Phytocompounds from *Zingiber zerum*bet can inhibit the DNA ligase activity of the pathogen *Haemophilus influenza*, an *in silico* approach

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Abstract:

*Haemophilus infl*uenza is a pathogenic bacterium responsible for causing various diseases, especially to the children. Due to the numerous reports of antibiotic resistance against this bacterium, the necessity of employing alternative therapy is emphasized by various researchers. Herbal medicines are consistently proven to be successful for managing various pathogen related conditions by numerous researchers and in this backdrop our study aimed to study the role of phytocompounds against *H. influenza*, using DNA ligase as a drug target. Phytocompounds namely α humulene, zeumbone and camphene, isolated from the Zingiberaceous plant *Z. zerum*bet were subjected to study their inhibitory potentials against the bacterial enzyme DNA ligase, using a rigid body *in silico* docking method (PatchDock). The result indicated that these phytochemicals especially α humulene, may be successfully employed as an anti-bacterial agent against *H. influenza*.

Keywords: *Haemophilus infl*uenza, Phytocopounds, Docking, DNA ligase

INTRODUCTION

Haemophilus influenza is a bacterium that belongs to the *Pasteurellaceae* family; it is a gram-negative Coccobacillus and is pleomorphic in nature. It was first discovered by Pfeiffer in the year 1892 (Turk, 1984). There are basically six types of *H.influenzae* based on their capsular composition (type a to f) (Loeb and Smith, 1980) and are capable of causing a number of human infections. They can cause serious systemic diseases in children especially due to their weak immune system. Out of all the varieties of capsular strains, the most virulent and pathogenic one *Haemophilus influenza* type b (Hib), which is responsible for about 95% of most of the invasive diseases (Turk and May, 1967). The presence or absence of the polysaccharide capsule on the bacterium further divides it into two types, the typeable form like that of Hib and the non-typeable forms (NTHi) that are non-capsular. NTHi mainly causes respiratory diseases whereas the systemic disease like meningitis is commonly caused by encapsulated strains (King, 2012).

The ability of *H. influenza* to colonize in the respiratory tract and cause infection is attributed to its pathogenic properties and mechanism. It has the ability to interact and attach to the mucosal layer with the help of the outer membrane proteins such as P5, P2 and protein D (King, 2012). A number of researchers have demonstrated the use of adhenins like HMW1 and HMW2 for the bacterial attachment to the epithelial cell (St Geme et al., 1993). Other pathogenic property of the bacterium includes its ability to secrete endopeptidases called IgA proteases ,which in turn act against human antibody (IgA) thereby inhibiting antibody activation (Mulks et al., 1982). One of the important

features of *H. influenza* is its capacity to survive within the cells especially the NTHi types. Studies have shown that they can survive for about 72 hours within cells like macrophages and epithelial cells (King, 2012).

Due to the increasing problem of antibiotic-resistant microorganisms, the need for new and better alternative for antibiotics has become highly relevant. The utilization of natural compounds such as from plant extracts, used in the earlier ages, is coming into existence again. The sequencing of microbial genome has led to new target ideas such as DNA synthesis and its reaction components (Brötz-Oesterhelt et al., 2003). Many enzymes and proteins carry out the process of replication in prokaryotic organisms. One of the most important enzymes for polynucleotide ligation is executed by DNA and RNA ligase. These have become an area of interest as a chemotherapeutic and drug targets in order to treat a variety of bacterial diseases (Swift and Amaro, 2009). **Pyridochromanones, Pyridopyrmidines and Arylamino are some of the compounds discovered against DNA ligase in different microorganisms** (Swift and Amaro, 2009)**.**

A significant number of plants and their chemical derivatives are already reported due to their antimicrobial activities (Gibbons, 2004). Plant compounds like phenols, terpenes, tannins, flavonoids etc. are known to have a variety of antimicrobial potentials (Sibanda and Okoh, 2007). *Zingiber zerumbet*,of the botanical family Zingiberaceae is commonly known as shampoo ginger and of high medicinal importance (Yob et al., 2011). Various studies have shown its potential property as a pharmacological agent for its anti- inflammatory (M. N. Somchit, 2012), antioxidant(Hamid et al., 2018) and anti-bacterial activities(Sutardi et al., n.d.). They are capable of producing volatile oils constituting natural compounds such as α humulene, zeumbone and camphene (M. N. Somchit, 2012).

 Zerumbone belongs to the chemical class of monoterpenoid which can be isolated from the rhizome of the plant *Zingiber zerumbet*, and is highly used as a pain killer for its anti-inflammatory and analgesic properties(M. N. Somchit, 2012). Its antimicrobial and anticancer activity was studied extensively (Hamid et al., 2018).On the other hand, camphene is an alkaloid and α humulene, is a naturally occurring sesquiterpene . The activity of camphene and alpha-humulene are reported by earlier investigator as a natural insecticide (Benelli et al., 2018). On the other hand anti-inflammatory property of α -humulene in mice and rats was also reported earlier (Fernandes et al., 2007).

The development of new drugs and their analysis as to how they react with a specific receptor or rotein has become much easier in the present era due to extensive use of *in silico* or computational biology methods (Nag and Mukherjee, 2014). This study aims to investigate and study the comparative inhibitory effect of three phytocompounds (reported from *Zingiber* zerumbet) namely humulene, zeumbone and camphene against *H. influenze* DNA ligase (PDB id 3PN1). The computational and comparative study of these compounds with the help of PatchDock server algorithm stipulated zerumbone as a prospective inhibitor of DNA ligase.

METHODOLOGY

Selection of Ligands

Ligands were selected based on the therapeutic principles through literature survey and three dimensional structures of three phytocompounds (reported from the plant *Zingiber zerumbet*) namely Humulene (CID: 5281520), Zerumbone (CID: 440966) and Camphene (CID: 440966) were downloaded from the IMPPAT database (Mohanra et al., 2018).

Protein Structure:

The three dimensional structure of NAD+-dependent DNA ligase (Lig A of *Haemophilus influenzae*, 3PN1) was downloaded from RCSB PDB (Protein Data Bank, Berman et al., 2000). Protein structure was refined using the software UCSF Chimera (Pettersen et al., 2004).

Docking:

Protein-Ligand docking was performed using the server PatchDock (Duhovny et al., 2002).

RESULT AND DISSCUSSION

Effective biological processes are dependent upon the receptor-ligand interactions and hence the structure is required to be stable, both chemically and biologically, to be an active compound (Duhovny et al, 2002). To understand the structural stability of a ligand binding at the active site of a target enzyme, molecular docking technique is helpful to computationally predict the optimal structure (Singh et al, 2018).

A high-throughput screening campaign carried out by the AstraZeneca corporate library tested nearly 517 compounds to identify inhibitors of *H. influenzae* LigA (DNA ligase) by using a FRET-based DNA ligation assay. The results confirmed the activity of various analogues of adenosine acts against the DNA ligase LigA, particularly IC_{50}s (Mills et al, 2011). Further, studies involving gene knockout has shown that Lig A is has significant role in maintaining the integrity of the DNA in several bacteria, including *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, and M. tuberculosis* (Srivastava et al, 2005). Since the DNA ligase (Lig A) is exclusive to bacterial system, it is therefore a novel and attractive drug target site (Srivastava et al, 2005).

The rhizome of *Zingiber zerumbet* is rich in essential oils, which have been reported to possess various therapeutic properties such as anti-inflammatory, analgesic, anticancer, antioxidant, antinociceptive, antitussive etc (Munda et al, 2018). PatchDock was used to compare bacterial NAD+-dependent DNA ligase (Lig A of *Haemophilus influenzae,* 3pn1) against three compounds from *Zingiber zerumbet* (Zerumbone, Camphene and α Humulene). These compounds were tested using a rigid body docking method (Patchdock) to understand the possible structural configurations to inhibit the active sites on DNA Lig A.
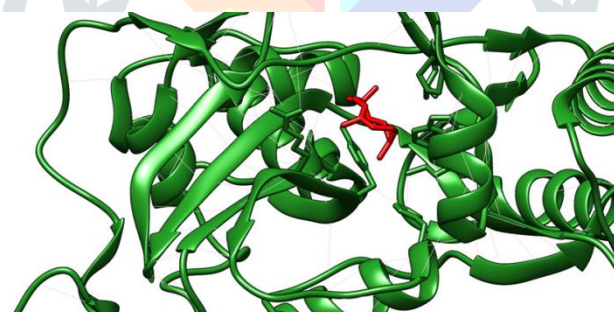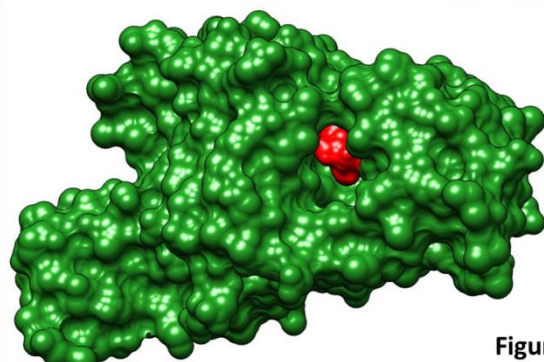

Figure: 1A

Figure: 1B

Figure: 1C

Figure 1:

Figure 1A: 3D dimensional structure of α Humulene

Figure 1B: Ligand (α Humulene)-Protein Docking (DNA ligase), Ribbon view (Green-Receptor; Red-Ligand)

Figure 1C: Ligand (α Humulene)-Protein Docking (DNA ligase), Surface view, Ribbon view (Green-Receptor; Red-Ligand)

According to the PatchDock analysis, α Humulene (CID: 5281520) shows the highest affinity to the DNA ligase (LigA) with an ACE value of -116.56 and the score of 3832 (Fig. 1), followed by the Zerumbone (CID: 440966) showing the second highest affinity towards the compound with an ACE value of -113.41 and score of 3788, whereas the least affinity towards the DNA ligase in the complex setting is shown by Camphene (CID: 440966) with an ACE value of -23.22. Zerumbone has comparatively less interface area than the Humulene that is 398.60 and 405.30 respectively (Zhang et al, 1997).

CONCLUSION

It can be concluded form the current study that α Humulene (CID: 5281520) is the potential drug candidate against *H. influenzae* LigA (DNA ligase) protein.

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