Comparative Study of Antibacterial Activity of Tulsi, Garlic and Commercially used Antibiotics against Bovine Bacterial Endometritis using Molecular Docking Analysis

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ABSTRACT

The present study was conducted with the objective to find effective drug against bacterial bovine endometritis using Molecular docking. The antibacterial property of selected plants was studied and its comparison with conventional antibiotics was done. The plants selected for study were Tulsi and Garlic on the basis of their potent antibacterial activity. Selection of bacteria (*S. aureus* and *E. coli*) for the present study was done on the basis of most predominant bacteria causing bovine endometritis as observed in previous studies. The selected bacterial proteins were docked against the phytochemicals of plants. Vital proteins of *Staphylococcus aureus* are Gyrase B (3G75), FtsA (3WQU), DNA ligase (3JSN), Dehydrosqualene synthase (3TFN) and *Escherichia coli* are FtsZ (6UMK), DNA gyrase (1KZN) and ZapD (5DKO). The docking procedure was carried out using Autodock. It was concluded that, gentamicin (-6.95 Kcal/mol), enrofloxacin (-6.97 Kcal/mol) and Tulsi (-6.40 Kcal/mol) showed better binding affinity followed by garlic (-3.89 Kcal/mol). Apigenin, a phytoconstituent found in tulsi, demonstrated the highest binding affinity(ranging from -7.2 Kcal/mol to -9.2 Kcal/mol) of all the phytochemicals found in garlic and tulsi against the targeted proteins found in bacteria. Hence, Tulsi alone or in combination with garlic can be used for further *in vivo* or *in vitro* trials in the direction of effective drug discovery against bacterial bovine endometritis.

Key words: Autodock, Bovine endometritis, Docking, Garlic, Tulsi.

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INTRODUCTION

There are many factors affecting reproduction but the presence of unwanted microbes in the genital tract results into pathological conditions and act as a main reason for infertility (Patel *et al.*, 2009). Reproductive disorders such as endometritis (Sharma, 2017), metritis, retained foetal membrane (RFM), pyometra, and some non-specific infections in dairy animals can all have an impact on reproduction, which in turn can result in infertility in animals (Deori and Arundhati, 2015). The inflammation of endometrium refers to endometritis which is mainly observed after ≥ 21 days of parturition and identified by the presence of pus cells (>50%) (purulent and mucopurulent) in the vaginal secretions originating mainly from uterus with or without any systemic illness (Sheldon *et al.,* 2006). Clinically, it is characterized by the presence of pus flakes in the uterine discharge. The reported incidences of clinical and subclinical endometritis in crossbred cows were 2% and 29.69%, respectively (Pillai, 2012).

In bovines, bacterial infection is one of the most common causes of endometritis, which leads to a repeat breeding (Purohit, 2008). Gram-negative endometrial pathogenic *Escherichia coli*, gram-positive *Trueperella pyogenes*, and numerous anaerobes are all associated with clinical endometritis (Bicalho *et al.*, 2012).

The treatment using antimicrobials showed varying degrees of success with various limitations like its milk disposal, inconsistent recovery rate, emergence of microbial resistance, and reduced phagocytic activity of polymorphonuclear leukocytes (PMN cells). The emergence of bacterial resistance results due to indiscriminate usage of antimicrobials without any antibiotic sensitivity tests (Gupta and Deopurkar, 1993).

Hence to minimize the antibacterial resistance and other problems related to the use of antibiotics, scientists are approaching towards the herbal therapy as it is cost-effective and has no harmful effects. In the early stages of drug discovery, *in silico* models and experimentation can be a vital part of the new drug development process. Using in silico technology for early drug development can help to save both time and money. *In silico* models pose no potential harm to animals as experiments or research is conducted via computers. *In silico* trials are also called virtual trials. Computer-aided drug screening with molecular design software and *in silico* trials can reduce drug discovery costs and improve efficiency in the drug development process. There are number of computational methods among which most efficient is 'Molecular Docking' which is used to predict the resultant effect of any drug with the help of software. As it reduces experimental cost, performance time, skilled personnel requirement, laboratory set-up, use of experimental animals etc.

Therefore, in the present study, antibacterial property of selected plants was analyzed on the basis of results obtained after the 'Molecular Docking' of phytochemicals of Tulsi and Garlic to target proteins of *Staphylococcus aureus and E. coli*. Various vital proteins i.e. Gyrase B, DNA ligase, dehydrosqualene synthase and FtsA protein from *S. aureus* and DNA gyrase, FtsZ and ZapD protein from *E. coli* were selected. Similarly, Phytochemicals such as Thymol, Eugnol, Linalool, Carvacrol, Apigenin and Caryophyllene of Tulsi and Allicin, Ajoene, Diallyldisulphide (DADS) and Diallyltrisulphide (DATS) of Garlic were considered for docking.

MATERIALS AND METHODS

Selection of target proteins of *Staphylococcus aureus* and *Escherichia coli*: In previous studies, it was reported that bacteria obtained causing endometritis were *Staphylococcus aureus* and *E. coli spp.* at Instructional Dairy Farm, Nagla (IDF), Pantnagar (Painuly, 2018) as predominant and most pathogenic microbes. The target proteins of *E. Coli* and *Staphylococcus aureus* is mentioned in Table 1.

Table 1: Target proteins with their respective PDB (Protein Data Bank) IDs

Sr. no.	Target proteins	PDB IDs
A	***Staphylococcus aureus***	
	Gyrase B	3G75
	FtsA	3WQU
	DNA ligase	3JSN
	Dehydrosqualene synthase	3TFN
B	***Escherichia coli***	
	FtsZ	6UMK
	DNA gyrase	1KZN
	ZapD	5DKO

Selection of ligands (Phytochemicals of Garlic): The phytochemicals as listed by Batiha *et al.* (2020) are mentioned in table 2. These were considered for the antibacterial activity against targeted proteins. The 3-dimensional structures were obtained from ChemSpider database and each phytochemical is having their respective IDs

Table 2: Phytochemicals of Garlic and their respective ChemSpider IDs

Sr. no.	Compounds	ChemSpider ID
1.	Allicin	58548
2.	Ajoene	4533332
3.	Diallyldisulphide (DADS)	15730
4.	Diallyltisulphide (DATS)	15481

Phytochemicals of Tulsi: The phytochemicals as described by Tyagi *et al.* (2021) are mentioned in table 3 and examined for their antibacterial activity against targeted proteins. The 3-D structures were obtained from ChemSpider database, each phytochemical is having their respective IDs.

Table 3: Phytochemicals of Tulsi with their respective ChemSpider IDs

Sr.no.	Compounds	ChemSpider IDs
1.	Thymol	21105998
2.	Eugenol	13876103
3.	Linalool	13849981
4.	Carvacrol	21105867
5.	Apigenin	4444100
6.	Caryophyllene	4444848

Antibiotics for comparative study

Table 4: Antibiotics with their respective PubChem IDs

Sr. no.	Antibiotic	PubChem IDs
1.	Gentamicin	72395
2.	Enrofloxacin	71188

Target Protein Preparation: The 3D structures of protein retrieved from PDB were obtained as complex structures with other ligand molecules. So, the unwanted ligand molecules as well as water molecules were cleaved from complex protein structure using Chimera software.

Ligand Preparation: The 3D structures from ChemSpider and PubChem database were downloaded in JSmol format and SDF format respectively. Autodock was used for molecular docking. Autodock only accepts the molecular structure in PDB format, so to convert the JSmol and SDF format to PDB, 'Open babel' software was used to change the format. Geometric optimization and ligand preparation were done using the Autodock tool and the ligand structure was also converted into pdbqt format.

Molecular Docking: Molecular docking was conducted to analyse the binding energy as well as to study the interaction between protein and ligand. All the selected phytochemicals and conventional antibacterial agents were docked against the selected target bacterial proteins and for each docking nine poses were defined in Autodock as a result. Among them one with the least binding energy was considered as best docked structure.

In Autodock, the ligands and receptors were prepared in pdbqt format and the configuration file having the X, Y and Z coordinate values where the ligand has been docked i.e., the active site of receptor was generated. The Autodock tool was used for grid box preparation of various dimensions according to the docking. The grid spacing of 1 Å was set and centre X, Y and Z was also set accordingly for different docking structure.

The final docked structures were analysed using LIGPLOT (Sidhu *et al.,* 2020). The 3-D docked structure of protein and ligand were represented in 2-D to estimate the number of hydrogen bonds, distance between hydrogen bonds and amino acid residues. The docked structures were visualized using PyMOL software.

RESULTS AND DISCUSSION

Among all the phytochemicals of Garlic, the highest efficacy was shown by Ajoene against all targeted bacterial proteins of *S. aureus* and *E. coli*. As mentioned in table 5. The overall efficacy of Garlic was found satisfactory which was also reported by other researchers, as Arunachalam (1980) also demonstrated that garlic has antibacterial activity against a wide range of bacteria of genital tract found in repeat breeder cattle. Kumar (2008) reported in their study that, the crude extract of garlic has broad-spectrum antibacterial activity and zone of inhibition was shown against all the cervical mucus bacteria of repeat breeder. Singh, (2016) found that ciprofloxacin (93%) results into clear discharge in cows followed by garlic (88.33%) and neem (86.67%) and found a significant difference in PMN%, TLC, and lymphocyte and monocyte counts.

Table 5: Binding energy (Kcal/mol) of Garlic against targeted bacterial proteins of *E coli* and *S. aureus*

Bacterial proteins	Phytochemicals of Garlic				
S. aureus	**Allicin**	**Ajoene**	**DATS**	**DADS**	**Average**
Gyrase B	-4.0	-4.7	-3.3	-3.1	-3.58
DNA ligase	-4.4	-4.4	-3.9	-3.9	-4.15
Dehydrosqualene synthase	-4.5	-4.9	-3.9	-4.0	-4.33
FtsA	-3.9	-5.1	-3.1	-3.4	-3.88
E. coli					
DNA gyrase	-3.9	-4.7	-3.7	-3.6	-3.98
FtsZ	-3.3	-4.2	-3.1	-3.2	-3.45
ZapD	-4.0	-3.6	-3.6	-3.4	-3.65
Average					-3.89

Table 6: 2-D representation of FtsA-Ajoene complex

Protein	Ligand	Binding energy (Kcal/mol)	No. of hydrogen bond	Amino acid involved in H-Bond	Amino acid residues
FtsA	Ajoene	-5.1	1	Lys77	Asp185, Tyr189, Ser361, Glu358, Lys17, Ser14, Tyr37, Glu209, Gly208, Ser13, Gly325, Gly12, Asp10, Asp206

Table 7: Binding energy (Kcal/mol) of Tulsi against bacterial proteins of *E coli* and *S. aureus*

Bacterial proteins	Phytochemicals of Tulsi					
S. aureus	Thymol	Eugenol	Linalool	Carvacrol	Apigenin	Caryophyllene
Gyrase B	-5.7	-5.7	-5.4	-5.8	-8.1	-7.2
DNA ligase	-6.6	-6.5	-4.9	-6.8	-8.7	-6.4
Dehydrosqualene synthase	-6.5	-6.5	-5.5	-7.0	-9.0	-8.4
FtsA	-6.3	-5.9	-5.1	-5.7	-9.2	-7.1
E. coli						
DNA gyrase	-5.8	-5.6	-5.5	-6.1	-8.9	-6.2
FtsZ	-6.1	-5.9	-4.9	-6.1	*-7.2*	-6.2
ZapD	-5.1	-5.0	-5.0	-5.7	*-7.2*	-6.1

Table 8: 2-D representation Dehydrosqualene synthase-Apigenin complex

Protein	Ligand	Binding energy (Kcal/mol)	No. of hydrogen bond	Amino acid involved in H-bond	Amino acid residues
Dehydrosqualene	Apigenin	-9.0	6	Asp48, Arg45, Gln65, Asn168	Leu164, Leu141, Phe76, Tyr41, Phe22

Ajoene of garlic shows binding energy of -5.1 Kcal/mol when docked with FtsA, and considered best among other phytochemicals of garlic. The 2-D presentation of docked structure i.e., FtsA and Ajoene is mentioned in table 6 and plate 1. The average binding affinity of garlic against vital proteins of pathogenic bacteria is -3.89 Kcal/mol.
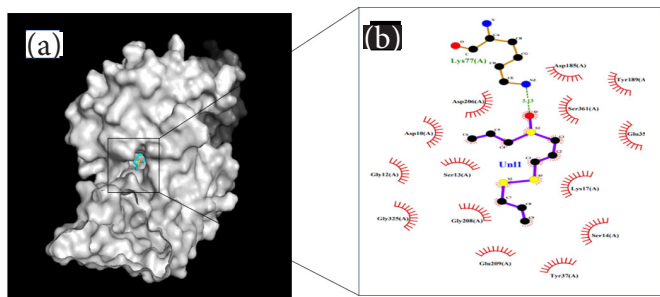

Plate 1: (a) Docked 3D structure of FtsA with Ajoene (space filling model). (b) Interaction of protein-ligand docked complex predicted by LIGPLOT+: 2D representation of 3D structure, showing H-bond and interacting amino acids.

Docking results of various phytochemicals of Tulsi against vital targeted proteins of *S. aureus* and *E. coli* are presented in table 7. Among all the phytochemicals of Tulsi, the highest efficacy was shown by Apigenin against all the targeted pathogenic proteins of selected bacteria. Overall efficacy of Tulsi was found good which was also reported by other researchers as Kulkarni *et al.* (2018) reported that extract of Tulsi leaves is effective against both gram positive and gram-negative bacteria.

The bacterial protein when docked with phytochemicals of Tulsi, shows maximum affinity with apigenin with binding energy of -9.0 Kcal/mol, followed by caryophyllene, carvacrol, eugenol, thymol and linalool. The ligand bonded to the receptor by six hydrogen bonds viz. one at Asp48, other at Gln165, two at Arg45 and remaining two at Asn168. Other than hydrogen bonds the amino acids involved in interaction are Leu164, Leu141, Phe76, Tyr41 and Phe22, as shown in plate 2 and table 8.

The binding energy of antibiotics against targeted bacterial proteins of *E. coli* and *S. aureus* is presented in table 9.
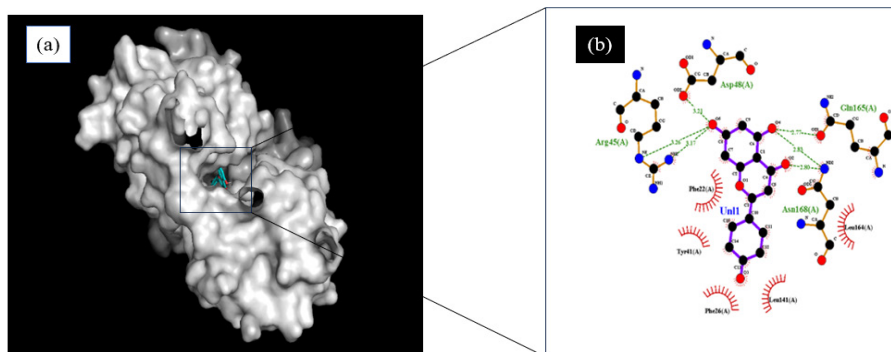
Plate 2: (a) Docked 3D structure of Dehydrosqualene synthase with Apigenin (space filling model). (b) Interaction of protein-ligand docked complex predicted by LIGPLOT+: 2D representation of 3D structure showing H-bonds and interacting amino acids.

Table 9: **B**inding energy (Kcal/mol) of Antibiotics against targeted bacterial proteins of *E. coli* and *S. aureus*

Bacterial proteins	Binding energy (Kcal/mol) of Antibiotics against targeted bacterial proteins	
Staphylococcus	**Gentamicin**	**Enrofloxacin**
Gyrase B	-6.3	-7.7
DNA ligase	-7.7	-7.3
Dehydrosqualene synthase	-6.6	-6.5
FtsA	-7.9	-6.7
E. coli		
DNA gyrase	-6.8	-7.8
FtsZ	-6.8	-6.3
ZapD	-6.6	-6.5
Average	**-6.95**	**-6.97**

It was noted that the binding energy of Apigenin and Ajoene the respective phytochemicals of tulsi and garlic was least and showed better binding efficacy. As compared to antibiotics (Table-9), the binding efficacy of Apigenin (Tulsi) was better.

CONCLUSIONS

Based on the total binding affinity or docking results, Apigenin component of tulsi was found more effective in treating endometritis. Apigenin has demonstrated the best binding affinity amongst all the screened phytochemicals of tulsi, garlic, and antibiotics against all of the targeted bacterial proteins. After being extracted, apigenin can be utilised alone or in combination with other phytoconstituents as an antibacterial agent. Phytochemicals may be invegitate further with *in vivo* or *in vitro* trials in the direction of effective drug discovery against bacterial bovine endometritis.

CONFLICT OF INTEREST
No conflict of Interest.

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