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Research Article

PHYTOCHEMICAL EVALUATION AND *INVITRO* ANTIBACTERIAL SCREENING OF *WRIGHTIA TINCTORIA* (ROXB.) R. BR. AGAINST ENTERIC PATHOGENS

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ABSTRACT

The enteric pathogens are responsible for major health problems in most of the countries. Pathogens develop resistance against the antibiotics, and its continuous use may cause severe side effects. This urged to find alternate means to control the infection. The leaves of *Wrightia tinctoria (Roxb.) R. Br.* taken from an herbal garden was evaluated against five enteric pathogens. Antibacterial activities of the extracts were determined by the agar disc diffusion method. Chlortetracycline (5 mcg/disc) was used as positive control for comparison of the inhibition zones. Phytochemical screening of the plant leaves revealed the presence of steroids, reducing sugar, alkaloids, phenolic compounds, flavonoids, saponins and tannins. The extract showed efficient antibacterial activity against *Staphylococcus aureus* with maximum zone of inhibition. 10µg/ml of the methanol extract was observed to be the minimum inhibitory concentration against *Staphylococcus aureus*, *Shigella dysenteriae* and *Shigella boydii*.

Keywords: *Wrightia tinctoria,* Enteric pathogens, Phytochemicals, Antibacterial activity.

INTRODUCTION

The control of bacterial infection has been remarkably effective since the discovery of antibacterial drugs. However some of the pathogens rapidly become resistant to many effective drugs. The development of drug resistance as well as appearance of undesirable side effects of certain antibiotics has led to the search of new antibacterial agents in particular from medicinal plants [1]. Higher plants have been shown to be a potential source for new anti-microbial agents [2]. There has been gradual revival of interest concerning the use of medicinal and aromatic plants in developed as well as in developing countries, because plant derived drugs have been reported to be safe and without side-effects [3].

Enteric or diarrheal infections are major public health problems in developing countries and contribute to the death of 3.3 to 6.0 million children, annually. Enteric bacteria comprised *Salmonella* spp., *Shigella* spp., *Proteus* spp., *Klebsiella* spp., *Escherichia coli, Pseudomonas* spp., *Vibrio cholerae* and *Staphylococcus aureus* which are major etiologic agents of sporadic and epidemic diarrhea both in children and adults. WHO reported that 80% populations rely mainly on traditional therapies, involving the use of plant extracts or their active constituents [4].

Wrightia tinctoria (Roxb.) R. Br. belonging 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 [5]. The five flavonoid compounds, Indigotin, Indirubin, tryptanthrin, isatin and rutin were isolated and identified from the leaves [6]. The plant has been reported for antimicrobial in psoriasis, wound healing and hepatoprotective activity [7, 8, 9]. A decoction of the leaves and bark is taken as a stomachic and in the treatment of abdominal pain.

With this background, antibacterial activity of *Wrightia tinctoria* was studied against selected human pathogens and examined their phytochemical constituents.

MATERIALS AND METHODS

Plant Material

The leaves of *Wrightia tinctoria (Roxb.) R. Br.* belonging to Apocynaceae were collected from the herbal garden of St. Xavier's College, Tirunelveli district, Tamilnadu.

Microorganisms Used

The pathogens *Salmonella typhi*, *Staphylococcus aureus, Klebsiella vulgaris, Shigella dysenteriae* and *Shigella boydii* were obtained from Microbial Type Culture Collection (MTCC), Chandigarh. The bacterial strain was cultured in nutrient broth at 37°C and maintained on nutrient agar (HiMedia) slant at 4°C.

Crude Methanolic Extract

The dried powdered leaf material was macerated with 95% methanol (100 g dried powder sample/500 ml of 95% methanol) for 7 days at room temperature. The filtrated solvent of each species was removed under vacuum at 40°C by using a rotary evaporator. The obtained crude extract was stored at 4°C.

Preliminary Screening

The condensed extracts were used for preliminary screening of phytochemicals such as alkaloids, steroids, reducing sugars, catechins, anthroquinones, flavonoids, terpenoids, sugars, phenols, saponins, tannins and aminoacids. The presence of phytochemicals from methanol extract of all the samples was qualitatively determined [10].

Determination of Antibacterial Activity

Antibacterial Activity

Antibacterial assay was carried out by disc diffusion method using microorganisms cell suspension whose concentration was equilibrated to 0.5 McFarland standards. For this, 0.1ml (10^{-5} cfu /ml) of 24 hrs old bacterial culture was placed on Mueller Hinton agar medium and spread throughout the plate by spread plate technique [11]. The sterile filter paper discs of 6mm diameter were loaded with 5, 10, 15, 20 and 25µg/ml of plant extract dissolved in DMSO were placed on the surface of the medium and incubated at 37°C for 24hrs. Antibacterial activity was recorded by measuring the diameter of zone of inhibition. Chlortetracycline (5 mcg / disc) was used as positive control, negative controls were done using paper disc loaded with 25 µl of DMSO. The entire test was performed in triplicate. The Minimum Inhibitory Concentration (MIC) of methanol extract was determined as the lowest concentration of the plant extract inhibiting the visible growth of organism.

Determination of % of Relative Inhibition Zone Diameter

The percentage of antibacterial activity was calculated by applying the expression: % RIZD = (IZD sample - IZD negative control) / IZD antibiotic standard × 100, where RIZD is the relative inhibition zone diameter and IZD is the inhibition zone diameter (mm) [12].

Statistical analysis

All values are expressed as mean ± standard deviation. The Inhibition Zone Diameter data of each concentration was analyzed using one way analysis of variance (ANOVA). P value < 0.05 was considered as significant. The software OriginPro 7.5 was employed for the statistical analysis.

RESULTS AND DISCUSSION

The methanol leaf extract of *Wrightia tinctoria* consisted of steroids, reducing sugar, alkaloids, phenolic compounds, flavonoids, saponins and tannins (Table 1). The plants are the vital source of innumerable number of antimicrobial compounds. Several phytoconstituents like flavonoids [13], phenolics and polyphenols [14], tannins [15], terpenoids [16], sesquiterpenes [17], etc., are effective antimicrobial substances against a wide range of microorganisms.

The zone of inhibition ranged from 7.0 to 15.6 mm. The methanol leaf extract of *Wrigtia tinctoria* showed efficient activity against all the bacteria studied. The highest inhibition zone 15.6 mm was formed against *Staphylococcus aureus* at the highest concentration with 67.1% of RIZD, followed

by 13.6 mm of inhibition zone against *Salmonella typhi* with 51.3% of RIZD. The least inhibition zone (10.3 mm) was found for *Klebsiella vulgaris* at the highest concentration. 10µg/ml of the methanol extract was observed to be the minimum inhibitory concentration against *Staphylococcus aureus*, *Shigella dysenteriae* and *Shigella boydii* with 7.3, 7.6 and 7.0 mm of inhibition zone respectively. The inhibition zone diameter and % of RIZD of the methanol extract of *Wrightia tinctoria* against the studied microorganism is tabulated in table 2.

Using Originpro software the values of the inhibition zone was statistically analysed through one way analysis of variance (ANOVA) followed by Tukey's test. Values of $P<0.05$ were considered statistically significant. The antibacterial activity of the leaf extract was almost significant with the positive control used against all the bacteria with a certain concentration and the data are given in table 3.

Similar studies have been carried out with various solvent extracts of bark of *W. tinctoria* and *W. arborea* against gram positive and gram negative organisms [18]. The chloroform extracts of *W. arborea* showed broader spectrum of antibacterial activity when compared with *W. tinctoria*. However, *Wrightia tinctoria* is a widely used medicinal plant. It has been reported that the methanolic and ethanolic leaf extract of *Wrightia tinctoria* were active against bacteria and the hexane extract was active against dermatophytic fungi, they also suggested that the active principles may be useful in the topical treatment of superficial skin infections [19]. Methonolic extract of *Wrightia tinctoria* exhibited good inhibitory efficacy against both *Staphylococcus* and *Salmonella* spp [20].

An important characteristic of plant extracts and their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeability. Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death [21]. It has been suggested that phytochemical extracts from plants hold promise to be used in allopathic medicine as potential sources of antimicrobial agents[22].

CONCLUSION

The results of present investigation show that the methanolic leaf extracts of *Wrightia tinctoria* have efficient antimicrobial activity against *Staphylococcus aureus, Salmonella typhi* and *Shigella dysenteriae*. It is therefore suggested that this active extract could be analyzed for bioassay to characterize the novel antibacterial agents.

Table 1: Qualitative analysis of the phytochemical constituents of leaf of *Wrightia tinctoria*

S.No	Phytochemicals	Methanol extract
1.	Steroids	+
2.	Triterpenoids	-
3.	Reducing sugar	+
4.	Sugars	-
5.	Alkaloids	+
6.	Phenolic compounds	+
7.	Flavonoids	+
8.	Catechin	-
9.	Saponins	+
10.	Tannins	+
11.	Anthroquinones	-
12.	Amino acids	-

(+) Present; (-) absent

Table 2: Antibacterial activity of the methanol leaf extract of *Wrightia tinctoria*

No	Bacteria	Inhibition zone diameter (mm)/RIZD (%)					
		Methanol extract					Positive control
		5µg	10µg	15µg	20µg	25µg	
1	B_1	-	-	8.3±0.3/15.5	12.6±0.3/44.5	13.6±1.4/51.3	14.8
2	B_2	-	7.3±0.3/9.1	9.6±0.3/25.1	13.0±0.5/48.9	15.6±0.3/67.1	14.3
3	B_3	-	-	6.6±0.3/5.1	8.0±0.5/17.2	10.3±0.3/37.0	11.6
4	B_4	-	7.6±0.6/9.8	8.2±0.3/13.4	10.5±0.5/27.6	13.3±0.3/44.7	16.3
5	B_5	-	7.0±0.5/7.1	7.6±0.3/11.4	9.6±0.8/25.7	12.3±0.3/45.0	14.0

B_1 - Salmonella typhi; B_2 - Staphylococcus aureus; B_3 - Klebsiella vulgaris; B_4 - Shigella dysenteriae; B_5 - Shigella boydii

Table 3: Statistical analysis of the antimicrobial activity

Bacteria	Concentration	MS value	F value	P value	Significance
B_1	5µg	-	-	-	-
	10µg	-	-	-	-
	15µg	54.0000	64.8000	0.0012	Yes
	20µg	4.1666	5.0000	0.0890	No
	25µg	0.6666	0.1739	0.6980	No
B_2	5µg	-	-	-	-
	10µg	73.5000	55.1250	0.0017	Yes
	15µg	32.6666	24.5000	0.0077	Yes
	20µg	2.6666	1.6000	0.2745	No
	25µg	2.6666	2.0000	0.2302	No
B_3	5µg	-	-	-	-
	10µg	-	-	-	-
	15µg	37.5000	112.5000	0.0004	Yes
	20µg	20.1666	30.2500	0.0053	Yes
	25µg	2.6666	8.0000	0.0474	Yes
B_4	5µg	-	-	-	-
	10µg	112.6666	135.2000	0.0003	Yes
	15µg	112.6666	338.0000	0.00005	Yes
	20µg	60.1666	90.2500	0.0006	Yes
	25µg	13.5000	40.5000	0.0031	Yes
B_5	5µg	-	-	-	-
	10µg	73.5000	73.5000	0.0010	Yes
	15µg	60.1666	90.2500	0.0006	Yes
	20µg	28.1666	16.9000	0.0147	Yes
	25µg	4.1666	6.2500	0.0667	No

B_1 - Salmonella typhi; B_2 - Staphylococcus aureus; B_3 - Klebsiella vulgaris; B_4 - Shigella dysenteriae; B_5 - Shigella boydii

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