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Antimicrobial Studies of Methanolic Extracts of Leaves, Stem bark and Roots of *Holoptelea Integrifolia* Planch.

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ABSTRACT: The methanolic extract of leaves, stem bark and roots of *Holoptelea integrifolia* Planch. were examined for antibacterial and antifungal activity by using disc diffusion method. *Escherichia coli*, *Klebsiella aerogenes*, *Proteus vulgaris* and *Salmonella paratyphi B* as Gram -ve and *Staphylococcus aureus* as Gram +ve bacteria were selected for antibacterial efficacy and *Aspergillus flavus*, *Aspergillus niger*, *Fusarium moniliforme* and *Rhizoctonia bataticola* were selected for antifungal efficacy. The antibacterial activity of methanolic extract of *H. integrifolia* leaves and stem bark inhibited the growth of *S. aureus* maximum whereas root extract indicated pronounced activity against *S. paratyphi B*. A comparative study of the fungicidal activity of the three extracts indicated that although all of them exhibited activity against the select test fungi out of which the activity against *A. flavus* was most significant.

KEYWORDS: methanolic extract, antimicrobial, leaves, roots, stem bark.

I. INTRODUCTION

Since the beginning of mankind, plants have been used for both, food and medicine. The medicinal importance of plant(s), attracted the attention of chemists to study the natural products. Isolation of active principles which possess anticancer, antiinflammation, antileprosy, antifertility and a wide range of other biological properties from aromatic and medicinal plants and their successful utilization to alleviate human suffering have encouraged research workers to continue investigation of new drugs from the plant kingdom with an ever increasing zeal.

The present work deals with the investigation of antimicrobial activity of methanolic extract of roots, leaves and stem bark of *Holoptelea integrifolia* Planch. The plant *Holoptelea integrifolia* Planch. belongs to the family Ulmaceae, it is a small family comprising mainly of deciduous trees. *Holoptelea integrifolia* Planch. (syn. *Ulmus integrifolia* (Roxb.) Planch.) commonly known as "Chilbil". It is a large spreading glabrous deciduous roadside tree distributed throughout the country up to an altitude 600 m³. Leaves extract of this plant is used to treat ring worms, eczema and cutaneous diseases [1]. Paste of stem bark is applied on forehead to cure common fever and it is also used for intestinal cancer [2,3]. Paste of stem bark in coconut oil with garlic is applied externally to eczema and its paste with lime juice is used in case of weakness [4,5]. The stem fibers tied to the upper arm are useful in patients suffering from malarial fever and it is also useful in intestinal cancer and wound healing [6-8]. Seeds of *H. integrifolia* are used on ringworm and dried fruits are useful in polyurea and urinary disorders [9]. Fruit pulp with black salt is recommended for the treatment of menstrual disorders [10,11].

II. MATERIALS and METHODS

Plant Material: The plant parts of *H. integrifolia* (roots, leaves and stem bark) were collected from Rajasthan University campus, Jaipur and identified from the Department of Botany, University of Rajasthan, Jaipur (Herbarium sheet No. RUBL 4334).

Preparation of Extract: Powdered leaves, roots and stem bark of *Holoptelea integrifolia* were extracted with methanol. Later, each of these extract was filtered, the residue re-extracted (2X) for complete exhaustion, the extracts were pooled individually and dried in vacuo.

Test Bacteria: Pure culture of all test organisms, namely *Escherichia coli*, *Klebsiella aerogenes*, *Proteus vulgaris* and *Salmonella paratyphi B* as Gram -ve and *Staphylococcus aureus* as Gram +ve bacteria, the human pathogens were obtained through the courtesy of SMS Medical College, Jaipur, which 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, which were maintained on Potato Dextrose Agar (PDA) medium.

Bactericidal and Fungicidal Assay: Disc diffusion method was adopted for bactericidal and fungicidal assays because of re-productivity and precision. The different test organisms were proceeded separately using a sterile swab over previously sterilized culture medium plates and the zone of inhibitions were measured around sterilized dried discs of What man No:1 paper (6 mm in diameter), which were containing 500 µg and 1000 µg of the test extracts and control amikacin (10 µg/ml for bacteria) and nystatin (100 units/ml for fungi) as reference drugs separately. Such treated discs were air-dried at room temperature, to remove any residual solvent which might interfere with the determination, sterilized and inoculated. Before incubation, these plates were placed at low temperature for 1 hr, so as to allow maximum diffusion of the compound from the test discs into the agar plate and later, incubated at 37°C for 24 hrs in case of bacteria and 48 hrs for fungi, after which the zones of inhibition could be easily observed. Three replicates of each test extract were examined and the mean values were then referred [12,13].

III. RESULTS and DISCUSSION

In case of bactericidal activity methanolic extract of leaves demonstrated appreciable activity

Table 1: Bactericidal and Fungicidal activity of methanolic extract of leaves, stem bark and roots of *Holoptelea integrifolia*.

Test organism	Leaves		Stem bark		Roots	
	1000µg/disc	500 µg/disc	1000µg/disc	500 µg/disc	1000µg/disc	500 µg/disc
A. Bacteria						
<i>E. coli</i>	IZ	-	-	-	13	-
	AI				0.39	
<i>K. aerogenes</i>	IZ	-	±	-	-	-
	AI					
<i>P. vulgaris</i>	IZ	10	-	12	11	-
	AI	0.45		0.56	0.52	
<i>S. paratyphi B</i>	IZ	±	-	10	10	±
	AI			0.57	0.57	1.00
<i>S. aureus</i>	IZ	11	8	16	14	-
	AI	0.57	0.42	0.83	0.73	
B. Fungi						
<i>A. flavus</i>	IZ	14	12	14	10	15
	AI	0.73	0.63	0.73	0.53	0.78
<i>A. niger</i>	IZ	12	10	13	8	10
	AI	0.50	0.41	0.54	0.33	0.41
<i>F. moniliforme</i>	IZ	21	13	14	12	17
	AI	0.75	0.46	0.50	0.42	0.60
<i>R. bataticola</i>	IZ	20	18	15	12	14
	AI	0.76	0.69	0.58	0.46	0.54

against *S. aureus* and *P. vulgaris* and extract of stem bark showed activity against *S. aureus*, *P. vulgaris* and *S. paratyphi B* with maximum efficacy against *S. aureus* in both the concentration (AI=0.83 at 1000µg/disc, 0.73 at 500 µg/disc). However, the methanolic extract of roots showed pronounced activity against *S. paratyphi B* at 1000 µg/disc, in this case the activity was equal to that of standard (AI= 1:00), it also exhibited appreciated activity against *E.coli* at 1000 µg/disc (Table 1).

In case of fungicidal activity all three methanolic extracts inhibited the growth of all the selected fungi at both concentrations. The extract of leaves showed significant activity against *R. bataticola* (AI=0.76 at 1000 µg/disc, 0.69 at 500 µg/disc), *F. moniliforme* (AI=0.75 at 1000µg/disc) and *A. flavus* (AI=0.73 at 1000µg/disc), the methanolic extract of stem bark also exhibited pronounced activity against *A. flavus* (AI=0.73 at 1000µg/disc) but the maximum inhibition against *A. flavus* was demonstrated by root extract (AI=0.78 at 1000µg/disc) with significant activity (AI=0.60 at 1000 µg/disc) against *F. moniliforme* (Table-1).



IV. CONCLUSION

The screening data indicated that the antibacterial activity of methanolic extract of *H. integrifolia* leaves and stem bark inhibited the growth of *S. aureus* maximum whereas root extract indicated pronounced activity against *S. paratyphi* B. A comparative study of the fungicidal activity of the three extracts indicated that although all of them exhibited activity against the select test fungi out of which the activity against *A. flavus* was most significant.

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