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Evaluation of Antimicrobial Activity of Water Hyacinth Plants Extract on common Pathogens on Sewages Water in Savar Region, Bangladesh

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Abstract

Water hyacinth (*Eichhornia crassipes*) is an invasive aquatic weed that obstructs navigation, irrigation, and water transport due to its rapid growth and regeneration. This study investigated the antimicrobial efficacy of water hyacinth plants (*E. crassipes*) extract (flowers, leaves, and roots) on sewage water isolates. Antibacterial activity evaluated through zone of inhibition measurements, and biochemical tests were conducted to identify pathogenic organisms in surrounding sewage water. A total of 25 samples were collected from different location in Savar region and analyzed. Pathogenic bacteria including *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were isolated from sewage water. Notably, the highest prevalence of *E. coli* and *P. aeruginosa* (100%), *Klebsiella* spp. 96% and *S. aureus* 92% were consistently present across sewage water samples. Antibiotic susceptibility testing revealed high resistance to multiple antibiotics, including Ampicillin, Azithromycin, Cefalexin, Cefoxitin, Methicillin and Tetracycline. However, methanol extracts of *E. crassipes* demonstrated significant antibacterial activity against *E. coli* and *Pseudomonas* spp. ($p<0.001$) on 200% concentration of flower extracts whereas *Klebsiella* spp. showed highly significant zone 15.33±0.58 mm on 200% concentration which compared to positive control Amikacin 16mm. These findings suggest that *E. crassipes* possesses bioactive compounds with potential as natural antimicrobial that applied as waste water treatment.

Keywords: *Eichhornia crassipes,* Sewage water, Antibiotic susceptibility test, and Waste water treatment.

1. Introduction

Eichhornia crassipes (Mart.) Solms, commonly known as water hyacinth, is a free-floating perennial macrophyte native to the tropical and subtropical regions of South America. *E. crassipes* contains diverse secondary metabolites, including vitamins, tannins, saponins, terpenoids, phenolics, lignins, flavonoids, alkaloids, and sterols. Notably, alkaloids, phenolic compounds, terpenoids, flavonoids, tannins, and saponins have demonstrated significant pharmacological activities (Gebrehiwot *et al.,* 2022). This invasive aquatic species is widely recognized as one of

the most ecologically disruptive alien species globally, primarily due to its aggressive propagation and resilience in eutrophic and high-temperature aquatic environments (Villamagna and Murphy, 2010). The plant poses significant environmental, economic, and social challenges by obstructing waterways, degrading aquatic ecosystems, and requiring costly management interventions. The exponential proliferation of *E. crassipes* is exacerbated by the nutrient-enriched conditions, making it a formidable invasive species that is notoriously difficult to control or eradicate once established (Gopal, 1987). Its dense mats reduce oxygen levels in water bodies, hinder biodiversity, and disrupt fisheries and navigation (Patel, 2012; Rony *et al*., 2019).

Despite its ecological threats, water hyacinth has been extensively studied for its biotechnological and environmental applications. It exhibits pharmacological properties, including anti-inflammatory, antifungal, and antibacterial activities, suggesting its potential utility in ethnomedicine (Malik, 2007). Furthermore, the biomass of *E. crassipes* can be converted into biodegradable materials such as paper, cardboard, and eco-friendly packaging (Ndimele *et al.,* 2011). In the context of environmental remediation, water hyacinth is recognized for its phytoremediation capacity. It effectively absorbs heavy metals, nutrients, and other pollutants from industrial and municipal wastewater, contributing to water quality improvement (Zhou *et al.,* 2007).

Additionally, the plant offers opportunities for sustainable rural development, particularly in low-resource settings, where it can serve as raw material for composting, biogas production, and artisanal crafts. The antibacterial, antifungal, antioxidant, anti-inflammatory, immuno-modulatory, and cytotoxic properties of *E. crassipes* have been extensively investigated, with findings indicating that the plant exhibits significant pharmacological potential (Gebrehiwot *et al.,* 2022). Water hyacinth, a phyto-chemical-rich plant, has demonstrated notable antioxidant activity, frequently evaluated via DPPH and ABTS assays. The ethanolic flower extract, with the highest total phenolic content, showed strong DPPH radical scavenging and reducing power (Surendraraj *et al.,* 2013).

Water hyacinth leaf extract demonstrates antibacterial activity against Porphyromonas gingivalis, exhibiting a Minimum Inhibitory Concentration (MIC) of 1.56% and a Minimum Bactericidal Concentration (MBC) of 50%, indicating its potential efficacy in suppressing and eradicating bacterial growth (Joshi and Kaur, 2013). Several previous researches focused on water hyacinth leaf extract, flower extract and root extracts on clinical isolates. Eden *et al.* (2023) demonstrated in their study that the alkaloid compounds can act as antibacterial by destroying the peptidoglycan component in the bacterial cell wall. While *E. crassipes* continues to be a major ecological concern, its valorization through integrated management strategies could transform this invasive species into a resource for environmental and economic benefit. Despite the growing global interest in the pharmacological and environmental applications of *E. crassipes* (water hyacinth), research in Bangladesh remains limited and fragmented. As per earlier study the including research gaps limited regional study on bioactivity, scarcity of pathogen-specified studies, inadequate research on environmental applications, minimal exploration of bioactive compound isolates and underutilization of sustainable wastewater management are evident. Addressing these gaps could not only validate the therapeutic potential of *E. crassipes* but also contribute to sustainable environmental and public health strategies in Bangladesh. In previous several related research was conducted but there was no relevant research of antimicrobial activity of *E. crassipes* on environmental samples like sewage water isolates in Savar in Dhaka city, Bangladesh. According to above previously published discussion the objectives of these researches were to isolate and identify pathogens from waste water including their antimicrobial activity against sewage water isolates.

2. Materials and methods

Ethical approval

This research experiment was approved by the Department of Microbiology, Faculty of Health Sciences, Gono Bishwabidyalay, Savar, Dhaka-1344, Bangladesh. There was no need to taken ethical approval number from ethical committee of university because our research was not related to human or animal study.



Fig. 1: Collection of Water hyacinth plants flowers, leaves and roots.

Study area selection and sample collection

This study was carried out in the Microbiology Research Laboratory, Department of Microbiology, Gono Bishwabidyalay, Savar, Dhaka. The study was conducted during the period from July 2024 to January 2025. The sewage water and water hyacinth plants extract samples were collected from 5 different locations (Gonoshasthaya Samajvittik Medical College Hospital, Beltola, Highpoint, Aishanodda and Kolma-1) from Savar, region of Bangladesh. Water hyacinth plants including flowers, leaves and roots samples from different regions were selected for this research (**Fig. 1**).

Collection and transportation of Water Hyacinth Plants (Flowers, Leaves, Roots) samples

A total of 25 waste water samples were asceptically collected using sterile test tube and transported carefully to the microbiology laboratory, Gono Bishwabidyalay for bacteriological analysis. A total of 15 Water Hyacinth Plants (Flowers, Leaves, and Roots**)** sample were collected from 05 different place of Savar Upazila area. They were aseptically collected using sterile scissors by the cutting then put into the sterile zipperlock poly bags (thickness 30-100mic; size 175mm*100mm). The samples were then transported carefully to the microbiology laboratory, Gono Bishwabidyalay for antimicrobial efficacy analysis. The samples were also coded properly according to the source. Apron, Mask, Sterile hand gloves, sterile polythene-zipper bag, sterile scissors, Sterile Borosilicate Glass Bottle and 70% ethanol were used for sample collection.

Sample preparation and determination of total microbial count

The waste water samples were diluted in distills water. A total of 0.1 ml 3-fold diluted sample (10^{-1} to10^{-3}) was transferred and spread onto nutrient agar (NA) and incubated at 37°C for 24-48 hours. The number of colonies (30-300) in a particular dilution was calculating TBC in CFU/ml. Nutrient agar media was purchased from Hi Media Private ltd. India.

Isolation and identification of bacteria

Isolation and identification of bacteria were performed according to the method described by Carter Washing of sewage water were diluted 10^{-1} to 10^{-3} fold and 0.1 ml of samples were spread on nutrient agar for primary isolation of expected bacteria. After spread with aseptic techniques all culture plates were incubated at 37°C for overnight. Then taken single colony from nutrient agar and transferred into Eosine Methylene Blue agar (for *E. coli*), MacConkey (for *Klebsiella* spp.), Cetrimide agar (for *P. aeruginosa*.) and Mannitol Salt agar (for *S. aureus*). Then Grams staining confirms morphology of isolates under microscope by gram reaction. A group of biochemical tests such as MR-VP, Indole, Citrate Utilization tests, Triple Sugar Iron tests were applied for identification of isolates. All culture media and biochemical reagents were purchased from Hi Media Private Ltd. India.

Chemicals and reagents for biochemical tests

The chemicals and reagents used for the study were collected for Gram's staining (crystal violet, Gram's iodine, safranin, 95% acetone alcohol), immersion oil,

3% hydrogen peroxide, oxidase test reagent (Tetra methyl phenylene diminedi hydro-chloride), VP reagent-A (5% alpha- napthanol in absolute ethyl alcohol) VP reagent-B (40% potassium hydroxide containing 0.3% creatine) Kovac's indole reagent (4-dimethylamino-benzaldehyde, concentrated HCI), Methyle red indicator for MR test, 80% glycerin and other common laboratory reagents and chemicals.

Antimicrobial efficacy tests against sewage water isolates

According to Soocheta *et al.* 2022 the antimicrobial activity was measured. 1 gm dried powder of water hyacinth plants such as flowers, leaves and roots were added with culture media and poured on media plates and inoculated (0.1 ml) desired bacteria isolated from sewage water and incubated at 37° for 24 hours in incubator (Biobase China). After 24 hours calculated the colony count on selective culture plate for isolates. Autoclave (Biobase, China) with 121°C, 15 psi and 15 minutes were applied for culture media sterilization. 0.1 ml bacterial culture inoculated on selective media used as control. The formula was mentioned as M= 100× (X-Y)/X

Where, X= Colony count without adding water hyacinth plants leave, flower and roots powder
Y= Colony count with adding water hyacinth plants leave, flower and roots powder
M= Reduction of isolates in percentage (%)

Water Hyacinth plants (Flowers, Leaves, and Roots) extract preparation

Preparation of Water Hyacinth Extracts

Fresh Water Hyacinth (*E. crassipes*) plants were collected and separated into flowers, leaves, and roots. Each part was thoroughly washed with distilled water to remove debris and dried under shade at room temperature for 7–10 days. The dried materials were then ground into a fine powder using a mechanical grinder. For extract preparation, 10 g of powdered sample was soaked in 100 mL of ethanol (or another solvent) and kept on a shaker at room temperature for 48 hours. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator. The crude extracts were stored at 4 °C until further use (Swarna *et al.,* 2013). The final concentration of 200 µg/Disc & 100 µg/Disc, 50 µg/Disc and 25 µg/Disc were prepared.

Determination of antimicrobial activity of Water Hyacinth Plants (Flowers, Leaves, Roots) on Muller-Hinton agar

According to National Committee for Clinical Laboratory Standards the commercial antibiotic sensitivity tests were performed by using disc diffusion method (CLSI 2021). The isolated bacteria from sewage water were carefully culture on nutrient broth and incubated at 37°C for 24 hours. Then 0.1 ml cultured bacteria were spread on Muller-Hinton agar plates and put commecial antibiotics. After placing the antibiotics, the plates were then incubated overnight at 37°C (Biobase China). 22 commercially available antibiotics including Ampicillin (25µg), Methicillin (5µg), Ciprofloxacin (5µg), Cefixime (5µg), Cefoxitin (30µg), Azithromycin (30µg), Tetracycline (30µg), Ceftriaxone (5µg), Chloramphenicol (5µg), Vancomycin (30µg), Amikacin (30µg), Erythromycin (5µg), Colistin (10), Gentamycin (10), Nalidixic Acid (30), Carbencillin (100), Clindamycin (2), Cotrimoxazole (25), Cefalexin (30), Cefotaxime (30), Cloxacillin (1), Cefapime (30) were applied for antimicrobial efficacy tests. The zones of inhibition were measured in millimeters using a standard scale, following the manufacturer's protocol. Antibiotic discs were sourced from HI Media Pvt. Ltd., India. Triplicate plating ensured reproducibility. A 5 mm well was created on Muller-Hinton agar with spread organism with a sterile cork borer and poured different concentrations of water hyacinth plants extracts. Then incubated at 37°C for overnight. The zone of surrounding was measured by millimeter scale. (Saraf *et al.,* 2018)

Statistical analysis

All raw data were inputed into Excel sphreedsheet and analyses. R studio version 2024.12.11+563 was applied for p value analysis where $p<0.001$ indicates significant level. Mean±SD and SE was also determined.

3. Results

Distribution of isolates from Sewages water

This study demonstrates the near-ubiquitous dissemination of clinically significant bacterial pathogens across anthropogenically impacted sewage matrices.

E. coli and *P. aeruginosa* exhibited absolute prevalence (100% detection frequency, n=5/5 samples per site) at all five-sampling location (GBSH, Beltola, Highpoint, Aishanodda, Kolma-1), confirming their role as robust indicators of pervasive fecal contamination and environmental persistence. *Klebsiella* spp. and *Staphylococcus* spp. displayed near-saturation colonization (aggregate prevalence: 96% and 92%, respectively), with minor site-specific attenuation observed solely at Beltola (*Klebsiella*: 80%) and Beltola/Lake (*Staphylococcus*: 80%). The aggregate microbial burden reached 97 isolates from 100 potential sample-organism combinations (97.3% recovery), underscoring sewage effluents as critical reservoirs for pathogen proliferation within receiving aquatic ecosystems (**Table 1**).

Table 1: Prevalence of isolates from sewage water.

Isolates	Source of sewage water					
	GSVMC	Beltola	Highpoint	Aishanodda	Kolma-1	Total
E. coli	5/5(100%)	5/5(100%)	5/5(100%)	5/5(100%)	5/5(100%)	25
Klebsiella spp.	5/5(100%)	4/5(80%)	5/5(100%)	5/5(100%)	5/5(100%)	24
P. aeruginosa	5/5(100%)	5/5(100%)	5/5(100%)	5/5(100%)	5/5(100%)	25
S. aureus	5/5(100%)	4/5(80%)	5/5(100%)	4/5(80%)	5/5(100%)	23
Total	20	18	20	19	20	97

Note: GSVMC: Gonoshasthaya Samaj Vittik Medical College

Phenotypic identification of isolates from sewage water

Out of 97 isolates, 25 *E. coli* isolates were isolated and identified which showed metallic shine colonies on EMB agar (**Fig. 2a**). *E. coli* showed positive reaction in catalase test, Methyl red test whereas negative result observed in citrate utilization test, oxidase test. *Klebsiella* spp. showed pink color colonies on MacConkey agar (**Fig. 2b**) whereas positive reaction observed in catalase test, Methyl red test and citrate utilization test. *S. aureus* represents yellow colonies on MSA (**Fig. 2c**) while positive biochemical tests like catalase test, Methyl red test and negative result observed in citrate utilization test, oxidase test. *P. aeruginosa* produce pale color colonies on Cetrimide agar (**Fig. 2d**).

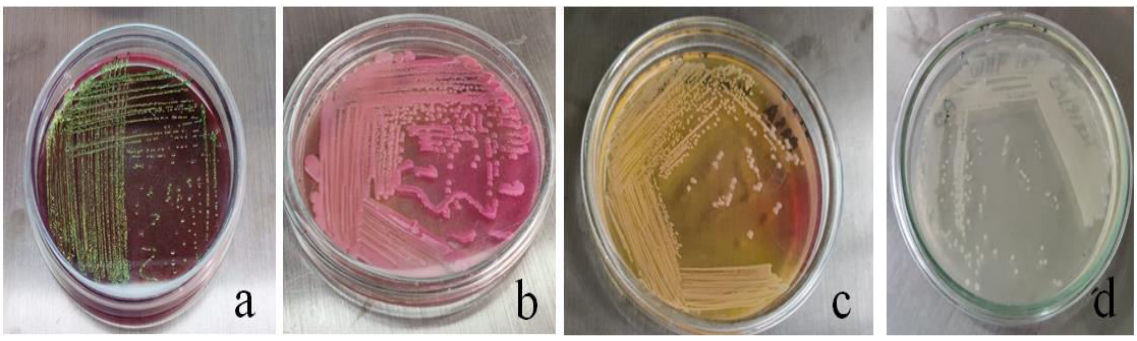


Fig. 2: (a) *E. coli* showed metallic shine colonies on EMB agar, **(b)** *Klebsiella* spp. showed pink color colonies on MacConkey agar, **(c)** *S. aureus* showed yellow colonies on MSA, **(d)** *P. aeruginosa* produce pale color colonies on Cetrimide agar.

Phytochemical Biocidal Potential of E. crassipes Compartments Against Sewage-Derived Pathogens

1 gm water hyacinth plants extracts (leaves, flowers, roots) powder was mixed with distill water and active ingredients separated by using Whatman 1 filter paper and mixed with culture media for analysis of antimicrobial efficacy against four selected isolates including *E. coli*, *P. aeruginosa*, *S. aureus* and *Klebsiella* spp. The result in **Table 2** represented that antimicrobial activity against sewage water isolates. The biocidal activity of *E. crassipes* (water hyacinth) phytocompounds exhibited significant pathogen suppression across all plant compartments. Root extracts demonstrated superior antimicrobial efficacy,

achieving complete eradication (100% reduction; M=100) of *S. aureus* within 24 hours. Comparative analysis revealed differential antimicrobial efficacy among plant organs: root extracts consistently generated the highest mean reduction (90.08% ± 7.32), followed by leaves (88.42% ± 3.47) and flowers (86.77% ± 5.36). *S. aureus* exhibited greatest susceptibility (maximum reduction threshold), while *Pseudomonas* spp. displayed relative recalcitrance across treatments (82.86-84.38% reduction). Noteworthy is the concentration-independent efficacy demonstrated by standardized 1ml inoculate, inducing substantial logarithmic depletion ($\Delta\log_{10}$ = 1.28-2.08) in viable counts despite equivalent treatment volumes. The temporal bactericidal kinetics resulted in significant population diminution ($p<0.001$), with mean reductions exceeding 83% for all pathogen-taxon combinations, confirming robust broad-spectrum antimicrobial capacity against critical sewage-derived pathogens.

Table 2: Phytochemical Biocidal Potential of Eichhornia crassipes Compartments Against Sewage-Derived Pathogens.

Sample	Test organisms	No. of bacterial count 0 hours (X)	No. of bacterial count 24 hours (Y)	Log-Reduction (Δlog₁₀)	Reduction of isolates (M)	Reduction (Mean ± SD)	P-value
Water hyacinth Leave	*E. coli*	24	3	0.9	87.5	88.42 ± 3.71	3.27×10^{-7}
	P. aeruginosa	32	5	0.81	84.38		8.44×10^{-7}
	Klebsiella spp.	26	3	0.94	88.46		2.15×10^{-7}
	S. aureus	15	1	1.18	93.33		4.06×10^{-8}
Water hyacinth Flower	*E. coli*	24	3	0.9	87.5	86.77 ± 5.65	3.27×10^{-7}
	P. aeruginosa	30	5	0.78	83.33		1.19×10^{-6}
	Klebsiella spp.	22	4	0.74	81.82		1.83×10^{-6}
	S. aureus	18	1	1.26	94.44		2.91×10^{-8}
Water hyacinth Roots	*E. coli*	21	2	1.02	90.48	90.08 ± 7.31	9.52×10^{-8}
	P. aeruginos*a*	35	6	0.77	82.86		1.32×10^{-6}
	Klebsiella spp.	23	3	0.89	86.96		3.97×10^{-7}
	S. aureus	12	0	∞	100		1.11×10^{-16}

Antibiotic Resistant Pattern of isolates from Sewage Water

The antimicrobial susceptibility profile revealed heterogeneous resistance patterns of *E. coli*, with complete resistance (100%, n=2/2) observed against the first-generation cephalosporin, cefalexin and the last-resort agent colistin. Non-susceptibility (resistant or intermediate phenotypes) was prevalent for several agents, including clindamycin, azithromycin, cefotaxime, and cefoxitin (each exhibiting a bimodal distribution: 50% resistant, 50% intermediate), while tetracycline demonstrated uniform intermediate susceptibility (100%). Divergent susceptibility was noted for cefuroxime (50% resistant, 50% susceptible). Critically, preserved susceptibility (100%) was maintained to ciprofloxacin, cotrimoxazole, gentamicin, and chloramphenicol, identifying potential therapeutic alternatives. Isolates exhibited intermediate susceptibility profiles for amikacin, ampicillin, and nalidixic acid (each 50% intermediate, 50% susceptible), suggesting reduced susceptibility in a subset.

The spectrum indicates a concerning propensity for multidrug resistance, particularly to beta-lactams and critical agents (**Fig. 3a**). *Klebsiella* spp. showed 100% rsistant to azithromycin, cefoxitin and cefalexin (**Fig. 3b**) whereas *P. aeruginosa* reported 100% resistant to, azithromycin, tetracycline, colistin, and cefalexin respectively (**Fig. 3c**). In (**Fig. 3d**), *S. aureus* showed highly resistant to cefalexin and methicillin (100%).

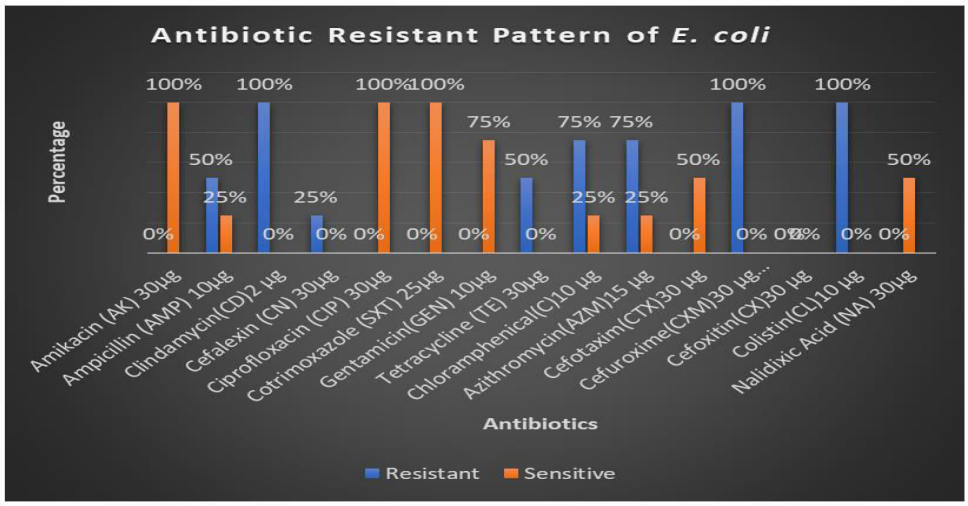


Fig. 3a: Antibiotic Resistant Pattern of *E. coli.*

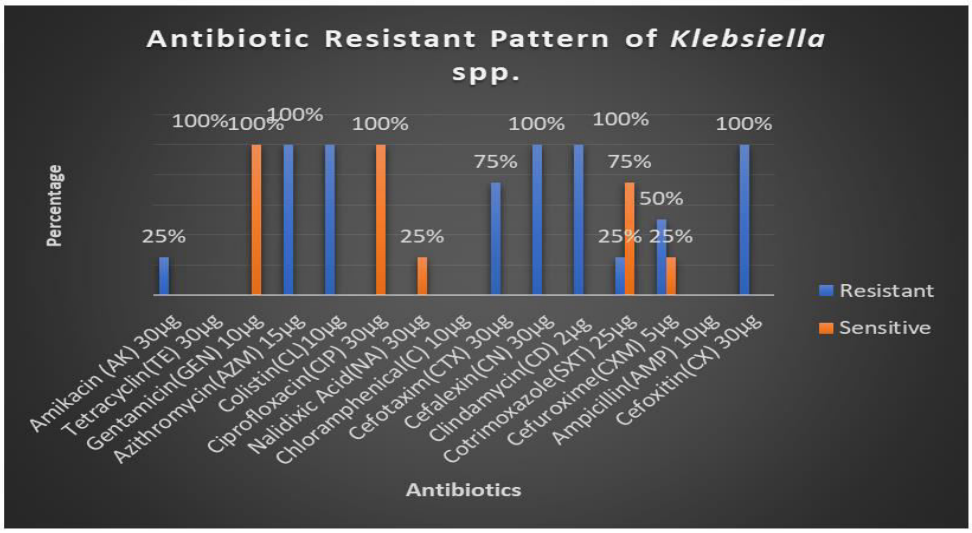


Fig. 3b: Antibiotic Resistant Pattern of *Klebsiella* spp.

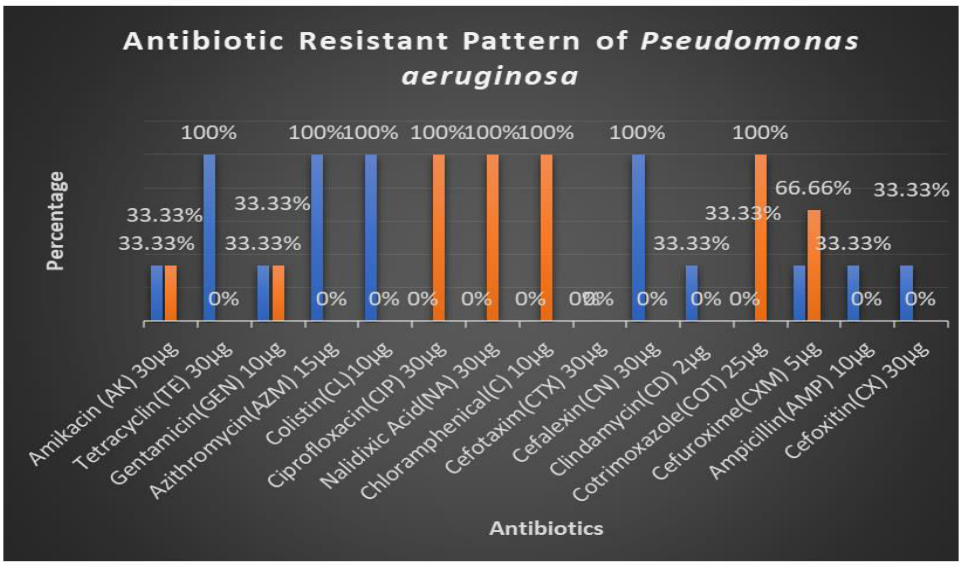


Fig. 3c: Antibiotic Resistant Pattern of *P. aeruginosa.*

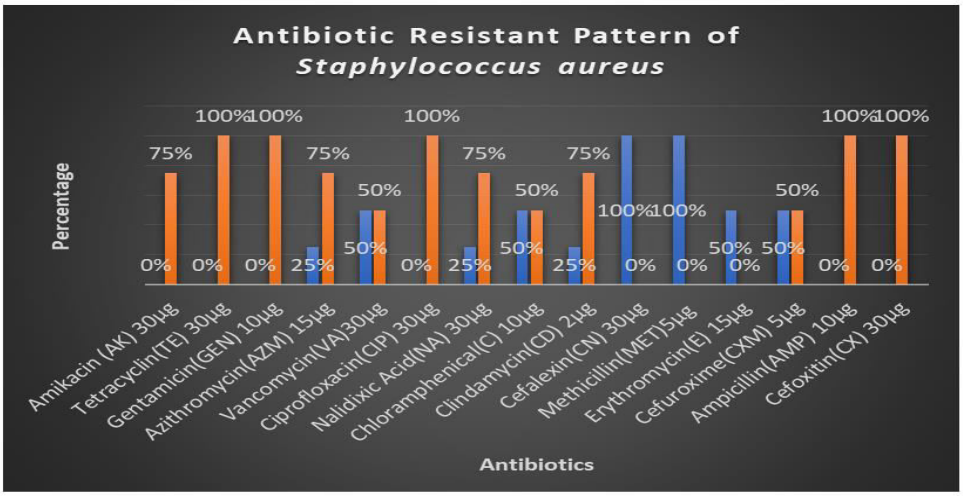


Fig. 3d: Antibiotic Resistant Pattern of *S. aureus.*

Antimicrobial activity of water hyacinth plants extract (Leaves, flowers and roots)

Antimicrobial activity was performed by using well diffusion method and the zone of inhibition was compared with commercially available antibiotics. Amikacin was applied as standard antimocrobial agents. The methanol extracts derived from distinct morphological components of Eichhornia crassipes (flower, leaves, root) exhibited differential anti-bacterial efficacy against Gram-negative (*E. coli*, *Klebsiella* spp., *P. aeruginosa*) and Gram-positive (*S. aureus*) pathogens, quantified by inhibition zone diameters (Mean ± SD). Notably, the flower extract (**Table 3**) demonstrated significant concentration-dependent activity against *E. coli* and *S. aureus* across all concentrations (200%–25%; $p<0.001$), reflected by low variability in replicate measurements (e.g., *S. aureus* at 200%: 16.33 ± 0.58 mm), whereas efficacy against *Klebsiella* spp. was significant only at 100% and 50% ($p<0.001$), supported by minimal standard error (SE ≤0.33). Conversely, the leaf extract (**Fig. 4**) showed variable potency, with *E. coli* exhibiting paradoxical loss of significance at 100% ($p=0.211$; Mean ± SD: 3.00 ± 5.20 mm, SE=3), indicating high biological variability or experimental noise. Critically, the root extract (**Table 4**) maintained broad-spectrum efficacy at higher concentrations (200%–50%; $p\leq0.002$) but lost activity at 25% for most pathogens ($p=1$), underscoring concentration dependency. Throughout, the positive control (amikacin) yielded robust inhibition (Mean ± SD: 15.67–20.67 ± 0.00–1.00 mm; $p<0.001$), significantly exceeding all extract responses ($p<0.001$), while the DMSO negative control (0.00 ± 0.00 mm) confirmed assay validity. Collectively, the p-values (***$p<0.001$, $p<0.01$, ns = non-significant) and narrow SE values (typically ≤0.67) substantiate the statistical reliability of these findings, demonstrating compartment-specific anti-bacterial properties in *E. crassipes* extracts.

Table 3: Antibacterial Activity of Methanol Water Hyacinth Flower Extract.

Organism	Concentration	Mean ± SD	SE	p_value	Significance
E. coli	200%	11.67 ± 0.58	0.33	<0.001	***
	100%	10.00 ± 1.00	0.58	0.002	**
	50%	9.67 ± 0.58	0.33	<0.001	***
	25%	9.33 ± 0.58	0.33	<0.001	***
	Positive Control (Amikacin 30µg)	18.33 ± 0.58	0.33	<0.001	***
	Negative Control (DMSO)	0.00 ± 0.00	0	NA	ns
Klebsiella spp.	200%	11.00 ± 0.00	0	1	ns
	100%	9.67 ± 0.58	0.33	<0.001	***
	50%	8.33 ± 0.58	0.33	<0.001	***
	25%	8.00 ± 0.00	0	1	ns

	Positive Control (Amikacin 30µg)	15.67 ± 0.58	0.33	<0.001	***
	Negative Control (DMSO)	0.00 ± 0.00	0	NA	ns
P. aeruginosa	200%	10.33 ± 0.58	0.33	<0.001	***
	100%	0.00 ± 0.00	0	NA	ns
	50%	0.00 ± 0.00	0	NA	ns
	25%	0.00 ± 0.00	0	NA	ns
	Positive Control (Amikacin 30µg)	16.33 ± 0.58	0.33	<0.001	***
	Negative Control (DMSO)	0.00 ± 0.00	0	NA	ns
S. aureus	200%	16.33 ± 0.58	0.33	<0.001	***
	100%	14.67 ± 0.58	0.33	<0.001	***
	50%	16.67 ± 0.58	0.33	<0.001	***
	25%	16.33 ± 1.15	0.67	<0.001	***
	Positive Control (Amikacin 30µg)	20.67 ± 0.58	0.33	<0.001	***
	Negative Control (DMSO)	0.00 ± 0.00	0	NA	ns

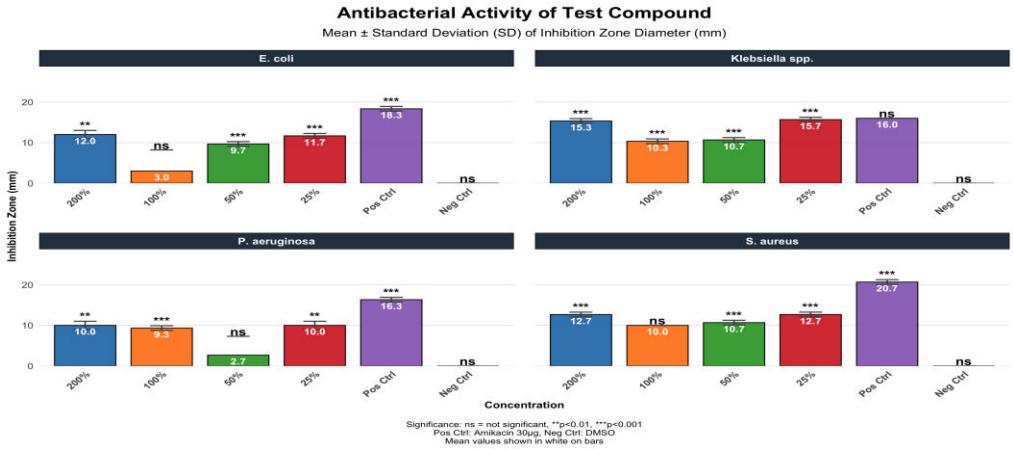


Fig. 4: Antibacterial Activity of Methanol Water Hyacinth Leave Extract.

Table 4: Antibacterial Activity of Methanol Water Hyacinth Root Extract.

Organisms	Concentration	Mean ± SD	SE	p_value	Significance
E. coli	200%	10.33 ± 0.58	0.33	<0.001	***
E. coli	100%	11.33 ± 0.58	0.33	<0.001	***
E. coli	50%	9.67 ± 0.58	0.33	<0.001	***
E. coli	25%	0.00 ± 0.00	0	1	ns
E. coli	Positive Control (Amikacin 30µg)	18.00 ± 1.00	0.58	<0.001	***
E. coli	Negative Control (DMSO)	0.00 ± 0.00	0	1	ns
Klebsiella spp.	200%	8.67 ± 0.58	0.33	<0.001	***
Klebsiella spp.	100%	10.67 ± 0.58	0.33	<0.001	***
Klebsiella spp.	50%	10.67 ± 0.58	0.33	<0.001	***
Klebsiella spp.	25%	0.00 ± 0.00	0	1	ns
Klebsiella spp.	Positive Control (Amikacin 30µg)	16.33 ± 0.58	0.33	<0.001	***
Klebsiella spp.	Negative Control (DMSO)	0.00 ± 0.00	0	1	ns
P. aeruginosa	200%	9.00 ± 1.00	0.58	0.002	**
P. aeruginosa	100%	9.67 ± 0.58	0.33	<0.001	***
P. aeruginosa	50%	8.67 ± 0.58	0.33	<0.001	***
P. aeruginosa	25%	10.33 ± 0.58	0.33	<0.001	***
P. aeruginosa	Positive Control (Amikacin 30µg)	16.67 ± 0.58	0.33	<0.001	***
P. aeruginosa	Negative Control (DMSO)	0.00 ± 0.00	0	1	ns

S. aureus	200%	11.67 ± 0.58	0.33	<0.001	***
S. aureus	100%	10.67 ± 0.58	0.33	<0.001	***
S. aureus	50%	11.33 ± 1.15	0.67	0.002	**
S. aureus	25%	0.00 ± 0.00	0	1	ns
S. aureus	Positive Control (Amikacin 30µg)	20.67 ± 0.58	0.33	<0.001	***
S. aureus	Negative Control (DMSO)	0.00 ± 0.00	0	1	ns



Fig. 5: Effect on Sewages Water of antibacterial activity of different concentration of methanol Extracts of Water Hyacinth Plants (Flowers, Leaves, Roots).

4. Discussion

The water hyacinth plants extracts (Flower, Leaves, and Roots) had a high value of antimicrobial active ingredients compared to other plant extract that was compared with previously published studies. Due to environmental conditions flowers, leaves and roots extract have different efficacy value against sewage water associated bacterial pathogens. The results were in well agreement with previously published research in Iran (Rufchaei *et al.,* 2021). In previous, several researchers have also evaluated the antimicrobial efficacy of water hyacinth plants extracts. In one study, revealed that the water hyacinth plants extract significantly reduce the growth of *E. coli* on sewage water. Beside the significant antimicrobial activity was reported for water hyacinth plants extracts such as flowers, leaves and roots against several microbial strains. Out of three extracts leave extract delivered a higher antimicrobial activity than roots (Fareed *et al.,* 2008). Similarly, water hyacinth plants extract has higher antimicrobial activity against *E. coli*, *S. aureus*, *P. aeruginosa* and *Klebsiella* spp. In another findings, Zhou *et al.* also demonstrated in their study the activity was depend on pH, concentration and dose exposure (Zhou *et al.,* 2009).

In accordance with other research, our results aggreged with previous findings and *E. coli* isolates showed highly significant zone of inhibition on water hyacinth plants extract. Saraf *et al.* (2018) revealed that they found less effective antimicrobial activity of water hyacinth plants extracts against *E. coli*, *Klebsiella* spp. *S. aureus*. *E. coli* isolates exposed highest zone of inhibition with 10.56mm which is comparatively higher than the previous study 8.3mm in (Haggag *et al.,* 2017). In accordance with Madge *et al.* (2023) findings, 20 mm zone of inhibition found in *E. coli* against water hyacinth plants extract whereas our research revealed 12mm zone of inhibition of water hyacinth plants extract (roots, flower and leaves). Similar findings were recorded in Iran; the highest antimicrobial activities were showed. In another research, Joshi and Kaur *et al.* (2013) exhibited that the zone of inhibition was resistance whereas our study recorded *P. aeruginosa* produce zone of inhibition 10.33mm in 200% concentration of water hyacinth plants extract (flower), 9.33mm zone of inhibition on 100% concentration of leaf extract of water hyacinth plants and 10.33mm found in 25% concentration of root extracts.

According to Sharaf *et al.* (2018) *P. aeruginosa* reported good antimicrobial activity with 17mm to 20 mm zone of inhibition in water hyacinth plants extracts which support our findings respectively. *Klebsiella* spp. the highest zone of inhibition 15mm of extract which is similar to previously published studies. Very limited research was conducted on water hyacinth plants extract on waste water treatment in Bangladesh. Our study strongly focused antimicrobial

efficacy of water hyacinth plants extract (flower, leaves and roots) on sewage water isolates which will further applied on waste water treatment. Water hyacinth plants can easily grow in sewage water and reduce water pH and uptake all forms of pathogenic bacteria that causes significant human and animal health hazard. If we applied water hyacinth plants in sewage water it will be beneficial for fish and other animals in water.

5. Conclusion

Our obtained result suggests that, water hyacinth plants extract have many active bioactive components that work on antibiotic resistance isolates from sewage water to remove or kill pathogen rapidly. Additionally, antimicrobial efficacy of water hyacinth plants extract such as leaves, flowers and roots actively working to remove heavy metals and toxic metals from sewage water. Hervesting water hyacinth plants in local area will have significant effects on pharmaceutical products as well as animal feed production as rich nutrients. Due to eco-friendly nature and easily availability the water hyacinth plants can be used as sewage water treatment options which drastically absorb harmful substances from environment.

6. Author Contributions

Study design and conceptualization: R.A. & M.R.A.: Laboratory experiment: M.R.A.; M.S.A.; A.A.; P.S.: Sample collection: M.R.A.; M.S.A.: Manuscript drafting and editing: M.R.A.; M.A.H.; Z.A.S.: Table and figure preparation: M.A.H.; Z.A.S.: Data analysis: Z.A.S. Final Review and editing: M.A.H.; R.A.: All authors checked the full manuscript and gave their approval befor final publication.

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8. Conflicts of Interest

The authors have no conflicts of Interest.

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