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IN VITRO EFFICACY OF PADIGALINGA CHENDOORAM, A TRADITIONAL SIDDHA MEDICINE AGAINST BACTERIAL PATHOGENS

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ABSTRACT

Infectious diseases are the main human killers, particularly in developing countries. Many traditional siddha medicines are used in the traditional treatment of Infectious diseases, but there has not been a sufficient focus on evaluating their antimicrobial properties. This study aimed to evaluate the antimicrobial properties of Padigalinga chendooram, a traditional herbo-mineral siddha medicine against bacterial pathogens. The zone of inhibition of the aqueous extract of Padikaraparpam was examined by using the agar well diffusion protocol. All tested concentrations showed considerable antibacterial activity. The highest activity showed by the 200 µg/ml concentration followed by 100 µg /ml compared to 50 µg/ml. There is no zone of inhibition in the lowest concentrations viz 25 µg/ml, 12.5 µg/ml against all pathogens. Thus, the study backs up the therapeutic claims that Padigalinga chendooram has strong antibacterial properties and provides significant therapeutic advantages against bacterial pathogens.

Keywords: Padigalinga chendooram, antimicrobial, siddha medicine, nanomedicine

INTRODUCTION

The most important among all other medical systems in the world is the Siddha System of

Medicine (Traditional Tamil System of Medicine), which was prominent in the ancient Tamil region. Its beginnings are between BC 10,000 and BC 4,000. Despite many of its contemporaries have long ago gone extinct, the Siddha System has continuously served humanity for more than 5000 years, both in the fight against disease and in sustaining its physical, mental, and moral health. One of the oldest medicinal systems of India is siddha medicine. The Dravidians and Tamils of ancient peninsular India used Siddha as their primary medical system. Siddham is an acronym for established truth (1)**.** Ayurveda, Siddha, and Unani are the three most prevalent traditional medical systems in contemporary India. Siddha medicine can be found in Tamil Nadu and some sections of Kerala, whereas Unani, which is derived from Arabic medicine, is present across India, primarily in urban areas. Ayurveda is primarily practiced in northern India and Kerala in the south. To examine the parallels and contrasts between Siddha medicine and Ayurveda, this study concentrates on South Indian Siddha medicine including its history and current practices (2)**.**
Numerous herbo-mineral medicines have been used in India for a very long time in a safe manner. However, there are increasing concerns about the security and effectiveness of these herbal and mineral medicines. The insufficiency or absence of standards is one of the main barriers preventing the wider acceptance of these pharmaceuticals from poor countries. Medicines made by manufacturers are authentic and of high quality according to standardization, which also brings consumers relief and satisfaction in prescribing physicians. In the present investigation, validation of a Siddha herbo-mineral preparation of padigalinga chendooram was executed by analyzing the characterization and anti-microbial properties. Though, this medicine has been traditionally used, till now there is no scientific validation reported for this formulation. 'Chendooram' is a category of medicine with reddish colour powder and it retains potency for 75 years. From ancient time padigalinga chendooram used for gingivitis, eye diseases, gastric ulcer, diarrhea, dysentery, children's vomiting, diarrhea, whooping cough, spittle cough with expectorant, pharyngitis, and gonorrhea.

MATERIALS AND METHOD

Test drug

The test drug Padigalinga chendooram was procured from IMPCOPS was used in the present study. The test drug contains ingredients like Lingam (Red sulphide of mercury), padikaram (alum), Kadukkai poo (*Terminalia chebula* gall) and Kataththi poo (*Woodfordia fruticosa*).

Chemicals for antimicrobial assay

As a reference antibiotic Ciprofloxacin, Mueller Hinton agar (Himedia, Mumbai) is used.

Test microorganisms

Collection of samples

Wound pathogens were collected from Anand Hospital, Manali, Chennai, and Tamil Nadu. The pathogens were inoculated in Cary-Blair transport medium until processed for Gram staining and culturing. The samples were aseptically inoculated on blood agar (with 5% sheep blood) and MacConkey agar plates, and incubated aerobically at 35°C–37°C for 24–48 h (3-12). Using standard microbiological, biochemical and molecular biology methods, identification and characterization of isolates were performed based on the Gram staining, microscopic and

biochemical characteristics, and 16s rRNA sequencing. The sequences were submitted to NCBI (13-15).

METHODOLOGY

Stock solution was prepared by dissolving 2 gm of each drug in 10 ml of distilled water. Then placed on the rotary shaker at 190-220 rpm for 24 hr. The extracts were filtered using Whatman filter paper (125mm) and stored at 4˚C in air-tight bottles until further use. Bacterial isolates were first grown in nutrient broth for 18hrs before use. One hundred microlitres of the standardized bacterial suspension were evenly spread onto the sterile Mueller-Hinton agar plates. Wells were then bored into the agar medium using a sterile 6-mm cork borer and the wells filled with different concentrations of the extract obtained by double serial dilution. Then the plates were incubated at 37 ◦C for 24 hrs (16-22). The effects of the extract on the test bacterial isolates were compared with the commonly used antibiotic ciprofloxacin (15 µg). The zone of inhibition was noted.

RESULTS AND DISCUSSION

The zone of inhibition of aqueous extract of padigalinga chendooram was examined by using agar well diffusion method. All the tested concentrations showed considerable antibacterial activity except the least concentrations 25 µg/ml, and 12.5 µg/ml. The highest activity explored by the 200 µg/ml concentration followed by 100 µg /ml compared to 50 µg/ml. There is no zone of inhibition in the lowest concentrations viz 25 µg/ml, 12.5 µg/ml against all pathogens..Table 1. Shows Antimicrobial Zone of inhibition (in mm) of Padigalinga chendooram against clinical isolates and Table 2. Shows the 16s rRNA of pathogens submitted NCBI Accession number.

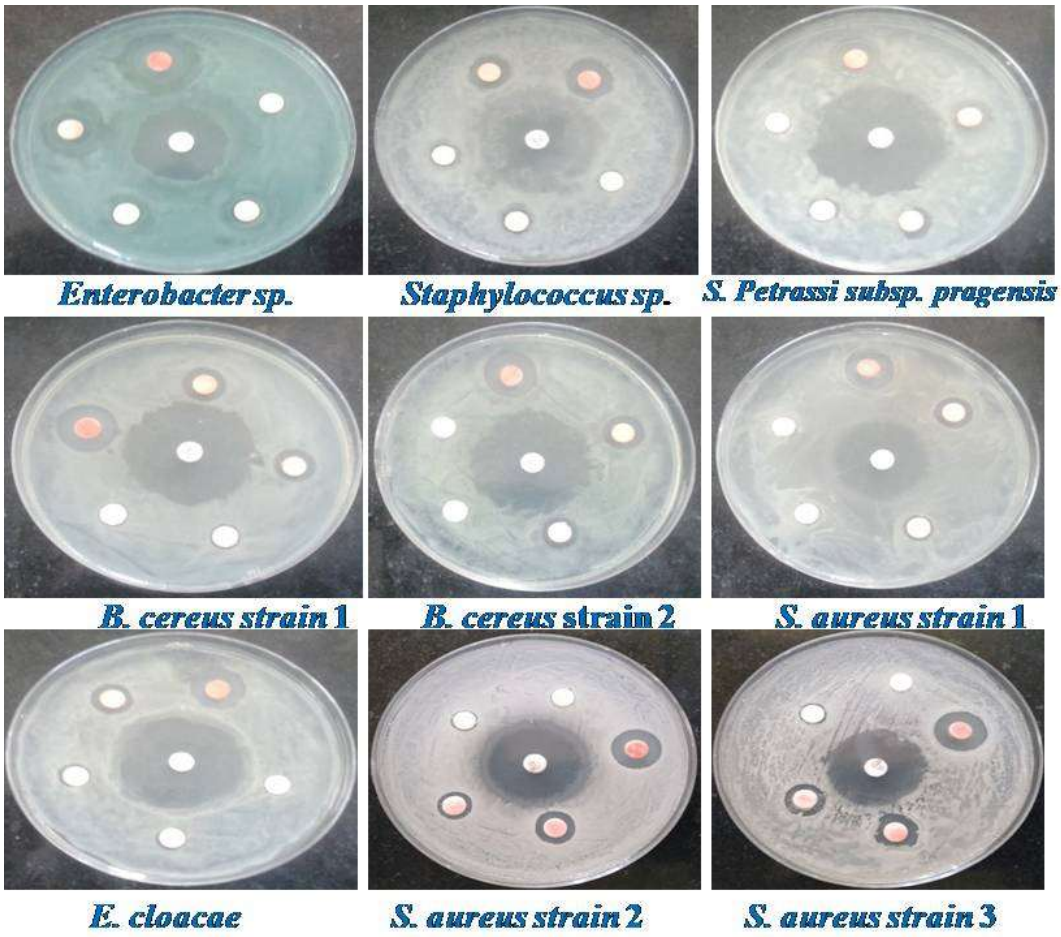

Figure 1. Antimicrobial Zone of inhibition (in mm) of Padigalinga chendooram against the pathogens

Table 1. Antimicrobial Zone of inhibition (in mm) of Padigalinga chendooram against the pathogens

Pathogen name	**Different concentration of Padigalinga Chendooram (Zone of inhibition in mm)**					**ciprofloxacin (15mcg)**
	12.5µg/ml	**25 µg/ml**	**50 µg/ml**	**100 µg/ml**	**200 µg/ml**	
Enterobacter sp.	NIL	NIL	5	11	15	24 mm
Staphylococcus sp.	NIL	NIL	5	11	13	15 mm
Staphylococcus petrassisubsp.pragensis	NIL	NIL	10	11	12	34 mm
Bacillus cereus strain1	NIL	NIL	10	12	16	32 mm
Bacillus cereus strain2	NIL	NIL	NIL	5	13	16 mm
Staphylococcus aureus strain1	NIL	NIL	10	12	15	32 mm
Enterobacter cloacae	NIL	NIL	8	11	14	24 mm

Staphylococcus aureus strain 2	NIL	NIL	NIL	11	15	31 mm
Staphylococcus aureus strain 3	NIL	NIL	10	11	14	28 mm

Table 2. Bacterial pathogens and their NCBI Accession number

Bacterial pathogens	**NCBI Accession Number**
Enterobacter sp.	MG763134
Staphylococcus sp.	MG774417
Staphylococcus petrassisubsp.pragensis	MG970131
B.cereus strain1	MH393374
B. cereus strain2	MH393401
S. aureus strain1	MH431700
E.cloacae	MH553000
S. aureus strain 2	MH552992
S. aureus strain 3	MH552991

UV-Spectrum

UV-Vis spectroscopy could be used to examine the size and shape-controlled nanoparticles in aqueous suspensions. The absorption spectrum was recorded for the sample in the range of 200-500 nm and the maximum absorbance was recorded at 220 nm (Fig.2). The results reveal that exposure to padigalinga chendooram was observed. Earlier research findings also state that there are no other peaks seen in the medicine.

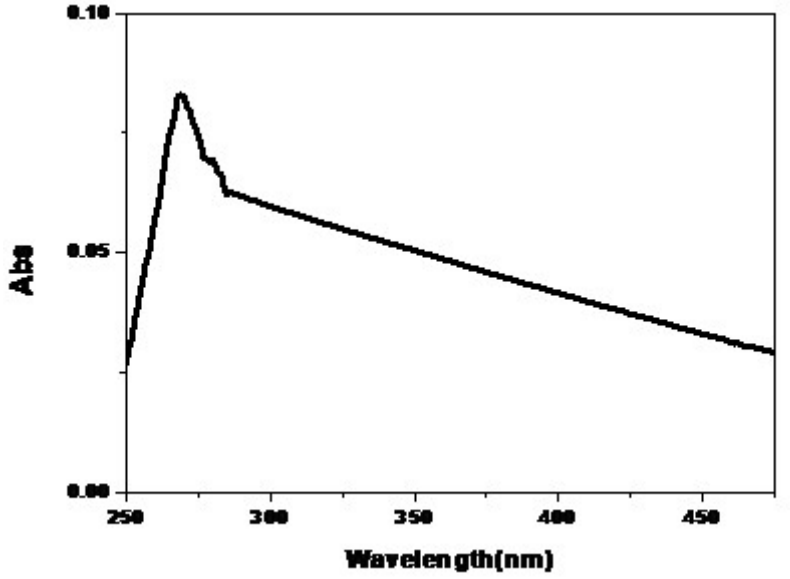

Figure 2. UV Visible spectrum of padigalinga chendooram

XRD analysis

The XRD pattern of chendooram extract findings recorded in the present work was in corroborated with the previous observation. The sharp Bragg's reflections might be due to the crystalline nanoparticles and the intense peaks suggesting that, the strong X-ray scattering centers in the crystalline phase. Independent crystallization of the capping agents was ruled out because of the process of centrifugation and redispersion of the pellet in Millipore water and hence here in this there is no formation of the nanoparticle peak is seen (Fig.3).

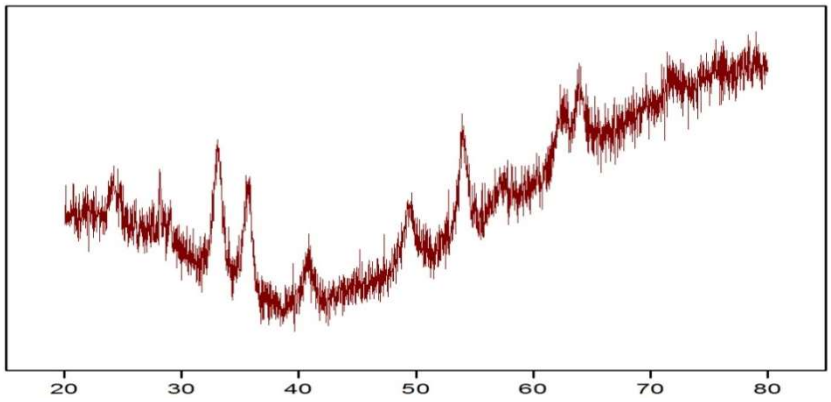

Figure 3. XRD analysis of padigalinga chendooram

FTIR study

FTIR result obtained from chendooram and studied which ranges from 1000 to 4000nm. FTIR helps to identify possible biomolecules present in the extract which acted as reducing agents and are responsible for their capping and stabilization. Graphs obtained for FTIR are shown in figure 4. The bands were seen at 3739cm^{-1}, 2978cm^{-1}, 2354cm^{-1}, 1516cm^{-1}, 1039cm^{-1}, 805cm^{-1} and 665cm^{-1} (Fig. 4). While that at 3739cm^{-1}, 2978cm^{-1}, 2354cm^{-1}, 1039cm^{-1}, 805cm$^{-1,}$ and 665cm^{-1}suggests the presence of biomolecules present in chendooram which acted as a reducing agent and also acted as capping and stabilizing agents. Similarly, the band observed at 1516cm^{-1} is due to the asymmetric and symmetric stretching of carboxylate the carboxylate probably comes from the reactive carbon-containing plasma species. As the size of nanoparticles increases the contents of carboxylate groups in the sample decrease.

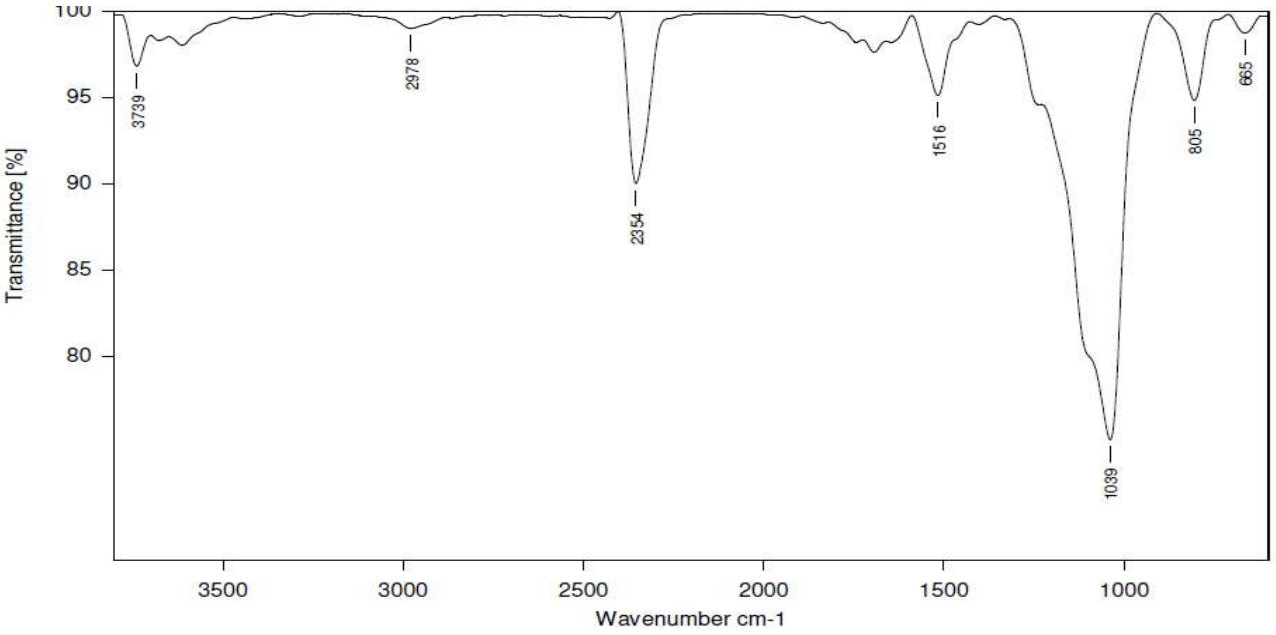

Figure 4. FTIR analysis of padigalinga chendooram

TEM

Transmission electron microscopy (TEM) has been used to identify the size, shape, and morphology of nanoparticles. The measured average size of chenduranam was 200nm (Fig.5). Occasional and full agglomeration of the chendooram has been observed. From the TEM images, it is evident that most of the particles were spherical or pencil heads and irregular in shape. In other studies, the shape was recorded as spherical or pentagons, however, the particle size ranges were within the limits. The variations in shape may be because of the variations in concentration, pH of the reaction mixture, reaction time, incubation temperature, concentration, and electrochemical potential of a metal ion.

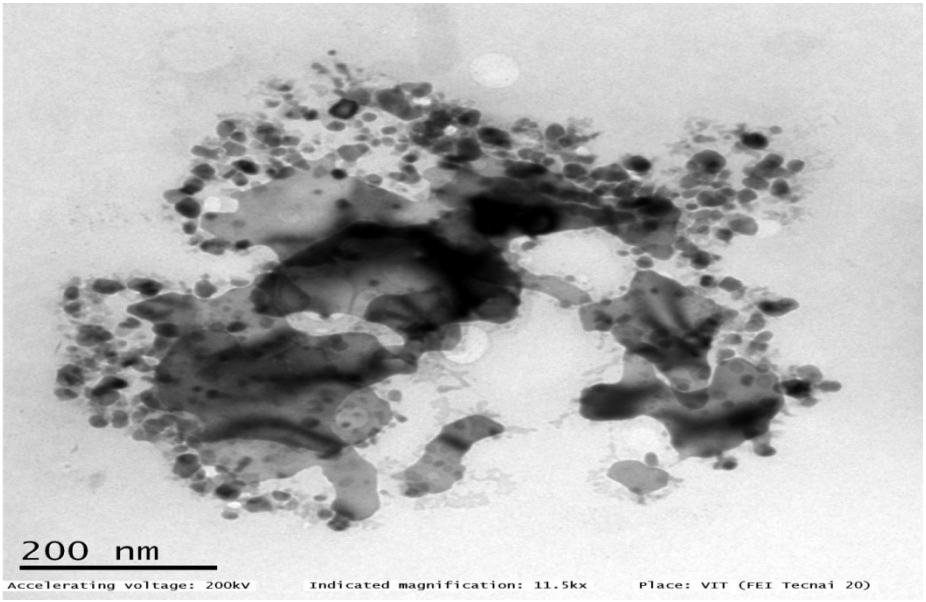

Figure 5. TEM analysis of padigalinga chendooram

Various dosages of test drugs against microorganisms revealed the increasing inhibitory effect with incremental dosages. This result suggests that the test drug Padigalinga chendooram is efficacious against pathogens. The development of resistance against the presently available antibiotics arises the necessity for the rediscovery of new antibacterial agents in traditional systems of medicine (23-26). The findings reveal that Siddha herbo-mineral drug - Padigalinga chendooram has antimicrobial potency against bacterial pathogens and can be used in the treatment of infectious diseases.

Conclusion

The data obtained in these studies justify and support the usage of this drug in biomedical applications. Further research in my topic of interest will be the detection of the compounds responsible for the observed antibacterial activity.

The authors report no financial or any other conflicts of interest in this work.

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Competing interests

Authors declare no competing interest.

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