

Evaluation of Antibacterial Activity and Phytochemical Screening of *Wrightia tinctoria* L.

S. Ravi Shankar^{a*}, R. Rangarajan^a, D.V.L. Sarada^b and C. Sreenath Kumar^c

^aDepartment of Plant Biology and Plant Biotechnology, Madras Christian College, Chennai – 600 059, India. ^bDepartment of Bioinformatics, SRM University, Chennai – 600 089, India. ^cDepartment of Plant Biology and Biotechnology, Loyola College, Chennai – 600 034, India.

ABSTRACT

Wrightia tinctoria L. leaf hexane, methanol, chloroform, ethyl acetate and water extracts were screened against plant pathogenic bacteria by *in vitro*. The extracts were tested using disc diffusion method and minimum inhibitory concentration. Ethyl acetate and methanol extracts showed significant antibacterial activity against gram negative bacteria; the MIC was 50 µg/ml for *Xanthomonas campestris* and *Erwinia* sp. Study shows *Wrightia tinctoria* leaves possessed potent antimicrobial properties against plant pathogenic bacteria suggesting that the active principles may be useful in the control of plant disease.

Key words: *Wrightia tinctoria*, antibacterial, phytochemicals, MIC

INTRODUCTION

Medicinal plants are an important therapeutic aid for various ailments. Around 15% of angiosperms have been investigated chemically and from that nearly 74% of pharmacologically active bioactive substances were identified.^[1] This evidence contributes to support and quantify the importance of further screening of medicinal plants.

Medicinal plants are known to contain innumerable biologically active substances which possess antibacterial properties.^[2] Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century. These natural plant products, known as biopesticides, have long been used in the control of microorganisms causing plant diseases.

Plant pathogenic bacteria cause many serious diseases of plants throughout the world. Most plants, both economic and wild, have innate immunity or resistance to many pathogens but certain food crops and ornamental plants are susceptible to diseases caused by bacteria which are

difficult to control and often result in sudden, devastating financial losses to farmers. Synthetic pesticides are commonly employed in agriculture to control phytopathogenic microorganisms.^[3] Although it is highly effective, synthetic pesticide often have undesirable side effects such as toxicity to mammals and causing environmental pollution.^[4-6] Most importantly, many phytopathogenic bacteria have acquired resistance to synthetic pesticides.^[7]

Considering the harmful effects of synthetic pesticides on life supporting systems, there is an urgent need to search for alternative approaches for the management of plant pathogenic microorganisms.^[8]

The use of plant-derived alternative pesticides seems to be regaining popularity and could play a vital role in meeting the demand for organically produced plants. Biopesticides has been suggested as an effective substitute for chemicals.^[9] Many authors have contributed only on the use of several plant by-products, which possess antimicrobial properties, on several human pathogenic bacteria and fungi;^[10-15] but information is obscure on the inhibitory activity of plant extract on phytopathogenic bacteria which are known to cause many diseases in a wide variety of crops, causing considerable losses in yield and quality.

Wrightia tinctoria is a well known potential medicinal plant distributed in tropical region belongs to the family

Address for correspondence:

E-mail: botanyravi@gmail.com

DOI: ****

Apocynaceae. The leaves of this plant are traditionally used in the treatment against Psoriasis and non-specific dermatitis in Siddha and Ayurvedic systems of medicine. This plant is good for treatment of dandruff, various scalp and skin disorders.^[16-17] In the present study an attempt has been made to evaluate the antibacterial activity and to test the efficacy of the leaf solvents extracts on important plant pathogenic bacteria with the aim of discovering new bioactive substances that can be used as for controlling diseases caused by them in economically important crops.

MATERIALS AND METHODS

Source of the Plant material

Fresh healthy leaves of *Wrightia tinctoria* L, were collected from the locality near the Chengalpattu, Tamil Nadu, India.

Sample Preparation for Phytochemical analysis

Healthy leaf samples were washed thoroughly 2-3 times with running tap water and followed by sterile water, shade-dried and ground into uniform powder, extracted in distilled water and organic solvents like ethyl acetate and methanol (1:2 w/v) for 12 hr. The extract was filtered using Whatman No. 1 filter paper and used for the screening of phytochemicals. Qualitative tests were carried out using solvent extract and powdered sample according to standard procedures for identification of major secondary metabolites such as alkaloids, glycosides, flavonoids, tannins, phlobatannins, saponins and terpenoids.^[18-20]

Preparation for water and solvent extractions

Fifty grams of shade dried, leaf powder of *W. tinctoria* was extracted in 200 ml of each hexane, ethyl acetate, chloroform, methanol and water using a Soxhlet extractor for 48 hours. The collected extracts were concentrated using rotary flash evaporator and stored in refrigerator at 4° C for further use.

Plant Pathogens

Pure isolates of phytopathogenic bacteria, *Erwinia* sp. MTCC 2760, *Xanthomonas campestris* MTCC 2286, *Xanthomonas citri* and *Xanthomonas oryzae* pv. *oryzae* MTCC 5156 were obtained from Institute of Microbial Technology, Chandigarh, India.

Antibacterial activity assay

Antibacterial activity of water and solvent extracts were determined by Disc diffusion method. Leaf powdered extract was added with 5% Dimethyl Sulfoxide (DMSO) and 100 µg of each was loaded on to sterile disc (Himedia) and placed assay plates containing on Muller Hinton Agar spread with over night grown bacterial pathogens.^[21] Inhibition zones around the discs were measured after 24h. Discs loaded with 5% DMSO served as negative control and Ampicillin, a synthetic antibiotic served as positive

control. Triplicates were maintained and the results were expressed in mean ± standard error, using statistical software SPSS 10.1.

MIC assay

Minimum Inhibitory Concentration was determined by the broth microdilution method.^[22-24] All tests were performed in Mueller Hinton agar broth (Himedia). The concentrations of maximum active leaf extract of *W. tinctoria* was dissolved in water + DMSO (95: 5) and serial double dilutions were prepared that were added to a 96-well microtiter plate over the range of 7 – 3,125 µg/ml. Overnight broth cultures of each strain were prepared and the final concentration of the microbe in each well was adjusted to 2×10^3 cfu/mL. Plates were incubated at 37° C for 24 h. The MIC was determined by reading the absorbance of each well using an automatic ELISA tray reader adjusted at 630nm (SLT Spectra). The samples were analyzed in duplicate and the assay was repeated twice. The wells showing complete absence of growth were identified and 10 µL of each well were transferred to Mueller Hinton agar plates and incubated at previously mentioned times and temperatures. Values are expressed as mean ± standard error and statistical significance was set at $P < 0.05$.

RESULTS

Phytochemical Screening

The preliminary phytochemical screening indicated ethyl acetate, methanol and water leaf extracts of *Wrightia tinctoria* showed the presence of bioactive constituents such as alkaloids, terpenoids, glycosides, flavanoids, saponin and phlobatannin (Table 1). These substances are said to be responsible for the antimicrobial activity of this plant.

Antibacterial activity of leaf extract of *W. tinctoria*

The result of the antimicrobial screening crude leaf extracts is shown in the Table 2. The maximum inhibitory activity was recorded in methanolic extract followed by ethyl acetate extract against the growth of all tested gram-negative plant pathogenic bacteria such as *Erwinia* sp., *Xanthomonas campestris*, *X. citri* and *X. oryzae*. Hexane and water extracts

Table 1: Phytochemical analysis of different solvent leaf extracts of *Wrightia tinctoria*

Phytochemicals	Ethyl acetate	Methanol	Water
Alkaloids	+	+	–
Terpenoids	+	+	+
Glycosides	+	+	+
Flavanoids	+	+	+
Saponin	+	+	+
Tannin	–	–	–
Phlobatannin	+	+	+

(+); Present, (–); Absent

Table 2: Antibacterial activity of *Wrightia tinctoria* against the plant pathogenic bacteria

Plant pathogen	Zone of inhibition* (mm in diameter)						Ampicilin
	Hexane	Ethyl Acetate	Chloroform	Methanol	Water	Control (5% DMSO)	
<i>Erwinia</i> sp.	0.00 ± 0.00	26.59 ± 0.00	0.00 ± 0.00	29.59 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	10.33 ± 0.69
<i>Xanthomonas campestris</i>	0.00 ± 0.00	24.00 ± 0.59	0.00 ± 0.00	25.67 ± 0.33	0.00 ± 0.00	0.00 ± 0.00	12.33 ± 0.33
<i>Xanthomonas citri</i>	0.00 ± 0.00	8.00 ± 0.33	0.00 ± 0.00	8.33 ± 0.33	0.00 ± 0.00	0.00 ± 0.00	12.69 ± 0.69
<i>Xanthomonas oryzae</i>	0.00 ± 0.00	10.00 ± 0.00	8.33 ± 0.33	18.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	10.00 ± 0.00

*Disc potency 100 µg.

Mean ± Standard error; Standard deviation $P \leq 0.05$

did not show activity. The commercial antibiotic ampicilin used as positive control also showed activity against all the tested plant pathogens.

Minimum Inhibitory Concentration (MIC) of the leaf extract of *W. tinctoria*

Minimum Inhibitory Concentrations of the methanol and ethyl acetate leaf extracts showed maximum activity against all the four pathogens are depicted in *Table 3*. The MIC of the leaf extracts studied ranged between 50 µg/ml and 180 µg/ml. Minimum concentrations of ethyl acetate and methanol extracts were effective in controlling the growth of *Erwinia* sp and *Xanthomonas campestris*.

DISCUSSION AND CONCLUSION

Natural products are considered an important source of new antibacterial agents.^[25] Plants have been formed the basis of natural pesticides, that make excellent leads for new pesticide development.^[26] Recently, researches have attempted in the screening of diverse plants for the antimicrobial potential in the management of disease caused by various phytopathogens.^[27-31] These evidences contributes to support and quantify the importance of screening natural products for exploitation of naturally available chemicals from plants, which would be an effective and eco-friendly approach for plant protection. It will also have a prominent role in the development of future commercial pesticides.^[32]

Table 3: MIC of *Wrightia tinctoria* against the plant pathogenic bacteria

Pathogens	Extracts	MIC (µg/ml) ^a	Ampicilin MIC (µg/ml) ^a
<i>Erwinia</i> sp.	Ethyl acetate	50	170
	Methanol	50	
<i>Xanthomonas campestris</i>	Ethyl acetate	50	160
	Methanol	50	
<i>Xanthomonas citri</i>	Ethyl acetate	180	160
	Methanol	180	
<i>Xanthomonas oryzae</i>	Ethyl acetate	170	170
	Methanol	75	
	Chloroform	180	

^a Values are the mean of 3 replicates using 1×10^3 cells of each culture

In the present study, results show that antibacterial activity of methanol and ethyl acetate leaf extracts exhibited more inhibitory activity indicating the effective extraction than the other extracts. The absence of antibacterial activity in the Hexane and aqueous extracts indicates the insolubility of the active substances in these solvents.

In conclusion, the present investigation forms a good basis in the selection of *W. tinctoria* for further TLC separation and characterization of bioactive substances. This plant could be successfully exploited for management of the diseases of plant pathogenic bacteria preventing considerable losses in yield and quality in an eco-friendly way for commercially important crops.

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