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Investigation of Therapeutic and Medicinal Potential of *Heliotropium indicum* on *Pseudomonas aeruginosa* from Samples of Patients Accessing Care at Alex-Ekwueme Federal University, Ndufu-Alike, Clinic, Ebonyi State, Nigeria

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Abstract

The increasing prevalence of multidrug-resistant pathogens such as *Pseudomonas aeruginosa* necessitates the exploration of alternative therapies. This study investigated the phytochemical composition and antimicrobial activity of *Heliotropium indicum* leaf extracts against *P. aeruginosa* isolates obtained from urine and stool samples at Alex Ekwueme Federal University Ndufu Alike, clinic, Ebonyi State. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, phenols, terpenoids, and glycosides, compounds widely associated with antimicrobial, antioxidant, and anti-inflammatory properties. Morphological and biochemical tests confirmed the isolates as *P. aeruginosa*, showing classical Gram-negative rod characteristics. Antimicrobial susceptibility testing demonstrated that the ethanol extract exhibited stronger inhibitory activity than the aqueous extract, with inhibition zones of 20–30 mm at 200 mg/ml, in some cases comparable to ciprofloxacin (23–28 mm). The aqueous extract showed moderate activity, with inhibition zones ranging from 10–24 mm. These findings indicate that ethanol effectively extracts more potent bioactive compounds than water, corroborating previous studies that reported enhanced activity of organic solvent extracts of medicinal plants. The results suggest that *H. indicum* possesses significant therapeutic potential against *P. aeruginosa*, a pathogen known for its multiple antibiotics resistance property and clinical importance. The findings contribute to the growing evidence supporting medicinal plants as sources of novel antimicrobial agents. However, further work is needed to isolate and characterize the active constituents, assess their toxicity and safety, and evaluate in vivo efficacy. This study underscores capability of *H. indicum* as a complementary strategy in addressing antimicrobial resistance.

Keywords: *Multi Resistant, Pathogens, Pseudomonas aeruginosa, Heliotropium indicum, Antimicrobial Susceptibility.*

I. INTRODUCTION

➢ *General Review*

The rise of bacteria with multidrug resistance signifies a need to source additional therapeutic options from medicinal plants (El-Saadony *et al.,* 2025). *Heliotropium indicum*, Indian heliotrope, is one of many plants that traditional medicine across cultures have used for its potential value in health benefits (Sarkar *et al.,* 2021). This project will investigate the potential medicinal value of *H. indicum* against *Pseudomonas aeruginosa*, a notoriously dangerous germ with high capability for infection and multidrug resistance.

P. aeruginosa is a type of bacteria which requires oxygen that does not stain purple in a Gram stain (Gram-

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negative). It can employ several ways to resist many antibiotics (bacterial efflux/shedding their outer membrane; making enzymes to digest the antibiotic; biofilm formation) (Obasi *et al.,* 2024). It can infect fast, and it can infect with diversity (could be a skin injury, urinary tract infection, respiratory tract in people with compromised immune systems). *P. aeruginosa* presents itself as an opportunity for infection and does not respond appropriately to conventional antibiotics, so finding new agents for killing *P. aeruginosa* is appropriate (Bassetti *et al.,* 2018). *Heliotropium indicum* is part of the *Boraginaceae* family. Research shows it has many active plant chemicals like alkaloids, flavonoids, saponins, tannins, and terpenoids (Adetuyi *et al.,* 2021). These substances have an impact on germs, inflammation, oxidation, and wound healing. Many studies prove *H. indicum* extracts work well against both Gram-positive and Gram-negative bacteria hinting at its use as a natural germ-fighter. This research project looks at various crude *H. indicum* extracts (methanol, ethanol, and water extracts) of *H. indicum* for the ability to fight *P. aeruginosa* bacteria isolated from clinical samples. The project could determine the presence of phytochemicals, compare the antimicrobial activity of extracts against standard tests such as disc diffusion or broth dilution, or if it demonstrates a strong enough negative effect, it could be compared to commonly used antibiotics such as gentamicin and ciprofloxacin offset.

In previous related research indicating and suggesting similar findings found that *H. indicum* extracts: methanol extracts were encouraging. The authors found methanol extracts produced large zones of negative growth against clinical isolates (Ragasa *et al.,* 2014). The preliminary studies suggest that there may be a synergistic effect when plant phytochemicals are tested with standard antibiotics, in other words, they may offer enhanced killing of bacteria. This exciting avenue of study may hold the key to combatting to some of the most resistant strains of infectious germs.

At the end of the day, this study produces results consistent beyond verifying the clinical abilities of alternative and traditional healing. These results could help the search for novel antimicrobial properties from a natural source, if *H. indicum* demonstrates clear comparison due to negative effect against *P. aeruginosa* and could validate further drug studies on *H. indicum*. With the aim toward more plant-derived treatment protocols, the study shows impacts in the respect both documenting the efficacy of traditional medicine and helping further delineate means of bacterium assembly inhibition.

➢ *Aim*

The purpose of this study is to discover the therapeutic and medicinal effects of *Heliotropium indicum* by assessing its antibacterial effects against Pseudomonas aeruginosa, isolated from the AE-FUNAI clinic, which aims to explore its potential as a natural alternative or adjunct treatment of infections cause by drug resistant pathogens.

➢ *Objectives*

- To isolate and identify *Pseudomonas aeruginosa* isolated from clinical samples found at the AE-FUNAI clinic.
- To prepare various solvent extracts of *Heliotropium indicum* (methanol and aqueous dissolutions).
- To evaluate the antibacterial activity of *H. indicum* extracts against *Pseudomonas aeruginosa* using a standard antimicrobial susceptibility test.
- To confirm the potency of the *H. indicum* extracts on *P aeruginosa* in comparison to standard antibiotics, such as gentamicin or ciprofloxacin.
- To determine the inhibitory potential of *Heliotropium indicum* on Multi- resistant strains *P aeruginosa*

II. METHODOLOGY

➢ *Plant Material*

For this study, we used fresh leaves of *Heliotropium indicum*. The plant was harvested from the natural vegetation surrounding the campus of Alex Ekwueme Federal University, Ndufu-Alike Ikwo (AE-FUNAI), Ebonyi State, Nigeria.

The collected plant materials were taken to the Department of Plant Science and Biotechnology, AE-FUNAI, where it was properly placed for taxonomic identification and authenticated by a plant taxonomist.

Following identification, leaves were washed several times with clean distilled water to remove dust and other debris after which they were air dried at ambient temperature (25-28°C) for 7-10 days in a shaded area away from dust to ensure active constituents were preserved. Once dried the leaves were ground into powder form using a mechanical blender and stored in an airtight container at room temperature until it was time for extraction.

Fig 1 *Heliotropium indicum* Plant

➢ *Plant Extraction*

- *Ethanol (75%):*

The primary solvent for extraction of bioactive compounds from *Heliotropium indicum* leaves.

- *Distilled water:*

Washing plant materials and preparing aqueous solutions when necessary.

- *Plant Processing and Extraction*

The plant processing and extraction procedure was carried out using standard laboratory methods for the preparation of crude plant extracts. Fresh leaves of *Heliotropium indicum* were first collected, cleaned thoroughly to remove dirt and debris, and subsequently air-dried under room temperature conditions until a constant weight was achieved (Albarka, 2023). The dried leaves were then pulverized into fine powder using a mechanical blender or grinder. Pulverization of the plant material increases the surface area, thereby enhancing efficient extraction of the bioactive constituents. An analytical weighing balance was used to accurately measure the quantity of the powdered plant material and reagents employed during the extraction process. Laboratory glassware, including beakers, conical flasks, and measuring cylinders, were utilized for measuring solvents and mixing the plant material during extraction. Exactly 60 g of the powdered leaves were dissolved separately in 300 mL of distilled water and 300 mL of 75% ethanol. The ratio was determined from the standard relationship in which 600 g corresponds to 3000 mL, giving a proportional ratio of 60 g to 300 mL of solvent. The mixtures were allowed to stand for adequate extraction of the phytochemical constituents, after which the extracts were filtered using a funnel and Whatman No. 1 filter paper to remove plant residues and obtain clear filtrates. The filtrates obtained were subsequently concentrated using a rotary evaporator or water bath to evaporate the solvent and obtain concentrated crude extracts.

➢ *Phytochemical Screening of Heliotropium Indicum*

Phytochemical screening was carried out on the ethanol extract of *Heliotropium indicum* leaves for secondary metabolites exhibiting therapeutic and antimicrobial properties. The screening was conducted based on standard qualitative methods referenced in Idowu, (2023).

- *Alkaloids Test (Mayer's Test):*

For the detection of alkaloids, 2 mL of the plant extract was treated with a few drops of Mayer's reagent, which consists of potassium mercuric iodide solution. The formation of a creamy white precipitate indicated the presence of alkaloids in the extract.

- *Flavonoids Test (Alkaline Reagent Test):*

Flavonoids were tested using the alkaline reagent method. In this procedure, 2 mL of the extract was mixed with 1 mL of 10% sodium hydroxide solution. The development of a yellow coloration, which disappeared upon the addition of dilute hydrochloric acid, confirmed the presence of flavonoids.

- *Tannins Test (Ferric Chloride Test):*

The presence of tannins was determined using the ferric chloride test. About 2 mL of the extract was treated with a few drops of 1% ferric chloride solution. The formation of a blue-black or greenish precipitate indicated the presence of tannins.

- *Saponins Test (Frothing Test):*

Saponins were identified using the frothing test. Five millilitres of the extract were mixed with 20 mL of distilled water and vigorously shaken. The persistence of froth for at least 10 minutes was taken as evidence for the presence of saponins.

- *Phenolic Test (Ferric Chloride Test):*

Phenolic compounds were detected by mixing 2 mL of the extract with a few drops of 5% ferric chloride solution. The appearance of a deep bluish-green coloration indicated the presence of phenolic compounds.

- *Terpenoids Test (Salkowski's Test):*

The Salkowski's test was used for the detection of terpenoids. In this test, 2 mL of the extract was mixed with 2 mL of chloroform, after which 3 mL of concentrated sulfuric acid was carefully added along the side of the test tube. The formation of a reddish-brown coloration at the interface confirmed the presence of terpenoids.

- *Glycosides Test (Keller-killiani Test):*

Cardiac glycosides were determined using the Keller–Killiani test. Two millilitres of the extract were mixed with glacial acetic acid containing one drop of ferric chloride solution, followed by the addition of concentrated sulfuric acid. The formation of a brown ring at the interface indicated the presence of cardiac glycosides in the extract.

➢ *Microbiological Analysis*

- *Study Area*

This study was carried out at the AE-FUNAI Clinic located within the premises of Alex Ekwueme Federal University Ndufu-Alike (AE-FUNAI), Ebonyi State, Nigeria. The university is situated in Ikwo Local Government Area of Ebonyi State and serves a large population of undergraduate students, staff, and surrounding communities. The clinic provides routine healthcare services, medical consultations, laboratory investigations, and treatment for various infections and health conditions among students and staff of the institution.

- *Study Population*

The study population consisted mainly of AE-FUNAI students between the ages of 17 and 26 years. Urine and stool samples were collected randomly from consenting students attending the clinic for medical examination and laboratory analysis. Sample collection

was carried out under aseptic conditions using sterile swab sticks and sterile sample containers to avoid contamination and ensure the reliability of laboratory results.

- *Sample Collection and Processing*

A total of 60 samples were collected (30 Urine and 30 stool samples) randomly from consenting students of Alex Ekwueme Federal University Ndufu-Alike (AE-FUNAI) within the age range of 17–26 years attending the university clinic. Sample collection was carried out under strict aseptic conditions to prevent contamination and ensure the accuracy of microbiological analysis. Clean-catch midstream urine samples were collected into sterile universal sample containers, while stool samples were collected using sterile swab sticks and transferred into sterile specimen containers (Nanyi *et al.,* 2025). Each sample container was properly labeled with relevant identification details and transported immediately to the AE-FUNAI microbiology laboratory for processing. Upon arrival at the laboratory, the samples were processed within 24 hours of collection. Urine samples were mixed gently before inoculation, while stool samples were homogenized in sterile normal saline to obtain uniform suspensions.

- *Preparation of Media*

All culture media used in this study, including Mueller Hinton Agar, Pseudomonas Agar, Nutrient Agar, and Peptone Broth, were prepared according to the manufacturer's instructions. The appropriate quantities of each dehydrated medium were weighed and dissolved in distilled water to obtain the required concentrations. The prepared media were sterilized by autoclaving at 121°C for 15 minutes to ensure the elimination of contaminants and unwanted microorganisms. After sterilization, the media were allowed to cool to approximately 50°C before being aseptically poured into sterile Petri dishes. The plates were then allowed to solidify and gel under sterile conditions prior to use for microbial culture and analysis.

- *Isolation of Pseudomonas aeruginosa*

For urine samples, sterile pipette tips were used to collect 0.1 mL of urine, then inoculated onto Pseudomonas Agar plates using the streak plate technique according to Karah *et al.,* (2020). Stool samples were first homogenized in sterile normal saline, after which a loopful of the suspension was inoculated onto Pseudomonas Agar by streaking to obtain discrete colonies. The inoculated plates were incubated aerobically at 37°C for 18- 24 hours (Cheesbrough, 2006). Following incubation, the plates were examined for characteristic colonies of *Pseudomonas aeruginosa*. Colonies appearing large, flat, irregular, and producing bluish-green pigmentation with a characteristic fruity odour were suspected to be *P. aeruginosa* (Hana & Abdeen, 2024). Pure isolates were obtained by repeated subculturing on freshly prepared Nutrient Agar plates to ensure colony purity. Pure cultures of confirmed isolates were maintained on Nutrient Agar slants and stores at 4ºC for other microbiological analyses.

- *Characterisation and Identification of the Isolates*

✓ *Identification of Bacterial Isolates*

The isolates were identified by using standard microbiological methods, starting from, cultural characterization on different selective media, gram staining, and biochemical tests such catalase, oxidase, citrate, Indole, and methyl red assays were all conducted according to Dabban *et al.,* (2024).

✓ *Gram Staining*

Gram reaction was performed on the overnight growth of each bacterial isolate on nutrient agar. A loop-full of culture was picked up using a sterile inoculating loop and applied on clean slides to form a thin layer, forming a smear. The slide will be left to air dry before being heat-fixed by passing it over a Bunsen burner flame. After being drenched with crystal violet for 60 seconds, the slide was rinsed with running water and then flooded with Lugol's iodine for 60 seconds to fix it with mordant. The slide was decolorized with a 50 percent acetone and 50 percent ethanol solution for 15 seconds, and then it was rinsed with water. The slide is then counter-stained with safranin, left for 60 seconds, and then rinsed off with running water. The slides will be left to air dry before being seen under a light microscope equipped with a ×100 objective lens (oil immersion).

✓ *Catalase Test*

A loop full of the microbe was emulsified with a loop full of a drop of 3% v/v hydrogen peroxide solution on a sterilized glass slide. The presence of effervescent gas bubbles indicates a positive result, whereas the lack of bubbles indicates a negative result.

✓ *Oxidase Test*

The reagent is made by dissolving 0.1g in 10ml of distilled water. The test organisms were picked up with a sterile stick onto filter paper soaked in 1% oxidase reagent (diamine hydrochlorine). After then, the organisms were observed for the development of a bright blue hue within ten seconds, which signifies a favorable response.

✓ *Citrate Utilization Test*

The test, which measures an organism's ability to use citrate as its only carbon source, was conducted by inoculating the test organism on Simon citrate agar slopes for 48 hours at 37 °C. Citrate is greenish, thus when it turns blue, it indicates a positive result.

✓ *Indole Test*

After pipetting five milliliters of peptone water broth into test tubes and properly corking them, the broth was autoclaved for 15 minutes at 15 psi to sterilize it. It was allowed to cool to 40°C before the test organism was added and allowed to grow for 72 hours. Kovac's reagent was then added in three drops to the culture, and it was left to stand for 30 minutes. Red development at the surface layer is a sign of a positive response.

✓ *Methyl Red Test*

After adding two drops of methyl red to five milliliters of peptone water that contained the isolates, the combination was incubated at 37°C for twenty-four hours. A change in color was seen after the incubation period. A positive methyl red test yielded a red color, whereas a negative test generated a yellow color.

➢ *Antimicrobial Susceptibility Testing of* Heliotropium indicum *Extract*

The antimicrobial activity of *Heliotropium indicum* extracts against *Pseudomonas aeruginosa* isolates was determined using the agar well diffusion method as described by Osungunna *et al.,* (2011). Mueller Hinton Agar plates were uniformly inoculated with standardized bacterial suspensions adjusted to correspond with the 0.5 McFarland turbidity standard. Wells measuring approximately 6 mm in diameter were aseptically bored into the inoculated agar plates using a sterile cork borer. Measured volumes of the plant extracts were carefully introduced into the wells, while sterile distilled water and standard antibiotics served as negative and positive controls, respectively. The inoculated plates were allowed to stand for a period to facilitate proper diffusion of the extracts into the agar medium before incubation at 37°C for 24 hours. Following incubation, the antimicrobial activity of the extracts was evaluated by measuring the diameters of the zones of inhibition surrounding each well using a transparent ruler. The results obtained were recorded in millimetres (mm) and interpreted as an indication of the susceptibility of the bacterial isolates to the plant extracts (Forbes *et al.,* 2016).

III. RESULTS

➢ *Qualitative Phytochemical Screening of Heliotropium indicum (Ethanol Leaf Extract)*

The ethanol leaf extract of *Heliotropium indicum* was subjected to qualitative phytochemical test and the result showed the presence of various bioactive compounds such as alkaloids, flavonoids, tannins, saponins, phenols, terpenoids, glycosides etc. The different test methods produced positive reactions, which confirmed the presence of the phytochemicals that showcase the medicinal and antimicrobial potential of the plant.

Table 1 Qualitative Phytochemical Screening of *Heliotropium indicum* (Ethanol Leaf Extract)

Phytochemical Compound	Test Method	Observation	Inference
Alkaloids	Mayer's Reagent Test	Creamy White Precipitate	Present (+)
Flavonoids	Alkaline reagent Test	Yellow Colouration that Disappears with Acid	Present (+)
Tannins	Ferric chloride Test	Blue-Black or Green Precipitate	Present (+)
Saponins	Frothing Test	Persistent Froth Lastin Over 10 Minutes	Present (+)
Phenols	Ferric chloride Test	Deep Bluish-Green Colouration	Present (+)
Terpenoids	Salkowski's Test	Reddish-Brown Interface Layer	Present (+)
Glycosides	Keller-Killiani Test	Brown Ring at Interface	Present (+)

➢ *Distribution of Positive Urine and Stool Samples among Different Age Groups*

The microbial analysis of urine and stool samples revealed positive microbial growth across almost all age groups examined. The highest number of positive urine samples was recorded among participants aged 21 years with seven positive cases, followed by age groups 20 and 22 years with five positive cases each. Similarly, stool samples showed the highest positivity among individuals aged 21 years with five positive cases. No stool samples were recorded for participants aged 24 and 25 years, while no urine sample was obtained from participants aged 27 years. 30 urine samples and 19 stool samples tested positive for microbial growth.

Table 2 Distribution of Positive Urine and Stool Samples among Different Age Groups

Age Group (Years)	Urine Samples Examined	Urine Positive Samples	Stool Samples Examined	Stool Positive Samples
17	1	1	1	1
18	3	3	1	1
19	3	3	4	4
20	5	5	4	4
21	7	7	5	5
22	5	5	2	2
23	1	1	1	1
24	2	2	0	0
25	1	1	0	0
26	1	1	1	1
27	0	0	–	–
28	1	1	–	–
Total	**30**	**30**	**19**	**19**

- *Key:*

Positive samples indicate the presence of microbial growth in the analyzed urine and stool specimens. "–" indicates no sample collected.

➢ *Colonial Morphological Characteristics of Pseudomonas aeruginosa Isolates*

The colonial morphology of *Pseudomonas aeruginosa* isolates obtained from urine and stool samples showed relatively similar characteristics. Most isolates produced white colonies with round shapes, irregular edges, and raised elevations. However, isolate U3 from urine exhibited a spreading colony shape with scalloped edges, while isolate S2 from stool showed round colonies with scalloped edges. The predominance of round, white, raised colonies with irregular margins suggests common morphological features among the isolates obtained from both sample sources.

Table 3 Colonial Morphological Characteristics of *Pseudomonas aeruginosa* Isolated from Urine and Stool Samples

Sample Source	Isolate Code	Colour	Shape	Edge	Elevation
Urine	U1	White	Round	Irregular	Raised
Urine	U2	White	Round	Irregular	Raised
Urine	U3	White	Spreading	Scalloped	Raised
Urine	U4	White	Round	Irregular	Raised
Urine	U5	White	Round	Irregular	Raised
Stool	S1	White	Round	Irregular	Raised
Stool	S2	White	Round	Scalloped	Raised
Stool	S3	White	Round	Irregular	Raised
Stool	S4	White	Round	Irregular	Raised
Stool	S5	White	Round	Irregular	Raised

Legend: U = Urine isolate**; S =** Stool isolate.

➢ *Biochemical and Microscopic Identification of Pseudomonas aeruginosa* Isolates

The biochemical and microscopic characterization of the bacterial isolates revealed similar features among all urine and stool isolates. All isolates were Gram-negative rods arranged in clusters and showed positive reactions for catalase, oxidase, and citrate utilization tests, while negative reactions were recorded for indole and methyl red tests. These biochemical characteristics are consistent with the standard identification profile of *Pseudomonas aeruginosa*, confirming the presence of the organism in both urine and stool samples analyzed.

Table 4 Biochemical, Gram Reaction, and Microscopic Characteristics of *Pseudomonas aeruginosa* Isolates

Isolate Code	Gram Reaction	Cell Shape	Arrangement	Catalase Test	Oxidase Test	Indole Test	Methyl Red Test	Citrate Test	Probable Organism
U1	–	Rod	Clusters	+	+	–	–	+	*Pseudomonas aeruginosa*
U2	–	Rod	Clusters	+	+	–	–	+	*Pseudomonas aeruginosa*
U3	–	Rod	Clusters	+	+	–	–	+	*Pseudomonas aeruginosa*
U4	–	Rod	Clusters	+	+	–	–	+	*Pseudomonas aeruginosa*
U5	–	Rod	Clusters	+	+	–	–	+	*Pseudomonas aeruginosa*
S1	–	Rod	Clusters	+	+	–	–	+	*Pseudomonas aeruginosa*
S2	–	Rod	Clusters	+	+	–	–	+	*Pseudomonas aeruginosa*
S3	–	Rod	Clusters	+	+	–	–	+	*Pseudomonas aeruginosa*
S4	–	Rod	Clusters	+	+	–	–	+	*Pseudomonas aeruginosa*
S5	–	Rod	Clusters	+	+	–	–	+	*Pseudomonas aeruginosa*

Legend: S- Stool Samples and U- Urine Samples

➢ *Antibacterial activity of Heliotropium indicum extracts against Pseudomonas spp.*

Ethanolic and aqueous extracts of *Heliotropium indicum* showed concentration-dependent inhibition of *Pseudomonas* isolates, with strongest activity at 200 mg·mL⁻¹ and absent effects at 50 mg·mL⁻¹. The ethanolic extract produced the largest zone (30 mm, U2), while the aqueous extract peaked at 24 mm (U3). Ciprofloxacin (10 µg) remained more potent across all isolates.

Table 5 Antimicrobial Susceptibility Profile of *Pseudomonas* Species to Ethanolic Extract of *Heliotropium indicum*

Isolate	200 mg·mL⁻¹ Mean ± SD (n=2)	100 mg·mL⁻¹ Mean ± SD (n=2)	50 mg·mL⁻¹ Mean ± SD (n=2)	Positive control (Cpx 10 µg)
S1	22.50 ± 3.54	14.50 ± 0.71	12.00 ± 1.41	15
S2	12.00 ± 1.41	11.00 ± 0.00	5.00 ± 7.07	25
U1	12.00 ± 1.41	10.00 ± 0.00	0.00 ± 0.00	25
U2	30.00 ± 0.00	12.00 ± 0.00	0.00 ± 0.00	23
U3	23.00 ± 2.83	15.00 ± 0.00	10.00 ± 0.00	28

Legend: S- Stool Samples and U- Urine Samples

Table 6 Antimicrobial Susceptibility Profile of *Pseudomonas* Species to Aqueous Extract of *Heliotropium indicum*.

Isolate	200 mg·mL⁻¹ Mean ± SD (n=2)	100 mg·mL⁻¹ Mean ± SD (n=2)	50 mg·mL⁻¹ Mean ± SD (n=2)	Positive control (Cpx 10 µg)
S1	12.00 ± 0.00	11.00 ± 0.00	0.00 ± 0.00	15
S2	20.00 ± 0.00	18.00 ± 0.00	12.00 ± 0.00	25
U1	17.00 ± 1.41	14.00 ± 1.41	0.00 ± 0.00	25
U2	15.00 ± 0.00	10.00 ± 0.00	0.00 ± 0.00	23
U3	24.00 ± 0.00	20.00 ± 0.00	16.00 ± 0.00	28

Legend: S- Stool Samples and U- Urine Samples

IV. DISCUSSION, CONCLUSION AND RECOMMENDATION

➢ *Discussion*

The emergence and persistence of multidrug-resistant pathogens such as *Pseudomonas aeruginosa* remain a major global health concern, especially in clinical settings with immunocompromised patients (Elfadadny *et al.,* 2024). Listed by WHO as a critical priority pathogen (WHO, 2017), *P. aeruginosa's* growing antibiotic resistance has driven renewed interest in alternative therapies, including medicinal plants (Karaiskos and Giamarellou, 2014). This study evaluated phytochemical composition and antibacterial activity of *Heliotropium indicum* leaf extracts against *P. aeruginosa* isolates from urine and stool at the AE-FUNAI clinic. *H. indicum*, used traditionally to treat wounds, respiratory and gastrointestinal ailments, eye and skin infections, is known for antibacterial, anti-inflammatory, antifungal, antioxidant, and wound-healing properties (Nandhini *et al.,* 2018).

Phytochemical screening detected alkaloids, flavonoids, tannins, saponins, phenols, terpenoids, and glycosides compounds associated with antimicrobial activity. Mechanistically, alkaloids may intercalate DNA, inhibit topoisomerases, and block protein synthesis (Abookleesh *et al.,* 2022); flavonoids serve as antioxidants and disrupt membranes; tannins precipitate proteins and inhibit essential enzymes (Lobiuc *et al.,* 2023); phenols and terpenoids permeabilize membranes; glycosides can inhibit quorum sensing, a key *P. aeruginosa* virulence mechanism (Bouyahya *et al.,* 2022). Prior analyses similarly link H. indicum's traditional uses to its secondary metabolites (Darshini *et al.,* 2024).

Isolates displayed classical *P. aeruginosa* morphology and biochemical profiles (catalase+, oxidase+, indole−, methyl red−, citrate+), consistent with standard descriptions (Cheesbrough, 2006).

Antibacterial testing showed concentration-dependent inhibition by both ethanol and aqueous extracts. The ethanolic extract produced stronger activity, particularly at 200 mg·mL−1, with inhibition zones up to 30 mm (notably U2) and several isolates showing zones comparable to or exceeding ciprofloxacin (23–28 mm). This superior performance aligns with reports that ethanol better extracts both polar and nonpolar bioactives, yielding broader phytochemical profiles and greater antibacterial potency (Akinmoladun *et al.,* 2019). The aqueous extract showed lower activity overall (200 mg·mL−1 zones 10-24 mm), with weaker or absent inhibition at 50 mg·mL−1 for several isolates consistent with observations that water extracts fewer active constituents or concentrates them less effectively (Oloyede *et al.,* 2017).

Comparative literature supports these findings: ethanolic and methanolic extracts of *H. indicum* and other medicinal plants have demonstrated inhibitory effects against Gram-negative pathogens, including *P. aeruginosa* (Nandhini *et al.,* 2018; Babalola *et al.,* 2020). Reviews emphasize that organic solvents typically yield extracts with higher antimicrobial activity than aqueous preparations due to phytochemical solubility differences (Balouiri *et al.,* 2016).

The study's results are particularly relevant given *P. aeruginosa* diverse resistance strategies efflux pumps, biofilm formation, enzymatic inactivation, and reduced membrane permeability (Breidenstein *et al.,* 2011). The

demonstrated activity of *H. indicum* extracts suggests the plant contains compounds that may circumvent conventional resistance mechanisms. Flavonoids and terpenoids can disrupt biofilms and quorum sensing, thereby lowering virulence (Mulat *et al.,* 2025). Thus, *H. indicum* represents a promising source of antibacterial leads or adjuncts that merit further isolation, characterization, and mechanistic study.

➢ *Limitations of the Study*

While the results are promising, several limitations must be acknowledged:

- The toxicity profile of the extracts was not assessed; some phytochemicals in *H. indicum* (pyrrolizidine alkaloids) have been associated with hepatotoxicity.
- The extracts were crude and not fractionated; thus, the specific compounds responsible for the antimicrobial activity remain unidentified.
- A limited number of isolates were tested; broader sampling may reveal more variability in susceptibility.

➢ *Conclusion*

This study has demonstrated that *Heliotropium indicum* possesses significant antibacterial activity against *Pseudomonas aeruginosa* isolates obtained from clinical samples at AE-FUNAI clinic. The ethanolic extract was more potent than the aqueous extract, producing inhibition zones comparable to ciprofloxacin in some isolates. The rich phytochemical composition of the plant, including alkaloids, flavonoids, tannins, phenols, terpenoids, and glycosides, underpins its antimicrobial activity.

The findings contribute to the growing body of evidence supporting the use of medicinal plants as alternative or complementary therapies in the fight against antibiotic-resistant pathogens. However, further research is required to isolate, characterize, and evaluate the specific active compounds, as well as to assess their safety and efficacy in vivo.

➢ *Recommendations*

- *Phytochemical Fractionation and Characterization:*

Future studies should isolate and identify specific compounds responsible for the antibacterial activity of *H. indicum*.

- *Toxicological Studies:*

Safety assessments are essential to evaluate potential side effects, especially considering the hepatotoxic potential of some alkaloids in *H. indicum*.

- *In Vivo Studies:*

Animal models and clinical trials are required to validate the efficacy and safety of the extracts in treating *P. aeruginosa* infections.

- *Synergistic Studies with Antibiotics:*

Investigating the potential of *H. indicum* extracts to enhance the activity of existing antibiotics could provide valuable strategies for overcoming resistance.

- *Formulation Development:*

Standardized herbal formulations, including ointments or oral suspensions, should be developed for therapeutic use against infections.

➢ *Contribution to Knowledge*

This study contributes to knowledge by:

- Demonstrating the comparative efficacy of ethanol versus aqueous extracts of *H. indicum* against clinical isolates of *P. aeruginosa*.
- Providing evidence that ethanol extract can produce inhibitory effects on *P. aeruginosa* comparable to ciprofloxacin.
- Supporting the ethnomedicinal use of *H. indicum* as an antibacterial agent, while highlighting the need for scientific validation and safety assessment.
- Adding to the growing literature on medicinal plants as viable alternatives in the global effort to combat antimicrobial resistance.

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