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Virtual Screening to Identify the Protein Network Interaction of Berberine with Red Complex Pathogens

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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

Introduction: Periodontal disease is an infection of the tissues that hold your teeth in place. It's typically caused by poor brushing and flossing habits that allow plaque, a sticky film of bacteria, to build up on the teeth and harden. Elimination of these pathogens from the site of infection remains a perplexing task, which demands the use of antibiotics. The emergence of drug resistant forms has spurred interest into identifying novel therapeutic targets against these pathogens.
Aim: The present study employs virtual screening method to identify the protein network interaction of berberine with red complex pathogens.
Materials and Methods: Computational tools were used to identify the targets, assess their functional role and virulence property. Further, the peptide epitopes present in the virulence factors were identified using the BepiPred tool. The subcellular location of the virulence proteins was also elucidated using PSORTb.
Results: Berberine was found to target vital protein transporters such as TetR family transcriptional

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regulator and MerR family transcriptional regulator, which is known to play a crucial role in the survival of bacterial cells.
Conclusion: Hence the present study provides preliminary data on the protein targets of berberine against red complex pathogens. However, *in vitro* studies using the compound is warranted to further confirm the efficacy of the compound.

Keywords: Berberine; periodontal disease; red complex pathogens; novel targets; virtual screening; green synthesis; innovative technology.

1. INTRODUCTION

Periodontal diseases are complex infections exerted by a multiplex of bacterial species that interchange with host tissues and cells, causing the release of an extensive array of inflammatory cytokines, chemokines and mediators, some of which lead to the destruction of the periodontal structures, including the tooth-supporting tissues, alveolar bone, and periodontal ligament [1]. Bacteria are analogous with periodontal disease that trigger inflammatory responses in the immune cells, which in later stages of the disease causes loss of both soft and hard tissue structures supporting the teeth [2]. Until now, only a handful of bacteria have been characterized as infectious agents of periodontal disease. Periodontitis is a dysbiotic disease resulting from deviation in subgingival gram-positive bacteria to gram-negative bacteria. The development of periodontal dysbiosis occurs over a broadened timeframe, which slowly revolves the mutual association of the host and the microbe to pathogenic form. Among microbial complexes, the first complex is a red-complex consisting of *Tannerella forsythia*, *Treponema denticola,* and *Porphyromonas gingivalis* [3]*.* The red complex, which appears later during biofilm development, comprises species that are considered periodontal pathogens.

Porphyromonas gingivalis has long been considered to be an important member of periodontopathic microbiota interacting in periodontal disease progression with bone and tissue destruction [2,4]. When grown on a blood agar surface, they produce black pigmented colones. *Treponema denticola* are long, thin, corkscrew-like gram-negative anaerobic bacteria whose characteristic motility and morphology can readily be identified by darkfield and phase contrast microscopic examination [5]. The spiral-shaped cells of *T. denticola* are covered with an outer sheath consisting of a fragile envelope-like structure [6]. *Tannerella forsythia* are anaerobic gram-negative members of the Cytophaga bacteroides family and were initially named *Bacteroides forsythus* [7]. *T. forsythia* is associated, more frequently and at higher levels, with various forms of periodontal disease, including gingivitis, chronic, and aggressive periodontitis, than with healthy individuals [8].

The emergence of drug resistant microbes has become a common observation in recent years in dental settings also. The *in silico* approach has helped researchers to drastically cut down their time and resources in screening bioactive compounds with the ability to destroy a broad spectrum of microbial pathogens [9]. Berberine is a chemical present in plants European barberry, goldenseal, and Oregon grapes. It is most commonly taken for diabetes, high levels of cholesterol or other fats in hyperlipidemia, and high blood pressure [10]. Our team has extensive knowledge and research experience that has translate into high quality publications [3–7] The aim of this study is to analyze to identify the protein network interaction of berberine in red complex pathogens employing the virtual screening methods.

2. MATERIALS AND METHODS

2.1 Study Design

The present study follows an observational study design that aims to virtually screen and identify the protein network interaction of berberine in red complex pathogens. The interaction of drugs with bacteria was analyzed using STITCH five pipeline and the virulence properties of the interacting proteins were deduced by VICMPred and VirulentPred softwares. The strains are included in the STITCH database, and the query was user defined [11].

2.2 Prediction of Protein-drug Interactions

The STITCH database provides an exhaustive platform for known and predicted interactions between drugs and proteins. The interactions

include physical and functional associations which stem from computational prediction and interactions aggregated from primary databases [11].

2.3 Virulence Prediction

VICMpred [12] and VirulentPred [13] pipelines were used for the identification of virulence factors targeted by berberine among the red complex pathogens. These tools employed a support vector machine based five fold cross-validation process to validate results. Virulence factors were screened based on amino acid composition using the VirulentPred tool, which classified them into two groups, that is virulent and avirulent. VICMpred groups proteins into four major classes, namely, proteins involved in cellular processes, metabolism, information storage, and virulence.

2.4 Prediction of Subcellular Localization of the Virulent Proteins

Computational prediction of subcellular localization of proteins aids in designing novel drug targets or for substantiating the role of an antimicrobial drug which targets the virulent protein. An algorithm which assigns a probable localization site to a protein from an amino acid sequence is pSORTb V3.0 [14].

2.5 Prediction of Epitopes

For the prediction of B-cell epitopes from a protein sequence, the server BepiPred-2.0 was used. To be part of an epitope, the residues with scores above the threshold (>0.5) [8,9,15].

3. RESULTS AND DISCUSSION

Our team has extensive knowledge and research experience that has translated into high quality publications [10,16-21,11]. Red complex pathogens, which include *P. gingivalis, T. denticola*, and *T. forsythia,* are vital contributors of periodontal infection. Berberine was found to interact with a plethora of crucial proteins in *P. gingivalis, T. denticola* and *T. forsythia.* They also possess strong mechanisms of defence, including efflux pumps, drug resistant genes, etc., to combat the selective pressure created by antibiotics. Hence, non-antibiotic drugs can be the drug of choice for multiple ailments associated with microbial infections.

The present study documents several types of metabolism molecules such as hypothetical protein, TetR family transcriptional regulator and MerR family transcriptional regulator are domain protein of *T. denticola* and membrane protein of *T. forsythia* targeted by berberine (Table 1; Fig. 1). In addition to it, many peptide epitopes were also identified in the virulence proteins, which can justify the use of berberine as an antimicrobial agent (Fig. 2). Most of the interacting proteins were found in the cytoplasm (Table 2). Several synthetic, semisynthetic and phytocompounds have been virtually screened for the presence of protein targets which can be further probed into to ascertain the mode of action of these compounds [17-20,12]. In line with these facts, berberine was chosen for the study to identify its potential targets in red complex pathogens. Our research team has embarked on identifying the potential molecular targets of different plant compounds against dental pathogens with a special emphasis on periodontal pathogens and emerging drug resistant species [22–34].

Berberine hydrochloride (BH) has been checked for the antimicrobial activity against *Candida albicans.* Huang *et al.,* recently report the anti-fungal effect of BH against *C. albicans.* The BH was found to alter the HOG-MAPK pathway with the simultaneous upregulation of core genes such as *SLN1, SSK2, HOG1* and *PBS2* [35]. A previous report by the same research group documented that BH could inhibit the formation of biofilm by destroying the cells and the adjacent structures. The molecular mechanisms involved may be the down regulation of expression of *EFG1, HWP1, ECE1* and *ALS1* which are involved the formation of hyphae and morphological transformation of *C. albicans* [36,37-38].

Table 1. Protein network interaction of red complex pathogens interacting with berberine

Organism	Identifier	Proteins which interacts with berberine	VICMPred Functional Class	VirulentPred	Virulent Pred Score
Porphyromonas gingivalis	PGN_0285	Pyridine nucleotide-disulphide oxidoreductase	Virulence factors	Non-Virulent	-1.001
	PGN_1301	Transcriptional regulator	Cellular process	Non-Virulent	-0.709
	PGN_1758	v-type ATPase subunit K	Cellular process	Non-Virulent	-0.936
	PGN_0631	Cell division protein	Cellular process	Non-Virulent	-1.025
Treponema denticola	**TDE_0100**	**Hypothetical protein**	**Metabolism Molecule**	**Virulent**	**0.0839**
	TDE_1953	TetR family transcriptional regulator	Metabolism Molecule	Non-Virulent	-1.081
	TDE_1365	**TetR family transcriptional regulator**	**Metabolism Molecule**	**Virulent**	**1.0437**
	TDE_0521	Carboxylesterase	Cellular process	Non-Virulent	-1.083
	TDE_0246	TetR family transcriptional regulator	Metabolism Molecule	Non-Virulent	-0.345
	TDE_0820	TetR family transcriptional regulator	Metabolism Molecule	Non-Virulent	-0.978
	TDE_2627	**TetR family transcriptional regulator**	**Metabolism Molecule**	**Virulent**	**1.0962**
	TDE_0082	**MerR family transcriptional regulator**	**Metabolism Molecule**	**Virulent**	**1.0062**
	TDE_1679	V-type ATP synthase subunit K	Cellular process	Non-Virulent	-0.739
	TDE_1204	Cell division protein	Cellular process	Non-Virulent	-1.006
Tannerella Forsythia	BFO_0553	Putative V-type sodium ATPase, K subunit	Metabolism Molecule	Non-Virulent	-1.042
	BFO_0753	**MerR family transcriptional regulator**	**Cellular process**	**Virulent**	**0.9968**
	BFO_2981	Kinase domain-containing protein	Metabolism Molecule	Non-Virulent	-0.764
	BFO_1419	Papain family cysteine protease	Virulence factors	Non-Virulent	-1.011
	BFO_1755	Pyridine nucleotide-disulfide oxidoreductase	Metabolism Molecule	Non-Virulent	-0.958
	BFO_2675	**Peptidase C13 family**	**Cellular process**	**Virulent**	**1.0166**
	BFO_0397	Cell division protein	Virulence factors	Non-Virulent	-0.932

Table 2. Subcellular localization of virulent proteins in *Treponema denticola* and *Tannerella forsythia*

Virulent Protein	Subcellular localization of protein	Score
Conserved hypothetical protein	Cytoplasmic	9.26
Transcriptional regulator, TetR family	Cytoplasmic	8.96
Transcriptional regulator, TetR family	Cytoplasmic	8.96
Transcriptional regulator, MerR family	Unknown	0.00
Transcriptional regulator	Cytoplasmic	9.97
Teptidase C13 family	Unknown	-

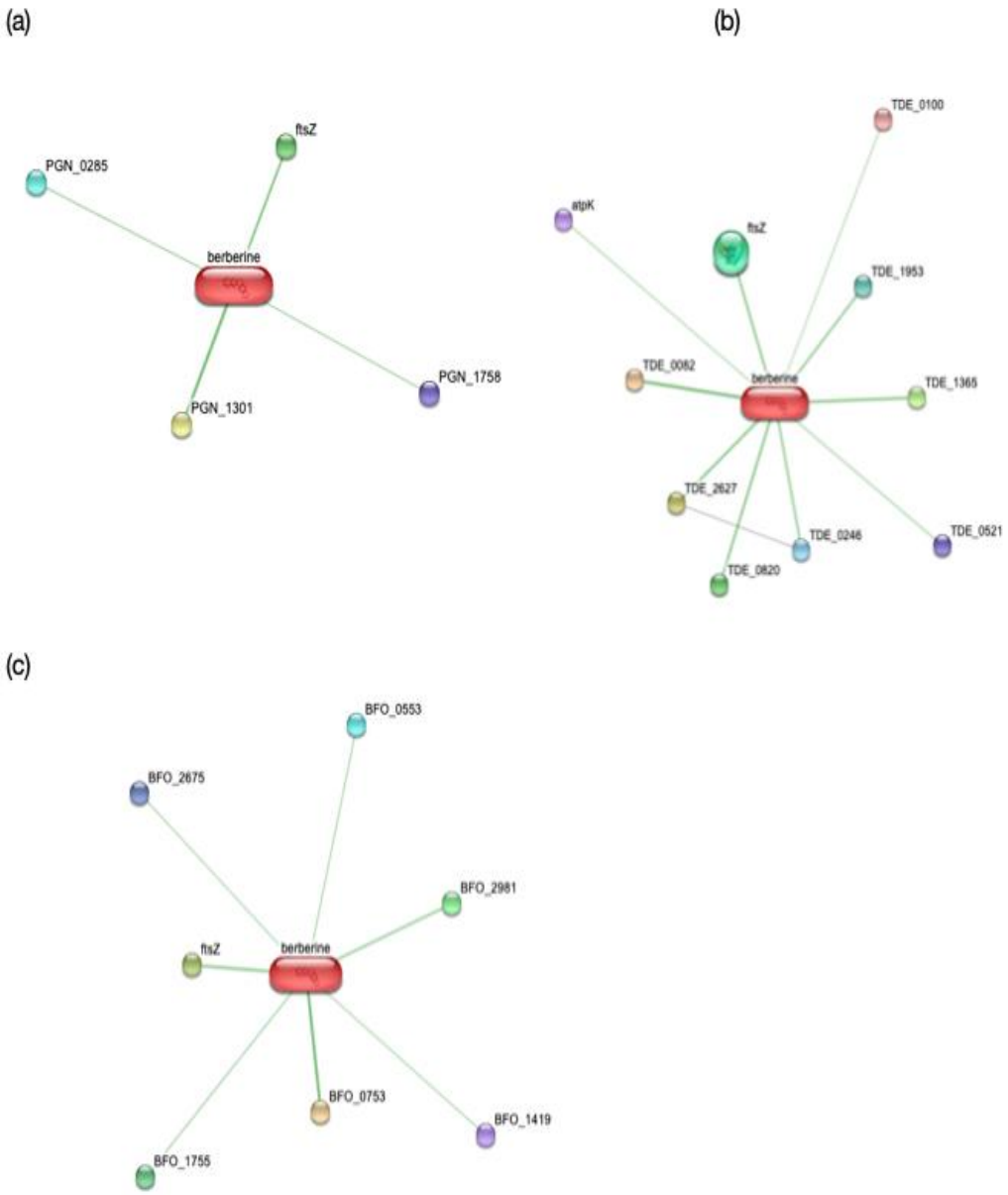

Fig. 1. Protein interaction network of (a) *Porphyromonas gingivalis (b) Treponema denticola and (c) Tannerella forsythia* with berberine

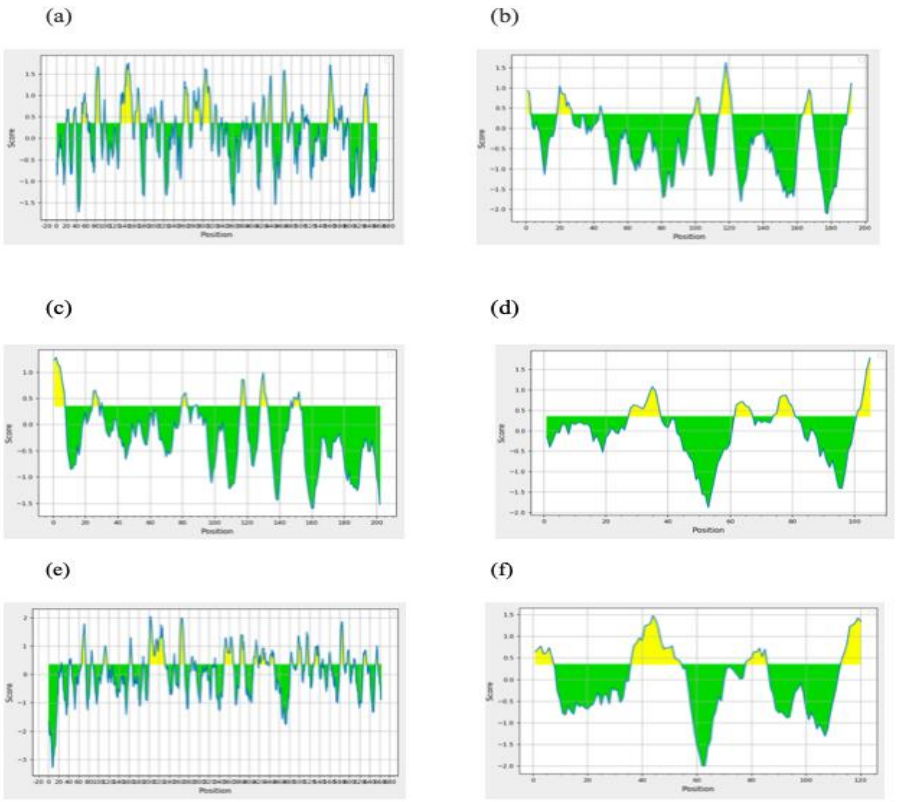

Fig. 2. Predicted epitopes in the virulent proteins identified (a) Hypothetical protein [TDE_0100], (b) TetR family transcriptional regulator [TDE_1365], (c) TetR family transcriptional regulator [TDE_2627], (d) MerR family transcriptional regulator [TDE_0082], (e) MerR family transcriptional regulator [BFO_0753] and (f) Peptidase C13 family [BFO_2675]

4. CONCLUSION

Berberine, with all its potential targets addressed, can be used as an antimicrobial agent to exterminate dental pathogens which are unmanageable to treatment. The mode of action of berberine as an MerR family transcriptional regulator and TerR family transcriptional regulator will produce it a perfect drug for therapeutic uses and applications.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company; rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

We conducted our research after obtaining proper IEC approval.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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