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Natural antibacterial gel to fight tooth decay: An in-silico modeling approach

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Abstract

This study developed an antibacterial oral gel to inhibit *Streptococcus mutans*, a bacterium that causes tooth decay. The gel was formulated using methanol extract of Piper betel leaves, hydroxypropyl methylcellulose and glycerin at different concentrations. The formulated gel was evaluated for pH, viscosity and antibacterial activity against S. mutans using standard methods. The methanol extract showed potent antibacterial activity, supporting its use in the gel formulation. In silico molecular docking studies targeted key S. mutans protein residues involved in substrate binding. Eleven bioactive compounds identified in the Piper betel extract via GC-MS analysis were docked against protein 1EUH, an NADP-dependent aldehyde dehydrogenase from S. mutans. All 11 compounds exhibited favorable docking scores, suggesting strong binding affinity for the target protein. The docking results correlated with the antibacterial activity of the formulated gel, which inhibited S. mutans growth more effectively than sodium fluoride, a standard dental product.

Keywords: Aldehyde dehydrogenase; Antibacterial agents; Molecular docking; Oral gel; Piper betel; *Streptococcus* mutans

1. Introduction

Piper betel leaves, belonging to the Piperaceae family, have been traditionally used in medicine due to their numerous health benefits [1]. These leaves contain various bioactive compounds, such as alkaloids, phenols, flavonoids, and tannins, which demonstrate antimicrobial, antioxidant, anti-inflammatory, and analgesic properties [2]. Chewing betel leaves is a common practice that is believed to promote oral hygiene, freshen breath, and improve digestion [3].

Tooth decay is a prevalent issue affecting individuals of all ages worldwide [4]. The condition is caused by acidproducing bacteria, particularly Streptococcus Mutans, which can erode the enamel and dentin layers of teeth, leading to cavities [5]. Sodium fluoride is a widely used dental care product that can prevent dental caries by inhibiting S. Mutans growth; however, its overuse can result in harmful side effects such as fluorosis [6].

In this study, our objective was to develop an antibacterial oral gel using Piper betel leaves as a natural alternative to sodium fluoride. We employed an in-silico and docking process to predict the binding energies of bioactive compounds in betel leaves with the target protein of S. Mutans. Our results revealed that the betel leaf compounds exhibited high binding energies and were more effective against S. Mutans than sodium fluoride.

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This paper presents the development of an antibacterial oral gel using Piper betel leaves as a natural and safe alternative to sodium fluoride for preventing dental caries. The incorporation of betel leaves in oral care products can provide several health benefits while minimizing the adverse effects of synthetic chemicals.

2. Materials and Methods

2.1. Materials

Hydroxy Propyl Methyl Cellulose (HPMC), Propylene Glycol (PG), Triethanolamine, Menthol, Glycerine, Sodium Sachharine, Peppermint oil are procured from Molychem chemicals Pvt Ltd, Hyderabad.

2.2. Extraction of Piper betel leaves

The *Piper betel* leaves were first washed thoroughly to remove any dirt or debris. They were then wiped dry with a towel and shade dried until completely dry to remove any excess moisture. The dried leaves were converted into a fine powder using a grinder or mixer. The leaf powder was weighed and then macerated in methanol for 5 days for extraction. During maceration, the leaf powder was soaked and mixed continuously in methanol to facilitate the extraction of active compounds [1].

After 5 days, the macerated leaf-methanol mixture was filtered to separate the leaf particles. The resulting filtrate containing the leaf extract was then subjected to distillation to remove the methanol solvent. Finally, the leaf extract was concentrated using a rotary evaporator to obtain the crude methanol extract of Piper betel leaves. The concentrated extract was used for formulating the antibacterial oral gel [7]. The schematic process of extraction is shown in the Figure 1.

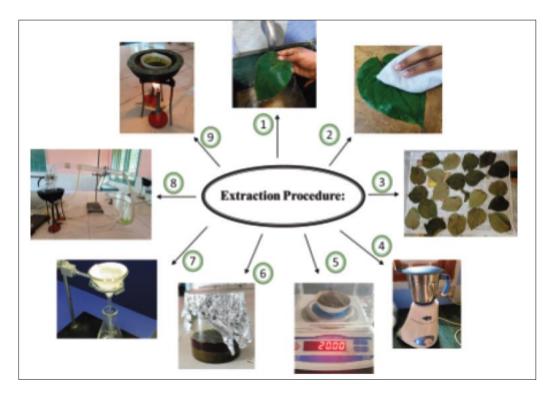


Figure 1 Extraction procedure of *Piper betel* leaves

2.3. Phytochemical tests of the obtained extract

The qualitative chemical tests were performed as follows to detect the presence of various phytochemicals in the Piper betel leaf extract:

Flavonoids: The extract was treated with sodium hydroxide solution, which resulted in a yellow coloration. Upon addition of dilute hydrochloric acid, the yellow color disappeared, indicating the presence of flavonoids [8].

Terpenes: The extract was mixed with chloroform and concentrated sulfuric acid. The formation of a reddish-brown coloration confirmed the presence of terpenes [9].

Phenols: Ferric chloride solution was added to the extract. The appearance of a violet color complex indicated the presence of phenolic compounds [10].

Cardiac glycosides: The extract was dissolved in chloroform and concentrated sulfuric acid was carefully added to form a layer. The observation of a deep reddish brown color at the interface indicated the presence of cardiac glycosides [11].

Tannins: The addition of ferric chloride solution to the extract resulted in the formation of a brownish green color, confirming the presence of tannins [10].

Alkaloids: Wagner's reagent was added to the extract and a brownish precipitate formed, indicating the presence of alkaloids [12].

2.4. Gas Chromatography Mass spectroscopy (GC-MS) evaluation of the plant extract

The Piper betel leaf extract was sent to VIT lab in Chennai for gas chromatography-mass spectrometry (GCMS) analysis to identify its phytochemical constituents. GCMS is an analytical method used for separation, identification and quantification of compounds in a mixture [13]. In this study, GCMS was employed to detect and characterize the bioactive compounds present in the leaf extract. The VIT lab performed GCMS analysis on the extract using an Agilent Technologies GCMS equipped with a capillary column and electron ionization source. The column temperature was maintained as per the standardized temperature program for optimal separation of compounds in the extract. The GCMS analysis yielded a chromatogram showing 11 distinct peaks, each corresponding to a unique compound based on its retention time. The total ion chromatogram revealed the relative concentrations of the compounds based on their peak areas. The mass spectra of each peak were analyzed to determine the probable structures of the compounds based on their fragmentation patterns. This allowed characterization of the 11 compounds identified in the extract.

2.5. Molecular docking

The molecular docking study was performed using AutoDock 1.4.6 software [14]. The protein used was 1EUH, an NADPdependent aldehyde dehydrogenase from Streptococcus mutans ATCC 25175. The full crystallographic information for this protein is available from the RCSB Protein Data Bank. The ligand SO4 was cocrystallized with the 1EUH protein structure. The protein has enzymatic activity as a glyceraldehyde-3-phosphate dehydrogenase and belongs to EC number 1.2.1.9. The three-dimensional structure of the 11 phytoconstituents identified in the Piper betel leaf extract through GCMS analysis were obtained from the PubChem database. These compounds served as the ligands for docking with the 1EUH protein. The protein structure was prepared by adding polar hydrogens, assigning Kollman charges and merging non-polar hydrogens. The grid box size was set to encapsulate the entire protein with adequate space for ligand movement. AutoGrid was used to create grid maps representing the interaction energy of the protein active site with different atom types. Autodock parameters like population size, number of generations and lamarckian genetic algorithm runs were set. The docked conformations obtained were ranked according to their docking free energy of binding. The conformation with the most favorable free energy of binding and appropriate interactions with key active site residues was selected. In this way, molecular docking of the 11 phytoconstituents identified in the Piper betel leaf extract was performed against the 1EUH protein using AutoDock software [15]. The grid values for docking are shown in Table 1. The docked conformations were evaluated based on their binding free energy and interactions with active site residues. The same process is done by taking Sodium Fluoride as the ligand and processed

 Table 1 Grid Values for Docking

Co-Ordinates	Center	Size
Х	-2.333	40
Y	3.505	40
Z	3.824	40

2.6. Formulation of antibacterial oral gel

The gel formulation was prepared using the ingredients in the specified quantities mentioned in Table 2. The ingredients were weighed accurately based on their percentage composition in the formulation table. Hydroxypropyl methylcellulose and glycerin were first dissolved in part of the propylene glycol. The extract of Piper betel was added to this mixture and stirred homogeneously. Menthol and sodium saccharin were dissolved separately in the remaining propylene glycol. The two propylene glycol solutions were mixed well and triethanolamine was added. Peppermint oil was added last and mixed to obtain the homogenous gel formulation. The gel components were selected based on their known functional roles in topical formulations, including viscosity modification, pH adjustment, flavor masking and preservative effects. Their quantities were optimized to achieve the desired consistency and properties in the final gel formulation [16], [17,][18].

Sl.No.	API/Excipient	Qty (1%)	Qty (g)	Category
1	Piper betel extract	1%	1gm	Antibacterial agent
2	Hydroxy Propyl Methylcellulose	1%	1gm	Thickeners, binders, film formers, and water retention
3	Propylene Glycol	5%	5ml	Solvent, humectant and viscosity enhancer
4	Triethanolamine	1.2%	1.2ml	pH adjuster, emulsifier and buffering agent
5	Menthol	0.1%	0.1ml	Flavoring agent
6	Glycerine	1.5%	1.5ml	Polyols or sugar alcohols
7	Sodium Saccharin	0.5%	0.5mg	Sweetener
8	Peppermint Oil	Q.S	Q.S	Flavoring agent

Table 2 Composition of antibacterial oral gel

2.7. Evaluation of antibacterial oral gel

The following evaluation tests are carried out for the prepared oral gel [16]:

- **Color:** The color of the gel was judged visually against a white background under natural daylight.
- **Odor:** The odor of the gel was assessed by smelling the gel formulation.
- Consistency: The consistency of the gel was judged by touch, observing how viscous and sticky it felt.
- Taste: The taste of the gel was assessed by placing a small amount on the tongue.
- **Percentage Yield:** The percentage yield was calculated as the ratio of the practical yield to the theoretical yield, multiplied by 100.
- Measurement of pH: The pH of 1% gel solution in water was measured using a calibrated digital pH meter.
- **Extrudability:** The extrudability of the gel from the tube was assessed on a 3-point scale (good, moderate and poor).
- **Gel Strength:** The gel strength was measured using a texture analyzer, which applies a constant stress and measures the strain on the gel.
- Spreadability: Spreadability (S) was calculated using the formula:
- $S = M \times L / T$
- where M is the weight in grams required to move the two slides of the spreadability apparatus apart, L is the length moved by the slides and T is the time in seconds taken to separate the slides.

3. Results and discussion

3.1. Phytochemical evaluation

The obtained extract showed the presence of flavonoids, terpenoids, alkaloids, anthraquinones, cardiac glycosides, tannins, and saponins which could be responsible for the antibacterial activity. The results are shown in Table 3.

Table 3 Results of phytochemical tests

Sl.No	Chemical test	Observation	Result
1	Flavonoids	Yellowish Green	Positive
2	Terpenoids	Reddish Brown	Positive
3	Alkaloids	Brownish Precipitate	Positive
4	Anthraquinones	Rosepink	Positive
5	Cardiac glycosides	Reddish Brown ring	Positive
6	Tannins	Brownish Green	Positive
7	Saponins	Blue black	Positive

3.2. GC-MS evaluation

The GCMS analysis revealed the presence of 11 compounds in the sample. Benzonitrile, 4-formyl- was the major component present at 27.331% area. This compound is known to have a strong aromatic odor. 1-Bromo-3-(2-bromoethyl)-octane was the second most abundant compound present at 21.329%. This compound has antimicrobial properties and is used as a biocide. The results of GC-MS evaluation are shown in Table 4 and the structures of each compound as per GC-MS analysis are shown in Figure 2.

Table 4 Results of the GC-MS evaluation

S.No	Name	Area %	Molecular Formula	Molecular Weight (gm)
1	BENZONITRILE, 4-FORMYL-	27.331	C ₈ H ₅ NO	131.04
2	1-BROMO-3-(2-BROMOETHYL)OCTANE	21.329	C10H20BR2	300.07
3	3-NITROSTYRENE	20.005	C ₈ H ₇ NO ₂	149.15
4	5-METHOXY-2-ALLYLPHENOL	15.144	C ₁₂ H ₁₆ O	176.12
5	ISOPROPYL LINOLEATE	7.8	$C_{21}H_{38}O_2$	322.29
6	3,7,11,15-TETRAMETHYL-2-HEXADECEN-1-OL	3.149	C ₂₀ H ₄₀ O	296.53
7	PHYTOL	1.563	C ₂₀ H ₄₀ O	296.31
8	HEXANE, 2,2,3,3-TETRAMETHYL	1.176	C ₁₀ H ₂₇	142.17
9	SULFUROUS ACID, BUTYL ISOHEXYL ESTER	0.982	C10H22O3S	222.34
10	HEPTANE, 1-NITRO-	0.835	C ₇ H ₁₅ NO ₂	141.20
11	4'-(2-METHYLPROPYL)ACETOPHENONE	0.715	C ₁₂ H ₁₆ O	176.12

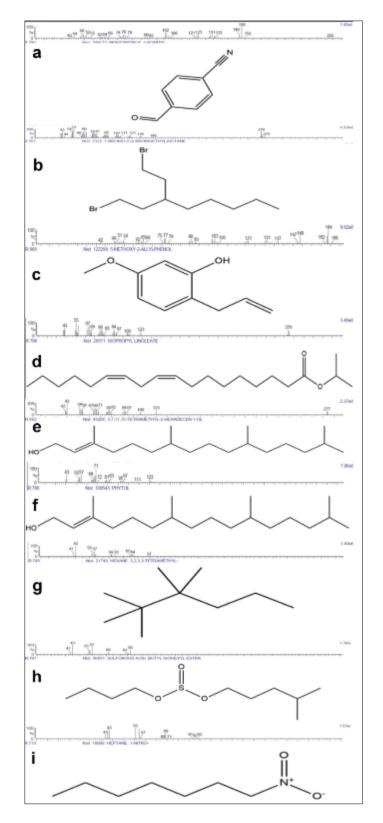


Figure 2 GC-MS analysis showing structures of a. benzonitrile, 4-formyl- b. 1-bromo-3-(2-bromoethyl)octane c. 5methoxy-2-allylphenol d. isopropyl linoleate e. 3,7,11,15 tetramethyl-2-hexadecen-1-ol f. Phytol g. hexane, 2,2,3,3tetramethyl h. sulfurous acid, butyl isohexyl ester i. 1-nitro-heptane

3.3. Molecular docking

The molecular docking studies revealed that all compounds in the extract showed higher binding energies and interactions with multiple amino acids in the active site compared to the standard drug sodium fluoride. The increased

number of active site interactions likely explains the higher binding energies of the extract compounds. 3-nitrostyrene showed the highest binding energy of -6.4 kcal/mol through interactions with 5 amino acids in the active site. The increased number of interactions compared to sodium fluoride, which interacted with only 4 amino acids, indicates 3-nitrostyrene may form a more stable complex with the target protein. Benzonitrile-4 formyl and 4'- (2-methylpropyl)acetophenone, despite their relatively lower areas, showed high binding energies of -5.4 kcal/mol and - 6.7 kcal/mol respectively through interactions with 2 and 1 amino acids. This indicates that compounds with fewer interaction sites can still form stable complexes through strong individual interactions. It can be observed that the constituents in the plant extract showed characteristics that may confer higher binding affinity and efficacy compared to the standard drug, possibly due to their interactions with multiple amino acids in the active site and higher binding energies. These results indicate the potential of the extract as an alternative therapeutic agent [14]. The summary of the molecular docking studies is shown in Table 5 while the docking parameters are shown in Figure 3 and 4. The interaction of the protein with the ligands is shown in Figure 5.

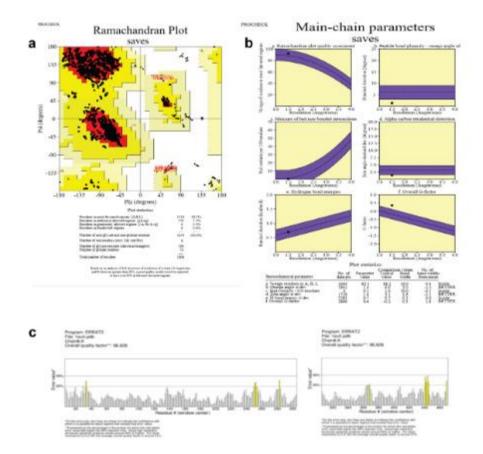


Figure 3 Docking parameters a. Ramachandran plots b. main chain parameters c. quality analysis of the protein sites

Table 5 Results of Molecular docking studies showing binding site interactions for all the compounds found in theextract

S.no	Name	Area		ids in	Total amino involved	no.of acids	0
1	SODIUM FLUORIDE (Standard)	(Standard)	GLY252		4		-2.2
			LEU251				
			LEU405				
			SER450				

2	Benzonitrile-4, Formyl	27.331	PHE379 PHE153	2	-5.4
3	1-BROMO-3-(2- BROMOETHYL)OCTANE	21.329	PHE379 CYS284 PRO162 PHE153 GLY230	5	-4.6
4	3-NITROSTYRENE	20.005	ARG437 LEU159 THR285 ARG103 TYR155	5	-6.4
5	5-METHOXY-2-ALLYLPHENOL	15.144	PHE379 GLU377 GLY230	3	-5.6
6	ISOPROPYL LINOLEATE	7.8	PRO152 PHE153 PHE379 GLY230 SER231 THR232 ILE150 PHE228 GLU377	9	-5.8
7	3,7,11,15-TETRAMETHYL-2- HEXADECEN-1-OL	3.149	PHE379 PHE163 SER231 PR0179 PHE228 ILE150 LYS177	7	-6.2
8	PHYTOL	1.563	PHE379 SER231 GLY230 PHE153 TYR333 SER330	6	-5.4
9	HEXANE, 2,2,3,3-TETRAMETHYL	1.176	PHE379 SER330	2	-4.7
10	SULFUROUS ACID, BUTYL ISOHEXYL ESTER	0.982	PHE379 SER231 THR232	3	-4.9
11	HEPTANE, 1-NITRON	0.835	ARG437 LEW159	2	-4.9

12	4'-(2-	0.715	PHE379	1	-6.7
	METHYLPROPYL)ACETOPHENONE				

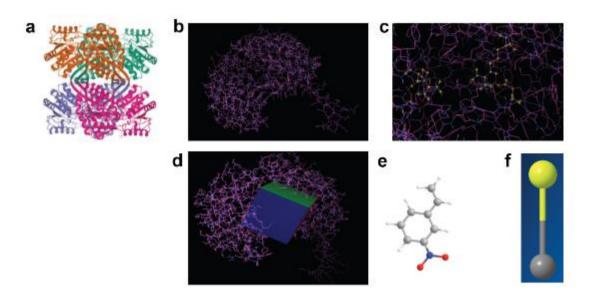


Figure 4 Proteins and ligands a. 1EUH protein molecule b. 3D image of chain A of protein c. binding sites d. construction of grid e. Active phytoconstituent 3-Nitrostyrene f. Sodium fluoride

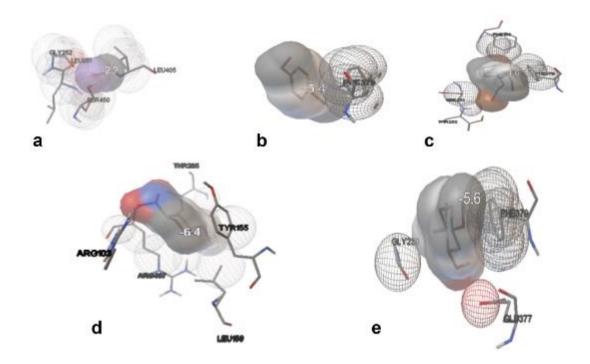


Figure 5 Interaction of amino acids of protein with ligands a. Standard-Sodium Fluoride b. Benzonitrile 4-formyl c. Bromo-3-(bromoethyl)-octane d. 3-nitrostyrene e. 5-methoxy-2-allylphenol

3.4. Evaluation of oral gel containing extract:

The developed herbal antibacterial oral gel had a green colour and mint odour imparted by the herbs used in the formulation. The consistency of the gel was good and it had a sweet taste. The percentage yield was 98.05 gm indicating

that loss of product during processing was minimal. The pH of the gel ranged from 7.75 to 7.32 over the 10 days evaluation period. The slightly alkaline pH is suitable for oral use and helps combat bacterial growth. The pH decreased gradually over time likely due to leaching of components into the medium. The extrudability of 95.2% and gel strength of 27 indicate that the gel has good extrudability through the collapsible tube while also maintaining its shape and consistency. The spreadability of 8 inches shows that the gel spreads easily on application, aiding uniform coverage in the oral cavity. Overall, the herbal oral gel formulation exhibited desirable properties in terms of colour, consistency, taste, pH and rheological characteristics. The high percentage yield, good stability and favourable properties indicate that the formulation has the potential to be developed as an effective antibacterial oral gel [18]. Further testing of antimicrobial activity and organoleptic properties is needed to confirm its efficacy and acceptability.

4. Conclusion

The study found that 11 phytoconstituents in piper betel leaves ethanolic extract were effective against *Streptococcus Mutans*, with greater binding energy than Sodium Fluoride. Purified compounds from the extract could be used as a better antibacterial dental agent, and a gel based on this activity passed all evaluation tests. This suggests that piper betel leaves ethanolic extract could be a natural and effective alternative for the prevention and treatment of tooth decay. Further research and clinical trials are needed to validate these findings. The use of herbal formulations in dental care is increasing, and the establishment of herbal oral gel from piper betel leaves extract is a promising development.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

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