ANTIBACTERIAL EVALUATION AND PHYTOCHEMICAL ANALYSIS OF *WRIGHTIA TINCTORIA* (Roxb.) R. BR. LEAVES

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Summary

Antibacterial and phytochemical activity of leaves of *Wrightia tinctoria* R.Br. was investigated. Antibacterial activity was tested against gram +ve and gram -ve organisms. The chloroform leaves extract exhibited broad-spectrum antibacterial activity against tested organisms. Maximum activity was exhibited against *Staphylococcus aureus* followed by *Micrococeus luteus* and *Salmonella typhi*. Acetone, methanol and water extract exhibited less activity, while petroleum ether showed negative inhibition The phytochemicas showed the presence of alkaloids, cardiac glycosides, flavonoids, leucoanthocyanins, simple phenolics, saponins , steroids and tannins.

Kew words: *Wrightia tinctoria*, leaves, antibacterial, phytochemical

Introduction

Herbal medicine represents one of the most important fields of traditional medicine all over the world. To promote the proper use of herbal medicine and to determine their potential as a source of new drug, it is essential to study medicinal plants which have folklore reputation in a more intensified way (1). Plant extracts have been used for a wide variety of purposes for many thousands of years (2). In particular, the antimicrobial activity of plant extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (3).The potential for the developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (4). On the other hand, research in herbal medicine has increased in developing countries as a way to rescue ancient traditions as well as an alternative solution to health problems in cites. Therefore, with the increasing acceptance of traditional medicine as an alternative form of healthcare, the screening of plants for active compound has become very important.

Wrightia tinctoria (Roxb.) R. Br. belongs to family apocynaceae commonly called as "Jaundice curative tree" in south India (5). The juice of the tender leaves is used efficaciously in jaundice; also crushed fresh leaves when filled in the cavity of decayed tooth relieve toothache (5, 6, 7, 8, 9). In Siddha system of medicine, it is used for psoriasis and other skin diseases (5, 7). An oil 777 prepared out of the fresh leaves of the plant has been assigned to analgesic, anti-inflammatory and antipyretic activities (10), and to be effective in the treatment of psoriasis (11). Leaves indicated the presence of flavonoids, glycoflavones-iso-orientin and phenolic acids in the leaves (12, 13). The current in vitro antibacterial study was aimed at validating the effect of *Wrightia tinctoria* for their antibacterial activity against eight bacteria. In addition, we also evaluated their phytochemical profiles to correlate possible biological activity of the plant.

Materials and Methods

Collection of plant materials

The fresh leaves of *Wrightia tinctoria* (Roxb.) R.Br. were collected in the month of October (2006) from Aurangabad District, (M.S.) India. The plant was identified with the help of Flora of Marathwada (14) and a voucher specimen has been deposited at the Botany department of the University. Plant samples were washed, shade dried at room temperature for 15 days.

Preparation of extracts and phytochemical screening

The dried plant material was pulverized into fine powder using a grinder (mixer). About 50 gm of powdered material was extracted in soxhlet extraction apparatus with 250 ml of each of the following solvents; Petroleum ether, chloroform, Acetone and Methanol (15). The extracts obtained with each solvent were filtered through Whatman filter paper No. 1 and the respected solvents were evaporated (at 40ºC) with the help of heating mantle. The sticky greenish-brown substances were obtained and stored in refrigerator and were suspended in dimethyl sulphoxide (DMSO) for prior to use (16).

Some of the extracts of each solvent were used for the qualitative phytochemical screening for the identification of the various classes of active chemical constituents, using standard prescribed methods (17,18,19,20). The positive tests were noted as weak (+), moderate (++), strong (+++) and absent (-).

Tested microorganisms

Various cultures of human pathogenic, gram positive and gram negative bacteria were used. These are *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella typhi, Micrococeus luteus, Pseudomonas aeruginosa, Klebsiella planticola* and *Bacillus megaterium*. The cultures were obtained from Microbial Type culture Collection (MTCC), IMTEC, Chandigarh, India. The microorganisms were repeatedly subcultured in order to obtain pure isolates. A loop full test organism was inoculated on nutrient broth and incubated for 24 h at 37±1ºC and maintained in sterile condition.

Screening for antibacterial properties

Antibacterial activities of plant extracts were tested by Agar well diffusion method (21). The culture plates were prepared by pouring 20 ml of sterile nutrient agar.1 ml inoculum

suspension was spread uniformly over the agar medium using sterile glass rod to get uniform distribution of bacteria. A sterile cork borer (6 mm) was used to make wells in each plate for extracts. These plates were labeled and 100µl of each plant extracts (at concentration of 100mg/ml) was added aseptically into the well. Then the plates were incubated for 24 h at 37ºC during which the activity was evidenced by the presence of zone of inhibition surrounding the well. Each test was repeated three times and the antibacterial activity was expressed as the mean of diameter of the inhibition zones (mm) produced by the plant extracts when compared to the controls.

Results and Discussion

Preliminary phytochemical analysis revealed the presence of alkaloid, cardiac glycosides, flavonoids, leucoanthocyanins, simple phenolics, saponins, steroids and terpenoids in the extracts of *Wrightia tinctoria* extracts (table 1). The antimicrobial potency of the plant may be attributed to single or combined effect of the mentioned chemical groups.

Table 1. Phytochemical constituents of leaves extracts of *Wrightia tinctoria*

Phytochemical constituents	**Petroleum ether**	**Chloroform**	**Acetone**	**Methanol**	**Water**
Alkaloids	--	+++	+	+++	+
Anthraquinone	--	--	--	--	--
Cardiac glycoside	--	--	++	++	--
Coumarins	--	--	--	--	--
Flavonoids	--	++	++	++	+
Leucoanthocyanins	--	--	--	--	--
Simple phenolics	--	++	++	+++	+
Steroids	++	++	--	--	--
Saponins	--	++	++	+++	+++
Tannins	--	++	++	+++	--
Terpenoid	--	++	++	++	--

Different solvent and aqueous extracts tested at 100µl concentration against eight important pathogenic bacteria are presented in table 2. Among four solvent extracts viz. petroleum ether, chloroform, acetone and methanol tested against all the strains of bacteria; chloroform and acetone extracts recorded considerable antibacterial activity followed by methanol extracts against all the test pathogens. Antibacterial activity was not observed in petroleum ether extracts against all the strains of bacteria.

Table 2: Antibacterial efficacy of different solvent extracts of *Wrightia tinctoria* leaves

Organisms	Gram + / -	Petroleum ether	Chloroform	Acetone	Methanol	Water	DMSO	Ampicillin (40 µg/ml)
Staphylococcus aureus	+	0	24	19	11	12	0	23
Bacillus subtilis	+	0	20	16	11	15	0	21
Bacillus megaterium	+	0	19	17	13	14	0	25
Micrococeus luteus MTCC 106	+	0	23	19	16	15	0	30
Escherichia coli	-	0	19	18	13	10	0	17
Salmonella typhi	-	0	22	21	11	16	0	19
Pseudomonas aeruginosa MTCC2488	-	0	17	16	10	10	0	16
Klebsella planticola	-	0	14	13	0	0	0	21

A-100mg/ml(100µl/well);Antibiotic-40µg/ml (100µl/well);0 -no inhibition; Figures are diameter of zone of inhibition (in triplicates)

Among chloroform, acetone and methanol extracts; chloroform extracts recorded significant antibacterial activity followed by acetone and methanol. *S. aureus* found highly susceptible to chloroform extracts, whereas *K. planticola* was less susceptible to acetone and negative to methanol extracts at tested concentration. In different solvent extracts both chloroform and acetone extracts recorded highly significant antibacterial activity against *S. aureus, E.coli, S. typhi P. aeruginosa* compared to standard (table 2). Antibacterial activity of acetone extracts against *P. aeruginosa* is almost equal when compared to ampicillin. The antibacterial activity of aqueous extracts varied among the different test bacteria. Highest activity was observed against *S. typhi* followed by *B. subtilis, M. luteus* and *B. megaterium.*

Based on these results, it is possible to conclude that, of the three solvents and aqueous extracts exhibited a broad range of antibacterial activity to varying degrees. Particularly, chloroform extract of showed significant antibacterial activities and could be used as an antibacterial agents in new drugs for therapy. Lastly to conclude, the chloroform extracts revealed greater activity when compared to antibiotics, so further evaluation and asalysis of chemical compounds are needed to form novel antimicrobial agents. Also, the present study justifies the claimed uses of *Wrightia tinctoria* in the traditional system of medicine to treat various infectious diseases caused by microbes.

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