

IN-VITRO ASSESSMENT OF ANTIMICROBIAL ACTIVITY OF ALCOHOLIC STEM EXTRACTS OF *WRIGHTIA TOMENTOSA*

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Summary

The ethanol extract of the stem of *Wrightia tomentosa* was evaluated for antimicrobial activity against Gram positive (*S. aureus*, *S. fecalis* and *B. subtilis*) and gram negative (*E. coli* and *P. aeruginosa*) organisms and the fungi *Candida albicans* by disc diffusion method. The extracts (200 µl) does not show any significant degree of activity against all pathogenic microbes. The results obtained were compared with standard drugs ciprofloxacin (10µg/disc) and clotrimazole (10µg/disc). Among the various microbial strains tested, the extract has shown complete resistance against *S. aureus*, *B. subtilis*, *P. aaeruginosa*, *C. albicans* and partial resistance against *S. fecalis* (2 mm) and *E.coli* (1mm) respectively. The probable mechanism of void antimicrobial activity of *Wrightia tomentosa* stem extract was mainly due to the effect of mutations at specific binding sites of bacterial DNA gyrase, which ultimately decreases the effectiveness of stem extract. Hence the stem extract of *Wrightia tomentsa* may also be accompanied with some other herbal drug species as combination therapy to avoid the problem of drug resistance in near future.

Key words: Extracts, *Wrightia tomentosa*, stem, disc method, antimicrobial activity.

Shortened Title: Anti-microbial activity of stem extract of *Wrightia tomentosa*

Introduction

The importance of plants as a source of novel compounds is probably related in large measure to the fact that they are not mobile, and hence must defend themselves by deterring or killing predators, whether insects, micro organisms, animals, or even other plants (1). Traditional uses of plants have led to investigating their bioactive compounds through screening programmes, which have resulted in the detection of a significant number of therapeutic properties (2). The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection combating strategies and new effective therapeutic agents (3). Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new, antimicrobial compounds (4). Plants are used medicinally in different countries and are a source of many potent and powerful drugs (5). Therefore, the development of alternative antimicrobial drugs from medicinal plants for the treatment of infectious diseases has become necessary.

Wrightia tomentosa Roem. and Schult. belonging to the family, Apocynaceae is a small deciduous tree, up to 12m high, found through out the warmer parts of India, ascending to an altitude of 600m in the Himalayas and to 1,200m in the Nilgiris. The bark is greyish yellow to rust-coloured, corky, with light coloured specks; leaves elliptic, often tomentose, 7.5-15.0 cm long (6). The bark and root-bark are believed to be useful in snake-bite and scorpion-stings (7). The phytochemical studies on this plant have revealed the presence of unsaturated sterols, triterpenes, flavonoids, phenolic acids and alkaloids (8). A novel isoflavone, wrightiadiene isolated from the plant possess cytotoxic activity against the murine P 388 lymphocytic leukemia cell line (9). The leaves and bark isolates of *Wrightia tomentosa* have already shown remarkable antimicrobial activity (10). The ethanolic bark extract of *Wrightia tomentosa* was found to possess maximum antihyperglycemic activity in streptozotocin induced diabetic rats (11). The ethanolic bark and leaf extract of *Wrightia tomentosa* possesses significant anti-allodynic effects (12) with no observable signs of toxicity (13). The alcoholic extract of *Wrightia tomentosa* dried bark was reported to exhibit markedly high anti-oxidant activity (IC₅₀ value of 75.0 mg/ml from DPPH radical scavenging assay), suitable for prevention of human disease (14). The leaf extract (100 mg) of *Wrightia tomentosa* has proved to be extremely useful against non-tuberculous mycobacterium infections (15), which are becoming a major concern for hospitals and medical clinics. The leaf extract of *Wrightia tomentosa* showed potent antitumour effect than the bark extract against Ehrlich ascites carcinoma (EAC) tumour bearing mice (16). An alkaloidal pure component was isolated from leaf of *Wrightia tomentosa*, identified as N-methyl pyrrole and tested *in-vitro* against *Mycobacterium tuberculosis* using Versa TREK rapid culture system, which showed time for positivity in 19.9 hours with respect to control (17). In view of the widest pharmacological activity of *Wrightia tomentosa*, the present study has been undertaken to investigate the effect of ethanolic extracts from the stem on different strains of clinical pathogenic bacteria and fungi by employing disc diffusion technique.

Materials & Methods

Plant Collection:

The stem of *Wrightia tomentosa* was collected from the hills of Yercaud forest. The plant identity was confirmed (18-19) and a specimen voucher was made with the authentication of an acknowledged Botanist. The present study was carried out at the Deptt. of Pharmacy, IIMT College of Medical Sciences, Meerut. The stem portion were dried under shade and then powdered. The powdered stem was extracted with Ethanol by continuous hot extraction using soxhlet apparatus for 16 hrs separately. The extract was concentrated to remove the solvent using Rotary Vacuum Evaporator (Buchi rota vapour) and dried on dessicator.

Phytochemical Studies:

The powdered materials (stem bark and leaves) were subjected to qualitative tests (20) for the identification of various plant constituents like alkaloids, glycosides, steroids, terpenoids, flavanoids, tannins, gums and mucilages, fixed oils and fats and saponins.

Antimicrobial Assay:

The ethanol extract of stem was evaluated by agar disc diffusion method (21). Mueller Hinton Agar No. 2 was used as an assay medium.

Inoculum size was maintained as 10^8 cells ml^{-1} for all the bacterial strains studied. The disc (7mm, Himedia) was saturated with 200 μl of the test compound extract, allowed to dry and was introduced on the upper layer of the seeded agar plate. The plates were incubated overnight at 37°C. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain controls were maintained where pure solvents were used instead of the extract. The control zones were subtracted from the rest zones and the resulting zone diameter is shown in Table 2. Similarly for antifungal screening, sabouraud dextrose agar was used as an assay medium. Ciprofloxacin (10 μg /disc) and Clotrimazole (10 μg /disc) were used as a standard for anti-bacterial and antifungal screening.

TABLE 1: Results of preliminary phytochemical tests for the presence of active constituents in Stem of *Wrightia tomentosa*.

S.No.	Constituents	Alkaloids	Glycosides	Steroids	Terpenoids	Flavonoids	Ethanol	Proteins	Tannins	Gums & Mucilages	Fats, Oils, waters	Saponins
1.	Pet. Ether	-	-	-	-	-	-	-	-	-	-	-
2.	CHCl_3	+	-	-	-	-	-	-	-	-	-	-
3.	Benzene	-	-	-	-	-	-	-	-	-	-	-
4.	n-butanol	-	-	-	-	+	-	-	-	-	+	-
5.	Acetone	-	-	-	-	-	-	-	-	-	-	-
6.	Ethanol	+	-	-	-	+	-	-	-	-	+	-

TABLE 2: Antimicrobial Activity of *Wrightia tomentosa* Stem extract against Gram+Ve and Gram -Ve bacteria and fungi.

S.No.	Organism used	Sample loaded/disc	Zone of Inhibition Diameter (mm)	
			Standard	Ethanol stem extract
1.	<i>Strep. faecalis</i> (ATCC 19433)	200 μl	38	02
2.	<i>Staph. aureus</i> (MTCC 29737)	200 μl	37	NS
3.	<i>B. subtilis</i> (ATCC 6633)	200 μl	34	NS
4.	<i>E. coli</i> (MTCC 739)	200 μl	35	01
5.	<i>P. aeruginosa</i> (MTCC 424)	200 μl	40	NS
6.	<i>C. albicans</i> (MTCC 10231)	200 μl	45	NS

NS: No zone of inhibition.

Results

Preliminary phytochemical screening of the ethanolic stem extract of *Wrightia tomentosa* reveals the presence of moderate quantity of alkaloids, flavonoids, fats, oils and waxes. The results of antimicrobial activity with stem extract against all pathogenic microbes were found to be invalid as none of the organisms responds to optimum zonal inhibition diameter. These negative results showed that, the stem extract does not have significant antimicrobial activity against all the strains tested. Stem extract was found to be partially resistant against gram positive *Streptococcus faecalis* (± 2 mm) and gram negative *E. coli* (± 1 mm).

All these evidences are shown in figures A-F and suggest that the active constituents are present in very minute quantity or sometimes having interaction over one another when they are in bulk as crude extract.

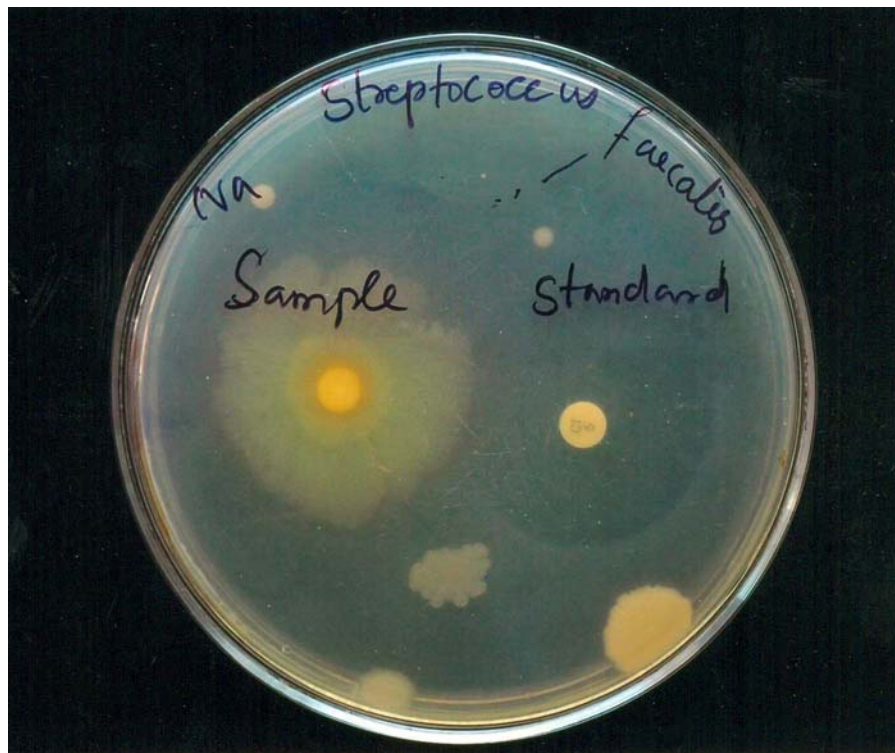


Fig. A: The disk represents the antibacterial activity with their zone of inhibition of the test sample I (± 2 mm) against *S. faecalis* in comparison to the standard ciprofloxacin (± 38 mm).



Fig. B: The disk represents the antibacterial activity with their zone of inhibition of the test sample I against *S. aureus* in comparison to the standard ciprofloxacin (± 37 mm).



Fig. C: The disk represents the antibacterial activity with their zone of inhibition of the test sample I against *B. subtilis* in comparison to the standard ciprofloxacin (± 34 mm).



Fig. D: The disk represents the antibacterial activity with their zone of inhibition of the test sample I (± 1 mm) against *E. coli* in comparison to the standard ciprofloxacin (± 35 mm).

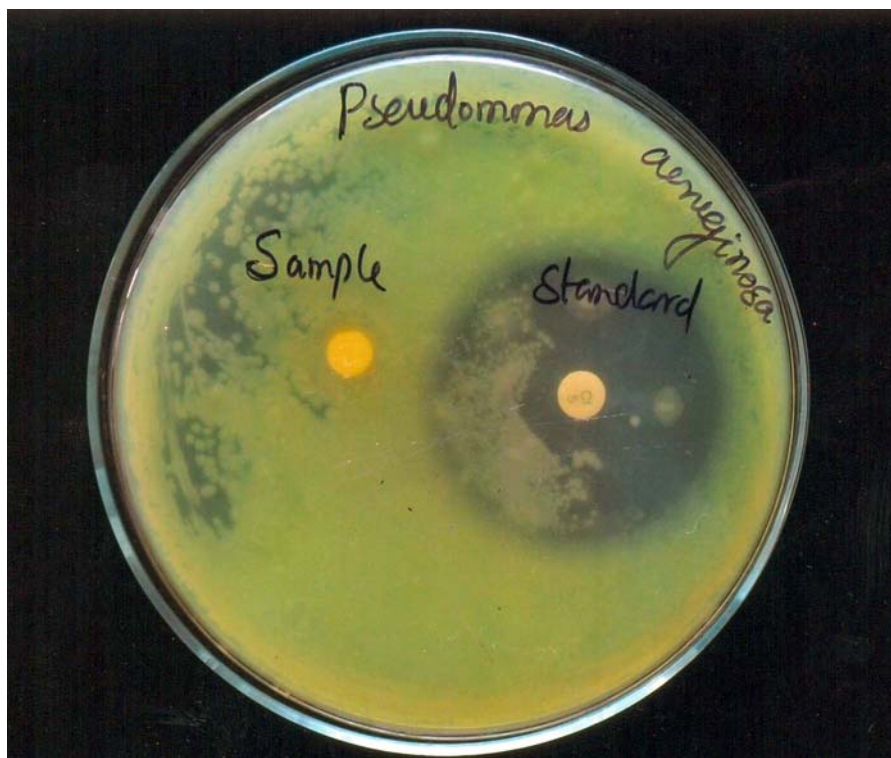


Fig. E: The disk represents the antibacterial activity with their zone of inhibition of the test sample I against *P. aeruginosa* in comparison to the standard ciprofloxacin (± 40 mm).



Fig. F: The disk represents the antifungal activity with their zone of inhibition of the test sample I against *C. albicans* in comparison to the standard ciprofloxacin ($\pm 45\text{mm}$).

Discussion

The results obtained indicated the non-existence of antimicrobial compounds in the crude ethanolic stem extracts of *Wrightia tomentosa*. It is possible that better therapy for many microbial diseases can be found the leaf extracts (22). The high percentage of positive results were found in the previous antimicrobial studies with the leaf and the bark portion (10) and this present approach is not promising for antimicrobial activity due to lack of many phytoconstituents in the stem portion.

Drug resistance can be a result of horizontal gene transfer (23) and also of unlinked point mutations in the pathogen genome and a rate of about 1 in 10^8 per chromosomal replication. As for example, there are three known mechanisms of fluoroquinolone resistance. Some types of efflux pumps can act to decrease intracellular quinolone concentration. In gram-negative bacteria, plasmid-mediated resistance genes produce proteins that can bind to DNA gyrase, protecting it from the action of quinolones. Finally, mutations at key sites in DNA gyrase or Topoisomerase IV can decrease their binding affinity to quinolones, decreasing the drug's effectiveness (24). Research has shown that the bacterial protein LexA may play a key role in the acquisition of bacterial mutations giving resistance to quinolones and rifampicin (25). The probable mechanism of void antimicrobial activity of *Wrightia tomentosa* stem extract was mainly due to the effect of mutations at specific binding sites of bacterial DNA gyrase, which ultimately decreases the effectiveness of stem extract.

A number of phyto-agents appear to have structures and modes of action that are distinct from those of the antibiotics in current use, suggesting that cross-resistance with agents already in use may be minimal.

For example the combination of 5'-methoxyhydnocarpine and berberine in herbs like *Hydrastis canadensis* and *Berberis vulgaris* can block the MDR-pumps that cause multidrug resistance. This has been shown for *Staphylococcus aureus* (26). In a similar fashion, the stem extract of *Wrightia tomentosa* may also be accompanied with some other herbal drug species as combination therapy to avoid the problem of drug resistance in near future.

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