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Virtual Screening to Identify the Protein Network Interaction of Hypericin 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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Short Research Article

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ABSTRACT

Introduction: Hypericin is the anthraquinone derivative and has many properties like antiviral, antifungal and antibacterial. The red complex pathogens which include *Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia* in association with other microbes found in the periodontal pockets, cause severe inflammation resulting in periodontitis. Novel bioactive agents from several sources have been tested against the microbial pathogens to deduce antimicrobial activity.
Aim: The aim of the study is to virtually screen and identify the protein network interaction of hypericin in red complex pathogens.
Methodology: The STITCH v5.0 pipeline was primarily used to identify the drug-protein interactions. The VirulentPred and VICMPred software were used for elucidating the functional class of the proteins and virulence property. The sub cellular localization of virulent proteins was analysed with pSORTb v3.0 software. Further, the epitopes in virulent proteins were identified using BepiPred v1.0 linear epitope prediction tool.

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Results: Heat shock protein 90 of *Porphyromonas gingivalis* were found to involve in the cellular process and DNA topoisomerase IV subunit B, heat shock protein 90, DNA gyrase subunit A and DNA gyrase subunit B of *Treponema denticola* were found to be the virulent factors. The virulent proteins were located in the cytoplasm, which would further increase the potential effect of the drug to serve as antimicrobial agents. Finally, epitopes were predicted on the virulent proteins which can be specifically docked to further ascertain their interactions with the phytocompound.
Conclusion: Hypericin with all its potential and biological benefits can be addressed, can be used as an antimicrobial agent to eradicate dental pathogens which are recalcitrant to treatment. The mode of action of hypericin is, it is targeting crucial proteins in red complex pathogens. Further *in vitro* studies should be performed on a wide range of pathogens to substantiate the true interactions between the drugs and the protein repertoire of pathogens.

Keywords: Red complex pathogens; phytocompounds; hypericin; novel targets; periodontitis; epitopes.

1. INTRODUCTION

Hypericin is one of the naturally occurring substances that is commonly found in the St. John's Wort (Hypericum species) and synthesized from the anthraquinone derivative emodin [1]. Hypericin has also become a product which is intensively used in biochemical research. It acts as a multifunctional drug in several medicinal applications for the last three decades. Recent research reports that hypericin has many properties like antitumor, antidepressant, antineoplastic, antiviral (human immunodeficiency and hepatitis C virus) activities [2]. Although hypericin is tested against many pathogens the mechanism of action of hypericin still remains largely unexplained. The current trend that is largely observed in most of the clinical settings is the emergence and resurgence of drug resistance in microbes which has immensely contributed to increased mortality rate. Oral microbes like red complex pathogens that are encountered in the dental settings are considered to be a menace as they are recalcitrant to treatment due to development of resistance and formation of biofilms [3]. The red complex pathogens which includes *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* (formerly *Bacteroides forsythus*), are recognized as the most important pathogens in causing periodontitis [4]. More than 700 bacterial species are present in the subgingival plaque and they are considered to be causative agents of periodontal diseases [5,6]. Additionally *Peptostreptococcus micros, Prevotella* species, *Fusobacterium nucleatum*, *Eikenella corrodens*, and *Campylobacter rectus* are increased in deep periodontal pockets and are implicated as possible periodontopathogens [7]. These bacteria are not usually not seen individually but are always associated with other pathogens in the periodontal pockets, suggesting that some bacteria may cause destruction of the periodontal tissue in a cooperative manner [8,9].Our team has extensive knowledge and research experience that has translate into high quality publications[10–14].

In view of the above facts, novel phytocompounds are assessed to deduce their role in treating oral diseases. Computational tools have been considered to be among the most cost-effective procedures to screen for potential phytocompounds, targeted against microbial pathogens. The prominent photosensitizer hypericin, is a natural pigment of hypericum plants. The property of photochemical cytotoxicity is because of its special and unique quinone structure of hypericin [15,16]. The inhibitor, hypericin, shows significant antileishmanial activity, and the mode of death showed necrotising-like features [17]. Hence, the rationale of the study lies in identifying the potential targets of hypericin in the red complex pathogens, which would further add-on to the knowledge about the pathways being targeted by the drug to elicit an antibacterial response. The research topics related to phytochemistry are concentrated on plant extracts, which could contain some biologically active compounds with antibacterial [18,19].

2. MATERIALS AND METHODS

2.1 Study Design

The present study follows an observational study design which aims to screen for the interaction of hypericin in red complex pathogens. The interaction was analysed using STITCH v.5 pipeline [20]. The functional class of proteins identified were assessed using VICMPred

(https://webs.iiitd.edu.in/raghava/vicmpred/help.html) [21] and VirulentPred (http://bioinfo.icgeb.res.in › virulent) softwares [22]. 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. These organisms were selected from the STITCH database.

2.1.1 Prediction of protein-drug interactions

To predict the interactions between proteins and chemicals STITCH database (Version 5; 2016) is used. The interactions include associations of direct or physical and indirect or functional is used for the computational prediction and from the responses the data is aggregated. The repertoire of proteins which interacts with *T. forsythia, P. gingivalis* and *T. denticola* and were further used for predicting virulence [23].

2.2 Virulence Prediction

For the identification of virulence factors the software used was VICMPRED [21] and Virulent Pred [24], pipelines. All these tools are employed to support vector machines (SVM) - based five-fold cross-validation processes for the validification of results. There are two groups of virulence factors that were screened using the Virulent Pred tool based on amino acids that are virulent and a virulent. VICMpred group's 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.

2.2.1 Prediction of subcellular localization of the virulent proteins

The novel drug targets play 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 [25].

2.2.2 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) [26,27].

3. RESULTS

The stitch pipeline is completely utilized to identify the protein interaction with red complex pathogens like *Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia* and drug interaction. The protein interaction depicts a score produced and given by the algorithms and confirmation of the nature of the proteins are given. Based on this results they were grouped as virulent or avirulent.

3.1 Drug Protein Interaction in *Porphyromonas gingivalis*

The proteins identified were found to be involved in cellular processes followed by virulence factors and metabolism. Interestingly the scores that were obtained from Virulent Pred marked it as an avirulent factor. But with some exceptions the interaction of hypericin with proteins associated with cellular and metabolism are virulence factors. All other proteins that are subjected and analysed were avirulent.

3.2 Drug Protein Interaction in *Treponema denticola*

STITCH prediction for hypericin indicated that proteins are mainly associated with virulence factors followed by cellular processes. Proteins that are identified as virulence factors such as DNA topoisomerase IV subunit B, heat shock protein 90 and DNA gyrase subunit A and DNA gyrase subunit B by VICM Pred and are falling into avirulent group as assessed by Virulent Pred scores.

3.3 Drug Protein Interactions in *Tannerella forsythia*

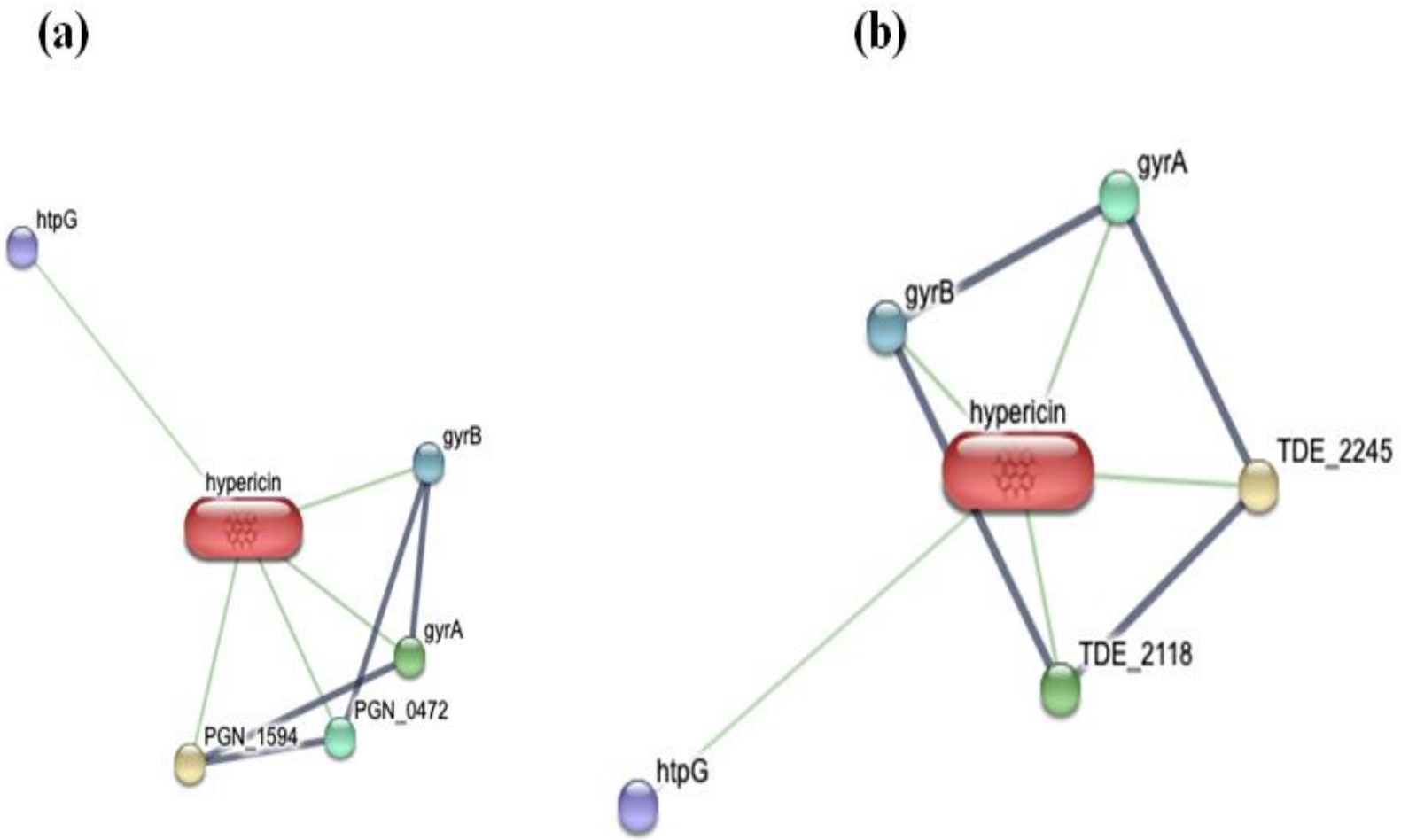
Major drug protein interaction seen in *Tannerella forsythia* falls in the category that involves metabolism such as putative DNA gyrase B subunit, DNA gyrase subunit A and DNA gyrase subunit B followed by cellular process such as DNA gyrase/ topoisomerase IV, A subunit and information and storage (Hsp 90) protein. All protein interaction was found to result as virulent factors using Virulent Pred.

Table 1. Proteins of red complex pathogens interacting with hypericin

Organism	Identifier	Proteins which interacts with hypericin	VICMPred Functional Class	Virulent Pred	Virulent Pred Score
Porphyromonas gingivalis	PGN_0472	DNA topoisomerase IV subunit A	Cellular process	Avirulent	-0.988
	PGN_1594	DNA topoisomerase IV subunit B	Cellular process	Avirulent	-0.929
	PGN_0041	Heat shock protein 90	Virulence factors	Avirulent	-1.003
	PGN_0875	DNA gyrase A subunit	Metabolism Molecule	Avirulent	-0.995
	PGN_0413	DNA gyrase B subunit	Metabolism Molecule	Avirulent	-1.020
Treponema denticola	TDE2118	DNA topoisomerase IV subunit A	Cellular process	Avirulent	-1.029
	TDE2245	DNA topoisomerase IV subunit B	Virulence factors	Avirulent	-1.006
	TDE2480	Heat shock protein 90; Molecular chaperone	Virulence factors	Avirulent	-1.023
	TDE0295	DNA gyrase subunit A	Virulence factors	Avirulent	-1.023
	TDE0002	DNA gyrase subunit B	Virulence factors	Avirulent	-1.021
Tannerella forsythia	BFO_1739	Hsp90 protein	Information and storage	Avirulent	-1.042
	BFO_1082	Putative DNA gyrase, B subunit	Metabolism Molecule	Avirulent	-1.002
	BFO_0740	DNA gyrase/topoisomerase IV, A subunit	Cellular process	Avirulent	-1.004
	BFO_2872	DNA gyrase subunit A	Metabolism Molecule	Avirulent	-1.009
	BFO_1695	DNA gyrase subunit B	Metabolism Molecule	Avirulent	-0.985

Table 2. Subcellular location of virulent proteins targeted by Hypericin

Organism	Proteins which interacts with hypericin	Subcellular location	Score
Porphyromonas gingivalis	Heat shock protein 90	Cytoplasm	9.97
Treponema denticola	DNA topoisomerase IV subunit B	Cytoplasm	9.97
Treponema denticola	Heat shock protein 90	Cytoplasm	9.97
Treponema denticola	DNA gyrase subunit A	Cytoplasm	9.97
Treponema denticola	DNA gyrase subunit B	Cytoplasm	9.97


(a)
htpG
hypericin
gyrB
gyrA
PGN_0472
PGN_1594
(b)
gyrA
gyrB
hypericin
TDE_2245
TDE_2118
htpG

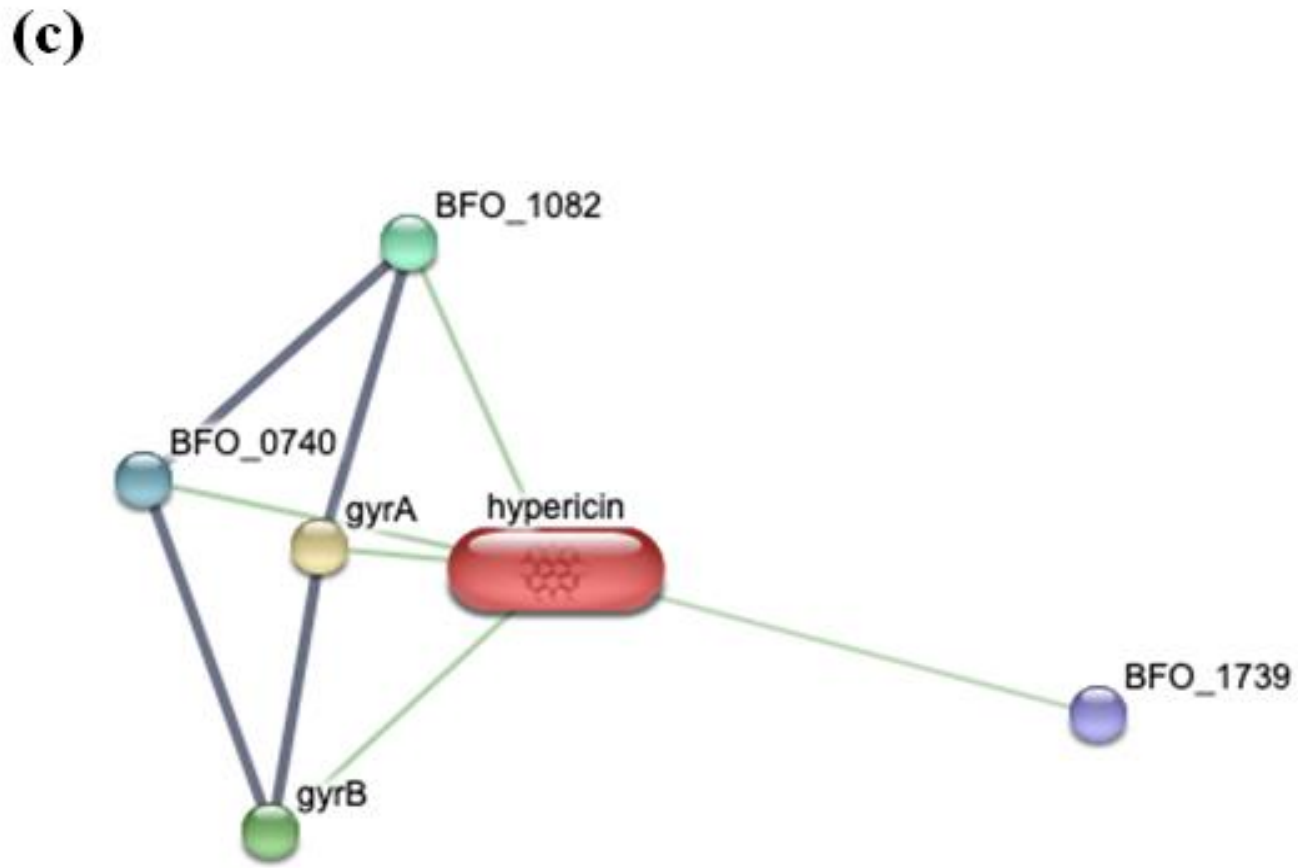
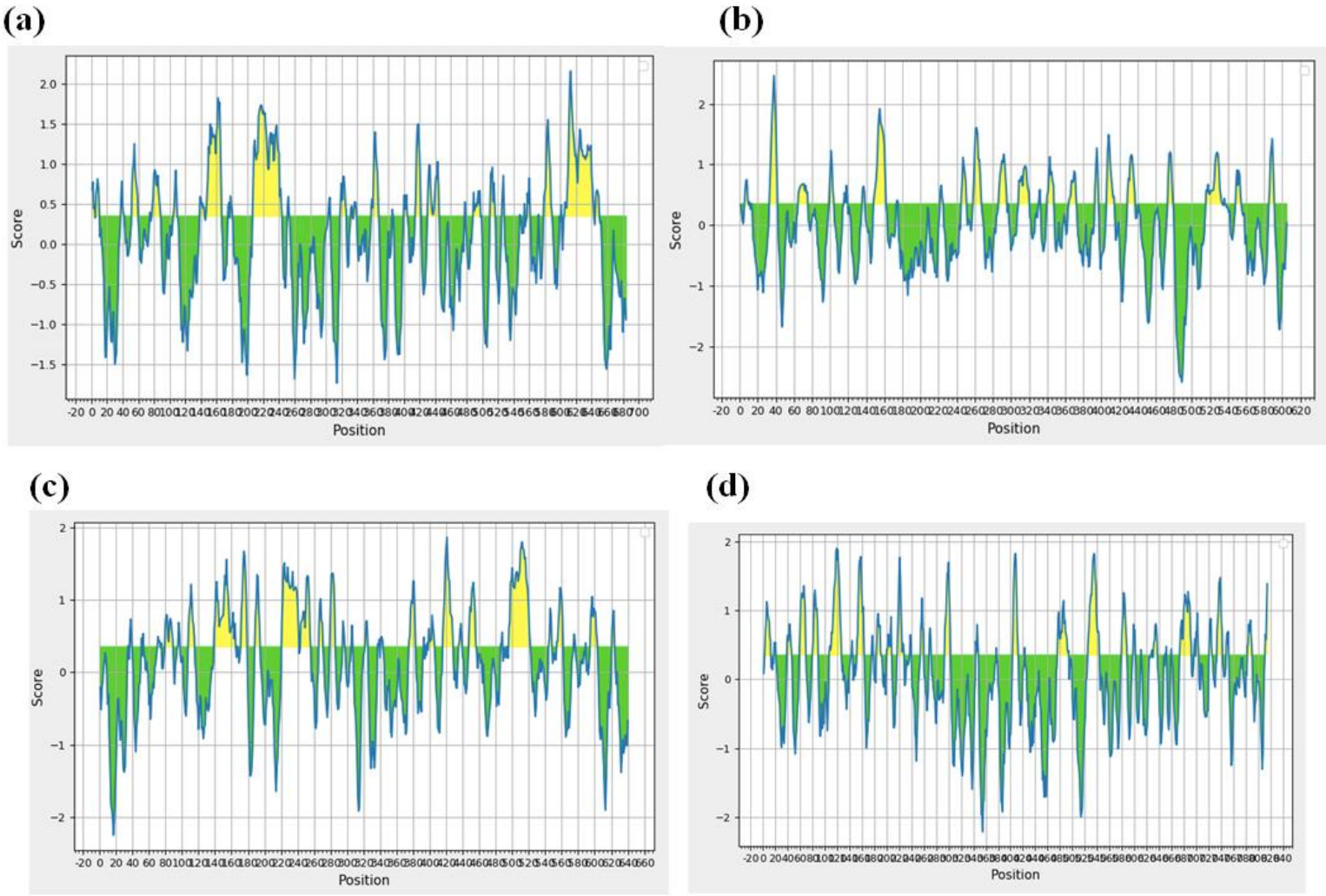


Fig. 1. Protein interaction network of (a) Porphyromonas gingivalis (b) Treponema denticola and (c) Tannerella forsythia with hypericin


(a)
(b)
(c)
(d)
Score
Position

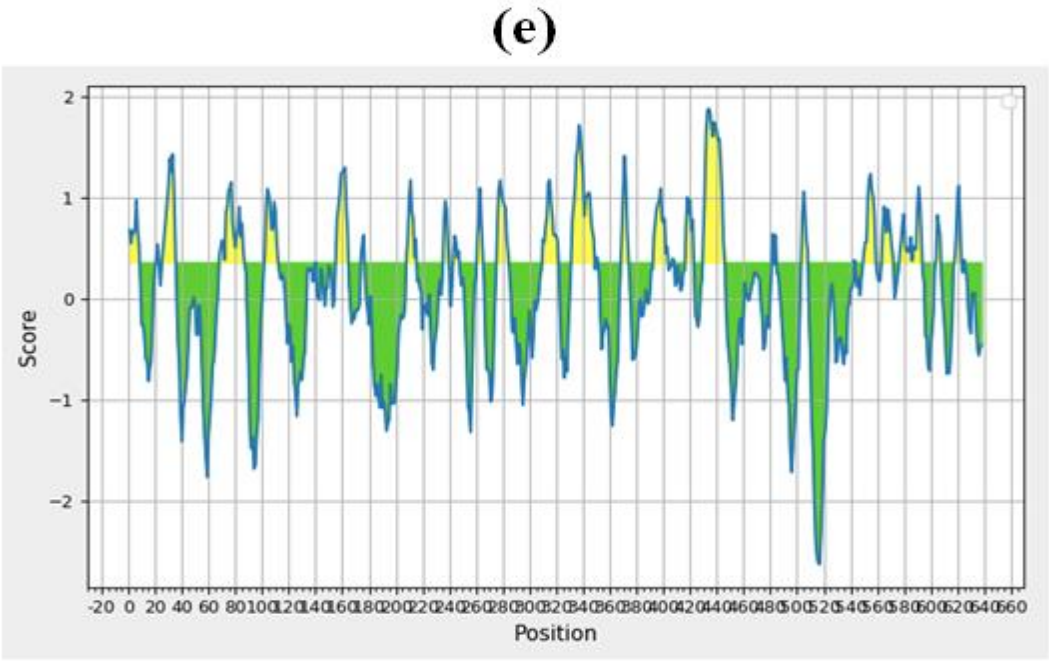


Fig. 2. Predicted epitopes for virulence factors (a) Heat shock protein 90 of Porphyromonas gingivalis, (b) DNA topoisomerase IV subunit B, (c) Heat shock protein 90, (d) DNA gyrase subunit A and (e) DNA gyrase subunit B of Treponema denticola

Prediction for the subcellular location of virulent proteins targeted by hypericin is scored as 9.97 for cytoplasmic location of heat shock protein 90, DNA gyrase subunit A and DNA gyrase subunit B of *Treponema denticola.* Additionally a number of epitopes or antibody binding sites in the virulent proteins were also identified. There were 34, 27, 39, 37 and 31 epitopes identified in the virulent proteins (data not shown), (a) Heat shock protein 90 of *Porphyromonas gingivalis*, (b) DNA topoisomerase IV subunit B, (c) Heat shock protein 90, (d) DNA gyrase subunit A and (e) DNA gyrase subunit B of *Treponema denticola* respectively. Comparatively heat shock protein 90 of *Treponema denticola* possesses more epitopes than heat shock protein 90 of *Porphyromanas gingivalis.*

4. DISCUSSION

Computational biology has helped biologists and clinical researchers to cut down on cost and time, by extending possible predictions of value which can be used as a preliminary data [23,24,28,29]. Validation for an *in silico* procedure is inevitable, while choosing a drug or a protein and to test *in vivo* or *in vitro* laboratory conditions. To conduct these experiments, *insilico* reports not only cut down the cost, but it provides clear ideas about the pathways or specific mechanism that is targeted during preliminary screening which reduces the time [30–34]. Present study concentrated on the protein network of red complex pathogens being targeted by hypericin.

Red complex pathogens that comprises Porphyromonas *gingivalis* [33] *Treponema denticola*, and *Tannerella forsythia* are the main causative organisms for the periodontic and endodontic problems [35]. In the previous studies done by Vijayshree, et al 2019., based on effect of non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens, the results that was obtained was APAP and IB were found to target vital proteins involved in the cellular process, metabolism, and virulence of red complex pathogens and it was similar to our study [35]. Several inflammatory diseases including the recent SARs-CoV2 epidemic demands rapid investigation and identification of markers [36]. In another research done by Ushantika, et al, 2019, identified the virulence factors targeted by reserpine in red complex pathogens, and the results obtained was reserpine was found to target vital protein transporters such as ABC transporter and efflux pumps [37]. Another study performed by Balamithra, et al in the year 2020, targeted proteins on the dental pathogens which were shown to interact with glycyrrhizin which was similar to our study [38]. Other research was done to see the protein interaction of red complex pathogens with Catechin, menthol and genistein [39–41].

Eradicating these organisms is really a challenge because it exhibits drug resistant genes etc. In this study many peptide epitopes were also identified in the virulence proteins which can be used as evidence to justify hypericin as an antimicrobial agent. Protein interaction with hypericin of heat shock protein 90 of *Porphyromonas gingivalis* and DNA topoisomerase IV subunit B, heat shock protein 90, DNA gyrase subunit B of *Treponema denticola* makes it an ideal drug target [18,42,43]. Recent study by Christinine *et al,* revealed that when a bacterial cell wall is incubated with hypericin followed by light irradiation of wavelength 600-800 nm and 5-3 nm, its effectiveness of hypericin which is mediated photodynamically. Mode of eradication strongly affects the cellular structure of bacterial cell structure, significant killing of Gram positive methicillin sensitive resistant *Staphylococcus aureus* cells but not effective against Gram negative *E.coli [44]*. In the study done by Thomas, *et al* concluded that in hypoxic conditions there is no effect of hypericin. Inhibitory effect of hypericin varies with enzymes and this is engaged in regulation for cell survival and proliferation [45]. The accumulated evidence and studies performed earlier by our team has helped us in conducting the present study [46–48].

While the *in silico* tool used provides preliminary data on the underlying molecular interaction between the protein network of red complex pathogens and the compound, there are some limitations: (a) the proteins of red complex bacteria could mimic host proteins that are targeted by the compound; (b) the interactions observed between the pathogen and compound may be purely physical and (c) drug interactions in a complex biological setting are not the same as observed *in silico*. To further confirm the effectiveness of the bioactive compound hypericin, it is critical to perform *in vivo* or *in vitro* studies. This could allow us to obtain accurate results and gain clarification on the safe use of phyto-compounds on human hosts.

5. CONCLUSION

Hypericin with all its potential and biological benefits as addressed can be used as an antimicrobial agent to eradicate dental pathogens which are recalcitrant to treatment. Further *in vitro* studies on a wide range of pathogens are warranted to substantiate the true interactions between the drugs and the protein repertoire of pathogens. The dosage of the drug, minimum inhibitory concentration, and minimum bactericidal concentration should be ascertained by in vitro and in vivo studies.

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.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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