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An insilico molecular docking, DFT analysis and ADMET approach towards the use of phenolic and substituted benzyl compounds of Salvadora persica against Streptococcus mutans

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Aim:
The present study employed a computational in silico approach to validate the mode of interaction of phenolic and substituted benzyl derivatives from Salvadora persica, for antibacterial activity against Streptococcus mutans, elucidating their ADMET-drug-likeness and quantum chemical properties..
Methods:
The 2D and 3D structures of all compounds were drawn and analyzed using ChemDraw 16.0 and Chem3D 16.0 software respectively. The molecular docking studies were performed with Glucansucrase (3AIC) from *S.mutans* using AutoDockTools (ADT), employing a ligand docking approach. The SwissADME tool was used to estimate the pharmacokinetic and other molecular properties of the compounds, utilizing their canonical SMILE structures. The organ toxicities and toxicology properties of the ligands were predicted using the ProTox-II tool. The DFT analysis was performed using Gaussian 09 and visualized through Gauss view 5.0. The structural coordinates of the lead compounds were optimized using B3LYP/6-31G basis levels.
Results and discussion:
The docking study indicates that the selected compounds have potential as inhibitors of bacterial glucansucrase of S. mutans, thereby suggesting anti-cariogenic activity. Results from docking and SwissADME prediction demonstrate that the carvacrol and kaempferol of Salvadora persica comply with Lipinski's rule of five. Toxicological predictions suggest that the primary compounds are non-carcinogenic, non-mutagenic, and non-immunotoxic, while all compounds are non-hepatotoxic and non-cytotoxic.
Conclusion:
The results predict a development of an inhibitor molecule acting against the 3AIC protein of *S.mutans* that could bring about the antibacterial activity.
Keywords: *Salvadora persica*, *Streptococcus mutans*, glucansucrase 3AIC, docking studies, DFT analysis

INTRODUCTION:

Herbal medicine has historically held a marginalized place in the healthcare system. Because of its effectiveness in treating a variety of disorders, modern trends indicate a growing acceptance

of herbal medicine as a substitute for traditional medical interventions (1). The tendency to use natural treatments, particularly those made from plants, has grown more pronounced. This rise in interest in plant-based medicines is caused by several factors (2). These factors include a wide variety of unstudied chemical and biological plants as well as the long history of traditional medicine, which suggests the dependability and effectiveness of using natural products. Within the field of dentistry, there has been a promotion of herbal remedies as a means to address or preempt particular microorganisms that may be responsible for dental-related diseases (3). The World Health Organization (WHO) emphasizes that a significant portion of the world's general population, particularly in emerging countries, relies heavily on natural medicine and traditional herbal therapies for primary healthcare across various conditions. Consequently, the WHO encourages developing nations to integrate therapeutic herbs as an additional resource to enhance the effectiveness of their healthcare systems (4,5).

In numerous developing nations, people continue to adopt traditional tooth-cleaning practices by using chewing sticks crafted from twigs, stems, or roots of various plant species. This is primarily due to their accessibility, affordability, and simplicity (6). Approximately 182 plant species are utilized as chewing sticks around the world (7). Among them, Salvadora persica stands out as the most widely employed plant for this purpose (8). Salvadora persica belongs to the Salvadoracea family and has a broad geographic distribution extending from India, Nepal, and Malaysia in the east to Pakistan, Iran, Iraq, Saudi Arabia, and Egypt, and further westward to Mauritania (9). This plant, commonly known as the miswak tree, is known to contain a diverse range of organic and inorganic compounds in its extract. Among the organic compounds are glycosides, saponins, flavonoids, alkaloids, tannins, benzyl derivatives, phenolic compounds, and organic acids. In terms of inorganic compounds, anionic compounds like fluoride, chloride, sulfate, thiocyanate, and nitrate have been identified (10). Miswak has been found to harbor diverse bioactive components that contribute to oral health (11). For example, the silica, present in miswak, plays a role in removing stains and plaque, while sodium bicarbonate imparts a mild abrasive effect (12). Many researchers have documented S.persica to possess antibacterial (13), antiplaque (14), antifungal (12), and anti-cariogenic properties (8).

Dental caries is a prominent oral health challenge, primarily arising from the demineralization of the tooth, induced by acid production from bacteria within dental plaque (15). The development of dental plaque on teeth is a significant characteristic of Streptococcus mutans (S.mutans), a bacterium that plays a pivotal role in the onset of dental caries in humans (16). Acid-producing species like mutans streptococci and lactobacilli play a role in caries by creating a cariogenic biofilm, maintaining a low pH around the tooth, leading to demineralization and caries formation. Streptococcus mutans, especially, is closely linked to caries development due to its acid production, glucan synthesis, and biofilm formation (17). In the presence of sucrose, S. mutans produce glucosyltransferases (Gtfs) that boost glucan synthesis, forming extracellular polysaccharides (EPSs) crucial for biofilm structure. These EPSs facilitate the adhesion of cariogenic bacteria, resulting in the formation of complex biofilm communities (18,19).

In silico studies have emerged as a promising avenue for research and advancement in the realm of dentistry. These studies involve computer-based simulations and modeling to explore various aspects of oral health, dental treatments, and related biological processes. In silico methodologies present invaluable prospects for the discovery and optimization of novel molecules, evaluation of their binding affinities to particular targets, and anticipation of essential pharmacokinetic parameters, potential toxicity, and physicochemical characteristics (20). The current in silico study aims to evaluate the interaction between cariogenic bacteria, Streptococcus mutans, and the phenolic and benzyl compounds present in Salvadora persica. The findings of the study could potentially support the use of Salvadora persica as an alternative for maintaining oral health, where it is more readily available and accessible.

MATERIALS AND METHODS:

Synthesis and Characterization of Ligands and Macromolecules

ChemDraw 16.0 was utilized to sketch and assess the 2D structures of all compounds, which were then converted to 3D structures using Chem3D 16.0. Subsequently, Chem3D was employed to load the 3D coordinates of each molecule for energy minimization. The protein target designated as the docking receptor was obtained from the RCSB Protein Data Bank (PDB

code: 3AIC), with all bound ligands and water molecules eliminated from the active site of the receptor.

Molecular Docking Analysis

The molecular docking study was carried out using AutoDockTools (ADT). Employing a standardized protocol, compounds 1-15 were docked against the active site of the Streptococcus mutans target protein (PDB code: 3AIC). Each ligand underwent docking to generate nine different conformations. Post-docking analysis was conducted using AutoDockTools and BIOVIA Discovery Studio Visualizer 2021. Conformations with the most favorable free binding energy were selected for further analysis of interactions between the target receptor and the ligands.

Insilico Drug-likeness and Toxicity predictions

Canonical simplified molecular input line entry system (SMILES) structures for compounds 1-15 were retrieved from the PubChem database. These SMILES structures were then analyzed using the SwissADME tool to determine various pharmacokinetic parameters and molecular properties following the methodology outlined by Amina et al. in 2016. SwissADME allows for the computation of essential physicochemical, pharmacokinetic, and drug-like parameters for individual or multiple molecules (21). The predictions provided insights into the numbers of hydrogen donors, hydrogen acceptors, and rotatable bonds, as well as the total polar surface area and synthetic accessibility of the compounds. Additionally, the drug-likeness of the ligands was assessed using screenings based on criteria given by Lipinski et al. via the SwissADME predictor. The ProTox II tool was utilized to predict potential toxicological outcomes and associated organ toxicities of the ligands. Drug-likeness is determined based on the Lipinski rule of five, which predicts poor absorption or permeation for compounds with more than 5 hydrogen-bond donors, 10 hydrogen-bond acceptors, a molecular weight exceeding 500, or a calculated LogP (CLogP) greater than 5.

Quantum Mechanical Studies

The lead compounds were analyzed using Gaussian 09 software via DFT (density functional theory), and their structures were visualized using GaussView 5.0. Optimization of structural coordinates was performed without symmetrical constraints at the B3LYP/6-31G(d,p) level basis set. Electrostatic potential and molecular energies were derived from the optimized geometry. The HOMO-LUMO energy gap was estimated using Koopman's approximation.

RESULTS:

Molecular Docking Analysis:

Compounds 1-15 were docked against the target binding protein 3AIC, exhibiting binding energies ranging from -0.6 to 4.5 kcal/mol (Table 1). A more negative value indicates higher affinity for the target protein.

SwissADME and Lipinski's Rule of Five:

The compounds demonstrate log Kp values spanning from -4.18 to -11.74 cm/s (Table 2). The majority of compounds exhibit high gastrointestinal (GI) absorption, suggesting they may not require a carrier molecule. Additionally, most compounds show no blood-brain barrier (BBB) permeability. Those compounds lacking GI absorption and BBB permeability are considered suitable for drug design assessment according to Lipinski's Rule of Five. Table 3 illustrates the drug-likeness properties of compounds using SwissADME.

Toxicity Profiling:

The compounds exhibit toxicity ranging from class 4 to class 6 (Table 4). All compounds except 2 and 6 display similar LD50 values (1000 mg/kg). Moreover, compounds 1-15 are deemed non-cytotoxic.

Table 1: Molecular Docking Scores and Residual Amino Acid Interactions of Compounds against glucansucrase of S. mutans (PDB ID 3AIC)

S.no.	Molecule	Affinity (kcal/mol)	H-bond	Residual Hydrophobic/Pi-Cation/Pi-Anion/Pi-Alkyl/unfavourable Interactions
1	Benzyl isothiocyanate	-5.4	Glu-515, Asp-477	Asp-909
2	N-Benzylbenzamide	-7.2	Gln-960, Asp-477	Leu-433, Asp-588, Asp-909, Tyr-916
3	Carvacrol	-5.6	Gln-592	Tyr-916, His-587, Phe-907, Asp-909, Asp-588, Leu-382, Leu-434, Leu-433
4	Sabine	-2.9	Tyr-430	Trp-517, Val-479, Phe-907
5	Trans-anethol	-4.8	Gln-592	Tyr-916, Val-957, Phe-907, Tyr-610, Asp-588
6	Methyl chavicol	-4.9	-	Tyr-916, His-587, Trp-517, Ala-478, Leu-433, Asp-588
7	Beta-pinene	-6	-	Ala-478, Leu-433, Tyr-916
8	Rutin	-3.8	Asp-477, Glu-515, Asn-481	Asp-588, Trp-517, Asp-480, Leu-434
9	Quercetin	-8.8	Tyr-430, Glu-515, Asp-477, Gln-592	Asn-481, Asp-588, Asp-909, Tyr-916, Ala-478, Leu-433

10	Kaempferol	-8	Asp-909	Glu-515, Asp-480, Trp-517, Ala-478, Asn-481, Leu-433
11	Beta-D-Glucopyranoside	-6.6	Asp-909, Asp-588, His-587, Gln-592	Trp-517, Ala-478, Asp-477, Asn-481, Glu-515
12	Salvadoraside	1.6	Asp-909, Gln-592, Asp-480	Trp-517, Gln-960, Glu-515, Tyr-430, Trp-517, Asn-481, Asp-588
13	Syringin	-7.1	Asp-909, Asp-480	Asn-862, Asp-588, Asp-477, Glu-515, Leu-433
14	Liriodendrin	4.5	Asn-481, Tyr-916, Gln-960, Asp-959	Ala-478, Glu-515, Asp-588, Leu-433, Gln-592, Trp-517
15	Beta-Sitosterol 3-O-beta-D-galactopyranoside	-0.6	-	Phe-907, Tyr-916, Ala-478, Leu-433, Gly-429, Asp-477

Table 2: ADME Predictions of Compounds computed by SwissADME

S. No.	Molecule	log Kp (cm/s)	GI absorption	BBB permeant	Inhibitor interaction (SwissADME/PreADMET)				
					CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
1	Benzyl isothiocyanate	-4.97	High	Yes	No	No	No	No	No
2	N-Benzylbenzamide	-6.41	High	Yes	No	No	No	No	No
3	Carvacrol	-4.74	High	Yes	Yes	No	No	No	No
4	Sabine	-9.59	Low	No	No	No	No	No	No
5	Trans-anethol	-4.86	High	Yes	Yes	No	No	No	No
6	Methyl chavicol	-4.81	High	Yes	Yes	No	No	No	No
7	Beta-pinene	-4.18	Low	Yes	No	No	Yes	No	No
8	Rutin	-10.26	Low	No	No	No	No	No	No
9	Quercetin	-7.05	High	No	Yes	No	No	Yes	Yes
10	Kaempferol	-6.7	High	No	Yes	No	No	Yes	Yes
11	Beta-D-Glucopyranoside	-9.34	Low	No	No	No	No	No	No

12	Salvadoraside	-11.74	Low	No	No	No	No	No	No
13	Syringin	-9.5	Low	No	No	No	No	No	No
14	Liriodendrin	-11.81	Low	No	No	No	No	No	No
15	Beta-Sitosterol 3-O-beta-D-galactopyranoside	-4.32	Low	No	No	No	No	No	No

Table 3: Drug-Likeness Predictions of Compounds computed by SwissADME

S. No.	Molecule	Molecular weight	NHD	NHA	NRB	TPSA	Consensus Log P	Lipinski's rule of five violation
1	Benzyl isothiocyanate	149.21	0	1	2	44.45	2.91	0
2	N-Benzylbenzamide	211.26	1	1	3	43.09	2.41	0
3	Carvacrol	150.22	1	1	1	20.23	2.82	0
4	Sabine	495.65	7	8	0	144.85	0.55	1
5	Trans-anethol	148.2	0	1	2	9.23	2.79	0
6	Methyl chavicol	148.2	0	1	3	9.23	2.78	0
7	Beta-pinene	136.23	0	0	0	0	3.42	1
8	Rutin	610.52	10	16	6	269.43	-1.12	3
9	Quercetin	302.24	5	7	1	131.36	1.23	0
10	Kaempferol	286.24	4	6	1	111.13	1.58	0
11	Beta-D-Glucopyranoside	372.32	3	9	6	153.96	-3.16	0
12	Salvadoraside	744.73	9	18	14	265.14	-1.28	3

13	Syringin	372.37	5	9	7	138.07	-0.67	0
14	Liriodendrin	742.72	8	18	12	254.14	-1.26	3
15	Beta-Sitosterol 3-O-beta-D-galactopyranoside	576.85	4	6	9	99.38	5.51	1

Table 4: Prediction of Toxicological properties of Compounds computed by Pro-Tox II

S. No.	Molecule	Predicted LD50	Predicted toxicity class	Organ Toxicity				
				Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
1	Benzyl isothiocyanate	900mg/kg	4	No	No	No	Yes	No
2	N-Benzylbenzamide	4920mg/kg	5	No	Yes	No	No	No
3	Carvacrol	810mg/kg	4	No	No	No	No	No
4	Sabine	1370mg/kg	4	No	No	Yes	No	No
5	Trans-anethol	150mg/kg	3	No	Yes	No	No	No
6	Methyl chavicol	1230mg/kg	4	No	Yes	No	No	No
7	Beta-pinene	4700mg/kg	5	No	No	No	No	No
8	Rutin	5000mg/kg	5	No	No	Yes	No	No
9	Quercetin	159mg/kg	3	No	Yes	No	Yes	No
10	Kaempferol	3919mg/kg	5	No	No	No	No	No
11	Beta-D-Glucopyranoside	8000mg/kg	6	No	No	No	No	No

12	Salvadoraside	3000mg/kg	5	No	No	Yes	No	No
13	Syringin	4000mg/kg	5	No	No	Yes	No	No
14	Liriodendrin	3000mg/kg	5	No	No	Yes	No	No
15	Beta-Sitosterol 3-O-beta-D-galactopyranoside	8000mg/kg	6	No	No	Yes	No	No

DISCUSSION:

Streptococcus mutans plays a significant role in the development of dental caries (16). Dental caries begins with the formation of a sticky biofilm, made up of a glucose polymer called glucan produced by Streptococcus mutans (22). This biofilm traps oral bacteria, food debris, and salivary components. Fermentation of dietary sugars like sucrose, fructose, and glucose by these bacteria produces acids, leading to demineralization of tooth structure and the onset of dental caries (23). Glucans are sticky substances that play a crucial role in causing dental caries. They are synthesized from sucrose by enzymes called glucansucrases produced by S. mutans. Targeting these enzymes offers promising prospects for developing effective treatments against dental caries (19,24).

Drug design is the process of developing novel medications through understanding biological targets (25). Essentially, it entails designing molecules that match the shape and charge of the molecular target they interact with and bind to (26). Contemporary drug discovery encompasses identifying screening hits, medicinal chemistry, and optimizing these hits to enhance affinity, selectivity, efficacy/potency, metabolic stability, and oral bioavailability (27,28). The in-silico pharmacology approach entails leveraging information to develop computational models or simulations (29), enabling predictions, hypothesis generation, and ultimately contributing to medical and therapeutic advancements and discoveries (30–33).

The present investigation explores the compounds of toothbrush tree, Salvadora persica for the development of potential inhibitors targeting the protein glucansucrase. The ligands were prepared using ChemDraw 16.0 software for 2D structure evaluation and subsequently converted to 3D structures. For macromolecule preparation, the target protein , glucansucrase encoded as 3AIC, was obtained from the RCSB Protein Data Bank (34).

Phenolic compounds are renowned for their potent antioxidant activity, attributed to their redox properties, which enable them to scavenge and neutralize free radicals through electron donation. The presence of phenolic compounds, encompassing flavonoids, tannins, and phenolic acids, in plant extracts indicates their antioxidant potential (35). In this study, various phenolic and benzyl compounds sourced from different parts of Salvadora persica were evaluated for their potential in inhibiting dental caries. Compounds 1-15 underwent molecular docking against the active site of the target protein 3AIC using AutoDockVina software. Post-docking analysis, conducted with BIOVIA Discovery Studio Visualizer 2021, focused on identifying conformations with the most favorable free binding energy to elucidate interactions between the target receptor and ligands.

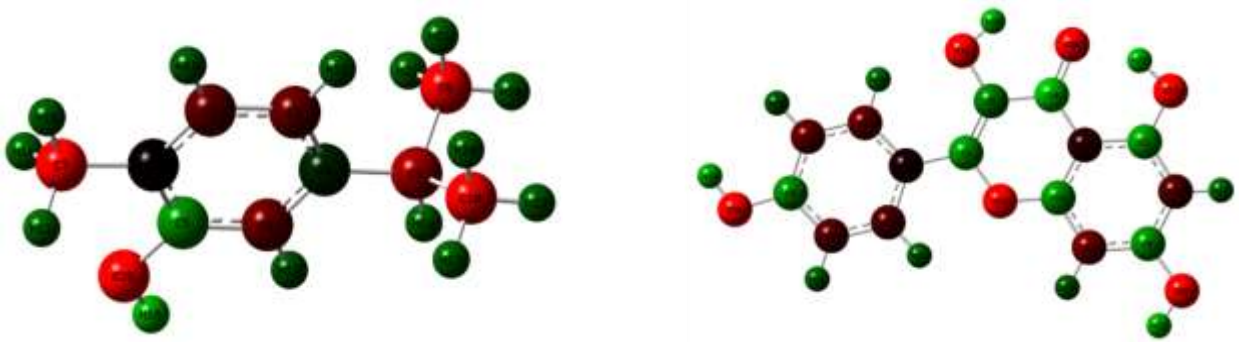
In-silico drug-likeness and toxicity predictions involved obtaining canonical simplified molecular input line entry system (SMILE) structures from PubChem. Drug-likeness serves as a critical criterion during the screening of drug candidates in the early phases of drug discovery and development. It involves correlating the physicochemical properties of a compound with its biopharmaceutical characteristics in the human body, particularly its impact on oral bioavailability (36). SwissADME tool assessed the molecular and pharmacokinetic properties.

Toxicological predictions were conducted using Pro Tox II, revealing that all compounds were non-cytotoxic and non-hepatotoxic. Moreover, the compounds were predominantly found to be non-carcinogenic, non-mutagenic, and non-immunotoxic. Specifically, carvacrol, beta-pinene, and kaempferol exhibited no toxicity across all assessed toxicity outcome parameters according to the Pro-Tox results.

Quantum computational studies were conducted based on density functional theory (DFT) analysis of lead compounds. Structural optimization was performed without symmetrical

constraints, and electrostatic potential and energies were calculated. Figure 1 illustrates the atomic charge distribution in individual atoms.

Figure 1: The DFT calculated Mulliken's atomic charges of compounds – Carvacrol (left) and Kaempferol (right)



The HOMO (highest occupied molecular orbital, electron donor) and LUMO (lowest unoccupied molecular orbital, electron acceptor) of the isolated compounds were determined to be -0.2092 eV and -0.0136 eV for carvacrol, and -0.2053 eV and -0.0711 eV for kaempferol, respectively (Figure 2,3). The energy gap (ΔE) between HOMO and LUMO energies reflects the chemical reactivity and potential for intramolecular charge transfer. A smaller energy gap indicates higher reactivity and significant intramolecular charge transfer, which is crucial for the molecule's affinity to the target (37). Negative electrostatic potentials are represented by red/yellow regions, positive by blue, and neutral by green (Figure 4).

Figure 2: Molecular orbitals and energies for the HOMO and LUMO of isolated compound - Carvacrol

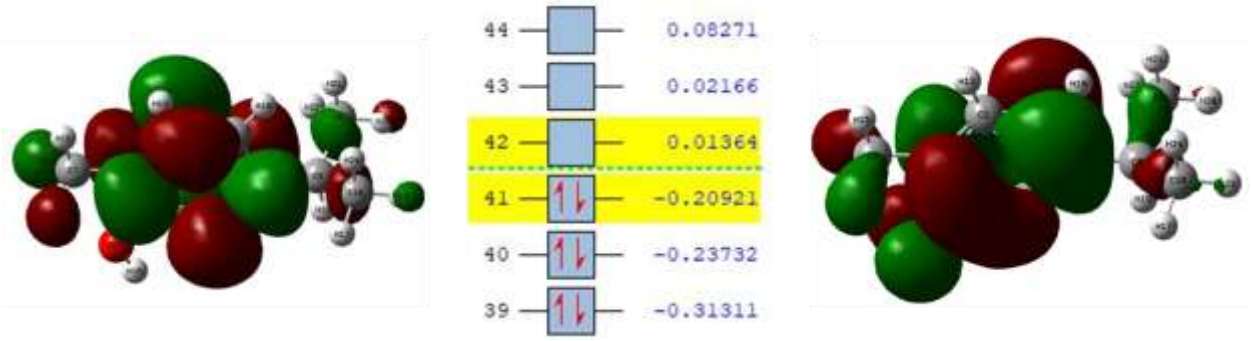


Figure 3: Molecular orbitals and energies for the HOMO and LUMO of isolated compound - Kaempferol

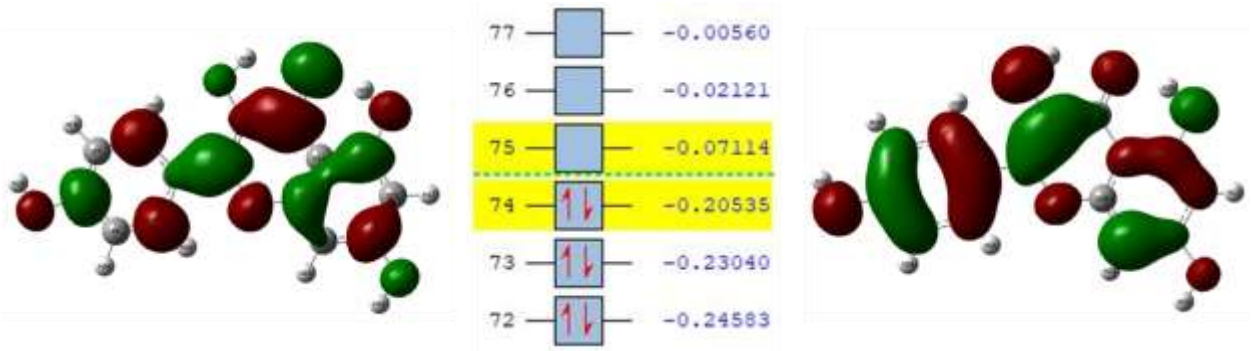
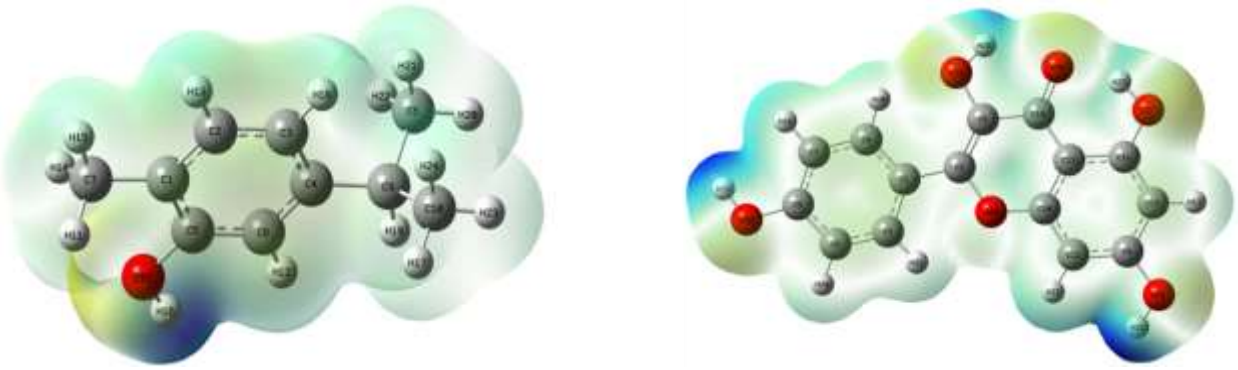


Figure 4: Molecular electrostatic potential surface of compounds – Carvacrol (left) and Kaempferol (right)



This study employed an insilco approach to validate the mode of binding of potential inhibitors, derived from Salvadora persica, to target protein, elucidate their drug-likeness and quantum chemical properties. Out of the 15 compounds, nine compounds were found to be following the Lipinski's rule of 5. Based on the results of present investigation, the compounds Carvacrol and Kaempferol were found to be the potential lead molecules for the development of novel antibacterial agents against 3AIC protein of Streptococcus mutans.

This research employs a holistic strategy that incorporates molecular docking, in-silico predictions, and quantum computational analyses to gain a comprehensive insight into ligand interactions with the target receptor. The study not only facilitates the identification of potential therapeutic candidates but also provides valuable insights into pharmacokinetic properties and potential toxicological consequences. The findings significantly contribute to the exploration and advancement of novel compounds with promising biological activities. While in silico approaches offer valuable insights and serve as an effective initial screening tool, it is imperative to conduct experimental validation to confirm the findings and assess the efficacy and safety of the compounds in real-world situations. In vitro studies can validate the observed ligand-protein

interactions from in silico analyses, and subsequent clinical trials are essential to evaluate the potential therapeutic benefits and overall safety profile of the compounds.

CONCLUSION:

Miswak is widely recognized as a crucial herb for promoting dental health, attributed to its active chemical components that play a pivotal role in its potent bioactive properties. The therapeutic characteristics of miswak can be attributed to its antioxidative, anti-carcinogenic, anti-fungal, anti-microbial, anti-bacterial, antimycotic, and anti-candida properties. In this study, Carvacrol and Kaempferol emerged as promising lead molecules for the potential development of novel antibacterial agents targeting the 3AIC protein of Streptococcus mutans. The findings suggest that further in-vitro analysis could pave the way for the creation of innovative antibacterial agents, making them valuable tools for oral hygiene in the future.

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