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Screening and *In-silico* approach to evaluate the Diabetic wound healing of phytocompounds against the target protein and Antibacterial activity

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Abstract

Diabetic foot infections can be caused by the ubiquitous gram-negative bacterium *Pseudomonas aeruginosa*. 16.7% of people with diabetes have diabetic foot infections. A gram-negative bacterium called *Klebsiella oxytoca* was found in a diabetic foot infection. It has a decreased susceptibility to chlorhexidine and is a common and dangerous consequence in diabetes individuals. Gram-positive *Staphylococcus aureus* and gram-negative *Enterococcus* sp. bacteria are frequently found in diabetic foot ulcer patients. *Escherichia coli* is one of these bacteria. Anti-bacterial resistance bacteria are a global concern when it comes to the use of different plant-based herbal medication formulations. This study is to found the phytocompound of herbal plant through in-silico molecular docking and in-vitro antibacterial analysis. The structure of the phytocompounds from the traditional medicinal herb *Andrographis paniculata* was obtained from PubChem, and their study and manifestation were required. Following in-silico docking to the target protein, generated phytocompounds were screened using Lipinski's rule in ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiles. Because MMP-9 is highly expressed during diabetic foot ulceration, it presents an intriguing molecular target for diabetic wound repair. Following molecular docking, Diethyl Phthalate, a naturally occurring molecule sourced from *Andrographis paniculata*, exhibited a moderate binding affinity, achieving a glide score of -4.43 Kcal/mol. The study on the antibacterial properties of plant leaf extract in methanol solvent was conducted against microorganisms.

Key words : Target protein MMP-9, *Andrographis paniculata*, Diethyl Phthalate, Gram-negative and Gram-positive bacteria.

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Diabetes is a metabolic disorder associated with the endocrine system that characterized by chronic hyperglycaemia[2]. Uncontrolled diabetes leads to many types of diseases, also referred to as diabetic complications, including diabetic retinopathy, neuropathy, nephropathy, foot ulcers, and delayed wound healing[1]. For the treatment of diabetes and wound, several synthetic drugs are available in the market, but they are of high cost and have adverse side effects. The major limitation of these drugs is an allergic reaction and drug resistance[11]. Traditionally, plants and their phytochemicals were used throughout the world because of their effectiveness, relative abundance, low cost, and fewer side effects. Thus, the use of such natural therapeutic drugs with the absence of any side effects appears promising towards chronic wound healing[9,12]. Natural products contain a wide variety of chemicals, including flavonoids, polyphenols, saponins, steroids, and vitamins that have several therapeutic roles like anti-diabetic, antioxidant, anti-inflammatory, and cell synthesis modulating properties. The phytochemicals influence various metabolic pathways and thereby increase the release of insulin, it's production, and efficacy[6,13]. The reduction in wound healing time is crucial for diabetes especially to lower the chance of infection and decrease complications and costs. Herbal products and their active compounds may inhibit bacterial growth and may have a significant clinical value in the treatment of resistant microbial strains[4]. some herbal products affect wound healing activities through anti-inflammatory and antioxidant activities, cell proliferation, and angiogenesis[8,10]. Diabetic foot ulcers has been associated with diabetic patients, causing ulceration, infection and eventually the need for amputation. (ADS 2004). *Andrographis paniculata* wall (family Acanthaceae) is one of the most popular medicinal plants used traditionally for the treatment of array of diseases such as cancer, diabetes, high blood pressure, The plant has bitter taste hence the name king of bitters. It possesses several phytochemical constituents with unique and interesting properties. Diterpenes, flavonoids, xanthones, and other miscellaneous compounds have been isolated from the plant[5]. In-silico studies help to overcome these by predicting the protein target interactions, and prior optimization of the drug can be carried out at an early stage of development from the most suitable drug compound. The present study focuses on the structure-based identification of plants whose phytocompounds can actively target the protein MMP-9 and the bacterial activity followed by their *in-vitro* study.

In-silico studies : Molecular docking is an efficient and expanding method for developing prospective lead drugs. Molecular docking involves various computational procedures, including preparing receptors and ligands, docking and post-docking analyses, etc.[7]. The computational software maestro Schrodinger version 9.0.211 was utilized for ADME profiling, LigPrep, Protein preparation, Glide grid generation, and G scoring function.

Structure retrieval : This study aimed to evaluate the antibacterial activity of phytocompounds from different natural plants. After conducting a literature survey, GC-MS identified 16 phytocompounds from Andrographis paniculata plant considered ligands. The PubChem database was used to derive the

chemical structures of phytocompounds 16. The three-dimensional structure of the protein to be targeted, MMP-9 was retrieved from the PDB- Protein Data Bank database. The target protein's active site region was determined using the LigSite online tool (http://projrct. biotec.tudresden.de/pocket/), which predicts the amino acids with binding pockets[16].

ADME Profiling of Phytocompounds :

To assess the drug-likeness of Particular ligands, the ADME properties, which include Absorption, Distribution, Metabolism, Excretion, and Toxicity of phytocompound were analyzed. The Schrodinger program's QikProp module version 4.4 was used to predict the ADME properties including, the number of rotatable bonds, molecular weight, number of donor hydrogen bonds, number of acceptor hydrogen bonds and octanal water partition coefficient logP, etc as per Lipinski rule of five 4 and the Pass online way2drug online tool was used to further Analyze the biological properties of the ligand[3].

Ligand and Receptor preparation: Before molecular docking, ligands were prepared using the LigPrep module, optimized by bond ordering and angles. In contrast, GLIDE's Protein preparation wizard was used to prepare proteins. In which water molecules were removed from the structure for preparation, hydrogen bonds were optimized and energy was minimized, further structure-based virtual screening was performed[14].

Molecular Docking : Identification of new therapeutic compounds is a critical step in the in-silico investigation and is accomplished through molecular docking, where structure-based virtual screening was performed for each screened phytocompound against MMP-9 The glide module of the Schrodinger program was used to simulate receptor-ligand interaction and binding affinities. Effective ligands against the target protein will be identified due to molecular docking based on the least glide score value and by the formation of hydrogen bonds and hydrophobic interactions. The PyMol visualization tool was further used to see the hydrogen bond interaction between the ligand and the target protein, where the interaction between the amino acid residues and the hydrogen bonds with bond length can be evaluated[15].

Sample Collection and Authentication :

The plant species *Andrographis paniculata*, were chosen for in-vitro antibacterial and the same sample plant as a result of in-silico studies. The plants are authenticated the Botanical Survey of India (BSI) in Coimbatore, Tamil Nadu, identified those plant s as *Andrographis paniculata* (BSI/SRC/5/23/ 2022/Tech/520).

Sample preparation : The plant parts of (leaves and seed) were manually separated and used by handpicking method then dried in a hot air oven at 50 °C. This plant material was utilised in the extractor of a Soxhlet apparatus after being dried into a good powder of the leaves and seed parts (20 g). Starting with Ethanol, Methanol, Aqueous, Chloroform, and Acetone, the pure form of plant extract was carried out in a sequential solvent system with variable polarity solvents. In a 24-hour period, 250 ml of various solvents were used to extract the material. The extracts were lyophilized at 40 °C under reduced pressure in

their respective solvent systems.

Anti-bacterial activity :

The Agar well diffusion method was used to determine the antibacterial activity of various extracts *Staphylococcus aureus* culture was purchased from MTCC (MTCC-96). The bacterial culture was swabbed into MHA plates, followed by 6 mm wells that were created. Used 50 µl to 150µl of crude extract. Plates were further kept for overnight incubation at 37 °C. After the incubation period, the sensitivity of the test plates was assessed using a zone of inhibition, with the diameter of the zone surrounding the well determined in millimetres.

Structure Retrieval and Active site prediction:

3D Structure of the target protein MMP-9 was retrieved from Protein Data Bank with a PDB ID of 5CUH (Fig. 1), and its active site pockets HIS 405 525was discovered via the LigSite online tool.

Fig. 1. Structure of target protein MMP-9

ADME Screening using QIKPROP Module:

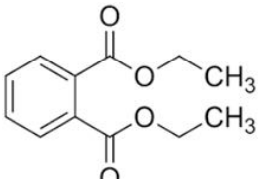
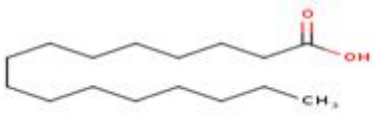
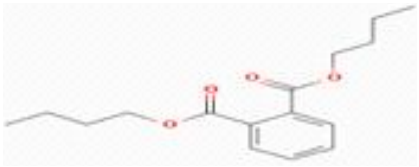
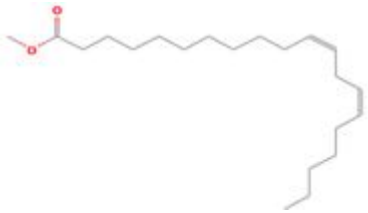
The bioavailability of selected phytocompounds was predicted using ADME profiling, which is a crucial step in the discovery of potential lead compounds. Out of 16 phytocompounds, only 4 compounds satisfy the Lipinski rule of five and are considered to be drug-likeness. Parameters like lipophilicity, permeability in octanol/ water partition coefficient and brain/ blood barrier along with these, properties like Number of rotatable bonds, Number of metabolic reactions, Molecular weight, Hydrogen bond donor, Hydrogen bond acceptor and Skin

Table-1. pharmacological properties of ADME-cleared compounds

Molecule Name	No. of rotatable bonds	Mole-cular weight	Dipole mom-ent	SASA	Donor Hydro-gen bonds	Accepgen tor Hydro-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
Diethyl Phthalate	4	222.24	5.429	502.036	0	4	10.438
n-Hexadecanoic acid	14	256.428	5.844	676.824	1	2	11.6
Dibutyl phthalate	8	278.347	5.083	623.903	0	4	12.381
cis-11,14-Eicosadi-enoic acid, methyl ester	16	322.53	2.908	697.831	0	2	11.622

Molecule Name	QPlogP Water /Gas	QPlogP Octanol /Water	QPlog BB for brain /Blood	No. of Meta-bolic reac-tions	QPlogKp for skin-permea-bility	Human Oral Absor-ption	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 - 1.0	1,2 (or) 3 L, M, H	Max 4	Max 3
Diethyl Phthalate	**5.638**	**2.305**	**-0.48**	**1**	**-2.165**	**3**	**1**	**1**
n-Hexadecanoic acid	**2.425**	**5.292**	**-1.474**	**1**	**-2.156**	**3**	**1**	**0**
Dibutyl phthalate	**5.002**	**3.859**	**-0.736**	**0**	**0.268**	**3**	**0**	**0**
cis-11,14-Eicosadi-enoic acid, methyl ester	**0.499**	**6.308**	**-1.007**	**4**	**1.126**	**1**	**1**	**1**

Table-2. 2D Structure of adme cleared phytocompounds

S.No	Phytocompounds	Structure
1	Diethyl Phthalate	
2	n-Hexadecanoic acid	
3	Dibutyl phthalate	
4	cis-11,14-Eicosadienoic acid, methyl ester	

permeability were evaluated. The compounds that satisfied the Lipinski rule of five were tabulated in Table-1 and the pharmaco-logical properties of ADME-cleared compounds were validated using PASS online Way2Drugand are reported in Table 2.

Molecular Docking studies :

Molecular docking investigation predicts the interaction of bioactive compounds against the target protein. Molecular docking was carried out using the Glide module of maestro Schrodingerr software. The docking result interprets the active site and binding efficiency of phytocompounds against the target protein MMP-9. Phytoconstituents from *Andrographis paniculata* were found to be potential lead compounds against MMP-9. Phytocompounds were allowed to dock with the target protein and the binding efficiency that is the formation of hydrogen bonds was visualized using the PyMol visualization tool. The bioactive compound Diethyl Phthalate showed efficient binding interaction against the target protein with the least Glide score is -4.43 Kcal/mol and residues interacted were HIS 405 (0-H) with bond length of 1.2 Å.

The binding interactions with the glide score value was reported in Table-3. Fig. 2 represents the binding efficiency of Diethyl Phthalate with the target protein MMP-9. Therefore, the molecular docking studies reveal that phytocompounds from Andrographis paniculata have significant inhibition against MMP-9 and further in-vitro antibacterial studies were carried out to investigate its antibacterial activity.

Table-3. Molecular Docking of Phytocompounds against Target Protein 5CUH

S.no	Name of the Ligand	Residues Interaction	Bond Length (Å)	No. of Hydrogen Bonds	G-Score (Kcal/mol)
Andrographis paniculata					
1	Diethyl Phthalate	HIS 405(O-H)	1.7	1	-4.43

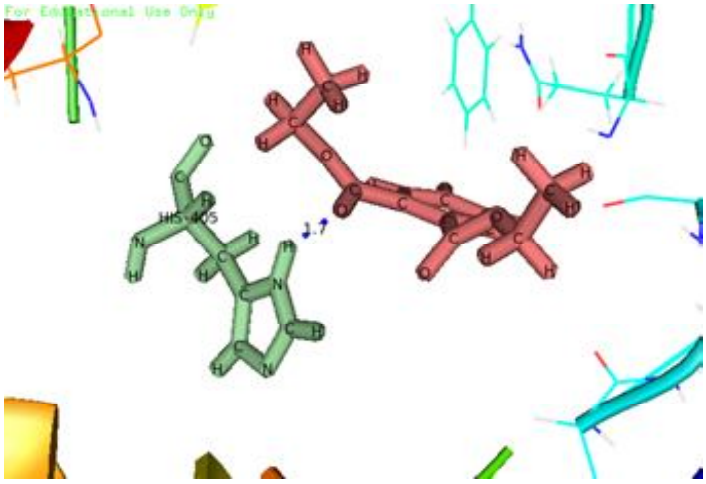


Fig. 2. Molecular interaction of Diethyl Phthalate with the target Protein.

Note: The creamy green colour represents the target protein and the deep salmon colour indicates the Diethyl Phthalate. Blue dots represent the hydrogen bond interaction of Diethyl Phthalate with the active site region of the target protein.

Antimicrobial activity :

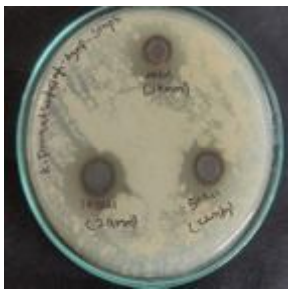


Figure 3

Figure 4

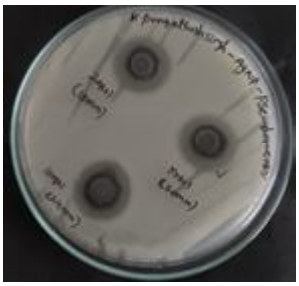


Figure 5

Table-4. Zone of Inhibition

Test organisms	50µl	100µl	150µl
Staphylococcus aureus	17mm	19mm	23mm
Escherichia coli	13mm	18mm	20mm
Pseudomonas aeruginosa	14mm	17mm	19mm

The study was clearly shown the phytocompounds from the *Andrographis paniculata* are found to be good and potential source of the MMP-9 target protein. The phytocompound of Diethyl Phthalate from *Andrographis paniculata* good binds to the MMP-9 target protein with the least Glide score is -4.43 Kcal/mol. The antibacterial activity of the plant extract and zone of Inhibition, *Staphylococcus aureus* showed a good result with comparative to other two bacteria's *Escherichia coli* and *Pseudomonas aeruginosa*, in that 150µl of sample can measured the zone was 23mm and 100µl sample zone was measured 19mm. based the antibacterial activity the *Staphylococcus aureus* have the good potential.

The research has carried from Hindustan College of Arts & Science. Coimbatore. The Anti-bacterial and Anti-fungal activity test done by Department of Microbiology, Hindustan College of Arts & Science. Coimbatore. An in-silico study has carried from Kongunadu Arts and Science College, Coimbatore.

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