COMPARATIVE ANTI – MICROBIAL EVALUATION STUDIES OF THE EXTRACTS AND ISOLATES OF LEAVES & BARK OF *WRIGHTIA TOMENTOSA*

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ABSTRACT :

The Butanol and Ethanol extract of the leaves and bark of Wrightia tomentosa along with its seven pure component isolates (**BLF_{28}, BLF_{29*}, BBF_{29}, ELF_3, ELF_7, ELF_{17*}, EBF_7**) after fractionation by column chromatography were evaluated for antimicrobial activity against Gram positive (S. aureus, S. fecalis, S.albus and B.subtilis) and Gram negative (Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris & Klebsiella aerogenes) bacteria and the fungi Candida albicans by disc diffusion method. The extracts and isolates showed different degree of activity against pathogenic microbes. The results obtained were compared with standard drugs Ciprofloxacin (10µg) and Clotrimazole (10µg). The isolates of butanol bark extract (BBF_{29}) followed by leaf extract(**BLF_{29*}**) were considerably more effective than the ethanol leaf and bark extract in inhibiting all the microbial strains.

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]. 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 [2]. Therefore, the development of alternative antimicrobial drugs from medicinal plants for the treatment of infectious diseases has become necessary.

Wrightia tomentosa Roem. & Schult. belonging to the family, Apocynaceae is a small deciduous tree, up to 12m high, found throughout the warmer parts of India, ascending to an altitude of 600m in the Himalayas and to 1,200 m 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 [3]. The bark and root-bark are believed to be useful in snake-bite and scorpion – stings [4]. A novel isoflavone, wrightiadione isolated from the plant possess cytotoxic activity against the murine P 388 lymphocytic leukemia cell line[5]. The objective of the present investigation is to assess the antimicrobial activity of the leaf & bark extract of this plant in solvents like ethanol & butanol.

MATERIALS AND METHODS:

The leaves and stem bark of Wrightia tomentosa were collected from the hills of Yercaud forest. The plant identity was confirmed[6,7] and a specimen voucher was made with the authentication of an acknowledged Botanist. The present study was carried out at the Dept. of Pharmaceutical Chemistry, Periyar college of Pharmaceutical Sciences for Girls, K. Sathanoor Main Road, Trichy, Tamil Nadu. The leaves and bark were dried under shade and then powdered. The powdered bark & leaves were extracted with Ethanol and Butanol 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 for the identification of various plant constituents like alkaloids, glycosides, steroids, terpenoids, flavanoids, tannins, gums and mucilages, fixed oils and fats and saponins.

ISOLATION OF PURE COMPONENTS BY COLUMN CHROMATOGRAPHY :

A part of the total ethanol leaf extract (TEL), total ethanol bark extract (TEB), total butanol leaf extract (TBL) and total butanol bark extract (TBB) was chromatographed separately over silica gel (60-120 mesh, CDH, Mumbai). The column was eluted to yield the pure fractions. The individual pure components were identified by monitoring of TLC and chemical tests.

The ethanol pure components of the leaf fraction, ELF3, ELF7 and ELF17* were eluted with 100% ethyl acetate, 60% ethylacetate – ethanol & 50% ethanol – water respectively. Similarly, the ethanol pure component of the bark fraction, EBF7 was eluted with 60% ethyl acetate – ethanol.

In addition, the butanol pure

components of the leaf fraction, BLF28 & BLF29* were eluted with 9:1:2 – Ethylacetate – methanol – formic acid and 80% chloroform – methanol. Similarly, the butanol pure components of the bark fraction, BBF29 was successfully eluted with Ethyl acetate – hexane – water (65:25:10).

ANTIMICROBIAL ASSAY:

The ethanol and butanol extracts of leaf and bark were evaluated by agar disc diffusion method [8]. 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 200µl and 100µl of the test compound extracts & isolates, 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 extracts or isolates [9]. The control zones were subtracted from the test 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 and Clotrimazole were used as a standard for anti-bacterial & antifungal screening.

RESULTS AND DISCUSSION:

Preliminary Phytochemical analysis of the bark revealed the presence of alkaloids & fats and oils in butanol fraction whereas the leaf extract of butanol showed the presence of terpenoids and flavanoids as active constituents. The ethanolic bark extract of the plant was rich in content of gums and mucilages along with fats and oils with moderate quantity of alkaloids whereas the ethanol leaf extract contains more amount of alkaloids, fats & oils and Gums & mucilages (Table 1).

The total extracts from leaf and bark of ethanol (TEL, TEB) & the extracts of butanol (TBL,TBB) along with seven isolated pure component fractions from ethanol & butanol of leaf and bark (EBF_7, ELF_3, ELF_{17*}, ELF_7,BLF_{29*},BLF_{28},BBF_{29}) were tested against 9 clinically important microbial strains for their antimicrobial efficacy and are presented in Table 2 & 3.

Among the tested components, ethanolic leaf extract fraction (ELF_{17*}) was ineffective against all the organisms used except Gram positive Staphylococcus aureus and Staphylococcus albus. The pure component butanol isolates, BBF_{29} and BLF_{29*} was found to be more potent against all the Gram positive and Gram negative organisms used. Pure component fraction BBF_{29} showed maximum antibacterial activity against the pathogenic Gram negative

Klebsiella aerogenes with a zone of inhibition of 28mm. (Ciprofloxacin – 35mm). Similarly, BLF_{29*} was found to be highly sensitive against the Gram positive organisms, Staphylococcus aureus and Streptococcus fecalis tested with a zonal inhibition of 22mm each (Ciprofloxacin – 37 & 38 mm).

In comparing various parts of the plant for antimicrobial potency, the bark extract of butanol showed maximum activity against all organisms used. The second most potent compound for antimicrobial activity identified was butanol leaf extract . The predominant antimicrobial action was mainly due to the presence of alkaloids, terpenoids and flavanoids.

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TABLE 1
Results of preliminary phytochemical tests for the presence of active constituents in leaves and bark of Wrightia tomentosa.

S.No.	Constituents	Leaf extract		Bark extract	
		Ethanol	Butanol	Ethanol	Butanol
1.	Alkaloids	++	-	+	++
2.	Glycosides	-	-	-	-
3.	Steroids	-	-	-	-
4.	Terpenoids	-	++	-	-
5.	Flavanoids	-	++	-	-
6.	Tannins	-	-	-	-
7.	Gums & Mucilages	++	-	++	-
8.	Fats & Oils	++	-	++	++
9.	Saponins	-	-	-	-

++ high, + medium and – absence.

TABLE – 2.

Antimicrobial Activity of Wrightia tomentosa Leaves & Bark extract against Gram +Ve and Gram –Ve bacteria and fungi.

S.No.	Organism used	Sample loaded / Disc	Zone of inhibition diameter (mm)						
			Standard	Toluene Leaf Extract	DMSO Leaf Extract	Ethanol Leaf Extract	Butanol Leaf Extract	Ethanol Bark Extract	Butanol Bark Extract
1.	Staph. aureus	200 µl	37	NS	NS	22	NS	NS	15
2.	Strep. fecalis	200 µl	38	23	NS	NS	NS	NS	22
3.	Staph. albus	200 µl	35	30	NS	22	NS	10	25
4.	Bacillus subtilis	200 µl	34	NS	NS	18	NS	14	16
5.	E. coli	200 µl	35	NS	NS	16	NS	12	26
6.	Pseudo. aeruginosa	200 µl	40	20	NS	NS	NS	NS	20
7.	Proteus. vulgaris	200 µl	38	NS	NS	12	NS	15	20
8.	Kleb. aerogenes	200 µl	35	20	NS	22	20	22	NS
9.	Cand. albicans	200 µl	45	NS	NS	25	NS	NS	NS

NS = No Zone of Inhibition. **DMSO** = Di-Methyl Sulphoxide

TABLE – 3.

Antimicrobial Activity of Wrightia tomentosa Leaves & Bark (Pure Components Isolated) against Gram +Ve and Gram –Ve bacteria and fungi.

S. No.	Organism used & its zone of inhibition against std. antibiotics (mm)	Sample Loded / Disc	Zone of inhibition diameter (mm)								
			EBF_7 1	ELF_3 2	$ELF_{17,18}$ 3	ELF_7 4	$BLF_{29,30}$ 5	BLF_{28} 6	BBF_{29} 7	Solvent control	
										ESC 8	BSC 9
1.	Staph. aureus. (37).	100 µl	16	08	07	13	22	15	16	NS	NS
2.	Strep. fecalis. (38).	100 µl	18	NS	NS	16	22	12	23	NS	08
3.	Staph. albus. (35).	100 µl	13	08	10	12	18	09	17	11	08
4.	Bacillus subtilis. (34).	100 µl	08	10	NS	12	22	14	25	15	12
5.	E. coli. (35).	100 µl	16	NS	NS	11	21	14	22	10	12
6.	Pseudo. aeruginosa. (40).	100 µl	15	08	NS	14	16	12	15	NS	NS
7.	Proteus. vulgaris. (38).	100 µl	13	08	NS	13	15	12	18	09	10
8.	Kleb. aerogenes. (35).	100 µl	18	12	NS	NS	18	13	28	08	NS
9.	Cand. albicans. (45).	100 µl	18	NS	NS	14	23	16	24	12	08

NS = No Zone of Inhibition
ELF = Ethanolic Leaf Fraction
BLF = Butanolic Leaf Fraction
BSC = Butanolic Solvent Control
EBF = Ethanolic Bark Fraction
BBF = Butanolic Bark Fraction
ESC = Ethanolic Solvent Control

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