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Natural compounds for the drug targets of Sexually Transmitted Diseases (STD) using virtual screening

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Abstract: *The Sexually transmitted diseases are the major health problem along with other diseases like cancer and diabetes. The present study involves obtaining of the 44 molecular drug targets of the STD which have been predicted and launched at www.bioresearch asia.com. The targets in this site are mainly predicted by the comparative genomics method, where the genomes of the STD bacteria are compared with the human to obtain only the unique bacterial molecular targets relative to human. The main objective of the present study is to find the natural compound that shows high binding affinity to the drug targets of the Sexually transmitted diseases. The methodology includes creation of the library of the natural compounds collected from various literature sources. The molecular targets of the STD organisms are docked with the natural compound to know the binding affinity of the ligand with their respective drug targets by using autodock vina and the admet-Tox properties of the compounds were also studied along with the antibiotic as a reference drug. The studies indicate certain compounds showing affinity to the targets of the STD: they include compounds such as hypericin, plantanoside, silybin, procyanidin, plumbagin, anonaine and withaferin A.*
Keywords: *Drug targets, lead compounds, STD, natural drug molecules, docking*

I. Introduction

For several reasons such as the expensive dead ends, alarming increase in the rise of multiple resistances in the bacteria and costly time consuming experimental methods has shifted the drug discovery to the genomics approach. One of such approaches is the subtractive genomics that systematically identifies the drug targets by differential genomics [1]. The comparative genomic approach has also been applied to various organisms such as *Helicobacter pylori*) [2], *Toxoplasma gondii* [3], *Vibrio cholera* [4], *Mycobacterium leprae* [5], *Borelia burdorferi* [6] and many more studies are underway. Natural compounds of the drug targets of *Ureaplasma urealyticum* has been studied [7]. In the present study natural compounds for other bacteria causing the STD have be focused. The drug targets of the STD organisms are available from its source www.bioresearch asia.com [8]. The targets were used to find the natural compounds showing high binding affinity by virtual screening.

1.1 Molecular drug targets:

The identification of the molecular drug targets is the crucial steps in the drug discovery process. The molecular target should play a crucial role in the metabolic reactions. Other features of the targets are: it should be druggable, previously unknown and should show binding properties to the drug molecules. Upon binding to the drug molecules it should bring the positive clinical effect. The molecular targets are much larger when compared to the drug molecules and possess the binding site called the active site. Target should modify the disease state, physiology as well as the pathology of the disease. The modifications should ultimately result in the disappearance of the disease and its associated conditions. Other important properties of the targets are the availability of its PDB structure; assayability; widely distributed in the body; it should act as a target disease biomarker. Huge amount of money is spent by pharmaceutical industries to identify the drug targets. Studies indicate 483 drug targets [9] which were further reduced to 120 based on the lipinskis rule of five [10]. 6000 drug targets are listed in the drug bank database which was narrowed down from the previous 14000 targets [11]. 218 drug targets mainly belonging to enzyme family, receptor family and the ion channel family were identified by Imming [12]. The therapeutic drug target database consists of the successful drug targets [13]. The important drug targets are the hydrolases, GPCRs, voltage gated ion channels.

The pharmacophores of the catalytic site of the targets can be studied with an aim to find the drug molecules that bind to various other targets [14]. Several factors help in identification of the drug targets, one factor is the homology between the target and the host proteins should be minimum or should be non- existent [15]. One of the important methods for identification of the drug targets are the biochemical methods, genetic interactions and computational interference. The *insilico* methods primarily are based on the structure, ligand and the profile. The major portions of the human drug targets belong mainly to the receptors (GPCRs) and the data is available drug bank database [16]. The drug targets bind to either the natural compounds or the presently available therapeutics. The proteins having the protein folds have the affinity for the drug –like small molecules.

Localization studies indicate that 60% of the drug targets are membrane- bound and 92% of them share similarity with the PDB structures [17]. Interaction of the drug molecules to the binding site of the drug targets results in the dominos effect. The inter-molecular interactions involve primarily the non- covalent forces. The functional groups modify the electronic properties of the targets; as a result they have the potential to block the metabolic pathway or restraint the conformation. Different methods have been used to predict the drug targets interaction based on QSAR [18], reverse docking [19], [20], [21]. The interactions can also be interpreted by, side effect similarity, drug based similarity, and target based similarity, based on interpretation of the networks [22], [23].

1.2 Drug molecules:

The pharmaceutical drug is derived from the Greek word 'pharmakeia'. This means making medications and vitamins. The drug molecule has the effector function to change the activity and the function of the drug targets. Modulatory functions include either enhancement or suppression of the activity of the protein. Pharmacophore constitutes the hydrophobic centre, H-bond acceptor and H-bond donor as a pharmacophoric points for better interaction with the targets.

There are different ways in which the drug or the pharmaceutic drugs are classified. In 1970s classification of the drug was mainly focused on the primary elements, functional groups and the class of the organic substance. Recently the classification of the drug is focused on the chemical constitution and the structure. A number of databases are now available with an objective of creating a substance library and understand the molecular recognition. At present the system of classification of the drug is the ATC system, based on the organ or the system on which the drugs act [24]. Drugs may be classified based on whether they act on viruses, bacteria or the parasites. Drugs may also be classified based on the condition of the health and is named as antipretic, antimalarial, analgesics etc. Drugs are also classified based on the mechanism of action. The drug –like molecules have acceptable ADME and toxicity properties. The medicinal substance should be optimized for the drug-like properties [25]. Physiochemical properties such as absorption, lipophilicity, solubility, etc. are essential for determining the drug-like properties. Absorption efficiency of the drug depends on the permeability and lipophilicity. The distribution of the drug mainly depends on the activity of the transporter proteins [26]. The dosage regimen is primarily dependent on the binding affinity of the drug molecules to the plasma proteins and PPA is determined by the lipophilicity which is mainly based on the calculation of the logP. Several programs have been developed to predict the logP value of the drug molecules [27]. The oral absorption is determined mainly by the solubility of the drug [28]. Molecular size of the drug limits the oral obsorption and larger size molecules undergo biliary excretion and do not cross blood brain barrier. Databases are available to give information about the ligands such as the Therapeutic drug Target Database (TTD) [29]. Pubchem of the NCBI provides the comprehensive data regarding the properties of the natural as well as the synthetic drugs. The drug likeliness of the drug molecules can be assessed by the Lipinski rule of five where drug having good absorptive and permeability properties should have MW<500, H-bond donor<5, LogP <5 and H-Bond acceptor<10.The drug molecule causes the bactericidal activity mainly by inhibition of the cell-wall as may be observed in the β-lactams. Drugs like the fluoroquinolones exhibit anti-bacterial activity due the inhibition of the nucleic acids [30]. Drugs like aminogylcosides, macrolides, ketolides, hiscosamides, streptogramins, oxazolidinones and glycylcyclines cause an inhibition of the protein synthesis. Drugs like daptomycin cause a change in the membrane potential and ultimately lead to the death of the bacteria [31].

Natural compounds are the best starting materials in the drug discovery process [32] and new drugs can be built from these molecules by using various computational techniques [33]. The other sources of the raw material includes microorganisms and marine invertebrates whose body constituents (metabolites) are tested for bioactivities. Many of the drugs that are now available are directly or indirectly derived from the natural compounds.

1.3 Comparative genomics:

Pathogens differ from genome size and number which reflects the environment in which the organism is living in an ecological niche. The free living organisms are armed with the large genome size as they live in diverse environmental conditions. The obligate parasites on other hand have smaller genome size since most of the nutrients are taken from the host. Many interactions determine the virulence properties of the pathogen before the infection is rooted; these factors destroy the host tissues. Some factors help in persistence of the pathogen in the host environment. Currently many of the researchers are focusing on the finding of the pathogenicity islands in the bacteria and the factors that involve in signal transduction to outwit the immune system [34]. The pathogenicity island should be present in the pathogenic but absent in the non- pathogenic bacteria. Genome analysis can also provide clue regarding change in the surface antigens. The region of the bacterial chromosome showing high rate mutations called the contingency loci have been identified [35]. The

size of the contingency locus may range from 1Kbps to 8 Kbps. In its most basic sense comparative genomics can be performed by comparing the protein sequence with another in the database. The availability of the complete genomic sequence of various organism enable scientist to compare between the genomes for making the large scale observation and setting hypothesis. In 1995 the complete genomes of the, *Haemophilus influenza* and *Mycoplasma genitalium* were deciphered. The important requirements for the comparative genomics are the huge relevant data and the computational tools for implementing and analyzing the data stored. Comparative genomics between the pathogenic and the non-pathogenic once can reveal the region of the genome that is responsible for the pathogenesis. This in fact is true even when comparing between two organisms at the sub species level [36]. The cross genome similarities that arise from the comparative genomics are useful in the evolutionary studies and functional analysis of the proteins at a higher level. One of the best tools necessary for the functional analysis is the COGS. One of the best ways to use the functional information stored in the genome is to transfer the functional information from a well characterized genome to the less characterized which is enabled by the COGS.

The drug discovery process is increasingly shifting from the conventional to the genomic approaches. Subtractive genomics is one such the differential comparative genomics approach that compares the genome of the pathogen with that of humans. The pathogens suitable for the subtractive genomics are those that are resistant to the antibiotics and for which the vaccines are not available or difficult to design. Organisms that are fastidious to grow and difficult to subject to experimental approaches are also suitable for the subtractive genomics approach. Availability of the complete genomes of the pathogens provides an opportunity for the data mining and derives useful data such as the drug targets. One of the criteria for mining the new drug targets is to find the genes that are non- homologous to the human proteins. At the same time they are critical or essential for the survival of the bacteria. These are the various reasons why the scientific community needs to focus on the pathogenic bacteria. Resistance has been exhibited by many organisms such as *Streptococcus* [37], *Enterococcus* [38] and *Pseudomonas* [39]. Resistance lead to ineffectiveness of the antibiotic treatments and may have critical consequences in the ill patients. Almost all the bacteria shows resistance to most of the antibiotics, but of main concern is the bacteria being initially susceptible becomes resistant later. The mode of action of antibiotics is mainly centered on blockage of cell-wall synthesis, protein synthesis, nucleic acid synthesis and the metabolic pathway. However, there are several mechanisms due to which the bacteria counteract the antibiotics. They include; production of the antibiotic degrading β-lactamase, presence of efflux pump to remove antibiotics, change in structure of cell-wall, genetic mutation that result in the lesser number of pores in the outerlayer and acquiring of the antibiotic-resistant genes. The acquisition of the antibiotic-resistance genes enhances the factors mentioned above in order to exhibit the resistance. Non-homology with the human protein provides the opportunity for further analysis and such proteins are good drug target candidates. Homologous sequence indicated by higher sequence identity shows common ancestry. While the low sequence identity indicates the occurrence of divergence long back [40]. Sequences of 20-30% identity is non- homology [41], [42] however sequences above 35% do not always predict homology.

Essential genes are the one necessary for the basic function of the pathogens and mutations in such genes lead to the death of the bacteria. The essential genes are more conserved in the evolution and evolve slowly when compared to the non- essential genes [43]. Most of the essential genes are studied with an aim to find the drug targets. Since experimental methods are time consuming and costly, *insilico* methods are used for finding the drug targets. Now the experimental essential genes are stored in the database for comparison with the query sequence to predict the essential gene *insilico* [44]. Functions encoded by the essential genes are critical for all cells and some of the basic functions are same all the cellular life on earth [45].

Different proteins are situated in different location of the cell and knowledge of the subcellular localization of proteins provides clues regarding the function of the protein [46]. The membrane associated proteins trigger humoral branch of immune system. Therefore the membrane proteins are ideal vaccines drug targets. Triggering of the immune response of the surface proteins have been studied in various organism such as *Staphylococcus* [47], *Plasmodium falciparum* [48] and *E. coli* [49].

Finding the metabolic pathway of the essential genes is a step further in refining the approach involved in the mining of the drug targets among the huge amount of the data. The good drug targets will be involved in the unique pathway. Since the unique pathway is present only in the pathogen and not in human, such targets are less likely to cross react with the human proteins.

The drug targets thus identified can be further be subjected to computational tools such as structure prediction and the identification of those natural compounds that show best binding affinity to the predicted drug targets.

1.4 Molecular recognition:

Receptors contain active site that binds to the ligand through the intermolecular interactions. Docking forms the simulation process of the molecular recognition between the protein and ligand. Natural compounds

suitable for the docking can belong to two categories first one is the natural compounds selected based on the lipinski's rule of five and the other is the one based on the literature search where the natural compound is known to have the anti-microbial activity. The binding of the protein to the ligand is analogous to the fitting of the key to the lock due to the complementary structure between the ligand and the active site of the protein. The other theory is the 'induced fit' where the proteins and the ligands make adjustment in their conformation to result in the best electronic fit [50]. Thus the optimized conformation between ligand and the targets (here proteins) results in the orientations between them, showing minimum energy. Screening has also been applied to the homology modeled protein and has proven to be a success in several cases [51], [52], [53].

II. Methodology

Putative drug targets of the STD pathogens by differential subtractive genomics have been reported. The 3-D structures of the putative drug targets were retrieved from 'Sexually transmitted diseases putative drug target database' (available at http://biomedresearchasia.org/) and saved as the .pdb files. The drug targets files obtained are further used for the virtual screening. The 3-d structure of the protein was then analyzed to find the proportion of amino acids in the allowed and the additionally allowed regions.

2.1 Natural compound library preparation:

Data regarding 150 natural compounds are collected from various literature sources and these compounds are known to have the anti-microbial activity. The compounds are mainly of the plant sources. These compounds are the good lead compounds for the above targets since they exhibit antimicrobial activity. The three dimensional structures of the natural compounds were retrieved from the pubchem database, by using the unique chemical identifier of the natural compounds. All the Sdf files were converted to pdb files since softwares used further in this research require this format. The druglikeliness index of the compounds was also calculated from the DrugMint server.

2.2 Energy minimization:

Energy minimization of the natural compounds was performed in order to release the internal constraint of the compounds. Marvin sketch was used to generate the 10 conformers with the low energy values. The protein conformation with the lowest energy values was used for the further steps and format of the files was the pdb.

2.3 Dock –based virtual screening:

The prospective drug targets of the STD pathogens and the natural compound library were docked, to find the compound that binds to their respective targets with minimum energy. Autodock vina (PyRx) finds the active site of the targets and docks the compounds to give the dock score that reflects the molecular recognition between the target and compound [54].

2.4 ADME-Tox properties:

The important pharmacokinetic and pharmacodynamic properties of the top scoring compounds were calculated. The logS were calculated, as solubility indicates the absorptive properties. The distribution, metabolism, excretion and toxicity were studied by the LD50 values. AdmetSAR is the cheminformatics tool used to predict the above properties [55]. ADME- Tox properties of the top scoring natural compounds of each of the targets and the reference antibiotic of the respective bacteria is predicted.

III. Results And Discussion

Virtual screening with the putative drug targets of *Chlamydia trachomatis A/HAR-13* showed the top scoring compounds for each of the drug targets along with the drug likeliness index as given in Table 1 and Table 2. The compounds showing affinity to the drug targets are the hypericin, withaferin and robustaflavone. However keeping in view with the prioritization of the targets based on the percent of amino acids in the allowed and additionally allowed regions, N-acetylglucosaminyl transferase and its corresponding compound hypericin were selected for the *Chlamydia trachomatis A/HAR-13*. The natural compounds showing high drug likeliness index were also prioritized in each case. The two way prioritization yielded hypericin showed good binding affinity to the N-acetylglucosaminyl transferase. Fig 1 indicates N-acetylglucosaminyl transferase showing hydrogen bond interaction with the hypericin at the Aspargine 124 and the threonine 261. Hpericin is a secondary metabolite and an anti-inflammatory ingredient of the *H.perforatum* [56] and belongs to the compounds called the napthodianthrones.

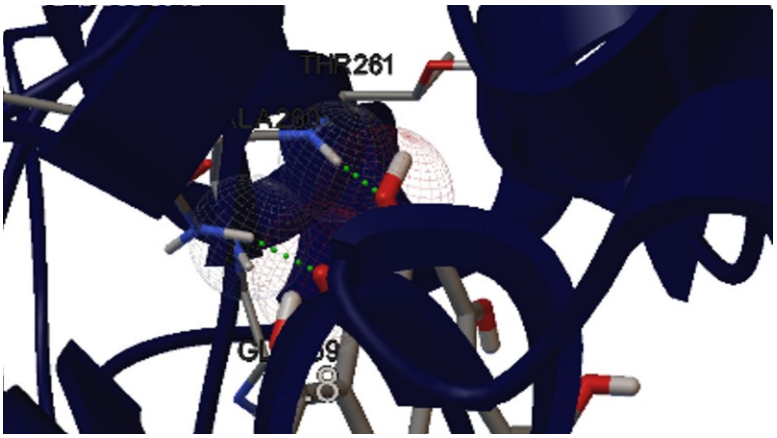

Figure 1 : Drug target interaction showing Hydrogen bond formation between N-acetylglucosaminyl transferase of the *Chlamydia trachomatis A/HAR-13* and the hypericin.

Table 1: Ligands showing good binding affinity with the targets of *Chlamydia trachomatis A/HAR-13.*

Lipid-A-disaccharide synthase (YP 328228.1)		
Name of the compound	**Dock score**	**Drug likeliness index**
Hypericin	-9.6	0.46666369
Amentoflavone	-9.1	-0.60523173
Robustaflavone	-9.1	-0.60523173
Hinokiflavone	-8.9	-0.60014507
Diosmin	-8.8	0.084577358
Hypothetical protein CTA_0677 (YP_328449.1)		
Withaferin A	-12.6	0.89623109
Amentoflavone	-12.5	-0.60523173
Lupeol	-11.7	1.3117783
Robustaflavone	-11.7	-0.60523173
Hesperidin	-11.4	0.084577358
Phospho-N-acetylmuramoyl-pentapeptide-transferase (YP 328585.1)		
Robustaflavone	-6	-0.60523173
Amentoflavone	-5.7	-0.60523173
Diosmin	-5.7	0.084577358
Hinokiflavone	-5.7	-0.60014507
Calotropin	-5.5	1.1013567
Hypothetical protein CTA_0830 (YP 328588.1)		
Robustaflavone	-10	-0.60523173
Amentoflavone	-9.7	-0.60523173
Hinokiflavone	-9.3	-0.60014507
Agathisflavone	-9.1	-0.60523173
Tiliroside	-8.9	0.095186246
N-acetylglucosaminyl transferase (YP 328589.1)		
robustaflavone	-10.1	-0.60523173
Hypericin	-9.7	0.46666369
agathisflavone	-9.4	-0.60523173
hinokiflavone	-9.4	-0.60014507
amentoflavone	-9.2	-0.60523173

Table 2: The ADMET-tox properties of the best fitting ligands for the targets of *Chlamydia trachomatis A/HAR-13* and the reference drug rifampin.

Ligand ID	**Aqueous solubility (logS)**	**Caco-2Permeability (logPapp)**	**Rat Acute Toxicity (LD50)**
CID 5381226 (rifampicin)	-2.9021	0.5468	2.7003
CID 5281051	-3.2198	0.8964	2.6122
CID 265237	-4.2028	0.7051	3.5404
CID 16142	-4.3298	0.2244	4.0114
CID 5320686	-3.094	-0.9415	2.8825
CID 5281051	-3.2198	0.8964	2.6122

Virtual screening with the targets of *Chlamydophila pneumoniae CWL029* showed three compounds having good binding affinity with their respective targets. They are Fumigaclavin, silybin and hypercin binding to Phospho-N-acetylmuramoyl-pentapeptide-transferase, Cell division protein FtsW and lipid-A-disaccharide synthase respectively. Prioritization yielded hypericin and lipid-A-disaccharide synthase as good compound and

target respectively. Interaction between the hypericin and lipid-A-disaccharide synthase showed H-bond at LYS348. Most of the other targets showed the possible non-covalent interactions belonging to the non-hydrogen bond category. Fig 2 shows the interaction between hypericin and lipid-A-disaccharide synthase. Table 3 and Table 4 shows the dockscore of the compounds showing affinity to the drug targets of *Chlamydophila pneumoniae CWL029* and the ADME-Tox properties of the top scoring compounds.

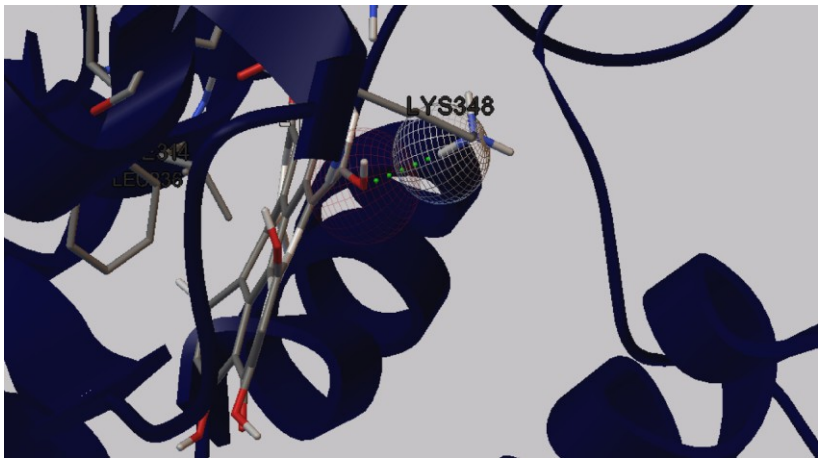

Figure 2: Drug target interaction between Lipid-A-disaccharide synthase and hypericin in *Chlamydia pneumonia CWL029.*

Table 3: The natural ligands showing binding affinity to the drug targets of *Chlamydia pneumonia CWL029.*

Name of the compound	Dock score	Drug likeliness index
Phospho-N-acetylmuramoyl-pentapeptide-transferase (NP 225095.1)		
Amentoflavone	-7.5	-0.60523173
Fumigaclavine	-7.3	0.7465329
Resveratrol	-7.1	0.30787494
Withaferine A	-6.9	0.89623109
Agathisflavone	-6.9	-0.60523173
Cell division protein FtsW (NP 225098.1)		
amentoflavone	-7.1	-0.60523173
silybin	-6.6	0.32390607
Hypericin	-6.6	0.46666369
Cynaroside	-6.4	0.00852429
apigetrin	-6.3	0.008524294
Lipid-A-disaccharide synthase (NP 225158.1)		
Hypericin	-10.8	0.46666369
hinokiflavone	-10.6	-0.60014507
withaferin A	-10.5	0.89623109
amentoflavone	-10.4	-0.60523173
robustaflavone	-9.8	-0.60523173

Table 4: ADMET-tox properties of the top scoring compounds along with reference drug *Chlamydia pneumonia CWL029.*

Ligand ID	Aqueous solubility (logS)	Caco-2Permeability (logPapp)	Rat Acute Toxicity (LD50)
Moxyfloxaxine (ref)	-3.0793	0.7706	2.3267
CID 173878	-3.1964	1.5627	2.9466
CID_31553	-2.6488	0.4354	2.2206
CID_265237	-4.2028	0.7051	3.5404

The target of the *Haemophilus ducreyi 35000HP* is the two sensor protein showing affinity to Dehydrosoyasaponin I. Two component systems aid the organism in sensing the surrounding environment. Two component system is the source of proteins that form the potential drug targets in the pathogens **[57]** Dehydrosoyasaponin I has the antibacterial activity and is the derivative of the saponins [58]. Fig 3 shows the interaction between the two component sensor protein and the dehydrosoyasaponin I showing interaction at ASN 364, THR 424 and LEU 427. Table 5 and Table 6 show the dockscore of the natural compounds and the ADME-Tox properties.

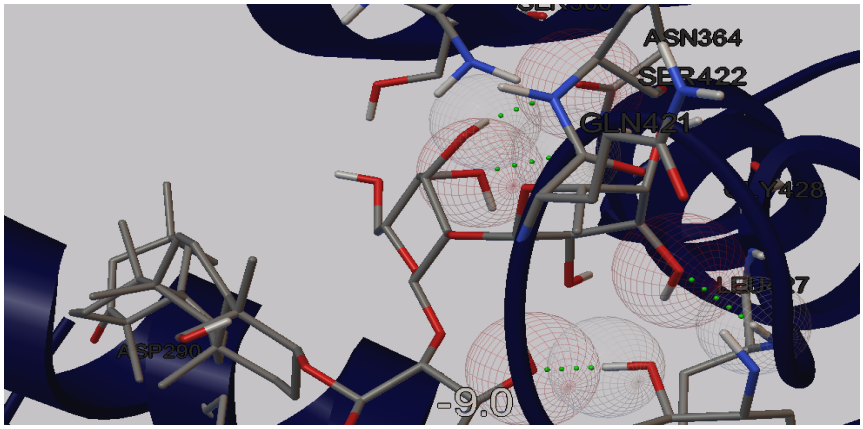

Figure 3: Figure showing the interaction between Two-component sensor protein with dehydrosaponin I in *Haemophilus ducreyi 35000HP.*

Table 7: The natural ligands showing binding affinity to the drug targets of *Haemophilus ducreyi 35000HP.*

Name of the compound	Dock score	Drug likeliness index
Tetraacyldisaccharide 4'-kinase (NP 872818.1)		
Catechin	-10	0.12117655
Quercetin	-10	-0.90181562
Robustaflavone	-10	-0.60523173
Flavone	-9.8	-0.78814486
Gossypetin	-9.8	-0.90181562
Phospho-N-acetylmuramoyl-pentapeptide-transferase (NP 872841.1)		
Hypericin	-7.5	0.46666369
Amentoflavone	-7.3	-0.60523173
Chelidonine	-6.7	0.15462575
Silybin	-6.7	0.32390607
Agathisflavone	-6.7	-0.60523173
Cell division protein FtsW (NP 872843.1)		
Hypericin	-5.9	0.46666369
Amentoflavone	-5.7	-0.60523173
Hesperidin	-5.6	0.084577358
Agathisflavone	-5.6	-0.60523173
Hinokiflavone	-5.5	-0.60014507
Lipid A biosynthesis (KDO)2-(lauroyl)-lipid IVA acyltransferase (NP 872980.1)		
Hypericin	-10.5	0.46666369
Robustaflavone	-10.5	-0.60523173
Tiliroside	-9.6	0.095186246
Hinokiflavone	-9.3	-0.60014507
Withaferin a	-9.1	0.89623109
pH-dependent sodium/proton antiporter (NP 873268.1)		
Anonaine	-8.5	0.13319882
Baicalein	-8.1	-0.90181562
Galangin	-8	-0.81772575
Ellagic acid	-7.9	-0.59069329
Chrysin	-7.8	-0.83382578
Undecaprenyldiphospho-muramoylpentapeptide beta-N-acetylglucosaminyltransferase (NP_873332.1)		
Hypericin	-11.2	0.46666369
Robustaflavone	-10.8	-0.60523173
Hinokiflavone	-10.5	-0.60014507
Amentoflavone	-10.3	-0.60523173
Plumbagin	-10.2	0.8813386
Lipid A biosynthesis lauroyl acyltransferase (NP 873583. 1)		
hypericin	-6.9	0.46666369
amentoflavone	-6.8	-0.60523173
diosmin	-6.7	0.084577358
withaferin A	-6.6	0.89623109
Dehydrosoyasaponin I	-6.6	0.45827078
Protease EcfE (NP 873653.1)		
Robustaflavone	-10.3	-0.60523173
Plumbagin	-10	0.8813386
Hinokiflavone	-9.6	-0.60014507
Withaferin a	-9.1	0.89623109

Diosmin	-9.1	0.084577358
Two-component sensor protein (NP 873883.1)		
Dehydrosoyasaponins	-9	0.45827078
Hinokiflavone	-9	-0.60014507
Agathisflavone	-8.7	-0.60523173
Robustaflavone	-8.7	-0.60523173
Plantanoside	-8.5	0.21371555
MviN virulence factor (NP 874315.1)		
Robustaflavone	-12.6	-0.60523173
Plantanoside	-12.1	0.21371555
Hypericin	-12	0.46666369
Hinokiflavone	-12	-0.60014507
Amentoflavone	-11.7	-0.60523173

Table 8: ADMET-Tox properties of the ligands showing good affinity with respective targets of *Haemophilus ducreyi 35000HP.*

Ligand ID	Aqueous solubility (logS)	Caco-2Permeability (logPapp)	Rat Acute Toxicity (LD50)
CID447043 (Azithromycin)	-2.0602	0.2710	2.5423
CID9 064	-3.1015	-0.5189	1.8700
CID 5281051	-3.2198	0.8964	2.6122
CID 160597	-2.4944	1.3197	2.7790
CID 10205	-3.5106	1.5940	3.2704
CID 656760	-4.0861	-0.3214	2.9436
CID 6451113	-3.9468	0.0899	2.8906

The post dock analysis of the targets of *Mycoplasma genitalium G37* showed Chromosomal replication initiation protein and rosmarinic acid have good electronic fit and also the druglikeliness index. Chromosomal replication initiation protein is an attractive drug target due its critical nature of initiating DNA replication. Rosmarinic acid is a phenolic compound showing the anti-inflammatory properties and its anti-bacterial property have also been reported [59]. Fig 4 shows the interaction between the chromosomal replication initiation protein and the rosmarinic acid interacting at THR 148, HIS 149 and ARG 307. Table 7 and Table 8 show the summary of the dockscore of the targets and the compounds showing high affinity and ADMETox properties respectively.

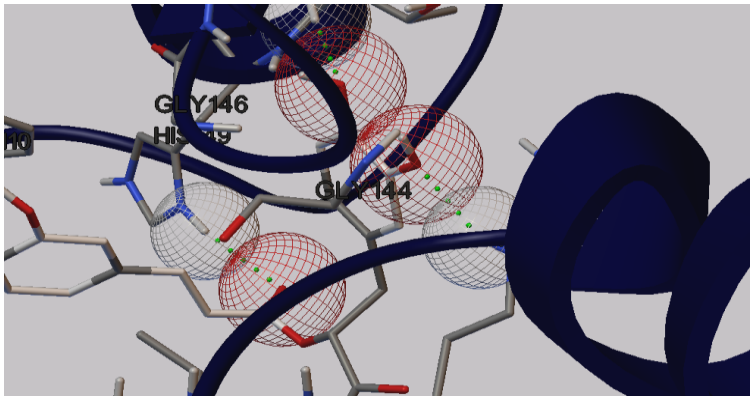

Figure 4: Drug target interaction between Chromosomal replication initiation protein of *Mycoplasma genitalium G37* and rosaminic acid.

Table 7: The natural ligands showing binding affinity to the drug targets of *Mycoplasma genitalium G37.*

Name of the compound	Dock score	Drug likeliness index
Cell division protein FtsZ (NP 072890.1)		
Ellagic acid	-7.4	-0.59069329
Hopane	-7.4	-0.86304953
Curcumin	-6.8	0.6758249
Caffeoylquinic acid	-6.7	0.29403503
Capsaicin	-6.4	0.56744161
ATP-dependent protease La (NP 072905.1)		
Amentoflavone	-9.3	-0.60523173
Silybin	-8.9	0.32390607
Robustaflavone	-8.9	-0.60523173
Withaferin A	-8.8	0.89623109
Tiliroside	-8.8	0.095186246

Elongation factor Tu (NP 073121.1)		
Robustaflavone	-8.7	-0.60523173
Procyanidin	-8.6	0.090156427
Hypericin	-8.5	0.46666369
Amentoflavone	-8.5	-0.60523173
Agathisflavone	-8.4	-0.60523173
Chromosomal replication initiation protein (NP 073140.1)		
Robustaflavone	-10	-0.60523173
Rosmarinic acid	-9.5	0.40160978
Naringin	-9.5	0.030521709
Hinokiflavone	-9.2	-0.60014507
Dehydrosoyasaponins	-9.2	0.45827078

Table 8: ADMET -Tox properties of the ligands showing binding affinity to the drug targets of *Mycoplasma genitalium G37*

Ligand ID	Aqueous solubility (logS)	Caco-2Permeability (logPapp)	Rat Acute Toxicity (LD50)
CID447043 (Azithromycin)	-2.0602	0.2710	2.5423
CID_969516	-3.3641	0.6485	2.5468
CID_31553	-2.6488	0.4354	2.2206
CID_107876	-3.4598	-0.9817	2.2049
CID_5281792	-3.2050	-0.5513	2.3234

Based upon the drug likeliness index and the 3-D structure of the protein Undecaprenyl pyrophosphate phosphatase and plantanoside were selected for the *Neisseria gonorrhoeae FA 1090*. Undecaprenyl pyrophosphate phosphatase plays an important role in peptidogylcan synthesis. Plantanoside is a flavanoid known for its antibacterial property [60]. No hydrogen bond interactions were noted therefore, involvement of other non-covalent forces is implicit. Fig 5 shows interaction of Undecaprenyl pyrophosphate phosphatase with plantanoside, The Table 9 and Table 10 shows the top scoring natural compounds and the ADMETox properties respectively.

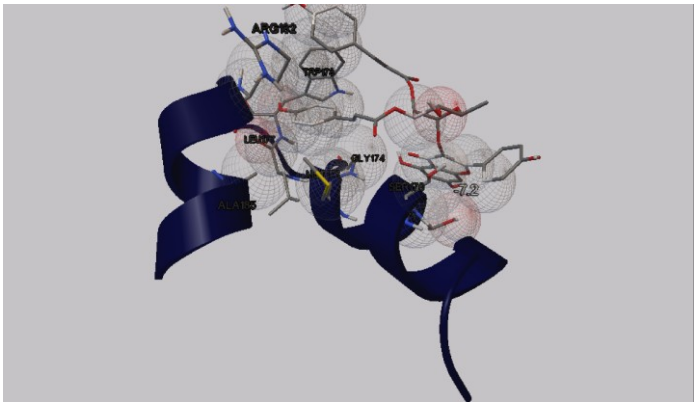

Figure 5 Drug target interaction between undecaprenyl pyrophosphate phosphatase and plantanoside in *Neisseria gonorrhoeae FA 1090.*

Table 9: The natural ligands showing binding affinity to the drug targets of *Neisseria gonorrhoeae FA 1090.*

Name of the compound	Dock acore	Drug likeliness index
Hypothetical protein NGO1534 (YP 208582.1)		
Hinokiflavone	-6.9	-0.60014507
Hypericin	-6.8	0.46666369
Amentoflavone	-6.8	-0.60523173
Glyceollin	-6.6	0.61551873
Agathisflavone	-6.4	-0.60523173
Phospho-N-acetylmuramoyl-pentapeptide-transferase (YP 208585.1)		
Hinokiflavone	-6.5	-0.60014507
Robustaflavone	-6.2	-0.60523173
Hypericin	-6.2	0.46666369
Agathisflavone	-6	-0.60523173
Amentoflavone	-5.8	-0.60523173
Undecaprenyl pyrophosphate phosphatase(YP_208595.1)		
Hinokiflavone	-7.6	-0.60014507
Amentoflavone	-7.3	-0.60523173

Robustaflavone	-7.3	-0.60523173
Plantanoside	-7.2	0.21371555
Agathisflavone	-7	-0.60523173
Hypothetical protein NGO1718 (YP_208751.1)		
Agathisflavone	-11.7	-0.60523173
Robustaflavone	-11	-0.60523173
Withaferin A	-10.8	0.89623109
Amentoflavone	-10.6	-0.60523173
Fumigaclavine C	-10.6	0.746533
Hypothetical protein NGO1800 (YP_208830_1)		
Hinokiflavone	-9.8	-0.60014507
Robustaflavone	-9.3	-0.60523173
Silybin	-9.2	0.32390607
Glyceollin	-9.2	0.61551873
Agathisflavone	-9.1	-0.60523173

Table 10: ADME-Tox properties of the ligands showing binding affinity to the drug targets in *Neisseria gonorrhoeae FA 1090.*

Ligand ID	Aqueous solubility (logS)	Caco-2Permeability (logPapp)	Rat Acute Toxicity (LD50)
CID 149096(ofloxacin)	-3.5105	1.1297	2.1639
CID 5281051	-3.2198	0.8964	2.6122
CID 6451113	-3.2198	0.8964	2.6122
CID 265237	-4.2028	0.7051	3.5404
CID 31553	-2.6488	0.4354	2.2206

In *Streptococcus agalactiae 2603V/R* Sensor hisdine kinases and narigin showed good interaction. Sensor hisdine kinases are the surface proteins that receive signals from the surrounding environment and tranduce the signal to activate the transcription factors. In the present study sensory box histidine kinase is known to be playing a role in two- component system by the biochemical annotation. Naringin is a secondary metabolite found in the grape fruit and has antibacterial activity [61]. Fig 6 shows interaction of Sensor hisdine kinases with naringin by three hydrogen bonds ARG246, ASN 300 and ARG 304. Table 11 represents the top 5 compounds showing affinity with their respective targets and the Table 12 shows adme-Tox properties of the top scoring compounds with respect to the reference.

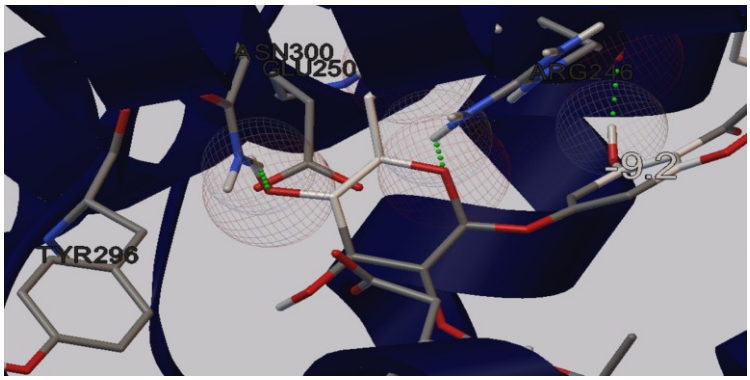

Figure 6: Drug target interaction between Sensor histidine kinase (NP_687355.1) of *Streptococcus agalactiae 2603V/R* and naringin.

Table 11: The natural ligands showing binding affinity to the drug targets of *Streptococcus agalactiae 2603V/R.*

Name of the compound	Dock score	Drug likeliness index
Sensor histidine kinase (NP 687160.1)		
Apigetrin	-11.5	0.008524294
Tiliroside	-11.3	0.095186246
Robustaflavone	-10.9	-0.60523173
Luteolin-7-glucoside	-10.6	0.0085243
Anonaine	-10.6	0.13319882
Undecaprenyl pyrophosphate phosphatase (NP 687174.1)		
Amentoflavone	-7.4	-0.60523173
Agathisflavone	-6.9	-0.60523173
Robustaflavone	-6.9	-0.60523173
Anonaine	-6.9	0.13319882
Fumigaclavine C	-6.8	0.7465329

Penicillin-binding protein 2X (NP 687322.1)		
Astilbin	-8.1	-0.003564923
1-Caffeoylquinic acid	-7.9	0.29403503
Anonaine	-7.9	0.13319882
Luteolin-7-glucoside	-7.8	0.0085243
Protocatechuic acid	-7.7	-0.9458831
Phospho-N-acetylmuramoyl-pentapeptide-transferase (NP 687323.1)		
Hinokiflavone	-9.2	-0.60014507
Ursane	-9.1	-0.76022747
Amentoflavone	-8.9	-0.60523173
pisatin	-8.9	-0.60523173
Brassicasterol	-8.8	0.24222149
Sensor histidine kinase (NP_687355.1)		
Naringin	-9.2	0.030521709
Poncirin	-9.1	0.014155298
Amentoflavone	-8.9	-0.60523173
Hinokiflavone	-8.9	-0.60014507
Robustaflavone	-8.8	-0.60523173
Sensor histidine kinase (NP 687428.1)		
Hinokiflavone	-9.3	-0.60014507
Robustaflavone	-9.3	-0.60523173
Hypericin	-9.2	0.46666369
Amentoflavone	-9.2	-0.60523173
Dehydrosoyasaponin I	-9.1	0.45827078
Sensory box histidine kinase (NP 687735.1)		
Amentoflavone	-9.1	-0.60523173
Robustaflavone	-8.8	-0.60523173
Plantanoside	-8.6	0.21371555
Hinokiflavone	-8.4	-0.60014507
Poncirin	-8.4	0.014155298
Cell cycle protein FtsW (NP 687776.1)		
Plantanoside	-8.3	0.21371555
Robustaflavone	-8.2	-0.60523173
Fumigaclavine C	-8.2	0.7465329
Xylopine	-8.1	0.30326379
Gamma-sitosterol	-8	0.11099078
Zinc metalloprotease (NP 688903.1)		
Naringenin	-8.2	-0.59069329
Eriodictyol	-8.1	0.16994626
Xylopine	-7.7	0.30326379
1-Caffeoylquinic acid	-7.4	0.29403503
Dihydrochalcone	-7.4	0.053720466
Sensor histidine kinaseb (NP 688947.1)		
Robustaflavone	-10.7	-0.60523173
Procyanidin	-10.5	0.090156427
Hypericin	-10.5	0.46666369
Hinokiflavone	-10.2	-0.60014507
Hesperidin	-10.1	0.084577358
Sensor histidine kinase (NP 689041.1)		
Plumbagin	-11.1	0.8813386
Amentoflavone	-10.9	-0.60523173
Hypericin	-10.8	0.46666369
Hinokiflavone	-10.6	-0.60014507
Robustaflavone	-10.6	-0.60523173
Sensor histidine kinase (NP_689108_1)		
Sanguinarine	-8.5	0.30787494
Hypericin	-8.4	0.46666369
Amentoflavone	-8.4	-0.60523173
Robustaflavone	-8.4	-0.60523173
Silybin	*-8.3*	0.32390607

Table 12: ADMET tox properties of the ligands showing binding affinity to the drug targets of *Streptococcus agalactiae 2603V/R.*

Ligand ID	Aqueous solubility (logS)	Caco-2Permeability (logPapp)	Rat Acute Toxicity (LD50)
CID_6249 (ampicillin)	-2.8487	0.0398	1.5620
CID 5385553	-2.3316	-0.9232	2.3755
CID 160597	-2.4944	1.3197	2.7790
CID 6451212	-2.4572	-0.6124	2.5685
CID 5281327	-4.6917	1.6384	2.6528

CID 442428	-4.3443	0.2440	2.9313
CID 5281051	-3.2198	0.8964	2.6122
CID 6451113	-3.9468	0.0899	2.8906
CID 440735	-3.4456	0.0595	3.3340
CID 107876	-3.4598	-0.9817	2.2049
CID 10205	-3.5106	1.5940	3.2704
CID 5154	-3.233	1.2338	2.3612

The putative drug target of the *Treponema pallidum subsp. pallidum SS14* the cell division protein is annotated to be involved in the cell-cycle caulobacter. The cell cycle caulobacter is a complex transcription regulation system that regulates the cell division. It showed best binding interaction with lupeol, a triterpene known to have the antibacterial activity [62]. Lupeol is the natural compound isolated from fruits and the vegetables. Fig 7 depicts the molecular interaction between the cell division protein and the lupeol. The summary of the dockscore and the ADMETox properties is given in Table 13 and Table 14 respectively.

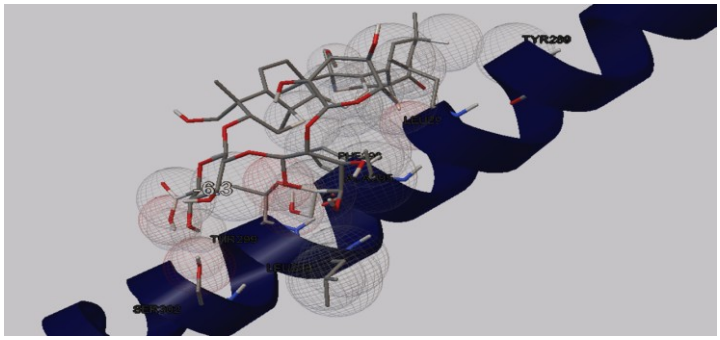

Figure 7: Drug target interaction between cell division protein of *Treponema pallidum subsp. pallidum SS14* and lupeol.

Table 13: The natural ligands showing binding affinity to the drug targets of *Treponema pallidum subsp. pallidum SS14.*

Name of the compound	Dock score	Drug likeliness index
Cell division protein (YP 001933392.1)		
Lupeol	-6.3	1.3117783
Dehydrosoyasaponin I	-6.3	0.45827078
Amentoflavone	-6.3	-0.60523173
Robustaflavone	-6.3	-0.60523173
Agathisflavone	-6.2	-0.60523173
Virulence factor (YP 001933518.1)		
Plantanoside	-12.1	0.21371555
Amentoflavone	-11.9	-0.60523173
Tiliroside	-11.9	0.095186246
Robustaflavone	-11.5	-0.60523173
Withaferin a	-11.4	0.89623109
Dicarboxylate transporter (YP 001933953.1)		
robustaflavone	-7.5	-0.60523173
Hypericin	-7.3	0.46666369
hinokiflavone	-7.3	-0.60014507
amentoflavone	-7.2	-0.60523173
diosmin	-7.2	0.084577358

Table 36: ADMET-Tox properties of the ligands showing binding affinity to the drug targets of *Treponema pallidum subsp. pallidum SS14.*

Ligand ID	Aqueous solubility (logS)	Caco-2Permeability (logPapp)	Rat Acute Toxicity (LD50)
CID447043 (Azithromycin)	-2.0602	0.2710	2.5423
CID 259846	-4.4139	1.6517	3.3838
CID 6451113	-3.9468	0.0899	2.8906
CID 5281051	-3.2198	0.8964	2.6122

IV. Conclusion

Natural compounds are good source of the therapeutic agents for various diseases. The drug targets as well as the natural compounds can be used in various experimental designs to pave way for the experimental process. The targets and the therapeutic agents provide a raw material for the experiments to validate their critical nature in survival and pathogenicity. The computational techniques and tools reduce the time required

for the identification of the therapeutic compounds to avoid huge amount of time required to test toxicities and other cul-de-sacs in the drug discovery process. Protein belonging to both the structural and the non- structural type, the non-structural type is ideal to be studied as a drug targets due to its functional role. The *insilico* approaches of target identification can also be used for other disease classifications for which no vaccines have been developed and for which the vaccines are inffective. Similar algorithms can also be applied to other targets to find the natural therapeutic agents by virtual screening. Active regions of the target called the epitopes can also be identified leading to use of epitope mapping approaches. Knowledge of the epitopes can also aid in development of the subunit vaccines such as the peptide vaccines that are specific and easy to develop. Therapeutic natural compounds themselves can be used to derive the analogous compounds by structural changes. Such compounds can act as potential therapeutic drugs against the known diseases.

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Conflict of interest

The conflict of interest between the authors is declared nil.

References:

[1]. K. Sakharkar, M. Sakharkar and V. Chow, A novel genomics approach for the identification of drug targets in pathogens, with special reference to Pseudomonas aeruginosa, *In silico biology, 4(3),* 2004, 355-60.
[2]. A. Dutta , S. Singh, P. Ghosh , R. Mukherjee , S. Mitter S and D. Bandyopadhyay. In silico identification of potential therapeutic targets in the human pathogen Helicobacter pylori, *In silico biology, 6(1-2)*, 2006, 43-7.
[3]. S. Saremy, M. Boroujeni, B. Bhattacharjee, V. Mittal and J. Chatterjee, Identification of potential apicoplast associated therapeutic targets in human and animal pathogen Toxoplasma gondii ME49, *Bioinformation, 7(8),* 2011, 379-83.
[4]. P. Katara, A. Grover, H. Kuntal and V. Sharma, In silico prediction of drug targets in Vibrio cholera, *Protoplasma, 248(4),* 2011, 799-804.
[5]. A. Shanmugam and J. Natarajan, Computational genome analyses of metabolic enzymes in Mycobacterium leprae for drug target identification, *Bioinformation, 4(9),* 2010, 392-5.
[6]. S. Madagi, V MaliPatil, S. Sadegh, A. Singh, B. Garwal, A. Banerjee, et al. Identification of membrane associated drug targets in Borrelia burgdorferi ZS7- subtractive genomics approach, *Bioinformation, 6(9),* 2011, 356-9.
[7]. M. Vijayakumari and M. Shivkumar, Putative drug targets in *Ureaplasma urealyticum* serovar 10 str ATCC 33699 by *In silico* genomic approach and virtual screening, *International Journal of Pharma and Biosciences, 4(4),* 2013, 214-21.
[8]. M. Vijayakuari , M. Shivkumar and B. Bhattacharjee , Sexually transmitted diseases putative drug target database: A comprehensive database of putative drug targets of sexually transmitted diseases pathogens identified by comparative genomics, *Indian Journal of Pharmacology, 45(45),* 2013, 434-38.
[9]. J. Drew, Genomic sciences and the medicine of tomorrow, *Nature biotechnology, 14(11),* 1996, 1516-8.
[10]. A. Hopkins, C.Groom, The druggable genome, Nature reviews Drug discovery, 1(9), 2002, 727-30.
[11]. D. Wishart, C. Knox, A. Guo, S. Shrivastava, M. Hassanali, P. Stothard, et al, DrugBank: a comprehensive resource for in silico drug discovery and exploration, Nucleic *acids research. 34(Database issue)*, 2006, D668-72.
[12]. P. Imming, C. Sinning, A. Meyer, Drugs, their targets and the nature and number of drug targets, *Nature reviews Drug discovery, 5(10),* 2006, 821-34.
[13]. C. Zheng, L. Han, C. Yap, B. Xie, Y. Chen, Progress and problems in the exploration of therapeutic targets, Drug *discovery today, 11(9-10)*, 2006, 412-20.
[14]. G. Paolini, R. Shapland, W. van Hoorn, J. Mason and A. Hopkins, Global mapping of pharmacological space, *Nature biotechnology, 24(7),* 2006, 805-15.
[15]. C. Freiberg, Novel computational methods in anti-microbial target identification, *Drug Discovery Today, 6(15)*, 2001, S72-S80.
[16]. M. Rask-Andersen, M. Almen and H. Schioth,Trends in the exploitation of novel drug targets, *Nature reviews Drug discovery, 10(8),* 2011, 579-90.
[17]. H. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. Bhat, Weissig H, et al, The Protein Data Bank. *Nucleic acids research, 28(1),* 2000, 235-42.
[18]. H. Gonzalez-Diaz, F. Prado-Prado, X. Garcia-Mera, N. Alonso, P. Abeijon, O. Caamano, et al, MIND-BEST: Web server for drugs and target discovery; design, synthesis, and assay of MAO-B inhibitors and theoretical-experimental study of G3PDH protein from Trichomonas gallinae, *Journal of proteome research, 10(4),* 2011, 1698-718.
[19]. L. Xie , T. Evangelidis, L. Xie and P.Bourne, Drug discovery using chemical systems biology: weak inhibition of multiple kinases may contribute to the anti-cancer effect of nelfinavir*, PLoS computational biology, 7(4),* 2011, e1002037.
[20]. H. Li, Z. Gao, L. Kang, H. Zhang, K. Yang, K. Yu, et al, TarFisDock: a web server for identifying drug targets with docking approach, *Nucleic acids research, 34(Web Server issue),* 2006, W219-24.
[21]. L. Yang , K. Wang , J. Chen, A. Jegga, H. Luo, L. Shi, et al. Exploring off-targets and off-systems for adverse drug reactions via chemical-protein interactome--clozapine-induced agranulocytosis as a case study, *PLoS computational biology, 7(3),* 2011, e1002016.
[22]. M. Campillos, M. Kuhn, A. Gavin, L. Jensen and P. Bork, Drug target identification using side-effect similarity, *Science, 321(5886),* 2008, 263-6.
[23]. F. Cheng, C. Liu, J. Jiang, W. Lu, W. Li, G. Liu et al, Prediction of drug-target interactions and drug repositioning via network-based inference, *PLoS computational biology, 8(*5), 2012, e1002503.
[24]. U. Schwabe, ATC-Code (Wissenschaftliches Institut derAOK, Bonn, Germany, 1995).
[25]. R. Borchard, *pharmaceutical profiling in drug discovery for lead selection* (Arlington, VA:AAPS press, 2004).

[26]. H. van De Waterbeemd, D. Smith, K. Beaumont and D.Walker, Property-based design: optimization of drug absorption and pharmacokinetics, *Journal of medicinal chemistry, 44(9),* 2001, 1313-33.
[27]. K. Jacek, H. Popielarska, A. Myka, B. Drabińska and M. Bernard, The log P Parameter as a Molecular Descriptor in the Computer-aided Drug Design – an Overview, *Computational Methods in Science and Technology18(2),* 2012, 81-88.
[28]. C. Bergstrom, Computational models to predict aqueous drug solubility, permeability and intestinal absorption, Expert *opinion on drug metabolism & toxicology, 1(4),* 2005, 613-27.
[29]. F. Zhu, Z. Shi, C. Qin, L. Tao, X.Liu, F. Xu, et al, Therapeutic target database update 2012: a resource for facilitating target-oriented drug discovery, *Nucleic acids research, 40(Database issue),* 2012, D1128-36.
[30]. K. Drlica and X. Zhao, DNA gyrase, topoisomerase IV, and the 4-quinolones, *Microbiology and molecular biology reviews : MMBR, 61(3),* 1997, 377-92.
[31]. H. Chambers, The changing epidemiology of Staphylococcus aureus?, *Emerging infectious diseases, 7(2),* 2001, 78-82.
[32]. M. Feher, J. Schmidt, Property distributions: differences between drugs, natural products, and molecules from combinatorial chemistry, *Journal of chemical information and computer sciences, 43(1),* 2003, 218-27.
[33]. F. von Nussbaum, M. Brands, B. Hinzen, S. Weigand and Habich D, Antibacterial natural products in medicinal chemistry--exodus or revival?, *Angewandte Chemie, 45(31),* 2006, 5072-129.
[34]. J. Hacker, J. Kaper, Pathogenicity islands and the evolution of microbes, *Annual review of microbiology, 54,* 2000, 641-79.
[35]. E. Moxon, P. Rainey, M. Nowak, R. Lenski, Adaptive evolution of highly mutable loci in pathogenic bacteria, Current biology : CB, 4(1), 1994, 24-33.
[36]. N. Perna, G. Plunkett, V. Burland, B. Mau, J. Glasner, D. Rose, et al, Genome sequence of enterohaemorrhagic Escherichia coli O157:H7, *Nature, 409(6819),* 2001, 529-33.
[37]. H. Chambers, The changing epidemiology of Staphylococcus aureus*?, Emerging infectious diseases, 7(2),* 2001, 178-82.
[38]. W. Martone, Spread of vancomycin-resistant enterococci: why did it happen in the United States? *Infection control and hospital epidemiology: the official journal of the Society of Hospital Epidemiologists of America, 19(8),* 1998, 539-45.
[39]. R. Jones, J. Kirby, M. Beach, D. Biedenbach and M. Pfaller, Geographic variations in activity of broad-spectrum beta-lactams against Pseudomonas aeruginosa: summary of the worldwide SENTRY Antimicrobial Surveillance Program (1997-2000), *Diagnostic microbiology and infectious disease, 43(3),* 2002, 239-43.
[40]. V. Memisevic, T. Milenkovic and N. Przulj, Complementarity of network and sequence information in homologous proteins, *Journal of integrative bioinformatics, 7(3),* 2010, doi: 10.2390/biecoll-jib-2010-135.
[41]. B. Rost, Twilight zone of protein sequence alignments, *Protein engineering, 12(2),* 1999, 85-94.
[42]. R. Doolittle, Similar amino acid sequences: chance or common ancestry?, Science, 214(4517), 1981, 149-59.
[43]. Jordan, I. Rogozin, Y. Wolf and Koonin E, Essential genes are more evolutionarily conserved than are nonessential genes in bacteria, Genome research, 12(6), 2002, 962-8.
[44]. R. Zhang, H. Ou, C. Zhang, DEG: a database of essential genes, *Nucleic acids research, 32(Database issue),* 2004, D271-2.
[45]. S. Peterson, C.Fraser, The complexity of simplicity, Genome biology, 2(2), 2001, COMMENT2002.
[46]. L. Jensen, R. Gupta, N. Blom, D. Devos , J. Tamames, C. Kesmir, et al, Prediction of human protein function from post-translational modifications and localization features, *Journal of molecular biology, 319(5),* 2002, 1257-65.
[47]. M. Plaunt, C. Patrick, Identification of the innate human immune response to surface-exposed proteins of coagulase-negative staphylococci, *Journal of clinical microbiology, 29(5),* 1991, 857-61.
[48]. H. Muller, K. Fruh, A. von Brunn, F. Esposito, S. Lombardi, A. Crisanti, et al, Development of the human immune response against the major surface protein (gp190) of Plasmodium falciparum, *Infection and immunity, 57(12),* 1989, 3765-9.
[49]. I. Roy, S. Bartels, F. Qadri and J. Fleckenstein, Enterotoxigenic Escherichia coli elicits immune responses to multiple surface proteins, *Infection and immunity, 78(7),* 2010, 3027-35.
[50]. B. Wei, L. Weaver, A. Ferrari, B. Matthews and B. Shoichet, Testing a flexible-receptor docking algorithm in a model binding site, *Journal of molecular biology, 337(5),* 2004, 1161-82.
[51]. P. Desai, A. Patny, Y. Sabnis, B. Tekwani, J. Gut, P. Rosenthal, Identification of novel parasitic cysteine protease inhibitors using virtual screening. 1. The ChemBridge database. *Journal of medicinal chemistry, 47(26),* 2004, 6609-15.
[52]. C. Bissantz, P. Bernard, M. Hibert and D. Rognan, Protein-based virtual screening of chemical databases. II. Are homology models of G-Protein Coupled Receptors suitable targets? *Proteins, 50(1),* 2003, 5-25.
[53]. G. Ramasamy, D. Gupta, A. Mohmmed, V. Chauhan, Characterization and localization of Plasmodium falciparum homolog of prokaryotic ClpQ/HslV protease. *Molecular and biochemical parasitology, 152(2),* 2007, 139-48.
[54]. O. Trott and A.Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, *Journal of Computational Chemistry, 31 (2),* 2010, 455-461.
[55]. F. Cheng, C. Liu, J. Jiang, W. Lu, W. Li, G. Liu, et al, Prediction of drug-target interactions and drug repositioning via network-based inference, PLoS computational biology, 8(5), 2012, e1002503.
[56]. Panossian, E. Gabrielian, V. Manvelian, K. Jurcic and H. Wagner, Immunosuppressive effects of hypericin on stimulated human leukocytes: inhibition of the arachidonic acid release, leukotriene B(4) and Interleukin-Ialpha production, and activation of nitric oxide formation, *Phytomedicine : international journal of phytotherapy and phytopharmacology, 3(1),* 1996, 19-28.
[57]. R. Utsumi and M. Igarashi M. [Two-component signal transduction as attractive drug targets in pathogenic bacteria], Yakugaku zasshi *: Journal of the Pharmaceutical Society of Japan, 132(1),* 2012, 51-8.
[58]. R. Helal and M. Melzig, In vitro Effects of Selected Saponins on the Production and Release of Lysozyme Activity of Human Monocytic and Epithelial Cell Lines, Scientia pharmaceutica, 79(2), 2011, 337-49.
[59]. J.Wang, X.Pan, Y. Han, D. Guo, Q. Guo and R. Li, Rosmarinic acid from eelgrass shows nematicidal and antibacterial activities against pine wood nematode and its carrying bacteria, Marine drugs, 10(12), 2012, 2729-40.
[60]. Edewor-Kuponiyi and Theresa Ibibia, A Review on Antimicrobial and Other Beneficial Effects of Flavonoids, International Journal of Pharmaceutical Sciences Review and Research, 21(1), 2013, 20-33.
[61]. G. Celiz, M. Daz and M. Audisio, Antibacterial activity of naringin derivatives against pathogenic strains, Journal of applied microbiology, 111(3), 2011, 731-8.
[62]. Pharmacogenomics. Indian journal of pharmacology, 40(Suppl 2), 2008, S57-9.