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Research Article

ANTIMICROBIAL STUDIES OF DIFFERENT EXTRACTS OF *HOLOPTELEA INTEGRIFOLIA* (ROXB.) LEAVES

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ABSTRACT

Objective: The aim of this study was to examine various extracts of leaves of *Holoptelea integrifolia* against some test bacteria and test fungi.

Methods: Disk diffusion method was adopted for the assessment of antimicrobial activity. Amikacin and nystatin were used as standard drugs for antibacterial and antifungal activity, respectively.

Results: The screening data indicated that all four extracts showed antibacterial activity against *Staphylococcus aureus*, but the growth of this bacteria was inhibited the most by the aqueous extract. In the case of antifungal efficacy, all the extracts inhibited the growth of almost all the test fungi. Petroleum ether and benzene extracts showed maximum efficacy against *Aspergillus flavus*, whereas methanolic extract and aqueous extract inhibited the growth of *Rhizoctonia bataticola* significantly.

Conclusion: Different extracts of leaves of *H. integrifolia* were significantly active against selected test fungi and they can be a harmless alternative of expensive conventional medicines.

Keywords: *Holoptelea integrifolia*, Leaves, Extracts, Antimicrobial efficacy.

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INTRODUCTION

Medically important plants are the best gift of nature to human beings to make their life healthier. The medicinal importance of plant(s) attracted the attention of chemists to study natural products. Plant products are being increasingly tried upon and utilized for various purposes in the field of pharmaceuticals. It is believed that the extracts and products of plants being natural are harmless to human beings and their environment.

In ancient times, the crude plant extracts had been used for the treatment of human infectious diseases [1-3]. Later on, chemists isolated the active principles and established their structures, including tannins, terpenoids, alkaloids, and flavonoids which have antimicrobial properties [4,5]. Therefore, the present work was undertaken with the antibacterial and antifungal studies of different extracts (petroleum ether, benzene, methanol, and water extract) of leaves of *Holoptelea integrifolia* (Roxb.) Planch. This plant belongs to the family Ulmaceae and common name is "Chilbil." *H. integrifolia* is a large spreading glabrous, deciduous roadside tree distributed throughout the country up to an altitude of 600 m^3. Only a few plants of the family Ulmaceae are known, which have medicinal importance [6,7].

Fruits of *Celtis australis* (syn. *C. caucasian*) are used in amenorrhoea and colic, *Coltricia cinnamomea, Commiphora wightii*, and *Citrus reticulata* (syn. *Gironniera reticulata*) are used as a blood purifier in itch and other cutaneous eruptions [6]. A review of literature revealed that *H. integrifolia* is also medicinally important, the juice of boiled mucilaginous bark is applied to rheumatic swellings, the stem fibers tied to the upper arm are useful for the patients suffering from malarial fever [6-9], and the ethanolic extract of the bark also showed significant inhibition of breast cancer formation [10]. The crude leaf sap of *H. integrifolia* was found to be mildly active against bean common mosaic virus [11], it is also useful in colic pain, intestinal worms, filaria, piles, pox, vitiligo, in wound healing [12,13], and petroleum ether extract and methanolic extract of leaf delayed onset of convulsion and also prolonged the onset of tonic convulsion in mice [14]. Keeping the medicinal importance in view, the antibacterial and antifungal investigation of different extracts of leaves of *H. integrifolia* was undertaken.

METHODS

Plant materials

The leaves of *H. integrifolia* (Roxb.) Planch. were collected from the Campus of Rajasthan University, Jaipur, and identified from the Botany Department of Rajasthan University, Jaipur (Herbarium sheet No. RUBL 4334). The shade dried and powdered leaves were stored in an airtight container.

Extraction

Powdered leaves of *H. integrifolia* were extracted for 24 h on a steam bath with pet.ether, benzene, methanol, and water separately. Later, each of these extracts was filtered and re-extraction (2×) of each residue was done for complete exhaustion. The extracts were collected, concentrated in vacuum, and stored in a dark-colored bottle at 4°C separately.

Sources of test organisms

Test bacteria

In vitro antibacterial activity was evaluated against most common pathogenic bacteria such as *Escherichia coli*, *Klebsiella aerogenes*, *Proteus vulgaris,* and *Pseudomonas aeruginosa* as Gram -ve and *Staphylococcus aureus* as Gram +ve. All the test organisms were obtained from SMS Medical College, Jaipur and were maintained on Nutrient Broth Medium.

Test fungi

The pure cultures of test fungi, namely *Aspergillus flavus, Aspergillus niger, Fusarium moniliforme,* and *Rhizoctonia bataticola* were obtained from the Seed Pathology Laboratory, Department of Botany, University of Rajasthan, Jaipur and were maintained on Potato Dextrose Agar (PDA) medium.

Table 1: Antibacterial activity

Extract	Dose µg/disk	Test bacteria									
		E. coli		*K. aerogenes*		*P. vulgaris*		*P. aeruginosa*		*S. aureus*	
		IZ*	AI*	IZ	AI	IZ	AI	IZ	AI	IZ	AI
Pet.Ether	1000	-	-	-	-	-	-	-	-	8	0.42
	500	-	-	-	-	-	-	-	-	±	-
Benzene	1000	-	-	-	-	-	-	-	-	10	0.52
	500	-	-	-	-	-	-	-	-	±	-
MeOH	1000	-	-	-	-	10	0.45	±	-	11	0.57
	500	-	-	±	-	-	-	-	-	8	0.42
Aqueous extract	1000	-	-	-	-	11	0.49	±	-	15	0.78
	500	-	-	±	-	8	0.36	-	-	10	0.52

IZ: Zone of inhibition (in mm) including the diameter of disk (6 mm), AI: Activity index=(inhibition zone of sample/inhibition zone of standard),
Standard: Amikacin = 10 µg/ml. (±) Trace activity: (-) No activity. *E. coli: Escherichia coli, K. aerogenes: Klebsiella aerogenes, P. vulgaris: Proteus vulgaris, S. aureus: Staphylococcus aureus*

Table 2: Antifungal activity

Extract	Dose µg/disk	Test fungi							
		A. flavus		*A. niger*		*F. moniliforme*		*R. bataticola*	
		IZ*	AI*	IZ	AI	IZ	AI	IZ	AI
Pet.Ether	1000	16	0.83	18	0.75	12	0.42	11	0.39
	500	14	0.72	15	0.62	±	-	±	-
Benzene	1000	15	0.78	14	0.58	17	0.60	14	0.50
	500	12	0.62	11	0.46	12	0.42	±	-
MeOH	1000	14	0.73	12	0.50	21	0.75	20	0.76
	500	12	0.63	10	0.41	13	0.46	18	0.69
Aqueous extract	1000	14	0.72	10	0.41	18	0.64	20	0.76
	500	10	0.52	8	0.33	15	0.54	16	0.57

IZ: Zone of inhibition (in mm) including the diameter of disk (6 mm), AI: Activity index=(inhibition zone of sample/inhibition zone of standard),
Standard: Nystatin=100 units/disk. (±) Trace activity: (-) No activity. *A. flavus: Aspergillus flavus, A. niger: Aspergillus niger, F. moniliforme: Fusarium moniliforme, R. bataticola: Rhizoctonia bataticola*

Culture of test microbes

For the bacteria cultivation, the nutrient agar plates were seeded with the suspension of the bactericidal strain and incubated at 37°C for 24 h.

However, for the cultivation of fungi, the test fungi were incubated at 37°C for 48 h and the cultures were maintained on PDA medium by regular sub-culturing.

Test plates for both bacteria and fungi were prepared by pouring 10–15 ml of the respective medium in the Petri-dishes and used for screening. For antibacterial activity, a fresh saline suspension of the test bacteria was prepared from a freshly grown agar slant, while for antifungal activity, the test fungi were spread using a sterile swab.

Bactericidal and fungicidal assay

Disk diffusion method [15,16] was adopted for bactericidal and fungicidal efficacy because of re-productivity and precision. The different test organisms were preceded separately over previously sterilized culture medium plates using a sterile swab. Sterilized filter paper disks of 6 mm diameter (Whatman no.1) containing 500 µg and 1000 µg dose of test compounds were placed on the agar surface along with disks impregnated with standard drugs (Amikacin for bacteria and Nystatin for fungi)in the concentration of 10 µg/ml and 100 units/disk respectively.

Before incubation, these plates were placed at 4°C for 1 h for the maximum diffusion of the test compound from the test disks into media and thereafter were incubated at 37±2°C for 24 h for bacteria and for 48 h for fungi, then the diameters of inhibition growth zones could be easily observed. The experiment was performed 3 times to minimize the error and the mean values were referred.

RESULTS AND DISCUSSION

Bactericidal activity

In the case of bactericidal activity against *E. coli, K. aerogenes, P. vulgaris, P. aeruginosa*, and *S. aureus,* all four extracts (pet.ether, benzene, methanol, and aqueous) inhibited the growth of *S. aureus*. The aqueous extract exhibited marked activity against *S. aureus* (activity index [AI]=0.78, 1000 µg/disk and 0.52, 500 µg/disk). Methanolic and aqueous extracts also exhibited moderate activity against *P. vulgaris* (Table 1).

Fungicidal activity

In the case of antifungal activity against *A. flavus, A. niger, F. moniliforme,* and *R. bataticola*, all four extracts demonstrated inhibition against all the test fungi. The pet.ether extracts exhibited marked activity against *A. flavus* (AI=0.83, 1000 µg/disk and 0.72, 500 µg/disk) and significant activity against *A. niger* (AI=0.75, 1000 µg/disk and 0.62, 500 µg/disk). The benzene extract was also effective at both concentrations (AI=0.78, 1000 µg/disk; AI=0.62, 500 µg/disk) against *A. flavus* and showed average activity against *F. moniliforme* (AI=0.60, 1000 µg/disk; AI=0.42, 500 µg/disk). The methanolic extract of leaves of *H. integrifolia* demonstrated maximum activity against *R. bataticola* (AI=0.76, 1000 µg/disk and 0.69, 500 µg/disk) and significant activity (AI=0.75, 1000 µg/disk) against *F. moniliforme*. Likewise, the aqueous extract was found to have maximum activity against *R. bataticola* (AI=0.76, 1000 µg/disk; AI=0.57, 500 µg/disk (Table 2).

The study of the literature clearly indicates the medicinal importance of crude extracts of different plant parts of *H. integrifolia*. The leaf extracts exhibited antiviral activity [11], wound healing activity [13], anticonvulsant activity [14], and this research work also indicates the pronounced activity against some test fungi. Further research on the isolation of active principles can be very useful for the mankind.

CONCLUSION

As per the experimental data above, it can be successfully concluded that different extracts of leaves of *H. integrifolia* displayed remarkable activity against all the test fungi. Thus, the leaves may be a source of effective and novel antifungal drugs.

AUTHOR'S CONTRIBUTIONS

The author declares that this entire work was done by the author named in this article.

CONFLICTS OF INTEREST

The author declares no conflicts of interest.

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