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ORIGINAL ARTICLE

Antimicrobial Activity of Chloroform & Methanolic Extracts of Bark & Leaves of *Holoptelea integrifolia*

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ABSTRACT
Plant derived medicine or herbs have made a huge contribution to human health. Holoptelea integrifolia was significantly proven to have antifungal and antibacterial effects. In the present study, antibacterial & antifungal activity of Holoptelea integrifolia was investigated against selected bacteria & fungus by using disk diffusion method. Holoptelea integrifolia leaves & Bark extract prepared in methanol & chloroform. For the antibacterial screening, the extracts were tested against four selected bacteria; Staphylococcus aureus (MTCC 7443), Escherichia coli (MTCC-111), Bacillus subtilis (MTCC-121) and Pseudomonas aeruginosa (MTCC-1934) whereas for antifungal screening the extracts were tested against two selected fungus i.e, Aspergillus niger (MTCC No-2196) and Aspergillus flavus (MTCC No-1783). This is followed by Minimum Inhibitory Concentration (MIC) test at 0.5mg/ml, 0.25 mg/ml, 0.125 mg/ml anf 0.62 mg/ml to identify the lowest concentration which can inhibit the bacteria and fungus. The result showed that both extractions had antibacterial & antifungal potential against bacteria & fungus. Thus, it is suggested that Holoptelea integrifolia leaves & Bark extracts has a potential to kill gram positive and gram negative pathogenic bacteria & fungus.
Keywords: *Holoptelea integrifolia, Zone of Inhibition, Antibacterial activity*

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INTRODUCTION

Since the dawn of civilization, Man utilized plants for their medicinal and edible value. By trial and error, Man distinguished between the beneficial and poisonous plants. Man also observed that in large quantities medicinal and edible plants may be poisonous, and learned about the usefulness of plants by observing animals. Sick animals utilise certain plants that they usually ignore. Today, this method is used by scientists to isolate active compounds from medicinal plants.

Holoptelea integrifolia have been used in traditional healthcare, particularly among the tribal communities system, from time immemorial. *Holoptelea integrifolia*, commonly known as Indian Elm Tree, is a large deciduous tree distributed throughout the India up to an altitude of 2,000 feet. The bark of the tree is grey, pustular, exfoliating in corky scales [1]. The family Ulmaceae is comprised of 200 species belonging to 15 genera distributed in tropical temperate regions of Northern Hemisphere. The plant is found in India, Nepal, Sri Lanka, Indo-China, Cambadia, Laos, Myanmar, Vietnam, Burma and China. It is also found in the lower Himalayan belt to Tranvancore, the southern part of India. In Pakistan 3 genera and 7 species of the drug are found in Karachi and in some other parts of Sindh [2,3]. Holoptelea integrifolia is the only species of this genera found in this region [4].

For pregnant women decoction of the bark of the plant is externally used in oxytocic, rheumatism and intestinal tumors whereas Decoction of the leaves is used to regulate fat metabolism, treat ringworm, eczema and cutaneous diseases. Tablet is prepared by mixing a leaf of the garlic (Allium sativum) and black pepper (Piper nigrum). One tablet is enough for the patients suffering from jaundice. Stem bark powder of *Holoptelea integrifolia* is externally applied on the forehead of the patient suffering from common fever. In Nepal the bark is externally used to relieve rheumatic, swellings. Bark and leaf paste of Holoptelea integrifolia are applied externally on the white patches or leucoderma. Some toxicological

studies of this plant have been reported but the toxicological manifestations are to be investigated by different tests [5,6]. The bark and leaves of the plant are used medicinally in Ayurvedic system of medicine. The mucilaginous juice squeezed from the boiled bark is used on external application to relieve rheumatic swelling. Leaves of the plant are used as external application of the wound. The fruit pulp pounded with black salt is recommended for the treatment of menstrual disorders [7].

MATERIAL AND METHODS

Collection of Plant material

Different parts of the plant of *Holoptelea integrifolia* (Roxb) Plunch was collected from Agra, Dehradun, Delhi, Hathras and Jaipur and was confirmed by Dr. J.S. Dhakre and Dr. A. K. Singh (Plant Taxonomist) comparision with Voucher specimen kept in Botanical Depatrtment of R.B.S. Collage, Agra and flora of Agra of BSI Dehradun. *Holoptelea integrifolia* was shade dried & finely Powdered to particle size and further used to carry out the extraction and isolation of phyto constituents from selected extracts.

Solvent Used: Methanol & Chloroform

Preparation of extracts

Crude plant extract was prepared by Soxhlet extraction method [8]. About 200 gm of powder material was uniformly packed in to a thimble and run in Soxhlet extractor. It was exhaustible extracted with 500 ml methanol for the period of about 48 hour or 22 cycles or till the solvent in the siphon tube of an extractor become color less. After that extracts were filtered with the help of filter paper and solvent evaporate from extract in Rotary evaporator to get the syrupy consistency. The residue was dried over anhydrous sodium sulphate to remove trace of alcohol. Then extract kept in refrigerator at 4°C for detect antibacterial activity and analyzed their physical and chemical property.

Micro- organism used:

Clinical isolates of *Staphylococcus aureus* (MTCC 7443), *Escherichia coli* (MTCC-111), *Bacillus subtilis* (MTCC-121), *Pseudomonas aeruginosa* (MTCC-1934), *Aspergillus niger* (MTCC No-2196) *and Aspergillus flavus* (MTCC No-1783) were isolated from soil. The bacterial strains were re-identified on the basis of morphological, cultural and biochemical characteristics [9]**.**

Determination of Minimum Inhibitory Concentration (MIC)

Antibacterial Activity

Disc diffusion method was used to test antibacterial activity of different extracts. **(10).**The sensitivity testing of the extracts was determined using agar well diffusion method. The MIC of the extract was also determined using a two-fold dilution method. The bacterial were first grown in nutrient agar for 18 hour before use. The inoculum suspensions were standardized. It was performed using an 18 h culture at 37°C in 10 ml of Mueller Hinton Broth. The cultures were adjusted to approximately 105 CFU/ml with sterile saline solution. Five hundred micro liters of the suspensions were spread over the plates containing Mueller-Hinton agar using a sterile cotton swab in order to get a uniform microbial growth on test plates and then tested against the effect of the plant extracts at the concentration of 500 mg/ml, 250 mg/ml, 125 mg/ml. All petridishes were sealed with sterile laboratory parafilms to avoid eventual evaporation of the test samples. These plates were incubate for 24 hour at 37°C and measured the zone of inhibition in millimeter the plates later incubated at 37°C± 0.5°C for 24 hours after which they were observed for zones of inhibition (Table 1 & 2 & Fig A,B,C,D). The effects were compared with that of the standard anti biotic Streptomycin at a concentration of 1mg/ml. [12]**.**

Antifungal Assay

The antifungal activity was tested by disc diffusion method [12]. The potato dextrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation. The filter paper discs (5 mm in diameter) impregnated with 100 µg ml concentrations of the extracts were placed on test organism-seeded plates. Methanol was used to dissolve the extract and was completely evaporated before application on test organism-seeded plates. Blank disc impregnated with solvent methanol followed by drying off was used as negative control and Nystatin (10 µg disc) used as positive control. The activity was determined after 72 h of incubation at 28°C. The diameters of the inhibition zones were measured in mm.

RESULT AND DISCUSSION

The antibacterial and antifungal activity of *Holoptelea integrifolia* (Roxb) stem bark and leaves was studied by Disc Diffusion method against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis, Pseudomonas aeruginosa, Aspergillus niger* and *Aspergillus flavus.* The results of Minimum Inhibitory Concentration are given in Table 1 and 2.

Table 1: Antibiotic activity of Bacteria & fungus

Antibiotic	Symbol	*B. subtilis* (Zone of inhibition)	*S.aureus* (Zone of inhibition)	*E. coli* (Zone of inhibition)	*P. aeruginosa* (Zone of inhibition)	*A.niger* (Zone of inhibition)	*A.flavus* (Zone of inhibition)
Gentamicin	G	30 mm	18 mm	22 mm	25 mm	-	-
Ketoconazole	KET	-	-	-	-	17 mm	15 mm

Table 2. Antimicrobial activity of Methanolic extracts of *Holoptelea integrifolia* (Leaves & Bark) against Different organisms (mm).

Diameter of growth of inhibition zones (mm) extracts	MINIMUM INHIBITORY CONCENTRATION (MIC) mg/ml (mm)							
	Leaves				Bark			
Methanol	0.5	0.25	0.125	0.62	0.5	0.25	0.125	0.62
B.subtilis	9	6	4	2	8	6	4	-
S. aureus	8	5	3	-	7	4	2	-
E. coli	9	7	4	2	10	8	5	3
P. aeruginosa	7	4	2	-	6	3	-	-
A. niger	14	10	7	4	10	7	5	3
A. flavus	13	11	9	6	11	9	6	4

Table 3. Antimicrobial activity of Chloroform extracts of *Holoptelea integrifolia* (Leaves & Bark) against Different organisms (mm).

Diameter of growth of inhibition zones (mm) extracts	MINIMUM INHIBITORY CONCENTRATION (MIC) mg/ml (mm)							
	Leaves				Bark			
Chloroform	0.5	0.25	0.125	0.62	0.5	0.25	0.125	0.62
B.subtilis	10	7	4	2	11	9	8	4
S. aureus	11	9	6	4	12	10	7	5
E. coli	10	8	3	-	9	7	4	2
P. aeruginosa	7	3	-	-	9	6	3	-
A. niger	8	5	2	-	6	3	-	-
A. flavus	6	2	-	-	7	4	-	-

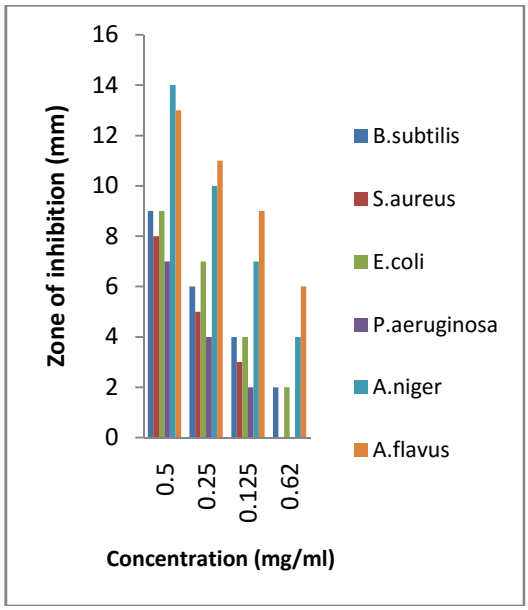

Fig A- Graphical representation of Methanolic Leaves Extract against different microorganisms

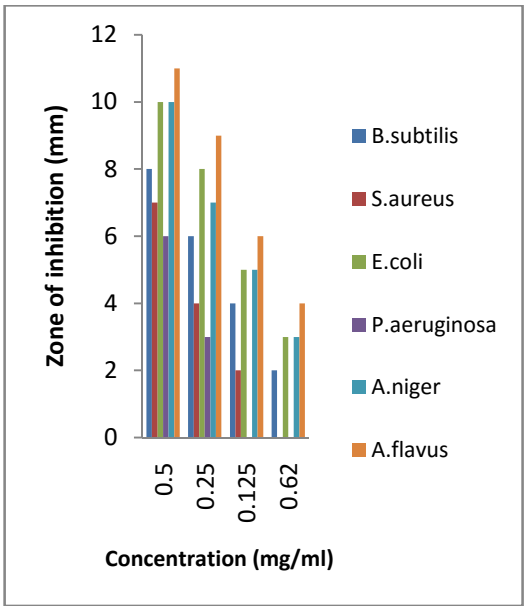

Fig B- Graphical representation of Methanolic Bark Extract against different microorganisms

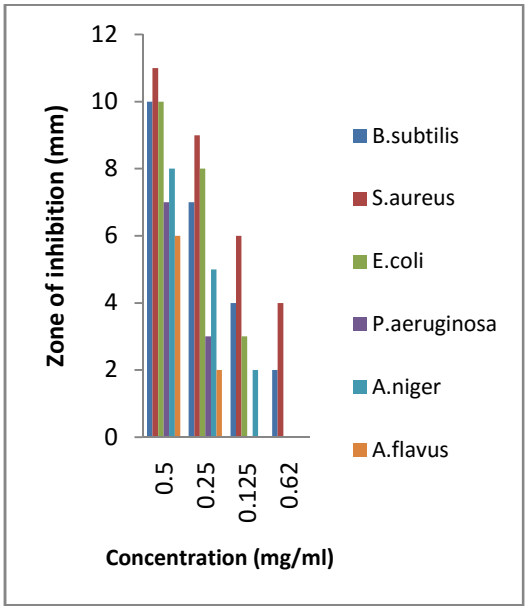

Fig C- Graphical representation of Chloroform Leaves Extract against Different microorganisms

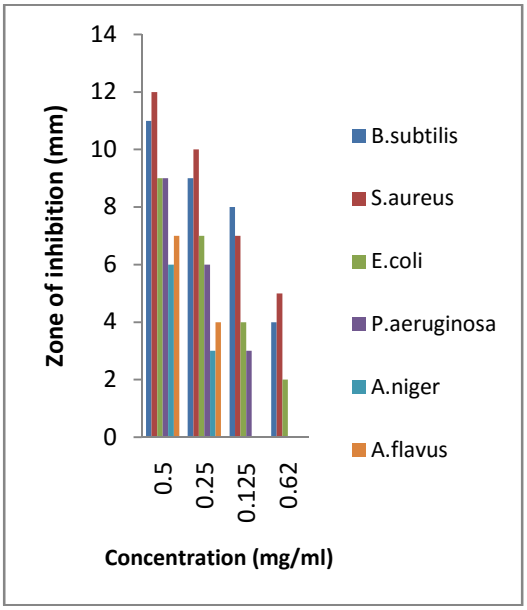

Fig D- Graphical representation of Chloroform Bark Extract against different microorganisms

In the present investigation, the antimicrobial activity of chloroform and methanol extract of *Holoptelea integrifolia* was evaluated in which the antimicrobial activity of chloroform Bark Extract of *Holoptelea integrifolia* showed maximum antibacterial activity against *S.aureus with* zone of inhibition of 12 mm and Methanolic leaves extract showed maximum antifungal activity against *A.niger* with zone of inhibition of 14 mm.The summarized finding of Methanolic Leaves Extract showed maximum zone of inhibition of 14 mm against *A.niger* & Methanolic Bark extract showed maximum zone of inhibition of 10 mm against *E.coli* & *A.flavus* whereas Chloroform Bark & Leaves Extract showed maximum zone of inhibition of 11 mm & 12 mm against *S.aureus* respectively. Gentamicin was used as positive control as an antibacterial antibiotic which produced the inhibition zone of 30 mm for *B. subtilis*, 18 mm for *S. aureus* and 22 mm for *E. coli* and for 22 mm for *P. aeruginosa* whereas *A. Niger* & *A.flavus* was found to show a zone of inhibition of 17 mm & 15 mm respectively to Ketoconazole which was used as positive antifungal antibiotic.

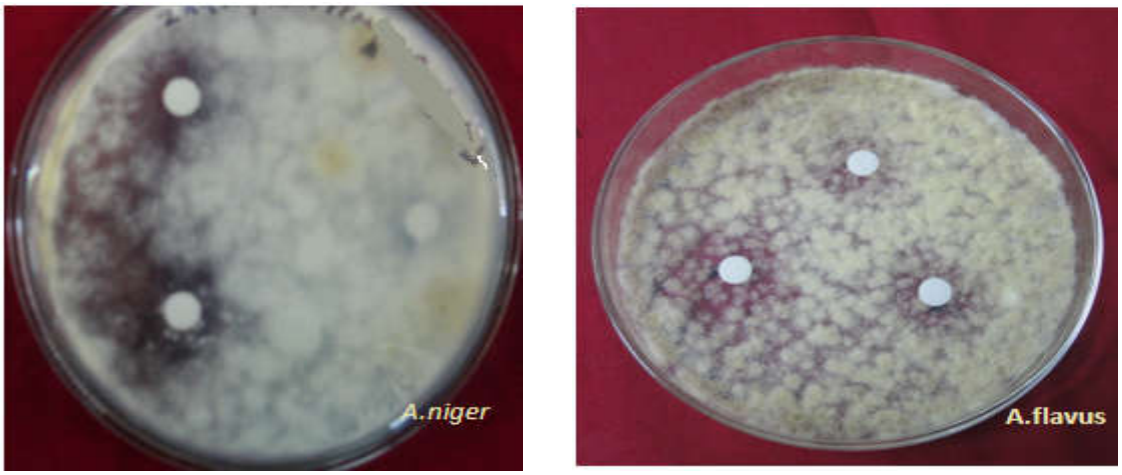

Fig E- Showing zone of inhibition of *Holoptelea integrifolia Leaves & Bark* Extract

CONCLUSION

Based on these results, we may conclude that both Leaves & Bark showed antibacterial & antigungal activity against all tested organisms and had large inhibition against *A.niger*. The varying degrees of sensitivity of the bacterial test organisms may be due to the intrinsic tolerance of microorganisms. Based on our findings, we envision that the discovery of novel antibacterial agent from natural sources (plants) will help to minimize the adverse effects of synthetic drugs.

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