



SCREENING OF ANTI-MRSA ACTIVITY OF SOME SELECTED TRADITIONAL DRUG FORMULATIONS AGAINST DIABETIC FOOT ULCER ISOLATES

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ABSTRACT

Methicillin resistant *Staphylococcus aureus* (MRSA) is a well known superbug commonly responsible for wound-related infections and most ominously for its life-threatening conditions. The MRSA infections are great medical challenges in diabetic patients, especially causing ulcer foot infections and related complications which might lead to amputation and ultimately to death. The alarming increase in drug-resistant bacteria makes it a global concern and the need to find novel drugs imperative. In the present study, an anti-MRSA screening was performed to substantiate the antibacterial activity of some selected ayurvedic traditional preparations used as a topical application for skin infections and wound healing. Jatyadi taila, kanjunniadi taila, durvadi taila, and murivenna were subjected for this study. Among the studied samples, jatyadi taila and kanjunniadi taila have shown significant anti-MRSA activity.

KEYWORDS: *Staphylococcus aureus*, MRSA, drug resistance, jatyadi taila, kanjunniadi taila, well diffusion method



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INTRODUCTION

The development of antibiotic resistance is an unavoidable phenomenon, since it is associated with the natural evolution of the highly flexible bacterial genome¹. Consequently, physicians are deprived of solutions to combat infections caused by highly resistant pathogenic strains¹. *Staphylococcus* species are the predominant representing organism in life threatening infections like bacteremia, diabetic foot ulcer infections etc². Foot ulcer infections are one of the major challenges in patients with diabetes mellitus which is associated with significant morbidity, disability and impairment in a diabetic patient's life quality. It is accounting for up to 20% of diabetes-related hospital admissions and often the proximate cause of lower extremity amputation unless prompt treatment strategies are ensured. Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a serious and common problem in patients with diabetic foot ulcers and the increased prevalence may be due to indiscriminate antibiotic use³. These chronic wound infections not responding to conventional treatment modality are the important cause of disability and mortality⁴. So the current medical scenario put stress on the discovery of new antimicrobial agents with diverse chemical structures and novel pharmacological responses. In Indian traditional medical system, there are several ayurvedic formulations like taila which have been claimed as potential wound healing agents. Taila are medicated oils in ayurveda and prepared by boiling oils with mixture of herbs which are the source of innumerable bioactive agents. Since all the traditional medicines in use are comprised of multiple components, each containing multiple active phytochemicals, their synergetic action has a main role in the effectiveness of the preparation. In the present study, an attempt was made to develop simple and effective treatment against MRSA by using different traditional formulations such as jatyadi taila, kanjunniadi taila, durvadi taila and murivenna which are used for topical application on skin, hair and wound infections. There are some reports of the wound healing

efficiency of jatyadi taila in excision wound model⁵ and partial thickness burn wound model⁶. The ingredient plants of jatyadi taila have been proven scientifically for their antimicrobial properties⁷. The other taila hardly posses scientific evidence for their antibacterial potency. Therefore, the aim of this study was to further explore their antibacterial activity against life threatening infections of MRSA.

MATERIALS AND METHODS

Materials

Four different traditional formulations such as jatyadi taila, kanjunniadi taila, durvadi taila, murivenna were tested in this study. The gingelly oil was the base substance of all selected samples. The triplicate samples of each formulation were collected from three different reliable resources (traditional healers) of Kerala, India. Gentamicin (10µg/100ul) was used as the standard antibiotic in this study.

Microbial strains

Total 50 isolates of *S.aureus* were collected from various diabetic foot ulcer patients. The strains were characterized by gram staining and biochemical tests. The methicillin sensitivity test was performed by Kirby Bauer disc diffusion method.

Medium

Dehydrated Mueller Hinton Broth (MHB) and Mueller Hinton Agar (MHA) were purchased from HiMedia Laboratories for the anti-staphylococcal studies.

Methods

In vitro anti-MRSA activities of collected samples were examined by agar well diffusion method^{8,9}. Well-isolated colonies of MRSA strains were grown in at 37°C for 18–24 h. The culture density was adjusted to McFarland standards No. 0.5 and re-suspended in MHB to obtain a final concentration of 1×10^6 cfu/ml prior to spread plating. The MHA plates were inoculated with 50µl of the inoculum (1×10^6

cfu/ml). For agar well diffusion method, wells were prepared in the plates with the help of a cork-borer (8mm). 100 µl of the test samples, gingelly oil (control) and gentamicin (positive control) were poured individually into each of the wells. The plates were incubated overnight at 37°C. The antimicrobial actions were determined by measuring the zone of inhibition in millimeters and recorded.

RESULTS AND DISCUSSIONS

S.aureus is a major pathogen in nosocomial and community acquired infections as a chief cause of wound suppuration¹⁰. Most of the clinical isolates of *S. aureus* are resistant to a number of antibiotics, especially to β-lactum antibiotics which are widely used for antibiotic therapy¹¹. The mortality due to *S.aureus* bacteremia remains approximately 20–40% despite the availability of effective antimicrobials¹². The increasing drug resistance of this organism makes serious problems especially in patients with diabetic ulcers which deleteriously lead to amputations. In this present study, out of total 50 strains of *S.aureus* collected from different diabetic foot ulcers, 42

(84%) were found as methicillin resistant (MRSA) and remaining 8 were found obviously as methicillin sensitive (MSSA). The previous reports are also show the seriousness in emergence of β-lactum resistance strains¹³. Such strains are capable to develop resistance to almost all classes of antibiotics, including the best known antibiotic, vancomycin, and are called as Vancomycin Resistant *Staphylococcus aureus* (VRSA)¹⁴. The increased incidence of MRSA/VRSA seeks serious attention to search for novel means for fighting against them. In this study, 4 different ayurvedic oil preparations having bioactive properties were included namely, jatyadi taila, kanjunniadi taila, durvadi taila and murivenna. Gingelly oil is also considered as base oil of the above said test samples. Anti-MRSA activities of the test samples and gentamicin (10µg/100µl) were screened in vitro by agar well diffusion method against 42 MRSA strains and 8 MSSA strains collected from diabetic ulcer foots. The experiment was done with triplicates of test samples and the mean values and standard errors of the zone of inhibitions are presented in the table I.

Table I
Anti - MRSA activity of different taila and gentamicin

Test samples	Zone of inhibitions(mm)*							
	MRSA				MSSA			
	1	2	3	Mean	1	2	3	Mean
Jatyadi taila	18.6 ±0.27	18.2 ±0.27	18.7 ±0.28	18.53 ±0.21	19.0 ±0.26	18.6 ±0.26	18.9 ±0.29	18.83 ±0.11
Kanjunniadi taila	12.8 ±0.35	12.8 ±0.32	12.8 ±0.37	12.84 ±0.30	16.0 ±0.26	15.6 ±0.26	15.9 ±0.29	15.82 ±0.18
Durvadi taila	8.2 ±0.11	8.2 ±0.10	8.4 ±0.13	8.29 ±0.85	7.9 ±0.12	8.0 ±0.00	8.1 ±0.12	8.00 ±0.07
Murivenna	8.1 ±0.09	7.9 ±0.11	8.2 ±0.14	8.09 ±0.88	8.0 ±0.00	7.8 ±0.16	7.8 ±0.16	7.88 ±0.12
Gingelly oil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Gentamicin	18.5 ±0.16	18.6 ±0.19	18.6 ±0.19	18.57 ±0.18	19.8 ±0.16	20.0 ±0.18	19.6 ±0.18	19.88 ±0.12

* Mean ± Standard error

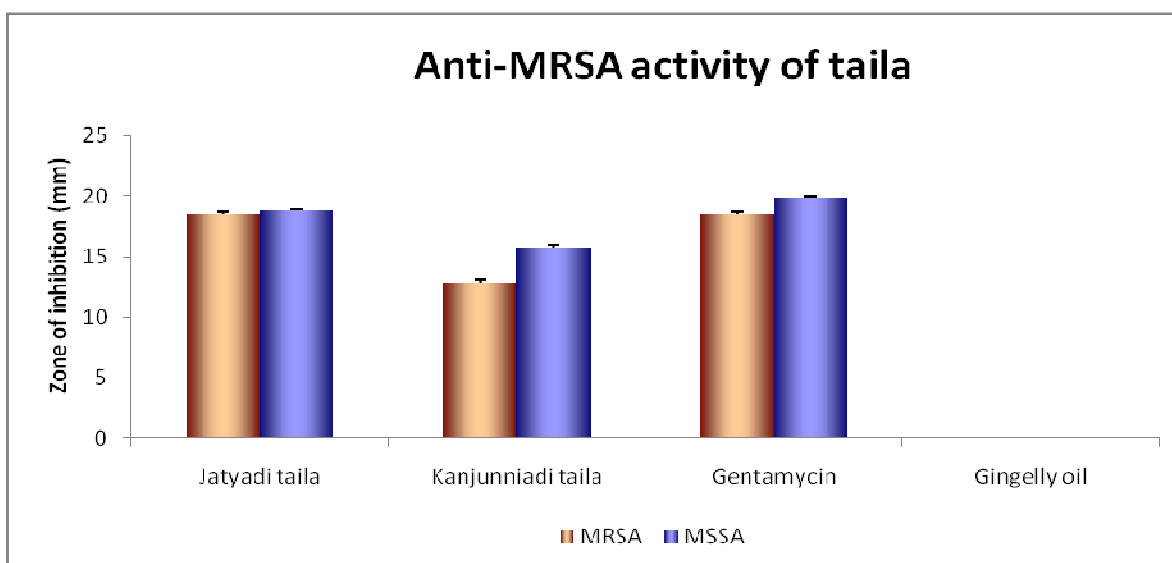
Out of the 4 test samples, jatyadi taila and kanjunniadi taila have shown significantly higher anti-MRSA activity. The Out of the 4 test samples, jatyadi taila and kanjunniadi taila have shown significantly higher anti-MRSA activity.

Zones of inhibition of jatyadi taila and kanjunniadi taila were 18.5±SE 0.21 and 12.8±SE 0.30 respectively. Jatyadi taila showed a range of zone of inhibition from 19 mm to 13 mm and that of kanjunniadi taila was 15mm to

12mm, against 42 MRSA strains. Hence the maximum antibacterial activity was shown by jatyadi taila, followed by kanjunnadi taila. The antibacterial efficacy of jatyadi taila was exactly similar to the standard antibiotic used, gentamicin (10µg/100µl) with 18.6±SE 0.18 mm of zone of inhibition. But durvadi taila and murivenna did not show anti-staphylococcal activity. The base substance of all test samples, gingelly oil was included as a control and it had

no activity against tested strains. Jatyadi taila showed similar antibacterial activity against MRSA and MSSA while kanjunnadi taila was found more active against MSSA. (Graph I). This result points towards another direction of research in mode of action of potential ligands in jatyadi and kanjunnadi taila against target molecules of *S. aureus* other than that of Methicillin.

Graph I
Anti-staphylococcal of activity of taila vs. antibiotics



The results support the traditional usage of the jatyadi taila and kanjunnadi taila against MRSA infections especially diabetic foot ulcers. This present study suggests the jatyadi taila as an antidote for diabetic ulcer foot because of its synergetic effect of wound healing efficacy⁵ and antibacterial activity. It is evident that jatyadi taila and kanjunnadi taila posse's compounds with antimicrobial properties that can be used as new antimicrobial agents against infectious diseases. Further research is necessary to determine the identity of the antibacterial compounds from within these ayurvedic preparations and also to determine their full spectrum of efficacy. However, the present study of *in vitro* antimicrobial evaluation of these

taila forms a primary platform for further phytochemical and pharmacological studies.

CONCLUSION

This in-vitro study shows promising results of jatyadi taila and kanjunnadi taila as anti MRSA agents and jatyadi taila is comparable in effectiveness to gentamicin. Scientific evaluation of these herbal preparations gives better information regarding the antibacterial efficacy of ayurvedic medicines available in India. Further research is needed to isolate the compounds responsible for the observed antibacterial activity.

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