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Research Article

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In Silico Studies of Various Bioactive Phytochemicals Present in *Andrographis Paniculata* as Potential Antimicrobial Candidates

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ABSTRACT

Introduction: *Andrographis paniculata*, commonly known as "King of Bitters," is a prominent medicinal plant belonging to the *Acanthaceae* family. It is indigenous to South Asian countries such as India, Sri Lanka, and Thailand, and is widely cultivated in other regions including China and Southeast Asia. The plant is renowned for its bitter taste and has been utilize extensively in traditional medicine systems like Ayurveda, Traditional Chinese Medicine (TCM), and Thai medicine for centuries.

Materials and Methods: The current research emphasized on exploring the inhibitory perspectives of phytochemicals present in *A. paniculata* such as andrographolide (1), bis-andrographolide (2), carvacrol (3), eugenol (4), α-guaiene (5), wogonin (6), oroxylin A (7), chlorogenic acid (8), myristic acid (9), caffeic acid (10), hentriacontane (11), triacontane (12), ninandrographolide (13), 14-deoxyandrographolide (14), and neoxyandrographiside (15) against *E. coli* Topoisomerase-IV co-complexed with inhibitor (PDB ID: 3FV5) by molecular docking approach using the Schrodinger software as anti-bacterial agent by using *in-silico* approaches with the help of published literature on downregulation of enzyme expression and combining this information in order to recognize drug target [PDB ID: 3FV5; Crystal Structure of *E. coli* Topoisomerase-IV co-complexed with inhibitor].

Result: The *in silico* studies revealed that the phytoconstituents successfully inhibited bacterial topoisomerase at varied degree, which suggested plausible utilization as antimicrobial.

Conclusion: This leads to the ultimate conclusion inhibiting *E. coli* topoisomerase-IV is the key to solving all naturally occurring problems, which will aid humanity in overcoming difficult circumstances.

Keywords: *Andrographis paniculata*, Antibacterial, Target, Phytochemicals, Inhibition, *In silico*

INTRODUCTION

Andrographis paniculata, commonly known as "King of Bitters," is a prominent medicinal plant belonging to the *Acanthaceae* family. It is indigenous to South Asian countries such as India, Sri Lanka, and Thailand, and is widely cultivated in other regions including China and Southeast Asia.[1] The plant is renowned for its bitter taste and has been utilized extensively in traditional medicine systems like Ayurveda, Traditional Chinese Medicine (TCM), and Thai medicine for centuries. Traditionally, *A. paniculata* has been employed to treat a variety of ailments. Its leaves and roots have been used to manage fever, liver disorders, digestive issues, and respiratory infections. The herb is particularly famous for its potent anti-inflammatory, antiviral, and immunostimulatory properties. In Ayurveda, it is often referred to as *Kalmegh*, highlighting its use in treating liver-related conditions and enhancing overall immune function.[2]

The therapeutic potential of *A. paniculata* is primarily attributed to its bioactive compounds. The most significant among these is andrographolide, a diterpenoid lactone that exhibits a broad spectrum of biological activities. Other notable compounds include neoandrographolide, deoxyandrographolide, and andrographiside. These constituents have been the focus of numerous pharmacological studies aiming to elucidate their mechanisms of action and therapeutic efficacy.[3] Recent scientific investigations have provided substantial evidence supporting the diverse pharmacological properties of *A. paniculata*. It has demonstrated remarkable anti-inflammatory,

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anticancer, antidiabetic, hepatoprotective, and cardiovascular protective effects in various *in vitro* and *in vivo* studies. Its immunomodulatory effects are particularly noteworthy, with research indicating its potential to enhance immune response and provide prophylactic benefits against infectious diseases, including respiratory tract infections and the common cold.[4] The clinical applications of *A. paniculata* have been expanding, with several studies highlighting its effectiveness in managing acute upper respiratory tract infections and reducing the severity of symptoms associated with common cold and influenza. Moreover, its role as an adjunct therapy in chronic diseases such as diabetes and cardiovascular disorders is gaining attention, suggesting its potential utility in integrative medicine.[5]

Current research trends focus on exploring the molecular mechanisms underlying the pharmacological actions of *Andrographis paniculata*. There is also a growing interest in developing novel formulations and drug delivery systems to enhance the bioavailability and therapeutic efficacy of its active constituents. Additionally, the safety profile and potential side effects of long-term use are being rigorously evaluated to ensure its suitability for widespread clinical use.[6]

Despite the extensive body of literature highlighting the pharmacological potential of *A. paniculata*, a critical research gap exists in the domain of computational-oriented antibacterial studies. A thorough search across various scientific databases, including PubMed, Scopus, Web of Science, and Google Scholar, reveals a significant absence of comprehensive computational analyses specifically targeting the antibacterial properties of *A. paniculata* and its bioactive compounds. To identify relevant studies, a systematic search strategy was employed using keywords such as "*Andrographis paniculata*," "computational studies," "antibacterial," "molecular docking," "molecular dynamics," and "*in silico*." Boolean operators and advanced search techniques were utilized to refine the search results and ensure the inclusion of studies from diverse scientific disciplines. The search results indicate a substantial number of experimental and clinical studies exploring the antibacterial effects of *A. paniculata*. These studies primarily focus on *in vitro* and *in vivo* methodologies, evaluating the efficacy of the plant extracts and isolated compounds against various bacterial strains. However, when it comes to computational studies, particularly those employing molecular docking, molecular dynamics simulations, quantitative structure-activity relationship (QSAR) modeling, and other in silico techniques, the findings are notably sparse.

The current research emphasized on exploring the inhibitory perspectives of phytochemicals present in *A. paniculata* such as andrographolide **(1)**, bis-andrographolide **(2)**, carvacrol **(3)**, eugenol **(4)**, α-guaiene **(5)**, wogonin **(6)**, oroxylin A **(7)**, chlorogenic acid **(8)**, myristic acid **(9)**, caffeic acid **(10)**, hentriacontane **(11)**, triacontane **(12)**, ninandrographolide **(13)**, 14-deoxyandrographolide **(14)**, and neoxyandrographiside **(15)** against *E. coli* Topoisomerase-IV co-complexed with inhibitor (PDB ID: 3FV5) by molecular docking approach using the Schrodinger software.

MATERIALS AND METHODS

Preparation of Ligand

The structures were created using the Schrodinger Software suite 2021-2's 2D-sketcher module. For docking analysis, the Maestro environment version 12.8 was employed. The stereoisomers of these ligands were created using the LigPrep programme. Using the Epik ionizer, a maximum of four poses with correct protonation states were created for each ligand at a target pH of 7.0. The OPLS 2005 force field was utilized to construct tautomerized, desalted ligands while keeping the input files' requisite chiralities, resulting in an optimized low energy 3D ligand.[7,8]

Preparation of Protein

Crystal Structure of *E. coli* Topoisomerase-IV co-complexed with inhibitor; https://doi.org/10.2210/pdb3FV5/pdb (PDB ID: 3FV5) was downloaded from the Protein Data Bank (PDB) (http://www.rcsb.org/pdb) and optimized later by assigning the bond orders, removing the crystal water molecules beyond 5 Å distance from any heterogroup, addition of hydrogen atoms, assigning the tautomeric states, heterogroup ionization, and application of Kollman charges. The pre-processed and inspected structures were taken when developing the biological target. To get the right shape, the disulfide bonds, bond ordering, and formal charges were assigned using the Protein Preparation Wizard module of the Schrodinger Maestro 9.1. Co-factors, metal ions, water molecules in crystal formations beyond a distance of 5A°, and the hetero group were all removed. The "impref utility" tool was used to optimize hydrogen atoms by keeping all heavy atoms in their original positions, while the "H-bond assignment" tool was used to optimize the hydrogen-bonding network. Molecular docking was used to define the receptor grids for the protein structure, allowing a variety of ligand poses to bind at the anticipated active site. Grids were built and placed at the ligand's centroid in such a way that they covered the whole ligand in a cubic box of defined measurement with the following characteristics: 1.00 Van der Waals scale factor and 0.25 charge cut off. The docking was done in XP mode, and only the energy-minimized postures were scored, which was expressed as a Glide score. The best-docked posture with the lowest Glide score value for each ligand was considered after the highest-scoring ligands were docked.[9,10]

Docking Procedure

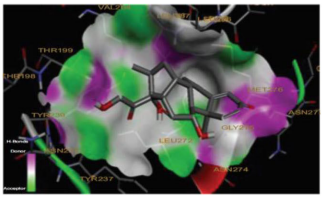
The structure-based drug design process was restarted after the target protein's structure was understood. The stiff receptor was docked with the low-energy ligands, and the fit into the active site was evaluated, as well as the predicted binding mechanism. In receptor-based computational techniques, the ligand interacting with the macromolecule protein (receptor) was represented using a molecular docking methodology. With low energy levels, IFD predicted that the ligand would have a good contact with the target. The method facilitates in the finding of low-free-energy conformations as well as the complete removal of steric conflicts. With 0.7 Van der Waals scaling for the receptor and 0.5 Van der Waals scaling for the ligand, side chains were eliminated, and a 0.18 RMSD value cut off, the maximum number of poses for each ligand remained at 20. The chemicals were ranked based on the information obtained, and a selection was tested for biological activity experimentally. The Glide Score was determined for each ligand.[11,12]

RESULTS

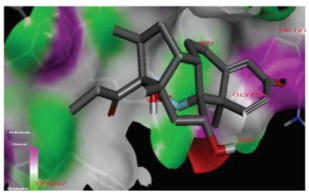
In this docking study, several compounds were analyzed for their binding affinities to a target molecule. The compounds and their corresponding docking scores are described in **Table 1**. Andrographolide and their near relative analogs such as Bis-Andrographolide, Ninandrographolide, 14-Deoxyandrographolide, and Neoxyandrographisside demonstrated high affinity binding with the antimicrobial target with docking score ranging from -9.1 Kcal/mol to -9.9 Kcal/mol by binding with amino acid residues like LEU A:272, LEU A:271, and GLY A:272. In contrast, Triacontane, Caffeic Acid, Oroxylin A, Wogonin, Alpha-Guaiene, and Eugenol demonstrated low binding affinities in the range of -4.7 Kcal/mol to -5.6 Kcal/mol by binding with varied amino acid residues such as LEU A:287, PHE A:103, etc (**Figure 1**).

Table 1: Docking scores of phytoconstituents present in *Andrographis paniculata*.

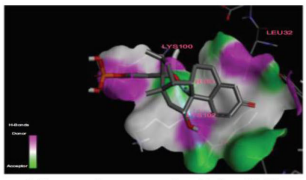
S. No.	Compound	Gscore (Kcal/mol)	Hydrogen bonding	Amino acid residues
1	Andrographolide	-9.946	3	LEU A:272, LEU A:271, GLY A:272
2	Bis-Andrographolide	-9.146	2	LYS A:100, GLU A:178
3	Carvacrol	-7.349	2	MET A:275, ASN A:277
4	Eugenol	-5.667	1	LEU A:287
5	Alpha-Guaiene	-5.197	0	-
6	Wogonin	-4.486	1	PHE A:103
7	Oroxylin A	-4.224	0	-
8	Chlorogenic Acid	-6.112	1	LEU A:272
9	Myristic Acid	-5.991	1	TYR A:103
10	Caffeic Acid	-4.897	0	-
11	Hentriacontane	-7.283	2	GLU A:178, LEU A:271
12	Triacontane	-4.769	0	-
13	Ninandrographolide	-9.573	2	LYS A:102, PHE A:103
14	14-Deoxyandrographolide	-9.558	3	LEU A:272, LEU A:271, GLY A:272
15	Neoxyandrographiside	-9.482	3	LEU A:272, LEU A:271, GLY A:272



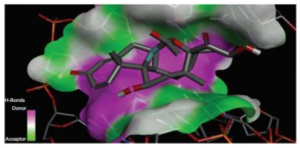
Andrographolide (1)



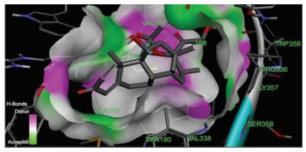
Bis-andrographolide (2)



Ninandrographolide (13)



14-Deoxyandrographolide (14)



Neoxyandrographiside (15)

Figure 1: Docking poses of Top-5 compounds present in *A. paniculata* against *E. coli* Topoisomerase-IV.

DISCUSSION

The results of the induced-fit molecular docking studies validated and clarified the antibacterial effect of the drugs. To this day, scientists utilised this time-tested technique to determine which chemicals bind to which proteins and how to arrange ligands so that complexes have the lowest possible energy. A binding affinity model for the inhibitors was constructed using the molecular docking approach.[13] Docking score, hydrogen bonds, Van der Waal's interactions, π-cation interaction, and π-π stacking interactions were the main parameters used to forecast the results. We calculated the ligand affinity for proteins using these parameters. A positive binding affinity is indicated by a negative docking score. According to the docking findings, there are a number of crucial features: Increased Van der Waals interactions and the presence of additional hydrogen bonding connections are indicators of ligands with many bulky groups and a high binding affinity for the molecular target, respectively, which is indicative of antagonist activity. As a result, we can predict inhibitor binding sites to proteins using the IFD module.[14]

As was clearly visible, both compounds were ringed by hydrophobic amino acid residues. The inability of either chemical to evaporate into the solvent suggests that it was unable to penetrate the binding cleft of the protein's active site. Due to their tiny size, the ligands may have the ability to penetrate further into the cavity.[15] Because amino acid residues entirely masked the ligand, using surface representation made it impossible to see it at the cavity. The stable fitting of ligands causes Van der Waals interactions, which attain maximum stability. It seems that the compounds were able to access the active region of the protein, as there was no solvent exposure. Strong interactions with the ligand are made possible by amino acid residues forming a deep bent-shaped cleft in the target's active site. A possible explanation for the improved topoisomerase-IV inhibition might lie mostly with the amino function.[16] Research has shown that the placement of interacting oxygen groups is very important. One may argue that the active site will be more effectively interacted with if the keto group is closer to the scaffold and the hydroxyl group is further away. Steric, hydrophobic, electrostatic, and hydrophilic interactions may all have a role in the stability of the drug-target association. Molecule docking studies, which successfully foretold the ligands' hypothetical binding to the biological target, confirmed their connections.[17]

CONCLUSION

Inhibitors that aid in the recovery of patients with bacterial infections and in their prevention were the focus of the current work. This is an early stage in the process of discovering effective and safe inhibitors that may alleviate the complicated symptoms of nosocomial infections or septicemia. Just when scientists are starting to think that natural compounds may hold the key to ending bacterial resistance and mitigating the pandemic's devastating effects, this study will remind them that there is still hope: small molecular weight ligands. In contrast, the findings of this intriguing investigation make it clear that all of these natural chemicals performed well. This leads to the ultimate conclusion that inhibiting *E. coli* topoisomerase-IV is the key to solving all naturally occurring problems, which will aid humanity in overcoming difficult circumstances.

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CONFLICT OF INTEREST

No conflict of interest is declared.

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ETHICAL DISCLOSURES

None required

AUTHORS' CONTRIBUTION:

Meena Chouksey: Performing docking

Kajaj Lal: Interpretation of data

Shiv Sagar Mahapatra: Obtaining data

Rashmi Gangotri: Literature, Discussion

Divya Pujari: Analysis of Data

Pratibha Sahu: Writing and revision of manuscript

REFERENCES

1. Akbar S. *Andrographis paniculata*: a review of pharmacological activities and clinical effects. Altern. Med. Rev. 2011;16(1):66-77.
2. Mishra SK, Sangwan NS, Sangwan RS. Phcog rev.: Plant review *Andrographis paniculata* (*Kalmegh*): A review. Pharmacogn. Rev. 2007;1(2):283-98.
3. Dai Y, Chen SR, Chai L, Zhao J, Wang Y, Wang Y. Overview of pharmacological activities of *Andrographis paniculata* and its major compound andrographolide. Crit. Rev. Food Sci. Nutr. 2019;59(1):S17-29.
4. Nyeem MA, Mannan MA, Nuruzzaman M, Kamrujjaman KM, Das SK. Indigenous king of bitter (*Andrographis paniculata*): A review. J. Med. Plant Stud. 2017;5(2):318-24.
5. Hossain MS, Urbi Z, Sule A, Rahman KH. Andrographis paniculata (Burm. f.) Wall. ex Nees: a review of ethnobotany, phytochemistry, and pharmacology. Sci. World J. 2014; 2014(1):274905.

6. Worakunphanich W, Thavorncharoensap M, Youngkong S, Thadanipon K, Thakkinstian A. Safety of *Andrographis paniculata*: A systematic review and meta-analysis. Pharmacoepidemiol. Drug Safety. 2021;30(6):727-39.
7. Asati V, Bajaj S, Mahapatra DK, Bharti SK. Molecular modeling studies of some thiazolidine-2, 4-dione derivatives as 15-PGDH inhibitors. Med. Chem. Res. 2016;25:94-108.
8. Chhajed SS, Chaskar S, Kshirsagar SK, Haldar GA, Mahapatra DK. Rational design and synthesis of some PPAR-γ agonists: Substituted benzylideneamino-benzylidene-thiazolidine-2, 4-diones. Comp. Biol. Chem. 2017; 67:260-5.
9. Joseph TM, Suresh AM, Mahapatra DK, Haponiuk J, Thomas S. The Efficacious Benefit of 25-Hydroxy Vitamin D to Prevent COVID-19: An *In-Silico* Study Targeting SARS-CoV-2 Spike Protein. Nutrients. 2022;14(23):4964.
10. Asati V, Bharti SK, Rathore A, Mahapatra DK. SWFB and GA strategies for variable selection in QSAR studies for the validation of thiazolidine-2, 4-dione derivatives as promising antitumor candidates. Indian J. Pharm. Educ. Res. 2017;51(3):436-51.
11. Pandey R, Dubey I, Ahmad I, Mahapatra DK, Patel H, Kumar P. *In-Silico* Study of Some Dexamethasone Analogs and Derivatives against SARS-CoV-2 Target: A Cost-Effective Alternative to Remdesivir for Various COVID Phases. Curr. Chin. Sci. (Bioinformatics). 2022;2(4):294-309.
12. Singh RK, Mishra AK, Kumar P, Mahapatra DK. Molecular Docking and In Vivo Screening of Some Bioactive Phenoxyacetanilide Derivatives as Potent Non-Steroidal Anti-Inflammatory Drugs. Int. J. Curr. Res. Rev. 2021;13(10):189.
13. Zehra A, Nisha R, Kumar A, Nandan D, Ahmad I, Mahapatra DK, Patel H, Maity B, Kumar P. Computational Approaches Molecular Docking and MD Simulation Establishes the Potential COVID-19 Main Protease Inhibitors from Natural Products. Curr. Chin. Sci. (Bioinformatics). 2024;4(2):114-34.
14. Joseph TM, Mahapatra DK. Exploration of two natural inhibitors for treating COVID-19 from simple natural products: nature probably has all the Solutions. J. Public Health Sanit. 2020;1(1):17-24.
15. Dadure KM, GM Haldar A, Kar Mahapatra D. Molecular Docking Study of Substituted Chalcone Compounds as Potential Cyclin-Dependent Protein Kinase 2 (Cdk-2) Inhibitors. Adv. Mater. Proceed. 2020; 5(4):1-3.
16. Mahapatra DK, Dadure KM, Haldar AG. *In silico* Computation of Theoretical Physicochemical Properties and Pharmacokinetic Profiles of Some Novel Substituted Amodiaquine Based Compounds. Int. J. Cheminformat. Res. 2020;6(2):9-16.
17. Singh RK, Mishra AK, Kumar P, Mahapatra DK. Molecular Docking and In Vivo Screening of Some Bioactive Phenoxyacetanilide Derivatives as Potent Non-Steroidal Anti-Inflammatory Drugs. Int J Cur Res Rev. 2021;13(10):189-96.