

Inhibition of RagB gene using herbal compounds in the treatment of primary endodontic pathogenesis caused by *Porphyromonas* species - An *in silico* study

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ABSTRACT

Herbal compounds taken from plant extracts are identified having numerous medicinal values and put into use for various treatments. Such compounds are also pave way and helpful in manufacturing such synthetic compounds for commercial use. In this article, about five herbal compounds, namely, allicin, nimbin, mangiferin, punicalin, and alpha-pinene were taken and studied for their efficacy in the treatment of primary endodontic pathogenesis. Almost all of them have antibacterial efficacy and alpha-pinene, outstands having varied application in the field of disease control. The aim of the study was to evaluate the efficacy of different herbal compounds on inhibition of RagB gene in the treatment of primary endodontic pathogenesis caused by *Porphyromonas* species. The three-dimensional (3D) structure of RagB gene protein was downloaded from the RCSB Protein Data Bank and was corrected using “what if” server. Then, the 3D chemical structures of allicin, nimbin, mangiferin, punicalin, and alpha-pinene were downloaded from PubChem compound database which was followed by analysis of target active binding site; the active binding sites of targets proteins were analyzed using the Bravio Discovery Studio version 2016. Finally, molecular docking analysis was carried out by computerized ligand-target docking approach was used to analyze structural complex of the adhesion protein with allicin, nimbin, mangiferin, punicalin, and alpha-pinene to understand the structural basis of this protein target specificity. Docking was carried out with the help of iGEMDOCK software 2.1 based version. The binding energy indicated the affinity of adhesion protein docked with herbal components. Among the five compounds, alpha-pinene showed the highest negative value which indicates active binding to the target site and also showed the best interaction with target protein based on the root-mean-square deviation values as compared to standards. *Porphyromonas gingivalis*, an anaerobic, Gram-negative pathogen and the bacterium most associated with chronic periodontitis, demonstrated highest sensitivity to alpha-pinene among the five herbal compounds. Further investigation should be done on more herbal compounds for the prevention and treatment of various endodontic diseases.

Keywords: RagB gene, herbal compounds, *Porphyromonas* species, docking

Introduction

For decades and even centuries back to historic age, herbal treatment has played a very prominent role in treatment of various diseases, burns, surgical care and dental care is no exception.

Dental plaque was a common occurrence leading to dental caries and periodontal diseases which are considered to be one of the most important global oral health problems.^[1] The dental plaque is yet another health issue caused by bacteria formed as a mixed microbial biofilm growing on teeth.^[2] Serving as a media or adhesive for the bacteria to proliferate and thus forming a biofilm over tooth surface as well as spreading to adjoining cells. The biofilm or microbial adhesion is now considered as a crucial step in the pathogenesis of wide range of bacterial diseases. Plaques are organized bacterial colonies that adhere strongly to the tooth, making their removal very difficult. Newly formed bacteria attach to old bacteria, resulting in a continuous enlargement of the plaque mass.^[3]

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More than 400 distinct bacterial species are reported in the plaque which digest carbohydrates producing acid substances, thus resulting in dental caries. Among the bacterial species comprising the plaque, there are aerobic Gram-positive bacteria (*Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus sobrinus*, *Streptococcus salivarius*, and *Actinomyces viscosus*) (*Actinobacillus*, *Actinomyces*, *Capnocytophaga* and *Eikenella corrodens*). In addition, the bacterial plaque also consists of anaerobic Gram-negative species (*Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Prevotella intermedia*, *Bacteroides forsythus*, and *Campylobacter rectus*) and spirochetes^[4,5] (*Treponema denticola*, among other species). Periodontal diseases are subgingival conditions that have been linked to anaerobic Gram-negative bacteria such as *P. gingivalis*, *Actinobacillus* sp., *Prevotella* sp., and *Fusobacterium* sp.^[6,7]

Ras-homologous GTPases constitute a large family of signal transducers that alternate between an activated GTP-binding state and an inactivated GDP-binding state. Ras-related GTP-binding B is a protein-coding gene. The rag locus of *P. gingivalis* is comprised receptor antigen RagA and RagB. RagA spans the outer membrane forming a beta-barrel-like structure and is believed to be linked to a TonB-like protein, whereas RagB is exposed on the outer surface of the membrane in a complex with RagA.^[8] While host-RagB interactions are poorly understood, study using *P. gingivalis* cell lysate and serum from patients with periodontitis has shown that RagB is the most immunodominant *P. gingivalis* antigen and is clearly recognized by the immune system.^[9] The rag operon may be essential for survival *in vitro* to *P. gingivalis* using a mariner transposon system.^[10]

RagB is frequently found in *P. gingivalis* from periradicular lesions^[11] and is mostly commonly found in the strains isolated by deeper pocket depths using polymerase chain reaction analysis of subgingival plaque samples from periodontal patients and healthy control individuals.^[8] Eukaryotic expression vector (pIRES) gene linked is used for RagB as a DNA vaccine and showed mice inoculated intramuscularly with a RagB-linked DNA vaccine produced a strong anti-RagB antibody response, which were strongly resistant to *P. gingivalis* infection and displayed a significant reduction in lesion size compared to the control group.^[12]

Materials and Methods

First, the three-dimensional (3D) crystallographic structure of RagB gene was downloaded from RCSB Protein Data Bank. Protein Data Bank archive-information about the 3D shapes of proteins, nucleic acids, and complex assemblies is aimed at assisting us to figure out all aspects of biomedicine and agriculture from protein synthesis to health and disease and then the protein structure was corrected using “What if”

Table 1: Fitness table

Compound	Energy	VDW	HBond	Elec
RagB_1_TG6-allicin	-44.8554	-44.8554	0	0
RagB_1_TG6-alpha-pinene	-48.7735	-48.7735	0	0
RagB_1_TG6-mangiferin	159.539	159.539	0	0
RagB_1_TG6-nimbin	27.6811	27.6811	0	0
RagB_1_TG6-punicalin	210.48	210.48	0	0

VDW: Vanderwaals Interaction

Table 2: Interaction table

Cluster ID	Top of cluster	Compound	Energy	E (pharma) Z-score=> W (pharma)=>	V-M-GLN-43	V-M-TYR-49	V-S-TYR-49	V-S-ARG-53	V-S-TYR-229	V-M-PHE-292	V-S-PHE-292
1	1	Alpha-pinene	-48.8	0	0	0	0	0	0	0	0
1	0	Allicin	-44.9	0	0	-4.8662	-3.2024	-6.4745	-4.11096	-4.56902	-4.56902
1	0	Nimbin	27.7	0	0	-3.85474	-2.11404	-4.69906	-3.22054	-3.17293	-3.17293
1	0	Mangiferin	159.5	0	0	0	0	-0.158583	0	0	0
1	0	Punicalin	210.5	0	0	1.02217	-5.83653	-8.38577	0.398933	-5.12312	-5.12312
1	0			0	-5.13086	0	0	-0.981425	0	-1.07327	-1.07327

server. This server checks, builds, and repairs the protein structure and completes it with straight chain, side chains, and hydrogen atoms.

Then, the five herbal compounds to be analyzed in this study are chosen they are:

1. Allicin,
2. Nimbin,
3. Mangiferin,
4. Alpha-pinene,
5. Punicalin.

Allicin

It is an organosulfur compound obtained from medicinal plant garlic (*Allium sativum*).

Allicin (thio-2-propene-1-sulfinic acid S-allyl ester) has been identified as the most important medicinal compound of freshly crushed garlic.^[13,14] Garlic extract is effective against many dental and periodontal pathogens and inhibits arginine–gingipain, a cysteine protease that acts as a major virulence factor of *P. gingivalis*, the oral pathogen most associated with chronic periodontal disease.^[15]

Mangiferin

A xanthone derivative,^[16] mangiferin, is a pharmacologically active phytochemical present in large amounts in the bark, fruits, roots, and leaves of Mango (*Mangifera indica*). Mangiferin possesses antibacterial activity *in vivo* against specific periodontal pathogens such as *P. intermedia* and *P. gingivalis*.^[17] Mango stem bark extract is useful in preventing the hyperproduction of reactive oxygen species, and their oxidative tissue damage *in vivo* was more active than Vitamin C, Vitamin E, and β -carotene.^[18]

Alpha-pinene

Alpha-pinene is an organic compound of the terpene class, one of two isomers of pinene.^[19] Alpha-pinene proved extensively as strong antimicrobial substance^[20] and is an effective antimicrobial agent against persistent endodontic microorganisms. It is also reported that alpha-pinene as a component of essential oil, along with other allied ingredients extracted from aromatic plants, can be used in the treatment of oral intractable infections caused by *Enterococcus faecalis*, especially persistent endodontic infections, and they are found to be strong in biofilm state.^[21]

It is also used as anti-inflammatory through prostaglandin E1 (PGE1)^[22] and also reported to be a broad-spectrum antibiotic.^[23]

Nimbin

Neem, *Azadirachta indica*, an Indian origin tree, reported to possess many natural medicinal substances which are antihyperglycemic,^[24] immunomodulatory,^[25] anti-inflammatory,^[26] antimalarial,^[26] antioxidant,^[27] antiviral,^[28] antimutagenic,^[29] and anticarcinogenic^[30] properties. Neem also exhibits antibacterial,^[31] antifungal,^[32] hepatoprotective,^[32] and antiulcer.^[33]

The phytochemical constituents present in neem are nimbidin, nimbin, nimbolide, azadirachtin, gallic acid, epicatechin, catechin, and margolone. Nimbin is one of the many substances found in neem seeds and is reported to have several medicinal properties and it can also be used as an antibiotic.

For treating inflammation, infections, and skin diseases, the neem leaves, flowers, seeds, roots, bark, and fruits are utilized which have been proved to be also useful in dental care. For maintaining healthy gums and teeth, neem has been used. There are various compounds found in neem which include nimbin, nimbidin, ninbidol, sodium nimbidate, and azadirachtin are also found in neem acting as anti-inflammatory, antipyretic, antihistamine, antifungal, antimalarial, vasodilator, analgesic, antibacterial, and antiulcer agents.^[34,35]

Punicalin

Punica granatum (Pomegranate) contains seven highly active inhibitors of carbonic anhydrase (CA), i.e., punicalin, punicalagin, etc. Punicalin is an ellagitannin and was found to significantly inhibit nitric oxide production and suppress inflammatory cytokine expression.^[36] Oral health promotion can be obtained by pomegranate components that reducing the risk of gingivitis is among the items. Thrice daily mouth rinsing with pomegranate extract dissolved in water increased the levels of antioxidant activity and decreased activities of aspartate aminotransferase.^[37] Aspartate aminotransferase is considered an effective indicator of cell injury and is elevated among periodontitis patients.^[38] *Punica granatum* extract can be used to control the adherence of different microorganisms in the oral cavity.^[39]

Then, the 3D chemical structures of compounds such as allicin, nimbin, mangiferin, alpha-pinene, punicalin were obtained from the PubChem compound database. The PubChem compound database contains validated chemical depiction information provided to describe substances in PubChem substance. It was prepared using Biovia Discovery Studio 2016 where the standard delay format of this ligand was converted to PDBQT file using PyMol version 1.7.4.5 tool to generate atomic coordinates. The active sites are the coordinates of the ligand in the original target protein grids, and these active binding sites of target protein were analyzed using the Biovia Discovery Studio 2016.

For understanding the structural basis of this protein target specificity, a computational ligand-target docking approach was used to analyze structural complexes of adhesion protein with allicin, nimbin, mangiferin, alpha-pinene, punicalin. Docking was carried out by iGEMDOCK option based on scoring functions. Molecular docking can be considered as an optimization problem, which would describe the “best-fit” orientation of a ligand that binds to a particular protein of interest. However, since both the ligand and the protein are flexible, a “hand-in-glove” analogy is more appropriate than “lock-and-key.”^[40] During the course of the docking process, the ligand and the protein adjust their conformation to achieve an overall “best fit” and this kind of conformational adjustment resulting in the overall binding is referred to as “induced fit.”^[41] The energy of interaction of herbal components with the adhesion protein is assigned.

The binding energy indicated that affinity of adhesion protein docked with herbal components. Among the five compounds, alpha-pinene showed the highest negative value which indicates active binding to the target site and also showed the best interaction with target protein based on the root-mean-square deviation (RMSD) values as compared to standards.

The human oral cavity has a wide variety of microbial colonization where the oral tissues and tooth structure are being constantly exposed to their products. Extensive researches have carried out to inhibit the causative agents to prevent the oral diseases by formulating newer drug formulations and designs.

The active sites are the coordinates of the ligand in the original target protein grids, and these active binding sites of target protein were analyzed using the Biovia Discovery Studio 2016.

Results

The binding energy indicated the affinity of adhesion protein.

Among the five compounds, alpha-pinene showed the highest negative value of -48.8 (Table 1) which indicates active binding to the target site and also showed the best interaction with target protein based on the RMSD values as compared to standards (Table 2).

Discussion

All the five herbal compounds are found to be effective though each one has its own specific activity. The organosulfur compound from garlic, allicin, is virulent against oral pathogen *P. gingivalis* associated with periodontal disease whereas mangiferin proved to have additional control over *P. intermedia*.^[17] References are plenty for nimbin with large applications in general and dental care. Punicalin known for its active inhibitors of carbonic anhydrase has antioxidant and control microorganisms in oral cavity.^[39] Alpha-pinene, the terpene class organic compound, proved to be an extensively strong antimicrobial substance.^[20] It is also found to be effective in persistent endodontic microorganisms even if they are found in biofilm state,^[21] besides, broad-spectrum antibiotic.

Conclusion

P. gingivalis, an anaerobic, Gram-negative pathogen, and the bacterium most associated with chronic periodontitis, demonstrated highest sensitivity to alpha-pinene among the five herbal compounds. Alpha-pinene proved extensively as a strong antimicrobial substance²¹ and is an effective antimicrobial agent against persistent endodontic microorganisms. It is also reported that alpha-pinene as a component of essential oil, along with other allied ingredients extracted from aromatic plants, can be used in treatment of oral intractable infections caused by *E. faecalis*, especially persistent endodontic infections, and they are found to be strong in biofilm state.^[21] Alpha-pinene has been used medicinally in human history. Alpha-pinene is a bronchodilator in humans at low exposure levels and is

highly bioavailable with 60% human pulmonary uptake with rapid metabolism or redistribution.^[22] Alpha-pinene is an anti-inflammatory through PGE1^[22] and seems to be a broad-spectrum antibiotic.^[23] Further investigation should be done on more herbal compounds for the prevention and treatment of various endodontic diseases.

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