Role of *Moringa oleifera* irrigation solution on the cell metabolism change of *Streptococcus mutans*

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Abstract

The principal etiological agent responsible for dental caries is *Streptococcus mutans (S. mutans)*. The *Moringa oleifera* (*M. oleifera*) possesses antioxidant and antibacterial properties that function through the response to oxidative stress, which affects bacterial cell metabolism. This research examined *M. oleifera* impact on *S. mutans* growth, toxicity, glucan-binding protein (GBP) expression, and nucleic acid structure. Methods included spectrophotometry for growth analysis, enzyme-linked immunosorbent assay for GBP quantification, the (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) MTT assay for cytotoxicity, Fourier transform infrared for nucleic acid changes, and docking simulation for ligand-receptor affinity. Results showed that *M. oleifera* significantly inhibited *S. mutans* growth at all concentrations over 24 and 48 h (optical density <0.1), comparable to <300 CFU/mL. At 72 h, 6.25% and 3.125% concentrations were most effective, with chlorhexidine also showing stability at these times. A 3.125% concentration of *M. oleifera* notably reduced GBP production to below 15% and caused cell toxicity. Furthermore, 25% and 3.125% concentrations significantly altered *S. mutans* nucleic acids, and *M. oleifera* showed high binding affinity to the GBP gene receptor. Thus, *M. oleifera* can inhibit *S. mutans* growth and GBP production, cause nucleic acid deformation, and strongly bind to the GBP receptor, highlighting its potential in dental caries prevention.

Key words: Glucan-binding protein, metabolism change, *Moringa oleifera*, nucleic acid, *Streptococcus mutans*

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INTRODUCTION

Caries is a multifactorial disease influenced by the host, bacteria, oral environment, and time, with *Streptococcus mutans* playing a key role in its development.[1] This bacterium produces dextran through dextransucrase, enabling it to adhere to tooth surfaces and form biofilms.[2] These biofilms contribute to plaque formation, a critical factor in caries pathogenesis. *S. mutans* utilizes various metabolic processes for survival and adaptation in fluctuating environmental conditions.[3]

S. mutans produce glucan-binding protein (GBP) and glucosyltransferase to attach to host cells and form

biofilms, enhancing their growth and creating a protective exopolysaccharide matrix.[4] This bacterium metabolizes carbohydrates into organic acids such as lactic, acetic, and propionic acid, lowering pH levels and leading to enamel demineralization when pH drops below 4.5.[5] This process accelerates dental cavity formation. *S. mutans* adapt to environmental changes through various metabolic strategies to maintain its survival and spread.[6]

Chlorhexidine (CHX), an oral antibacterial mouthwash, can irritate the oral mucosa and, with prolonged use, lead to *S. mutans* resistance, increasing caries risk.[7] Conversely, *Moringa oleifera* has shown promise as an alternative, with its array of secondary metabolites such as tannins, saponins, terpenoids, phenols, alkaloids, and flavonoids demonstrating antibacterial, anti-inflammatory, and antioxidant properties.[8] *M. oleifera's* fatty acids also possess antibacterial solid effects, highlighting its potential in oral health applications.[9,10]

Previous research found that *M. oleifera's* ethanolic extract has antibacterial effects against *Enterococcus faecalis* and inhibits biofilm formation.[11] In addition, it was shown to hinder *Candida albicans* growth and disrupt its biofilm production, attributed to *M. oleifera's* bioactive compounds, which can also interfere with *S. mutans* metabolism, particularly in glucan synthesis inhibition.[12]

This study introduces the novel use of *M. oleifera* as an antibacterial against *S. mutans*, a major dental caries pathogen. It focuses on *M. oleifera's* effects on bacterial metabolism, GBP production, and cell structure integrity, which were previously unexplored in caries prevention. In addition, it investigates *M. oleifera's* potential as an endodontic irrigation solution, showcasing its application in dentistry beyond traditional uses. The study assesses *M. oleifera's* ability to inhibit *S. mutans* growth, reduce GBP protein production, cause nucleic acid deformation, and examine its binding affinity with the GBP gene, highlighting its efficacy in combating dental caries.

MATERIALS AND METHODS

This study explored the antibacterial effects of *M. oleifera* against *S. mutans* ATCC 25175, focusing on its ability to inhibit bacterial growth, reduce GBP production, and cause nucleic acid deformation. *M. oleifera* was sourced from Aceh, Indonesia, and prepared in the Chemical Laboratory at Universitas Syiah Kuala, Banda Aceh, under voucher C243.

Preparation of extract and gas chromatography–mass spectrometry analysis of *Moringa oleifera*
The *M. oleifera* leaf ethanol extract was adopted protocol by Gani,[12] and gas chromatography–mass spectrometry (GC-MS) evaluated the chemical compound on a Shimadzu Japan QP2010PLUS with a polymethyl silicon-lined GC column (0.25 mm × 50 m). Analysis conditions included a temperature range of 80°C–200°C, escalating at 5°C/min to hold at 200°C for 20 min. The flame ionization detector (FID) temperature was 300°C, and the injection temperature was 220°C, and nitrogen as the carrier gas at a flow rate of 1 mL/min and a split ratio of 1:75. Pressure was set at 116.9 kPa, with the column specifications being 30 m long, 0.25 mm in diameter, and a flow rate of 50 mL/min.

Culture and growth assay
S. mutans ATCC 25175 from a 50% glycerol stock was re-cultured in tryptic soy broth media to refresh the culture. The culture was standardized to McFarland 0.5 (1.5×10^8 CFU). Moringa leaf extracts at various concentrations were added to 96-well plates (100 μL/well) and incubated at room temperature for 30 min. A 10 μL solution of *S. mutans* was then added and homogenized at 200 rpm for 15 min. The samples underwent incubation periods of 24, 48, and 72 h. The antibacterial activity was assessed using spectrophotometry (Bio-Rad, USA) at an optical density (OD) of 620 nm, correlating a 0.08–0.1 nm OD with McFarland 0.5 (1.5×10^8) or 300 CFU/mL.[13]

Cytotoxicity assay (MTT Assay).[14]
The (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) MTT assay was used to evaluate *S. mutans* cytotoxicity. In this process, 96-well plates containing 100 μL of Moringa leaf extract were incubated at 37°C for 30 min, followed by adding 50 μL of *S. mutans* (10^6 cells/mL) and further incubation. After a 90-min settlement, wells received 50 μL of MTT solution for a 3-h incubation at 37°C. Then, 100 μL of acidified isopropanol was added, shaken at 50 rpm for an hour, and OD measured at 550 nm. To examine *S. mutans* toxicity, samples with varying *M. oleifera* concentrations were incubated, cultured in nutritional media, and subjected to gram staining for morphological analysis using microscopy at ×400.

Fourier transform infrared assessment of nucleic acid
The stress response of *S. mutans* to Moringa leaf extract was analyzed using Fourier transform infrared (FTIR) spectroscopy, focusing on the 4000 cm^{-1}–400 cm^{-1} wave number range. Cell extract was adopted by Cut Soraya *et al.*[11] The method involved positioning the cell extract of *S. mutans* on an infrared prism with a higher refractive index (1.39 for bacterial cells) and employing attenuated total reflectance (ATR) for enhanced measurement of the light intensity and absorption spectrum.[15,16] This ATR-FTIR technique, adapted from Zarnowiec *et al.*, analyzed the stress response by examining specific windows for lipids (3000–2800 cm^{-1}), proteins (1700-1500 cm^{-1}), nucleic acids and carbohydrates (1500–1200 cm^{-1}), and the fingerprint region (900-500 cm^{-1}), effectively capturing the functional groups affected in the sample.[17]

Enzyme-linked immunosorbent assay

The GBP levels in *S. mutans* were determined using the sandwich enzyme-linked immunosorbent assay method with Cusabio kits.[18] The procedure involved transferring the protein sample to a 1.5 mL Eppendorf tube, adding 50 µL of both standard and samples, and then 50 µL of antibody into each well. After a 40-min incubation at 37°C and three washes, 100 µL of horseradish peroxidase (HRP)-conjugate was added, followed by a 30-min incubation and five washes. The wells received 90 µL of 3,3',5,5'-Tetramethylbenzidine (TMB) substrate for a 20-min incubation in the dark at 37°C, then 50 µL of stop solution before measuring the absorbance at 450 nm.

Docking assessment

The molecular docking study followed the molecular operating environment (MOE) 2020 protocol,[19] starting with converting ligand "SMILES" structures into three-dimensional "mdb" format, while receptors were in "pdb" format. Both ligand and receptor structures were optimized for geometry and energy. Validation used the native ligand as a reference binding site, targeting a root mean square deviation (RMSD) value of <2Å. Docking of the experimental ligand involved the triangle-matching algorithm for placement over 1000 iterations, using the London dG scoring function and displaying the top 30 results. Refinement was conducted with a force field, with a population size of 1000 per the MOE default settings, culminating in an optimal dataset saved in "mdb" format.

Statistical analyses

S. mutans growth data and GBP titers of *S. mutans* cells were analyzed using the Kruskal–Wallis test. Meanwhile, toxicity data on *S. mutans* cells was analyzed using one-way ANOVA, and data on changes in nucleic acids from *S. mutans* cells were analyzed descriptively, with significance analysis $P < 0.05$.

RESULTS AND DISCUSSION

Chemical content and spectrum of *Moringa oleifera*

The chromatogram of *M. oleifera* leaf extract [Figure 1] shows peaks between 4 and 41.225 min, with components identified via GC-MS and matched against the national institute of standards and technology (NIST) chemical database. Seventeen compounds were identified, including antioxidants and antibacterials such as glycerol and benzeneacetonitrile [Table 1]. These compounds combat *S. mutans* by inhibiting cell wall synthesis, disrupting cell membranes, and interfering with protein and nucleic acid synthesis, thereby preventing GBP protein release and biofilm formation. This suggests *M. oleifera's* potential to halt *S. mutans* growth and pathogenicity.[20]

Table 1 shows the 17 compounds in the ethanol extract of Moringa leaves analyzed using GC-MS. Of the 17 compounds, 5 of them are alkanone class compounds (1, 2, 7, 8, and 10), two phenol and alcohol class compounds (3 and 17), two acid class compounds (14 and 15), and six aromatic compounds (6, 11–13, 16, and 17), as shown in Figure 2, whereas Among the 17 compounds presented in the ethanol extract of moringa leaves, compound number 14 with IUPAC name: 1,3,4,5-tetrahydroxy-cyclohexane carboxylic acid referred to trivially as quinic acid, is the main component reaching 43.04%.

Inhibition growth of *Streptococcus mutans*

Table 2 reveals that *M. oleifera* leaf ethanol extract significantly

Table 1: Chemical contents of *Moringa* leaf ethanol extracts based on gas chromatography-mass spectrometry spectra

Compounds	Retention time (min)	Percentage
Alpha-butyrolactone	5.452	2.06
1,3-cyclopentanedione	5.645	4.90
Glycerol	6.411	8.48
Cis-1,2,6-trimethylpiperidine	6.797	1.33
1,2-epoxy cyclohexane	6.914	2.34
benzeneacetaldehyde	7.803	3.05
Isobutyraldehyde, propylhydrazone	8.141	1.83
2-pyrrolidinone	8.700	2.02
2-butenamide, 2-cyano-3-hydroxy	8.817	3.20
2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	10.603	8.00
2-trideuteromethoxy-3-methyl pyrazine	22.904	1.39
Benzeneacetonitrile, 4-hydroxy-	23.008	4.87
1,2,3,3a,4,8b-hexahydrocyclopenta[b]indole	26.131	1.11
1,3,4,5-tetrahydroxy-cyclohexane carboxylic acid	27.834	43.04
Hexadecanoic acid	30.475	3.04
n-cbz-beta-alanine	31.054	2.05
3-(2,2-dimethyltetrahydrofuran-3-yl)phenol	33.826	1.24

Table 2: Distribution and frequency of growth of *Streptococcus mutans*

M. oleifera	*S. mutans* growth (turbidity OD 600 nm)												P*
	24 h (*n*=18)				48 h (*n*=18)				72 h (*n*=18)				
	Mean	SDV	CFU/mL	Frequency (%)	Mean	SDV	CFU/mL	Frequency (%)	Mean	SDV	CFU/mL	Frequency (%)	
Concentrations$_{25\%}$	0.09	0.091	<300	5	0.11	0.012	<300	10	0.15	0.044	300–600	5	0.045
Concentrations$_{12.5\%}$	0.11	0.074	<300	6	0.11	0.009	<300	10	0.14	0.026	300–600	5	
Concentrations$_{6.25\%}$	0.10	0.006	<300	6	0.12	0.006	<300	11	0.10	0.005	<300	4	
Concentrations$_{3.125\%}$	0.11	0.031	<300	6	0.11	0.015	<300	10	0.11	0.006	<300	4	
CHX	0.10	0.053	<300	6	0.10	0.007	<300	10	0.14	0.007	300–600	5	
S. mutans	1.21	0.002	≥600	71	0.51	0.002	600–1200	48	2.21	0.003	>1200	78	
P*			0.0611				0.105				0.0724		

*Kruskal–Wallis test. OD: Optical density, SDV: Standard deviation, *M. oleifera: Moringa oleifera*, *S. mutans: Streptococcus mutans*
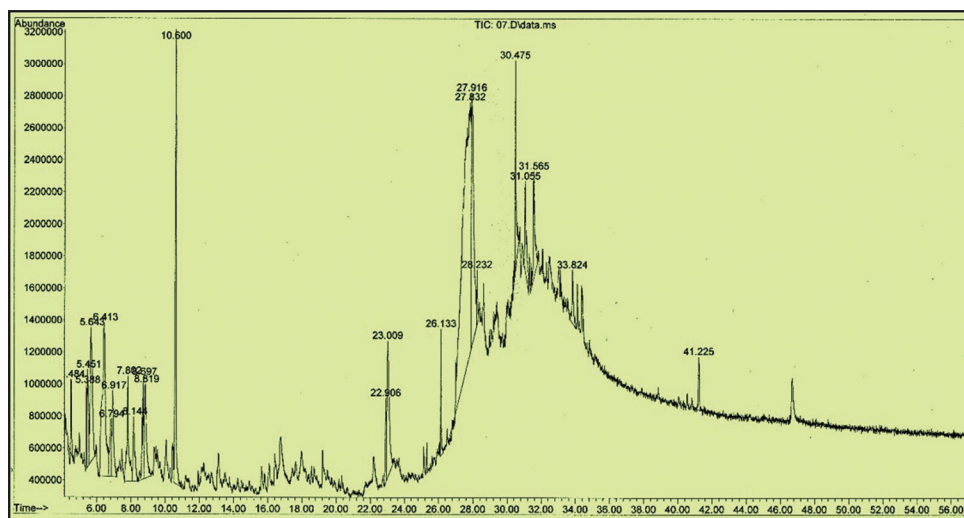
Figure 1: General characteristics of *Moringa oleifera* leaf extract. Compounds No. 3, 10, and 14, as indicated in Table 1, have a quantity exceeding 5%. Among these components, quinic acid is the most prevalent, with a retention duration of 27.9 min

inhibits *S. mutans* growth across all tested concentrations, with the most pronounced effects seen after 72 h, particularly at 6.25% and 3.125%. CHX served as a consistent positive control. This inhibition suggests *M. oleifera's* potential to disrupt *S. mutans* growth phases, likely due to its antibacterial components, such as the toxicity-inducing quinic acid against *E. faecalis*, as identified by Cut Soraya *et al.*[11] Kruskal–Wallis analysis highlighted a significant time-dependent effect on *S. mutans* suppression (*P* < 0.05), benchmarked against the McFarland 0.5 standard, indicating *M. oleifera's* bacteriostatic capabilities.[13]

Cytotoxicity cell of *Streptococcus mutans*

Figure 3a shows the alteration in the number of *S. mutans* cells due to the interaction of *M. oleifera* compounds. It showed significant changes in *S. mutans* cells. Morphological changes of *S. mutans* cells that could be observed were. (1) Intercellular chain septum detached, (2) Cells agglutinated, (3) Lysis cells, (4) Failure of cell cleavage, (5) Nucleo-toxicity occurred. This process shows that several active compounds possessed by *M. oleifera* can damage cell membranes and protein synthesis as an

indicator of the release of the nucleus from the cell membrane. Figure 3b reports the best toxicity of *M. oleifera* solution at a concentration of 3.125%, especially at 72 h. Moringa's antibacterial components, such as quercetin, kaempferol, and isothiocyanates, disrupt bacterial growth by affecting cell membrane integrity and inhibiting DNA replication.[21,22] This interference with essential bacterial functions and metabolic pathways halts the spread of infections, positioning Moringa as a potent natural source for combating bacterial proliferation.[23,24] This aligns with antibacterial strategies that target cell walls and protein synthesis, including inhibiting crucial GBP secretion for adhesion.[25] Thus, *M. oleifera* exhibits potent antibacterial effects by compromising *S. mutans* cell integrity and hindering its growth.[26] One-way ANOVA analysis shows that there is a significant difference between concentration and incubation time in causing toxicity to *S. mutans* cells (*P* < 0.05).

Glucan-binding protein expression of *Streptococcus mutans*

M. oleifera effectively reduces GBP production in *S. mutans*,
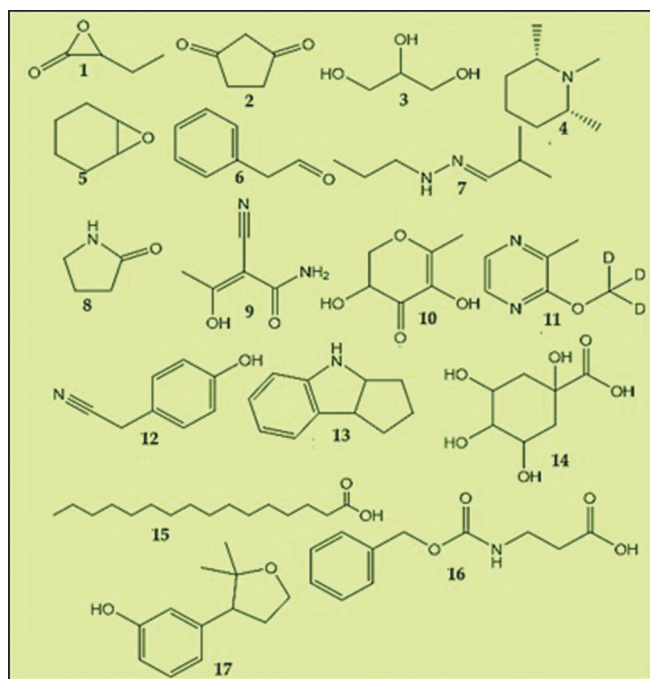
Figure 2: The structure of chemical compounds contained in the ethanol extract of Moringa leaves was analyzed using a gas chromatography–mass spectrometry instrument

a critical factor in its adherence to host cells in sucrose-rich environments. Testing showed all concentrations of *M. oleifera* decreased GBP levels, with 3.125% being the most effective [Figure 4]. This is likely due to *M. oleifera's* active compounds inhibiting protein formation, supported by observed nucleotoxic effects on *S. mutans* cells [Figure 3].

M. oleifera disrupts *S. mutans'* crucial GBP,[27] impairing tooth adhesion and biofilm formation.[28] Its antioxidants may affect GBP genes, while compounds such as saponins and terpenoids could change cell membrane permeability. Phenols and flavonoids might block GBP synthesis enzymes, and the plant's extracts could hinder bacterial communication and nutrient uptake, weakening *S. mutans'* virulence.[29] This comprehensive action demonstrates *M. oleifera's* potential as an effective antibacterial against the GBP pathway in *S. mutans*.[30]

Table 3 shows that Moringa leaf ethanol extract consistently reduced *S. mutans* GBP levels across all concentrations, with an average decrease of below 20%, highlighting its potential in preventing GBP formation, a critical aspect of dental caries development. The Kruskal–Wallis test revealed a significant variation in GBP suppression by incubation time ($P = 0.0312$), confirming the extract's effectiveness varies with the duration of exposure.

Nucleic acid deformation of *Streptococcus mutans*

FTIR data show *M. oleifera* leaf ethanol extract, particularly at 25% and 3.125% concentrations, destabilizes nucleic
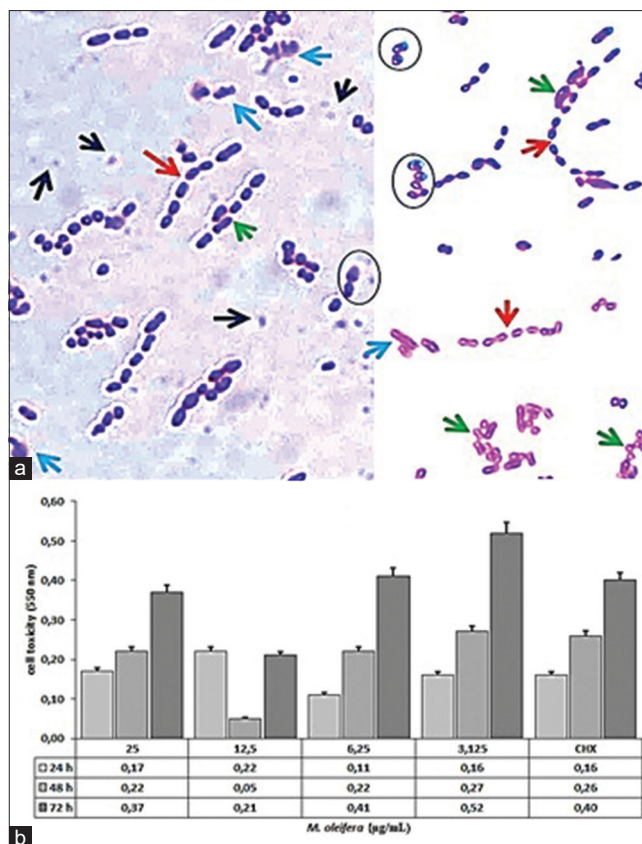
Figure 3: The effects of *Moringa oleifera* on *Streptococcus mutans*. (a) Compares the 3.125% *M. oleifera* group (left) to chlorhexidine (right), indicating cell lysis (blue arrows), septum loss (red arrows), agglutination (green arrows), nucleus detachment (black arrow), and failed cleavage (circled) at ×1000 magnification, (b) Reveals Moringa extract's toxicity towards *S. mutans* increases over time, with notable effects at 25%, 6.25%, and 3.125% after 72 h

acids in *S. mutans*, akin to the control (CHX). These concentrations affect cell membrane potential and disrupt genetic mechanisms, hindering cell growth. Such nucleic acid deformation leads to DNA damage [Table 4], increased replication stress, and impaired cell functions,[31,32] showcasing *M. oleifera's* ability to hinder bacterial proliferation by affecting nucleic stability.[33,34] In addition, this impacts *S. mutans* by inhibiting GBP synthesis,[35,36] essential for its adhesion and biofilm formation under optimal conditions, thereby affecting its virulence and biofilm integrity.[37]

Several research results have described the strength of the Moringa leaf (*M. oleifera*), which was formulated as a solution to be used as an active material for preventing the development of *S. mutans* on the tooth surface from preventing dental caries.[38] In addition, the bacteriostatic properties of Moringa leaves against *S. mutans* were found by assessing growth and GBP interactions, which were in line with changes in the properties of *S. mutans* DNA after being influenced by Moringa leaf extract.[39]

Table 3: Distribution and frequency of *Streptococcus mutans* glucan-binding protein titers after interaction with *Moringa oleifera* solution

| *M. oleifera* | Titer GBP of *S. mutans* (ng/mL), OD 450 nm | | | | | | | | | | | | P* |
| | 24 h (*n*=18) | | | | 48 h (*n*=18) | | | | 72 h (*n*=18) | | | | |
	Mean	SDV	Frequency (%)	Scale	Mean	SDV	Frequency (%)	Scale	Mean	SDV	Frequency (%)	Scale	
Concentrations$_{25\%}$	19.93	2.11	18	Moderate	12.35	0.58	13	Low	21.97	2.46	16	Moderate	0.0312
Concentrations$_{12.5\%}$	18.47	1.00	17	Moderate	14.22	3.45	15	Low	19.81	3.02	15	Moderate	
Concentrations$_{6.25\%}$	19.07	0.76	17	Moderate	12.70	0.55	14	Low	15.42	1.33	11	Low	
Concentrations$_{3.125\%}$	11.43	2.04	10	Low	12.43	0.15	13	Low	22.57	1.42	17	Moderate	
CHX	16.63	7.88	15	Low	14.01	4.02	15	Low	22.57	2.33	17	Moderate	
S. mutans	24.37	2.02	22	High	27.03	0.97	29	High	33.67	3.45	25	High	
P			0.042				0.021				0.042		

*Kruskal–Wallis test. OD: Optical density, SDV: Standard deviation, GBP: Glucan-Binding protein, *M. oleifera: Moringa oleifera*, *S. mutans: Streptococcus mutans*

Table 4: Nucleic acid deformation of *Streptococcus mutans* after being affected by *Moringa oleifera*

| *M. oleifera* | Peak (cm⁻¹) | Absorbance (A) | Band | Nucleic acid changes | |
				Frequency (%)	Total (%)
Concentrations$_{25\%}$	1.238	0.291	4	4.99	21
	1.315	0.286		4.92	
	1.396	0.309		5.30	
	1.465	0.323		5.54	
Concentrations$_{12.5\%}$	1.238	0.294	3	5.04	16
	1.396	0.308		5.29	
	1.465	0.323		5.54	
Concentrations$_{6.25\%}$	1.238	0.294	3	5.04	16
	1.396	0.308		5.28	
	1.465	0.322		5.52	
Concentrations$_{3.125\%}$	1.238	0.290	4	4.98	21
	1.315	0.286		4.92	
	1.396	0.310		5.32	
	1.465	0.324		5.56	
CHX	1.253	0.295	5	5.06	27
	1.315	0.293		5.02	
	1.396	0.316		5.43	
	1.462	0.328		5.63	
	1.492	0.328		5.63	

M. oleifera: Moringa oleifera, CHX: Chlorhexidine
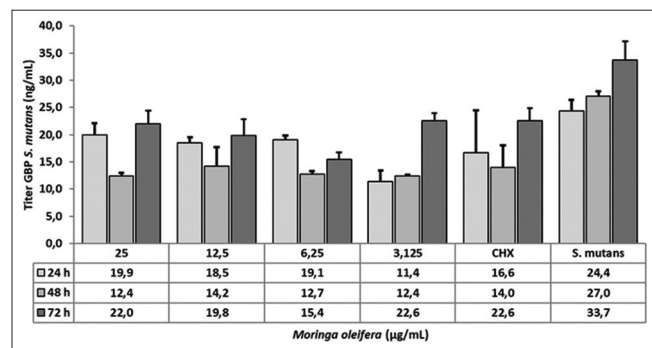
Figure 4: Inhibition of *Streptococcus mutans* glucan binding protein (GBP) after interaction with Moringa leaf ethanol extract solution. The concentration of 3.125% significantly reduces *S. mutans* GBP production by Moringa leaf extract at 24 h and 72 h. Bar (GBP titer) error bar (standard deviation)

Table 5 highlights that out of 17 chemical compounds identified via GC-MS, three from *M. oleifera*, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, hexadecanoic acid, and n-cbz-beta-alanine show high-binding affinity to the *S. mutans* GBP receptor. Specifically, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one interacts with serine at positions 134 (two bonds) and 139 (two bonds) and Asparagine at position 175 (no bonds). Hexadecanoic acid and n-cbz-beta-alanine interact with serine 134, with n-cbz-beta-alanine forming two bonds with asparagine, indicating a significant interaction with key amino acids.

Figure 5 and Table 5 illustrate the binding affinity between *M. oleifera* ligands and the *S. mutans* GBP receptor, with

Table 5: Docking ligands of *Moringa oleifera* for glucan-binding protein receptors

Compound	Free energy (ΔG) (kcal/mol)	Hydrogen bonds with amino acids
Alpha-butyrolactone	−4.74	Ser 134
1,3-cyclopentanedione	−6.03	Asn 175(2)
Cis-1,2,5-trimethylpiperidine	−5.19	-
2-pyrrolidinone	6.51	Tyr 155
2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	−8.64	Ser 134(2), Ser 139(2), Asn 175
2-trideuteromethoxy-3-methyl pyrazine	−5.35	-
Hexadecanoic acid	−9.09	Ser 134
n-cbz-beta-alanine	−8.72	Ser 134(2), Asn 175(2)
3-(2,2-dimethyltetrahydrofuran-3-yl)phenol	−7.50	Arg 142
Myricetin (native ligan as controls)	−17.41	Tyr 107, Ser 134(2), Pro 136, Ser 139(2), Ile 140, Arg 142, Tyr 307
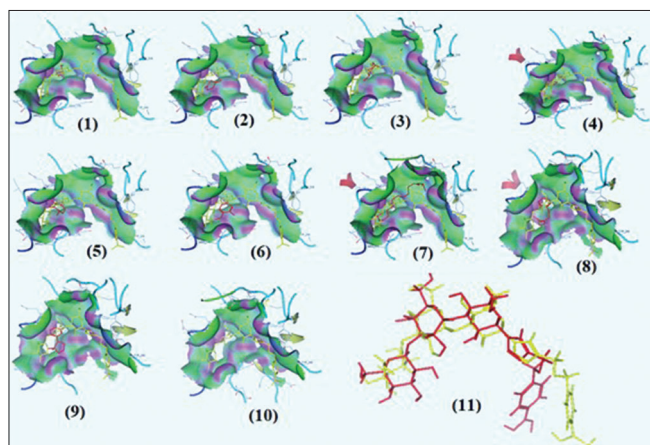
Figure 5: Visualization of three-dimensional interactions of test (red) and control (yellow) ligands resulting from docking hydrogen bonds with amino acids of *Moringa oleifera* ligands with the glucan binding protein receptor

interactions facilitated by specific amino acids. While all tested *M. oleifera* ligands showed potential to inhibit GBP function, their binding affinities were lower than the control (native ligand with an RMSD value of 2.66Å). The docking simulations highlighted serine's role in forming hydrogen bonds between the ligands and the GBP receptor,[40] emphasizing its importance in the biofilm formation process of S*. mutans* and the potential of serine to strengthen bonds between antioxidant compounds from Moringa leaves and the GBP gene.[41]

Asparagine and serine amino acids enhance the interaction between *M. oleifera's* compounds and *S. mutans'* GBP receptors, potentially altering GBP structure to prevent dental plaque formation.[42,43] *M. oleifera*, with its rich content of polyphenols and flavonoids, is being studied for its ability to block GBP binding, a key factor in plaque development.[44] Further research is needed to identify the most effective compounds for utilizing *M. oleifera's* antibacterial benefits in oral health.[45,46] In addition, *M. oleifera's* potential as an endodontic irrigation solution to modify metabolic activities and inhibit protein synthesis suggests promising applications in dental care. However, its safety and efficacy, particularly in root canal treatments, require comprehensive investigation to determine the optimal concentration and formulation for therapeutic use.

CONCLUSION

The *M. oleifera* solution can generally reduce the growth of *S. mutans*, and increase toxicity toward *S. mutans* cells. In addition, it can cause the deformation of nucleic acids. The active compounds (ligands) of *M. oleifera* have a stable binding affinity to the GBP gene (receptor) involved in forming dental pellicle surface biofilm.

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Conflicts of interest
There are no conflicts of interest.

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