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In Silico Screening of Phytochemicals Targeting SmdCD of *Streptococcus mutans* using Molecular Docking Approach

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Abstract

Introduction: Dental caries is a chronic infectious disease caused by bacteria forming a biofilm formation on the tooth surface. Drug-resistant bacteria *Streptococcus mutans* (*S. mutans*) pose a vital public health issue. To overcome this, the development of effective drugs with a novel mechanism of action is very important. Drug repurposing is considered a viable alternative approach to overcome the above issue. In the present study, we have attempted to select unique and traditional source used as in traditional medicine. To continue this effort, in this study we used phytochemical of selected to find an effective cure for dental caries. **Materials and methods:** Inhibition of *S. mutans* deoxycytidylate deaminases (SmdCDs) is a promising drug development strategy against *S. mutans*, responsible for biofilm formation. Since no effective drugs are available against *S. mutans*, we conducted a virtual screening of phytochemicals to find novel compounds against this bacterium. Hence, we created a library of 871 phytochemicals from 13 plants with reported antimicrobial activity. The phytochemical library was subjected to virtual screening against the SmdCD target. **Results and discussion:** Traditionally, many cultures use chewing sticks for oral hygiene maintenance. In the present work, out of 871 phytochemicals, 211 phytochemicals showed the most druggable substance with zero violation from any of the drug-likeness rules. Further, the binding energy indicates the affinity of adhesion of protein structure docked with 2 hit potential herbal compounds of which Cyclocurcumin and Androsta-1,4,6-triene-3,17-dione showed the best docking with SmdCD. **Conclusions:** Based on the binding energy score, we suggest that hit compounds can be used for dental caries and further could be developed as an organic mouthwash.

Keywords: Dental caries, *Streptococcus mutans*, Medicinal plant, Drug-likeness rule, Virtual screening

Introduction

Oral hygiene is influenced by numerous factors, mainly diet and host immune competence, promoting the virulence and adhesion of microorganisms [1,2]. About 700 microbial species have been identified from oral microbiomes [3]. The main causative agent for the formation of biofilm has been identified as the gram-positive bacteria *Streptococcus mutans* (*S. mutans*) associated with dental plaque formation [4]. Generally, dental plaques occur when the oral microbial is low pH, thereby creating the presence of increased acid-producing and acid-tolerant bacteria in a structurally and functionally organized biofilm formation [5]. This has been attributed to the consumption of dietary free sugars (sucrose), either as additives or preservatives, and they are linked to biofilm-induced tooth decay [6,7]. Microbial biofilms display increased tolerance to host defenses and antimicrobial agents [5-8], challenging the clinical management of dental plaque. The commonly used method for caries prevention was mechanical plaque control, such as tooth brushing and flossing. Excessive use of antimicrobials is considered an appropriate combinatory measure for the control of dental caries [9]. Several different approaches have been developed to prevent dental caries such as Chlorhexidine (CHX) is one of the most common antimicrobial agents. It is recognized as the principal agent for chemical plaque control [10]. However, CHX has cytotoxic effects on a wide variety of human cells including oral mucosal cells, blood

cells, keratinocytes, osteoblasts, and osteoclasts [11,12]. Besides, CHX can cause taste confusion, tooth staining, and drug resistance. Therefore, it is important to investigate alternative antibacterial candidates against dental disease. The search for new drug candidates against tooth disease has led to the discovery of molecular targets, the development of a drug, and explores of new bioactive substances. Despite this, there is no effective treatment available in the market. Hence in this study, we used small molecule phytochemicals against putative deoxycytidylate deaminase (dCD) alloenzyme of *S. mutans* because several efforts have been devoted to characterizing the mechanisms of action of these phytochemicals. Numerous studies have been carried out on the antibacterial effects of natural medicinal plants. Several reports suggest that phytochemicals possess remarkable inhibitory activities against this bacteria [13,14].

In gram-positive bacteria and eukaryotic organisms, deoxycytidine-5′-monophosphate (dCMP) deaminases, dCDs catalyze the conversion of dCMP to deoxyuridine monophosphate (dUMP) in the pyrimidine salvage pathway. A crystal structure of dCD complexed with deoxycytidine triphosphate (dCTP) and a substrate analogue from *Streptococcus mutans* (SmdCD-dCTP) indicates an activation mechanism triggered by dCTP [15]. dCTP can allosterically bind to SmdCD and induce a conformational change to activate deamination. The deoxythymidine triphosphate (dTTP) bound complex adopts an inactive conformation that is consistent with its inhibitory role. To clarify the significance of the variability of the regulatory mechanism, it is necessary to compare a pair of activator-bound and inhibitor-bound SmdCD structures from the same species. As dTTP increases, dCTP can be replaced by dTTP from SmdCD and the deamination activity decreases, and vice versa. Furthermore, SmdCD reduces the efficiency of anticancer and antimicrobial drugs [16,17], indicating that SmdCD inhibitors have a potential application for drug discovery. Currently, The effects of this COVID-19 crisis on dental services, such as the restriction of dental practices to emergencies, the shutdown of many dental centers, and the risks of infection transmission, were major concerns of dental care providers [18]. In this situation, our strategies can help oral and dental health workers move toward different approaches during and post COVID-19. These new approaches can vary from optimizing dental healthcare standards to enabling positive attitudes and introducing constructive changes in oral and dental health workers [19]. Now, herbal medicines have received greater attention because of their multiplicity of curing diseases, safety, and being well-tolerated remedies when compared with conventional drugs. Plants are known to produce a variety of bioactive compounds to protect themselves against a variety of pathogens. Therefore, the use of compounds present in medicinal plants plays a crucial role in destroying the cross-links of the biofilm matrix [20-22]. Focus on the search for new drug candidates against periodontal diseases has led to the discovery of molecular targets and explores of new bioactive inhibitors. The objective of our study is, to carry out virtual screening against the SmdCD target of *S. mutans* using 871 compounds of medicinal plants and analyze molecular docking results to find out hit inhibitors for the discovery of potential drug candidates. This study may enable the identification of potential therapeutic against *S. mutans* shown in graphical abstract.

Material and methods

Construction of phytochemical library

Text mining analysis of plants by using Carrot2 and PubTator server showed selected plant compounds with potential antimicrobial properties. Hence, to find out an antibacterial activity against the SmdCD enzyme, a library of 871 phytochemicals was constructed from 13 plants through searching various databases. Further, the 3D structure of each phytochemical was retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov) in SDF format and further converted of them into PDB format by using the Open Babel tool [23].

Enzyme preparation

The 3D crystal structure of the SmdCD target with PDB ID 5C2O was retrieved from Protein Data Bank (https://www.rcsb.org). All water molecules, charged ions, and extra ligands were removed by using PyMOL software [24]. After those adding hydrogen atoms to the enzyme was carried out by using MG Tools of AutoDock Vina software [25]. The crystal structure of the allosteric protein was then saved in PDB format for further analysis.

Ligand preparation

The 3D structure of each phytochemical was retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov) in SDF format and then converted into PDB files using Open Babel open-source software. The crystal structure of SmdCD complexed with dTTP (Compound CID: 64968) is

presented at 2.35 Å resolution. The structure of the reference molecule was retrieved from Protein Data Bank (https://www.rcsb.org).

Drug-likeness prediction

Analysis of molecular properties and drug-likeness of screened phytochemicals is the most important step in drug discovery. To be effective as a drug, potent phytochemicals must reach their target in the body in sufficient concentration, and stay there in a bioactive form long enough for the expected biological events to occur. Swiss ADME web tool [26], was used to generate predictive models for physiological properties, pharmacokinetics, drug-likeness, and physicochemical properties. Easy efficient input and interpretation are ensured through the login-free website http://www.swissadme.ch [27]. All screened phytochemicals were evaluated for their drug-like nature under different rules: Lipinski's rules of 5; 'RO5 [28], Ghose filter, PAINS filter, and Verber filter. Drug-likeness property of hit molecules was checked by the SwissADME web tool.

Molecular docking

Molecular Docking is a computational technique used in drug discovery. It is a method to predict prefer orientation of ligands to bind at the active site of the receptor domain to form a stable complex by using AutoDock Vina software in PyRx open-source software (GUI version 0.8 of AutoDock) [25]. Before docking, the starting directory was set to the desired folder. Firstly, docking was performed using SmdCD, a reference molecule to validate the docking protocol. Pdbqt format of receptor and ligands were dragged into their respective columns in form of pdbqt formate. Assigning the grid parameters is the most important step in molecular docking since it navigates the ligand to the binding site of the protease. Grid spacing was set to 0.375 Å (default). Grid box center for docking was set as X, Y, Z and with dimensions of the grid box for SmdCD target. Docking was performed to obtain a population of possible orientations and conformations for phytochemical at the binding site. Finally, the binding energy table was extracted from the software. Once the analysis is completed the results were checked for best different confirmation with the lowest binding energy or docking score than that of positive control was chosen after the docking search was completed. At the end of docking, the best conformations were compared with rigid docking for binding energy (kcal/mol), and the lowest binding energy complex was saved in PDB format for further analysis.

Docking validation

The docking procedure was validated by using the important method. In this method, the dTTP inhibitor from the SmdCD was removed and re-docked into the active site using AutoDock Vina. It was done manually by opening the co-crystallized complex in a notepad, removing the inhibitor heteroatoms from the SmdCD, pasting it into a new notepad, and saved as an inhibitor in PDB file format. The same protocol including the grid parameters was unchanged in the process. It was done to ensure the inhibitor binds exactly to the active site cleft and must show less deviation compared to the actual co-crystallized complex. The re-docked complex was then superimposed onto the reference co-crystallized complex using PyMOL software and the root mean square deviation (RMSD) was calculated and the 2-dimensional image showing the superimposed amino acid residues were highlighted using LigPlot v.2.2 software. This was done to validate the docking procedure to ensure the validation of docking.

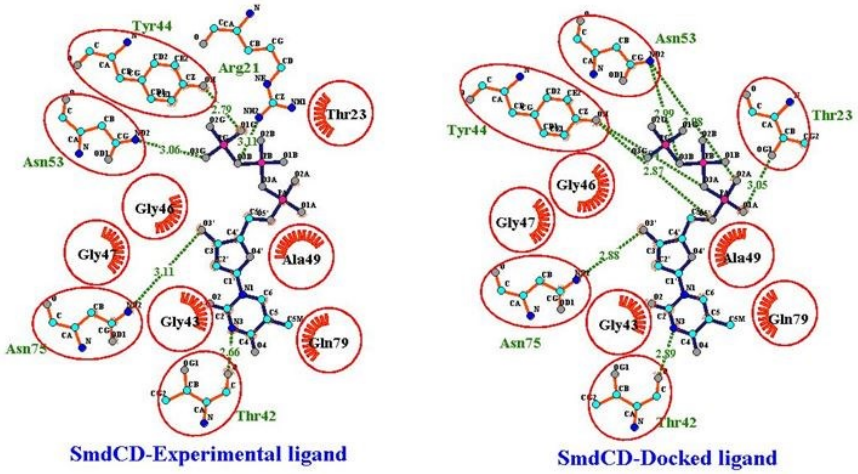

Figure 1 Binding pocket of SmdCD showing experimental and docked reference ligand (Blue color) binds to modulator site residues. Modulator site residues are in orange circles representation. Hydrogen bonds and that formed between protein and ligand are shown by green dotted lines and other residues are hydrophobic bond-forming residues.

Toxicity prediction

Screened phytochemicals obtained from molecular docking were used for toxicity prediction. Toxicity analysis of phytochemicals with good binding affinity towards the protein was carried by using OSIRIS property explorer program [29]. US Food and drug administration toxicity risk predictor tool OSIRIS evaluated various toxicity risks properties phytotochemicals such as tumorigenicity, mutagenicity, irritation, and reproductive development toxicity.

ADME/tox study

ADME/tox is the vital pharmacological property that deals primarily with Absorption, Distribution, Metabolism, Excretion, and Toxicity of ligands in the human body which is essential for drug development. Hit compounds that are screened to all protein targets were chosen for the evaluation of ADME/tox using the admetSAR (http://lmmd.ecust.edu.cn/admetsar1/predict) servers [30]. These servers compared query phytochemicals to the recent and most comprehensive manually compiled data for a variety of chemicals associated with known ADME/tox profiles. The canonical SMILES of the compounds were used to calculate ADME/tox properties using the default parameters of the server. For this analysis, the following properties of the compound were considered: Absorption properties (Human Intestinal Absorption (HIA), Blood-Brain Barrier Penetration (BBB), Caco-2 Permeability, Distribution properties, Metabolism properties (Cytochrome P450 (CYP450) substrate and inhibitor), Human ether-a-go-go-related gene (HERG) inhibition, and Toxic properties.

Visualization

Now, we were able to see our phytochemicals have been docked in the substrate active site. The result can be visualized and analyzed by the bioinformatics tool LigPlot + v.2.2.5 software. The 2 D depiction of hydrogen-bond interactions of the complex receptor-ligand structure was done by LigPlot + v.2.2.5 software [31], to identify the interactions of amino acid between protein and ligand complex. LigPlot depicted hydrophobic bonds, hydrogen bonds, and their bond lengths in each docking pose.

Results and discussion

S. mutans is gram-positive pathogenic bacteria causing dental caries. Treatments for bacteria in the form of antibiotics and synthetic mouthwash are available to treat dental diseases. The major problem with these chemicals is that they are toxic in nature which is harmful to human health and has side effects. Another problem is drug resistance which arises due to mutation in target enzymes. To solve these problems, research is focused mainly on discovery and identification of phytochemicals against SmdCD target to identify potential therapeutic phytochemicals.

Construction of phytochemical library

This process takes more advantage in biomedical research and drug discovery. In this process, useful information is extracted from data sources via recognition and exploration of interesting patterns. However, hunting through a large number of medicinal plants is a laborious yet time-consuming process. In this study, we prepared a library of 13 medicinal plants through text mining analysis Carrot2 and PubTator servers showed that these plants have potential anti-bacterial activity and phytochemicals of these plants can have anti-bacterial properties. Hence to find out natural phytochemicals with an anti-bacterial activity against *S. mutans*, a library of 871 phytochemicals was constructed from 13 plants, namely, *Acacia nilotica, Acacia catechu, Allium sativum, Aloe vera, Azadirachta indica, Curcuma longa, Embelia ribes, Glycyrrhiza glabra, Hemidesmus indicus, Juglans regia, Mangifera indica, Mimusops elengi* and *Phyllanthus emblica* through searching scientific literature and PubChem server. The 3D structures of phytochemicals were generated from PubChem Server in sdf format and then convert into pdb format by using Open Babel software.

Drug-likeness prediction

Advancement in computer science, lot of successful findings drugs from natural products using computer aided drug design methods for example the development of Dazamide, Imatinib, Dasatinib and Ponatinib etc. [32]. The rationale behind *in silico* approaches are due to relatively lower cost time factor involved compared to standard ADMET profiling [33,34]. In the present study SwissADME online software tool was used to evaluate the ADME properties.

Results obtained from *in silico* studies clearly indicate 211 phytochemicals showed druggable substance with a zero violation from any of drug likeness rules. It was interesting to note that results from SwissADME predictor values of Log P with important rules of drug likeness. eg. Lipinski, Ghose, Veber, Egan etc. Though the phytochemicals exhibited good hydrophilic lipophilic balance and same predicted bioavailability, hydroxy derivative with high lipophilicity was expected to show decent GI absorption. Hydroxyl derivative with a higher value of probability of antibacterial activity and non-carcinogenic and mutagenic properties were predicted as lead in the study.

Molecular docking

All 211 selected phytochemicals were docked with SmdCD (PDB ID (5C2O)) using PyRx software by selecting AutoDock Vina as the docking engine to find the reasonable binding geometry and discover the protein-ligand complex, and it was found that 3 phytochemicals had a good binding affinity to receptors compared to reference molecule. Before performing the virtual screening, validation of protocol was carried out by re-docking reference compound (dTTP) into the modulator site of SmdCD. The result showed that the docked dTTP was completely superimposed with co-crystallized dTTP in PDB ID (5C2O). Further, all selected inhibitors were in the pocket of the target SmdCD, exhibiting a possible interaction and ranked-based binding energies with SmdCD on a specific binding pocket (**Table 1**). Binding energies of the screened phytochemicals were found to be in the range of –7.9 to –7.5 kcal/mol and indicate good inhibition of the enzyme. 3 phytochemicals were observed to better fit strong binding in the allosteric substrate pocket. The binding energy of screened phytochemicals was in the order Cyclocurcumin (–7.9 kcal/mol) = Elatin (–7.9 kcal/mol) > Androsta-1,4,6-triene-3,17-dione (–7.6 kcal/mol) = SmdCD reference ligand (–7.5 kcal/mol) (**Table 1**). Docking results are ranked based on binding energies. After docking, 3 successful phytochemicals were screened for toxicity prediction.

Table1 Molecular docking scores of various screened Phytochemicals with the SmdCD.

S. No	Protein-ligand complex	Compound ID	SMILES structure of compound	Binding affinity (kcal/mol)
1	SmdCD - Reference	64968	CC1=CN(C(=O)NC1=O)C2CC(C(O2)COP(=O)(O)OP(=O)(O)OP(=O)(O)O)O	–7.5
2	SmdCD - Cyclocurcumin	69879809	COc1cc(/C=C/C2=CC(=O)C[C@H](O2)c2ccc(c(c2)OC)O)ccc1O	–7.9
3	SmdCD - Androsta-1,4,6-triene-3,17-dione	104880	O=C1C=C[C@]2(C(=C1)C=C[C@@H]1[C@@H]2CC[C@]2([C@H]1CCC2=O)C)C	–7.6
4	SmdCD -Elatin	44257938	Oc1ccc(cc1)c1oc2cc(O)cc(c2c(=O)c1O)O	–7.9

Re-docking and superimposition

The re-docking was done to examine the docking procedure and efficiencies. The same methodology that was used previously was used in the re-docking process. The dTTP inhibitor bound exactly to the active site with the good binding energy of –7.5 kcal/mol. Gly43, Gly46, Gly47, Ala49, Gln79, Thr23, Asn53, Tyr44, Asn75 and Thr42 are the interacting amino acids in the active site pocket and a totally 7 hydrogen bonds were formed with a threshold distance of 3.05 Å. The re-docked complex was then superimposed onto the native co-crystallized dTTP-SmdCD from PDB using PyMOL and a low RMSD of 0.725 Å was observed. The re-docked complex was then superimposed onto the native co-crystallized dTTP-SmdCD using LigPlot+ v.1.4.5 interestingly re-docked complex was superimposed completely onto the native co-crystallized complex without any adjustments. All the atoms of amino acids of both complexes were superimposed without any constraints. On the whole, there were a total of 10 amino acid residues superimposed. The superimposed 2-dimensional structure is shown in **Figure 2**, the superimposed amino acids of the complexes are encircled in red.

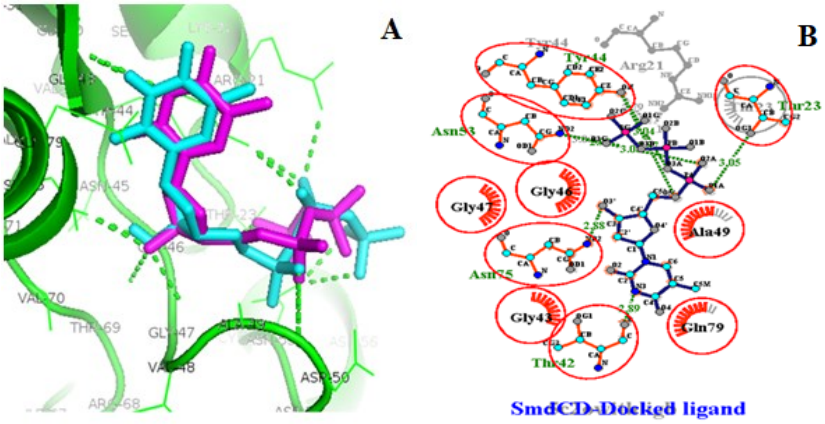

Figure 2 (A) Superimposition of experimental reference in pink color and docked reference in Cyan color with 3-D structure of the protein. (B) The 2 D structure of superimposition of the docked reference molecule (dTTP) with its X-ray crystal structure (Dotted green lines indicate the hydrogen bond and brick red sparked arc represent hydrophobic interaction.

Toxicity prediction

OSIRIS tool

The 3 phytochemicals were predicted for tumorigenicity, mutagenicity, irritation, and reproductive toxicity by OSIRIS tool. The predicted toxicity of 3 phytochemicals is shown in **Table 2**. Accordingly, Elatin showed a high risk of toxicity, while, Cyclocurcumin and Androsta-1,4,6-triene-3,17-dione, were showed no risk. Drug-score show ranges between 0 to 1, where the value 1 indicates the possibility of a compound to be a drug molecule, whereas score value 0 indicates that compounds have no possibilities of drug candidates. The drug score combines drug-likeness, cLogP, logS, molecular weight and toxicity risks in 1 handy value than may be used to judge the compound's overall potential to qualify for a drug. This value is calculated by multiplying contributions of the individual properties with Eq. (1):

$$ds = \prod \left(\frac{1}{2} + \frac{1}{2} s_i\right) \cdot \prod t_i \quad (1)$$

$$s = \frac{1}{1 + e^{ap+b}} \quad (2)$$

ds is the drug score. si are the contributions calculated directly from of cLogP, logS, molweight and druglikeness (pi) via Eq. (2) which describes a spline curve. Parameters a and b for cLogP, logS, molweight and druglikeness, respectively. ti are the contributions taken from the 4 toxicity risk types. Therefore, 2 phytochemicals (Cyclocurcumin, Androsta-1,4,6-triene-3,17-dione,) were evaluated for binding interaction with SmdCD.

Table 2 Toxicity profile of the reference molecule and hit phytochemicals.

S. No.	Compound name	TUM	MUT	IRR	REP	cLogP	Solubility (LogS)	Mol weight	Drug-score
1	Reference(TTP)	Non Toxic	Non Toxic	Non Toxic	Non Toxic	–9.41	2.62	482	0.39
2	Cyclocurcumin	Non Toxic	Non Toxic	Non Toxic	Non Toxic	3.34	–3.47	368	0.73
3	Androsta-1,4,6-triene-3,17-dione	Non-Toxic	Non-Toxic	Non-Toxic	Non-Toxic	3.09	–3.66	282	0.75
4	Elatin	Toxic	Non-Toxic	Non-Toxic	Non-Toxic	1.84	–2.79	286	0.46

AdmetSAR server

For any drug or candidate to achieve optimum therapeutic efficacy, it must possess a high degree of potency and selectivity for interaction with a biological target as well as the ability to attain target tissue concentrations that are above a certain threshold value. Absorption, distribution, metabolism, and excretion (ADME) processes play a pivotal role in defining the disposition of a drug candidate, and thus its therapeutic efficacy. Optimizing the chemical structure of lead candidates with respect to the ADME processes has become an integral part of drug discovery [35]. Using admetSAR server, we extracted a few more data of screened compounds. The results of ADME of screened compounds were shown in **Table 3**. From the output, it was concluded that these compounds have higher BBB permeability and HIA analysis than the reference compound, it is well known that Greater HIA suggests that the compound could be better absorbed from the intestinal tract upon oral administration. In terms of predicting the efflux by P-glycoprotein from the cell, both compounds come out to be the substrate of P-glycoprotein except reference compound which was nonsubstrate. P-glycoprotein inhibitor indicates that the drug will inhibit the efflux process from the cell and increase bioavailability. Whereas a non-inhibitor of P-glycoprotein means that the drug will efflux from the cell by the P-glycoprotein and be pumped back into the lumen, limiting bioavailability and promoting the elimination of that drug in bile and urine [36]. In terms of metabolism, Cytochrome P450 monooxygenase (CYP) enzymes play important roles in drug metabolism and have been extensively studied particularly 2D6, 2C9 and 3A4, which are most important in humans. For the CYP-2D6 and 2C9 we observed that both hit compounds including the reference molecule were nonsubstrate. Whereas, in the case of CYP-3A4, compound Androsta-1,4,6-triene-3,17-dione was shown to be metabolized by CYP450 since it comes out to be a substrate. For CYP-1A2 and 2C19 both compounds were shown as a non-inhibitor (**Table 3**). A non-inhibitor of CYP450 refers that the molecule will not interfere with the biotransformation of drugs metabolized by CYP450 enzyme [37]. AMES Toxicity reveals that both compounds were non- AMES and can be used as a drug candidate. According to the carcinogenic profile, both the ligands were come out as non-carcinogenic similar to the reference molecule. For acute oral toxicity both the compounds including reference molecule were shown as a type III acute oral toxicity. The data of LD50 dose in rat model also computed by admetSAR server. The median lethal dose (PLD 50) is a specific measure of acute toxicity (dose that causes 50 % mortality in treated animals when given over a period of time) that is used to compare the relative toxicity of various compounds. By computing this property, we observed that all the compounds showed similar values as compared to reference molecule. Overall, these ADMET predictions suggest that some properties of compounds are associated with alerts. But a little modification in the structure can make these compounds more potent anti-bacterial drugs.

Table 3 The ADMET profile of screened compounds obtained from admetSAR server.

Parameters	dTTP	Cyclocurcumin	Androsta-1,4,6-triene-3,17-dione
Absorption			
BBB probability	+/0.6582	+/0.6818	+/0.9793
HIA probability	+/0.7735	+/0.9747	+/1.0000
Caco-2 permeability probability	+/0.7847	+/0.8473	+/0.8011
Distribution			
P-glycoprotein Substrate	Non-substrate/0.6088	substrate/0.6193	substrate/0.5526
P-glycoprotein Inhibitor	Non-inhibitor/0.7734	inhibitor/0.7064	Non-inhibitor/0.6615
Renal Organic Cation Transporter	Non-substrate/0.9216	inhibitor/0.5611	Non-inhibitor/0.6632
Metabolism			
CYP-2C9 substrate/inhibitor	Non-substrate/0.6086	Non-substrate/0.7903	Non-substrate/0.8548
CYP-2D6 substrate/inhibitor	Non-substrate/0.8509	Non-substrate/0.8847	Non-substrate/0.9131
CYP-3A4 substrate/inhibitor	Non-substrate/0.5396	Non-substrate/0.5154	substrate/0.7193
CYP-1A2 inhibitor	Non-inhibitor/0.8866	Non-inhibitor/0.9553	Non-inhibitor/0.9046
CYP-2C19 inhibitor	Non-inhibitor/0.8733	Non-inhibitor/0.5623	Non-inhibitor/0.8138
CYP inhibitory promiscuity	Low	High	Low
Toxicity			
AMES Toxicity	Non AMES toxic	Non AMES toxic	Non AMES toxic
Carcinogens	Non- carcinogens	Non- carcinogens	Non- carcinogens
Acute Oral Toxicity	III/0.5521	III/0.5362	III/0.7373
Rat LD50	2.3754	2.7862	1.5360

Visualization

LigPlot + v.1.4.5 was used to visualize the protein-ligand interactions. The docked poses of these 2 compounds with SmdCD are shown in **Figure 3**. SmdCD -reference the docked dTTP showed interaction with the amino acid residues by hydrogen and hydrophobic bonds as found in the experimental structure shown in **Figure 1**. It forms 7 hydrogen bonds with 5 residues Thr42, Tyr44, Asn53, Thr23 and Asn75 of SmdCD. It also formed the 5 hydrophobic bonds with residues Ala49, Gly47, Gly46, Gly43 and Gln79 of SmdCD as shown in **Figure 3**. According to **Figure 3(A)**, SmdCD - Cyclocurcumin formed one hydrogen bond with Thr42 which have a bond distance 3.08 Å and it also formed 9 hydrophobic bonds with Ala49**,** Gln79, Ala41, Gly47, Gly46, Asn75, Tyr44, Val48, and Lys82. SmdCD - Androsta-1,4,6-triene-3,17-dione interacted with Tyr44 that make 1 hydrogen bond having a bond distance 3.14 Å and 7 hydrophobic bonds with Asn53, Gly47, Asn75, Gly43, Gln79, Ala49, and Asp50, residues were found to participate in SmdCD- Androsta-1,4,6-triene-3,17-dione complex showed in **Figure 3(B)**.

Table 4 2D details Interactions between the SmdCD and top hits after the molecular docking. The bold residues represent the common interacted residues between SmdCD-reference complex and SmdCD - screened phytochemical complex.

S. No	Compounds name	Number of H-bonds	Interacted residues with SmdCD	Common active site residues
1	Reference(TTP)	7	**Ala49,** Gly46, **Gly47, Gln79,** Gly43, Thr23, **Tyr44,** Asn53, **Asn75,** Thr42	**Ala49, Gly47, Gln79, Tyr44, Asn75**
2	SmdCD - Cyclocurcumin	1	**Tyr44,** Gly46, **Gly47, Asn75, Ala41,** Thr42, **Gln79, Ala49,** Val48, Lys82	
3	SmdCD - Androsta-1,4,6-triene-3,17-dione	1	**Ala49, Gly47, Gln79, Asn75, Tyr44,** Asn53, Gly43, Asp50	

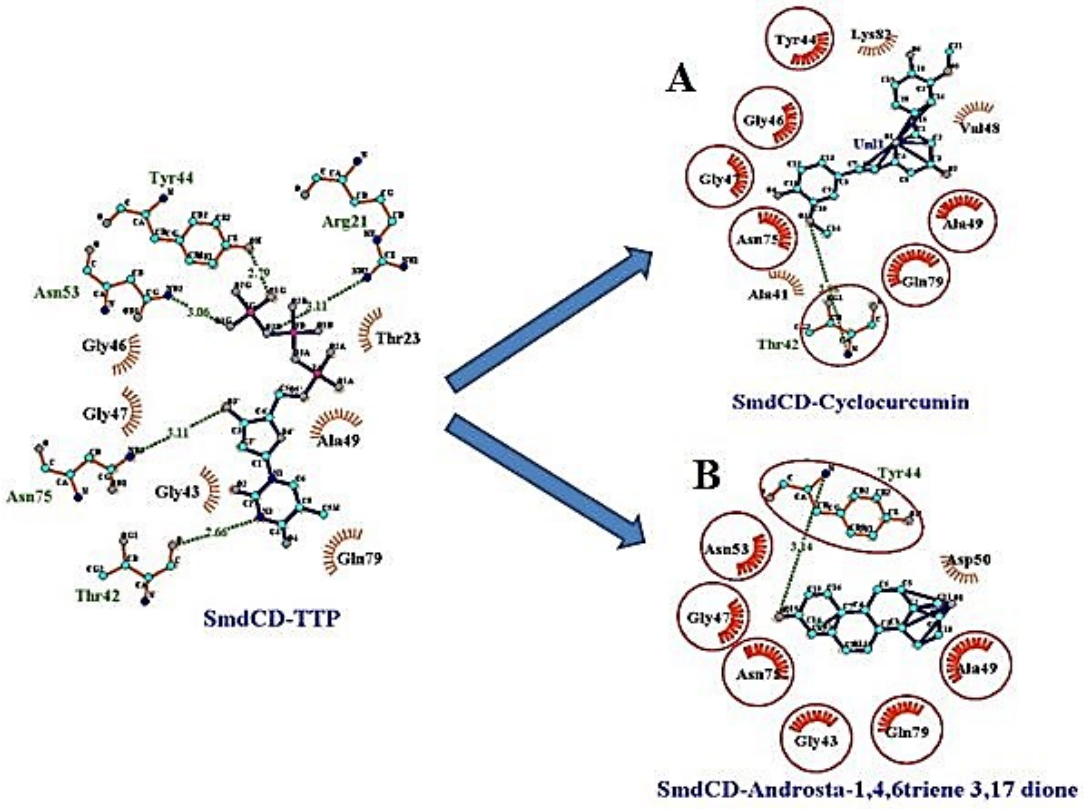

Figure 3 2D interaction of protein-ligand complexes with Hydrogen and Hydrophobic-bonds between hit phytochemicals (reference ligand, Cyclocurcumin, and Androsta-1,4,6-triene-3,17-dione) and dotted green lines denote hydrogen bonds, red half arcs indicate hydrophobic interactions.

Conclusions

Despite improvement in pharmacology and conventional chemistry in developing rapidly new synthetic antibiotics, altering existing antibiotics or finding suitable protein targets against which inhibitors can be developed. Present global drug development programs may not be able to afford new effective antibiotics for the next decade [38]. In a similar study, potential inhibitors targeting Streptococcus mutans sortase were screened using in-silico approaches [39]. In our study, we used different compounds from selected medicinal plants against the allosteric substrate of SmdCD. These

plants are used in Ayurveda and ancient medicinal systems with antibacterial, anti-inflammatory, antiviral, antioxidant, anticancer, and antidiabetic activities. Glycyrrhiza glabra is an extensively used herb in the ancient medicinal history of Ayurveda. The antimicrobial activity of G. glabra has been exploited medicinally for many years. Natural Plant Products (NPPs) of G. glabra are considered antitussive, mucolytic, expectorant, antimicrobial, immunostimulant as well as a flavoring agent [40]. Saponins have also been reported to possess antimicrobial activity due to the detergent-like nature they can cause leakage in the membrane by interacting with enzymes from bacterial cell [41]. Rationale based selection of G. glabra against P. aeruginosa by employing bioprospection, *in silico* and *in vitro* study is reported previously [42,43]. Curcumin is an active component of *C. longa* is having properties such as antioxidant, anti-inflammatory, anti-viral, antibacterial, antifungal, and anticancer activities, and also works against various malignant diseases such as diabetes [44], arthritis, Alzheimer's [45], and other chronic diseases have been reported. Cyclocurcumin, a curcumin derivative of *C. longa*, exhibits immune-modulating ability and is a potential phytochemical for the treatment of rheumatoid arthritis. TNF-α is a key factor in a variety of inflammatory diseases. The role of cyclocurcumin in overcoming p38α-induced production of TNF-α and hence can be used as a therapeutic agent to target rheumatoid arthritis [46]. Cyclocurcumin can be used as a herbal drug or proven to be a good lead compound for oral and cervical cancers [47,48]. The information is quite significant in drug development for oral and cervical cancers. In this study, we employed *in silico* techniques to investigate natural compounds Cyclocurcumin and Androsta-1,4,6-triene-3,17-dione as possible to control biofilm formation. To find out potential phytochemicals, a library of phytochemicals of 13 selected medicinal plants and buildup a library of 871 phytochemicals. Further, filtered 211 phytochemicals were subjected to molecular docking against SmdCD of *S.mutans*. Based on the virtual screening of phytochemicals against SmdCD, namely Cyclocurcumin, Androsta-1,4,6-triene-3,17-dione, Elatin showed good binding energy with SmdCD as compared to reference compounds. After the virtual screening, we checked the toxicity prediction of screened phytochemicals through OSIRIS software. Finally, out of 3 phytochemicals, 2 phytochemicals were non-toxic. These phytochemicals viz. Cyclocurcumin and Androsta-1,4,6-triene-3,17-dione can be used against the SmdCD target. In our drug repurposing study, both phytochemicals were found to inhibit the SmdCD and these phytochemicals may be used against *S. mutans* infection. Finally, it is suggested that Cyclocurcumin and Androsta-1,4,6-triene-3,17-dione may be used for the development of organic mouthwash as potent anti-biofilm drugs for preventing dental caries.

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