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ANALYSIS OF THE MECHANISM OF ACTION BY MOLECULAR DOCKING STUDIES OF ONE ETHNO VETERINARY HERBAL PREPARATION USED IN BOVINE MASTITIS

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ABSTRACT

Mastitis, a disease in dairy cattle characterized by inflammation due, but not necessarily limited, to microbial infection in the mammary gland. Mastitis leads to loss in terms of reduction in milk, milk discards, early culling, increasing labour cost and veterinary services. The mammary tissue damage decreases the number and the activity of epithelial cells, caused by bacterial factors and host immune system through necrosis or apoptosis, which can be differentiated by the changes in morphological, biochemical and molecular characters of the dying cells. However, bacteria and their products contribute to the initial development of the disease. *Staphylococcus aureus* is a pathogen with a broad range of hosts and mastitis is a major disease caused by it. It has the ability to colonize the host tissue, causing more acute relapsing infection than other *staphylococcus* species. Many strategies have been employed in the treatment among which the topical application of herbal paste comprising *Aloe Vera*, turmeric powder and lime has been effective against the infection though the mechanism of action is not fully understood. The present study uses the *in silico* approach to find the effect of the herbal preparation against the infection. The bioactive compounds were tested for its effect against the target proteins of *S. aureus* using molecular docking studies.

KEYWORDS: Mastitis – in-Silico – Computer Aided Drug Discovery

INTRODUCTION

Bovine mastitis is a disease of economic importance, because of the reduced milk quality and quantity; veterinary services and drug usage. It is characterized by the inflammation of the mammary gland, commonly caused by microbes and recognized by the abnormalities in the udder and milk (Dodd and Jackson, 1971). When the physical barriers such as teat is disturbed, pathogenic bacteria enter the sterile environment of the mammary gland (Atiken, 2011; Sordillo, 1997). In spite of the controversy, the pathogens causing mastitis are grouped into environmental and contagious (Zadoks, 2014). Occurrence of mastitis depends on age, lactation stage and somatic cell score (SCC) history (Kehrli, 1994; Steeneveld, 2008). As the immune response depends on the type of pathogen, the host requires a pathogen specific response for protection (Bannerman, 2008; Wellnitz, 2011).

REVIEW OF LITERATURE

Mastitis can be subclinical or clinical. At subclinical level, there are no visual signs of inflammation (De Vliegher, 2012). On the other hand, clinical mastitis causes inflammation in the mammary gland tissue and abnormality in milk. In mild or moderate clinical mastitis, swelling, pain, heat and redness of the udder is observed. Severe case of clinical mastitis, the inflammation response includes systemic involvement, causing anorexia, fever and shock

(Ballou, 2011; Zhao, 2007). The mammary gland of bovine is equipped with anatomical non-immune barrier and adaptive and innate immune responses which are specific and non-specific, respectively (Borghesi et al., 2007).

Even though there have been over 130 microorganisms known to cause the infection, *Staphylococcus aureus*, *Streptococcus agalactiae, Strep. dysgalactiae, Strep. uberis* are the common types of pathogens among the Gram-negative bacteria (Watts, 1988). Though *S. aureus* causes relatively low grade mastitis, co-infections may increase the severity and may lead to death. Early in the dairy business, this pathogen was the predominant cause of clinical mastitis (Hillerton and Berry, 2005).

The use of antibiotics for the treatment for mastitis, limited to some kind of severe cases, includes bacterial selection by diagnostic-techniques and selection of antibiotics (Roberson, 2012). The regular use of antibiotics lead to production of milk with antibiotic residues and development of antibiotics resistance strains (Gao et al, 2012; Oliver and Murinda, 2012; Wang et al., 2012; Nosanchuk et al., 2014). In spite of the use of antibiotics as the conventional treatment, alternative herbal, and homeopathic approaches also contribute to the treatment (Hillerton and Berry, 2005). One such treatment is that the topical application of aloe Vera, turmeric and lime as a paste. This treatment is clinically proved, though the exact mechanism of action is not known.

In this study, the probable mechanism of action through which the herbal preparation targets the *S. aureus* is studied using molecular docking. In the field of molecular modelling, molecular docking predicts the preferred orientation of one molecule with another when in a stable complex by binding to each other. The binding conformation of the complex is used to predict the binding affinity using scoring functions. Docking is frequently used to predict the binding conformation of drug molecules with their protein targets to study the binding affinity of drug molecule to the proteins (Jubieet al, 2011). In the current study, the bioactive molecules from turmeric (Li et al., 2011), *Aloe Vera* (Saljooghianpour et al, 2013) and lime were docked against 6 protein targets. Biotin protein ligase (BPL) is a potential target for antibiotics for drug resistant pathogens, as it is a bifunctional protein possessing biotin ligase activity and transcriptional repressor activity. Blocking of BPL results in disruption in some key metabolic pathways (Pendini et al, 2013). Inhibiton of DNA gyrase results in inhibition of DNA synthesis (Fournier et al., 2000). OpuCB is a probable glycine betaine/carnitine/choline ABC transporter. Sir A functions as airon-regulated ABC transporter siderophore-binding protein (Balaji et al., 2014). Penicillin-binding proteins (PBP) are targets for β-lactam antibiotics (Stapleton and Taylor, 2002). Sortase A (Srt A) is a surface protein, which mediates the adhesion to specific organ, tissue and host immune system invasion (Wang et al, 2015).

MATERIALS AND METHODS

3D structures of the target proteins BPL, DNA gyrase, opuCB, sirA, SrtA and PBP were retrieved from Protein Data Bank (PDB) (www.rcsb.org) with PBD ID 3V7S, 3G7B, 3O66, 3MWF, 1T2W and 3VSL respectively. The structure of the bioactive components of turmeric, *Aloe Vera* and lime was retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in SDF file format (*. sdf). A total of 178 components were used in this study, including 24 components from *Aloe Vera*, 148 components from turmeric and 6 components from lime.

The ligands were prepared for docking by removing the geometry to facilitate the flexible docking. The additional molecules, including ligands and water molecules in the target protein structures were removed to avoid their interference in docking followed by the addition of CHARM mforcefield. The binding pockets in the target proteins were predicted using tools in Accely's Discovery Studio 4.0. Molecular docking for the ligands and target proteins were performed using

the LIGANDFIT algorithm as explained by Ophelia et al, 2016. The binding affinity for each interaction was given as Dock Score.

RESULTS AND DISCUSSIONS

The 3D structures of the target proteins are shown in Table 1, along with their respective numbers of binding sites and PDB ID.

Table 1

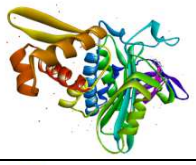
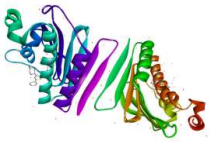
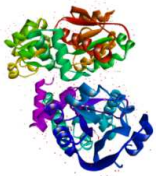
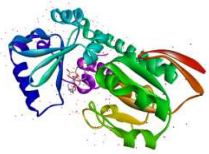
Target	PDB ID	Structure of Target	Total Binding Sites
BPL	3V7S		7
DNA gyrase	3G7B		5
opuCB	3O66		14
sirA	3MWF		7
SrtA	1T2W		14
PBP	3VSL		44

Table 2: Lists Out the Components Having Highest Dock Score and their Respective Dock Score. In Addition to that it Lists the 2D Diagram of the Interaction Between the Particular Component and its Orientation with the Protein Whose Surface Indicates the Hydrophobicity

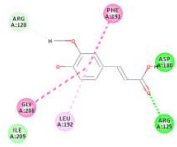
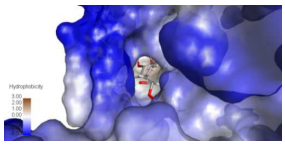
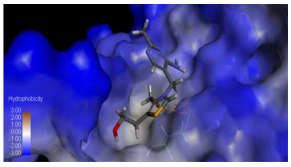
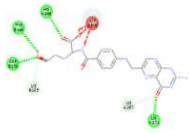
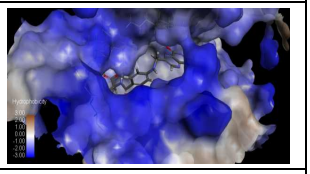
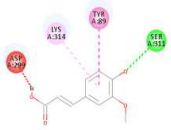
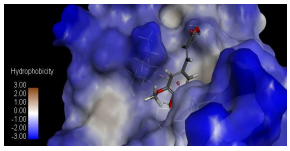
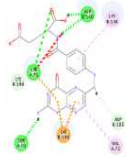
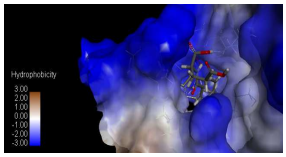
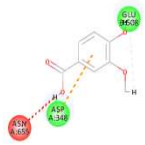
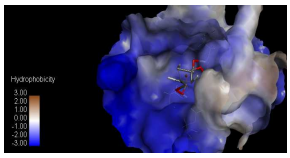
Target	Component Having Highest Dock Score	Interacting Site	Dock Score	2D Diagram of the Interaction	3D Diagram of the Interaction
BPL	Ferulic Acid	1	87.484		
DNA gyrase	Thiamine	4	59.892		
Opu CB	Folic Acid	1	73.989		
Sir A	Ferulic Acid	7	59.065		
SrtA	Folic Acid	1	62.437		
PBP	Vanillic Acid	32	120.15		

Table 3: Indicates the Name, Distance, Category and Type of the Favourable Bonds Formed Between the Component and the Target

Target	Component	Interacting Site	Distance	Category	Type
BPL	Ferulic Acid	1	2.02453	Hydrogen Bond	Conventional Hydrogen Bond
BPL	Ferulic Acid	1	0.431391	Hydrogen Bond	Conventional Hydrogen Bond
BPL	Ferulic Acid	1	1.95041	Hydrogen Bond	Carbon Hydrogen Bond
BPL	Ferulic Acid	1	4.31418	Hydrophobic	Amide-Pi Stacked
BPL	Ferulic Acid	1	5.43498	Hydrophobic	Amide-Pi Stacked
BPL	Ferulic Acid	1	5.11655	Hydrophobic	Pi-Alkyl
DNA gyrase	Thiamine	4	2.95112	Electrostatic	Attractive Charge
DNA gyrase	Thiamine	4	3.25899	Electrostatic	Pi-Anion
opuCB	Folic Acid	1	1.73886	Hydrogen Bond	Conventional Hydrogen Bond
opuCB	Folic Acid	1	2.95952	Hydrogen Bond	Conventional Hydrogen Bond
opuCB	Folic Acid	1	1.94424	Hydrogen Bond	Conventional Hydrogen Bond
opuCB	Folic Acid	1	2.56994	Hydrogen Bond	Conventional Hydrogen Bond
opuCB	Folic Acid	1	2.27906	Hydrogen Bond	Carbon Hydrogen Bond

Table 3: Contd.,

Target	Component	Interacting Site	Distance	Category	Type
opuCB	Folic Acid	1	3.06675	Hydrogen Bond	Carbon Hydrogen Bond
opuCB	Folic Acid	1	2.65844	Hydrogen Bond	Carbon Hydrogen Bond
sirA	Ferulic Acid	7	2.02083	Hydrogen Bond	Conventional Hydrogen Bond
sirA	Ferulic Acid	7	2.49786	Hydrogen Bond	Carbon Hydrogen Bond
sirA	Ferulic Acid	7	4.25847	Hydrophobic	Pi-Pi Stacked
sirA	Ferulic Acid	7	4.56039	Hydrophobic	Pi-Alkyl
Target	**Component**	**Interacting Site**	**Distance**	**Category**	**Type**
SrtA	Folic Acid	1	2.04678	Hydrogen Bond	Conventional Hydrogen Bond
SrtA	Folic Acid	1	1.48943	Hydrogen Bond	Conventional Hydrogen Bond
SrtA	Folic Acid	1	1.99631	Hydrogen Bond	Conventional Hydrogen Bond
SrtA	Folic Acid	1	2.58823	Hydrogen Bond	Carbon Hydrogen Bond
SrtA	Folic Acid	1	2.9175	Hydrogen Bond	Carbon Hydrogen Bond
SrtA	Folic Acid	1	4.98558	Electrostatic	Pi-Cation
SrtA	Folic Acid	1	3.45965	Electrostatic	Pi-Cation
SrtA	Folic Acid	1	2.41772	Hydrogen Bond; Electrostatic	Pi-Cation; Pi-Donor Hydrogen Bond
SrtA	Folic Acid	1	5.45351	Hydrophobic	Pi-Alkyl
SrtA	Folic Acid	1	4.30438	Hydrophobic	Pi-Alkyl
SrtA	Folic Acid	1	4.39216	Hydrophobic	Pi-Alkyl
SrtA	Folic Acid	1	4.58205	Hydrophobic	Pi-Alkyl
SrtA	Folic Acid	1	4.85875	Hydrophobic	Pi-Alkyl
PBP	Vanillic Acid	32	0.19645	Hydrogen Bond	Conventional Hydrogen Bond
PBP	Vanillic Acid	32	0.594321	Hydrogen Bond	Conventional Hydrogen Bond
PBP	Vanillic Acid	32	2.3703	Hydrogen Bond	Carbon Hydrogen Bond
PBP	Vanillic Acid	32	3.31063	Electrostatic	Pi-Anion

The results from the molecular docking showed that many bioactive components from aloe Vera and turmeric interact with the target proteins. The components of lime had interaction with lesser affinity. Among the interactions 74 interactions were having Dock Score above 50. From these results, it is evident that the proteins are targeted by many bioactive components. Among the bioactive components, three were able to target all the proteins. They are vanillic acid, Ferulic acid and cur cumin III from turmeric.

Pharmacodynamic Study

All the active ingredients of *Aloe Vera*, turmeric and lime were subjected to pharmacodynamic study, using the online server PASS. The server reveals that the compounds in the herbal preparation process, anti-inflammatory, anti-healing and anti-bacterial properties.

CONCLUSIONS

The study shows that many of the bioactive components of turmeric and *Aloe vera* are effective against the target proteins. The proteins are targeted by many components and three components target all the proteins. Thus, the infection caused by *S. aureus* can be treated by targeting its essential proteins. Hence, the tropical application of turmeric, *Aloe Vera* and lime can be used to treat bovine mastitis. Though the mechanism of action is studied, the significance of the particular quantity ratio of the turmeric, *Aloe Vera* and lime is important, and further studies have to be conducted.

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