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Phytochemical and Antimicrobial Evaluation of *Eichhornia crassipes* Extracts Against Clinical and Hospital Effluent Pathogens in Savar, Bangladesh

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Abstract

Water hyacinth plants are available in rural areas and have antimicrobial, antifungal and antioxidant activities. A study was conducted at Savar, Bangladesh, to evaluate the phytochemical composition and antimicrobial activity of *Eichhornia crassipes* (water hyacinth) extracts against pathogens derived from clinical and hospital wastewater. The study aimed to assess the potential of *E. crassipes* as a source of natural antimicrobial agents, particularly against bacteria and fungi isolated from clinical and hospital effluent samples. A total of 15 clinical samples and 15 hospital derived effluents samples were selected for identification of isolates and *E. crassipes* leave, roots and flower extract were collected for antimicrobial efficacy and antioxidants determination from Savar region, Bangladesh. Selective culture media like Mannitol salt agar (MSA, Eosine Methylene Blue agar (EMB) and Cetrimide agar was applied for isolation of bacteria whereas phytochemical properties was evaluated by using different selective methods. Out of 15 clinical isolates highest 12(80%) was found *Staphylococcus* spp. whereas 93.33% *Pseudomonas* spp. were detected in hospital derived effluents. The total phenolic compounds of metanolic extracts of water hyacinth plants (flower) were 892.201±295.535 gm, 552.264±14.877 mg (leaves) and 224.591±17.638 mg (root) measured compared with gallic acid standard. The total flavonoids components of water hyacinth plants (flower) 329.211 ± 9.846 mg higher than leave extract 263.421 ± 4.297mg and 119.561 ± 5.407 mg of root extracts. The highest antimicrobial activity of water hyacinth plant flower extract at 100% concentration was found against both clinical and hospital derived effluents isolates. Water hyacinth plant extracts (flower, leaves and roots) have antimicrobial and antioxidants activity against *E. coli*, *Pseudomonas* spp. and *Staphylococcus* spp.

Keywords: *Eichhornia crassipes*, Phenolic compounds, Flavonoids, Gallic acid, and Clinical isolates.

1. Introduction

Eichhornia crassipes, commonly known as water hyacinth, is a fast-growing aquatic weed notorious for UniversePG | www.universepg.com

its negative impact on aquatic ecosystems and related human activities. It has been recorded in Egypt, India, Australia, and many other parts of the world, where it

rapidly covers water bodies, adversely affecting fisheries, irrigation systems, navigation, and hydroelectric projects (Taqi, 2019). *E. crassipes* is a potent reservoir of bioactive phytochemicals such as alkaloids, flavonoids, phenolic compounds, and tannins, which exhibit various biological activities including anticancer, antiviral, antibacterial, and antifungal effects (Abedin *et al.*, 2020; Piya *et al.*, 2022; Rufchaei *et al.*, 2022).

Nature has served as a valuable source of medicinal agents for thousands of years, with many modern drugs being derived from natural products based on traditional medicinal uses (Kirubakaran *et al.*, 2023). Approximately 80% of the world's population still relies primarily on traditional plant-based medicines for their primary healthcare needs (Zemede *et al.*, 2024). However, the indiscriminate and extensive use of commercial antimicrobial drugs has led to the emergence of resistant human pathogenic microorganisms, which has become a serious global health challenge (Irfan *et al.*, 2022). This alarming rise in antimicrobial resistance, coupled with the side effects of certain antibiotics and the appearance of previously uncommon infections, has urged the scientific community to search for new and effective antimicrobial agents. Both chemical synthesis and the exploration of natural products from living organisms are major avenues for discovering novel bioactive compounds (Kiristos *et al.*, 2018).

Among natural sources, plants are particularly rich in biologically active compounds with potential chemotherapeutic applications. Herbal medicines have long been the foundation of healthcare in developing countries, and their popularity is now increasing globally (Kiristos *et al.*, 2018). The vast diversity of medicinal plants provides an alternative and promising strategy in the search for new drugs which will be effective on several infectious diseases. Furthermore, the plant is rich in enzymatic antioxidants like catalase, peroxidase, and superoxide dismutase, as well as non-enzymatic antioxidants such as flavonoids, phenolics, and carotenoids. These constituents contribute significantly to the plant's ability to combat oxidative stress by scavenging reactive oxygen species (ROS) and free radicals, thus protecting cellular biomolecules from damage (Priya and Jeyanthi, 2024).

Oxidative stress disrupts the prooxidant-antioxidant balance and leads to lipid peroxidation, protein oxidation, and DNA damage, ultimately resulting in cell dysfunction and death. Previously published studies in Iran, first researchers observed in Guilan University, water hyacinth plants rapidly increasing on water and a threat for environment, decreasing water transfer capacity, and fishing respiration problems (Rufchaei *et al.*, 2022). In contrast, water hyacinth plants have some beneficial roles if the plants extract chemical composition explored it can used as cattle feed which reduce the feed cost and increase milk and meat production (Rufchaei *et al.*, 2022; Talukder *et al.*, 2020). It also has capabilities to remove heavy metals from water. Savar, a rapidly urbanizing area near the capital Dhaka, is home to a growing number of hospitals, clinics, and industrial zones, which collectively contribute to significant environmental pollution, particularly in the form of untreated or poorly treated effluents. These wastewaters often harbor multidrug-resistant (MDR) pathogenic microorganisms, posing a serious public health threat to the surrounding communities and ecosystems. Despite the urgent need for alternative antimicrobial strategies, limited research has been conducted on environmentally sustainable, locally available resources with antimicrobial potential.

E. crassipes (commonly known as water hyacinth) is an invasive aquatic plant widely distributed in the water bodies of Savar and other parts of Bangladesh. While often considered an environmental nuisance due to its rapid growth and obstruction of waterways, this plant also contains a rich diversity of phytochemicals with documented antimicrobial and antioxidant properties. However, the bioactivity of its extracts against pathogens specific to hospital and clinical effluents in the Savar region remains underexplored. This study aims to bridge that gap by evaluating the phytochemical profile and antimicrobial potential of *Eichhornia crassipes* extracts specifically against MDR pathogens isolated from local effluent sources. The findings could offer a cost-effective, eco-friendly solution to combat antimicrobial resistance and promote wastewater bio-remediation using a plant already abundant in the region. Furthermore, the study aligns with national priorities in Bangladesh to address

AMR challenges and improve environmental health through sustainable, indigenous resources.

2. Materials and Methods

Ethical Approval

This study was approved by the Department of Microbiology, Faculty of Health Sciences, Gono Bishwabidyalay, Savar, Dhaka-1344, Bangladesh. Ethical clearance was not required, as the research did not involve human or animal subjects.

Study Area Selection and Sample Collection

The study was conducted at the Microbiology Research Laboratory, Department of Microbiology, Gono Bishwabidyalay, Savar, Dhaka, between July 2024 and January 2025. Aerial (flowers, leaves) and subterranean (roots) parts of *Eichhornia crassipes* were aseptically collected from five distinct locations - Birulia, Nilachor, Usubpur, Beribadh, and Kheyaghat - using sterile zip-lock polyethylene bags (thickness: 30–100 µm; dimensions: 175 mm × 100 mm). Samples were promptly transported to the laboratory under controlled conditions for bacteriological evaluation and were systematically coded based on their respective collection sites.

Preparation of Plant Extracts

Water hyacinth (flowers, leaves, and roots) samples underwent initial rinsing with tap water to remove superficial contaminants. Subsequently, the botanical specimens were air-dried over several days, followed by a 24-hour period of convective oven drying at a temperature not exceeding 50°C. This precisely controlled dehydration regimen was implemented to enhance the efficiency of subsequent grinding. The prepared, dried plant material was then mechanically ground into a coarse particulate fraction using a high-capacity Walton grinding apparatus. All preparation procedures were conducted within the Phytochemical Research Laboratory, Department of Pharmacy, Gono Bishwabidyalay (Ahmad *et al.*, 2018; Rufchaei *et al.*, 2022).

Methanol Extracts

A 300g sample of pulverized Water Hyacinth (flowers, leaves, and roots) was accurately weighed and introduced into a conical flask containing approximately 2.5L, 1L, or 1.5L of methanol (depending on

the specific part). This suspension was left for 14 days with periodical stirring to allow for the extraction of active ingredients. Following the maceration, the methanolic supernatant was separated from the plant residue by filtration through a clean, white cloth. The resultant filtrate was then concentrated using a rotary evaporator (Bibby RE-200, Sterilin Ltd, UK) connected to a water bath, operating at a rotation speed of 5-6 rpm under low temperature to prevent the degradation of thermolabile constituents. Upon complete evaporation of the solvent, the resulting crude extract, characterized as a dark, viscous concentrate, was collected and subsequently stored at +4°C to ensure its preservation for future research applications (Al-Snafi 2022; Jahan *et al.*, 2023).

Isolation and Identification of Bacteria

Bacteria were isolated from 15 clinical and 15 hospital effluent samples from different locations in the Savar region of Bangladesh as per standard microbiological protocols. For primary isolation, samples were initially cultured on nutrient agar. Subsequent subcultures were performed on selective and differential media, including MacConkey agar and specialized selective agars such as Eosin Methylene Blue (EMB) agar for *Escherichia coli* and Mannitol Salt Agar (MSA) for *Staphylococcus* spp., Cetrimide agar for *Pseudomonas* spp. The morphology of the bacterial isolates was confirmed through microscopic examination. Further identification was achieved via a series of biochemical tests. All culture media utilized in this study were procured from HI Media Private Ltd., India (Cheesbrough, 2006).

Chemicals and Reagents

The chemicals and reagents for this study were sourced from several suppliers. For Gram's staining, crystal violet, Gram's iodine, safranin, and 95% acetone alcohol were used. Other essential reagents included immersion oil, 3% hydrogen peroxide, oxidase test reagent (Tetramethyl-p-phenylenediamine dihydrochloride), VP reagent-A (5% alpha-naphthol in absolute ethyl alcohol), VP reagent-B (40% potassium hydroxide containing 0.3% creatine), Kovac's indole reagent (4-dimethylamino-benzaldehyde, concentrated HCl), Methyl red indicator for MR test, 80% glycerin, and other common laboratory reagents and chemicals. All chemicals were purchased from HiMedia Private

Ltd., India. Methanol and ethanol were purchased from Merck Co. (Germany). Folin-Ciocalteu reagent (FCR), gallic acid, sodium carbonate, aluminum chloride, sodium nitrate, sodium phosphate, ammonium molybdate, and NaOH were purchased from Sigma-Aldrich (St. Louis, MO, USA) (Rufchaei *et al.*, 2022).

Determination of Total Phenolics

The content of total phenolics of Water Hyacinth plants (flowers, leaves, roots) was determined according to the Folin-Ciocalteu reagent (FCR) method, where this reagent was used as an oxidizing agent and Gallic Acid (GA) was used as a standard (Rufchaei *et al.*, 2022). The reaction mixture was prepared by mixing 0.4 mL of plant extract with 2 mL of Folin-Ciocalteu reagent, 3 mL of 7.5% NaHCO₃, and 10 mL of distilled water. After mixing the extracts with water, the mixture was incubated at 25°C for 30 minutes, and the absorbance was measured at 760 nm with a UV-spectrophotometer (Shimadzu UV-1800, Japan). All tests were performed in triplicate for accuracy. The total content of phenolic compounds in methanolic extracts and its four fractions was calculated as gallic acid equivalent (GAE) by the following formula:

$$C = (x \times V) / M$$

Where,

C = total content of phenolic compounds as mg GAE in each gram of dried extract.

x = GAE concentration in mg/mL, present in that particular sample concentration.

V = Final volume of the solution in mL.

M = Mass of the sample in the final solution in grams.

Determination of Total Flavonoids

The total flavonoid content was determined by the aluminum chloride colorimetric method, as described by Rufchaei *et al.*, 2022, with slight modifications. Quercetin was used as a standard, and the total flavonoid content of the extractives was expressed as mg of Quercetin Equivalent (QE) per gram of dried extract. The reaction mixture was prepared by mixing 0.5 mL of plant extract with 150 µL of 5% sodium nitrate and 2.5 mL of distilled water in each test tube. After 5 minutes, 0.3 mL of 10% AlCl₃ was added. Subsequently, 1 mL of 4% NaOH was added to the mixture within the next 60 seconds. The test tubes

were then incubated at room temperature for 15 minutes to complete the reaction. Absorbance was measured at 510 nm using a spectrophotometer (Shimadzu UV-1800, Japan). All tests were performed in triplicate for accuracy. The total content of flavonoid compounds in the methanolic extract and its four fractions was calculated as Quercetin equivalent (QE) using the following formula:

$$C = (x \times V) / M$$

Where,

C = total content of flavonoid compounds as mg QE in each gram of dried extract.

x = QE concentration in mg/mL, present in that particular sample concentration.

V = Final volume of the solution in mL.

M = Mass of the sample in the final solution in grams.

Evaluation of Antioxidant Activity

The total antioxidant capacity of different extractives from Water Hyacinth (*E. crassipes*), specifically from its flowers, roots, and leaves, was determined according to the method of Prieto *et al.* (1999) with some modifications. The phosphomolybdenum method is based on the reduction of Molybdenum (VI) to Molybdenum (V) by antioxidant compounds, followed by the subsequent formation of a green phosphate/Mo (V) complex at an acidic pH. For the assay, 0.5 mL of plant extract was mixed with 3 mL of the reaction mixture, which consisted of 0.6 M sulfuric acid, 28 mM sodium phosphate, 1% ammonium molybdate.

The reaction tubes were then incubated at 95°C for 10 minutes. Absorbance was measured at 695 nm using a spectrophotometer (Shimadzu UV-1800, Japan).

DPPH (1, 1-diphenyl-2-picrylhydrazyl) Free Radical Scavenging Assay

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of various compounds and medicinal plant extracts was evaluated. A 1.6 mL methanol solution of the plant extract was added to 2.4 mL of a 0.1 mM DPPH methanol solution. The mixture was then incubated at room temperature for 30 minutes in a dark place. Absorbance was measured at 517 nm using a spectrophotometer (Shimadzu UV-1800, Japan) (Jahan *et al.*, 2023). The percentage (%)

scavenging activity of DPPH radicals was calculated using the following equation:

$$\% \text{ Scavenging Activity} = [(Ac - As) / Ac] \times 100$$

Where,

Ac = Absorbance of the control (DPPH solution without extract)⁴

As = Absorbance of the extract/standard solution

Antimicrobial Activity Evaluation

According to the Clinical and Laboratory Standards Institute (CLSI) guidelines (2021), the antimicrobial activity of water hyacinth plant extracts was measured by the agar well diffusion method against clinical and hospital effluent *E. coli* and *Staphylococcus* spp. and *Pseudomas* spp. isolates. For this purpose, 0.1 mL of each diluted bacterial suspension was aseptically swabbed onto Muller-Hinton agar plates. Subsequently, 6 mm wells were created using a sterile cork borer, and 50 µL of each plant extract at various concentrations (e.g., 25 mg/mL, 50 mg/mL, 100 mg/mL, 200 mg/mL) were introduced into the wells. After loading the extracts, all plates were incubated at 36°C for 24 hours. Following the incubation period, the diameters of the zones of inhibition were measured in millimeters (mm) according to CLSI guidelines. For the positive control, an Amikacin antibiotic disc was applied as a standard (Rathod and Pande, 2018;

Kebede and Zewde, 2023). A 10% Dimethyl Sulfoxide (DMSO) solution served as a negative control. Antibiotic discs were purchased from HI Media Private Ltd., India.

Statistical Analysis

All experiments will be performed in triplicate, and the result was expressed as the mean ± standard deviation (SD). Statistical analysis was performed using appropriate software (e.g., GraphPad Prism, SPSS). One-way analysis of variance (ANOVA), followed by Tukey's post-hoc test, was used to determine significant differences between groups. A p-value of < 0.05 was considered statistically significant. Antimicrobial activity was analysis by using R studio version 2024.12.11+563.

3. Results

Determination of Total Phenolics

Table 1 represent the absorbance values (mean± standard deviation) of water hyacinth plant extracts determined by Folin-Ciocalteu (FCR) assay for total phenolic compound with different concentrations and compared with standard curve for GA (Gallic Acid) as shown in Fig. 1. The highest absorbance value (2.187±0.089) was recorded in 200 concentrations followed by 100 concentration (1.11±0.062) which support strong antioxidant activity.

Table 1: Absorbance of GA (standard) at different concentrations after treatment with FCR.

Conc. (µg/ml)	Absorbance			(Mean ± STD)
	a	b	c	
25	0.354	0.347	0.349	0.359 ± 0.007
50	0.618	0.567	0.592	0.605 ± 0.021
100	1.104	0.986	1.127	1.116 ± 0.062
200	2.187	1.993	2.174	2.181 ± 0.089

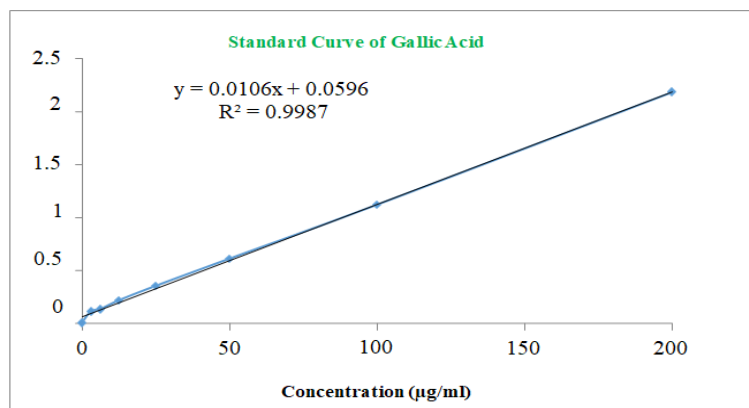


Fig. 1: Standard curve of GA for the determination of total phenolics.

The phenolic content was found in methanolic extract, water hyacinth plant flower extracts (892.201±295.535mg of GAE/gm of dried extract) at a concentration of 100 µg/mL followed by water hyacinth plant

leave extract (552.264±14.877 mg of GAE / gm of dried extract), water hyacinth plant root extracts (224.591±17.638mg of GAE/gm of dried extract) respectively (Table 2).

Table 2: Determination of total phenolic contents of Water Hyacinth Plant Extracts ((Flowers, Leaves, Roots).

Sample Name	Conc. (µl/ml)	Absorbance			mgGAE/gm of dried sample			mg GAE/gm of dried sample (Mean± STD)
		a	b	c	a	b	c	
W. H.F.	100	1.443	0.846	0.727	1305.094	741.8868	629.6226	892.201±295.535
W. H.L.	100	0.624	0.662	0.649	532.4528	568.319	556.0377	552.264±14.877
W. H.R.	100	0.272	0.305	0.316	200.3774	232%	241.8868	224.591±17.638

Determination of Total Flavonoids

Table 3 revealed that total flavonoids of Water Hyacinth Plant Extracts ((Flowers, Leaves, Roots) were determined using well known aluminum chloride

colorimetric method using Quercetin as standard. The highest absorbance a value (0.749±0.062) was reported in 100 concentrations with compared to standard curve of Quercetin (Fig. 2).

Table 3: Absorbance of Quercetin (standard) at different concentrations for quantitative determination of total flavonoids.

Conc. (µg/ml)	Absorbance			Absorbance (Mean ± STD)
	a	b	c	
3.125	0.115	0.095	0.009	0.073 ± 0.046
6.25	0.111	0.099	0.025	0.078 ± 0.038
12.5	0.147	0.119	0.103	0.123 ± 0.018
25	0.207	0.206	0.202	0.205 ± 0.002
50	0.391	0.356	0.462	0.403 ± 0.044
100	0.746	0.675	0.827	0.749 ± 0.062

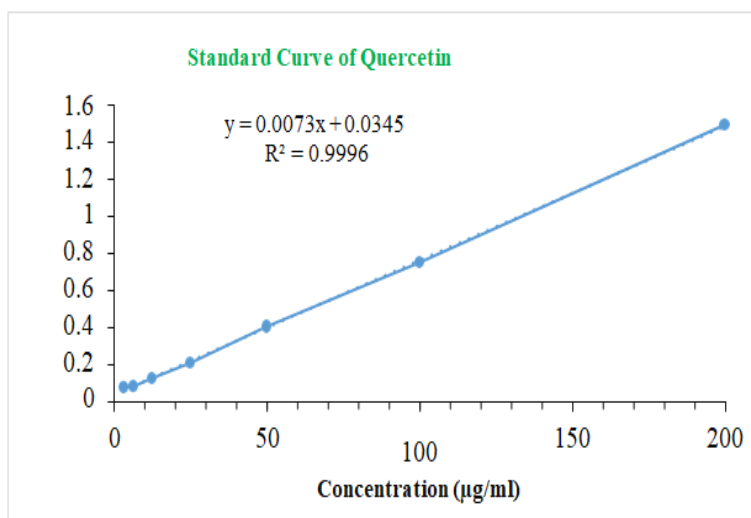


Fig. 2: Standard curve of Quercetin for the determination of total flavonoids.

The flavonoids content of Water Hyacinth Plant Extracts ((Flowers, Leaves, Roots) were 329.211 ± 9.846, 263.421 ± 4.297, 119.561 ± 5.407 mg of

QE/gm of dried extractives at a concentration of 100µg/mL, respectively (Table 4).

Table 4: Determination of total flavonoid contents of Water Hyacinth Plant Extracts ((Flowers, Leaves, Roots).

Sample Name	Conc. (µl/ml)	Absorbance			mgGAE/gm of dried sample			mg GAE/gm of dried sample (Mean± STD)
		a	b	c	a	b	c	
W. H.F.	100	0.177	0.174	0.183	326.579	318.684	342.368	329.211 ± 9.846
W. H.L.	100	0.151	0.153	0.155	258.158	263.421	268.684	263.421 ± 4.297
W. H.R.	100	0.101	0.096	0.098	126.579	113.421	118.684	119.561 ± 5.407

Total Antioxidant Activity

The total antioxidant activity of different extractives was assessed by phosphomolybdenum method, based on the reduction of Mo (VI) to Mo (V) by the standard and the formation of green phosphate/ Mo (v) complex with a maximal absorption at 695 nm. The absorbance of AA at a concentration of 100 (µg/mL) were 2.368 respectively. Comparing the results, it is observed that W.H.P. had considerable total antioxidant activity between AA. Total antioxidant activity of Water Hyacinth Plant Extracts ((Flowers, Leaves, Roots) and standard (AA) was depicted in **Table 5**.

DPPH Free Radical Scavenging Assay

Table 6 represents the DPPH free radical scavenging activity of three Water Hyacinth extracts - W.H.F. (flower), W.H.L. (leaf), and W.H.R. (root) - compared to a standard antioxidant (BHT) at different concentrations (5–40 µg/ml). W.H.F. exhibits the strongest activity among plant extracts (IC₅₀ = 35.5 µg/ml), with scavenging increasing from 7.20% to 56.77% as concentration rises. Consequently, W.H.L. shows moderate activity (IC₅₀ = 85 µg/ml), peaking at 22.57% at 40 µg/ml and W.H.R. demonstrates the lowest antioxidant effect (IC₅₀ = 155 µg/ml), with lower scavenging % whereas BHT (butylated hydroxytoluene) standard reported with the lowest IC₅₀ (9.8 µg/ml), indicating strong antioxidant potential.

Table 5: Determination of total antioxidant capacity of Water Hyacinth Plant Extracts ((Flowers, Leaves, Roots).

Sample	Conc. (µg/mL)	Absorbance			Absorbance (Mean ±STD)
		a	b	c	
AA	3.125	0.117	0.155	0.145	0.139
	6.25	0.177	0.236	0.228	0.214
	12.5	0.31	0.42	0.403	0.378
	25	0.579	0.801	0.771	0.717
	50	1.135	1.547	1.567	1.416
	100	2.315	2.413	2.376	2.368
W.H. F.	3.125	0.069	0.069	0.053	0.064
	6.25	0.088	0.079	0.077	0.081
	12.5	0.105	0.106	0.09	0.1
	25	0.148	0.134	0.121	0.134
	50	0.194	0.164	0.182	0.18
	100	0.267	0.229	0.261	0.252
W.H.L.	3.125	0.082	0.081	0.087	0.083
	6.25	0.105	0.104	0.104	0.104
	12.5	0.145	0.148	0.147	0.147
	25	0.231	0.233	0.232	0.232
	50	0.413	0.399	0.406	0.406
	100	0.614	0.605	0.634	0.618
W.H.R.	0.077	0.074	0.076	0.076	0.077
	0.085	0.084	0.086	0.085	0.085
	0.115	0.109	0.111	0.112	0.115
	0.17	0.162	0.158	0.163	0.17
	0.272	0.264	0.267	0.268	0.272
	0.266	0.473	0.493	0.411	0.266

Table 6: Determination of DPPH free radical scavenging activity of Water Hyacinth Plant Extracts ((Flowers, Leaves, Roots) and BHT at different concentrations.

Sample Name	Conc. (µg/ml)	% of Scavenging			% of Scavenging (Mean ± STD)	IC ₅₀ (µg/mL)
		a	b	c		
BHT	5	0.077	0.074	0.076		9.8
	10	0.085	0.084	0.086	21.51±0.846	
	20	0.115	0.109	0.111	32.442±0.923	
	30	0.17	0.162	0.158	45.993±0.257	
	40	0.272	0.264	0.267	61.333±0.922	
W.H.F.	5	8.271	7.528	5.808	7.202 ± 1.032	35.5
	10	14.035	14.555	16.288	14.959 ± 0.963	
	20	31.83	34.253	36.364	34.149 ± 1.852	
	30	44.737	37.892	46.212	42.947 ± 3.625	
	40	55.514	58.344	56.439	56.766 ± 1.178	
W.H.L.	5	1.377	6.008	5.882	4.422 ± 2.154	85
	10	3.129	3.129	5.757	4.005 ± 1.239	
	20	24.03	18.899	17.897	20.275 ± 2.686	
	30	16.896	16.395	18.648	17.313 ± 0.966	
	40	21.902	23.905	21.902	22.57 ± 0.944	
W.H.R.	5	2.381	2.46	2.819	2.553 ± 0.191	155
	10	3.008	3.69	4.289	3.662 ± 0.523	
	20	11.278	7.872	5.515	8.222 ± 2.366	
	30	8.271	11.193	7.966	9.143 ± 1.455	
	40	13.91	14.637	14.093	14.213 ± 0.309	

Antimicrobial activity of methanol Extracts of Water Hyacinth Plants (Flowers, Leaves, and Roots)

The highest antimicrobial activity of *E. crassipes* flower extract against *Staphylococcus* spp. Demonstrated at 200% concentration with 4.47 ± 5.68 mm

(Mean ± SD), followed by 3.67 ± 4.70 mm at 100% concentration whereas no activity was observed at 50% (0.00 ± 0.00 mm) concentration. Additionally, sample S2 & S3 exhibited moderate zone at 200% and 100% concentration while remaining samples showed no zone and compared with Amikacin (**Fig. 3**).

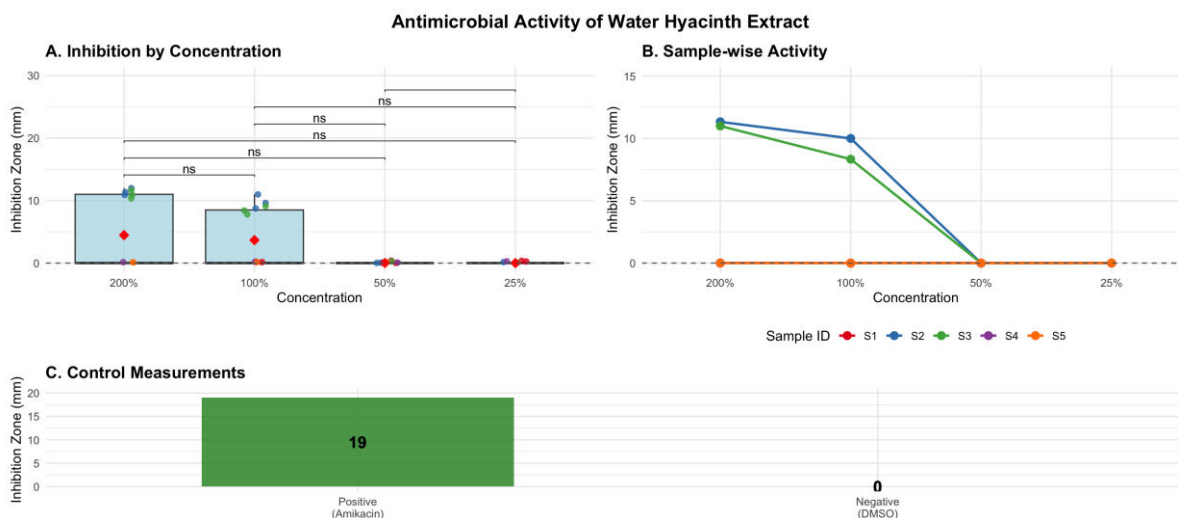


Fig. 3: Antimicrobial activity of water hyacinth flower extract on *Staphylococcus* spp. from clinical samples.

Fig. 4 demonstrated the significant antimicrobial efficacy of W.H.F extract against clinical *E. coli* isolates, with significant concentrations (200%: 5.53 ± 4.91 mm; 100%: 4.07 ± 5.20 mm) whereas isolates UniversePG | www.universepg.com

isolates, with significant concentrations (200%: 5.53 ± 4.91 mm; 100%: 4.07 ± 5.20 mm) whereas isolates

S1–S3 exhibited exclusive responsiveness to 200% extract (S1: 9.33±0.58 mm; S2: 11.33±0.58 mm; S3: 7.00±1.00 mm), and S4–S5 demonstrated sensitivity solely to 100% extract (S4: 11.00±1.00 mm; S5:

9.33±0.58 mm) respectively. The zone of inhibition was compared with positive control Amikacin (9mm) and negative control (DMSO: 0 mm).

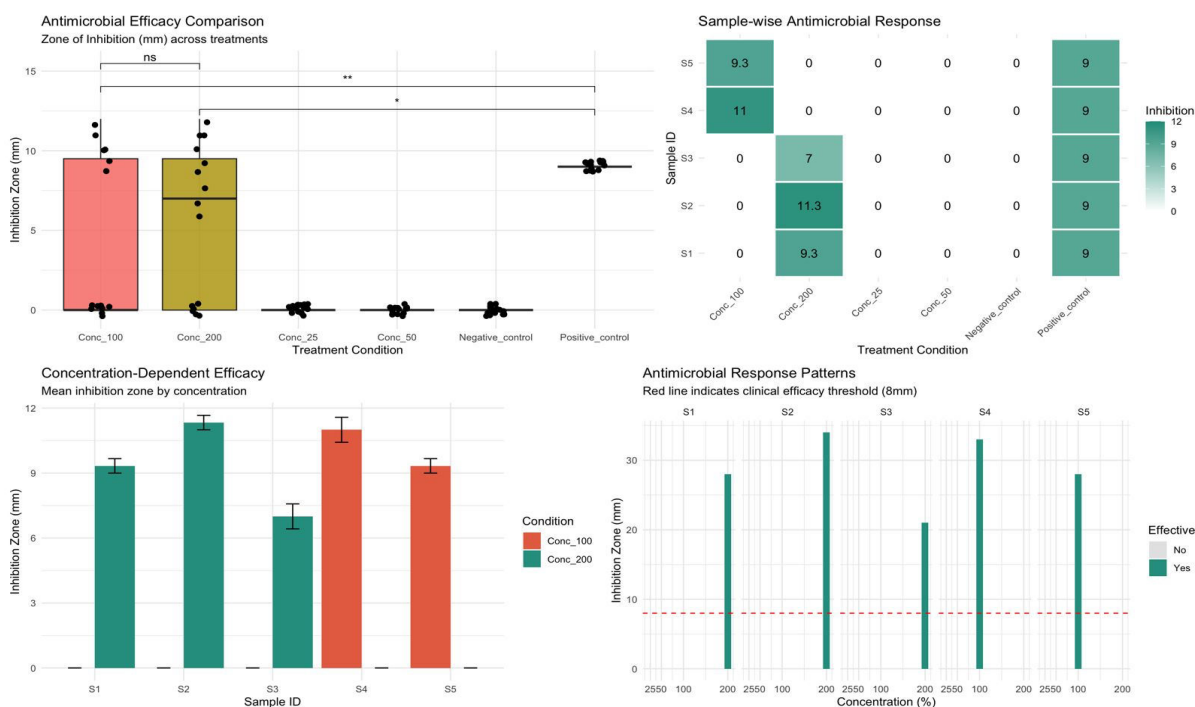


Fig. 4: Antimicrobial activity of water hyacinth flower extract on *E. coli* from clinical samples.

The root extract of *E. crassipes* demonstrated highest antimicrobial efficacy against *Staphylococcus* spp. clinical isolates, with mean inhibition zones declining from 13.53 ± 2.30 mm (200%) to 7.07 ± 1.10 mm (25%) - revealing a significant statistics below 50% concentration (*p* < 0.001). While higher concentrations (200%–50%) exhibited statistically comparable inhibition (WH_200 vs WH_100: *p* > 0.05).

Striking inter-isolate variability was observed at 200% (95% CI: 12.26–14.80) and 100% (95% CI: 11.59–14.54), evidenced by expanded confidence intervals and elevated standard deviations (SD ≥ 2.30), suggesting strain-specific susceptibility patterns - notably, isolate S5 exhibited exceptional responsiveness (200%: 17.00±1.00 mm) (**Fig. 5**).

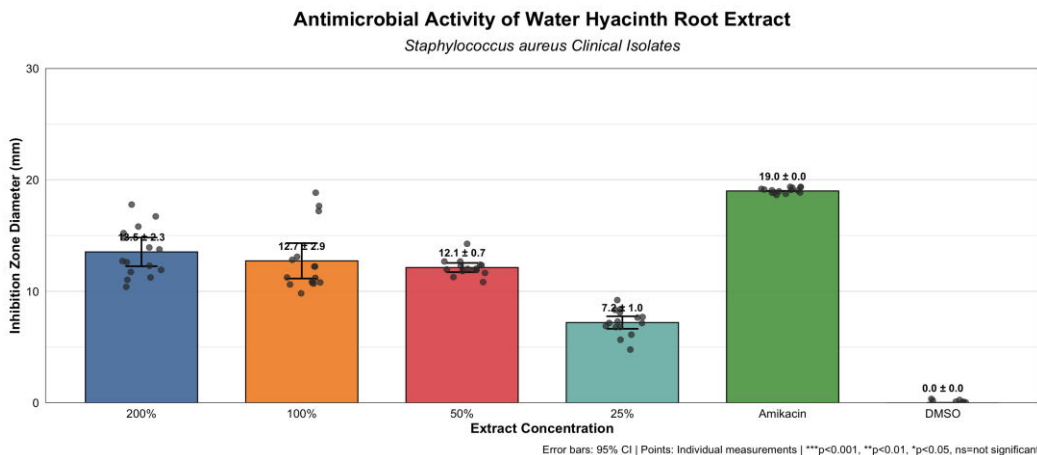


Fig. 5: Antimicrobial activity of water hyacinth Root extract on *Staphylococcus* spp. from clinical samples.

Fig. 6 illustrated the antimicrobial activity of root extract of *E. crassipes* against clinical *E. coli* isolates, with statistically significant inhibition zones across all tested concentrations (200%–25%; 11.40–11.87 mm, *p* > 0.05). Statistical analysis revealed differential extract stability: WH_50 demonstrated optimal repro-

ducibility (CV=5.5%, 95% CI: 11.05–11.75), whereas WH_25 exhibited marked dispersion (CV=11.4%, 95% CI: 10.99–12.47) while Amikacin's inhibition (9.00±0.00 mm) use as positive control and DMSO's use as negative control.

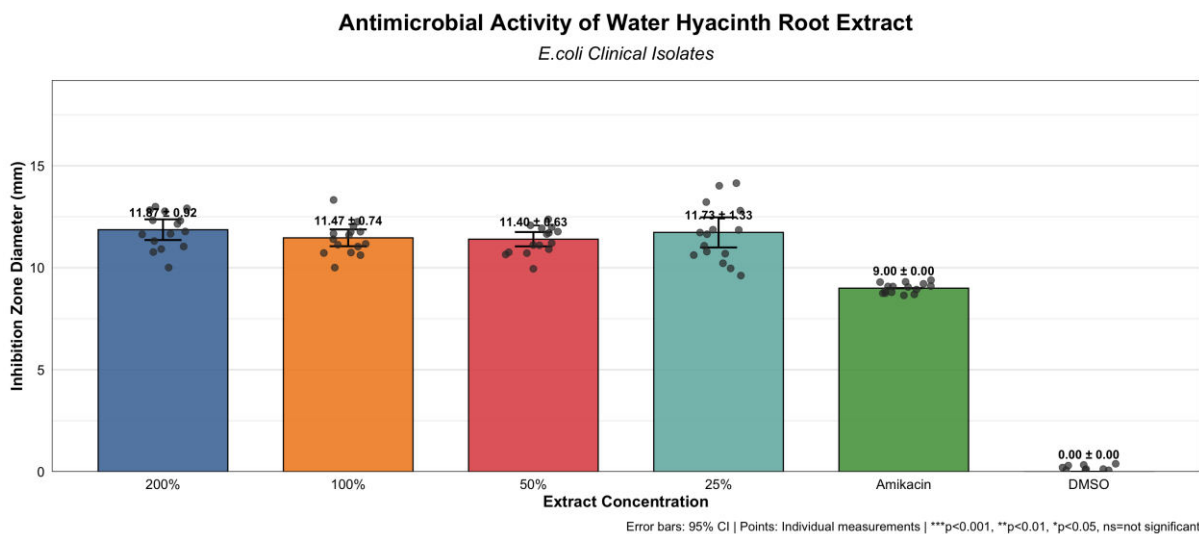


Fig. 6: Antimicrobial activity of water hyacinth Root extract on *E. coli* from clinical samples.

The leaf extract of *E. crassipes* exhibited significant concentration-dependent antimicrobial activity against clinical *E. coli* isolates, with mean inhibition zones progressively declining from 13.80 ± 0.77 mm at 200% to 10.60 ± 0.63 mm at 25% (*p* < 0.001). These

results demonstrate that water hyacinth leaves possess concentration-modulated antimicrobial compounds with clinically relevant efficacy against *E. coli*, though isolate-specific resistance mechanisms may necessitate phytochemical optimization (Fig. 7).

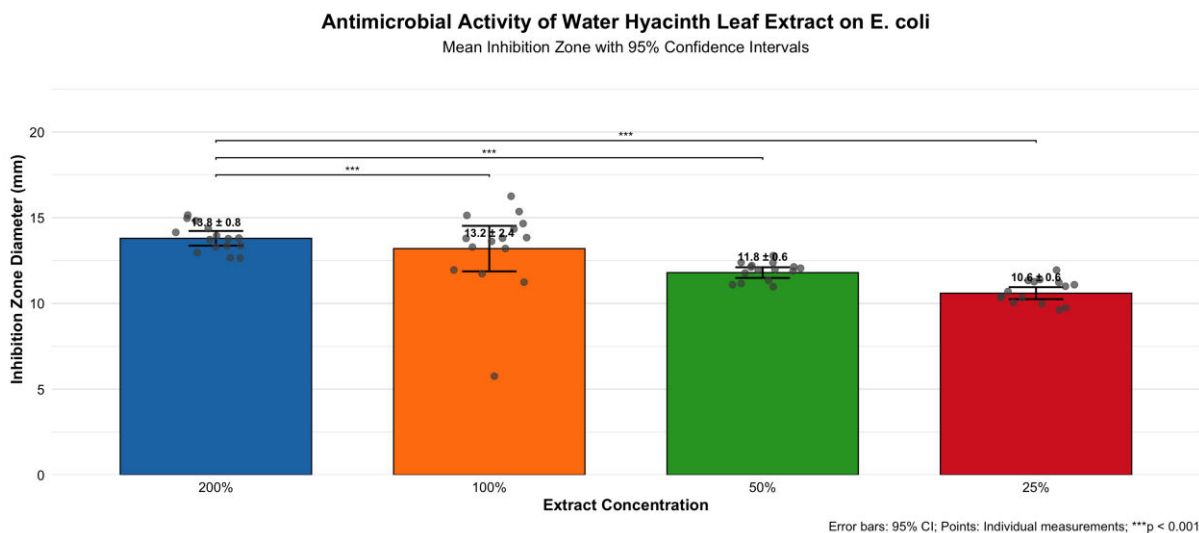


Fig. 7: Antimicrobial activity of water hyacinth leaves extract on *E. coli* from clinical samples.

The highest zone of inhibition was measured at 200% concentration (23.20 ± 1.26 mm; *p* < 0.001) of leave

extract of water hyacinth plants against *Staphylococcus* spp. of clinical isolates whereas the least zone

of inhibition was measured at 25% concentration (14.87±0.64 mm) respectively. These findings reveal water hyacinth leaves possess superior efficacy power against *Staphylococcus* spp. (Fig. 8).

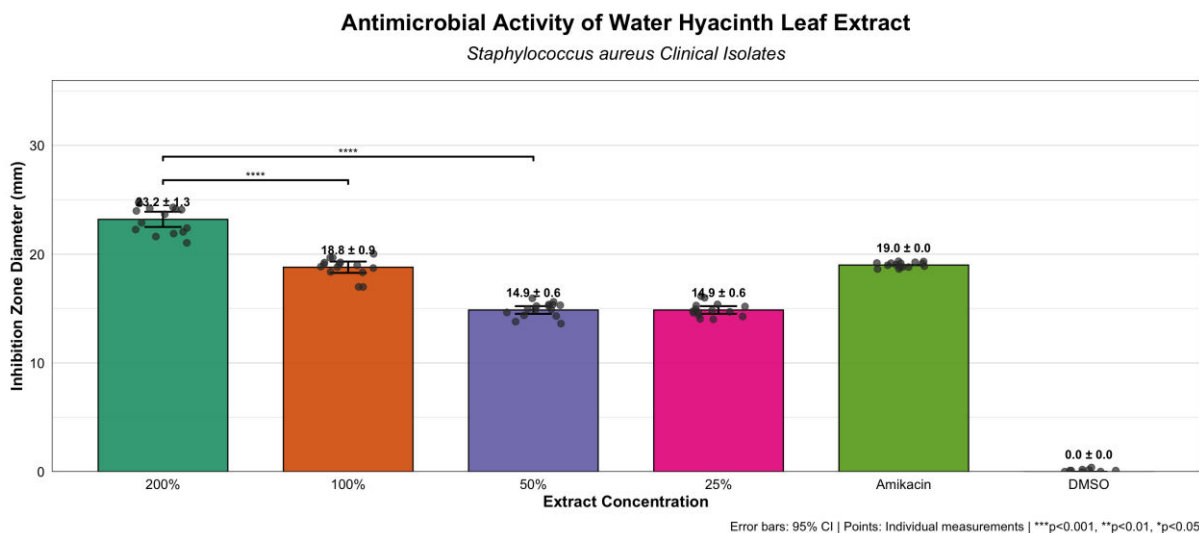


Fig. 8: Antimicrobial activity of water hyacinth leaves extract on *Staphylococcus aureus* from clinical samples.

The antimicrobial efficacy of water hyacinth flower extracts against *Pseudomonas* spp. demonstrated a clear concentration-dependent trend across all five sample groups (S1–S5). At the highest concentration (200%), all samples exhibited notable zones of inhibition (ranging from 19.3 ± 3.8 mm to 22.0 ± 2.5 mm), comparable to or exceeding the positive control

(Amikacin, 17 mm). Particularly, S4 showed the greatest antimicrobial potency with a mean inhibition of 22.0 ± 2.5 mm, suggesting a robust bactericidal effect (Fig. 9). These findings underscore the potential of concentrated water hyacinth floral extracts as a viable natural antimicrobial agent.

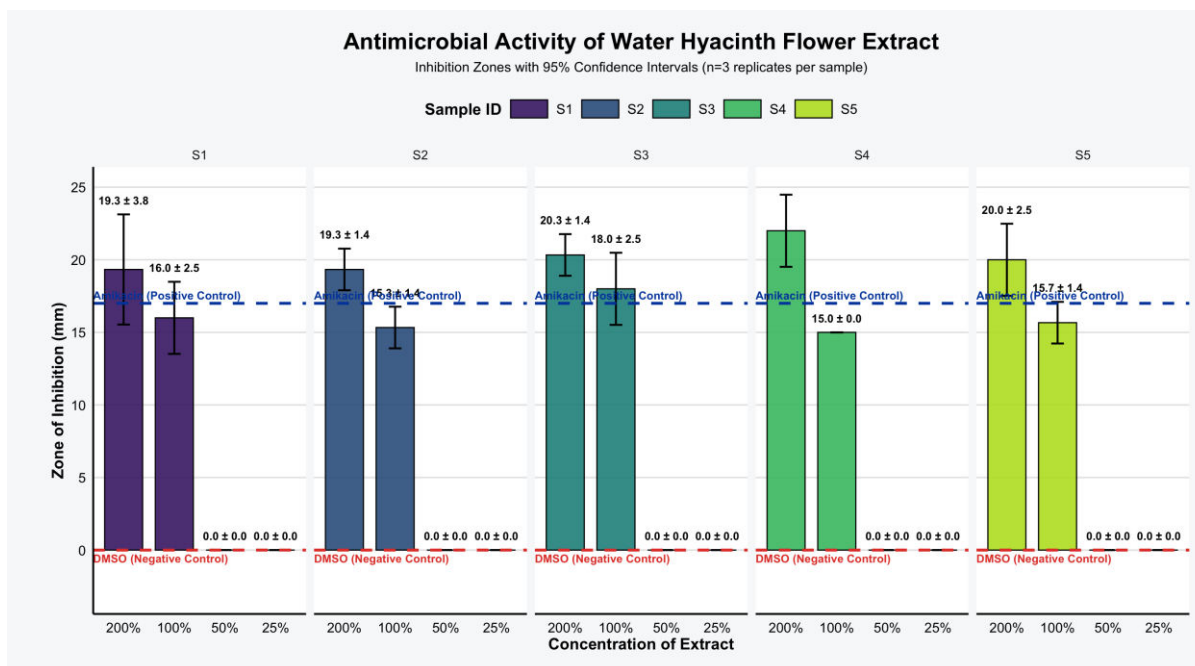


Fig. 9: Antimicrobial activity of water hyacinth flower extract on *Pseudomonas* spp from Hospital derived.

According to Figure 10, the highest zone of inhibition of water hyacinth leaf extracts against *Pseudomonas* UniversePG | www.universepg.com

spp. with measurable inhibition observed only at the highest concentration (200%) across all sample groups

(S1–S5) with 11.0 ± 2.5 mm (S1), 11.3 ± 1.4 mm (S2), 10.0 ± 2.5 mm (S3), 10.7 ± 3.8 mm (S4), and 13.0 ± 2.5 mm (S5) indicating highest activity. In contrast, all

lower concentrations (100%, 50%, and 25%) failed to elicit any detectable inhibitory effect, aligning with the performance of the negative control (DMSO).

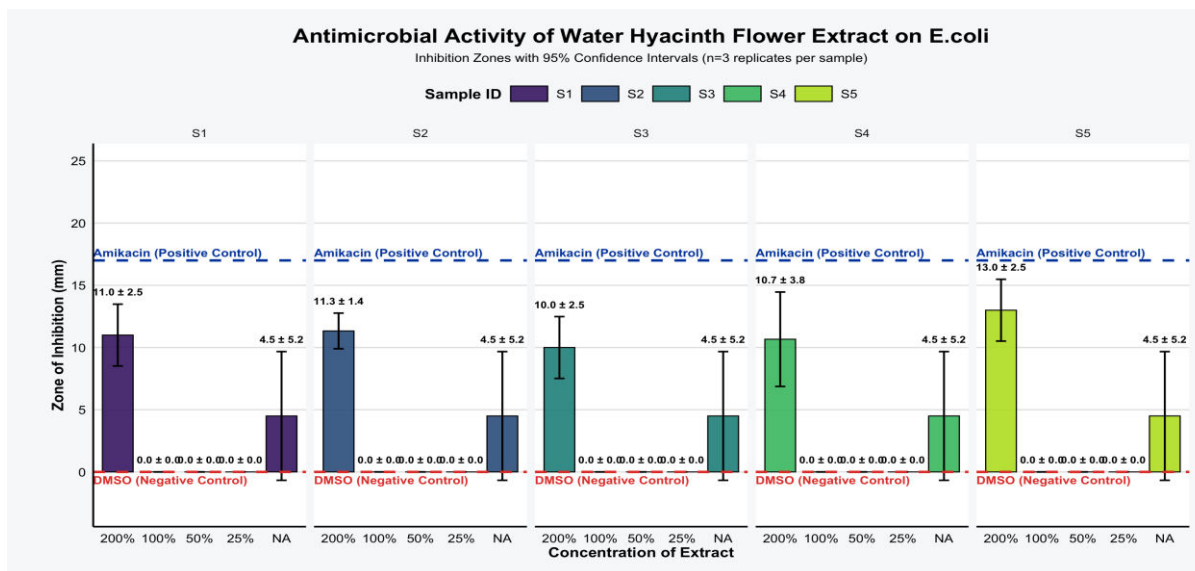


Fig. 10: Antimicrobial activity of water hyacinth flower extract on E.coli from Hospital derived.

According to Figure 11, the highest zone of inhibition of water hyacinth root extracts against *Pseudomonas* spp. at 200% concentration with 18.0 ± 2.5 mm (S1), 19.0 ± 0.0 mm (S2), 18.0 ± 2.5 mm (S3), 17.0 ± 2.5 mm (S4), and 17.3 ± 2.9 mm (S5) indicating highest activity.

In contrast, all lower concentrations (100%, 50%, and 25%) failed to elicit any detectable inhibitory effect, aligning with the performance of the negative control (DMSO).

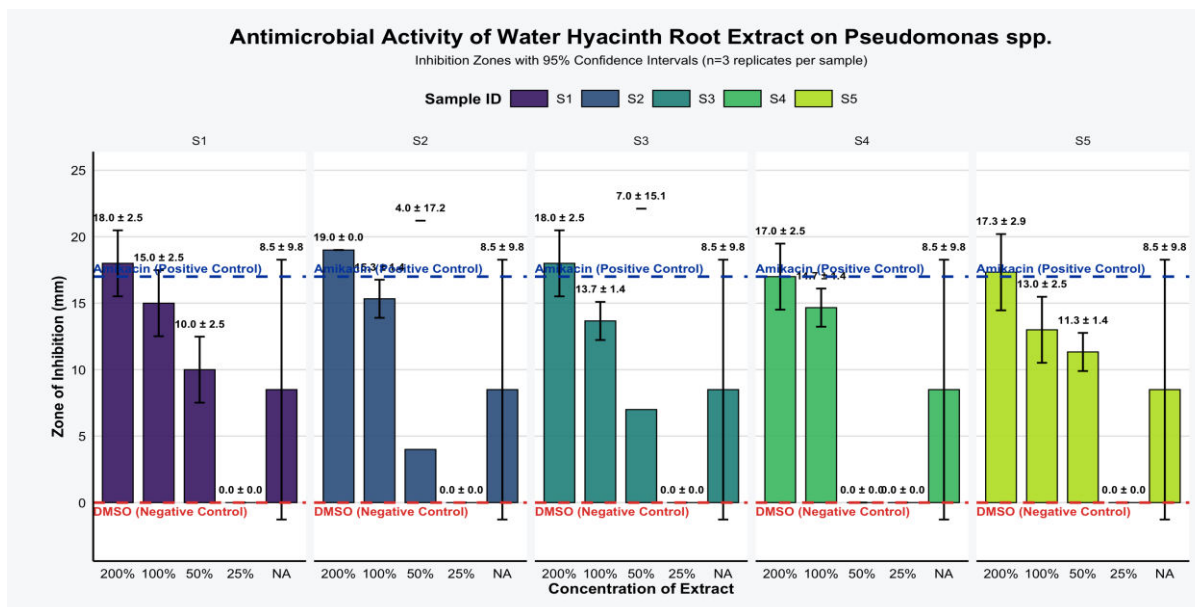


Fig. 11: Antimicrobial activity of water hyacinth root extract on *Pseudomonas* spp. from Hospital derived.

The water hyacinth root extract exhibited statistically significant antimicrobial activity against hospital effluents *E. coli*, with inhibition zones consistently exceeding the DMSO control (4.5 ± 5.2 mm; CI

spanning -0.7 – 9.7 mm). Maximal efficacy was observed at 200% concentration (11.7 – 12.3 mm; CI ± 1.4 – 2.5 mm), demonstrating robust bioactivity across all replicates (Fig. 12).

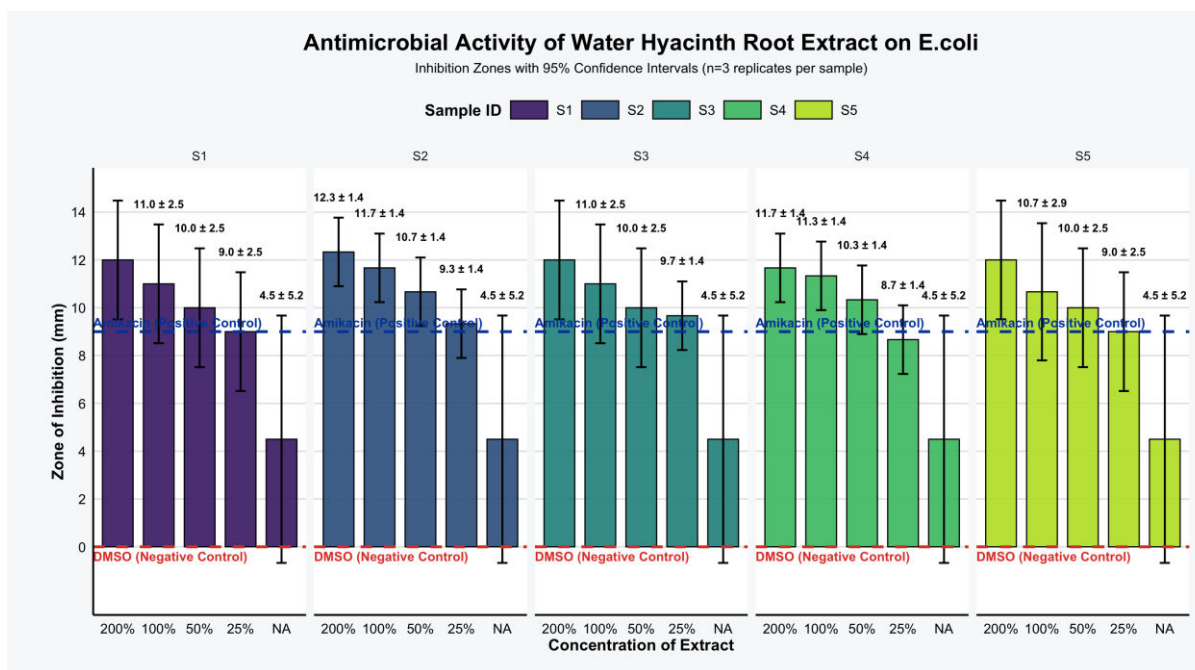


Fig. 12: Antimicrobial activity of water hyacinth root extract on *E. coli* from Hospital effluents.

The data delineate a pronounced concentration-dependent response across samples (S1-S5), with maximal bioactivity observed at 200% and 100% concentrations (mean range: 19.0–21.3 ± 0.0–3.8). Notably,

these elevated concentrations exhibited robust reproducibility, evidenced by minimal standard deviations (SD ≤ 1.53) and narrow confidence intervals (CI width ≤ 3.8) (Fig. 13).

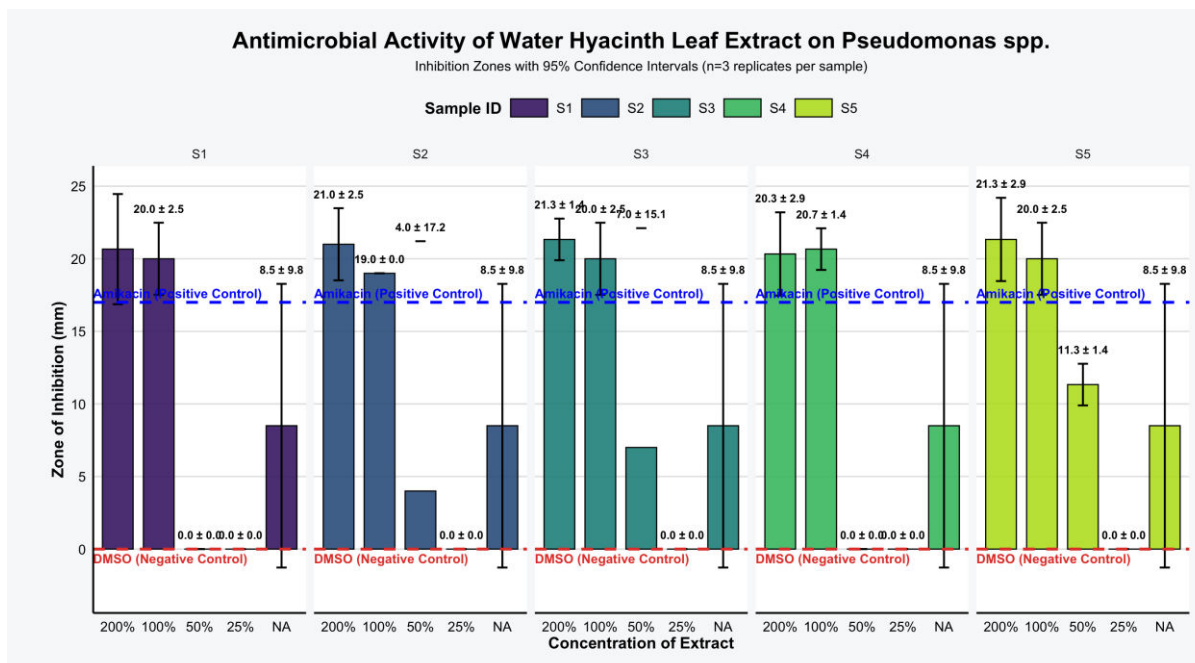


Fig. 13: Antimicrobial activity of water hyacinth leaf extract on *Pseudomonas* spp from Hospital derived.

The data demonstrate a concentration-dependent agonist response across biological replicates (S1-S5), with supra-maximal concentrations (200%) eliciting peak mean activity (19.3–22.0 ± 0.6–1.5 SD), signi-

ficantly exceeding physiological levels (100%: 15.0–18.0 ± 0–1.0 SD). Negative controls (NA) exhibit stochastic baseline noise (mean = 8.5 ± 9.3 SD; 95% CI: -1.3–18.3), indicating non-significant signal.

Replicate consistency is evidenced by low dispersion at active concentrations, while Label_Y values (Upper_CI + 0.7) suggest vertical offset for graphical

representation. The response profile confirms a sigmoidal dose-efficacy relationship with an estimated EC₅₀ between 50–100% concentrations (**Fig.14**).

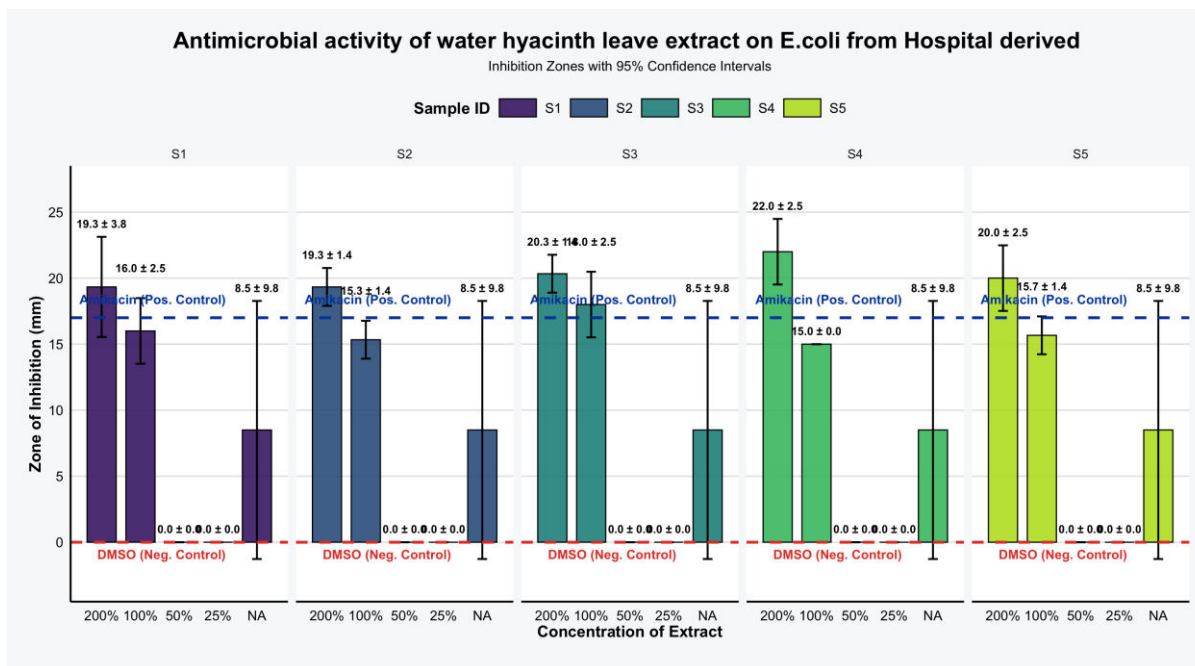


Fig. 14: Antimicrobial activity of water hyacinth leaves extract on *E. coli* from Hospital effluents.

4. Discussion

Multi-drug resistance pathogens isolated from different clinical and hospital derived effluents have significant public health concern beside water hyacinth plant extracts like as flower, leaves and roots have antimicrobial efficacy against this isolates. The total phenolic and flavonoid contents of plant extracts are significantly influenced by the polarity of the extraction solvent. Polar solvents exhibit a greater capacity to dissolve phenolic and flavonoid compounds, thereby enhancing their extraction efficiency (Mohsen and Ammar 2009). In the present study, the aerial parts of the plant were subjected to extraction using two polar solvents: distilled water and a hydro-methanolic mixture. The results demonstrated that the hydro-methanolic extract contained higher concentrations of total phenolics and flavonoids compared to the aqueous extract. These findings are consistent with those previously reported by Ho *et al.* and Rorong *et al.*, (Shanab *et al.*, 2010; HO *et al.*, 2012; Rorong *et al.*, 2012). In the present study, the presence of phenolic compounds, flavonoids, and DPPH radical scavenging activity was confirmed in the extracts of *E. crassipes* (water hyacinth). These

findings are in concordance with a similar investigation conducted in Iran, where Rufchaei *et al.*, (2022) also reported comparable results. Previous literature has documented the antimicrobial properties of *E. crassipes* extracts, with several studies highlighting its inhibitory effects against clinical and hospital-derived bacterial isolates.

Among the various studies, one notably demonstrated the potent inhibitory effects of methanolic extracts of water hyacinth against pathogenic bacteria such as *E. coli*, *Staphylococcus* spp., and *Pseudomonas* spp., particularly those isolated from clinical and hospital effluents. Furthermore, significant antimicrobial activity was also observed for ethanolic, methanolic, and aqueous extracts derived from the roots and leaves of the plant, which closely aligns with the results obtained in the current research (Rufchaei *et al.*, 2022). Supporting evidence from Fareed *et al.* (2008) suggested that aqueous leaf extracts exhibited superior antimicrobial efficacy compared to root extracts. Similarly, Zhou *et al.*, (2009); Saraf *et al.*, 2018 documented strong antimicrobial activity of water hyacinth extracts against *E. coli*, *Staphylococcus* spp., and *Pseudomonas* spp. Notably, flavonoids have been

reported to possess antimicrobial mechanisms that can disrupt bacterial resistance pathways, thereby enhancing the efficacy of antibacterial agents (Daglia *et al.*, 2012; Rufchaei *et al.*, 2022). Additionally, consistent with our observations, Ho *et al.*, (2012) reported higher concentrations of total phenolics and flavonoids in methanolic extracts compared to aqueous extracts. Furthermore, Savar, an industrial suburb of Dhaka, is a known hotspot for untreated hospital and industrial effluent discharge. The release of pharmaceutical waste and pathogenic microbes into surrounding water bodies poses a severe risk to both the environment and public health. Your study's findings provide critical insight into the possibility of utilizing *E. crassipes*, an abundant aquatic plant, as a low-cost, eco-friendly antimicrobial agent to reduce microbial load in such contaminated environments. *E. crassipes* extracts are proven effective and they could be integrated into wastewater treatment protocols for healthcare institutions to curb the spread of hospital-derived pathogens. In another concept, the rise of antibiotic resistance in Bangladesh, especially in urban clinical settings like those in Dhaka and Savar, has created an urgent need for alternative treatments. Your study supports the use of natural plant-based antimicrobials, which may help alleviate the pressure on conventional antibiotic use and offer new solutions against multidrug-resistant (MDR) pathogens.

Despite these promising findings, the current study faced certain limitations due to the lack of advanced laboratory infrastructure, which precluded the use of analytical techniques such as High-Performance Liquid Chromatography (HPLC) or Gas Chromatography-Mass Spectrometry (GC-MS) for the precise characterization and quantification of secondary metabolites. Therefore, further studies employing these advanced analytical methodologies are essential to elucidate the detailed phytochemical profile and bioactive potential of *E. crassipes* extracts.

5. Conclusion

This study elucidates the potent phytochemical and antimicrobial potential of *E. crassipes* against clinically relevant and hospital effluent-derived bacterial strains in the Savar region of Bangladesh. Hydro-methanolic and aqueous extracts from leaves and roots demonstrated high levels of phenolics and

flavonoids, underpinning notable antioxidant and antibacterial activities. Methanolic fractions exhibited superior efficacy, significantly inhibiting *E. coli*, *Staphylococcus* spp., and *Pseudomonas* spp. These outcomes underscore *E. crassipes* as a promising, low-cost reservoir of bioactive metabolites for antimicrobial applications, particularly in resource-constrained contexts. Further investigation employing advanced analytical platforms such as HPLC and GC-MS is warranted to elucidate and standardize its pharmacologically active constituents. Moreover, the study emphasizes the biopharmaceutical and bioremediation potential of valorizing invasive aquatic flora.

6. Author contributions

Conception and design: M.R.A.; and M.R.A. Methodology: Z.A.S.; and T.A. Software: M.A.H. Original draft preparation: Z.A.S., Z.M.S. Review and editing: T.S.; M.J.M.; T.A.; L.K.R.B. Supervision: M.R.A. All authors read and approved the final version of the manuscript.

7. Acknowledgment

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8. Conflicts of Interest

The authors declare that they have no competing interests.

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