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Antibacterial Activity of Acetone Coconut Husk (Cocos Nucifera Linn) Extract Against Staphylococcus Aureus and Escherichia Coli Bacteria

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Abstract. Coconut plant (Cocos nucifera Linn) is an annual plant that is most beneficial to the people of Indonesia, because almost all parts of the plant can be utilized and used for medicine and daily needs. Part of the coconut plant, namely coconut fiber which is considered as waste, can be used as an antibacterial agent because it contains tannin compounds or also called tannic acid which is a complex chemical compound consisting of several polyphenolic compounds which act as antibacterial and antiviral. The purpose of this study was to determine the antibacterial activity of coconut coir acetone extract (Cocos nucifera Linn) against the growth of Staphylococcus aureus and Escherichia coli bacteria. The method used is disc diffusion (Kirby and Bauer test). Coconut coir powder (Cocos nucifera Linn) was macerated with acetone for 3 x 24 hours. Furthermore, the antibacterial test of coconut coir extract (Cocos nucifera Linn) was carried out against Staphylococcus aureus and Escherichia coli bacteria. Chloramphenicol as a positive control and dimethyl sulfoxide (DMSO) was used as a negative control. The results of antibacterial activity that have been carried out show that coco coir extract (Cocos nucifera Linn) with a concentration of 60% is the highest concentration of inhibition zone diameter between 20% and 40% with an average inhibition zone diameter of 11.7 mm against Staphylococcus aureus bacteria and Escherichia coli and belongs to the category of strong sensitivity.

Keywords: Coconut Husk, Antibacterial, Zone Of Inhibition

INTRODUCTION

Coconut (Cocos nucifera Linn) is the most beneficial plant for the people of Indonesia because all parts of the plant can be used and used for treatment and daily needs. Coconut coir is part of the coconut plant that can be used as an antibacterial ingredient because it contains tannin compounds or also called tannic acid which is a complex chemical compound consisting of several polyphenolic compounds (Sumarni, 2019; Oktaviani, 2015). Find in coconut husk ethanol extract contains polyphenol compounds and tannins. In coconut husk water extract there are several compounds, including ellagic acid, gallic acid, tannins and catechins (Silva, 2013; Wulandari, 2018).

Tannin compounds are known to inhibit bacterial growth by inactivating bacterial enzymes and inhibiting the formation of proteins in the inner layer of bacterial cells. Tannins work on the polypeptide wall of the bacterial cell wall so that the formation of the cell wall becomes less perfect and will result in bacterial cells dying (Sapara, 2016). Coconut coir can be used for various diseases such as excessive menstrual drugs, hemorrhoids, vomiting due to bile disorders, excessive acid in the stomach, sore throat and peptic ulcers, roundworm and tapeworm exterminators, and anti-vomiting drugs during pregnancy or cravings. In addition, it can be efficacious as an anti-diarrheal (Ahmad, 2012).

Research examining the antibacterial activity of coconut husk extract (Cocos nucifera Linn) against bacteria Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli) has so far used polar solvents, very few uses semipolar solvents such as acetone. Therefore, in this study, coconut husk extraction (Cocos nucifera Linn) was carried out using maceration method with acetone solvent to determine the antibacterial activity of coconut husk acetone extract (Cocos nucifera Linn) against the growth of S. aureus and E. coli bacteria.

LITERATURE

Coconut coir (Cocos nucifera Linn) is one of the agricultural wastes produced in Indonesia. Coconut coir has various benefits, one of which is as a source of bioactive compounds that have antibacterial activity. The antibacterial activity of coconut husk can be used to overcome infections caused by pathogenic bacteria, such as Staphylococcus aureus and Escherichia coli (Silva et al., 2013; Silva et al., 2015; Nwachukwu et al., 2015; Kumar & Singh, 2019; Temikotan et al., 2021).

Staphylococcus aureus and Escherichia coli are two types of bacteria that often cause disease in humans, both mild and severe. Staphylococcus aureus can cause skin infections, abscesses, osteomyelitis, endocarditis, and toxic shock syndrome. Escherichia coli can cause diarrhea, urinary tract infections, neonatal meningitis, and sepsis. Both of these bacteria also have the ability to develop resistance to available antibiotics, thus posing challenges in medicine (Godijk et al., 2022; Silva et al., 2020; Kumar & Singh, 2019)

Several studies have been conducted to evaluate the antibacterial activity of coconut husk extract against Staphylococcus aureus and Escherichia coli. These studies used various organic solvents and water to extract bioactive compounds from coconut husk, such as methanol, ethanol, acetone, n-hexane, chloroform, ethyl acetate, and n-butanol. These studies also used various methods to test the antibacterial activity of coconut husk extract, such as disc diffusion

method, agar well method, liquid microdilution method, and time-kill method (Lu et al., 2019; Silva et al., 2013; Silva et al., 2015; Nwachukwu et al., 2015; Kumar & Singh, 2019; Temikotan et al., 2021).

The results showed that coconut husk extract has antibacterial activity that varies depending on the type of solvent, extract concentration, type of bacteria, and test method used. In general, coir extract has higher antibacterial activity against Staphylococcus aureus than Escherichia coli. Coir extract also has higher antibacterial activity if it uses organic solvents rather than water. Coir extract also showed increased antibacterial activity as the extract concentration increased (Silva et al., 2013; Silva et al., 2015; Nwachukwu et al., 2015; Kumar & Singh, 2019; Temikotan et al., 2021).

Several bioactive compounds contained in coconut husk extract have been successfully isolated and identified using chromatographic and spectrometric techniques. These bioactive compounds include gallic acid, ellagic acid, catechins, epicatechins, two procyanidin dimers, and condensed tannins. These bioactive compounds are thought to play a role in providing antibacterial activity from coconut husk extract. Several mechanisms of action of biocides from coconut husk extract on bacteria have also been studied, such as damage to bacterial cytoplasmic membranes, leakage of proteins, potassium ions, and nucleotides from bacterial cells (Silva et al., 2015; Belwal et al., 2022; Temikotan et al., 2021).

These studies provide scientific evidence of coconut husk's potential as a source of bioactive compounds that have antibacterial activity against Staphylococcus aureus and Escherichia coli. These studies have also contributed to the development of natural medicines to overcome antibiotic resistance by several pathogens that are currently a problem in agriculture and health. However, these studies still have some limitations, such as variations in the quality and quantity of coconut husk, variations in extraction and test methods, and lack of toxicity tests and in vivo tests. Therefore, further studies are still needed to optimize the antibacterial activity of coir extract and to explore its clinical potential (Silva et al., 2013; Silva et al., 2015; Nwachukwu et al., 2015; Kumar & Singh, 2019; Temikotan et al., 2021; Lu et al., 2019; Belwal et al., 2022).

One analytical method that is often used in chemistry and pharmacy is *Kromatografi Lapis Tipis* (KLT). KLT is a type of liquid chromatography whose stationary phase is a thin layer of uniform particle sorbent in the form of a glass plate, aluminum foil, or plastic (Industrial Pharmacy, 2019). KLT has many advantages, such as simple, cheap, fast, and can analyze many samples at once (Afriansyah, 2019). TLC can also be used for various purposes, such as

compound identification, compound purification, compound purity determination, and compound biological activity determination (Lestari, 2021; Asfiyah, 2020)

METHOD

The tools used in this study were IC55 incubator, autoclave, analytical balance, Buchi rotary evaporator, laminar air flow (LAF), caliper, laboratory glassware, micropipette, petri dish, Buchner funnel, and blender. The materials used in this study were coconut husk extract (Cocos nucifera Linn), S. aureus and E. coli test bacteria, MHA media (Mueller Hinton Agar), technical acetone which was first distilled to remove water, aquadest, and DMSO (Dimethyl sulfoxide) p.a (Merck) which was used as a solvent in antibacterial tests.

The plant material used is coconut husk (Cocos nucifera Linn) obtained from the Karangwangun area, Babakan District, Cirebon Regency. Fresh coconut husk is wet sorted, washed under running water, dried in direct sunlight, dried dry. Next, it is displayed and blended then sifted with mesh 60. Coconut husk simplicial powder (Cocos nucifera Linn) was obtained.

Coconut husk powder (Cocos nucifera Linn) is put into a maceration container and added acetone until all submerged (in a ratio of 1:12). Extraction is carried out for 3x24 hours while stirring occasionally. The maceration results are then filtered using Whatman filter paper. The fiber is then put together and evaporated using a rotary evaporator at 50 ° C and concentrated with a water bath to evaporate acetone solvent so that a thick extract of 25 grams is obtained

Phytochemical screening is carried out to determine the secondary metabolites contained in coconut husk extract (*Cocos nucifera* Linn). These qualitatively tested secondary metabolites include alkaloids, flavonoids, phenols, and tannins.

1. Alkaloid test: A total of 2 mL of coconut husk extract is added HCl. The formation of brown or orange deposits at the base of the test tube indicates the presence of alkaloid compounds (Fachiroh, 2021).
2. Flavonoid test: A total of 2 mL of coconut husk extract added a few drops of 10% NaOH solution. The occurrence of changes in the intensity of orange / orange indicates the presence of flavonoid compounds (Iklinus, 2015).
3. Phenol test: A total of 2 mL of coconut husk extract in a test tube was added 10 drops of 1% FeCl3 solution. The formation of green, red, bluish-black, or purple indicates the presence of phenol compounds (Fachiroh, 2021).

4. Tannin test: Coconut husk extract is put into a test tube as much as 2 mL then added a few drops of 1% FeCl3 solution. The formation of blackish-green or dark blue or blackish color indicates the presence of tannin compounds (Fachiroh, 2021).
5. Saponin test: Coconut husk extract is put into a test tube as much as 2 mL, then added 10 mL hot water and cooled. Shake vigorously for 10 seconds, then add 1 drop of HCl 2 N. If a stable foam as high as 1-10 cm is formed for no less than 10 minutes, then the sample is positive for saponins (Fachiroh, 2021).

Analysis of coconut husk acetone extract (Cocos nucifera Linn) with *Kromatografi Lapis Tipis* (KLT) analyzed using mobile phase n-butanol: acetic acid: water (4: 1: 5) stationary phase silica gel 60 F254 coating plate and as a spot viewer used 1% FeCl3 reagent (Rohaeni, 2016). The mobile phase is fed into a vessel and then saturated using filter paper. The extract solution is tolerated at the lower limit line on the silica gel 60 F254 plate that has previously been activated, then put into a vessel that has been saturated with developer steam and tightly closed, after the elution is complete the plate is removed from the vessel and dried in air, then the plate is sprayed with a spotting appearance solution. The color of the spots that occur is calculated the Rf price.

This antibacterial test is carried out using the disc diffusion method (Kirby and Bauer test) with an indication of inhibition of microorganism growth by antibacterial agents, the surface of the media in order is a clear area. The test bacteria used were Staphylococcus aureus and Escherichia coli.

The sterilized MHA media is put into sterile petri dishes of 20 mL each and allowed to solidify at room temperature. Test bacteria aged 24 hours were evenly etched on the surface of MHA media. Sterile disc paper with a diameter of 6 mm dripped with coconut husk acetone extract (*Cocos nucifera* Linn) as much as 10 µL each concentration of 20%, 40%, and 60% (which has been dissolved with DMSO), then placed on solid agar media that has been applied with test bacteria. The negative control used was pure DMSO solution and the positive control was chloramphenicol. Then incubated at a temperature of 37°C for 24 hours and after incubation, the diameter of the inhibitory zone formed was measured which was characterized by the presence of a clear zone on MHA media using a caliper (Fachiroh, 2021).

DISCUSSION

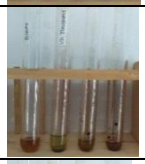
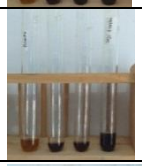
The coconut palm plant has been known in human civilization and is known to grow in the tropics. Part of this plant, namely coconut husk, in the modern era is widely used for research because it is considered potential as medicine. Extraction aims to attract chemical compounds or

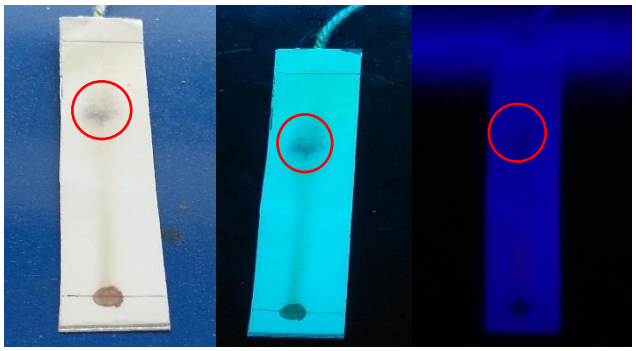
active substances present in the sample. Based on the extraction process, a thick extract of 25 grams was obtained (Figure 1). Coconut husk extract (*Cocos nucifera* Linn) is then carried out phytochemical screening which aims to analyze qualitatively as an initial stage of knowing the secondary metabolite compounds contained in the extract (Agustina, 2021). The phytochemical screening results of coconut husk can be seen in Table 1. Based on the results obtained, it can be seen that coconut husk acetone extract (*Cocos nucifera* Linn) positively contains several compounds, namely alkaloids, phenols, tannins, saponins and shows negative results on flavonoid compounds. This is in line with the results of Nugraha (2020) which states that the ethyl acetate extract of coconut coir varieties in positive contains phenol compounds, tannins, and saponins, but does not contain a group of flavonoid compounds.

Figure 1 Coconut husk acetone extract (*Cocos nucifera* Linn)

KLT is a method of separating chemical compounds based on the difference in the distribution of two phases, namely the stationary phase and the mobile phase. This KLT method is carried out to ensure qualitative test results from the tube test that coconut husk contains polyphenol compounds through UV light. The spots obtained from the KLT test seen by chromatogram profiles under UV light of 254 nm and 366 nm are purple-black (Figure 2). These results show that coconut husk extract positively contains tannin compounds with an Rf value of 0.68 which indicates that compounds with an Rf value are tannin compounds because close to the results of research conducted by Nuraini (2002) states that tannin compounds have an Rf value of 0.7 with a standard tannin Rf value of 0.737.

Table 1 Phytochemical screening test results of *Cocos nucifera* Linn

Classes of compounds	Reagent	Result	Research results	
			Picture	Information
Alkaloid	HCl, Pereaksi Wagner	Positive		Brown deposits are formed
Flavonoid	NaOH 10%	Negative		Green discoloration occurs
Fenol	$FeCl_3$ 1%	Positive		The formation of black color
Tanin	$FeCl_3$ 1%	Positive		Formed blackish-green color
Saponin	air panas dan HCl 2N.	Positive		A stable foam 1 cm high forms for more than 10 minutes

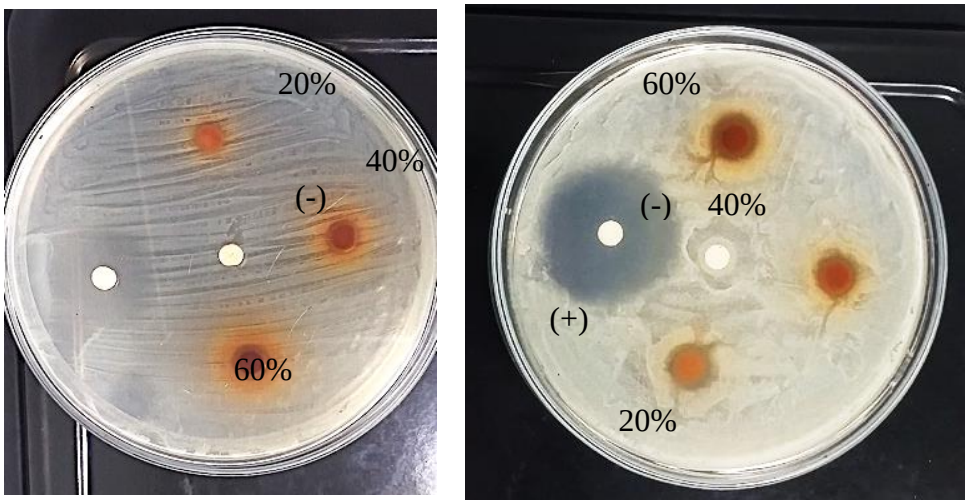


Visible light UV 254 nm UV 366 nm

Figure 2 Thin Layer Chromatographic Profile of *Cocos nucifera* Linn

Determination of the diameter of the inhibitory zone is carried out using the agar diffusion method, namely by looking at the clear zone and measuring the diameter of the clear zone. Based on the results of this study, it can be seen that coconut coir acetone extract *(Cocos nucifera* Linn) has antibacterial activity characterized by the presence of a clear zone in the treatment (Figure 3) with the average value of the diameter of the inhibitory zone listed in table 2 against *Staphylococcus aureus* and *Escherichia coli bacteria.* The diameter of the inhibitory zone was

obtained because based on phytochemical screening tests coconut coir extract (*Cocos nucifera* Linn) contains alkaloid compounds, phenols, tannins, and saponins which are believed to be antibacterial. From the inhibition zone diameter data, it can be seen that the higher the concentration of coconut husk acetone extract, the greater the diameter of the inhibitory zone produced, the smaller the extract concentration, the smaller the diameter of the inhibitory zone, this is because the higher the concentration of coconut husk extract, the higher the active substance in the extract.



S. aureus *E. coli*

Figure 3 Antibacterial Activity of Cocos nucifera Linn Against S. aureus and E. coli Bacteria

Table 2 Diameter of phytochemical inhibitory zone of Cocos nucifera Linn Against S. aureus and E. coli bacteria

Extract concentration	Average diameter of the inhibitory zone (mm)	
	Staphylococcus aureus	*Escherichia coli*
20%	0	10,9
40%	9,5	9,7
60%	10,7	11,7
Positive control (Kloramfenikol)	22	32
Check negative (DMSO)	0	0

The concentration of 60% coconut husk extract is the best concentration in inhibiting the growth of *S. aureus* and E. coli *bacteria* with the highest average inhibitory zone diameter value compared to 40% and 20% concentrations, namely 10.7 mm and 11.7 mm. Coconut husk acetone extract (*Cocos nucifera* Linn) is able to inhibit *Escherichia* coli bacteria greater than Staphylococcus *aureus bacteria because judging from the average result of* E. coli inhibitory zones *greater than* S. aureus. *E. coli* bacteria are gram-negative bacteria that have thin peptidoglycan so that they are easily damaged by antibacterial compounds such as alkaloids, tannins, phenols, and saponins contained in coconut husk extract (*Cocos nucifera* Linn). This is in line with Wulandari's research (2018) which states that young, half-old, and old coconut husk ethanol extract has a high inhibitory power against gram-negative bacteria compared to gram-positive bacteria.

CONCLUSION

The results of the study found that coconut husk acetone extract (Cocos nucifera Linn) was positive for alkaloid compounds, phenols, tannins, saponins and showed negative results on flavonoid compounds. Coconut husk acetone extract (Cocos nucifera Linn) with a test extract concentration of 60%, 40%, 20% has antibacterial activity characterized by the presence of clear zones in treatment.

Coconut husk acetone extract (Cocos nucifera Linn) is able to inhibit Escherichia coli bacteria greater than Staphylococcus aureus bacteria because judging from the average results of the inhibitory zone of Escherichia coli bacteria the highest diameter of the inhibitory zone is between 20% and 40% with an average value of 11.7 mm inhibitory zone diameter against Staphylococcus aureus and Escherichia coli bacteria and is included in the strong sensitivity category.

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