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Phytochemical Screening and Antibacterial Activities of *Oroxylum indicum* (Linn.): A Threatened Tree of India

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Abstract

Oroxylum indicum belongs to the family Bignoniaceae. It is used as a traditional medicine from a very long time and because of over exploitation comes under threatened category. It is traditionally used against inflammation and bacterial infections. Keeping this in view, an attempt has been made to evaluate its pharmacological value through phytochemical analysis and antibacterial activities against four pathogenic bacteria. Results revealed that leaf and fruit extracts are rich in diverse types of bioactive compounds and showed inhibition potential against selected microbes. The present study highlights the importance of pharmacological potential of *O indicum* against antimicrobial resistance.

Keywords: Antimicrobial, Inflammation, *Oroxylum indicum*, Phytochemical

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Introduction

Since time immemorial plants have been used in Indian villages as folk medicine by traditional healers and Shamans. Even today, in remote villages, healers still use plant parts for healing purposes which has also been proven by scientific research. Today, plants are used as a great source of natural health healing products by humans. In the past few decades, extensive research work is going on across the globe with regard to the finding of various antimicrobial effects and activities of compounds of many different plant species against various diseases. Uses of many plants are due to their antimicrobial traits, which are due to the compounds synthesized during secondary metabolism of the plants.[1] Thousands of compounds found in plants are used as therapeutic compounds for different kinds of diseases. Many therapeutic agents have been identified and used from their natural origin, in traditional medicine.[2] Microbial infections till date remain a scourge of humanity due to lack of vaccine against some infections, emergence of drug resistant phenotypes, and the resurgence of infections among others.[3] Plant extracts and essential oils have been widely explored for their therapeutic activities against most microbial infections.[4] Plants have an amazing ability to produce a wide variety of secondary metabolites such as alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinones, and coumarins,[5] some of these natural products are highly efficient in the treatment of bacterial infections.[6] Medicinal plants are traditionally used worldwide as remedies for the treatment of various diseases including asthma, gastrointestinal symptoms, skin disorders, respiratory and urinary problems, and hepatic and cardiovascular disease.[7] Studies done by Ushimaru *et al*. where *in vitro* antimicrobial activity of methanolic extracts of some medicinal plants such as *Allium sativum*, *Zingiber officinale*, *Caryophyllus aromaticus*, *Cymbopogon citratus, Mikania glomerata,* and *Psidium guajava* against *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Enterococcus* spp. showed that methanolic extract of *C. aromaticus* presented the highest anti *S. aureus* activity and was effective against all bacterial strains tested.[8] Such types of antimicrobial activity from compounds of various plants have been reported in several research papers. *Oroxylum indicum* (Linn.) is one among the many plants widely used in Ayurvedic system of medicine. Both the stem and root are useful parts in many formulations as per Ayurvedic classics.[9] Many studies have been done regarding the roots and the bark of this plant. However, very little work has been done on the fruit and leaves of this plant. Our present study was conducted to determine the antibacterial activities of *O. indium* fruits and leaves.

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Methodology

Collection of Fruits and Leaves for Experimental Work

The sample (Fruits and leaves) was collected from different parts of Odisha and kept in poly bags tagged with the botanical name and sorted out as per standard sampling procedure and passport description.[10]

Preparation of Extracts

Soxhlet method and percolation were adopted to obtain different extracts.[11,12] The collected experimental plant materials were

dried at room temperature under shade and were powdered after drying using mechanical devices. The powdered material of the experimental plant was kept in a thimble and extraction was carried out using the Soxhlet apparatus. The residues were collected and left for air drying and dried crude extracts were stored in refrigerator for further phytochemical analysis and antibacterial activities.

Phytochemical Analysis

Phytochemical analysis was carried out using standard procedure to identify the bioactive compounds.[13-15]

Antibacterial Activity

The extracts of experimental plant parts were screened for antibacterial activity against two Gram-positive bacteria *Streptococcus mutans* Microbial Type Culture Collection (MTCC 497) and *Streptococcus pyogenes* (MTCC 1926); three Gram-negative bacteria *Vibrio cholerae* (MTCC 3906), *Shigella flexneri* (MTCC 1457), and *Salmonella* Typhi (MTCC 1252). All used MTCC bacterial strains (Plate 1e) were collected from Institute of Microbial Technology, Chandigarh. Antibacterial activity was done using slight modification of standard methods of agar well diffusion assay;[16] disc diffusion method,[17] and broth dilution assay.[18]

Agar Well Diffusion Assay

Agar well diffusion method[16] was followed to test the antibacterial activity of extracts of experimental plant parts against the five bacterial strains. Nutrient agar plates were prepared as per instructions of manufacturer. 100 µl of nutrient broth cultures of the test microbes prepared a day before were poured over the plates uniformly and a lawn culture was prepared using a sterile spreader in a laminar hood. Wells (6 mm) were made using a sterile borer. Stock solutions of samples were prepared in 100% DMSO (Sigma) and two-fold serial dilutions were made in amount of 100 µl per well at concentration of 0.25 and 0.5 mg/mL. 100 µl of samples were added by sterile syringes into the wells in three above mentioned concentration and allowed to diffuse at room temperature for 2 h. Plates were incubated at 35 ± 2°C for 18–24 h. Kanamycin served as standard antibiotics control. Triplicates were maintained and the experiment was repeated thrice. For each replicate, the readings (diameter of zone of inhibition in cm) were taken and the mean ± SD values (diameter of zone of inhibition) were recorded.

Disc Diffusion Assay

Antibacterial activity using disc diffusion assay was done using the 6 mm of disc prepared from Whattman filter paper.[17] Each extract was dissolved in dimethyl sulfoxide. The sets of dilutions (10 µg/disc and 50 µg/disc) of crude extracts and standard drugs were prepared. 6 mm of discs were kept in the drugs for 12 h before placing on the agar plates. The zones of growth inhibition around the discs were measured after 18–24 h of incubation at 37°C for bacteria. The sensitivities of the microbial species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disc) on the agar surface around the disc and values <8 mm were considered as not active against microorganisms.

Minimum Inhibitory Concentration (MIC) using Broth Dilution Assay

All the extracts of experimental plant were screened for their antibacterial activity (modified method).[18,19] Antibacterial activity was assessed by MIC by a serial dilution method. Selected colonies of aforesaid bacteria were picked off from a fresh isolated plate and inoculated into corresponding tubes containing 5 ml of trypticase soy broth. The broth was incubated for 8 ± 1 h at 35 ± 2°C until there was a visible growth. McFarland No. 5 Standard and Phosphate Buffer Saline were used to adjust the turbidity to get 10^5 cfu/mL.

Data Interpretation

After the incubation, the tubes showing no visible growth after 8 h till 12 h were considered to be inhibition of bacteria which represent MIC values of a respective concentration. Inoculum control showed visible growth due to no antimicrobial agents, whereas the broth control showed no growth due to absence of bacteria. Triplicates were maintained and the experiment was repeated thrice, for each replicate. The readings were taken as foresaid.

Results and Discussion

Phytochemical Analysis

The plant extracts were screened for the presence of biologically active chemicals. From Table 1, it signifies that secondary compounds present in aqueous extract of leaves were tannins and phenolic compounds. More secondary compounds in methanolic extracts were tannins, phenolic compounds, and terpenoids. In petroleum ether extract, flavonoids were present. In n-hexane extract, terpenoids were present. Aqueous and methanolic extracts of fruits showed more compounds such as tannins, saponins, phenolic compounds, and flavonoids.

Evaluation of Antibacterial Activity

Disc diffusion assay

The antibacterial activity of aqueous and methanolic extract of leaves and fruits was carried out with *S. mutans*, *S. pyogenes*, *V. cholerae*, *and S. flexneri*. It was observed that the aqueous extract of leaf of *O. indicum* showed a zone of inhibition in disc diffusion assay. It was noted that the highest zone of inhibition was 1.2 mm against *S. mutans* and *S. pyogenes* and methanolic extract of leaf *O indicum* showed highest zone of inhibition of 1.3 mm against *S. mutans* and *S. pyogenes*. From the result, it is evident that the aqueous extract of fruit of *O. indicum* showed maximum zone of inhibition of 0.9 mm against test bacteria (*S. mutans*, *S. pyogenes*) whereas methanolic extract of fruit of *O. indicum* showed zone of inhibition of 1.1 mm against *S. pyogenes and V. cholerae* [Tables 2 and 3, Figure 1].

Agar well diffusion assay

The antibacterial activity of aqueous and methanolic extract of leaf of *O indicum* showed highest zone of inhibition against *S. mutans* and *S. pyogenes*. Fruit extracts of aqueous and methanolic extracts showed highest zone of inhibition against *S. mutans* and *S. pyogenes* [Tables 2 and 3, Figure 1].

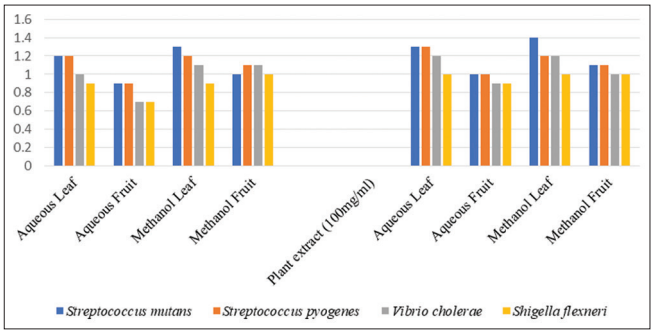

Figure 1: Comparison between disc diffusion assay and agar well diffusion assay

MIC

This study reports that the activity of aqueous extract of leaf of *O. indicum* showed MIC at 300 mg/ml against *S. mutans* and *S. pyogenes* and aqueous extract of fruits showed equal result of MIC at 500 mg/ml. Methanolic extract of leaf showed MIC at 300 mg/ml, whereas methanolic extract of fruit showed MIC at 350 mg/ml [Table 4].

Discussion

Various recent studies have reported that the phytochemicals in leaf of *O. indicum* showed the presence of bioactive compounds such as saponins, alkaloids, flavonoids, and tannins.[20] Flavonoids

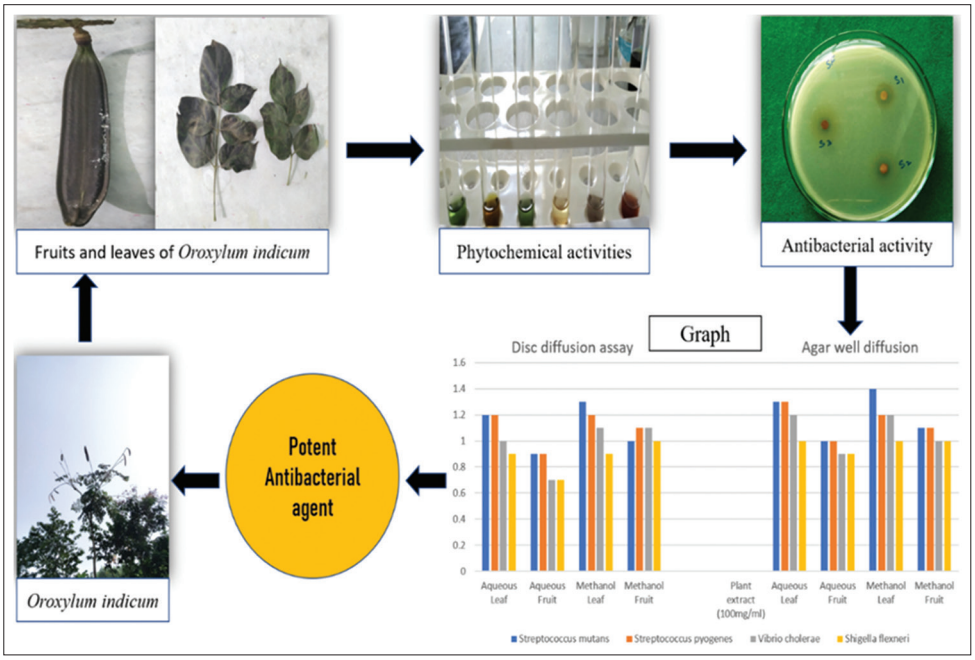

Figure 2: Illustration for *Oroxylum indicum* a potent antibacterial agents

Table 1: Phytochemical analysis of leaves and fruits of *Oroxylum indicum*

Plant extract	*Solvent*	*Tannin*	*Saponin*	*Phenolic compounds*	*Terpenoids*	*Alkaloids*	*Flavonoids*
Leaf	Aqueous	+	-	+	-	-	+
	Methanol	+	-	+	+	-	-
	Petroleum ether	-	-	-	-	-	+
	n-Hexane	-	-	-	+	-	-
Fruit	Aqueous	+	+	+	-	-	+
	Methanol	+	+	+	-	-	+
	Petroleum ether	-	-	-	-	-	-
	n-Hexane	-	-	-	-	-	-

Table 2: Antimicrobial activity of aqueous and methanolic extract of leaves and fruits of *Oroxylum indicum* using disc diffusion assay and agar well diffusion assay

Disc diffusion assay				
Extract (100 mg/ml)	*Streptococcus mutans*	*Streptococcus pyogenes*	*Vibrio cholerae*	*Shigella flexneri*
Aqueous leaf	1.2	1.2	1.0	0.9
Methanol leaf	1.3	1.2	1.1	0.9
Aqueous fruit	0.9	0.9	0.7	0.7
Methanol fruit	1.0	1.1	1.1	1.0
Agar well diffusion assay				
Extract (100 mg/ml)	*Streptococcus mutans*	*Streptococcus pyogenes*	*Vibrio cholerae*	*Shigella flexneri*
Aqueous leaf	1.3	1.3	1.2	1.0
Methanol leaf	1.4	1.2	1.2	1.0
Aqueous fruit	1.0	1.0	0.9	0.9
Methanol fruit	1.1	1.1	1.0	1.0

Table 3: Comparison between aqueous and methanolic extract of leaves and fruits of *Oroxylum indicum* (100 mg/ml)

Disc diffusion assay					
Extract (100 mg/ml)	*Plant extract*	*Streptococcus mutans*	*Streptococcus pyogenes*	*Vibrio cholerae*	*Shigella flexneri*
Aqueous	Leaf	1.2	1.2	1.0	0.9
	Fruit	0.9	0.9	0.7	0.7
Methanol	Leaf	1.3	1.2	1.1	0.9
	Fruit	1.0	1.1	1.1	1.0
Agar well diffusion assay					
Extract (100 mg/ml)	*Plant extract*	*Streptococcus mutans*	*Streptococcus pyogenes*	*Vibrio cholerae*	*Shigella flexneri*
Aqueous	Leaf	1.3	1.3	1.2	1.0
	Fruit	1.0	1.0	0.9	0.9
Methanol	Leaf	1.4	1.2	1.2	1.0
	Fruit	1.1	1.1	1.0	1.0

Table 4: MIC of leaf and fruit extracts of *Oroxylum indicum* against selected bacterial pathogens (50–500 mg/ml)

Extract (50–500 mg/ml)	*Streptococcus mutans*	*Streptococcus pyogenes*	*Vibrio cholerae*	*Shigella flexneri*
Aqueous leaf	300 mg/ml	300 mg/ml	350 mg/ml	350mg/ml
Aqueous fruit	500 mg/ml	500 mg/ml	500 mg/ml	500 mg/ml
Methanol leaf	250 mg/ml	250 mg/ml	300 mg/ml	300 mg/ml
Methanol fruit	350 mg/ml	350 mg/ml	400 mg/ml	400 mg/ml
Inoculum	Growth	Growth	Growth	Growth
Broth	No growth	No growth	No growth	No growth

play a major role as antioxidants and in cell signaling pathways. They also have antiallergic, anti-inflammation, anticancer, and antiviral properties.[21] The stem, bark, and root extracts of *O. indicum* are known for its antimicrobial activity.[22] Mature fruits are acrid, sweet, antihelmintic, and anti-stomachic. The leaf decoction is used to treat rheumatic pain, enlarged spleen, ulcer, cough, and bronchitis.[23] The chemical constituents of leaves are flavones, glycosides, baicalein, scutellarein, anthraquinones, and aloe emodin. The chemical constituents of fruits are oroxylin, chrysin and ursolic acid, and aloe emodin.[24] The studies till date have focused on screening of phytochemicals and antimicrobial activity of *O. indicum* from the extracts of bark, stem, roots, and seeds. None of the studies have targeted the leaves and fruits of *O. indicum* as a potent source of phytochemicals and antimicrobial activities [Figure 2].

Conclusion

The result showed that *O. indicum* could be a potent source for the treatment of bacterial infections and *O. indicum* has biologically active compounds which could be useful for the formulations of the future drugs.

References

1. Janssen AM, Scheffer JJ, Svendsen AB. Antimicrobial activity of essential oils: A 1976-1986 literature review. Aspects of test methods. Planta Med 1987;53:395-8.
2. Cragg GM, Newman DJ. Natural product drug discovery in the next millennium. Pharma Biol 2001;39:8-17.
3. Nijmoh DL, Assob JC, Mokake SE, Nyhalah DJ, Yinda CK, Sandjon B. Antimicrobial activities of a plethora of medicinal plant extracts and hydrolates against human pathogens and their potential to reverse antibiotic resistance. Int J Microbial 2015;38:717-9.
4. Nawab A, Yunus M, Mahdi AA, Gupta S. Evaluation of anticancer properties of medicinal plants from the Indian sub-continent. Mol Cell Pharmacol 2011;3:21-9.
5. Das K, Tiwari RK, Shrivastava DK. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. J Med Plants Res 2010;4:104-11.
6. Eloff JN. Which extractant should be used for the screening and isolation of antimicrobial components from plants. J Ethnopharmacol 1998;60:1-8.
7. Tian XR, Feng JT, Ma ZQ, Xie N, Zhang J, Zhang X, *et al.* Three new glycosides from the whole plant of clematis lasiandra Maxim and their cytotoxicity. Phytochem Lett 2014;10:168-72.
8. Ushimaru PI, Nogueira da Silva MT, Di Stasi LC, Barbosa L, Junior AF. Antibacterial activity of medicinal plant extracts. Brazil J Microbiol 2007;38:717-9.
9. Radhika LG, Meena CV, Peter S, Rajesh KS, Rosamma MP. Phytochemical and antimicrobial study of *Oroxylum indicum*. Anc Sci Life 2011;30:114-20.
10. Taherdost H. Sampling methods in research methodology; How to choose a sampling technique for research. Int J Acad Res Manage 2016;5:18-27.
11. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: A review. Int Pharma Sci 2011;1:98-106.
12. Kumar S, Mahanti P, Rath SK, Patra JK. Qualitative phytochemical analysis and antibacterial activity of *Dioscorea alata* L. A nutraceutical tuber crops of rural Odisha. J Altern Med Res 2017;3:122.
13. Trease GE, Evans WC. Pharmacognosy. 13th ed. London: WB Scanders Company, Ltd.; 1989. p. 89-300.
14. Sofowara A. Medicinal Plants and Traditional Medicine in Africa. Ibadan, Nigeria: Spectrum Books Ltd.; 1993. p. 191-289.
15. Misra RC, Kumar S, Pani DR, Bhandari DC. Empirical tribal claims and correlation with bioactive compounds: A study on *Celastrus paniculata* Willd, a vulnerable medicinal plant of Odisha. Indian J Tradit Know 2012;11:615-22.
16. Allen KL, Molan PC, Reid GM. A survey of the antibacterial activity of some New Zealand honeys. J Pharm Pharmacol 1991;43:817-22.
17. Driscoll AJ, Bhat N, Karron RA, O'Brien KL, Murdoch DR. Disk diffusion bioassay for the detection of antibiotic activity in body fluids: Application for the pneumonia etiology research for child health project. Clin Infect Dis 2012;54:159-64.
18. Rai US, Isloor AM, Shetty P, Vijesh AM, Prabhu N, Isloor S, *et al.* Novel chromeno (2,3-b)-pyrimidine derivates as potential anti-microbial agents. Eur J Med Chem 2010;45:2695-9.
19. Nayak K, Vailanka MS, Sheeba E. Antibacterial activity of different extracts of medicinal plant *Swertia chirata*. Int J Curr Microbiol App Sci 2015;4:889-97.
20. Eswari JS, Dhagat S, Naik S, Dibya S. Phytochemical and antimicrobial studies of *Oroxylum indicum* extracts pharmaceutical bioprocessing.

Pharm Bioproc 2018;6:7-14.
21. Panche AN, Diwan AD, Chandra SR. Flavonoids: An overview. J Nutr Sci 2016;5:e47.
22. Ali RM, Houghton PJ, Raman A, Hoult JR. Antimicrobial and antinflammatory activities of extracts and constituents of *Oroxylum indicum* (L.) Vent. Phytomedicine 1998;5:375-81.
23. Bhattacharje SK. Use of flavours and fragrance. In: Bhattacharje SK, editor. Handbook of Aromatic Plants. 2nd ed. Jaipur: Pointer Publishers; 2005.
24. Padgilwar S, Manwar J, Vohra M, Banginwar Y. Traditional uses, phytochemistry and pharmacology of *Oroxylum indicum*: A review. Int J Pharma Phytopharmacol Res 2014;3:483-6.