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In *silico* Screening of Potential Compounds from Medicinal Plants by Targeting *Streptococcus mutans* Deoxycytidylate Deaminase

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Dental caries is the chronic infectious disease caused by bacteria to from a biofilm formation on the tooth surface of man. Drug-resistant *Streptococcus mutans* (*S. mutans*) poses a vital public health issue. To overcome this, the development of effective drugs with novel mechanism of action is requisite. Drug repurposing is considered a viable alternative approach to overcome the above

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issue. In the present study, we have attempted to selected unique and traditional source use as in traditional medicine. Traditionally, many cultures use chewing sticks for oral hygiene maintenance. When properly used, these chewing sticks are found to be efficient due to the combined effect of mechanical cleaning, enhanced salivation and the antimicrobial action of leached out plant. Inibition of the *S. mutans* deoxycytidylate deaminases (SmdCDs) is the most promising drug development strategy against the *S. mutans*, responsible for the biofilm formation. In the present work, out of 871 phytochemicals 211 phytochemicals were showed the most druggable substance with zero violation from any of druglikeness rule. Further, the binding energy indicates the affinity of the adhesion of protein structure docked with the 2 hit potential herbal compounds of which Cyclocurcumin and Androsta-1,4,6-triene-3,17-dione showed best docking with the SmdCD.

Keywords: Streptococcus mutans; medicinal plants; phytochemicals; in silico screening.

1. INTRODUCTION

The 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 are identified from oral microbiome” [3]. “Dental caries is one of the most common chronic infectious and diseases that occur at any age of humans” [1]. 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” [4]. “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” [5,6]. The main causative agent is gram-positive bacteria *Streptococcus mutans* (*S. mutans*) associated with dental caries and dental plaque formation [7]. “Unlike microbial biofilms display increased tolerance to the host defenses and antimicrobial agents [8,4], 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 as an appropriate combinatory measure for the control of dental caries, particularly in the highrisk population” [9]. “Microbes within the 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 confusions, tooth staining, and drug resistance. Thereby, alternative antibacterial agents are still needed for the control of dental plaque and caries.

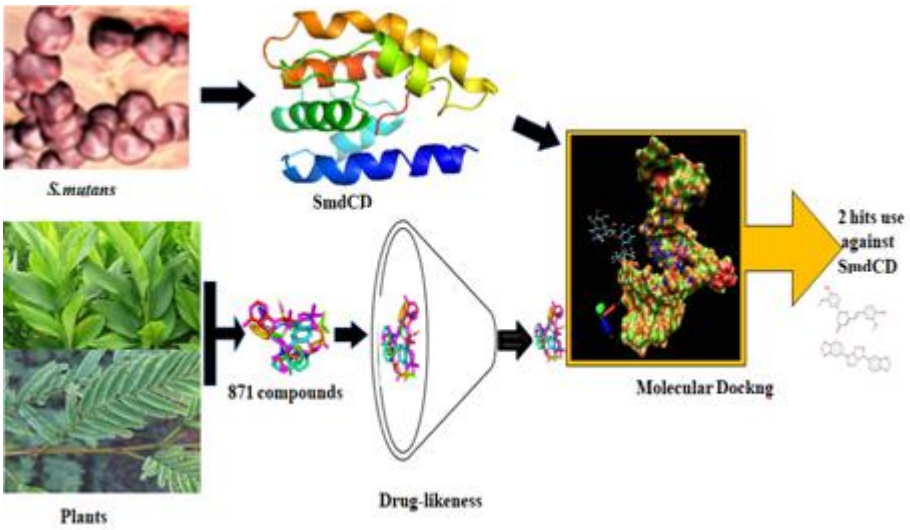

Fig. 1. Outline of the study

"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 *S. mutans* (SmdCD–dCTP) indicates an activation mechanism triggered by dCTP" [13]. "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" [14,15], which indicates that SmdCD inhibitors have a potential application for drug discovery.

Currently, herbal medicines have received greater attention because of their multiplicity of curing diseases, safety and being well tolerated remedies when compared with the conventional drugs. Plants are known to produce a variety of phytochemicals to protect themselves against a variety of pathogens. The use of phytochemicals present in the medicinal plants plays a crucial role in destroying the cross-links of the biofilm matrix. If we 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,

i. To carry out virtual screening against the SmdCD target using 871 phytochemicals from medicinal plants.
ii. To predict toxicity of compounds by using OSIRIS softwere to find out 2 inhibitors for the discovery of potential drug candidates. This study may enable the identification of potential therapeutic against the dental caries.

2. MATERIALS AND METHODS

Since there are no effective drugs available against dental disease, we conducted virtual screening of phytochemicals to find novel compounds against *S.mutans* bacteria. Hence, inclusion and exclusion criteria in our methodology, we created a phytochemical library of phytochemicals which have been reported as antibacterial, antiviral, and antifungal activity. The phytochemical library was subjected to virtual screening against molecular targets; SmdCD.

2.1 Construction of Phytochemical Library

Text mining analysis of plants by using Carrot2 and PubTator server which showed that selected plants photochemical had potential antimicrobial properties. Hence to find out an antibacterial activity against SmdCD enzyme, a library of 871 phytochemicals was constructed from 13 plants through searching the scientific literature and PubChem database. Further, 3D structure of each phytochemicals retrieved from PubChem https://pubchem.ncbi.nlm.nih.gov) in SDF format and further converted all of them into PDB format by using Open Babel tool [16].

2.2 Enzyme Preparation

The 3D crystal structure of SmdCD target with PDB ID 5C2O was retrieved from the Protein Data Bank (https://www.rcsb.org). Here, the all water molecules, charge ions, and extra ligands were removed from the enzyme using PyMOL software [17]. After that the adding of hydrogen atoms to the enzyme was carried out by using MG Tools of AutoDock Vina software [18]. The crystal structure of allosteric protein was then saved in PDB format for further analysis.

2.3 Ligand Preparation

The 3D structure of each phytochemical was retrieved from Pub Chem (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 reference molecule was retrieved from Protein Data Bank (https://www.rcsb.org).

2.4 Drug-likeness Prediction

Analysis of molecular properties and drug-likeness of the screened phytochemicals is an

important step in drug discovery. To be effective as a drug, a potent phytochemicals must reach its target in the body in sufficient concentration, and stay there in a bioactive form long enough for the expected biologic events to occur. Here, we used the new Swiss ADME web tool [19] that gives free access to a pool of fast yet robust predictive models for physiyological property pharmacokinetics, drug-likeness and physicochemical properties. Easy efficient input and interpretation are ensured through the login-free website http://www.swissadme.ch. [20]. Therefore, all screened phytochemicals were evaluated for their drug-like nature under different rules: Lipinski's rules of five; 'RO5 [21], Ghose filter, PAINS filter and Verber filter. The drug-likeness property of the hit molecules was checked by SwissADME web tool.

2.5 Molecular Docking

Molecular Docking is the computational technique use a drug discovery. Molecular Docking is the method which predict of prefer orientation of the phytochemicals 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) [18]. Firstly docking was performed using SmdCD, reference molecule to validate docking protocol. Pdbqt format of the receptor and phytochemicals were dragged into their respective columns in form of pdbqt formate. The grid box centere for docking set as X, Y, Z and with the dimensions of the grid box for SmdCD target. Now, we can run docking with Vina. Docking was performed to obtain a population of possible orientations and conformations for the phytochemical at the binding site. The finally, the binding energy table was extracted from the software. Once the analysis is completed we can check the result of best different confirmation with the lowest binding energy pose or docking score than that of the positive control was chosen after the docking search was completed. The binding affinities of phytochemicals for SmdCD target were recorded. At the end of the docking, the best conformations were compared with the rigid docking for binding energy (kcal/mol). The best conformation of the phytochemicals which had lower binding energy as compare to reference molecule were chosen for further toxicity prediction analysis.

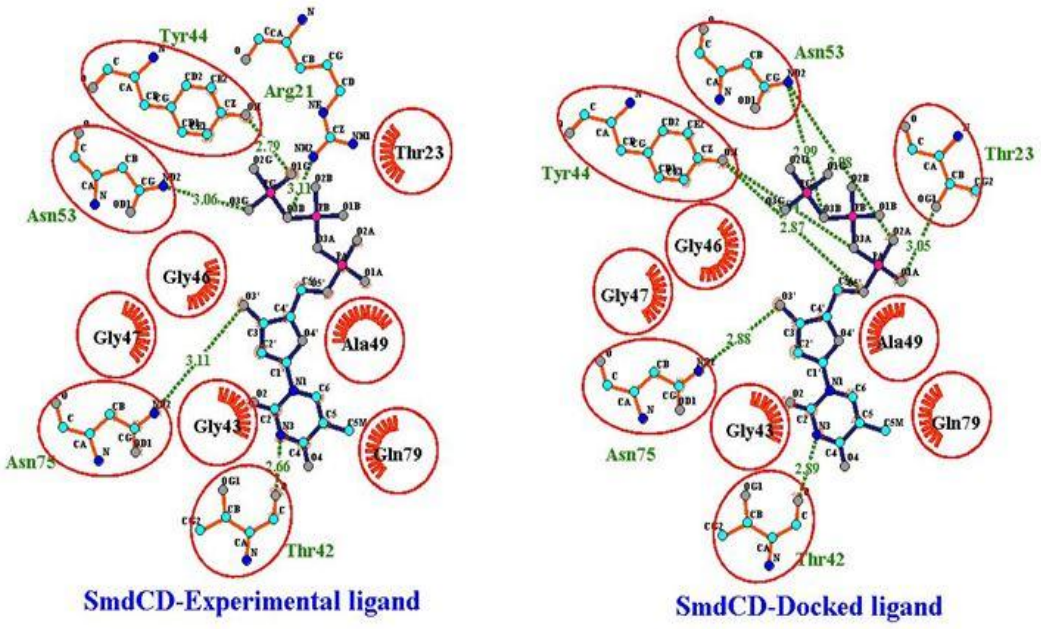

Fig. 2. Binding pocket of SmdCD showing experimental and docked reference ligand (Blue color) binds to modulator site residues. Modulator site residues are in orange circles representation. Hydrogen bonds and that formed between protein and ligand are shown by green dotted lines and other residues are hydrophobic bond-forming residues

2.6 Toxicity Prediction

Screened phytotochemicals obtained from molecular docking were preceded for toxicity prediction. The toxicity analysis of phytotochemicals which have a good binding affinity with protein was carried by using OSIRIS property explorer program [22]. The main aspect of 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.

2.7 Visualization

Now, we were able to see our phytochemicals have been docked in the substrate active site. The result can be visualized and analysed different bioinformatics tool LigPlot + v.2.2.5 softwere. The 2 D depiction of hydrogen-bond interactions of the complex receptor-ligand structure was done by LigPlot + v.2.2.5 software [23] 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.

3. RESULTS

3.1 Drug-likeness Prediction

Currently, due to continuous 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 [24]. The rationale behind these *In silico* approaches are due to relatively lower cost time factor involved compared to standard ADMET profiling [25,26]. In the present study we used SwissADME online software tool which is available free for the users to evaluate the ADME properties.

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

3.2 Molecular Docking

All the 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 the 3 phytochemicals have good binding affinity to the receptors as campared to reference molecule. Before performing the virtual screening, validation of the protocol was done by re-docking the reference compound (TTP) into the molulator site of SmdCD. The result showed that the docked TTP was completely superimposed with co-crystallized TTP in PDB ID (5C2O). The results showed that all selected inhibitors were in the pocket of the target SmdCD, exhibiting a possible interaction and ranked based binding energies with SmdCD on specific binding pocket (Table 1). The binding energies of the screened phytochemicals were found to be in the range −7.9 kcal/mol 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 the screened phytochemicals was found in the following order; Cyclocurcumin(−7.9kcal/mol) = Elatin(−7.9kcal/mol) > Androsta-1,4,6-triene-3,17-dione(−7.6kcal/mol) = SmdCD reference ligand(−7.5kcal/mol). The results obtained were given in Table 1. Docking results are ranked based on binding energies. Now, after docking studies all these 3 successful natural screened phytochemicals further proceeded for toxicity prediction.

3.3 Toxicity Prediction

The above three phytochemicals were also further proceeded to predict their tumorigenicity, mutagenicity, irritation, and reproductive toxicity by the OSIRIS tool. The predicted toxicity of three phytochemicals is shown in Table 2. According to Table 2, the 1 phytochemical i.e. Elatin showed a high risk of toxicity, while, 2 phytochemicals i.e. Cyclocurcumin, Androsta-1,4,6-triene-3,17-dione, were non-toxic (non-mutagenic, non-tumorigenic,non-irritant, and no reproductive effect). The drug-score show ranges

between 0 to 1, where as the value 1 indicates the possibility of a compound to be drug molecule, whereas, the score value 0 indicates that compounds having no possibilities of drug candidates. Therefore, these 2 phytochemicals (Cyclocurcumin, Androsta-1, 4,6-triene-3,17-dione,) can be further subjected to study their binding interaction with SmdCD.

3.4 Visualization

LigPlot+ v.1.4.5 was used to visualize the protein-ligand interactions. The docked poses of these two compounds with SmdCD is shown in Fig. 3. SmdCD -reference the docked TTP showed interaction with the amino acid residues by hydrogen and hydrophobic bonds as found in the experimental structure shown in Fig. 2. It forms seven hydrogen-bonds with five residues Thr42, Tyr44, Asn53, Thr23 and Asn75 of SmdCD. It also formed the five hydrophobic bonds with residues Ala49, Gly47, Gly46, Gly43 and Gln79 of SmdCD as shown in Fig. 3. According to Fig. 2(A), SmdCD - Cyclocurcumin formed one hydrogen bonds with Thr42 which have the bond distance 3.08 Å and It also formed nine 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 one hydrogen bonds having the bond distance 3.14 Å and seven 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 Table 3.

Table 1. 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

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

S. No.	Compound Name	TUM	MUT	IRR	REP	Drug-Score
1	Reference (TTP)	Non Toxic	Non Toxic	Non Toxic	Non Toxic	0.39
2	Cyclocurcumin	Non Toxic	Non Toxic	Non Toxic	Non Toxic	0.73
3	Androsta-1,4,6-triene-3,17-dione	Non-Toxic	Non-Toxic	Non-Toxic	Non-Toxic	0.75
4	Elatin	Toxic	Non-Toxic	Non-Toxic	Non-Toxic	0.46

Table 3. 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	

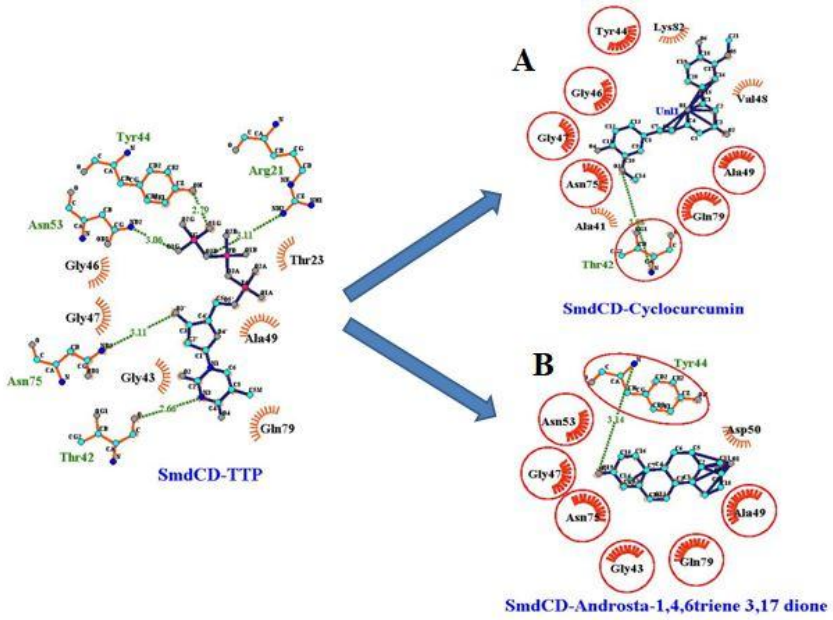

Fig. 3. 2D interaction of protein-ligand complexes with Hydrogen and Hydrophobic-bonds between hit phytochemicals (reference ligand, Cyclocurcumin, and Androsta-1,4,6-triene-3,17-dione) and dotted green lines denote hydrogen bonds, red half arcs indicate hydrophobic interactions

4. DISCUSSION AND CONCLUSION

The search for new drug candidates against tooth disease has led to the discovery of molecular targets, the development of 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 done on the antibacterial effects of natural medicinal plants. Several reports suggest that phytochemicals possesses remarkable inhibitory activities against bacteria. Present global drug development programs may not be able to afford new effective antibiotics for the next decade" [27]. In our study, we used different compounds from selected medicinal plant against allosteric substrate of SmdCD. These are all of the plants used in Ayurveda and the ancient medicinal system with antibacterial, anti-inflammatory, antiviral, antioxidant, anticancer, and antidiabetic activities. "*Glycyrrhiza glabra* is one of the extensively used herbs from the ancient medicinal history of Ayurveda. The antimicrobial activity of *G. glabra* has been both researched and exploited medicinally for many years. Natural Plant products (NPPs) of *G. glabra* is considered as antitussive, mucolytic, expectorant, antimicrobial, immunostimulant as well as a flavoring agent" [28]. "Saponins have also been reported to possess antimicrobial activity due to their detergent like nature they can cause leakage in the membrane by interacting with proteins and certain enzymes from the bacterial cell" [29]. "Rationale based selection of *G. glabra* against P. aeruginosa by employing bioprospection, *In silico* and *In vitro* study is reported previously" [30,31]. Curcumin is a active component *C. longa* is having many properties such as antioxidant, anti-inflammatory, anti-viral, antibacterial, antifungal and anticancer activities and also works against various malignant diseases such as diabetes [32], arthritis, Alzheimer's [33] and other chronic diseases has 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" [34]. Cyclocurcumin can be used as a herbal drug or proved to be a good lead compound for oral and cervical cancers [35,36]. The information is quite significant in drug development for oral and cervical cancers.

Here in this study, we employed *in silico* techniques to investigate the natural compound Cyclocurcumin and Androsta-1,4,6-triene-3,17-dione as possible to control biofilm formation.

Therefore, to find out potential phytochemicals, we prepared a library of phytochemicals of 10 selected medicinal plant and buildup a library of 871 phytochemical. Afterthat, It is used to filtered a library of 871 phytochemical through Swiss ADME web tool. Further filtered 211 phytochemical were subjected to molecular docking against SmdCD. Based on Virtual screening of Elatin phytochemical of selected pants we found the top 3 phytochemicals against SmdCD, namely Cyclocurcumin, Androsta-1,4,6-triene-3,17-dione, Elatin, and all these compounds showed good binding energy with SmdCD as compared to reference compounds. After virtual screening we were checked toxicity prediction of screened phytochemicals through OSIRIS softwere. Now, we get the result out of 3 phytochemicals, 2 phytochemical were non toxic in nature. Through these results, we can suggest Cyclocurcumin, and Androsta-1,4,6-triene-3,17-dione phytochemicals can be used against SmdCD target. Our drug repurposing study, both phytochemicals were found to inhibit the SmdCD and these phytochemicals may be used against the *S. mutans* microbial infection. Finally, we suggest based on a future perspective on the *In vitro* and *In vivo* research, that these phytochemicals namly Cyclocurcumin, and Androsta-1,4,6-triene-3,17-dione may become development of organic mouthwash and potent anti biofilm drugs to oral care.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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