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# MOLECULAR DOCKING ANALYSIS OF PLANT PHYTOCOMPOUNDS AS PROMISING INHIBITORS AGAINST LASB VIRULENCE PROTEIN - AN *IN-SILICO* AND *IN-VITRO* APPROACH

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**Keywords:**

*Pseudomonas aeruginosa*, Elastase, Antibacterial activity, Drug discovery, Phytocompounds, ADMET properties, Molecular docking

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**ABSTRACT:** *Pseudomonas aeruginosa* is a Gram-negative pathogenic bacterium that can cause a variety of hospital-acquired diseases. It is a multidrug-resistant bacterium and can survive a wide range of antibiotics; hence there is a concern about its limited therapy options. In this scenario, the discovery and development of novel antibiotics are in high demand. In our research work, molecular docking a Bioinformatics technique, is applied to find the ideal lead molecule from plants. For this study, we have extracted literature-proven phytocompounds from a variety of traditional medicinal plants and their structures were retrieved from the PubChem database. Lipinski and ADME/TOX screening were used to evaluate drug likeliness, followed by docking against the target protein. *Pseudomonas aeruginosa* elastase, or LasB is a virulence protease that hydrolyzes host tissue and is thus considered the target protein. Elastase degrades human skin extracellular protein in wound infections, including tissue damage and inflammation. Thus, elastase could be a promising target for the discovery of novel lead compounds. As a result of docking studies, Eupalitin 3-o-galactoside, a natural phytocompounds present in *Boerhavia diffusa*, showed a strong binding affinity with the least glide score of -6.72 kcal/mol followed by Ephedrine and Quercetin from *Sida rhombifolia* and *Clerodendrum infortunatum* with G Score of -5.84 and -5.84 kcal/mol respectively. The plants that contained phytocompounds with a substantial gliding score were chosen for the antibacterial analysis. The ethyl acetate leaf extract of all three plants *Boerhavia diffusa*, *Sida rhombifolia*, and *Clerodendrum infortunatum* had the strongest anti-bacterial activity against *Pseudomonas aeruginosa*.

**INTRODUCTION:** *Pseudomonas aeruginosa* is a Gram-negative, opportunistic bacterium that can cause severe infections in immunocompromised patients, including pneumonia, cystic fibrosis, urinary tract infections, and skin and soft tissue infections [1]. It is the third most prevalent cause of hospital infections and the second leading cause of harmful bacteria in surgery [2].

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The multidrug-resistant bacteria *Pseudomonas aeruginosa* already threatens global public health since it produces a wide range of virulence factors that contribute to the development of antibiotic resistance, encompassing all important antibiotic classes such as quinolones, lactams, aminoglycosides, and polymyxins [3, 4].

The emergence of the multidrug-resistant organism occurs due to the change in the regulatory system, which controls the expression of the resistant mechanism by mutation, membrane permeability, and inactivating enzymes that promotes target alteration along with the extensive use of the synthetic drug [1]. Even though multidrug-resistant strains are still evolving, a constant search for the

development of new medications is required [5]. The traditional strategy for drug development frequently fails during clinical trials due to a lack of understanding of multi-target pharmaceutical interactions, cross-reactivity, and adverse side effects. Computational methods can anticipate the prediction of drug-likeness and protein-ligand interactions beforehand [6]. The current study focuses on discovering new therapeutic compounds through molecular docking studies. Bacterial virulence factors were used as the target protein in the docking analysis. In contrast, phytocompounds from medicinal plants, *Boerhavia diffusa*, *Clerodendrum infortunatum*, *Sida rhombifolia*, *Scoparia dulcis*, *Breynia retusa*, *Euphorbia heterophylla*, *Hemigraphis alternata*, *Imperata cylindrica*, *Hedyotis corymbosa*, and *Tephrosia purpurea* were used as the ligands. Since plants have been utilized as medications and treatment options for numerous diseases for centuries and are considered to be safe when compared to synthetic drugs [7].

Various plant species are used to treat a wide range of diseases, including minor infections, diarrhoea, skin issues, asthma, malaria, and a host of other ailments [8]. As noted in Ayurveda, *Boerhavia diffusa* is a well-known medicinal plant that is used to cure a wide range of human illnesses. It has been reported that the entire plant or its portions, such as the aerial part or root, have a variety of medical characteristics, including anti-bacterial, anti-inflammatory, anti-stress, anti-convulsant, and so on [9]. *Clerodendrum viscosum* has been reported to be used to treat skin disorders. Similarly, the plant *Sida rhombifolia* possesses various biological activities like antibacterial and anti-inflammatory properties, and it is also used to treat hypertension [10]. The *Pseudomonas aeruginosa* elastase also known as Pseudolysin or LasB, is a proteolytic virulence factor released by *P. aeruginosa* that plays a crucial role in Pseudomonal infections. Elastaseis a zinc-dependent metalloprotease regulated by the las, rhl quorum-sensing transcription system and encoded by the LasB gene [11]. LasB disrupts host cells-extracellular matrix materials and can induce tissue damage via hydrolysis, altering the immune response and allowing the evasion of host defenses, resulting in inflammation. The extensive production of LasB in *P. aeruginosa*, as well as the negative effects of its activity on host tissue and immune response, has made LasB a potential therapeutic target [12]. The ultimate purpose of this research is to use docking studies to develop efficient therapeutic compounds from recognized medicinal plants for bacterial virulence protein LasB and to provide appropriate in-vitro antibacterial evidence for the biological activity of selected plants.

## MATERIALS AND METHODS:

**Structure-Based Virtual Screening:** Structure-based virtual screening is the most straightforward way of identifying and ranking the best lead compound against the target protein. Virtual screening involves a set of computational processes such as receptor and ligand preparation, docking and post-docking analysis, *etc.* [13] all computational analyses, including ADME screening, Ligand, and Protein Preparations, GLIDE grid generation, and docking, were performed with maestro Schrodinger version 9.0.211

**Lead Identification:** Biologically active phytocompounds from different natural plants such as *Boerhavia diffusa, Sida rhombifolia, Clerodendrum infortunatum, Scoparia dulcis, Tephrosia purpurea Hemigraphis alternata, Imperata cylindrica, Breynia retusa, Hedyotis corymbosa*, and *Euphorbia heterophylla* were considered as ligands, through a literature search. The PubChem database (http://pubchem.ncbi.nlm.nih.gov) was utilized to retrieve the chemical structure of all phytocompounds [14], and the biological properties of the ligand were evaluated using the PASS online way 2 Drug online program (http://way2drug.com/PassOnline/predict.php) [15].

**Lead Compound Screening: ADME/Toxicity:** The ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) characteristics of phytocompounds are a significant factor in determining the drug-likeness of certain ligands. The number of rotatable bonds, molecular weight, hydrogen bond acceptor, hydrogen bond donor, and octanal water partition coefficient logP are some of the important parameters in ADME/TOX profiling and were predicted using Schrodinger's program QikProp module version 4.4 by the Lipinski rule of five [16].

**Target Identification and Binding Site Prediction:** The target protein *Pseudomonas aeruginosa* elastase's three-dimensional structure (PDB ID: 3DBK) was obtained from the Protein Data Bank (PDB) (http://www.rcsb.org/pdb). Additionally, using the LigSite online tool (http://projrct.biotec.tudresden.de/pocket/), active binding site pockets of target protein were predicted [17].

**Ligand Preparation:** The preparation of ligands was then applied to all phytocompounds from the selected plants, which had fulfilled ADMET validation. High-quality 3D structures of phytocompounds were generated using the LigPrep program, which is included in the maestro Schrodinger software. The structures were subsequently optimized by hydrogen bond order [18].

**Protein Preparation:** Protein preparation was carried out using the Protein preparation wizard module, where all water molecules were removed by default in a pre-processing step. Further proteins were prepared by correcting errors in protein, such as assigning bond orders, adding hydrogen and removing water molecules, and filling loops near the active site followed by optimization and minimization of all atoms [19].

**Glide Grid Generation:** The GLIDE tool in Schrodinger software accomplishes GLIDE grid generation, which is a key step in molecular docking. It looks for ligand-protein interactions which are beneficial. It incorporates filters to determine the most optimal ligand binding position in the active site of a protein [18].

**Molecular Docking:** Molecular docking studies were carried out to explore the binding affinities of phytocompounds (ligands) against *Pseudomonas aeruginosa* elastase (target protein). The extra precision flexible mode was used to dock ligands against the target protein's active site. The gliding score, hydrogen bonding, and hydrophobic interaction between docked ligands and the target protein were assessed based on the docked complex. The ligands with the lowest G Score were the most effective against the target protein [20].

**Visualization of Ligand-Receptor Interaction:** The PyMol visualization tool can be used to analyze the hydrogen bond interaction of phytocompounds (ligands) against the target protein (3DBK) in a docked complex along with the bond length and amino acid residues interacted [21].

***In-vitro* Anti-Bacterial Studies:**

**Plant Sample Collection and Authentication:** The leaves, stem, roots, and flowers of plants *Boerhavia diffusa, Clerodendrum infortunatum*, and *Sida rhombifolia* were selected for *in-vitro* analysis of antibacterial activity. The plants were collected within the Kanyakumari district of Tamil Nadu and authenticated by The Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, (*Boerhavia diffusa:* BSI/SRC/5/23/2022/Tech/520, *Clerodendrum infortunatum:* BSI/SRC/5/23/2022/Tech/518, *Sida rhombifolia:* BSI/SRC/5/23/2022/Tech/519).

**Preparation of Plant Extract:** The parts of the plants were washed under tap water, shade dried for two weeks, and pulverized. Further. The pulverized materials of 50 grams were extracted with the solvents like methanol, ethyl acetate, and hexane using the Soxhlet apparatus [22]. The crude form of extracts was obtained using a rotary evaporator. Final concentrated extracts were stored for further use.

**Anti-Bacterial Analysis:** The agar well diffusion method was used to test the antibacterial activity of the crude plant extracts *against P. aeruginosa*. This assay was determined according to the method described previously [23]. Gram-negative *Pseudomonas aeruginosa* culture was purchased from MTCC (MTCC No: 1748) for antibacterial screening. Bacterial culture was previously prepared by allowing the bacteria to grow overnight in Muller Hinton Broth at 37°C.

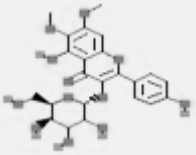
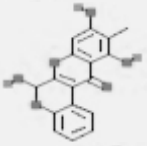
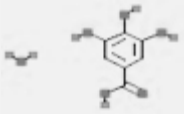
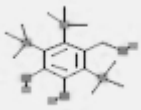
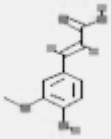
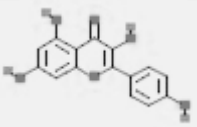
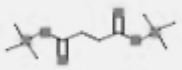
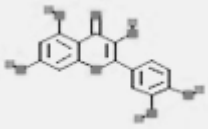
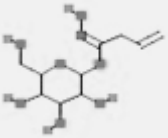
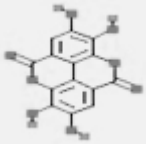
Muller Hinton Agar plates were swabbed uniformly with 100µl bacterial suspension. A sterilized cork borer was used to pierce 6mm wells in each plate. And 50µl of crude extract from each plant sample was added to each well, and 50µl of Neomycin sulfate was utilized as a positive control. Organic solvents such as methanol, ethyl acetate, and hexane were considered as negative controls and kept for incubation at 37°C for 24 hrs. The diameter of the zone of inhibition surrounding the well was measured after incubation and compared

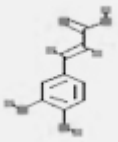
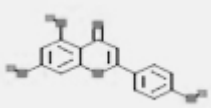
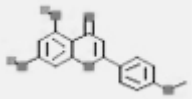
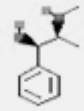
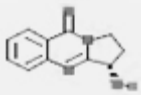
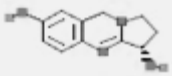
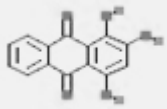
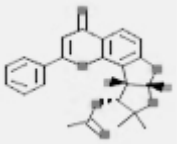
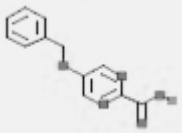
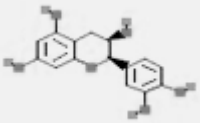
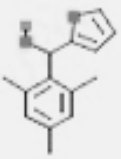
to the standard to determine the sensitivity of the test plates.

## RESULT:

**Ligand Identification and Pass Prediction:** **Table 1** summarizes the 2D structure of reported phytocompounds from the selected plants *Boerhavia diffusa*, *Clerodendrum infortunatum*, *Sida rhombifolia*, *Scoparia dulcis*, *Breynia retusa*, *Euphorbia heterophylla*, *Hemigraphis alternata*, *Imperata cylindrica*, *Hedyotis corymbosa*, and *Tephrosia purpurea* that are retrieved from the PubChem database. The biological characteristics such as antibacterial, and anti-inflammatory activities of phytocompounds were predicted using the pass online way 2 drug online tool.

**TABLE 1: 2D STRUCTURE OF PHYTOCOMPOUNDS WITH PUBCHEM ID**

| S. no. | Phytocompounds | PubChem Id | 2d structure |
|---|---|---|---|
| | ***Boerhavia diffusa*** | | |
| 1 | Eupalitin 3-O- galactoside | 44259727 |  |
| 2 | Boeravinone B | 14018348 |  |
| 3 | Gallic acid monohydrate | 24721416 |  |
| 4 | 3'4-dihydroxy-benzyl alcohol | 101663520 |  |
| 5 | Ferulic acid | 445858 |  |
| 6 | Kaempferol | 5280863 |  |
| 7 | Succinic acid | 520988 |  |
| | ***Clerodendrum infortunatum*** | | |
| 8 | Quercetin | 5280343 |  |
| 9 | Desulphosinigrin | 9601716 |  |
| 10 | Ellagic acid | 5281855 |  |

| | | | |
|---|---|---|---|
| 11 | Caffeic acid | 689043 |  |
| 12 | Apigenin | 5280443 |  |
| 13 | Acacetin | 5280442 |  |
| | ***Sida rhombifolia*** | | |
| 14 | Ephedrine | 9294 |  |
| 15 | Vasicinone | 442935 |  |
| 16 | Vasicinol | 442934 |  |
| | ***Tephrosia purpurea*** | | |
| 17 | Purpurin | 6683 |  |
| 18 | Semiglabrin | 156341 |  |
| | ***Scoparia dulcis*** | | |
| 19 | Benzofuran 2,3 dihydro | 20209882 |  |
| 20 | 5- Benzyloxypyrimidine | 561874 |  |
| | ***Breynia retusa*** | | |
| 21 | Epicatechin | 72276 |  |
| | ***Euphorbia Herterophylla*** | | |
| 22 | 2,4,6-trimethylphenyl) furan-2-ylmethanol | 49962474 |  |

**Structure Retrieval and Active Site Prediction:** The 3D Structure of protein *Pseudomonas aeruginosa* elastase **Fig. 1** was retrieved from the Protein Data Bank (PDB ID: 3DBK). Its active site pockets are predicted using the LigSite online tool and the residues were HIS 223, TRP 115, GLU 141, HIS 140, and ARG 198.

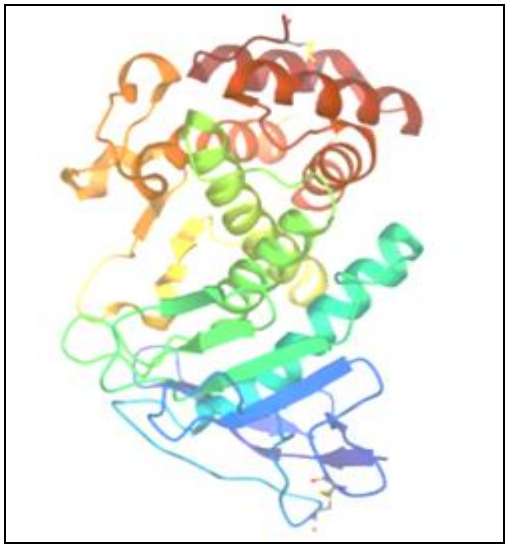


**FIG. 1: 3D STRUCTURE OF PROTEIN *PSEUDOMONAS AERUGINOSA* ELASTASE**

**ADME Prediction Using Qikprop Module:** ADME screening is an important criterion in the discovery of novel lead compounds, where the bioavailability of phytocompounds was determined using the QIKPROP module. It determines the pharmacokinetic parameters like molecular weight, number of metabolic reactions, number of rotatable bonds, donor hydrogen bond, acceptor hydrogen bonds, lipophilicity, permeability in octanol/ water partition coefficient, brain/ blood barrier skin permeability, and so on. According to ADME studies, only 22 compounds out of 125 satisfied Lipinski's rule of five **Table 2**. The compounds eupalitin 3-o-galactoside, boeravinone b, gallic acid monohydrate, 3'4-dihydroxy-benzyl alcohol, ferulic acid, kaempferol, and succinic acid from the plant *Boerhavia diffusa*, as well as quercetin, desulphosinigrin, ellagic acid, caffeic acid, apigenin, and acacetin from the plants *Clerodendrum infortunatum* has fulfilled the Lipinski rule of five. Apart from these, the compounds ephedrine, vasicinone, vasicinol from *Sida rhombifolia* and purpurin, and semiglabrin from *Tephrosia purpurea* also cleared all the ADME parameters. Along with these benzofuran 2,3 dihydro, 5- benzyloxypyrimidine from *Scoparia dulcis*, epicatechin and 2,4,6-trimethylphenyl) furan-2-ylmethanol from the plants *Breynia retusa and Euphorbia herterophylla* also satisfied Lipinski rule of 5. Only molecules that satisfied the Lipinski rule of 5 are examined for docking investigations.

**TABLE 2: ADME PROFILING OF PLANT COMPOUNDS USING QIKPROP MODULE**

| Molecule Name | No. of rotatable bonds | Molecular weight | Dipole moment | SASA | Donor Hydrogen bonds | Acceptor Hydrogen bonds | QPlogP for Octanol/gas |
|---|---|---|---|---|---|---|---|
| **Normal Range** | **0-15** | **130.0-725.0** | **1.0-12.5** | **300.0-1000.0** | **0.0-6.0** | **2.0-20.0** | **8.0-35.0** |
| 3,4-Dihydroxy-benzyl alcohol | 7 | 356.683 | 2.689 | 618.238 | 3 | 3.2 | 17.553 |
| 5-Benzyloxypyrimidine-2-carboxylic acid | 4 | 230.223 | 9.473 | 475.807 | 1 | 4.75 | 13.762 |
| Acacetin | 3 | 284.268 | 6.881 | 517.54 | 1 | 3.75 | 13.963 |
| Apigenin | 3 | 270.241 | 6.07 | 537.139 | 2 | 3.75 | 13.963 |
| Benzofuran 2,3, dihydro | 2 | 199.224 | 7.011 | 385.618 | 2 | 5.25 | 12.53 |
| Boeravinone B | 3 | 312.278 | 4.885 | 516.43 | 2 | 5.45 | 16.195 |
| Caffeic acid | 5 | 180.16 | 7.175 | 392.531 | 3 | 3.5 | 12.706 |
| Desulphosingrin | 11 | 279.307 | 6.977 | 491.931 | 5 | 11.2 | 20.811 |
| Ellagic acid | 4 | 302.197 | 4.916 | 446.65 | 4 | 8 | 18.761 |
| Epicatechin | 5 | 290.272 | 2.921 | 509.455 | 5 | 5.45 | 19.681 |
| Eupalitin 3-o-galactoside | 11 | 492.435 | 8.321 | 695.424 | 5 | 13.75 | 29.318 |
| Ephedrine | 4 | 165.235 | 1.941 | 408.626 | 2 | 3.2 | 10.372 |
| Ferulic acid | 5 | 194.187 | 6.295 | 420.153 | 2 | 3.5 | 11.367 |
| Furon-2-ylmethanol | 3 | 216.279 | 2.385 | 455.662 | 1 | 2.2 | 10.219 |
| Gallic acid monohydrate | 4 | 170.121 | 5.716 | 342.782 | 4 | 4.25 | 13.283 |
| Kaempferol | 4 | 286.24 | 5.622 | 501.402 | 3 | 4.5 | 16.695 |
| Purpurin | 3 | 256.214 | 3.14 | 445.607 | 1 | 4.25 | 12.061 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Quercetin | 5 | 302.24 | 3.533 | 512.235 | 4 | 5.25 | 18.32 |
| Semiglabrin | 1 | 392.407 | 8.159 | 572.912 | 0 | 6 | 17.46 |
| Succinic acid | 5 | 262.452 | 3.975 | 587.602 | 0 | 4 | 11.448 |
| Vasicinol | 2 | 204.228 | 5.644 | 422.555 | 2 | 3.95 | 12.316 |
| Vasicinone | 1 | 202.212 | 1.481 | 412.623 | 1 | 5.7 | 11.374 |

| **Molecule Name** | **QPlogP Water /Gas** | **QPlogP Octanol /Water** | **QPlog BB for brain /Blood** | **No. of Metabolic reactions** | **QPlogKp for skin permeability** | **Human Oral absorption** | **Rule of Five** | **Rule of Three** |
|---|---|---|---|---|---|---|---|---|
| **Normal Range** | **4.0-45.0** | **-2.0-6.5** | **-3.0-1.2** | **1.0-8.0** | **-8.0 to -1.0** | **1,2 (or)3 L, M, H** | **Max 4** | **Max 3** |
| 3,4-Dihydroxy-benzyl alcohol | 17.553 | 7.834 | -0.519 | 3 | -2.169 | 3 | 0 | 0 |
| 5-Benzyloxypyrimidine-2-carboxylic acid | 13.762 | 9.154 | -1.033 | 4 | -2.84 | 3 | 0 | 0 |
| Acacetin | 13.963 | 8.351 | -0.976 | 3 | -3.002 | 3 | 0 | 0 |
| Apigenin | 13.963 | 8.351 | -0.976 | 3 | -3.002 | 3 | 0 | 0 |
| Benzofuran 2,3, dihydro | 12.53 | 10.124 | -0.679 | 1 | -3.441 | 3 | 0 | 0 |
| Boeravinone B | 16.195 | 11.412 | -1.073 | 3 | -3.386 | 3 | 0 | 0 |
| Caffeic acid | 12.706 | 9.871 | -1.569 | 2 | -4.524 | 2 | 0 | 1 |
| Desulphosingrin | 20.811 | 18.74 | -2.122 | 6 | -4.733 | 2 | 0 | 0 |
| Ellagic acid | 18.761 | 16.688 | -2.333 | 4 | -6.753 | 2 | 0 | 1 |
| Epicatechin | 19.681 | 15.562 | -1.845 | 7 | -4.686 | 2 | 0 | 1 |
| Eupalitin 3-o-galactoside | 29.318 | 22.69 | -2.488 | 8 | -4.47 | 1 | 2 | 1 |
| Ephedrine | 7.535 | 1.252 | 0.25 | 3 | -3.603 | 3 | 0 | 0 |
| Ferulic acid | 11.367 | 1.378 | -1.189 | 2 | -3.697 | 2 | 0 | 0 |
| Furon-2-ylmethanol | 10.219 | 3.179 | 0.087 | 5 | -1.206 | 5 | 0 | 0 |
| Gallic acid monohydrate | 13.283 | -0.567 | -1.669 | 3 | -5.486 | 3 | 0 | 1 |
| Kaempferol | 16.695 | 1.06 | -1.803 | 4 | -4.533 | 4 | 0 | 0 |
| Purpurin | 12.061 | 1.025 | -1.39 | 3 | -4.32 | 3 | 0 | 0 |
| Quercetin | 18.32 | 0.387 | -2.309 | 5 | -5.422 | 5 | 0 | 1 |
| Semiglabrin | 17.46 | 3.368 | -0.144 | 1 | -1.889 | 1 | 0 | 0 |
| Succinic acid | 11.448 | 3.446 | -0.332 | 2 | -2.15 | 2 | 0 | 0 |

**Molecular Docking Studies:** The molecular docking studies were carried out for elastase protein with the compounds satisfying Lipinski's rule of five using the Glide module of maestro Schrodinger software. The results were interpreted based on the docking score, the number of interactions with active site residues and the number of H-bond formed, and the length of the bond **Table 3**. The interactions were observed using the PyMol visualization tool. Eupalitin 3-o-galactoside from *Boerhavia diffusa* showed the least docking score with the target protein **Fig. 2**. The Glide scoreis -6.72 Kcal/mol and the residues interacted were TRP 115(O-H), TRP 115 (O-H), GLU 141 (H-O), GLU 141 (O-O), GLU 141 (O-O), GLU141 (H-O), HIS 140 (O-N), ALA 113 (O-O), ARG 198 (O-H), HIS 223 (O-H) with a bond length of 2.3 Å, 2.7 Å, 2.1 Å, 3.5 Å, 3.4 Å, 2.3 Å, 3.3 Å, 3.3 Å, 1.8 Å, and 2.8 Å respectively. Phytocompounds ephedrine and quercetin from the plant's *Sida Rhombifolia and Clerodendrum infortunatum* respectively showed a significant binding interaction with a least G Score of -5.84, and -4.62 **Fig. 3** and **Fig. 4**. Phytocompound purpurinfrom *Tephrosia purpurea* had substantial G scores of -4.05 Kcal/mol. Benzofuran 2,3 dihydro from the plant *Scoparia dulcis* also had higher binding efficiency against the target protein, with G Scores of -3.23 Kcal/mol. Epicatechin and 2,4,6-trimethylphenyl) furan-2-ylmethanol from the plants *Breynia retusa* and *Euphorbia Herterophylla* had significant Glide scores of -3.24 and -2.65 Kcal/mol. Among all other phytocompounds, succinic acid from the plant *Boerhavia diffusa*

showed poor binding interaction with a G Score of -0.65 Kcal/mol. Docking studies revealed that Eupalitin 3-O-galactoside, Quercetin, and Ephedrine from plants *Boerhavia diffusa, Sida rhombifolia,* and *Clerodendrum infortunatum* have significant binding interactions with the target protein 3DBK when compared to all other phytocompounds. *In-vitro* investigations with *Boerhavia diffusa, Sida rhombifolia,* and *Clerodendrum infortunatum* revealed a similar outcome of enhanced antibacterial activity and thus justified *in-silico* studies.

**TABLE 3: DOCKING SCORE OF PHYTOCOMPOUNDS AGAINST TARGET *PSEUDOMONAS AERUGINOSA ELASTASE***

| S. no. | Name Of The Ligand (Pubchem Id) | Residues Interaction | Bond Length (Å) | No. of Hydrogen Bonds | G-Score (Kcal/mol) |
|---|---|---|---|---|---|
| | | ***Boerhavia diffusa*** | | | |
| 1 | Eupalitin 3-O- galactoside (44259727) | TRP 115(O-H) | 2.3 | 10 | -6.72 |
| | | TRP 115 (O-H) | 2.7 | | |
| | | GLU 141 (H-O) | 2.1 | | |
| | | GLU 141 (O-O) | 3.5 | | |
| | | GLU 141 (O-O) | 3.4 | | |
| | | GLU141 (H-O) | 2.3 | | |
| | | HIS 140 (O-N) | 3.3 | | |
| | | ALA 113 (O-O) | 3.3 | | |
| | | ARG 198 (O-H) | 1.8 | | |
| | | HIS 223 (O-H) | 2.8 | | |
| 2 | Boeravinone B (14018348) | ARG 198 (O-H) | 2.1 | 5 | -4.36 |
| | | ARG 198 (O-H) | 2.4 | | |
| | | ARG 198 (O-H) | 2.2 | | |
| | | GLU 141 (H-O) | 2.0 | | |
| | | TRP 115 (O-H) | 2.1 | | |
| 3 | Gallic acid monohydrate (24721416) | ARG 198 (O-H) | 2.5 | 4 | -3.88 |
| | | HIS 140 (H-N) | 2.9 | | |
| | | GLU 141 (H-O) | 1.9 | | |
| | | HIS 223 (O-H) | 2.7 | | |
| 4 | 3'4-dihydroxy-benzyl alcohol (101663520) | ASN 112 (O-H) | 2.0 | 2 | -3.73 |
| | | GLU 141 (H-O) | 2.1 | | |
| 5 | Ferulic acid (445858) | ASN 112 (O-H) | 2.4 | 2 | -2.58 |
| | | GLU 141 (H-O) | 1.9 | | |
| 6 | Kaempferol (5280863) | TRP 115 (H-O) | 2.1 | 4 | -0.94 |
| | | TRP 115 (O-H) | 2.6 | | |
| | | ASN112 (H-O) | 2.0 | | |
| | | ASN 112 (O-H) | 2.7 | | |
| 7 | Succinic acid (520988) | HIS 223 (O-H) | 2.1 | 2 | -0.65 |
| | | TRP 115 (O-H) | 2.1 | | |
| | | ***Clerodendrum infortunatum*** | | | |
| 8 | Quercetin (5280343) | ASN 112 (O-H) | 2.5 | 5 | -4.62 |
| | | ASN 112 (O-H) | 2.4 | | |
| | | ARG 198 (O-H) | 2.1 | | |
| | | TRP 115 (H-O) | 2.1 | | |
| | | TRP 115 (H-O) | 2.2 | | |
| 9 | Desulphosinigrin (9601716) | GLU 141 (H-O) | 1.8 | 5 | -4.40 |
| | | TRP 115 (O-H) | 2.4 | | |
| | | GLU 164 (H-O) | 1.9 | | |
| | | HIS 223 (O-H) | 1.9 | | |
| | | HIS 223 (O-H) | 2.7 | | |
| 10 | Ellagic acid (5281855) | GLU 141 (H-O) | 1.8 | 2 | -3.73 |
| | | ASN 112 (O-O) | 2.9 | | |
| 11 | Caffeic acid (689043) | GLU 141 (H-O) | 1.7 | 3 | -4.05 |
| | | HIS 140 (H-O) | 2.0 | | |
| | | ARG 198 (O-H) | 1.9 | | |

| | | | | | |
|---|---|---|---|---|---|
| 12 | Apigenin (5280443) | HIS 223 (OH) | 2.8 | 3 | -3.22 |
| | | ASN 112 (OH) | 2.6 | | |
| | | TRP115 (O-O) | 1.8 | | |
| 13 | Acacetin (5280442) | HIS 223 (O-H) | 2.5 | 3 | -3.21 |
| | | TRP 115 (O-H) | 2.1 | | |
| | | TRP 115 (O-O) | 3.5 | | |
| | | ***Sida rhombifolia*** | | | |
| 14 | Ephedrine (9294) | ARG 198 (O-H) | 1.8 | 5 | -5.84 |
| | | HIS 140 (H-O) | 1.7 | | |
| | | GLU 141 (H-O) | 2.4 | | |
| | | GLU 141 (H-O) | 2.2 | | |
| | | ALA 113 (H-O) | 1.9 | | |
| 15 | Vasicinone (442935) | ARG 198 (N-H) | 2.4 | 4 | -4.00 |
| | | ARG 198 (O-H) | 2.0 | | |
| | | HIS 140 (H-N) | 2.1 | | |
| | | GLU 111 (O-O) | 2.9 | | |
| 16 | Vasicinol (442934) | ARG 198 (O-H) | 2.0 | 2 | -3.80 |
| | | HIS 140 (H-N) | 2.0 | | |
| | | ***Tephrosia purpurea*** | | | |
| 17 | Purpurin (6683) | GLU 141 (H-O) | 1.7 | 2 | -4.05 |
| | | HIS 223 (O-H) | 2.0 | | |
| 18 | Semiglabrin (156341) | ASN 112 (O-H) | 2.3 | 2 | -2.45 |
| | | GLU 141 (O-O) | 3.3 | | |
| | | ***Scoparia dulcis*** | | | |
| 19 | Benzofuran 2,3 dihydro (20209882) | HIS 140 (O-N) | 3.1 | 3 | -3.23 |
| | | ALA 113 (H-O) | 2.3 | | |
| | | ARG 198 (H-O) | 2.5 | | |
| 20 | 5- Benzyloxypyrimidine (561874) | ASN 112 (N-H) | 2.0 | 3 | -3.20 |
| | | ARG 198 (O-H) | 1.7 | | |
| | | ASN 112 (O-H) | 2.7 | | |
| | | ***Breynia retusa*** | | | |
| 21 | Epicatechin (72276) | HIS 140 (H-N) | 2.8 | 6 | -3.24 |
| | | HIS 144 (O-N) | 3.2 | | |
| | | GLU 164 (H-O) | 2.6 | | |
| | | HIS 223 (O-H) | 1.9 | | |
| | | GLU 164 (H-O) | 1.7 | | |
| | | ASN 112 (O-H) | 2.4 | | |
| | | ***Euphorbia Herterophylla*** | | | |
| 22 | 2,4,6-trimethylphenyl) furan-2-ylmethanol (49962474) | ARG 198 (O-H) | 2.2 | 3 | -2.65 |
| | | ALA 113 (O-H) | 2.6 | | |
| | | GLU 141 (H-O) | 2.1 | | |

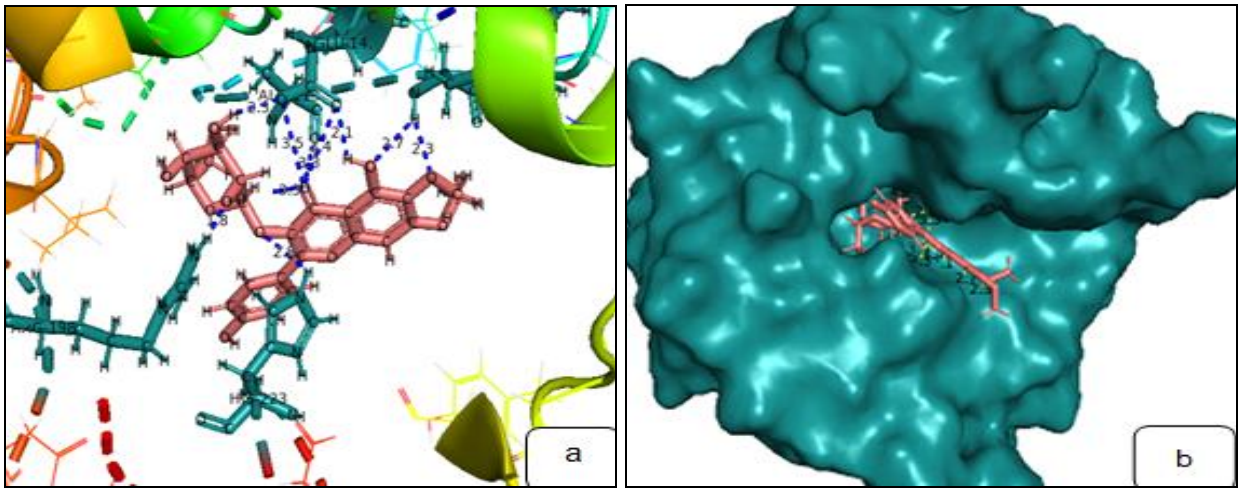



**FIG. 2: MOLECULAR INTERACTION OF EUPALITIN 3-O- GALACTOSIDE WITH THE TARGET PROTEIN 3DBK (A) AND DOCKING COMPLEX (B)**

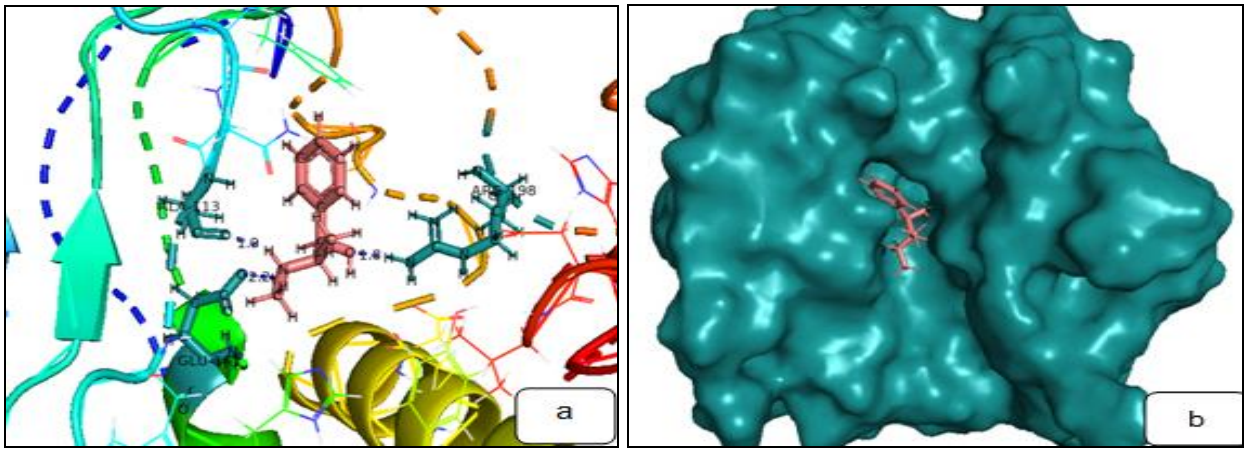



**FIG. 3: MOLECULAR INTERACTION OF EPHEDRINE WITH THE TARGET PROTEIN 3DBK (A) AND DOCKING COMPLEX (B)**

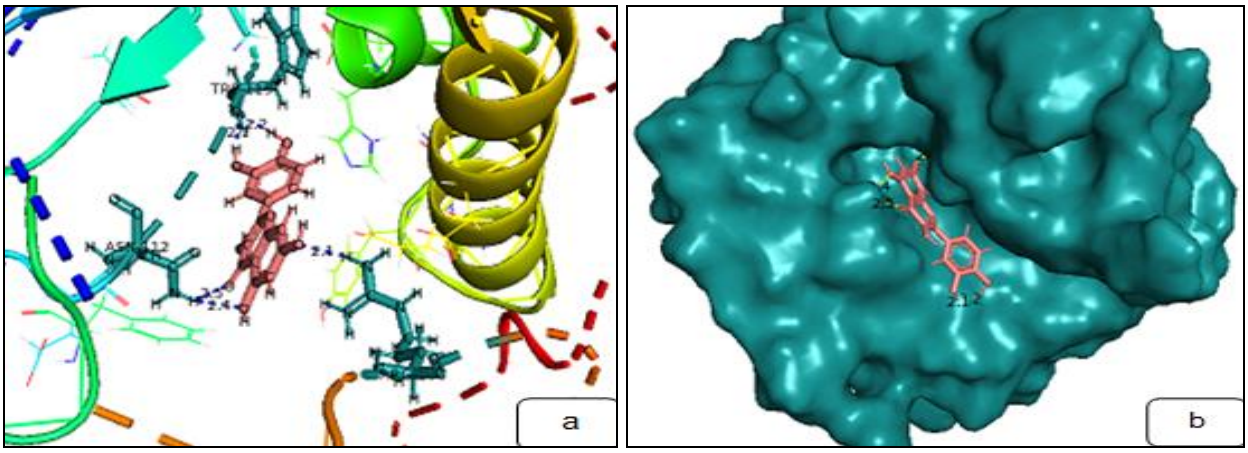



**FIG. 4: MOLECULAR INTERACTION OF QUERCETIN WITH THE TARGET PROTEIN 3DBK (A) AND DOCKING COMPLEX (B)**

**Note:** The deep teal color represents the target protein, whereas the phytocompound is represented by the deep salmon color. Blue dots depict the lead compound's hydrogen bond interaction with the target protein (a), while docked complex (b) ligands bind to the target protein's active site pockets.

***In-vitro*** **Antibacterial Screening:** The *in-silico* docking studies indicated that compounds from *Boerhavia diffusa, Sida rhombifolia,* and *Clerodendrum infortunatum* had significant interactions with the target protein. Therefore, these plants alone were selected for *in-vitro* antibacterial activity.

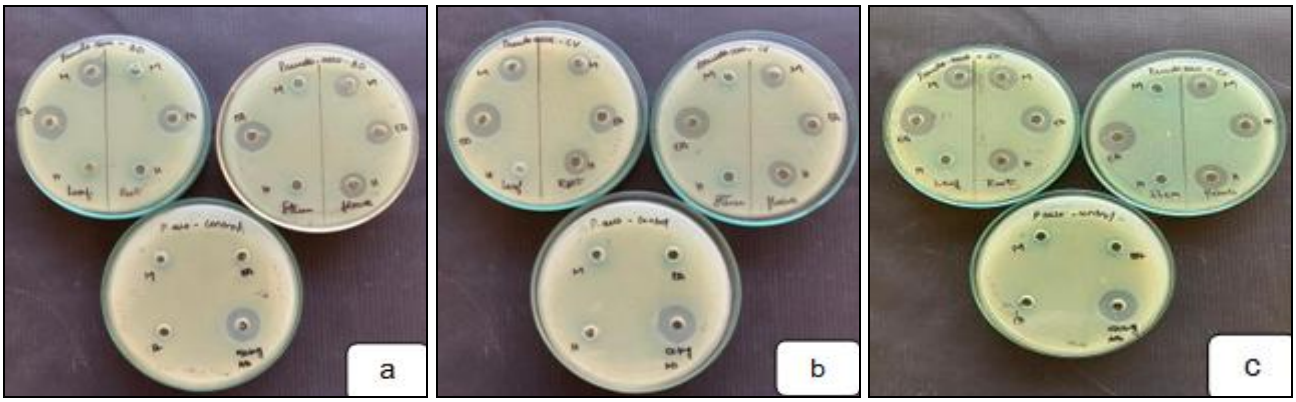



**FIG. 5: ANTIBACTERIAL ANALYSIS OF DIFFERENT EXTRACTS OF BOERHAVIA DIFFUSA (A), CLERODENDRUM INFORTUNATUM (B), AND SIDA RHOMBIFOLIA (C) AGAINST P.AERUGINOSA – WELL DIFFUSION METHOD**

The well-diffusion method was used to study the inhibitory effect of extracts obtained from leaves, stem, root, and floral parts of the above-mentioned plants. The zone of inhibition was observed and shown in Fig. 5. Among leaf, stem, root, and flower extract, the leaf extract of all three plants showed significant bacterial inhibition. Positive control neomycin sulphate was used and has shown efficient antibacterial activity with a zone of inhibition of 18 mm. Likewise, ethyl acetate leaf extracts of all three plants have shown a similar zone of inhibition of 18 mm tabulated in **Table 4**. Followed by methanol leaf extracts. Hexane leaf extract showed no activity against *P. aeruginosa*. Organic solvent hexane, ethyl acetate, and methanol were used as negative control and showed no antibacterial activity.

**TABLE 4: ZONE OF INHIBITION OF DIFFERENT PLANT EXTRACTS AGAINST *P. AERUGINOSA* AN AGAR WELL DIFFUSION METHOD**

| S. no. | Sample (100 µL) | | Zone Of Inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Leaf | Stem | Root | Flower | Negative Control | Antibiotic (NS) 50µg |
| 1 | *Boerhavia diffusa* | M | 16 | - | - | 13 | - | 18 |
| | | EA | 18 | 18 | 14 | 13 | - | |
| | | H | - | - | - | 13 | - | |
| 2 | *Clerodendrum infortunatum* | M | 14 | - | 12 | 13 | - | 18 |
| | | EA | 18 | 18 | 14 | 13 | - | |
| | | H | - | - | 13 | 13 | - | |
| 3 | *Sida rhombifolia* | M | 16 | - | 15 | 14 | - | 18 |
| | | EA | 18 | 17 | 14 | 14 | - | |
| | | H | - | - | 13 | 14 | - | |

(M): Methanol, (EA): Ethyl acetate, (H): Hexane, (NS): Neomycin sulphate. For each extract's average zone of inhibition, the diameter was calculated from the triplicates.

**DISCUSSION:** Most Gram-negative bacteria persist with multidrug resistance and constitute a global threat. The most common bacteria encountered in clinical implications is *P. aeruginosa* which has been chosen for the present study. The complicated relationship between pathogenicity and antibiotic resistance and the connection between resistance mechanisms and virulence has made many pseudomonal infections more challenging to treat [24].

The current study emphasizes the importance of developing a novel potential therapeutic antibacterial drug molecule to combat *Pseudomonas aeruginosa* infections. Bacteria typically produce virulence proteins, which can damage host tissues in addition to bacterial infections [25]. A variety of virulence proteins are produced by *Pseudomonas aeruginosa*, with elastase (LasB) being one of the most extremely toxic to the host tissue since it affects both the innate and adaptive immune system [26]. The present study focuses on the *in-silico* analysis of phytocompound to identify promising small molecules by targeting the LasB virulence protein. *Pseudomonas aeruginosa* elastase induces infection by generating virulence proteins, including LasA protease and LasB elastase, which is mediated by quorum sensing [27]. A molecular docking investigation targeted the pathogenicity proteins LasR and RhlR carried out and they screened roughly 1920 chemicals against these proteins using Glide version 5.5. Additionally, it noted that the natural plant compounds rosmarinic acid, naringin, chlorogenic acid, and morin were effective against elastase inhibition [28].

Similarly, in our study phytochemicals eupalitin 3-O-galactoside, ephedrine, and quercetin from the plants *Boerhavia diffusa*, *Sida rhombifolia*, and *Clerodendrum infortunatum* have demonstrated good binding interactions with the target protein elastase. Another objective of this study is to analyze the antibacterial activity of *Boerhavia diffusa*, *Sida rhombifolia*, and *Clerodendrum infortunatum* against *P. aeruginosa*. Ethyl acetate leaf extract of *Boerhavia diffusa* has shown significant antibacterial activity. The aerial part of the plant *Boerhavia diffusa*has significant antibacterial properties against *Pseudomonas aeruginosa* [23]. Similarly, in Ayurveda, it is mentioned that *Sida rhombifolia* is effective against

many diseases and can be used to treat fever, heart diseases, burning sensations, antibacterial, and all kinds of inflammations [29]. Additionally, it was reported that various extracts from the leaves of *Sida rhombifolia* are effective against a variety of Gram-positive and Gram-negative bacteria, including *Pseudomonas aeruginosa.* Compared to the other extracts, it was discovered that the ethyl acetate extract had the best zone of inhibition [30].

Supporting our results, ethyl acetate leaf extracts of *Boerhavia diffusa* and *Sida rhombifolia* had a greater inhibitory impact against *Pseudomonas aeruginosa*. As well as *Clerodendrum infortunatum* had promising antibacterial and antioxidant activity [31]. *Clerodendrum infortunatum* leaf extracts in ethyl alcohol, chloroform, and ethyl acetate had good antibacterial action against *Pseudomonas aeruginosa* [32]. Likewise, in our findings, ethyl acetate leaf extract of *C. infortunatum* showed a better zone of inhibition. Comparable to our finding, it was reported that *Pseudomonas aeruginosa* was most effectively inhibited by *Clerodendrum infortunatum* leaf extracts [33].

Altogether, using molecular docking studies, we were able to find natural plant compounds that have the potential to inhibit the metalloprotease target protein LasB. Thus, these bioactive compounds can be further utilized to develop efficient inhibitors against 3DBK. Similarly, *in-vitro* investigations confirmed the *in-silico* studies that plant extracts from all three plants have a strong ability to inhibit the growth of *Pseudomonas aeruginosa*.

**CONCLUSION:** In this study, *in-silico* investigations revealed that phytocompounds from the plants *Boerhavia diffusa, Sida rhombifolia,* and *Clerodendrum infortunatum* have effective binding abilities against the target protein 3DBK. With a minimum G score of -6.72, -5.84, and -4.62 Kcal/mol, respectively, docking data showed that eupalitin 3-O-galactoside, ephedrine, and quercetin had excellent binding interactions with the target protein. According to antibacterial investigations, all three plants' leaf extracts significantly inhibit *Pseudomonas aeruginosa* growth.

When compared to antibiotics (neomycin sulphate) ethyl acetate leaf extract showed similar antibacterial activity. Therefore, the current study reveals that the traditional plants used here have antibacterial properties against *P. aeruginosa*, and phytocompounds from those plants can be used to develop promising antibacterial agents.

**Declarations:**

**Ethics Approval and Consent to Participate:** This article does not contain any authors' studies involving animals and human participants.

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**CONFLICTS OF INTEREST:** The authors declared no competing interest in the studies.

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