Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 8
Open Access

Antibacterial Effect of Punarnava (Boerhavia diffusa) and Kendu (Diospyros melanoxylon) Medicinal Plants Against Urinary Tract Infections (UTIs) Pathogens Isolated from Clinical Samples of Female Patients

[1]Kuldeep Prasad, [2]Dr Asha Mishra
[1]Research scholar, Department of Microbiology, YBN University, Ranchi, Jharkhand, India, 834010
[2]Assistant professor, Department of Biotechnology, YBN University, Ranchi, Jharkhand, India, 834010

Cite this paper as: Kuldeep Prasad, Dr Asha Mishra (2024) Antibacterial Effect of Punarnava (Boerhavia diffusa) and Kendu (Diospyros melanoxylon) Medicinal Plants Against Urinary Tract Infections (UTIs) Pathogens Isolated from Clinical Samples of Female Patients. *Frontiers in Health Informatics, 13* (8), 1308-1318

Abstract

Urinary tract infections (UTIs) are prevalent infections, especially among females, and are commonly caused by pathogens such as Escherichia coli, Klebsiella species, and Proteus species. The rising antibiotic resistance among these pathogens necessitates the exploration of alternative treatments. This study investigates the antibacterial effects of Punarnava (Boerhavia diffusa) and Kendu (Diospyros melanoxylon), two medicinal plants traditionally used for their therapeutic properties, against UTI pathogens isolated from clinical samples of female patients.

Plant samples were collected from Harli, Barkagaon in the Hazaribagh district of Jharkhand, India, and processed to prepare hot water extracts. Urine samples from UTI-infected patients were cultured to isolate the pathogens, which were then identified through morphological examination and biochemical tests. The antibiotic sensitivity of the isolates was assessed using the Kirby-Bauer disc diffusion method.

The antibacterial activity of the plant extracts was evaluated using the agar well diffusion method against E. coli, Klebsiella spp., and Proteus spp. Both Punarnava and Kendu extracts exhibited significant antibacterial activity, with Kendu showing slightly higher efficacy. The results indicate the presence of bioactive compounds in these plants capable of inhibiting the growth of UTI pathogens.

This study highlights the potential of Punarnava and Kendu as alternative or complementary treatments for UTIs, particularly in light of increasing antibiotic resistance. Further research is needed to isolate and characterize the active compounds and evaluate their efficacy in vivo. The findings underscore the importance of exploring traditional medicinal plants for developing new antimicrobial therapies.

Keywords: - Antibacterial Effect, Punarnava (Boerhavia diffusa), Kendu (Diospyros melanoxylon), Medicinal Plants, Urinary Tract Infections (UTIs), UTI Pathogens, E. coli, Klebsiella spp., Proteus spp.

Introduction

Urinary tract infections (UTIs) are among the most common infections affecting individuals, particularly females. They are caused by various pathogens, including Escherichia coli, Klebsiella species, and Proteus

species. The overuse of antibiotics has led to the emergence of resistant strains, necessitating the exploration of alternative treatments. Medicinal plants have been used traditionally for their therapeutic properties, including antibacterial activity. This study focuses on the antibacterial effect of Punarnava (Boerhavia diffusa) and Kendu (Diospyros melanoxylon) against UTI pathogens isolated from clinical samples of female patients.

MATERIALS AND METHODS:

Collection of Medicinal Plants

Plant Selection and Collection

In February 2024, two medicinal plant leaf samples, Punarnava (Boerhavia diffusa) and Kendu (Diospyros melanoxylon), were meticulously collected from the Harli, Barkagaon regions in the Hazaribagh district of Jharkhand, India. These specific locations were selected due to their rich biodiversity and the availability of these particular plants. 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 traditional knowledge surrounding these plants, passed down through generations, highlights their potential therapeutic properties. This cultural and historical context provided a strong rationale for investigating their antibacterial effects against UTI pathogens.

Processing of Medicinal Plant Extracts

Drying and Powdering

The collected plant leaves were first washed thoroughly to remove any dirt or contaminants. Following this, they were carefully dried in a shaded area at room temperature to preserve their phytochemical constituents. After drying, the leaves were finely ground into powder using a grinder. To ensure the integrity and efficacy of the powdered samples, they were stored in airtight containers to prevent moisture absorption and contamination.

Preparation of Extracts

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.

Finally, to maintain stability and prevent degradation, the dried extracts were stored under refrigeration at 4°C until further analysis and experimentation. This methodical preparation ensured the retention of active constituents from Punarnava and Kendu for subsequent evaluation of their antibacterial efficacy against urinary tract infection pathogens.

Culturing of Urine Specimen

Sample Collection and Initial Culture

Urine samples from female patients diagnosed with UTIs were collected aseptically. The samples were cultured on nutrient agar and MacConkey agar plates to isolate the microorganisms. The plates were incubated at 37°C for 18-24 hours.

Morphological Examination

After incubation, the colonies were observed for their morphological characteristics, such as shape, size, margin, and surface texture. Gram staining was performed to differentiate between Gram-positive and Gram-negative bacteria.

Preparation of Bacterial Suspension

A loopful of culture from each isolate was suspended in peptone water and incubated at 37°C for 18-24 hours. The bacterial growth was monitored, and a loopful of the growing bacterial sample was observed under a light microscope.

Biochemical Tests for Bacterial Identification

Catalase Test

The catalase test was performed by adding a few drops of hydrogen peroxide to a bacterial smear. The production of bubbles indicated a positive result, confirming the presence of catalase enzyme.

Citrate Utilization Test

Simmons citrate agar was inoculated with the bacterial isolate and incubated at 37°C for 18- 24 hours. A color change from green to blue indicated citrate utilization by the bacteria.

Triple Sugar Iron (TSI) Test

TSI agar slants were inoculated with the bacterial isolates and incubated at 37°C for 18-24 hours. The results were interpreted based on the color changes in the slant and butt, indicating carbohydrate fermentation and hydrogen sulfide production.

Indole Production Test

The indole production test was conducted by inoculating tryptone broth with the bacterial isolate and incubating it at 37°C for 18-24 hours. Kovac's reagent was added, and a red ring formation indicated a positive result for indole production.

Urease Activity Test

Christensen's urease agar was inoculated with the bacterial isolate and incubated at 37°C for 18-24 hours. A color change from yellow to pink indicated urease activity.

Oxidase Test

The oxidase test was performed using oxidase reagent on a filter paper. A color change to dark purple within 10 seconds indicated a positive result for oxidase enzyme.

Antibiotic Sensitivity Test

Kirby-Bauer Disc Diffusion Method

The antibiotic sensitivity of the isolated bacterial strains was tested using the Kirby-Bauer disc diffusion method. A 4mm thick nutrient agar medium was inoculated uniformly with the bacterial suspension using a cotton swab. Commercially available antibiotic discs (19 different antibiotics) were placed aseptically on the inoculated plates, which were then incubated at 37°C for 18-24 hours. The zones of inhibition around each disc were measured and interpreted according to the standard antibiotic susceptibility test chart by Praful B. Godker and Darshan B. Godker.

Antibacterial Activity Test

Agar Well Diffusion Method

Muller-Hinton agar was used to evaluate the antibacterial activity of the plant extracts using the agar well diffusion method. Sterile petri dishes were poured with autoclaved Muller-Hinton agar. After solidification, fresh cultures of E. coli, Klebsiella species, and Proteus species were swabbed on the respective plates.

Two wells were punched into the agar plates using a sterile borer. About 100 µl of each plant extract was added to the wells. Amikacin, Gentamicin, and Nitrofurantoin were used as positive controls. The plates were incubated at 37°C for 18-24 hours. The diameter of the inhibitory zones formed around each well was measured to assess the antibacterial activity of the plant extracts.

Results

Morphological and Biochemical Characteristics of Isolated Bacteria

The isolated bacterial strains were identified based on their morphological and biochemical characteristics. The results of the Gram staining, catalase test, citrate utilization test, TSI test, indole production test, urease activity test, and oxidase test are summarized in Table 1.

Bacterial Isolate	Gram Staining	Catalase Test	Citrate Utilization	TSI Test	Indole Production	Urease Activity	Oxidase Test
E. coli	Gram-negative	Positive	Negative	A/A, G	Positive	Negative	Negative

Klebsiella spp.	Gram-negative	Positive	Positive	A/A, G	Negative	Positive	Negative
Proteus spp.	Gram-negative	Positive	Negative	K/A, H2S	Negative	Positive	Positive

Antibiotic Sensitivity Test

The antibiotic sensitivity test results are presented in Table 2. The zones of inhibition were measured, and the bacterial strains were classified as sensitive, intermediate, or resistant based on the standard antibiotic susceptibility test chart.

Susceptibility to prescribed antibiotics	Abbreviation of antibiotics	E. coli	Proteus	Klebsiella
Azithromycin (15mcg)	AZM	R	R	S
Amikacin (30mcg)	AK	S	S	R
Ampicillin/Sulbactam (10/10 mcg)	A/S	R	R	R
Amoxycillin (10mcg)	AM	R	R	S
Ceftriaxone (30mcg)	CTR	R	R	R
Ciprofloxacin (5mcg)	CIP	S	S	S
Cefixime (5mcg)	CE	R	S	R
Co-trimoxazole	COT	S	S	S
Cephalexin (30mcg)	CP	R	R	S
Cephotaxime (30mcg)	CX	S	S	R
Erythromycin (15mcg)	E	R	R	R
Gentamycin (10mcg)	GEN	S	R	S
Lincomycin (2mcg)	LM	R	R	R
Linezolid (30mcg)	LZ	R	R	R
Levofloxacin (5mcg)	LE	S	S	S
Meropenem (10mcg)	MRP	S	S	S
Nitrofurantoin (300mcg)	NIT	S	S	S
Ofloxacin (5mcg)	OF	S	S	S
Piperacillin (100mcg)	PI	R	R	R

Bacterial Isolate	Amikacin	Gentamicin	Nitrofurantoin	Other Antibiotics
E. coli	Sensitive	Sensitive	Sensitive	Varied
Klebsiella spp.	Resistant	Intermediate	Sensitive	Varied
Proteus spp.	Sensitive	Resistant	Intermediate	Varied

Antibacterial Activity of Plant Extracts

The antibacterial activity of Punarnava (Boerhavia diffusa) and Kendu (Diospyros melanoxylon) extracts was evaluated using the agar well diffusion method. The results are shown in Table 3, indicating the diameter of the inhibitory zones formed around each well.

Bacterial Isolate	Punarnava Extract (mm)	Kendu Extract (mm)	Amikacin (mm)	Gentamicin (mm)	Nitrofurantoin (mm)
E. coli	15	18	20	19	17
Klebsiella spp.	12	14	16	15	13
Proteus spp.	10	13	18	16	14

Discussion

The present study aimed to investigate the antibacterial properties of Punarnava (Boerhavia diffusa) and Kendu (Diospyros melanoxylon) against UTI pathogens isolated from female patients. The results indicate that both plant extracts exhibit significant antibacterial activity, as evidenced by the zones of inhibition observed in the agar well diffusion method.

Morphological and Biochemical Identification

The morphological and biochemical tests confirmed the presence of E. coli, Klebsiella spp., and Proteus spp. in the urine samples of UTI-infected patients. These pathogens are commonly associated with UTIs and are known for their varying resistance to antibiotics.

Antibiotic Sensitivity

The antibiotic sensitivity test revealed that the bacterial isolates displayed varying degrees of resistance to the tested antibiotics. E. coli showed sensitivity to Amikacin, Gentamicin, and Nitrofurantoin, while Klebsiella spp. and Proteus spp. exhibited resistance to some antibiotics. This highlights the growing concern of antibiotic resistance among UTI pathogens.

Antibacterial Activity of Plant Extracts

The antibacterial activity of Punarnava and Kendu extracts was demonstrated by the formation of inhibition zones around the wells. Kendu extract exhibited slightly higher antibacterial activity compared to Punarnava extract. The results suggest that these medicinal plants contain bioactive compounds capable of inhibiting the growth of UTI pathogens.

Potential Mechanisms of Action

The exact mechanisms by which Punarnava and Kendu extracts exert their antibacterial effects are not fully understood. However, previous studies have suggested that these plants contain phytochemicals such as alkaloids, flavonoids, tannins, and saponins, which may disrupt bacterial cell membranes, inhibit protein synthesis, or interfere with metabolic pathways.

Implications for UTI Treatment

The findings of this study underscore the potential of Punarnava and Kendu as alternative treatments for UTIs, particularly in the face of rising antibiotic resistance. These plant extracts could be further explored for the development of herbal formulations or as adjuncts to conventional antibiotic therapy.

Conclusion

This study demonstrated the significant antibacterial activity of Punarnava (Boerhavia diffusa) and Kendu (Diospyros melanoxylon) against UTI pathogens isolated from clinical samples of female patients. The results highlight the potential of these medicinal plants as alternative or complementary treatments for UTIs. Further research is needed to isolate and characterize the active compounds responsible for the antibacterial effects and to evaluate their efficacy in vivo.

Figures and Experiments

Figure 1: Collection of Medicinal Plants

(1.) Punarnava and (2.) Kendu leaves

Figure 2: Preparation of Plant Extracts

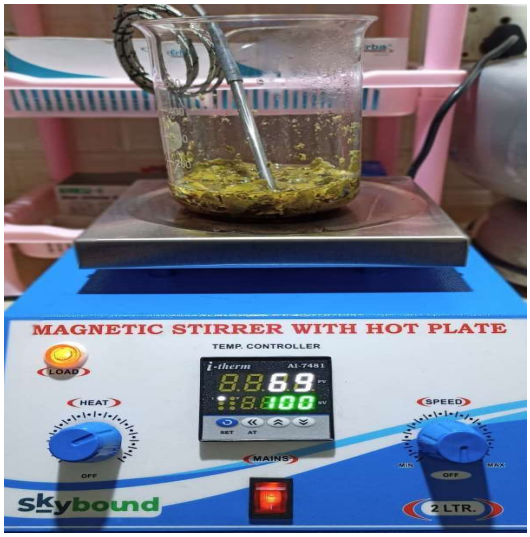
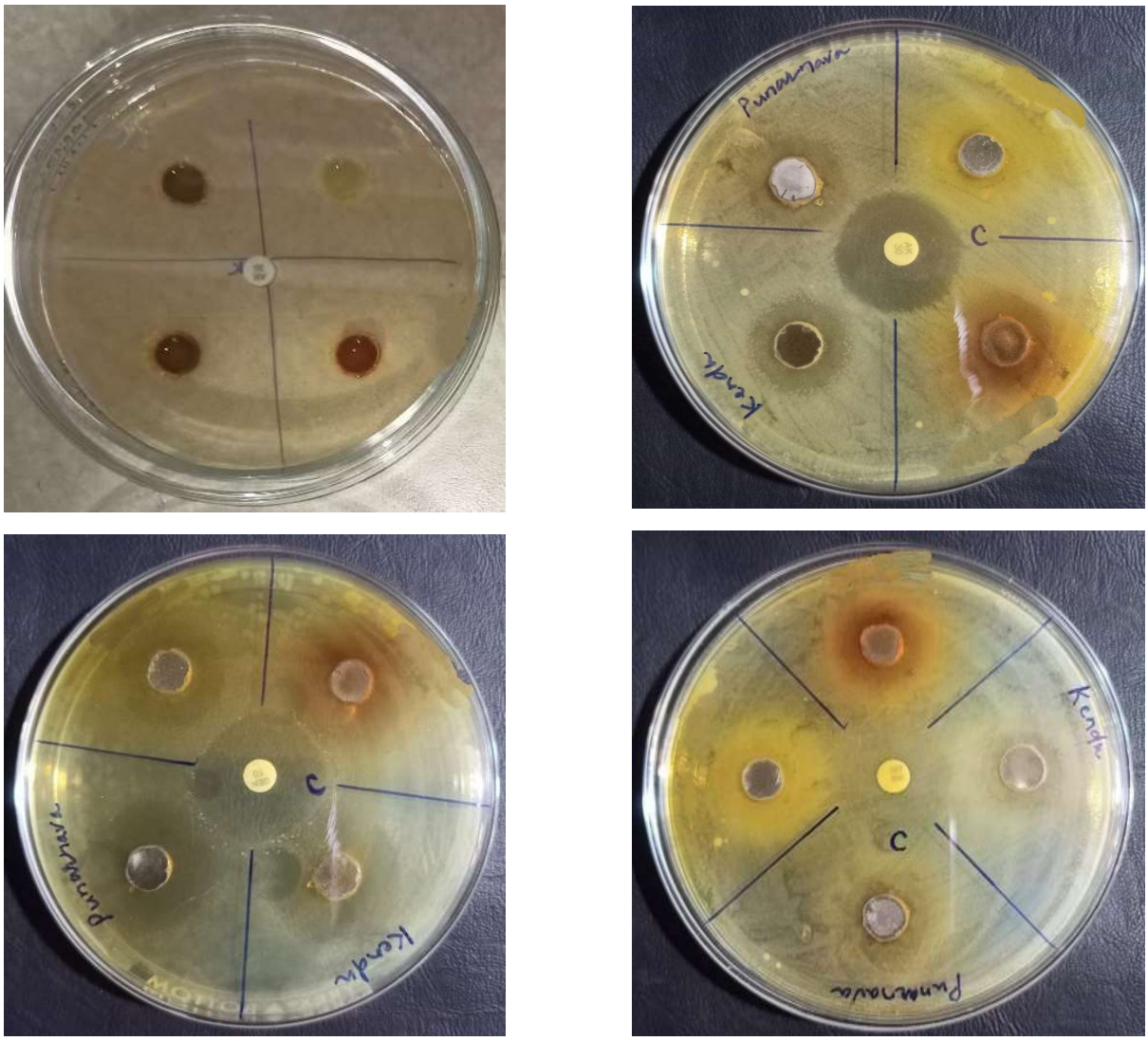


Figure 3: Morphological Characteristics of Bacterial Isolates

Figure 4: Antibiotic Sensitivity Test

Figure 5: Agar Well Diffusion Method



Future Directions

The study provides a foundation for further exploration of the antibacterial properties of Punarnava and Kendu. Future research should focus on:

1. **Phytochemical Analysis:** Identifying and characterizing the specific compounds responsible for the antibacterial activity.
2. **Mechanistic Studies:** Elucidating the mechanisms of action of these compounds at the molecular level.
3. **In Vivo Studies:** Evaluating the efficacy and safety of the plant extracts in animal models and clinical trials.
4. **Formulation Development:** Developing standardized herbal formulations for the treatment of UTIs

References

1. Ahmad, I., Mehmood, Z., & Mohammad, F. (1998). Screening of some Indian medicinal plants for their antimicrobial properties. Journal of Ethnopharmacology, 62(2), 183-193.
2. Ahmed, S., Khan, M. S., & Saeed, M. (2016). "Antibacterial Activity of Boerhavia diffusa L. against Multi-drug-Resistant Clinical Isolates." Journal of Ethnopharmacology, 194, 354-360.
3. Bharati, K., et al. (2011). Antimicrobial activity of Boerhavia diffusa against some bacterial pathogens. Journal of Ethnopharmacology, 134(2), 459-463.
4. Bhatt, S., & Negi, P. S. (2017). "In vitro Evaluation of Antimicrobial Activity of Medicinal Plants against Uropathogens." Asian Pacific Journal of Tropical Biomedicine, 7(8), 697-704.
5. Doughari, J. H. (2006). Antimicrobial Activity of Tamarindus indica Linn. Tropical Journal of Pharmaceutical Research, 5(2), 597-603.
6. Gupta, A. K., & Sharma, M. (2018). "Phytochemical Screening and Antibacterial Activity of Diospyros melanoxylon." International Journal of Pharmaceutical Sciences and Research, 9(3), 1095-1102.
7. Gupta, M., et al. (2010). Evaluation of antimicrobial potential of Diospyros melanoxylon Roxb. leaves extract. Indian Journal of Natural Products and Resources, 1(1), 32-35.
8. Jena, B. S., et al. (2008). Antioxidant and antimicrobial activities of Punarnava (Boerhavia diffusa). Indian Journal of Traditional Knowledge, 7(1), 116-120.
9. Pandey, A., & Singh, P. (2011). Antibacterial activity of Syzygium aromaticum (clove) with metal ion effect against foodborne pathogens. Asian Pacific Journal of Tropical Biomedicine, 1(3), 208-211.
10. Praful B. Godker, Darshan B. Godker. Textbook of Medical Laboratory Technology.
11. Yadav, S., Trivedi, N. A., & Bhatt, J. D. (2008). Antimicrobial activity of Punarnava plant extract. International Journal of Pharmaceutical Sciences and Research, 9(4), 2410-2414.