Original Article

Molecular Docking Studies Of Solanum Xanthocarpum Against Streptococcus Mutans In Dental Caries

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Abstract

Dental caries is one of the prevalent persistent disease conditions in humans. It is caused by the bacteria *Streptococcus mutans*, a major pathogen which when infected, creates an acidic environment in the mouth leading to the formation of biofilm thereby ends up in tooth decay. Dental caries can be managed by oral hygiene practices, fluoride application, pit and fissure sealants as the primary treatments today with temporary solution. But, Siddha medicine is emerging in treating the Dental caries which remains as the mainstay after all these years for the large population. In this study, Solanum xanthocarpum is identified as the Siddha form of medicine which consists of phytochemicals such as Carpesterol, Oleic acid, Solasodine, Diosgenin, Lupeol, Apigenin, Tomatidenol, Caffeic acid, Scopoletin that interacts with the binding site of target Glucosyltransferase (GFT) – PDB – 3AIC core amino acid residues (Asp588, Gln960, Glu515 and Asp477)of the *S. mutans*, binds with them and inhibit the synthesis of Extracellular polysaccharides through which the biofilm formation is suspended. The docking studies were carried out using identified phytocomponents against GFT proving the binding efficiency with all 4 amino acid residues by Carpesterol, Oleic acid, Solasodine followed by Diosgenin, Lupeol, Tomatidenol, Caffeic acid, Scopoletin bound with 3 amino acid residues.

Keywords: Dental caries, *S. mutans,* Siddha system, *Solanum xanthocarpum*, phytocomponents, Molecular docking.

INTRODUCTION

Dental caries is caused by the formation of a complex interaction of factors like host susceptibility and the bacteria. It gets activated by bacteria on the tooth which breakdowns food particles, producing acid which leads to tooth decay [1]. The most susceptible to this infection are Children of 6-11 years of age. It is also a fact that the number of adolescents with dental decay is four times greater than those with asthma in the 14-17 age groups [2].

CAUSES:

- Certain foods & drinks
- Sugary drink
- Inadequate brushing
- Bedtime infant feeding
- Eating disorders
- Younger/older age

Solanum xanthocarpum

Siddha medicine is a renowned traditional Indian Medicinal System which is being practiced for over thousands of years that provides solution in various aspects of pharmacognosy, chemistry, pharmacology and clinical therapeutics. Natural products such as plants, animals and minerals have been used as the source for treatment of human ailments [3].

Taxonomic classification

- Kingdom - Plantae
- Subkingdom - Tracheobionta
- Division - Magnoliophyta
- Class - Magnoliopsida
- Subclass - Asteridae
- Order - Solanales
- Family - Solanaceae
- Genus – Solanum

USES:

There is an array of bitter, pungent, digestive, alternative astringent properties associated with *S. xanthocarpum* plants, including its stems, flowers, fruits and roots. The root decoction is used to treat fever, as a diuretic, and as an expectorant and it is specific to cough and asthma. Children Bangasena with chronic cough are prescribed Linctuses made from flower stamens [4].

The whole plant is used to cure a variety of ailments. Gonorrhea is treated with the plant extracts; Pain is relieved by applying leaves mix paste; catarrhal fever, coughs, asthma and chest pain can be treated with roots and seeds that are expectorant and diuretic [5]. Pest repellent properties and mollusicidal properties make the plant useful as a contact poison and molluscicide. The above medicinal properties make the plant an extensively used one in Indian system of medicine.

Throat infections, inflammatory diseases and infections are treated by its edible fruits. Vesicular eruptions and its accompanying burning sensation are cured by its stem, flowers and fruits. Moreover, the plant is also reported to have spasmodic, antitumor, cardiotonic, hypotensive, antianaphylactic and cytotoxic activities. Its fruit juice is useful in treating sore throats and rheumatism while the fruits it selves are eaten as it is anthelmintic and for indigestion [6].

Phytocomponents of S. xanthocarpum

S.N	Name of the Herb	Phyto components
	Solanum xanthocarpum	• Carpesterol • Oleic acid • Solasodine • Diosgenin • Lupeol • Apigenin • Tomatidenol • Caffeic acid • Scopoletin

9 phytocomponents have been identified in the selected plant. Out of those, Carpesterol, Solasodine are identified as the major leads for binding the target enzyme of *S.mutans* [7].

CARPESTEROL:

Carpesterol was the first substance to be isolated from the plant's lipid fraction. Its structural details could provide insight into the biogenetic process leading to solasodine, the main alkaloid that coexists with carpesterol in *S. xanthocarpum* [7].

SOLASODINE:

Solasodine alkaloid, which is found in large quantities in *S. xanthocarpum* and is employed as a steroidal precursor in the steroid medicine industry to make corticosteroids, infertility medications, anabolic steroids, etc. The majority of the berries of plants in the genus Solanum contain it in the form of a glycoside, and the glycoalkaloids are variously called as solasonine, solamargine, etc. with the common spiroaminoketal alkaloid or aglycon namely solasodine. The berries of SX are reported to contain Solasodine varying from 1.1% to 4.6% [8].

S. mutans and its Infection

According to reports, *Streptococcus mutans* is the primary cause of dental caries. It is an anaerobic bacterium and lactic acid production is a component of its metabolism. The ability of *S. mutans* to adhere to tooth surfaces in the presence of sucrose results in the formation of a polysaccharide by the production of water-insoluble glucans. This polysaccharide helps the bacterium adhere to the tooth. *S. mutans*, in contrast to other oral bacteria, thrives in an acidic environment and emerges as the dominating bacterium [9].

Transmission of dental caries

The bacteria *Streptococcus mutans* tends to break down the sucrose in meals, using one of the sugars to construct its capsule and turning sticky to cling firmly to the tooth. The other sugar is used by the bacteria that are imprisoned in the capsule to fuel their metabolism and cause the infection [9].

Binding site

Dental caries is typically the result of the metabolic acids produced by *S. mutans* demineralising the tooth surface. The enzyme glucosyltransferase (GTF) produced by *S. mutans* is the key factor in this process which when utilized produces an extracellular polysaccharide possessing α (1-3) glucose linkage which helps in the attachment of the bacterium [10]. Additionally, *S. mutans* produces lipo teichoic acid, which bind to the exterior enamel and speeds up colonisation. It superficially adheres to the tooth, breaks down sugar for energy, lowers pH, makes the environment acidic, and this results in demineralization of the tooth like enamel, leading to the development of dental caries [11] [12].

Siddha Therapeutics for Dental Caries:

In recent years, the ancient Indian medical system Siddha has gained recognition on a global scale. The eighteen Siddhars who promoted this system of medicine had already developed a number of medications and lines of therapy, specifically 32 exterior and 32 interior medications, for a total of 4448 disorders, both present day and future illnesses. Siddha prescribes both single herb and polyherbal formulations as therapeutics for many ailments.

Dental caries is the most prevalent and common health disease in all ages caused by major pathogen *S. mutans* with the help of its virulence factore glucosyltransferase (GTF) in the formation of its biofilm. In Siddha, Solanum xanthocarpum (known as Kandankathiri in tamil) is prescribed for dental caries. According to Siddha text, fumes of *S. xanthocarpum* seeds are used in the treatment of Pat Pulu (Dental caries) and Pal vali (Tooth ache). According to Siddha text Theraiyar tharu, pugai (Fumigation) is placed at 8th place in the external therapes series. Fumigation denotes the artificial impregnation of the atmosphere with the fumes or the smoke of any vegetables or aromatic substance. Fumigation is done by burning the raw drugs as wicks in small earthen lamps which contain neem oil or castor oil. For disease of the oral cavity, dental caries and jaws, fumes are taken in by mouth and it is carried out for 7 consecutive days as a treatment for dental caries.

In Classical Siddha text Gunapadam Mooligai Vaguppu Part-1 by Dr. Ka. Sa. Murugesa Mudaliar, the description on the use of the seeds of *Solanum xanthocarpum* for dental caries has been mentioned in detail [13]. The Siddha book Siddhar Aruvai Maruthuvam by Dr. Ka. Su. Uthamarayan, also states the explicit use of the fumigation of Solanum xanthocarpum seeds for the treatment of dental caries [14].

Aim & Objective:

Docking is a Bio-informative technique that predicts the 3-D structure of any complex molecule depending on the binding properties of ligand and target. Molecular docking provides a variety of potential structures by employing the scoring feature in the software that are ranked and grouped together. Based on the system's total energy, docking simulations forecast an optimum docked conformer [15] [16]. Hence, the present study aims at establishing the docking simulations for the antimicrobial activity of the phytocomponents present in *S. xanthocarpum* upon the virulence factor GTF of *S. mutans*, thereby acknowledging the fumigation method prescribed by Siddha, an effective method for the treatment of dental caries.

RECEPTOR STUDY:

Crystalline structure of the target protein Glucosyltransferase (GFT) of Streptococcus mutans - PDB 3AIC was retrieved from protein data bank. Subsequently, protein clean-up process was done and essential missing hydrogen atom was being added. The Autodock algorithm assessed various lead molecule orientations with regard to the target protein, and the best dock posture was chosen based on the interaction study results [17] [18].

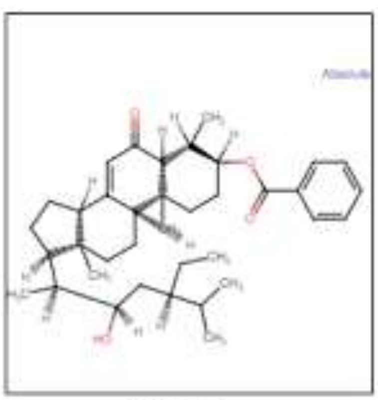
METHODOLOGY

Docking calculations were carried out for retrieved phytocomponents against target protein Glucosyltransferase (GFT) of *Streptococcus mutans* - PDB 3AIC. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools (Morris, Goodsell et al., 1998*).* Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program (Morris, Goodsell et al., 1998). AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method (Solis and Wets, 1981). Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied [11].

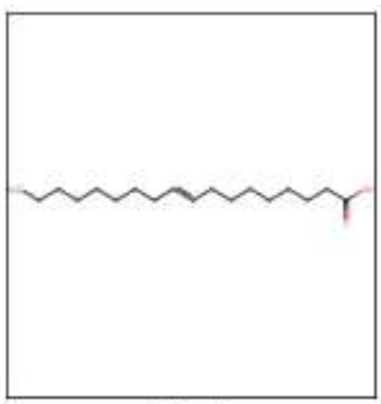
RESULTS AND DISCUSSION:

Figure 1. 3D- Structure of Glucosyltransferase (GFT) of *Streptococcus mutans*

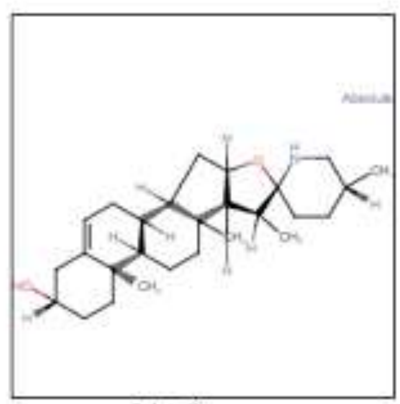
Crystalline structure of the target protein Glucosyltransferase (GFT) of Streptococcus mutans - PDB 3AIC was identified and the structure was selected for the Interaction study analysis for the inhibition efficiency of S.xanthocarpum



Carpesterol



Oleic acid



Solasodine

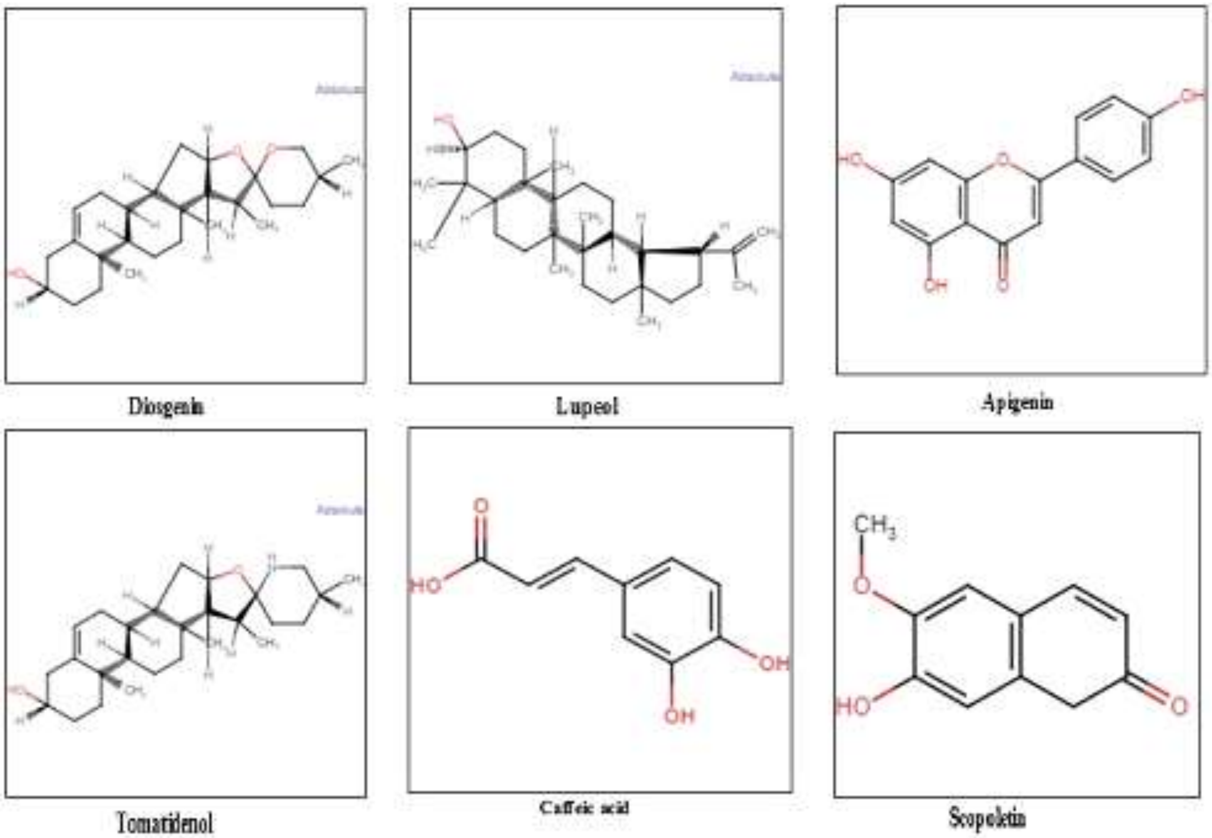
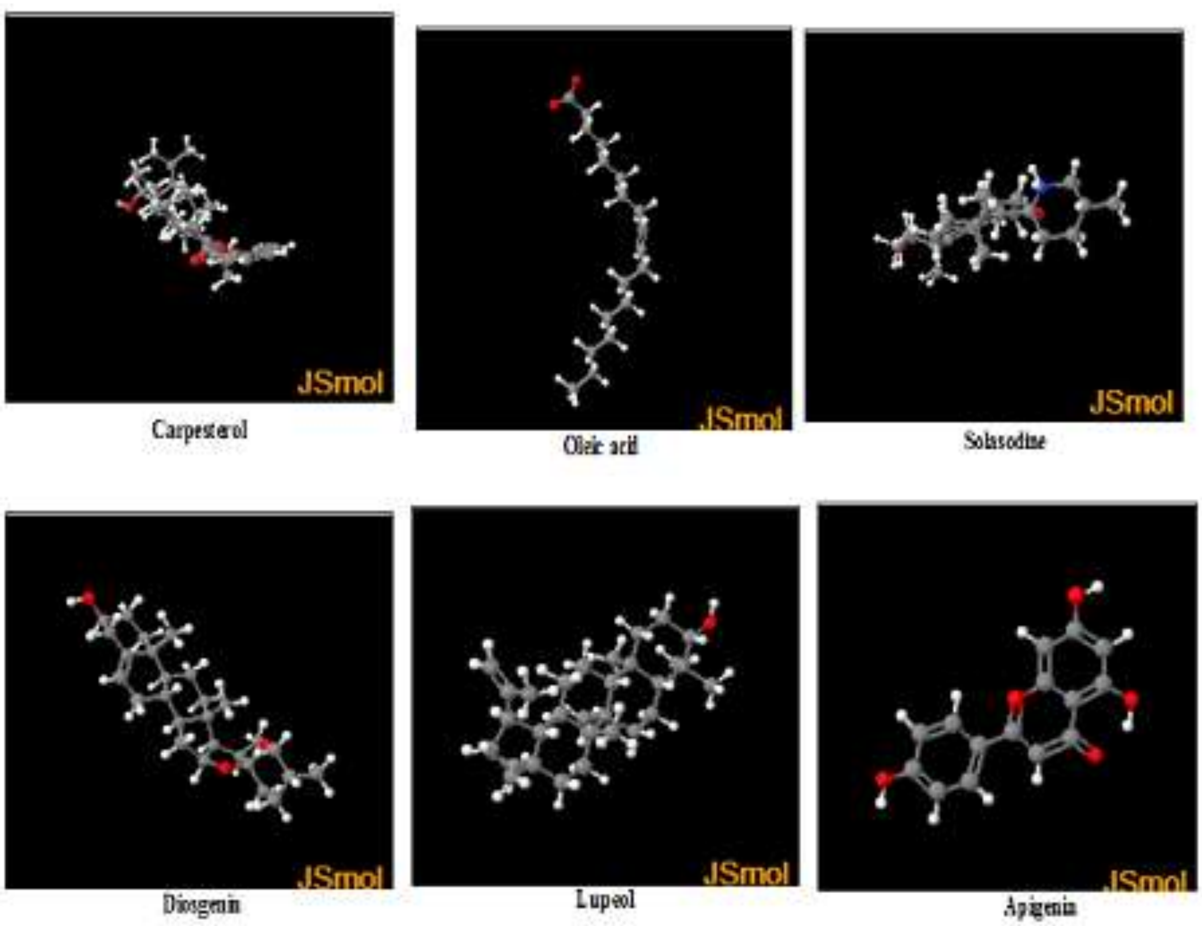


Figure2. 2D Structure of the Ligands



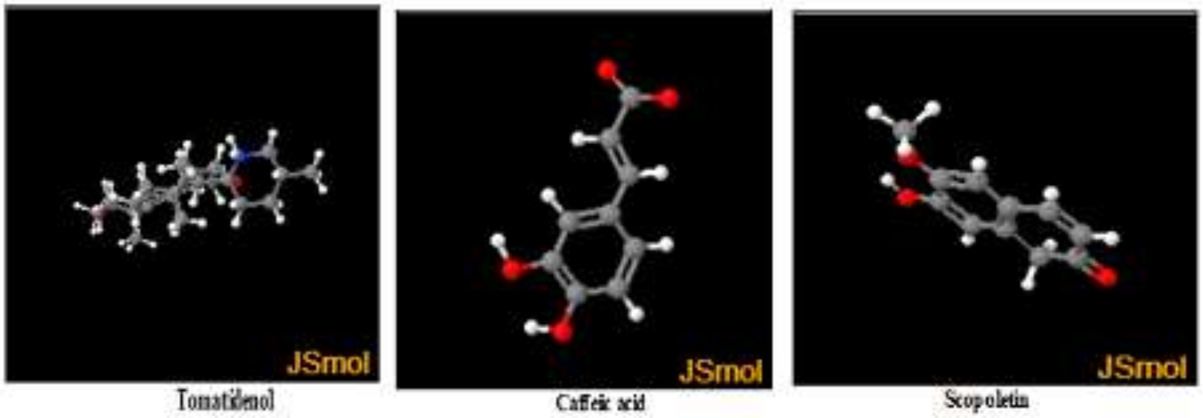


Figure 3. 3D Structure of the Selected Ligands

Table 1. Ligand Properties of the Compounds Selected for Docking Analysis

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Carpesterol	562.8 g/mol	$C_{37}H_{54}O_4$	1	4	9
Oleic acid	282.5 g/mol	$C_{18}H_{34}O_2$	1	2	15
Solasodine	413.6 g/mol	$C_{27}H_{43}NO_2$	2	3	0
Diosgenin	414.6 g/mol	$C_{27}H_{42}O_3$	1	3	0
Lupeol	426.7 g/mol	$C_{30}H_{50}O$	1	1	1
Apigenin	270.24 g/mol	$C_{15}H_{10}O_5$	3	5	1
Tomatidenol	413.6	$C_{27}H_{43}NO_2$	2	3	0
Caffeic acid	180.16	$C_9H_8O_4$	3	4	2
Scopoletin	192.17 g/mol	$C_{10}H_8O_4$	1	4	1

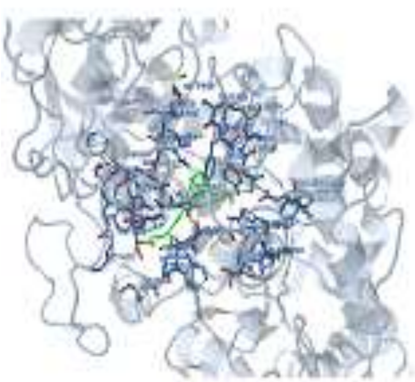
Table 2. Summary of the molecular docking studies of compounds against Glucosyltransferase (GFT) of Streptococcus mutans - PDB 3AIC

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki µM (*mM)(**nM)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Carpesterol	-9.56	98.67	-0.27	-11.32	946.63
Oleic acid	-6.88	9.06	-0.45	-7.18	778.50
Solasodine	-11.00	8.70**	-0.68	-11.29	845.00
Diosgenin	-8.88	309.74**	-0.48	-9.18	828.69
Lupeol	-9.07	224.79**	-0.18	-9.66	866.44
Apigenin	-5.80	56.38	-0.30	-6.32	641.55
Tomatidenol	-11.00	8.71**	-0.67	-11.29	843.45
Caffeic acid	-3.98	1.20*	-0.42	-4.02	407.89
Scopoletin	-3.37	3.39**	-0.36	-3.41	400.56

Table 3. Amino acid Residue Interaction of Lead against Glucosyltransferase (GFT) of Streptococcus mutans - PDB 3AIC

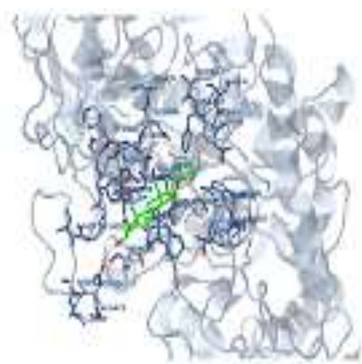
Molecule	Interaction	Amino Acid Residue- Binding															
Campesterol	4	382 LEU	430 TYR	433 LEU	434 LEU	477 ASP	480 ASP	481 ASN	515 GLU	517 TRP	588 ASP	592 GLN	907 PHE	909 ASP	916 TYR	960 GLN	
Oleic acid	4	430 TYR	433 LEU	434 LEU	477 ASP	478 ALA	480 ASP	481 ASN	515 GLU	517 TRP	588 ASP	592 GLN	862 ASN	907 PHE	909 ASP	916 TYR	960 GLN
Solasodine	4	434 LEU	477 ASP	515 GLU	517 TRP	588 ASP	592 GLN	909 ASP	916 TYR	960 GLN							
Diosgenin	3	382 LEU	430 TYR	433 LEU	475 ARG	477 ASP	515 GLU	517 TRP	587 HIS	588 ASP	916 TYR						
Lupeol	3	430 TYR	433 LEU	475 ARG	477 ASP	480 ASP	481 ASN	515 GLU	517 TRP	587 HIS	588 ASP	592 GLN	907 PHE	909 ASP	916 TYR		
Apigenin	2	430 TYR	433 LEU	480 ASP	481 ASN	515 GLU	517 TRP	587 HIS	588 ASP	592 GLN	909 ASP	914 ASN	916 TYR				
Tomatidenol	3	424 ASP	430 TYR	433 LEU	434 LEU	477 ASP	480 ASP	481 ASN	515 GLU	517 TRP	588 ASP	592 GLN	907 PHE	909 ASP			
Caffeic acid	3	433 LEU	434 LEU	477 ASP	478 ALA	481 ASN	515 GLU	517 TRP	907 PHE	909 ASP	916 TYR	960 GLN					
Scopoletin	3	433 LEU	477 ASP	478 ALA	481 ASN	515 GLU	517 TRP	907 PHE	909 ASP	916 TYR	957 VAL	960 GLN					

The Docking poses of the phytocomponents were estimated and the images are below which helps in the confirmation of inhibition capacity of the phytocomponents of *Solanum xanthocarpum.*



Oleic acid

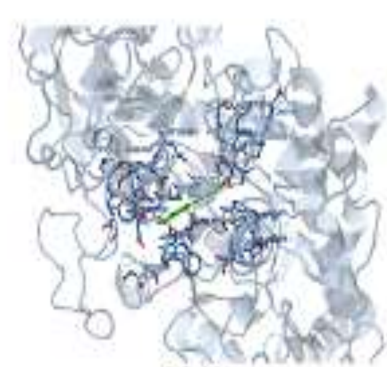
Oleic acid



Solasodine

Diosgenin

Lupeol



Apigenin

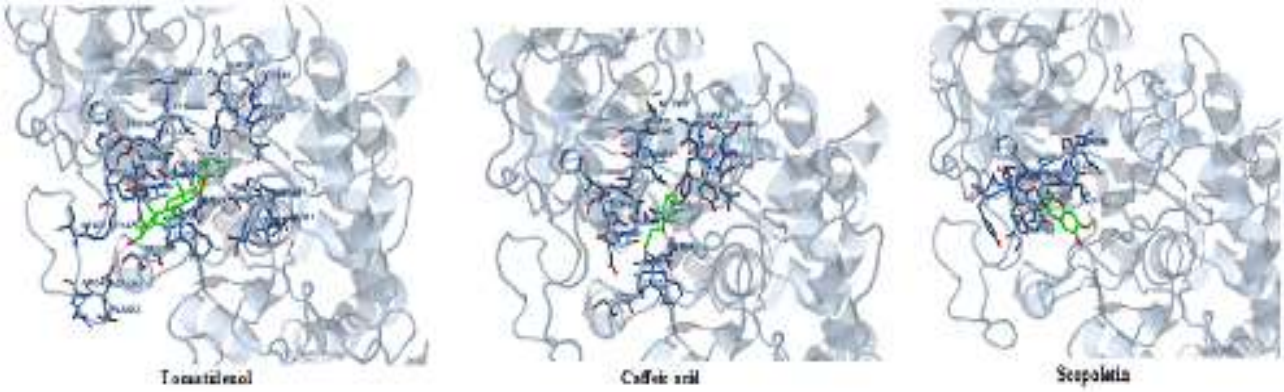


Figure 4. Docking Pose of Ligands with Glucosyltransferase (GFT) of Streptococcus mutans - PDB 3AIC

2D INTERACTION PLOT ANALYSIS OF THE LIGANDS

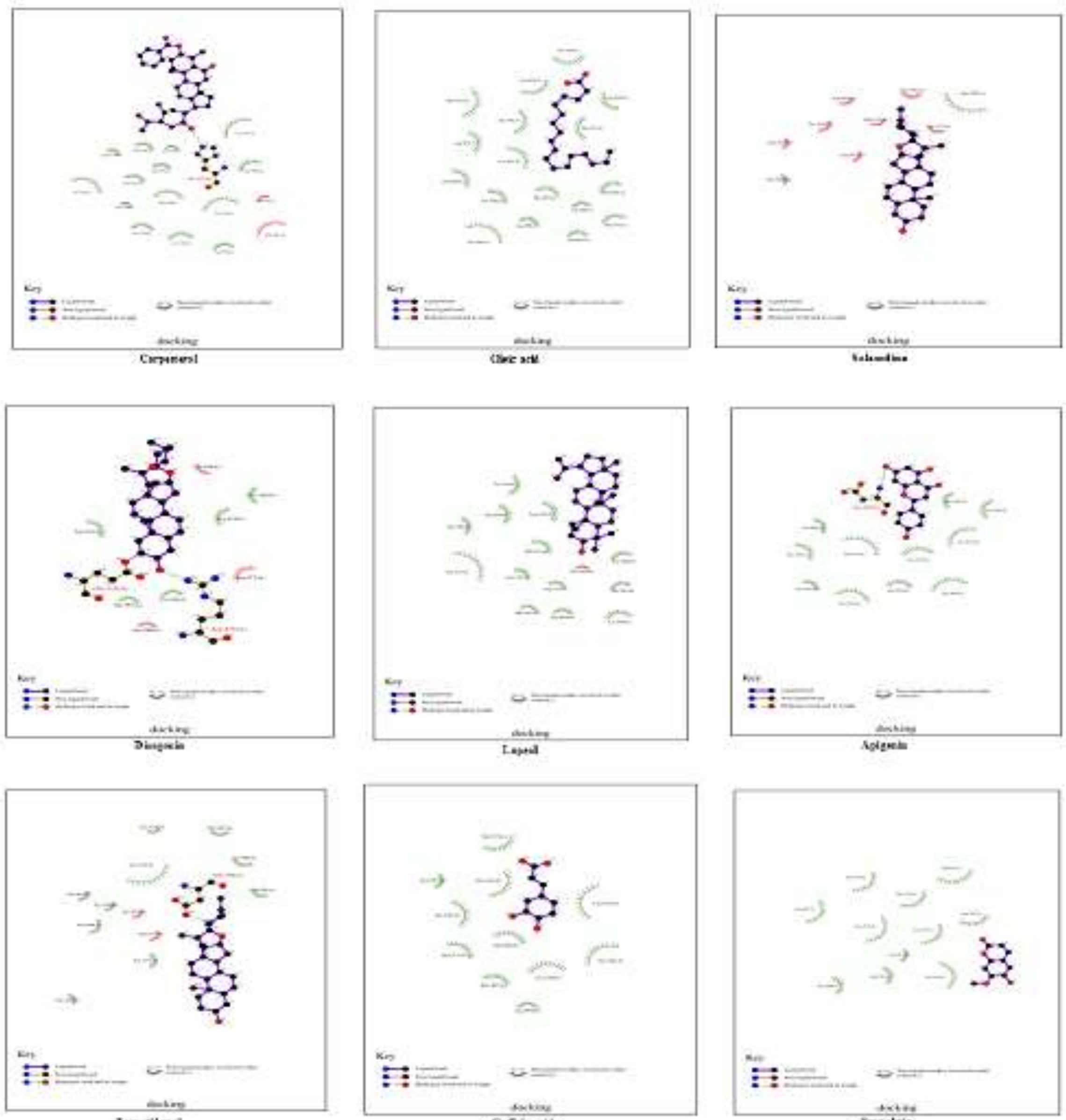


Figure 5. 2D INTERACTION PLOT ANALYSIS OF THE LIGANDS

In this study, it was observed that among the total of nine bioactive lead compounds that were retrieved from the herb *Solanum xanthocarpum* from the reports, the leads such as Carpesterol Oleic acid and Solasodine bound with all four bioactive amino acids (Asp588, Gln960, Glu515 and Asp477) present on the target Glucosyltransferase (GFT) of Streptococcus mutans that plays a critical role in synthesize of adhesive extracellular polysaccharides (EPS) which is essential for biofilm formation in Streptococcus mutans, followed by which other phytocompounds like Diosgenin, Lupeol, Tomatidenol, Caffeic acid and Scopoletin ranked second by occupying 3 out of four prominent binding residues.

CONCLUSION:

Based on the findings of the present computational analysis it was concluded that the bio-active compounds like Carpesterol, Oleic acid and Solasodine present in *Solanum xanthocarpum* reveals significant binding against Glucosyltransferase (GTF) of *Streptococcus mutans* that plays a significant role in synthesize of adhesive extracellular polysaccharides (EPS) which is essential for biofilm formation in dental caries. Thereby it may be inferred that the plant *Solanum xanthocarpum* contains phytochemicals that may inhibit the growth of Streptococcus mutans and serve as a viable treatment for dental caries in Siddha.

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DECLARATIONS:

HUMAN AND ANIMAL RIGHTS ETHICAL STATEMENT:

This article does not contain any studies with human or animal subjects performed by any one of the authors.

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CONFLICTS OF INTEREST/COMPETING INTERESTS

The authors declare that there is no conflict of interest to disclose.

REFERENCES:

1. Amiri, M., Jalali-Javaran, M., Haddad R., Ehsani P, In silico and in vivo analyses of the mutated human tissue plasminogen activator (mtPA) and the antithetical effects of P19 silencing suppressor on its expression in two Nicotiana species. Sci Rep 8,14079,2018 https://doi.org/10.1038/s41598-018-32099-6.
2. T. A. Halgren,Merck molecular force field. I. Basis, form, scope, parametrization, and performance of MMFF94. Journal of Computational Chemistry. 17:(5-6), 490-519,1996.
3. G. M. Morris, D. S. Goodsell, et al. (1998). Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. Journal of Computational Chemistry 19 (14), 1639-1662
4. F. J. Solis, R. J- B. Wets. (1981). Minimization by Random Search Techniques. Mathematics of Operations Research. 6(1): 19-30.
5. Bikadi, Z., Hazai, E. (2009). Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. J. Cheminf. 1, 15
6. Siva Kumar Tekuri et al. (2019). Phytochemical and pharmacological activities of Solanum surattense Burm. f.–A review. Journal of Applied Pharmaceutical Science. 9 (3), 126-136.
7. Okram M.S. and Thockhom P. S. (2010). Phytochemistry of Solanum xanthocarpum an amazing traditional healer. Journal of Scientific and industrial research.;69:732-740
8. Said Mohamed Daboor et al. A review on Streptococcus mutans with its diseases dental caries, dental plaque and endocarditis. Indian J Microbiol Res. 2(2):76-82 (2015).
9. Sachin Parmar et al. (2010). Solanum xanthocarpum (Yellow Berried Night Shade): A review. Der Pharmacia Lettre. 2(4): 373-383
10. K S Devulapalle and G Mooser. (2001). Glucosyltransferase inactivation reduces dental caries. J Dent Res. 80(2), 466-469
11. Mahalakshmi P., Ramesh Kumar A., Sudha G., Dineshkumar T., Vinoth H., Malar AD. A. (2019). Evaluation of antimicrobial properties of Solanum xanthocarpum and Pistacia lentiscus extracts on Streptococcus mutans, Lactobacillus species and Actinomyces viscosus: An in vitro study. Journal of Oral and Maxillofacial Pathology: JOMFP 23: 383 – 388.
12. Ryota Nomura et al. (2020). Conntribution of Severe Dental Caries Induced by Streptococcus mutans to the Pathogenicity of Infective Endocarditis. Infection and Immunity.88 (7)
13. Dr. Ka. Sa. Murugesa Mudaliar, Gunapadam Mooligai Vaguppu part-1, (1936), Directorae of Indian Medicine and Homoeopathy, Chennai, Tamil Nadu, India. Edition 1, Pg. 214.
14. Dr. Ka. Su. Uthamarayan, Siddhar Aruvai Maruthuvam, (1968), Directorate of Indian Medicine and Homoeopathy, Chennai, Tamil Nadu, India. Edition. 1, Pg. 64-65.
15. Ghazal T.S. et al. (2018). Dental Caries: A New Statistical Modeling Approach. Caries Res. 52, 246–252.
16. Wbeimar Andrey Rivera-Pérez et al. (2019). Molecular docking and in silico studies of the physicochemical properties of potential inhibitors for the phosphotransferase system of Streptococcus mutans. Arch Oral Biol. 98, 164-175.
17. Hao Luo et al. (2017). In silico identification of potential inhibitors targeting Streptococcus mutans sortase A. Int J Oral Sci. 9(1), 53-62.
18. Sofia D. Forssten et al. (2010). Streptococcus mutans, Caries and Simulation Models. Nutrients. 2(3), 290–298.