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Plasmid curing activity of plumbagin and its application in bacterial antibiotic resistance

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***Plumbago zeylanica*, a medicinal plant, contain plumbagin a naphthoquinonic compound. Partially purified plumbagin was tested for its plasmid curing activity. *E. coli*, isolated from pathological samples showed multiple antibiotic resistance. Plumbagin showed antibacterial activity at MIC value; 80 µg ml^{-1}. Antibiotics and plumbagin together decreased the antibiotic resistance pattern, suggesting plasmid curing by plumbagin. Thus, plumbagin can be used as an adjuvant to antibiotic therapy for treating infectious diseases caused by antibiotic resistant organisms.**

Keywords: *Plumbago zeylanica,* plasmid curing, plumbagin, antibiotic resistance**.**

The use of antibiotics should have created a catastrophic situation for microbial populations but the genetic flexibility allowed bacteria to survive and multiply under the antibiotic pressure. Bacteria can resist antibiotics as a result of chromosomal mutation or by exchange of genetic materials, which carry resistance genes, through transformation, transduction or conjugation by plasmids. The resistance pattern was developed because of the antibiotic modification, prevention of antibiotic entry, antibiotic efflux and alternation of antibiotic target[1].

Enterobacters, *E. coli, Klebciella* sp., *Salmonella* sp., *Shigella* sp., *Proteus* sp. etc causes diarrhea, typhoid like diseases. These microorganisms have developed resistance to large number of antibiotics. Antimicrobial agents represent the great advancement in the modern curative medicine. Recent evidences, however, point to an inexorable increase in the prevalent of microbial drug resistance apparently paralleling the expansion of the antimicrobial usage in various fields.

Medicinal plants have been traditionally used for different kinds of ailments including infectious diseases. Plumbagin, a natural medicinal compound synthesized by acetate-malonate pathway in different plants such as *Plumbago zeylanica, P. europea, P. rosea*[2], *P. indica, Dionaea muscipula* Elli[3], *Drosophyllum lusitanicum, Drosera gigantean, D. natalensis*, *D. capensis, D. rotundifblia, D. intermedia*[4], *Diospyros mespiliformis*[5], *Nepenthes rafflesiana* Jack[6], etc. Roots are the major source of this compound[7]. Many of the natural compounds have antibacterial, antifungal, antiviral etc. properties and used in traditional Indian medicine practice like Ayurveda. The roots of plant *Plumbago zeylanica* contain bioactive compound plumbagin, having potential as a chemotherapeutic agent[8]. Plumbagin has cardiotonic action[9], insecticidal activity[10], preimplementationary loss and abortion on uterine luminal proteins in albino rats[11],

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inhibitory activity of carcinoma[12] and antimicrobial activity[13,14]. The metal chelates of plumbagin with aluminium, cobalt and uranium showed considerable antibacterial activity[15]. The present work is the study of plasmid curing activity of plumbagin, isolated from *P. zeylanica* on the multiple antibiotic resistant *E. coli*.

MATERIAL AND METHODS

Microorganisms

Bacterial species, isolated from blood of a patient from pathology laboratory, Jalgaon, India. Identification and characterization was carried out according to Bergey's manual of Systematic Bacteriology[16].

Antibiotic resistance

Response of *E. coli* against multiple antibiotics was checked using antibiotic octa disk (Himedia, Bombay) on Muller Hinton agar medium at 37°C [17]. The isolates showing zone of clearance around the octa disc are sensitive while the growth around the disc indicated resistant against that antibiotic.

Plant material

The roots of *P. zeylanica* plant were collected from the campus of Charak Medicinal Plantation Field, Jalgaon (India). The extraction of plumbagin was carried out in 95% ethanol followed by diethyl ether in soxhlet extractor[18].

Minimum Inhibitory Concentration (MIC)

MIC of partially purified plumbagin was determined to test the extent to which the isolate was capable to tolerate the plumbagin. Solutions of partially purified plumbagin 10-100 μgml^{-1} were taken and inoculated with 0.1 ml of 24 h old culture of *E. coli*. The tubes were incubated at 37°C for 24h. MIC was reported as the lowest growth of *E. coli* as compared to control.

Plasmid isolation

Plasmid isolation was done by the method of alkali lysis[19].

Curing

Overnight logarithmically grown culture was used for curing experiment. The culture was inoculated in Nutrient broth embedded with different concentrations of an ethanolic extract of root powder of *P. zeylanica*, dissolved in DMSO solvent and incubated overnight at 37°C. The lowest concentration inhibiting the growth of *E. coli* was taken as minimum inhibitory concentration (MIC) value. The sub-inhibitory concentration (SIC) value was used for serial dilution and plated on nutrient agar and incubated at 37°C. Individual colonies were then replica plated on Nutrient agar medium containing tetracycline antibiotic to screen loss of plasmid encoded trait of antibiotic resistance. The loss of plasmid was confirmed by agarose gel electrophoresis of DNA of *E. coli* [20].

RESULTS AND DISCUSSION

E. coli, a pathogenic microorganism was isolated from a patient blood and identified by routine biochemical tests (Table 1).

The antibiotic sensitivity- resistance profile is summarized in Table 2 and the *E. coli* strain was found to be resistant to multiple antibiotics.

The crude extract of plumbagin from *Plumbago zeylanica* had potential antibacterial activity. 0.67g% of crude plumbagin was extracted from dried root powder.

The antibacterial activity against *E. coli* was evident by the zone of clearance of 20 mm in diameter. The crude plumbagin showed antibacterial activity against *E. coli* and other gram-negative bacteria[21].

Table 1. Biochemical characteristics of pathological isolate.

S. No.	Test	Observation
1	Gram nature	Gram negative
2	Motility	Motile
3	Indole production	+
4	Methy Red (M.R.)	+
5	Voges- Praskaur (V.P.)	-
6	Citrate utilization	-
7	Glucose utilization	Acid & gas production
8	Lactose utilization	Acid & gas production
9	Sucrose utilization	Acid production
10	Urease production	-
11	Nitrate reduction	-
12	H_2S production	+
13	Growth on MacConkey	+

+ Positive test, - Negative test

The MIC value of ethanolic extract for *E. coli* was 80 µg ml^{-1} and the SIC value was 70 µg ml^{-1}. The antibiotic resistance shown by *E. coli* may be at plasmid level. Likewise antibiotic resistance at plasmid level was observed by Shakibale *et al*.,[20]. Farr *et al*.[22] reported toxicity and mutagenicity of plumbagin and the induction of a possible new DNA repair pathway in *E. coli*. In another report plumbagin was able to modify the lactose carrier of *E. coli* and affect the binding of galactoside [23]. It was confirmed by curing the plasmid and visualizing the plasmid before and after curing under the UV light. Before curing, the one band of plasmid DNA was observed and the band was absent in the cured *E. coli* strain, in agarose gel electorphoretic pattern Fig. 1. The strain which was previously resistant to tetracycline was sensitive to the adjuvant mixture of tetracycline- plumbagin. This indicates that the partially purified plumbagin acts on plasmid DNA to inhibit the resistance potency of microorganism.

The results from the current study revealed that the naphthoquinone plumbagin could be the main constituent from root responsible for plasmid curing activity. Likewise, sensitivity index of antimicrobial agents as a simple solution for multidrug resistance in *Salmonella typhi* was reported earlier[24]. Toxicological studies on this naphthoquinone and other compounds must be performed to ensure the safety for their use.

The discovery of a potent remedy from plant origin will be a great advancement in bacterial infection therapies. Plumbagin is potent in eliminating stringent, conjugative R-plasmids at high frequency with excellence reproducibility. It is hoped that this study points another way to meet the growing problem of drug resistance and in the moment of spread of diseases like typhoid, dysentery, and diarrhoea.

Table 2. Antibiotic sensitivity- resistivity profile of *E. coli*

S. No.	Antibiotics	Antibiotic code	Concentration (µg)	Results
1	Cephotaxime	Ce	30	-
2	Cephalexin	Cp	30	+
3	Chloramphenicol	C	30	-
4	Furazolidone	Fr	50	-
5	Norfloxacin	Nx	10	+
6	Oxytetracyclin	O	30	+
7	Ampicillin	A	10	+
8	Carbenicillin	Cb	100	+
9	Co-trimazole	Cm	25	+
10	Gentamycin	G	10	+
11	Amikacin	Ak	30	-
12	Oxacillin	Ox	5	+
13	Cephoxitin	Cn	30	-
14	Ceftazidime	Ca	30	-
15	Ceftriaxone	Ci	30	-
16	Piperacillin	Pc	100	-
17	Cephalothin	Ch	30	-
18	Clindamycin	Cd	2	+
19	Erythromycin	E	15	+
20	Vancomycin	Va	30	+
21	Co-trimaxazole	Co	25	+
22	Nalidixic acid	Na	30	+

+ Resistance, - Sensitive

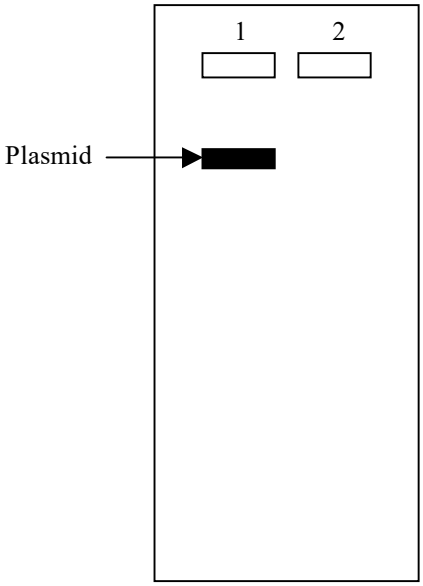

Fig. 1. Agarose gel electrophoresis pattern (sketch) of *E. coli*

1. Uncured *E. coli* plasmid DNA,
2. Cured *E. coli* plasmid DNA

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