https://doi.org/10.33472/AFJBS.6.9.2024.2896-2911

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Insilico Validation and Evaluation of ADMET properties of Cinnamaldehyde and Apigenin as Anti-biofilm agents against Glucosyltransferase enzyme

Dr. Ipsitha Vejendla, Dr. Jogikalmat Krithikadatta, Prof. Dr. Mukesh Doble

Department of Conservative dentistry and Endodontics,
Saveetha Dental college and hospitals
Saveetha Institute of Medical and Technical sciences (SIMATS)
Saveetha University,
Chennai - 600077, Tamil Nadu, India
Email id: vipsitha1997@gmail.com

Department of Cariology,
Saveetha Dental college and hospitals,
Saveetha Institute of Medical and Technical sciences (SIMATS),
Saveetha University,
Chennai - 600077, Tamil Nadu, India
Email id: krithikadatta.sdc@saveetha.com

Department of Cariology,
Saveetha Dental college and hospitals,
Saveetha Institute of Medical and Technical sciences (SIMATS),
Saveetha University,
Chennai - 600077, Tamil Nadu, India
Email id: mukesh.doble0@gmail.com

Corresponding author:Dr. Jogikalmat Krithikadatta

Volume 6, Issue 9, May 2024

Received: 09 March 2024

Accepted: 10 April 2024

Published: 20 May 2024

doi:10.33472/AFJBS.6.9.2024.2896-2911

Abstract:

Aim: In the current study, a computational insilico approach was used to confirm mode of interaction for antibacterial activity against glucosyltransferase enzyme of *Streptococcus mutans*, elucidating quantum chemical properties and ADMET-drug-likeness of cinnamaldehyde and apigenin.

Materials and methods: Using an online database called ZINC15, which enables virtual screening by downloading the database subset for the two natural compounds that were chosen, the features of the selected compounds were examined.The molecular docking studies were performed with Glucansucrase (3AIC) from *S.mutans* using swissdock database, employing a ligand docking approach and their binding energies are determined. SwissADME tool was used to estimate the pharmacokinetic and other molecular properties of the compounds, utilizing their canonical SMILE structures.

Results and discussion: The docking study reveals the potential of the selected compounds as an inhibitor of bacterial glucosyltransferase of *S.mutans*, thereby predicting anti-cariogenic activity. The SwissADME prediction results showed that cinnamaldehyde and apigenin satisfy Lipinski's rule of five with zero violations.

Conclusion: The results predict a development of an inhibitor molecule acting against the glucosyltransferase enzyme of *S.mutans* that could bring about the antibacterial activity.

Keywords: Cinnamaldehyde, apigenin, glucosyltransferase, dental caries, docking studies.

Introduction

Dental caries, a prevalent oral health issue worldwide, is primarily caused by the demineralization of tooth enamel due to the production of acids by bacteria present in the dental plaque. The development of dental plaque on teeth is a significant characteristic of Streptococcus mutans, a bacterium that is primarily responsible for the occurrence of dental caries in humans[1],[2]. Species such as mutans streptococci and lactobacilli, known for their ability to produce acids, contribute to the development of dental caries by creating a cariogenic biofilm that maintains a low pH environment on the tooth surface [3]. This acidic environment leads to the demineralization of the tooth and the formation of caries. The presence of these bacteria, Streptococcus mutans, in particular, is strongly associated with the development of dental caries due to its acid-producing characteristics, along with its capacity to synthesize glucans and create biofilms[4]. In the presence of sucrose, S. mutans produce glucosyltransferases (Gtfs) that enhance the synthesis of glucans, thereby generating extracellular polysaccharides (EPSs), which contribute to the structure and integrity of the biofilm. The EPSs produced by Gtfs promote the adhesion of cariogenic bacteria, enabling them to form robust biofilm communities[5],[6].

In light of the microbial resistance and drug toxicity observed in conventional antimicrobial therapy, there has been significant exploration of natural products possessing therapeutic properties as potential alternatives. Utilizing natural products, such as essential oils and their constituents, offers several advantages including decreased expenses, reduced toxicity, and enhanced affordability (in contrast to prescription medications). However, it is crucial to acknowledge that the effectiveness of alternative therapy in terms of antimicrobial activity may be compromised by microbial resistance and challenges in isolating the active principle[7],[8]. In traditional Chinese medicine, cinnamaldehyde (CA), one of the most significant bioactive components in C. cassia, is frequently employed[9]. Cinnamaldehyde, a prominent compound found in cinnamon, is an aromatic aldehyde known for its diverse therapeutic properties [10,11]. Many biological processes carried out by CA include antioxidant, anti-inflammatory, anti-diabetic, and anti-cancer properties. Flavonoids such as luteonin, quercetin, and CA are potent natural anti-cancer agents that work by inhibiting histone deacetylase (HDAC)[12,13]. Cinnamaldehyde, a widely recognized HDAC inhibitor and anti-tumor agent, demonstrates remarkable anti-cancer properties by inducing apoptosis and cell death in various cancer types[14]. Previous studies have highlighted the potential of cinnamaldehyde as a basis for the development of a new anti-cancer medication. However, a comprehensive understanding of the specific biological and molecular mechanisms of cinnamaldehyde remains limited. He et al. reported that cinnamaldehyde possesses antimicrobial properties against S. mutans biofilm formation by influencing its

hydrophobicity, aggregation, acid production, acid tolerance, and the expression of virulence genes[15]. Additionally, cinnamaldehyde has demonstrated effectiveness against biofilms formed by Gram-positive and Gram-negative bacteria, including Pseudomonas aeruginosa and Staphylococcus aureus[16],[17]. These findings highlight the potential of cinnamaldehyde as a promising candidate for alternative approaches in preventing and managing dental caries.

Another natural derivative also known for its therapeutic properties is apigenin. Apigenin, a naturally occurring, non-mutagenic, non-toxic bioflavonoid ubiquitously found in many herbs, fruits, and vegetables, is a well-known phenolic compound renowned for its numerous nutritional and organoleptic characteristics. Apart from its sensory attributes, apigenin possesses beneficial health properties that make it a potential candidate for inclusion in nutraceutical formulations[18]. The antioxidant properties of apigenin are widely recognized, and it also exhibits therapeutic potential in addressing various conditions such as inflammation, autoimmune disorders, neurodegenerative diseases, and certain types of cancers. Notably, apigenin demonstrates a relatively lower intrinsic toxicity on normal cells compared to cancerous cells, setting it apart from other structurally related flavonoids[19,20]. Apigenin plays a significant role in the antibacterial actions against oral pathogenic agents. Koo et al. demonstrated that apigenin exhibited a pronounced impact on the biomass and total polysaccharide levels in S. mutans biofilms[21].

The advancement of *in silico* analysis models has made significant contributions to pharmacology research. These computational tools enable various analytical tasks, including the study of quantitative structure-activity relationships, defining pharmacophores, and conducting molecular modeling. *In silico* approaches offer valuable opportunities for searching and enhancing new molecules, analyzing their affinity towards specific targets, as well as predicting pharmacokinetic factors, toxicity, and physico-chemical properties[22–25]. Moreover, employing computational screening to identify potential, well-tolerated molecules before laboratory, preclinical, and clinical investigations can help reduce costs associated with materials, equipment, and personnel during the initial stages of drug discovery.

Materials and methods

Ligands identification

In our study, we employed a ligand identification approach to explore the potential bioactive molecules, cinnamaldehyde and apigenin. To identify these compounds, we utilized the Zinc15 database, a

comprehensive collection of commercially available compounds. The Zinc15 database (https://zinc15.docking.org/) contains a vast array of chemical structures, making it a valuable resource for virtual screening and ligand identification[26]. A search within the Zinc15 database using the chemical names "cinnamaldehyde" and "apigenin" as queries was done. This allowed us to retrieve potential compounds with structural similarities to our target molecules. Subsequently, we filtered the results based on various criteria, such as molecular weight, lipophilicity, and drug-like properties, to ensure the selection of relevant ligands.

To facilitate further analysis and computational simulations, it was necessary to convert the identified ligands from the Zinc15 database into an appropriate file format. Open Babel, an open-source chemical toolbox designed for chemical file format interconversion, was used. Using Open Babel, we converted the ligands of interest from their original formats to the widely used mol2 format. The mol2 format is well-suited for molecular docking, molecular dynamics simulations, and other computational analyses. Converting the ligands to mol2 format allowed us to maintain their chemical information, while ensuring compatibility with various molecular modeling software packages. The resulting mol2 files contained the necessary structural information of cinnamaldehyde and apigenin, enabling subsequent computational analyses and simulations in our study.

By employing the ligand identification approach and leveraging the Zinc15 database along with Open Babel, we successfully retrieved cinnamaldehyde and apigenin compounds and converted them into the mol2 file format. These transformed ligand structures served as the foundation for our subsequent investigations. To identify three-dimensional pharmacophores from our ligands, the selected structures were sent to the pharmaGist programme (http://bioinfo3d.cs.tau.ac.il/PharmaGist/php.php) which utilizes a ligand-based approach for pharmacophore detection. Through this analysis, we aimed to uncover key molecular features and spatial arrangements that contribute to the ligands' pharmacological activity.

Target Protein structure

The target protein, 3AIC, a protein encoding the crystal structure of Glucansucrase (also known as glucosyltransferase) from Streptococcus mutans, was selected for the current investigation. The 3AIC protein was obtained from the Protein Data Bank (PDB) database (https://www.rcsb.org/), a widely recognized online resource that houses experimentally determined protein structures (Figure 1).

By accessing the PDB database, we were able to retrieve the necessary structural information of 3AIC, enabling us to study its interactions with the ligands cinnamaldehyde and apigenin[27]. The structure of ligands are described in figures 2 and 3.

Pharmacophore detection

Pharmacophore is a molecular framework that carries the essential features responsible for the biological activity of a drug[28]. Using the PharmaGist and ZINCPharmer (http://zincpharmer.csb.pitt.edu/) programmes, the pharmacophore detection is carried out by inputting the evaluated two ligands downloaded inputs in mol2 format. The program generated numerous pharmacophore hits, and from the thousands obtained, we selected 20 outputs through Pharmacophore virtual screening. The swissADME software (http://www.swissadme.ch/) was used to gain insights into the characteristics of these selected pharmacophores. This software evaluates drug-likeness parameters and assesses the compounds' potential as therapeutic agents. The obtained results were carefully examined, and the relevant traits and properties of the 20 selected pharmacophores were tabulated for a comprehensive analysis.

Docking

Docking is a computational technique that plays a crucial role in understanding ligand-protein interactions and aiding in the rational design of new therapeutics. The SwissDock program (http://www.swissdock.ch/docking) was used to perform individual docking of the 20 pharmacophores, which were selected from the hits obtained through the pharmacophore analysis on the Pharmagist website. Using this database, each pharmacophore was docked with the target protein, and the resultant were analyzed to determine the binding energies and evaluate the strength of the ligand-protein interactions (Figure 4). The binding energy of each pharmacophore obtained was noted and the values were tabulated.

Fig 1: Protein structure of 3AIC

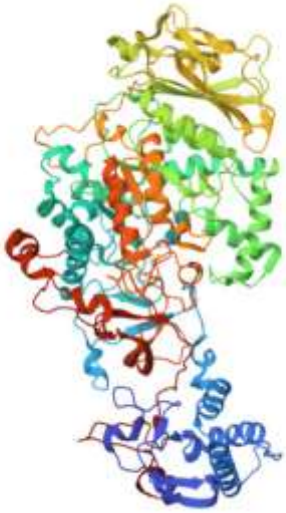

Fig 2: Structure of Cinnamaldehyde

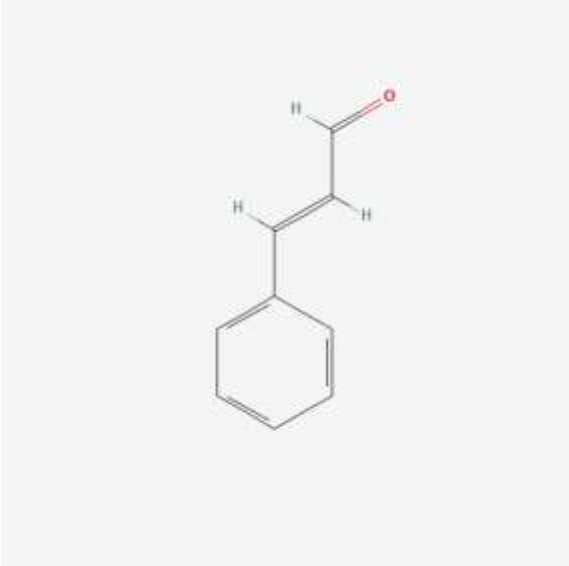

Fig 3: Structure of Apigenin

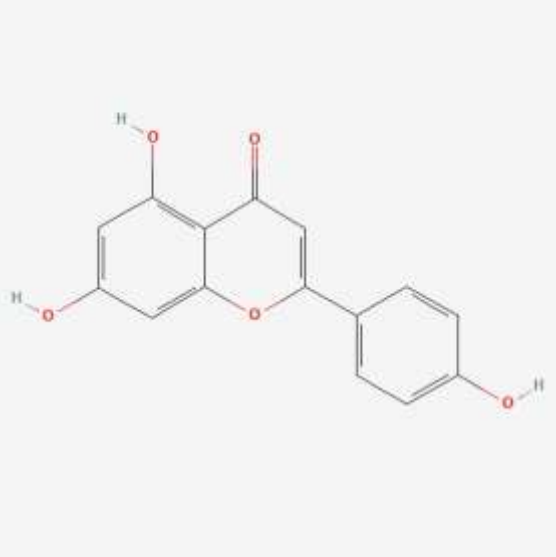

Fig 4: 3AIC protein structure with binding sites (Structure obtained after docking)

Results

The binding energies of cinnamaldehyde and apigenin were determined to be -6.44 and -9.10 respectively. Among the 20 newly derived outputs selected from the hits, ZINC36099457 exhibited the highest binding energy, with a value of -9.13. This indicates a strong interaction between ZINC79496741 and the target protein. Following closely is ZINC40845897, which displayed a binding energy of -8.70, suggesting a favorable binding affinity (Table 1 and 2).

Table I: The selected 20 Pharmacophores from PharmaGist Database

Molecule ID	Lipinski rule	Ghose rule	Veber rule	Egan rule	Muegge rule	Topological polar surface area (TPSA)	Log P
ZINC02565488	Yes	Yes	Yes	Yes	Yes	46.53	3.94
ZINC77379257	Yes	Yes	Yes	Yes	Yes	58.36	2.49
ZINC36026131	Yes	Yes	Yes	Yes	Yes	95.15	3.06
ZINC00136197	Yes	Yes	Yes	Yes	Yes	46.53	3.71
ZINC01184733	Yes	Yes	Yes	Yes	Yes	46.53	3.89
ZINC00828704	Yes	Yes	Yes	Yes	Yes	49.33	4.53
ZINC00828703	Yes	Yes	Yes	Yes	Yes	49.33	4.54
ZINC36099457	Yes	Yes	Yes	No	Yes	133.25	1.2

ZINC00795937	Yes	Yes	Yes	Yes	Yes	58.56	3.87
ZINC34854552	Yes	Yes	Yes	Yes	Yes	92.35	2.31
ZINC00795936	Yes	Yes	Yes	Yes	Yes	58.56	3.86
ZINC36026133	Yes	Yes	Yes	Yes	Yes	95.15	3.14
ZINC00395504	Yes	Yes	Yes	Yes	Yes	46.53	3.68
ZINC04045277	Yes	Yes	Yes	Yes	No	49.77	4.27
ZINC57457384	Yes	Yes	Yes	Yes	Yes	92.08	2.53
ZINC16490612	Yes	Yes	Yes	Yes	Yes	112.11	2.67
ZINC00402525	Yes	Yes	Yes	Yes	No	46.53	4.2
ZINC15011684	Yes	Yes	Yes	Yes	Yes	128.73	2.16
ZINC15011070	Yes	Yes	Yes	Yes	Yes	130.92	2.26
ZINC40845897	Yes	Yes	Yes	Yes	Yes	123.74	2.49

Table II: Molecules with Highest Binding Energies with 3AIC

Molecule ID	Lipinski rule	Ghose rule	Veber rule	Egan rule	Muegge rule	TPSA	Log P	Bioavailability	GI absorption	Binding energy
ZINC36099457	Yes	Yes	Yes	No	Yes	133.25	1.2	0.55	High	-9.13
ZINC40845897	Yes	Yes	Yes	Yes	Yes	123.74	2.49	0.55	High	-8.70

Notably, ZINC36099457 not only demonstrated a significant binding energy but also complied with all five guidelines for drug-likeness. This suggests that it possesses favorable physicochemical properties and structural characteristics that are typically associated with successful drug candidates. Therefore, ZINC36099457 holds promise as a potential medication targeting the 3AIC protein.

The high binding energy, combined with adherence to drug-likeness guidelines, makes ZINC36099457 a compelling candidate for further investigation and potential development as a therapeutic agent. Additional studies are warranted to explore its efficacy, selectivity, and safety profile in relation to the targeted 3AIC protein.

Discussion

The management of oral bacterial infections requires a comprehensive approach that goes beyond simply reducing the population of bacteria. Virulence factors are molecular mechanisms employed by bacteria to establish and maintain infections. One promising strategy is to identify and target specific virulence factors involved in the pathogenesis of oral infections[29–31]. Glucosyltransferases, produced by Streptococcus mutans, play a crucial role in the formation of dental biofilms and the production of polysaccharides [32]. Inhibiting Gtfs can prevent biofilm formation and limit the ability of bacteria to adhere to tooth surfaces, thus reducing the risk of dental caries[33].

The current study aimed to explore the potential of cinnamaldehyde and apigenin as ligands for human health and dental hygiene applications, and also to determine potential compounds that act against the target protein, 3AIC, of Streptococcus mutans. Firstly, the in silico analysis using the Pharmagist and ZINCPharmer database allowed us to identify and analyze the pharmacophoric features of cinnamaldehyde and apigenin.

The pharmacophore, which refers to the spatial configuration of essential features necessary for a chemical compound to interact with a specific target receptor, holds significant importance in the field of drug design. In this study, we utilized the Pharmagist program to detect pharmacophores by inputting the mol2 format files of the two evaluated compounds. The program generated numerous pharmacophore hits, and from the thousands obtained, we selected 20 outputs through Pharmacophore virtual screening. The obtained results

were carefully examined, and the relevant traits and properties of the 20 selected pharmacophores were tabulated for a comprehensive analysis. Subsequent analysis using swissADME software offered a systematic and efficient approach to identify and evaluate potential pharmacophores for drug design and development.The SwissDock program was used to perform individual docking of the selected 20 pharmacophores. The docking process generates multiple predicted binding modes for each ligand within the active site. These poses are ranked based on scoring functions that estimate the binding affinity or energy of each ligand-protein complex. The poses with the lowest energy or highest binding affinity are considered the most favorable and likely represent the biologically relevant binding mode.

Cinnamaldehyde, a prominent component found in cinnamon, is an aromatic aldehyde that has garnered considerable attention due to its diverse range of potential therapeutic activities. Numerous studies have highlighted its beneficial properties and its potential applications in various fields. The ability of cinnamaldehyde to target and disrupt bacterial biofilms is of great importance, as biofilms pose a significant challenge in various clinical settings[34]. Cinnamaldehyde has been shown to be effective against both Gram-positive and Gram-negative bacterial biofilms, including those formed by Pseudomonas aeruginosa and Staphylococcus aureus[16],[17].

Apigenin, chemically referred to as 4',5,7-trihydroxyflavone, is a naturally occurring compound found abundantly in plants, propolis, and honey. It possesses a wide range of bioactivities, making it a subject of considerable scientific interest[35]. One of the notable bioactivities of apigenin is its potent antioxidant property. It acts as a scavenger of reactive oxygen species (ROS) and helps mitigate oxidative stress, which is associated with various diseases and aging processes[18]. Apigenin has been found to be the most effective agent present in propolis for inhibiting the activity of glucosyltransferases (Gtfs), enzymes produced by S. mutans that are involved in biofilm formation and polysaccharide production. Apigenin can suppress the expression of gtf genes, leading to the inhibition of biofilm accumulation and polysaccharide production by S. mutans[36]. The retrieved pharmacophores provided valuable insights into the spatial configuration of chemical properties required for interaction with specific target receptors. This information is crucial for understanding the ligands' mode of action and their potential as therapeutic agents.

Upon docking of cinnamaldehyde and apigenin with the selected target protein, their binding energies were determined to be -6.44 and -9.10, respectively. The binding energies that have been noted from the outputs of the pharmacophore modeling were shown to be superior compared to the selected two ligands. The

binding energies of ZINC36099457 and ZINC40845897 were noted to be -9.13 and -8.70. These phytocompounds hold promise as potential alternatives or improvements over existing inhibitors. However, further research is necessary to fully understand their therapeutic potential and conduct comprehensive assessments of their pharmaceutical properties, including in vitro experiments and clinical studies.

It is important to acknowledge the limitations of this study. While in silico methods provide valuable insights and serve as a valuable initial screening tool [37], experimental validation is essential to confirm the findings and evaluate the compounds' efficacy and safety in real-world settings. In vitro studies can help validate the ligand-protein interactions observed in silico, and subsequent clinical trials are necessary to assess their potential therapeutic benefits and evaluate their overall safety profile.

Conclusion

Within the set of 20 compounds analyzed in this study, two compounds, namely ZINC36099457 and ZINC40845897, exhibited superior binding energy compared to the two selected inhibitors. These compounds displayed features that outperformed the previously identified inhibitors, indicating their potential as promising candidates for further investigation. In order to advance the pharmaceutical development of these newly discovered phytocompounds, it is imperative to conduct comprehensive studies assessing their pharmacodynamics and pharmacokinetics. The combination of in vitro and clinical investigations will contribute to a deeper understanding of its properties and guide future development strategies. Ultimately, these studies may pave the way for potential therapeutic applications and provide the necessary evidence for the advancement of these phytocompounds as novel drugs.

Conflict of interest: The authors had no conflict of interest to declare.

Financial disclosure: The authors declared that they have received no financial support.

References

1. Hamada S, Slade HD. Biology, immunology, and cariogenicity of Streptococcus mutans. Microbiol Rev. 1980 Jun;44(2):331–84.

2. Kawabata S, Hamada S. Studying biofilm formation of mutans streptococci. Methods Enzymol. 1999;310:513–23.

3. Pandiyan I, Rathinavelu PK, Arumugham MI, D S, Balasubramaniam A. Efficacy of Chitosan and

Chlorhexidine Mouthwash on Dental Plaque and Gingival Inflammation: A Systematic Review. Cureus. 2022 Mar;14(3):e23318.

4. Takahashi N, Nyvad B. The role of bacteria in the caries process: ecological perspectives. J Dent Res. 2011 Mar;90(3):294–303.

5. Klein MI, Hwang G, Santos PHS, Campanella OH, Koo H. Streptococcus mutans-derived extracellular matrix in cariogenic oral biofilms. Front Cell Infect Microbiol. 2015 Feb 13;5:10.

6. Zhang Q, Ma Q, Wang Y, Wu H, Zou J. Molecular mechanisms of inhibiting glucosyltransferases for biofilm formation in Streptococcus mutans. Int J Oral Sci. 2021 Sep 30;13(1):1–8.

7. Atanasov AG, Zotchev SB, Dirsch VM, International Natural Product Sciences Taskforce, Supuran CT. Natural products in drug discovery: advances and opportunities. Nat Rev Drug Discov. 2021 Mar;20(3):200–16.

8. Doddanna SJ, Patel S, Sundarrao MA, Veerabhadrappa RS. Antimicrobial activity of plant extracts on Candida albicans: an in vitro study. Indian J Dent Res. 2013 Jul-Aug;24(4):401–5.

9. Nagai H, Shimazawa T, Matsuura N, Koda A. Immunopharmacological studies of the aqueous extract of Cinnamomum cassia (CCAq). I. Anti-allergic action. Jpn J Pharmacol. 1982 Oct;32(5):813–22.

10. Anandhi P, Tharani M, Rajeshkumar S, Lakshmi T. Antibacterial activity of cinnamon and clove oil against wound pathogens. J Popul Ther Clin Pharmacol. 2022 Jan 6;28(2):e41–6.

11. Monica K, Rajeshkumar S, Ramasubramanian A, Ramani P, Sukumaran G. Anti-inflammatory and antimicrobial effects of herbal formulation using karpooravalli, mint, and cinnamon on wound pathogens. J Adv Pharm Technol Res. 2022 Dec;13(Suppl 2):S369–73.

12. Schoene NW, Kelly MA, Polansky MM, Anderson RA. Water-soluble polymeric polyphenols from cinnamon inhibit proliferation and alter cell cycle distribution patterns of hematologic tumor cell lines. Cancer Lett. 2005 Dec 8;230(1):134–40.

13. Khan A, Safdar M, Ali Khan MM, Khattak KN, Anderson RA. Cinnamon improves glucose and lipids of people with type 2 diabetes. Diabetes Care. 2003 Dec;26(12):3215–8.

14. Tian F, Yu CT, Ye WD, Wang Q. Cinnamaldehyde induces cell apoptosis mediated by a novel circular RNA hsa_circ_0043256 in non-small cell lung cancer. Biochem Biophys Res Commun. 2017 Nov 25;493(3):1260–6.

15. He Z, Huang Z, Jiang W, Zhou W. Antimicrobial Activity of Cinnamaldehyde on Streptococcus mutans Biofilms. Front Microbiol [Internet]. 2019 Sep 25 [cited 2023 May 21];10. Available from: http://dx.doi.org/10.3389/fmicb.2019.02241

16. Zhang H, Zhou W, Zhang W, Yang A, Liu Y, Jiang Y, et al. inhibitory effects of citral, cinnamaldehyde, and tea polyphenols on mixed biofilm formation by foodborne Staphylococcus aureus and Salmonella enteritidis. J Food Prot. 2014 Jun;77(6):927–33.

17. Topa SH, Subramoni S, Palombo EA, Kingshott P, Rice SA, Blackall LL. Cinnamaldehyde disrupts biofilm formation and swarming motility of Pseudomonas aeruginosa. Microbiology. 2018 Sep;164(9):1087–97.

18. Hostetler GL, Ralston RA, Schwartz SJ. Flavones: Food Sources, Bioavailability, Metabolism, and Bioactivity. Adv Nutr. 2017 May;8(3):423–35.

19. Ali F, Rahul, Naz F, Jyoti S, Siddique YH. Health functionality of apigenin: A review. Int J Food Prop [Internet]. 2016 Dec 18 [cited 2023 May 21]; Available from: https://www.tandfonline.com/doi/abs/10.1080/10942912.2016.1207188

20. Lotha R, Sivasubramanian A. FLAVONOIDS NUTRACEUTICALS IN PREVENTION AND TREATMENT OF CANCER: A REVIEW. Asian J Pharm Clin Res. 2018 Jan 1;42–7.

21. Koo H, Hayacibara MF, Schobel BD, Cury JA, Rosalen PL, Park YK, et al. Inhibition of Streptococcus mutans biofilm accumulation and polysaccharide production by apigenin and tt-farnesol. J Antimicrob Chemother. 2003 Nov;52(5):782–9.

22. Ekins S, Mestres J, Testa B. In silico pharmacology for drug discovery: applications to targets and beyond. Br J Pharmacol. 2007 Sep;152(1):21–37.

23. Sankar S. In silico design of a multi-epitope Chimera from Aedes aegypti salivary proteins OBP 22 and OBP 10: A promising candidate vaccine. J Vector Borne Dis. 2022;59(4):327.

24. Ushanthika T, Smiline Girija AS, Paramasivam A, Vijayashree Priyadharsini J. An in silico approach towards identification of virulence factors in red complex pathogens targeted by reserpine. Nat Prod Res [Internet]. 2021 Jun 3 [cited 2024 May 8]; Available from: https://www.tandfonline.com/doi/abs/10.1080/14786419.2019.1641811

25. Mathivadani V, Smiline AS, Priyadharsini JV. Targeting Epstein-Barr virus nuclear antigen 1 (EBNA-1) with Murraya koengii bio-compounds: An in-silico approach. Acta Virol. 2020;64(1):93–9.

26. Sterling T, Irwin JJ. ZINC 15--Ligand Discovery for Everyone. J Chem Inf Model. 2015 Nov 23;55(11):2324–37.

27. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, et al. The Protein Data Bank. Nucleic Acids Res. 2000 Jan 1;28(1):235–42.

28. Güner OF. History and evolution of the pharmacophore concept in computer-aided drug design. Curr Top Med Chem. 2002 Dec;2(12):1321–32.

29. Hasan S, Danishuddin M, Adil M, Singh K, Verma PK, Khan AU. Efficacy of E. officinalis on the cariogenic properties of Streptococcus mutans: a novel and alternative approach to suppress quorum-sensing mechanism. PLoS One. 2012 Jul 5;7(7):e40319.

30. Girija S, A. S. Delineating the Immuno-Dominant Antigenic Vaccine Peptides Against gacS-Sensor Kinase in Acinetobacter baumannii: An in silico Investigational Approach. Front Microbiol. 2020 Sep

8;11:511551.

31. Varghese RM, Aravind KS, Shanmugam R. Antimicrobial Activity of Zinc Oxide Nanoparticles Synthesized Using Ocimum Tenuiflorum and Ocimum Gratissimum Herbal Formulation Against Oral Pathogens. Cureus [Internet]. 2024 Feb 4 [cited 2024 May 8];16(2). Available from: https://www.cureus.com/articles/211030-antimicrobial-activity-of-zinc-oxide-nanoparticles-synthesized-using-ocimum-tenuiflorum-and-ocimum-gratissimum-herbal-formulation-against-oral-pathogens

32. Suresh S, Arumugham IM, Doraikannan S, Rathinavelu PK, Prabakar J, Balasubramaniam A. Comparing the Effectiveness of Herbal and Conventional Dentifrices in Reducing Dental Plaque and Gingivitis: A Systematic Review. J Int Soc Prev Community Dent. 2021 Nov 30;11(6):601–8.

33. Bowen WH. Dental caries - not just holes in teeth! A perspective. Mol Oral Microbiol. 2016 Jun;31(3):228–33.

34. Doyle AA, Stephens JC. A review of cinnamaldehyde and its derivatives as antibacterial agents. Fitoterapia. 2019 Nov;139:104405.

35. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents. 2005 Nov;26(5):343–56.

36. Gazzani G, Daglia M, Papetti A. Food components with anticaries activity. Curr Opin Biotechnol. 2012 Apr;23(2):153–9.

37. Virtual screening of mutations in antioxidant genes and its putative association with HNSCC: An in silico approach. Mutat Res/Fundam Mol Mech Mutag. 2020 May 1;821:111710.