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Genistein binding protein targets in dental pathogens

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Abstract:
Oral pathogens have created a menace in recent years due to biofilm formation and antimicrobial drug resistance. The current treatment strategy works well with antibiotics. However, constant use of antibiotics creates a selective pressure, which increases adaptability of the pathogens. Therefore, it is of interest to analyze the potential targets of genistein in dental pathogens using computer aided prediction tools.

Keywords: Genistein, phytochemicals, anti-microbial, dental pathogens, virulence proteins.

Background:
Antimicrobial agents have long been used to target pathogenic microorganisms. In many instances the antibiotics administered fail to act upon the pathogens due to increasing antimicrobial resistance. A potent antibiotic against drug resistant microbes is critical. Hence, an alternative measure has to be adopted to combat such menace in the clinical settings. Several dental pathogens have also been reported to harbor drug resistant genes, which tend to hamper the treatment procedure **[1-2]**. Alternative medicine using herbs and bioactive compounds from plants is of significance. Isoflavones are flavonoids which are found to exhibit antioxidant, antimicrobial, anti-inflammatory and anticancer properties. Several reports have confirmed the effect of isoflavones in preventing chronic inflammatory diseases **[3]**. One of the important isoflavone present in soy is genistein and it is a phytoestrogen. The biological activities of genistein include inhibition of inflammation, modulation of steroidal hormone receptors, promotion of cell death by apoptosis and regulation of metabolic pathways **[4]**. Since these pathways are directly or indirectly related to inflammatory, metabolic diseases and malignant transformation, genistein is an excellent alternative for synthetic drugs used to treat such disorders. Genistein being a protein kinase inhibitor exhibits antibacterial activity by preventing the invasion of pathogenic bacteria in mammalian epithelial cells **[5]**. Oh *et al.* showed the antimicrobial activity of genistein against a model organism for bacterial septicaemia *Vibrio vulnificus.* They found that genistein inhibited the adhesion of *V. vulnificus* to HeLa cells and prevented cell death **[6]**. Therefore, it is of interest to analyze the potential targets of genistein in dental pathogens using compter aided prediction tools.

Materials and Methods:
Strains used in the study:
The following strains available in the STITCH database were used for the study. *Streptococcus mutans UA159, Enterococcus faecalis V583, Porphyromonas gingivalis ATCC 33277, Treponema denticola ATCC 35405 and Tannerella forsythia ATCC 43037.7*

Analysing protein interaction network:

STITCH is an exhaustive pipeline used for predicting the interactions between chemicals and proteins. The interactions are of two types (a) direct or physical and (b) indirect or functional associations which arise from data accumulated in the primary databases. The repertoire of proteins interacting with *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia* were used for predicting virulence **[7]**. The FASTA format of protein sequences was retrieved from the National Centre for Biotechnology Information (NCBI) **[8]**.

Prediction of functional class of interacting proteins:

VICMpred server is used for the classification of pathogenic microbial proteins into four major classes namely, virulence factors, information and storage processing, cellular process and metabolism. The principal virulence factors such as adhesins, toxins and haemolytic molecules are identified using the support vector machine (SVM) algorithm, which classifies proteins based on their amino acid composition and sequence pattern **[9]**.

Prediction of virulence properties of interacting protein:

The identification of virulent bacterial protein targeted by a drug or phytocompound helps in substantiating the antimicrobial activity of the compound. VirulentPred is a yet another SVM based method, used for automated prediction of virulent proteins based on the sequences (bioinfo.icgeb.res.in/virulent). The scores with positive predicted values are more often categorised into virulent protein and those with negative predicted values are categorised as avirulent proteins **[10]**.

Prediction of B-cell epitopes in the virulence proteins:

Epitopes are small regions on the antigens recognized by antibodies. Identification of B-cell epitopes on the virulence proteins identified adds advantage to the phyto compound selected. BepiPred 2.0 server was used to identify the peptide epitopes on the virulent proteins. The peptide molecules, which scored above a threshold greater than 0.5 are predicted to be part of the epitope and are, colored yellow in Figure 2 **[11-12].**

Prediction of sub-cellular localization of proteins:

The identification of the subcellular localization of virulence proteins is of prime importance as the efficiency of the compound lies in target identification. Cell surface proteins are readily targeted, whilst, the cytoplasmic or nuclear proteins need proper drug delivery systems to target the protein of interest. Hence, PSORTb V.3.0 was used for identification of sub-cellular location of virulence proteins **[12].**

Table 1: List of proteins of common dental pathogens, which interacts with genistein

Organism	Identifier	Proteins which interacts with geinstin	VICMPred Functional Class	Virulent Pred	VirulentPred Score
Enterococcus faecalis	EF_1311	Hypothetical protein EF_1311	Metabolism	Avirulent	-1.058
	EF_3120	Serine/threonine protein kinase	Virulent protein	Avirulent	-0.28
	EF_0841	Hypothetical protein EF_0841	Information and storage	Avirulent	-1.033
	pyrA	Carbamoyl phosphate synthase small subunit	Cellular process	Avirulent	-1.056
	pyrB	Aspartate transcarbamoylase	Cellular process	Avirulent	-1.037
	gyrA	DNA gyrase subunit A	Cellular process	Avirulent	-1.009
	gyrB	DNA gyrase subunit B	Cellular process	Avirulent	-1.041
	parC	DNA topoisomerase IV subunit A	Metabolism	Avirulent	-1.021
	parE	DNA topoisomerase IV subunit B	Metabolism	Avirulent	-1.039
Porphyromonas gingivalis	PGN_1449	Inosine-5'-monophosphate dehydrogenase	Metabolism	Virulent	1.1379
	PGN_1443	Carbamoyl-phosphate synthase large subunit	Virulence factor	Avirulent	-1.021
	PGN_0387	Putative O-methyltransferase	Metabolism	Avirulent	-0.924
	PGN_0472	DNA topoisomerase IV A subunit	Metabolism	Avirulent	-0.988
	PGN_1594	DNA topoisomerase IV B subunit	Virulence factor	Avirulent	-0.929
	gyrA	DNA gyrase subunit A	Metabolism	Avirulent	-0.999
	gyrB	DNA gyrase subunit B	Cellular process	Avirulent	-1.034
	carA	Carbamoyl-phosphate synthase small chain	Cellular process	Avirulent	-1.088
	pyrB	Aspartate carbamoyltransferase.	Cellular process	Virulent	0.749
	hisS	Histidyl-tRNA synthetase	Metabolism	Avirulent	-1.067
Streptococcus mutans	glmS	Glutamine--fructose-6-phosphate aminotransferase	Metabolism	Avirulent	-1.044
	carA	Carbamoyl-phosphate synthase small chain	Information and storage	Avirulent	-1.028
	carB	Carbamoyl-phosphate synthase large chain	Virulence factor	Avirulent	-1.008
	pknB	Serine/threonine protein kinase	Metabolism	Virulent	0.912
	pyrB	Aspartate carbamoyltransferase	Cellular process	Avirulent	-1.009
	hisS	Histidine--tRNA ligase	Cellular process	Avirulent	-0.88
	parC	DNA topoisomerase IV subunit A	Metabolism	Avirulent	-1.015
	parE	DNA topoisomerase IV subunit B	Metabolism	Avirulent	-1.012
	gyrA	DNA gyrase subunit A	Cellular process	Avirulent	-1.01
	gyrB	DNA gyrase subunit B	Metabolism	Avirulent	-1.001
Treponema denticola	TDE_2693	Ankyrin repeat protein	Cellular process	Avirulent	-0.4
	TDE_2450	Ankyrin repeat protein	Metabolism	Avirulent	-1.057
	TDE_2245	Topoisomerase IV, B subunit, putative	Virulence factor	Avirulent	-1.006
	TDE_2118	Topoisomerase IV, A subunit, putative	Metabolism	Avirulent	-1.029
	TDE_0492	Sensor histidine kinase/response regulator	Metabolism	Virulent	1.134
	pyrB	Asparate carbamoyltransferase	Cellular process	Avirulent	-1.051
	guaB	Inosine 5-monophosphate dehydrogenase	Cellular process	Avirulent	-0.789
	hisS	Histidyl-tRNA synthetase	Metabolism	Avirulent	-1.015
	gyrA	DNA gyrase subunit A	Virulence factor	Avirulent	-1.023
	gyrB	DNA gyrase subunit B	Virulence factor	Avirulent	-1.021
Tannerella forsythia	BFO_2044	Inosine-5-monophosphate dehydrogenase	Metabolism	Avirulent	-1.053
	BFO_2981	Kinase domain protein	Metabolism	Avirulent	-0.907
	BFO_2007	ATPase/histidine kinase/DNA gyrase B/HSP90 domain protein	Virulence factor	Avirulent	-0.172
	BFO_0740	DNA topoisomerase IV subunit A	Metabolism	Avirulent	-1.004
	BFO_1082	Putative DNA gyrase, B subunit	Metabolism	Avirulent	-0.997
	carB	Carbamoyl-phosphate synthase large chain	Virulence factor	Avirulent	-1.008
	pyrB	Aspartate carbamoyltransferase catalytic chain	Cellular process	Virulent	0.904
	gyrA	DNA gyrase subunit A	Cellular process	Avirulent	-1.008
	gyrB	DNA gyrase subunit B	Metabolism	Avirulent	-0.985

Table 2: Sub-cellular localization of virulence encoded proteins

Organism	Virulent protein	Subcellular localization
Porphyromonas gingivalis	Inosine-5'-monophosphate dehydrogenase	Cytoplasmic
	Aspartate carbamoyltransferase	
Streptococcus mutans	Serine/threonine protein kinase	Cytoplasmic membrane
Treponema denticola	Sensor histidine kinase/response regulator	Cytoplasmic membrane
Tannerella forsythia	Aspartate carbamoyltransferase catalytic chain	Cytoplasmic

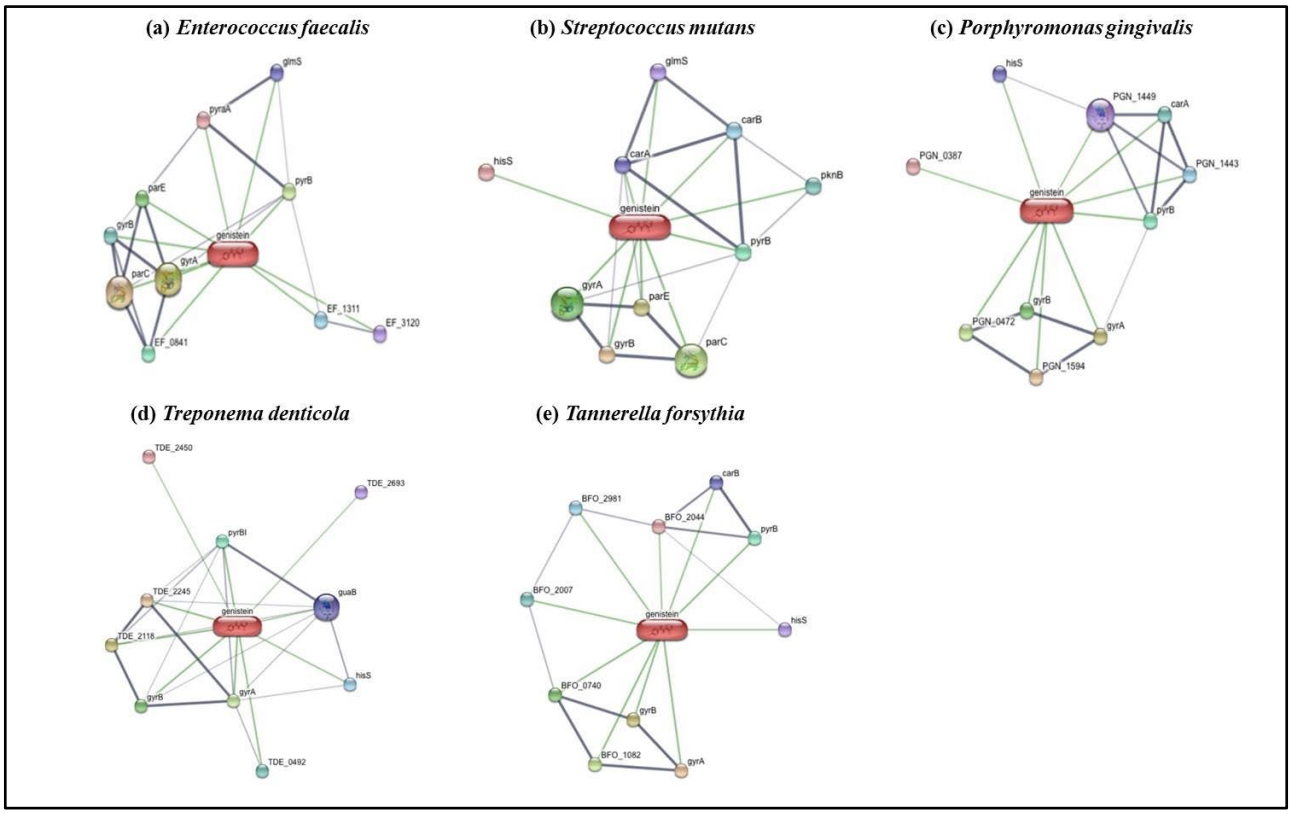

Figure 1: Interaction of genistein with the protein repertoire of common dental pathogens. (a) *Enterococcus faecalis,* (b) *Streptococcus mutans*, (c) *Porphyromonas gingivalis*, (d) *Treponema denticola*, (e) *Tannerella forsythia*

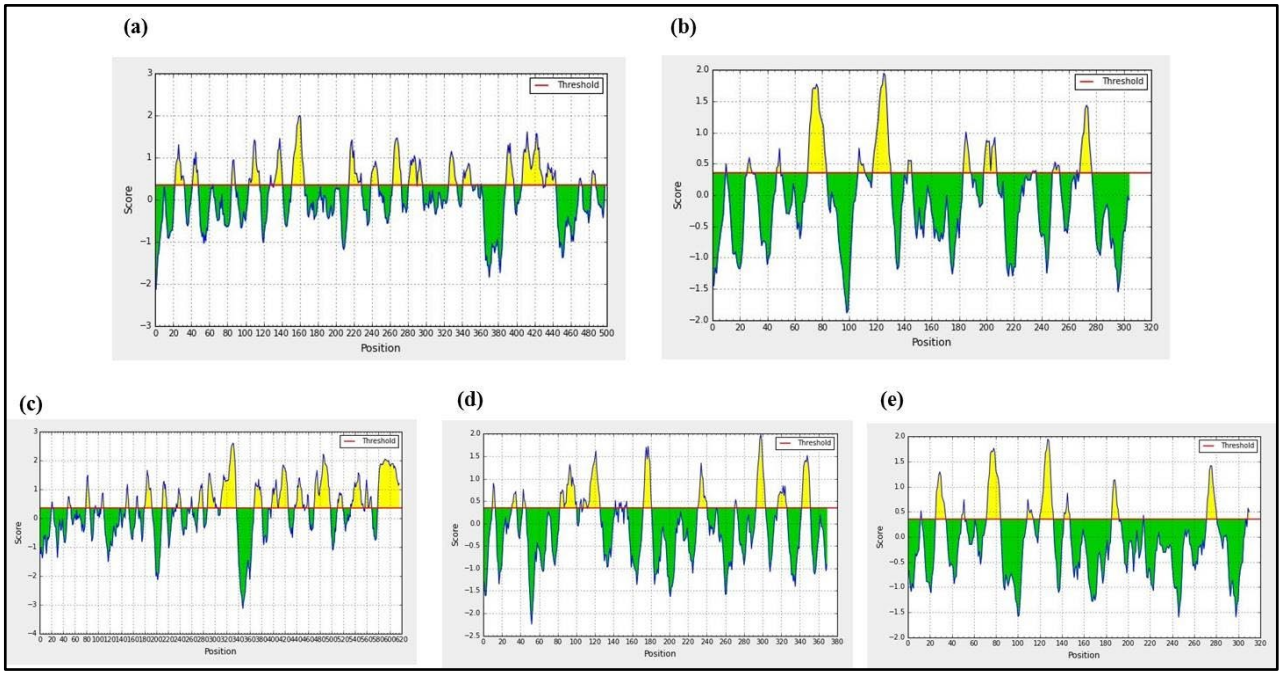

Figure 2: Predicted epitopes on the virulence protein (a) Inosine-5'-monophosphate dehydrogenase and (b) aspartate carbamoyltransferase of *Porphyromonas gingivalis,* (c) Serine/threonine protein kinase of *Streptococcus mutans,* (d) sensor histidine kinase/response regulator of *Treponema denticola*, (e) aspartate carbamoyltransferase catalytic chain of *Tannerella forsythia*

Results and Discussion:

The computer aided prediction tools were used to find the potential targets of genistein in *Enterococcus faecalis, Porphyromonas gingivalis, Streptococcus mutans, Treponema denticola* and *Tannerella forsythia* of which some of them were virulence. Software like STITCH, VICMPred, PSORTb and BepiPred were used to identify the drug protein interactions, virulent nature of the proteins, subcellular localization and putative epitopes in the proteins identified **(Figure 1).** Inosine-5'-monophosphate dehydrogenase and aspartate carbamoyltransferase of *Porphyromonas gingivalis*, Serine/threonine protein kinase of *Streptococcus mutans*, Sensor histidine kinase/response regulator of *Treponema denticola,* Aspartate carbamoyltransferase catalytic chain of *Tannerella forsythia* were identified as virulence factors **(Table 1).** These virulence factors were involved in metabolism and cellular processes. Enzymes involved in DNA replication processes such as gyrase and topoisomerase were targeted by genistein in all the five species of bacterial pathogens. The subcellular location of virulence proteins was in the cytoplasm, with an exception of serine/threonine protein kinase and sensor histidine kinase/response regulator, which were found in the cytoplasmic membrane (**Table 2**). The location of drug targets is very vital in drug designing, pharmacokinetics and dynamics of the compound used for analysis. Moreover, putative peptide epitopes were identified in the virulence proteins targeted by genistein **(Figure 2).** These epitopes are antigen-determining sites on the virulence proteins identified by the host immune system. Targeting an epitope on the virulence protein is known to improve the efficacy of the compound.

Bacteria associated with dental diseases are present as a polymicrobial community. *S. mutans, E.faecalis, Lactobacillus salivarius, L. plantarum, Actinomyces naeslundii* are a few bacteria, which colonize and form biofilm. Although there are mechanical and chemical methods available to get rid of these pathogens, complex structures like biofilms are difficult to manage due to their refractoriness to antibiotic treatments. The existing procedures at times exhibit adverse effects such as allergic reactions, tissue damage etc., also, they have not guaranteed complete removal of pathogenic bacteria **[13]**. Numerous compounds have been tested from bacteria, plants and other sources to identify the best compound with an efficacy to target and eradicate pathogens leaving the host tissues unharmed **[14],[15]**. Antibiotic dressing employing triple antibiotic paste is usually done to facilitate endodontic regeneration **[16]**. An acute or chronic exposure to such antibiotics will eventually lead to selective pressure that aids in the transformation of antibiotic susceptible strain into antibiotic resistant strains. Hence, such exposure can be avoided by use of alternative compounds, which do not impose selective pressure on pathogens.

Genistein and derivatives of ginestein have been identified in *Pterospartum tridentatum*, a plant used in folk medicine to treat diseases related to inflammatory processes. *P. tridentatum* extract was shown to exhibit the highest antibacterial activity in a dose dependent manner against methicillin-resistant (MRSA) and methicillin-sensitive *Staphylococcus aureus* MSSA. This antibacterial activity is due to high content of flavonols, flavones and isoflavones, which act synergistically to exert their role against this type of bacteria **[17,18]**. The antibacterial effect of genistein upon pathogens like *Escherichia coli, Shigella sonnei,* and *Staphylococcus aureus* as well as *Klebsiella pneumoniae* and the non-pathogenic organism, *Bacillus anthracis* (Sterne) is known. Subsequently, significant reductions in colony forming units were recorded for *S. aureus* and *B. anthracis* when cultured in the presence of 100-µM genistein. Modified derivatives of genistein were screened for their antibacterial and antifungal activities by MTT method against gram positive bacteria such as *Bacillus subtilis, Staphylococcus aureus,* gram negative bacteria such as *Escherichia coli, Pseudomonas fluorescens* and fungal species *Trichophyton rubrum, Candida albicans*. Promising results supports the design of the present study **[19]**. A similar study by Li *et al.* used deoxybenzoin derivatives from genistein against *B. subtilis, E. coli, P. fluorescence, S. aureus, A. niger, C. albicans and T. rubrum.* Most of the derivatives tested showed significant antibacterial effects **[20]**. However, information is not available on the molecular mechanisms related to the antimicrobial activity of genistein. A few studies have provided evidence on the inhibitory role of the compound. Verdrengh *et al*. showed the growth inhibition of *S. aureus* that is mediated by inhibition of topoisomerase IV **[21]**. Data shown here is consistent with the above report where genistein was found to act on a common target DNA topoisomerase IV A and B. Thus, we report the data on the potential targets of genistein in dental pathogens using computer aided prediction tools. The virulence-encoded proteins were differentiated using specific computational tools and the epitopes associated with those proteins.

Conclusion:

We document the potential targets of genistein in dental pathogens using computer aided prediction tools.

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Conflict Of Interest: The authors declare no conflict of interest.

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