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Original Research Paper

ANTIMICROBIAL EVALUATION OF CHIRATTAI THAILAM

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ABSTRACT

The antimicrobial activity of the wound healing Siddha drug, Chirattai thailam was assayed by the agar plate disc diffusion, well diffusion method, pour plate method, direct drop method and nutrient broth dilution techniques. Test microorganisms were *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*. All organisms were laboratory isolates from pus samples. The drug showed significant inhibited growth of test organisms especially against *S. aureus*. This study has justified the traditional use of this drug for the treatment for wound healing.

Keywords: Antimicrobial, Siddha drugs, Wound infection, Chirattai thailam.

INTRODUCTION

Wounds, resulting from microbial infection, are the most common public health problems. The common wound pathogens includes bacteria, fungi, protozoa and viruses among which the most common are Streptococcus pyogens, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus*, *Escherichia coli*, *Enterococcus*, *Acinetobacter*, *Klebsiella*, and other *Coliforms*. Although wounds may heal through the body's natural process of regenerating dermal and epidermal tissues, chronic forms cause significant impact on health and economic growth. Current methods used to treat chronic wounds include debridement, irrigation, antibiotics, tissue grafts and proteolytic enzymes, which have major drawbacks and unwanted side effects. Topical antimicrobials may be indicated when the clinical signs and symptoms of an active infection are present. Complications of deep tissue infections such as bacteremia can be treated with systemic antibiotic. However, the increase in antibiotic resistant strains together with the lack of and high cost of new generation antibiotics increased wound-related morbidity and mortality. A wider range of plants are being used in the treatment of wounds and other diseases in the traditional health care system. Crude extracts of plants and others used elsewhere revealed strong antibacterial activities indicating that these plants can serve as sources of effective drugs against wound-causing bacteria.

Siddha system of medicine is one of the oldest systems of medicine in Tamil Nadu, India. Herbal medicines are being used by about 80% of the world population mostly in the developing countries for primary health care. These medicines have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. Indian medicinal drugs and their derivatives have been an invaluable source of therapy due to their antibacterial, antihelmintic, anti ulcer, anti-inflammatory and even anticancer, antioxidant and anti-inflammatory activity. In recent years, among the world population, there is an increasing trend towards the usage of herbal medicines especially Siddha medicines and

Ayurvedic medicines (Indian system of Medicine) which may be probably due to the side effects and enormous cost involved in modern medicines. And with an increased incidence of resistance to antibiotics, herbal medicines and natural products from plants could be interesting alternatives. So it is necessary to scientifically evaluate these medicines and their phytoconstituents. The objective of this study was therefore to evaluate the activity of Chirattai thailam traditionally used Siddha drug for skin ailments.

According to the Siddha system of medicine, one of the most important external medicines is Chirattai Thailam (coconut shell oil), easily obtainable in South India. Coconut shells are the only 'drug content' used in the preparation of Chirattai Thailam. It is a very effective medicine for eczema, ringworm infections, plantar warts, warts, and various other skin diseases. As per the Siddha practitioners it is applied on the skin for treatment of fungal infections and planter warts, or warts. As it may cause mild corrosion, this oil is mixed with an equal quantity of coconut oil before application on the skin. It is best suited for corns on the foot. In the case of corns, Chirattai Thailam is applied with Croton seed and three to four drops of coconut oil. For best results, it is better to use at night. Apply the oil on the affected area at night and wrap it with a cotton cloth. Remove the cloth only in the morning. If the treatment is followed for three consecutive days, one can be completely cured of warts, corns, and other skin problems. As this oil is corrosive, it should not be applied near the eyes. There was no extensive report on the presence of active ingredients and compounds from this drug. In this investigation, the in vitro antimicrobial effects of the Chirattai thailam against the organisms found commonly in pyogenic infections were investigated.

MATERIALS AND METHODS

Test Drugs

Chirattai thailam was purchased from The Indian Medical Practitioner's Co-operative Pharmacy & Stores Ltd (IMCOPS), Pondicherry, India. Amoxycillin and Ciprofloxacin ready to use paper discs of HI-Media and tablets from Government Pharmacy were used. Chirattai thailam is prepared through the 'Kuzhi Thalika' method. Under this method, thin wires are inserted into a clay pot through small circular holes. These thin wires are pulled out of holes on the bottom of the pot and tied into a knot so that it holds firm. After that, the upper ends of the pot are filled with pure, well-dried coconut shell pieces. Once the pot is sealed, the sides and top of the pot are covered by cow dung cakes, which are then burnt. The resultant heat produces medicinal oil from the coconut shell pieces inside the pot. Expert pharmacists of Siddha medicine then collect the oil through the 'destructive distillation method' by placing a vessel below the pot's bottom hole.

Test Organisms

Staphylococcus aureus, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Most of the isolates from pus samples were obtained from the Department of Microbiology, Indira Gandhi Government General Hospital, Pondicherry, India.

Methods of Studying Antibacterial Effects

First of all, a suspension was made out of the bacteria. Mueller-Hinton broth matrix was poured into the test tubes equal to the number of bacteria and was then sterilized. Next, through a swap sanitization, some of the bacteria were transferred from the Petri dish to the test tubes that contained the Mueller-Hinton broth matrix and eventually the suspension was provided. In the next 24 hours, the tubes were held in the incubator with the degree of 37 degrees Centigrade to let the bacteria grow. After that, the tube murkiness was measured by the McFarland witnessed murkiness. The murkier the microbial suspension is, the weaker it would be. When the suspension is provided, it is evenly spread all over the Mueller-Hinton broth cultural matrix by a swap suspension in three directions and with a 60-degree-rotation each time on the cultural matrix in the Petri dish. It is all done in sterile conditions. The antibacterial effect of the Siddha drug was studied by the following methods: Minimum inhibitory concentrations (MIC), well, pour plate, disc paper and direct drop methods.

Disc Paper Method

Under Laminar Airflow, 150 microliters of Chirattai thailam were poured on the blank discs (for three successive times, 50 microliters each time). The blank discs had been sterilized with autoclave. When the drug were dried in the proper time, the discs, which now contain the drug, were put on certain spots of the cultural matrix on which the bacteria were previously inoculated by lawn culture method. Standard antimicrobial disc used were Amoxycillin and Ciprofloxacin. The Petri dishes were put in an incubator at a temperature of 37 degrees Celsius for 24 hours. Later, the results were recorded.

Well Method

In some parts of the matrix on which the bacteria were cultured, some wells with the diameter of 3 millimeters made. Therefore, about 150micro-liters of Chirattai thailam was poured by a micropipette and the Petri dishes were kept in the fridge for 2 hours until the drug properly permeated the cultural matrix around the wells. It was repeated three times (every two hours). Then, the Petri dishes were carefully put in a 37-Centigrade incubator for 24 hours. When the bacteria grew in the proper time, the impact of drug on the bacteria growth was studied and recorded.

Pour Plate Method

Before the cultural matrix completely cooled down and was covered (40°C), 1ml of the Chirattai thailam was added to the 9 millimeters of the cultural matrix. Then, the Petri dishes were slowly shaken in different directions until the extract properly and evenly spread over the cultural matrix. When the cultural matrix was covered, the bacteria were cultured over them using a standard wire loop of diameter 0.01 mm carrying 0.001ml of liquid culture of bacterial strains and the samples were kept in 37 degrees Celsius in incubator for 24 hours. Colony counts were noted.

Direct Drop Method

150 micro liters Chirattai thailam is directly dropped on single spots of the cultural matrix surface. The drops were left to permeate the matrix. Finally, The Petri dishes were put in an incubator at a temperature of 37 degrees Celsius for 24 hours. Later, the results were recorded.

Minimum Inhibitory Concentration (MIC) Method:

100 micro-liters of the microbial suspension from the 24-hour bacteria culture were added to the tubes numbered 1 to 9. The tubes were put in the incubator with the degree of 37 degrees C for 24 hours. The relevant results were recorded. MIC was defined as the lowest concentration where no visible turbidity was observed in the test tubes. The MIC was determined for the micro-organisms that showed reasonable sensitivity to the test drug. In this test, the micro-organisms were prepared using the broth dilution technique. The stock drug concentration of 100 mg/ml was made by dissolving 1 g of the drug in 10 ml of sterile distilled water and the working concentrations prepared by two-fold serial dilution technique that ranged from 0.195 mg/ml to 50 mg/ml using nutrient broth and later inoculated with 0.2 ml suspension of the test organisms. After 24 h. incubation at 37^{o}C, the tubes were observed for turbidity. The lowest concentrations where no turbidity was observed was determined and noted.

RESULTS AND DISCUSSION

The results of studying the impact of Siddha drug on the six bacterial strains in the disc diffusion Method, Well Method and direct drop method are summarized in Table1, Table 2 and Table 3 respectively. Both well and direct drop methods provided the same results as that of disc diffusion. But due the oily nature of the drug, it was difficult to demarcate the zone of inhibition and record the results. Chirattai thailam showed activity against all the tested organisms. It was more active against *S. aureus* (15 mm) and *A. baumannii* (16mm). Least activity was recorded against *P. aeruginosa* (08 mm).

The results of studying the impact of Siddha drug on the six bacterial strains in the pour plate Method is summarized in Table 4. A semi quantitative colony count was measured based on the standard wire loop method. A significant reduction in the colony count was noted in all the

test plates. The control plate without drug showed colony count of >100000 for all bacterial strains. It was clear that, the drug have an inhibitory impact on bacterial strains isolated from wound infections especially against *A. baumannii* (<25000 CFU/ml). Minimum Inhibitory Concentration (MIC) of Chirattai thailam is recorded in Table 5. The drug was active more around 6.25mg/ml for *S. aureus* and *Esch. coli*. The MIC for *A. baumannii* was recorded least (3.125mg/ml).

Table 1: Antimicrobial activity of Chirattai thailam (By Disc Paper Method)

Name of the drug	Organisms and Zone of inhibition in mm					
	S. aureus	*E. faecalis*	*P. aeruginosa*	*Esch. coli*	*K. pneumoniae*	*A. baumannii*
Chirattai thailam	15	12	08	12	12	16
Amoxycillin	21	22	18	16	18	25
Ciprofloxacin	10	24	10	22	24	21

Table 2: Antimicrobial activity of Chirattai thailam (By Well Diffusion Method)

Name of the drug	Organisms and Zone of inhibition in mm					
	S. aureus	*E. faecalis*	*P. aeruginosa*	*Esch. coli*	*K. pneumoniae*	*A. baumannii*
Chirattai thailam	15	12	08	12	12	16
Amoxycillin	21	22	18	16	18	25
Ciprofloxacin	10	24	10	22	24	21

Table 3: Antimicrobial activity of Chirattai thailam (By Direct Drop Method)

Name of the drug	Organisms and Zone of inhibition in mm					
	S. aureus	*E. faecalis*	*P. aeruginosa*	*Esch. coli*	*K. pneumoniae*	*A. baumannii*
Chirattai thailam	15	12	08	12	12	16
Amoxycillin	21	22	18	16	18	25
Ciprofloxacin	10	24	10	22	24	21

Table 4: Antimicrobial activity of Chirattai thailam (By Pour Plate Method)

Name of the drug	Organisms and colony count(CFU/ml of drug)					
	S. aureus	*E. faecalis*	*P. aeruginosa*	*Esch. coli*	*K. pneumoniae*	*A. baumannii*
Chirattai thailam	<70000	<80000	<70000	<80000	>70000	<25000
Amoxycillin	<25000	<25000	<25000	<25000	<25000	<25000
Ciprofloxacin	>100000	<25000	>100000	<25000	<25000	<25000

CFU= colony forming units

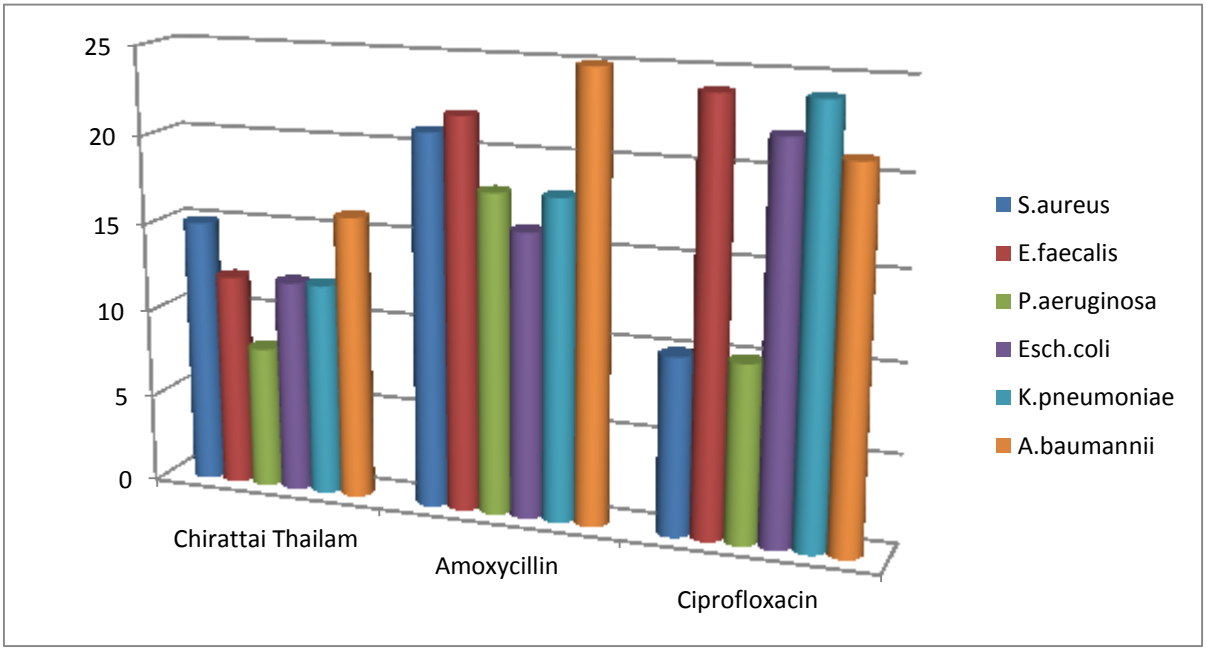

Figure 1: Comparative Antimicrobial effect of Chirattai thailam

Table 5: Minimum Inhibitory Concentration (MIC) of Chirattai thailam

Organisms	Concentration (mg/ml)									
	0.098	0.195	0.390	0.780	1.563	3.125	6.250	12.500	25.00	50.00
S. aureus	+	+	+	+	+	+	*	#	#	#
E. faecalis	+	+	+	+	+	+	+	*	#	#
P. aeruginosa	+	+	+	+	+	+	#	#	*	#
Esch. coli	+	+	+	+	+	+	*	#	#	#
K. pneumoniae	+	+	+	+	+	+	+	+	*	#
A .baumannii	+	+	+	+	+	*	#	#	#	#

Key: +=turbidity seen; #=No turbidity seen;* MIC value

The advantage of herbo-mineral preparations is their stability over a period, easy storability and sustained availability. Herbs mixed with minerals and metals make them more effective against the target pathogens. It was observed that the selected wound healing Siddha drug viz. Chirattai thailam showed significant antimicrobial activity against the common pathogens of wound infection such as Staphylococcus aureus, Acinetobacter baumannii, and Escherichia coli when compared to the standard antibiotics Amoxycillin and Ciprofloxacin which are taken as positive control. The disc diffusion method provided more clear and recordable zone of inhibition compared to well and direct drop methods. But all the three methods provided the same results.

CONCLUSIONS

The drug, Chirattai thailam showed significant antibacterial activity mostly against the common pyogenic Bacteria, Staphylococcus aureus and Acinetobacter baumannii. The activity was dose dependent and was highest at 6.25 mg/ml. and lower concentrations were not effective. When the results were compared with the antibacterial activity of Amoxycillin and Ciprofloxacin, the drugs showed comparable antibacterial activity. In general, the present screening of Siddha formulation Chirattai thailam showed antibacterial activities. This indicates the potential of the Siddha drug to be used as antibacterial agents against wound causing pathogens. However, further studies should be conducted with different extraction solvents and toxicity and phytochemical analysis should also be performed on this drug to

use them as sources and templates for the synthesis of drugs to control wound and other disease-causing bacteria.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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