

EXPLORING THE ANTI MICROBIAL ACTIVITY OF ASHTA CHOORANAM

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ABSTRACT

As per the World Health Organization, approximately 80% of the global population primarily relies on traditional medicines for their healthcare needs. The utilization of herbal medicines dates back to ancient Chinese, Greek, Egyptian, and Indian medicinal practices, making the history of herbal remedies as ancient as human civilization itself. Siddha stands as a distinct medicinal system with its origins in Tamil Nadu and deeply rooted in the Tamil language. In its literal sense, the term "Siddha" signifies "established truth". According to Siddha medicine, diseases result from an imbalance in the three biological components of the human body, known as Vatha, Pitha, and Kapha. These imbalances are rectified through the use of herbs, metals, and minerals. Siddha preparations are categorized into various medicinal forms, consisting of 32 internal and 32 external types, with "chooranam" being one of the internal medicinal forms. It is generally considered safe with minimal side effects; the treatment duration may

be extended.

KEYWORDS: Vatha, Pitha, and Kapha.

INTRODUCTION

Ashta Chooranam is a traditional Ayurvedic formulation renowned for its therapeutic properties, particularly in supporting digestive health and maintaining overall well-being. The term "Ashta Chooranam" comes from Sanskrit, where "Ashta" means eight, and "Chooranam" means powder. This formulation is characterized by its blend of eight distinct

herbs and spices, each selected for their complementary effects on the body. Its use is rooted in ancient Ayurvedic practices and continues to be valued in contemporary holistic health approaches.

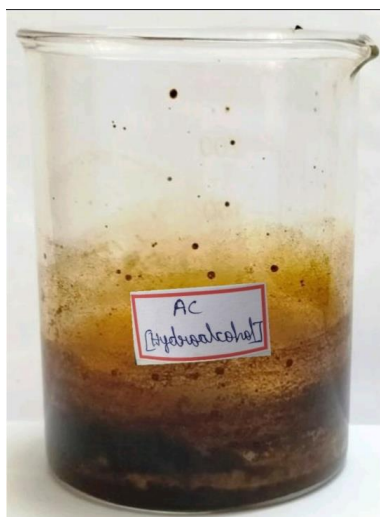
MATERIALS

Equipments	Apparatus
Isomantle	Soxhlet extractor
Mortar and Pestle	Porous cellulose thimble
Stands and Clamps	Round bottom flask
Petriplates	Test tubes
Spirit lamp	Beakers
Autoclave	Conical flasks
	Siphon

EXTRACTION OF ASHTA CHOORANAM

Soxhlet extraction is a very useful tool for preparative purposes in which the analyte is concentrated from the matrix as a whole or separated from particular interfering substances. Sample preparation of environmental samples has been developed for decades using a wide variety of techniques. Solvent extraction of solid samples, which is commonly known as solid-liquid extraction (also referred to as leaching or Lixiviation in a more correct use of the physicochemical terminology), is one of the oldest methods for solid sample pretreatment. Conventional Soxhlet extraction remains as one of the most relevant techniques in the environmental extraction field.





ANTIBACTERIAL ACTIVITY OF ASHTA CHOORANAM.

Petri plates containing 20 ml nutrient agar medium were seeded with 24 hr culture of bacterial strains were adjusted to 0.5 OD value according to McFarland standard. Wells were cut and concentration of test sample (500, 250, 100 and 50 µg/ml) was added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Gentamicin was used as a positive control. The values were calculated using Graph Pad Prism 6.0 software (USA).

ANTIFUNGAL ACTIVITY OF ASHTA CHOORANAM.

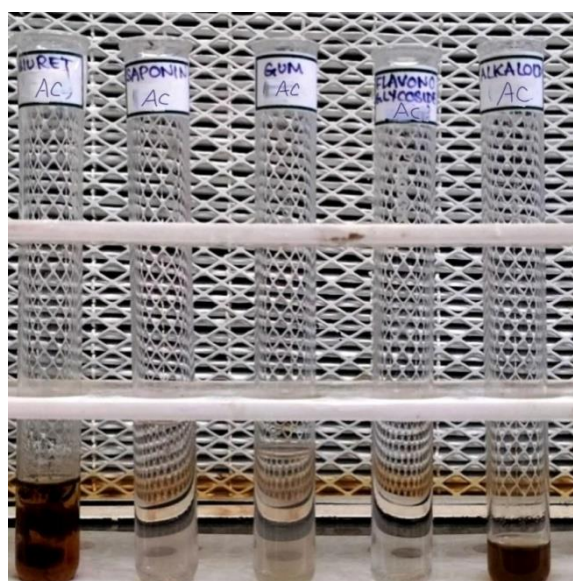
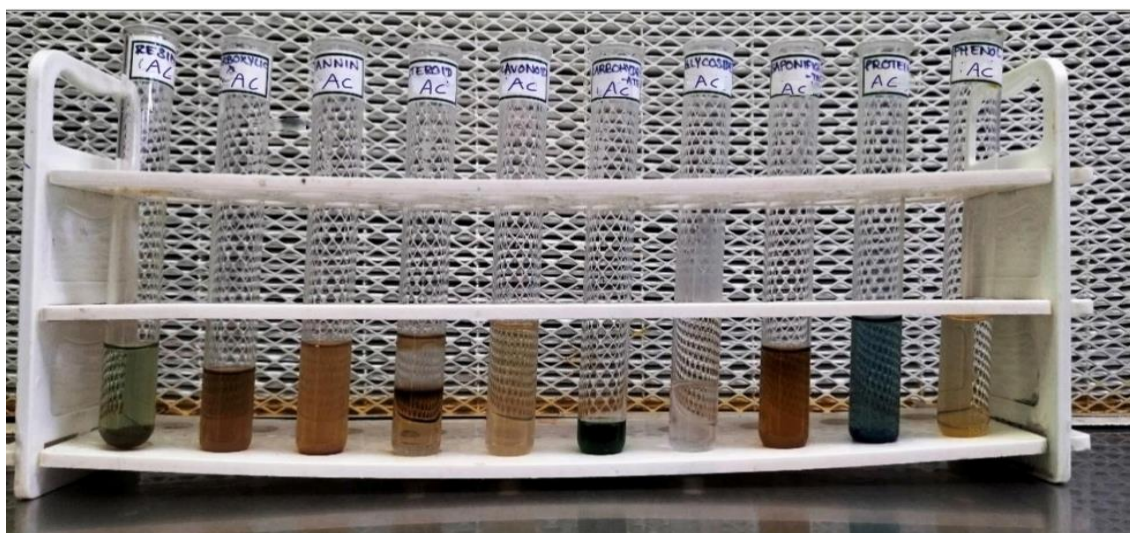
Petri plates containing 20ml Potato dextrose agar medium was seeded with 72 hr culture of fungal strains (*Aspergillus niger*, *Candida albicans*,) different concentration of sample PM (500, 250, 100 and 50 µg/ml) was added. The plates were then incubated at 28°C for 72 hours. The Antifungal activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Amphotericin B was used as a positive control. The values were calculated using Graph pad prism 6.0 software (USA).

RESULTS

Results of Qualitative Phytochemical Screening Methods of AC.

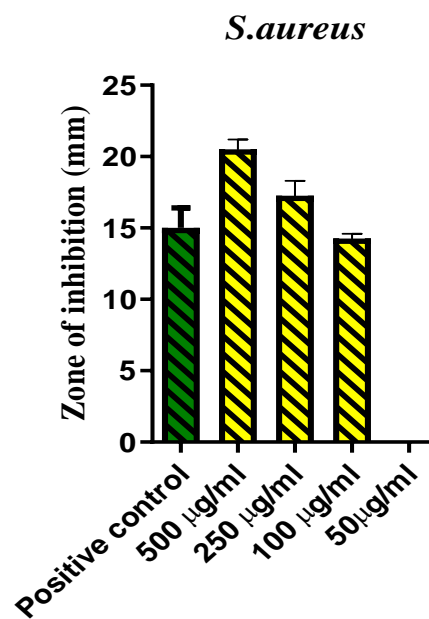
S.No.	Name of the Sample	Phytochemical compound	Result
1.	AC	Resins	+
2.		Carboxylic acid	-
3.		Tannins	-
4.		Steroids	-
5.		Flavonoid	+
6.		Carbohydrates	+
7.		Glycosides	-

8.		Saponification	+
9.		Protein	+
10.		Phenol	-
11.		Biuret	-
12.		Soponin	-
13.		Gum	+
14.		Flavanoglycosides	-
15.		Alkaloids	-

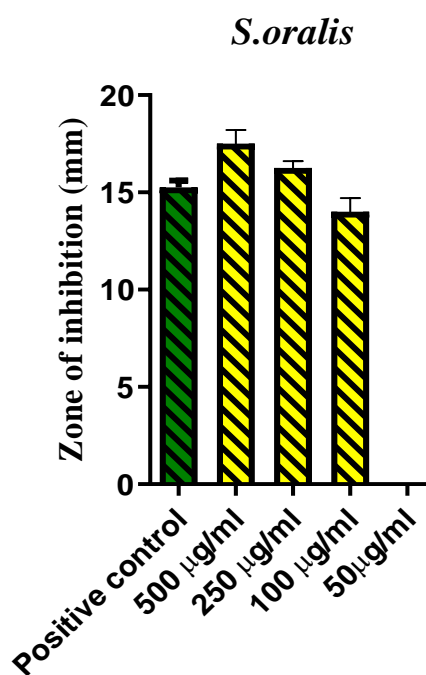
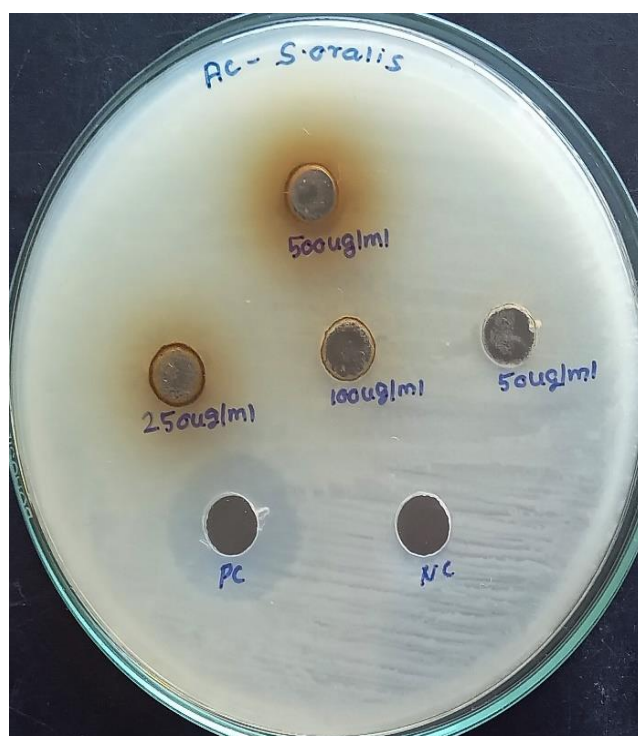


ANTIBACTERIAL ACTIVITY OF ASHTA CHOORANAM

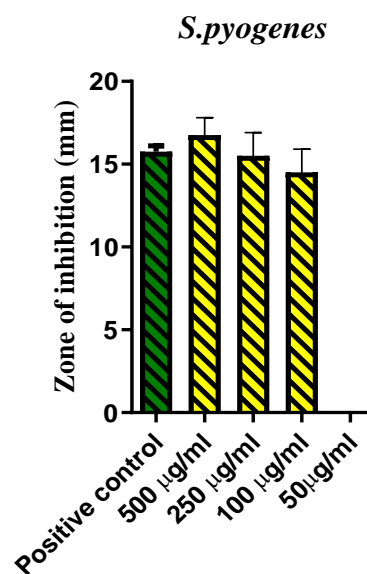
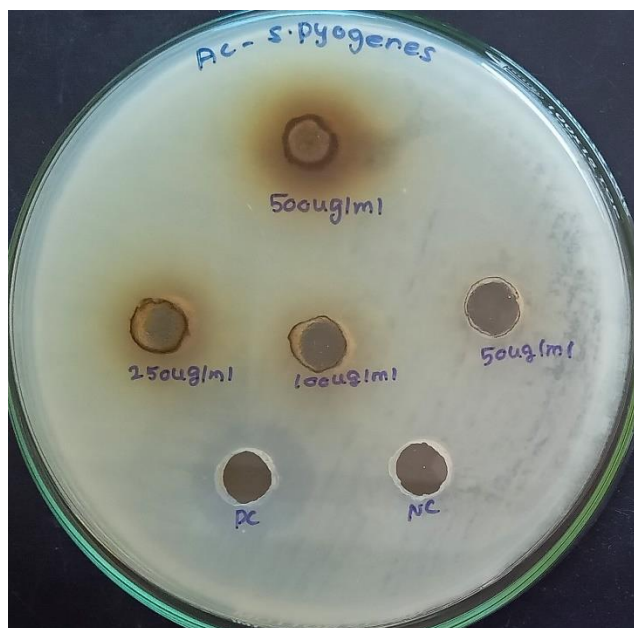
Effect of sample AC against *S.aureus*.



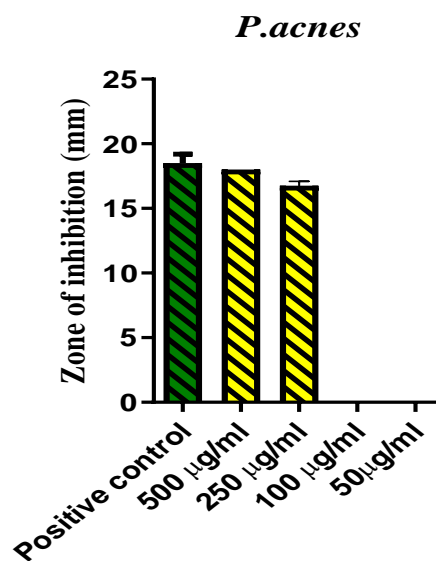
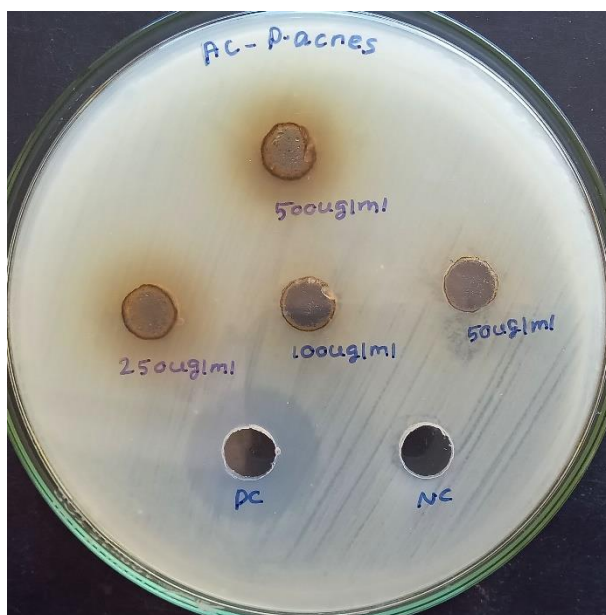
Effect of sample AC against *S.oralis*.

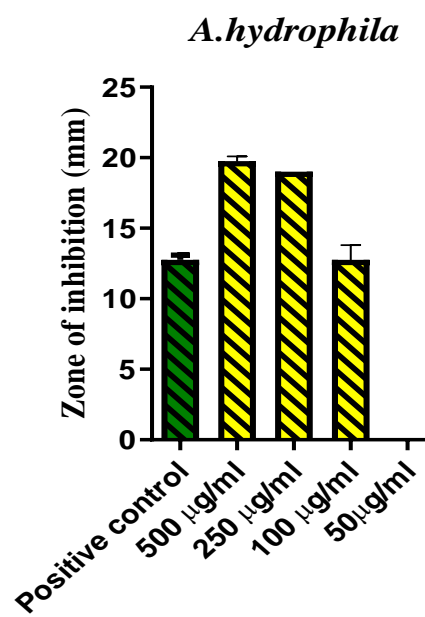
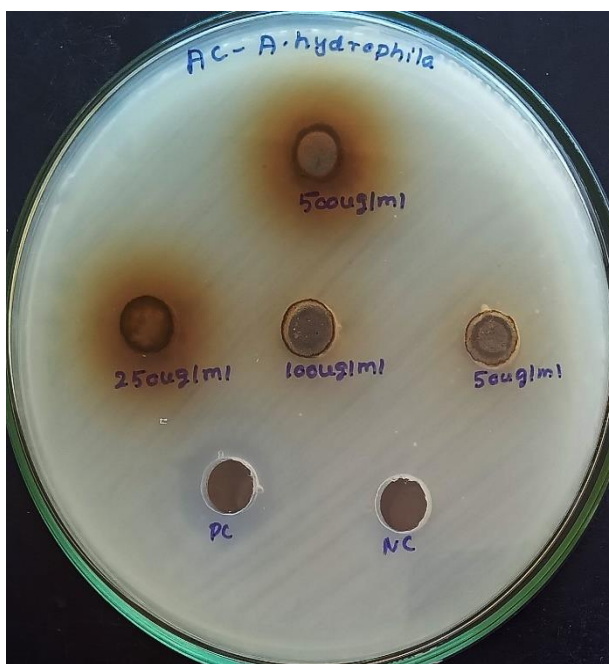
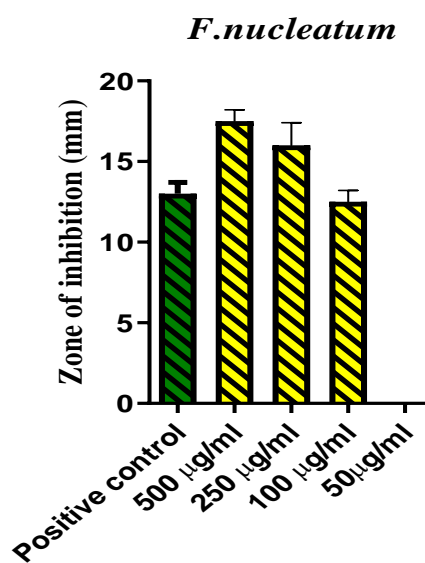


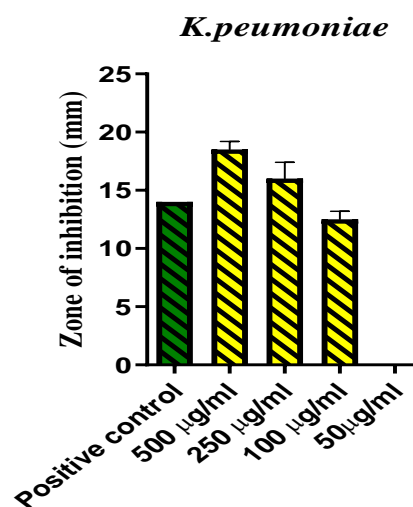
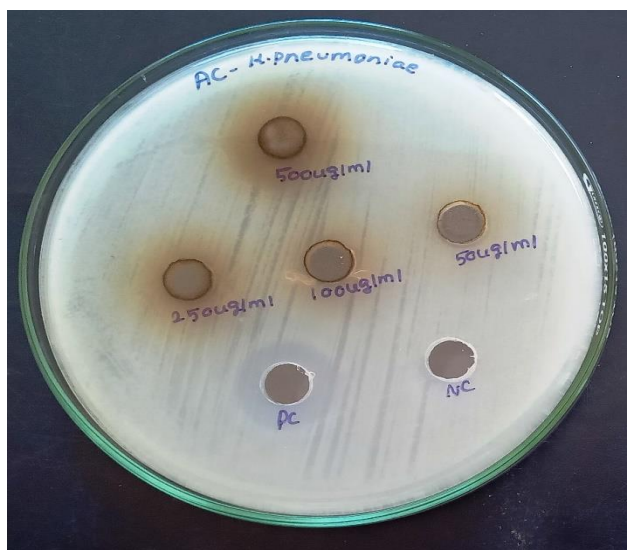
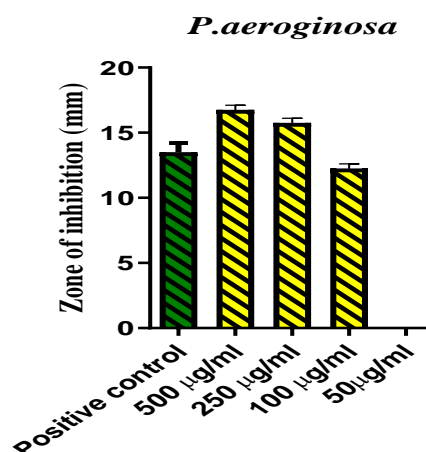
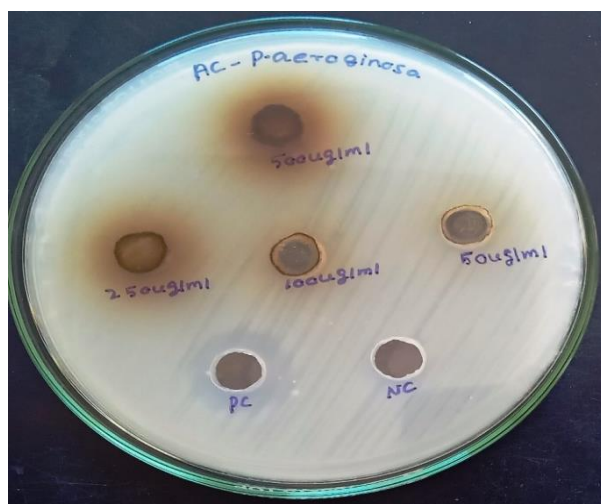
Effect of sample AC against *S.pyogenes*.



Effect of sample AC against *P.acnes*.



Effect of sample AC against *A.hydrophila*Effect of sample AC against *F.nucleatum*

Effect of sample AC against *K.Pneumoniae*Effect of sample AC against *P.aeruginosa*

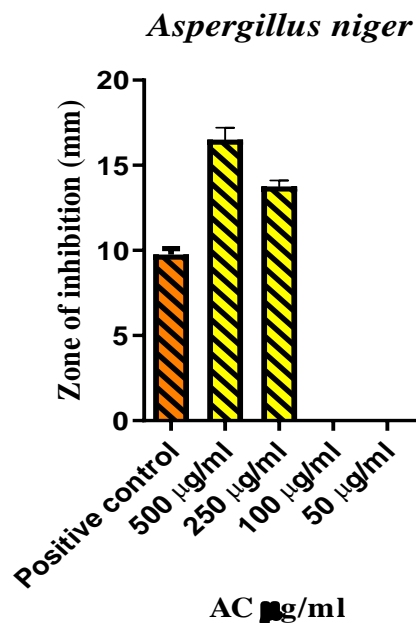
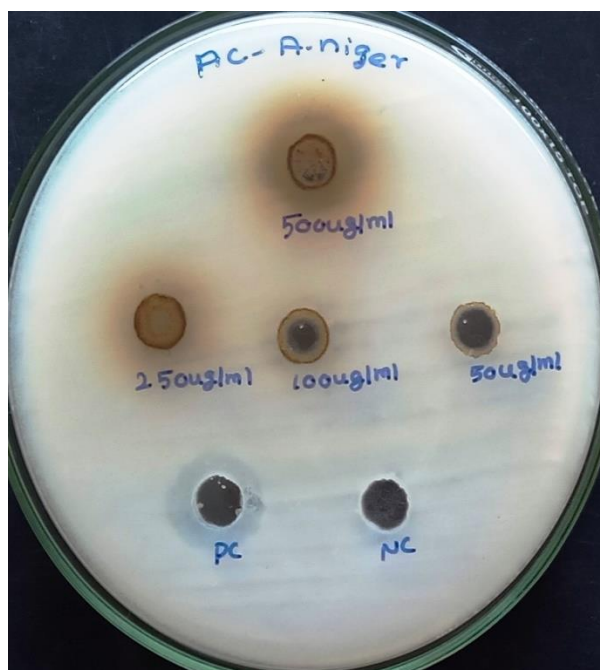
Means ± SD of zone of inhibition obtained by sample against Test organ

S. No	Name of the test organism	Name of the test sample	Zone of inhibition (mm)				
			Mean±SD				
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC
1.	<i>S.aureus</i>	AC	20.5±0.70	17.25±1.06	14.25±0.35	0±0	15±1.41
2.	<i>S.oralis</i>		17.5±0.7	16.25±0.35	14±0.70	0±0	15.25±0.35
3.	<i>S.pyogenes.</i>		16.75±1.06	15.5±1.41	15.5±1.41	0±0	15.75±0.35
4.	<i>P.acnes.</i>		18±0	16.75±0.35	0±0	0±0	16.75±1.76
5.	<i>A.hydrophila</i>		19.75±0.35	19±0	0±1.06	0±0	12.75±0.35
6.	<i>F.nucleatum</i>		17.5±0.70	16±1.41	12.5±0.70	0±0	13±0.70
7.	<i>K.Pneumoniae</i>		18.5±0.70	16±1.41	12.5±0.70	0±0	13.25±1.06
8.	<i>P.aeruginosa</i>		16.75±0.35	15.75±0.35	12.25±0.35	0±0	13.5±0.70

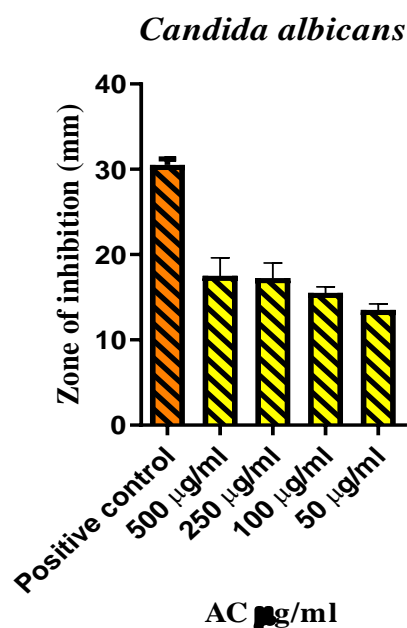
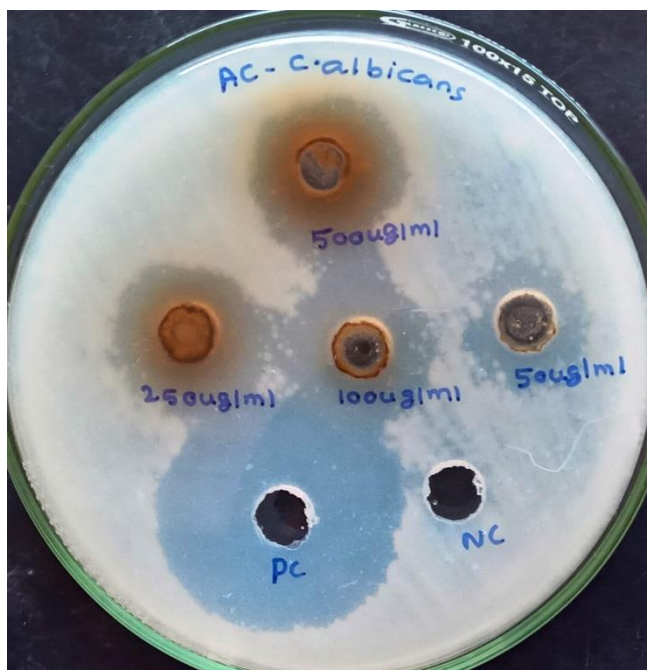
SD – Standard Deviation, *Significance - $p < 0.05$

ANTIFUNGAL ACTIVITY OF ASHTA CHOORANAM

Effect of sample AC against *Aspergillus niger*.



Effect of sample AC against *Candida albicans*.



SD \pm Means of zone of inhibition obtained by sample AC against *Candida albicans*, *Aspergillus niger*

S. NO	Name of the test organism	Name of the test sample	Zone of inhibition (mm) SD \pm Mean				
			500 μ g/ml	250 μ g/ml	100 μ g/ml	50 μ g/ml	PC
1.	<i>Candida albicans</i>	AC	17.5 \pm 2.12	17.25 \pm 1.76	15.5 \pm 0.70	13.5 \pm 0.70	30.5 \pm 0.70
2.	<i>Aspergillus Niger</i>	AC	16.5 \pm 0.70	13.75 \pm 0.35	0	0	9.75 \pm 0.35

SD – Standard Deviation, *Significance - $p < 0.05$

DISCUSSION

The Anti-bacterial activity of the extract was measured by observing bacterial free zones formed around the discs. The Anti-bacterial study was carried out for Ashta chooranam against different strain of bacteria (Gram +ve such as *Streptococcus pyogenes*, *Streptococcus oralis*, *Staphylococcus aureus*, *Propionibacterium acnes* and four Gram -ve such as *Pseudomonas aeruginosa*, *Fusobacterium nucleatum*, *Klebsiella pneumoniae*, *Aeromonas hydrophila*) are known to cause infection in human and plants by agar- well diffusion method. Gentamicin was used as a positive control. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells. The zone of inhibition was observed for both Gram +ve and Gram -ve bacteria. The maximum zone of inhibition (20.5mm) was observed for *Staphylococcus aureus* for the Gram +ve organisms and Gram-ve organisms the maximum zone of inhibition (19.75mm) was found for *Aeromonas hydrophila pneumonia*. Thus Ashta chooranam was observed to have significant antibacterial activity. Ashta chooranam were found to have anti-bacterial activity with MIC of 500 μ g/ml for both gram positive and gram negative organisms.

The Anti-fungal activity of the extract was measured by observing fungal free zones formed around the discs. The anti-fungal study was carried out for Ashta chooranam against different strain of fungi (*Aspergillus niger* *Candida albicans*), that are known to cause infection in human and plants by disc diffusion method. Amphotericin B was used as a positive control. The antifungal activity was assayed by measuring the diameter of the inhibition zone formed around the wells. The maximum zone of inhibition (17.5mm) was found for *Candida albicans*. Ashta choornam were found to have anti-fungal activity with MIC of 500 μ g/ml.

CONCLUSION

The individual antimicrobial studies of Ashta Chooranam unveil their respective abilities to inhibit the growth of various microorganisms. Ashta Chooranam demonstrates efficacy against bacteria and viruses, antimicrobial activity with a focus on febrile illnesses. The

nuanced differences in their antimicrobial spectra suggest a complementary approach in addressing diverse microbial challenges.

The Ashta Chooranam introduces a novel dimension to this study. The synergistic effects observed in the combined formulation hint at a potential enhancement of antimicrobial activity. The diverse array of bioactive compounds from the different herbs in each formulation may act collaboratively, potentially broadening the spectrum of effectiveness against a wider range of pathogens. This synergy underscores the holistic nature of traditional herbal medicine, where multiple components work in concert to yield therapeutic benefits.

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