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ANTIBACTERIAL ACTIVITY OF AQUEOUS EXTRACTS OF KENDU (*DIOSPYROS MELANOXYLON*) AND PUNARNAVA (*BOERHAVIA DIFFUSA*) LEAVES AGAINST URINARY TRACT INFECTIONS (UTI) CAUSING PATHOGENS.

Biotechnology

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ABSTRACT

Urinary Tract Infection (UTI) is a common yet one of the serious health concerns afflicting women in India. It is caused by the pathogens that generally originate the digestive tract and get passed on to urethra and start to multiply. However, UTI can be caused by various other reasons, most prominent being lack of hygiene. The first line of treatment generally includes antibiotics that can sometimes prove to be ineffective due to cases of Antimicrobial Resistance (AMR) or Multi Drug Resistance (MDR). The situation creates a need to explore newer sources of drugs that can bring relief to affected population. The current study explores the antibacterial activity of Kendu (Diospyros melanoxylon) and Punarnava (Boerhavia diffusa) leaf extract against common UTI causing pathogens.

KEYWORDS

UTI, pathogens, antibacterial activity, antimicrobial resistance, etc.

INTRODUCTION:

Cases of Urinary Tract Infection (UTI) among women have seen a steady increase in India. Women are most affected by the UTI primarily because the length of urethra in women is shorter. Rate of infection among women is generally high due various reasons that include lack of hygiene, diabetes, accidental invasion of gut bacteria into urethra, and some more. UTI forms 25% of all the clinical bacterial infections that affect women. Reports suggest that up to 60% of women suffer from UTI once in their life time.

The front line treatment for UTI generally involves antibiotics like combination drug trimethoprim and sulfamethoxazole, fluoroquinolones, trimethoprim, β-lactams, nitrofurantoin, and fosfomycin tromethamine, ciprofloxacin, gentamicin (Jancel and Dudas, 2002, MacGowan and Albur, 2013). However, various independent studies have revealed that the efficacy of front line antibiotics has deteriorated due to Anti Microbial Resistance (AMR) among pathogens (Kothari and Sagar, 2008, Mohapatra *et al*., 2022, Bhuiya *et al*., 2025).

To counter the scenario of AMR UTI it is crucial to explore newer sources of antibacterial drugs. Certain plants have long been known to be medicinal with various health benefits. Current study aims to test the antibacterial activity of Kendu (*Diospyros melanoxylon Roxb*) and Punarnava (*Boerhavia diffusa*) leaf extract against common UTI causing bacteria like *E.coli*, *Klebsiella pneumonia, and Pseudomonas aeruginosa*.

MATERIALS AND METHODS:

Sample Collection And Processing: Kendu (*Diospyros melanoxylon Roxb*) and Punarnava (*Boerhavia diffusa*) *leaf samples were collected from a location near Harli in Hazaribagh, Jharkhand (Geo-location: 23.824816, 85.274400 and 23.824030, 85.274726 respectively) and brought to laboratory.* Punarnava and Kendu were chosen for this study due to their longstanding use in traditional medicine for treating a variety of health conditions, including infections. *The leaves were* cleaned with running tap water to remove dust and other impurities, followed by air drying indoor at room temperature (Kokate *et al*., 2015). After drying, the leaves were finely ground into powder using a mortar and pestle. To ensure the integrity and efficacy of the powdered samples, they were stored in airtight containers to prevent moisture absorption and contamination.

Extract Preparation:

To prepare the hot water extracts, 10 grams of each powdered plant sample was meticulously dissolved in 100 ml of distilled water. The mixtures were carefully heated on a hot plate until they reached a vigorous boil, ensuring thorough extraction of the plant's bioactive compounds. Subsequently, the solutions were allowed to simmer gently for 30 minutes, facilitating the release and dissolution of phytochemicals into the solvent. After cooling to room temperature, the extracts underwent filtration using Whatman No. 1 filter paper to eliminate any solid particulates and obtain clear filtrates. To concentrate the extracted compounds, the filtrates were then subjected to drying in a hot air oven set at 40°C. This drying process aimed to remove excess water while preserving the potency of the phytochemicals present in the extracts.

Extract was collected in a centrifuge tube and centrifuged at 8,000 rpm to remove the solid particles. The supernatant was collected in fresh centrifuge tube and concentrated using desiccator. The final yield of the extract was approximately 5.73% relative to the dry leaf powder.

Phytochemical Analysis:

The phytochemical composition of the Kendu and Punarnava aqueous leaf extracts were assessed separately. Standard qualitative methods were employed for the detection of alkaloids, flavonoids and phenolic phytochemicals (Harborne, 1998; Wagner and Bladt, 2001, Banu and Catherin, 2015): (a) alkaloid test: Wagner's Test - Kendu and Punarnava extracts were treated with a few drops of Wagner's Reagent to test the presence of alkaloid. (b) phenol test: Ferric Chloride Test - Kendu and Punarnava extracts were treated with a few drops of Ferric Chloride to test the presence of phenols, and, (c) flavonoids test: Alkaline Reagent test - Kendu and Punarnava extracts were treated with a few drops of Sodium Hydroxide and dilute Hydrochloric Acid to test the presence of flavonoids.

Sourcing Bacterial Isolates:

Urine samples were collected from female patients clinically diagnosed with urinary tract infections (UTIs) and processed to isolate the causative bacterial pathogens (Cheesbrough, 2006). Clinical samples were cultured on CLED agar and MacConky agar media. The plates were incubated at 37°C for 18-24 hours.

Biochemical Identification:

The isolated bacteria were identified using biochemical methods: Citrate test, Triple Sugar Iron (TSI) test, Urease test and Indole test (Godkar and Godkar, 2014).

Antibiotic Sensitivity Test:

Antibiotic sensitivity testing was performed to evaluate the susceptibility of the isolated UTI-causing bacteria (*E. coli*, *Klebsiella pneumoniae and Pseudomonas aeruginosa*) against the Kendu and Punarnava *leaf* extract. The Kirby-Bauer disc diffusion method was employed on Luria Bertani agar, a standard medium for antimicrobial susceptibility testing.

RESULTS:

Phytochemicals analysis of Kendu and Punarnava revealed significant presence of alkaloids, flavonoids and phenol group of phytochemicals (Table – 1).

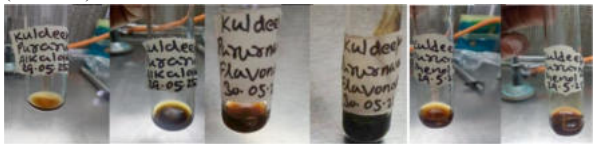

Figure: 1 - Punarnava leaf extract phytochemical analysis – A:

Alkaloid test, B: Flavonoid test, C: Phenol test.

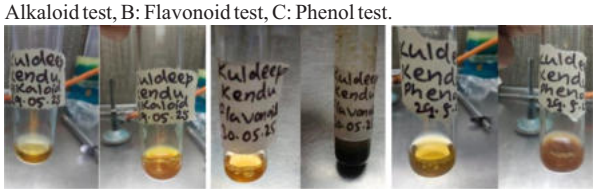

Figure: 2 - Kendu leaf extract phytochemical analysis – A: Alkaloid test, B: Flavonoid test, C: Phenol test.

Table – 1: Phytochemical analysis		
Phytochemical Compound	**Kendu**	**Punarnava**
	Presence (+/−)	
Alkaloids	+	+
Flavonoids	+	+
Phenols	+	+

On the basis of biochemical tests, the isolates were identified to be *E.coli* as the bacteria tested negative for Citrate and Urease tests and positive for Indole and TSI tests; *Klebsiella pneumoniae* was identified by positive Citrate and Urease tests, acidic pH and gas production in TSI test and negative for Indole test; and *Pseudomonas aeruginosa* was identified by characteristic positive tests for Citrate and TSI tests and negative for Urease and Indole tests. The identification criteria and test have been summarized in the Table – 2.

Table – 2: Biochemical tests				
Bacteria	**Citrate**	**TSI**	**Urease**	**Indole**
E. coli	Negative	Positive/H_2S	Negative	Positive
Klebsiella pneumoniae	Positive	A/A, G	Positive	Negative
Pseudomonas aeruginosa	Positive	Positive	Negative	Negative

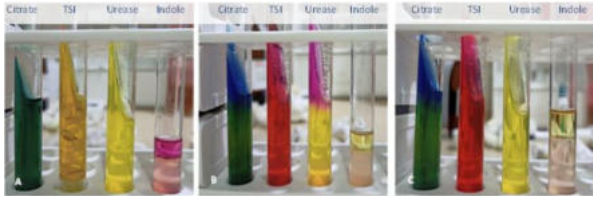

Figure: 3 – Biochemical based identification of bacteria. A: *E. coli*, B: *Klebsiella pneumoniae*, C: *Pseudomonas aeruginosa*

Antibacterial Activity: Aqueous extract of Kendu and Punarnava leaf extracts significantly inhibited the growth of the three bacterial isolates (Table – 3).

Table – 3: Antibacterial Activity Of Kendu And Punarnava Leaf Extracts		
Kendu		
Pathogen	**Antibacterial activity**	**Inhibitory concentration (in mg)**
E. coli	Positive	20
Klebsiella pneumoniae	Positive	20
Pseudomonas aeruginosa	Positive	20
Punarnava		
E. coli	Positive	0.1
Klebsiella pneumoniae	Positive	20
Pseudomonas aeruginosa	Positive	1.0

Figure: 4 - Antibacterial activity of Punarnava leaf extracts against *E.coli* (A), *P. aeruginosa* (B) and *Klebsiella pneumoniae* (C).

Figure: 5 - Antibacterial activity of Kendu leaf extracts against *E.coli* (A), *P. aeruginosa* (B) and *Klebsiella pneumoniae* (C).

DISCUSSION:

Kendu and Punarnava plants have long been known for various medicinal properties. However, their application as treatment options against Urinary Tract Infections (UTI) has been scantily explored. In this study aqueous extracts of Kendu and Punarnava leaves have shown significant antibacterial activity against UTI pathogens like *E. coli*, *Klebsiella pneumoniae and Pseudomonas aeruginosa with Punarnava extract being more efficient than Kendu extract in inhibiting the growth of E.coli and Pseudomonas aeruginosa*.

CONCLUSION:

The study clearly demonstrates the effective antibacterial activity of Kendu and Punarnava leaf extracts against common UTI causing pathogens *E. coli*, *Klebsiella pneumoniae and Pseudomonas aeruginosa.* However, more research needs to be done to determine the active bio-molecule responsible for antibacterial activity.

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