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The Utilisation of Coconut Water Waste into Nanocellulose and Potential Synergistic Effect of Patikan Kebo (*Euphorbia hirta L.*) Extract as an Antibacterial Agent

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Abstract

Skin infections contribute to one of the most significant global health issues. Acne, blisters, and abscesses are examples of skin infections that can be induced by Propionibacterium acnes, Staphylococcus aureus, and Staphylococcus epidermidis. Treatment can be attempted through the development of nanotechnology in the form of nanocellulose. Nanocellulose, an organic substance, can be produced through the conversion of coconut water waste and Acetobacter xylinum bacteria. Future applications of nanocellulose formulated with a blend of extracts derived from natural sources, including Patikan Kebo (Euphorbia hirta L.), are anticipated to include the treatment of wound dressings (patches). Determining the antibacterial potential of nanocellulose was the objective of this study. The experimental research technique is implemented in phases. The extraction process was conducted by means of maceration, while the parallel streak method was utilized for antibacterial testing. The findings of the study indicated that the mean zone of inhibition for each treatment variable was as follows: 0 mm, 15.1 mm, 23.53 mm, and 23.06 mm for nanocellulose (serving as the negative control), nanocellulose and 0.5% extract, 1% extract, 1.5% extract, and clindamycin, respectively. Conclusion: Antibacterial activity was observed in nanocellulose extracts containing 1% and 1.5%

Keywords: *Antibacterial, Euphorbia hirta L, Coconut Water Waste, Nanocellulose*

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INTRODUCTION

Infectious infections are prevalent in both developing and developed countries. Infectious diseases are the result of various microorganisms, including bacteria, parasites, viruses, and fungi (Novaryatiin *et al.*, 2018). Pathogenic microorganisms, including *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Propionibacterium acnes*, have the potential to induce skin infections, leading to the development of conditions such as boils and acne (Abu & Tandah, 2015).

Nanotechnology harnesses the characteristics of molecules or atomic structures at the nanoscale scale to develop novel technologies with numerous advantages (Sijabat *et al.*, 2017). Nanocellulose is a notable outcome of the application of nanotechnology. Nanocellulose is a product of nanotechnology that has a high concentration of cellulose. It serves as a versatile material, functioning as a filler, biodegradable substance, reinforcement, thickener, composite, adsorbent, and drug delivery system. Nanocellulose is distinguished by its enhanced crystallinity, aspect ratio, surface area, and dispersing ability (Effendi *et al.*, 2015). The raw material for making nanocellulose comes from sago pulp waste (Myrra *et al.*, 2018), coconut water (Sijabat *et al.*, 2017), ambon banana peel (Nugraha *et al.*, 2021). Most of the coconut water used is thrown away because it has no price. However, another use of coconut water has been used in making *nata de coco*.

Nata de coco is a culinary product that is created through the process of fermenting coconut water using *Acetobacter xylinum* bacteria. Coconut water is a safe beverage to eat directly due to its high fiber content, amino acids, minerals, and low calorie count (Tinentang *et al.*, 2021). Utilizing bacterial cellulose (*Acetobacter xylinum*) in the healthcare industry is feasible by combining nanocellulose with *Patikan Kebo* extract, which possesses the capability to function as an antibacterial agent.

Patikan Kebo plant (*Euphorbia hirta L.*) is extensively utilized in traditional medicine. *Patikan Kebo* plant (*Euphorbia hirta L.*) possesses numerous therapeutic properties for treating a range of ailments including diarrhea, asthma, bronchitis, and inflammation (Zulkarnain *et al.*, 2021). This plant possesses chemical components, including flavonoids, alkaloids, tannins, and phenolic compounds, which enable it to treat a wide range of illnesses. *Patikan Kebo* plant (*Euphorbia hirta L.*) extract possesses inhibitory effects on the growth of *Staphylococcus epidermidis*. The presence of tannins and flavonoids in patikan kebo can lead to its antibacterial properties (Simanjuntak & Rahmiati, 2021). Nanocellulose may be generated by fermenting *Acetobacter xylinum* with coconut water waste, whereas the extract of *Patikan Kebo* plant (*Euphorbia hirta L.*) exhibits promising antibacterial properties. Therefore, this research aimed to determine the potential of patikan kebo extract combined with nanocellulose from coconut water waste as an antibacterial.

METHOD

Instruments and materials

The instruments used in this research were petri dishes, ovens, incubators, autoclaves, bunsens, sieves, trays, Erlenmeyers, magnetic stirrers, stirrers, analytical balances, wire meshes, measuring cups, and calipers.

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The materials were coconut water waste, granulated sugar, ZA (*Zwavelzure ammonium*), 5% NaOH, DMSO, filter paper, plastic wrapping, aluminum foil, newsprint, Aquades, *Acetobacter xylinum*, NA, *Patikan Kebo* plant (*Euphorbia hirta L.*), and *Staphylococcus epidermidis*.

Making *Patikan Kebo* Extract (*Euphorbia hirta L.*) Maceration Method

600 grams of *Patikan Kebo* herbal simplicia were weighed and then soaked in 6000 ml of ethanol in a glass bottle for ± 3 days, protected from light, with occasional stirring. Then, it was filtered with filter paper to produce macerate. Then the macerate was concentrated using a rotary evaporator at a temperature of 60^{o}C until a thick extract was produced.

Making Nano cellulose from Cellulose Bacteria

1 liter of coconut water waste was used, then filtered with a cloth to remove dirt, and then put into a glass beaker. 25 grams of granulated sugar and 5 grams of ZA were added, then homogenized with a magnetic stirrer. It was heated on the stove until it boiled. It was left to cool and cover to prevent contamination. After that, it was poured into a sterile tray and add 10 ml of *Acetobacter xylinum* starter. Then it was covered by the container with sterile newspaper, and then ferment for 7 days at room temperature. the nata layer was observed by that forms.

Making Test Materials

Nanocellulose Preparation

The nanocellulose formed was soaked in 5% NaOH for 3 hours with the aim of neutralizing the acidic atmosphere. Nanocellulose was cut to a size of 5 x 2.5 cm. Then, it was dried in the oven at 40^{o}C for one day.

Preparation of Patikan Kebo Extract

Variations in extract concentration used consisted of 0.5%, 1% and 1.5%. It was weighed 0.5 grams of extract dissolved in 100 ml DMSO (0.5%), 1 gram of extract dissolved in 100 ml DMSO (1%), 1.5 grams of extract dissolved in 100 ml DMSO (1.5%) .

Treatment variables consisted of:

a. Nanocellulose (negative control)
b. Nanocellulose + 0.5% Extract
c. Nanocellulose + 1% Extract
d. Nanocellulose + Extract 1.5%
e. Nanocellulose + Clindamycin (as positive control)

Parallel Streak Method Antibacterial Testing

It was poured for 1 ml of *Staphylococcus epidermidis* bacterial suspension into a sterile petri dish. Then, it was poured for 20 ml of NA media and homogenize. It was left to be cool and solidify. Nanocellulose treatment (negative control nanocellulose, nanocellulose with clindamycin, nanocellulose extract 0.5%, 1% and 1.5%) were placed on NA media. Then, 1 cycle of *Staphylococcus epidermidis* bacterial culture was taken and then scratched on the surface of each nanocellulose 5 times). It was wrapped for the petri dish in plastic wrapping and then incubate for 1 x 24 hours at 37°C in the incubator. The inhibition zone formed around the nanocellulose was calculated.

RESULT AND DISCUSSION

Nanocellulose Formation

Nanocellulose was formed on the 7th day. The formation of nanocellulose is characterized by the formation of a layer of cellulose (nata) on the surface of the media which is cloudy white in color, has a chewy texture and has a sour aroma.

Figure 1. Formation of Nanocellulose (Nata)

Antibacterial Activity Test Results

Observations of the antibacterial activity test were seen from the absence of growth of *Staphylococcus epidermidis* bacteria on the nanocellulose surface which can be seen in table 1.

Table 1. Nanocellulose Clear Zone Measurement Results

Variable	Clear Zone (mm)			Average (mm)
	Test 1	Test 2	Test 3	
Nanocellulose (negative control)	0	0	0	0
Nanocellulose + 0.5% Extract	0	0	0	0
Nanocellulose + 1% Extract	0	20,3	25	15,1
Nanocellulose + Extract 1.5%	20,6	25	25	23,53
Nanocellulose + Clindamycin	23,5	21	24,7	23,06

Ns (Nanocellulose) NS+Clindamycin Ns+Extract 0.5% Ns+Extract 1% Ns+Extract 1.5%

Based on the table and image above, it can be explained that nanocellulose without extract shows the growth of *Staphylococcus epidermidis* bacteria. Nanocellulose mixed with clindamycin and a mix of 1.5% and 1% patikan kebo extract stopped bacteria from growing on nanocellulose. This means that it can kill *Staphylococcus epidermidis* bacteria.

The combination of clindamycin and nanocellulose exhibited antibacterial efficacy. Clindamycin, an antibiotic developed from lincomycin, is utilized to treat many infectious disorders affecting the skin, lungs, blood, female reproductive systems, and internal organs. Clindamycin inhibits protein synthesis by attaching to the 50S ribosomal subunit, which explains why it has this effect. Furthermore, clindamycin functions by impeding the growth of Gram-positive bacteria and anaerobic bacteria, but it does not affect Gram-negative or aerobic bacteria (Hartanto *et al*., 2020). The combination of nanocellulose and clindamycin exhibited complete inhibition of bacterial growth on nanocellulose.

The combination of nanocellulose and patikan kebo extract at concentrations of 1% and 1.5% exhibited antibacterial properties, as evidenced by the absence of bacterial growth on the nanocellulose substrate. The presence of chemical components in patikan kebo gives it antibacterial properties. The patikan kebo extract contains flavonoids, alkaloids, saponins, tannins, steroids, and triterpenoids (Simanjuntak & Rahmiati, 2021). Flavonoids function as antibacterials by creating intricate compounds that target extracellular proteins. These compounds disturb the structure of bacterial cell membranes by altering the structure of proteins and causing harm to the cells (Haryati *et al*., 2015). Alkaloid compounds exhibit antibacterial properties by triggering the degradation of bacterial cell wall components, leading to more effective cell wall breakdown and subsequent cell death (Amelia *et al*., 2020). Furthermore, alkaloid chemicals have the ability to disrupt the process of nucleic acid synthesis in bacteria, as demonstrated by Wijaya *et al*., (2019). Saponin compounds exert their antibacterial effect by destabilizing the surface tension of cell walls, facilitating the entry of the antibacterial substance into the cell. Once inside, the antibacterial substance disrupts the bacteria's metabolism, ultimately leading to their demise. Saponin is a bactericidal compound that hinders the activity of microbial cell membranes. Saponins create intricate compounds with cell membranes via hydrogen bonding, disrupting the permeability characteristics of cell walls, resulting in the liberation of cellular contents and subsequent cell demise (Mathematics, 2016).

Tannin exhibits antibacterial properties by inducing cell lysis. This occurs due to the affinity of tannin for the polypeptide walls of bacterial cells, leading to impaired synthesis of the cell walls and subsequent cell death. Tannins also have the ability to inactivate bacterial enzymes and impede the flow of proteins in cell layers (Ngajow *et al*., 2013). Triterpenoid chemicals exert their antibacterial effects by interacting with the active site of bacterial cell membranes, causing harm either through chemical reactions or by disrupting the lipid components and enhancing their permeability. The composition of bacterial cell membranes mostly comprises phospholipids and protein components. Enhanced permeability allows antibacterial chemicals to penetrate the cell and either rupture the cell membrane or solidify the cytoplasm of the bacterial cell (Situmorang *et a*l., 2019).

According to Barja, (2021), explains that nanocellulose can be used in regenerative medicine (replacing damaged tissue or organs) if combined with other components needed to accelerate healing, through mechanisms that facilitate adhesion, proliferation, migration and differentiation such as in membranes for wound dressings. . Previous research explains that Nanocellulose with Ag provides great benefits in avoiding infectious bacteria such as *E.coli*, *S.aureus*, *K.pneumoniae*, *B.subtilis*, and *P.aeruginosa*. In this case, the properties of nanocellulose act as a composite, adsorbent and drug delivery. Where the phytochemical compounds contained in the extract are composited in nanocellulose, thereby optimizing the work of the phytochemical compounds as antibacterials.

CONCLUSION

The research findings indicate that nanocellulose, when combined with 1% and 1.5% Patikan Kebo extract, exhibits antibacterial properties.

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