

ANTIMICROBIAL ACTIVITY OF *WRIGHTIA TINCTORIA* (Roxb.) R.Br METHANOLIC EXTRACT

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ABSTRACT

Methanolic extract of Indian medicinal plant *wrightia tinctoria*, (Roxb.) R.Br. was examined for their antimicrobial potential against selected bacteria and fungi. The purpose of screening is to justify and authenticate the use of Indian medicinal plants in ethno medicinal or folklore as traditional treasure to cure various ailments. In present investigations attempts were made to screen the Indian medicinal plants as antimicrobial agent. The extracts were tested against selected test bacteria and fungi through disc diffusion assay where Tetracycline and Mycostatin were used as standard. Indian medicinal plants have a traditional background that they have potentials to use as antimicrobial

agents. The results showed that methanolic extract have excellent antimicrobial activity against selected test bacteria and fungi. The present results therefore suggest a scientific basis for traditional use of the methanolic extract of *Wrightia tinctoria* (Roxb.) R.Br.

KEYWORDS: *wrightia tinctoria*, Antimicrobial, Indian Medicinal Plants, Disc diffusion assay

INTRODUCTION

Many efforts have been made to find out new antimicrobial compounds from various kinds of sources such as micro-organisms, animals, and plants. One of such resources is folk medicines. Systematic screening of them may result in the discovery of novel effective compounds.^[1]

The beneficial medicinal effects of plant materials typically result from the combinations of secondary Products present in the plant. In plants, these compounds are mostly secondary

metabolites such as alkaloids, steroids, tannins and phenol compounds flavonoids, steroids, resins fatty acids gums which are capable of producing definite physiological action on body.^[2] the rise in the collapse of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has lead to the screening of several medicinal plants for their potential antimicrobial activity. (Colombo *et.al.*, 1996; Okunji *et.al.*,1999). There are several standard methods used for the Phytochemicals screening of medicinal plants. They are as described for alkaloids (Harborne *et. al.*, 1973), steroids (Trease *et. al.*, 1989), phenolics and flavonoids (Awe *et. al.*, 2001), saponins and cardiac glycosides (Sofowora *et. al.*, 1993), tannins (Odebiyi *et. al.*, 1978).

Methods for quantitative analysis of Phytochemicals are as described for phenolics (Edeoga *et al.*, 2005), flavonoids (Boham *et. al.*, 1974), alkaloid (Harborne, *et. al.*, 1973), saponins (Obadoni *et. al.*, 2001) and glycosides (El-Olemy *et al.*, 1994).

MATERIALS AND METHODS

Collection

A Plant sample (*Wrightia tinctoria*(Roxb.) R.Br.) Was collected from various tribes living in tribal pockets of Mt. Abu, arid zone of Rajasthan. The plant was used by these tribes in their daily lives to cure various ailments.

Identification

The entire sample was authenticated and was given identification number .The sample was authenticated and submitted in Ethno medicinal Herbarium, Centre of Excellence funded by DST, MGiaS, Jaipur (Rajasthan).

Sources of Test Organisms

Pure culture of all test organisms, bacteria's namely *Enterobacter aerogenes*, *Staphylococcus aureus*,*Bacillus subtilis*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Shigella flexneri*,and *Chryseobacterium gleum* and fungi *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus* were obtained through the courtesy of Mahatma Gandhi Institute of applied Sciences (MGiaS), Jaipur, which were maintained on Nutrient broth media.

Culture of Test Microbes

For the cultivation of bacteria, Nutrient Agar Medium (NAM) was prepared by using 20 g Agar, 5 g Peptone, 3 g beef extract and 3 g NaCl in 1 L distilled water and sterilized at 15 lbs pressure and 121°C temperature for 25-30 min. Agar test plates were prepared by pouring approximately 15 mL of NAM into the Petri dishes (10 mm) under aseptic conditions. A saline solution was prepared (by mixing 0.8% NaCl) in distilled water, followed by autoclaving and the bacterial cultures were maintained on this medium by regular sub-culturing and incubation at 37°C for 24-48 h. To prepare the test plates, in bacteria, 10-15 mL of the respective medium was poured into the Petri plates and used for screening. For assessing the bactericidal efficacy, a fresh suspension of the test bacteria was prepared in saline solution from a freshly grown Agar slant.

Preparation of Test Extracts

Crushed powder (50 g) of all the species were successively soxhlet extracted with ethanol. Later, each of the homogenates was filtered and the residue was re-extracted twice for complete exhaustion, the extracts were pooled individually. Each filtrate was concentrated to dryness *in vitro* and redissolved in respective solvents, out of which 80 mg/10 discs i.e. 8 mg/disc concentration were stored at 4°C in a refrigerator, until screened for antibacterial activity.

Antimicrobial Assay by Disc Diffusion Method

For both, bactericidal *in vitro* Disc diffusion method was adopted.^[3] because of Reproducibility and precision. The different test organisms were proceeded separately using a sterile swab over previously sterilized culture medium plates and the zone of inhibition were measured around sterilized dried discs of Whattman No. 1 paper (5 mm in diameter), which were containing 1mg, 5mg and 10mg of the text extracts and reference drugs (tetracycline and Mycostatin for bacteria and fungi, respectively) separately. Such treated discs were air-dried at room temperature to remove any residual solvent, which might interfere with the determination, sterilized and inoculated.

These plates were initially placed at low temperature for 1 h so as to allow the maximum diffusion of the compounds from the test disc into the agar plate and later, incubated at 37°C for 24 h in case of bacteria and °C in case of fungi, after which the zones of inhibition could be easily observed. Five replicates of each test extract were examined and the mean values were then referred. The Inhibition Zone (IZ) in each case were recorded and the Activity

Index (AI) was calculated as compared with those of their respective standard reference drugs (AI = Inhibition Zone of test sample/Inhibition zone of standard).

RESULTS AND DISCUSSION

The report of the medicinal plants used in the present investigation. The results of antimicrobial activity of the crude extracts of Selected Indian Medicinal Plant (*Wrightia tinctoria* (Roxb.) R.Br. show good Antimicrobial activity against selected test bacteria and fungi (Table-1,2,3). Overall, this extract showed appreciable activity against selected test bacteria and fungi and hence, it justifies their use in our traditional system of medicine to cure various diseases.

Table 1. Shows the results of inhibitions zones *Wrightia tinctoria* (Roxb.) R.Br stem against above seven microorganisms at different concentrations.

<i>Wrightia tinctoria</i> (Roxb.) R.Br stem				Inhibition zone(m.m)				
Extractconcentration		<i>E.aerogenes</i>	<i>S.aureus</i>	<i>C. gleum</i>	<i>P.vulgaris</i>	<i>B.subtilis</i>	<i>K.pneumoniae</i>	<i>S.flexneri</i>
Methanol extract	1mg/disc	5	5	0	6	5	4	3
	5mg/disc	7	7	6	9	7	4	9
	10mg/disc	10	9	10	12	10	6	10

Table 2. Shows the results of inhibitions zones *Wrightia tinctoria* (Roxb.) R.Br leaf against above seven microorganisms at different concentrations.

<i>Wrightia tinctoria</i> (Roxb.) R.Br leaf				Inhibition zone(m.m)				
Extractconcentration		<i>E.aerogenes</i>	<i>S.aureus</i>	<i>C.gleum</i>	<i>P.vulgaris</i>	<i>B.subtilis</i>	<i>K.pneumoniae</i>	<i>S.flexneri</i>
Methanol extract	1mg/disc	5	6	0	4	7	6	0
	5mg/disc	7	10	5	8	9	10	6
	10mg/disc	8	20	9	9	12	12	7

Table 3. Shows the results of inhibitions zones *Wrightia tinctoria* (Roxb.) R.Br fruit against above seven microorganisms at different concentrations.

<i>Wrightia tinctoria</i> (Roxb.) R.Br fruit				Inhibition zone (m.m)				
Extract concentration		<i>E.aerogenes</i>	<i>S.aureus</i>	<i>C.gleum</i>	<i>P.vulgaris</i>	<i>B.subtilis</i>	<i>K.pneumoniae</i>	<i>S.flexneri</i>
Methanol extract	1mg/disc	5	8	6	7	0	4	0
	5mg/disc	8	12	12	9	0	7	3
	10mg/disc	12	17	19	10	5	15	6

Table 2. showing the results of *Wrightia tinctoria* (Roxb.) R.Br (leaf) methanol extract for antimicrobial efficacy against selected test microorganisms. Methanol extract showed appreciable inhibition against all the selected test microorganisms (bacteria). The maximum efficacy of methanol extract A3 (10mg concentration) was against *Staphylococcus aureus* (I.Z.-20 mm) even A1 (1mg concentration) was also active against *Bacillus subtilis* (I.Z.-7mm), A2 (5mg concentration) was also active against *Klebsiella pneumoniae*, *Staphylococcus aureus* (I.Z.-10mm), whereas no activity against *Chryseobacteria gleum*, *Shigella flexneri* A1 (1mg concentration) have been screened. The maximum efficacy of leaf Methanol extract A3 (10mg concentration) was against *Aspergillus fumigates*, (I.Z.-15 mm) A2 (5mg concentration) was also active against *Aspergillus fumigates* (I.Z.-12mm), *Aspergillus niger*, *Aspergillus flavus* not have activity against A1 (1mg concentration).

Table 1. showing the results *Wrightia tinctoria* (Roxb.) R.Br (stem) methanol extract for antimicrobial efficacy against selected test microorganisms. Methanol extract was showed appreciable inhibition against all the selected test microorganisms (bacteria). The maximum efficacy of methanol extract A3 (10mg concentration) was against *Proteus vulgaris* (I.Z.-12 mm), even A1 (1mg concentration) was also active against *Proteus vulgaris* (I.Z.-6mm), A2 (5mg concentration) was also active against *Proteus vulgaris*, *Shigella flexneri* (I.Z.-9mm), whereas no activity against *Chryseobacteria gleum* A1 (1mg concentration) have been screened. The maximum efficacy of Methanol extract A3 (10mg concentration) was against *Aspergillus fumigatus*, (I.Z.-8 mm) A2 (5mg concentration) was also active against *Aspergillus fumigatus* (I.Z.-6mm), *Aspergillus niger*, *Aspergillus flavus* not have activity against A1 (1mg concentration).

Table 3. showing the results *Wrightia tinctoria* (Roxb.) R.Br (fruit) methanol extract for antimicrobial efficacy against selected test microorganisms. Methanol extract was possessed appreciable inhibition against all the selected test microorganisms (bacteria). The maximum efficacy of methanol extract A3 (10mg concentration) was against *Staphylococcus aureus* (I.Z.-17 mm) even A1 (1mg concentration) was also active against *Staphylococcus aureus* (I.Z.-8mm), A2 (5mg concentration) was also active against *Staphylococcus aureus*, *Chryseobacteria gleum* (I.Z.-12mm), whereas no activity against *Shigella flexneri*, *Bacillus subtilis* A1 (1mg concentration) have been screened. In the case of fungi the maximum efficacy of Methanol extract A3 (10mg concentration) was against *Aspergillus fumigates*, (I.Z.-8 mm) A2 (5mg concentration) was also active against *Aspergillus fumigates* (I.Z.-

7mm), *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* not have activity against A1 (1mg concentration).

CONCLUSION

Many medicinal plants have been found effective in the curing of bacterial disease. Methanolic extract of Indian Medicinal Plants *Wrightia tinctoria* (Roxb.) R.Br. (leaf, stem, Fruit) was examined for their anti-microbial, potentials against selected bacteria and fungi. The purpose of screening is to justify, authenticate and validate the use of Indian Medicinal Plants in Ethno-medicinal or folklore as traditional treasure to cure various ailments and disease caused by Environmental pollutions. The various extracts from traditional medicinal plants with folklore reputation have been examined to identify the source of therapeutic drugs; therefore the drugs were tested against selected test bacteria and fungi as antimicrobial assay through disc diffusion assay, where standard drugs were tetracycline, Mycostatin and Streptomycin were used. Indian Medicinal Plants have a traditional background that they have potentials to use as antimicrobial agents. The results showed that methanolic extract possess good antimicrobial activity against selected test bacteria and intermediate against fungus, therefore offer a scientific basis for traditional use of methanolic extracts of *Wrightia tinctoria* (Roxb.) R.Br. (leaf, stem, Fruit) it justifies their use in our traditional system of medicine to cure various diseases.

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