



An *in silico* Analysis of Protein Targeted by Glycyrrhizin in Common Dental Pathogens

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Authors' contributions

This work was carried out in collaboration among all authors. Author JVP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SG managed the analyses of the study. Author SB managed the literature searches and performed certain computational analysis. All authors read and approved the final manuscript.

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ABSTRACT

Glycyrrhizin is a phytochemical which is derived from *Glycyrrhiza glabra*. It is used in treating the upper respiratory tract disease like cough, bronchitis, laryngitis, sore throat, etc. It has various medicinal uses in rheumatism, peptic ulcers, asthma, allergies, and inflammation. Glycyrrhizin has been reported to possess antibacterial, antiviral, antioxidant, anti-inflammatory properties. In view of the above facts, the present *in silico* study was designed to demonstrate the molecular mechanism underlying the antimicrobial activity of glycyrrhizin against common dental pathogens such as *Streptococcus mutans*, *Porphyromonas gingivalis*, *Treponema denticola*, *Enterococcus faecalis* and *Tannerella forsythia*. The STITCH tool was used to identify the drug-protein interaction. The functional class of the protein was deduced using VICMPred, followed by the identification of epitopes on the virulence factors using BepiPred. Further, the subcellular location of the virulence factors were also studied using PSORTb software. The computational analysis

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performed identified several virulence factors viz., short chain dehydrogenase/reductase family oxidoreductase of *Treponema denticola* and D-mannonate oxidoreductase of *Tannerella forsythia* which were found to interact with glycyrrhizin. Interestingly, phosphopyruvate hydratase was found to be the protein present in all the five genera was shown to interact with glycyrrhizin. Thus the present study reveals the target proteins on the dental pathogens which were shown to interact with glycyrrhizin. Furthermore, experimental validation of the results are warranted to provide substantial details on the anti-microbial activity of glycyrrhizin against common dental pathogens.

Keywords: Glycyrrhizin; *in silico* analysis; STITCH; VICMPred; virulence.

1. INTRODUCTION

The emergence of drug resistant microbes is regarded as a threat to mankind. Multidrug resistant in different microbial species underscores the need for alternate drugs for its eradication. Several bioactive compounds from plants, marine and animal sources have been identified to possess antimicrobial property. The present *in silico* study is one such attempt to identify the protein targets of a phytocompound known as glycyrrhizin in common dental pathogens. Generally, antimicrobial susceptibility assays have been performed to assess the susceptibility or resistance of a specific drug to a pathogen being tested. Although it provides details on the variations in drug response by the microorganism, the test does not identify the potential molecular targets of the organism being tested. In view of the above fact, the present study has been designed to assess the antimicrobial effect of glycyrrhizin against common dental pathogens. Glycyrrhizin is derived from the root of the plant *Glycyrrhiza glabra*. It is the main biological active constituent of licorice and *Glycyrrhiza glabra*. It is a saponin used as an emulsifier and gel forming agent in foodstuffs and cosmetics. The roots of *Glycyrrhiza glabra* contain high concentration of triterpene saponin glycyrrhizin (about 2.5%-9%) [1]. Glycyrrhizin are grown in subtropical regions of Europe, Middle East and Western Asia. The compound is known for its use in folk medicine to treat upper respiratory ailments including cough, hoarseness, sore throat, and bronchitis. It is also used to treat gastric ulcer, to suppress cough, and used in treatment of Addison disease [2]. Glycyrrhizin not only shows antibacterial effect, but also possesses antiviral property as it prevents the entry of viruses into cells, by suppressing the fluidity of plasma membranes. A study has reported that glycyrrhizin inhibited non-syncytium inducing variant of HIV replication by triggering the production of beta-chemokines [3]. Glycyrrhizin is herbal which cures the peptic ulcers as it acts as an anti ulcer agent. It also

thus helps in healing the peptic ulcer which is caused by the hydrochloric acid [4]. Oral administration of glycyrrhizin treats liver diseases which leads to conversion of glycyrrhetic acid. It is known to enhance the cortisol in eyes [5]. Glycyrrhizin inhibits the colonic metabolism of methylprednisolone [6]. A study reported that glycyrrhizin prevents melanogenesis by inhibiting the B16 melanoma cells, which induces cell cycle arrest in the G1 phase of cell cycle. The compound also has potent free radical scavenging property, hence has been used as an antioxidant [7]. Despite the fact that there was no effect on organic metabolism it was noticed that the compound induces the retentions of sodium, potassium, chloride and increased potassium in urine [8]. Glycyrrhizin alleviate the symptom of the acute sinusitis within the three days. Therefore it reduces the inflammation within the air cell sinus and relieves the pain doubling up on its role as an anti inflammatory agent [9]. To a lesser extent high doses of glycyrrhizin have been shown to induce hypertension and hypovolemia [10].

2. MATERIALS AND METHODS

2.1 Strains Used in the Study

Phytocompound glycyrrhizin was tested against dental pathogens namely *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia*, *Streptococcus mutans*, and *Enterococcus faecalis*. STITCH tool [11] was used to reveal the interaction between the compound and protein targets in the pathogen.

2.2 Protein Network Interaction Analysis

STITCH is an exhaustive pipeline which can be used for predicting the interaction between chemicals and proteins. The interactions are of two types (a) direct or physical (b) indirect or functional association which arise from data accumulated in the primary databases. The repertoire of protein from *Porphyromonas*

gingivalis, *treponema denticola*, *Tannerella forsythia*, *streptococcus mutans* and *Enterococcus faecalis* interacting with glycyrrhizin were used for predicting virulence. The FASTA format sequence was retrieved from the National Centre of Biotechnology information domain and used for predicting the functional class of protein and their virulence properties (<https://www.ncbi.nlm.nih.gov/protein/?term=>).

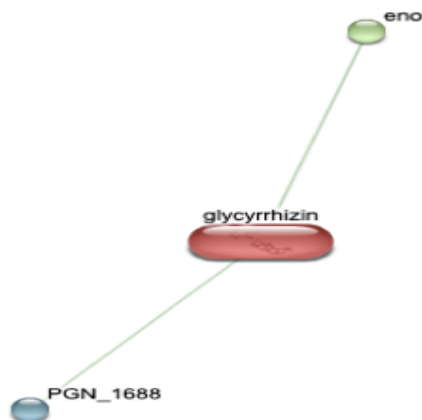
2.3 Prediction of Functional Class of Interacting Protein

VICMpred server classifies the protein identified into four major classes namely virulence factor, information and storage processing, cellular process and metabolism. Anchorage dependent

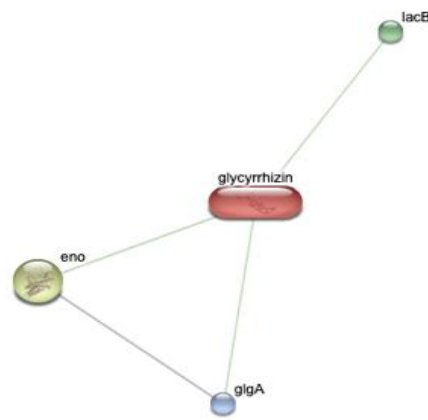
protein effluent pumps, transporters, toxins and hemolytic molecules are identified based on the support vector machine (SVM) algorithm that classifies the protein based on their amino acid composition pattern [12].

2.4 Epitope Prediction

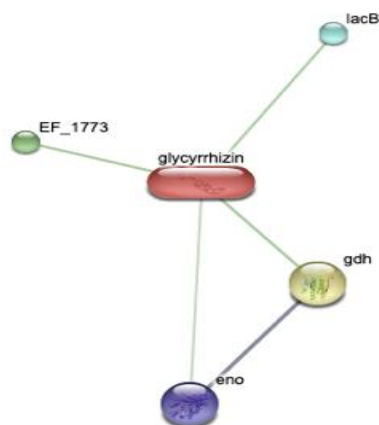
Epitope is an antigen determining site on the virulent proteins capable of eliciting an immune response in the host. The identification of those B cell epitopes on virulence protein adds merit to the compound. The peptide molecule which score above a threshold >0.5 are predicted to be part of the epitope and are coloured yellow in the graph (Figs. 2a and 2b) [13,14].



Porphyromonas gingivalis



Enterococcus faecalis



Streptococcus mutans



Tannerella forsythia

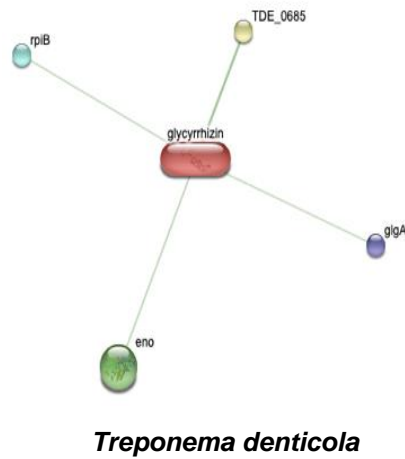
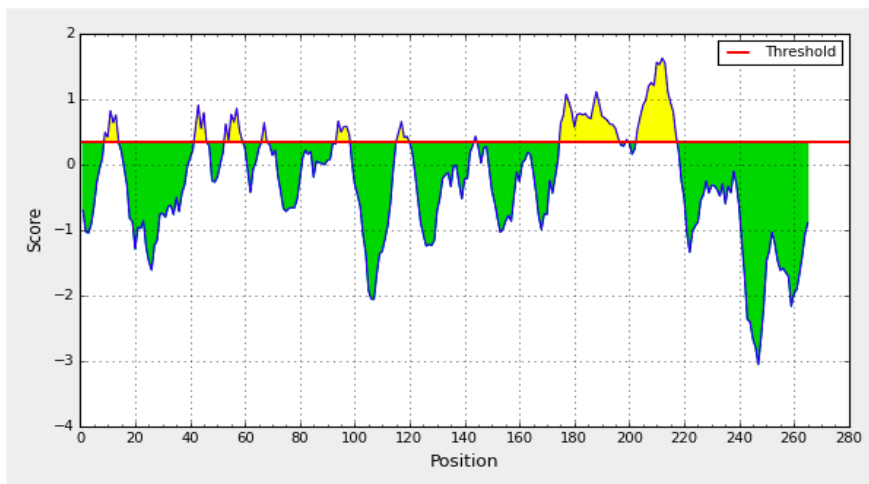


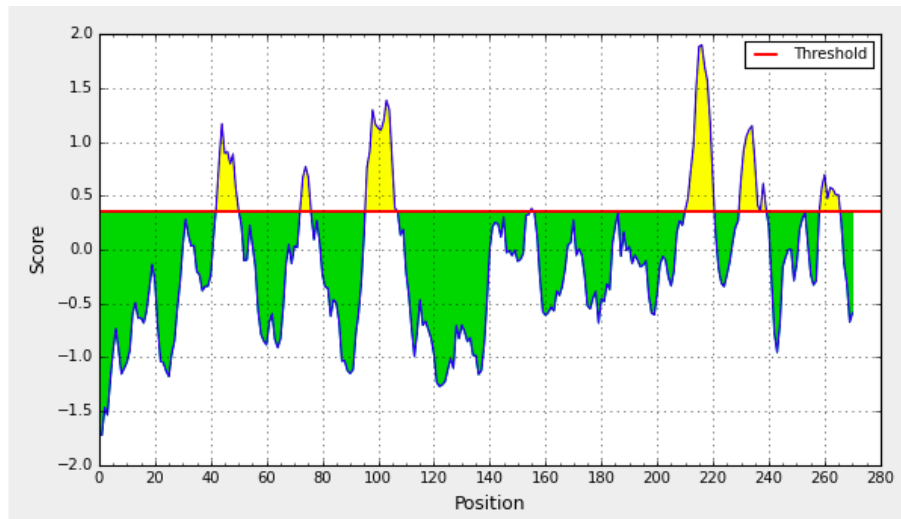
Fig. 1. Interaction of glycyrrhizin with the protein repertoire of common dental pathogens



Predicted peptides:

No. ↕	Start ↕	End ↕	Peptide ↕	Length ↕
1	9	13	GGSSG	5
2	42	45	QEKS	4
3	53	59	TDEENLT	7
4	66	67	WE	2
5	94	98	EAKKQ	5
6	116	119	YMRP	4
7	144	144	S	1
8	175	196	DVATPFTAARNKSNLGDDVYNG	22
9	199	199	S	1
10	203	217	SKMEKDEQKGMNPEK	15

Fig. 2a. Predicted epitopes and the corresponding peptide sequence in short chain dehydrogenase/reductase family oxidoreductase of *Treponema denticola*



Predicted peptides:

No. ◆	Start ◆	End ◆	Peptide ◆	Length ◆
1	42	50	NAEKGNRLK	9
2	72	75	QLEE	4
3	96	107	GNMPGATIAPDQ	12
4	155	155	S	1
5	210	220	MTNADGTPTER	11
6	230	239	YGRLGKPEEL	10
7	259	265	IPVDGGF	7

Fig. 2b. Predicted epitopes and the corresponding peptide sequence in D-Mannosate oxidoreductase of *Tannerella forsythia*

2.5 Prediction of Subcellular Localisation of Protein

Cell surface proteins are readily targeted, while the cytoplasmic or nuclear protein needs a proper drug delivery system to target the protein of interest. Hence, PSORTb was used for identification of subcellular locations of virulence protein [15].

3. RESULTS AND DISCUSSION

Glycyrrhizin has maximum inhibitory effect on bacteria. Some studies have shown that glycyrrhizin has antiviral properties by inhibiting

the viral gene expression and replication. In the present study glycyrrhizin is targeted towards the common dental pathogens like *Treponema denticola*, *Porphyromonas gingivalis*, *Streptococcus mutans*, *Enterococcus faecalis* and *Tannerella forsythia*. Glycyrrhizin was found to interact with galactose 6 phosphate isomerase subunit, phosphopyruvate hydratase and glycogen synthase of *Streptococcus mutans*. The protein targets of glycyrrhizin on other dental pathogens have been tabulated in Table 1. Interestingly, the phytochemical was found to interact with phosphopyruvate dehydratase in all the five genera which were chosen for the present study. Glycyrrhizin shows many activity

like chemopreventive, hepatoprotectant [16], oxidoreductase inhibitor, wound healing agent, lipid peroxidase inhibitor, lipid metabolism regulator, antiviral (influenza), antiulcerants, anaphylatoxin receptor antagonists, and hypolipidemic. These properties are estimated through the Pass prediction test. Pass Prediction test is the one which establishes the properties of any compound. Glycyrrhizin helps in treating liver cancer which is the second most cause of cancer death worldwide [17].

The VICMPred algorithm identified short chain dehydrogenase/reductase family oxidoreductase and D-Mannonate oxidoreductase as virulence

factors in *Treponema denticola* and *Tannerella forsythia* respectively. These organisms are included in the group of red complex pathogens which are known to cause periodontal infection which in turn can lead to systemic illness like cardiovascular disease. *G. glabra* extract treated the corneal tissue showed that it has recovered to normal with blood vessels nearly diminished and collagen. There was a sign of epithelial hypertrophy and it was observed in fewer blood vessels. Extract *G. glabra* showed the inhibition of growth of a new vessel on the corneal surface and it is also indicated in CNV treatment [18]. Many previous studies have shown that the glycyrrhizin has extensive antimicrobial and

Table 1. List of proteins of common dental pathogens which interact with glycyrrhizin. The VICMPred, predictions on the functional class of proteins are also given

Organism	Identifier	Proteins which interacts with glycyrrhizin	VICMPred Functional Class
<i>Streptococcus mutans</i>	lacB	galactose-6-phosphate isomerase subunit lacB	Cellular process
	eno	phosphopyruvate hydratase	Metabolism
	glgA	glycogen synthase	Metabolism
<i>Enterococcus faecalis</i>	EF_1773	3-ketoacyl-ACP reductase	Cellular process
	lacB	galactose-6-phosphate isomerase subunit lacB	Cellular process
	eno	phosphopyruvate hydratase	Metabolism
<i>Porphyromonas gingivalis</i>	gdh	glucose 1-dehydrogenase	Metabolism
	eno	phosphopyruvate hydratase	Metabolism
	PGN_1688	ribose 5-phosphate isomerase B	Metabolism
<i>Treponema denticola</i>	TDE_0685	Short chain dehydrogenase/reductase family oxidoreductase	Virulence factor
	rplb	ribose 5-phosphate isomerase B	Cellular process
	eno	phosphopyruvate hydratase	Metabolism
<i>Tannerella forsythia</i>	glg	glycogen synthase	Cellular process
	BFO_1071	short chain dehydrogenase/reductase family oxidoreductase	Metabolism
	BFO_3307	D-Mannonate oxidoreductase	Virulence factor
	eno	phosphopyruvate hydratase	Metabolism
	rplb	ribose-5-phosphate isomerase B	Metabolism

Table 2. The sub-cellular localization of virulent proteins identified in dental pathogens

Organism	Protein identifier and Virulence factor	Subcellular localisation of the protein with score
<i>Treponema denticola</i>	TDE_0685 Short chain dehydrogenase/reductase family oxidoreductase	Cytoplasmic 9.6
<i>Tannerella forsythia</i>	BFO_3307 D-Mannonate oxidoreductase	Cytoplasmic 9.97

antiviral properties. A study reported the antibacterial activity of glycyrrhizin against the methicillin resistant *Staphylococcus aureus* which causes nosocomial infection [19]. Antimicrobial activity of licorice root extract prepared with alcohol and aqueous solution was found to be effective against the *Streptococcus mutans*. It was also reported that alcoholic licorice root extract showed more inhibitory effect than chlorhexidine which is a dental mouthwash [20]. *P. gingivalis* is anaerobic coccobacilli bacteria which affects the oral epithelial cell, human gingival fibroblast and number of immune cells like macrophage and monocytes. It is a major opportunistic bacteria in destructive periodontitis [21]. Licorice extract containing the active component glycyrrhizin demonstrated the ability to inhibit the biofilm formation in *P. gingivalis* with attenuation of *P. gingivalis* virulence to cause periodontal disease [22]. It has shown that *T. forsythia* induces the pro inflammatory cytokines like IL-1 and 8 whereas *P. gingivalis* induces the gingival pain which in turn affects the host immune system by degradation of immunoglobulin and complement system. These red complex pathogens have been shown to cause nasopharyngeal cancer [23].

The glycyrrhizin has been used as a root canal medication. While sodium hypochlorite and chlorhexidine shows the limited eradication of *E. faecalis* in the root canal, glycyrrhizin destroys the complete presence of root canal pathogen [24]. Glycyrrhizin shows potential beneficial effects in orodental diseases. These effects are associated with anti adherence anti microbial and anti inflammatory properties of the compound [25]. A study has shown that the glycyrrhizin were used in treatment of neurological disorders such as schizophrenia, dementia and Alzheimer's disease [26]. The antiviral mechanism of glycyrrhizin includes inhibition of viral replication and immune regulation. It affects the cellular signaling pathway such as protein kinase C and casein kinase 2 and transcription factor such as activator protein 1. The anti inflammatory properties of glycyrrhizin used in treatment of hepatitis. It also inhibits the SARS virus replication [27]. Glycyrrhizin was also investigated for its antiviral action on varicella zoster virus. It was effective against the replication of varicella zoster virus. It inactivated more than 90% of virus particles within the 30 minutes at 37 degree Celsius [28]. A study has shown that glycyrrhizin is effective in decreasing the severity of head and neck cancer [29]. Unlike

other mouthwashes, glycyrrhizin mouthwash was not associated with the discolouration of tooth or unpleasant taste and was effective in reducing plaque accumulation and gingival inflammation [30]. Numerous studies have been designed by researchers using *in silico* methods to identify potential protein targets on dental pathogens [31,32]. The emergence and resurgence of microbial pathogens into pan-drug resistant forms have created a threat in the community [33,34]. Hence, there is an urgent need for identifying novel drugs to combat these species. Computational analysis aids in the identification of drug molecules by providing preliminary data on the mode of action of the drugs against target organisms.

4. CONCLUSION

In conclusion, the present study has unraveled the potential targets of glycyrrhizin on common dental pathogens, which adds to the evidence gathered to substantiate the role of the phytocompound to fight microbial infections. An exhaustive research designed using experimental and clinical set up can be used to further validate the preliminary results that is reported in the present study.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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