



## Research Article

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### EXPLORATION OF ANTIBACTERIAL ACTIVITY OF VARIOUS EXTRACTS OF *EPALTES PYGMAEA* DC. AGAINST FEW BACTERIAL ORGANISMS

Murugammal S<sup>1</sup>, Meeradevi Sri P<sup>2</sup>, Shakila R<sup>3</sup>, Ilavarasan R<sup>4\*</sup>

<sup>1</sup>Research Scholar, Captain Srinivasa Murthi Regional Ayurveda Drug Development Institute (Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH, New Delhi), Arignar Anna Hospital Campus, Arumbakkam, Chennai, India

<sup>2</sup>Consultant (Microbiology), Regional Research Institute for Unani Medicine (Central Council for Research in Unani Medicine, Ministry of AYUSH, New Delhi), Royapuram, Chennai, India

<sup>3</sup>Research Officer (Chemistry), Siddha Central Research Institute, (Central Council for Research in Siddha, Ministry of AYUSH, Chennai), Anna Hospital Campus, Arumbakkam, Chennai, India

<sup>4</sup>Assistant Director (Scientist-III) I/c., Captain Srinivasa Murthi Regional Ayurveda Drug Development Institute (Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH, New Delhi), Arignar Anna Hospital Campus, Arumbakkam, Chennai, India

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**\*Corresponding author**

E-mail: arilavarasan@yahoo.co.in

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#### ABSTRACT

The present investigation is aimed to provide a tentative antibacterial activity of successive n-hexane, chloroform, ethyl acetate and ethanol extracts of the plant *Epaltes pygmaea* DC. The plant was collected and extracted using Soxhlet apparatus by successive method. The microbial cultures were procured from National Chemical Laboratory (NCL), Pune and few were randomly collected from the clinical laboratories in Chennai. Antibacterial activity was assayed in duplicates by agar well diffusion method for the test organisms. The plant showed good inhibitory activity against the organisms *Bacillus cereus*, *Klebsiella pneumoniae* and *Staphylococcus aureus* even at microgram levels. It is suggested that the plant can be used as an alternative medicine after the clearance of various pharmacological issues in controlling certain medical problems caused due to the organisms *Bacillus cereus*, *Klebsiella pneumoniae* and *Staphylococcus aureus*.

**Keywords:** *Epaltes pygmaea*, Hexane, Ethanol, *Klebsiella pneumoniae*, *Bacillus aureus*.

#### INTRODUCTION

In modern era, the phytomedicines have become potential medicines to cure variety of diseases. The usage of phytomedicine has increased in both developing and developed countries<sup>1</sup>. *E. pygmaea* is a weed plant belonging to the family Asteraceae, annual herb, grows up to a height of 20 cm with branched stem and aromatic root. People all over the world relies on novel herbal based medicines for treating many ailments since they believe that the natural origin drugs are safer and have lesser side effects with equal or nearly equal potency compared to allopathy drugs. The genus *Epaltes* is used in traditional Ayurvedic medicine in Srilanka to alleviate jaundice. Literature survey reveals that the plant of the genus has the therapeutic action of diaphoretics, diuretics, stimulant, expectorant and are used in urethral discharges and acute dyspepsia. In India two species of the genus *Epaltes* namely *Epaltes divaricata* (L.) Cass. and *Epaltes pygmaea* DC. are seen, of which the plant *Epaltes pygmaea* DC. remains unexplored. Only few research articles denoting the phytoconstituents and pharmacological activity stating the hepato-protectivity against paracetamol induced hepatotoxicity in rats and diuretic effect of alcoholic and aqueous extracts of the plant are available. With this view, the present study was designed to evaluate the antibacterial property of the plant *Epaltes pygmaea* DC. against certain gram positive and gram-negative organisms with the aim to exploit the potency of various extracts of the plant.

#### MATERIALS AND METHODS

##### Collection of Plant

Fresh whole plant of *Epaltes pygmaea* DC. was collected from Tirunelveli District in September 2016 was identified and authenticated by Prof. P. Jeyaraman, Director, Institute of Herb Botany, Plant Anatomy Research Centre, Tambaram, Chennai, India, where a voucher specimen no PARC/2014/2071 was also deposited. Plant was dried in shade and powdered in a pulverizer.

##### Preparation of various extracts

The plant material (10 g) was extracted with n-hexane, chloroform, ethyl acetate and ethanol solvents in a successive manner using Soxhlet apparatus. All the obtained extracts were filtered using sterile Whatman filter paper no.2, dried using Rotavapor R-300 and stored separately for further use.

##### Collection of microorganism

To evaluate the microbial activities, few cultures were procured from National Chemical Laboratory (NCL) Pune and few were randomly collected from the clinical laboratories in Chennai. The organisms used were two gram-positive organisms namely *Staphylococcus aureus*, *Bacillus cereus* (NCIM 2458) and five gram-negative organisms namely *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella*

*typhimurium* (NCIM 2501) and *Escherichia coli*. All the organisms were confirmed using specific biochemical tests<sup>2</sup>.

#### Inoculum preparation

A lag phase uniform suspension of all the organisms listed above were prepared by inoculating a loopful of each culture in 10 ml of peptone water, incubated at 37°C for about 6 to 8 hours.

#### Preparation of plates and inoculation of microbial cultures

The required quantities of the Muller Hinton agar plates were prepared and uniformly swabbed with sterile cotton swabs dipped with each microbial culture, covering the entire surface of the plate by rotating the plates in all the directions. After solidification, wells of 6 mm diameter were punched over each agar plates. Plates were then allowed to set for few minutes.

#### Drug concentration

All the extracts were accurately weighed and dissolved in DMSO solvent<sup>3</sup> to make the stock solution from which further working dilutions were made for the experiments performed to detect minimum inhibitory concentration (MIC).

Hexane extract (yield 5.296%): stock solution – 100 µg/ml; diluted to 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.06 µg/ml and 1.53 µg/ml.

Chloroform extract (yield 3.750%): stock solution – 600 µg/ml; diluted to 300 µg/ml, 150 µg/ml, 75 µg/ml, 37.5 µg/ml and 18.75 µg/ml.

Ethyl acetate extract (yield 4.511%): stock solution – 100 µg/ml; diluted to 50 µg/ml, 25 µg/ml, 12.5 µg/ml and 6.125 µg/ml.

Ethanol extract (yield 6.510%): stock solution – 1000 µg/ml; diluted to 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml and 15.62 µg/ml.

#### Antibacterial assay and determination of MIC

Antibacterial activity was assayed in duplicates by agar well diffusion method<sup>4</sup> using the mentioned test organisms. The well was loaded with 60 µl of each extract of various concentrations. The commercially available drug ampicillin (10 µg/disc) was used as control. The plates were incubated at 37°C for 24 hours. The diameter of the clearing zones was measured in mm using the calipers.

The minimum inhibitory concentration assessment of the drug against the susceptible bacteria was achieved by determining the MIC with varying concentration of each extract<sup>5</sup>. The plates were

incubated at 37°C for 24 hours and were observed for the MIC, which was read as the lowest concentration of the extract required to completely inhibit the growth of the organism (Plate 1 to 4).

#### RESULTS AND DISCUSSION

The antibacterial activity was studied against seven bacterial cultures of which two were gram positive organisms namely *Staphylococcus aureus* and *Bacillus cereus* (NCIM 2458) and five were gram negative organisms namely *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhimurium* (NCIM 2501) and *Escherichia coli*. *K. pneumoniae* is an opportunistic pathogen involved in nosocomial infections like urinary tract infections, pneumonia, septicemia and infantile meningitis<sup>6</sup>. *S. aureus* is a major pathogen which colonizes and infects both patients with less insusceptibility and healthy immuno-competent people. It is found commonly in the environment and on the human skin. It causes infections of the skin, nose, urethra, vagina and gastrointestinal tract<sup>7</sup>. *B. cereus* is responsible for food borne diseases such as nausea, vomiting and diarrhea; *P. vulgaris* creates urinary tract infections; *E. coli* causes diarrheal and urinary tract infections<sup>8</sup>. The results are presented in Table 1. A significant growth inhibition was shown by most of the organisms tested indicating the profound potency of the plant *Epaltes pygmaea* DC. Among the various extracts tested, the extracts obtained in nearly polar solvents namely ethyl acetate and ethanol were higher and found to possess most of the phytoconstituents that can inhibit growth of majority of bacteria. The nonpolar solvent hexane extract showed inhibitory activity against the bacteria *Klebsiella pneumoniae*, *Escherichia coli* and *Proteus vulgaris* with minimum inhibitory concentration of 3.06 µg/ml for *Klebsiella pneumoniae*, 25 µg/ml for *Escherichia coli* and 50 µg/ml for *Proteus vulgaris*. The chloroform extract did not show inhibitory action other than *Bacillus cereus* against any of the organisms tested which might be due to the presence of medium polar phytoconstituents in chloroform solvent which could not inhibit bacteria. The ethyl acetate and the ethanol extract of the plant showed profound activity against the organism *Klebsiella pneumoniae* followed by *Staphylococcus aureus* and *Bacillus cereus*. Among the tested organism, *Proteus vulgaris* and *Escherichia coli* were inhibited by most of the extracts tested and the organism *Salmonella typhimurium* was not inhibited by any of the extract. The study suggests that the plant *Epaltes pygmaea* DC. could be an effective drug against the organism *Klebsiella pneumoniae* followed by *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* whereas it is least effective against *Salmonella typhimurium*. The extracts of *Epaltes pygmaea* showed inhibition of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhimurium* and *Escherichia coli* which are responsible for urinary tract infections, gastrointestinal infections and typhoidal fevers. Hence this plant could be a better remedy for the infections caused by the above organisms.

Table 1: Antibacterial activity of various extracts of *Epaltes pygmaea*

Sl. No	Organism	Name of the extract	Concentration in µg/ml																				
			1000	600	500	300	250	150	125	100	75	62.5	50	37.5	31.25	25	15.62	12.5	6.125	3.06	1.53	Std.	
1	SA	H, C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		EA	-	-	-	-	-	-	-	16	15	14	13	12	11	10	-	-	-	-	-	28	
		Et	19	18	17	16	15	14	12	-	-	-	-	-	-	-	-	-	-	-	-	28	
2	BC	H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		C	16	14	13	12	11	10	9	8	8	7	6	6	-	-	-	-	-	-	-	20	
		EA	23	21	20	20	19	18	18	17	16	15	14	13	12	12	11	10	-	-	-	25	
		Et	18	17	16	15	14	-	-	-	-	-	-	-	-	-	-	-	-	-	20		
3	ST	H, C, EA, Et	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
4	KP	H	22	21	21	20	19	19	18	18	17	17	16	16	15	14	13	12	12	10	-	-	
		C	--	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		EA	21	20	20	19	18	18	17	17	17	16	16	15	15	15	14	14	-	-	-	-	
		Et	25	24	23	22	21	20	20	19	18	17	17	16	16	13	-	-	-	-	-	-	
5	PA	H, C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		EA	18	17	16	15	15	14	13	13	--	--	-	-	-	-	-	-	-	-	-	-	
		Et	25	23	21	19	17	15	13	-	--	-	--	-	-	-	-	-	-	-	-	-	-
6	EC	H	16	16	16	15	15	15	14	14	14	13	13	12	12	10	--	-	-	-	-	17	
		C, EA	--	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Et	22	20	19	18	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16
7	PV	H	16	15	15	14	13	13	12	12	11	11	10	-	-	-	-	-	-	-	-	-	
		EA	25	23	22	22	20	19	18	18	--	--	-	-	-	-	-	-	-	-	-	-	-
		C, Et	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

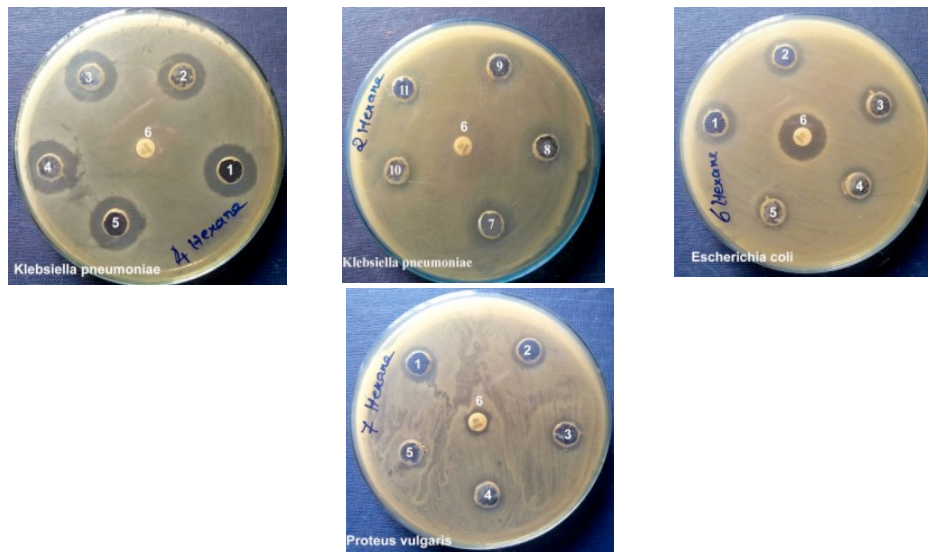


Figure 1: Plate 1 showing the zone of inhibition by Hexane extract

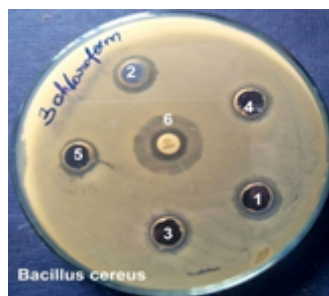


Figure 2: Plate 2 showing the zone of inhibition by Chloroform extract

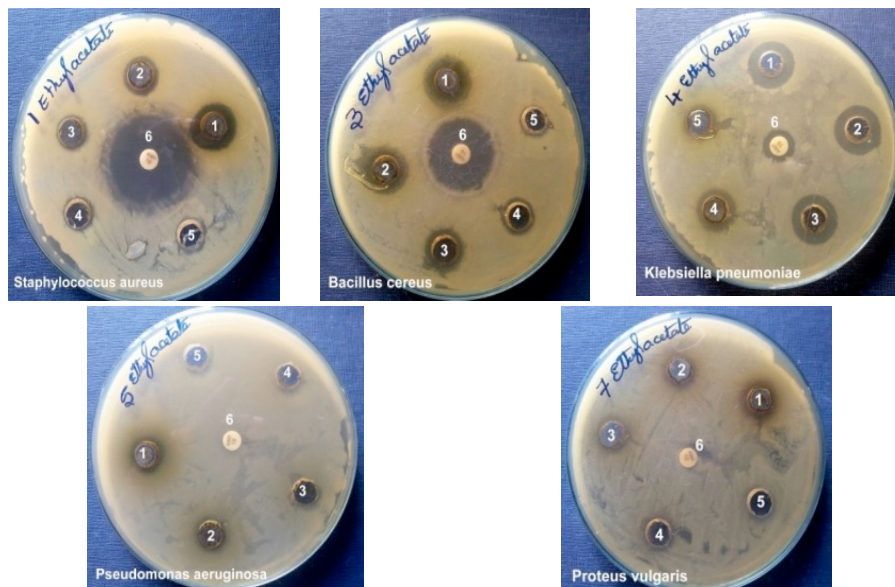


Figure 3: Plate 3 showing the zone of inhibition by Ethyl acetate extract

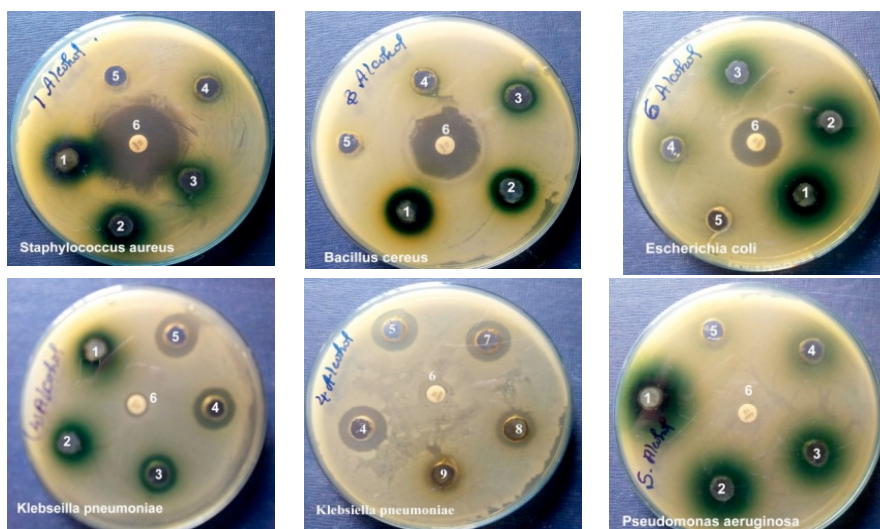


Figure 4: Plate 4 showing the zone of inhibition by Ethanol extract

## CONCLUSION

The results of the present investigation, provides a tentative antibacterial idea about the plant *Epaltes pygmaea* DC. Since the plant shows good inhibitory activity against the organism *Bacillus cereus*, *Klebsiella pneumonia* and *Staphylococcus aureus* even at minimum concentration of microgram level, it is suggested that the plant can be used as an alternative medicine after the clearance of various pharmacological issues in controlling certain medical problems caused due to the organism *Klebsiella pneumoniae* and *Staphylococcus aureus*.

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