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Molecular docking studies of *aloe vera* for their potential antibacterial activity using Argus lab 4.0.1

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Abstract
Antibiotic resistance is an earnest and progressive phenomenon in coeval medicine and has emerged as one of the supreme public health concerns in current scenario. The use of alternative medicines practiced for years and remained as constituent part of many cultural and technological developments around globe. The aim of the present study was to evaluate the antibacterial activity of *Aloe vera* (L) via molecular docking studies using 17 phytoconstituents already reported in multifarious scientific reports i.e. acemannan, aloe emodin, aloesin, aloin A, aloin B or isobarbaloin, anthracene, anthranol or anthranol benzoate, auxin or 3 indoleacetate, campesterol, chrysophanic acid, dermatan 4 sulphate, dermatan 6 sulphate, emodin or archin, hyaluronic acid, lupeol, salicylic acid and sitosterol beta against the rate limiting enzyme involved in cell wall synthesis of bacteria i.e. glucosamine 6 phosphate synthase respectively. The docking procedure was carried out using ArgusLab 4.0.1 and post docking analysis was carried out by using PyMOL molecular viewer. Among the ligands screened sitosterol beta, campesterol and anthracene showed highest binding energy i.e. -12.419, -11.764 and -11.038 with better inhibition properties respectively. From the current *insilico* studies, it can be concluded that the above three drugs showed good binding energy and druggish property. Further, bioactivity guided fractionation and isolation of constituents is required for *in vitro* and *in vivo* studies.

Keywords: Antibiotic resistance, *Aloe vera*, sitosterol beta, *in silico* studies, Argus Lab

1. Introduction
The new age predominant impugn challenges of therapeutics is the care and governance of bacterial infections, in as much as origin of bacteria with multiple resistance to antibiotics (Joray *et al*., 2013) [1] with resultant implications on mortality and morbidity (Frieri *et al*., 2017) [2]. The center for Disease Control and Prevention (CDC) reported more than two million people were diagnosed with infectious disease every year due to antibiotic resistance and about 20,000 people die due to therapy failure (Li *et al*., 2016) [3]. At a low estimate antibiotic resistance is currently causing 7, 00,000 deaths worldwide annually, with this figure projected to reach 10 million by 2050.
Now a day's treating the bacterial infection is challenging, due to the ability of bacteria to develop resistant in contra to antimicrobial agents. The classification of antimicrobial agents is based on the varied mechanism of action i.e., inhibition of protein synthesis, metabolic pathways, cell wall synthesis, RNA and DNA synthesis. The bacteria will produce resistance by multifarious processes i.e., efflux pump, antibiotic inactivation, modification of target etc. So, the currently available treatment is not effective to combat resistance developed by certain bacterial species. However, the phytoconstituents of plants having antimicrobial activity has prodigious potential to battle bacterial diseases without any side effects. However, plant-based antimicrobials have immense potential to combat bacterial, diseases without any known side effects. Such plant metabolites include quinines, alkaloids, lectins, polypeptides, flavones, flavonoids, flavonols, coumarin, terpenoids, essential oils and tannins. *Aloe vera* (L.) Burm. f. (Family: Liliaceae), a xerophytic plant, adjusted to multitude of climatic conditions including temperate and sub-tropical areas. The exact origin of *A. vera* is not known, but studies and documentation infer that it is materialized from the Arabian Peninsula (Reynolds, 2004) [4], North Africa and Mediterranean countries (Sahu *et al*., 2013; Manvitha & Bidya, 2014) [5-6]. The plant is commonly called aloe, lily of desert, plant of burn and elephant's gall, which cannot survive freezing temperature. The juicy content of *Aloe vera* has two constituents; yellow exudate and clear mucilaginous gel. The yellow exudate has extravagant concentration of anthraquinone type compounds *viz* aloin, barbaloin, aloe emodin, isobarbaloin and chrysophanic acid known to possess antibacterial, fungicidal, diuretic, laxative, antiviral,

hepatoprotective and vasorelaxant activities (Salah *et al*., 2017; Drudi *et al*., 2018) [7, 8]. Mucilaginous gel used for the therapy of burns and wounds (Fox *et al*., 2017; Reynolds and Dweck, 1999) [9, 10]. Acemannan, the major sugar residue, has been extensively investigated and proven to stimulate wound healing and hard tissue regeneration by inducing cell proliferation and stimulating Vascular Endothelial Growth Factor (VEGF) and type I collagen synthesis (Chantarawaratit *et al*., 2014) [11]. *Aloe vera* can inhibit the inflammatory process by reduction of leukocytes adhesion as found by Duansak *et al*. (2009) [12]. The administration of aloe has been demonstrated to result in an increase in phagocytic and proliferative activity by inhibiting the cyclooxygenase pathways and reducing prostaglandin E_2 production, which play a role in inflammation (Im *et al*., 2005; Park *et al*., 2009) [13, 14].

To identify a reliable and potent drug for protein target protein-ligand docking modules are used. The aim of docking is to predict the binding energy of the protein-ligand complex given the atomic coordinates. Recent enhancements in search algorithms and energy functions, computational docking strategies became a valuable tool to explore the interaction between protein and its inhibitors. The binding energy between the protein and its ligand is calculated by a simplified, often grid-based force field (Luty *et al*, 1995) [15]. The overall cost of drug development could be reduced by as much as 50% through extensive use of *in silico* technologies in drug discovery procedure (White, 2007) [16].

Bacterial shape is attributed to the peptidoglycan layer, while permeability is determined by both the inner and outer membranes (Demchick and Koch 1996; Forsberg *et al*. 1970; Nakae and Nikaido 1975) [17-19]. Glucosamine-6-phosphate synthase (GlmS) catalyzes the rate-limiting step in the synthesis of uridine diphosphate (UDP)-N-acetylglucosamine, an important precursor for peptidoglycan and lipopolysaccharides, by binding fructose-6-phosphate and glutamine to produce glutamate and glucosamine-6-phosphate (Milewski 2002) [20].

The present study was designed to screen the 17 bioactive constituents present in *Aloe vera i.e.* acemannan, aloe emodin, aloesin, aloin A, aloin B or isobarbaloin, anthracene, anthranol or anthranol benzoate, auxin or 3 indoleacetate, campesterol, chrysophanic acid, dermatan 4 sulphate, dermatan 6 sulphate, emodin or archin, hyaluronic acid, lupeol, salicylic acid and sitosterol beta against glucosamine 6 phosphate synthase, a rate limiting enzyme involved in peptidoglycan synthesis using molecular docking software ArgusLab 4.0.1 and the docking results were visualized with open source modern visualization tool, PyMOL.

2. Materials and Methods

2.1 Selection and retrieval of protein structure from database

In the present study, the crystal structure of enzyme Glucosamine 6 phosphate synthase (PDB ID 4VF5) obtained from RCSB Protein Data Bank (http://www.pdb.org) containing resolution about 2.9 A° respectively (Hetal *et al*., 2013) [21].

2.2 Processing of target proteins

The water molecules present will disturb the binding nature of the compounds to the active site thereby reducing the efficiency of the compound against the target proteins. Thus, by using Argus lab, crystallographic water molecules and other unwanted ligands were cleaved and were removed from the protein. Crystallographic disorders and void atomic spaces were corrected to improve binding energy. Then, the protein was subjected to energy minimization and on the final stage, addition of hydrogen atoms to the target protein molecule before docking was performed (Naganathan, 2016) [22] and geometric optimization was performed according to Hartree – Fock (HF) calculation method by ArgusLab 4.0.1 software (Tripathy and Sahu,)

2.3 Binding site detection

PDBsum (http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl?pdbcode=index.html) is a web based database providing a largely pictorial summary of the key information on each macromolecular structure deposited at protein data bank (PDB), which provides the summary of the molecule contained in each PDB entry together with annotations and analysis of their key structural features. So, each PDB entry there is a corresponding web page in PDBsum, accessible by the four character PDB identifier. PDBsum was used for the identification of most potent active site for binding and interaction of target protein and ligand (Laskowski 2001) [24].

2.4 Selection and retrieval of ligand structure

The compounds reported in *Aloe vera* majorly anthraquinones and anthrones like acemannan, aloe emodin, aloesin, aloin – A, aloin – B (isobarbaloin), anthracene, anthranol, auxin or 3-indoleacetate, campesterol, chrysophanic acid, dermatan 4 sulphate, dermatan 6 sulphate, emodin or archin, hyaluronic acid, lupeol, salicylic acid and sitosterol beta, the structure of the phytoconstituents were retrieved from Chemspider, a chemical structure database providing fast search and structure search access and saved in JSmol format.

2.5 Processing of ligand structure

The structures were visualized in 3D in Chemspider database and were saved in JSmol format for Argus lab. The geometric optimization was performed using Argus Lab 4.0.1 software. Molecular Mechanics (MM) method UFF was used for refining initial geometries, using the "Clean Geometry" option in the ArgusLab. Hydrogens were added using "Add Hydrogens" option under edit column of ArgusLab 4.0.1 respectively.

2.6 Molecular docking using Argus Lab 4.0.1

All the computational docking studies were performed using Argus lab 4.0.1, which is a computerized structure program, generally based on the quantum mechanics and is used to predict the potential energies, molecular structures; geometrical optimization of structure, vibrational frequencies of various atom coordinates, bond length and reactions pathway. Target proteins i.e. Glucosamine 6 phosphate synthase (PDB ID – 2VF5) were docked against the 17 ligands (Obtained from the chem spider) using Argus Lab 4.0., to find the reasonable binding geometries and explore protein ligand interactions. The docking was mainly targeted only on to the predicted active site. Simulations for docking were performed by selecting "Argus Dock" as the docking engine. The residues (In the receptor) that have been selected were defined to be a part of the binding site. A 0.4 Å spacing was used between the grid points and an comprehensive search was performed by enabling "High precision" option in Docking precision menu, "Dock" was chosen as the

calculation type, "flexible" for the ligand and the A Score was used as the scoring function. The A Score function was generally used to calculate the binding energies of the resulting docked structures. All the compounds present in the data file were docked into the active site of antimicrobial target, using the same protocol. The molecular visualization of the ligand-protein interactions were analyzed by PyMOL software to examine the type of interactions. The docking poses saved for each compound were ranked according to their dock score function. The pose having the highest dock score was selected for further analysis.

3. Results

Table 1: Characteristic of phytoconstituents of *Aloe vera* (L)

Sl. No.	Compound name	Chemspider ID	Molecular formula	Average mass (in Dalton)	Polar surface area (in $^0A^2$)
1	Acemannan	65033	$C_{66}H_{101}NO_{49}$	1692.485	711
2	Aloe emodin	9792	$C_{15}H_{10}O_5$	270.237	-
3	Aloesin	140797	$C_{19}H_{22}O_9$	394.373	154
4	Aloin-A	24534069	$C_{21}H_{22}O_9$	418.394	-
5	Aloin – B or Isobarbaloin	14269	$C_{21}H_{22}O_9$	418.394	-
6	Anthracene	8111	$C_{14}H_{10}$	178.229	-
7	Anthranol or Anthranol Benzoate	32699606	$C_{21}H_{14}O_2$	298.335	26
8	Auxin or 3-Indoleacetate	779	$C_{10}H_8NO_2$	174.177	56
9	Campesterol	151215	$C_{28}H_{48}O$	400.680	-
10	Chrysophanic acid	9793	$C_{15}H_{10}$	254.238	-
11	Dermatan 4 Sulphate	30361	$C_{14}H_{21}$	475.380	273
12	Dermatan 6 Sulphate	58163730	$C_{14}H_{23}$	477.395	
13	Emodin or Archin	3107	$C_{15}H_{10}$	270.237	-
14	Hyaluronic acid	2341173	$C_{28}H_{44}$	776.649	400
15	Lupeol	50645939	$C_{25}H_{26}$	390.471	67
16	Salicylic acid	331	$C_7H_6O_3$	138.121	-
17	Sitosterol- Beta	192962	$C_{29}H_{50}$	414.707	-

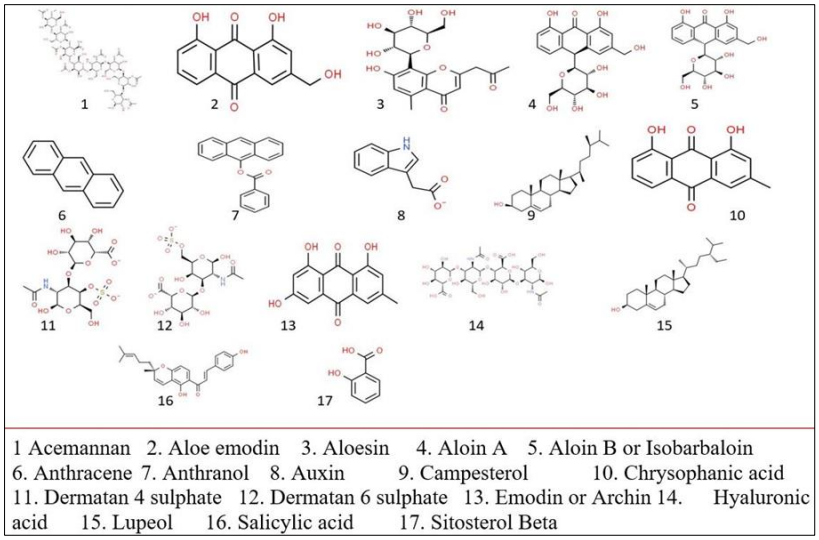

Fig 1: Chemical structure of Ligands of *Aloe vera* (L):

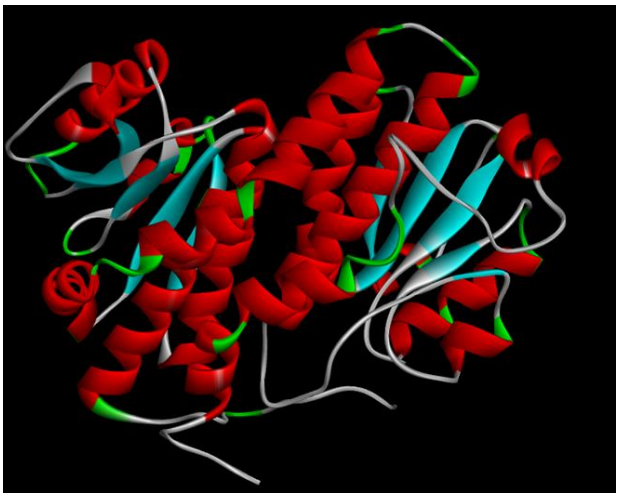

Fig 2: Crystal structure of Glucosamine 6 Phosphate Synthase (PDB ID – 4VF5)

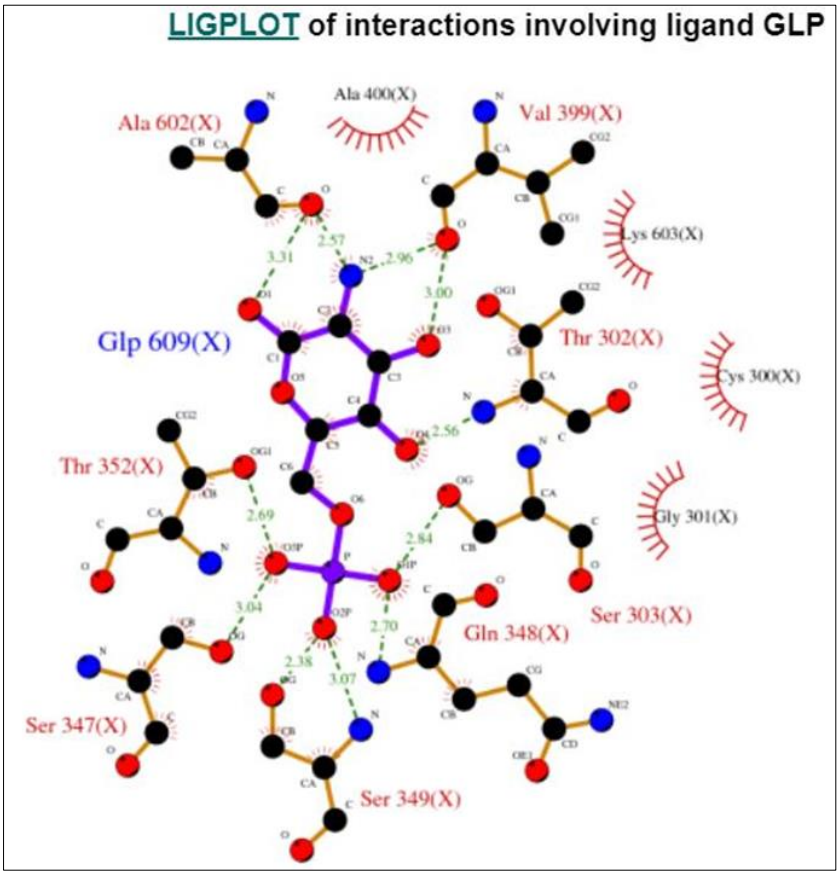

Fig 3: PDB sum calculation on active site residue of Glucosamine 6 phosphate synthase:

Table 2: PDB sum calculation on active site residue of Glucosamine 6 phosphate synthase:

Sl. No.	Protein module	Contacts	Active site residues
1	Glucosamine 6 Phosphate Synthase PDB ID – 4VF5	Non bonded contacts	ALA602, ALA 400, CYS300, GLY301, GLN348, LYS603, SER303, SER347, SER349, SER401, THR352, THR302, VAL399.
2	Glucosamine 6 Phosphate Synthase PDB ID – 4VF5	Hydrogen bonds	ALA602, GLN348, SER303, SER347, SER349, SER349, THR302, THR352, VAL399.

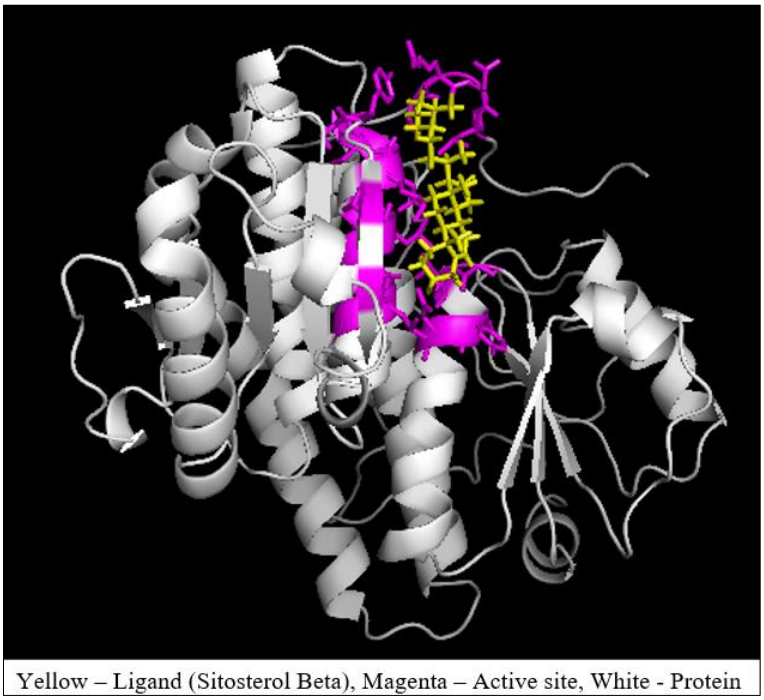

Fig 4: Docking interactions of Glucosamine 6 Phosphate Synthase with Sitosterol Beta

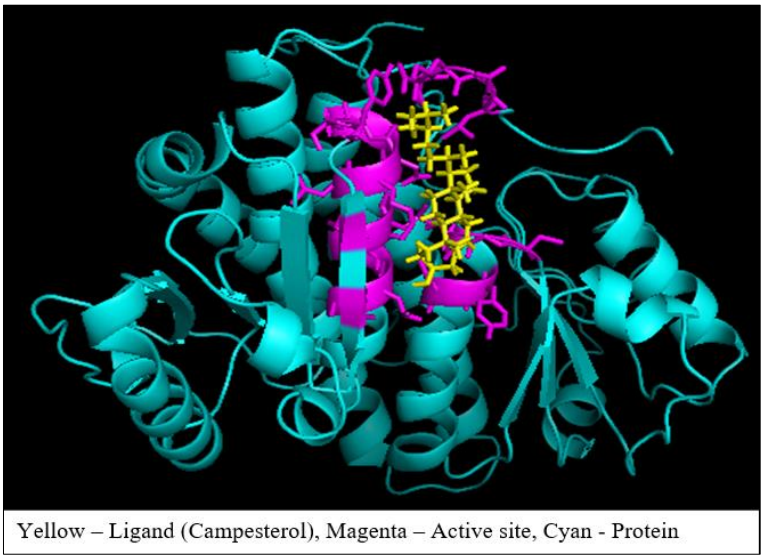

Fig 5: Docking interactions of Glucosamine 6 Phosphate Synthase with Campesterol

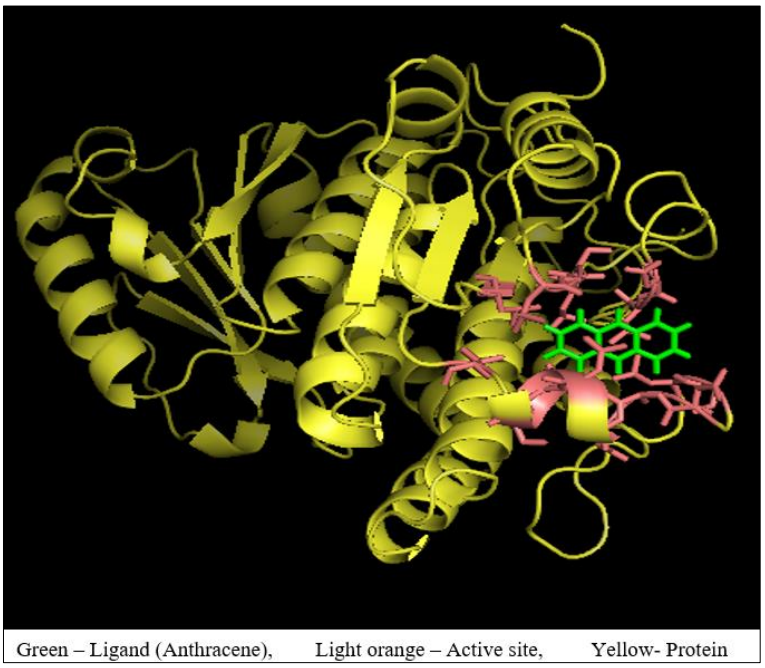

Fig 6: Docking interactions of Glucosamine 6 Phosphate Synthase with Anthracene

Table 3: Docking results of protein (4VF5) with ligands of *Aloe vera* (L):

Sl. No.	Drug / Ligands	Maximum number of poses	Number of target torsions	Number of ligand torsions	Precision	Best ligand pose energy (kcal/mol)
1	Acemannan	150	0	54	Regular	0
2	Aloe emodin	150	0	6	Regular	-9.396
3	Aloesin	150	0	10	Regular	-8.745
4	Aloin A	150	0	10	Regular	-8.591
5	Aloin B	150	0	11	Regular	0
6	Anthracene	150	0	0	Regular	-11.038
7	Anthranol Benzoate	150	0	3	Regular	-10.701
8	Auxin or 3 indole	150	0	2	Regular	-8.924
9	Campesterol	150	0	6	Regular	-11.764
10	Chrysophanic acid	150	0	4	Regular	0
11	Dermatan 4 sulphate	150	0	13	Regular	-7.051
12	Dermatan 6 sulphate	150	0	12	Regular	-7.405
13	Hyaluronic acid	150	0	22	Regular	-5.834
14	Lupeol	150	0	8	Regular	-10.943
15	Salicylic acid	150	0	2	Regular	-7.935
16	Emodin or Archin	150	0	4	Regular	-5.328
17	Sitosterol Beta	150	0	7	Regular	-12.419

4. Discussion

Medicinal plants have varied clinical and curative implication because of biopharmaceutical components (Merken *et al*., 2001; Zheng and Wang, 2001) [25-26], especially antibacterial constituents (Hemaiswaya, *et al*., 2008, Buhner, 2012 & Brown, 2015, Nychas, 1995) [27-29]. *Aloe vera* is known as a healing plant and has been used in several cultures for the treatment of skin injuries (Hashemi *et al*., 2015) [30]. Glycoproteins and lectins present in *Aloe vera* have found to have cell proliferation activities (Winters *et al*., 1981; Danof *et al*., 1983; Reynolds & Dweck, 1999) [31-33].

The concept of docking is important in the study of various properties associated with protein-ligand interactions such as binding energy, geometric optimization, electron distribution, hydrogen bond donor acceptor properties, hydrophobicity and polarizability (Girija *et al*., 2010) [34]. Since molecules in nature have a tendency to be found in their low energy form, the final configuration should also be of low energy (Pyne and Gayathri, 2005) [35].

ArgusLab is free distributed molecular docking software in Windows platform. This program is developed as molecular modeling software. It provides us with molecular building analyses, the ability to perform various molecular calculations, molecular docking and molecular structure visualization capabilities. ArgusLab is an easy to use program and has an accessible user-interface even by beginners in molecular docking. For new researchers in molecular docking, ArgusLab provides a fast and robust method of a binding site optimization which means the program can locate binding site automatically which make the docking process fast. Furthermore, ArgusLab program does not need to do blind docking which normally need a lot of time for calculation and sometime obtains the wrong binding site. Many researchers used ArgusLab to perform their molecular docking researches (Hafeez *et al*., 2013, Naz *et al*. 2009, Oda and Takahasi, 2009, Tanguenyongwatana, 2016) [36-39].

The present study describes the antibacterial potential of multifarious phytoconstituents of *Aloe vera*. A total of 17 phytoconstituents of the plants *viz*. acemannan, aloe emodin, aloesin, aloin A, aloin B or isobarbaloin, anthracene, anthranol or anthranol benzoate, auxin or 3 indoleacetate, campesterol, chrysophanic acid, dermatan 4 sulphate, dermatan 6 sulphate, emodin or archin, hyaluronic acid, lupeol, salicyclic acid and sitosterol beta were screened for antibacterial activity against glucosamine 6 phosphate synthase (PDB ID – 4VF5) as protein target. ArgusLab version 4.0.1 is used for docking ligand and protein, further PyMOL used for visualizing the molecular connection between the two.

The chemical structure of the ligands were identified by using chemspider, a chemical structure database, the structure and molecular characteristics were obtained and are depicted in table and figure 01 respectively. The crystal structure of target protein (fig 02) and their active sites (fig 03 and table 02) were retrieved from PDBsum database. The docking was carried out using ArgusLab 4.0.1, among the 17 ligands docked, the highest binding energy shown in the descending order was sitosterol beta > campesterol > anthracene > lupeol > anthranol benzoate > aloe emodin > auxin > aloesin > aloin A > salicylic acid > dermatan 6 sulphate> dermatan 4 sulphate > hyaluronic acid > archin respectively. The phytoconstituents like sitosterol beta followed by campesterol and anthracene showed highest inhibition of the enzyme, proving as potent bactericidal agents.

This study provides a new paradigm for the use of specific phytoconstituents in *Aloe vera* as antibacterial agent in place of their synthetic counterparts. One must investigate further in detail by isolation and purification techniques of phytoconstituents, however more preclinical and clinical trials are also required to investigate the further utility of these phytopharmaceutical agents either alone or in combination with existing therapy.

5. Conclusion

In silico modeling is a modern approach for faster and quicker analysis of efficient binding. Molecular docking is a method to confirm and locate the binding mode and interaction energy for the ligands with the target protein without any complex wet lab analysis and screening. It has been shown in the literature that these computational techniques can strongly support and help the design of novel, more potent inhibitors by revealing the mechanism of drug-receptor interaction. In this study, an *in silico* approach to study the binding orientations of several *Aloe vera* derived phytoconstituents against specific enzyme involved in cell wall synthesis. It was concluded from the docking simulation studies that among multifarious ligands, sitosterol beta, campesterol and anthracene found to be best inhibitors. The results obtained from this study would be useful in both understanding the inhibitory mode of the plant-derived natural products in rapidly and accurately predicting the activities of newly designed inhibitors on the basis of docking scores. These models also provide some beneficial clues in structural modification for designing new inhibitors.

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