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

C. Pratheebha¹, Jayaseelan Vijayshree Priyadharsini²*, A. S. Smiline Girija¹, P. Sankar Ganesh¹ and Nidhi Poddar²

¹Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-77, Tamil Nadu, India. ²Clinical Genetics Lab, Cellular and Molecular Research Centre, Chennai -77, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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.
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.

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.
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 VirulentPred [24], pipelines. All these tools are employed to support vector machines (SVM) - based five-fold cross-validation processes for the validation 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  |
Fig. 1. Protein interaction network of (a) Porphyromonas gingivalis (b) Treponema denticola and (c) Tannerella forsythia with hypericin
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 *Porphyromonas 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 Vijaysree, 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 Christine, 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.

FUNDING

We thank Saveetha Institute of Medical and Technical Science (SIMATS), Saveetha Dental College and Hospital, Saveetha University, Chennai and Seiko Book Centre, Thiruvallur for funding this study.

CONSENT

It is not applicable.

ETHICAL APPROVAL

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

ACKNOWLEDGEMENT

We thank Saveetha Dental College and Hospitals for providing us the support to conduct the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kubin A, Werrani F, Burner U, Alth G, Grünberger W. Hypericin—the facts about a controversial agent. Curr Pharm Des.2005;11:233–53.
2. Priyadharsini JV, Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. In silico analysis of virulence genes in an emerging dental pathogen A. baumannii and related species. Archives of Oral Biology. 2018;94:93–8. Available:https://doi.org/10.1016/j.archoralbio.2018.07.001.
3. Priyadharsini VJ, SmilineGirija AS, Paramasivam A. Enterococcus faecalis an Emerging Microbial Menace in Dentistry-An Insight into the In-Silico Detection of Drug Resistant Genes and Its Protein Diversity. J Clin Diagn Res.2018;12.
4. Suzuki N, Yoneda M, Hirofuji T. Mixed red-complex bacterial infection in periodontitis. Int J Dent. 2013;2013:587279.
5. Paster BJ, Olsen I, Aas JA, Dewhirst FE. The breadth of bacterial diversity in the human periodontal pocket and other oral sites. Periodontol.2000 2006;42:80–7.
6. Colombo APV, Boches SK, Cotton SL, Goodson JM, Kent R, Haffajee AD, et al. Comparisons of subgingival microbial profiles of refractory periodontitis, severe periodontitis, and periodontal health using the human oral microbe identification microarray. J Periodontol.2009;80:1421–32.
7. Lourenço TGB, Heller D, Silva-Boghossian CM, Cotton SL, Paster BJ, Colombo APV. Microbial signature profiles of periodontally healthy and diseased patients. J Clin Periodontol.2014;41:1027–36.
8. Jayaseelan VP, Arumugam P. Exosomal microRNAs as a promising theragnostic tool for essential hypertension. Hypertens Res.2020;43:74–5.
9. Darveau RP, Tanner A, Page RC. The microbial challenge in periodontitis. Periodontol. 2000 1997;14:12–32.
10. Rajendran R, Kunjusankaran RN, Sandhya R, Anilkumar A, Santhosh R, Patil SR. Comparative Evaluation of Remineralizing Potential of a Paste Containing Bioactive Glass and a Topical Cream Containing Casein Phosphopeptide-Amorphous Calcium Phosphate: An in Vitro Study. Pesquisa Brasileira Em Odontopediatria E Clinica Integrada.2019;19:1–10.
Ashok BS, Ajith TA, Sivanesan S. Hypoxia-inducible factors as neuroprotective agent in Alzheimer's disease. Clin Exp Pharmacol Physiol. 2017;44:327–34.

Sureshbabu NM, Selvarasu K, Jayanth KV, Nandakumar M, Selvam D. Concentrated growth factors as an ingenious biomaterial in regeneration of bony defects after periapical surgery: A report of two cases. Case Reports in Dentistry. 2019;2019:1–6. Available: https://doi.org/10.1155/2019/7046203.

Mohan M, Jagannathan N. Oral field cancerization: an update on current concepts. Oncol Rev. 2014;8:244.

Menon S, Ks SD, R S, S R, S VK. Selenium nanoparticles: A potent chemotherapeutic agent and an elucidation of its mechanism. Colloids Surf B Biointerfaces. 2018;170:280–92.

Vijayashree Priyadharsini J. In silico validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens. J Periodontol. 2019;90:1441–8.

Theodossiou TA, Hothersall JS, De Witte PA, Pantos A, Agostinis P. The multifaceted photocytotoxic profile of hypericin. Mol Pharm. 2009;6:1775–89.

Singh S, Sarma S, Katiyar SP, Das M, Bhardwaj R, Sundar D, et al. Probing the molecular mechanism of hypericin-induced parasite death provides insight into the role of spermidine beyond redox metabolism in Leishmania donovani. Antimicrob Agents Chemother. 2015;59:15–24.

Ramalingam AK, Selvi SGA, Jayaseelan VP. Targeting prolyl tripeptidyl peptidase from Porphyromonas gingivalis with the bioactive compounds from Rosmarinus officinalis. Asian Biomed. 2019;13:197–203.

Vukovic N, Milosevic T, Sukdolak S, Soljuc S. Antimicrobial Activities of Essential Oil and Methanol Extract of Teucrium montanum. Evid Based Complement Alternat Med. 2007;4:17–20.

Szklarczyk D, Santos A, von Mering C, Jensen LJ, Bork P, Kuhn M. STITCH 5: augmenting protein-chemical interaction networks with tissue and affinity data. Nucleic Acids Res. 2016;44:D380–4.

Saha S, Raghava GPS. VICMPred: an SVM-based method for the prediction of functional proteins of Gram-negative bacteria using amino acid patterns and composition. Genomics Proteomics Bioinformatics. 2006;4:42–7.

Garg A, Gupta D. Virulent Pred: A SVM based prediction method for virulent proteins in bacterial pathogens. BMC Bioinformatics. 2008:9:62.

Kumar SP, Girija ASS, Priyadharsini JV. Targeting NM23-H1-mediated inhibition of tumour metastasis in viral hepatitis with bioactive compounds from Ganoderma lucidum: A computational study. Pharmaceutical-Sciences 2020;82. Available: https://doi.org/10.36468/pharmacutical-sciences.650.

Samuel SR, Kuduruthullah S, Khair AMB, Shaye MA, Elkaseh A, Varma SR. Dental pain, parental SARS-CoV-2 fear and distress on quality of life of 2 to 6 year-old children during COVID-19. Int J Paediatr Dent.2021:31:436–41.

Yu NY, Wagner JR, Laird MR, Melli G, Rey S, Lo R, et al. PSORTb 3.0: improved protein subcellular localization prediction with refined localization subcategories and predictive capabilities for all prokaryotes. Bioinformatics.2010;26:1608–15.

Larsen JEP, Lund O, Nielsen M. Improved method for predicting linear B-cell epitopes. ImmunoRes.2006:2:2.

Jespersen MC, Peters B, Nielsen M, Marcitlili P. BepiPred-2.0: improving sequence-based B-cell epitope prediction using conformational epitopes. Nucleic Acids Res.2017:45:W24–9.

Teja KV, Ramesh S. Is a filled lateral canal - A sign of superiority? J Dent Sci.2020;15:562–3.

Barma MD, Muthupandiyan I, Samuel SR, Amaechi BT. Inhibition of Streptococcus mutans, antioxidant property and cytotoxicity of novel nano-zinc oxide varnish. Arch Oral Biol. 2021;126:105132.

Girija AS. Fox3 (+) CD25 (+) CD4 (+) T regulatory cells may transform the nCoV's final destiny to CNS! Comment 2021.

Verbanac D. Predictive methods as a powerful tool in drug discovery. Biochem Med. 2010;20:314–8.

Priyadharsini JV, Girija ASS, Paramasivam A. An insight into the emergence of Acinetobacter baumannii as an oro-dental pathogen and its drug resistance gene profile--An in silico approach. Heliyon. 2018;4:e01051.
33. Paramasivam A, Vijayashree Priyadharsini J, Raghunandhakumar S. N6-adenosine methylation (m6A): a promising new molecular target in hypertension and cardiovascular diseases. Hypertens Res. 2020;43:153–4.

34. Mathivadani V, Smiline AS, Priyadharsini JV. Targeting Epstein-Barr virus nuclear antigen 1 (EBNA-1) with Murraya koengii bio-compounds: An in-silico approach. Acta Virol. 2020;64:93–9.

35. Reddy P, Krithikadatta J, Srinivasan V, Raghu S, Velumurugan N. Dental Caries Profile and Associated Risk Factors Among Adolescent School Children in an Urban South-Indian City. Oral Health Prev Dent. 2020;18:379–86.

36. Girija ASS, Shankar EM, Larsson M. Could SARS-CoV-2-Induced HyperInflammation Magnify the Severity of Coronavirus Disease (CoViD-19) Leading to Acute Respiratory Distress Syndrome? Front Immunol. 2020;11:1206.

37. Ushanthika T, Smiline Girija AS, Paramasivam A, Priyadharsini JV. An in silico approach towards identification of virulence factors in red complex pathogens targeted by reserpine. Nat Prod Res. 2021;35:1893–8.

38. Balamithra S, Girija S, Vijayashree Priyadharsini J. An in silico Analysis of Protein Targeted by Glycyrrhizin in Common Dental Pathogens. Journal of Pharmaceutical Research International. 2020:170–8.

39. Thaslima Nandhini JS, Smiline Girija AS, Vijayashree Priyadharsini J. Virtual Screening to Identify the Protein Targets in Common Dental Pathogens Interacting with Menthol. Journal of Pharmaceutical Research International. 2020:25–31.

40. Vivek Babu B, Smiline Girija AS, Vijayashree Priyadharsini J. An in silico approach to identify the protein targets of common dental pathogens targeted by Genistein. J Toxicol Environ Health B Crit Rev. 2020;7:3340–8.

41. Cibikkarthik T, S GSA, J VP. An In silico approach to detect potential targets of catechin in red complex pathogens. J Toxicol Environ Health B Crit Rev. 2020;7:3328–31.

42. Paramasivam A, Vijayashree Priyadharsini J. Novel insights into m6A modification in circular RNA and implications for immunity. Cell Mol Immunol. 2020;17:668–9.

43. Paramasivam A, Priyadharsini JV, Raghunandhakumar S. Implications of m6A modification in autoimmune disorders. Cell Mol Immunol. 2020;17:550–1.

44. Yow CMN, Tang HM, Chu ESM, Huang Z. Hypericin-mediated photodynamic antimicrobial effect on clinically isolated pathogens. Photochem Photobiol. 2012;88:626–32.

45. Thomas C, Pardini RS. Oxygen dependence of hypericin-induced phototoxicity to EMT6 mouse mammary carcinoma cells. Photochem Photobiol. 1992;55:831–7.

46. Iswarya Jaisankar A, Smiline Girija AS, Gunasekaran S, Vijayashree Priyadharsini J. Molecular characterisation of csgA gene among ESBL strains of A. baumannii and targeting with essential oil compounds from Azadirachta indica. Journal of King Saud University – Science. 2020;32:3380–7.

47. Jayaseelan VP, Paramasivam A. Emerging role of NET inhibitors in cardiovascular diseases. Hypertens Res. 2020;43:1459–61.

48. Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? Int J Paediatr Dent. 2021;31:285–6.