6 | | | |
| 425 | 6 | 6 | | | |
| 400 | 6 | 6 | | | |
| 375 | 6 | 6 | | | |
| 350 | 6 | 6 | | | |
| 325 | 6 | 6 | | | |

3.11 Results of Monitoring Fraction By Thin Layer Chromatography: Monitoring fraction of n-hexane, ethyl acetate, and methanol using a developer of n-hexane: ethyl (7: 3). Identification was done at UV 254 and UV 366 with 10% H2SO4 spray reagen. Results fraction of n-hexane detected under UV light 366 shows the 6 spot with Rf 0.13; 0.32; 0.35; 0.50; and 0.55. At the ethyl acetate fraction showed the 12 spot with Rf 0.08; 0.13; 0.23; 0.28; 0.32; 0.38; 0.42; 0.47; 0.50; 0.55; 0.60;and 0.63. While the methanol fraction showed the presence of 9 spot with $R_f 0.12$; 017; 0.20; 0.25; 0.28; 0.35; 0.47; 0.49; and 0; 50. When detected in UV light at 366 shows a yellow spot with $R_f 0.50$ in fractions of n-hexane, ethyl acetate, and methanol. While at the same spot when detected in UV light 254 shows a blackish green color. This showed the presence of the flavonoid ⁹.

3.12 Results Bioautography Bacteria: In the test results bioautografi, ethyl acetate fraction providing the antibacterial activity in the presence of clear zone is the result of TLC ethyl acetate fraction of the bacteria *Escherichia coli, Staphylococcus epidermidis, Propionibacterium acnes.* For TLC results ethyl acetate fraction of the bacteria *Escherichia coli* provide clear zone on the spot 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, and 12 with a value of Rf 0.08; 0.13; 0.23; 0.28; 0.32; 0.38; 0.42; 0.50; 0.55; 0.60; and 0.63. For TLC results ethyl acetate fraction against *Propionibacterium acnes* bacteria provide clear zone on the spot 1, 2, 3, 4, 5, 6, 9, 10, 11, and 12 with a value of Rf 0.08; 0.13; 0.23; 0.28; 0.32; 0.38; 0.13; 0.23; 0.28; 0.32; 0.38; 0.13; 0.23; 0.28; 0.32; 0.38; 0.13; 0.23; 0.28; 0.32; 0.38; 0.13; 0.23; 0.28; 0.32; 0.38; 0.13; 0.23; 0.28; 0.32; 0.38; 0.50; 0.55; 0.60; and 0.63.

For TLC results ethyl acetate fraction against *Staphylococcus epidermidis* provide clear zone on spot 2, 8, and 9 with the value of Rf 0.13; 0.47; and 0.50. For the TLC fractions of methanol only provide antibacterial activity against *Escherichia coli* at the 4 spot with Rf value of 0.25 and to *Propionibacterium acnes* at 5 spot with Rf value of 0.28. As for the results of the TLC fraction of n-hexane does not provide antibacterial activity is suspected steroids because when the results of the TLC given spray reagen Lieberman-Burchard gives brownish red color in UV lamp 366 and blackish green color in UV lamp 254.

3.13 Results Bioautograph Fungi: Bioautografi on the test results, the results of TLC fractions of methanol and n-hexane fraction does not provide the antifungal activity against *Candida albicans* Fungi growth, while the TLC results fractions of ethyl acetate giving the antifungal activity with the clear zone on third spot with Rf value of 0.23. Spot antifungal activity identified as steroids because when the results of the TLC given spray reagen Lieberman-Burchard gives brownish red color in UV lamp 366 and blackish green color in UV lamp 254.

CONCLUSION: Variations methanol extract of green leaves of breadfruit, fermented green, yellow leaves, fallen yellow and fallen dry has antibacterial activity against the growth of *Staphylococcus epidermidis, Escherichia coli,* and *Propionibacterium acnes*. But only the methanol extract of leaves of green fermented breadfruit who have antifungal activity against *Candida albicans* fungal growth.

Variations breadfruit leaves which have antibacterial activity against the growth of *Staphylococcus epidermidis, Escherichia coli, Propionibacterium acnes*, and have antifungal activity against the growth of *Candida albicans* Fungi is fermented breadfruit leaf green.

The test results Minimum Inhibitory Concentration (MIC) of methanol extract of fermented green breadfruit leaf against *Staphylococcus epidermidis*, *Escherichia coli*, and *Propionibacterium acnes* in a row is 47.5 mg/mL; 15 mg/mL; and 20 mg/mL. While the results of MIC green breadfruit leaf methanol extract of the fermentation of the *Fungi Candida albicans* growth is 475 mg/mL.

Fraction of fermented green breadfruit leaves have antibacterial activity against the growth of *Staphylococcus epidermidis, Escherichia coli, Propionibacterium acnes*, and have antifungal activity against the growth of *Candida albicans* Fungi is a fraction of ethyl acetate. Methanol fraction having antibacterial activity against the growth of bacteria *Escherichia coli* and *Propionibacterium acnes*. While the n-hexane fraction has no antibacterial activity against the growth of *Staphylococcus epidermidis*, *Escherichia coli*, *Propionibacterium acnes* as well as antifungal activity against *Candida albicans*

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