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Research Article
Potential Antibacterial Activity of *Oroxylum indicum* (L.) Kurz Extracts Against Antibiotic-Resistant Bacteria Isolated from Roi Et Hospital

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Abstract

Background and Objective: *Oroxylum indicum*, a plant commonly used in traditional medicine to address various human ailments, has recently gained attention as a promising candidate in this regard due to its rich phytochemical composition and potential antibacterial properties. This study was undertaken to evaluate the antibacterial efficacy of *O. indicum* extracts, specifically from its leaves and stems, against antibiotic-resistant bacteria. **Materials and Methods:** Stems and leaves of *O. indicum* were extracted using ethanol, hexane and dichloromethane. The antibacterial activity of the extracts was initially evaluated through the disc diffusion method, while the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were determined using the broth microdilution method. The inhibition zone diameters (mm) were statistically analyzed using Duncan's Multiple Range Test (DMRT) in SAS software at a p-value threshold of <0.05. **Results:** The ethanolic stem and leaf extracts of *Oroxylum indicum* produced the largest inhibition zone of 11 mm against *Proteus mirabilis*, highlighting a significant antibacterial response. Further analysis showed that the lowest minimum inhibitory concentration (MIC) was recorded at 1.56 mg/mL in these ethanolic extracts, effective against both *Acinetobacter baumannii* and *Proteus mirabilis*. The minimum bactericidal concentration (MBC) was observed at 6.25 mg/mL for these bacteria, indicating a potent bactericidal effect. **Conclusion:** This study represents the first documented instance of *O. indicum* extracts effectively targeting antibiotic-resistant bacteria, thereby underscoring their potential as a foundation for developing new antibiotics. The findings pave the way for further research and development, contributing to the critical pursuit of novel therapeutic agents to combat resistant bacterial infections.

Key words: *Oroxylum indicum* (L.) Kurz extracts, antibiotic-resistant bacteria, antibacterial activity, *Acinetobacter baumannii*, *Proteus mirabilis*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Oroxylum indicum (L.) Kurz, commonly known as trumpet tree and is a member of the Bignoniaceae family. It is one of the medicinal plants that has been used traditionally across various regions of the world, particularly in Asia[1,2]. In various parts of Southeast Asia, *O. indicum* has been used for centuries in ethnomedicinal systems for the prevention and treatment of diseases[3]. In Thailand *O. indicum*, known locally as "Pekha or Linpha" is traditionally used throughout Thailand for its medicinal properties[4] and edible plant, especially in the North and Northeast areas[5,6]. Research on *O. indicum* in Thailand has explored various aspects of the plant's medicinal properties and applications. *Oroxylum indicum*, a prominent medicinal plant in traditional medicine systems, has been extensively utilized for the prevention and treatment of a wide range of ailments. These include jaundice, arthritis, rheumatism, gastric ulcers, tumors and respiratory disorders, as well as chronic conditions such as cancer and diabetes. Additionally, it is employed to address gastrointestinal issues, including diarrhea and dysentery, owing to its anti-inflammatory, antioxidant and antimicrobial properties. The therapeutic applications of *Oroxylum indicum* are deeply rooted in its phytochemical composition, which includes bioactive compounds such as flavonoids, alkaloids, tannins and saponins, making it a subject of growing interest in modern pharmacological research for its efficacy and safety in disease management[7,8]. Previously reports were presented that Pekha has various pharmacological properties such as antitumor[9], anti-inflammatory[10], anti-arthritic, anticancer, antioxidant[2], analgesic[10], anti-ulcer[11], anti-mutagenic[12], anti-helminthic[13], hepatoprotective, immunostimulating[14], antiviral and antimicrobial[1,15].

Antibiotic-resistant bacteria are strains of bacteria that have developed the ability to withstand the effects of antibiotics, rendering these medications less effective or entirely ineffective in treating infections caused by such bacteria[16]. The emergence of antibiotic resistance is a growing global concern, as it compromises the effectiveness of commonly used antibiotics and presents a serious threat to public health[17]. The proliferation of drug-resistant pathogens is chiefly attributed to the inappropriate utilization and excessive application of antibiotics in human, animal and plant contexts. Infections caused by drug-resistant bacteria not only necessitate prolonged and financially burdensome hospitalization but also elevate the likelihood of mortality associated with the infection[18,19].

According to a review presented by Mancuso *et al.*[20], it was shown that the highest priority status due to their significant threat to human health was the *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species. *Acinetobacter baumannii* is a Gram-negative bacillus[21] and an opportunistic pathogen recognized for its capacity to cause diverse infections, particularly in healthcare settings. It is a significant contributor to healthcare-associated infections, including pneumonia, bloodstream infections, urinary tract infections and wound infections, primarily affecting individuals with weakened immune systems or pre-existing health conditions[22]. Colistin-resistant *Pseudomonas aeruginosa* is an opportunistic Gram-negative pathogen which is a major cause of acute pulmonary healthcare-associated infections[23]. *Proteus mirabilis* is a Gram-negative bacterium which most frequently associated with infections of the urinary tract[24]. The rise of antibiotic-resistant bacteria further underscores the ongoing challenge and highlights the need for vigilant surveillance, judicious antibiotic use and the development of alternative therapeutic strategies to address such resistant strains[25].

Antibacterial activity of *O. indicum* was a few reports. The *O. indicum* bark was extracted with dichloromethane and hexane showed an inhibition zone against *Klebsiella pneumoniae* ATCC 25955 and *Staphylococcus aureus* ATCC 25923[26]. The methanolic extract of *O. indicum* leaves presented the inhibition zone against *Pseudomonas aeruginosa* and *Bacillus subtilis*[27]. The stem bark and root of *O. indicum* extracted using dichloromethane presented antimicrobial activity against *Candida albicans*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*[28]. The antibacterial activity of *O. indicum* extracts against antibiotic-resistant bacteria is limited. This research aimed to evaluate the antibacterial activity of *O. indicum* against antibiotic-resistant bacteria (*Acinetobacter baumannii*, Colistin-resistant *Pseudomonas aeruginosa* and *Proteus mirabilis*). The findings from this study will furnish crucial insights into the advancement of novel antibiotics aimed at addressing infections induced by antibiotic-resistant bacterial strains.

MATERIALS AND METHODS

Study area: The research was conducted from May to October, 2024 in the Microbiology Laboratory, Department of Science and Technology, Faculty of Liberal Arts and Science, Roi Et Rajabhat University, Roi Et, Thailand.

Plant sample collection and extraction: Two kilograms of *Oroxylum indicum* leaves and stem were collected from Ban Tha Muang, Tha Muang, Selaphum, Roi Et, Thailand. The plant samples were washed twice using tap water and cut into small pieces before being dried at 50°C using hot air over (POL-EKO-APARATURA company, Wodzisław Śląski, Poland) until completely dry. The dried *O. indicum* leaves and stems were powered using a blender (WF-20B THAIGRINDER, Thailand). The *O. indicum* leaves and stems powder were extracted using 3 different polarities of the solvent including ethanol, dichloromethane and hexane. The extracted solutions were filtered and dry at 50°C for 2 days. The plant extracts were mixed with Dimethyl Sulfoxide (DMSO, Sigma) to a final concentration of 500 mg/mL before use.

Human antibiotic-resistant bacteria preparation: Three human antibiotic-resistant bacteria including *Acinetobacter baumannii*, Colistin-resistant *Pseudomonas aeruginosa* and *Proteus mirabilis* were obtained from the Department of Clinical Microbiology, Roi Et Hospital, Roi Et, Thailand. Antibiotic-resistant bacteria were streaked onto a nutrient agar (NA) plate and incubated overnight at 37°C. The single colony of each antibiotic-resistant bacteria was inoculated into 5 mL nutrient broth (NB). The culture tube was incubated with shaking at 150 rpm at 37°C for 18 hrs. Each antibiotic-resistant bacterial concentration has adjusted to the concentration at OD_{600} to 0.1 before use.

Antibacterial activity primary screening: The disc diffusion assay was used for antibacterial activity primary screening. The pathogenic bacteria have adjusted the concentration at OD_{600} to 0.1[29]. One hundred microliters of each pathogenic bacteria were spread onto an NA plate and a sterile paper disc with a diameter of 0.6 mm was placed onto an NA plate. Ten microliters of each extract were dropped onto a sterile paper disc. The NA plates were incubated at 37°C for 18 hrs. The inhibition zone that occurred was measured and recorded.

MIC and MBC values determination: Minimum inhibitory concentration (MIC) is the lowest concentration of extract that inhibits bacterial growth. Minimum bactericidal concentration (MBC) is the lowest concentration of extract that eliminates bacteria. The MIC and MBC values are important values in microbiology and antimicrobial susceptibility testing. These values are used to assess the effectiveness of antimicrobial agents. The broth microdilution and colorimetric assay were used to determine the MIC and MBC values[30,31]. The extracts that presented the positive result from the disc diffusion assay 2-fold serially diluted in a 96-well plate containing 100 µL NB. One hundred microliters of antibiotic-resistant bacteria were added into a 96-well plate containing various *O. indicum* extract concentrations. The 96-well plates were incubated overnight at 37°C. The iodonitrotetrazolium chloride (INT) solution was added to 96-well plates and incubated at 37°C for 30 min. The MIC value denotes the lowest concentration of the *O. indicum* extract at which inhibition of antibiotic-resistant bacterial growth is observed. The MBC value represents the lowest concentration of the *O. indicum* extract necessary to eradicate antibiotic-resistant bacteria that exhibit no color change after the addition of iodonitrotetrazolium chloride[32-34].

Data analysis: The inhibition zone diameter was analyzed using SAS software version 5.0. The experiment was conducted following a Completely Randomized Design (CRD) with three replicates per treatment, each replicate comprising 3 plates. Statistical analysis involved Analysis of Variance (ANOVA) to evaluate differences, with mean comparisons performed using Duncan's Multiple Range Test (DMRT). Statistical significance was determined at a p-value threshold of <0.05.

RESULTS AND DISCUSSION

Agar disc diffusion assay: The agar disc diffusion assay was employed as the primary screening method for antibacterial activity. Extracts from the stem and leaf of *O. indicum* were obtained using solvents such as ethanol, hexane and dichloromethane. These extracts were subsequently applied to the surface of nutrient agar plates inoculated with antibiotic-resistant bacteria. The results demonstrated that the most substantial inhibition zone, measuring 11 mm, was observed with ethanolic extracts from the stem and leaf against *Proteus mirabilis*. This was followed by ethanolic extracts from the leaf and stem against *Acinetobacter baumannii* and Colistin-resistant *Pseudomonas aeruginosa*, as well as hexane extracts from the stem against *Acinetobacter baumannii* and Colistin-resistant *Pseudomonas aeruginosa*, both of which exhibited an inhibition zone of 10 mm (Table 1).

MIC and MBC values: The broth microdilution assay was employed to ascertain the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of *O. indicum* extracts. This method conventionally involves the testing of twofold dilutions of various antimicrobial agents in a 96-well culture plate. The iodonitrotetrazolium (INT) reagent was employed as an indicator in the colorimetric

Table 1: Diameter of the inhibition zone of *Oroxylum indicum* (L.) Kurz extracts against human antibiotic-resistant bacteria

Plant sample	Bacterial strain	Solvent	Average of Inhibition zone diameter (mm)*
Leave	*Acinetobacter baumannii*	E	10^{ab}
		H	9^{b}
		D	9^{b}
Stem	*Acinetobacter baumannii*	E	9^{b}
		H	10^{ab}
		D	9^{b}
Leave	Colistin-resistant *Pseudomonas aeruginosa*	E	9^{b}
		H	9^{b}
		D	7^{c}
Stem	Colistin-resistant *Pseudomonas aeruginosa*	E	10^{ab}
		H	10^{ab}
		D	0^{d}
Leave	*Proteus mirabilis*	E	11^{a}
		H	9^{b}
		D	11^{a}
Stem	*Proteus mirabilis*	E	11^{a}
		H	0^{d}
		D	0^{d}
	p-value		0.0001

*Means (n = 3) in the column followed by the same common letter were not significantly different (DMRT, p>0.05), E: Ethanol, H: Hexane and D: Dichloromethane

Table 2: MIC and MBC values of *Oroxylum indicum* (L.) Kurz extracts against human antibiotic-resistant bacteria

Plant sample	Bacterial strain	Solvent	MIC (mg/mL)	MBC (mg/mL)
Leave	*Acinetobacter baumannii*	E	3.125	6.25
		H	12.5	25
		D	6.25	12.5
Stem	*Acinetobacter baumannii*	E	1.56	12.5
		H	6.25	25
		D	6.25	25
Leave	Colistin-resistant *Pseudomonas aeruginosa*	E	6.25	12.5
		H	12.5	25
		D	6.25	12.5
Stem	Colistin-resistant *Pseudomonas aeruginosa*	E	6.25	12.5
		H	12.5	25
		D	-	-
Leave	*Proteus mirabilis*	E	1.56	12.5
		H	12.5	25
		D	12.5	25
Stem	*Proteus mirabilis*	E	1.56	6.25
		H	-	-
		D	-	-

E: Ethanol, H: Hexane, D: Dichloromethane, MIC: Minimum inhibitory concentrations and MBC: Minimum bactericidal concentrations

assay designed for the quantification of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The findings demonstrated that the MIC of 1.56 mg/mL was observed in the ethanolic extracts from the stem and leaf of *O. indicum* against *Acinetobacter baumannii* and *Proteus mirabilis*. Furthermore, the MBC of 6.25 mg/mL was recorded for the same extracts against *Acinetobacter baumannii* and *Proteus mirabilis* (Table 2).

Oroxylum indicum, locally known as Phekaa in Thailand, is a plant with a multitude of medicinal properties. It possesses anti-inflammatory attributes, enhances immunity, aids digestion, reduces stress and promotes cardiovascular health. Additionally, it assists in controlling cholesterol and blood sugar levels, treats allergic diseases and contains antitumor constituents. Its antifungal and antibacterial properties contribute to the prevention and treatment of various diseases. This plant's extensive health benefits make it a valuable addition to the local Thai diet[7,35]. Radhika *et al.*[36] documented that the stem bark of *O. indicum*, when extracted with 80% methanol, demonstrated inhibitory effects on *Bacillus cereus* KUCC 23, *Bacillus subtilis* KUCC 17, *Staphylococcus aureus* MTCC 96 and *Staphylococcus albus* MTCC 96 with inhibition zone diameter range of 4-11.7 mm.

The findings of this study revealed an inhibition zone diameter of 10 mm against Colistin-resistant *P. aeruginosa*, as reported by Moirangthem *et al*.[37]. Their research highlighted the antibacterial activity of *O. indicum* bark, extracted with petroleum ether, dichloromethane and methanol, against a range of bacteria including *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Bacillus cereus* and *Staphylococcus aureus*. The results indicated that the largest inhibition zone diameter of 14.5 mm was achieved with a methanolic extract against *Escherichia coli*, followed closely by *Pseudomonas aeruginosa* and *Staphylococcus aureus*, both with diameters of 14.1 mm. In their 2023 study, Getahun *et al*.[38] examined the antibacterial activity of both methanol extract and essential oil derived from *Acanthus polystachyus* against *A. baumannii*. The findings indicated that when ethanolic *A. polystachyus* extracts were utilized at concentrations ranging from 50 to 400 mg/mL, the inhibition zone diameter varied between 7.3 and 12.3 mm. Furthermore, when essential oil was applied in volumes of 10 to 40 µL, the inhibition zone diameters against *A. baumannii* ranged from 6.8-9.9 mm.

This study demonstrated that the lowest minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values, observed at 1.56 and 6.25 mg/mL, respectively, were found in the ethanolic extracts from the stem and leaf of *O. indicum* against *Acinetobacter baumannii* and *Proteus mirabilis*. However, a contrasting study by Mishra *et al*.[39] suggested that the extraction of *O. indicum* with a methanol and dichloromethane combination was ineffective against the growth of *P. mirabilis*. The findings of this study were consistent with the MIC values of ethanol extracts from traditional Thai remedies against *A. baumannii*, as reported by Chusri *et al*.[40]. They found that the MIC values of these ethanol extracts against multidrug-resistant *A. baumannii* strains (NPRC AB002, NPRC AB004, NPRC AB005, NPRC AB034) ranged from 500 to over 1000 µg/mL. Similarly, Getahun *et al*.[38] reported on the MIC and MBC of methanol extract and essential oil from *A. polystachyus* against *A. baumannii*. Their results indicated that the MIC of the methanol extract and essential oil were at 0.5 and 0.31 mg/mL, respectively, while the MBC values were at 4 and 1.25 mg/mL. Future research should focus on the quantitative analysis of the active compound present in the stem and leaf extracts of *O. indicum*.

CONCLUSION

In the present study, the antibacterial potency of extracts from the stems and leaves of *Oroxylum indicum*, obtained through ethanol, hexane and dichloromethane, was assessed against antibiotic-resistant bacteria. The extracts derived from the stems and leaves of *O. indicum* exhibited significant antibacterial activity against *Acinetobacter baumannii*, Colistin-resistant *Pseudomonas aeruginosa* and *Proteus mirabilis*. Of the solvents employed for extraction, ethanol emerged as the most effective for the stems and leaves of *O. indicum*.

SIGNIFICANCE STATEMENT

This study investigates the antibacterial activity of *Oroxylum indicum* leaf and stem extracts against three antibiotic-resistant human pathogens. By exploring this previously uncharted area, the research highlights the potential of *Oroxylum indicum* extracts as natural antibacterial agents, paving the way for novel applications harnessing their therapeutic properties.

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