Original Research Article

VIRTUAL SCREENING TO IDENTIFY THE PROTEIN NETWORK INTERACTION OF THEOPHYLLINE IN RED COMPLEX PATHOGENS

ABSTRACT

Background: Plants, herbs and plant derived compounds have been used since decades in folk and traditional medicine. These compounds were found to be non-toxic and compatible with the human cells. Screening of this exhaustive collection of compounds seems to be a herculean task. Hence, virtual screening methods have been developed to predict the potential targets of these compounds enabling researchers to acquire preliminary data on the compound intended to be tested in vitro.

Aim: The present study aims to identify protein targets of theophylline in red complex pathogens which are mainly associated with periodontitis.

Methods: The STITCH v5.0 pipeline was mainly used to classify drug-protein interactions, while VirulentPred and VICMPred were used to determine the proteins' virulence properties and functional classes. PSORTb v3.0 was used to determine the subcellular localization of viral proteins, and BepiPred v1.0 Linear Epitope Prediction was used to classify epitopes.

Results: Proteins of red complex pathogens implicated in cellular process, metabolism, and virulence were identified to interact with theophylline. The virulent proteins attacked by the drugs were present in the cytoplasm, which would improve the drugs' antimicrobial efficacy even more. Finally, epitope prediction showed a number of epitopes in virulent proteins that can be targeted.

Conclusions: Proteins involved in the cellular process, metabolism, and virulence of red complex pathogens targeted by theophylline were identified. To substantiate the true interactions

between the drugs and the protein arsenal of pathogens, further in vitro experiments on a broad range of pathogens are required.

KEYWORDS: Theophylline, antimicrobial, periodontitis, virulence, novel targets

INTRODUCTION

Antimicrobial drug resistance has emerged as a great menace in the hospital settings. Novel drugs are being screened for their activity against microbial pathogens. One common and the most cost effective method of drug screening is using silico tools. Several studies have been designed previously to assess the role of bioactive compounds against common dental pathogens (1–3) In view of the facts, existing bioactive compounds are being tested to identify the potential targets in common pathogens. Theophylline is a bioactive component in black tea *(Camellia sinensis).* It has been found in green coffee beans and trace amounts were detected in cacao cotyledon (https://www.ncbi.nlm.nih.gov/books/NBK507021/). Several existing antibiotics or non-steroidal anti-inflammatory drugs have been repurposed for use against dental pathogens (4). Singh and team reported that theophylline possesses antifungal activity against *Candida albicans.*Theophylline has no discernible pre-systemic elimination, readily distributes into fat-free tissues, and is substantially processed in the liver. Renal excretion of unaltered theophylline in newborns is around 50% of the dosage, compared to around 10% in children older than three months and adults.(5)

The mode of action was damage to the membrane exerted by the elevation in flippase activity which resulted in membrane fluidity.Theophylline relaxes the smooth muscle of the bronchial airways and pulmonary blood vessels, lowering airway reactivity to histamine, methacholine, adenosine, and allergens.(6) Theophylline inhibits type III and type IV phosphodiesterase (PDE), the enzyme responsible for breaking down cyclic AMP in smooth muscle cells, perhaps leading to bronchodilation.Theophylline also binds to the adenosine A2B receptor, preventing adenosine-induced bronchoconstriction.(7) Theophylline stimulates histone deacetylase in inflammatory situations to block transcription of inflammatory genes that require histone acetylation to initiate transcription. Another study conducted by Borkowski *et al.,* 2016 demonstrated the cytotoxic effects of theophylline based ionic liquids towards *Bacillus cereus* and *Escherichia coli.* These

results provide evidence on the use of theophylline as a potent antimicrobial drug. But the lacunae which is encountered is the information on the molecular targets.(8) The aim of this study is to identify protein network interaction of theophylline in red complex pathogens using virtual screening. Our team has extensive knowledge and research experience that has translate into high quality publications (9–12), other articles about cytotoxic effects like (13–16) studies are also done about antioxidant (17–20), other studies about antibacterial activity was also done (21–24), and also other studies about microbes were also done (25–27)

MATERIALS AND METHODS:

Study design :

The present study follows an observational study design which aims to screen for the interaction of theophylline in red complex pathogens. The interaction was analysed using STITCH v.5 pipeline (28). The functional class of proteins identified were assessed using VICMPred (29) and VirulentPred softwares (30). The microbial pathogens *Treponema denticola ATCC 35405, Tannerella forsythia ATCC 43037, Porphyromonas gingivalis ATCC 33277* are the strains of red complex pathogens that are included in the present study.

Prediction of protein-drug interactions

To predict the interactions between proteins and chemicals STITCH database (Version 5; 2016) is used. The repertoire of proteins which interacts with *T. forsythia*, *P. gingivalis* and *T. denticola* and were further used for predicting virulence (28).

Virulence prediction

For the identification of virulence factors the software used was VICMpred (29) and VirulentPred (30) pipelines. There are two groups of virulence factors that were screened using the VirulentPred tool based on amino acids that are virulent and avirulent. VICMpred groups proteins are classified into four major classes : proteins involved in metabolism, information storage, virulence and cellular processes. The overall accuracy of VirulentPred servers and VICMpred were 86% and 70.75% , respectively.

Prediction of subcellular localization of the virulent proteins

The novel drug targets plays an important role in an antimicrobial drug which targets the virulent protein. The subcellular localization of proteins aids in designing using the Computational prediction. The great interest is that cell surface proteins can be used in making vaccines. An algorithm which assigns a probable localization site to a protein from an amino acid sequence is pSORTb V3.0 (31).

Prediction of B-cell epitopes in the virulent proteins

For the prediction of B-cell epitopes from a protein sequence the server BepiPred-2.0 was used. It employs the Random Forest algorithm, which discriminates between epitopes and non-epitope amino acids determined by its crystal structures. To be part of an epitope the residues with scores above the threshold (>0.5) (32,33).

RESULTS AND DISCUSSION

Numerous proteins were identified in the red complex pathogens being targeted by theophylline. Proteins identified in *P. gingivalis* are mostly involved in cellular processes. The proteins belonging to three functional classes except for virulence factors were identified in *T.denticola*. The proteins of *T.forsythia* were mostly avirulent and of the functional class cellular process and metabolism. Only one protein was identified as a virulence factor viz., hypothetical protein [BFO_2902]. The subcellular location of type I phosphodiesterase-nucleotide pyrophosphatase, and cardiolipin synthase were found in the cytoplasmic membrane. The acetyl hydrolase identified in *T. denticola* was found to be the virulent protein. Among all the proteins identified, type I phosphodiesterase-nucleotide pyrophosphatase [PGN_0239], alkaline phosphatase [PGN_1457], cardiolipin synthase [PGN_0466] of *Porphyromonas gingivalis* and acetyl hydrolase [TDE_2263] of *Treponema denticola* were found to be the virulent proteins. Multiple epitopes were identified in the alkaline phosphatase enzyme of *P. gingivalis.*

Polymer mixing is a simple yet appealing way for obtaining polymer physical and mechanical characteristics that are blended. In this research, three different types of blend hydrogels were created by physically mixing two distinct natural polymers, and a model drug, theophylline (TPH), was adsorbed into these hydrogels for drug release investigations, comparatively this study is based on the effect of the red complex pathogens.A cost-effective method to fast-track

development of drugs is drug repurposing. Some of the drugs have been already tested against dental pathogens using in silico procedures (34). These drugs when used with other existing antibiotics act synergistically to enhance the therapeutic spectrum. Biofilm formers are especially important in dental settings because the combination of pathogens are found to do more harm to the host tissues than when they are seen individuallyThis complex demonstrates how interlocking structural motifs may be used to provide a highly selective ligand-binding site with high affinity and molecular selectivity,same with the red complex pathogens.. Targeting biofilm formers renders pathogens more vulnerable to antibiotic treatment and immune responses of the host. Theophylline, a xanthine analog, causes abnormal cells (35). There are not many reports which can relate to the antimicrobial effect of theophylline against dental pathogens. In this context, the present study has identified targets of theophylline which are potential virulent proteins of the red complex pathogens. Further experimental analysis is required to establish this fact.

CONCLUSION

The research discovered molecular targets of theophylline on red complex pathogens, which must be verified in order to validate the essential mechanism caused by the drugs in physiological conditions.To the best of our knowledge, this is the first research of its kind that seeks to understand the molecular targets of theophylline in red complex pathogens. Further, *in vitro* and *in vivo* tests can be used to determine the drug's dose, minimum inhibitory concentration and minimum bactericidal concentration.

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Table 1: Protein Network interaction of red complex pathogens interacting with theophylline

Organism	Identifier	Proteins which interacts with theophylline	VICMPred Functional Class	VirulentPred	Virulent Pred Score
Porphyromonas	PGN_0120	Hypothetical protein	Cellular process	Avirulent	-0.978

gingivalis	PGN_1181	Thiol:disulfide oxidoreductase	Cellular process	Avirulent	-1.064
	PGN_2017	Hypothetical protein	Cellular process	Avirulent	-1.074
	PGN_0488	Hypothetical protein	Metabolism	Avirulent	-0.820
	PGN_0239	**Type I phosphodiesterase-nucleotide pyrophosphatase**	**Cellular process**	**Virulent**	**0.1068**
	PGN_1457	**Alkaline phosphatase**	**Virulence factors**	**Virulent**	**0.2650**
	PGN_1081	Hypothetical protein	Cellular process	Avirulent	-0.597
	PGN_0373	Thioredoxin	Metabolism Molecule	Avirulent	-0.915
	PGN_0367	Exodeoxyribonuclease III	Cellular process	Avirulent	-0.992
	PGN_0466	**Cardiolipin synthetase**	**Cellular process**	**Virulent**	**1.1591**
Treponema denticola	**TDE_2263**	**Acetyl hydrolase**	**Cellular process**	**Virulent**	**1.1463**
	TDE_2372	Hypothetical protein	Metabolism Molecule	Avirulent	-1.045
	TDE_2369	Hypothetical protein	Metabolism Molecule	Avirulent	-0.820
	TDE_1734	Exodeoxyribonucleas	Information and	Avirulent	-0.993

		e III	storage		
	TDE_0957	Glycerophosphoryl diester phosphodiesterase	Metabolism Molecule	Avirulent	-0.904
	TDE_0799	Glycerophosphoryl diester phosphodiesterase	Cellular process	Avirulent	-0.997
	TDE_1370	Hypothetical protein	Cellular process	Avirulent	-1.079
	TDE0238	Thioredoxin, selenocysteine-containing	Cellular process	Avirulent	-0.981
	TDE1641	Ribose-5-phosphate isomerase A	Metabolism Molecule	Avirulent	-1.032
	TDE0744	Thioredoxin	Cellular process	Avirulent	-0.942
Tannerella forsythia	BFO_2118	YjeF C-terminal domain-containing protein	Metabolism	Avirulent	-0.997
	BFO_3041	Type I phosphodiesterase/nucleotide pyrophosphatase	Metabolism	Avirulent	-0.951
	BFO_2171	AhpC/TSA family antioxidant protein	Metabolism	Avirulent	-1.089

	BFO_2902	Hypothetical protein	Virulence factors	Avirulent	-1.068
	BFO_1753	Thioredoxin	Metabolism	Avirulent	-0.980
	BFO_1832	Putative cardiolipin synthetase	Cellular process	Avirulent	-1.034
	BFO_0550	Glycerophosphodiester phosphodiesterase family protein	Metabolism	Avirulent	-0.595
	BFO_2690	Glycerophosphodiester phosphodiesterase family protein	Cellular process	Avirulent	-1.014
	BFO_1118	Hypothetical protein	Cellular process	Avirulent	-1.022
	BFO_1729	Thioredoxin	Metabolism	Avirulent	-0.962

Table 2: Subcellular localization of virulence protein in *Porphyromonas gingivalis* and *Treponema denticola*

Virulent Protein	Subcellular location	Score
Putative type I phosphodiesterase-nucleotide pyrophosphatase	Cytoplasmic Membrane	9.82
Alkaline phosphatase	Unknown	-
Putative cardiolipin synthetase	Cytoplasmic Membrane	10.00
Acetyl hydrolase, putative	Cytoplasmic	8.96

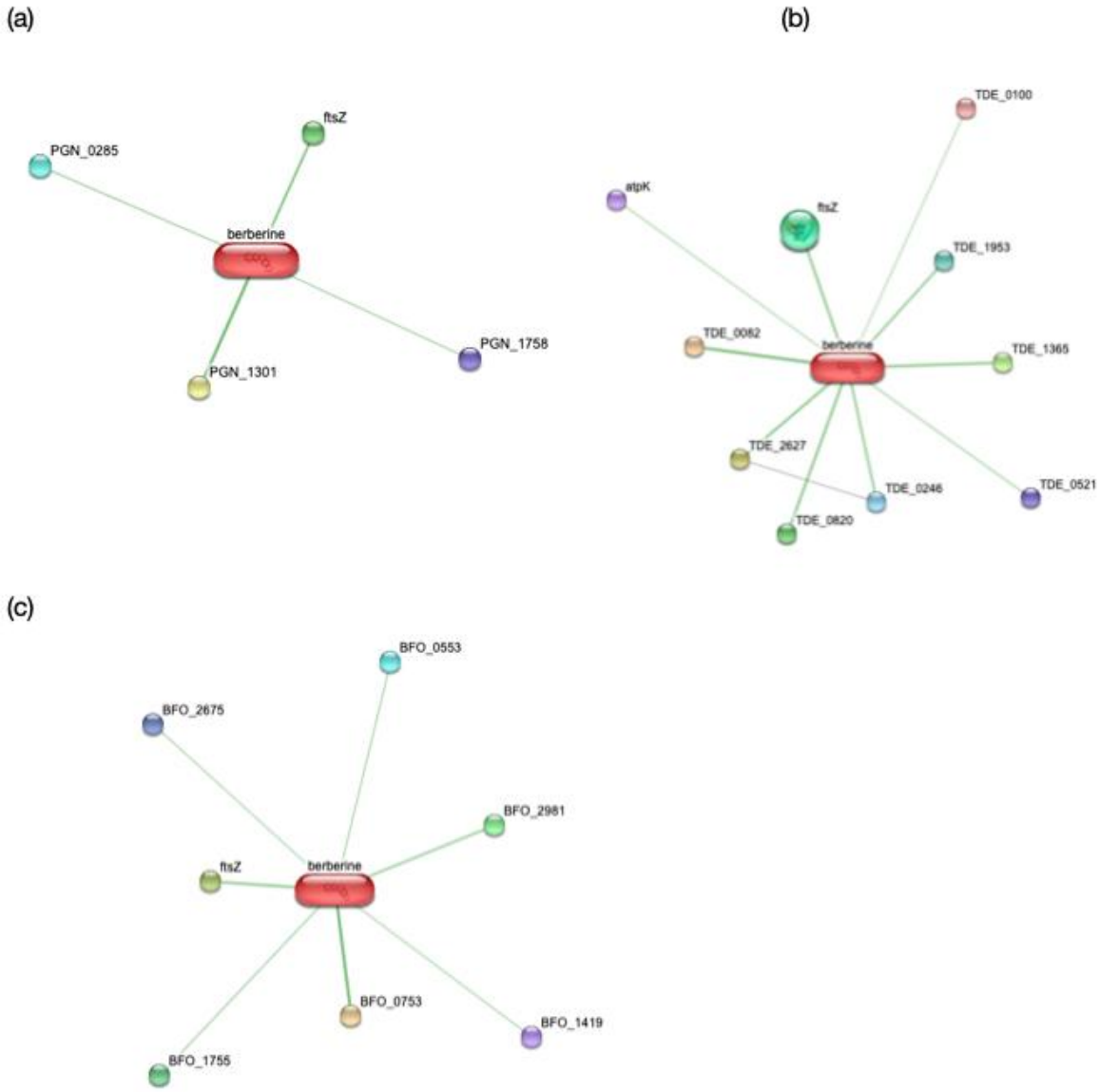

Figure 1: Protein interaction network of (a) *Porphyromonas gingivalis (b) Treponema denticola and (c) Tannerella forsythia* with theophylline

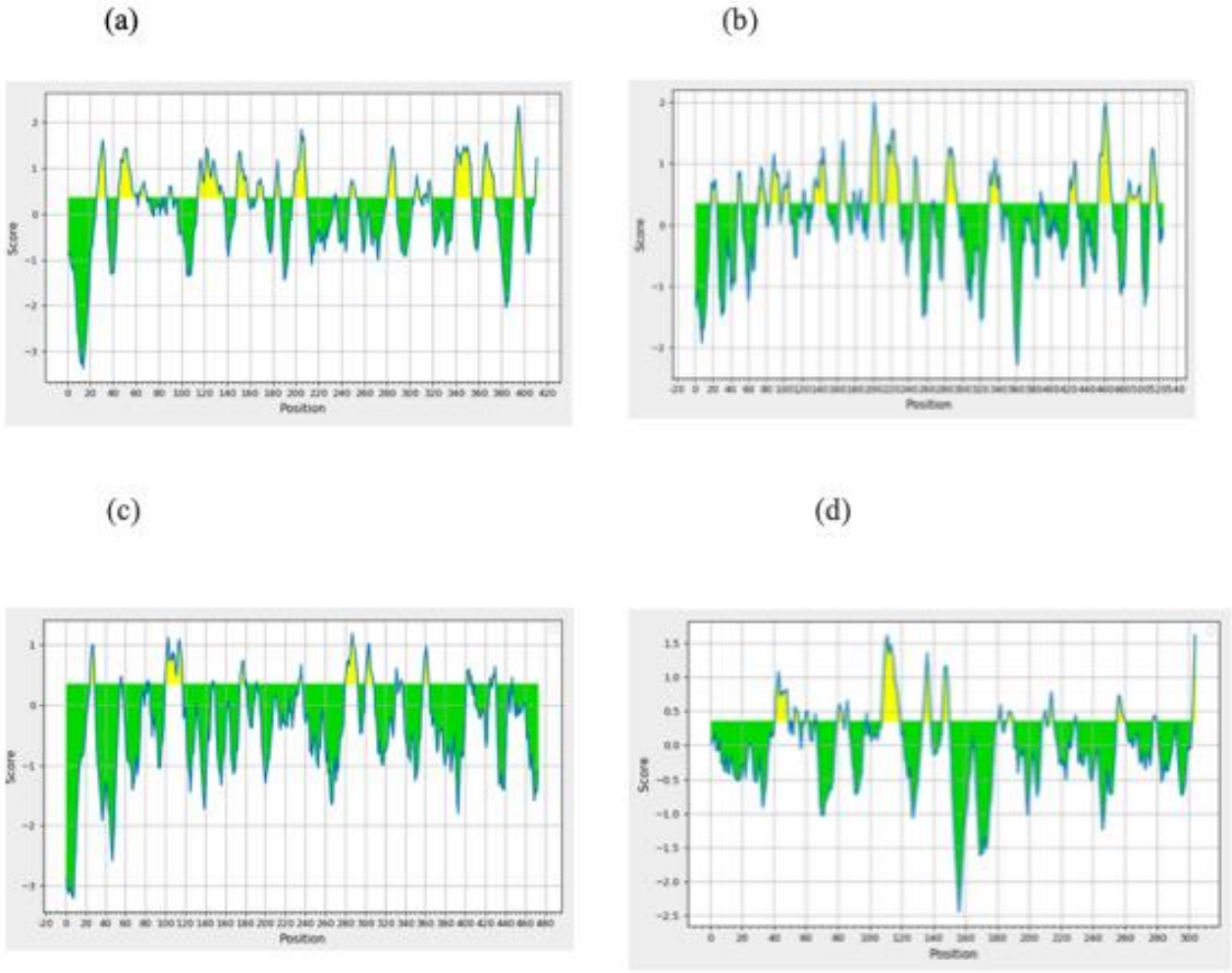

Figure 2: Predicted epitopes on virulent proteins identified using computational tools. (a) Type I phosphodiesterase-nucleotide pyrophosphatase [PGN_0239] (b) Alkaline phosphatase [PGN_1457] (c) Cardiolipin synthetase [PGN_0466] of *Porphyromonas gingivalis* and (d) Acetyl hydrolase [TDE_2263] of *Treponema denticola.*