Antimicrobial Activity of Aqueous Extract of *Holoptelea Integrifolia* (Roxb.) Leaves: an *In vitro* Study

Sharma, M.C.[1], Nigam, V.K.[2], Behera, B.[1] and Kachhawa, J.B.S.[1]

[1] Department of DravyaGuna, National Institute of Ayurveda, Madhav Vilas Palace, Jaipur-302002 (INDIA)

[2] Birla Institute of Scientific Research, Statue Circle, Jaipur-302001 (INDIA).

Summary

The aqueous extract of *Holoptelea integrifolia* Roxb. (Ulmaceae) was evaluated for antimicrobial activity against various bacteria viz. *Streptococcus pyogenes* MTCC No. 1928, *Staphylococcus aureus* MTCC No. 3160, *Escherichia coli* MTCC No. 901, *Pseudomonas aeruginosa* MTCC No. 424 and *Salmonella typhi* MTCC No. 733. Agar well diffusion method was adopted for the assessment of *invitro* antimicrobial activity. The antibacterial activity of aqueous extract of *Holoptelea integrifolia* in different concentration were evaluated where zone of inhibition was observed against all most resistant bacterial strains.

Key-words: *Holoptelea integrifolia*, Ayurveda, antimicrobial activity, inhibition zone

Introduction

The history of Ayurvedic medicine goes back to at least three thousand years, in which centuries after centuries rolled by witnessing varied fluctuations in human life. In Ayurvedic science, visible or invisible minute animals that affect on living and non-living things of biosphere are described very efficiently. Under the heading 'Krimi' they have been described worms, insects, bacteria, viruses & parasites.

In Ayurvedic system of medicine, a large number of plants are being used for treating diseases of bacterial origin and good results have been obtained with some of them. However, scientific investigation and information of the therapeutic potential of these plants is limited. In the same concern the present study was conducted to assess the antimicrobial activity of aqueous leaf extract of *Holoptelea integrifolia.*

It is a large deciduous tree, distributed through out the greater part of India upto on altitude of six hundred meter and belongs to family Ulmaceae. It is useful in cholic pain, intestinal worms, filaria, piles, pox, vitiligo (1) and also in wound healing (2).

Material and Methods

Plant material

Fresh leaves of *Holoptelea integrifolia* was collected from National Institute of Ayurveda campus, Jaipur in the month of

Correspondence to: Dr. Banshidhar Behera, Department of DravyaGuna, National Institute of Ayurveda, Madhav Vilas Palace, Jaipur-302002
E. Mail banshidharbehera@yahoo.co.in; Phone: 09784742206

January. The plant material was identified and authenticated by Prof. M.C. Sharma, Deptt. of DavyaGuna, National Institute of Ayurveda, Jaipur (Rajasthan). After the collection of plant material they were washed with running tap water and shade dried.

Preparation of extracts

For the aqueous extraction of plant material the 'Hot extraction method' was used recommended by W.H.O. (Quality Control Methods for Medicinal Plant Materials).

30 gms of shade dried macerated material of *Holoptelea integrifolia* was added to 300 ml. of sterile distilled water in a glass-stoppered round bottom flask. The mixture was then shacked well and allowed to stand for 1 hour. Then reflux condenser was attached to the flask and boiled gently for 1 hour, cooled, shacked well and filtered through a dry whattman filter paper. The filtrate was then poured into sterile beaker and evaporated to dryness on a water bath at 60°C. It was cooled in desiccators for 30 minutes and weighed without delay, where 30.14% extractive value was found. This residual was brown colored, water-soluble, which was transferred to a moisture free test tube and made it air tight by means of a cork.

Microorganisms

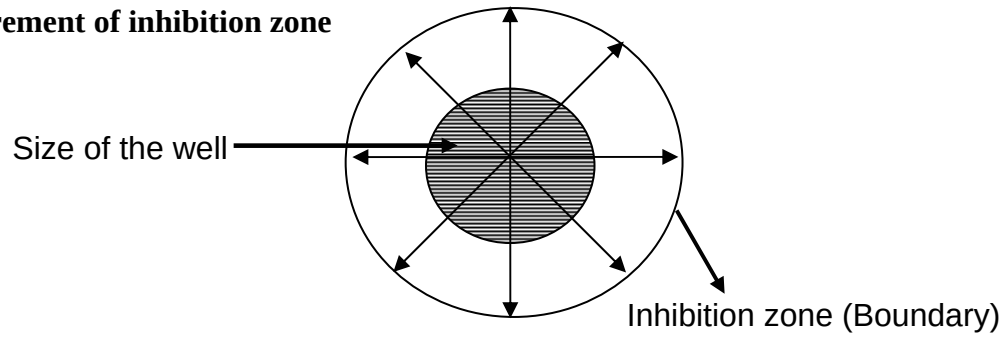
Microorganisms for the present study were obtained from Institute of Microbial Technology (IMTECH), Chandigarh (INDIA). They were maintained at 4°C on nutrient agar

Antimicrobial assay

The antimicrobial assay was performed by agar well diffusion method (3)

The disc was saturated with 100 µl of the test compound, allowed to dry. For agar well diffusion method, a well of 8 mm diameter was prepared in the plate. 100 µl of the extract was then introduced into the well. The plate was incubated at 37^{o}C for 24 hrs. and then observed for presence of inhibition zone.

Measurement of inhibition zone



The zone of inhibition of bacteria growth around the well was measured in mm, with the help of a scale. The readings were taken at 4 different planes as shown in figure below: -

Then the mean was calculated of the four readings taken.

Results and Observations

The results were based upon the scale developed by Arora and Bhardwaj (4). The zone of inhibition and result of drug sensitivity was described as below.

Table-1: Relation between zone of Inhibition drug sensitivity

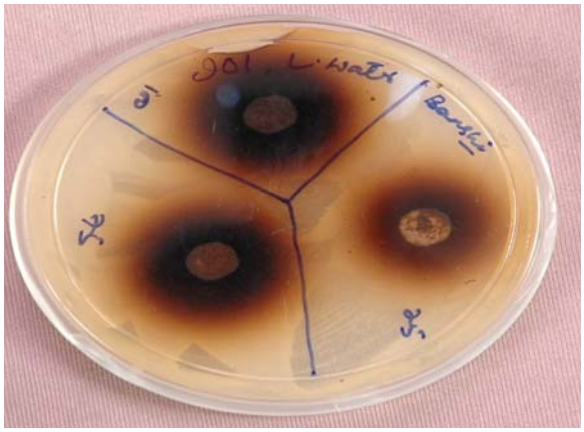
S. No.	Zone of Inhibition (m.m.)	Drug Sensitivity
1.	N.I. (below 6)	Insensitive
2.	6 < 9	Less sensitive
3.	9 < 12	Moderate sensitive
4.	> 12	Highly sensitive

Table-2: Zone of inhibition of Water extract of leaves of *Holoptelea integrifolia*

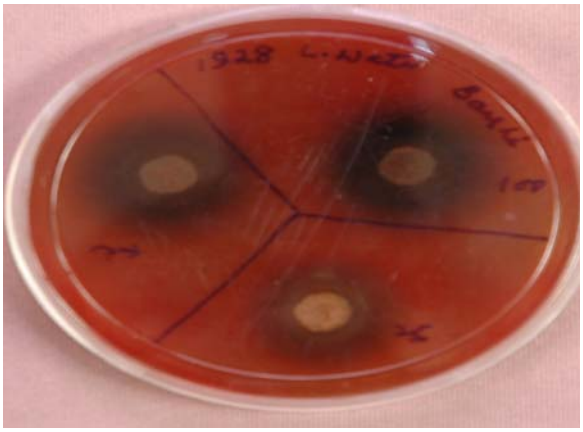
Concentration of leaf extract	M.T.T.C Bacteria code				
	Streptococcus pyogenes **(1928)**	***Staphylococcus aureus*** **(3160)**	***Escherichia coli*** **(901)**	***Pseudomonas aeruginosa*** **(424)**	***Salmonella typhi*** **(733)**
50 mg/100 µl	20,20,20,19 M = 20	7,8,8,8 M = 8	12,12,12,12 M = 12	12,12,12,12 M = 12	6,7,7,6 M = 6.5
75 mg/100 µl	20,20,20,20 M = 20	9,9,9,9 M = 9	16,16,17,17 M = 16.5	14,12,14,13 M = 13	9,9,9,9 M = 9
100 mg/100 µl	21,21,22,22 M = 21.5	10,10,11,11 M = 10.5	20,20,18,18 M = 19	15,16,16,16 M = 16	10,11,11,10 M = 10.5

Zone of inhibition:

The inhibition zone with most significant results



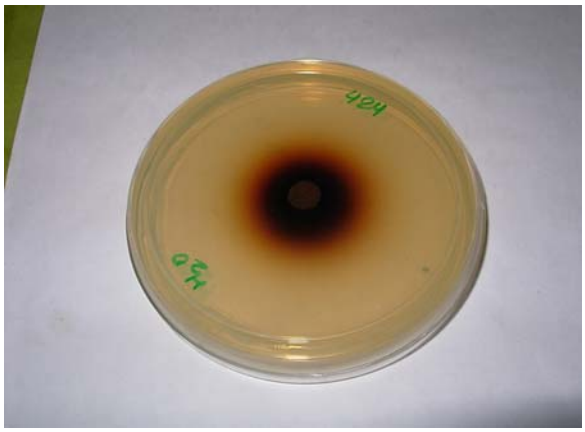
H_2O Ext. (50, 75 an 100 mg/100 µl) on *E. Coli*



H_2O Ext. (50, 75 an 100 mg/100 µl) on *S. pyogenes*

H_2O Ext. (100 mg/100 µl) on *E. coli*

H_2O Ext. (100 mg/100 µl) on *S. pyogenes*



H_2O Ext. (100 mg/100 µl) on *P. aeruginosa*

Streptococcus pyogenes (1928): Aqueous extract of leaves of *Holoptelea integrifolia* shows highly sensitivity at all dose levels i.e. 50mg, 75 mg and 100 mg.

Staphylococcus aureus (3160): *H. integrifolia* water leaf extract shows less sensitivity at 50 mg and 75 mg, however moderate sensitivity was found at 100 mg dose level. The similar results were also observed in ***Salmonella typhi*** (733) strain.

Escherichia coli (901): Aqueous extract shows highly sensitivity at all dose levels i.e. 50mg, 75 mg and 100 mg.

Pseudomonas aeruginosa (424): *H. integrifolia* leaf extract shows moderate sensitivity at 50 mg whereas high sensitivity was found at 75 and 100 mg dose level.

Discussion

When we put a view on research work done previously, we found that different active ingradients of plants like Terpenes (5**)**, Tannins (6), Saponins (7), Phenols (8**)** shows antimicrobial activity. As this experimental plant contains the same active ingredient (9), so it is probably the cause of antimicrobial activity. However, further study is also required to know which component is most responsible for this.

The experimental study only tells that different concentrations plant extract are effective against Microorganism, where further study is required to explore that how much it is potent and efficacious as compared to a standard anti-microbial agent like ciprofloxacin, Roxithromycin

etc. because no standard comparator was taken during the present experiment.

All the experiments were carried out in well method; therefore further analysis is required to explore the antimicrobial activity by disc diffusion method.

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