Antibacterial Effect of Moringa Oleifera Gel to Prevent the Growth, Biofilm Formation, and Cytotoxicity of Streptococcus Mutans

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Abstract

To evaluate the antibacterial of Moringa oleifera gel in preventing the growth, biofilm formation, and cytotoxicity of S. mutans cells.

The M. oleifera were used as test material and S. mutans ATCC 25175 as the subject of analysis. The evaluation of the growth of S. mutans using spectrophotometry while toxicity to S. mutans cells using MTT assay. The measurement of the S. mutans biofilm using the Crystal violet.

At 48 hours, the concentration of 12.5% had a better ability to reduce the growth of S. mutans. At 24 hours, the 6.25% and 3.125% had an excellent ability to reduce the growth of S. mutans. The concentration of 6.25% had a better ability to reduce the biofilm formation of S. mutans. The M. oleifera gel extract provided an opportunity for S. mutan as a commensal to form a biofilm below 30% with an average S. mutans biofilm inhibition value above 70%, which was indicated by the biofilm mass not developing. At all concentrations, M. oleifera can cause toxicity to S. mutans cells. Insilico, the antibacterial power of M. oleifera gel was predicted by the bioactivity of 1,3,4,5-tetrahydroxy-cyclohexane carboxylic acid, Hexadecanoic acid, and n-cbz-beta-alanine compounds.

The M. oleifera ethanol extract gel can reduce the growth and formation of S. mutans biofilm on the tooth surface and cause toxicity to S. mutans cells which antibacterial compounds may influence.

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Introduction

Dental caries are caused by the intensity of the development of pathogenic bacteria on the dental surface. Several bacteria are involved in the pathogenesis of dental caries, *Streptococcus mutans* (*S. mutans*) was reported as the primary agent triggering the development of dental caries[1]. Its potential is related to the ability to cellularly ferment carbohydrates as a source of nutrition and a medium for growth, forming quorum sensing and biofilms on tooth surfaces. These bacteria are easily attached to the tooth surface because a thin layer is formed from salivary glycoproteins[2]. Gani (2006) explained that carbohydrate fermentation is a strategy to create quorum sensing to increase colonization on the tooth surface[3]. If it lasts for a long time, this activity causes demineralization of tooth enamel and forms cavities or dental caries[4].

S. mutans bacteria initiate plaque growth on the tooth surface by producing polysaccharide-based dextran utilizing the enzyme dextransucrase (hexocyltransferase: mediation of the dextransucrase enzyme to support plaque formation). With this product, *S. mutans* adheres and colonizes the tooth surface[5]. Meanwhile, the impact of the cariogenic activity of *S. mutans* occurs in the accumulation of biofilm. It produces a polymer matrix to support the biological attachment of *S. mutans* to tooth enamel[6]. Untreated tooth infection will continue to root canal infection, inducing an immunoinflammatory response that can cause apical tissue damage[7]. The progression of this disease begins with the condition of the normal pulp experiencing inflammation or pulpitis. Inflammatory changes occur from acute to

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chronic and from reversible to irreversible until the pulp undergoes necrosis leading to periapical disease leading to abscess formation[8].

Intercellular communication or quorum sensing through peptide signal transduction influences biofilm formation. This activity significantly contributes to biofilm communities that can support increased growth[9]. Efforts to prevent the attachment of bacteria to the tooth surface will prevent the formation of biofilms and inhibit growth. Several natural ingredients from tropical plants are reported to work as antibacterial. Specifically, Mubarak (2018) said that *Gracilaria verucosa* could prevent the adhesion and growth of *Candida albicans* in vitro[10]. In addition, Soraya reported that *M. oleifera* had a good effect on biomass formation and viscosity stability on the impact of biological activity of *Enterococcus faecalis* in changes in incubation time[11].

This study strengthens the hypothesis of the possibility of using active material from *M. oleifera* to be applied to the treatment of dental caries by reducing the population and virulence of *S. mutans* as a minimal intervention approach strategy. This study aimed to evaluate the biological properties of the ethanolic extract of *M. oleifera*, which was formulated in the form of a gel to inhibit the growth and biofilm formation and was toxic to *S. mutans*.

Materials and methods

The research was approved by ethics No.284/KE/FKG/2021 from the Faculty of Dentistry, Syiah Kuala University, Darussalam, Banda Aceh. Indonesia. The subject of this study was *S. mutans* ATCC 25175. At the same time, the test material for this study was the ethanol extract of *M. oleifera*, which was formulated in a gel.

Plant Material

The plant was collected from Aceh Besar District, Aceh Province, Indonesia (5.603444, 95.405863). The *M. oleifera was* extracted in the Chemical Laboratory, Faculty of Mathematics and Natural Science, Universitas Syiah Kuala, Darussalam Banda Aceh, Indonesia. Voucher Number Co2021. Its assay material was collected in the Laboratory of Oral Biology, Dentistry Faculty, Universitas Syiah Kuala, Darussalam, Banda Aceh, Indonesia.

Extract and GC-MS Assay of *Moringa oleifera*

The Moringa leaves (*Moringa oleifera*) that have been separated from the stems are collected as much as 1 kg and then washed with water. Drying was carried out for two days until wilted, then dried in an oven at 50 ^{0}C for 48 h. Moringa leaves are crushed with a blender to obtain Moringa leaf powder. Moringa leaf powder received is then stored in an airtight container. The powder is placed in a clean flat bottomed glass container which is then closed and soaked in 100 mL of 96% ethanol. Separation of residue and filtrate was carried out for three days by changing the solvent. The filtrate is collected together and concentrated with a rotary vacuum evaporator at a temperature of 50 °C and a pressure of 75 mmHg so that the extract is obtained.[11]

The extract obtained was then examined for chemical compounds that have antibacterial properties. GC-MS analysis of the ethanol extract of *M. oleifera* leaves was performed using Shimadzu Japan gas chromatography QP2010PLUS with a fused GC column (2010) coated with polymethyl silicon (0.25nm x 50m). We set up the following conditions: 80–200 °C, and the flow rate was 5 °C/min and 200 °C for 20 min. The FID temperature was 300 °C, injection temperature was 220 °C, and nitrogen carrier gas at a 1 mL/min flow rate, split ratio of 1:75. The pressure is at 116.9 kPa. The column length was 30 m with a diameter of 0.25 mm and a 50 mL/min flow rate[12].

Preparation of *Moringa oleifera* gel

The gelling of *M. oleifera* extract was accomplished in three stages. A gel base is created in the first stage. Gel basis is made by dispersing carbopol in 50 mL of distilled water, dissolving methylparaben in boiling water separately, and then pouring into a mortar containing carbopol, homogenizing it, and making it up to volume with the remaining 50 ml of distilled water. The gel is then created by combining 50 mL of methylparaben with distilled water, followed by propylene glycol, glycerin, and triethanolamine, which is then homogenized to form a gel. The preparation of *M. oleifera* extract gel at 25%, 12.5%, 6.25%, and 3.125% began with adding the test material to a gel base and stirring until homogeneous. Table 1 summarizes the sample's composition[13].

The composition of gel formula extract of *M. oleifera* is below.

Material	Formula of *M. oleifera* gel (%)				Function
	I	II	III	IV	
M. oleifera extract	25%	12.%	6.25%	3.125	Active compound
CMS-Na	3	3	3	3	Gelling agent
Propylene glycol	15	15	15	15	humectant
Glycerin	10	10	10	10	humectant
Methyl paraben	0.025	0.025	0.025	0.025	Preservative
Aquadest	100	100	100	100	solvent

Spectrometry Growth Assay of *Streptococcus mutans*

The growth inhibition test of *S. mutant* used a spectrophotometric assessment approach adopted from Soraya (2020)[14]. Moringa leaf extract gel with various concentrations was added to 100 µL 96 well plates and then incubated at room temperature for 30 min. Then 10 µL of *S. mutans* bacterial solution was added, which had been equalized with 0.5 Mc Farland. Then it was vortexed for 15 minutes at 200 rpm. They were then incubated for 24 h, 48 h, and 72 h. The growth inhibitory activity of *S. mutans* was measured by spectrophotometry at wavelength 520 based on turbidity using a standard suspension of *S. mutans* ($0.5x10^8$). The data was made three times repetition.

Cytotoxicity of *Streptococcus mutans* Assay

The cytotoxic activity of *S. mutans* cells was assessed based on the effect of adaptation time in the *M. oleifera* extract gel for 24 h, 48 h, and 72 h. In the first stage, *S. mutans* cells were cultured for 48 hours at 37 on TYS20B selective media. The toxicity of *M. oleifera* gel against *S. mutans* was assessed based on the principle of the MTT assay (4,5-Dimethyitliiazol-2-yl), 2,5-diphenyl tetrazolium bromide assay)[15]. In the first step, the 96-well plate was put in 100 µL of saliva and then incubated for 30 min at 37 ^{0}C. Then the saliva was removed, then 50 µL of *S. mutans* cell samples (106 cells/mL) were added to the well and incubated for 90 min. At 37 ^{0}C, then observed under a microscope, then the supernatant was removed. Each well was added 70 µL of nitrogen base (NB) medium and 50 µL of *M. oleifera* extract gel solution with 25%, 12.5%, 6.25%, and 3.125%. Treatment with MTT Assay started by removing the supernatant from each well and then washing it with PBS. 50 µL of MTT solution was added to each well and incubated for 3 h at 37 ^{0}C. Then 100 µL of acidified isopropanol 0.04 N was added to the well and placed in an orbital shaker at 50 rpm for 1 h. The MTT test results were read with a microplate reader at a wavelength of 550 nm to obtain the optical density (OD).

Quantity of Biofilm formation Assay

25 µL of saliva was coated on a 96-well plate for 15 min. Then discarded and continued with the addition of 30 L *S. mutans* on the bottom, covered with saliva on each well plate, and allowed to stand for 15 min. Then, *M. oleifera* extract gel was added to each well of 100 µL, then adapted to room temperature for 10 min at 300 rpm. The interaction ability of *S. mutans* biofilm formation was assessed after incubation for 24 h, 48 h, and 72 h at 37 ^{0}C. Assessment of the inhibition of biofilm formation begins by removing the mixed solution of *S. mutans*, the leaves of the test material. Then added 1% SDS solution 100 µL 15 min and then discarded also washed once with PBS. Then, 150 µL of glycerol was added to each well plate, after which it was washed once with 200 µL of PBS. Visualize the biofilm mass by adding 150 µL of 1% crystal violet for 10 min, then washing with 76% ethanol (150 µL) and PBS. Quantitative data reading with ELISA reader at 520 nm[16].

Bioactivity Score Analysis

The molinspiration property engine software calculates bioactivity and molecular properties. The compound structure of *M. oleifera* extract was made using ChemDraw professional v.16© Cambridge soft software. Furthermore, the bioactivity value and molecular score of *M. oleifera* were calculated using the molinspiration properties engine software v.2018.10[17].

Statistical Analysis

Data on growth, biofilm formation, and the relationship between *S. mutans* virulence factor variables were examined by Kruskal-Wallis. Meanwhile, the toxicity of *M. oleifera* gel on *S. mutans* cells was examined by One Way Anova. The probability value ($p<0.05$) with the coefficient correlation value ($r = 1$).

Results

This study reported several interrelated results to determine the quality and quantity of the role of *M. oleifera* in inhibiting growth, biofilm formation, and toxicity to *S. mutans* cells using incubation time as an analytical variable. Figure 1 shows that all concentrations of Moringa leaf ethanol extract gel could reduce the growth of *S. mutans*. The 24 hours and 48 hours were shown to have the most practical effect in reducing the

growth of *S. mutans* which was influenced by all concentrations of the ethanol extract of Moringa leaf gel. Meanwhile, within 72 hours, only 25% and 12.5% concentrations had a better effect than other concentrations. Positive control (CHX) gave a good product at 24 hours, 48 hours, and 72 hours. Based on the Kruskal-Wallis analysis showed that the growth of *S. mutans* under the influence of Moringa leaf ethanol extract gel had a significant difference ($p < 0.05$; 0.050) based on incubation time with a moderate correlation ($r = 0.653$), but based on the concentration, did not show a significant difference ($p > 0.05$; 0.608) and there was no relationship ($r = -0.044$).

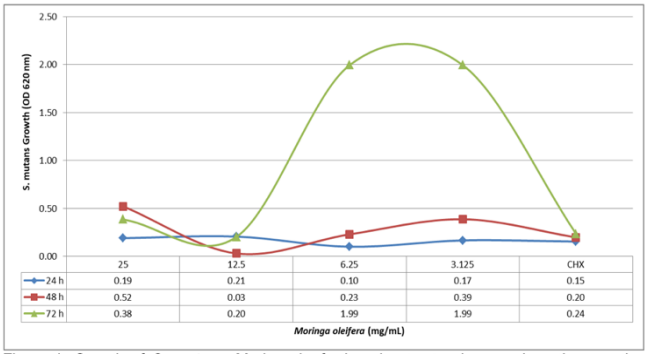

Figure 1. Growth of *S. mutans*. Moringa leaf ethanol extract gel can reduce the growth of *S. mutans,* especially for 24 hours and 48 hours. At 48 hours, the concentration of 12.5% had a better ability to reduce the growth of *S. mutans*. Meanwhile, at 24 hours, the concentrations of 6.25% and 3.125% had an excellent ability to reduce the growth of *S. mutans*. Data is taken three times through repetition.

Table 1 shows the incubation times of 24, 48, and 72 hours showing the growth intensity of *S. mutans* with an average OD of 0.47 nm (minimum 0.03 and maximum 1.99). The Mc Farland standard can be classified as < 300 CFU or Mc. Farland 0.5 (1.5×10^8). Optical Density 0.05 nm (<150 CFU/mL), 0.08-0.1 nm (Mc Farlan 0.5; <300 CFU), OD 0.11-0.29 nm (Mc Farland 1; 300-600 CFU); OD 0.3-0.49 nm (Mc Farland 2; 600-1200 CFU). These scales were adopted by Mc Farland Standard for in vitro use only, Catalog No. TM50-TM60, Scott Sutton, Measurement of Microbial Cells by Optical Density, 2011)[18].

Figure 2. shows the formation of *S. mutans* biofilm after being affected by the ethanol extract gel of Moringa leaves. Concentrations of 12.5% and 6.25% decreased the formation of *S. mutans* biofilm, which was better at 24 and 48 hours of incubation. In addition, CHX as a positive control tends to stabilize the formation of *S. mutans* biofilms. Based on the analysis of Kruskal Wallis, it was shown that there was no significant difference in the effect of Moringa leaf ethanol extract gel on biofilm formation based on incubation time ($p > 0.05$; 0.152) but had a relatively moderate relationship ($r = 0.501$). While, based on concentration there was also no significant difference ($p > 0.05$;0.11) which did not show a relationship ($r = -0.022$).

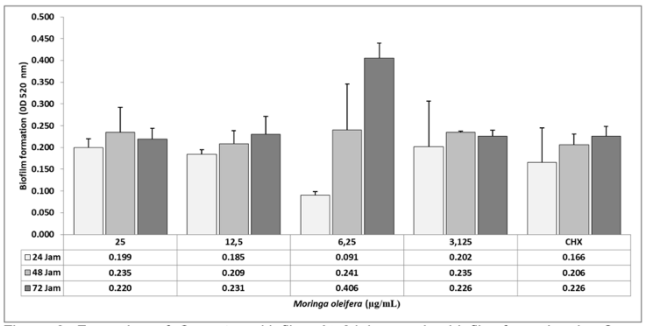

Figure 2. Formation of *S. mutans* biofilm. At 24 hours, the biofilm formation by *S. mutans* decreased after being influenced by Moringa leaf extract gel. A concentration of 6.25% has a better effect than other concentrations. Data was taken three times repetition. Bar (biofilm formation) Bar error (Standard deviation).

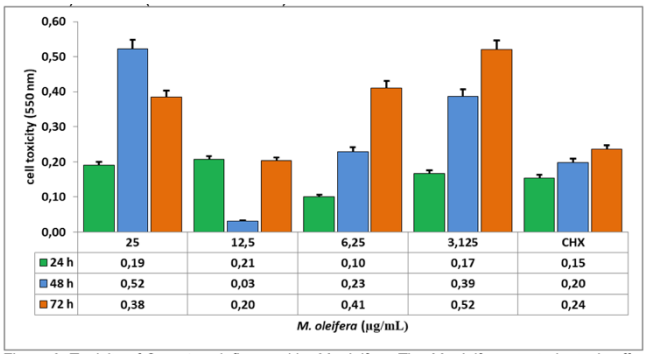

Figure 3. Toxicity of *S. mutans* influenced by *M. oleifera*. The *M. oleifera* exerted a toxic effect on *S. mutans* cells. The *M. oleifera* gel of 25%, 6.25%, and 3.125% gave a better effect than the concentration of 12.5%. Bar (Toxicity) Bar error (error bars with percentage).

Table 2 shows the distribution and frequency of decreasing *S. mutans* biofilm formation after interaction with Moringa leaf ethanol extract gel. Moringa leaf ethanol extract gel provides growth opportunities below 30%.

The ability to inhibit the formation of *S. mutans* biofilms is above 70% on average. This value can be justified because this test material provides an excellent phyto-response effect to prevent the development and spread of *S. mutans* biofilm. The ethanolic extract gel of Moringa leaves a good impact on reducing *S. mutans* biofilm with concentrations of 12.5%, 6.25%, and 3.125%. They are having a better effect on preventing the development of S mutans biofilm. It indicates that the biofilm mass did not develop. At the same time, the negative control (*S. mutans*) without the influence of the test material showed that the quantity and quality of the biofilm mass had progressed. Figure 3. shows that *M. oleifera* had an excellent effect in causing toxicity to *S. mutans* cells at all concentrations, except the concentration of 12.5% at 48 hours. Based on this figure, CHX as a positive control had a relatively stable toxic level based on incubation time. While the test material had a higher poisonous effect on *S. mutans* cells than the positive control, it had a response that changed based on incubation time. Based on One Way Anova analysis, it showed that the toxicity of *M. oleifera* based on incubation time had no significant difference (p>0.07) but had a moderate relationship (r=0.58). Based on the concentration, there was also no significant difference (p>0.479), and there was no correlation between concentration effect on the toxicity of *M. oleifera* to *S. mutans* cells (-0.145).

M. oleifera (µg/mL)	N	Growth of *S. mutans*											
		24 h				48 h				72 h			
		OD	SDV	colony (CFU/mL)	Freq	OD	SDV	colony (CFU/mL)	Freq	OD	SDV	colony (CFU/mL)	Freq
25%	3	0,19	0,171	<600	23%	0,52	0,138	<1200	38%	0,38	0,015	<900	8%
12.5%	3	0,21	0,063	>600	25%	0,03	0,048	<200	2%	0,20	0,103	<600	4%
6.25%	3	0,10	0,011	<300	12%	0,23	0,019	<600	17%	1,99	0,000	>1500	41%
3,125%	3	0,17	0,023	<600	20%	0,39	0,066	<900	28%	1,99	0,000	>1500	41%
CHX	3	0,15	0,004	<600	19%	0,20	0,015	<600	14%	0,24	0,007	<600	5%

Table 1. Distribution and growth frequency of *S. mutans* after being affected by *M. oleifera* extract gel.

Figure 4 reported that based on the percentage of similarity in the action of the toxicity properties of *M. oleifera* gel with various concentrations on the growth and formation of *S. mutans* biofilms. At 24 hours, a concentration of 25% and a concentration of 6.25% had a better ability to suppress the toxicity that impacted growth retardation and biofilm formation. Meanwhile, at 72 hours of incubation, the toxicity of *S. mutans* cells was very closely correlated with growth, especially at concentrations of 25%, 12.5%, and CHX as a positive control. The same thing happened at 72 hours of incubation. Based on the correlation of the percentage of toxicity of the *M. oleifera* gene to *S. mutans*, growth, while the formation of *S. mutans* biofilms was only more sensitive to *S. mutans* cells which were affected by *M. oleifera* gel for 24 hours. Based on the Kruskal-Wallis analysis, the inhibitory power of the *M. oleifera* extracts gel against the three virulence factors of *S. mutans* (growth, biofilm formation, and toxicity) showed that there was no significant difference between the virulence variables of *S. mutans* (p>0.05;0.782), and between the effects of incubation of *S. mutans* (p>0.05;0.607).

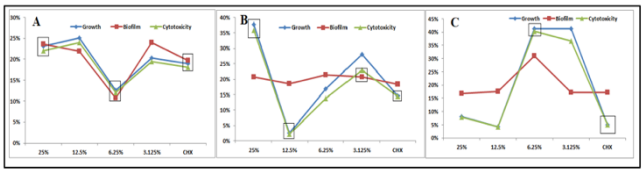

Figure 4. Effect of *M. oleifera* on the virulence activity of *S. mutans*. (A) 24 hours, (B) 48 hours, and (C) 72 hours). *M. oleifera* gel has an interconnected effect suppressing virulence properties (growth, biofilm formation, and toxicity to *S. mutans* cells. Y-axis (correlation of virulence activity), and X-axis (concentration of *M. oleifera* gel).

M. oleifera (µg/mL)	N	Biofilm formation of *S. mutans*								
		24 h			48 h			72 jam		
		OD	Freq	Scale	OD	Freq	Scale	OD	Freq	Scale
25	3	0,20	24%	Moderate	0,23	21%	Moderate	0,22	17%	Moderate
12.5	3	0,18	22%	Moderate	0,21	19%	Moderate	0,23	18%	Moderate
6.25	3	0,09	11%	Strong	0,24	21%	Moderate	0,41	31%	Weak
3.125	3	0,20	24%	Moderate	0,23	21%	Moderate	0,23	17%	Moderate
CHX	3	0,17	20%	Moderate	0,21	18%	Moderate	0,23	17%	Moderate

Table 2. Distribution and frequency of *S. mutans* biofilm formation after interaction with gel of *M. oleifera.*

Table 3 reports that the biological activity of *M. oleifera* compounds involved in affinity with GPCR ligands, nuclear receptor ligands, kinase inhibitors, protease inhibitors, and enzyme inhibitors from *S. mutans* cells to reduce growth biofilm formation and increase toxicity to S cell*S. mutans*. Based on the bioactivity score, the most promising *M. oleifera* compounds in Table 3 are 1,3,4,5-tetrahydroxy-cyclohexane carboxylic acid, Hexadecanoic acid, and n-cbzbeta-alanine

compounds. A number of these compounds are thought to act in more than three mechanisms to prevent the development of *S. mutans*. It is based on the value of the bioactivity score. If it is more than 0.00, it indicates a relatively sizeable biological activity. If the value is -0.50 to 0.00, it is pretty active. If the score is less than -0.50, it is not happening[19].

No	Compounds	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1	Alpha-butyrolactone	-3.61	-3.61	-3.68	-3.54	-3.53	-3.52
2	1,3-cyclopentanedione	-3.48	-3.07	-3.70	-3.26	-3.21	-3.04
3	Cis-1,2,6-trimethylpiperidine	-2.90	-2.58	-3.41	-3.39	-2.75	-2.86
4	2-pyrrolidinone	-3.56	-3.58	-3.81	-3.97	-3.34	-3.60
5	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	-1.59	-0.96	-2.25	-1.60	-1.53	-0.65
6	1,3,4,5-tetrahydroxy-cyclohexanecarboxylic acid	-0.55	**0.17**	-0.84	-0.18	**0.06**	**0.29**
7	Hexadecanoic acid	**0.02**	**0.06**	-0.33	**0.08**	-0.04	**0.18**
8	n-cbz-beta-alanine	**0.23**	**0.38**	-0.40	-0.10	**0.43**	**0.37**

Table 3. Bioactivity score of *M. oleifera* compounds.

Discussion

Based on phytochemical screening, *M. oleifera* contains alkaloid compounds, flavonoids, saponins, phenolics, steroids, and tannins. These compounds are reported to be antibacterial and have antioxidant properties. A number of these compounds are reported to be very commonly found in natural plants, including *M. oleifera*[20]. In addition, *M. oleifera* contains several chemical compounds with antibacterial potential with various sites and targets of action on GPCR ligands, ion channel modulators, kinase inhibitors, nuclear receptor ligands, protease inhibitors, and enzyme inhibitors (Table 3).

In Figure 1 and Table 1 was explained that *M. oleifera* gel was able to reduce the growth of *S. mutans*, especially at 24 and 48 hours. Meanwhile, within 72 hours, only 25% and 12.5% concentrations had a better effect than other concentrations. In principle, the ability of antibacterial materials has two aspects of work, namely bacteriostatic or bacteriocidal[21]. Based on the findings of this study, it was shown that *M.oleifera* gel has bacteriostatic properties. This phenomenon indicates that this test material indirectly limits the growth of *S. mutans*. This concept is highly expected as a commensal antibacterial because it can maintain and control the overgrowth of *S. mutant*s. This bacteriostatic property is highly desirable in the tolerance mechanism of the immune system[22].

Cellularly, the ability of *M. oleifera* gel to suppress the growth of *S. mutans* can be described from the activity of several active compounds contained in it, which function as antibacterial. The nature of this compound is intracellular damage through the membrane charge mechanism[23]. Antibacterial chemical compounds are generally capable of carrying a negative charge. These negatively charged molecules have a higher affinity for positive ions on the bacterial cell membrane. It caused the accumulation and increased absorption of ions, which then caused intracellular damage. This phenomenon is reported as one of the strategies of active plant compounds to suppress or interfere with bacterial growth[24]. Gonelimali (2018) reported that several plant extracts had a significant effect on the cell membranes of Gram-positive and Gram-negative bacteria, as indicated by a decrease in pH and hyperpolarization of the cell membrane[25].

Prajapati (2021) reported that the surface of bacterial membranes is not wholly impervious because the presence of porin proteins on the membrane surface can create channels large enough to allow the passage of molecules with a molecular mass below 600 kDa, such as substituted phenolics in herbal extracts and essential oils, allowing their slow penetration into the periplasmic space and cytoplasmic membrane[26].

Figure 2 reports that *M. oleifera* gel concentrations of 12.5% and 6.25% had a better effect on decreasing the formation of *S. mutans* biofilms at incubation times of 24 hours and 48 hours. In addition, the data from Table 2 clarify the findings from Figure 2, where the *M. oleifera* extract gel provides an opportunity to grow *S. mutans* biofilm below 30%. The ability to inhibit the formation of *S. mutans* biofilms is above 70% on average. The growth inhibitory activity and biofilm formation of *S. mutans* had a good correlation at 24 hours at 25% and 12.5% (Figure 3.).

The ability to inhibit the biofilm of *S. mutans* bacteria by several natural extracts can occur by lowering the action potential of the biofilm mass so that the biofilm does not develop and is degraded from the membrane surface[27]. In addition, antibacterial agents also inhibit the production of extracellular substances, including proteins and exopolysaccharides, the extracellular matrix. This extracellular matrix

secures bacteria together in a multicellular community[28]. So that it can disrupt the ion channel communication between bacterial species involved in the formation of biofilms, it reduces access for bacterial attachment and decreases biofilm formation[29].

This study also reported that *M. oleifera* gel also gave a tolerance for biofilm formation of 30%. This ability correlated with the bacteriostatic response to *S. mutans*. In the concept of biofilm resistance mechanism, pathogens may be able to resist antimicrobial action more when they are present in the biofilm, and their infection can survive on different biotic and abiotic surfaces[30]. In addition, factors contributing to resistance in biofilms include the presence of an extracellular polymeric matrix leading to strong adhesion of microbes to feelings and low penetration of antibiotics or increased activity of efflux pumps that remove antimicrobial agents from cells[31].

Figure 2 and Table 2 explain that morphologically, *M. oleifera* gel can damage the morphology of *S. mutans* biofilms, where concentrations of 12.5%, 6.25%, and 3.125% have a better effect in preventing the development of S. biofilm *S. mutans*. According to Roy (2018), this ability is that several antibacterial compounds can reduce oxygen levels in the biofilm mass, thereby interfering with interactions with cell surface bases. Static interactions between biofilm protein molecules and host cell receptors are disrupted, causing the biofilm area to become hydrophilic. This property can decompose the biofilm mass into degradation.[29] In addition, the ability of antibacterial compounds found in plants to respond to oxidation on the surface of bacterial cells, thereby interfering with the production of the enzyme GTF (glucosyltransferase), breaks down carbohydrates into simple sugars, a source of nutrition for other quorum-sensing bacteria[32].

In the hydrophobicity pathway, active compounds from plants interfere with cell surface hydrophobicity activities. The hydrophobicity of plant extracts and their bioactive contributes to the breakdown of lipid cell membranes and supports a more permeable membrane[33]. Furthermore, the bioactive compounds in the section can inhibit the synthesis of essential metabolites (folic acid) by preventing enzymatic reactions. Protein synthesis in microorganisms can also be hindered if it forms bioactive and changes the shape of the ribosome. Disturbances can cause errors in reading the genetic code in the mRNA[34].

In Figure 3, it is explained that the toxicity of *M. oleifera* gel on *S. mutans* cells has the same action (inline) in inhibiting the growth and formation of biofilms. At 24 hours, a concentration of 25% and a concentration of 6.25% had a better ability to cause toxicity, resulting in decreased growth and biofilm formation (Figure 4). *S. mutans* biofilm is very difficult to eliminate because it naturally becomes the point for the development of other commensal bacteria in the formation of quorum sensing on the tooth surface. In addition, this test did not have a working relationship that coincided with the toxicity of the test material with the formation of biofilms because the intensity of activity of protein receptors involved in carbohydrate fermentation has a high sensitivity to environmental changes[35]. Gani (2006) reported that glucosyltransferase, fructosyl-transferase, maltose, and sucrose proteins are significant determinants of quorum sensing formation as prerequisites for biofilm development.[3]

The toxic nature of *M. oleifera* is predicted to have a role in influencing the mechanism of action and protein synthesis of peptidoglycan that can affect growth[36]. Several antibacterial agents, when working to inhibit growth and biofilm formation, can occur through the peptidoglycan interaction pathway, activate autolysis genes on the cell wall/membrane, or alter the structure of cell surface proteins by increasing the negative charge and increasing oxygen solubility through the mechanisms of ROS and RES[37]. In addition, *S. mutans* has a mosaic sequence of anionic surface domains. The high potential for binding of several antibacterial active compounds possessed by *M. oleifera* in this negative anionic domain can increase the relatively high toxicity of cells[38]. In addition, some ROS, such as negatively charged hydroxyl radicals, easily penetrate cell membranes. Xu (2020) reported that higher extract concentrations tend to cause pressure on cell membranes so that they leak into the cytoplasm, which triggers cell death[39]. It was also speculated that the high concentration of *M. oleifera* gel extract contributed to the toxicity of *S. mutans* cells.

Table 3 reports that many active antibacterial compounds in *M. oleifera* have biological activities in affinity with GPCR ligands,

nuclear receptor ligands, kinase inhibitors, protease inhibitors, and enzyme inhibitors against *S. mutans* cells. Based on the results of bioactivity analysis, three compounds represented all cellular affinity groups. The compounds 1,3,4,5-tetrahydroxy-cyclohexane carboxylic acid, Hexadecanoic acid, and n-cbzbeta-alanine contain relatively high bioactivity in reducing all cellular activities of *S. mutans* cells. The action of these three compounds is assumed to be in silicon, able to block the protease enzyme so that it can prevent the degradation of gelatin and collagen substrates in the host tissue[40]. In addition, active antibacterial compounds from *M. oleifera* can also be predicted to inhibit the work of enzymes (virulence proteins) involved in growth (YidC1 and YidC2) proteins[41], and biofilm formation, enzymes such as Als1 and Als3, and SpaP[42]. The activity of antibacterial compounds of *M. oleifera* also shows active action on the surface of bacterial cell membranes, including S. mutant, by modulating ion channel modulators that work to increase channel and ion permeability so that active drug compounds can enter membrane channels to affect the cytoplasm of organelles through increased responsiveness of G protein-coupled receptors (GPCRs) involved in cell transmembrane responses[43].

Conclusions

M. oleifera gel can reduce the growth and formation of *S. mutans* biofilm based on the incubation time and extract concentration approach. In addition, *M. oleifera* can be toxic to *S. mutans* cells. The toxicity activity of *M. oleifera* correlated with the growing frequency of *S. mutans*. Based on the bioactivity test, 1,3,4,5-tetrahydroxy-cyclohexane carboxylic acid, Hexadecanoic acid, and n-cbzbeta-alanine compounds have high bioactivity and may be involved in efforts to reduce growth and biofilm formation and affect toxicity to *S. mutans* cells.

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Declaration of Interest

The authors report no conflict of interest.

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