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## STUDIES ON ANTIMICROBIAL ACTIVITY, PHYSIO-CHEMICAL AND PHYTOCHEMICAL ANALYSIS OF *WRIGHTIA TINCTORIA* R.BR.

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### ABSTRACT

The dried leaf of *Wrightia tinctoria* R.BR. were subjected to successive extraction using the solvents methanol and water. The prepared extracts were then subjected to preliminary phytochemical analysis. It was found that the plant possesses steroids, alkaloids, carbohydrates, flavonoids, tannins & phenols, saponins and proteins in varying concentration. The methanol and aqueous extracts were selected for further study. They were tested against six bacteria; three Gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes* and *Leuconostoc lactis*) and three Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*) and against four fungi (*Aspergillus niger*, *Aspergillus flavus*, *Rhizopus indicus* and *Mucor indicus*). For antimicrobial test, well diffusion technique was used and the zone of inhibition of microorganisms was measured in mm. The pattern of inhibition varied with the solvent used for extraction and the microorganism tested. Among these extracts, aqueous extract showed significant antibacterial activity against most of the tested bacteria but in the case of fungus against methanol extract showed some result. The most susceptible microorganism was *Leuconostoc lactis* (32 mm) followed by *Pseudomonas aeruginosa* (31 mm zone of inhibition in aqueous extract) and *Mucor indicus* (32 mm zone of inhibition in methanol extract).

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extract, aqueous extract,  
antibacterial, well diffusion

## INTRODUCTION

The use of higher plants and their preparations to treat infectious diseases is an age-old practice and in the past possibly the only method available. However, the systematic study of higher plants for detecting antimicrobial activity is of comparatively recent origin<sup>1</sup>. These investigations have been triggered by the emergence and spread of antibiotic resistant microorganisms causing the effective life-span of existing antibiotics limited<sup>2</sup>. The five flavonoid compounds, Indigotin, Indirubin, tryptanthrin, isatin and rutin were isolated and identified from the leaves<sup>3</sup>.

*Wrightia tinctoria* (Roxb.) R. Br. Belongs to family Apocynaceae is a small deciduous tree, generally up to 1.8m tall and often under 60cm girth, sometimes up to 7.5m high, distributed all over India.. The wrightial, a new terpene and other phytoconstituents such as cycloartenone, cycloeucaleanol were isolated identified by fractionation of methanol extract of the immature seed pods<sup>4</sup>. 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<sup>5,6</sup>.

Leaves indicated the presence of flavonoids, glycoflavones-iso-orientin and phenolic acids<sup>7</sup>. Bark and seeds are antidiarrhetic, carminative, astringent, aphrodisiac and diuretic, used in flatulence, stomach pain and bilious affections. The plant is very useful as stomachic, in the treatment of abdominal pain, skin diseases, antidiarrhoeal and antihaemorrhagic<sup>8,9</sup>. The plant is also reported for antimicrobial in psoriasis, wound healing and hepatoprotective activity<sup>10,11</sup>.

Over the past 20 years, there has been a lot of investigation on the plants as sources of new antimicrobial agents. But still there is an immediate need to identify novel substances active towards pathogens with high resistance<sup>12,13</sup>. Recent studies have shown that several alcoholic extracts of various traditional medicinal plants exhibit antibacterial activity.

## MATERIALS AND METHODS

### Preparation of plant extracts

Fresh Plant leaf of *Wrightia tinctoria* was collected from Nolambur village, Villupuram district, Tamil Nadu, India; they were identified with the help of Gamble's flora.

### Preparation of powder

The leaves of plants were collected and dried under shade. These dried materials were mechanically powdered sieved using 80 meshes and stored in an airtight container. These powdered materials were used for further physicochemical, phytochemical and fluorescent analysis<sup>14</sup>.

### Extraction of plant material

Various extracts of the study plant was prepared according to the methodology of Indian Pharmacopoeia<sup>15</sup>. The leaves were dried in shade and the dried leaves were subjected to pulverization to get coarse powder. The coarse powder material was subjected to Soxhlet extraction separately and successively with methanol and distilled water. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50°C). Both the extracts were stored in a refrigerator in air tight containers. Both the extracts were analyzed for phytochemical screening of compounds, antimicrobial and pharmacological activity.

### Qualitative phytochemical studies

Qualitative phytochemical analyses were done by using the procedures of Kokate *et al.* (1995). Alkaloids, carbohydrates, tannins, phenols, flavonoids, gums and mucilages, proteins, amino acids, fixed oils, fats, volatile oil and saponins were qualitatively analyzed.

### Test organisms

The stored culture of *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Leuconostoc lactis* and *Salmonella typhi* were collected from the Microbial Type Culture Collection (MTCC), The Institute of microbial Technology. Sector 39-4, Chandigarh, India.

The pathogenic fungal strains *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus indicus* and *Mucor indicus* were collected from the Microbiological Lab, Christian Medical College, Vellore, Tamil Nadu, India.

## Antibacterial Studies

### Bacterial Media (Muller Hindon Media)

Thirty Six grams of Muller Hindon Media (Hi-Media) was mixed with distilled water and then sterilized in autoclave at 15lb pressure for 15 minutes. The sterilized media were poured into petridishes. The solidified plates were bored with 6mm dia cork porer. The plates with wells were used for the antibacterial studies.

### Antifungal studies

#### Fungal media (PDA)

Two Hundred gram of potato slices were boiled with distilled water. The potato infusion was used as water source of media preparation. 20g of dextrose was mixed with potato infusion. 20g of agar was added as a solidifying agent. These constituents were mixed and autoclaved. The solidified plates were bored with 6mm dia cork porer.

### Well diffusion method

Antibacterial and Antifungal activity of the plant extract was tested using well diffusion method<sup>17</sup>. The

prepared culture plates were inoculated with different bacteria and fungus by using plate method. Wells were made on the agar surface with 6mm cork borer. The extracts were poured into the well using sterile syringe. The plates were incubated at  $37\pm 2^{\circ}\text{C}$  for 24 hours for bacterial activity and 48 hours for fungal activity. The plates were observed for the zone formation around the wells.

The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in three different fixed directions in all 3 replicates and the average values were tabulated.

## RESULTS

Physico-chemical analysis of powdered leaf viz. ash values and extractive values are presented in Table 1. The fluorescence analysis of *W. tinctoria* leaf is presented in Table 2.

**Table 1:** Pharmacognostic characters of leaf of *Wrightia tinctoria*

Sl. No	Parameters	Percentage yield (%)
1	Total ash value	29
2	Acid insoluble ash value	21
3	Acid soluble value	79
4	Solubility % in alcohol	42
5	Solubility % in water	28

**Table 2:** Analysis of fluorescence characters of leaf powder of *Wrightia tinctoria* in different chemical reagents

Sl. No	Chemical reagent	Appearance
1	Powder colour	Green
2	5% NaOH	Dark green
3	10% NaOH	Dark green
4	Con. H <sub>2</sub> SO <sub>4</sub>	Dark green
5	Acetic Acid	Dark green
6	1N NaOH in H <sub>2</sub> O	Dark green
7	5% KOH	Dark green
8	50% HNO <sub>3</sub>	Brown
9	5% FeCl <sub>2</sub>	Green
10	1N HCl	Green
11	Con.HNO <sub>3</sub>	Light brown

12	1N NaOH in Ethanol	Dark green
13	50% H <sub>2</sub> SO <sub>4</sub>	Green
14	50% HCl	Dark green
15	Con. HCl	Dark green

The results of the phytochemical screening of the leaves of *Wrightia* are shown Table 3. It indicates the presence of carbohydrates, alkaloids, steroids, phenols, saponins, tannins, flavonoids and proteins.

**Table 3:** Results of phytochemical screening of aqueous leaf extracts of *Wrightia tinctoria*

S.No.	Name of the compounds	Name of the test	Status of the substances	
			Aqueous extract	Methanol extract
1	Carbohydrates	Fehling's	++	+
		Benedict's	+	+
2	Alkaloids	Mayer's	++	++
		Hager's	++	+++
		Wagner's	++++	+++
		Dragen Dorfff's	++++	++++
3	Steroids	Chloroform + Acetic acid + H <sub>2</sub> SO <sub>4</sub>	+++	+++
4	Tannins & Phenols	10% Lead acetate	++	++
		5% Ferric chloride	++	++
		1% gelatin	+	+
5	Saponins	Foam test	+	+
6	Fixed oils & Fats	Spot test	-	-
7	Gums & Mucilage	Alcoholic precipitation	-	-
8	Proteins	Biuret test	++	+
9	Flavonoids	NaOH / HCl	+++	++
10	Volatile oils	Hydro distillation method	-	-

++++	-	High rich amount
+++	-	Rich amount
++	-	Moderate amount
+	-	Minimum amount
-	-	Absent

The antibacterial activities of *W. tinctoria* in aqueous and methanol extracts examined in the current study and their potency were qualitatively assessed by the presence or absence of inhibition zones. The results showed that the extracts of *W. tinctoria* mediated high

degree of activity against bacteria. Except, *Escherichia coli* against methanol extract other strains like

*Staphylococcus aureus*, *Leuconostoc lactis*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Streptococcus pyogenes* are inhibited by both aqueous and methanol extracts (Table 4).

**Table 4:** Inhibition zone of Aqueous and Methanol extracts of *Wrightia tinctoria* against bacterial pathogens

Sl. No.	Name of the organisms	Zone of inhibition					
		Aqueous extract			Methanol extract		
		50mg	100mg	200mg	50mg	100mg	200mg
1	<i>Staphylococcus aureus</i>	15±2.8	18±2.8	30±6.5	16±2.8	18±2.8	22±2.4
2	<i>Escherichia coli</i>	13±3.7	18±2.4	21±1.4	-	-	-
3	<i>Leuconostoc lactis</i>	18±1.4	28±5.7	32±2.8	12±3.7	24±5.1	26±1.4
4	<i>Salmonella typhi</i>	16±2.8	18±1.4	22±2.8	16±3.7	19±5.1	29±5.1
5	<i>Pseudomonas aeruginosa</i>	16±4.2	24±4.9	31±2.4	18±3.7	21±2.4	24±2.8
6	<i>Streptococcus pyogenes</i>	13±3.7	23±2.8	28±2.4	17±2.8	18±2.8	21±3.7

The antifungal activity of *W. tinctoria* extracts were not showed much prominent in the activities against all fungus tested at test concentration. The methanol extract of *W. tinctoria* exhibited significant

antifungal activities against *Aspergillus flavus* and *Mucor indicus* tested but, the aqueous extract did not respond the test fungus (Table 5).

**Table 5:** Inhibition zone of Aqueous and Methanol extracts of *Wrightia tinctoria* against fungal pathogens

Sl. No.	Name of the organisms	Zone of inhibition					
		Aqueous extract			Methanol extract		
		50mg	100mg	200mg	50mg	100mg	200mg
1	<i>Aspergillus flavus</i>	-	-	-	12±3.7	18±3.7	22±1.4
2	<i>Mucor indicus</i>	-	-	-	11±2.8	16±4.2	18±3.7
3	<i>Aspergillus niger</i>	-	-	-	-	-	-
4	<i>Rhizopus indicus</i>	-	-	-	-	-	-

## DISCUSSION

The physico-chemical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs by African Pharmacopoeia<sup>18</sup>. Equally important in the evaluation of crude drugs, is the ash value and acid insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e., the presence or absence of foreign organic matter such as metallic salts and/or silica<sup>19</sup>. The total ash content of the *W. tinctoria* leaf is 29%. The water Solubility is less than that of alcohol Solubility at 28% and 42% respectively. Ash value is useful in determining authenticity and purity of drug and also these values are important quantitative standards<sup>20</sup>.

The presence of alkaloids, steroids, saponins and flavonoids is indicated only in *Wrightia tinctoria*. However, steroids, triterpenoids, flavonoids and glycosides were reported to occur<sup>21</sup> in *W. tinctoria* and triterpenoids, cardiac glycosides in *W. arborea*<sup>19</sup>.

*In vitro* antibacterial studies of the leaf extracts of *W. tinctoria* revealed that the methanol extracts had significant activity against most of the organism and followed by aqueous extracts<sup>22</sup>. But in our study aqueous extract had significant activity against the tested bacteria and followed by the methanol extract. *W. tinctoria* leaves extracts possessed potent antimicrobial properties against bacteria and dermatophytic fungi. In particular methanol and ethanol extracts were active against bacteria and hexane extract was active against dermatophytic fungi, suggesting that the active principles may be useful in the topical treatment of superficial skin infections<sup>23</sup>. In this work the methanol extract was active against *Aspergillus flavus* and *Mucor indicus* but in the case of aqueous extract was not active against the fungus.

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