Al-Hayat: Journal of Biology and Applied Biology
Volume 7, No 1 (2024): 81-92
DOI. 10.21580/ah.v7i1.18877

GC-MS Study of Bioactive Compounds of *Peperomia pellucida* and Its Antibacterial Activity against *Streptococcus mutans*

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Abstract

Streptococcus mutans is the primary bacterium causing dental caries. Pepper elder (*Peperomia pellucida*) might prevent this disease due to its antibacterial bioactive content. Correspondingly, the present study aimed to identify the bioactive profile of pepper elder ethanol extract and its antibacterial activity against *Streptococcus mutans*. Bioactive compounds were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The antibacterial activity was tested through an inhibition test using the well-diffusion method, the Minimum Inhibitory Concentration (MIC) test, and the Minimum Bactericidal Concentration (MBC) test. The most detected compounds in pepper elder ethanol extract were n-Eicosane, n-Hexadecane, and glycerol. There were also antibacterial bioactives such as phenols, flavonoids, alkaloids, and terpenoids. The inhibition test of 500 mg/mL extract revealed a clear zone of 8.25 mm diameter. The MIC and MBC values were 50 mg/mL and 100 mg/mL, respectively. Based on the results, pepper elder ethanol extract demonstrated potential as an antibacterial, although its inhibitory effectiveness still needed to be improved.

Keywords: Antibacterial, GC-MS, *Peperomia pellucida*, *Streptococcus mutans*

Introduction

Dental caries, an infectious disease attacking the dental hard tissues, is mainly caused by the interaction between certain bacteria and a high-sugar diet (Bradshaw & Lynch, 2013; Oliveira et al., 2019). The primary bacterium involved is *Streptococcus mutans* (Karpiński & Szkaradkiewicz, 2013; Shetty et al., 2016). It is part of the oral microflora that has evolved pathogenic characteristics due to its ability to adhere to tooth surfaces, form dental plaque, and thrive in an acidic environment (Alejandra & Daniel, 2020). These factors make it a significant contributor to the development of dental caries. The acid produced by *Streptococcus mutans* damages the dental layer and promotes the formation of cavities.

One way to prevent dental caries is by administering natural or synthetic antibacterials. However, synthetic or chemical antibacterials are known to have long-term side effects (Chen et al., 2020). Therefore, to minimize the side effects of synthetic drugs, research development is needed to explore alternative agents that can prevent dental caries. One such alternative is natural medicines made from herbs. The pepper elder plant (*Peperomia pellucida*) is a wild herbaceous plant with various medicinal properties, including anti-inflammatory, analgesic, antipyretic, antimicrobial, anticancer, and antidiabetic activities (Sheikh et al., 2013; Soboyejo & Ade-Ademilua, 2017; Teoh et al., 2021). The stems and leaves of pepper elder are reported to contain bioactive secondary

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metabolites such as terpenoids, diterpenoids, and phenolic compounds that can act as antibacterials against various gram-positive (*M. smegmatis*, *L. ivanovii*, *S. aureus*, and *S. uberis*) and gram-negative (*E. coli*, *E. cloacae*, and *V. parahaemolyticus*) bacteria (Okoh et al., 2017).

Given the medicinal potential of pepper elder plant, determining the profile of bioactive compounds contained in pepper elder ethanol extract through GC-MS is essential to support its bioactivity as an antibacterial. Likewise, compound profiling using GC-MS is recognized and widely used to identify various metabolites from plant extracts (Ghimire et al., 2017). Furthermore, GC-MS has several advantages, including high sensitivity, identifying compounds with low volatility, and providing faster results compared to other methods (Torras-claveria et al., 2010).

Research Methods

Preparation of Pepper Elder Ethanol Extract

The pepper elder ethanol extract was prepared utilizing a modified maceration method adapted from Pebrian et al. (2021). 100 g of pepper elder stem and leaf simplicia were added to an extraction container containing 1 L of 70% ethanol (1:10 ratio). The solution was allowed to stand for 3 days and was stirred at 24-hour intervals. Subsequently, the extract solution was filtered using filter paper to obtain the first filtrate. The residue from the first filtration was re-macerated using 70% ethanol solvent in a ratio of 1:5 for 2 days. The result of this re-maceration was filtered to obtain the second filtrate. The first filtrate from the initial maceration and the second filtrate from the re-maceration were combined until homogeneous. Next, the combined filtrate was evaporated using a rotary evaporator (Stuart®) at 50°C until a pure extract was obtained. The extract was then placed in an oven incubator (Equitron®) at 50°C until it became a paste extract, which was subsequently stored in a glass cup in a refrigerator (Polytron®).

GC-MS Analysis

The analysis of bioactive compounds in the pepper elder ethanol extract was conducted using Gas Chromatography-Mass Spectrometry (GC-MS) QP2010 Plus (Shimadzu®). The compound analysis began with sample preparation and derivatization. The solution was injected into the GC column through the heated injection port. In the column, the compounds in the sample were separated based on their chemical properties. Next, the extract components passed through the GC detector, producing a recorded signal. These compounds were then directed into the MS, which measured the molecular mass of each compound and produced a characteristic mass spectrum. These mass spectra, used as unique molecular 'fingerprints,' were then matched against the Wiley 7 Library database to identify the compounds precisely.

Preparation of Bacterial Suspension

Streptococcus mutans ATCC 25175 bacteria obtained from the Bioscience Laboratory, Dental and Oral Hospital (*RSGM*), University of Jember, were cultured on Mueller-Hinton Agar (MHA) medium (Merck®) in a Petri dish. A bacterial suspension of *Streptococcus mutans* was prepared by taking bacterial colonies from the MHA medium using an inoculating loop and dissolving them in 5 mL of 0.9% NaCl. The suspension solution was homogenized using a KMC-1300V vortex mixer (Vision Scientific®). The bacterial suspension was then compared to the McFarland 0.5 standard suspension, equivalent to a bacterial concentration of 1.5×10^8 CFU/mL.

Antibacterial Activity Test

Antibacterial activity testing was carried out using the inhibition test, the MIC determination test, and the MBC determination test (Magdalena & Kusnadi, 2015). The inhibition test was conducted using the well-diffusion method. A total of 1,000 µL of *Streptococcus mutans* bacterial suspension was pipetted and added to a test tube containing 20 mL of MHA medium that had not yet solidified. The media solution and suspension were homogenized using a vortex mixer and poured into a Petri dish. After solidification, the agar medium was perforated to create seven holes

using an 8 mm diameter cork borer. Next, 30 µL of the test solution, namely pepper elder ethanol extract at five concentration levels (100 mg/mL, 200 mg/mL, 300 mg/mL, 400 mg/mL, and 500 mg/mL), positive control solution (chloramphenicol 1 mg/mL), and negative control solution (distilled water), were added to the wells using a micropipette. The Petri dishes were then sealed with plastic wrap and incubated at 37°C for 24 hours.

The MIC determination test was conducted to ascertain the lowest concentration of pepper elder ethanol extract that could inhibit the growth of *Streptococcus mutans*. The microdilution method, involving serial dilutions with liquid media, bacteria, and test compounds in small amounts, was employed for MIC determination (Kolarević et al., 2016). A 96-well microplate (Biologix®) was used for the test. In the MIC test, Mueller-Hinton Broth (MHB) medium (Merck®) was utilized. 100 µL of MHB medium was loaded into 10 columns in 4 rows of microplate wells, each representing one repetition of the assay. A series of dilutions of the pepper elder ethanol extract was performed with an initial concentration of 200 mg/mL. 100 µL of the 200 mg/mL extract concentration was homogenized into the first column. Then, 100 µL from the first column was transferred to the second column, resulting in a 100 mg/mL concentration. This step was repeated until reaching the seventh column with a final concentration of 3.125 mg/mL. The eighth column was filled with 100 µL of distilled water as a negative control, while the ninth column received no treatment and served as the untreated control. The tenth column was filled with 100 µL chloramphenicol as a positive control. Next, 10 µL of *Streptococcus mutans* suspension equivalent to McFarland 0.5 was added to each well. The microplate was then incubated at 37°C for 24 hours. The absence of turbidity after incubation indicates the lowest concentration that can inhibit bacteria (Jannah et al., 2018).

The MBC determination test was carried out to establish the minimum concentration of pepper elder ethanol extract needed to kill *Streptococcus mutans*. This test employed the streak plate method (Kusuma et al., 2017). In the MBC test, Mueller-Hinton Agar (MHA) media was utilized. Determination of MBC was conducted at a concentration considered to have the MIC value and was capable of inhibiting bacterial growth. MHA media was poured into a Petri dish and allowed to solidify. The dish was then divided into 4 quadrants for 4 repetitions. Samples that could inhibit bacterial growth were taken and streaked on the surface of the MHA media using an inoculating loop in all four quadrants. Next, the dish was incubated at 37°C for 24 hours. If the results showed no bacterial growth, the concentration was considered the MBC. Conversely, if bacterial growth occured, the concentration only inhibited the growth but did not kill the bacteria.

Data Analysis

The results of the inhibition test data were analyzed utilizing the One-Way ANOVA method and the Post Hoc Duncan test with SPSS version 25. Meanwhile, the GC-MS results, the MIC determination test, and the MBC determination test were analyzed descriptively.

Research Results and Discussion

Bioactive Compound Profile

The name, retention time, percentage peak area, molecular formula, molecular weight, and class of each active compound in the pepper elder ethanol extract are presented in Table 1.

Table 1
Profiling of bioactive compounds of secondary metabolites of pepper elder ethanol extract by GC-MS

Compound Name	Retention Time	Area (%)	Molecular Formula	Molecular Weight	Compound Groups
Piperazine	3.067	0.2	$C_4H_{10}N_2$	86	Alkaloids
Pyrazine, methyl- (CAS: Methylpyrazine)	3.883	1.48	$C_5H_6N_2$	94	Alkaloids
1,2,3-propanetriol (CAS: Glycerol)	15.739	9.8	$C_3H_8O_3$	92	Sugar alcohol

Compound Name	Retention Time	Area (%)	Molecular Formula	Molecular Weight	Compound Groups
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	19.765	0.62	$C_6H_8O_4$	144	Flavonoids
Phenol, 4-ethenyl-2-methoxy-	24.661	0.56	$C_9H_{10}O_2$	150	Phenol
Phenol, 3-methoxy-4-(Phenylmethoxy)-	25.28	0.57	$C_{14}H_{14}O_3$	230	Phenol
Phenol, 2,6-dimethoxy- (CAS: 2,6-Dimethoxyphenol)	25.986	1.13	$C_8H_{10}O_3$	154	Phenol
Lyxitol, 1-O-Decyl-	29.761	6.73	$C_{15}H_{32}O_5$	292	Sugar alcohol
Heneicosane (CAS: n-Heneicosane)	30.504	2.42	$C_{21}H_{44}$	296	Terpenoids
9-Octadecene, (E)- (CAS)	33.008	4.87	$C_{18}H_{36}$	252	Terpenoids
Nonacosane (CAS: n-Nonacosane)	35.235	6.44	$C_{29}H_{60}$	408	Fatty acid
Hexadecane (CAS: n-Hexadecane)	36.108	5.4	$C_{16}H_{34}$	226	Alkane
1-Nonadecene (CAS: n-Nonadecene)	37.513	4.1	$C_{19}H_{38}$	266	Fatty acid
Tetradecanoic acid (CAS: Myristic acid)	39.78	3.45	$C_{14}H_{28}O_2$	228	Fatty acid
Eicosane (CAS: n-Eicosane)	42.494	15.64	$C_{20}H_{42}$	282	Terpenoids
Hexadecane (CAS: n-Hexadecane)	46.254	6.4	$C_{16}H_{34}$	226	Alkane

Based on these data, it is known that eicosane (CAS: n-Eicosane) was the bioactive compound with the highest content in the pepper elder (*Peperomia pellucida*) ethanol extract. Eicosane and two other compounds, octadecene and heneicosane, are classified as terpenoid compounds (Sumayya et al., 2020). Terpenoids are dehydrogenated and oxidized derivatives of terpene compounds (hydrocarbon groups). Eicosane (CAS: n-Eicosane) is known to act as a wax that protects plants from physical damage and prevents water loss, and it can exhibit antimicrobial activity against several bacteria (Ahsan et al., 2017; Chuah et al., 2018; Sumayya et al., 2020). Octadecene is also reported to act as an antimicrobial (Kumar et al., 2011). Likewise, heneicosane (CAS: n-Heneicosane) is known to have antimicrobial activity against bacteria and fungi (Ghavam et al., 2021; Kawuri & Darmayasa, 2019).

The mechanism of terpenoids as antibacterials involves membrane damage by lipophilic compounds. Terpenoids can form strong polymeric bonds with porins (transmembrane proteins) on the outer membrane of the cell wall, ultimately causing porin damage (Heni et al., 2015). The damaged porins reduce the permeability of the cell wall, leading to nutrient deprivation and the inhibition or death of bacterial cells.

Hexadecane (CAS: n-Hexadecane) was the second most abundant compound after eicosane. It is a plant metabolite that belongs to the alkane group. It is reported to have antibacterial and antioxidant activities (Osman et al., 2023; Yogeswari et al., 2012).

The next most abundant compounds in the pepper elder ethanol extract were sugar alcohols, including 1,2,3-propanetriol (CAS: Glycerol) and lyxitol, 1-O-decyl-. Glycerol, a compound with three hydroxyl groups, is hydrophilic (can bind to water) and hygroscopic (can absorb water). It is known to be a precursor in lipid and carbohydrate metabolism. Additionally, glycerol has antimicrobial activity against nine microorganisms isolated from primary dental caries: *S. mutans*, *S. salivarius*, *S. mitis*, *S. pneumoniae*, *N. mucosa*, *S. epidermidis*, *L. species*, *C. xerosis*, and *C. albicans* (Hill et al., 1991). Its antimicrobial effect is due to its viscous nature, which deprives microorganisms of oxygen (Hill et al., 1991). In addition, glycerol can disrupt the osmotic balance in bacterial cells, causing water loss and inhibiting bacterial growth and reproduction.

Lyxitol, 1-O-decyl- is a sugar alcohol that generally acts as a carbohydrate reserve in some angiosperms. No studies have shown that it could act as an antibacterial. However, previous studies suggest that oral bacteria cannot metabolize sugar alcohols and do not contribute to tooth decay (Awuchi & Echeta, 2019; Grembecka, 2015).

Nonacosane (CAS: n-Nonacosane) is a fatty or carboxylic acid with a long aliphatic chain. It is reported to have antibacterial activity

against gram-negative (*E. coli* and *P. vulgaris*) and gram-positive bacteria (*B. subtilis*, *S. aureus*, and *S. epidermidis*) (Carev et al., 2023). 1-Nonadecene (CAS: n-Nonadecene) is a long-chain fatty acid that acts as a plant metabolite and has biological activity as an antibacterial agent (Ghavam et al., 2021; Kotagiri et al., 2018). Tetradecanoic acid (CAS: Myristic acid) is a saturated fatty acid synthesized from the malonic acid pathway. It is known to have antimicrobial activity against a broad spectrum of microorganisms (Rehman et al., 2017). Fatty acids can damage plasma membranes or cell walls and disrupt intracellular processes such as energy production and oxidative phosphorylation (Desbois & Smith, 2010). Additionally, saturated fatty acids can inhibit bacterial growth by absorbing nutrients present in bacteria, inhibiting water entry, and blocking enzyme functions.

The next group of compounds found in the pepper elder ethanol extract was phenol compounds. Specifically, the extract contained three phenol compounds: phenol, 4-ethenyl-2-methoxy-; phenol, 3-methoxy-4-(phenylmethoxy)-; and phenol, 2,6-dimethoxy-. Phenol is a class of compounds with a hydroxyl group attached to an aromatic ring (benzene) (Simatupang et al., 2021). Phenol compounds can act as antioxidants and antimicrobials (Hutagalung et al., 2023). Based on Alghamdi et al. (2018), phenol, 4-ethenyl-2-methoxy- has antimicrobial, antioxidant, anti-inflammatory, and analgesic activities. Phenol, 3-methoxy-4-(phenylmethoxy)- acts as an antimicrobial agent by inhibiting the growth of bacteria and fungi. Meanwhile, phenol, 2,6-dimethoxy- can be applied as an antibacterial and antioxidant material (Fauziati & Sampepana, 2021).

The mechanism of phenol compounds as antibacterials involves denaturing cell proteins. The hydrogen bonds formed between phenol and protein cause damage to the protein structure (Bontjura et al., 2015). Cell walls and cytoplasmic membranes composed of proteins will change their permeability due to these hydrogen bonds. The disruption of the permeability of the cell wall and cytoplasmic membrane results in an imbalance of macromolecules and ions in the cell, leading to bacterial cell lysis.

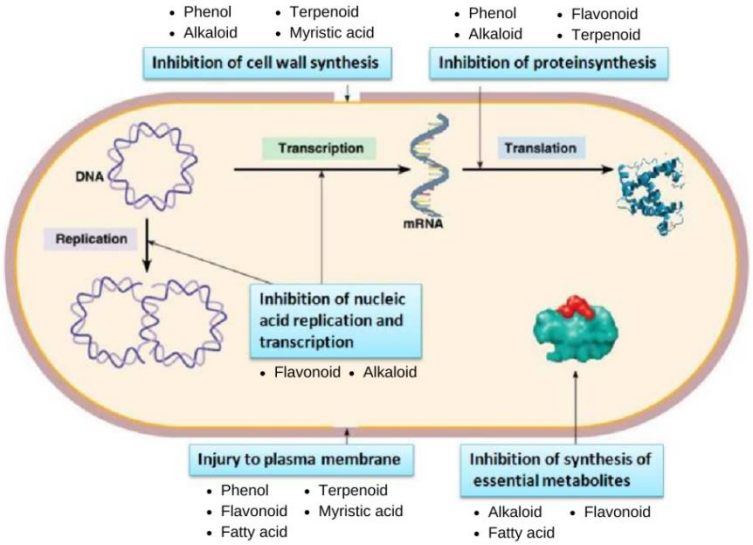
The flavonoid group contained in the pepper elder ethanol extract included 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, which is reported to have strong antioxidant, antibacterial, and anticancer activities (Chen et al., 2021; Naibaho et al., 2021). The mechanism of flavonoids as antibacterials involves interfering with nucleic acid synthesis, cell membrane function, and energy metabolism of bacteria (Bontjura et al., 2015). The interaction between flavonoids and bacterial DNA can damage the permeability of bacterial cell walls, microsomes, and lysosomes. Flavonoids damage bacterial cell membranes by forming complex compounds on extracellular proteins until they dissolve and cause bacterial lysis.

Alkaloid compounds found in the pepper elder ethanol extract included piperazine and pyrazine, methyl-. Alkaloids are secondary metabolite compounds of plants that contain nitrogenous bases in their structure (Resti & Parbuntari, 2022). They have biological activities as antibacterials, antioxidants, anti-inflammatories, anticancer, and antimalarials (Bai et al., 2021; Belyagoubi-Benhammou et al., 2019; Yan et al., 2021). Piperazine is an azacycloalkane with a six-membered ring containing two nitrogen atoms in opposite positions. According to Shaquiquzzaaman et al. (2015), piperazine has antimicrobial activity. Pyrazine, methyl- (CAS: Methylpyrazine) is a compound in which one of the hydrogens is replaced by a methyl group. Pyrazine is known to act as an antibacterial (Jelita et al., 2019). Meanwhile, alkaloids can damage bacterial DNA and RNA polymerase enzymes, inhibiting the synthesis of these nucleic acids. In addition, alkaloids can interfere with the formation of peptidoglycan in the cell wall, causing cell lysis (Milanda et al., 2021).

Based on the above explanation, the antibacterial action of the bioactive compounds found in the pepper elder ethanol extract can be seen in Figure 1.

Al-Hayat: Journal of Biology and Applied Biology
Volume 7, No 1 (2024): 81-92
DOI. 10.21580/ah.v7i1.18877

Figure 1
Antibacterial mechanism of bioactive compounds in pepper elder ethanol extract (modified from Barzic & Ioan, 2015)



Antibacterial Activity

The inhibition test results indicated the presence of an inhibition zone (clear zone) around the wells, suggesting the inhibition of the growth of *Streptococcus mutans* bacteria. Based on the results of One-Way ANOVA analysis with a 99% confidence level, the significance value of the inhibition zone was 0.000 ($P < 0.05$), signifying a significant difference in the diameter of the inhibition zone produced by the test and control extracts against bacteria. The average diameter of the inhibition zone can be seen in Table 2. The inhibition response category from the diameter measurement results was adjusted based on the classification by Davis and Stout (1971).

Table 2
Average diameter of the inhibition zone for Streptococcus mutans *growth*

Test Group	Average Diameter of Inhibition Zone (mm) ± SD	Inhibition Response Categories
K- (aquadest)	0.00 ± 0.000	No response
P_1 (extract: 100 mg/mL of *P. pellucida*)	2.75 ± 0.500	Weak
P_2 (extract: 200 mg/mL of *P. pellucida*)	4.00 ± 0.408	Weak
P_3 (extract: 300 mg/mL of *P. pellucida*)	5.38 ± 0.479	Moderate
P_4 (extract: 400 mg/mL of *P. pellucida*)	6.88 ± 0.250	Moderate
P_5 (extract: 500 mg/mL of *P. pellucida*)	8.25 ± 0.500	Moderate
K^+ (1 mg/mL of chloramphenicol)	11.50 ± 0.578	Strong

These data demonstrate a positive relationship between the concentration of ethanol extract used and the diameter of the inhibition zone (clear zone) formed. In this regard, the higher the concentration of the ethanol extract, the larger the diameter of the inhibition zone. This indicates that higher concentrations of pepper elder ethanol extract have stronger potential antibacterial activity against *Streptococcus mutans* because they contain more bioactive compounds with antibacterial properties.

According to Jawetz et al. (1996), four factors can affect antibacterial activity: the type of bacteria inhibited, the diffusion power of the extract, the concentration used, and the content of antibacterial compounds. In this study, the pepper elder (*Peperomia pellucida*) ethanol extract inhibited bacterial growth by diffusing into the media that had been inoculated with

test bacteria. Accordingly, the type of bacteria and the concentration of the extract could affect the diffusion. *Streptococcus mutans* are gram-positive bacteria with a simpler cell wall structure than gram-negative bacteria. According to Brooks et al. (2010), the structure of the bacterial cell wall can determine the penetration ability, bonding, and activity of an antibacterial compound. *Streptococcus mutans* have a cell wall of thick peptidoglycan and teichoic acid connected to peptidoglycan through covalent bonds. Based on Rahman et al. (2017), teichoic acid is hydrophilic (soluble in water) and functions as a transport medium for positively charged ions to enter and exit the cell wall. The water-soluble nature causes the cell wall of gram-positive bacteria to be more polar, so polar antibacterial compounds will more easily diffuse and penetrate the cell wall of *Streptococcus mutans*.

The pepper elder ethanol extract at a concentration of 100 mg/mL could still inhibit the growth of *Streptococcus mutans* with an inhibition zone diameter of 2.75 mm. To determine the minimum concentration of the extract that could inhibit bacterial growth, the Minimum Inhibitory Concentration (MIC) determination test was carried out. The test results revealed that the solution with the smallest concentration could inhibit the growth of *Streptococcus mutans* at 50 mg/mL. This could be seen from the test solution, which remained clear (bacteria did not grow) in the well containing the extract. Therefore, the pepper elder ethanol extract solution with a concentration of 50 mg/mL still contained enough antibacterial metabolite compounds to inhibit the growth of *Streptococcus mutans*. The MIC test results of the ethanol extract of *Peperomia pellucida* against the growth of *Streptococcus mutans* are presented in Table 3.

Table 3

The MIC test results of pepper elder ethanol extract on the growth of Streptococcus mutans

Test Group	Bacterial Growth			
	1st Repetition	2nd Repetition	3rd Repetition	4th Repetition
Extract: 200 mg/mL of *P. pellucida*	(-)	(-)	(-)	(-)
Extract: 100 mg/mL of *P. pellucida*	(-)	(-)	(-)	(-)
Extract: 50 mg/mL of *P. pellucida*	(-)	(-)	(-)	(-)
Extract: 25 mg/mL of *P. pellucida*	(+)	(+)	(+)	(+)
Extract: 12.5 mg/mL of *P. pellucida*	(+)	(+)	(+)	(+)
Extract: 6.25 mg/mL of *P. pellucida*	(+)	(+)	(+)	(+)
Extract: 3.125 mg/mL of *P. pellucida*	(+)	(+)	(+)	(+)
K^- (aquadest)	(+)	(+)	(+)	(+)
K^+ (1 mg/mL of chloramphenicol)	(-)	(-)	(-)	(-)

Description:
The (+) sign indicates the growth of *Streptococcus mutans*, characterized by the turbidity of the solution in the well, while the (-) sign indicates the absence of *Streptococcus mutans* growth, characterized by the clarity of the solution in the well.

The Minimum Bactericidal Concentration (MBC) was determined on test solution that showed visual media clarity from the MIC test, precisely test solutions with ethanol extract concentrations of 200 mg/mL, 100 mg/mL, and 5 mg/mL. These three concentrations demonstrated the MIC test's ability to inhibit bacterial growth. The MBC value was obtained by observing whether there was colony growth of *Streptococcus mutans* on MHA media. After incubation for 24 hours, the test results for determining the MBC value were obtained, as shown in Figure 2. The results of the MBC test observations are depicted in Table 4.

Figure 2
The MBC test results of pepper elder ethanol extract on the growth of Streptococcus mutans *bacteria at concentrations of a) 200 mg/mL, b) 100 mg/mL, and c) 50 mg/mL*

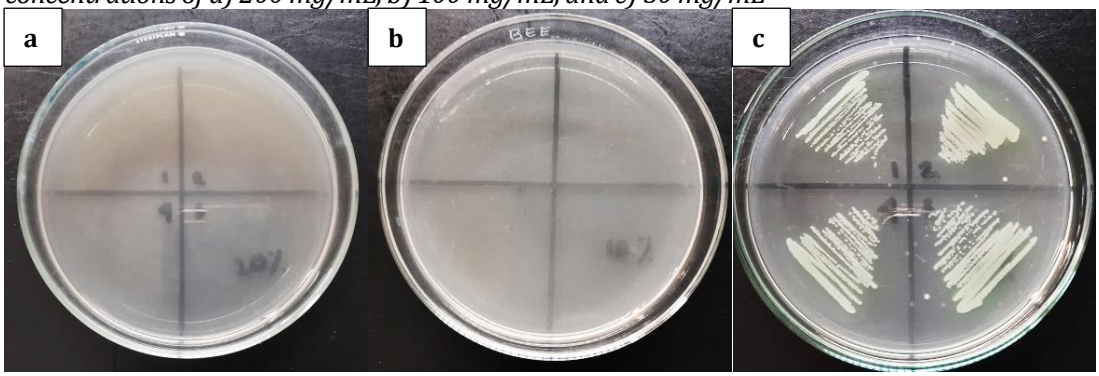


Table 4
The MBC test results of pepper elder ethanol extract on the growth of Streptococcus mutans

Test Group	Bacterial Growth			
	1st Repetition	2nd Repetition	3rd Repetition	4th Repetition
Extract: 200 mg/mL of *P. pellucida*	(-)	(-)	(-)	(-)
Extract: 100 mg/mL of *P. pellucida*	(-)	(-)	(-)	(-)
Extract: 50 mg/mL of *P. pellucida*	(+)	(+)	(+)	(+)

Description:
The (+) sign indicates the growth of *Streptococcus mutans* colonies, while the (-) sign indicates the absence of *Streptococcus mutans* colony growth.

The results of the MBC determination test revealed that a cup containing MHA media scratched using a test solution with a concentration of 50 mg/mL showed the growth of *Streptococcus mutans* colonies. In contrast, the media scratched with test solutions at 100 mg/mL and 200 mg/mL indicated no growth of *Streptococcus mutans* colonies. Accordingly, the ability of pepper elder ethanol extract to kill *Streptococcus mutans* bacteria is inseparable from bioactive compounds in the form of phenols, flavonoids, and alkaloids that are bactericidal (able to kill bacteria).

Conclusion

The pepper elder ethanol extract contains bioactive compounds from the terpenoid group (eicosane, heneicosane, and octadecene), alkaloids (piperazine and methylpirazine), phenols, flavonoids, sugar alcohols (glycerol and lyxitol, 1-O-decyl-), fatty acids (myristic acid, nonacosane, and nonadecene), and alkanes (hexadecane), which exhibit antibacterial activity. The antibacterial activity analysis through the inhibition test showed a medium inhibition zone category at an extract concentration of 500 mg/mL, while the MIC value was 50 mg/mL, and the MBC was 100 mg/mL. This indicated that the pepper elder ethanol extract had potential as an antibacterial agent against *Streptococcus mutans*, but it was still not optimal. For further studies, researchers can explore higher concentrations to find the most effective extract concentration as an antibacterial for *Streptococcus mutans* and investigate the antibacterial activity of pepper elder ethanol extract against other microorganisms that cause dental caries.

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