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Evaluation of the Antibacterial Activity of the Leave Extract of *Oroxylum Indicum* Against Multidrug-Resistant Bacteria Causing Urinary Tract Infection

Debasmita Dubey¹, Mahesh Chandra Sahu², Sadhni Inudar³, Swaraj Babu⁴, Pradeep Kumar Naik⁵

¹Post Doctoral Fellow, Centre of Excellence in Natural Products and Therapeutics, Department of Biotechnology and Bioinformatics, Sambalpur University, Jyoti Vihar, Burla, Sambalpur-768019, Odisha, India; ²Scientist B, Department of Microbiology, National Institute of Occupational Health - ICMR, Meghani Nagar, Ahmedabad-380016, Gujarat, India; ³Inspire Fellow, Centre of Excellence in Natural Products and Therapeutics, Department of Biotechnology and Bioinformatics, Sambalpur University, Jyoti Vihar, Burla, Sambalpur-768019, Odisha, India; ⁴Rajiv Gandhi Fellow, Centre of Excellence in Natural Products and Therapeutics, Department of Biotechnology and Bioinformatics, Sambalpur University, Jyoti Vihar, Burla, Sambalpur-768019, Odisha, India; ⁵Professor, Centre of Excellence in Natural Products and Therapeutics, Department of Biotechnology and Bioinformatics, Sambalpur University, Jyoti Vihar, Burla, Sambalpur-768019, Odisha, India.

ABSTRACT

Aim: Evaluation of antibacterial efficacy of the methanolic leaf extract of *Oroxylum indicum* against isolated MDR strains viz. *Acinetobacter* sp., *Citrobacter* sp., *Enterobacter* sp., *Escherichia coli*, *Klebsiella pneumonia*, *Proteus Vulgaris*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus fecalis* causing urinary tract infections (UTI).

Methods: Pathogenic bacteria were isolated and identified using the routine microbiological procedure. All the bacteria were MDR as determined from the antibiotic sensitivity test. Agar well diffusion and microbroth dilution methods were used for determining antibacterial efficacy and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *O. indicum* methanolic leaf extract were determined. Cord blood lymphocytes were used to determine the *in vitro* toxicity of the leaf extracts.

Results: The leaf extract of *O. indicum* exhibited good antibacterial activity against both Gram-positive and Gram-negative MDR bacteria. The MIC value of the plant extract ranges from 0.19 to 0.78 mg/ml and the MBC value ranges from 0.78 to 1.56 mg/ml with all the isolated bacterial pathogens. Plant extracts were found to be very effective against these MDR strains. The plant extract also revealed no toxicity even at a very high concentration of 1800 mg/l with lymphocytes isolated from the cord blood. Surprisingly, the MIC value was found to be 600 mg/l with lymphocytes.

Conclusion: *O. indicum*'s methanolic leaf extract can be used as a potential antibacterial drug. Similarly, other medicinal plants can also be explored for their possible antibacterial efficacy over the antibiotic resistance problem.

Key Words: UTI (Urinary Tract infection), *O. indicum*, Antibacterial Activity, Toxicity Study, Alternative Medicine

INTRODUCTION

Urinary tract infections (UTI) caused by both "Gram-negative (GN) and Gram-positive (GP) pathogenic bacteria increases the rate of morbidity and mortality throughout the globe. The first antibiotic, penicillin, or 6-aminopenicillins acid (6-APA) control bacterial infections, however, certain bacteria have developed resistance against it.^{1,2} Further, pathogenic bacteria have been shown to developed resistance to newly introduced antibiotics.²⁻⁴ Thus, multidrug-resistant (MDR) bacteria must be controlled with an iron hand, which has been giving impetus to search for newer antibacterial

from conventional and non-conventional sources, apart from a chemical modification of existing antibiotics. Several phytochemicals were reported to have antibacterial and antifungal activities and lend themselves to the preparation of mainstream medicine.^{5,6}

Oroxylum indicum (L.) Vent. is commonly known as shyonaka or sonpatha, is a small to medium-sized deciduous tree of the family Bignoniaceae. It is distributed throughout India, upto an altitude of 1200 M and found mainly in the ravine and moist places in the forests and also in the Himalayan foothills, Eastern and the Western Ghats, and North-East India.^{7,8}

Corresponding Author:

Pradeep Kumar Naik, Centre of Excellence in Natural Products and Therapeutics, Department of Biotechnology and Bioinformatics, Sambalpur University, Jyoti Vihar, Burla, Sambalpur-768019, Odisha, India; Phone: +91-9479268802; E-mail: pknaik7879@gmail.com

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Different plant parts of *O. indicum* being used in Ayurveda and traditional medicine for the treatment of different ailments such as fevers, cough, cancer, diarrhoea, ulcer, dropsy, jaundice and preventing other respiratory disorders.⁹ Root extract has been used for ayurvedic preparations like Dashmularisht and Chyawanprash.¹⁰ It is also one of the important ingredients in an ayurvedic formulation such as Amartarista, Dantadyarista, Narayana taila, Dhanawantaraghrita, Brahma Rasayana and Awalwha.¹¹ It possesses antioxidant, antifungal, antimicrobial, anti-inflammatory, antibacterial, anti-arthritic and anti-cancer properties.¹² The leaves and stem bark are reported to contain flavonoids namely chrysin, oroxylin-A, scutellarin, baicalein.^{13,14} Root bark is reported to contain chrysin, baicalein, biochanin-A, and ellagic acid.¹⁵ Seeds are reported to use in the perfume industry.⁹ Baicalein is reported to possess an anti-inflammatory, anti-ulcer, antioxidant, hepatoprotective, and immunomodulatory activity.¹⁶⁻²⁰ Baicalein is also reported to check the proliferation of human breast cancer cell line MDA-MB-435.¹⁶⁻²¹

In the present study, we have attempted to investigate the antibacterial activity of the leaf extract of *O. indicum* against both the GP and GN MDR strains of bacteria isolated from the clinical samples of patients suffering from UTI.

MATERIALS AND METHODS

Collection, processing, storage and solvent extract preparation

Leaf of *O. indicum* was collected from Bhubaneswar, Western Odisha. The plant species were identified with the help of regional flora books (Haines, 1921-25, Saxena and Brahman, 1994-96). Voucher specimens were deposited in the herbarium of Centre of Excellence in Natural Products and Therapeutics, Dept. of Biotechnology and Bioinformatics, Sambalpur University, Odisha, India. The collected leaf samples were processed and solvent extracts were made and stored as described previously.⁴

Bacterial Strain isolation, identification and antibiogram

Two GPs, *Enterococcus faecalis*, and *Staphylococcus aureus*, as well as 8 GNs viz. *Acinetobacter sp.*, *Citrobacter sp.*, *Enterobacter sp.*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, and *Pseudomonas aeruginosa* were isolated from the patients with UTI from a private hospital, Odisha, India. The bacteria were identified based on the conventional colony and biochemical characteristics. Further, Kirby-Bauer's disk diffusion method was utilized to evaluate the antibiotic sensitivity of the isolated bacteria.^{22,23}

Evaluation of antibacterial activity and determinations of MIC and MBC of plant

Agarwell diffusion method was used for determining the antibacterial efficacy of the methanolic leaf extract against the isolated UTI MDR bacterial strains.²⁴ Further, the microbroth dilution method was used for determining the MIC and MBC values of the methanolic leaf extract, following the protocol described previously.²⁴

Phytochemicals screening

Phytochemical screening tests of the methanolic leaf extract was done according to the protocol described earlier.²⁴

Evaluation of toxicity of plant extract with lymphocytes

Toxicity of the methanolic leaf extract of *O. indicum* was evaluated based on *in vitro* lymphocyte culture isolated from umbilical cord blood as described before. The proliferation of lymphocytes in the presence of graded concentrations of the plant extract was monitored by MTT assay as reported earlier.⁴ The toxicity of plant extracts to isolated lymphocytes was also studied by comet assay as reported earlier.⁴

Statistical Analysis

The observed percentage of the lethality of the plant extract was determined based on probit analysis.

RESULTS

Antibiotic susceptibility screening of the isolated bacterial strains

All the isolated bacteria were screened with 18 antibiotics from 6 different groups. The antibiogram of the isolated bacteria is collated in (Table 1). It was found that all the bacterial strains have shown resistance to most of the antibiotics.

Antibacterial activity of the plant extract

The results of the antibacterial activity of the methanolic leaf extract of *O. indicum* against isolated MDR strains viz. *Acinetobacter sp.*, *Citrobacter sp.*, *Enterobacter sp.*, *E. coli*, *K. pneumoniae*, *P. vulgaris*, *P. mirabilis*, *P. aeruginosa*, *S. aureus* and *enterococcus faecalis* from patients suffering from UTI infection are presented in (Table 2). The plant extract could effectively inhibit the growth of the bacteria as indicated by the zone of inhibition. The methanolic leaf extract was used for qualitative evaluation of the composition of various secondary metabolites using traditional biochemical characterization.

Table 1: Antibiogram of the isolated bacteria from the patients suffering from UTI infection against 18 antibiotics used in the clinic.

Strains	Susceptibility to prescribed antibiotics																	
	Amino-Glycosides				β-lactams		Cephalosporins			Fluoro-quinolones				Glyco-peptide	Sul-fona-mide	Stand-alone		
	Ac	Ge	Am	Ak	Ox	Pt	Ce	Cf	Ci	Gf	Na	No	Of	Va	Cot	Ch	Nf	Te
<i>Acinetobacter</i> sp.	-	-	-	-	-	-	-	-	+	-	-	-	-	n	-	+	-	+/-
<i>Citrobacter</i> sp.	-	-	-	-	-	-	-	-	+	-	-	-	-	n	+	+	-	-
<i>Enterobacter</i> sp.	-	-	-	-	-	-	-	-	+	-	-	-	-	n	+	+	-	-
<i>E. coli</i>	-	+	-	-	n	-	-	-	-	-	-	-	-	n	-	+	-	-
<i>K. pneumonia</i>	+	+	-	-	n	+	-	-	+	-	+	+	+	n	-	+	+/-	+
<i>P. vulgaris</i>	-	-	-	-	n	-	-	-	+/-	-	-	-	-	n	-	+	-	-
<i>P. mirabilis</i>	-	-	-	-	n	-	-	-	+/-	-	-	-	-	n	-	+/-	+/-	-
<i>P. aeruginosa</i>	-	+/-	-	-	n	-	-	-	+	-	-	-	-	n	-	+	-	-
<i>S. aureus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	+/-	+/-
<i>E. faecalis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	+

Note: +/-: intermediately sensitive; +: sensitive; -: resistant

Table 2: The inhibition zone size and MIC, MBC values of the leaf extract (30 mg/mL) against bacteria isolated from patients suffering from UTI infection.

Sl. No.	Bacteria	Crude extract		
		Size of zone of inhibition (mm)	MIC(mg/mL)	MBC(mg/mL)
1	<i>Acinetobacter</i> sp.	28	0.78	1.56
2	<i>Citrobactersp.</i>	26	0.19	0.78
3	<i>Enterobactersp.</i>	26	0.78	1.56
4	<i>E. coli</i>	29	0.78	1.56
5	<i>K. pneumonia</i>	22	0.78	1.56
6	<i>P. vulgaris</i>	19	0.19	0.78
7	<i>P mirabilis</i>	26	0.78	1.56
8	<i>P. aeruginosa</i>	28	0.78	1.56
9	<i>S aureus</i>	29	0.78	1.56
10	<i>E. faecalis</i>	26	0.19	0.78

Toxicity evaluation of the plant extract

We have used human cord blood lymphocyte culture to evaluate the toxicity of the crude leaf extract. No evidence of toxicity was found even at the concentration of 1800 mg/l

leaf extract using comet assay (Figure 1). The experimental MIC value of the crude extract was determined to be 600 mg/l and the computed LC₂₅ value was found to be 1698.24 mg/l during cytotoxicity (Figure 2, Table 3).

Table 3: Lethality values during leaf extract toxicity to human lymphocytes growing in DMEM, assessed by AO/EB staining with probit values.

Methanolic leaf extract concentration (mg/L)	Log ₁₀ concentrations	Lethality (%)	Probitvalues
0	-	-	-
300	2.47	-	-
600	2.77	6.8	3.51
900	2.95	8.2	3.61
1200	3.07	13.4	3.89

Table 3: (Continued)

Methanolic leaf extract concentration (mg/L)	Log ₁₀ concentrations	Lethality (%)	Probitvalues
1500	3.17	17.6	4.06
1800	3.25	21.4	4.21
2100	3.32	24.8	4.32
2400	3.38	31.2	4.51
2700	3.43	32.4	4.54
3000	3.47	33.1	4.56

DMEM, Dulbecco's modified Eagle's medium; MTT, 3-[4, 5-dimethylthiazol-2-yl] 2,5-diphenyl tetrazolium bromide; PL, Percent lethality; -, Not applicable"

DISCUSSION

UTI is considered one of the major problems for hospitalized patients. It is due from the advancement of UTI from the faecal issue, it is more promptly in females in comparison to males.^{25,26} The bacterial pathogens involved in UTI are mostly *Klebsiella*, *E. coli*, *Pseudomonas* and *Enterobacter*. Additionally, *Candida* sp. is also widely recognized as a pathogen that causes UTIs.^{27,28} In quest of finding bacterial pathogens from the patients suffering from UTI, we have identified 2 GP bacteria, *S. aureus* and *Enterococcus faecalis* as well as 8 GN bacteria viz. *Acinetobacter* sp., *Citrobacter* sp., *Enterobacter* sp., *E. coli*, *K. pneumonia*, *P. vulgaris*, and *P. mirabilis*. Most of these isolated bacterial strains revealed resistance to most of the antibiotics. These isolated bacterial strains were screened for their susceptibility against the methanolic leaf extract of *O. indicum*, which revealed good antibacterial activity against all the isolated bacterial strains. The MIC value ranges from 0.19 to 0.78 mg/ml and MBC value ranges from 0.78 to 1.56 mg/ml for crude extract. The antibacterial activity of *O. indicum* extracts was successfully proven previously against gram-positive bacteria.²⁹ Another Indian study also proved the antibacterial efficacy of the stem bark and root extracts against both gram-positive, gram-negative bacteria and pathogenic *Candida* species.³⁰ Similarly, Talaria et al, 2013, reported the antibacterial activity of stem bark extracts against clinically important Gram-positive bacteria.³¹ Further, Singh et al., 2002 in his study reported the antibacterial and antifungal efficacy of ethanolic extracts of various Himalayan medicinal plants.³² Toxicity evaluation of the methanolic leaf extract of *O. indicum* failed to reveal any toxicity using lymphocytes isolated from cord blood even at a very high concentration of 1800 mg/l. Parenthetically the MIC value was found to be 600 mg/l with lymphocyte which revealed no severe toxicity. The antibacterial activity of *O. Indicum* is mainly because of the presence of various phytochemicals in its extract. Bark extract showed the presence of various secondary metabolites in the alcoholic extracts of bark of *O. indicum*.³³ Similarly, many other studies revealed plants contains secondary metabolites and many other trace

elements, which can be attributed to their antibacterial and other therapeutic efficacy along with a possible treatment option for Covid -19 virus too.³⁴⁻³⁶

CONCLUSIONS

In conclusion, UTI in patients was caused by MDR bacteria, hence cannot be controlled by antibiotics. Methanolic leaf extract of *O. indicum* proved to be a potent crude drug in this study as it was able to inhibit the growth of all the MDR bacteria. Crude extract failed to reveal any toxicity at very high concentrations. Therefore, it is concluded that *O. indicum*'s methanolic leaf extract can be further used as a potential antibacterial drug. Similarly, other medicinal plants can also be explored for their possible antibacterial efficacy over the antibiotic resistance problem.

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Conflicts of Interest: None

Authors Contribution: D Dubey and MC Sahu conducted the experiments, collected the data and drafted the manuscript. PK Naik is finalized the data and manuscript.

Ethical Issues: This paper includes no human or animal subjects, hence no ethical approval is required.

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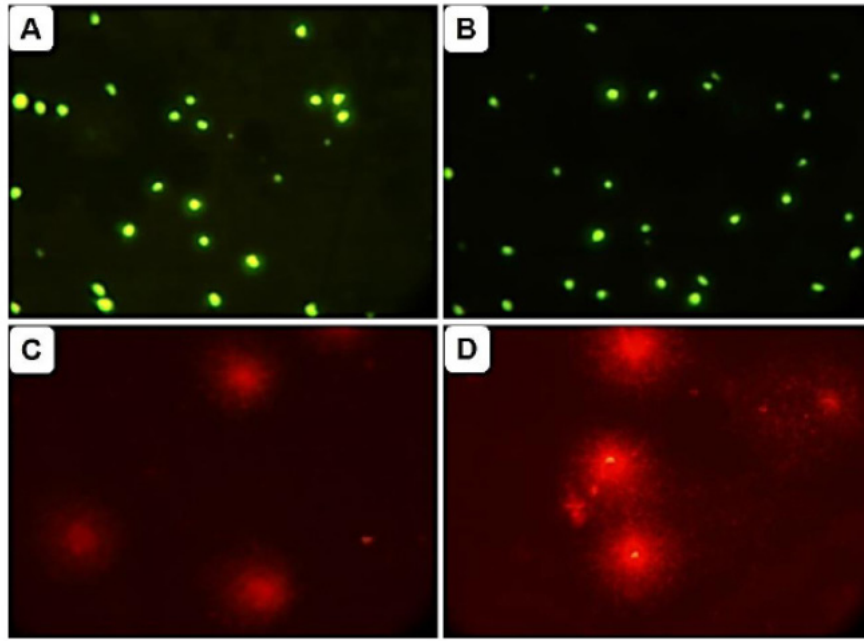


Figure 1: Comet assay with lymphocytes. A) Control cells, B) Cells after treatment with 300 mg/L plant extract.

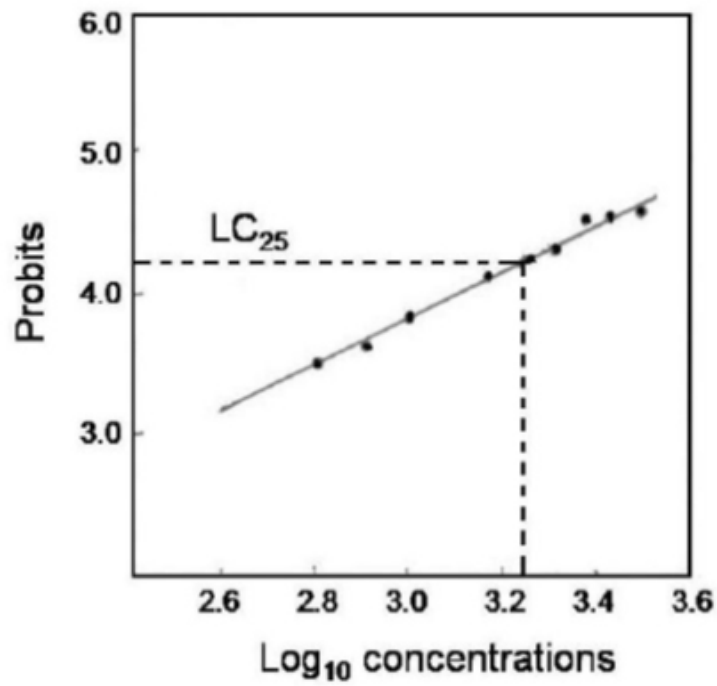


Figure 2: "Probits of percentage lethality values plotted against log₁₀ concentrations of plant extract in the toxicity study of lymphocytes.