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EVALUATION OF ANTI MICROBIAL ACTIVITY OF CHAUSHASTA PRAHARA PIPPALI- IN VITRO STUDY

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ABSTRACT

Microbial infections pose a significant challenge to global public health, The discovery and validation of effective antimicrobial agents are crucial for the proper management of infectious diseases. Pippali (the fruit of Piper longum) is one such well-known medicinal herb in traditional Ayurvedic medicine. Pippali is renowned for its broad therapeutic properties, including its role in managing systemic and immune-related disorders. it is believed to enhance vitality and treat both acute and chronic illnesses. Its efficacy is attributed to its rich phytochemical profile, which has been increasingly supported by experimental and clinical studies. A special formulation known as Chaushashta Prahara Pippali involves the repeated processing of Pippali over 64 praharas (each prahara being an 8-hour period, totaling approximately 21 days). This intensive method of preparation is believed to enhance the herb's potency, especially in treating respiratory ailments. The present study is designed to evaluate the antimicrobial properties of Chaushashta Prahara Pippali against selected pathogenic microorganisms. This work aims to bridge traditional Ayurvedic knowledge with contemporary scientific validation, potentially contributing to the development of novel antimicrobial agents.

KEYWORDS: Pippali, Anti microbial, Chaushasta prahara pippali, anti microbial.

INTRODUCTION

Microbial infections present a significant challenge to global health, and the emergence of drug-resistant strains of bacteria due to the overuse of antimicrobial agents has sparked an urgent need for alternative sources of antimicrobial compounds. One such promising source is Pippali, the fruit of Piper longum, which has been used in traditional medicine for centuries.

Pippali has been referenced in ancient texts, such as the Vedas, Samhitas and Nighantus where its medicinal properties were extolled. The pippali with its excellent qualities and phyto chemical constituents helps to cure many systemic disorders, immunity disorders and as rejuvenating drug when used as rasayana for acute and chronic illness. The detailed drug research with experimental and clinical study validates its traditional uses.

Pharmacognostical and phytochemical studies of Pippali (Piper longum) have revealed its diverse biological activities, including anti-inflammatory, hepatoprotective, antimicrobial, and antioxidant effects. This makes it a valuable component in numerous therapeutic formulations.

One such unique preparation is Chaushasta Praharapippali, a formulation where Pippali is used in two different forms through a process called Bhavana Samskara. This preparation exemplifies the versatility of Pippali in Ayurvedic practice, utilizing its full potential to enhance its therapeutic effects.

Thus, Pippali not only has a rich historical and cultural significance but also presents a scientifically backed option in the fight against microbial infections, especially in light of rising antimicrobial resistance. Its broad spectrum of therapeutic benefits further underlines its importance in modern pharmacology and medicine.

OBJECTIVE

To evaluate *In vitro* anti-microbial activity of Chaushastapraharapippali.

METHODOLOGY

a. Preparation of drug

CHAUSHASTA PRAHARA PIPPLI was procured from GMP certified pharmacy. Based on the solubility of above drugs stock of 375 mg / mL and 625 mg / mL were prepared using 0.9 % saline aseptically. Further different dilutions were prepared and used to assess the anti-microbial and anti-tubercular activities.

b. Procurement of different bacterial and fungal strains

Different bacterial and fungal strains were procured from Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh and National Centre for Microbial Resource (NCMR), Pune.

Mycobacterium sp. (MTCC 290) and MCC 2515 were procured from Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh and National Centre for Microbial Resource (NCMR), Pune respectively. All the strains were subcultured as per routine procedure.

c. Preparation of Medium, Inoculum and Well diffusion procedure for *K. pneumonae*

Nutrient agar medium was prepared by dissolving Beef extract (1 g), Yeast extract (2 g), Peptone (5 g) and Sodium Chloride (5 g) in 900 ml of distilled water. The pH was adjusted to 7.2 and the volume was made up to 1000 ml. Finally 15 g agar was added to the media and autoclaved at 121˚C for 20 minutes. *Klebsiella pneumonae*(MTCC 7407) was procured from Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh. Loopful of 24 h old culture from the slants was transferred to sterile saline and mixed well to prepare homogenous inoculums. The medium was cooled to around 45-55˚C, around 20 ml each was poured into sterile petriplates. One ml of the inoculum was immediately added to the plate, swirled for uniform distribution. Wells were bored using a sterile borer. The samples and the antibiotic were dispensed into the wells. Plates were incubated overnight at 37˚C and observed after 24 h.

d. Preparation of Medium, Inoculum and Well diffusion procedure for *P. aeruginosa*

Nutrient agar medium was prepared by dissolving Beef extract (1 g), Yeast extract (2 g), Peptone (5 g) and Sodium Chloride (5 g) were dissolved in 900 ml of distilled water. The pH was adjusted to 7.2 and the volume was made up to 1000 ml. Finally 15 g agar was added to the media and autoclaved at 121˚C for 20 minutes. *Pseudomonas aeruginosa* (MTCC 8077)

was procured from Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh. Loopful of 24 h old culture from the slants was transferred to sterile saline and mixed well to prepare a homogenous inoculum. The medium was cooled to around 45-55˚C, around 20 ml each was poured into sterile petriplates. One ml of the inoculum was immediately added to the plate, swirled for uniform distribution. Wells were bored using a sterile borer. The samples and the antibiotic were dispensed into the wells. Plates were incubated overnight at 37˚C and observed after 24 h.

e. Preparation of Medium, Inoculum and Well diffusion procedure for *S. aureus*

Nutrient agar medium was prepared by dissolving Beef extract (1 g), Yeast extract (2 g), Peptone (5 g) and Sodium Chloride (5 g) were dissolved in 900 ml of distilled water. The pH was adjusted to 7.2 and the volume was made up to 1000 ml. Finally 15 g agar was added to the media and autoclaved at 121˚C for 20 minutes. *Staphylococcus aureus* (MTCC 3160) was procured from Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh. Loopful of 24 h old culture from the slants was transferred to sterile saline and mixed well to prepare a homogenous inoculum. The medium was cooled to around 45-55˚C, around 20 ml each was poured into sterile petriplates. One ml of the inoculum was immediately added to the plate, swirled for uniform distribution. Wells were bored using a sterile borer. The samples and the antibiotic were dispensed into the wells. Plates were incubated overnight at 37˚C and observed after 24 h.

f. Preparation of Medium, Inoculum and Well diffusion procedure for *S. pyogenes*

Nutrient agar medium was prepared by dissolving Beef extract (1 g), Yeast extract (2 g), Peptone (5 g) and Sodium Chloride (5 g) were dissolved in 900 ml of distilled water. The pH was adjusted to 7.2 and the volume was made up to 1000 ml. Finally 15 g agar was added to the media and autoclaved at 121˚C for 20 minutes. *Streptococcus pyogenes* (MTCC 442) was procured from Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh. Loopful of 24h old culture from the slants was transferred to sterile saline and mixed well to prepare a homogenous inoculum. The medium was cooled to around 45-55˚C, around 20 ml each was poured into sterile petriplates. One ml of the inoculum was immediately added to the plate, swirled for uniform distribution. Wells were bored using a sterile borer. The samples and the antibiotic were dispensed into the wells. Plates were incubated overnight at 37˚C and observed after 24 h.

RESULT

A. Results (obtained with tables, charts, diagrams and photographs)

Table 1: *In vitro* antibacterial activity of Chaushasta Prahara Pippaliagainst *K. pneumonae*

Sample	Concentrations	Zone of inhibition (Radius in mm)
ChaushastaPraharaPippali (375 mg/ml)	10 mg	6
	20 mg	6
	50 mg	7
	100 mg	7
	200 mg	8
	300 mg	8
	375 mg	8
Control (0.9 % saline)	100 µl	0
Standard (Ampicillin) 5mg /0.5ml	100 µl	10

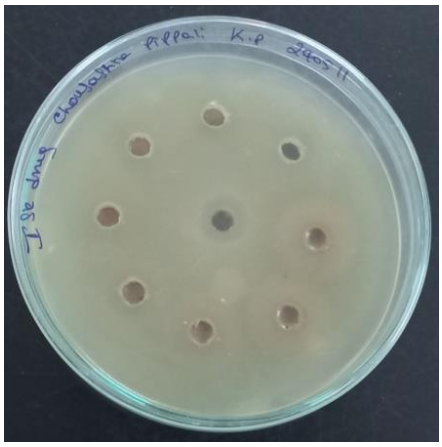


Table 2: *In vitro* antibacterial activity of ChaushastaPraharaPippaliagainst *P. aeruginosa.*

Sample	Concentrations	Zone of inhibition (Radius in mm)
ChaushastaPraharaPippali (375 mg/ml)	10 mg	0
	20 mg	0
	50 mg	0
	100 mg	7
	200 mg	7
	300 mg	8
	375 mg	8
Control (0.9 % saline)	100 µl	0
Standard (Gentamicin)-240 µg	30 µl	19

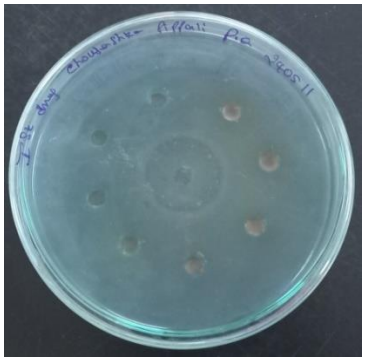


Table 3: *In vitro* antibacterial activity of ChaushastaPraharaPippaliagainst *S. aureus.*

Sample	Concentrations	Zone of inhibition (Radius in mm)
ChaushastaPraharaPippali (375 mg/ml)	10 mg	6
	20 mg	6
	50 mg	6
	100 mg	7
	200 mg	7
	300 mg	7
	375 mg	7
Control (0.9 % saline)	100 µl	0
Standard (Ampicillin) 5mg /0.5ml	50 µl	11

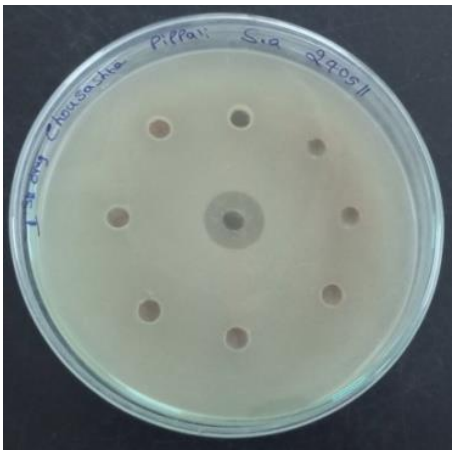


Table 4: *In vitro* antibacterial activity of ChaushastaPraharaPippaliagainst *P. aeruginosa.*

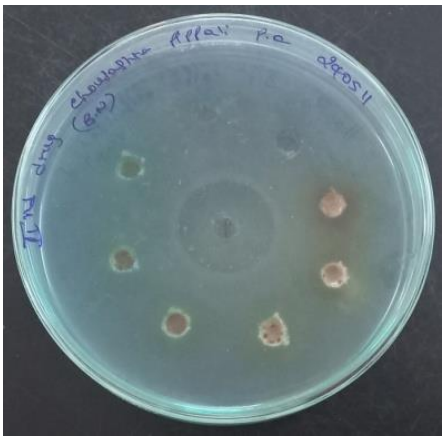
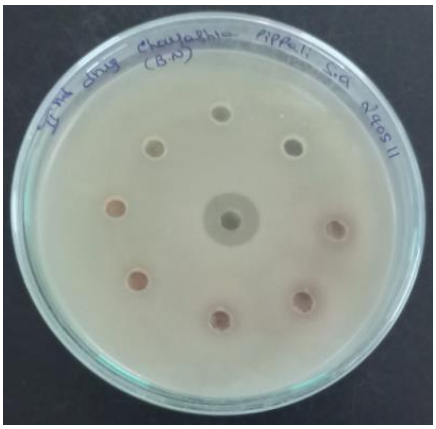
Sample	Concentrations	Zone of inhibition (Radius in mm)	
ChaushastaPraharaPippali (625 mg/ml)	10 mg	0	
	20 mg	0	
	50 mg	0	
	100 mg	0	
	200 mg	7	
	500 mg	7	
	625 mg	7	
Control (0.9 % saline)	100 µl	0	
Standard(Gentamicin-240 µg)	30 µl	20	

Table 5: *In vitro* antibacterial activity of ChaushastaPraharaPippaliagainst *K. pneumonae.*

Sample	Concentrations	Zone of inhibition (Radius in mm)	
ChaushastaPraharaPippali (625 mg/ml)	10 mg	7	
	20 mg	7	
	50 mg	7	
	100 mg	7	
	200 mg	8	
	500 mg	9	
	625 mg	9	
Control (0.9 % saline)	100 µl	0	
Standard (Ampicillin) 5mg /0.5ml	100 µl	10	

Table 6: *In vitro* antibacterial activity of ChaushastaPraharaPippaliagainst *S. aureus*

Sample	Concentrations	Zone of inhibition (Radius in mm)	
ChaushastaPraharaPippali (625 mg/ml)	10 mg	0	
	20 mg	7	
	50 mg	7	
	100 mg	7	
	200 mg	7	
	500 mg	7	
	625 mg	7	
Control (0.9 % saline)	100 µl	0	
Standard (Ampicillin) 5mg /0.5ml	50 µl	11	

B. DISCUSSION

Different concentrations (10-375 mg/mL) of ChaushastaPraharaPippali showed antibacterial activity against *K. pneumonae*(**Table 1**). The positive control Ampicillin (5mg /0.5ml; 100 µl)showed 10 mm of inhibition zone against *K. pneumonae.*

Different concentrations (100-375 mg/mL) of ChaushastaPraharaPippali showed antibacterial activity against *P. aeruginosa* (**Table 2**). The positive control Gentamicin (240 µg; 30 µl)showed 19 mm of inhibition zone against *P. aeruginosa*.

Different concentrations (10-375 mg/mL) of ChaushastaPraharaPippali showed antibacterial activity against *S. aureus* (**Table 3**). The positive control Ampicillin (5mg /0.5ml; 50 µl) showed 11 mm of inhibition zone against *S. aureus*.

Different concentrations (200-625 mg/mL) of ChaushastaPraharaPippali showed antibacterial activity against *P. aeruginosa* (**Table 4**). The positive control Gentamicin (240 µg; 30 µl)showed 20 mm of inhibition zone against *P. aeruginosa*.

Different concentrations (10-625 mg/mL) of ChaushastaPraharaPippali showed antibacterial activity against K. pneumonae(**Table 5).** The positive control Ampicillin (5mg /0.5ml; 100 µl)showed 10 mm of inhibition zone against K. pneumonae.

Different concentrations (20-625 mg/mL) of ChaushastaPraharaPippali showed antibacterial activity against *S. aureus* (**Table 6**). The positive control Ampicillin (5mg /0.5ml; 50 µl) showed 11 mm of inhibition zone against *S. aureus*.

CONCLUSION

All the concentrations (10-375 mg/mL) of ChaushastaPraharaPippali used showed antibacterial activity against *K. pneumonae*. Higher concentrations of ChaushastaPraharaPippali showed antibacterial activity against *P. aeruginosa*. Wide concentrations range of ChaushastaPraharaPippali showed antibacterial activity against *S. aureus.* Different concentrations of ChaushastaPraharaPippali not showed antibacterial activity against *S. pyogenes*. Overall different concentrations of ChaushastaPraharaPippali not showed activities against Aspergillus niger and C. albicans.

Further research could explore the mechanisms underlying these differences and their potential applications in antimicrobial therapies.

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