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EVALUATION OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIIES OF THE LEAF AND BARK EXTRACTS OF *WRIGHTIA TINCTORIA* R.Br.

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ABSTRACT

The antibacterial and antifungal activities of the leaf and bark extracts of *Wrightia tinctoria* (Pala Indigo) were investigated. The antibacterial potential of the leaf coconut oil extracts, petroleum ether, chloroform, ethyl acetate and methanol extracts of the leaves and bark of *Wrightia tinctoria* were studied against human pathogenic bacteria viz. *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Serratia marcescens* and antifungal activity against two fungi namely *Aspergillus niger* and *Penicillium chrysogenum* by 'agar well diffusion' method. Leaf and bark methanol extracts of *Wrightia tinctoria* exhibited pronounced activity against Gram-positive and Gram-negative bacteria and their activity is quite comparable with the standard antibiotics such as tobramycin and gentamycin sulphate screened under similar conditions. The results obtained showed that the leaf and bark methanol extracts of *W.tinctoria* can be considered as good sources of antimicrobial compounds and can be incorporated into the drug formulations.

Key words: *Wrightia tinctoria,* antibacterial activity, antifungal activity, agar well diffusion method, drug formulations

INTRODUCTION

Medicinal plants are a source of great economic value all over the world. Nature has given us a very rich botanical wealth and large number of diverse types of plants grows in different parts of the country. Ayurveda, Unani and Siddha are systematically used nearly 1500 plants in indigenous system of medicine. Medicinal plants are the oldest existing complete medical system in the world. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range

of substances that can be used to treat chronic as well as communicable diseases [1]. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Medical plants contain large varieties of chemical substances which possess important therapeutic properties that can be utilized in the treatment of human diseases. The studies of Medicinal plants used in folklore remedies have attracted the attention of many scientists in finding solution to the problems of multiple resistances to the existing synthetic antibiotics. Most of the synthetic antibiotics now available in the market have major setback due to the multiple resistance developed by pathogenic micro-organisms against their drugs [2]. Modern technique and pharmacological screening procedure results new plant drugs usually find their way into modern medicines. Now a day's maximum number of plant are being screened for their possible pharmacological value. The plant kingdom still hold many plant species containing substance of medicinal value which have yet to be discovered. The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on the human body. The most important of these phytocompounds of plants are alkaloids, flavonoids, tannins and phenolic compounds.

Wrightia tinctoria is a widely recognized medicinal plant [3] and considered to be therapeutically very effective jaundice plant in India in indigenous system of medicine. The juice of the tender leaves is used efficaciously in jaundice. Crushed fresh leaves when filled in the cavity of decayed tooth relieve toothache. In Siddha system of medicine, it is used for psoriasis and other skin diseases. Oil 777 prepared out of the fresh leaves of the plant has been assigned to analgesic and anti-inflammatory activities and to be effective in the treatment of psoriasis [4]. The seeds and bark of this plant are used in Indian traditional medicine as anti-diarrheal and anti-dysenteric [5].

Ethnomedically, the bark of this plant is used as a galactagogue to treat abdominal pain, skin diseases and wounds [6] as an anti-pyretic (Shah et al, 1988) and anti-hemorrhagic agents [7] and as an antidote for snake poison [8]. Seeds of this plant are also used as an aphrodisiac [9]. The methanolic and ethyl acetate extracts of *Wrightia tinctoria* R.Br. fruit possessed significant anti-diabetic activity [10].

MATERIALS AND METHODS

Plant Material

The leaves and bark of *Wrightia tinctoria* were collected from Thrissur district of Kerala, South India and authenticated by Dr. Kochuthressia M.V., HOD, Department of Botany, Vimala College, Thrissur. Voucher specimen is deposited in the specially maintained herbarium, Department of Botany, Vimala College, Thrissur.

Preparation of Plant Extracts

Fifty grams of the powered plant material were extracted successively with 150ml of petroleum ether, chloroform, ethyl acetate and methanol as solvents for 24hours by Soxhlet equipment. Leaf coconut oil extract was prepared by exposing the fresh leaves of *Wrightia tinctoria* in coconut oil to sunlight for 1, 4, 7 and 15 days.

Test microorganisms

The microorganisms used for antibacterial and antifungal activity evaluation were obtained from Microbial Type Culture Collection and gene bank (IMTECH, Chandigarh, India). They were bacteria such as *Bacillus cereus* (MTCC-1305), *Enterobacter faecalis* (MTCC-5112), *Salmonella paratyphi*, (MTCC-735), *Staphylococcus aureus* (MTCC-96), *Escherichia coli* (MTCC-729), *Proteus vulgaris* (MTCC-426), *Klebsiella pneumoniae* (MTCC-109), *Pseudomonas aeruginosa* (MTCC-647), *Serratia marcescens* (MTCC-86) and fungi such as *Aspergillus niger* (MTCC-2425) and *Penicillium chrysogenum* (MTCC-5108).

The Gram-positive bacterium *Staphylococcus aureus* is mainly responsible for post operative wound infection, toxic shock syndrome and food poisoning. The Gram-positive bacteria *Bacillus cereus* cause food borne illness in humans and *Enterobacter faecalis* cause inflammation of inner layer of the heart. The Gram-negative bacterium *E. coli* is present in human intestine and causes lower urinary tract infection, coleocystis or septicemia. Gram-negative bacteria such as *Klebsiella pneumoniae* cause pneumonia and urinary tract infections. *Proteus vulgaris*, the Gram-negative bacteria, cause wound infections and pneumonia. The Gram-negative *Salmonella paratyphi* causes bacterial enteric fever and *Pseudomonas aeruginosa* causes kidney infection. *Serratia marcescens* is responsible for septicemia, meningitis, wound and eye infections [11]. Crown rot of groundnut is the most serious plant disease caused by *Aspergillus niger* [12]. The airborne asexual spores of *Penicillium chrysogenum* are important human allergens [13].

Culture medium and inoculum

The stock cultures of microorganisms used in this study were maintained on Plate Count Agar slants at 4^0C. Inoculum was prepared by suspending a loop full of bacterial cultures into 10ml of nutrient broth and was incubated at 37^0C for 24hours. On the next day Muller-Hinton agar (MHA) (Merck) sterilized in a flask and cooled to 45-50^0C was distributed by pipette (20ml) into each sterile Petri dish and swirled to distribute the medium homogeneously. About 0.1ml of bacterial suspension was taken and poured into Petri plates containing 20ml nutrient agar medium. Using the L-shaped sterile glass spreader bacterial suspensions were spread to get a uniform lawn culture.

The fungi were grown on Potato Dextrose Agar (PDA) plate at 28^oC and maintained with periodic sub-culturing at 4^oC. Inoculum was prepared by suspending a loop full of fungal cultures into 10ml of potato dextrose broth and was incubated at 37^0C for 24hours.

Potato Dextrose Agar (PDA) Medium (pH 6.7)

Potato - 250g

Dextrose - 15g

Agar - 18g

Distilled water - 1000ml

Every 20ml of sterile potato dextrose agar medium was poured into sterile petridishes and the solution in each petridish was gently swirled and allowed to solidify. About 0.1ml of fungal suspension was taken and poured into Petri plates containing 20ml potato dextrose agar medium. Using the L-shaped sterile glass spreader fungal suspensions were spread to get a uniform lawn culture.

Antimicrobial activity assay

The agar diffusion method is used for the antimicrobial evaluations. Wells of 8mm (0.8cm) diameter were dug on the inoculated nutrient agar medium (antibacterial assay) and on potato dextrose agar medium (antifungal assay) with sterile cork borer and 50µl of the petroleum ether, chloroform, ethyl acetate and methanol extracts of the bark and leaf and coconut oil extract of the leaf of *Wrightia tinctoria* were added in each well. Wells introduced with 50µl of pure petroleum ether, chloroform, ethyl acetate, methanol and coconut oil served as negative controls. The plates were incubated at 37^0C over night and examined for the zone of inhibition. The diameter of the inhibition zone was measured in mm. The standard antibiotic drugs such as tobramycin, gentamicin sulphate, ofloxacin and ciprofloxacin were used for

antibacterial evaluation and standard drug chloramphenicol was used for antifungal study. An extract was classified as active when the diameter of the inhibition was equal to or larger than 8mm [14]. All the assays were performed in triplicate and expressed as average values.

Preliminary Phytochemical analysis

The sample extracts were analysed for the presence of various phytoconstituents like flavonoids, alkaloids, glycosides, steroids, phenols, saponins and tannins according to standard methods [15].

RESULTS

Antibacterial screening

The antibacterial and antifungal spectra of the leaf petroleum ether, chloroform, ethyl acetate, methanol and coconut oil extracts of *Wrightia tinctoria*, showing the zone of inhibition in millimeters, for Gram positive and Gram negative bacteria and fungi are summarized in Table 1. The antimicrobial activity of the bark extracts and negative controls are given in Table 2. In addition, the inhibition zones formed by standard antibiotics are listed in Table 3. The standard drug chloramphenicol (10µg) inhibited the growth of the fungus *Aspergillus niger* (26mm/50µl inhibition zone) and *Penicillium chrysogenum* (24mm/50µl inhibition zone).

Phytochemical screening

Phytochemical evaluation was performed with leaf coconut oil extract, methanol, ethyl acetate, chloroform and petroleum ether extracts of the leaves and bark of *Wrightia tinctoria* (Table 4 & Table 5).

Table 1: Inhibition zones formed by *Wrightia tinctoria* leaf extracts

Diameter of inhibition zones(mm/50µL)								
	***W. tinctoria* Leaf oil**				***W. tinctoria* Leaf extracts**			
Microorganisms	**15d**	**7d**	**4d**	**1d**	**A**	**B**	**C**	**D**
1. *Bacillus cereus*	19	17	15	14	28	24	23	20
2. *Enterobacter faecalis*	13	12	10	9	14	12	11	10
3. *Salmonella paratyphi*	14	12	11	10	20	18	15	13
4. *Staphylococcus aureus*	15	13	12	10	28	26	20	15
5. *Escherichia coli*	16	15	13	11	16	15	14	12
6. *Proteus vulgaris*	14	13	11	10	15	12	11	10
7. *Klebsiella pneumoniae*	15	14	13	11	14	13	12	11
8. *Pseudomonas aeruginosa*	14	13	12	10	20	18	16	14

9. *Serratia marcescens*	13	12	11	10	22	18	14	11
10. *Aspergillus niger*	13	12	12	9	14	13	12	11
11. *Penicillium chrysogenum*	12	11	10	8	18	16	12	11

A: methanol; B: ethyl acetate; C: chloroform; D: petroleum ether extracts

Coconut oil leaf extract (1, 4, 7 and 15 days of exposure to sunlight)

Used concentrations: 50µL of 10mg/mL of plant extracts

Table 2: Inhibition zones formed by *Wrightia tinctoria* bark extracts

Diameter of inhibition zones(mm/50µL)					
Microorganisms	***W. tinctoria* bark extracts**				**Control**
	A	**B**	**C**	**D**	**A, B, C, D & Coconut oil**
1. *Bacillus cereus*	23	20	18	16	--
2. *Enterobacter faecalis*	14	12	11	9	--
3. *Salmonella paratyphi*	18	16	12	10	--
4. *Staphylococcus aureus*	18	16	15	13	--
5. *Escherichia coli*	20	18	15	13	--
6. *Proteus vulgaris*	19	18	16	15	--
7. *Klebsiella pneumoniae*	24	18	15	13	--
8. *Pseudomonas aeruginosa*	22	20	18	15	--
9. *Serratia marcescens*	22	18	14	12	--
10. *Aspergillus niger*	20	18	14	12	--
11. *Penicillium chrysogenum*	20	18	15	14	--

Controls- A: methanol; B: ethyl acetate; C: chloroform; D: petroleum ether
Used concentrations: 50µL of 10mg/mL of plant extracts

Table 3: Inhibition zones formed by the standard antibiotics—tobramycin, gentamicin sulphate, ofloxacin, ciprofloxacin

Diameter of inhibition zones(mm/50µL)				
Microorganisms	**Tob 10µg**	**Gen 10µg**	**Oflo 10µg**	**Cip 10µg**
1. *Bacillus cereus*	28	32	34	30
2. *Enterobacter faecalis*	26	32	32	26
3. *Salmonella paratyphi*	25	30	28	30
4. *Staphylococcus aureus*	26	28	24	24
5. *Escherichia coli*	30	36	32	34
6. *Proteus vulgaris*	26	30	24	32
7. *Klebsiella pneumoniae*	26	32	32	36
8. *Pseudomonas aeruginosa*	26	24	32	28
9. *Serratia marcescens*	24	32	30	30

Tob: tobramycin, Gen: gentamicin sulphate, Oflo: ofloxacin, Cip:ciprofloxacin

Table 4: Phytochemical screening of *W.tinctoria* leaf extracts

Phytoconstituents	Leaf extracts							
	Mt. ext.	EA ext.	Chl. ext.	Pet. ether ext.	Coconut oil extract			
					15d	7d	4d	1d
Alkaloids	+++	_	_	_	_	_	_	_
Phenolics	+++	+	+	++	++	+	+	+
Saponins	+++	+++	+++	+++	++	+	_	_
Tannins	++	++	++	_	_	_	_	_
Glycosides	+++	++	++	++	_	_	_	_
Steroids	+++	++	++	+	+	_	_	_
Flavonoids	+++	_	_	++	++	_	_	_

+ Present *++ Moderately present* *+++ Appreciable amount*

Mt. ext: Methanol extract; EA ext.: Ethyl acetate extract; Pet. ether ext.: Petroleum ether extract; Chl. ext.: Chloroform extract.

Coconut oil extracts (1d, 4d, 7d &15d): Fresh leaves in coconut oil for1d, 4d, 7d and 15days of exposure to sunlight.

Table 5: Phytochemical screening of *W.tinctoria* seed and bark extracts

Phytoconstituents	Seed extracts				Bark extracts			
	Mt. ext.	EA ext.	Chl. ext.	Pet. ether ext.	Mt. ext.	EA ext.	Chl. ext.	Pet. ether ext.
Alkaloids	++	+	++	+++	+	+	_	_
Phenolics	+++	++	++	+	+++	++		
Saponins	+++	+	_	_	+++	+	_	_
Tannins	++	_	_	_	+++	++	+	
Glycosides	+++	++	+	++	++	+	_	_
Steroids	_	++	++	_	_	_	_	_
Flavonoids	+++	_	_	+++	+++	+	+	_

+ Present *++ Moderately present* *+++ Appreciable amount*

Mt. ext: Methanol extract; EA ext.: Ethyl acetate extract

Pet. ether ext.: Petroleum ether extract; Chl. ext.: Chloroform extract

DISCUSSION

Antibacterial screening of leaf and bark extracts

Among the leaf and bark extracts of *Wrightia tinctoria*, methanol extracts exhibited higher activity than the other extracts and petroleum ether extract showed least activity.

The leaf methanol, ethyl acetate, chloroform and petroleum ether extracts exhibited significant activity against *Bacillus cereus* (28-20mm/50µl inhibition zone) and

Staphylococcus aureus (28-15mm/50µl inhibition zone). Leaf methanol and ethyl acetate extracts showed pronounced activity against *Salmonella paratyphi* (20-18mm/50µl inhibition zone), *Serratia marcescens* (22-18mm/50µl inhibition zone) and *Pseudomonas aeruginosa* (20-18mm/50µl inhibition zone) and *Penicillium chrysogenum* (18-16mm/50µl inhibition zone). Among the leaf coconut oil extracts of *Wrightia tinctoria*, oil with 15days exposure to sunlight was found to be more active than the other extracts with one, four and seven days of exposure (Table1). Oil sample with 15days of exposure inhibited the growth of *Bacillus cereus* (19mm/ 50µl inhibition zone) and *Escherichia coli* (16mm/50µl inhibition zone). Bark and leaf extracts of *Wrightia tinctoria* were found to be more effective against the tested microorganisms than the coconut oil leaf extracts.

Wrightia tinctoria bark methanol and ethyl acetate extracts showed remarkable activity (Table 2) against all tested microorganisms such as *Bacillus cereus* (23-20mm/50µl inhibition zone), *Salmonella paratyphi* (18-16mm/50µl inhibition zone), *Staphylococcus aureus* (18-16mm/50µl inhibition zone), *Escherichia coli* (20-18mm/50µl inhibition zone), *Proteus vulgaris* (19-18mm/ 50µl inhibition zone), *Klebsiella pneumonia* (24-18mm/50µl inhibition zone), *Pseudomonas aeruginosa* (22-20mm/50µl inhibition zone), *Serratia marcescens* (22-18mm/50µl inhibition zone), *Aspergillus niger* (20-18mm/50µl inhibition zone) and *Penicillium chrysogenum* (20-18mm/50µl inhibition zone).

Bark and leaf chloroform extracts inhibited the growth of *P.aeruginosa* (18-16mm/50µl inhibition zone). Bark chloroform extract showed appreciable activity against *Bacillus cereus* and *Proteus vulgaris* (18-16mm/50µl inhibition zone).

The results obtained were compared with standard antibiotics (Table 3) and it was observed that *W. tinctoria* leaf methanol extract at a concentration of 10mg/ml showed marked activity against *Bacillus cereus* (28mm/50µl inhibition zone) and its activity was comparable with tobramycin (10µg). Leaf methanol and ethyl acetate extracts exhibited significant activity against *Staphylococcus aureus* (28-26mm/50µl inhibition zone) and activity was quite comparable with that of standard antibiotics such as tobramycin, gentamicin suphate (10µg each) screened under similar conditions. Bark methanol extract of W. *tinctoria* at a concentration of 10mg/ml was found to be effective against *Klebsiella pneumoniae* (24mm/50µl inhibition zone), *Serratia marcescens* (22mm/50µl inhibition zone), *Aspergillus niger* (20mm/50µl inhibition zone) and *Penicillium chrysogenum* (20mm/50µl inhibition zone) and their activity was comparable with the standard antibiotics such as tobramicin and

chloramphenicol (10µg each). The Minimum Inhibitory Concentration (MIC) of leaf and bark methanol extracts against *Bacillus cereus* and *Staphylococcus aureus* was found to be 0.5mg/ml.

Phytochemical analysis

Phytochemical studies revealed the presence of secondary metabolites in the leaf, bark and seed extracts of *W.tinctoria*. The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences [16]. Phytochemical evaluation was performed with leaf coconut oil extract, methanol, ethyl acetate, chloroform and petroleum ether extracts of *W.tinctoria* leaves, bark and seeds. Leaf methanol extract showed appreciable amount of phenolics, saponins, flavonoids, alkaloids, glycosides and steroids. Seed methanol extract was found to be rich in phenolics, saponins, flavonoids and glycosides. Bark methanol extract showed appreciable amount of tannins, saponins, flavonoids and phenolics. Leaf coconut oil extract gave positive test for saponins, flavonoids and phenolics. Antimicrobial potential of leaf and bark extracts of *W.tinctoria* can be attributed to the presence of these phytochemicals.

CONCLUSION

The leaf and bark methanol extracts of *W.tinctoria* showed pronounced activity against *Bacillus cereus* and their activity is quite comparable with the standard antibiotic tobramycin. Leaf methanol extract was also found to be effective against *Staphylococcus aureus* and its activity was comparable with the standard drugs screened under similar conditions. Hence, *W.tinctoria* leaf and bark methanol extracts can be incorporated into the drug formulations. The antimicrobial potency of the *W. tinctoria* leaf methanol extract can be attributed to the presence of flavonoids, phenolic compounds and saponins and the activity of bark methanol extract is due to the presence of phenolic compounds, saponins and tannins[17,18] . It is interesting to note that even crude extract of this plant showed prominent activity against various pathogenic bacteria where modern therapy has failed. The variation of the susceptibility of the tested microorganisms could be attributed to their intrinsic properties that are related to the permeability of their cell surface to the extracts [19]. The results of this study support the use of this plant for human diseases and reinforce the ethnobotanical importance of plant as a potential source of bioactive substances.

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