Swati Mohanty et al /J. Pharm. Sci. & Res. Vol. 9(7), 2017, 1246-1249

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Inhibition of purE Gene Using Herbal Compounds to Treat Oral Diseases Caused by Oral Pathogens – An in silico Study

Swati Mohanty
Postgraduate Student, Department of Conservative Dentistry and Endodontics
Saveetha Dental College, Saveetha University, Chennai, India.

Dr. Sindhu Ramesh
Professor and Head (Admin), Department of Conservative Dentistry and Endodontics
Saveetha Dental College, Saveetha University, Chennai, India.

Dr. Vishnu Priya V
Professor, Department of Biochemistry, Saveetha Dental College
Saveetha University, 162, Poonamallee High Road, Chennai 600077, India

Dr. Gayathri R
Assistant Professor, Department of Biochemistry, Saveetha Dental College
Saveetha University, 162, Poonamallee High Road, Chennai 600077, India

Abstract

Introduction
Oral diseases affect only a limited area but their consequences aren't limited to the mouth. It eventually affects the health of the entire body. Trepanoma denticola is a gram negative, obligate anaerobic bacterium that dwells in a complex and diverse microbial community within the oral cavity. purE enzyme inhibits conversion of AIR to CAIR thus preventing the formation of purine nucleotide essential for energy transformation in the micro organism.

Aim
This insilico study aims to analyze the inhibitory action of 5 different herbal compounds by computational docking studies to treat oral diseases caused by Treponema denticola.

Materials and Method
An insilico study was carried out using 5 herbal compounds: Allyl isothiocyanate, Eugenol, Cinnamaldehyde, Carvacrol and Thymol against the 3-D structure of purE protein.

Results
All the 5 compounds docked well with the target protein. Cinnamaldehyde used the least binding energy for docking with purE protein (-60.1) than the other 4 herbal compounds.

Conclusion
Docking studies of the 5 herbal compounds proved that all of these ligands are good molecules that docks well with the target purE protein. Out of these 5, Cinnamaldehyde showed the lowest interaction energy indicating that it has better docking ability and accuracy in interacting with the purE protein target.

Key Words: purE, Cinnamaldehyde, Eugenol, Thymol, Carvacrol, Docking, Insilco

INTRODUCTION

The human oral environment comprises of numerous microorganisms that grow and harbor in various parts of the oral cavity. Apart from these, oral tissues are constantly exposed to many environmental factors that eventually lead to tooth destruction. Dental caries and periodontal diseases are the most common and widely prevalent.

Oral diseases affect only a limited area but their consequences aren't limited to the mouth. It eventually affects the health of the entire body. WHO (World Health Organization) defines oral health as ' A state of being free from mouth and facial pain, oral and throat cancer, oral infection and sores, periodontal diseases, tooth decay, tooth loss and other diseases and disorders that limit an individual's capacity in biting, chewing , smiling, speaking and psychosocial well being'. Though optimum oral care plays a vital importance to prevent oral diseases, many new drug formulations and designs are under research aiming at the same purpose.

Trepanoma denticola is a gram negative, obligate anaerobic bacterium that dwells in a complex and diverse microbial community within the oral cavity. It has features needed for adherence, invasion and damage in the oral cavity. Extensive research has revealed that it primarily invades the periodontium. The ability of Treponema denticola to cause damage to the epithelial cells and human gingival fibroblasts may initiate a sequence of events that may lead to bacterial invasion of deeper layers of periodontal connective tissue as observed in humans and animal studies [1,2,3,4] .

It's a common knowledge that nucleotides serve as building blocks of nucleic acids. There are 2 pathways of synthesis

of purine nucleotides- the De Novo synthesis pathway and the Salvage pathway. The former is the main synthesis pathway for nucleotides [6].

De novo purine biosynthesis takes place by two divergent paths. In bacteria, yeasts and plants, AIR (5-aminoimidazole ribonucleotide) is converted to CAIR (4-carboxy-AIR) by two enzymes: N5-CAIR synthetase (PurK) and N5-CAIR mutase (Class I PurE). But in animals, it requires a single enzyme i.e., AIR carboxylase (class II PurE). Both types of PurE are mechanistically related but bind to different substrates[5]. Inhibiting this purE enzyme will further inhibit conversion of AIR to CAIR thus preventing the formation of purine nucleotide essential for energy transformation in the micro organism. Many insilico studies have been done using various chemical compounds, but we have come across no herbal compounds being used for this purpose.

This insilico study aims to analyze the inhibitory action of 5 different herbal compounds by computational docking studies to treat oral diseases caused by Treponema denticola.

MATERIALS AND METHOD

The 5 herbal compounds analyzed here are listed in the table below-

Table 1: Herbal products and their active compounds

Herbal Products	Active Compounds
1. Mustard, Horse radish	Allyl isothiocyanate
2. Cloves	Eugenol
3. Cinnamon	Cinnamaldehyde
4. Sage	Thymol
5. Oregano	Carvacrol

3D structures of the herbal compounds

Figure 1: Allyl isothiocyanate: (PubChem CID: 5971)

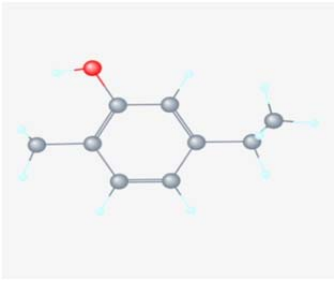

Figure 2: Eugenol: (PubChem CID: 3314)

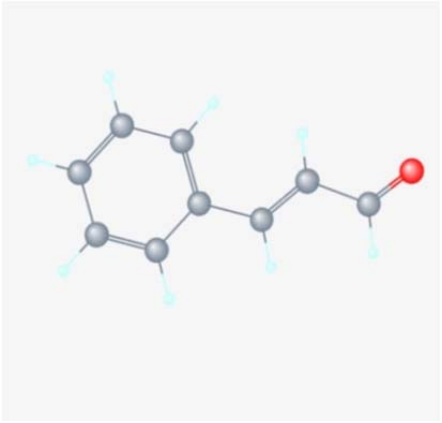

Figure 3: Cinnamaldehyde: (PubChem CID: 637511)

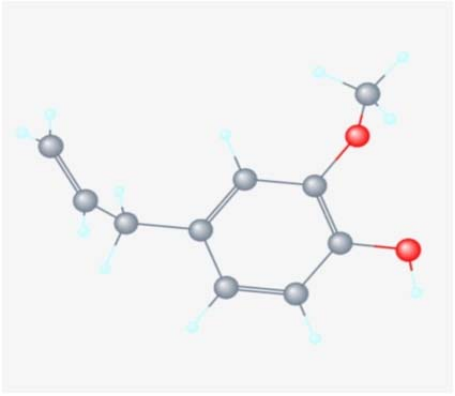

Figure 4: Carvacrol: (PubChem CID: 10364)

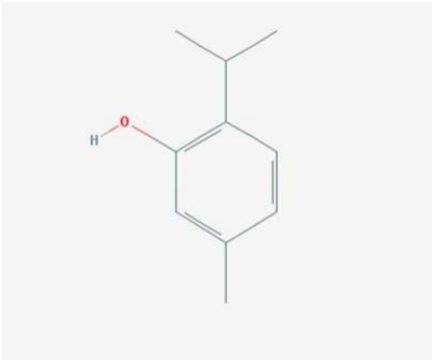

Figure 5: Thymol: (PubChem CID: 6989)

1. **Ligand selection**
   The Chemical structure of the above mentioned herbal compounds was obtained from PubChem compound database. It was prepared by ChemBio Draw and MOL SDF format of this ligand converted to PDBQT file using PyRx tool to generate atomic coordinates.
2. **Accession of Target Protein**
   The three dimensional structure of purE was downloaded from Protein Data Bank.

3. Target and Ligand Optimization

For Docking analysis, PDB coordinates of the target protein (purE) and the 5 herbal compound molecules were optimized using Discovery studio version 3.0 software. The Clean Geometry tool was applied to all 5 compounds to correct the structures. These coordinates

had minimal energy and stable structures. Protein correction was done using What if Server

4. Analysis of Target active Binding Sites

The active sites of the ligands and the binding sites of the target protein were analyzed using Drug Discovery Studio version 3.0.

5. Molecular Docking Analysis

A computational ligand-target docking approach was used to analyze structural complexes of PurE i.e., the target with the 5 herbal compounds i.e., Allyl isothiocyanate, Eugenol, Cinnamaldehyde, Thymol and Carvacrol. Docking was carried out using iGEm Dock software version 2.1. The positions were generated.

Table 2: Table depicting binding energy of the compounds

Top of cluster	Compound	Energy
1	cav3rgg_AIR-cinnamaldehyde-1.pdb	-60.1
0	cav3rgg_AIR-thymol-0.pdb	-58
0	cav3rgg_AIR-allyl isothiocyanate-0.pdb	-37.5
0	cav3rgg_AIR-carvacrol-0.pdb	-18.5
0	cav3rgg_AIR-eugenol-0.pdb	27.3

RESULTS AND DISCUSSION

Interaction Tables

Table 3(a): Energy Levels

Compounds	E(pharma)	V-M-GLY-10	V-S-HIS-40	V-M-ALA-66	V-M-GLY-67	V-S-ARG-68
	Z-score=>	0	0	0	0	0
	W(pharma)=>	0	0	0	0	0
cav3rgg_AIR-cinnamaldehyde-1.pdb	0	-6.081	-5.80684	-4.63601	-6.242	-0.785082
cav3rgg_AIR-thymol-0.pdb	0	-5.87164	-7.56685	-3.35989	-5.66462	-1.90797
cav3rgg_AIR-allyl isothiocyanate-0.pdb	0	-4.18385	-4.5461	-2.62251	-2.864	-0.0187479
cav3rgg_AIR-carvacrol-0.pdb	0	0	0	-0.128671	-3.21719	-5.89008
cav3rgg_AIR-eugenol-0.pdb	0	38.0129	-0.479792	-2.1676	-5.69564	10.1191

Table 3(b): Energy levels

Top of cluster	Compound	Energy	VDW	H Bond	Elec
1	cav3rgg_AIR-cinnamaldehyde-1.pdb	-37.482	-37.482	0	0
0	cav3rgg_AIR-thymol-0.pdb	-18.5247	-18.5247	0	0
0	cav3rgg_AIR-allyl isothiocyanate-0.pdb	-60.0705	-60.0705	0	0
0	cav3rgg_AIR-carvacrol-0.pdb	27.2935	27.2935	0	0
0	cav3rgg_AIR-eugenol-0.pdb	-58.0057	-58.0057	0	0

The binding energy indicated that PurE protein was successfully docked with the herbal compounds among which Cinnamaldehyde had the minimum binding energy (-60.1). The possible binding mode has been shown in Figure 6.

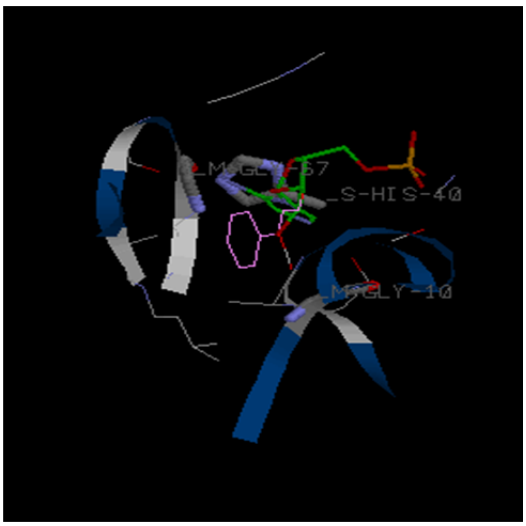

Figure 6

The antibacterial efficacy of cinnamaldehyde has been proved to show rapid inhibition of energy metabolism of bacterial cells. [7] The probable mechanism of cinnamaldehyde is its interaction with the cell membranes of the bacteria causing their disruption [7]. A study by Suxia

et al, 2015 [8] have shown that bacterial cell morphology, membrane integrity and permeability are damaged when E.coli and S. aureus are exposed to minimum inhibitory concentrations of cinnamaldehyde and the higher the cinnamaldehyde concentration, the more serious is the bacterial membrane damage. A study by Yossa et al [9] showed that cinnamaldehyde proved to be extremely effective against E.coli O157:H7 as well as Salmonella typhi. This result is in agreement with another study done by Kim et al [10] who observed complete inhibition of E.coli in 2 hours in cinnamaldehyde solution. Microscopic analysis revealed that cell membranes of the bacteria were severely affected rather than its cellular contents. Di Pasqua et al [11] stated that cinnamaldehyde had the ability to structurally alter the outer envelope of E.coli leading to its membrane disintegration. On the other hand, the studies have also stated that Salmonella typhi seemed to have been affected both internally and externally by cinnamaldehyde [9, 12]. Cinnamaldehyde also was observed to inhibit growth of Clostridium botulinum [17] and Staphylococcus aureus [18, 21].

There have been many studies reporting the antimicrobial activity of other aromatic molecules like Eugenol and Carvacrol. [13, 14, 15, 22, 23] Eugenol has been reported to inhibit E.coli and Listeria monocytogenes [16, 25]. As seen in Eugenol, the possible mechanism of cinnamaldehyde could be inhibition of synthesis of cell wall [19, 24] or inhibition of biosynthetic enzymes [20] is unlikely because of ATP inhibition [7].

This computational docking analysis revealed that all the 5 herbal compounds binding successfully with the target protein out of which Cinnamaldehyde had the lowest interaction energy proving its potential inhibitory effect against purE protein that is essential for survival of Treponema denticola. Further studies needs to be undertaken to put forward more concrete evidence of anti-bacterial action of cinnamaldehyde.

CONCLUSION

Docking studies of the 5 herbal compounds- Allyl isothiocyanate, Eugenol, Cinnamaldehyde, Carvacrol and Thymol proved that all of these ligands are good molecules that docks well with the target purE protein. Out of these 5, Cinnamaldehyde showed the lowest interaction energy indicating that it has better docking ability and accuracy in interacting with the purE protein target.

REFERENCES

1. Micheal N. Sela, Role of Treponema denticola in periodontal diseases.Crit Rev Oral Biol Med, 12(5):399-413, 2001.
2. Fenno JC, Wong GWK, Hannam PM, McBride BC. Mutagenesis of outer membrane virulence determinants of the oral spirochete Treponema denticola. FEMS Microbiol Lett 163:209-215, 1998.
3. Haapasalo M, Singh U, McBride BC, Uitto VJ. Sulfhydryl-dependant attachment of Treponema denticola to laminin and other proteins. Infct Immun 58:347-351,1991.
4. Dawson JR, Ellen RP. Clustering of fibronectin adhesions toward Treponema denticola tips upon contact with immobilized fibronectin. Infect Immun 62:2214-2221, 1994.
5. Sylvian Tranchimand, Courtney M. Starks, Irimpan I. Mathews, Susan C. Hockings, T. Joseph Kappock. Treponema denticola PurE is a bacterial AIR Carboxylase. Biochemistry, 50, 4623-4637, 2011.
6. Mc Curry JE, Begley TP. The organic chemistry of biological pathways. Roberts and Company, 2005.
7. Alexander O. Gill, Richard A. Holley. Mechanisms of Bactericidal action of Cinnamaldehyde against Listeria monocytogenes and Eugenol against L. monocytogenes and Lactobacillus sakei. Applied Environmental Microbiology, 70(10): 5750-5755, 2004.
8. Suxia Shen, Teihua Zhang, Yuan Yuan, Haiqing Ye. Effects of cinnamaldehyde on Escherichia coli and Staphylococcus membrane. Food Control 47: 196-202. January 2015.
9. Yossa Nadine, Patel Jitendra, Macarisin Dumitru, Millner Patricia, Murph Charles. Antibacterial Activity of Cinnamaldehyde and Sporan against Escherichia coli O157:H7 and Salmonella. Journal of Food Processing and Preservation. August, 2012.
10. Kim H.-O., Park,S.-W and Park, H.-D. Inactivation of Escerichia coli O157:H7 by cinamic aldehyde purified from Cinnamomum cassia shoot. Food Microbiol. 21, 105-110, 2004.
11. Di Pasqua R Betts G., Hoskins N, Edwards M, Ercolini D, Mauriello G. Membrane toxicity of antimicrobial compounds from essential oils. Journal of Agric. Food Chem, 55,4863-4870, 2007.
12. Smid EJ, De Bont J.A.M and Poolman B. Mechanisms of membrane toxicity of hydrocarbons.Microbiol.Rev. 59, 201-222, 1995.
13. Jay.J.J, G.M. Rivers. Antimicrobial activity of some food flavoring compounds .J. Food Saf. 6: 129-139, 1984.
14. Juven, B. J., J. Kanner, F. Schved, and H. Weisslowicz . Factors that interact with the antibiotic action of thyme essential oil and its active constituents. J. Appl. Bacteriol.76**:**626-631, 1994.
15. Moleyar, V., and P. Narasimham. Antibacterial activity of essential oil components. Int. J. Food Microbiol. 16**:**337-342. 1992.
16. Blaszyk, M., and R. A. Holley . Interaction of monolaurin, Eugenol and sodium citrate on growth of common meat spoilage and pathogenic organisms. Int. J. Food Microbiol.39**:**175-183, 1998.
17. Bowles, B. L., and A. J. Miller . Antibotulinal properties of selected aromatic and aliphatic aldehydes. J. Food Prot. 56**:**788-794, 1993.
18. Bowles, B. L., S. K. Sackitey, and A. C. Williams. Inhibitory effects of flavor compounds on *Staphylococcus aureus* WRRC B124. J. Food Saf. 15**:**337-347. 1995.
19. Kwon, J. A., C. B. Yu, and H. D. Park. Bactericidal effects and inhibition of cell separation of cinnamicaldehyde on *Bacilluscereus*. Lett.Appl. Microbiol. 37**:**61-65, 2003.
20. Wendakoon, C. N., and M. Sakaguchi . Inhibition of amino acid decarboxylase activity of *Enterobacter aerogenes* by active components in spices. J. Food Prot. 58**:**280-283, 1995.
21. Shelef, L. A. . Antimicrobial effects of spices. J. Food Saf. 6**:**29-44, 1983.
22. Ultee, A., E. P. W. Kets, and E. J. Smid. Mechanisms of action of Carvacrol on the food-borne pathogen *Bacillus cereus*. Appl. Environ. Microbiol. 65**:**4606-4610, 1999.
23. Ultee, A., M. H. J. Bennik, and R. Moezelaar. The phenolic hydroxyl group of Carvacrol is essential for action against food-borne pathogen *Bacillus cereus*. Appl. Environ. Microbiol. 68**:**1561-1568, 2002.
24. Walsh, S. E., J. Y. Maillard, A. D. Russell, C. E. Catrenich, D. L. Charbonneau, and R. G. Bartolo. . Activity and mechanisms of action of selected biocidal agents on Gram-positive and -negative bacteria. J. Appl. Microbiol. 94**:**240-247, 2003.
25. Parker, C., and R. W. Hutkins**.** *Listeria monocytogenes* Scott A transports glucose by high-affinity and low-affinity glucose transport systems. Appl. Environ. Microbiol.63**:**543-546, 1997.