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Screening of bactericidal activity of selected *Plumbago* species against bacterial pathogens

Abstract

The present study was undertaken to determine the antibacterial potential of *Plumbago* species viz., *Plumbago zeylanica* Linn., *Plumbago auriculata* Lam. and *Plumbago rosea* Linn. collected from various localities of South India. For the bio-efficacy studies six different extracts of *Plumbago* species with various concentrations viz., 20, 40, 60, 80 and 100µg/ml against gram positive and gram negative bacterial pathogens viz., *Staphylococcus aureus* (MTCC 737), *Streptococcus pyogenes* (MTCC 1928), *Bacillus subtilis* (MTCC 441), *Klebsiella pneumoniae* (MTCC 109), *Morganella morganii* (MTCC 662) and *Pseudomonas aeruginosa* (MTCC 1688) using well diffusion method. Among the eighteen different extracts of three different *Plumbago* species, highest frequency of antibacterial activity (54%) was recorded in *P. rosea*. The antibacterial activity of various extracts in different concentration of the selected *Plumbago* species are as follows: *P. rosea* (54%) > *P. zeylanica* (49%) > *P. auriculata* (40%). The ethanolic extract of all the *Plumbago* species revealed superior bactericidal activity compared to other tested extracts. The ethanolic extracts of *P. zeylanica, P. auriculata* and *P. rosea* showed 91%, 50% and 99% of activity against Gram positive pathogens and 66%, 26% and 89% of activity against Gram negative pathogens. The bioactive compound plumbagin and extract of aerial parts of *Plumbago species* show a wide spectrum of antibacterial activity. The compound shows promise as a new drug for various bacterial infectious diseases. Hence, this study offers a base of using *Plumbago* species as herbal alternative for the synthesis of antimicrobial agents.

Keywords: bactericidal, *Plumbago* species, antibacterial, extracts

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Introduction

Plants and plant derived metabolites are believed as rich sources of antimicrobial agents. A wide range of plants and their parts are used for their medicinal properties by local communities and folklore healers. Plants possess varieties of secondary metabolites with antimicrobial properties. The studies on plant derived polyphenolic compounds confirmed the antimicrobial, antioxidant, anticancer and apoptosis inducing properties of the plants and supplemented effective usage of medicinal herbs against microorganisms.[1,2] With the impact and less side effects, the herbal medicine is practiced as complementary and alternative medicine day by day in developing countries.[3,4] Plants and plant derived products are the cheapest, easily avialable and safer alternative sources of antimicrobials.[5-7] The phytochemical and pharmacological studies on the roots of *P. zeylanica* confirmed antiplasmodial,[8] antimicrobial,[9] antifungal,[10] anti inflammatory and anticancer,[11] antihyperglycemic,[12] hypolipidaemic and anti atherosclerotic,[13] properties of the plant.

The ethnobotanical knowledge and biological studies on the aerial parts or roots of *P. auriculata* suggested that root and aerial parts of *P. auriculata* are employed to control black water fever,[7] plant are used as anti feedant[14] and antifungal agents to control spore germination of *Macrophomina phaseolina.*[15] The ethanolic leaf extract of *P. rosea* is active against herpus simplex virus type I. The root of *P. rosea* was used to treat digestive problems, dyspepsia, colic cough and bronchitis.[16] Ibrahim et al.,[17] studied the antibacterial potentials of *P. indica*. Devi et al.,[18] screened the antibacterial efficacies of *P. zeylanica* leaf extracts. Vishnukanta & Rana[19] studied the anti convulsant activity *Plumbago zeylanica*, Jeyachandran et al.,[20] evaluated the antibacterial activity of plumbagin and root extracts of *Plumbago zeylanica*, Rahman & Anwar[21] screened the antimicrobial activity of *Plumbago zeylanica* root crude extract. Parekh & Chanda[22] studied the antibacterial potentials of *P. zeylanica*. Tharmaraj & Antonysamy[23] screened the antibacterial efficacy of *P. rosea* from Chankanachari, Kerala. Most of the studies were focused on the antimicrobial potentials of *P. zeylanica* roots only; very few reports are available on aerial parts of the selected *Plumbago* species of Tamil Nadu. There is no report on the antimicrobial actvities of *Plumbago auriculata.* There is no report on the antimicrobial actvities of *Plumbago auriculata.* To supplement the previous observations, an attempt has been made to reveal the bactericidal activity of three selected *Plumbago* species viz., *Plumbago zeylanica* Linn., *Plumbago auriculata* Lam. and *Plumbago rosea* Linn. aerial parts.

Materials and methods

Preparation of extracts

For bioefficacy studies, the aerial parts of *Plumbago zeylanica* Linn., *Plumbago auriculata* Lam. and *Plumbago rosea* Linn. were collected from Papanasam (Tamil Nadu), Tenkasi (Tamil Nadu) and Dana (Tamil Nadu) respectively. The collected *Plumbago* species were washed thoroughly with tap water followed by distilled water. The washed *Plumbago s*pecies were blotted on the blotting paper and spread out at room temperature in shade to remove the excess water contents. The shade dried plant samples were ground to fine powder using mechanical grinder. The powdered samples were stored at 4°C for further use.

The dried and powdered aerial parts of *Plumbago* species were extracted successively with 30g of plant powder and 180ml of petroleum ether, chloroform, acetone, ethyl acetate, ethanol and

water using soxhlet extractor for 8hr at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using Whattman filter paper (No.1) and then concentrated in vacuum at 40°C using rotary evaporator. The residues obtained were stored in a freezer until further tests. One gram of plant extracts were diluted with 1 ml of the respective solvents. They were used as stock solutions for the atibacterial assay.

Preparation of the test organisms

Staphylococcus aureus (MTCC 737), *Streptococcus pyogenes* (MTCC 1928), *Bacillus subtilis* (MTCC 441), *Klebsiella pneumoniae* (MTCC 109), *Morganella morganii* (MTCC 662) and *Pseudomonas aeruginosa* (MTCC 1688) were commercially purchased from Institute of Microbial Technology, Chandigarh, India. Stock cultures of different bacteria were grown in nutrient broth at 30 °C and were sub-cultured and maintained in nutrient broth at 4°C. Before swabbing, each culture was diluted (1:10) with fresh sterile nutrient broth.

Antibacterial assay

The antibacterial activity was determined by the agar well diffusion method Parekh & Chanda.[24] A suspension of the culture organism was swabbed above the solidified Muller Hinton agar medium. Wells were made using sterile cork borer under aseptic condition. One millgram of plant extracts were diluted with1 ml of the respective solvents. They were used as stock solutions for the atibacterial assay. From the stock the plant extracts (1mg/ml) various concentrations viz., 20, 40, 60, 80 and 100µg/ml were prepared and inoculated in the wells. The antibiotic amikacin (30µg /disc) was used as a standard to compare its effect on test organisms with the plant extracts. The plates were kept at room temperature for 2 h to allow diffusion of the test solution into the agar then they were incubated for 24h at 37°C. After the incubation period, the plates were observed and zone of inhibition was measured (mm) and the activities were recorded.

Results

Among the three species studied, highest frequency of antibacterial activity (54%) was recorded in *P. rosea*. The range of antibacterial activity of various extracts of *Plumbago* species are as follows: *P. rosea* (54%)>*P. zeylanica* (49%)>*P. auriculata* (40%). Major antibacterial activities were observed predominantly in ethanolic extracts (85%) of *P. zeylanica*. However the range of the inhibition zone varied with test organisms based on different concentration of extracts. Highest antibacterial activity was observed in ethanolic extract at 100µg/ml followed by petroleum ether and chloroform extract. There was minor difference on the size of the inhibition zone between ethanolic and other extracts of *P. zeylanica*. The range of inhibitory activity was less at lower concentration of extracts (Table 1). Ethanolic extracts of *P. zeylanica* was active against all the selected bacterial pathogens except *B. subtilis* which represents no zone of inhibition at 20-40µg/ml concentration. Compared to standard amikacin (30µg), 100µg/ml of ethanolic extract showed more antibacterial (90% and 5%) activity against *S. pyogenes* and *K. pneumonia* respectively (Table 1). The ethanolic extracts of *P. zeylanica* showed higher antibacterial activity against pathogens *K. pneumonia* and *S. pyogenes*. The petroleum ether extract of *P. zeylanica* also expressed maximum zone of inhibition (20±0.5 mm) against *S. pyogenes*. The acetone and aqueous extracts of *P. zeylanica* failed to show the antibacterial activity against *S. aureus*. Similar to that, the petroleum ether, acetone and aqueous extracts of *P. zeylanica* were also unsuccessful against *B. subtilis*. The antibacterial activity of *P. zeylanica* extracts at different concentrations are arranged as follows: ethanolic extracts (85%)>petroleum ether (69%)>chloroform (62%)>acetone (53%)>ethyl acetate (34%)>aqueous extract (31%). Ethanolic extracts of *P. zeylanica* showed 91% percentage of activity against Gram positive pathogens and 66% of activity against Gram negative pathogens.

Table 1 Antibacterial activity of *Plumbago zeylanica*

Pathogens	Zone of inhibition in mm							
	Extracts Conc. in µg	P	C	A	EA	E	AQ	Amikacin 30µg
S. aureus	20	10±0.2	6±0.3	nil	nil	6±0.3	nil	24
	40	15±0.3	9±0.3	nil	nil	10±0.5	nil	
	60	17±0.3	10±0.5	nil	3±0.3	15±0.3	nil	
	80	18±0.4	13±0.5	nil	4±0.3	18±0.5	nil	
	100	19±0.3	16±0.5	nil	6±0.5	20±0.5	nil	
B. subtilis	20	nil	5±0.3	nil	6±0.5	nil	nil	25
	40	nil	8±0.5	nil	8±0.3	nil	nil	
	60	nil	10±0.5	nil	10±0.4	5±0.3	nil	
	80	nil	12±0.3	nil	12±0.3	7±0.5	nil	
	100	nil	13±0.5	nil	16±0.3	9±0.3	nil	
S. pyogenes	20	14±0.3	nil	5±0.3	nil	15±0.3	nil	11
	40	16±0.3	nil	10±0.3	nil	18±0.5	nil	
	60	17±0.3	nil	12±0.5	nil	19±0.3	3±0.5	
	80	19±0.5	5±0.3	14±0.3	nil	20±0.5	4±0.5	
	100	21±0.5	7±0.5	18±0.5	nil	21±0.3	6±0.5	

Citation: Tharmaraj RJJM, Antonysamy JM. Screening of bactericidal activity of selected Plumbago species against bacterial pathogens. *J Microbiol Exp.* 2015;2(6):194–200. DOI: 10.15406/jmen.2015.02.00070

Table continued

Pathogens	Zone of inhibition in mm							
	Extracts Conc. in µg	P	C	A	EA	E	AQ	Amikacin 30µg
	20	3±0.3	5±0.3	3±0.3	4±0.3	17±0.3	nil	
	40	4±0.3	10±0.3	6±0.3	6±0.5	19±0.3	nil	
K. pneumoniae	60	6±0.3	12±0.5	8±0.3	7±0.5	20±0.5	3±0.5	21
	80	8±0.3	14±0.3	10±0.3	10±0.3	21	5±0.5	
	100	10±0.3	18±0.5	12±0.5	12±0.5	22±0.3	7±0.5	
	20	nil	12±0.3	nil	nil	8±0.3	8±0.2	
	40	nil	16±0.3	5±0.5	nil	12±0.3	9±0.2	
M. morganii	60	nil	17±0.5	8±0.5	nil	15±0.5	10±0.2	20
	80	3±0.3	19±0.5	9±0.2	nil	16±0.5	12±0.2	
	100	6±0.5	21±0.5	11±0.3	nil	19±0.5	14±0.5	
	20	7±0.3	nil	nil	3±0.5	2±0.3	nil	
	40	9±0.5	nil	2±0.3	4±0.3	4±0.3+	nil	
P. aeruginosa	60	11±0.5	nil	5±0.5	7±0.5	7±0.5	nil	24
	80	12±0.5	nil	8±0.3	8±0.5	10±0.3	nil	
	100	16±0.5	nil	10±0.3	10±0.3	12±0.3	nil	

Note: P, Petroleum ether; C, Chloroform; A, Acetone; EA, Ethylacetate; E, Ethanol; AQ, Water

Similar to *P. zeylanica*, highest activity was observed in ethanolic extracts (70%) of *P. auriculata* followed by petroleum ether and chloroform extracts. There was distinguished difference on the size of the inhibition zone between ethanolic and other extracts of *P.auriculata.* The range of inhibition was directly coincided with the concentrations of extracts tested, less inhibition was observed at lower concentration of extracts and maximum inhibition was obtained in higher concentrations (Table 2).

Table 2 Antibacterial activity of *Plumbago auriculata*

Pathogens	Zone of inhibition in mm							
	Extracts Conc. in µg	P	C	A	EA	E	AQ	Amikacin 30µg
	20	4±0.3	2±0.3	nil	4±0.3	nil	nil	
	40	6±0.3	4±0.3	nil	7±0.3	nil	nil	
S. aureus	60	8±0.3	7±0.7	nil	8±0.3	nil	nil	24
	80	10±0.3	9±0.5	nil	11±0.3	nil	3±0.5	
	100	12±0.3	11±0.3	nil	13±0.3	nil	4±0.3	
	20	6±0.3	nil	1±0.3	2±0.3	4±0.3	nil	
	40	8±0.4	nil	2±0.3	4±0.3	6±0.3	nil	
B. subtilis	60	10±0.3	6±0.5	4±0.3	5±0.3	7±0.5	1±0.3	25
	80	13±0.5	9±0.5	6±0.3	7±0.2	13±0.3	4±0.3	
	100	15±0.3	10±0.3	8±0.3	10±0.3	16±0.3	6±0.3	
	20	nil	nil	4±0.3	10±0.5	4±0.3	nil	
	40	nil	nil	6±0.3	13±0.3	6±0.5	nil	
S. pyogenes	60	nil	nil	7±0.3	14±0.3	7±0.5	3±0.5	11
	80	nil	nil	10±0.3	16±0.3	9 ±0.3	4±0.5	
	100	nil	nil	12±0.3	18±0.3	13±0.5	6±0.5	

Citation: Tharmaraj RJJM, Antonysamy JM. Screening of bactericidal activity of selected Plumbago species against bacterial pathogens. *J Microbiol Exp.* 2015;2(6):194–200. DOI: 10.15406/jmen.2015.02.00070

Table continued

Pathogens	Zone of inhibition in mm							
	Extracts Conc. in µg	P	C	A	EA	E	AQ	Amikacin 30µg
	20	7±0.5	4±0.3	nil	4±0.3	13±0.5	nil	
	40	9±0.7	6±0.3	nil	6±0.5	15±0.3	nil	
K. pneumoniae	60	14±0.3	8±0.3	3±0.3	7±0.5	17±0.3	3±0.5	21
	80	18±0.3	11±0.3	6±0.3	10±0.3	19±0.3	5±0.5	
	100	20±0.5	14±0.3	7±0.3	13±0.3	23±0.3	7±0.5	
	20	3±0.5	2±0.3	nil	nil	nil	nil	
	40	5±0.5	4±0.3	nil	nil	2±0.3	nil	
M. morganii	60	8±0.3	6±0.5	nil	nil	4±0.3	nil	20
	80	10±0.3	8±0.3	3±0.3	nil	6±0.3	nil	
	100	14±0.5	11±0.5	4±0.3	nil	8±0.3	nil	
	20	6±0.3	nil	11±0.3	nil	2±0.3	2±0.3	
	40	9±0.3	nil	14±0.3	nil	3±0.3	4±0.3	
P. aeruginosa	60	11±0.3	nil	16±0.3	nil	5±0.5	7±0.5	24
	80	14±0.3	nil	18±0.3	nil	7±0.3	10±0.3	
	100	16±0.3	nil	20±0.3	nil	10±0.3	12±0.3	

Note: P, Petroleum ether; C, Chloroform; A, Acetone; EA, Ethylacetate; E, Ethanol; AQ, Water

The ethanolic extracts of *P. auriculata* demonstrated maximum zone of inhibition (23±0.3 mm) against *M. morganii* (Table 2). *P. auriculata* ethanolic extracts was active against all the examined pathogens except *S. aureus*. Compared to all the tested pathogens, *K. pneumoniae* was highly sensitive to all the screened extracts of *P. auriculata.* The 40-100µg/ml of ethyl acetate extracts represented more percentage of activity (18, 27, 45 and 63%) against *S. pyogenes* than the standard amikacin. 100µg/ml ethanolic extracts represented 18% and 9% of more antibacterial activity against *S. pyogenes* and *K. pneumoniae* than the standard amikacin. Similar to ethyl acetate and ethnaolic extracts, acetone extracts also showed more activity against *S. pyogenes* than the standard amikacin.

The significant antibacterial activity was observed in ethanolic extracts compared to other tested extracts of *P. auriculata*. The activity of *P. auriculata* extracts as follows: ethanolic extracts 70%>petroleum ether 54%>ethyl acetate 53%>acetone 48%>chloroform 34%>water extract 31%. The ethanolic extracts of *P. auriculata* showed above 50% activity against Gram positive pathogens and 26% activity against Gram negative pathogens.

In *P. rosea,* highest activity was observed in ethanolic extracts (91%) followed by acetone (64%) and chloroform extracts (59%). The ethanolic extracts 100µg/ml showed 45% higher antibacterial activity against *S. pyogenes* and 10% more activity against *M. morganii* than the standard amikacin. There was prominent difference on the size of the inhibition zone between ethanolic and other extracts of *P. rosea*. All the screened extracts of *P. rosea* demonstrated the inhibition against *K. pneumoniae* (Table 3). Highest antibacterial activity was observed in ethanolic extracts against *B. subtilis* and *M. morganii* with 22±0.3 and 22±0.7 mm zone of inhibition. Except 20µg/ml of water extracts, all other extracts of *P. rosea* with various concentrations (20-100µg/ml) showed activity against *K. pneumonia* and maximum zone of inhibition (20±0.3 mm) was observed in ethanolic extracts of *P. rosea.* The 100µg/ml of *P. rosea* ethanolic extracts displayed higher activity against *M. morganii* with 22±0.5 mm zone of inhibition.

Table 3 Antibacterial activity of *Plumbago rosea*

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Pathogens	Zone of inhibition in mm							
	Extracts Conc. in µg	P	C	A	EA	E	AQ	An 30µg
	20	7±0.5	nil	2±0.5	5±0.3	nil	nil	
	40	11±0.5	nil	9±0.3	10±0.3	4±0.3	nil	
S. aureus	60	14±0.5	nil	10±0.2	11±0.3	6±0.5	nil	24
	80	15±0.7	nil	12±0.3	13±0.3	8±0.5	nil	
	100	18±0.3	nil	14±0.3	16±0.3	9±0.3	nil	

Citation: Tharmaraj RJJM, Antonysamy JM. Screening of bactericidal activity of selected Plumbago species against bacterial pathogens. *J Microbiol Exp.* 2015;2(6):194–200. DOI: 10.15406/jmen.2015.02.00070

Table continued

Pathogens	Zone of inhibition in mm							
	Extracts Conc. in µg	P	C	A	EA	E	AQ	An 30µg
	20	3±0.3	nil	4±0.5	6±0.5	15±0.3	nil	
	40	6.5±0.7	nil	6±0.5	8±0.3	19±0.3	3±0.3	
B. subtilis	60	7±0.4	nil	11±0.5	10±0.4	20±0.7	4	25
	80	11±0.5	nil	14±0.3	12±0.3	21±0.3	5±0.3	
	100	13±0.3	nil	17±0.3	14±0.3	22±0.3	7±0.3	
	20	nil	nil	8±0.3	4±0.1	3±0.3	nil	
	40	nil	4±0.3	10±0.5	7±0.5	6±0.3	nil	
S. pyogenes	60	nil	6±0.5	12±0.3	8±0.3	7±0.5	nil	11
	80	nil	8±0.3	14±0.3	12±0.5	9±0.5	nil	
	100	nil	9±0.5	16±0.3	13±0.5	10±0.5	nil	
	20	10±0.5	7±0.4	8±0.3	10±0.3	11±0.3	nil	
	40	13±0.5	12±0.3	12±0.3	12±0.3	14±0.3	5±0.3	
K. pneumoniae	60	16±0.8	14±0.3	13±0.5	13±0.5	16±0.5	6±0.3	21
	80	17±0.4	16±0.3	15±0.3	16±0.3	18±0.5	6±0.5	
	100	19±0.5	17±0.3	16±0.5	18±0.3	20±0.5	8±0.5	
	20	6±0.5	9±0.3	nil	nil	12±0.3	nil	
	40	11±0.5	12±0.3	nil	nil	16±0.3	2±0.3	
M. morganii	60	12±0.3	14±0.5	nil	nil	18±0.3	3	20
	80	14±0.3	16±0.4	nil	nil	20±0.3	4±0.5	
	100	16±0.3	18±0.3	nil	nil	22±0.7	5±0.5	
	20	nil	4±0.5	8±0.3	3±0.3	7±0.5	nil	
	40	nil	9±0.5	10±0.3	7±0.3	9±0.5	nil	
P. aeruginosa	60	nil	10±0.3	13±0.3	8±0.3	13±0.5	nil	24
	80	nil	13±0.3	15±0.2	11±0.5	14±0.5	nil	
	100	nil	16±0.5	18±0.4	13±0.4	17±0.5	nil	

Note: P, Petroleum ether; C, Chloroform; A, Acetone; EA, Ethylacetate; E, Ethanol; AQ, Water

Similar to other two studied plants, the highest zone of inhibition was observed in ethanolic extracts compared to other tested extracts of *P.rosea.* The activity of *P. rosea* extracts as follows: ethanolic extracts 91% >acetone; 64%>chloroform; 56%>ethyl acetate; 52%>petroleum ether; 50%>water extract. Ethanolic extract of *P. rosea* showed Gram 99% activity against positive pathogens and 89% activity against Gram negative pathogens.

Discussion

Due to the failure of synthetic drugs, adverse side effects of antibiotics and antibiotic resistance of the microorganisms led to the development of new plant derived antibiotic without any side effects.[3,6,25,26] Simonsen et al.,[8] documented the use of natural products as new antibacterial drugs. In the present study also we screened the antibacterial potentials of *P. rosea*, *P. zeylanica* and *P. auriculata* against various bacterial pathogens. The ethanolic extract of *P. zeylanica* and *P. rosea* showed high frequency of activity against Gram positive pathogens and Gram negative pathogens. The ethanolic extracts of *P. auriculata* showed leact activity against the tested pathogens. The antibacterial potentials of the *P. rosea*, *P. zeylanica* and *P. auriculata* against the tested pathogens may be due to the existence of alkaloids, phenolic substantces.[27]

Antimicrobial activities of *Plumbago* species have been reported by many workers, Ibrahim *et al*.,[17] evaluated the antibacterial activity using the methanolic extracts of *P. indica* against *S. aureus, S. typhi, S. dysenteriae, B. cereus P. aeruginosa, S. sonnei, V. cholera* and *E. coli.* The highest inhibition was observed against *S. aureus, E. coli* and *S. typhi* compared to other pathogens ranged from 15-27 mm. Devi et al.,[28] studied the antibacterial activity of *P. zeylanica* methanolic leaf, root and stem extract of against *Bacillus subtilis*. Antibacterial

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activity of methanolic and chloroform extracts of *P. zeylanica* against five different organisms viz., *S. pyogenes*, *S. aureus*, *Bacillus* sp., *P. aeruginosa* and *E. coli* were studied using disc diffusion method. The methanolic extracts were more active against all the tested organisms.[18] Vishnukanta & Rana[29] studied the antibacterial activity of *P. zeylanica* against *S. gallinarium, E. coli, P. vulgaris, S. typhimurium, P. aeruginosa* and *S. aureus*. Among the tested extracts methanolic extract exhibited higher antibacterial activity against all the pathogenic bacteria.

Jetty et al.,[30] evaluated the antimicrobial properties of compounds such as neoisoshinanolone and 1-epineo-isoshinanolone isolated from the roots of *P. zeylanica*. Among these 1-epineo-isoshinanolone is more active with a MIC of 12.5-25µg/mL whereas neoisoshinanolone has recorded a MIC of 50-100µg/mL. The activities are compared with plumbagin [0.78-3.13µg/mL] and standards streptomycin for bacteria and nystatin for fungi. Jeyachandran et al.,[20] revealed the minimum inhibitory concentration of methanolic, chloroform and aqueous extract of *P. zeylanica* root against *E. coli*, *S. typhi* and *S. aureus*. The zone of inhibition against *K. pneumoniae*, *S. marcescens* and *B. subtilis* were moderate and lower against *Proteus vulgaris* and *Pseudomonas aeruginosa*. The methanolic extract exhibited moderate activity and the aqueous extract weak activity against bacterial strains as assessed by disc diffusion assays.

Rahman & Anwar[21] studied the antimicrobial activities of ethanolic extracts of *P. zeylanica* root against 11 human pathogenic bacteria and 6 phytopathogenic fungi using disc diffusion method. *V. cholerae* was found to be the most sensitive. Parekh et al.,[24] studied the antibacterial potential of *P. zeylanica* using agar disc diffusion method and agar well diffusion method against five bacterial strains viz*., B. cereus, S. aureus, K. pneumoniae, E. coli* and *P. pseudoalcaligenes*. Preliminary screening revealed that methanolic extracts were more potent than the aqueous extracts. Wang & Huang[31] revealed the anti-*H. pylori* activity of ethanolic, ethyl acetate, acetone and aqueous extracts of *P. zeylanica*.

Paiva et al.,[14] evaluated the antimicrobial activity in the plumbagin isolated from the chloroform extract of *P. scandens* against *S. aureus*, *P. aeruginosa*, *B. subtilis*, *P. vulgaris* and against the yeast *C. albicans*. Plumbagin exhibited relatively specific antimicrobial activity. The growth of *S. aureus* and *C. albicans* was completely inhibited. Antibacterial activity of *P. zeylanica* alcoholic root extracts was studied against multidrug-resistant clinical isolates of bacteria (*S. paratyphi*, *S. aureus*, *E. coli*, *S. dysenteriae*). The extracts exhibited strong antibacterial activity against all test bacteria irrespective of their antibiotic resistance behaviour.[9] Except Wang & Hang et al.,[31] observation, all others were recorded the highest frequency of antibacterial activity in the methanolic extracts of *Plumbago* species. Tharmaraj & Antonysamy[23] observed the antibacterial activity of *P. rosea* against *K. pneumoniae*, *B. subtilis, S. aureus, P. aeruginosa* and *P. vulgaris* with maximum zone inhibition 20, 19, 17, 16 and 16 mm respectively. In the present study we observed better results then the previous observations. 100µg of ethanolic extracts of *P. rosea* showed maximum zone of inhibition against *B. subtilis* and *K. pneumoniae* with 22 and 20 mm respectively. 100µg of acetone extracts of *P. rosea* demonstrated maximum zone (18 mm) of inhibition against *P. aeruginosa* earlier 16 mm of inihibition was observed with 250µg of methanolic extracts. In addition, the ethanolic and acetone extracts of *P. rosea* illustrated the inhibition against *M. morganii* (22 mm) and *S. pyogenes* (16 mm) respectively.

Similar to that in the present study also we observed the high frequency of antibacterial activity in the ethanolic extracts of three *Plumbago* species. The antibacterial action of various aerial parts extracts of *Plumbago* species may indicate their potential as antibacterial herbal remedies. Further work is needed to locate the active principle from the various extracts and their phyto pharmaceutical studies. Research into the effects of local medicinal plants is expected to boost the use of these plants in the therapy against disease caused by the test bacterial species and other microorganisms.

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Conflicts of interest

Authors declare that there is no conflict of interest.

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