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Bio-active Spectra of Plumbagin

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The bio-active compound plumbagin is isolated from Chitraka, *Plumbago zeylanica* Linn. The anti-bacterial and anti-fungal activities of Plumbagin were studied by using the Disc-Assay Method against a series of pathogenic micro-organisms which are responsible for severe neasopharyngeal, respiratory, cardio-vascular, dermal, neuromuscular, gastro-intestinal and genito-urinary tract infections. Plumbagin shows broad spectral antimicrobial activity against selected pathogenic micro-organisms *in vitro* and *in vivo*.

INTRODUCTION

The plant *Plumbago zeylanica* (Chitraka) containing active compounds plumbagin (5-hydroxy, 2-methyl, 1, 4-napthaquinone) is the derivative of napthaquinones. Chitraka has been known in an ancient Indian medicine as digestive stimulant, appetiser and anti-coagulant.

The present study deals with antimicrobial spectra with variable susceptibilities to different pathogenic strains. A wide range of antimicrobial agents have been used in the treatment of naso-pharyngeal, gastro-intestinal, dermal, respiratory, cardio-vascular and genito-urinary tract infections.

During recent years there has been an increase in bacterial resistance to the most commonly used antibacterial drugs, but plumbagin (plant extract) was a more powerful chemotherapeutic agent than the routine antibiotics (ampicillin, norfloxacin, pencillin etc.) and synthetic plumbagin. Plum bagin shows antimicrobial activities.

EXPERIMENTAL

Herbal plumbagin (2-methyl, 5-Hydroxy, 1–4-napthaquinone) was isolated from roots of *Plumbago zeylanica*. The petroleum ether extract of *Plumbago* roots contains an active compound plumbagin isolated by using TLC and column chromatography method and the fraction containing the compound was identified with the help of NMR, IR, and mass spectrum.

The antimicrobial activities of Plumbagin were tested against gram-positive

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and gram-negative bacteria and fungi. The selected pathogenic stains were collected from bacteriological proven patients of the following types of infections, and were screened against plumbagin: naso-pharyngeal, respiratory, gastro-intestinal, cardio-vascular, dermal, neuro-muscular and urinary tract infection. The selected pathogenic organisms, *viz.*, *Staphylococcus* sp., *Stretococcus* sp., *E. coli*, *Proteus* sp., *Klebsiella pneumoniae*, *Pseudomonas* sp., *Acinetobacter* sp., *Candida albicans* and *Aspergillus niger* etc. were tested by using the Standard Disc Assay method.

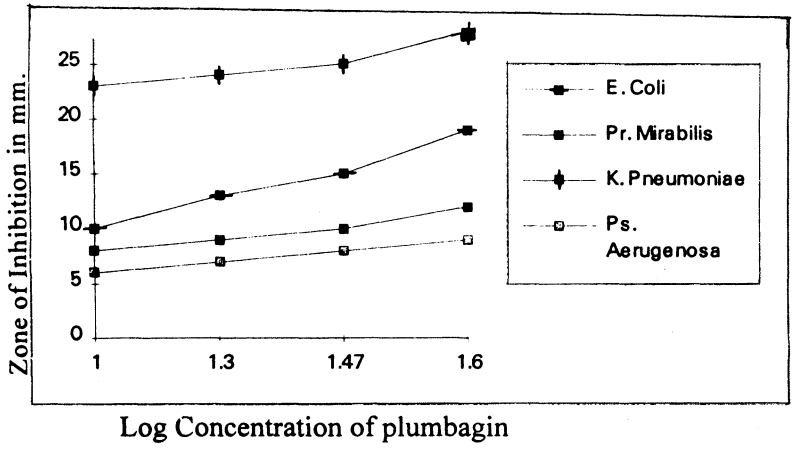
Nutrient agar and sabouraud agar were used as a test medium for bacteria and fungi respectively. Uniform lawn of each test bacterium was made on the nutrient agar plate(s) and then soaked discs (5 mm diameter) with the prepared compounds were placed over it.

The petridishes used for antimicrobial screening were incubated at 37°C for 24 h while the petridishes used for antifungal studies were incubated at 30°C or at room temperature for 48 h. The activity was measured in terms of inhibitory zones appearing around the discs by using vernier callipers (Table-1).

Minium inhibitory concentration (MIC) was determined by Serial Dilution Method with the incorporation of different known concentrations of the plumbagin in 18 h grown both cultures (10% inoculum). The inocula were incubated at different time intervals, *viz.*, 24, 48, 72 and 96 h., etc. The turbidity (optical density) was measured spectrophotometrically. Finally it was confirmed by streaking a loopful of culture on a plate(s) Table-2. It was observed that no colonies grown on a plate(s) were observed which means the values resemble for minimum inhibitory concentrations.

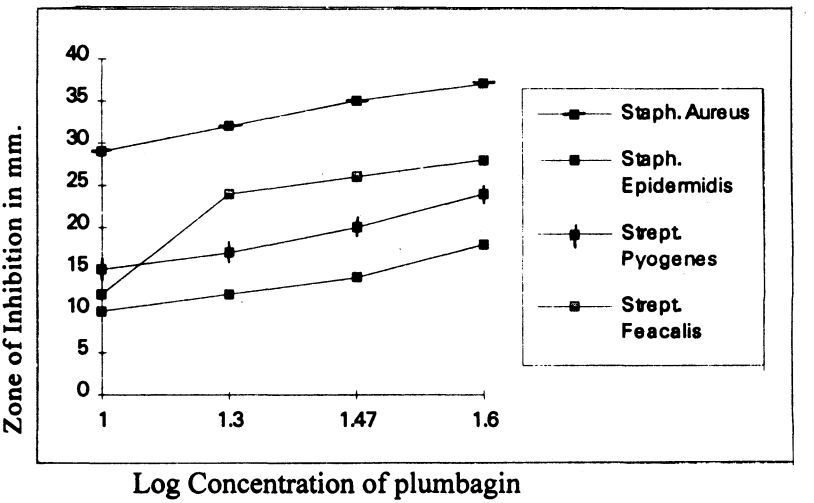
RESULTS AND DISCUSSION

It is quite evident from the present study that plumbagins are harmless and non-toxic to human and animal body, because they inhibit all the oral (perioden-

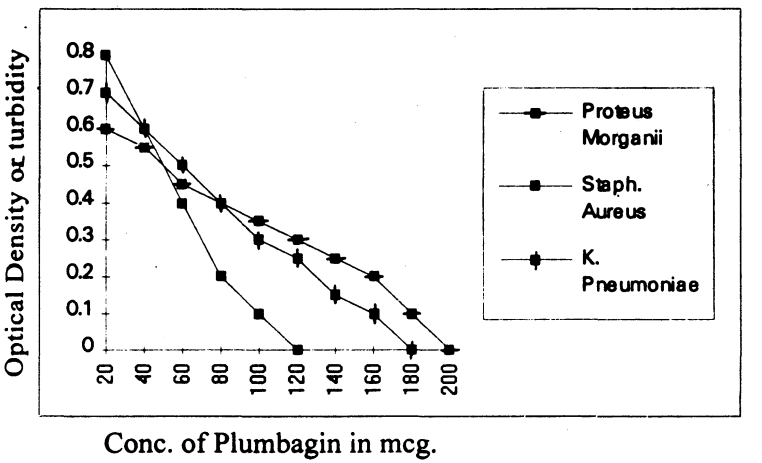


Graph 1: Log concentration of plumbagin *vs.* response Curve for four gram-negative microorganisms

tal), naso-pharyngeal, respiratory, cardio-vascular, dermal, neuro-muscular, gastro-intestinal and urinary tract infections and disorders. However, it does not affect the normal gastro-intestinal micro flora. The MIC study shows that *Staphylococcus aureus* was a more suceptible organism than *Klebsiella pneumoniae* and *Proteus morganii*.



Graph 2: Log concentration of plumbagin *vs.* response Curve for four gram-negative microorganisms



Graph 3: Minimum inhibitory conc. of plumbagin against *Pr. morganii, Staph. aureus and Klebsiella pneumoniae*

The overall study shows (Graph I–III) that the natural isolated plumbagin has broad antimicrobial activty against selected pathogenic strains *in vitro* as well as *in vivo*.

TABLE-1
ANTIBACTERIAL ACTIVITIES OF PLUMBAGIN AGAINST GRAM-NEGATIVE AND GRAM-POSITIVE BACTERIA

Disc Assay Method			
Test mdium	Nutrient agar	Inoculum	18–20 h. old broth culture
Diffusion	2 h at refrigeration temp.	Incubation	35 ± 2°C for 24 h.
Size of sterile disc	5 mm dia.		

Micro-organism	Conc. of plumbagin/zone of inhibition in mm			
	10 mcg	20 mcg	30 mcg	40 mcg
E. coli	10	13	15	19
Pr. mirabilis	08	09	10	12
Pr. morganii	12	13	15	17
Pr. rettegri	09	10	11	12
Pr. vulgaris	07	08	09	10
K. pneumoniae	23	24	25	28
Providenica sp.	13	15	17	18
Ps. aeruginosa-2521	06	07	08	09
Ps. fluorescens	00	00	07	09
Ps. aeruginosa-2496	00	00	08	09
Staph. aureus-2938	29	32	35	37
Staph. epidermidis	10	12	14	18
Staph. aureus-6571	30	32	34	36
Strept. pyogenes	15	17	20	24
Strept. viridans	20	22	24	26
Strept. faecalis	12	24	26	28
Acinetobacter sp.	20	21	22	23
Fungi *Candida albicans*	08	09	10	11

TABLE-2
MINIMUM INHIBITORY CONC. OF PLUMBAGIN AGAINST *Pr. morganii*, *Staph. aureus and Klebsiella sp.*

Stock solution	100 mcg/mL in DMSO		
Inoculum	18–20 h old broth culture	Incubation	35 ± 2°C for 24 h.
Nurtient Broth			
Lab lemco powder	10 mg	Yeast extract	10 mg
Peptone	10 mg	Sodium chloride	0.5 mg
Dist. water	1 litre	pH	7.4 to 7.5

Conc. in mcg	*Proteus morganii* OD	*Staphyloccus aureus* OD	*Klebsiella pneumoniae* OD
20	0.60	0.80	0.70
40	0.55	0.60	0.60
60	0.45	0.40	0.50
80	0.40	0.20	0.40
100	0.35	0.10	0.30
120	0.30	0.00	0.25
140	0.25	—	0.15
160	0.20	—	0.10
180	0.10	—	0.00
200	0.00	—	—

Antimicrobial study *in vivo*

Stock solution of plumbagin	100 mcg/mL in propylene glycol
Animal used	Albino mice
Wt. of mice	30–40 g
Mode of administration	ip. route
$I.D_{50}$ of plumbagin	4 mg/100 g
Pathogenic strain	0.1 mL proteus morganii 18 h Broth culture injected by ip

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