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Article

Antibacterial Effects of Green Tender Coconut Water (*Cocos nucifera L.)* as a Natural Root Canal Irrigation Material on the Growth of *Enterococcus Faecalis in Vitro*

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Abstract: Green coconut (*Cocos nucifera L.*) is a plant that has many benefits for medication. Green coconut water has antibacterial effects because of the presence of monolaurin and chemical agents such as tannins, flavonoids, saponins, phenolics, and terpenoids. Recognized as the most resistant pathogen in the oral cavity, *Enterococcus faecalis* commonly appears in cases following root canal treatment. The existence of *E. faecalis,* which is able to survive in extreme environments, will lead to endodontic failure, so the elimination of *E. faecalis* is required. This study aimed to determine the antibacterial effect of green coconut water (Cocos nucifera L.) on inhibiting Enterococcus faecalis growth through an in vitro study. This study is an experimental laboratory with *a* post-test only control group design and consisted of 3 control groups of coconut water, such as natural, pasteurized, and fermented coconut water; chlorhexidine as a positive control; and aquades as a negative control, with 5 repetitions. We performed phytochemical tests on the green coconut water before the inhibition test to identify flavonoids, alkaloids, tannins, saponins, phenolics, terpenoids, and steroids. The green coconut water phytochemical test showed only a positive value for flavonoids, whereas alkaloids, tannins, saponins, phenolics, terpenoids, and steroids showed a negative value. The diffusion test revealed only positive results in the fermented coconut water group at 2nd, 3rd, and 4th repetitions, with a positive control group at all repetitions. The study concluded that green coconut water did not inhibit Enterococcus faecalis growth.

Keywords: Green Coconut Water; Root Canal Irrigation Solution; *Enterococcus faecalis.*

1. Introduction

Caries that reach the pulp will cause pain that disrupts a person's quality of life because functional activities such as eating, brushing teeth, and even sleeping are disrupted [1]. The pain arises because caries continues, resulting in bacterial invasion through the dentin, which stimulates pulpitis and ends in infection and even pulp necrosis [2]. The root canal treatment procedure is a conservative treatment that needs to be carried out to maintain the function of a tooth that has an irreversible pulp infection, or pulp necrosis [3].

Root canal treatment, or endodontic treatment, is a procedure that aims to reduce pain, control infection of the pulp and surrounding periapical tissue, and restore the function of the tooth being treated [4]. Endodontic therapy consists of three main pillars (the endodontic triad), namely biomechanical preparation, including cleaning and shaping, disinfection, and root canal obturation. Cleaning and shaping is a very important phase to support the success of root canal treatment [5].

Cleaning and shaping is a procedure for removing vital and necrotic pulp tissue, microorganisms, and remaining residual. Cleaning and shaping procedures will cause accumulation of debris in the pulp chamber [6]. Based on this, a debridement procedure is required, by flowing irrigation solution into the root canal so that residual necrotic tissue from the settled pulp can be removed [7]. Inadequate cleaning can cause irritants to remain in the pulp area which can lead to endodontic treatment failure leading to secondary infection [8].

In secondary root canal infections, the bacteria considered to be the causative microorganism is Enterococcus faecalis. E. faecalis bacteria are gram-positive bacteria and facultative anaerobes. Even though it is considered a low-grade pathogen, the existence of E. faecalis which is able to survive in extreme environments can disrupt endodontic therapy so that elimination of this bacteria is necessary [9].

Irrigation is one of the most essential indicators of success in root canal treatment [10]. Root canal irrigation is ideally carried out to clean debris and necrotic tissue as well as bacteria that accumulate in the pulp chamber. The ingredients most often used are ethylene diamine tetraacetic acid (EDTA) and sodium hypochloride (NaOCL). However, it was found that the use of EDTA as an irrigation can cause significant damage, namely dentin erosion [11]. Sodium hypochloride has toxic side effects if it enters the periapical tissue accidentally, causing tissue damage accompanied by pain, swelling and bruising [12]. Chlorhexidine (CHX) is also used as an irrigation solution. CHX has broad spectrum antibacterial activity that is effective against gram-positive bacteria [13]. If the use of CHX as an irrigation is mistaken, it can trigger inflammatory reactions, tooth pigmentation, desquamative gingivitis and a metallic taste in the mouth [14].

The green coconut plant (Cocos nucifera L.) has many benefits in human life, including coconut water [15]. Green coconut water also has anti-toxic properties because it contains the most tannins compared to other types of coconut [16]. Tannins are antibacterial which inhibit the growth of bacteria [17]. Apart from tannins, there are also antibacterial agents in the form of monoglycerides (monolaurin) which are effective in destroying lipid-coated bacteria and a number of other polyphenols such as flavonoids, alkaloids, saponins and terpenoids[18, 19]. Based on its antimicrobial properties, young coconut water has been proven to be used as a root canal irrigation solution [15]. It was found that young coconut water was effective in removing the smear layer during endodontic treatment [20].

Research by Nasimuddin et al. (2016) shows that there is antimicrobial activity in coconut water which inhibits the growth of gram-positive and negative bacteria including S. aureus, E. coli, K. pneumoniae, and P. Aeruginosa [21]. However, the antibacterial effect of green coconut water on Enterococcus faecalis is not yet known. Based on this, researchers are interested in knowing the antibacterial effect of green coconut water in inhibiting the growth of E. faecalis as an alternative root canal irrigation solution which is much safer and has minimal side effects on the oral cavity.

2. Material and Method

This research is a laboratory experimental study with a post-test only control group design. This research was conducted from September to October 2023 at the Laboratory of Department of Chemistry, Faculty of Science, Univesitas Syiah Kuala and Fundament Science Laboratory Banda Aceh. The population of this study was a colony of Enterococcus faecalis bacteria.

The sample chosen for this research was coconut water obtained from 3 month old young green coconuts obtained from a green coconut plantation located in Gampong Jaboi, a highland area in the city of Sabang. In this study there were 5 treatment groups consisting of natural light green coconut water (unpasteurized & fermented), pasteurized light green coconut water, fermented light green coconut water, 2% chlorhexidine (CHX) as a positive control and distilled water as a negative control. The research consisted of Phytochemical Tests and Antibacterial Tests using the disc diffusion method (Kirby Bauer) with 5 repetitions.

2.1 Materials

The ingredients used in this research include: Tender green coconut water, Chlorhexidine 2%, Aquades, Enterococcus faecalis isolate, Mc Farland 0.5 solution, Brain Heart Infusion Broth (BHIB) media, Mueller-Hinton Agar (MHA) media, Dry yeast (dry baker's yeast), Aluminum foil.

2.2 Methods

The Enterococcus faecalis isolate used in this study was obtained from the Fundament Science Laboratory in Banda Aceh. The isolate was grown on slanted agar media. After that, it was incubated for 24 hours at 37°C. The colonies that grow are selected as good colonies, namely colonies that are round or ovoid, clear, and arranged in the form of a chain. Bacterial colonies resulting from growth for 24 hours were suspended in 0.5 ml

of liquid BHI and incubated for 5-8 hours at 37°C. Sterile distilled water was added to the bacterial suspension in BHI, so that the turbidity was in accordance with the Mc Farland bacterial concentration standard of 0.5, equivalent to 1.5,108 CFU/ml. Enterococcus faecalis which has been standardized with Mc Farland 0.5 solution is equivalent to 1.5,108 CFU/ml. taken with a sterile cotton swab, then smeared on Muller Hinton agar media. After that, place the paper disk (samir paper) which has been soaked in the treatment for 3 hours, then incubate the test media at a temperature of 37°C for 24 hours. After that, the results of the size of the bacterial growth inhibition zone are read, measured with calipers in millimeters (mm).

3. Results

The phytochemical test was carried out as a preliminary test to see the content of phytochemical compounds contained in green coconut water. Phytochemical test results can be seen in the following table:

Table 1. Phytochemical Test Results of Green Coconut Water

Metabolite Compounds	Results +/-
Alkaloids	-
Saponins	-
Flavonoids	+
Phenolic	-
Tannin	-
Steroids	-
Terpenoids	-

Note: (+) indicates a positive result and (-) indicates a negative result

Coconut water is said to contain several secondary metabolites that can act as antibacterial agents. Based on the results of the phytochemical screening test on green coconut water used as a research sample, the secondary metabolite compounds contained in green coconut water are flavonoids (Table 1). The research results show findings that are quite different from other previous studies. Research by Khasanah (2018) shows the presence of tannin content in the results of phytochemical screening of green coconut water [22]. Different results were also shown in Sartika's (2019) research, the green coconut water used for research did not show any secondary metabolite content [17].

In this study, the identification of flavonoids was said to be positive because a red or orange color appeared after adding Mg powder concentrated HCl solution. The results of testing flavonoids from green coconut water in this study were that the solution changed color to pink, which indicated that the sample contained flavonoid compounds. The formation of red or orange color in the flavonoid test is a reaction due to the addition of Mg and HCl metals resulting in reduction of the benzopyrone nucleus in the flavonoid structure and forming flavilium salts [23].

Table 2. The Inhibitory Power of Green Coconut Water (*Cocos nucifera L.*) against *Enterococcus faecalis* bacteria using the Kirby Bauer disc diffusion method

No	Sample	Clear Zone				
		1st time	2nd times	3rd times	4th times	5th times
1	Natural Green Coconut Water	-	-	-	-	-
2	Pasteurized Green Coconut Water	-	-	-	-	-
3	Fermented Green Coconut Water	-	+	+	+	-
4	Aquades	-	-	-	-	-
5	Chlorhexidine 2%	+	+	+	+	+

Note: (+) indicates a positive result and (-) indicates a negative result

Based on Table 2. The antibacterial effectiveness test of Green Coconut Water (Cocos nucifera L.) against Enterococcus faecalis bacteria shows that the results obtained are that coconut water is unable to inhibit the growth of Enterococcus faecalis bacteria as a whole, while the positive control in the form of 2% Chlorhexidine shows significant results in inhibiting the growth of Enterococcus faecalis bacteria.

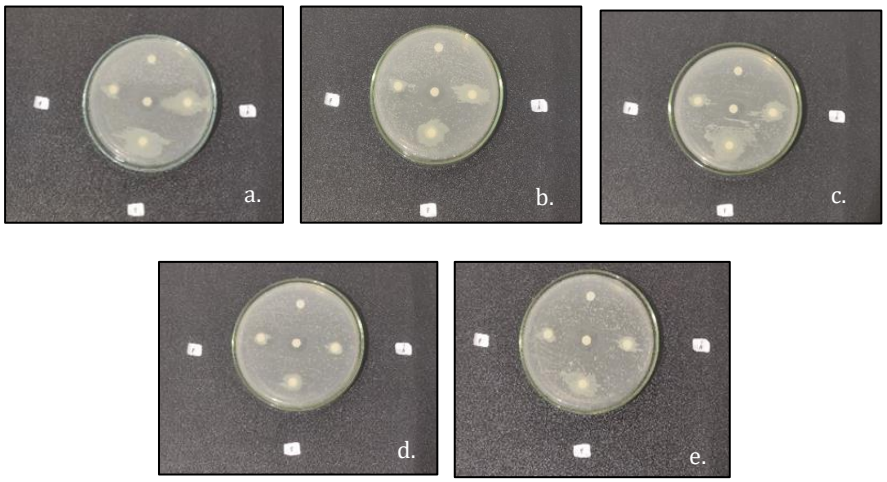

Figure 1. Inhibition zone formed in 1st repetition (a), Inhibition zone formed in 2nd repetition (b), Inhibition zone formed in 3rd repetition (c), Inhibition zone formed in 4th repetition (d), and the Inhibition zone formed in 5th repetition (e)

4. Discussion

The antibacterial testing of green coconut water was carried out using the disc diffusion method (Kirby Bauer test) with 3 different coconut water treatment groups; natural tender green coconut water, pasteurized tender green coconut water, and fermented tender green coconut water, with five repetitions of the test.

Observation of the test results was carried out after 24 hours of the research time, and showed that there was no inhibition zone formed around the paper disk in the natural and pasteurized tender green coconut water treatment group, while in the fermented tender green coconut water control group, an inhibition zone was found to be formed, thinly around the paper disc in the 2nd, 3rd and 4th repetitions (Figure 1).

Another study by Kurniah (2012), tested the antibacterial ability of 1-3 month old green coconut water on several test bacteria and positive results were only found on the bacteria Staphylococcus aureus, Pseudomonas aeroginosa, Vibrio sp and Bacilus subtilis, while the bacteria Salmonella thyposa and Escherichia coli showed negative results [24]. Different results were also shown by research by Sartika (2019) testing the antibacterial ability of 2.4 month old green coconut water on S. typhosa and E. coli bacteria, but the results obtained were that green coconut water was unable to inhibit the two tested bacteria [17].

In this study, there were differences in results between the fermented young green coconut water group. In this study, fermented young green coconut water experienced a decrease in pH reaching 4.6. Enterococcus faecalis is a very resistant pathogen, this bacteria can live in very extreme environments, even at an acidic pH environment so it is very difficult to eliminate [25]. Therefore, because the pH of fermented young green coconut water has reached an acidic pH and the sugar content in coconut water has decreased, it can partially inhibit the growth of E. faecalis bacteria, but its antibacterial power is still relatively low so it is not able to kill all the E. faecalis around the paper disc.

Apart from that, differences in the type of coconut and the age of the coconut have led to inconsistencies in test results in several studies. Khasanah's research (2018) shows that it has antibacterial capabilities against Salmonella thyposa and Escherichia coli bacteria, and the best is for the medicinal green coconut type [22]. The opinion that the age of the coconut can influence the antibacterial ability of coconut water was driven by research by Risda (2019) which used green coconut water as a blood agar solvent to accelerate the growth of Enterococcus faecalis. In his research, it was stated that the older the coconut water, the higher the fructose and glucose content in coconut water. Apart from that, the older the coconut water, the higher the nutrients in the coconut water [26].

In this research, the sample used was 3 month old green coconut water. So it can be concluded that 3 month old coconut water has a high sugar and nutrient content when compared to younger coconuts, so that coconut water is actually a source of energy for the development of Enterococcus faecalis, and did not inhibit the growth of the bacteria in this study.

5. Conclusion

The results showed that green coconut water (Cocos nucifera L.) had no effect in inhibiting the growth of Enterococcus faecalis bacteria. The inability of young coconut water to inhibit the growth of Enterococcus faecalis bacteria is thought to be due to the minimal content of secondary metabolites in green coconut water. Apart from that, differences in age and type of coconut strengthen the suspicion that not all coconuts have anti-bacterial capabilities.

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Conflicts of Interest: The authors declare no conflict of interest.

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