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Antibacterial Activity of Ethanol Extract of Aceh Patchouli Leaves (*Pogostemon cablin* Benth.) against *Enterococcus faecalis*: A Potential Alternative for Root Canal Infections

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Abstract

The persistence of bacterial infections and antibiotic resistance in secondary root canal infections necessitate effective antimicrobial approaches utilizing natural compounds. This study aimed to evaluate the extraction efficiency, phytochemical composition, and antibacterial activity of the ethanol extract of Aceh patchouli leaves (*Pogostemon cablin* Benth.) against *Enterococcus faecalis*. The maceration method was employed for extraction and phytochemical analysis was conducted using gas chromatography-mass spectrometry (GC-MS). Antibacterial activity was assessed using the Kirby-Bauer method, and the minimum inhibitory concentration (MIC) was determined using UV-vis spectrophotometry. The results indicated a total extract yield of 17.25 % and significant antibacterial activity against *E. faecalis*. The active compounds in the extract were primarily sesquiterpenes and fatty acids, with patchouli alcohol as the most prominent. The MIC value was 12.5 %, suggesting the potential of Aceh patchouli extract as an alternative or adjunctive treatment for root canal infections. This study has the potential to make Aceh's indigenous *Pogostemon cablin* Benth. plant as an alternative or adjunctive treatment for root canal infections leading to safe and effective treatments that minimize the risk of antibiotic resistance.

Keywords: *Enterococcus faecalis*, Antibacterial, Minimum inhibitory concentration (MIC), *Pogostemon cablin* Benth, Ethanol extract, Gas chromatography - mass spectrometry (GC-MS)

Introduction

Bacterial infections in the root canal present a significant challenge in endodontics, with research indicating a wide prevalence range of approximately 27 to 70 % in different populations [1]. The persistence of bacteria in these infections is a major obstacle in their management, as they can survive even after thorough cleaning and shaping of the root canal system [2]. The high prevalence of bacterial root canal infections and the challenges associated with bacterial persistence underscore the need for effective antimicrobial strategies and innovative approaches, including natural products, to improve treatment outcomes.

Secondary infections of the root canals, an outcome of caries, are the most common cause of endodontic infections, where a microorganism penetrates the pulp and colonizes the root canal system [1, 3]. *Enterococcus faecalis*, a gram-positive cocci bacterium, is notably refractory to root canals, even in environments devoid of nutritional intake [4]. Its ability to survive environmental hazards and eventually endure an antibiotic assault makes *E. faecalis* as one of the principal pathogens in endodontic infections.

Because most bacteria have become resistant to conventional modern drugs, researchers searching for new antimicrobial agents are encouraged to study natural agents coming from Asiatic or Pacific areas, including Aceh patchouli plants (*Pogostemon cablin* Benth) [5]. Patchouli (*Pogostemon cablin* Benth.) belongs to a plant that comes from the Lamiaceae family and can be found in tropical areas such as India, China, Indonesia, and Philippines [7]. Plantation of patchouli has long been done by local populations in Indonesia, namely in the provinces of Aceh, North Sumatra, Bali, West Sumatra, Lampung, West Java, Central Java, and East Java for the use of this plant as herbal medicine [8]. Patchouli are alkaloid and flavonoid compounds with antibacterial properties. Other antibacterial compounds found in Aceh patchouli are phenolics, triterpenoids, and glycosides, where alkaloids are the most abundant secondary metabolite compounds found in nature [9]. Plant essential oils, particularly patchouli alcohol, have been identified as key components with significant antimicrobial activities.

This study aims to determine the potency of extraction, phytochemical composition as well as antimicrobial activity of ethanol extract of Aceh patchouli leaves (*Pogostemon cablin* Benth.) against *Enterococcus faecalis*. The study determines the MIC value and assesses the possibility of using Aceh patchouli extract of Aceh patchouli leaves as an alternative or adjunctive treatment for root canal infections.

Materials and methods

Preparation of ethanol extracts

Patchouli leaf (*Pogostemon cablin* Benth.) the variety Lhokseumawe (Aceh) used in the present study were kindly provided by Nino Park, Atsiri Research Center (ARC-PUI PT), Universitas Syiah Kuala, Banda Aceh, Indonesia. Patchouli leaves were washed and dried at room temperature. The dried patchouli leaves were ground in a blender to obtain a powder and added to the funnel to filter for finer consistency. The coarse powder (2 kg) was weighed, and an ecopharmacological approach was used for verification; maceration was carried out using 96 % ethanol as the solvent. The fine powder obtained (1 kg) was soaked in 7 L of 96. % ethanol for 5 days. The mixture was covered with aluminum foil to avoid sunlight and stirred once a day. After 5 days, the mixture was filtered and the macerate and dregs were separated. Dregs were soaked again with another 3 litters macerating solution for 5 days. The 2 macerates were collected and added to a rotary vacuum evaporator for drying with ethanol at 40 °C to obtain a thick extract. The total extract yield was calculated using the formula [10].

$$\text{Total extract yield (\%)} = \frac{\text{Extract weight}}{\text{Dry simplicial weight}} \times 100 \quad (1)$$

The thick extract of Aceh patchouli leaves was dissolved in sterile distilled water to obtain concentrations of 1.56, 3.12, 6.25, 12.5, and 25 %.

Photochemical test using gas chromatography - mass spectrometry (GC-MS)

GC-MS analysis of the ethanol extract was conducted using a Shimadzu GC-2010 plus a gas chromatograph. The mixture was separated using a TG-5MS capillary column (30 m length; inner diameter 0.2 mm and film thickness 0.25 μm). Two microlitres of the ethanol extract were injected in a split mode (split ratio 1:10) into the GC apparatus. The temperature program included heating from 60 °C for 4 h to 150 °C for 4 h, and then to 250 °C. Helium was used as a carrier gas at a flow rate of 1.35 mL/min; and ionization of the sample was achieved in a post-column electron impact ion source at 70 eV. The obtained peaks were compared with the mass spectrum database based on NIST (NIST, Gaithersburg, MD, USA) using Chromeleon software.

Cultivation of *enterococcus faecalis* culture

E. faecalis cultures were cultivated on Tryptic Soy Agar (TSA) and Tryptic Soy Broth (TSB) media. The bacterial isolates were inoculated into TSA medium and incubated at 37 °C for 48 h. Subsequently, the isolates were transferred from TSA to TSB medium (50 mL) and incubated in an orbital shaker at room temperature for 24 h.

Antibacterial test using agar disc diffusion method

Antibacterial activity of the ethanol extract was assessed using the agar disc diffusion method (Kirby–Bauer method). A 10^{-5} dilution of the *E. faecalis* suspension was prepared. The bacterial suspension was inoculated onto TSA medium using the spread plate method. Paper discs (6 mm in diameter) were soaked in different concentrations of the extract (1.56, 3.12, 6.25, 12.5, and 25 %) and placed on inoculated TSA plates. Chlorhexidine (CHX 0.2 %) served as a positive control and sterile distilled water was used as a negative control. The plates were incubated at 37 °C for 24 h, and the diameters of the inhibition zones were measured using calipers. Each treatment was carried out in 4 replicates.

Minimum inhibitory concentration (MIC) testing

The microbial inhibitory concentration (MIC) was determined by UV-vis spectrophotometry at 610 nm. Suspensions of *E. faecalis* cultures, which were standardized to a McFarland turbidity of 0.5 (equivalent to 1.5×10^8 CFU/mL), were added to tubes containing 5 mL of media and variable extract concentrations (1.56, 3.12, 6.25, 12.5, and 25 %). The negative control contained the media, bacteria, and distilled sterile water, while the positive control received media, bacteria, and 0.2 % chlorhexidine (CHX) in water. The OD was determined at 610 nm before and after 24 h of incubation at 37 °C. The MIC was defined as the lowest concentration with an OD difference (OD after – OR before) ≤ 0 [11].

Statistical analysis

Statistical analysis of variance (ANOVA) followed by Duncan's multiple range test were used to calculate significant differences of inhibition zones and MIC values using the SPSS version 26.

Results and discussion

Extraction efficiency and phytochemical compositions

The yield percentage of ethanol extract of dry Aceh patchouli leaves (*Pogostemon cablin* Benth.) was 172.5 g with a weight of 1000 g, or 17.25 % extraction efficiency. This is higher than that of the Aceh patchouli leaf extract reported previously, which obtained a yield of 8.51 % from 300 g of dry powder [12]. Such a high yield shows that there was no significant loss of phytochemical compounds during the maceration and subsequent extraction processes, where the percentage yield has been shown to imply the phytochemical content of the plant material [13]. It has a strong impact on extraction efficiency and is dependent on the solvent and its concentration [14]. In this study, 96 % ethanol, which has good solubility for organic compounds owing to its polarity, was chosen as the solvent. The smaller particle size of the simplicia after drying could likely increase the extraction efficiency because it could enhance the surface area contact between the solvents and materials [15]. In addition, a maceration time of 5 days at room temperature (25 - 30 °C) also had a positive effect because this condition would provide a longer contact time for solvent-material interactions despite the cold extraction conditions applied [16].

GC-MS analysis revealed that the ethanol extract of Aceh patchouli leaves contained 30 phytochemical compounds, which are summarized in **Table 1** and were mainly composed of sesquiterpenes, fatty acids, and esters. Patchouli alcohol, a tricyclic sesquiterpene, was detected as the

bioactive dominant component with a peak area percentage of 20.03 % at a retention time of 27.64 % (**Figure 1**), which is in accordance with several studies that identified patchouli alcohol as the predominant constituent in Aceh patchouli essential oil [17,18]. Patchouli alcohol has been reported to possess anti-inflammatory, antiulcerogenic, antibacterial, and antifungal properties [19]. In addition to the sesquiterpenes identified, several fatty acids were also detected, including octadecanoic acid and n-hexadecanoic acid, which showed the highest peak areas at 12.71 and 11.54 %, respectively.

Table 1 Major phytochemical compounds (area percentage ≥ 3 %) identified in the ethanol extract of Aceh patchouli leaves (*Pogostemon cablin* Benth.) using Gas Chromatography-Mass Spectrometry (GC-MS) analysis, with their retention times, molecular formulas, molecular weights, compound types, and reported biological activities.

Peak	Retention time	Areas (%)	Component	Chemical formula	Molecular weight (g/mol)	Type of compound	Potential biological activity
11	27.64	20.03	Patchouli alcohol	$C_{15}H_{26}O$	222.37	Tricyclic sesquiterpenes	Anti-inflammatory, antiulcerogenic, antibacterial and antifungal [19]
17	32.87	3.11	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.50	Fatty acid methyl esters	Antioxidant, anti-inflammatory, hypocholesterolemic, and cancer prevention activities [20]
18	33.73	11.54	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	Fatty acid	Antibacterial and antioxidant [21]
19	36.15	3.24	6-Octadecenoic acid, methyl ester, (Z)-	$C_{19}H_{36}O_2$	296.50	Fatty acid esters	Antioxidant and antimicrobial [22]
21	36.61	3.82	Methyl stearate	$C_{19}H_{38}O_2$	298.50	Fatty acid methyl esters	Anti-inflammatory, intestinal lipid metabolism regulation, nematicidal, antinociceptive, antioxidant and antifungal [20]
22	37.02	8.31	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.50	Trans fatty acid	Antibacterial ad hypolipidemic effects in rats [23]
23	37.44	12.71	Octadecanoic acid	$C_{18}H_{36}O_2$	284.50	Saturated fatty acid	Antibacterial [24]
28	42.44	3.50	Glycidyl palmitoleate	$C_{19}H_{34}O_3$	310.50	Fatty acid	Therapeutic potential to augments neuron level, manipulate a subset of enzymes that control eCB signaling [25]
29	42.83	5.54	Glycidyl palmitate	$C_{19}H_{36}O_3$	310.50	Fatty acid	Inhibit apoptosis [25]

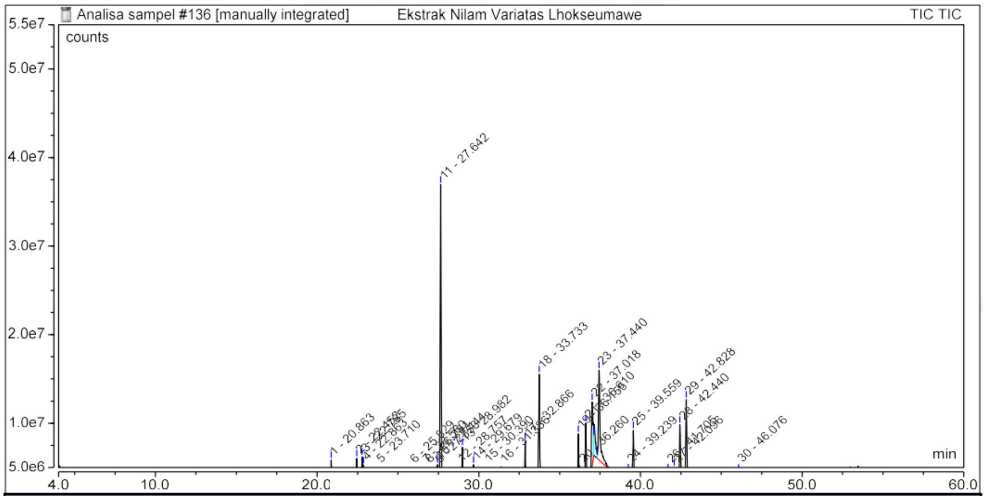

Figure 1 Gas Chromatography-Mass Spectrometry (GC-MS) chromatogram of the ethanol extract of Aceh patchouli leaves (*Pogostemon cablin* Benth.) with the major phytochemical compounds detected.

Antibacterial activity of Aceh patchouli leaf ethanol extract against *Enterococcus faecalis*

The results showed that the antibacterial activity of the extract increased with increasing concentration, as a larger inhibition zone diameter was observed at high concentrations (**Figure 2**). In result, at the concentration of 25 %, extract exhibited the highest inhibition effect at mean (± standard deviation), namely 9.36 (± 0.65) mm, significantly better than all the other treatments, including the positive control (CHX 0.2 %) which has an inhibition zone of 5.20 ± 0.42 mm. This suggests that 25 % extract concentration has a stronger effect than the common antiseptic drug chlorhexidine. The extracts of 1.56, 3.12, 6.25 and 12.5 %, the lower concentrations, appeared to have a much lower inhibitory activity, with inhibition zone diameters of 3.43 (± 0.36) mm, 3.49 (± 0.67) mm, 3.68 (± 0.67) mm, and 4.8 (± 0.58) mm, respectively. None of these concentrations was significantly different from each other. However, they were all significantly lower than those of the 25 % extract and the control.

The upward trend observed with the increase in the concentration of the extract can be partly attributed to the higher concentrations of biologically active compounds present within higher concentrations of the extract [22]. As shown in **Table 1**, Aceh patchouli leaves contain a number of phytochemical compounds with established antibacterial activity, including patchouli alcohol, fatty acids, and their derivatives. The greater the concentration of the ethanol extract, the more these metabolites were available to interact with and inhibit the growth of *E. faecalis*.

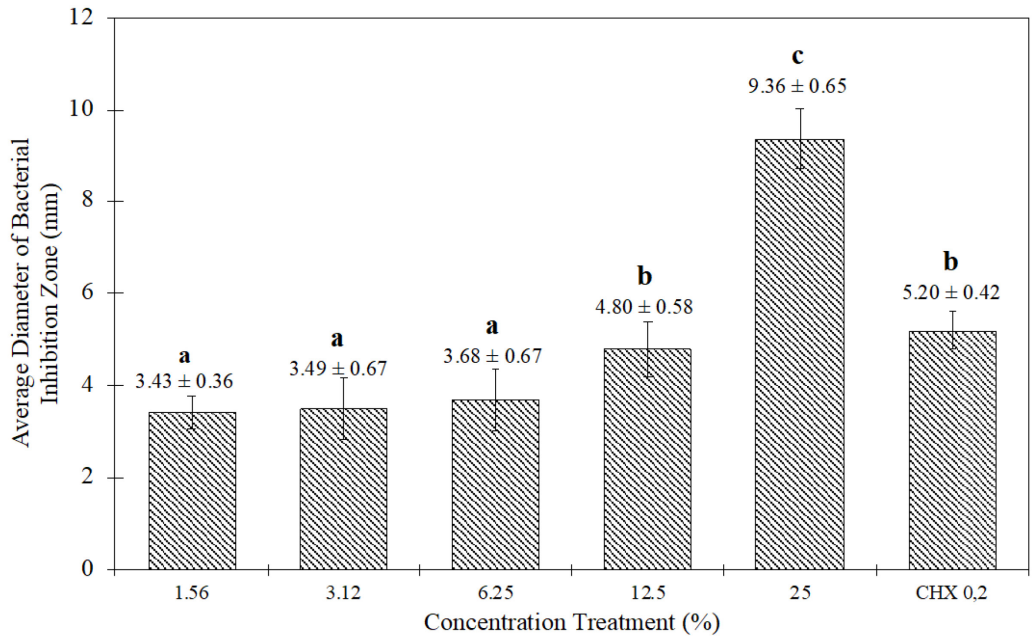

Figure 2 Inhibition zone diameters (mm) of the ethanol extract of Aceh patchouli leaves (*Pogostemon cablin* Benth.) at various concentrations and the positive control (chlorhexidine (CHX) 0.2 %) against *Enterococcus faecalis* was determined by the agar disc diffusion method. Different letters above the bars indicate statistically significant difference ($p \leq 0.05$).

The ethanol extract of Aceh patchouli leaves showed moderate inhibitory activity, only at 25 %. The inhibitory activities observed at 1.56, 3.12, 6.25, and 12.5 % concentrations were deemed weak (**Figure 3**). The inhibitory activity was categorized based on the size of the inhibition zone: ≥ 20 mm, very strong, 10-20 mm to have strong, 5 - 10 mm to have, moderate; and ≤ 5 mm, weak [26]. The ethanol extract of Aceh

patchouli leaves, as analyzed using GC-MS, contains primarily sesquiterpene compounds, which belong to the terpenoid group and are constructed from 3 isopropene units, including an acyclic and bicyclic framework with a naphthalene framework. Terpenoids have been found to reduce bacterial growth by exploiting their lipophilicity to puncture bacterial cell membranes. These compounds can invade the bacterial double layer and subsequently diffuse inward, thereby harming the integrity of the cell membranes for normal physiological activities of bacteria. In turn, membrane damage may lead to critical leakage of substances such as proteins and enzymes to achieve antimicrobial effects [27]. Although the ethanol extract was shown to be able to reduce *E. faecalis* growth, this bacterium is known to be capable of producing toxic compounds leading to a high level of resistance.

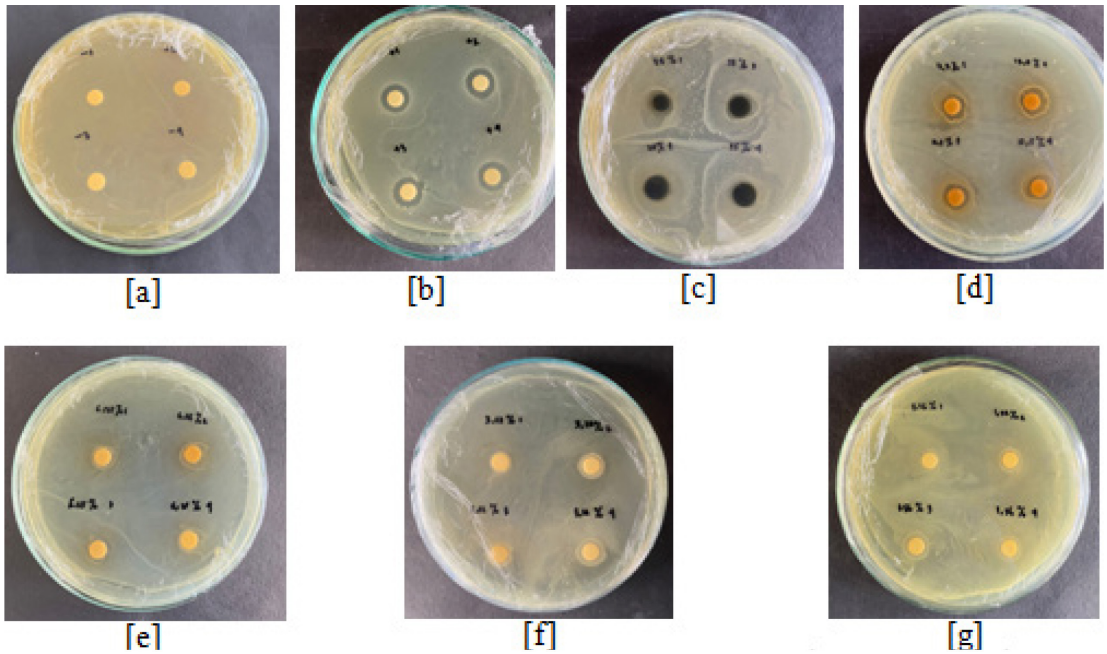

Figure 3 Inhibition zone activity of the ethanol extract of Aceh patchouli leaves (*Pogostemon cablin* Benth.) against *Enterococcus faecalis* determined by the agar disc diffusion method (a) negative control, (b) positive control, at various concentrations (c) 25, (d) 12.5, (e) 6.25, (f) 3.12 and (g) 1.56 %.

Other compounds that are dominant in the ethanol extract of Aceh patchouli leaves are fatty acids and esters. Fatty acids have been proven to be capable of antibacterial agents, such as glycidyl palmitate and glycidyl palmitoleate, which are monounsaturated fatty acids believed to be the most active antibacterial compounds [29]. **Table 1** shows that the fatty acid group in the ethanol extract of Aceh patchouli leaves consists of more than 12 double bonds (C atoms). A number of double bonds of more than 12 is more influential on the antibacterial activity of fatty acid compounds than fatty acids containing C double bonds less than 12 [29]. In addition, the geometric configuration of fatty acids that are active as antibacterials is the cis form, such as the cis-vaccenic acid component with a retention time of 37.02 [29]. Furthermore, alkaloid compounds, namely the acetamide component N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]. This alkaloid compound could damage the peptidoglycan component of the bacterial cell wall [30]. However, *E. faecalis* also has carbohydrate cell walls associated with cytoplasmic membranes that

contain a lot of peptidoglycan and teichoic acid. This is what causes *E. faecalis* to have a strong ability to withstand cytoplasmic osmotic pressure and provides strength for *E. faecalis* as a self-defense against alkaloid compounds contained in the ethanol extract of Aceh patchouli leaves; therefore, inhibition based on the diameter of the inhibition zone obtained is still weak.

Several studies on the antibacterial activity of the ethanol extract of Aceh patchouli leaves against *E. faecalis* have highlighted the potential of the patchouli extract as an antibacterial agent, although with varying levels of effectiveness. A study compared the antibacterial efficacy of extracts prepared using different solvents (hexane, ethanol, and methanol) against several bacterial species, including *E. faecalis*. The hexane extract of Aceh patchouli leaves showed the most obvious antibacterial activity, whereas the ethanol and methanol extracts showed a lower zone of inhibition [31]. Based on these results, it can be concluded that the ethanol extract showed antibacterial properties but was not as effective as the hexane extract [31].

The antibacterial activity associated with herbal extracts, including the leaves of Acehnese patchouli, was further studied. Pogostone, a secondary metabolite of patchouli, showed excellent antibacterial activity and was shown to disrupt bacterial cell membranes and reduce the activity of enzymes essential for the survival and reproduction of bacteria [32]. The present study, which focused on the ethanol extract of Acehnese patchouli leaves, also highlighted the measurable inhibitory effect of the ethanol extract on *E. faecalis*, although it was categorized as moderate. Compounds such as sesquiterpenes, fatty acids, and alkaloids are thought to play a role in this antibacterial activity. Although the ethanol extract of Acehnese patchouli leaves exhibited antibacterial activity against *E. faecalis*, its effectiveness varied. The main active components, such as sesquiterpenes, fatty acids, and alkaloids, contribute to this activity, although other solvent extracts such as hexane may show higher efficacy. The ability of this extract to disrupt bacterial cell membranes and inhibit important bacterial enzymes is at the heart of its antibacterial activity.

Minimum inhibitory concentration (MIC) determination

The *Minimum Inhibitory Concentration* (MIC) of the ethanol extract of Aceh patchouli leaves (*P. cablin* benth.) against *E. faecalis* was determined by measuring the optical density (OD) value using a UV-vis spectrophotometer at a wavelength of 610 nm. Chlorhexidine (CHX, 0.2 %) was used as the positive control. This is because CHX 0.2 % is an antiseptic used to fight bacterial infections found in the mouth and teeth. CHX 0.2 % was used to compare the OD results at each extract concentration.

The OD value obtained after incubation was subtracted from the OD value before incubation (OD after - OR before), and the ethanol extract of Aceh patchouli leaves inhibited the growth of *E. faecalis* at concentrations of 12.5 and 25 % (**Figure 4**). This is because at extract concentrations of 12 and 25 %, the average value (OD after - OR before) was negative, whereas for the average value (OD after - OR before) in the treatment with an extract concentration of 1.56, 3.12, and 6.25 % remained positive. These results show that the difference in OD values, which are negative, indicates that bacterial growth was inhibited. The MIC value was determined based on the lowest extract concentration in the treatment tube, with a difference in the OD value of ≤ 0 [11]. This means that based on the test treatment that has been carried out, the MIC value obtained is at an extract concentration of 12.5 %

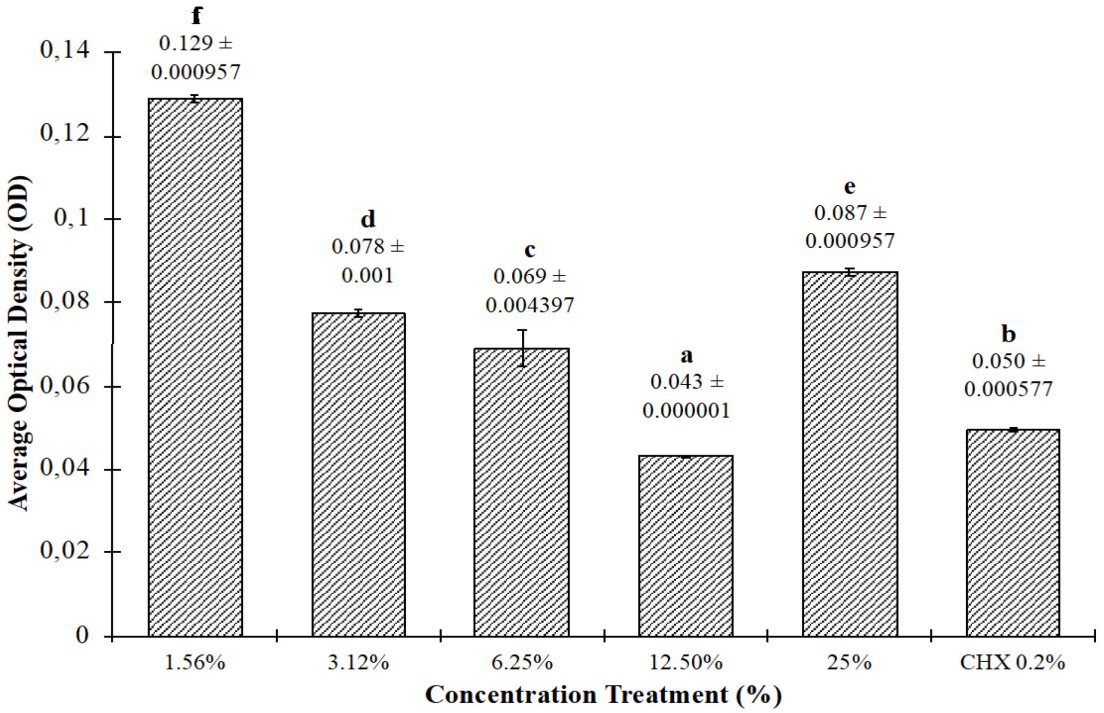

Figure 4 The Average Optical Density (OD) values show the Minimum Inhibitory Concentration (MIC) of the ethanol extract of Aceh patchouli leaves at various concentrations and the positive control (CHX 0.2 %) against *Enterococcus faecalis*. Different letters above the bars indicate statistically significant difference ($p \leq 0.05$).

Figure 4 shows the antibacterial activity of the ethanol extract of Aceh patchouli leaves at concentrations of 1.56, 3.12, 6.25, 12.5, and 25 % against *E. faecalis*. Based on the test results, the MIC value of Aceh patchouli leaf ethanol extract, which provides the greatest inhibitory power against *E. faecalis*, was 12.5 % with the lowest average OD value of 0.043, followed by a concentration of 6.25 % with an average OD value of 0.069, 3.12 % with an average OD value of 0.078, 25 % with an average OD value of 0.087, and 1.56 % with an average OD value of 0.129. The results of the analysis of variance (ANOVA) test showed that the ethanol extract of Aceh patchouli leaves had significant antibacterial activity against *E. faecalis*, based on the OD or turbidity value ($p \leq 0.05$). The antibacterial activity of the ethanol extract of Aceh patchouli leaves against *E. faecalis* was significantly different at each extract concentration. The MIC value is the lowest concentration of an antimicrobial compound that can inhibit the growth of microbes such as bacteria. The lower the turbidity value from the spectrophotometry results, the greater the ability of the extract to inhibit microbial growth [33,34]. An increase in the turbidity value in measurements with a UV-vis spectrophotometer indicates microbial growth, whereas a decrease in the turbidity value indicates an inhibitory effect of the extract on the growth of microbial cells, such that the number of microbes that grow is smaller [35]. However, increased turbidity does not always indicate microbial growth in the medium and can be influenced by the color density of the test material extract [36]. In addition, color changes can also affect light absorption in turbidity measurements; therefore, extracts with lower concentrations may have better values owing to more optimal light absorption and less excreted pigment [37].

The Minimum Inhibitory Concentration (MIC) of 12.5 % ethanol extract of Aceh patchouli leaves against *E. faecalis* can be considered moderate to strong based on existing research. For example, studies have shown that extracts with MIC values less than or equal to 20 % generally show good antibacterial activity. Therefore, the MIC of 12.5 % indicated that the extract had a significant inhibitory effect on *E. faecalis.* Several factors may contribute to this antibacterial activity, including the chemical composition of the extract, which is a rich source of bioactive compounds, such as sesquiterpenes, fatty acids, and alkaloids that can exert DNases and DNA enzymes, as well as by disrupting bacterial cell membranes and β-hemolysis [19-22,29,34]. Another external factor is related to the environment of the plants grown and harvested. Environmental factors and the approaches for plant extraction and testing are performed could affect the potency of biologically active ingredients. This is because climate, soil quality, microenvironment, and post-harvest processing, such as drying and milling, result in concentration and subsequent variation in the efficacy of the compounds contained in the extracts [38]. Such variation can be seen when some studies revealed strong antibacterial activity of Aceh patchouli leaf ethanol extract against *E. faecalis*, but with low MIC values compared to other studies (for instance, < 12.5 %), which could be due to differences in extraction methods, bacterial strains used, or even the geographic origin of the plant material [38].

Based on the results of this study, the ethanol extract of Aceh patchouli leaves appears to be a suitable alternative or additional treatment for infections caused by a root canal. The moderate to weak inhibitory activity at a wide range of concentrations could be further optimized by considering variables of the extraction process and the formulation, providing a stronger antimicrobial activity. Meanwhile, the presence of patchouli alcohol in the extract revealed its potential therapeutic activity against bacterial infections, whereby the proposed bioactive compound that is present in Acehnese patchouli is one of the important solutions in conventional medicine to combat problems caused by clinically significant pathogens. This study showed that the antimicrobial effect of the ethanol extract of Aceh patchouli leaves could be applied directly to treat infections caused by the root canal. In the future, the results of the systematic chemical composition in Acehnese patchouli could open up the possibility of future research on the synergy and mechanism of action of chemical compounds in the antimicrobial activity of Aceh patchouli using *in vitro* tests on animals, as well as clinical trials in humans. Furthermore, the biopotential of patchouli alcohol could be utilized as part of interactome-based compound discovery, thereby paving the way toward developing newer antimicrobial agents to be used in root canal therapy. Thus, it is possible to evolve treatments towards safe and effective ways to cure infections caused by a root canal, or any infection, by paying attention to the synergistic effects of various compounds in Aceh patchouli and optimizing the mechanism of delivery, while minimizing the risk of antibiotic-resistant pathogens.

Conclusions

In summary, this study demonstrated the possible antibacterial properties of the ethanol extract obtained from Aceh patchouli leaves (*Pogostemon cablin* Benth.) against *Enterococcus faecalis*, a major pathogen that causes root canal infections. The extract showed significant efficacy and contained a wide array of bioactive constituents, including patchouli alcohol, fatty acids, and their derivatives, which likely contributed to its antibacterial properties. Additionally, the extract exhibited varying levels of antibacterial activity based on its concentration with moderate-to-strong inhibitory effects at doses of 25 and 12.5 %, respectively. These findings highlight the potential of the Aceh patchouli extract as a viable alternative or complementary treatment for root canal infections. However, further investigation is necessary to improve the development and transportation of the extract, elucidate the exact processes through which its bioactive compounds function, and validate its efficacy and safety in the clinical setting. Despite its limitations, this

study contributes to the growing body of evidence supporting the use of natural products to address oral health problems and combat antibiotic resistance.

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