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Antimicrobial activity and phytochemical screening of *Wrightia tinctoria* (Roxb.) R.Br.

P. Vedhanarayanan; P. Unnikannan; P. Sundaramoorthy**ABSTRACT**

In the present investigation, antibacterial activity of different extracts (Chloroform, ethanol and methanol) of *Wrightia tinctoria* has been studied against the human pathogenic bacterial strains, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* by disc diffusion method on agar. The findings showed potential antibacterial properties of the extracts against the organisms tested. Among the three solvents tested, ethanol extract of leaf showed higher inhibition zone. Ethanol extract of *Wrightia tinctoria* exhibits maximum zone of inhibition against *Escherichia coli* (29 mm), *Bacillus subtilis* (24 mm) *Staphylococcus aureus* (30 mm) and *Pseudomonas aeruginosa* (24 mm). Preliminary phytochemical analysis of *Wrightia tinctoria* showed the presence of alkaloids, flavonoids, phenols, saponins, steroids and tannins.

Keywords: *Wrightia tinctoria*, Antimicrobial activity, Phytochemical screening.**1. 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 [3]. 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 phytochemicals of plants are alkaloids, flavonoids, tannins and phenolic compounds. *Wrightia tinctoria* (Roxb.) R.Br. (Sans: Asita-Kutaja) belonging to family Apocynaceae is a small deciduous tree with pale grey, smooth bark, distributed in tropical Africa and Asia. It is considered to be therapeutically very effective jaundice plant in Indian indigenous system of medicine. The juice of the tender leaves is used efficaciously in jaundice. It is reported to possess aphrodisiac, anthelmintic, anti-inflammatory, astringent and antimicrobial properties. The crushed fresh leaves when filled in the cavity of decayed tooth relieve toothache. Bark and seeds are used to cure bilious infections, psoriasis, leprosy, asthma and various skin diseases [4] 19.

It has anti-dandruff properties and hence is used in hair oil preparations. The leaves are a fodder for the cattle, goat and sheep. In south India the plant is used for green manuring rice fields. The major active constituents of the plant are saponins, β -sitosterol, triterpenoids, ursolic acid, lupeol, oleanolic acid, α and β -amyriins [5, 6]. The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants [7]. The present investigation designed to screen antimicrobial activity of *Wrightia tinctoria* against some microbial pathogens and its phytochemical constituents.

2. Materials and Methods

2.1. Preparation of Plant Extracts

Wrightia tinctoria (Roxb.) R.Br leaf was collected from Cuddalore district of Tamil Nadu. The plants were taxonomically identified by using Flora of Madras presidency [8]. In the laboratory, the different parts of *W. tinctoria* samples were washed 2-3 times with running fresh water, leaf material was then air dried under shade after complete shade drying, the plant material (500 g) was grinded with mechanical grinder, the powder was kept in small labeled plastic bags. The extract was prepared by using the soxhlet extraction at 60-80 °C. The leaves were extracted with chloroform, ethanol and methanol for 72 hours to get crude extract and it was filtered through whatman No.1 filter paper. The extract was concentrated under vacuum and dried at 45 °C for complete removal of the solvent and the residue was used in the assay of antimicrobial analysis [9].

2.2. Screening for Antimicrobial Activity assay

Pathogenic microorganisms *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *pseudomonas aeruginosa* were selected for the present investigation. The microorganisms were originally obtained from Microbial Germ Plasma Culture collection RMMCH Medical lab, Annamalai University. The antibacterial activities of the leaves were tested against the selected bacterial strains.

The 20 ml of sterilized agar medium was poured into each sterile Petri plates and allowed to solidify. The test bacterial cultures were evenly spread over the appropriate media by using a sterile cotton swab. Then a well of 0.5 cm was made in the medium by using a sterile cork borer, 150 μ l of each methanol, ethanol and chloroform plant extracts were transferred into separate wells. After these plates was incubated at 37 °C for 24-48 hours. After incubation period, the results were observed and measure the diameter of inhibition zone around the each well.

2.3. Phytochemical Analysis

The freshly prepared extracts were chemically tested for the presence of different phytochemical constituents such as alkaloids, flavonoids, Phenolics, steroids, saponins, tannins, etc using standard methods [10-12].

3. Results and Discussion

3.1. Antimicrobial Activity

The dried leaf extract of *Wrightia tinctoria* shown to possess both antibacterial as well as antifungal activity. The antimicrobial activity of chloroform, ethanol and methanol extracts of *Wrightia tinctoria* were inspected against the selected experimental pathogens such as *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *pseudomonas aeruginosa* by disc diffusion methods. The tested microbial organism shows varying degrees of antimicrobial activities in examined plant extracts. Chloroform extract showed maximum zone of inhibition against in *E. coli* (4 mm), *Bacillus subtilis* (1 mm), *Staphylococcus aureus* (1.5 mm) and *Pseudomonas aeruginosa* (5 mm). The ethanol extract of leaf extract of *Wrightia tinctoria* showed maximum zone of inhibition was observed *E. coli* (29 mm), in *Bacillus subtilis* (24 mm), *Staphylococcus aureus* (30 mm) and *Pseudomonas aeruginosa* (24 mm). The methanol extract was exhibited moderate activity against *E. coli* (26 mm), *Bacillus subtilis* (24 mm), *Staphylococcus aureus* (21 mm) and *Pseudomonas aeruginosa* (31 mm) at 500 μ g of leaf extract of *Wrightia tinctoria* (Table 1).

Table 1: Antimicrobial activity of extract from *Wrightia tinctoria* against clinical pathogens expressed as MIC (μ g/ml)

Bacterial strains	Gram	Chloroform Extract					Ethanol Extract					Methanol Extract				
		100 μ g	200 μ g	300 μ g	400 μ g	500 μ g	100 μ g	200 μ g	300 μ g	400 μ g	500 μ g	100 μ g	200 μ g	300 μ g	400 μ g	500 μ g
<i>E. coli</i>	-	0.9	0.9	2	2.7	4	6	14	17	19	29	5	7	14	19	26
<i>B. subtilis</i>	+	-	-	0.5	2	1	7	9	12	13	24	0	0	9	11	24
<i>S. aureus</i>	+	0.6	0.9	4	2	1.5	24	26	28	29	30	5	7	13	15	21
<i>P. aeruginosa</i>	-	-	05	-	4	5	17	19	22	22	24	8	14	17	20	31

Investigation of Badmanaban and Patel [13], reveals significant activity against bacterial strains and fungal strains showed more in (65%) ethanol extracts against when compared to that of chloroform extract. Antimicrobial activity of medicinal plants are being increasingly stated from different parts of the world and the plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens [14]. The changing unit of sensitivity of the bacterial strains may be due to the intrinsic tolerance of the bacterial and the nature and combinations of phytocompounds present in the extracts [15].

3.2. Phytochemical analysis

Methanolic extract revealed the presence of flavonoids, phenolics and steroids. Ethanol extract shown the presence of flavonoids, phenolics, steroids and tannins, whereas in chloroform extract

revealed the presence of alkaloids, saponins, steroids and tannins and the results were summarized in Table 2. Phytochemicals variation present in different solvents as shown in the result of phytochemical screening might be experienced to the ability of the solvents to dissolve into solution specific type of phytochemicals. Tannins bind to proline rich proteins and interfere with the protein synthesis [16-18]. Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls [19].

Table 2: Preliminary Phytochemical Analysis of Extracts

Chemical components	Chloroform Extract	Ethanol extract	Methanol Extract
Alkaloides	+	-	-
Flavanoids	-	+	+
Phenolics	-	+	+
Saponins	+	-	-
Stroids	+	+	+
Tannins	+	+	-

(+)= Present; (-) = Absent

4. Conclusion

The present research work concludes that *Wrightia tinctoria* is important medicinal plant with varied pharmacological spectrum. The plant shows the presence of many phytochemical constituents which are responsible for varied pharmacological and medicinal property. The evaluation needs to be carried out on *Wrightia tinctoria* in order to uses and formulation of the plant in their practical clinical applications, which can be used for the welfare of the mankind.

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