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STUDIES ON ANTIBACTERIAL AND PHYTOCHEMICAL POTENTIAL OF *PLUMBAGO ZEYLANICA* LINN. (PLUMBAGINACEAE) FROM JHARGRAM DISTRICT, WEST BENGAL, INDIA

DUTTA DAS S[1], DAS PK[2], SINHA SK[3] AND BANERJEE N[4*]

1: Centre for Life Sciences, Vidyasagar University, West Bengal

2: Department of Botany, Uluberia College (University of Calcutta), Uluberia, Howrah-711315, West Bengal

3: Department of Human Physiology, Vidyasagar University, Midnapore-721102, West Bengal

4: Department of Botany and Forestry, Vidyasagar University, Midnapore-721102, West Bengal

***Corresponding Author: Dr. Nilanjana Banerjee: E Mail: nilanjana@mail.vidyasagar.ac.in; soma.dbot@gmail.com**

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ABSTRACT

The antibacterial activity of *Plumbago zeylanica* Linn. (Plumbaginaceae) leaf, stem, flower and root extract was evaluated on microbial strains like gram positive species *Staphylococcus aureus* and gram-negative species *Pseudomonas aeruginosa.* The in vitro antibacterial assay was done by agar well diffusion method. Different solvents like ethanol, methanol, acetone, hexane and water were used for extraction of plant. The acetone and ethanol extracts of *Plumbago zeylanica* Linn. Root shows maximum antibacterial activity. Different phytoconstituents like alkaloid, phenols, flavonoids, and terpenoids were present in different solvent extracts of the plant. These phytochemicals may be responsible for antibacterial activity.

Keywords: Ethnomedicinal plants, antibacterial, gram positive, gram-negative, phytochemicals

INTRODUCTION:

India is rich in biodiversity and medicinal plant resources. According to WHO, about 80% of the world's population living in rural areas depend on traditional herbal medicines

for primary health care **[1]**. The age-old traditional system of medicine, namely Ayurveda, Unani, Siddha and Homoeopathy, etc. are based on herbal formulation **[2-4]**. Medicinal plants produce a huge range of bioactive molecules making them a wonderful source of herbal drugs. The Secondary metabolites are responsible for medicinal activity of plants **[5]**. According to WHO, the traditional medicine and the traditional formulation of herbal drugs mainly involved the use of plant extract or their active constituents **[6]**. Jhargram district is a wonderful source of medicinal plants. About 85% of the rural tribal people of Jhargram district are closely associated with nature and mainly depend on wild plants for the treatment of different human diseases. The plant *Plumbago zeylanica* belongs to the family Plumbaginaceae, commonly known as Swetchita and it is a very common medicinal plant in lateritic zone of Jhargram district. *Plumbago zeylanica* was used for the treatment of different human ailments like tumor, piles, wounds, boils, blister, carbuncle, skin disease, leucoderma and cancer. The different plant parts like leaf, stem, flower and root are widely used and play dominant role against different disease-causing pathogenic bacteria and fungi. The pharmacological and phytochemical studies have indicated that different solvent extracts of *Plumbago zeylanica* has antimicrobial **[7-10]**, antidiabetic, antiulcer, antiobecity, antihyperlipidemic, wound healing, nephroprotective, antiulcer, antifertility, anticancer and anthelmintic activities **[10]**, Antiinflamatory **[11]**, antioxidant **[12]**, hepatoprotective **[13]**. The present study was carried out to screen the phytochemical components present and to evaluate the antibacterial activity of ethanol, methanol, acetone, n-hexane and water extracts of leaves, stem, flower and root of *Plumbago zeylanica.* The plant *Plumbago zeylanica* was selected on the basis of their traditional medicinal importance.

MATERIALS AND METHODS:

Plant Sample collection

Plant samples (**Figure 1)** were freshly collected during flowering season from their wild habited at Jhargram district. It was washed under running tap water and shade dried for 6-7 days. The dried plant samples were grinded to make into fine powder and stored in airtight container at room temperature for future usage.

Preparation of plant extract

About 10 gm of grinded fine powder of different parts like leaf, stem, flower and root was soaked in 100 ml of different solvents like ethanol, methanol, acetone, n- hexane and

water and it was placed on a rotary shaker for 24 hours. The extract was filtered using Whatman no. 1 filter paper and the filtered extracts were evaporated to dryness using rotary flask evaporator. The aqueous extract was placed in a lyophilizer to yield crude plant extracts. Dried plant extracts were stored in airtight bottles and placed in a refrigerator for phytochemical analysis and antibacterial screening. The dried alcoholic extract was redissolved in DMSO and the other solvent extracts i.e. acetone and n-hexane were re dissolved in respective solvents and to get the final concentration of each stock solution in different extracts were 10 mg/ml which was used for antibacterial screening.

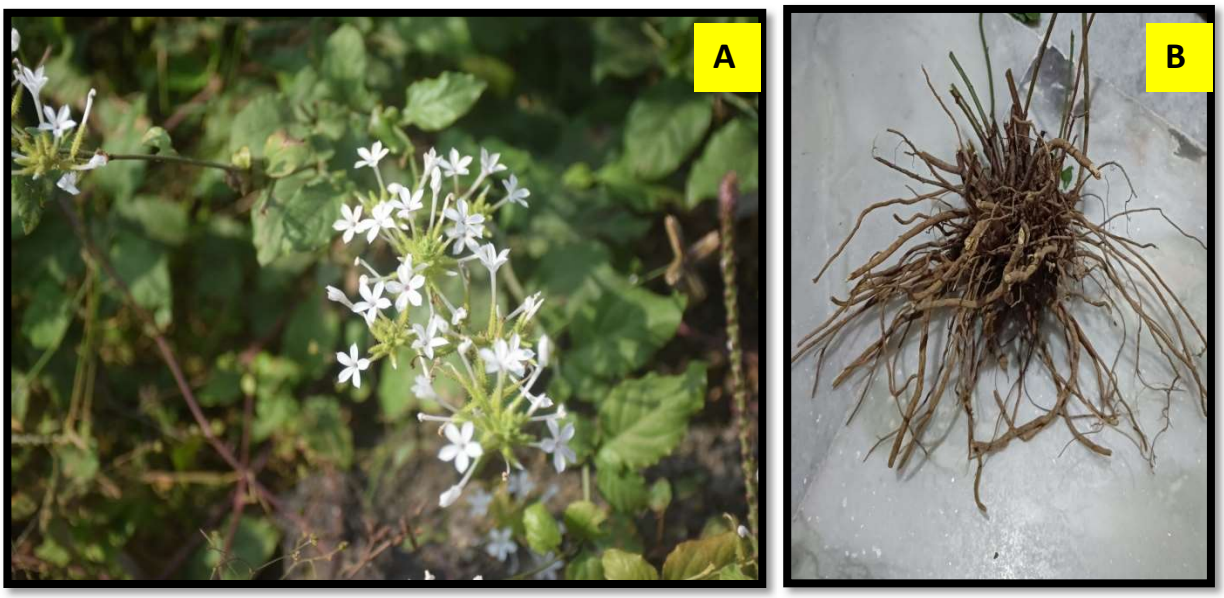

Figure 1: A. The plant *Plumbago zeylanica*; B. Root of *Plumbago zeylanica*

Phytochemical Screening:

A. Preliminary qualitative phytochemical analysis

Preliminary qualitative phytochemical analysis was carried out following the standard methods **[14, 5]**

B. Quantitative phytochemical analysis

Estimation of total phenolic content

Estimation of the total phenolic content was determined by Folin-ciocalteu reagent method using gallic acid as standard. Test tubes were filled with 1 ml of plant extract (1 mg/ml) and then mixed with 1 ml of the Folin-Ciocalteu reagent, 10% Na_2CO_3 (1ml) and 7 ml of DH_2O and the final volume was made up to 10 ml then incubated in dark condition at room temperature for 25 minute. Gallic acid used as a standard and it was prepared following same procedure. The OD value was measured by UV-VIS spectrophotometer at 765 nm against

blank. The total phenol content was determined in milligrams of gallic acid equivalent/ gm of sample.

Estimation of total flavonoids

The total flavonoids were determined by $AlCl_3$ method. Aluminium chloride method was used to determine the total flavonoids with using quercetin as standard. Test tubes were filled with 1 ml of plant extract and then mixed with 1 ml of 10% $AlCl_3$, 1 ml of $NaNO_3$ (5%), 6.7ml of 1mM NaOH was vortex and incubated in dark condition at room temperature for 25 min. Standard quercetin was prepared following same procedure. The OD value was measured by spectrophotometer at 540 nm against blank. The total flavonoids content was determined in milligram of quercetin equivalent/ gm of sample.

In-vitro Antibacterial assay:

Bacterial strains and culture conditions:

The bacterial strains like *Staphylococcus aureus* (*S. aureus* MTCC 87) and *Pseudomonas aeruginosa* (MTCC 741) were taken for antibacterial screening. They were cultured in nutrient broth media in aerobic condition at 37.9°C.

Antibacterial assay

Antibacterial activity assay was done by Agar well diffusion method (Bauer *et al.* 1966) **[15]**. A stock solution (10 mg/ml) was prepared by dissolving 100 mg of extract in 10 ml of their respective solvents. For alcoholic extracts 10% of DMSO was used. The wells were filled with separate concentrations (5µl,10µl,25µl,50µl,100µl) of the extract prepared from the stock solution. The sterile antibiotic disc i.e. Gentamycin (10mcg/disc) was placed on Mueller-Hinton Agar plate in an aseptic condition. It was used as a positive control for comparative study and respective solvents were used as a negative control and DMSO was used as a negative control for alcoholic extracts of the plant. The plates were placed on an incubator at 37˚C for 24 hours. The antibacterial activity was recorded by measuring the diameter (mm) of clear zone of inhibition.

RESULTS AND DISCUSSIONS:

Preliminary Qualitative phytochemical analysis

The results showed that the different solvent like ethanol, methanol, acetone, N-hexane and water extracts of leaf, stem, flower and root of *Plumbago zeylanica* contained phytochemically important compounds like alakaloids, flavonoid, phenol, saponin, tannin and terpenoids (**Table 1**). The Ethanol, methanol, acetone and n-hexane extracts of root and leaves were showed the presence of rich variety of secondary metabolites while stem and flowers showed the presence of less

variety of secondary metabolites. Water extracts of all the usable parts *P. zeylanica* showed the less variety of secondary metabolites. The presence of these phytoconstituents may be used as a novel source of modern medicine. The plant *P. Zeylanica* has rich variety of phytochemicals and it is a natural gift to human being **[16]**.

Table 1: Phytochemical analysis of different parts of *Plumbago zeylanica* by using different solvents

Plant species	Usable parts	Solvent used	Phytochemicals								
			Alk	Car	Fla	Phe	Phlo	Sap	Tan	Ter	Cou
Plumbago zeylanica	*Root*	Hexane	+	++	+	+	-	+	-	+	-
		Acetone	++	+++	+	+	-	-	-	++	-
		Ethanol	+++	+++	++	+++	-	+	-	+++	+
		Methanol	+++	++	++	++	-	+	++	+++	+
		Water	+++	+++	+	-	-	+++	+	-	+++
	Leaf	Hexane	-	+++	+	++	-	-	-	-	+
		Acetone	+	++	++	+++	+	-	++	-	-
		Ethanol	++	+	+	++	+	++	++	-	+
		Methanol	+++	-	+	++	-	-	++	-	-
		Water	+	+++	-	-	-	+++	++	-	-
	Stem	Hexane	-	+++	-	-	-	-	-	-	-
		Acetone	+	-	-	-	-	-	-	-	-
		Ethanol	++	+++	+++	-	-	-	-	-	+
		Methanol	++	-	-	+	-	+++	-	-	++
		Water	+++	+++	-	-	+	++	++	-	-
	Flower	Hexane	-	+++	-	-	-	-	-	-	-
		Acetone	+	-	-	-	-	-	-	-	-
		Ethanol	+	+	-	-	-	-	-	-	++
		Methanol	++	-	-	-	-	-	-	-	++
		Water	+	+++	+	-	+	+++	++	-	++

Quantitative phytochemical analysis of total phenol and flavonoid content

Quantitative analysis of the total phenol was determined using Folin-ciocalteu reagent, with galic acid as standard (**Figure 2)**. The result (**Table 2)** showed that the total phenol contents of different solvent extracts of *P.zeylanica* leaf, stem, flower and root were in the range of 5.43-61.2µg/mg dry weight of the extract. The highest amount of phenol (61.2 µg/mg extract) was present in methanol extracts of root and least amount of phenol (5.43µg/mg extract) was present in hexane extract of flower. Phenolic compounds are diverse compounds and it was reported to have potential antibacterial properties **[17-19]**. The total flavonoid contents were determined using by $AlCl_3$with Quercetin as a standard (**Figure 3)**. The results (**Table 2)** showed that the total flavonoid contents of different solvent extracts of *P. zeylanica* leaf, stem, flower and root were in the range of 1.42-62.73µg/mg dry weight of the extract. The highest amount of flavonoid (62.73µg/mg extract) are present in acetone extract of leaf and least amount of flavonoid (1.42µg/mg extract) are present in water extracts of root.

Flavonoids play a big role in plant defense system and are involved in various biological activities including antimicrobial, antitumor, anti-inflammatory, anti-carcinogenic activity **[17, 19]**. Quality and quantity of phytochemicals are significant in medicinal field.

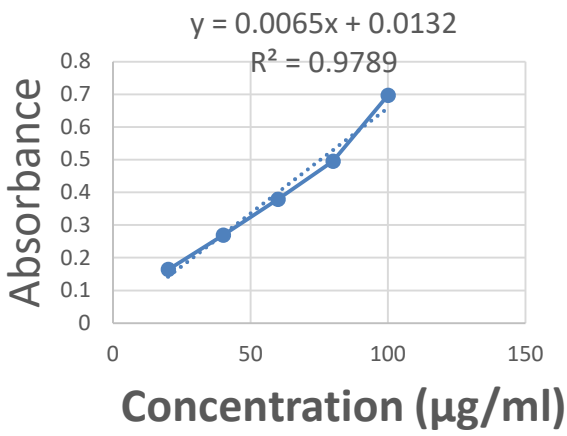

Figure 2: Gallic acid standard curve

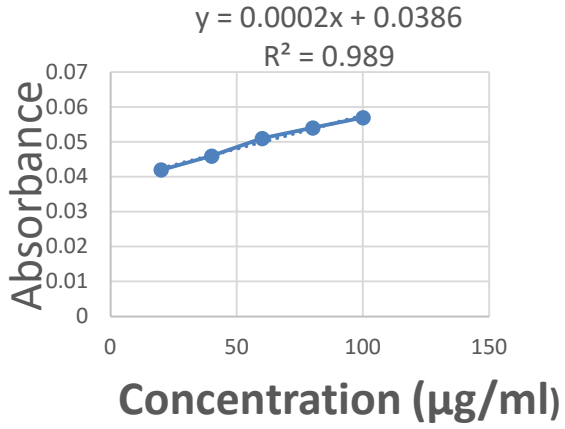

Figure 3: Quercetin standard curve

Table 2: Total phenol and flavonoid contents in different solvent extracts of *P. zeylanica*

Plant species	Usable parts	Solvent used	Total phenol content mg of GAE/gm sample	Total flavonoid content mg of QUE/gm sample
Plumbago zeylanica	*Root*	Ethanol	48.43	20.89
		Methanol	61.2	30.93
		Acetone	14.58	1.947
		Hexane	6.43	15.63
		Water	-	1.42
	Leaf	Ethanol	18.68	7.47
		Methanol	28.69	18
		Acetone	53.03	62.73
		Hexane	17.04	7.21
		Water		-
	Stem	Ethanol	16.58	11.94
		Methanol	12.58	-
		Acetone	11.03	-
		Hexane	7.58	-
		Water		-
	Flower	Ethanol	11.2	-
		Methanol	13.58	-
		Acetone	7.42	-
		Hexane	5.43	-
		Water	-	6.68

In-vitro antibacterial assay

The different solvent extracts (ethanol, methanol, acetone, N-hexane and water) of *Plumbago zeylanica* root, leaves, stem and flowers were screened for antibacterial activity against Gram- positive bacteria

named *Staphylococcus aureus* and gram-negative bacteria named *Pseudomonas aeruginosa* (**Figure 4)**. Among all the tested plant samples, highest antibacterial activity was observed in acetone extract of root (25.5 mm) followed by ethanol (24mm), n-hexane (18mm), methanol (17.5mm) and water (5mm) extract at 1000µg/ml against *Staphylococcus aureus*. The ethanol extract of root showed highest antibacterial activity (20.3 mm) followed by methanol (20mm) and acetone extract (16.5mm) at 1000µg/ml against *Pseudomonas aeruginosa* but n-hexane and water extract of root showed no inhibitory effects against *Pseudomonas aeruginosa.* The N-hexane extracts of leaf showed strongest antibacterial activity (23mm) against *Staphylococcus aureus* followed by Acetone extract of leaf (12.5mm), ethanol extract of leaf (10mm), methanol extract of leaf (7mm) and the least activity was found in water extract of leaf (6mm) while N-hexane extracts of leaf showed moderate activity(16mm) against *Pseudomonas aeruginosa* followed by ethanol extract of leaf (14mm), methanol extract of leaf (9mm), andacetone extract leaf (8mm). The water extract of leaf showed no inhibitory effects against *Pseudomonas aeruginosa.* The ethanol, methanol and acetone extract of stem and flowering spike showed lowest antibacterial activity against *Staphylococcus aureus* while hexane extract of stem and flowering spike showed no inhibitory effect against *Staphylococcus aureus*. Similar to the ethanol and methanol extracts of stem and flowering spike showed lowest activity against *Pseudomonas aeruginosa* but acetone and hexane extracts of stem and flowering spike showed no inhibitory effects against *Pseudomonas aeruginosa.* The results of the antibacterial activity in different solvent extracts are represented in **Table 3**. The ethanol, methanol, acetone and N-hexane crude extracts of *Plumbago zeylanica* leaves and root showed significant antibacterial activity against both the tested bacteria except N-hexane extract of root. The n-hexane extract of root showed good antibacterial activity against *Staphylococcus aureus* but no inhibitory effects against *Pseudomonas aeruginosa.* The strongest antibacterial activity was observed in ethanol, methanol, acetone extracts of root and n hexane extract of leaf against all the tested bacteria and the moderate antibacterial activity was observed in ethanol, methanol and acetone extract of leaf. Lowest antibacterial activity was observed in different solvent extracts of Stem and flowering spike against all the tested bacteria.

The methanolic extract of root showed significant activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* and aqueous extracts of root showed less activity against *Staphylococcus aureus* have been reported **[20]**. Another report showed that the alcoholic extract of leaves showed strongest Antimicrobial activity and having rich variety of phytochemical than petroleum ether and chloroform extracts of leaf **[7]**. The wide range of phytoconstituents like alkaloids, glucosides, flavonoids, terpenoids, saponins, tannins, triterpinoids, coumarins and phenolic compounds of *Plumbago zeylanica* showed significant role in prevention of various diseases including cancer, antimicrobial, antihyperglycemic, wound healing properties etc. have been reported **[10]**. The Antibacterial activity of methanolic extracts of *Plumbago zeylanica* root showed strongest activity against *B. subtilis* than others parts of the plant have been reported **[21]** and also reported that the antimicrobial properties of ethanolic extract of *Plumbago zeylanica* root against 11 (eleven) human pathogenic bacteria and 6 (Six) fungi **[22]**.

In the present study, we observed the presence of strongest antibacterial activity in acetone and ethanol extracts of root and N-hexane extract of leaf. These results indicate that the medicinal plants of *Plumbago zeylanica* have good potential antibacterial activity due to the presence of many phytoconstituents such as alkaloids, carbohydrates, phenols, flavonoids, tannin, terpenoids and saponins. The present study showed that the significant antibacterial activities against above-mentioned disease-causing bacteria. This study also revealed that the different extracts i.e. hexane, ethanol, methanol and acetone extracts of leaf and root can be used as novel source of antibacterial agents and also may be used as an herbal drug to the treatment of different diseases caused by above selected pathogenic bacteria.

Table 3: Antibacterial of the different solvent extracts of leaf, root, stem and flower of *P. zeylanica*.

Sl. No	Plant species	Plant Parts used	Microorganism	Solvent used	Zone of inhibition (mm)					
					Concentrations (µg/ml)					Standard drug- Gen (10mcg/disc)
					50	100	250	500	1000	
1.	*Plumbago zeylanica*	Leaf	*Staphylococcus aureus* (+ve)	Ethanol	9	4	6	7	10	26
				Methanol	4.5	4	4.6	5	7	22
				Acetone	6.5	8	9.5	11	12.5	29.5
				Hexane	12.5	14	14	21	23	25
				Water	-	-	-	-	6	
			Pseudomonus Sp (-ve)	Ethanol	-	19	15.5	13.5	14	20
				Methanol	-	4	5	7	9	24.5
				Acetone	-	-	-	4	8	34
				Hexane	12	12	12.5	15	16	24
				Water	-	-	-	-	-	
		Root		Ethanol	19	20.5	20	22.3	24	24.8

			***Staphylococcus aureus* (+ve)**	**Methanol**	**11.5**	**13**	**14.5**	**17.5**	**17.5**	**24.4**
				Acetone	**21.3**	**22.5**	**22.5**	**23.5**	**25.5**	**26**
				Hexane	**14.5**	**13**	**11**	**18**	**18**	**23.5**
				Water	**-**	**-**	**-**	**-**	**5**	
			***Pseudomonus* Sp (-ve)**	**Ethanol**	**14.3**	**18**	**17**	**18**	**20.3**	**21**
				Methanol	**15.5**	**15**	**17.5**	**17.3**	**20**	**24.6**
				Acetone	**14.5**	**15**	**17.5**	**16**	**16.5**	**25.5**
				Hexane	**-**	**-**	**-**	**-**	**-**	**17**
				Water	**-**	**-**	**-**	**-**	**-**	
		Stem	***Staphylococcus aureus* (+ve)**	**Ethanol**	**3**	**3.5**	**5**	**5**	**10**	**16.5**
				Methanol	**2**	**3**	**3**	**4**	**6**	**18**
				Acetone	**8**	**7**	**9**	**10**	**10**	**19**
				Hexane	**-**	**-**	**-**	**-**	**-**	**17**
				Water	**-**	**-**	**-**	**-**	**-**	
			***Pseudomonus* Sp (-ve)**	**Ethanol**	**-**	**-**	**-**	**3.5**	**4.5**	**19**
				Methanol	**4**	**4**	**4**	**5.6**	**8**	**16**
				Acetone	**-**	**-**	**-**	**-**	**-**	**19**
				Hexane	**-**	**-**	**-**	**-**	**-**	**14**
				Water	**-**	**-**	**-**	**-**	**-**	
		Flowering spike	***Staphylococcus aureus*(+ve)**	**Ethanol**	**-**	**-**	**-**	**3**	**4**	**14**
				Methanol	**3**	**3**	**4**	**5**	**6**	**20**
				Acetone	**2**	**2**	**2**	**3**	**3.5**	**20.5**
				Hexane	**-**	**-**	**-**	**-**	**-**	**17**
				Water	**-**	**-**	**-**	**-**	**-**	
			***Pseudomonus* Sp (-ve)**	**Ethanol**	**3**	**3**	**4**	**7**	**9**	**20**
				Methanol	**3**	**-**	**6**	**6.5**	**8**	**18**
				Acetone	**-**	**-**	**-**	**-**	**-**	**20**
				Hexane	**-**	**-**	**-**	**-**	**-**	**13**
				Water	**-**	**-**	**-**	**-**	**-**	

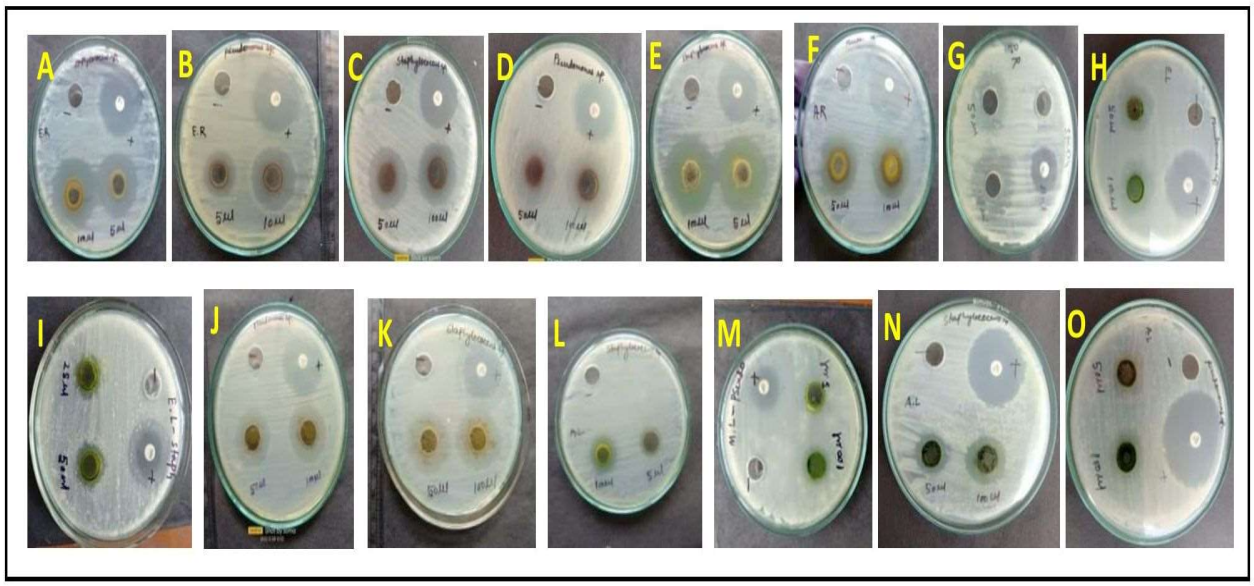

Figure 4: Antibacterial activity (A & B- Ethanol extract of root, C & D-Methanol extract of root,E & F- Acetone extract of root, G- hexane extract of root, H & I-Ethanol extract of leaf, J & K- Hexane extract of leaf, L & M- Methanol extract of leaf, N & O- Acetone extract of leaf.

CONCLUSION:

The results from present study indicate that *P. zeylanica* leaves and roots are potential source of phytoconstituents that could be used in herbal drug discovery. The different solvent extracts were showed good antibacterial

activity except water extract, but highest antibacterial activity was acetone &ethanol extract of root. This result indicates that *P. zeylanica* extracts have active compound and also have antibacterial agents which may be natural source of novel medicine against disease causing bacteria in human beings. Further work is required to characterize and isolat of active compound which may be used to develop antibacterial drugs.

CONFLICT OF INTEREST:

Authors do not have any conflict of interest.

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REFERENCES:

[1] Singh C, Saxena RK and Tripathi P. Antibacterial Activity of *Herpestis monniera* Against Some selected Antibiotics. Bulletin of Environment, Pharmacology and Life Sciences. 2021; 11(1): 79-85.

[2] Ravishankar, B and Shukla VJ. Indian Systems of Medicine: A Brief Profile. African Journal of Traditional, Complementary and Alternative. Medicines. 2007; 4(3): 319-337.

[3] Pandey MM, Rastogi S and Rawat AKS. Indian Traditional Ayurvedic System of Medicine and Nutritional Supplementation. Hindawi Publishing Corporation: Evidence-Based Complementary and Alternative Medicine. 2013; pp-1-12.

[4] Kala CP. Traditional Health Care Systems and Herbal Medicines. European Journal of Environment and Public Health. 2017; 1(1): 03: 1-6.

[5] Savithramma N, Linga Rao M. and Suhrulatha D. Screening of Medicinal Plants for Secondary Metabolites. Middle-East Journal of Scientific Research. 2011; 8 (3): 579-584.

[6] WHO. The use of herbal medicines in primary health care (NO. SEA-HSD-322), WHO Regional office for South-East Asia. 2009.

[7] Dhale DA and Markandeya SK. Antimicrobial and Phytochemical Screening of *Plumbago zeylanica* Linn. (*Plumbaginaceae*) Leaf. Journal of Experimental Sciences. 2011; 2 (3): 04-06.

[8] Singh MK, Pandey A, Sawarkar H, Gupta A, Gidwani B and Dhongade H. Methanolic extract of *Plumbago zeylanica*

-A remarkable antibacterial agent against many human and agricultural pathogens. Journal of Pharmacopuncture. 2017; 20 (1):18–22.

[9] Aleem M. Anti-Inflammatory and Anti-Microbial Potential of *Plumbago zeylanica* L.: A Review. Journal of Drug Delivery & Therapeutics. 2020; 10 (5-s): 229-235.

[10] Shukla B, Saxena S, Usmani S and Kushwaha P. Phytochemistry and pharmacological studies of *Plumbago zeylanica* L.: a medicinal plant review. Clinical Phytoscience. 2021; 7(34): pp-1-11.

[11] Arunachalam KD, Velmurugan P and Balaji R. Anti-inflammatory and Cytotoxic Effects of Extract from *Plumbago zeylanica.* African Journal of Microbiology Research. 2010; 4(12):1239-1245.

[12] Nile SH and Khobragade CN. Antioxidant Activity and Flavonoid Derivatives of *Plumbago zeylanic*a. Journal of Natural Products. 2010; 3: 130-133.

[13] Kumar R. Hepatoprotective Activity of Aerial Parts of *Plumbago Zeylanica* Linn against Carbon Tetrachloride-Induced Hepatotoxicity in Rats. International Journal of Pharmacy and Pharmaceutical Sciences.2009; 1(l): 171-173.

[14] Harborne JB. Phytochemical methods: a guide to modern techniques of plant analysis. Chapman and Hall, New York. 1998; pp.1-271.

[15] Bauer AW, Kirby WMM, Sherris, JC and Turck, M. Antibiotic susceptibility testing by a standardized single disk method. Amirican journal of Clinical Pathology. 1966; 45: 493-496.

[16] Kishore N, Mishra BB, Tiwari VK and Tripathi V. An account of phytochemicals from *Plumbago zeylanica* (Family: *Plumbaginaceae*): A natural gift to human being. Chronicles of Young Scientists. 2012; 3: 178-198.

[17] Saxena M, Saxena J, Nema R, Singh D and Gupta A. Phytochemistry of medicinal plants. Journal of Pharmacognosy and Phytochemistry. 2013; 1(6):168-182.

[18] Kabera JN, Semana, E, Mussa AR, and He X. Plant Secondary Metabolites: Biosynthesis, Classification, Function and Pharmacological Properties. Journal of Pharmacy and Pharmacology, 2014; 2: 377-392.

[19] Ndongo D. Antibacterial, Antioxidant and Phytochemical Investigation of *Acacia arenaria*, *Aloe esculenta*and *Pechuel-loeschealeubnitziae*. 2017; Doctoral dissertation University of Namibia.

[20] Jeyachandran R, Mahesh A, Cindrella L, Sudhakar S and Pazhanichamy K. Antibacterial Activity of Plumbagin and Root Extracts of *Plumbago zeylanica* L. Acta BiologicaCracoviensia Series Botanica. 2009; 51(1): 17–22.

[21] Devi MS and Thenmozhi M. Antibacterial Activity of *Plumbago zeylanica* leaf extracts. International Journal of Research in Biomedicine and Biotechnology. 2011; 1(1) 1-4.

[22] Rahman MS and Anwar MN. Antimicrobial Activity of Crude Extract Obtained from the Root of *Plumbago zeylanica*. Bangladesh Journal of Microbiology. 2007; 24 (1): pp73-75.