



## ANTI MICROBIAL ACTIVITY ON LEAF EXTRACT OF *CHLOROXYLONSWIETENIA*

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### ABSTRACT

The methanolic extract of *Chloroxylonswietenia* possess high anti-bacterial activity against all the test pathogenic bacterial species, the order of zone of inhibition in the following manner: methanolic extract > hexane fraction > ethyl acetate fraction.

**Keywords:** *Chloroxylonswietenia*, Antibacterial, Antifungal

### INTRODUCTION

Micro-organisms have proved to be beneficial to man in a number of ways and form an indispensable component of our ecosystem. In both terrestrial and aquatic systems, the micro-organisms enable the carbon, oxygen, nitrogen and sulphur cycles. They have, however, also proved to be harmful to mankind, specifically in their capacity to cause disease by growing on and or within other organisms. This results in microbial colonisation, which in turn may lead to disability and death. It is, therefore, critical to minimise the growth of these micro-organisms through effective prevention and treatment of disease. Therefore, the identification of the intrinsic characteristics of a particular pathogen, its source, mode of transmission and the susceptibility of the host and the exit mechanism of the pathogen will limit the spread of the pathogen<sup>1</sup>.

The development of drug resistance in human pathogens against commonly used antibiotics is resulting from the excessive and inappropriate use of antimicrobial agents, which in turn lead to potentially serious public health problems and has necessitated a search for new antimicrobial substances from other sources including plants. Plants are known to produce a variety of compounds to protect themselves against a variety of their pathogens and therefore considered as potential source for different classes of antimicrobial substances<sup>2-5</sup>. For centuries, plants have been used in the traditional treatment of microbial infections. This assembly of knowledge by indigenous people about plants and their products continue to play an essential role in health care of a great proportion of the population<sup>6</sup>.

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Plants used in traditional medicine contain a wide range of substances that can be used to treat chronic as well as acute infectious diseases. The substances that can either inhibit the growth of micro-organisms or kill them are considered as candidates for developing new drugs for treatment of various infectious diseases<sup>7</sup>. The use of plants in traditional system of medicine is well known in the rural areas of developing countries<sup>8-11</sup>. Herbal medicines have been used in developing countries as an alternative to allopathic medicines due to higher cost, chances of intake of spurious drugs and side effects of synthetic drugs. In addition there are more consumers nowadays who tend to question the safety of synthetic additives and prefer natural food preservatives<sup>12</sup>.

The efficacy of an antimicrobial agent can be estimated through the determination of the minimum inhibitory concentration (MIC), being the minimum concentration at which no microbial growth occurs after a specified exposure time to the antimicrobial agent<sup>13</sup>. In order to detect the antimicrobial substances present in very small quantities in plant extract, testing is carried out on the extracts in the form in which they are prepared or on concentrated extracts. The water insolubility of lipophilic samples (non-polar extracts) makes it necessary to use other solvents such as alcohol, acetone, chloroform, dimethyl sulphoxide, dioxane, glycerol and different emulsifiers such as macrogol ethers, sorbitan and cellulose derivatives. The solvent used for diluting the sample should be inert ie. It should not have any antimicrobial activity. The pH of the samples should be checked before testing the extracts are best adjusted to pH between 6.0-8.0 or dissolved in buffer solution such as physiological Tris buffer or others.

## MATERIAL AND METHODS

### Collection and Preparation of Plant

The leaves of *chloroxylonswietenia*DC were collected from nearby chowdavaram, Turuvalu village in Visakhapatnam, in the month of February 2013. The plant material (species) was taxonomically identified and authenticated by Prof. M.Venkaiah, Department of Botany, College of Science and Technology, Andhra University, Visakhapatnam. The herbarium voucher specimen (BGR/NA/CS-2013).

### Extraction and Isolation

The leaves (1kg) were air dried, coarsely powdered and extracted with hexane, ethyl acetate and methanol and concentrated under vacuum to a small residue.

### METHODS FOR EVALUATING ANTIMICROBIAL ACTIVITY

The term microbiological assay is a biological assay performed with micro-organisms like bacteria, yeast, moulds etc. This involves the measurement of the relative potency or activity of compounds by determining the amount of test material required for producing stipulated effect on suitable organism under standard conditions.

The procedures employed in microbial assay were, Cylinder plate method or cup plate method Turbidimetric or tube assay method (two fold serial dilution method). In the present study, antimicrobial screening was carried out using cup plate method. In cup plate method, the antimicrobial substance diffuses from the cup through a solidified agar layer in a Petri dish or a plate to some extent so that the growth of added micro-organism is inhibited entirely in a circular area or zone around the cavity containing the solution of a known quantity of antimicrobial substance.

The antimicrobial activity is expressed as the zone of inhibition in millimeters, which is measured with a zone reader. The hexane, chloroform and methanolic extracts of leaves of *Chloroxylonswietenia* were screened for antimicrobial activity against a wide spectrum of micro-organisms and the activity was compared with appropriate reference standards (amikacin for both gram-positive and gram-negative organisms). Micro-organisms were grown in nutrient agar medium. Dimethyl sulphoxide (DMSO) and distilled water were used as control and the drug vehicles for the plant extracts and reference standards respectively.

## EXPERIMENTAL DESIGN

### Test Organisms:

The microorganisms used for the experiment were procured from MTCC, IMTECH, Chandigarh.

### Gram-positive organisms:

*Staphylococcus aureus*, Rosenbach (MTCC 737),  
*Bacillus subtilis*.

### Gram-negative organisms:

*Escherichia coli* (Migula) Castellani and Chalmers (MTCC 443),

*Pseudomonas aeruginosa* (Schroeter) Migula (MTCC1688),

### Standardization of micro-organisms:

One loop-full of micro-organisms were inoculated into 100 ml of sterile medium and incubated for 24 h at 37°C for bacterial culture and for 48 h at 27°C for fungal culture. After 24 h/48 h of incubation, 1 ml of broth containing the micro-organisms was added to 9 ml of peptone water. 10 fold serial dilutions were made in the range of 10<sup>-1</sup> to 10<sup>-10</sup>. 100 µl of the dilutions ranging from 10<sup>-5</sup> to 10<sup>-8</sup> were spread over the sterile nutrient agar (SDA) plates and kept at 37 and 27°C for 24 / 48 hours respectively. The number of colony forming units (CFU) was counted and number of micro-organisms per 1 ml of stock culture was calculated.

### Preparation of test and standard solutions:

The stock solution of test compounds was prepared by dissolving the dried extracts at a concentration of 5 and 10 mg/ml in dimethyl sulphoxide (DMSO) respectively. The stock solution of reference standard (Amikacin) was prepared at a concentration of 0.6 mg/ml in sterile water. Antimicrobial activity was screened by adding 0.05 ml stock solution to each cup by micropipette.

### Culture medium:

The following media were used for the present antimicrobial studies.

### Nutrient broth for bacteria:

Beef extract	-	0.35%
Sodium chloride	-	0.5%
Peptone	-	0.5%

The above ingredients weighing 37 g were dissolved in distilled water (1000 ml). pH was adjusted to 7.2-7.4 and sterilized by autoclaving at 15 lbs for 20 min.

### Sterilization:

Sterilization of the media, water etc., were carried out by autoclaving at 15 lbs/inch<sup>2</sup> for 20 minutes. The glassware like syringes, petridishes, pipettes, empty test-tubes were sterilized by dry heat in an oven at a temperature of 160°C for one hour.

### Nutrient agar for bacteria:

Beef extract	-	0.3%
Sodium chloride	-	0.5%
Peptone	-	0.5%
Agar	-	2.0%
pH	-	7.2-7.4

The sterilized medium was cooled to 40°C and poured into the Petri dishes to contain 6 mm thickness. The media was allowed to solidify at room temperature.

## EVALUATION OF ANTIBACTERIAL ACTIVITY:

### Determination of zone of inhibition by cup plate method

The cylinder plate assay of drug potency is based on measurement of the diameter of zone of inhibition of microbial growth surrounding cylinders (cups), containing various dilutions of test compounds. A sterile borer was used to prepare four cups of 6 mm diameter in the agar medium spread with the micro-organisms and 0.1 ml of inoculum. These cups were spread on the agar plate by spread plate technique. Accurately measured (0.05 ml) solution of each concentration and reference standards were added to the cups with a micropipette. All the plates were kept in a refrigerator at 2 to 8°C for a period of 2 hours for

effective diffusion of test compounds and standards. Later, they were incubated at 37°C for 24 hours. The presence of definite zone of inhibition of any size around the cup indicated antibacterial activity. The solvent control was run simultaneously to assess the activity of Dimethyl sulphoxide and water which were used as a vehicle. The experiments were performed three times. The diameter of the zone of inhibition was measured and recorded.

## RESULTS

**Table 1:** Antibacterial activity of *Chloroxylonswietenia* leaf extracts

Plant material	Dose (µg/ml)	Zone of inhibition (diameter in mm)			
		Gram (+) ve		Gram (-) ve	
		<i>B.s</i>	<i>S.a</i>	<i>E.c</i>	<i>P.a</i>
Methanolic extract	25	10	12	13	16
	50	12	19	12	22
	100	22	28	15	28.8
Ethylacetate fraction	25	10	9	8.7	12.3
	50	13	14.2	10.8	13.9
	100	20	22.5	15.8	22.5
Hexane fraction	25	10.8	11	11.9	16
	50	12.2	12.6	18.5	18.5
	100	22	23	19.6	22.6
Amikacin	25	29	31.5	30	32
DMSO	-	-	-	-	-
Water	-	-	-	-	-

*B.s* = *bacillus substillis*, *S.a* = *Staphylococcus aureus*, *E.c* = *Escherichia coli*, *P.a* = *Pseudomonas aeruginosa*.

## DISCUSSION

The results of anti-bacterial screening of *Chloroxylonswietenia* were summarized and the averages of triplicates are shown in the above Table 1. By summarizing the results it was found that by adding a fixed volume of 50 µl per each cup, all the extracts *i.e.*, methanolic, ethyl acetate and hexane extracts, at concentrations of 25 µg/ml, 50 µg/ml, and 100 µg/ml exhibited considerable and significant anti-bacterial activity more or less in dose-dependent manner against one or more organisms. The methanolic extracts of *Chloroxylonswietenia* possess high anti-bacterial activity against all the test pathogenic bacterial species, the order of zone of inhibition in the following manner: methanolic extract > hexane fraction > ethyl acetate fraction.

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