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Study of Antibacterial Efficacy of *Andrographis paniculata* against TEM-1 Beta-lactamase Producing *Escherichia coli*

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Abstract

Aims – A large number of plants with medicinal properties have been used in the pharmacological researches for drug delivery against the infections caused by *Escherichia coli.* Such plants have always been known for their ability to produce secondary metabolites that have antimicrobial properties and are main components for drug design. The terpenes that occur naturally in the plant *Andrographis paniculata* have evidences of showing pharmacological activities against beta-lactamase producing *Escherichia coli*. TEM-1 beta-lactamase is the enzyme responsible for the resistance shown by bacteria against different types of potent antibiotics. The study was initiated to analyze the antimicrobial potential of the different terpenes which occur naturally in the plant.

Methods – Biochemical study was conducted on the plant extract to reveal the presence of terpenes in the extract. The receptor protein and the ligands were fetched. ADMET screening was conducted to analyse the drug likeliness of the terpenes. UCSF Chimera was used to remove the already available ligands from the receptor protein to vacate the active sites. AutoDock 4.2.6 was used to conduct the molecular docking experiments.

Results – ADMET screening revealed that the natural terpenes were non-toxic and can be taken as candidates for the discovery of new drugs. The binding affinity of the naturally occurring terpenes against TEM-1 beta-lactamase suggested that they had the potential to act against TEM-1 beta-lactamase enzyme-producing *Escherichia coli.*

Conclusion - Results indicated that the naturally occurring terpene andrographolactone show highest efficacy by inhibiting the activity of TEM-1 beta-lactamase.

Keywords – ADMET screening, Drug delivery, Inhibition constant (Ki), Secondary metabolites, Terpenes

INTRODUCTION

Escherichia coli, which is a gram-negative and facultative anaerobic type of bacteria is mostly found in the intestines of human body. It is a rod-shaped and coliform bacterium belonging to the genus *Escherichia* and family Enterobacteriaceae. Although most of the *Escherichia coli* strains are harmless and assist in digestion some strains are harmful and are causal agents of many diseases. Serious illnesses like enteritis, urinary tract infection, septicemia, and neonatal meningitis are being prominently caused by the bacteria *Escherichia coli* [1]. Among the harmful variants, those producing the enzyme beta-lactamase have shown resistance to several antibiotics like penicillin, cephalosporin and carbacephem [2]. The beta-lactamase group hydrolyses several antibiotics by degrading the β-lactam ring of the antibiotics. Among the types of beta-lactamase, TEM-1 is the most confronted beta-lactamase in Gram-negative bacteria. A huge percent *i.e.* 90% of antibiotic resistance in bacteria like *Escherichia coli* is because of TEM-1 [3]. The variant has been reported to confer resistance to amoxicillin and clavunalic acid which are important antibiotics for infections caused by *Escherichia coli* [4]. TEM-1 beta-lactamase is taken as the prime target for the study.

Andrographis paniculata also called Kalmegh by some locals is a plant of great importance found in South Asian countries of the world. It is placed in the family Acanthaceae [5]. It is a traditional cure for many underlying diseases like diabetes, cough, fever, cold, and respiratory tract infections. Evidences are also found that the plant has an inhibitory effect against fatal conditions like inflammation, cancer, obesity, diabetes, and other activities [6]. Many important phytochemicals like terpenes, flavonoids and phenols are found in the plant which have evidences of inhibiting the effect of several disease-causing microbes [7]. It has been reported that the compounds found in the plant can inhibit a certain extent of beta-lactamase-producing strains [8].

MATERIALS AND METHODS

Biochemical study

The fresh aerial parts of the plant *Andrographis paniculata* were collected from the Herbal Medicinal Garden of Assam Don Bosco University, Tapesia campus. The aerial parts of the plant were dried at room temperature for 30 days and then powdered. Methanol extract of the powder was prepared and Salkowski test was done to check for the presence of terpenes [9]. 2 ml of the extract was poured into a test tube and 1 ml of chloroform was added to it and mixed. After mixing 2 ml of concentrated Sulphuric acid (H_2SO_4) is added to the mixer.

The Ligands

Five naturally occurring terpenes from the aerial parts of the plant were selected based on extensive literature review and phytocompound database analysis [10, 11]. The phytocompounds were fetched in 3D structures from the NCBI PubChem database (https://pubchem.ncbi.nlm.nih.gov/). All the terpenes were downloaded in SDF format from the database and then converted to PDB format with the help of OpenBabel [12]. The natural terpenes taken as ligands for study were 14-deoxy-11,12-didehydroandrographolide, Andrograpanin, Andrographolactone,Andrographolide and Neoandrographolide. To analyze the efficacy of the above terpenes, known antibiotics which are reported to be effective against different types of beta-lactamase

enzymes are taken as references (Amoxicillin, Ampicillin, Piperacillin and Ticarcillin). All these reference antibiotics are drugs that are used in combination with beta-lactamase inhibitors like Tazobactum, Sulbactum and Clavulanic acid [13, 14]. 3D structures of all the reference drugs were downloaded from the NCBI PubChem database in SDF format and then similarly converted to PDB format.

ADME / Tox Screening

ADMET screening is the method of determining the drug likeliness of a particular ligand [15]. The name ADMET is referred to Absorption, Distribution, Metabolism, Excretion and Toxicity. The screening of the ligands was done with the help of the server "Mobyle@rpbs" [16]. All the selected ligands were imported to the server in SMILES format based on the following standard parameters –

Molecular weight:<500, logP: <5, Hydrogen donors: <5, Hydrogen acceptors: <10,

LogSw: <0, Polar Surface Area: 0 - 150.0, Lipinski violation: 0, Result: Accepted

The receptor

The crystal structure of the Imipenem inhibited TEM-1 Beta-lactamase from *Escherichia coli* with PDB ID – 1BT5 was fetched from RCSB Protein Data bank (http://www.rcsb.org). The protein has one chain (Chain A) of 263 residues determined by X-ray diffraction method at a resolution of 1.80 Å. It was deposited by Maveyraud et al., in the year 1998. Imipenem and Sulfate ions were present in the receptor protein as docked ligands. The docked ligands from the enzyme TEM-1 beta-lactamase were removed with the assistance of the tool UCSF Chimera [17]. The removal of the already present docked ligands from the enzyme made the active sites vacant for molecular docking study. The natural terpenes as well as reference antibiotics were taken as ligands for molecular docking study against the receptor enzyme TEM-1 beta-lactamase.

Preparation of the ligands and receptor protein

The crystal structure of TEM-1 beta-lactamase from *Escherichia coli* which was downloaded in PDB format was loaded in AutoDock-MGL Tools [18]. The water molecules were deleted as they are not involved in the binding process and they might block the binding site. Polar hydrogens and Kollman charges were added to the receptor. The standard processes were followed to obtain the PDBQT format of the protein.

The converted PDB format of the 3D structures of the ligands was also loaded in AutoDock-MGL Tools and optimized one by one. Non-polar hydrogens and Gasteiger charges were added to the ligands. The roots, torsions, and aromatic carbons were analyzed followed by the standard process to obtain PDBQT files of the ligands.

Protein-Ligand interaction using Autodock

Through molecular docking, we can predict the inhibitory effect of a certain ligand against a receptor protein. It has become a powerful approach for structure-based drug discovery [19]. The software used for molecular docking of the optimized receptor protein and the ligands was AutoDock 4.2.6 from The Scripps Research Institute [18]. As the proper active sites were unknown, blind docking was conducted maximizing the grid box. The results of the molecular docking were analyzed thoroughly which consists of different binding conformations between the ligands and receptor with the value of binding affinity in kcal/mol. The binding conformation with the highest binding affinity was selected. The interactions between the protein and the ligands were visualized with the help of the tool UCSF Chimera in 3D structures. In the same way, the 2D structures were visualized with the assistance of the software LigPlot plus [20].

RESULTS

The test for phytochemicals by Salkowski test verified the presence of terpenes in the plant extract. A reddish-brown coloration of the interface formed revealed positive results for the presence of terpenes [21]. The result of the test is given in Fig. 1. Here in the study, all the compounds taken for the study of antimicrobial properties were terpenes.

A molecule can only be promoted for use as a drug when it has no toxicity and allergenic effect. All the ADMET properties should also be present in the compound within the recommended value. The ADMET screening value of all the five compounds taken for the present study showed positive results. This represents the potential of the terpenes as drugs. The results of the ADMET screening are mentioned in Table 1.

The interaction between the receptor and the ligand plays a major role in drug designing. The TEM-1 Beta-lactamase structure was selected as the receptor and the five terpenes from the plant as ligands. The four other known antibiotics were also docked against the selected receptor. The molecular docking analysis of the terpenes is represented in Table 2. The terpene andrographolactone showed the best results with an inhibition constant (Ki) of 0.21 uM (micromolar) and binding energy of -9.11 Kcal/mol.

The molecular docking analysis of the known antibiotics which are reported to be used against beta-lactamase enzymes are represented in Table 3. Ampicillin exhibited the best result with an inhibition constant of 0.56 uM (micromolar) and binding energy of -8.52 Kcal/mol.

After completing the process of molecular docking, the different protein-ligand complexes formed against the receptor protein by the different natural terpenes taken as ligands were visualized. The 3D visualizations to show the location of the binding sites of the receptor protein against the naturally occurring terpenes used as ligands are illustrated in Fig. 3.

Reference antibiotics were also molecularly docked against the receptor protein, TEM-1 beta-lactamase. The different protein-ligand complexes formed against the receptor protein by the different reference antibiotics taken as ligands are visualized. The 3D visualization to show the location of the binding sites of the receptor

protein against the reference antibiotics taken as ligands is shown in Fig. 4.

It was essential to know the different types of interactions formed between the receptor protein and the ligands. The 2D visualization to show the different bonds formed between the various interacting amino acid residues of the receptor protein and the naturally occurring terpenes is given in Fig. 5. Both the hydrogen and the hydrophobic interacting amino acid residues are illustrated.

Similarly, the interaction between the receptor protein and the reference antibiotics is displayed in 2D visualization to show the different bonds formed between the amino acid residues of the receptor protein and the reference antibiotics. Here also, the hydrogen interacting as well as the hydrophobic interacting amino acid residues are displayed in Fig. 6

Table 1: ADMET screening of the Terpenes from *Andrographis paniculata*

Compound	MW	LogP	HBD	HBA	PSA	Lipinski violation	Result
Standard parameters	**<500**	**<5**	**<5**	**<10**	**0-150**	**0**	**Accepted**
14-deoxy-11,12-didehydroandrographolide	332.43	3.23	2	4	66.76	0	Accepted
Andrograpanin	318.45	4.22	1	3	46.53	0	Accepted
Andrographolactone	296.4	4.72	0	2	26.3	0	Accepted
Andrographolide	350.45	2.16	3	5	86.99	0	Accepted
Neoandrographolide	480.59	2.63	4	8	125.68	0	Accepted

a) MW: Molecular weight, b) LogP: Lipophilicity,
c) HBD: Hydrogen Bond Donor, d) HBA: Hydrogen Bond Acceptor, e) PSA: Polar Surface Area

Table 2: Molecular docking analysis of Terpenes

Name of the compound	Binding energy (Kcal/mol)	RMSD (Å)	Ki in µM	H bond	Amino acids involved in Hydrogen bonding
14-deoxy-11,12-didehydroandrographolide	-6.91	28.670	8.59	2	Glu212, Ile231
Andrograpanin	-7.73	42.542	2.16	1	Ile143
Andrographolactone	-9.11	32.536	0.21	1	Arg43
Andrographolide	-5.77	37.103	59.17	3	Ser235, Val216, Ser70
Neoandrographolide	-5.45	16.471	100.38	4	Glu63, Lys34, Glu58, Ser59

a) Ki: Inhibition constant, b) H Bond: Hydrogen bond

Table 3: Molecular docking analysis of known drugs

Name of the compound	Binding energy (Kcal/mol)	RMSD (Å)	Ki in µM	H bond	Amino acids involved in Hydrogen bonding
Ampicillin	-8.52	39.595	0.56	4	Ser70, Ser130, Asn132, Ala237
Amoxicillin	-6.71	47.701	12.04	1	Glu104
Piperacillin	-5.53	30.331	88.98	2	Arg222, Lys215
Ticarcillin	-5.27	31.113	136.59	2	Arg65, Asn175

a) Ki: Inhibition constant, b) H Bond: Hydrogen bond

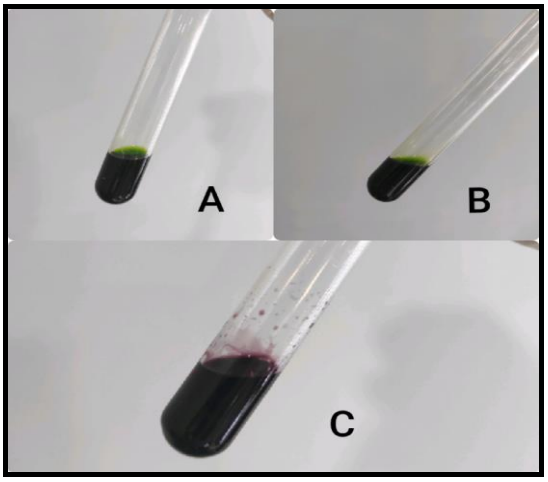

Fig. 1: Salkowski test verified the presence of terpenes in *A. paniculata*

A – Methanol extract of aerial part of the plant; B – Addition of chloroform to the extract;
C – A reddish-brown coloration showing a positive result

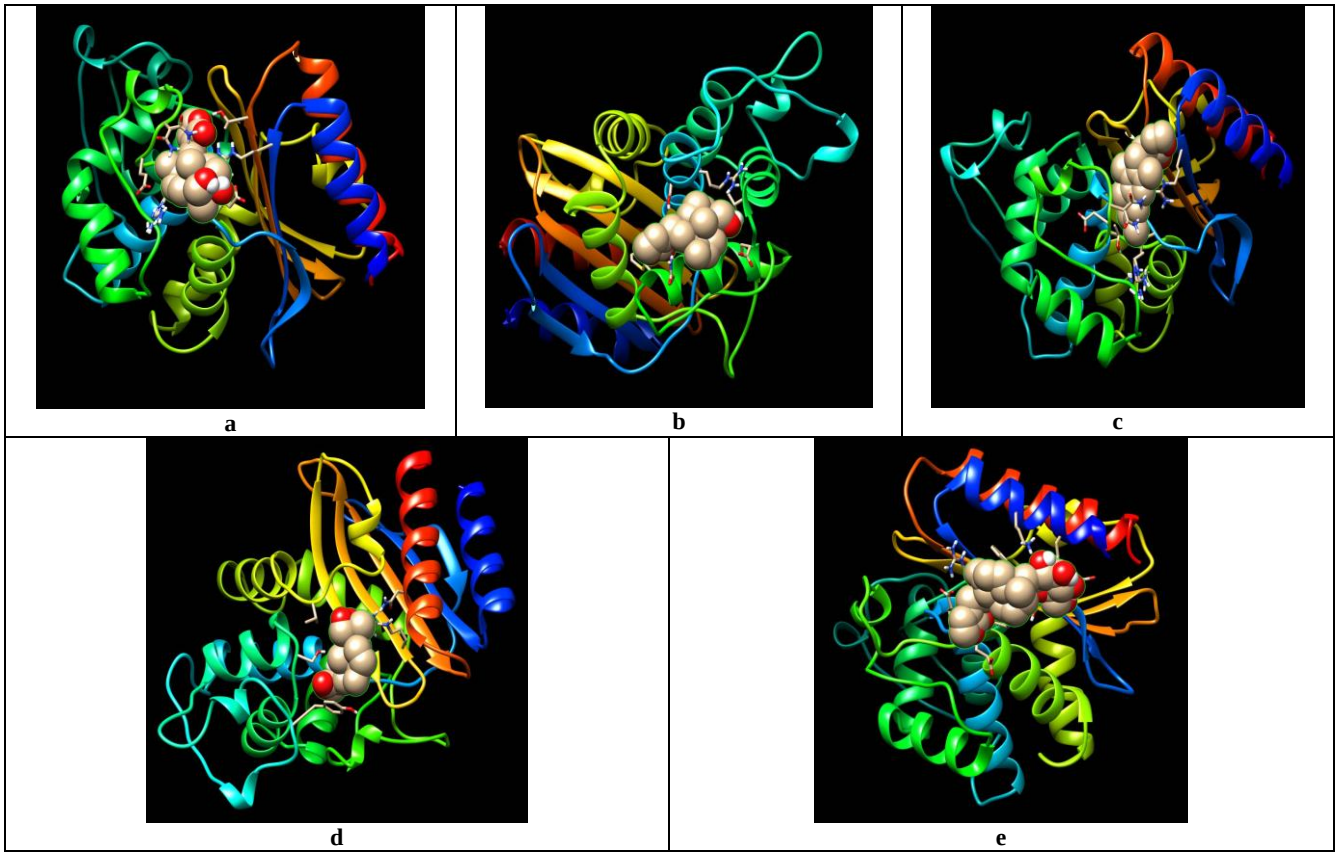

Fig. 3: 3D visualizations of molecular docking analysis of natural terpenes
a) 14-deoxy-11,12-didehydroandrographolide, b) Andrograpanin, c) Andrographolactone, d) Andrographolide, e) Neoandrographolide

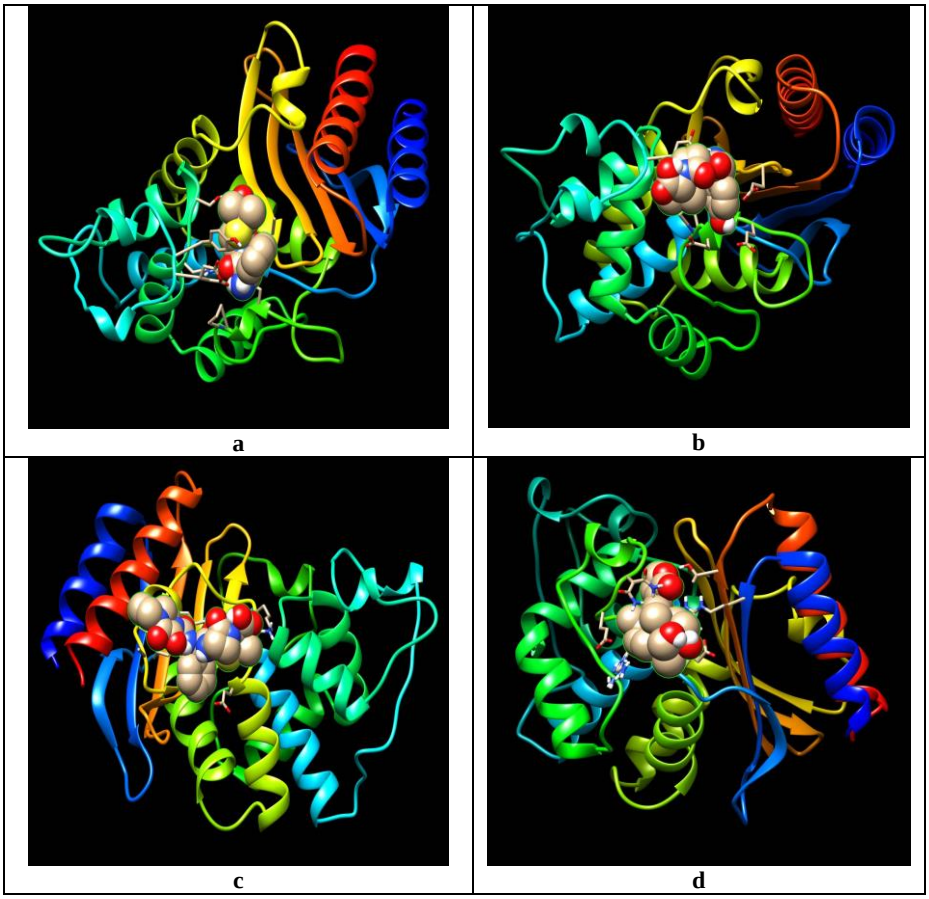

Fig. 4: 3D visualizations of molecular docking analysis of reference drugs
a) Ampicillin b) Amoxicillin c) Piperacillin d) Ticarcillin

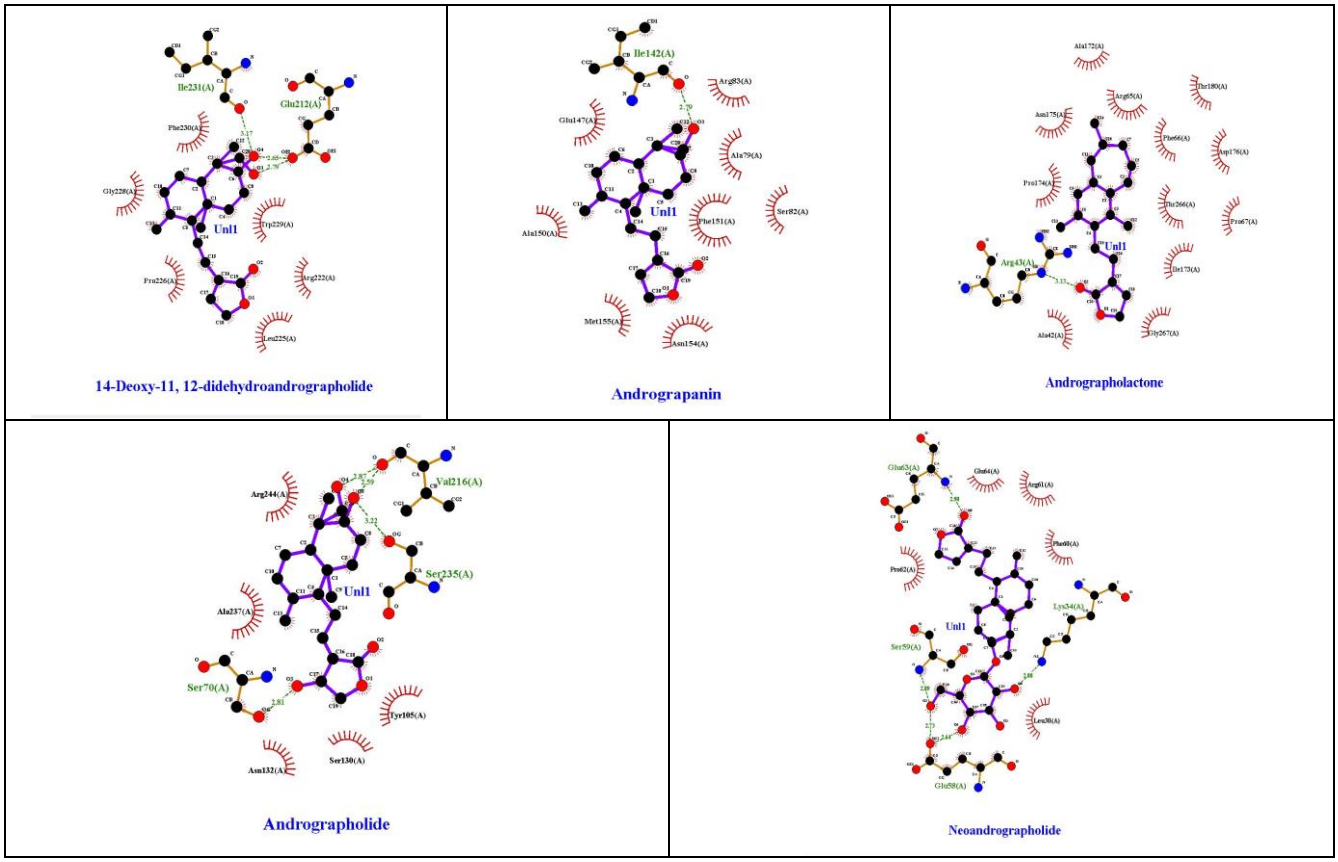

Fig. 5: Bonds formed between the amino acid residues and natural terpenes

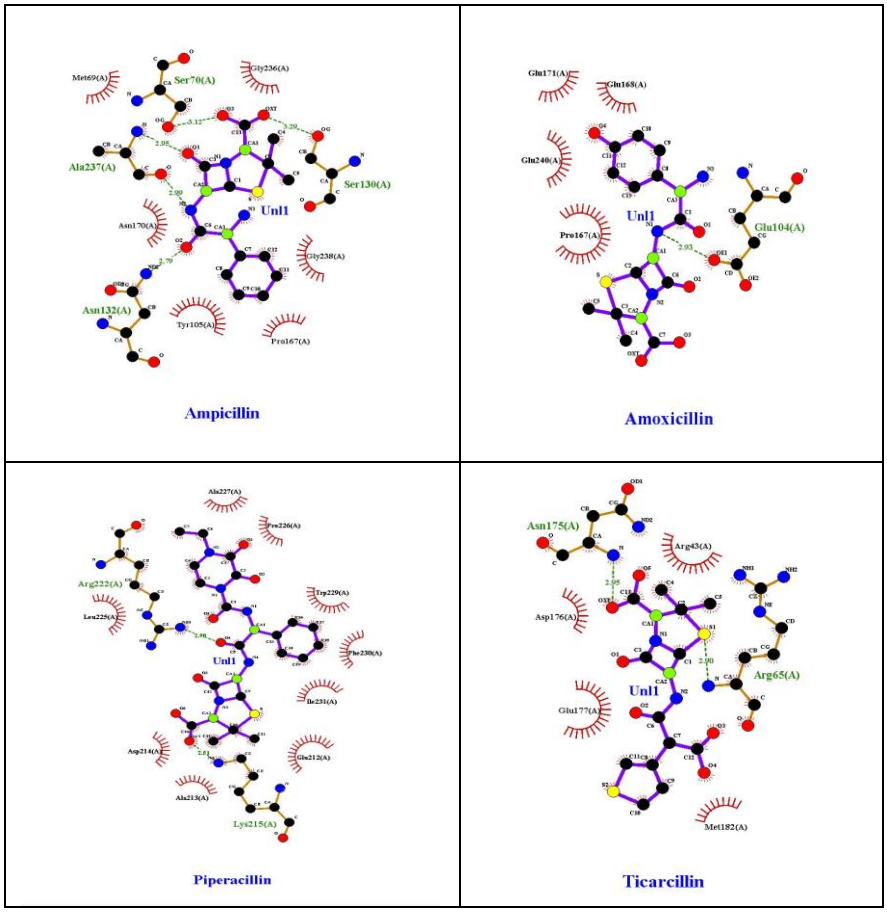

Fig. 6: Bonds formed between the receptor protein and reference antibiotics

DISCUSSION

After the ADMET screening of the terpenes, no negative results were found and thus it indicates the potentiality of the molecules to be served as drug-like compounds. All the terpenes taken for the study did not violate Lipinski's rule of 5 [22]. In molecular docking, the ligand with the least binding affinity was considered as it indicates more stability in binding. The binding affinity between the ligands and the receptor was determined based on the formation of different interactive bonds and contacts [23]. Also, lower the inhibition constant (Ki), the better is the potential of the inhibitor. LigPlot plus study visualized the 2D structures showing different hydrogen and hydrophobic bonds formed between the ligands and the receptors.

Terpenes from *Andrographis paniculta* and known antimicrobials were used for the inhibition of the receptor TEM-1 Beta-lactamase from *Escherichia coli*. Molecular docking analysis revealed that some of the naturally occurring terpenes from *Andrographis paniculata* and reference antimicrobials showed promising results. Among the naturally occurring terpenes, andrographolactone had shown the best result with an inhibition constant of 0.21 µM and binding efficacy of -9.11 Kcal/mol. Similarly, among the reference antimicrobials, ampicillin exhibited the best result with an inhibition constant of 0.56 µM and binding efficacy of -8.52 Kcal/mol. The overall best molecular docking result was shown by andrographolactone, which is a naturally occurring terpene from the plant *Andrographis paniculata.*

So, andrographolactone may be used to inhibit the TEM-1 beta-lactamase enzyme from *Escherichia coli* which is the main reason for the hydrolysis of several antibiotics by degrading the beta-lactam ring of the antibiotics. The compounds present in *Andrographis paniculata* may also be effective against certain other types of beta-lactamases like SHV beta-lactamases, CTX-M beta-lactamases, OXA beta-lactamases and other plasmid-mediated types like PER, VEB, GES and IBC beta-lactamases. It has been demonstrated that the gene encoding CTX-M-15 beta-lactamase of *Escherichia coli* showed downregulation when treated with 100 µg/ml of the extract of *Andrographis paniculate* [8]. Studies also justified that the extract of *Andrographis paniculata* inhibits growth, biofilm formation in multi drug-resistant strains of *Klebsiella pneumoniae* [24]. *Klebsiella pneumoniae* is also a beta-lactamase-producing bacteria. Serious illnesses like enteritis, urinary tract infection, septicaemia and neonatal meningitis are being prominently caused by the bacteria *Escherichia coli*. Urinary tract infection (UTI) shows increased resistance to antimicrobials. There have been evidences that the leaf extracts of *Andrographis paniculata* is effective against UTI causing bacteria [25]. Reports based on different extracts of the plant also proved that the ethanol extract shows potential bacterial and antifungal activity against UTI causing bacteria and dermatophytes like *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Trichophyton rubrum* and *Epidermophyton floccosum* [26]. Many such diseases may be cured or controlled with the help of new potent drugs discovered from natural compounds of plants with very little to no harmful effects on the body.

CONCLUSION

Based on the study conducted and the molecular docking of TEM-1 beta-lactamase from *Escherichia coli* with natural terpenes as well as reference antibiotics, the authors suggest that the terpene andrographolactone may be potent new drug against TEM-1 beta-lactamase enzyme-producing *Escherichia coli* for treating diseases like enteritis, UTI, septicemia and other clinical infections, such as neonatal meningitis. However, further studies and analysis of the compounds against the enzyme are required to validate the same *in vivo* or *in vitro.*

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REFERENCES

[1] Allocati, N., Masulli, M., Alexeyev, M. F., Di Ilio, C., *Escherichia coli* in Europe: An overview. *Int. J. Environ. Res. Public Health*. 2013, *10 (12)*, 6235–6254. doi: 10.3390/ijerph10126235

[2] Bush, K., Bradford, P. A., β-lactams and β-lactamase inhibitors: An overview. *Cold Spring Harb. Perspect. Med.* 2016*; 6 (8):* 1-22. doi: 10.1101/cshperspect.a025247

[3] Cooksey, R., Swenson, J., Clark, N., Gay, E., Thornsberry, C., Patterns and mechanisms of β-lactam resistance among isolates of *Escherichia coli* from hospitals in the United States. *Antimicrob. Agents Chemother*. 1990; *34 (5)*: 739–745. doi: 10.1128/AAC.34.5.739

[4] Stapleton, P., Wu, P. J., King, A., Shannon, K., French, G., Phillips, I., Incidence and mechanisms of resistance to the combination of amoxicillin and clavulanic acid in *Escherichia coli. Antimicrob Agents Chemother*. 1995 Nov; *39(11)*:2478-83. doi: 10.1128/AAC.39.11.2478

[5] Subramanian, R., Asmawi, M. Z., Sadikun, A., A bitter plant with a sweet future? A comprehensive review of an oriental medicinal plant: *Andrographis paniculata*. *Phytochemistry Reviews*. 2012; *11 (1)*: 39–75. doi: 10.1007/s11101-011-9219-z

[6] Dai, Y., Chen, S. R., Chai, L., Zhao, J., Wang, Y., Overview of pharmacological activities of *Andrographis paniculata* and its major compound andrographolide. *Crit. Rev. Food Sci. Nutr*. 2019; *59*: 17–29. doi: 10.1080/10408398.2018.1501657

[7] Okhuarobo, A., Ehizogie Falodun, J., Erharuyi, O., Imieje, V., Falodun, A., Langer, P., Harnessing the medicinal properties of *Andrographis paniculata* for diseases and beyond: A review of its phytochemistry and pharmacology. *Asian Pacific J. Trop. Dis*. 2014; *4 (3)*: 213–222. doi: 10.1016/S2222-1808(14)60509-0

[8] Rasool, U., Parveen, P. S. A., Sah, S. K., S, H. Efficacy of *Andrographis paniculata* against extended spectrum β-lactamase (ESBL) producing *E. Coli*. *BMC Complement. Altern. Med.* 2018; *18 (1)* 1–9. doi: 10.1186/s12906-018-2312-8

[9] Sheel, R., Nisha, K., Kumar, J., Preliminary Phytochemical Screening of Methanolic Extract Of *Clerodendron infortunatum*. *IQSR-JAC*. 2014; *7 (1)*: 10-13. Available: www.iosrjournals.orgwww.iosrjournals.org

[10] Swaminathan, K., Karunakaran, K. N., Manoharan, J. P., Vidyalakshmi, S., SARS-CoV2 multiple target inhibitors from *Andrographis Paniculata*: An in-silico report. *European Journal of Molecular & Clinical Medicine*. 2021; *8 (3)*: 1653-1685. Available: https://pubchem.ncbi.nlm.nih.gov/

[11] Eswari, J. S., Yadav, M., New perspective of drug discovery from herbal medicinal plants: *Andrographis paniculata* and *Bacopa*

monnieri (terpenoids) and novel target identification against *Staphylococcus aureus*. *South African J. Bot*. 2019; *124*: 188–198. doi: 10.1016/j.sajb.2019.05.013

[12] O'Boyle, N. M, Banck, M., James, C. A., Morley, C., Vandermeersch, T., Hutchison, G. R., Open Babel: An Open chemical toolbox. *J. Cheminform*. 2011; *3 (10)*: 1–14. doi: 10.1186/1758-2946-3-33

[13] Smith, B. R., LeFrock, J. L., Amoxicillin-potassium clavulanate: A novel β-lactamase inhibitor. *Drug Intelligence and Clinical Pharmacy*. 1985; *19 (6)*: 415–420. doi: 10.1177/106002808501900601

[14] Sader, H. S., Tosin, I., Sejas, L., Miranda, E., Comparative evaluation of the in vitro activity of three combinations of beta-lactams with beta-lactamase inhibitors: piperacillin/tazobactam, ticarcillin/clavulanic acid and ampicillin/sulbactam. *Braz. J. Infect.* Dis. 2000; *4 (1)*: 22–28.

[15] Yu, H., Adedoyin, A., ADME-Tox in drug discovery: Integration of experimental and computational technologies. *Drug Discovery Today*. 2003; *8 (18)*: 852–861. doi: 10.1016/S1359-6446(03)02828-9

[16] Lagorce, D., Maupetit, J., Baell, J., Sperandio, O., Tuffery, P., Miteva, M. A., Galons, H., Villoutreix, B. O., The FAF-Drugs2 server: A multistep engine to prepare electronic chemical compound collections. *Bioinformatics.* 2011; *27 (14)*: 2018–2020. doi: 10.1093/bioinformatics/btr333

[17] Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., Ferrin, T. E., UCSF Chimera – A Visualization System for Explainatory Research and Analysis. *J.Comput*. Chem. 2004; 25: 1605-1612. doi: 10.1002/jcc.20084

[18] Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., Olson, A. J., AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J. Comput*. Chem. 2009; *30 (16)*: 2785–2791. doi: 10.1002/jcc.21256

[19] Meng, X. Y., Zhang, H. X., Mezei, M., Cui, M., Molecular Docking: A Powerful Approach for Structure-Based Drug Discovery. *Curr. Comput. Aided-Drug Des.* 2012; *7 (2)*: 146–157. doi: 10.2174/157340911795677602

[20] Laskowski, R. A., Swindells, M. B., LigPlot+: Multiple ligand-protein interaction diagrams for drug discovery. *J. Chem. Inf. Model*. 2011; *51(10)*: 2778–2786. doi: 10.1021/ci200227u

[21] Kale, S., Screening of Phytochemical Components of Leaf Extracts. *Int. J. Curr. Microbiol. App. Sci*. 2020; *9 (9)*: 2394–2401. doi: 10.20546/ijcmas.2020.909.299

[22] Lipinski, C. A., Lombardo, F., Dominy, B. W., Feeney, P. J., Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev*. 2001; 46 *(1-3)*: 3-26. Available: www.elsevier.com/locate/drugdeliv

[23] Hsu, K. C., Chen, Y. F., Yang, J. M., Binding affinity analysis of protein-ligand complexes. In the proceedings of the 2nd International Conference on Bioinformatics and Biomedical Engineering, *iCBBE* 2008, pp. 167–171. doi: 10.1109/ICBBE.2008.46

[24] Sah, S. K., Rasool, U., S. H., Andrographis paniculata extract inhibit growth, biofilm formation in multidrug resistant strains of *Klebsiella pneumoniae*. *J. Tradit. Complement. Med*. 2020; *10 (6)* 599–604. doi: 10.1016/j.jtcme.2019.02.006

[25] Sahare, P., Moon, P., Shinde, G. B., *In Vitro* Phytochemical Analysis for Combating Urinary Tract Infection with *Andrographis paniculata*. *J. Pharm. Chem. Biol. Sci*. 2014; *2 (2)*: 93-103. Available: http://www.jpcbs.info

[26] Premanath, R., Lakshmi Devi, N., Antibacterial, antifungal and antioxidant activities of *Andrographis paniculata* Nees. leaves. *Int. J. Pharma. Sci. Res*. 2011; *2 (8)*: 2091–2099. Available: www.ijpsr.com