Pharmacogn. Res., 2025; 17(4):1315-1327.
https://www.phcogres.com

Original Article

Exploring the Pharmacological Potential of *Achyranthes aspera* (Latzeera): A Complete Investigation of its Anticancer, Anti-Microbial and Antioxidant Activities

Arijit Chaudhuri[1,*], Biswajit Dash[2], Ashish Sarkar[3], Afrin Alam[4], Ayush Garg[5], Shivani Devi[1], Ankita Sharma[1], Varsha Chauhan[6], Tanbeer Kaur[7], Yashna Bawa[8]

[1]Department of Pharmacology, Shiva Institute of Pharmacy, Luhnoo, Kanatain, Chandpur, Himachal Pradesh, INDIA.
[2]Department of Pharmaceutical Chemistry, Amity Institute of Pharmacy, Amity University, Kolkata, West Bengal, INDIA.
[3]Department of Pharmaceutical Chemistry, School of Pharmacy, YBN University, Ranchi, Jharkhand, INDIA.
[4]Department of Pharmaceutics, School of Pharmacy, YBN University, Ranchi, Jharkhand, INDIA.
[5]Department of Pharmaceutics, Venkateshwar Institute of Pharmacy, Sai Tirupati University, Udaipur, Rajasthan, INDIA.
[6]Department of Biotechnology, School of Science, Institute of Technology and Management University, Gwalior, Madhya Pradesh, INDIA.
[7]Department of Food Technology and Nutrition, School of Agriculture, Lovely Professional University, Phagwara, Punjab, INDIA.
[8]Department of Home Science, Sanatan Dharma College, Kurukshetra University, Ambala Cantt, Haryana, INDIA.

ABSTRACT

Background: A medicinal herb called *Achyranthes aspera* has been used for centuries to treat a wide range of illnesses. By assessing its antineoplastic, anti-microbial and antioxidant properties. This study sought to thoroughly explore *Achyranthes aspera* pharmacological potential. **Materials and Methods:** Antibacterial activity against a panel of bacterial strains was assessed using the disc diffusion method. To ascertain cell viability in cancer cell lines, anti-cancer activity was assessed using the MTT assay. The antioxidant activity of the plant extract was assessed by its capacity to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. **Results:** *Achyranthes aspera* extract showed encouraging antibacterial efficacy against specific bacterial strains, however not as strong as the common antibiotic, ciprofloxacin. The MTT assay indicated possible anti-cancer efficacy by showing a notable dose-dependent reduction in cancer cell lines' cell viability. Through the scavenging of free radicals, the extract from *Achyranthes aspera* also demonstrated strong antioxidant activity. **Conclusion:** The pharmacological potential of *Achyranthes aspera* is strongly supported by this investigation, confirming its historic use in treating a variety of illnesses. Promising antioxidant, antimicrobial and anti-cancer properties were demonstrated by the plant extract. In order to determine the bioactive components, clarify the underlying mechanisms of action, assess the protection and efficacy of *Achyranthes aspera* extracts in preclinical, clinical contexts, these findings call for additional research.

Keywords: *Achyranthes aspera*, Antioxidant, Anti-Cancer, Anti-Microbial.

Correspondence:
Dr. Arijit Chaudhuri
Department of Pharmacology, Shiva Institute of Pharmacy, Chandpur, Himachal Pradesh, INDIA.
Email: armanchaudhuri92@gmail.com

Received: 07-05-2025;
Revised: 29-07-2025;
Accepted: 12-09-2025.

INTRODUCTION

Achyranthes aspera is a well-known coarse straw that is a member of the Amaranthaceae family and is found in Asian nations such as Bangladesh, Pakistan, China, and India.[1] It is called Chirchira and Chirchitta in Hindi, Aghedo and Aghedi in Gujarati, and Apamarg in Sanskrit.[2] It is a little herb with important therapeutic qualities that is found throughout India. This perennial plant, which is classified as a weed, is found in southern United States, Africa, tropical Asia, and India.[3] It is a lifespan herb that can grow upright or procumbent, and it usually has a woody base. Its height ranges from one to two meters. Stems that are Sharp, ridged, simple, branched from the base, often with a hint of purple; terete or definitely quadrilateral, striped, pubescent branches; thick leaves measuring 3.8 - 6.3 × 22.5 - 4.5 cm, ovate-elliptic or obovate- rounded; entire, petiolate, petiole 6 - 20 mm long; greenish white, reddish brown flowers; subcylindric, truncate at the apex, rounded at the base, and with numerous armpit or terminal spikes up to 75 cm long.[4] Herbal remedies are essential for both preventing, treating cancer, medicinal plants are widely accessible and reasonably priced. A significant amount of the quality of herbal medications used to treat cancer has significantly increased because to pharmaceutical research conducted in technologically advanced nations including the USA, Germany, France, Japan and China. Another important source of both synthetic and herbal medications is medicinal

DOI: 10.5530/pres.20252314

plants. Pharmaceutical firms have evaluated over 25,000 plants for anti-cancer medications thus far.[5]

Traditional medicine uses *Achyranthes aspera* to treat conditions like snake bites, diabetes, inflammation and skin disorders. Alkaloids, flavonoids, steroids, glycosides, terepenoids, saponin, and more are all present in the plant. Given the exceptional therapeutic response of *Achyranthes aspera* leaf extract, no scientific research has been conducted to date on its hepatoprotective, nephroprotective or anti-venom action.[1] It is believed that oxygen radical- induced oxidative stress is the primary cause of many degenerative diseases, such as cancer, atherosclerosis, gastric ulcers, and others. When reactive oxygen species and free radicals, which have been connected to the emergence of various diseases as well as the deterioration and spoiling of food, come into touch with other molecules, antioxidants protect them from oxidation. Natural products are known to have potential in drug discovery, development are a rich source of physiologically active chemicals and are an example of molecular diversity.[6] Several medicinal plants have been shown to have antibacterial properties and therapeutic benefits. Predicting the circumstances in which an antimicrobial drug works best against particular microbes is crucial. The cytoplasmic membrane, enzymes, protein synthesis and the creation of bacterial and fungal cell walls can all be impacted by the antimicrobial principle.[7]

According to the WHO, traditional herbal medicine provides primary medical care for more than 80% of people globally. Plants are still a potential source of novel medications and compounds made from different plant sections.[8] Many different illnesses have traditionally been treated using herbal medicine. Medicinal herbs are the only easily accessible and reasonably priced source of primary healthcare, especially in situations where access to modern medical facilities is restricted. They are used to treat illness, maintain health, and advance well-being. Because they contain physiologically active chemicals, different plant parts-leaves, stems, roots, bark are utilized to prepare medicines that treat both acute and chronic illnesses.[9]

MATERIALS AND METHODS

Plant material

Achyranthes aspera is a well-known coarse straw that is a member of the Amaranthaceae family, is found in Asian nations such as Bangladesh, Pakistan, China and India. A specialist in the Government College Khimlasa, Sagar (M.P.) plant botany department has verified it (Ref. No. 2023051). Fresh *Achyranthes aspera* leaves were collected at Shiva Institute of Pharmacy's medicinal garden. The aerial portion of *Achyranthes aspera* showed (Figure 1) was ground into a coarse powder and shade-dried for extraction. The *Achyranthes aspera* plant profile is displayed in Table 1 below.[1]

Reagent and Chemicals

Plant powder, Dragondroff's reagent, chloroform, Sodium Hydroxide (NaOH), Ferric Chloride (FeCl3), phenol, ethanol, sodium carbonate, gallic acid, toluene, petroleum ether, sulfuric acid and catechins.[10]

Extract preparation

The dried plant's aerial parts were ground into a powder. In a conical flask, 300 grams of powdered plant material were suspended in two liters of ethanol. For three days, the suspension was maintained with sporadic tremors. On the fourth day, the suspension was filtered via Whatman filter paper. To achieve the full extraction of phytoconstituents, the same process was repeated twice more with the leftover marc. The filtrates from three different extractions were mixed together. The solvent was evaporated in a rotavapor apparatus to produce a dried sticky mass of *Latzeera* Methanolic Extract (MEUP).

Cell line

A human cancerous cell line called the human leukemia monocytic cell was supplied by the National Centre for Cell Science (NCCS), which is situated in Pune, Maharashtra. The cell line was cultured in RPMI-1640 medium (10% FBS) in a CO_2 incubator at 37°C and 5% CO_2 in a humidified environment.

In vitro Antimicrobic activity assay for *B. subtilis*

The Zone Inhibition Method (also known as the Kirby-Bauer method) was used to assess the antibacterial activity. 100 µL of bacterial culture, *B. subtilis, S. aureus, P. aeruginosa, E. coli, C. albicans*, and *A. niger* were spread out on the MHA plates to inoculate them. After preparing the inoculum by increasing the cell density by 0.5 McFarland Units (about 1.5 X 108 CFU/mL from Mueller-Hillinton Broth), 10 µL of various concentrations (0 to 100 mg/mL) were placed on the discs. Each plate had a single disc loaded with solvent only as a vehicle control, and a disc with 10 µg of ciprofloxacin was used as a positive control. For 24 hr, the plates of *B. subtilis, S. aureus, P. aeruginosa, E. coli, C. albicans*, and *A. niger* were incubated at 37°C (Basil Scientific Corp., India). The disc's surrounding clean zones were measured and noted.

MTT Assay

Samples were produced in DMSO as a stock solution, which was then further diluted to get various concentrations in The MTT Assay was used to assess the substances' cytotoxicity on the THP-1 (a human leukemia monocytic cell) cell line, which was purchased from NCCS Pune. 10% FBS (fetal bovine serum, HIMEDIA-RM 10432) and 1% antibiotic solution (Penicillin-Streptomycin-Sigma-Aldrich P0781) were added to the MEM medium (Minimum Essential Medium Eagle-AT154, HIMEDIA) in a 96-well plate, which was kept at 37°C with 5% CO_2 for 24 hr. Cells were treated with varying quantities the following

day (concentration as specified in the excel sheet) shown (Table 2). Cell culture medium that is not complete (without FBS). MTT Solution (concentration as specified in the excel sheet) was added to the cell culture after a 24-hr incubation period, and the cells were then incubated for an additional 2 hr using an air-jacketed CO_2 incubator (Heal Force HF90) shown (Figure 2). Control cells were those that received no treatment, while blank cells were those that did not receive MTT. Following the experiment, the cell layer matrix was dissolved in 100 µL of Dimethyl Sulfoxide (DMSO-SRL-Cat no. 67685), and the culture supernatant was withdrawn. The results were then read at 540 and 660 nm using an Elisa plate reader (iMark, Biorad, USA). The GraphPad Prism -6 software was used to determine the IC_{50}. Images were taken with an AmScope digital camera (10 MP Aptima CMOS) on an Olympus eK2 inverted microscope. The representation of the 50% inhibitory concentration (IC_{50}) was Mean±SEM (Standard Error of Mean).

DPPH Scavenging Assay

In a 96-well plate, 0.1 mL of 0.1 mM DPPH solution was mixed with 5 µL of a separate stock of the test chemical (as stated in the excel sheet). As stated in the excel sheet, the reaction was set up in quadruplicate, and duplicate blanks were made with 0.2 mL of methanol and 10 µL of standard/sample at various concentrations shown (Figure 3). Wells without reagent (DPPH) were regarded as blank, and wells without treatment were regarded as controlled shown (Tables 3 and 4). For 30 min, the plate was incubated in the dark. Using a microplate reader (iMark, BioRad), the decolorization was measured at 517 nm at the conclusion of the incubation. 20 µL of deionized water was added to the reaction mixture as a control. In relation to the control, the scavenging activity was displayed as "% inhibition." GraphPad Prism 6 software was used to determine the IC_{50}. A graph was created that contrasted the X (sample concentration) and Y (inhibition percentage relative to control).

RESULTS

Anti-Microbial Test

According to antimicrobial testing, Ciprofloxacin (PC) demonstrated significant inhibitory zones at a 10 µg dose and shown good activity against all tested bacterial strains (*B. subtilis, S. aureus, P. aeruginosa*, and *E. coli*). Out of all the agents examined, this molecule showed the strongest antibacterial activity. With inhibitory zones seen at a dose of 50 µg, amphotericin B (PC) demonstrated moderate to good efficacy against both *Candida albicans, A. niger Latzeera*, on the other hand, showed only modest effectiveness against every tested microbe. *Latzeera's* low effectiveness, Amphotericin B's activity against fungus and Ciprofloxacin's potent antibacterial action. It conveys the necessary information in a clear and professional manner showed (Figure 4).

The presence of clear zones around the antibiotic discs indicates the susceptibility of the microorganisms to the respective antibiotics. The size of the inhibition zones can be used to qualitatively assess the degree of sensitivity. This information is essential for choosing the right antimicrobial drugs to treat illnesses brought on by these microbes shown (Table 3).

MTT Assay

A definite dose-dependent response to the tested doses was shown by the cell culture analysis. A high degree of consistency across replicates was shown by the mean value of 100 at the lowest

Table 1: Taxonomic classification of *Actinidia deliciosa*.

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Caryophyllales
Family	Amaranthaceae
Genus	Achyranthes
Species	*aspera*

Table 2: Final Strength Values and Statistical Analysis for Sample Strength.

Final Strength Values						Stats			
Sample Strength	1	2	3	4	Sample Strength	Mean	SD	SEM	N
0	0.6391633	-1.45264	1.104009	-0.29053	0	0.00	1.128708	0.564354	4
0.78	1.2202208	-0.87159	0.755375	1.220221	0.78	0.58	0.992912	0.496456	4
1.56	3.7768739	0.755375	1.220221	0.290529	1.56	1.51	1.557697	0.778848	4
3.125	7.1470076	0.871586	5.520046	1.104009	3.125	3.66	3.158445	1.579223	4
6.25	17.37362	14.35212	11.33062	11.0982	6.25	13.54	2.955217	1.477608	4
12.5	37.013364	33.2946	32.3649	31.43521	12.5	33.53	2.445049	1.222524	4
25	79.662987	75.94422	78.03603	82.21964	25	78.97	2.65003	1.325015	4
50	96.513655	93.957	93.02731	91.40035	50	93.72	2.138629	1.069314	4

concentration (0 µg) and the comparatively low standard deviation (SD=9.27). Cell viability was shown to decrease in proportion to the concentration. The mean value fell to 23.34 at the maximum concentration (1000 µg), indicating a substantial decline in cell viability. At higher dosages, like 500 µg (SD=0.94), standard deviation values stayed continuously low, indicating consistent and repeatable results across repeats. Together, these results show that cell viability gradually decreased as the compound's concentration rose, with the lowest viability occurring at the greatest concentration (1000 µg) shown (Tables 5 and 6).

The cell distribution appears heterogeneous, with regions of clustering interspersed with more dispersed areas. Additionally, a visible scratch or line traversing the field of view suggests potential disruption to the culture surface showed (Figure 5).

DPPH Scavenging Assay

The evaluation of *Latzeera's* biological activity using 96-well microtiter plates reveals a clear dose-dependent response across the tested concentrations. At the lowest concentration (0 µg), no significant activity was observed, with a mean value of 0. As the concentration increased, there was a progressive rise in activity, beginning with a modest response at 0.78 µg (mean=0.58). The activity continued to increase at higher concentrations, with 1.56 µg, 3.125 µg showing mean values of 1.51 and 3.66, respectively. At concentrations of 6.25 µg, 12.5 µg, the activity intensified further, with mean values of 13.54 and 33.53. The highest concentrations (25 µg and 50 µg) demonstrated the most substantial activity, with mean values of 78.97 and 93.72 respectively. The homogeneity of the Standard Deviation (SD) and Standard Error of the Mean (SEM) values across all concentrations demonstrates the reliability and reproducibility of the results (Tables 1 and 7).

These plates consist of multiple sets (Set 1, Set 2, Set 3 etc.), each representing different concentrations of the test samples. The observed color changes in the wells serve as an indicator of the activity or reaction of the samples, with variations in hue intensity signifying differences in activity levels. Notably, the *Latzeera* sample exhibits a gradient of color changes, indicating a range of activity across the tested concentrations, while the standard sample provides a control benchmark for evaluating the efficacy of *Latzeera*.

Figure 1: *Achyranthes aspera.*

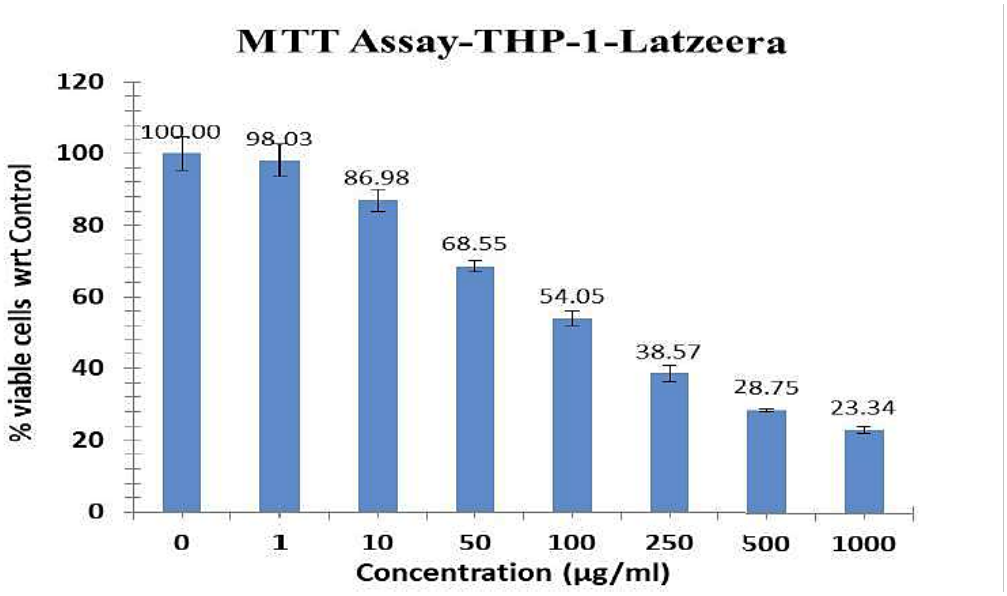

Figure 2: The chart illustrates the relationship between varying concentrations of *Latzeera* (in µg/mL) and the percentage of living cells relative to the control. The data highlights a concentration-dependent cytotoxic effect, with cell viability declining progressively from 100% at 0 µg/mL to approximately 23.34% at 1000 µg/mL. This demonstrates that *Latzeera* exhibits significant cytotoxic activity at higher concentrations, potentially indicating its therapeutic or inhibitory efficacy at elevated doses.

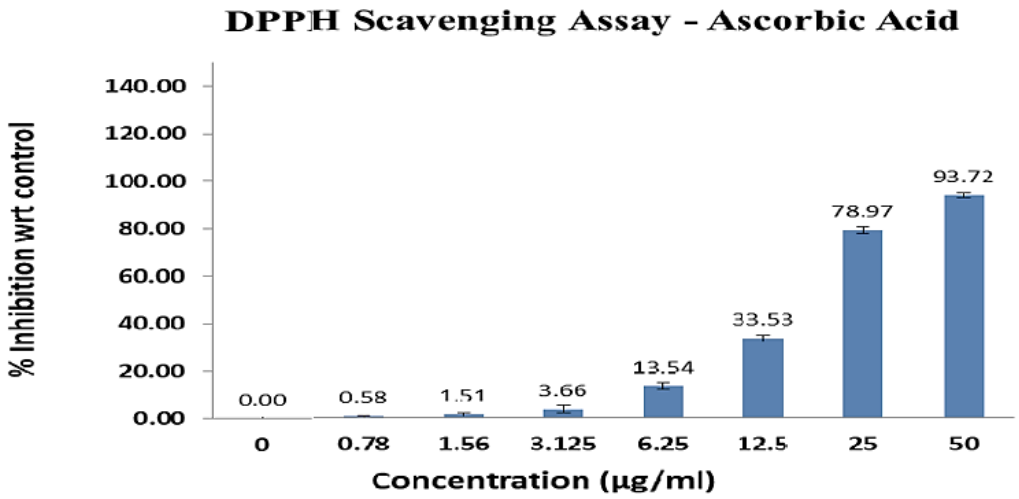

Figure 3: The following bar chart demonstrates the antioxidant capacity of ascorbic acid through the DPPH scavenging test. The x-axis represents the concentration of ascorbic acid (in µg/mL, ranging from 0 to 50 µg/mL,whereas the percentage of inhibition in relation to the control is shown on the y-axis. A concentration-dependent increase in antioxidant activity is evident. At lower concentrations, minimal inhibition is observed, such as 0.58% at 0.78 µg/mL and 1.51% at 1.56 µg/mL. The activity shows a marked increase at higher concentrations, reaching 78.97% at 25 µg/mL and peaking at 93.72% at 50 µg/mL. These results highlight ascorbic acid's potent antioxidant qualities at elevated levels.

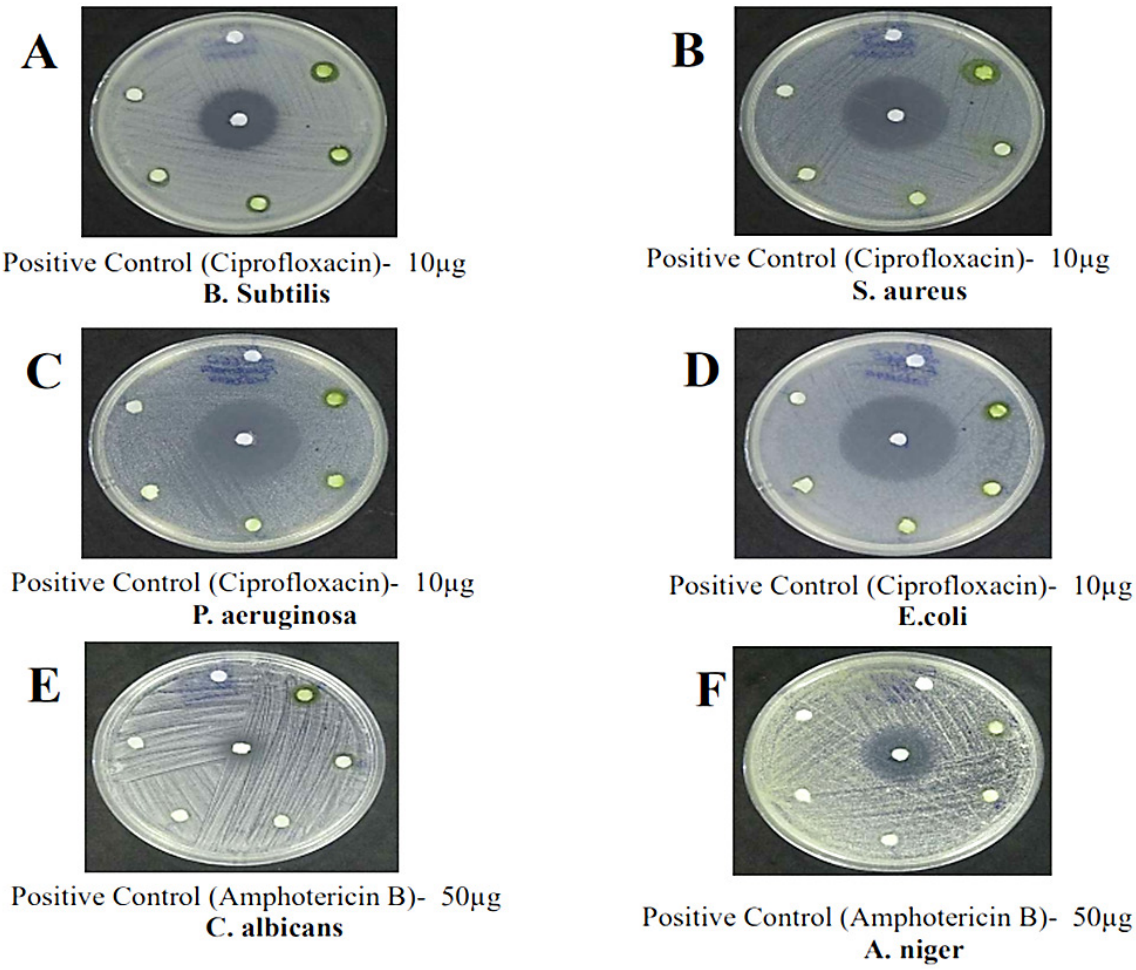

Figure 4: This figure demonstrates the results of antibiotic sensitivity testing for a panel of bacterial and fungal strains.

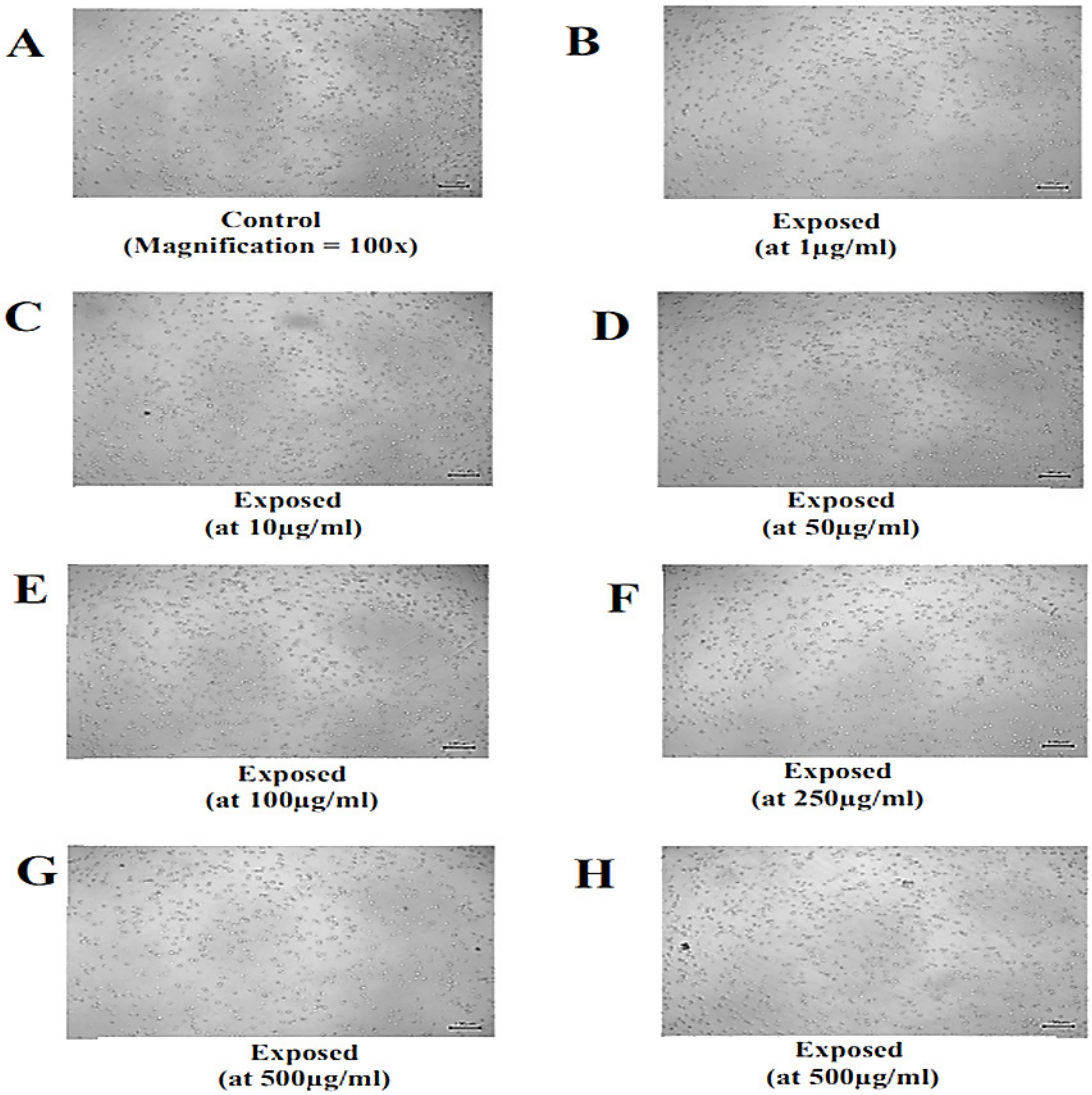

Figure 5: Under microscopic examination, the images reveal a cell culture characterized by a high density of small and round cells.

Table 3: Final Strength Values and Statistical Analysis of Sample Strength.

Final Strength Values					Stats				
Sample Strength	1	2	3	4	Sample Strength	Average	SD	SEM	N
0	99.262899	113.0221	91.40049	96.3145	0	100	9.267384	4.633692	4
1	99.262899	110.0737	89.43489	93.36609	1	98.0344	8.985152	4.492576	4
10	88.943489	94.84029	80.09828	84.02948	10	86.97789	6.369278	3.184639	4
50	66.830467	72.72727	65.84767	68.79607	50	68.55037	3.042453	1.521227	4
100	53.071253	57.98526	56.01966	49.14005	100	54.05405	3.848432	1.924216	4
250	37.346437	45.20885	35.38084	36.36364	250	38.57494	4.494815	2.247407	4
500	27.518428	29.48403	28.50123	29.48403	500	28.74693	0.94096	0.47048	4
1000	21.621622	24.57002	21.62162	25.55283	1000	23.34152	2.026096	1.013048	4

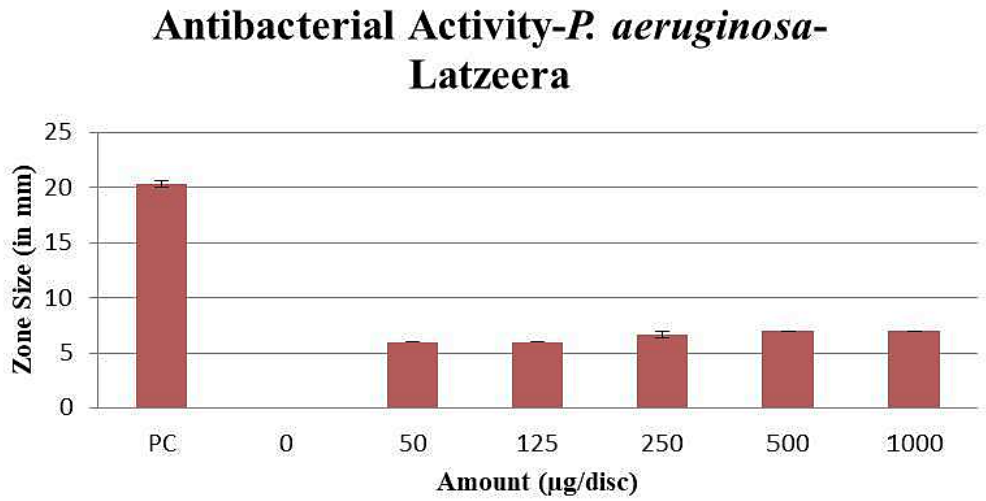

Figure 6: The bar chart illustrates the antibacterial activity of varying concentrations (in µg/disc) of the compound "*Latzeera*" against *P. aeruginosa*. The y-axis denotes the inhibition. zone size (measured in mm), reflecting the degree of bacterial susceptibility, while the x-axis represents the concentrations of the compound, ranging from 0 to 1000 µg/disc. This analysis provides insights into the potential efficacy of "*Latzeera*" as an antimicrobial agent.

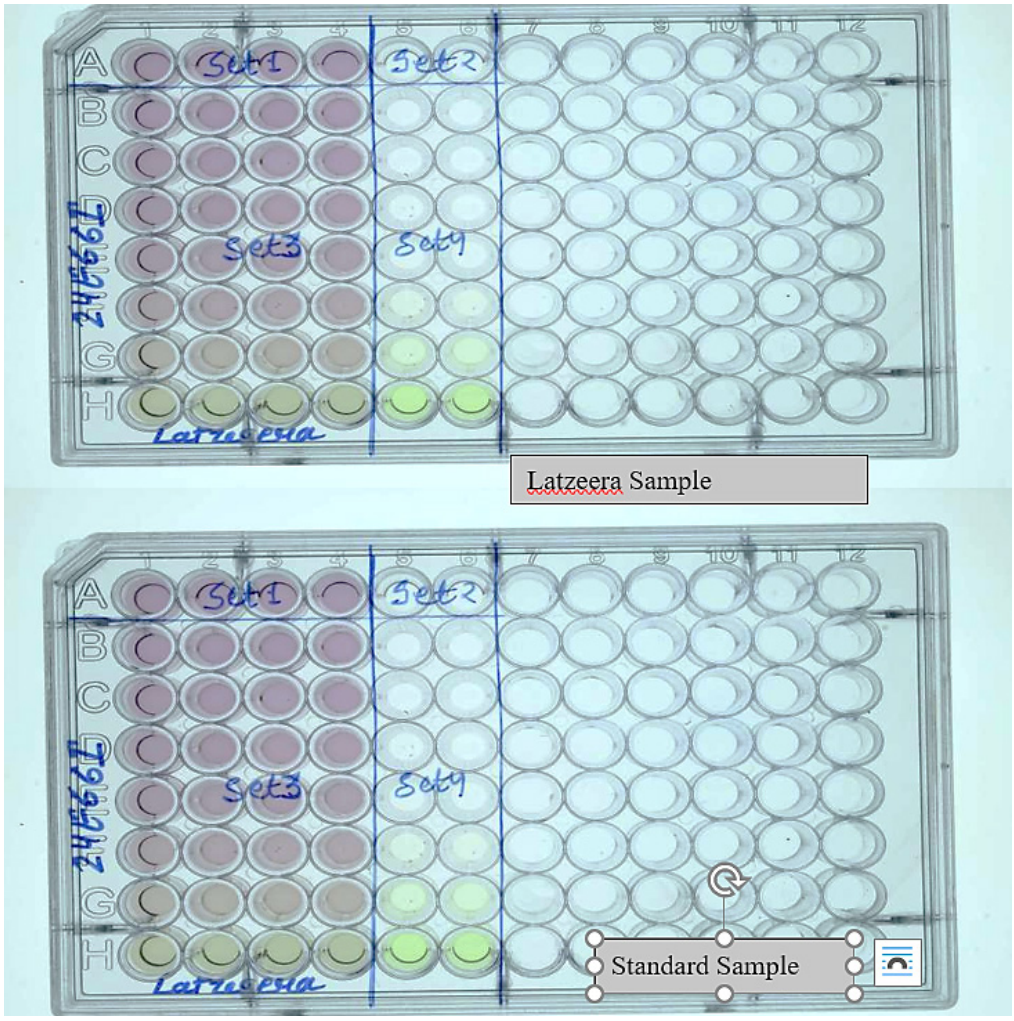

Figure 7: The following images present 96-well microtiter plates employed in the assessment of the biological activity of the compound "*Latzeera*" in comparison with a standard reference sample.

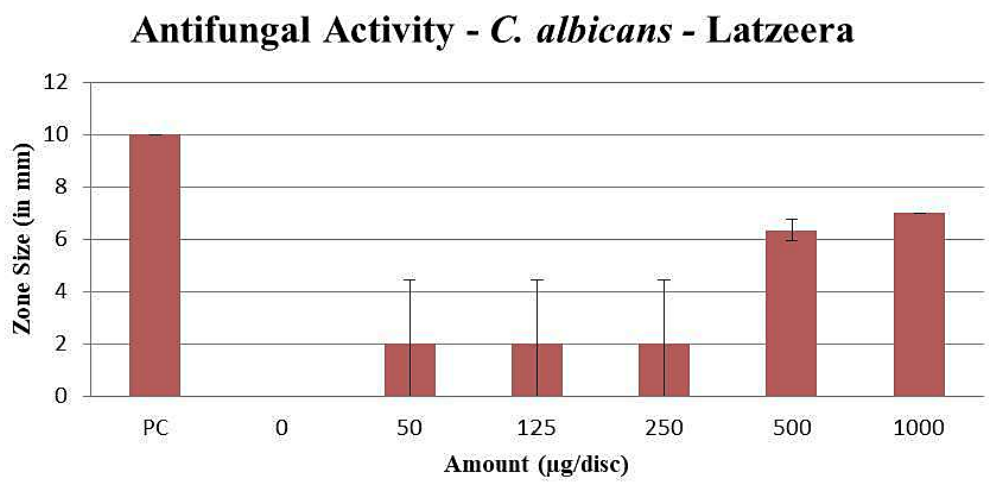

Figure 8: This bar chart illustrates the antifungal activity of different concentrations (in µg/disc) of the compound "*Latzeera*" against *C. albicans*. The y-axis represents the inhibition zone size (in mm), reflecting the degree of fungal susceptibility, while the x-axis shows the compound concentrations, ranging from 0 to 1000 µg/disc. Significant antifungal activity is observed at higher concentrations (500-1000 µg/disc), with inhibition zones increasing to approximately 8-10 mm. This analysis provides valuable insights into the potential antifungal efficacy of "*Latzeera*" against *C. albicans*.

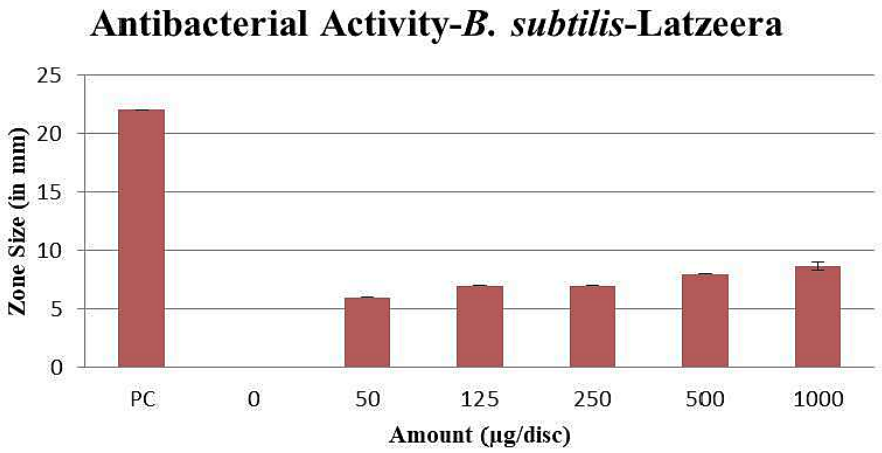

Figure 9: The graph demonstrates a concentration-dependent increase in antibacterial activity of *Latzeera* against B. subtilis. As the concentration of *Latzeera* applied to the discs increases, the zone of inhibition surrounding the discs expands, indicating enhanced antimicrobial efficacy. The Positive Control (PC) serves as a benchmark for comparison and exhibits the largest inhibition zone.

Table 4: Test Strength, Blank Values, and Corrected Values for Sample Strength.

Sample Strength	Test Strength				Blank		Corrected Values			
	1	2	3	4	1	2	1	2	3	4
0	0.473	0.482	0.471	0.477	0.047	0.044	0.4275	0.4365	0.4255	0.4315
0.78	0.462	0.471	0.464	0.462	0.037	0.037	0.425	0.434	0.427	0.425
1.56	0.449	0.462	0.46	0.464	0.033	0.037	0.414	0.427	0.425	0.429
3.125	0.441	0.468	0.448	0.467	0.046	0.037	0.3995	0.4265	0.4065	0.4255
6.25	0.388	0.401	0.414	0.415	0.032	0.033	0.3555	0.3685	0.3815	0.3825
12.5	0.307	0.323	0.327	0.331	0.036	0.036	0.271	0.287	0.291	0.295
25	0.137	0.153	0.144	0.126	0.048	0.051	0.0875	0.1035	0.0945	0.0765
50	0.084	0.095	0.099	0.106	0.06	0.078	0.015	0.026	0.03	0.037

Calculation % RSA = $((Abs_{Control} - Abs_{Sample})/Abs_{Control}) \times 100$ RSA = Radical Scavenging Activity.$Abs_{Control}$ = Absorbance of control Abs_{Sample} = Absorbance of sample.

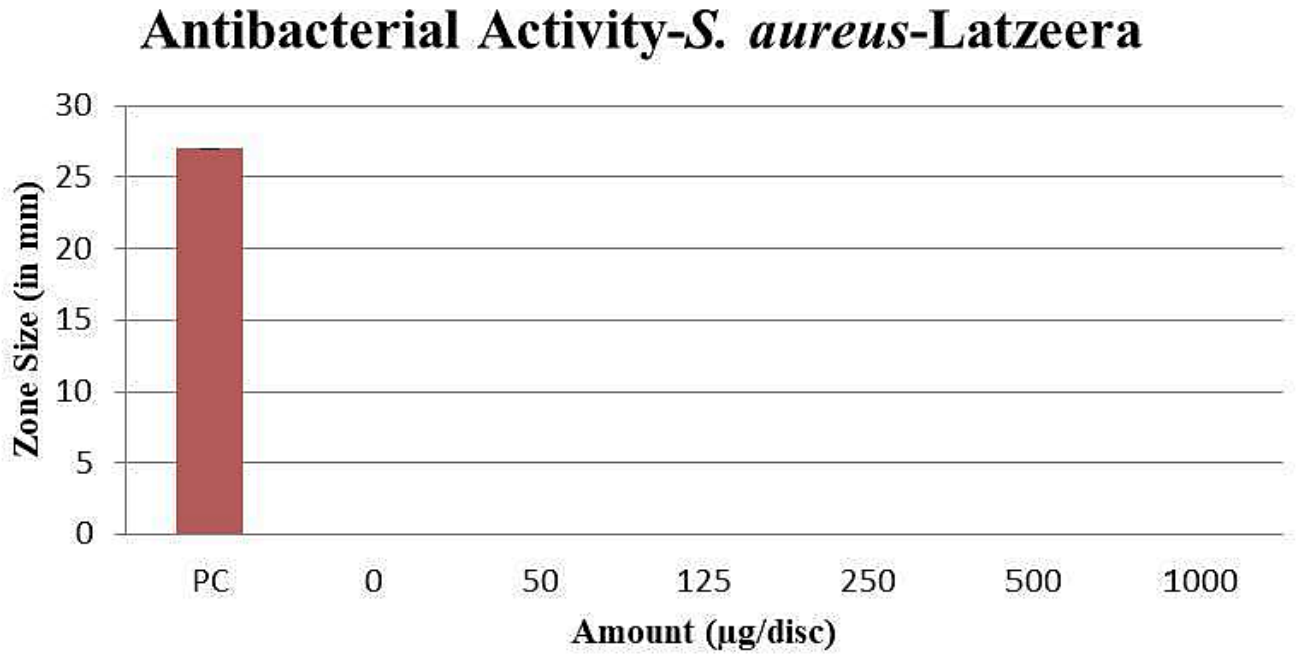

Figure 10: This graph presents the results of an antibacterial activity assay against *S. aureus* using Latzeera at various concentrations. A significant zone of inhibition is observed for the Positive Control (PC), indicating the effectiveness of the assay conditions.

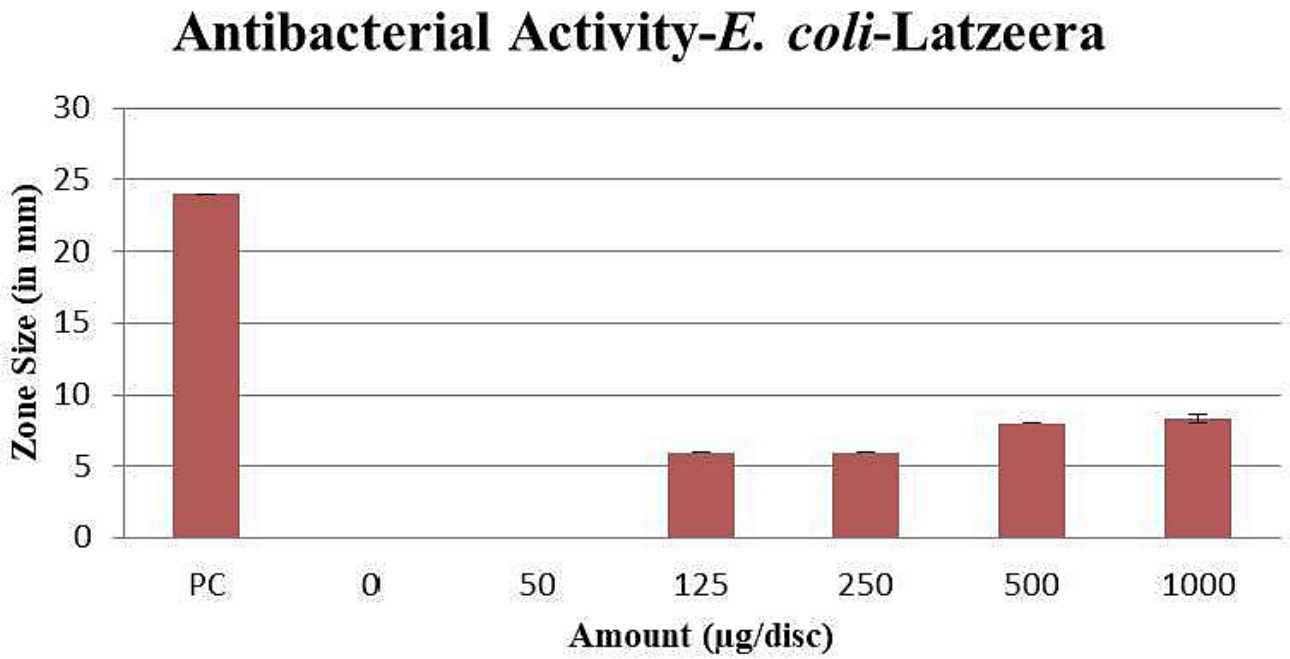

Figure 11: The bar chart depicts the antibacterial activity of various concentrations (in µg/disc) of the compound "*Latzeera*" against *E. coli*. The y-axis represents the size of the inhibition zone (measured in mm), which indicates the degree of bacterial susceptibility, while the x-axis displays the compound concentrations, ranging from 0 to 1000 µg/disc. This analysis provides a comprehensive assessment of the potential antimicrobial efficacy of "*Latzeera*" against *E. coli*.

DISCUSSION

This study assessed *Achyranthes aspera* anti-microbial, anti-cancer, an antioxidant qualities. A traditional medicinal herb, in order to explore its pharmacological potential. Our study's findings confirm the plant's traditional use in treating a range of illnesses by offering strong proof of its complex biological qualities. All investigated bacterial strains, including *B. subtilis, P. aeruginosa, S. aureus*, and *E. coli*, were significantly affected by the well-known antibiotic Ciprofloxacin, according to antimicrobial testing using a positive control. Although it was less effective than Ciprofloxacin, it's interesting to note that the *Achyranthes aspera* extract shown encouraging antibacterial action against particular bacterial strains. This result aligns with previous studies that reported the antibacterial properties of several *Achyranthes aspera* extracts against a variety of microorganisms, including bacteria and fungi. These results strongly imply that substances found in *Achyranthes aspera* have intrinsic antibacterial potential and should be studied further in order to create new antimicrobial drugs. To advance drug development efforts based on these natural substances, it is essential to identify the precise bioactive compounds that cause this activity, such as flavonoids, terepenoids, saponins, which are known to be found in *Achyranthes aspera*.

The MTT assay results showed a significant dose-dependent decrease in cell viability after treatment with the *Achyranthes aspera* extract, indicating a cytotoxic effect in the anti-cancer activity. This finding supports the plant extract's possible anti-cancer effects. One important feature of successful anticancer medications is their potential for selective toxicity towards cancer cells, which is suggested by the steady decline in cell viability with increasing extract concentrations. This selective toxicity may be explained by the way that the bioactive substances in *Achyranthes aspera* affect cancer cells differently than they do healthy cells showed (Figure 6). Additional research is necessary to understand the underlying mechanisms of this cytotoxic effect, which include cell cycle arrest, apoptosis induction and cell growth suppression. To maximize *Achyranthes aspera* therapeutic

Table 5: Zone of Inhibition (mm) of *Latzeera* and Standard Antibiotics against Different Microorganisms.

Sl. No.	Sample Id	Effective Amount	Zone at Effective Conc. (in mm) Avergae	Microbes
1	Ciprofloxacin (PC)	10 µg	22	*B. subtilis*
2	Latzeera	50 µg	6	
3	Ciprofloxacin (PC)	10 µg	27	*S. aureus*
4	Latzeera	-	-	
5	Ciprofloxacin (PC)	10 µg	20.33	*P. aeruginosa*
6	Latzeera	50 µg	6	
7	Ciprofloxacin (PC)	10 µg	20.33	*E. coli*
8	Latzeera	50 µg	6	
9	Amphotericin B (PC)	50 µg	10	*C. albicans*
10	Latzeera	50 µg	2	
11	Amphotericin B (PC)	50 µg	15.66	*A. niger*
12	Latzeera	250 µg	2	

Table 6: Microscopic Observations of Cell Culture Corrected for Blank Values.

Sample	Test Replicates				Blank		Corrected Values			
	1	2	3	4	1	2	1	2	3	4
0	0.151	0.165	0.143	0.148	0.05	0.05	0.101	0.115	0.093	0.098
1	0.15	0.161	0.14	0.144	0.05	0.048	0.101	0.112	0.091	0.095
10	0.142	0.148	0.133	0.137	0.051	0.052	0.0905	0.0965	0.0815	0.0855
50	0.119	0.125	0.118	0.121	0.049	0.053	0.068	0.074	0.067	0.07
100	0.108	0.113	0.111	0.104	0.053	0.055	0.054	0.059	0.057	0.05
250	0.091	0.099	0.089	0.09	0.053	0.053	0.038	0.046	0.036	0.037
500	0.082	0.084	0.083	0.084	0.054	0.054	0.028	0.03	0.029	0.03
1000	0.075	0.078	0.075	0.079	0.053	0.053	0.022	0.025	0.022	0.026

Calculation % Viable cells = (A_{test} / $A_{Control}$) *100 (A_{test} = Absorbance of test sample) ($A_{Control}$ = Absorbance of Control).

Table 7: **Antibacterial Activity of Different Microorganisms.**

Quantity (µg/disc)	Plate A	Plate B	Plate C	Mean	SD	SEM	Microbes
PC	22	22	22	22	0	0	*B. subtilis*
0	0	0	0	0	0	0	
50	6	6	6	6	0	0	
125	7	7	7	7	0	0	
250	7	7	7	7	0	0	
500	8	8	8	8	0	0	
1000	8	9	9	8.66667	0.57735	0.33333	
PC	27	27	27	27	0	0	*S. aureus*
0	0	0	0	0	0	0	
50	0	0	0	0	0	0	
125	0	0	0	0	0	0	
250	0	0	0	0	0	0	
500	0	0	0	0	0	0	
1000	0	0	0	0	0	0	
PC	21	20	20	20.3333	0.57735	0.33333	*P. aeruginosa*
0	0	0	0	0	0	0	
50	6	6	6	6	0	0	
125	6	6	6	6	0	0	
250	7	6	7	6.66667	0.57735	0.33333	
500	7	7	7	7	0	0	
1000	7	7	7	7	0	0	
PC	24	24	24	24	0	0	
0	0	0	0	0	0	0	
50	0	0	0	0	0	0	
125	6	6	6	6	0	0	*E. coli*
250	6	6	6	6	0	0	
500	8	8	8	8	0	0	
1000	9	8	8	8.33333	0.57735	0.33333	
PC	10	10	10	10	0	0	*C. albicans*
0	0	0	0	0	0	0	
50	0	0	6	2	3.4641	2.44949	
125	0	0	6	2	3.4641	2.44949	
250	0	0	6	2	3.4641	2.44949	
500	6	7	6	6.33333	0.57735	0.40825	
1000	7	7	7	7	0	0	
PC	15	16	16	15.6667	0.57735	0.3333	*A. niger*
0	0	0	0	0	0	0	
50	0	0	0	0	0	0	
125	0	0	0	0	0	0	
250	0	0	6	2	3.4641	2	
500	0	0	6	2	3.4641	2	
1000	6	6	7	6.33333	0.57735	0.3333	

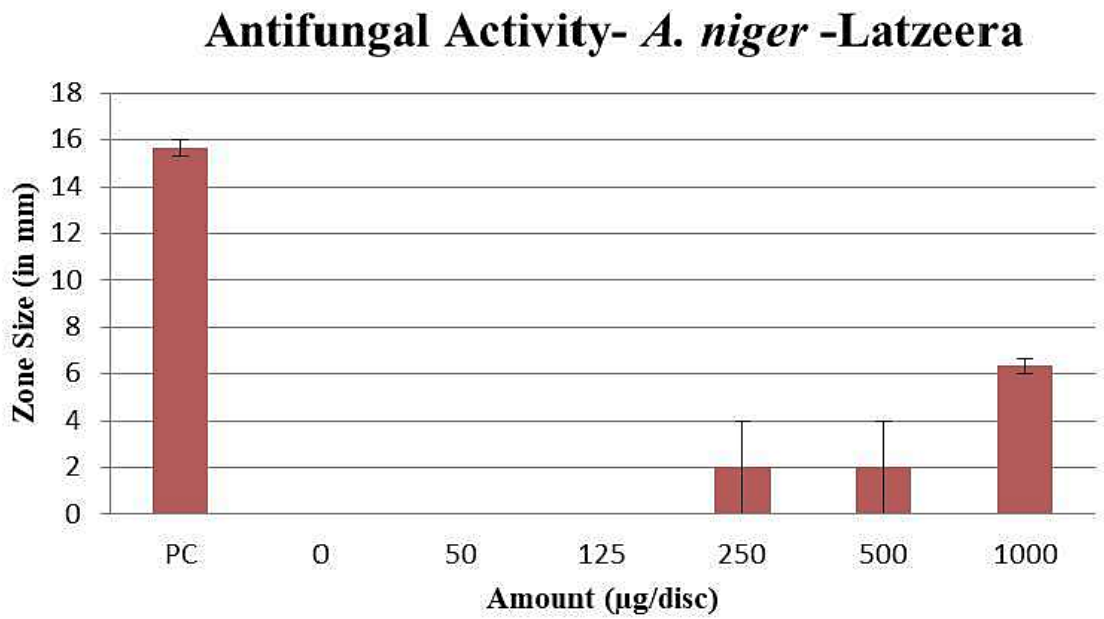

Figure 12: The chart highlights the antifungal activity of "*Latzeera*" across a concentration range from 0 to 1000 µg/disc. The y-axis represents the inhibition zone size in millimeters, serving as an indicator of fungal susceptibility, while the x-axis displays the different concentrations tested. Notably, the compound exhibits significant antifungal activity only at the highest concentration (1000 µg/disc), achieving an inhibition zone of approximately 12- 14 mm. In contrast, lower concentrations display negligible or no inhibitory effects. For comparison, the Positive Control (PC) achieves the highest inhibition zone, exceeding 15 mm, underscoring its superior antifungal efficacy.

potential, create tailored medicines, it will be essential to pinpoint the precise molecular targets and signaling pathways underlying its anti-cancer activity showed (Figure 7).

In the activity of antioxidants is the plant extract has strong antioxidant qualities, as demonstrated by the DPPH scavenging experiment, which showed a dose-dependent increase in antioxidant activity with increasing quantities of *Achyranthes aspera* extract shown (Figure 8). Scavenging free radicals is essential for avoiding oxidative stress, which is connected to the etiology of a number of illnesses, including as malignancy, heart disease and neurological conditions. These disorders are largely caused by oxidative stress, which damages lipids, proteins and DNA in cells. Many phytochemicals, including phenolic acids (like caffeic acid and chlorogenic acid), terpenoids (like quercetin and rutin), flavonoids (like rutin and quercetin), which have been shown to have strong antioxidant qualities, are responsible for *Achyranthes aspera* antioxidant activity. These chemicals can protect cells from oxidative damage and lower the risk of associated diseases by effectively neutralizing free radicals showed (Figure 9).

All things considered, the study's findings offer strong support for *Achyranthes aspera* pharmacological potential. The plant extract's historic use in a variety of illnesses was supported by its encouraging antimicrobial, anti-cancer and antioxidant properties. Additional investigation is necessary to clarify the fundamental mechanisms of action, pinpoint the precise bioactive substances in charge of the effects seen, thoroughly assess the protection, efficacy of *Achyranthes aspera* extracts in preclinical and clinical contexts showed (Figure 10).

CONCLUSION

This investigation assessed *Achyranthes aspera's* antimicrobial, anti-cancer, and antioxidant qualities. A traditional medicinal herb, in order to explore its pharmacological potential. The findings show that *Achyranthes aspera* has a complex pharmacological profile shown (Figure 11). As a positive control in the antimicrobial tests, the well-known drug ciprofloxacin shown strong action against every tested bacterial strain. Interestingly, although not as effective as ciprofloxacin, the extract from *Achyranthes aspera* showed encouraging antibiotic efficacy against specific bacterial strains. This implies that the plant extract contains antibacterial substances. A notable dose-dependent reduction in cell viability was observed in the MTT assay following treatment with *Achyranthes aspera* extract, suggesting potential anti-cancer activity. This finding suggests a possibility of selective toxicity towards cancer cells, as does the steady decline in cell viability with rising concentrations. Additionally, the powerful antioxidant qualities of *Achyranthes aspera* extract were indicated by the DPPH scavenging experiment, which

showed a dose-dependent increase in antioxidant capacity with increasing doses of the extract. This antioxidant capacity is a result of the plant's many phytochemicals, including terpenoids, flavonoids and phenolic acids. All things considered, these results offer compelling proof of *Achyranthes aspera* pharmacological potential. The plant extract's historic use in a variety of illnesses was supported by its encouraging antimicrobial, anti-cancer and antioxidant properties. To clarify the fundamental mechanisms of action, pinpoint the precise bioactive substances in charge of the effects seen, assess the efficacy, safety of *Achyranthes aspera* extracts in preclinical, clinical contexts and more investigation is necessary shown (Figure 12). This conclusion summarizes the study's main conclusions, *Achyranthes aspera* possible therapeutic uses and the need for more research to completely understand its pharmacological potential.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to Shiva Institute of Pharmacy for providing the necessary facilities and resources that made this research possible. I am also thankful to my friends and coworkers whose collaboration and encouragement greatly supported me throughout this process. My deepest appreciation goes to my family for their unwavering understanding and support during the course of this work.

I gratefully acknowledge the valuable contributions of the authors, scholars, and professionals whose research and insights guided and inspired this study.

This research was strengthened by the collaborative efforts of several individuals. Dr. Biswajit Dash contributed through data analysis, while Dr. Ashish Sarkar carried out the experimental studies. Dr. Afrin Alam[4] supported the work with literature review, and Dr. Ayush Garg[5] assisted in statistical analysis. Lab work collaboration was efficiently managed by Ms. Shivani Devi and Ms. Ankita Sharma[1]. The process of plant extraction was undertaken by Mrs. Varsha Chauhan, and Mr. Tanbeer Kaur provided valuable assistance in bioassays. Finally, Ms. Yashna Bawa extended her support in the preparation of the manuscript. Their contributions were instrumental in shaping and completing this work.

ABBREVIATIONS

***B. subtilis*:** *Bacillus subtilis*; ***S. aureus*:** *Staphylococcus aureus*; ***P. aeruginosa*:** *Pseudomonas aeruginosa*; ***E. coli*:** *Escherichia coli*; ***C. albicans*:** *Candida albicans*; ***A. niger*:** *Aspergillus niger*; **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

Achyranthes aspera is a traditional medicinal herb whose pharmacological potential was examined in this study by assessing its antioxidant, anti-microbial and anti-cancer properties. The findings revealed a complex pharmacological profile of *Achyranthes aspera* Strong antioxidant qualities, dose-dependent cytotoxicity in cell culture and promising antibacterial activity against specific bacterial strains were all displayed by the plant extract. These results validate the traditional usage of *Achyranthes aspera*, for more investigation to assess its therapeutic potential in preclinical, clinical contexts, discover bioactive chemicals and clarify the underlying mechanisms.

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Cite this article: Chaudhuri A. Exploring the Pharmacological Potential of *Achyranthes aspera* (Latzeera): A Complete Investigation of its Anticancer, Anti-Microbial and Antioxidant Activities. Pharmacog Res. 2025;17(4):1315-27.