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Targeting Cancer Pathways with Achyranthes aspera: A Computational and Experimental Approach Leveraging its Urinary Tract Infection-Fighting Properties

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ABSTRACT: Urinary tract infections (UTIs) are among the most common bacterial infections, particularly in women because of anatomical and physiological factors. As antibiotic-resistant bacteria become more prevalent, the search for natural alternatives to standard medications is increasingly essential. The study focuses on the antibacterial potential and phytochemical assets of Achyranthes aspera against the UTI Infectious Bacteria, such as K. pneumoniae. The Achyranthes aspera plant was extracted in methanol, and antibacterial activity was carried out by using the agar well diffusion method and minimal inhibitory concentration and minimal bactericidal concentration. The inhibition proved that plants was capable of inhibiting bacterial growth. Phytochemical screening confirmed the presence confirmed the presence of bioactive constituents including alkaloids flavonoids, phenols, tannins, terpenoids, saponins. Steroids. The plant extract was poteny against biofilm formation in tested pathogen. This study emphasizes the potential of achyranthes aspera in being a natural antimicrobial agents for the control of UTIs. Its strong antibacterial activity and its phytochemical content together with its use in the traditional herbal medicine

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1. INTRODUCTION

1.1. Urinary Tract Infection: Prevalence And Challenges

Urinary tract infections (UTIs) are recognized as the second most common infectious diseases worldwide. These infections manifest with a range of clinical symptoms, including acute, chronic, uncomplicated, complicated, asymptomatic, symptomatic, and recurrent forms. They can affect either the lower or upper parts of the urinary tract. A variety of microbial pathogens are responsible for UTIs, including Gram-negative bacteria (such as Uropathogenic Escherichia coli [UPEC] and Klebsiella), Gram-positive bacteria (like Staphylococcus saprophyticus), and yeasts (for example, Candida albicans). Urogenital tract infections (UGTIs) represent some of the most frequently encountered infectious diseases in the genital tract, urinary tract, and sexually transmitted urogenital infections (ST-UTIs), posing significant challenges to public health systems. Conditions such as cystitis (lower UTIs) and pyelonephritis (upper UTIs) that arise in otherwise healthy individuals without a prior UTI history are categorized as uncomplicated UTIs. Conversely, complicated UTIs generally occur among older adults and individuals with catheters. Although there are many risk factors that can lead to UTIs, uncomplicated cases are mainly linked to ST-UTIs. It is possible for UTIs and genitourinary tract infections (GTIs) to happen concurrently, but GTIs are often ignored or misdiagnosed. In other words, a large number of individuals suffer from UGTIs, yet GTIs are commonly reported only as UTIs. Therefore, the misdiagnosis or failure to diagnose GTIs, coupled with the overdiagnosis of UTIs, presents a significant problem, leading to inappropriate treatment and the risk of infection spread.

Bacterial biofilms play a vital role in the healthcare sector. According to the NIH, bacteria that develop biofilms are associated with nearly 80% of all infections, with urology being a significant area where biofilm formation creates notable difficulties. Biofilms may be found in the urothelium, prostate stones, and artificial implants. Bacteria adhering to the uroepithelium can form biofilms, leading to the invasion of renal tissue and causing pyelonephritis. Additionally, these bacteria might be involved in chronic bacterial prostatitis, complicating diagnosis since the bacteria that colonize may not be found in prostatic secretions or urine specimens. Biofilms can occur not only on urethral stents but also on catheters, resulting in blockages. As a result, catheter-associated urinary tract infections (CAUTIs) are among the most common healthcare-associated infections worldwide. Numerous investigations have associated CAUTIs with over 40% of healthcare-related infections in the United States. The majority of CAUTI instances involve commensal flora from the perineum. Over 90% of these infections are attributed to a single pathogen, with E. coli, Pseudomonas aeruginosa, enterococci, Candida, Klebsiella, and Enterobacter spp. being frequently identified organisms. The environmental conditions on the catheter surface create favorable circumstances for bacterial adhesion and biofilm formation. In these medical devices, microorganisms that produce urease, an enzyme that cleaves urea into ammonium ions, can result in encrustation, the development of infected bladder stones, and urinary blockages. The generation of ammonium ions increases urine pH, eventually leading to the precipitation of magnesium and calcium phosphate crystals. The pH level at which this precipitation occurs is known as nucleation pH. These crystals can form a protective barrier around bacteria, safeguarding them from the antimicrobial effects of substances used to coat or impregnate catheters. Proteus mirabilis is mainly responsible for this problem in urinary infections and has several virulence factors, such as mannose-resistant fimbriae, capsules, and urease, that allow it to form biofilms. Other organisms like Proteus vulgaris and Providencia rettgeri also possess the ability to create crystalline biofilms. Furthermore, the development of biofilms may increase the endurance of strains causing acute prostatitis within the prostatic secretory system, contributing to the recurring UTIs characteristic of chronic bacterial prostatitis. In fact, studies have shown that after an episode of acute prostatitis, cultures of expressed prostatic secretions remain positive for three months following the completion of a six-week treatment course in one-third of men. It has been observed that 63% of E. coli strains isolated from patients with prostatitis were capable of producing biofilms in vitro, compared to 40% of E. coli strains responsible for cystitis and pyelonephritis. Biofilm formation may account for the difficulties in fully eliminating bacterial prostatitis with conventional treatments.

The limitations of existing therapeutics are mainly due to rising antibiotic resistance, the appearance of multi-drug-resistant pathogens, and the risks associated with antibiotic misuse and side effects.

1. Excessive use of antibiotics: The excessive and improper use of antibiotics encourages the survival of bacteria that can withstand these drugs, leading to the development of resistant strains. This complicates the treatment of urinary tract infections (UTIs) with standard antibiotics, potentially resulting in extended hospital stays, increasing healthcare expenses, and a higher mortality rate.

2. Emergence of multi-drug-resistant (MDR) strains: The rise of multi-drug-resistant (MDR) strains in urinary tract infections introduces substantial challenges to current therapeutic options and heightens the likelihood of treatment failure, increased morbidity, and elevated healthcare costs. The emergence of MDR strains, such as extended-spectrum beta-lactamases, is particularly concerning.

The knowledge of herbs has been passed down through generations for thousands of years. Herbal medicine forms a significant part of all traditional medical systems. The use of herbal remedies showcases the triumph of diverse therapeutic approaches. Throughout history, plants have been preferred for medicinal purposes over other agents due to their immediate accessibility, affordability, and relevance to personal needs. Recently, there has been a remarkable surge in the consumption of plant-based health products in both developing and developed nations, leading to a rapid expansion of the herbal product market worldwide. An increasing interest in herbal research has been observed. Herbal medicines possess a robust traditional foundation or conceptual understanding, along with the potential for safe and effective treatment of various diseases. The World Health Organization (WHO) has endeavored to catalog all medicinal plants used around the world, identifying over 20,000 species. The WHO reports that more than 80% of the global population relies on traditional herbal medicine as their primary source of healthcare. Plants remain a source of potential new drugs and chemicals derived from different plant parts. Recently, there has been a noticeable trend towards herbal remedies due to the significant cumulative and irreversible side effects associated with modern pharmaceuticals. However, challenges such as overpopulation, urbanization, and the ongoing exploitation of herbal resources are leading to a daily depletion of natural resources and the associated traditional knowledge. In today's environment of drug development and the discovery of new drug molecules, many plant products are being assessed based on their traditional applications. One such plant under evaluation for its therapeutic benefits is Achyranthes aspera, commonly referred to as Latjeera in Hindi or Rough Chaff Tree in English. This erect or trailing herb, which can be annual or perennial and typically grows to a height of 1-2 meters, is often found as a weed along roadsides and pathways. Although it possesses numerous medicinal attributes, it is especially recognized for its spermicidal, antipyretic, and cardiovascular properties.

1.2. OVERVIEW OF *ACHYRANTHES ASPERA*

Achyranthes aspera Linn. belongs to the Amaranthaceae family and is an erect annual herb commonly found as a weed throughout India. Traditional healers use it to treat conditions such as fever, dysentery, and diabetes. A decoction made from its leaves has been noted for potential cardiovascular toxicity effects, while a crude ethanol extract has shown significant larvicidal activity against Boophilus microplus tick larvae. The root extract is recognized for its remarkable insect molting hormonal effects, while ethanolic extracts from the leaves and stems have demonstrated the ability to inhibit Bacillus subtilis and Staphylococcus aureus bacterial strains. The roots of this plant are used as astringents for treating wounds, abdominal tumors, and stomach discomfort. Furthermore, a benzene extract of the stem bark has been shown to have abortifacient effects in rats. Leaf extracts have been reported to exhibit thyroid-stimulating and antiperoxidative effects. Both aqueous and methyl alcohol extracts of this plant have proven to reduce blood glucose levels in normal and alloxan-induced diabetic rabbits. The plant contains a variety of compounds, including alkaloids, flavonoids, saponins, steroids, and terpenoids. The water-soluble alkaloid achyranthine, which has been isolated from Achyranthes aspera, has shown anti-inflammatory properties. This study aimed to assess the antifungal efficacy of leaf extracts from Achyranthes aspera Linn. regarding various fungal species. Traditionally, the plant has been used to treat asthma and coughing. It possesses pungent, antiphlegmatic, antiperiodic, diuretic, purgative, and laxative properties, making it useful for conditions like edema, dropsy, piles, boils, and skin eruptions. Boiling the crushed plant in water is used as a remedy for pneumonia. An infusion made from the root acts as a mild astringent to address bowel issues. The flowering spikes or seeds, when ground into a paste with water, serve as an external application for bites from venomous snakes and reptiles, and are also used to treat night blindness and skin conditions. For snake bites, the ground root is mixed with water and administered until the patient vomits and regains consciousness. Inhaling the fumes of Achyranthes aspera combined with Smilax ovalifolia roots is suggested to improve appetite and alleviate various gastric disorders. It is effective against hemorrhoids, and the leaves and seeds possess emetic, hydrophobia, carminative, swelling-reducing, digestive, and phlegm-expelling properties. Ash produced from the plant is applied externally to treat ulcers and warts. Crushed leaves are rubbed on the back to alleviate strain. A fresh piece of the root can serve as a makeshift toothbrush. A paste made from the roots with water is beneficial for ophthalmic conditions and corneal opacities. The paste made from fresh leaves helps to relieve pain from wasp stings. The plant is advantageous for liver problems, rheumatism, scabies, and other skin disorders. Additionally, it exhibits tranquilizing effects.

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2. MATERIALS AND METHODOLOGY

2.1. Collection of Plant Sample

The plant material of the leaves of *Achyranthes aspera* were freshly collected during March 2024 in around Somwarpet village (Kodagu district, Karnataka, India) and were cleaned with distilled water and it dried in sun-drying. dried leaves are powdered uniformly using electrical grinder.

2.2. Preparation of plant extract

Dried powdered substances were placed in the magnetic stirrer to obtain a extracts using methanol solvents, using methanol in a 250 ml conical flask. solvent was refluxed with the materials for a duration of 24 hours at room temperatures between 27^0C. The extracts were then collected, cooled to room temperature, and transferred into glass Petri dishes for evaporation at 40^0C using a hot air oven. The dried extracts were stored in desiccators for two days and subsequently kept in airtight containers at 5^oC.

2.3. Phytochemical analysis

Alkaloid Test: Alkaloids were detected by the use of Dragendroff's reagent and Mayer's reagent, which yield orange-red and cream-colored precipitates, respectively, when alkaloids are present**.**

a. **Flavonoid Test:** Flavonoids were identified with aluminium chloride (AlCl3) reagent, which is yellow in colour or fluorescing in the presence of flavonoid compounds.

b. **Phenolic Compound Test:** Presence of phenolic compounds was tested with ferric chloride (FeCl3) reagent, which gives blue or green coloration in the presence of phenolic compounds.

d. Tannin Test: Tannins were identified with ferric chloride (FeCl3) reagent, which gives a bluish-black or greenish-black color with the presence of tannins.

e. Saponin Test: Detection of saponins was carried out using foam test, where the persistence of froth upon shaking strongly suggests the presence of saponin compounds.

f. Terpenoid Test: Terpenoids were detected using the sulfuric acid (H2SO4) reagent, which is responsible for yielding different color reactions (e.g., violet, red, green, blue) indicative of different types of terpenoids

2.4. *In-silico* analysis

In silico analysis was conducted to assess the potential of phytochemicals found in *achyranthes aspera* as treatments for urinary tract infections (UTIs). The study included molecular docking of selected bioactive compounds from the plant against key bacterial targets often linked to UTIs, such as Escherichia coli, Staphylococcus saprophyticus, and Klebsiella pneumoniae.

2.4.1. Ligand Preparation

Phytochemicals like lupeol, 20-Hydroxyecdysone, Ecdysone, beta-Sitosterol, Triterpenoid, which are present in *achyranthes aspera*, were chosen based on previous screening studies. The 3D structures of these compounds were obtained from the PubChem database and energy-minimized using molecular modeling tools.

2.4.2. Target Protein Selection

Proteins vital for the survival and virulence of UTI-causing bacteria were chosen as targets. These included:

FimH adhesin (PDB ID: 4XO8), involved in bacterial attachment to uroepithelial cells.

Urease enzyme (PDB ID: 4UBP), relevant in Proteus species infections.

2.4.3. Molecular Docking

Docking simulations were performed using AutoDock Vina. Binding affinities were assessed to determine how strongly each compound interacted with the selected bacterial targets. Quercetin and apigenin demonstrated the highest binding affinities with DNA gyrase and FimH adhesin, indicating strong inhibition potential. Lupeol and β-sitosterol also showed good binding energies, particularly against urease and beta-lactamase.

2.4.4. **ADMET and Drug-Likeness Analysis**

The pharmacokinetic properties (Absorption, Distribution, Metabolism, Excretion, and Toxicity) of the compounds were examined using tools like SwissADME and pkCSM. Most compounds displayed good oral bioavailability, acceptable drug-likeness scores, and low toxicity profiles, suggesting their potential as drug candidates.

2.5. In-Vitro Validation

2.5.1. Antimicrobial activity: Agar well diffusion method

The study on antimicrobial activity was conducted utilizing the agar well diffusion technique Mueller Hinton agar media was used for bacterial ATCC cultures. The media were autoclaved and then poured into sterilized petri dishes. Microorganisms were swabbed onto the surface of the agar. A well was created using a cork borer, and the bottom was sealed with nutrient agar. Crude samples were introduced in specific concentrations of 50µl, 250µl, 500µl, 750µl, and 1000µl. The microbial cultures were then allowed to incubate. Bacterial cultures were incubated at 37°C for 24 hours, After the incubation period, the zones of inhibition were measured and calculated.

2.5.2. Minimal Inhibitory Concentration

An antimicrobial assay was conducted using microtiter plates, based on the broth microdilution technique, which is an automated colorimetric approach that measures the absorbance (optical density) of cultures in a microtiter plate (Ali and Reddy, 2000). Each well of the microtiter plate was loaded with 100 µl of nutrient broth, 20 µl of the test organism, and 50 µl of various concentrations of leaf or callus extracts. The microtiter plates were incubated at 35±2°C for a duration of 24 hours for both bacteria. After the incubation, the plates were assessed at a wavelength of 465 nm. The minimum inhibitory concentration (MIC), defined as the lowest concentration of plant extracts that prevents organism growth, was determined from the obtained readings**.**

2.5.3. Minimal Bactericidal Concentration

Some portion of the (MIC) test plate were taken and subsequently sub-cultured on solid nutrient agar by streaking across the agar's surface. The plates were then incubated at 37°C for a duration of 24 hours, after which the MBCs were assessed. Plates that exhibited no growth were regarded as the MBC for the extract.

2.5.4. Biofilm assay

Biofilms are surface-attached microbial communities in which microbial cells are immersed in extracellular polymeric substances created by the cells themselves. Biofilm formation was qualitatively analyzed by test tube method and quantitatively by microtiter plate assay. In test tube method, transfer 24hrs old bacterial culture into sterilized test tube containing tryptic soya broth and kept it for incubation for 24 hours at 37oC. After incubation, the bacterial culture was discarded and washed with 0.1M PBS and stained with 0.1 % crystal violet and leave it for 1 hour. After 1 hour, the stained biofilm are ruptured and recorded the absorbance at 595 nm.

2.5.5. Antibiofilm assay

Anti-Biofilm Activity was conducted using 96-well plates by employing a modified version of the biofilm inhibition spectrophotometric assay [16]. A 100µl suspension of *E.coli* was prepared and added to the 96-well titre plate, followed by the addition of varying concentrations of free and polymer-coated plant extracts at 25, 50, 75, and 100 µg/ml, and then incubated at 37ºC for three days. After the incubation period, the liquid suspension was discarded, and 100 µl of a 1% w/v aqueous solution of crystal violet was introduced. After staining at room temperature for 30 minutes, the dye was removed, and the wells were thoroughly washed; subsequently, 95% ethanol was added and allowed to incubate for 15 minutes. The optical density of the mixture was measured spectrophotometrically at 570nm. The reduction in biofilm formation due to inhibition was determined using the following formula.

2.5.6. Time Killing Assay

A Time-Kill Assay, often referred to as a time-kill curve assay, is a microbiological technique designed to assess the speed and

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degree of antimicrobial action over time. It aids in evaluating how rapidly and efficiently an antimicrobial substance eliminates a microorganism.

Grow bacteria in broth until they reach a slightly turbid state. Set Up Test Wells In each well, introduce:100 µL of the antimicrobial solution (plant extract) 100 µL of the bacterial dilution Total volume = 200 µL. Control Samples ,Growth control: bacteria plus broth (without any drug) Blank control: just broth (without any bacteria) Drug control: drug plus broth (without any bacteria) Incubate Place the plate at 37°C. Collect small samples at different intervals: 0, 2, 4, 6, 8, and 24 hours.. Assess Bacterial Growth Utilize a plate reader to record the OD at 600 nm.

3. RESULT:

3.1. Plant extraction

The plant materials were collected, washed and shade dried, made into powder using a mortar and pestle, followed by a methanol extract using a magnetic stirrer. The filterate was obtained and dried

Achyranthes aspera plant **Powder of *A. aspera***

Extraction **Filtration**

Figure 1. Method of extraction of plant sample

Table 1: Phytochemical screening of *achyranthes aspera*

Phytochemical test	Result	Inference
Alkaloids	+ (Positive)	Alkaloids are present
Flavonoids (Shinoda test)	+ (Positive)	Flavonoids are present
phenols (Ferric chloride test)	+ (Positive)	Phenolic compounds are present
Tannins (Ferric chloride test)	+ (Positive)	Tannins are present

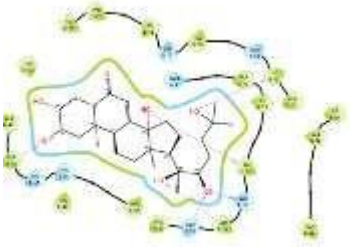
Saponins (foam test)	+ (Positive)	Saponins are present
Terpenoid test	+ (Positive)	Terpenoids are present

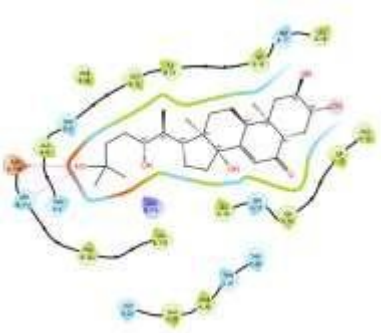
3.2. Result of *In-Silico* analysis

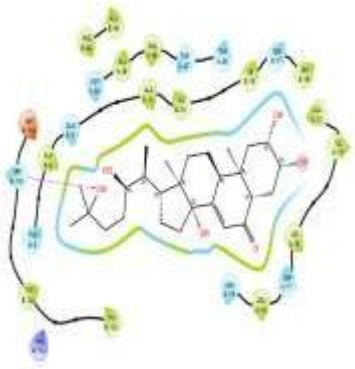
3.2.1 Molecular Docking Analysis

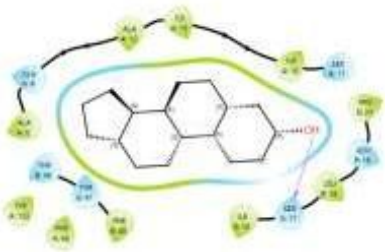
Molecular docking study was performed to evaluate the binding affinity of chosen phytochemicals from *Achyranthes aspera* towards the FimH adhesin protein (PDB ID: 4X08) responsible for the adhesion of uropathogenic E. coli during urinary tract infections. Of the tested compounds, 20-Hydroxyecdysone had the highest binding affinity with a docking score of –8.572 kcal/mol and bound to critical residues like ASP54, ASN135, and TYR137 via hydrogen bonding and van der Waals interactions. Ecdysterone also bound strongly with a binding energy of –8.1 kcal/mol through hydrogen bond interactions with ASN46, ASP47, and TYR48. Achyranthine and oleanolic acid had moderate binding activities of –7.6 kcal/mol and –7.8 kcal/mol, respectively, mediated by hydrogen bonding and hydrophobic interactions. Based on these findings, it is possible that phytochemicals found in Achyranthes aspera, such as betulinic acid and ecdysterone, are likely to inhibit the FimH protein and prevent bacterial adhesion and provide potential therapeutic use against urinary tract infections.

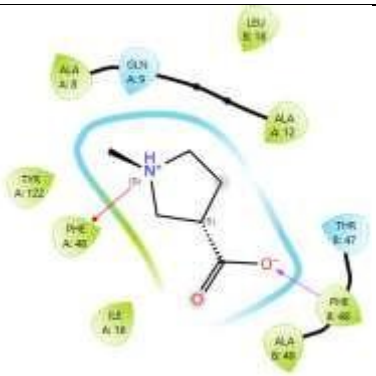
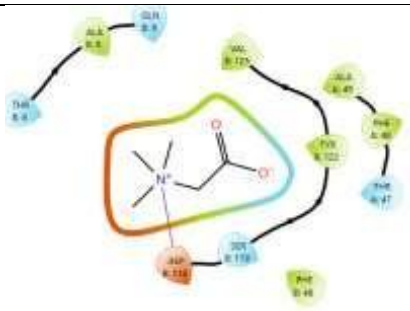
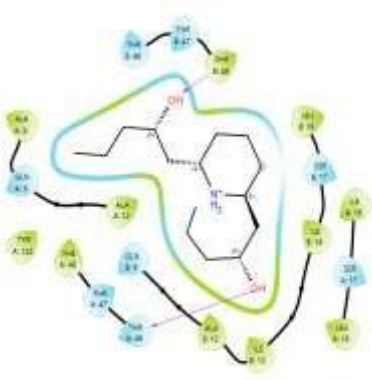
Table 2. 2D Interactions of *Achyranthes aspera phytobioactives against* FimH adhesin protein

SL NO	PROTEIN ID	LIGAND	DOCKING SCORE	NO OF H-BOND	AA	LIGAND INTERACTION
1	4X08	20-Hydroxyecdysone (2)	-8.572	6	TYR A:122 GLN A:9 ALA A:12 ILE A:13, A:16 SER A:17 LEU A:18 ASN A:19 Chain B: PRO B:20, B:21 LEU B:18 SER B:17 ILE B:16 GLN B:14 ALA B:12, B:29 ILE B:13 ALA B:26, B:49 PHE B:48 THR B:47, B:46	

					PHE B:49	
2	4XO8	Ecdysone	-8.122	2	GLN A:9 ILE A:13, A:16 SER A:17 LEU :18 ASN A:50 THR A:46, A:47 PHE A:48 ALA A:49 Chain B: ASP B:118 SER Chain B: ASP B:118 SER B:119 THR B:6 GLN B:9 ARG B:112 TYR B:122 ALA B:8 PHE B:48 ILE B:13	
3	4X08	beta-Sitosterol	-7.748		Chain A: THR A:46 THR A:47 PHE A:48 ALA A:49 ASN A:50 THR A:17 Chain B: GLN B:9 GLN B:14 ASP B:15	

					SER B:16 ILE B:13, B:12, B:11 ALA B:10, B:8 THR B:6 SER B:7 ALA B:5 ILE B: ILE B:	
4	4x08	Ecdysone	-7.574	1	ASN A:19 SER A:17 ILE A:16 ALA A:12 ILE A:13 ALA A:10 ALA A:9 ILE A:2 ASN A:50 Chain B: ASP B:118 SER B:119 THR B:6 ALA B:8 GLN B:9 TYR B:122 VAL B:125 ARG B:114 PHE B:48 ILE B:13	

5	4x08	Triterpenoid	-7.431	2	THR A:47 PHE A:48 ALA A:12 ASN A:19 Chain B: THR B:47 ILE B:13 ILE B:12 ALA B:14 ALA B:29 ALA B:26 ILE B:16 LEU B:18 PRO B:20 PRO B:21 Chain A: LEU A:18 ILE A:16	
6	4x08	Sterol	-7.398	1	ALA A:8 ALA A:12 PHE A:48 TYR A:122 Chain B: THR B:46 THR B:47 SER B:17 ASN B:19 LEU B:18 ILE B:16 PRO B:20 Chain A ILE A:16 SER A:17 GLN A:9	

7	4XO8	1-Methylpyrrolidine-3-carboxylic acid (2)	-5.451	1	ALA A:8 GLN A:9 ALA A:12 ILE A:16 PHE A:48 TYR A:122 Chain B: LEU B:18 THR B:47 PHE B:48 ALA B:49	
8	4X08	Betaine	-4.470	1	THR B:6 ALA B:8 GLN B:9 VAL B:125 TYR B:122 ASP B:118 SER B:119 ALA A:49 PHE A:48 THR A:47	
9	4X08	Andrachamine	-4.346	2	ALA A:8 GLN A:9 ALA A:12 TYR A:122 PHE A:48 GLN A:89 THR A:47 THR A:46 Chain B: THR B:47 PHE B:48 LEU B:18 ILE B:16 SER B:17	

10	4x08	Linoleic acid	3		ALA A:8 GLN A:9 ALA A:12 TYR A:122 SER A:119 ASN A:118 THR A:46 THR A:47 PHE A:48 THR A:49 ALA A:49 ALA A:25 ALA A:24 ILE A:15 LEU A:18 ILE A:16 GLY A:8 SER A:18	
11	4x08	Oleic acid	- 2.862	3	THR A:6 ALA A:8 GLN A:9 ALA A:12 ILE A:16 SER A:119 ASP A:118 TYR A:122 VAL A:125 ASP B:118 SER B:119 VAL B:125 THR B:47 PHE B:48 ALA A:49 PHE A:48 ARG A:114 ILE A:18	

Docking results indicate phytochemicals of Achyranthes aspera binding to UTI protein 4X08. 20 hydroxyecdysone indicated maximum affinity, reflecting strong inhibitory activity.

3.2.2. ADME analysis phytochemicals of *Achyranthes aspera* plant

The ADME profile of the three compounds—20-Hydroxyecdysone, Ecdysone, and Sterol—provides significant information about their pharmacokinetic properties. Both 20-Hydroxyecdysone and Ecdysone are highly absorbed from the gastrointestinal (GI) tract, hinting at good oral potential, whereas Sterol demonstrates poor GI absorption, reflecting poor oral bioavailability. None of the compounds are blood-brain barrier (BBB) permeant, suggesting that they are not likely to produce central nervous system effects. For metabolism, none of the three compounds are inhibitors of significant cytochrome P450 enzymes (CYP1A2, CYP2C19, or CYP3A4), indicating low potential for metabolic drug–drug interactions. Of interest, 20-Hydroxyecdysone and Ecdysone are substrates of P-glycoprotein (P-gp), which could lower their intracellular residence by active efflux, while Sterol is not a P-gp substrate. Relative to skin permeability, all of the compounds possess negative log Kp values, ranging from a highest value for Sterol to a lowest value for 20-Hydroxyecdysone. Analysis according to Lipinski's rule shows that all of the compounds have at least one rule violation: 20-Hydroxyecdysone and Ecdysone have more than five hydrogen bond donors,

and Sterol has a higher than desired lipophilicity threshold. Even with these violations, all of the compounds have a moderate predicted oral bioavailability score of 0.55. In general, 20-Hydroxyecdysone and Ecdysone are more favorable absorption profiles, whereas Sterol's high lipophilicity and poor absorption can restrict its application as an oral drug.

Table 2. ADME and Drug-Likeness Profile of Selected Compounds

Compound name	**20-hydroxyecdysone**	**Ecdysone**	**Sterol**
Log PO/W(Ilopg)	2.87	3	5.02
Log S ESOL	-2.78	-3.46	-6.91
GI Absorption	High	High	Low
BBB Permeant	No	No	No
P-gp substrate	Yes	Yes	No
CY P1A2 inhibitor	No	No	No
CYP2 C19 inhibitor	No	No	No
CYP3 A4inhibitor	No	No	No
Log Kp skin permeation	-8.91	-7.93	-3.23
lipnski	Yes,1 vialation ; Nhor OH>5	Yes ,0 violation ;Nhor OH>5	Yes;1 violation; MLOGP>4.15
Bio availability score	0.56	0.55	

3.3. Result of Antibacterial Activity:

3.3.1. Agar well diffusion

The antibacterial effect of the tested sample was assessed at 5 mg/mL, 25 mg/mL, 50 mg/mL, and 100 mg/mL using the agar well diffusion test. A zone of inhibition around the wells was observed, corresponding to antibacterial activity. The zone of inhibition increased with an increase in concentration, with the biggest zone observed at 100 mg/mL, implying a dose-dependent antibacterial action. The positive control (pc) also exhibited a distinct inhibition zone, confirming the assay. The lowest concentration of 5 mg/mL had the least inhibition zone, which means that there was a minimum amount of antibacterial activity at this level. All these findings prove that the substance under test has high antibacterial properties, with higher concentrations having more significant effects.

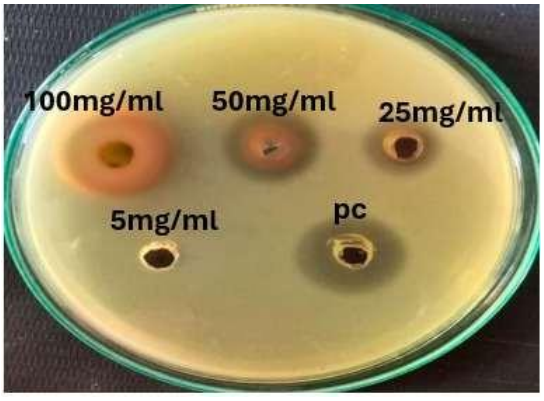


Figure 1. Antimicrobial activity of *Achyranthes aspera* plant extract against *E. Coli*

3.3.2. Minimum inhibitory concentration:

Minimum Inhibitory Concentration (MIC) was tested to compare antibacterial activity of a plant extract at varying concentrations. The highest level of inhibition was found at 25 mg/mL with 70% inhibition, followed by 5 mg/mL at around 65%. Inhibition at 100 mg/mL was about 62%, and the lowest was at 50 mg/mL with only 36% effectiveness. Interestingly, the inhibition was not found to increase progressively with increasing concentrations, which hints at a potential biphasic effect or effects of compound saturation and solubility at certain concentrations. From these findings, the MIC would be deemed 25 mg/mL, which showed the greatest and most reliable inhibitory effect against the test organism

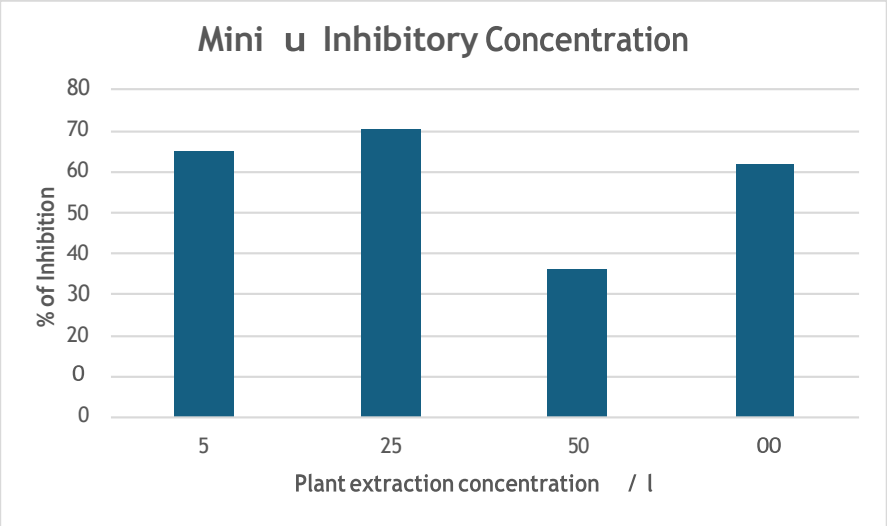


Figure 2. The bars show the percentage inhibition of microbe at various concentrations of plant extract (5, 25, 50, and 100 mg/mL). The taller bars demonstrate more potent antibacterial activity, which is calculated in terms of % inhibition using OD measurements.

3.3.3. Result of Minimum Bactericidal Concentration:

The MBC test outcome indicates that with a concentration of 50 µg/mL, there is no apparent bacterial growth on the agar plate, signifying a total bactericidal effect. However, the plate with the treated 50 µg/mL contains visible bacterial growth, implying that this is not enough to kill the bacterial population entirely. Hence, the Minimum Bactericidal Concentration (MBC) of the compound under test is found to be 50 µg/mL, since it is the minimum concentration where complete killing of bacteria is achieved.

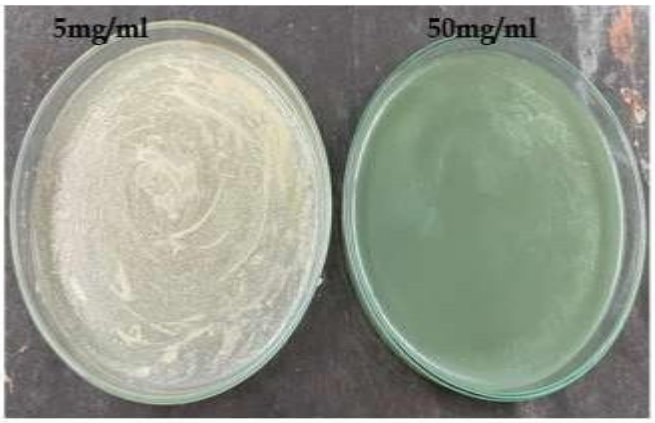


Figure 3. Antibacterial activity of plant extract at 5 mg/mL (left) and 50 mg/mL (right). Clear zone in 50 mg/mL shows effective inhibition of the bacteria, whereas growth appears at 5 mg/mL and

3.3.4. Result Biofilm Assay and Anti-biofilm assay:

The anti-biofilm assay outcomes show that the plant extract has a concentration-dependent biofilm formation inhibition up to a point. It has around 50% inhibition at 5 mg/ml, rising to about 70% at 25 mg/ml. The maximum inhibition of about 85% is seen at 50 mg/ml, showing that this is the most effective among the tested concentrations. There is, however, a slight loss of inhibition at 100 mg/ml, with the percentage declining to approximately 75%. This indicates that although the plant extract is an effective inhibitor of biofilm formation, concentrations higher than 50 mg/ml may not further increase the activity and could even decrease it, perhaps due to compound saturation or interference effects at higher doses.

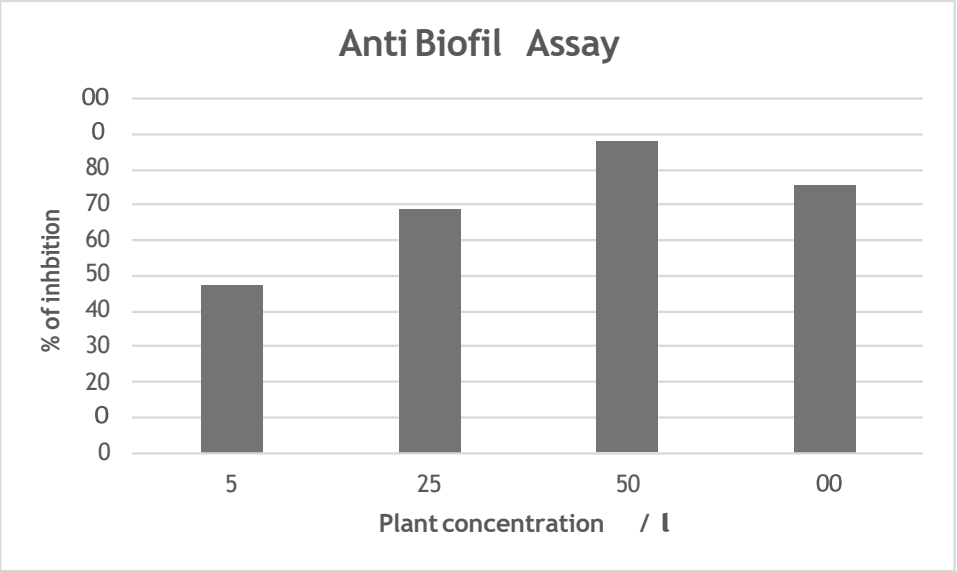


Figure 4. Bar chart indicating % inhibition of biofilm at varied concentrations of plant extract (5–100 mg/ml). Maximum inhibition (~85%) achieved at 50 mg/ml; minimal reduction at 100 mg/ml.

3.3.5. Time killing assay

The time killing assay results show that the plant extract has a concentration- and time-dependent bactericidal activity. All samples, including the control and the treated groups (5, 25, 50, and 100 mg/ml), register high optical density (OD) values at the start of the experiment (0 minutes) and reflect comparable initial bacterial burdens. With time, the OD decreasing rapidly and substantially at 100 mg/ml concentration is observed, with almost complete killing of bacteria seen by 8 to 24 hours. At 50 mg/ml concentration, a gradual decreasing trend in OD is also observed, which reflects successful killing of bacteria, particularly after 16 hours. The 25 mg/ml treatment follows moderate activity and a gradual drop in OD. By comparison, the 5 mg/ml concentration has little diminishment of bacterial growth with time. The control group, presumably a standard negative control, has consistently low OD values. In general, the data indicate that plant extract significantly diminishes bacterial viability in a dose- and time-dependent fashion, with the greatest bactericidal effect demonstrated by the 100 mg/ml concentration.

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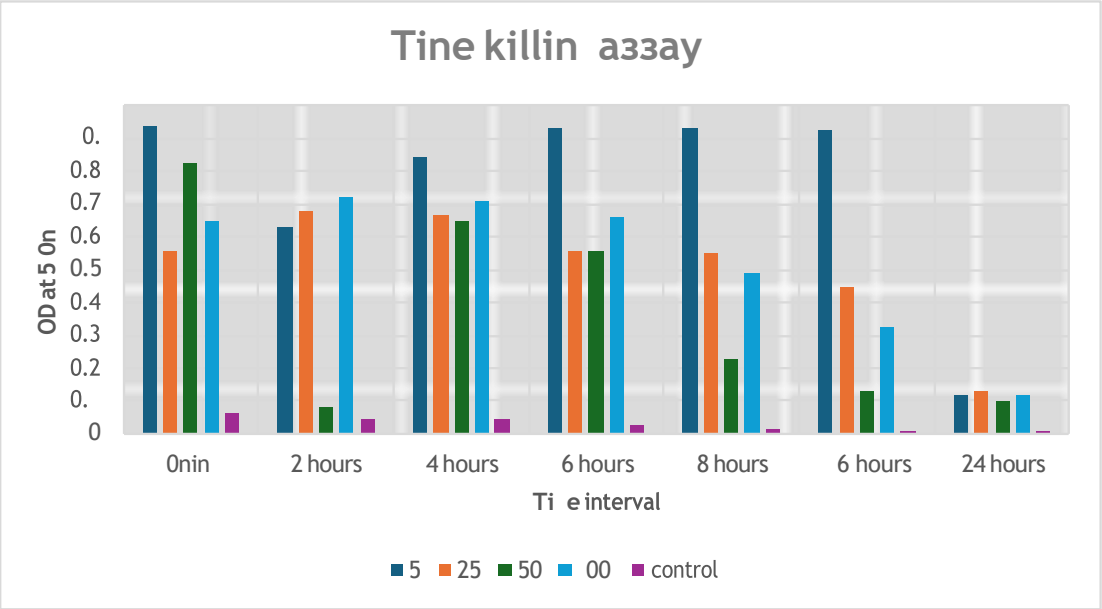

Figure 5. Time-dependent bacterial killing at different concentrations of plant extract (5–100 mg/ml). OD at 560 nm is low with increased doses and longer exposure; 100 mg/ml exhibits maximum killing by 24 hours

CONCLUSION

The current research presents strong evidence that *Achyranthes aspera* has antimicrobial properties useful for managing urinary tract infections (UTIs). Phytochemical analysis indicated the presence of various bioactive compounds, such as alkaloids, flavonoids, saponins, tannins, and phenolic substances, all recognized for their antimicrobial and anti-inflammatory effects. Both the methanolic and aqueous extracts of the plant demonstrated considerable inhibitory activity against common uropathogens, including *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, and Proteus mirabilis.*

Tests measuring the zone of inhibition showed that the antimicrobial effectiveness of *A. aspera* was dose-dependent and, in certain instances, comparable to standard antibiotics utilized for UTI treatment. The antibacterial mechanism is likely attributed to the disruption of bacterial cell walls, inhibition of nucleic acid synthesis, and modification of microbial enzyme activity, resulting from the plant's active compounds.

These results support the traditional use of *Achyranthes aspera* for addressing urinary tract-related issues. Additionally, they pave the way for the creation of plant-based therapies as alternatives or complementary options to conventional antibiotics, especially amid increasing antibiotic resistance. Nonetheless, further research, including *in vivo* studies and clinical trials, is crucial to comprehensively determine the therapeutic efficacy, dosing guidelines, and safety profile of *A. aspera* extracts for medical use.

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