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Antimicrobial activity of sungkai leaf extract (*Peronema canescens* Jack.) and antioxidants

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Abstract

Sungkai (*Peronema canescens* Jack.) is a plant that has straight or slightly curved stems, no buttresses, and branches covered with fine hairs. Sungkai (P. canescens) is one of the many medicinal plants that grow in Indonesia and is widely used, the part that is commonly used is the leaves, where the leaves of Sungkai (*P. canescens*) have been widely known as a cure for several diseases such as malaria, high fever and for maintaining health. Research on the antimicrobial potential of sungkai leaf extract (P. canescens) and its antioxidant activity was carried out from April to August 2022. This study used the 2-factor Nested pattern experimental method. This study aims to determine the antimicrobial activity, determine Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC) as well as determine the antioxidant activity of the fresh treatment, fresh infusion, dry infusion, dry infusion plus lime and dried decoction of sungkai leaves (*P. canescens*). The test microbes used in this study ware *Escherichia coli*, *Staphylococcus aureus,* and *Candida albicans*. The research results showed that sungkai leaf extract (P. canescens) could inhibit the growth of the tested microbes *S. aureus* and *E. coli* by producing significantly different inhibition zones and for the tested microbes *C. albicans s*were significantly different. MIC of fresh sungkai for *S. aureus* 25% and 50% *E. coli*. The highest antioxidant activity value was found in fresh samples with an IC50 value of 98.02 µg/mL which was classified as strong and the highest polyphenols in fresh treatment were 76.73 (mgGAE/G).

Keywords: Antioxidant; Antimicrobial; MIC; MBC; *Peronema canescens*

1. Introduction

Indonesia is a tropical country that is rich in medicinal plants and has great potential to be developed but has not been optimally managed [14]. Currently, infectious diseases are still a serious problem in Indonesia, coupled with the increasingly widespread resistance of microbes to the available antibiotic drugs. This encourages the importance of exploring sources of other antimicrobial drugs from natural ingredients. One of the many medicinal plants that grow in Indonesia which is widely used is the Sungkai plant (*Peronema canescens* Jack.). Until now, the Dayak tribe in East Kalimantan still maintains the tradition by utilizing the surrounding plants for treatment or health care, for example, the Sungkai plant (used as a bath for women after giving birth and as a mouthwash to prevent toothache. This is a typical Indonesian plant ideally found in Sumatra and Kalimantan [7].

Sungkai leaf infusion is believed to reduce blood pressure, overcome malaria, fever, flu, cough, and runny nose increase body immunity, because Sungkai leaves contain active compounds such as flavonoids, tannins, phenolics, saponins, steroids, and terpenoids. The content of flavonoids in Sungkai leaves is used to prevent hypertension. In addition, the body also needs antioxidants that can neutralize free radicals. Flavonoids and tannins act as antioxidants which can prevent oxidation the of body cells and flavonoid compounds, saponins, alkaloids, and phenols have anti-inflammatory activity [5].

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2. Material and methods

2.1. Materials

The materials used were fresh and dry samples of Sungkai leaves (Peronema canescens Jack.) Pure cultures of Staphylococcus aureus ATTC 25923, Escherichia coli ATTC 25922 and Candida albicans, Nutrient Agar (NA) medium, Potato Dextrose Agar (PDA), Mueller Hinton Agar (MHA), Sabouraud Dextrose Agar (SDA), Hinton Broth Medium (MHB), Saboroud Dextrose Broth (SDB), distilled water, alcohol, spirit, Phenol- Ciocalteu, Sodium Carbonate, DPPH solution, and methanol.

2.2. Methods

2.2.1. Preparation of Extraction

(Fresh Extract) Fresh leaves are washed with running water until clean. Then the sample is crushed to get the extract, then squeezed and filtered, (Fresh Brew) Fresh leaves are washed with running water until clean and wiped dry. Then the sample is processed to get the extract, then squeezed and filtered, (Dry Brewing) Prepare 2 grams of dried leaves (equivalent to 4 grams of fresh), then heat 100 ml of water to a boil, and soak the sample in heated water. After that, close the lid tightly and leave it to cool, (Dry Stew) Prepare 2 grams of dried leaves (equivalent to 4 grams of fresh), then heat 100 ml of water to a boil, and put the sample into the boiling water. After that, close the lid tightly and leave it to cool, (Dry Infusion) Add Lime Prepare 2 grams of dried leaves (equivalent to 4 grams of fresh), then heat 100 ml of water to a boil, and soak the sample in heated water. After that let stand until warm and add 5 ml of lime juice, cover tightly, and leave to cool.

2.2.2. Preparation of Test Microbial Suspension

A total of 2 doses of the rejuvenation test bacteria were suspended in 2 mL of physiological NaCl in a sterile test tube and homogenized with a vortex for 15 seconds, then the turbidity was seen by comparing the turbidity standard 0.5 Mc Farland (equivalent to 1.5 x 108 CFU). mL-1).

2.2.3. Determination of Microbial Free Areas Using the Disc (Diffusion)

Method The MHA/SDA media was poured aseptically into a petri dish and allowed to stand until it solidified. Then 1 ml of bacterial suspension was taken and inoculated evenly on the surface of the media using sterile cotton, then allowed to stand for a while at room temperature. After that, dip the 6 mm paper disc aseptically into the sample and place the paper disc on the surface of the media with sterile tweezers. Incubate for 24 hours and measure the diameter of the clear zone formed (Madigan et al., 2012). The positive control used was chloramphenicol (1 mg/mL) for antibacterial, fluconazole (1 mg/mL) for antifungal and the negative control was the solvent used in the extraction, namely aquadest.

2.2.4. Sample Testing on Test Microbes with Dilution Method

Provide 12 tubes, then tubes 1-10 are aseptically inserted with 2 ml of SDB/MHB media. Added 2 ml of sungkai leaf extract (P. canescens) in one tube, diluted in 10 tubes, and 1 ml of test microbe in each tube. All tubes were incubated at 37ºC for 1x24 hours, and the diameter of the clear zone formed around the paper disc was measured.

2.2.5. Determination of Antioxidant Activity with the DPPH Method

An antioxidant activity test was carried out using the free radical scavenging effect of DPPH (1,1-Diphenyl-2- Picryl-Hydrazine). The DPPH method refers to Molyneux [21]. Dissolve 1.9 mg of DPPH with 100 ml of methanol to obtain a DPPH solution with a concentration of 0.05 mM. This solution is known as a DPPH solution. Next, 4 ml of 0.05 mM DPPH solution was dissolved with 1 ml of test solution. Antioxidant activity was analyzed using a spectrophotometer with a wavelength of 517 nm. In the antioxidant activity test, Vit C/ascorbic acid was used as a comparison.

2.2.6. Gallic Acid Standard Curve

Gallic acid powder was measured as much as 0.025 g and put into a 100 ml beaker and filled up to 100 ml with distilled water, then homogenized. Gallic acid standard solutions were prepared at various concentrations of 0, 50, 100, 150, and 200 ppm. Pipette 1 ml of gallic acid standard solution and put it into a test tube then add 1 ml of Folin-Ciocalteu reagent and homogenize. After 5 minutes, 1 ml of sodium carbonate solution (Na2CO3) was added, then distilled water was added until the volume reached 10 ml, then homogenized and incubated for 90 minutes, and the absorbance value was measured.

2.2.7. Calculation of Total Polyphenol Content

P. canescens extract was carried out using the Folin-Ciocalteu method with some modifications. Dilute 1 ml of the extract with 4 ml of distilled water. Take 1 ml of *P. canescens* sample, then mix it with 1 ml of Folin- Ciocalteu reagent (a phenol reagent is made with a ratio of (0.25 ml) Folin-Ciocalteu reagent : (2.25 ml) sterile distilled water = 1:9). % was added to the mixture and made up with distilled water until the volume reached 10 ml. Each tube of the solution was filled with 10 ml of distilled water. The tube was stored in a dark place for 90 minutes and the absorbance value was measured using a spectrophotometer at 765 nm. For total activity, the polyphenol test used gallic acid as a comparison.

3. Results and discussion

3.1. Inhibition Zone Diameter

Based on the results of the antimicrobial activity test on fresh extract, fresh infusion, dry infusion, dry infusion plus lime, and decoction of dried *P. canescens* leaves using the diffusion method, the following results were obtained:

Table 1 The average diameter of the inhibition zone for each treatment of the tested microbes.

Diameter of Microbial Inhibition Zone (mm)						
Extract	*E. coli*		*S. aureus*		*C.albicans*	
Fresh extract	7.80	**a**	8.73	**a**	6.00	**a**
Fresh brew	6.03	**e**	6.00	**d**	6.00	**a**
Dry brew	6.10	**d**	6.03	**d**	6.00	**a**
Dry stew	6.96	**b**	7.73	**b**	6.00	**a**
Dry brew + lime	6.53	**c**	6.96	**c**	6.00	**a**

Description = numbers followed by unequal lowercase letters in the column are significantly different at the 5% level of Duncan's test.

From Table 1 it can be seen that all treatments of *P. canescens* leaves showed antimicrobial activity against *E. coli* and *S. aureus* bacteria, but had no effect on *C. albicans s s*. The average diameter of the inhibition zones of fresh extract, fresh brewing, dry brewing, dry brewing plus lime, and dry decoction of sungkai leaves for *E. coli* ranged from 6.1 - 7.8 mm, *S. aureus* ranged from 6 - 8.73 mm and *C. albicans s s* 6 mm. All treatments had significantly different effects on the growth of *E. coli* and *S. aureus* bacteria, but had no significantly different effects on *C. albicans s s*.

The antimicrobial activity of Sungkai leaves (*Peronema canescens*) against *E. coli*, *S. aureus*, and *C. albicans s s* is illustrated in the following figure:

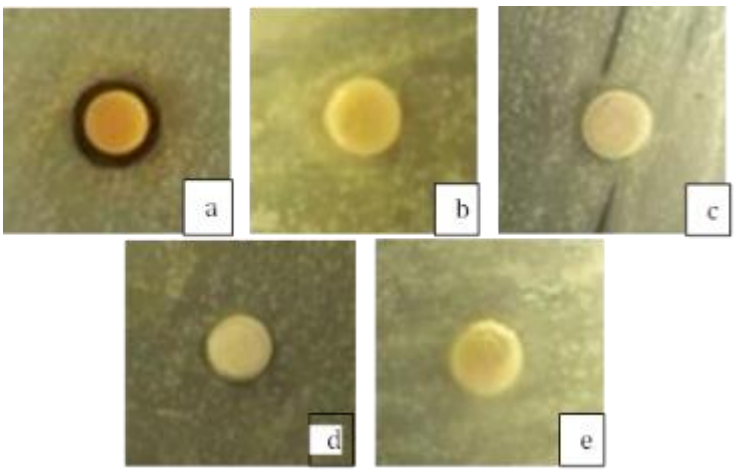

Figure 1 Antimicrobial Activity of Sungkai Leaves Against *Escherichia coli* Test Microbes. a) fresh extract, b) fresh infusion, c) dry infusion, d) dry infusion, e) dry infusion + lime

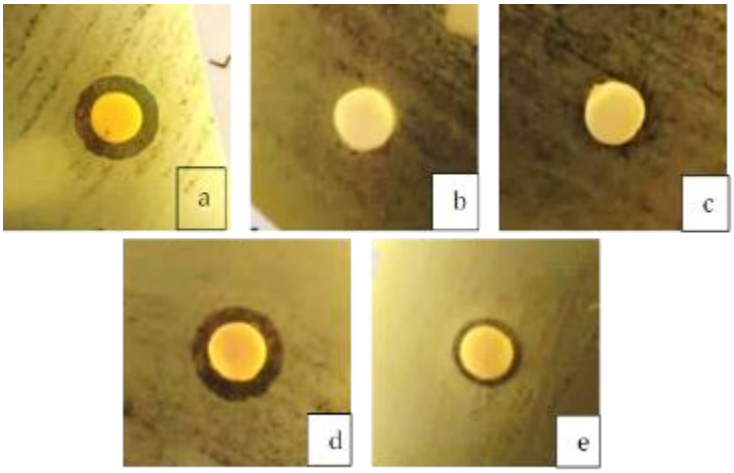

Figure 2 Antimicrobial Activity of Sungkai Leaves Against *Staphylococcus aureus* Test Microbes. a) fresh extract, b) fresh infusion, c) dry infusion, c) dry infusion, d) dry infusion + lime

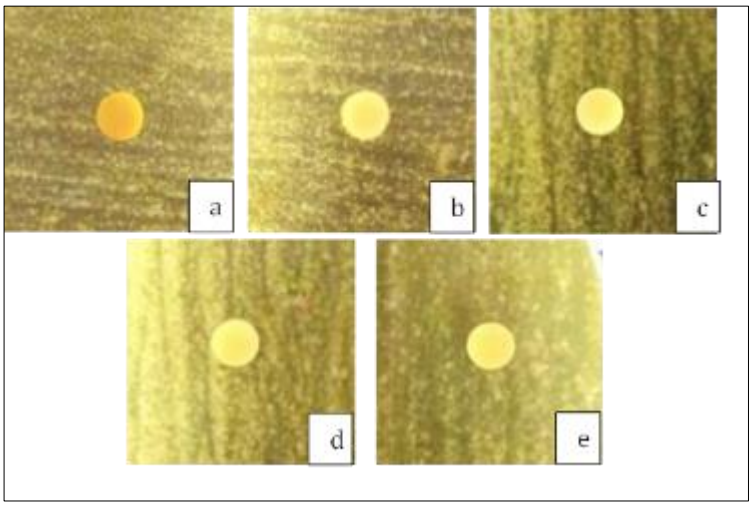

Figure 3 Antimicrobial Activity of Sungkai Leaves Against *Candida albicans* Microbes. a) fresh extract, b) fresh infusion, c) dry infusion, d) dry infusion, e) dry infusion + lime

The difference in the inhibition zone on each bacterium is thought to be influenced by the sensitivity of each test microbe to P. canescens. The sensitivity of the test microbe to antimicrobial substances is influenced by the permeability of the cell membrane. The defense ability of each microbe against the metabolites produced by each extract is indicated by the microbial inhibition zone depending on the type and ability of the antibacterial compounds of each extract component and the number of active components contained in the extract.

The treatment that produced the largest inhibition zone was obtained from the fresh extract, this was because the number of active compounds was sometimes more in the fresh extract than in the other treatments so the resulting inhibition zone was also larger. Some things that can affect antimicrobial substances include the concentration of antimicrobial substances, the content of antimicrobial compounds, the number of microorganisms, temperature, and pH [8]. Where the higher the concentration of antimicrobial substances, the greater the antimicrobial power. After the fresh treatment, the dried decoction also affected the test microbes. Compared to steeping, both dry brewing, and fresh brewing, the boiling treatment produces a larger inhibition zone because during the boiling process the heating of the sample takes place more intensely so that the process of withdrawing secondary metabolites is also more optimal. The higher the heating temperature causes the vacuoles to open, making it easier for the active compounds to exit the cells, especially polyphenols [2].

The dry infusion treatment added with lime also affected test tested mmicrobesaddition of lime in the sample icanascane solubility process of the active compound from the sample itself. Lime contains various kinds of ingredients

in the form of useful chemical compounds such as amino acids (tryptophan and lysine), citric acid, essential oils (limonene, linen acetate, gerliningetate, phellandrene, citral, kadinen, actiaacetaldehydeldehid), vitamin A, vitamins B1 and vitamin C [3]. The content of vitamin C in lime whi thought to help in the process of dissolving active compounds. The mechanism of action of the antimicrobial substances in *P. canescens* against *E. coli* is determined by the permeability of the cell membrane.

Based on Figure 1, the antibacterial activity of the fresh extract of *P. canescens* against *E. coli* resulted in a clear inhibition zone without any single bacterial colony, which was significantly different against dry infusion, fresh infusion, dry infusion, and dry, infusion with lime added, this indicates that the antibacterial activity of the extract fresh *P. canescens* to *E. coli* is bacteriostatic. There is a porin protein in the outer membrane of the cell wall of *E. coli* which functions as a channel for the ry and the d exit of the active compound so that tso that compound in the fresh extract of *P. canescens* will easily enter and damage cell enzyme activity which causes damage to *E. coli* cells [13].

In Figure 2, the antibacterial activity of the fresh extract of *P. canescens* against *S. aureus* produces the largest inhibition zone followed by the dry decoction and dry infusion of P. canescens, this shows that the fresh extract of *P. canescens* has the highest concentration of active antibacterial compounds due to fresh treatment only use water from the plant itself without adding solvent so that the secondary metabolite activity is higher and produces the largest inhibition zone. The higher the concentration of the extract, the greater the activity of the antibacterial compounds released, so the utility of oaabilityompounds to inhibit bacterial growth also increases [10].

In Figure 3 there is no inhibition zone on the *C. albicans s s* test microbe. This is presumably because the active compounds from the *P. canescens* treatment were unable to penetrate the cell walls of *C. albicans s* s. In *C. albicans s* s, chlamydospores are formed, namely asexual spores at the ends of the hyphae which form a thick wall that makes it difficult for antimicrobial compounds to penetrate [4]. The *C. albicans s s* cell wall is glucans, chitin, fatty monoproteinmannoproteinsc salts [12]. The thick fat content in the cell walls of *C. albicans s s* is thought to make it difficult for antimicrobial compounds to penetrate the cell walls. The cell wall is the target of some antimycotics. Candida albicans have highreachigh reactancemicrobials [11].

3.2. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

Based on research that has been done on MIC and MBC against *P. canescens* test microbes, the following results are obtained:

Table 2 MIC and MBC *P. canescens* against the test microbes

Microbes	Extract	MIC %	MBC%
E. coli	Fresh extract	50	-
S. aureus	Fresh extract	25	-

Note: (-) indicates there is no teaching and learning process.

Based on Table 2, it can be seen that the fresh extract of *P. canescens* has a MIC value in *E. coli* at 50% and *S. aureus* is 25%, because the turbidity observation, starting from the concentration of *E. coli* at 50% and *S. aureus* 25% treatment shows clarity close to the clarity of the media as control after incubation for 24 hours, this indicates that the extract can inhibit the growth of the tested microbes. While the MBC for fresh extracts of *P. canescens* for *E. coli* and *S. aureus* was not found. The difference in extract concentrations is thought to be a factor affecting the MIC and MBC *P. canescens* on the test microbes, the extract diffusion method is acan and an inhibition zone against *E. coli* and *S. aureus*, while the concentration of the extract from 0.1% to 50% cannot kill microbes test. The inhibition of bacteria increases as the concentration of an extract increases, because the higher the concentration of the extract, the more active antibacterial ingredients there are. Antimicrobial power is directly proportional to the high concentration of active substances contained in the extract [1].

3.3. Polyphenol and Antioxidant Test Results

Based on research that has been done on polyphenol levels and antioxidant activity of P. canescens, the following results are obtained:

Table 3 Total Content of Polyphenols and Antioxidants (IC50 Value) of P. canescens

Treatment	Polyphenol (mgGAE/ml)	Antioksidan IC50 (µg/ml)
Fresh extract	0.767	98,02
Dry Stew	0.694	115,67
Dry Brew + Lime	0.640	118,39
Dry Brewing	0.644	118,98
Fresh Brew	0.534	119,52

From Table 3 it can be seen that the highest polyphenol content of *P. canescence* was a fresh extract of *P. canescens* of 0.767 mgGAE/ml. The highest antioxidant activity of *P. canescens* was fresh extract at an IC50 value of 98.02 g/ml. The antioxidant activity of *P. canescens* in reducing free radicals (DPPH) is influenced by the content of polyphenols in the ex because polyphenolic compounds have hydroxyl groups that react with free radicals.

In Table 3 it can also be seen that the antioxidant activity of the fresh extract of *P. canescens* is classified as strong, while the antioxidant activity of fresh brew, dry brew, dry brew plus lime, and, dry decoction is moderate. The ability of antioxidants to scavenge free radicals is divided into several groups, namely very strong antioxidants if the IC50 value is less than 50 ppm, strong if the IC50 value is 51-100 ppm, moderate if the IC50 value is 101-150 ppm, weak if the IC50 value is 151-200 ppm, and very weak if the IC50 value is more than 200 ppm [6].

The lowest IC50 value was obtained from the fresh extract with a value of 98.02 µg/mL, this indicated that the fresh extract produced the highest antioxidant activity. The IC50 value can be identified as the concentration that can inhibit free radical activity by 50%, namely the smaller the IC50 value, the greater the antioxidant activity. The greater the concentration of the sample extract, the % value of the antioxidant activity increases as indicated by the lower the I value, this is because the higher the concentration of the sample extract, the more hydrogen donors are given their sample extract to free radicals, causing the antioxidant activity to increase as indicated by the lower the IC50 value [9].

4. Conclusion

Peronema canescens showed antimicrobial activity, where each treatment had a significantly different effect on *E. coli* and *S. aureus* and did not have a significantly different effect on *C. albicans s* s. MIC of *P. canescens* against *E. coli* and *S. aureus* was obtained by 50% and 25%, while MBC was not found. The antioxidant value of *P. canescens* leaves in the fresh extract was obtained IC50 (98.02 g/ml) where this value was included in the strong category of antioxidant activity.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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