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Original Article

STATISTICAL ANALYSIS OF THE ANTIMICROBIAL ACTIVITY OF WRIGHTIA *TINCTORIA* LEAF AND BARK EXTRACTS

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ABSTRACT

Objective: *Wrightia tinctoria* has been extensively used in Folk medicine. It has been reported to have good analgesic, anti-inflammatory, anthelmintic, antiulcer, antidysentric, antidiabetic, anticancer, antipyretic activities and also effective in the treatment of psoriasis. The present investigation was undertaken to statistically evaluate the antimicrobial activity of the leaf and bark extracts of *Wrightia tinctoria*.

Methods: The antibacterial and antifungal activities of leaf coconut oil extracts (1, 4, 7 and 15 days exposure to sunlight), bark and leaf methanol, ethyl acetate, chloroform and petroleum ether extracts of *Wrightia tinctoria* (Apocynaceae) against nine pathogenic bacteria such as *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Serratia marcescens* and two fungi viz. *Aspergillus niger* and *Penicillium chrysogenum* were statistically analyzed so as to evaluate the medicinal potential of these plant extracts.

Results: *Wrightia tinctoria* leaf and bark methanol extracts were found to be potent extracts and their activity is quite comparable with the standard antibiotics such as tobramycin and gentamycin sulphate screened under similar conditions.

Conclusion: Methanol extracts of the leaves and bark of *Wrightia tinctoria* can be used as a potential external antiseptic and can be incorporated into drug formulations.

Keywords: *Wrightia tinctoria*, Antibacterial activity, Statistical analysis, Standard antibiotics, Drug formulation.

INTRODUCTION

The universal role of plants in the treatment of diseases is established by their employment in all important systems of medicine. There are many herbs on earth which lies unexplored in the field of medicine or Science. *Wrightia tinctoria* (Syn. Pala indigo plant) of Apocyanaceae family is widely used in skin diseases, liver disorders and broad spectrum biological activities [1]. *Wrightia tinctoria* flower has been reported to have a good anti-inflammatory activity. Many compounds of plant origin have been identified that inhibit different stages in the replication cycle of virus [2, 3]. *Wrightia tinctoria* is an important medicinal plant used in the Indian system of medicine for the treatment of variety of diseases [4] and it was reported to possess analgesic [5] and cytotoxic activities. 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-amyrins [6]. The bark of *W. tinctoria* is considered for antidiarrhoeal, aphrodisiac, anthelmintic, febrifuge, stomachic, toothache, tonic and dog bite [7, 8]. It is employed in seminal weakness and flatulence, also used in piles and skin diseases [9].

Ethnomedically, the bark of this plant is used as a galactagogue to treat abdominal pain, skin diseases and wounds [10] as an anti-pyretic [11] anti-dysenteric, anti-diarrheal- and anti-hemorrhagic [12] agents, and as an antidote for snake poison[13]. Seeds of this plant are also used as an aphrodisiac [14].

MATERIALS AND METHODS

Plant material

The leaves and bark of *Wrightia tinctoria* were collected from Thrissur district of Kerala, South India and authenticated by Dr. Kochuthressia M.V., HOD, Department of Botany, Vimala College, Thrissur. Voucher specimen is deposited in the specially maintained herbarium, Department of Botany, Vimala College, Thrissur.

Preparation of Plant Extracts

Fifty grams of the powered plant material were extracted successively with 150ml of petroleum ether, chloroform, ethyl acetate and methanol as solvents for 24hours by Soxhlet equipment. Leaf coconut oil extract was prepared by exposing the fresh leaves of *Wrightia tinctoria* in coconut oil to sunlight for 1, 4, 7 and 15 days.

Test microorganisms

The microorganisms used for antibacterial and antifungal activity evaluation were obtained from Microbial Type Culture Collection and gene bank (IMTECH, Chandigarh, India). They were bacteria such as *Bacillus cereus* (MTCC-1305), *Enterobacter faecalis* (MTCC-5112), *Salmonella paratyphi,* (MTCC-735), *Staphylococcus aureus* (MTCC-96), *Escherichia coli* (MTCC-729), *Proteus vulgaris* (MTCC-426), *Klebsiella pneumoniae* (MTCC-109), *Pseudomonas aeruginosa* (MTCC-647), *Serratia marcescens* (MTCC-86) and fungi such as *Aspergillus niger* (MTCC-2425) and *Penicillium chrysogenum* (MTCC-5108).

Antimicrobial activity assay

The agar diffusion method is used for the antimicrobial evaluations. Wells of 8mm (0.8cm) diameter were dug on the inoculated nutrient agar medium (antibacterial assay) and on potato dextrose agar medium (antifungal assay) with sterile cork borer and 50µl of the petroleum ether, chloroform, ethyl acetate and methanol extracts of the bark and leaf and coconut oil extract of the leaf of *Wrightia tinctoria* were added in each well. Wells introduced with 50µl of pure petroleum ether, chloroform, ethyl acetate, methanol and coconut oil served as negative controls. The plates were incubated at 37^0C over night and examined for the zone of inhibition. The diameter of the inhibition zone was measured in mm. The standard antibiotic drugs such as tobramycin, gentamicin sulphate, ofloxacin and ciprofloxacin were used for antibacterial evaluation and standard drug chloramphenicol was used for antifungal study. An extract was classified as active when the diameter of the inhibition was equal to or larger than 8mm [15]. All the assays were performed in triplicate and expressed as average values.

RESULTS AND DISCUSSION

The antimicrobial activities of methanol, ethyl acetate, chloroform and petroleum ether extracts of the bark and leaves of *Wrightia tinctoria* and leaf coconut oil extracts (1, 4, 7 and 15 days exposure to sunlight) against nine pathogenic bacteria such as *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Serratia marcescens* and two fungi viz. *Aspergillus niger* and *Penicillium chrysogenum* were statistically evaluated.

Statistical analysis

Let us assume that the mean activity of *W. tinctoria* leaf methanol extract (Wtla) and W. *tinctoria* bark methanol extract (Wtba) are same. Also the alternate hypothesis is that the mean activity of Wtla is greater than Wtba. By applying student's t-test, it can be seen that the calculated t-value is less than tabled value at twenty degrees of freedom with significance level 0.05. Hence the assumption that two extracts having same activity is accepted and the mean activity of Wtba against eleven pathogenic microorganisms is found to be comparable with Wtla.

Similarly by applying t-test, the mean activities of W. tinctoria bark and leaf extracts in various solvents are compared. The mean activity of Wtba>Wtbd (activity of Wtba greater than Wtbd), Wtba>Wtbc, Wtba>Wtlc, Wtba>Wtld, Wtba>Wt15, Wtba>Wt 7, Wtba>Wt 4, Wtba>Wt 1 while that of Wtba-Wtla and Wtba-Wtbb are almost same.

The interval estimation of the mean activity of *W. tinctoria* bark and leaf extracts in various solvents are given in table 1.

Table 1: Interval estimation of the mean antimicrobial activity of *W.tinctoria* bark and leaf extracts

W. tinctoria bark and leaf extracts	Mean activity	Standard Deviation	Interval estimation at 5% level of significance
Wtba	20	2.79	20 + 1.65
Wtbb	17.45	2.21	17.45 + 1.30
Wtbc	14.82	2.14	14.82 + 1.26
Wtbd	12.91	2.12	12.91 + 1.25
Wtla	19	5.23	19 + 3.1
Wtlb	16.82	4.69	16.82 + 2.77
Wtlc	14.55	3.86	14.55 + 2.28
Wtld	12.55	2.94	12.55+ 1.74
Wt15	14.36	1.91	14.36 + 1.13
Wt7	13.09	1.70	13.09 + 1.00
Wt4	11.82	1.47	11.82 + 0.87
Wt1	10.18	1.54	10.18 + 0.91

Wtb: *W.tinctoria* bark; Wtl: *W.tinctoria* leaf

Wt1, Wt4, Wt7, Wt15: *W.tinctoria* leaf coconut oil extract with 1, 4, 7 &15 days of exposure to sunlight

a: methanol extract; **b**: ethyl acetate extract; **c**: chloroform extract **d**: petroleum ether extract The correlation studies suggest that Wtla-Wtlb (0.9824), Wtla-Wtlc (0.9263), Wtlb-Wtlc (0.9356), Wtlc-Wtld (0.9661) and Wt1-Wt15 (0.9619) are highly correlated and hence the mean activity of these extracts against eleven pathogenic microorganisms increases simultaneously.

Ethyl acetate (b) and chloroform (c) extracts of *W. tinctoria* bark and leaf also showed appreciable positive correlation (table 2).

From table 3, it can be seen that the mean activity of Wtla-Wtlb, Wtlb-Wtlc, Wtlc-Wtld and Wtla-Wtba are almost same as the t-value is less than tabled value. The correlation studies showed that standard antibiotics and bark extracts of *W.tinctoria* Oflo-Wtba, Oflo-Wtbb, Oflo-Wtbc, Oflo-Wtbd, Cip-Wtba, Cip-Wtbb, Cip-Wtbc and Cip-Wtbd showed low positive correlation. Gen- Wtba, Gen- Wtbb, Gen- Wtbc and Gen- Wtbd showed negative correlation and the mean activity of these extracts varies in an inverse manner.

The standard antibiotics and leaf extracts of *W.tinctoria* Cip-Wtla, Cip-Wtlb, Cip-Wtlc, Cip-Wtld, Gen- Wtla, Gen- Wtlb, Gen- Wtlc, Gen-Wtld, Oflo-Wtla, Oflo-Wtlb and Tob-Wtla exhibited negative correlation and they have inverse relationship.

Table 2: Student's t-test: Mean activities of *W. tinctoria* bark and leaf extracts

W.tinctoria bark and leaf extracts	Calculated value of 't'	Correlation coefficient
Wtba-Wtbb	2.261	0.8759
Wtba-Wtbc	4.66	0.7039
Wtba-Wtbd	6.40	0.5950
Wtbb-Wtbc	2.71	0.8675
Wtbb-Wtbd	4.70	0.8141
Wtbc-Wtbd	2.00	0.8434
Wtla-Wtlb	0.982	0.9824
Wtla-Wtlc	2.167	0.9263
Wtla-Wtld	3.40	0.8303
Wtlb-Wtlc	1.184	0.9356
Wtlb-Wtld	2.441	0.8338
Wtlc-Wtld	1.303	0.9661
Wt1-Wt15	5.390	0.9619
Wtla-Wtba	0.533	0.1984

Wtb: *W.tinctoria* bark; Wtl: *W.tinctoria* leaf; Wt1, Wt15: *W. tinctoria* leaf coconut oil extract with 1 &15 days of exposure to sunlight, a: methanol extract; b: ethyl acetate extract, c: chloroform extract d: petroleum ether extract

Table 3: Mean activities of *W. tinctoria* bark and leaf extracts

W.tinctoria leaf extracts and standard antibiotics	Correlation coefficient	*W.tinctoria* bark extracts and standard antibiotics	Correlation coefficient
Tob-Wtla	-0.0392	Tob-Wtba	0.09
Tob-Wtlb	0.030	Tob-Wtbb	0.24
Tob-Wtlc	0.240	Tob-Wtbc	0.35
Tob-Wtld	0.320	Tob-Wtbd	0.35
Gen- Wtla	-0.290	Gen- Wtba	-0.02
Gen- Wtlb	-0.30	Gen- Wtbb	-0.18
Gen- Wtlc	-0.210	Gen- Wtbc	-0.34
Gen- Wtld	-0.180	Gen- Wtbd	-0.25
Oflo-Wtla	-0.12	Oflo-Wtba	0.37
Oflo-Wtlb	-0.12	Oflo-Wtbb	0.20
Oflo-Wtlc	0.10	Oflo-Wtbc	0.14
Oflo-Wtld	0.25	Oflo-Wtbd	0.30
Cip-Wtla	-0.52	Cip-Wtba	0.58
Cip-Wtlb	-0.52	Cip-Wtbb	0.44
Cip-Wtlc	-0.38	Cip-Wtbc	0.17
Cip-Wtld	-0.25	Cip-Wtbd	0.23

Wtb: *W.tinctoria* bark; Wtl: *W.tinctoria* leaf a: methanol extract; b: ethyl acetate extract c: chloroform extract d: petroleum ether extract Tob: tobramycin; Gen: gentamicin sulphate; Oflo: ofloxacin; Cip: ciprofloxacin

CONCLUSION

Wrightia tinctoria bark methanol extract (Wtba) was found to be a potent extract and its activity is quite comparable with the standard antibiotics such as tobramycin screened under similar conditions. *W.tinctoria* leaf methanol extract (Wtla) was also found to be effective against *Staphylococcus aureus* and *Bacillus cereus* and its activity is comparable with the standard antibiotics tobramycin, gentamicin suphate, ofloxacin and ciprofloxacin (10µg each). Methanol extracts of the *Wrightia tinctoria* bark and leaf can be used as a potential external antiseptic in pharmaceutical preparations. The antimicrobial potency of the *W.tinctoria* leaf methanol extract can be attributed to the presence of flavonoids, phenolic compounds and saponins and the activity of bark methanol extract is due to the presence of phenolic compounds, saponins and tannins [16, 17].

It is interesting to note that even crude extract of this plant showed prominent activity against various pathogenic bacteria where modern therapy has failed. The variation of the susceptibility of the tested microorganisms could be attributed to their intrinsic properties that are related to the permeability of their cell surface to the extracts [18]. The results of this study support the use of this plant for human diseases and reinforce the ethnobotanical importance of plant as a potential source of bioactive substances.

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ABBREVATIONS

Wtba: *W.tinctoria* bark methanol extract; Wtla: *W.tinctoria* leaf methanol extract

Wtlc: *W.tinctoria* leaf chloroform extract; Wtlb: *W.tinctoria* leaf ethyl acetate extract

Wtbc: *W.tinctoria* bark chloroform extract

Wtbb: *W.tinctoria* bark ethyl acetate extract

Wtbd: *W.tinctoria* bark petroleum ether extract

Wtld: *W.tinctoria* leaf petroleum ether extract

Wt1: *W.tinctoria* leaf coconut oil extract (1d)

Wt4: *W.tinctoria* leaf coconut oil extract (4d)

Wt7: *W.tinctoria* leaf coconut oil extract (7d)

Wt15: *W.tinctoria* leaf coconut oil extract (15d)

CONFLICT OF INTERESTS

Declared None

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