Virtual Screening to Identify the Protein Targets in Common Dental Pathogens Interacting with Menthol

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Authors' contributions

This work was carried out in collaboration among all authors. Author JVP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author ASSG managed the analyses of the study. Author JSTN managed the literature searches and certain computational analysis. All authors read and approved the final manuscript.

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ABSTRACT

Deducing the molecular pathway underlying the antimicrobial effect of phytocompounds is an inevitable part of drug discovery. Selection of potential targets on the microbial pathogens will eventually lead to eradication of microbes and effective treatment. In this context, the present insilico study identifies vital targets in the dental pathogens interacting with menthol. The STITCH tool was used for identifying the protein drug interaction, VICTMPred and VirulentPred tools were used for identifying the functional class and virulence nature of proteins. PSORTb was used to locate the sub-cellular location of the virulent proteins. The study results indicate that menthol

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interacts with virulence factors of Treponema denticola. These factors play a crucial role in cell survival and hence can be a good target for further in vitro and in vivo studies. To conclude, menthol was found to interact with crucial proteins of dental pathogens which can be targeted to achieve promising results.

**Keywords:** Menthol; common dental pathogen; bacteria; DNA replication; in silico approach.

1. INTRODUCTION

Microbial pathogens in the oral cavity dwell in a poly-microbial environment which enhances their interaction with other pathogens. Eradication of microbes from the site of infection becomes complex due to several features, both natural and acquired exhibited by the pathogen. Biofilm formation, intrinsic or extrinsic drug resistance has more often hampered the treatment process [1,2]. Herbal medicine and phyto-compounds have revolutionized the treatment strategy and has taken a giant leap in the field of modern medicine, due to its safety, efficacy and cost-effectiveness. Common dental pathogens such as *Streptococcus mutans*, *Enterococcus faecalis*, *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia* have been studied extensively so as to reveal their virulence properties. Biofilm formation is one of the best characterized features which enable bacterial pathogens to survive selective pressure in the presence of antibiotics [3]. Antimicrobial drug resistance is yet another bewildering property of endodontic pathogens which usually turns treatments futile [4]. Novel antimicrobials are the need of this hour to combat the menace created by these organisms. Virtual screening of bioactive compounds is one method which can provide clues on the molecular targets within a short period of time. This reduces laborious wet lab procedures which are considered to be standard methods to study drug susceptibility or resistance of a pathogen.

In line with the above facts, menthol is a bioactive compound extracted from the plant *Mentha piperita*. Peppermint oil obtained from the plant has a variety of therapeutic applications and has been used in mouthwashes, toothpastes, bath and topical preparations [5]. Topical application of menthol provides an analgesic effect by inhibiting Ca\(^{2+}\) currents of neuronal membranes [6]. Essential oils (EO) extracted from *Mentha piperita* exhibited potential antimicrobial effect against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Micrococcus luteus* ATCC14452, *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* ATCC 6633, *Salmonella typhimurium*, *Bacillus cereus*, *Candida albicans* and *Candida tropicalis* [7]. A recent study also reported the effect of EO from different plant sources against oral pathogens. EO containing menthol, thymol and carvacrol were found to be more effective against *S. mutans* and *Lactobacillus* species, individually or in combination with chlorhexidine [8].

2. MATERIALS AND METHODS

In the present in silico study, the phytocompound menthol was tested against anaerobic oral pathogens viz., *Enterococcus faecalis*, *Porphyromonas gingivalis*, *Streptococcus mutans*, *Treponema denticola* and *Tannerella forsythia*. STITCH tool was used to reveal the interactions between the compound and the protein repertoire in the pathogen. VICMPred and VirulentPred softwares were subsequently used to check the virulence nature of the proteins targeted by menthol. PSORTb was used to identify the sub cellular location of the virulent protein. The methodology adopted and computational tools employed have been described in detail.

2.1 Strains Used in the Study

The following strains available in the STITCH database were used for the present study. *Streptococcus mutans* UA159, *Enterococcus faecalis* V583, *Porphyromonas gingivalis* ATCC 33277, *Treponema denticola* ATCC 35405, *Tannerella forsythia* ATCC 43037 [9].

2.2 Analysing Protein Interaction Network

STITCH is an exhaustive pipeline which can be 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 which interacts with *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia* were used for predicting
virulence [9]. The FASTA format of protein sequences were retrieved from the National Centre for Biotechnology Information [NCBI] domain and used for predicting the functional class of proteins and their virulence properties [https://www.ncbi.nlm.nih.gov/protein/?term=].

2.3 Prediction of Functional Class of Interacting Proteins
VICMpred server aids in 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 based on the support vector machine [SVM] algorithm which classifies proteins based on their amino acid composition and sequence pattern [10].

2.4 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 [11].

2.5 Prediction of Sub Cellular Localisation of Proteins
The identification of the sub cellular localisation of virulence proteins are 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 was used for identification of sub-cellular location of virulence proteins [12].

3. RESULTS AND DISCUSSION
The In silico approach employed identified the potential virulence factors targeted by menthol in Treponema denticola among all the pathogens investigated in the present study. Menthol was found to interact with topoisomerase IV, B subunit, putative, DNA gyrase subunit A, DNA gyrase subunit B of Treponema denticola, which were also identified as virulence factors by VirulentPred tool [Table 1]. Interestingly, menthol was found to bind to DNA gyrase and topoisomerase of all the pathogens studied and hence considered as a “topoisomerase poison” [Fig. 1]. Apart from this protein, ankyrin repeat protein was the second most common protein target of menthol. Glycosyltransferase, a crucial protein of S.mutans was found to be complex with menthol. The subcellular location of the virulence proteins identified in Treponema denticola was in the cytoplasm [Table 2].

![Fig. 1. Interaction of menthol with protein repertoire of dental pathogens](image-url)
Table 1. Protein interaction network of menthol against common dental pathogens

| Organism                   | Identifier | Proteins which interacts with menthol | VICMPred Functional | Virulent Pred | Virulent Pred Score |
|----------------------------|------------|----------------------------------------|---------------------|---------------|---------------------|
| *Enterococcus faecalis*    | EF_0841    | Hypothetical protein                   | Information and storage | Avirulent     | -1.033              |
|                            | EF_0377    | Ankyrin repeat family protein          | Cellular Process    | Avirulent     | -0.332              |
|                            | parC       | DNA topoisomerase IV subunit A         | Metabolism          | Avirulent     | -1.018              |
|                            | parE       | DNA topoisomerase IV subunit B         | Metabolism          | Avirulent     | -1.041              |
|                            | gyrA       | DNA gyrase subunit A                   | Cellular Process    | Avirulent     | -1.009              |
|                            | gyrB       | DNA gyrase subunit B                   | Cellular Process    | Avirulent     | -1.031              |
| *Porphyromonas gingivalis* | PGN_0472   | DNA topoisomerase IV A subunit         | Metabolism          | Avirulent     | -0.988              |
|                            | PGN_1594   | DNA topoisomerase IV B subunit         | Metabolism          | Avirulent     | -0.929              |
|                            | gyrA       | DNA gyrase A subunit                   | Cellular Process    | Avirulent     | -0.995              |
|                            | gyrB       | DNA gyrase B subunit                   | Cellular Process    | Avirulent     | -1.020              |
| *Streptococcus mutans*     | SMU_1806   | Glycosyltransferase                    | Cellular Process    | Avirulent     | -0.633              |
|                            | parC       | DNA topoisomerase IV subunit A         | Metabolism          | Avirulent     | -1.012              |
|                            | parE       | DNA topoisomerase IV subunit B         | Metabolism          | Avirulent     | -1.010              |
|                            | gyrA       | DNA gyrase subunit A                   | Cellular Process    | Avirulent     | -1.020              |
|                            | gyrB       | DNA gyrase B subunit                   | Cellular Process    | Avirulent     | -1.057              |
| *Treponema denticola*      | TDE_2450   | Ankyrin repeat protein                 | Metabolism          | Avirulent     | -0.400              |
|                            | TDE_2693   | Ankyrin repeat protein                 | Cellular Process    | Avirulent     | -1.043              |
|                            | TDE_0502   | Ankyrin repeat protein                 | Metabolism          | Avirulent     | -1.042              |
|                            | TDE_2118   | DNA topoisomerase IV, A subunit, putative | Metabolism | Avirulent     | -1.029              |
|                            | TDE_2245   | DNA topoisomerase IV, B subunit, putative | Virulence factor | Avirulent     | -1.006              |
|                            | gyrA       | DNA gyrase subunit A                   | Virulence factor    | Avirulent     | -1.023              |
|                            | gyrB       | DNA gyrase subunit B                   | Virulence factor    | Avirulent     | -1.021              |
| *Tannerella forsythia*     | BFO_0740   | DNA topoisomerase IV subunit A         | Metabolism          | Avirulent     | -1.004              |
|                            | BFO_1082   | Putative DNA gyrase, B subunit         | Metabolism          | Avirulent     | -0.997              |
|                            | BFO_0595   | Hypothetical protein DNA gyrase subunit A | Metabolism | Avirulent     | -0.986              |
|                            | gyrA       | DNA gyrase subunit A                   | Cellular Process    | Avirulent     | -1.008              |
|                            | gyrB       | DNA gyrase subunit A                   | Metabolism          | Avirulent     | -0.985              |
Table 2. Sub-cellular location of virulence proteins identified in Treponema denticola

| Organism            | Virulence factor                   | Subcellular localisation of the protein |
|---------------------|------------------------------------|----------------------------------------|
| Treponema denticola | Topoisomerase IV, B subunit, putative | Cytoplasm                              |
|                     | DNA Gyrase subunit A                | Cytoplasm                              |
|                     | DNA Gyrase subunit B                | Cytoplasm                              |

Endodontic regeneration is highly dependent on eradication of microbial pathogens at the site of infection. Antibiotic dressing is usually preferred to avoid microbial growth [13]. Nevertheless, this process creates a selective pressure on the microbes to adapt to the antibiotic laden environment transforming them into antibiotic resistant strains. Now the process of treatment becomes even more difficult as these strains are recalcitrant to antibiotic therapy. In this state of alert, an enormous number of phyto-compounds have been identified and their bioactive principles are revealed which can be utilized as potent antimicrobials. Screening of these compounds using agar well diffusion methods is used as a standard method to evaluate their efficacy against specific pathogens. This procedure is time consuming, expensive and does not provide any clue about the underlying mechanisms leading to the death of the pathogen.

An alternative strategy which is gaining a lot of appreciation is screening bioactive compounds using computational tools. This procedure virtually screens for the best compound and identifies molecular targets on the pathogens which can be utilized as a candidate protein. Numerous studies have substantiated on the mode of action of menthol against bacterial and fungal pathogens. Bacterial topoisomerase has been regarded as one of the best drug targets [14] owing to its role replication, transcription, repair and recombination [15]. The present study documents the interaction of menthol with gyrase and topoisomerase of all the bacterial pathogens tested.

Peppermint oil [PO] from Mentha piperita was shown to possess potent antimicrobial and antioxidant properties. Both gram positive positive [S. aureus ATCC 25923 and S. pyogenes ATCC19615], and gram negative bacteria [Escherchia coli ATCC 25922 and Klebsiella pneumonia ATCC 13883] were selected for the antimicrobial assay. The line of sensitivity observed was in the pattern S. aureus > S. pyogenes > K. pneumoniae > E.coli. Gram negative pathogens exhibited a minimal degree of resistance to antibacterial, which might be due to the presence of lipopolysaccharides. Interestingly, PO produced a greater zone of inhibition against S. aureus, S.pyogenes and K. pneumonia in comparison to the positive control gentamycin [10 IL of 10 lg/ml concentration] [16]. The inhibition of bacterial pathogens may be attributed to the presence of major components of PO which include menthol [29-48%], menthone [20-31%], menthofuran [6.8%] and menthol acetate [3-10%] [17].

Korkmaz et al. reported the use of menthol in toothpastes aids in the reduction of S. mutans and Staphylococcus aureus [18]. Bouyahya and colleagues tested the antimicrobial activity of Menthapulegium essential oil [MPEO] against several bacterial species. Bacillus subtilis, Proteus mirabilis and S.aureus were found to be inhibited by MPEO [19]. The effect of menthol solution and oral hygiene status of dental students were tested using chlorhexidine as control. Although, menthol did not produce similar results as that of chlorhexidine, menthol mouthwash exhibited a significant reduction in plaque, gingival and bleeding indices in mice model [20]. Several studies based on insilico methodology have aided in identifying molecular targets in dental pathogens against non-steroidal anti-inflammatory drugs [21] and phyto-compounds [22]. More investigations directed towards the molecular aspects of menthol could enrich its role as a potent antimicrobial agent.

4. CONCLUSION

The present study throws light on the molecular targets of menthol which is topoisomerase and gyrase. Thus, it can be used as a potential drug of choice against several oro-dental pathogens. Although the study has a lot of merits, the major limitations of the study are, [a] interaction of compounds may not be the same in a biological environment, [b] there can be homology between the targeted bacterial
proteins and host proteins, and [c] the binding observed may be physical and may not affect the functional properties. Hence, In vitro and In vivo studies are warranted to justify the effect of menthol on dental pathogens.

CONSENT AND ETHICAL APPROVAL

Not applicable

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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