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Antimicrobial and Antioxidants Activity Test of Fresh Extract and Decoction Extract of Areca Plant Roots (Areca catechu Linn.) Against Test Microbes

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Abstract—Research on the Antimicrobial and Antioxidant Activities of Several Areca (Areca catechu Linn.) Root Extracts which was conducted at the Microbiology Laboratory, Andalas University in April - June 2023. This study aims to determine the antimicrobial activity of several areca root extract treatments, determine the most effective extraction of the tested microbes and determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration(MBC) of areca root against the tested microbes, as well as the antioxidant activity and polyphenols of areca root extract. The method used in this study is the nested pattern experimental method. The results showed that the inhibition zone of areca root extract had a significantly different effect on S. aureus bacteria, but did not have a significantly different effect on E. coli bacteria. and the fungus C. albicans. The MIC of areca root extract against E. coli and S. aureus bacteria is 3.12% with MBC on E. coli 12.5% and S. aureus 25%, while the MIC for C. albicans is 25% and there is no MIC. Antioxidant value of fresh extract, fresh decoction and dry decoction of betel roots respectively with IC50 values of 22.37 µg/ml, 16.79 µg/ml and 84.01 µg/ml for very strong activity category and total polyphenols obtained from fresh extract , fresh decoction and dry decoction were 30.66 mgGAE/ml, 33.16 mgGAE/ml, and 19.89 mgGAE/ml, respectively.

Keywords—Antimicrobial, Antioxidant, Areca catechu L, Extraction, Polyphenols.

I. INTRODUCTION

The areca plant (*Areca catechu* L.) is a plant that is often found in Indonesia. Areca palm plants are widespread in Indonesia both individually and in groups. Areca palm plants have various benefits for human health. One of them is at the root. The roots of the areca plant contain secondary metabolite compounds such as: alkaloids, steroids, flavonoids, terpenoids, quinones, phlobatin, tannins and phenols which can be used as an antimicrobial. Root plant areca (*Areca catechu* L.) has special benefits for human health such as treating skin irritation and urinary tract diseases[1].

Antimicrobials are chemical compounds that can inhibit the growth of microbes and can even stop/kill bacterial growth[2]. Because many types of bacteria are resistant to antibacterials, we are looking for new alternative sources that have the potential to inhibit these bacteria. One alternative is to use the roots of the areca plant (*Areca catechu* L.) which from the compounds contained in the roots of the areca plant is thought to beantimicrobial. Antimicrobial activity testing uses infectious microbes representing each type, namely *Escherichia coli* as a group of Gram-negative bacteria, *Staphylococcus aureus* as a group of Gram-positive bacteria and *Candida albicans* as a group of fungi.[3].

Apart from being thought to be an antimicrobial, the roots of the areca plant are thought to be used as an anticancer because the roots of the areca plant have antioxidant compounds. Antioxidants can protect cells from free radical damage by donating one electron to the free radical or accepting an unstable electron so that it becomes stable. Free radicals are very reactive molecules, this is because free radicals have unpaired electrons in their outer orbitals so they can react with body cell molecules by binding

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the cell molecule's electrons.[4]. Naturally, plants containing antioxidants are distributed in various parts of plants such as roots, stems, skin, twigs, leaves, fruit, flowers and seeds.[5]. So it is suspected *A. catechu* roots has potential as an antioxidant.

Ethanol extract of areca root is known to have antimicrobial and antioxidant activity. However, so far no research has been carried out comparing the antimicrobial and antioxidant activity of fresh extracts and Decoction areca root plants against these test microbes. Based on the things mentioned above, the author is interested in testing the antimicrobial and antioxidant activity of several areca root extracts (*Areca catechu* Linn.) against the test microbe *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*.

II. MATERIALS AND METHODS

2.1 Research Methods

The method used is the nested pattern experimental method with 3 repetitions, where factor A is the treatment of root extract A. catechu (A1 = Fresh extract, A2 = Fresh decoction and A3 = Dry decoction) and factor B is the test microbe (B1 = E. coli, B2 = S.aureus, B3 = C. albicans).

2.2 Sample Preparation of Areca root (A. catechu)

Areca root plant samples were taken in Andalas University, South Limau Manis Village, Pauh District, West Sumatra. Sampling is done by digging the soil around the roots and then cutting the fresh roots. Then the sample is washed, put in plastic and taken to the laboratory. After washing, the root is then prepared for making fresh extracts and for drying in the air for 2-3 days. The drying process is carried out without sunlight.

2.3 Extraction method

2.3.1 Fresh extract

Fresh Areca root samples were weighed as much as 12 grams fresh, after which they were washed with running water and rinsed thoroughly, then the Areca root samples were mashed, squeezed and filtered through sterile gauze. The filter results were put into Eppendorf and centrifuged for 5 minutes at 10,000 rpm.

2.3.2 Decoction Fresh

The Areca root sample was cut into small pieces, then weighed as much as 12 grams of fresh (equivalent to 2 grams of dry), then heat 100 ml of water to a boil with an Erlenmeyer. Then boil the sample until it reaches a volume of 50 ml then cover tightly, allow it to cool.

2.3.3 Decoction Dry

Fresh Areca root samples were weighed as much as 12 grams fresh, after which they were washed with running water and rinsed thoroughly, then the Areca root samples were mashed, squeezed and filtered through sterile gauze. The filter results were put into Eppendorf and centrifuged for 5 minutes at 10,000 rpm.

2.4 Determination of Microbial-Free Areas Using the Disc (Diffusion) Method

The diffusion method used according to [6]. MHA media was poured into a petri dish aseptically and allowed to solidify. Then 1 ml of bacterial suspension was taken and inoculated on the surface of the medium and smoothed over with a sterile cotton swab. Then the paper disc with a diameter of 6 mm was aseptically dipped into the sample, and dried for a while then placedthe paper disc on the surface of the media with sterile tweezers. After that, it was incubated for 24 hours and the diameter of the clear zone was measured. The positive controls used were chloramphenicol (0.1 mg/mL) and fluconazole (0.1 mg/mL) and the negative controls used dissolution used in the extraction, namely distilled water. The sample treatment with the highest inhibition zone area was continued to determine the MIC and MBC values in the dilution method.

2.5 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) with Dilution method

A sterile test tube is provided which has been labeled 1-10 and tubes A, B, for control. Enter 2 ml of SDB/MHB medium aseptically into Tubes 1-10. Then 2 ml of fresh root extract was added to tube 1 then homogenized and transferred from tube 1 to tube 2 as much as 2 ml, homogenized, then from tube 2 to tube 3 and so on until tube 10. In tube 10, 2 ml was taken and thrown away. So that the volume in each tube is 2 ml. In order to obtain the concentration of root extract successively as follows 50%, 25%, 12.5%, 6.25%, 3.125%, 1.5%, 0.8%, 0.4%, 0.2% and 0.1%. Then in tube 1-10 added 1 ml of microbial suspension.

Tubes A and B served as controls, where Tube A was filled with 2 ml of Areca root extract (*Areca catechu* L.), then added 2 ml of SDB/MHB medium. Tube B was filled with 2 ml of SDB/MHB medium and 1 ml of the test microbial suspension was added. All tubes were incubated 130 at 37°C for 1x24 hours. After 24 hours, the cloudy tube was marked and the dilution value was recorded. The positive (clear) tube was taken as much as 0.1 ml of the test microbial suspension and cultured on SDA MHA medium, incubated at 37°C for 24 hours. It was observed whether there was growth of the test microbes, if there was, the number of colonies formed was recorded, then the MIC and MBC numbers were determined[7].

2.6 Determination of Antioxidant Activity with the DPPH

Method Antioxidant activity test was carried out using the DPPH (1.1-Diphenyl-2-Picryl-137 Hydrazine) method. 1 ml of areca root was diluted with 4 ml of methanol, then made various concentrations of 25, 50, 75, 100, and 125 ppm, then continued by dissolving 1.97 mg of DPPH with 100 mL of methanol to obtain a DPPH solution with a concentration of 0.05 mM . then cover with aluminum foil. 8 ml of areca root extract solution was reacted with 2 ml of 0.05 mM DPPH solution, homogenized with a vortex. Antioxidant activity was analyzed using a spectrophotometer with a wavelength of 517 nm. Methanol was used as a blank during the spectrophotometer process. The linear regression curve equation and the IC50 value are calculated using the equation: Y = ax+b. In the antioxidant activity test, Vit C was used as a comparison[8].

2.7 Determination of Polyphenol Levels

The areca root extract was carried out using the Folin-Ciocalteu Assay method with several modifications based[9]. 1 ml of areca root was diluted with 4 ml of distilled water, then 1 ml of the sample was mixed with 1 ml of Folin-Ciocalteu reagent. After 5 minutes, 1 ml of 13% Sodium Carbonate was added to the mixture and made up with distilled water until the volume reached 10 ml. The tube was stored in a dark place for 90 minutes and the absorbance value was measured using a spectrophotometer at 725 nm.

III. Results And Discussion

3.1 Microbial Activity Test using the Kirby-Bauer Method

Based on research that has been carried out regarding antimicrobial activity tests, the results of fresh extracts, fresh decoctions and dried decoctions of the roots of the areca plant (*Areca catechu* Linn.) were given against the test microbes, namely Esherichia coli bacteria, *Staphylococcus aureus* and *Candida albicans* fungi. The zone of inhibition of several areca root extracts against the test microbes can be seen in Table 1.

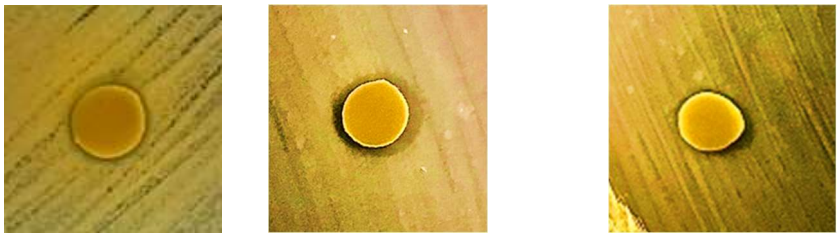
Table1.Average Antimicrobial Activity of Fresh Treatment, Fresh Decoction and Dry Decoction of Areca Plant Roots (*Areca catechu L.) against test microbes (Escherichia coli, Staphylococcus aureus and Candida albicans).*

No	Treatment	Diameter of Inhibition zone (mm)		
		E. coli	***S. aureus***	***C. albicans***
1	Fresh	6.53[c]	6.82[a]	6.32[a]
2	Fresh Decoction	7.90[a]	7.09[a]	6.33[a]
3	Dry Decoction	7.37[b]	7.18[a]	6.65[a]

Description: letters behind the number represent the notation from Duncan's test at 5% level, different letters represent significantly different values for each phatogenic microbe.

Table 1. It can be seen that fresh treatment, fresh Decoction and dried Decoction *A. catechu* roots have antimicrobial activity against *E. coli* and *S. aureus* bacteria but do not have a major effect on the fungus *C. albicans*. Based on ANOVA analysis and continued with the DMRT test which was carried out on the fresh, fresh Decoction and dry Decoction treatments gave significantly different effects on *S. aureus* bacteria but on *E. coli* bacteria and *C. albicans* fungi the fresh, fresh Decoction and dry Decoction treatments did not give any effect. significantly different effects.

The antimicrobial activity test of fresh extract, fresh decoction and dried decoction of areca nut roots against the test microbe *E. coli* can be seen in Figure 2 as follows:



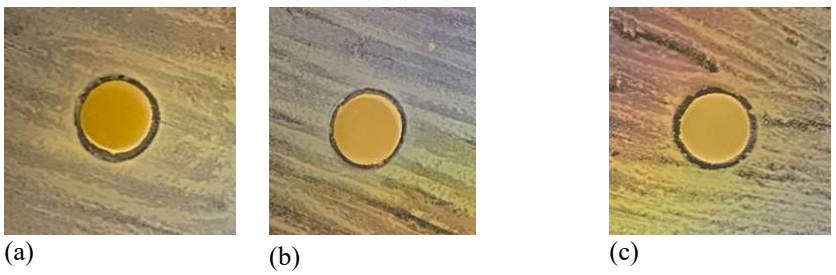
(a) (b) (c)

Figure 1. Antimicrobial activity produced by some areca root extracts

Description: (a) Fresh Extract, (b) Decoction Fresh, (c) Decoction Dry.

Treatment of fresh samples, fresh Decoction and dried Decoction samples was able to inhibit the growth of *E.coli* bacteria. The largest zone of inhibition is found in the fresh Decoction extract. The larger the inhibition zone formed, the greater the extract's ability to inhibit bacterial growth[10]. The fresh Decoction extract obtained a larger zone of inhibition compared to others and the compounds contained were still not damaged due to oxidation. This is different, namely that during extraction, compounds that are polar are more easily dissolved in water because water is a polar solvent. Water is a polar solvent, polar compounds that can dissolve in water are alkaloids, flavonoids, tannins, carbohydrates and steroids. These polar compounds are found in the roots of the *A. catechu* plant[11]. Regarding the phytochemical test of ethanol samples on the roots of the areca plant (*Areca catechu* L.), it was found that the secondary metabolites of the roots of the areca plant contain compounds alkaloids, steroids, flavonoids, terpenoids, quinones, phlobatin, tannins and phenols[1].

The antimicrobial activity test of fresh extract, fresh decoction and dried decoction of areca nut roots against the test microbe *S. aureus* can be seen in Figure 2 as follows:



(a) (b) (c)

Figure 2. Antimicrobial activity produced by some areca root extracts

Description: (a) Fresh Extract, (b) Decoction Fresh, (c) Decoction Dry.

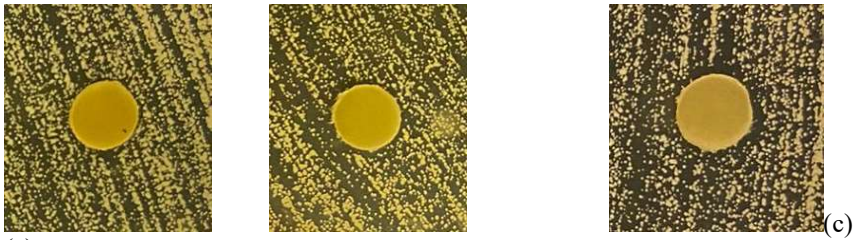
Treatment of fresh samples, fresh Decoction and dried Decoction samples was able to inhibit the growth of *S. aureus* bacteria. The largest zone of inhibition is found in the fresh extract.The fresh treatment used is water from the plant without adding

solvents, this indicates that what works in this treatment is the original content contained in the extract. The secondary metabolite content of areca roots which acts as an antimicrobial is alkaloids, flavonoids, terpenoids, quinones, phlobatin, tannins and phenols.Tannins have antibacterial activity by damaging cell membrane components, cell walls, genetic material, enzymes and other protein components.[12]..

Flavonoid compounds has antibacterial activity because flavonoid compounds have disinfectant properties which play a role in denaturing proteins so that they can reduce the metabolic activity of bacterial cells to a halt. Apart from being said to reduce the metabolic activity of bacterial cells, flavonoid compounds can also damage cell walls[13]. There are three types of antibacterial mechanisms of flavonoids, namely by inhibiting nucleic acid synthesis, inhibiting energy metabolism, and inhibiting cytoplasmic membrane function.[14].

Alkaloid compoundshas antibacterial activity Because has a mechanism for damaging cells which is thought to be by disrupting the components that make up the peptidoglycan in bacterial cells, so that the cell layer does not form completely and causes cell death [15]. Phenolic compounds have antibacterial activity, namely through inhibition of oxidizing compounds that react with sulfhydryl groups, through nonspecific interactions with proteins or bacterial cell membranes [16]. Meanwhile, tannin compounds Contains food components that work by inhibiting enzymes such as hyaluronidase and 5-lipoxygenase, which act as anti-inflammatory, keratolytic and antimicrobial agents [17].

The antimicrobial activity test of fresh extract, fresh decoction and dried decoction of areca nut roots against the test microbe *C. albicans* can be seen in Figure 3 as follows:



(a) (b) (c)

Figure 2. Antimicrobial activity produced by some areca root extracts

Description: (a) Fresh Extract, (b) Decoction Fresh, (c) Decoction Dry.

The treatment of fresh samples, fresh Decoction and dried Decoction samples was less able to inhibit the growth of the fungus *C. albicans*. The zone of inhibition formed was not clearly visible in all extracts. This is inIt is suspected that the compound is unable to penetrate the *C. albicans* cell wall. This is related to the components of the *C. albicans* cell wall which are more complex compared to the cell wall components of bacteria. The main components of the *C. albicans* cell wall are chitin, glucans, manoprotein while the minor components are fat and inorganic salts. The antifungal activity test on *C. albicans* can be influenced by various factors such as the root extraction method, the concentration of the extract used and the location where the sample grows as well as virulence factors that play a role in pathogenesis .[18].

Table 2. Microbial Inhibition Zone Test Area of Control Treatment

Treatment	Diameter of Inhibition zone (mm)		
	E. coli	***S. aureus***	***C. albicans***
positive control (mm)	24.42	22.33	20.9
negative control (mm)	0,00	0,00	0,00
Percentage positive control (%)	32.35	31.75	30.28

Based on research that has been done for the positive control treatment of *S. aureus* and *E. coli* using the antibiotic chloramphenicol 0.1 mg/ml and for the positive control treatment of *C. albicans* using fluconazole 0.1 mg/ml while for the negative control using distilled water. The diameter of the inhibition zone for the positive control of 0.1 mg chloramphenicol on *S. aureus* was 22.33 mm, when compared with the diameter of the inhibition zone for Decoction fresh extract on *S.aureus*, the percentage of positive control for chloramphenicol 0.1 mg was 31.75% strength in inhibiting microbial growth. Diameter of the inhibition zone for the positive control of 0.1 mg chloramphenicol on *E. coli* was 24.42 mm, when compared with the diameter of the inhibition zone for Decoction fresh extracts on *E. coli*, the percentage of positive control for chloramphenicol 0. 1 mg was 32.35% strength in inhibiting microbial growth. Chloramphenicol was used as a positive control in the antibacterial activity test which is a broad spectrum antibiotic. Chloramphenicol can inhibit bacterial protein synthesis on the ribosome and can inhibit the peptidyl transferase enzyme so that peptide bonds cannot be formed in the bacterial protein synthesis process .[19].

Diameter of the inhibition zone for the positive control of fluconazole 0.1 mg on *C. abicans* was 20.9 mm, when compared with the diameter of the inhibition zone for Decoction fresh extracts *C. albicans*, the percentage of positive control for fluconazole 0.1 mg was 30, 28% strength in inhibiting microbial growth. Fluconazole has activity as an antifungal both systemic and non-systemic which is effective against *C. albicans*. Fluconazole is an effective antifungal agent in the treatment of local and systemic candidiasis [20].

3.2 Value of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration(MBC)

Based on research that has been carried out regarding the Minimum Inhibitory Concentration (MIC) and Minimum Kill Concentration (MBC) using fresh *A. catechu* root extract against the test bacteria *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and Candida albicans, the following results were obtained:

Table 3. MIC and MBC values of fresh treated areca nut plant roots*S. aureus, Fresh Decoction Against E. coli and Dry Decoction Against C. albicans*

Test Microbes	MIC value (%)	MBC Value (%)
Escherichia coli	3.12%	12.50%
Staphylococcus aureus	3.12%	25%
Candida albicans	25%	-

Table 2. It is known that the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration(MBC) of fresh Decoction samples of areca nut roots are able to inhibit (MIC) the growth of *Escherichia coli* bacteria at a concentration of 3.12% and have the ability to Minimum Bactericidal Concentration(MBC) of 12. 50%. Then, fresh samples of areca plant roots were only able to inhibit (MIC) the growth of *Staphylococcus aureus* bacteria at a concentration of 3.12% and had the ability to Minimum Bactericidal Concentration(MBC) of 25%. Meanwhile, the dry Decoction samples of areca nut roots were only able to inhibit (MIC) the growth of *Candida albicans* fungus at a concentration of 25% and did not have the ability to Minimum Bactericidal Concentration(MBC).

Minimum Inhibitory Concentration (MIC) is the lowest inhibitory concentration in a tube that appears clear, in other words there is little visible microbial growth. Meanwhile, the Minimum Bactericidal Concentration (MBC) is the absence of microbial growth on solid media contained in a tube that looks clear (MIC). Minimum Inhibitory Concentration is indicated by culture results that contain little microbial growth or cultures that begin to appear clear. Meanwhile, the Minimum Bactericidal Concentration is characterized by clear culture results and no microbial growth[21].

3.3 Antioxidant Activity of Fresh Extracts of Areca Plant Roots (Areca catechu L.)

Based on research that has been carried out on the antioxidant activity of fresh *A. catechu* extract using the method of reducing effects on DPPH free radicals (1,1-Diphenyl-2-Picryl Hidracil), the following results were obtained:

Table 4. Antioxidant Activity of Fresh *A. catechu* L. Root Extract, Fresh Decoction and Dried Decoction.

Treatment	Antioxidant (IC50) (µg/mL)	Polyphenols(mgGAE/ml)
Fresh	22.37	30.66
Fresh Decoction	16.79	33.16
Dry Decoction	84.01	19.89

Table 4 shows that the antioxidant activation value of the fresh treatment is 22.37µg/mL The polyphenol content is 30.66 mgGAE/G, then the areca root extract treated with fresh Decoction value antioxidant activation 16.79 µg/mLfor its polyphenol content, namely 33.16 mgGAE/G, while the areca root extract treated with dry Decoction value antioxidant activation 84.01 µg/mL for its polyphenol content, such as 19.89 mgGAE/ml, so it can be said that the antioxidants from *A. catechu* roots are classified as very strong because the results of the research that has been carried out, the antioxidant activation value is within the IC50 value or less than 50ppm. *A. catechu* root ethanol extract showed a maximum DPPH scavenging activity of 95% at a concentration of 1000 g/ml with an IC50 value of 65.7, which is considered strong [1]. The ability of antioxidants to reduce free radicals into several groups, among others, antioxidants are said to be very strong if the IC50 value is less than 50 ppm, said to be strong if the IC50 value is 51-100 ppm, said to be moderate if the IC50 value is 101-150 ppm, said to be weak if the IC50 value is 151 -200 ppm, and is said to be very weak if the IC50 value is more than 200 ppm[8]. Meanwhile, IC50 itself is a concentration that can reduce 50% of DPPH free radicals, where the smaller the IC50 value, the greater the antioxidant activity.

Testing total phenol activity is the basis for testing antioxidant activity. This is because phenolic compounds play a role in preventing free radical oxidation events. Measurement of total antioxidants originating from plants can be done by measuring the total phenolic content using the Folin-Ciocalteau reagent. Most antioxidants that come from plants are polyphenolic compounds. Based on Table 4, the results showed that the polyphenol value in the fresh treatment was 30.66 mgGAE/mL, in the fresh Decoction treatment it was 33.16 mgGAE/mL, and in the dry Decoction treatment it was 19.89 mgGAE/mL. Total phenol testing aims to determine the total phenolic compounds contained in the sample[22].

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