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Insilico Identification of Potent Inhibitor from Andrographis Paniculata for ESBLs

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ABSTRACT

The study aimed at docking of TEM - 52 & modeled CTX – M -13 by ethanol extracted compounds from *Andrographis paniculata* and to predict their binding efficiency. BLAST-P was performed to retrieve suitable templates for homology-modeling using the bla CTX-M sequences obtained from Genbank. Protein Data Bank (PDB) IDs of these templates were retrieved. Models were prepared using Swiss-Model-Server and verified by Procheck and 3D programmes RAMPAGE was used to prepare Ramachandran plots. The 13 - Hexyl - oxa - cyclotridec - 10 - En- 2 - one was selected as ligand for this docking study from the data generated by Mass spectrometry analysis of methanol extracted compounds from *Andrographis paniculata*. The structure was drawn manually with aid of JME and its drug likeliness & molecular properties was calculated using Pre Admet server. Docking was performed by using docking server. Since the docking results proved that the amino acid residues Ser70,Ser 130,Ser237,Arg 276 and Thr235 of CTX-M-13 (enzyme) make important contacts with the ligand, researchers are expected to duly utilize this information for designing more potent and CTX - M inhibitors.

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1. INTRODUCTION

Emerging resistant bacterial strains are the most serious global threats to treatment to the infectious diseases. While in 1975 just 3% of resistance isolates were registered by 2003 it had grown to 59.5%. More people have died of the bacterial infection caused by various microbes rather than HIV. The resistant organisms are now a worldwide problem. The wide spread use of antibiotics in hospitals has led to emergence of multidrug resistant organisms of low virulence like *Klebsiella* causing serious opportunistic infections. The resistant strains can be found in a variety of Enterobateriaceae species; however the majority of multi-drug resistant strains are *K.pneumoniae, K.oxytoca* and *Escherichia coli*. Among the several drug resistant bacteria β-lactamase production is the most important mechanism of resistance to penicillin and cephalosporins. The mechanism of resistance was the production of Extended Spectrum of β-Lactamases (ESBLs). The antibiotic resistance is conferred by several mechanisms in bacteria. The most common mode of resistance is enzymatic inactivation of the antibiotic. When the enzyme is modified it will not affect the microorganism. (Todar, 2008). The other mechanism is by alteration of antibiotic target site.

1.1. β-Lactamases

β - lactamases are enzymes produced by some bacteria and are responsible for their resistance to beta-lactam antibiotics like penicillins, cephalosporins (are relatively resistant to beta lactamase), cephamycins and carbapenems (ertapenem).These antibiotics have a common element in their molecular structure: a four atom ring known as a beta-lactam. The lactamase enzyme breaks the ring open and deactivates the molecule's antibacterial properties. The mechanism of action of β-lactamases includes attacks on amide bond in the β-lactam ring of pencillin and cephalosporin to produce pencillinoic acid and cephalosporic acid respectively, ultimately rendering the compound inactive (Philippon *et al*., 1989).

1.2. Extended Spectrum of β-Lactamases (ESBLs)

Extended Spectrum Beta Lactamases (ESBLs) have become a challenge both from the diagnostic as well as on the management point of view. (Mirza *et al.,*2006). The first Extended-spectrum β-lactamase isolates were discovered in Western Europe in the mid 1980's and subsequently in US in the late 1980s (Nathisuwan *et al*., 2001). They can be found in a wide variety of Enterobacteriaceae species however, the majority of ESBL producing strains are *Klebsiella pneumoniae*, *Klebsiella oxytoca* and later in *Escherichia coli*, *Pseudomonas aeruginosa* and *Serratia marcescens* and other gram-negative bacilli (Kiratisin *et al*., 2008: Cheng *et al*., 2008 and Morris *et al*., 2003). ESBLs are also able to hydrolyze 3rd and 4 th generation cephalosporins and monobactams. ESBL producing strains are inhibited by β-lactamase inhibitors (Clavulanic acid, Sulbactam and tazobactam) (Bradford, 2001: Pitout *et al.*, 2007 and Bali *et al*, 2010). Treatment of infections caused by these resistant bacteria has become very difficult since they are resistant to many antibiotics. This limits therapeutic options. Extended spectrum β-lactamases are plasmid mediated, TEM and SHV derived enzymes, most commonly found in *Klebsiella spp.*, followed by *Escherichia coli* (Poole, 2004). Single amino acid substitutions at positions 104, 164, 238, and 240 produce the ESBL phenotype, but ESBLs with the broadest spectrum usually have more than a single amino acid substitution. Based upon different combinations of changes, currently 140 TEM-type enzymes have been described.

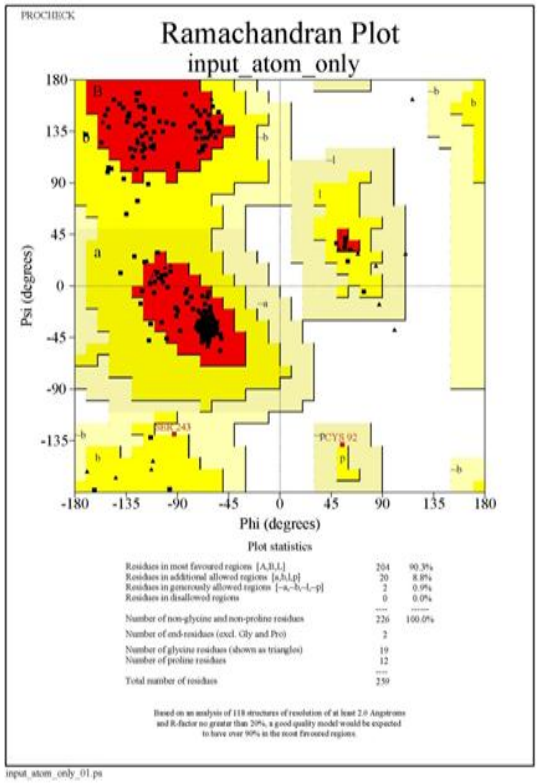

Figure 1. Procheck analysis results shows more favouring regions are present in modeled protein through their Ramachandran Plot analysis

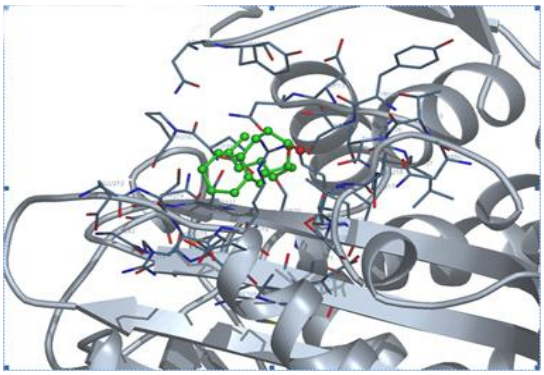

Figure 2. Interaction of 13 Hexyl oxa cyclo tridec 10 – en – 2- One & TEM_{52}

Many β-lactamase genes have been found in *Klebsiella pneumoniae* plasmids including those encoding extended spectrum β -lactamases (ESBLs). They are encoded in large plasmids with sizes of 80-100kb. Members of the family *Enterobacteriaceae* commonly express plasmid encoded β-lactamase (eg) TEM-1, TEM-2 and SHV-1) which confer resistance to penicillins but not to expanded spectrum of cephalosporins.

In the mid 1980s a new group of enzymes, the extended – spectrum β-lactamases (ESBLs), were detected. The resistance is derived from genes for TEM-1, TEM-2 or SHV-1 by mutations that alter the amino acid configuration around the active site of β-lactamases. This extends the spectrum of β-lactam antibiotics susceptible to hydrolysis by these enzymes. Carbapenems are the treatment of choice for serious infections due to ESBL producing organisms, yet carbapenem resistant isolates have recently been reported. However, treatment with such antibiotics has been associated with high failure rates in recent years CTX-M extended-spectrum β-lactmases (ESBLs) have very rapidly disseminated and are now frequently reported from countries all over Europe and much of Asia. (Kapil, 2005). In India, the very first report of the presence of CTX-M- producing Enterobacteriaceae came from New Delhi. Six strains isolated in the year 2000 were investigated, all the strains were found to be unrelated and all produced CTX-M-15. Since then several Indian surveys have reported the presence of ESBLs in clinical isolates based on phenotypic tests. Recently reported rates vary widely (12.6-71%), with most studies reporting prevalence rate of around 50% and upwards. Considering a population of approximately 1.1 billion in India, it represents a very large reservoir for resistance gene.

The problem of emerging bacterial resistance traditionally has been solved by the discovery of new antimicrobial drugs. Unfortunately, there is no assurance that the development of new antibiotics can keep pace with the ability of bacterial pathogens to develop resistance. The increased use of antibiotics encourages the emergence of resistant strains of bacteria. There are geographic variations in antibiotic resistance depending on local prescribing trends. Resistance of bacteria is continuing to increase, both in number and in variety, but not significantly different newer antibiotics are yet available. Thus today there is an urgent need for new antibacterial drugs due to fast multiplication of drug resistance bacterial strains and also new, emerging pathogens. The ultimate goal to offer appropriate and efficient antimicrobial drugs to the patient is through continuous studies to develop new drugs, either synthetic or natural. Natural products, either as pure compounds or as standardized plant extract provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Traditional medicines are used by about 60 percent of the world's population. These are used for primary health care not just in rural areas of developing countries, but also in developed countries where modern medicines are predominantly used (Rout *et al*, 2012).

This study explores the possibility of utilizing the plant *A. paniculata* active principles in medicine to reduce the drug resistance problem by ESBL pathogens. The plant is reported to possess protective activity against various liver disorders. It is used to treat gastrointestinal tract and upper respiratory tract infections, fever, herpes, sore throat, hepatitis and a variety of other chronic and upper respiratory tract infections (Chopra *et al*., 1956). It exhibits antibacterial, filaricidal, antidiarrhoeal, improves cardiovascular activities, fertility and gives protection to liver and gall bladder. The herb and its isolates like andrographolide, neographolide, etc. are reported to possess anti-inflammatory, hepato-protective, astringent, anodyne, tonic, alexiphormic and anti pyretic properties and helps in arresting dysentery, cholera, diabetes, influenza, bronchitis, swellings and itches, piles and gonorrhoea (Prajapati *et al*.,2003). Flavonoids present in plant showed potent inhibition of collagen, arachidonic acid, thrombin and platelet aggregation (Wu *et al.,* 2008). The primary medicinal constituents of *A. paniculata* are andrographolide and related compounds which are diterpeniods showing antipyretic, anti-inflammatory, immuno stimulatory and anti cancerous activities (Mishra *et al*., 2009; Saxena, 2000; Kumar *et al*., 2004).

The present study undertaken to investigate the plant active compound of fractions whose binding properties was studied *Insilico* provides an initial step towards the development of a novel drug molecule that can competively bind to the active site of ESBL genes TEM $_{52}$, CTX- M $_{13}$ and SHV 1. The antimicrobial activity potential of *A. paniculata* has been determined and the active principles responsible for the activity have been isolated and characterized by FT IR and GC MS spectrum analysis.

2. MATERIALS AND METHODS

In order to determine the binding energy of active compounds characterized by FT IR and GC MS spectrum analysis responsible for the antibacterial activity against ESBL s TEM $_{52}$, CTX – M $_{13}$ & SHV$_{12}$ a molecular docking calculation was done in DOCKING WEB SERVER (www.dockingserver.com). The steps carried out as the protein structure TEM $_{52}$ required for docking procedure were downloaded from Protein Data Bank CTX – M $_{13}$structure was homology built by using SWISS protein model server,

SHV12 structure was obtained from Protein Model Portal (Protein Model Portal:www.proteinmodelportal.org/quey/uniprot/A6TIK7).

The active compounds shown in highest peak characterized by FTIR and GC Analysis is 13 – Hexyloxacyclotridec 10 – En – One, the structure of this compound was drawn with chemical drawing tools such as saved in 'mol' file format and the biological activity of the compound was predicted and Absorption Distribution Metabolism Excretion and Toxicity (ADMET) properties were analyzed through computational methods ADMET server (http://preadmet.bmdrc.org/). Drug likeness of this compound was tested with WDI rule and Lipinski's rule 5 and revealed that $_{13}$-hexyl oxy cyclo tridec-10-en-2-one, would serve as an effective compound against ESBLs.

The MMFF94 (Halgren, 1998) was used for energy minimization of ligand molecule. Gasteiger partial charges were added to the ligand atoms. Non polar hydrogen atoms were merged and rotatable bonds were defined. Docking calculations were carried out on TEM_{52},CTX-M_{13} and SHV_{12} protein models. Essential hydrogen atoms, Kollman united atom type charges and solvation parameters were added with the aid of AutoDock tools implemented in docking server. Affinity (grid) maps of 20x20x20 A^o grid points and 0.375 A^o spacing were generated using the Autogrid program. AutoDock parameter set and distance-dependent dielectric functions were use in the calculation of the Vander Waals and the electrostatic terms respectively. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250000 energy evaluations and the binding energies in different poses of interaction of 13 – Hexyloxacyclotridec - 10 – En – One to ESBLs were calculated.

3. RESULTS AND DISCUSSIONS

The three dimensional structure of beta lactamase $TEM_{52}$was determined using X-ray crystallography method. Homology modeling of CTX- M-$_{13}$ Blast-P was performed against Protein Data Bank to retrieve suitable templates for homology modeling the CTX- M-$_{13}$ sequence obtained from Genbank accession numbers AF252623. Based on the E-score PDB-IYSA was chosen as template for modeling. Models were prepared using Swiss Model Server (Arnold *et al*., 2006) and verified by Procheck. RamPage was used to prepare Ramachandran plots (Fig.1).Over 90% of the amino acid residues in the protein structure modeled from CTX- M-$_{13}$ gene were found to be present in the most favourable regions as revealed by their respective Ramachandran plots. Modeled Structure of SHV_{12} was obtained from Protein Model Portal (Protein Model Portal: www.proteinmodelportal.org/quey/uniprot/A6TIK7).

The ligand molecule 13-hexyl oxa cyclo tridec-10-en-2-one included in this study was obtained from ethanol extract of *Andrographis paniculata* which showed the highest peak value in the Mass Spectrometry analysis. The structure of this compound was drawn with chemical drawing tools such as saved in 'mol' file format. The biological activity of the compound was predicted and Absorption Distribution Metabolism Excretion and Toxicity (ADMET) properties were analyzed through computational methods ADMET server (http://preadmet.bmdrc.org/) Drug likeness of this compound was tested with WDI rule and Lipinski's rule 5 and revealed that $_{13}$-hexyl oxa cyclo tridec-10-en-2-one would serve as a drug –like compound. The predicted properties of 13-hexyl oxa cyclo tridec-10-en-2-one are shown in Table 1.

Table 1. Predicted Properties of 13 – Hexyloxacyclotridec -10 – En –one

Calculated Properties	13 – Hexyloxacyclotridec - 10 – En –one
Molecular Formula	$C_{18}H_{32}O_2$
Molecular Weight	289
Molar Volume	325.39
Log P	4.29
Hydrogen bond acceptor site	3
Hydrogen bond donor site	1
Partial positive area	324.59
Partial negative area	160.35
Hydrophobic surface	446.521
Surface area on donor Hydrogen atoms	10.324
Charge on donatable Hydrogens	0.340706
Polar surface area	34.162
Hydrophobic surface area	249
Wiener Index	1031
Lipinski's Rule of Five	Suitable

Docking stimulations were performed using the Lamarckian Genetic Algorithm (LGA) and the Solis and Wets local method. Initial position, orientation and torsions of the ligand (Srivastava *et al*., 2010) molecules set randomly. Each docking experiment was derived from 10 different runs that were set to

terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 and quaternion and torsion steps of 5 were applied (Table 2).

Table 2. Best Rank Pose Results

Protein Ligand	Estimation of free energy of binding	Estimation of inhibition constant Ki	VanderWals+ Hydrogen bond +Dissolving energy	Electrostatic energy	Total Internal energy	Interacting Surface
TEM $_{52}$ to13 Hexyl oxa cyclotridec 10-en 2-one	-3.27 kcal/mol	3.99uM	- 4.17kcal/mol	-0.33kcal/ mol	-4.50kcal/ mol	600.373
CTX – M_{13} to 13 Hexyl cyclotridec10-en 2-one	-4.95 kcal/mol	236.43uM	-5.75 kcal/mol	-0.44kcal/ mol	-6.19kcal/ mol	638.323
SHV_{12} to 13 Hexyl cyclotridec10-en 2-one	-4.67 kcal/mol	379.65 uM	-5.11 kcal/mol	-0.95 kcal/ mol	-6.06 kcal/ mol	-

The molecular basis of interaction and affinity of binding of TEM_{52} ,CTX – M_{13} and SHV_{12} to the potent ligand 13 -Hexyl cyclotridec-10 – 2 - en- one from *Andrographis paniculata.* These were docked into the active site of of TEM $_{52}$, CTX -M_{13} and SHV_{12}. Fig. 2,3 & 4 represents interaction of 13 Hexyl oxa cyclo tridec 10- en- 2- One & TEM_{52}.

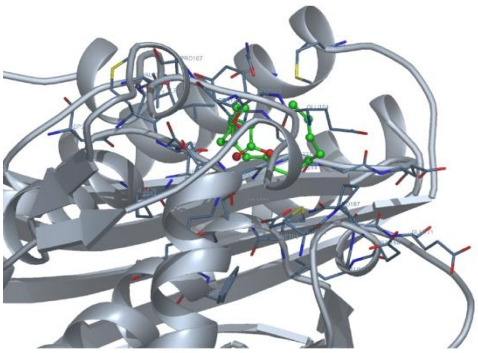

Figure 3. Interaction of 13 Hexyl oxa cyclotridec 10 – en – 2- One & CTX-M_{13}

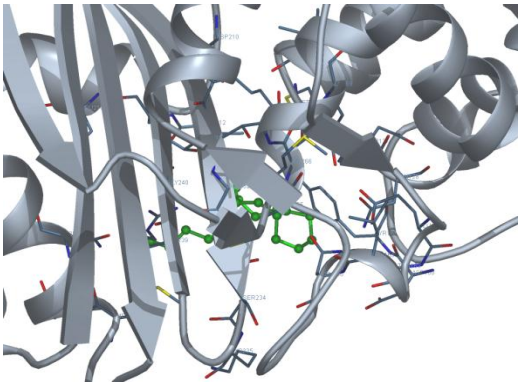

Figure 4. Interaction of 13 Hexyl oxa cyclo tridec 10 – en – 2- one & SHV_{12}

The docking results proved that the aminoacid residues Ser 70, Ser 130, Ser 126,Arg 276 and Thr 231, Thr 235 of TEM $_{52}$, CTX-M $_{15}$ and SHV_{12} make important contact with the ligand. In a docking study conducted by Shakil *et al*.,(2010), the amino acid residues Asn132, Glu166, Pro167,Val172,Lys234 and Thr235 make important contact with cefotaxime thereby resist the third generation cephalosporin drugs. The present investigation also gave a very good idea about the presence of amino acids in most favourable regions revealed by the respective Ramachandran plot (and the amino acid residues Ser 70, Ser 130 Ser 126,Arg 276, Thr 231 and Thr 235 of TEM_{52}, CTX-M_{15} and SHV_{12} make important contact with 13 Hexyl oxa cyclo tridec 10 – en – 2- one. Since 13 Hexyl oxa cyclo tridec 10 – en – 2- one is able to contacts with these amino acids and this could be a potent drug against ESBL strains.

4. CONCLUSION

In an investigation by Daisy *et al*., (2008), the protein autolysin from ESBL strain *Staphylocoocus aureus* was docked with the terpenoid from *Elephantopus scaber* and found that terpenoid can inhibit the activity of autolysin by forming a strong atomic interaction with the active site residues. In the present study also ESBL *E.coli* and *K.pneumoniae* were taken for docking study which posses the ESBL enzymes. The Log P value of 13 -Hexyl cyclotridec-10-en-2one was found to be 4.29 which resembles terpenoid from *Elephantopus scaber* and so because of this partition coefficient this ligand can break up the cell wall of the organisms and penetrate them.

In present scenario, the ongoing fight against multidrug resistant microorganisms has received a set back to generate a potent drug. Therefore, studies are being carried out in various parts of world to find an alternative viable drug which can come into rescue. This ligand may be such a potent one. Experimental investigations are still carried out to validate the findings from insilico approaches. Hence the compound 13 -Hexyl cyclotridec-10-en- 2- one from *Andrographis paniculata* in the present study can act as drug for ESBL pathogens and could be potential drug against multidrug resistant and ESBL organisms.

Further investigations can be carried out to predict the activity on other ESBL pathogens. This active molecule whose binding property was studied in silico provides an initial step towards the development of a novel drug molecule that can competitively bind to the active site of the ESBL enzymes thereby inhibiting beta lactamase resistance. Through our work we have tried to provide an insilico based investigation on the same by using most reliable and sophisticated Docking algorithm to provide an insilico testimony and basis to the experimental investigations. Hence the present investigation gives a clear insilico indication and evidence that 13 -Hexyl cyclotridec-10 -en- 2- one from *Andrographis paniculata* could be a potential drug for generation drug development against multidrug resistant and ESBL organisms. Further investigations can be carried out to predict the activity of 13 - Hexyl cyclotridec -10 -en- 2- one with the other target ESBL enzymes.

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