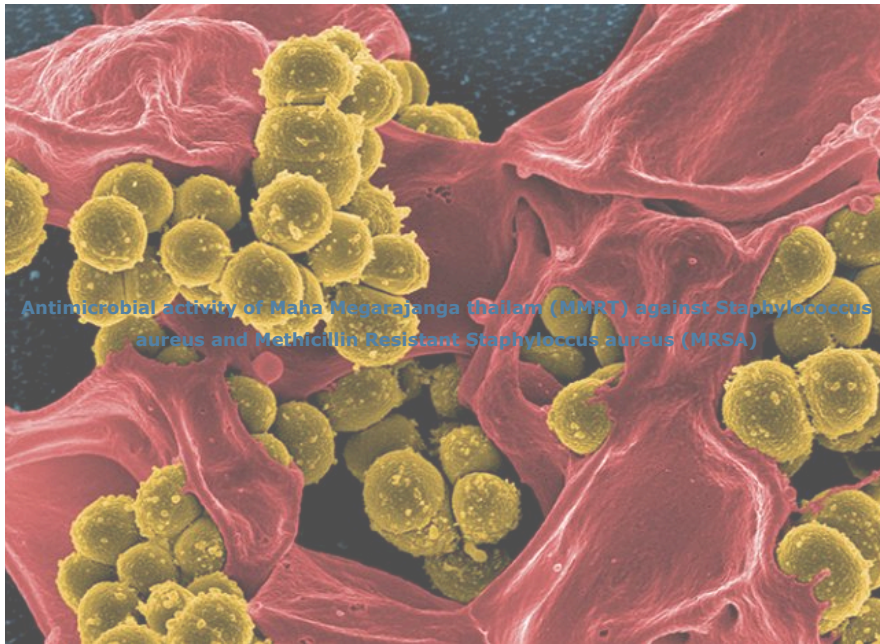


# Antimicrobial activity of Maha Megarajanga thailam (MMRT) against *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus* (MRSA)

Subramanian S<sup>1</sup>, Gnanamani A<sup>2</sup>, Pushkala VP<sup>3</sup>, H Subhashree<sup>4\*</sup>, K Yazhini<sup>5</sup>



## Abstract

*Staphylococcus aureus* is primarily responsible for the persistent infections in chronic wounds. From 2015 to 2019 the generic prevalence of MRSA was found to be 37%. There has been a gradual increase in the prevalence rate of MRSA over a period of time. There is uniform reporting of prevalence rate in all zones of India. One of the most serious global public health concern of this century is Antimicrobial resistance (AMR). World Health Organization (WHO) has emphasized that MRSA is one of the high priority pathogen among multidrug-resistant organisms. Maha Megarajanga Thailam mentioned in the text Theraiyar Taila Varga Surukkam is an unique preparation used externally as well as internally. It has castor oil as base with more than 180 plant parts and minerals as ingredients. It has got unique therapeutic values against skin lacerations, burns, contusions, and diabetic wounds. Vaidyar Sachidananda Swamy has prepared this unique preparation for the past 40 years and has implemented it clinically. The agar well diffusion assay and broth dilution assays were used to determine the antibacterial activity of MMRT. The former is considered to be the gold standard of susceptibility testing which permits detection of contamination and microbial heterogeneity readily whereas broth dilution enables the reproducibility and economy of reagents. The results prove that MMRT is potent against *Staphylococcus aureus* and MRSA. It inhibits bacterial growth and has been proven to be effective in management of diabetic wounds and skin infections.

**Keywords:** Antimicrobial resistance, MMRT(Maha Megarajanga Thailam), MRSA, Agar well diffusion, Broth dilution

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## Declaration

**15.59%** Plagiarism. Authors state no conflict of interest. Non Funded. The conducted research is not related to either human or animals use. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

## Evidence in Context

**What Know:** World Health Organization (WHO) has emphasized that MRSA is one of the high priority pathogen among multidrug-resistant organisms.

**What New:** In this era of increase in transmission of communicable diseases and anti-microbial resistant strains it is becoming increasingly difficult to combat the same.

## To view

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## Introduction

*Staphylococcus aureus* is primarily responsible for the persistent infections in chronic wounds[1]. According to the results of a meta-analysis, the pooled prevalence of MRSA in India between 2015 to 2020 is 37%. There has been a gradual increase in the prevalence rate of MRSA over a period of time. Highest prevalence of MRSA was found to be 55% in Jammu and Kashmir. In all zones of India, there is uniform reporting of prevalence rate[2].

World Health Organization (WHO) has emphasized that MRSA is one of the high priority pathogen among multidrug-resistant organisms[3]. It is capable of producing recurrent infections in wound tissue. This property is attributed to its sturdy biofilm formation. The manifestations range from a simple skin infection to fatal bacteraemia. The pathogen is generally found in urine, blood, sputum and other body fluids. However, it is highly prevalent in wounds[4].

A case control study has arrived to the conclusion that prolonged hospitalization and surgical procedures are risk factors for MRSA infection[5]. Maha Megarajanga Thailam is a unique preparation used externally as well as internally. This formulation includes castor oil as a base added with more than 180 plant parts and minerals. Vaidyar Sachidananda Swamigal had this unique preparation in his clinical practice for the past 40 years. This formulation is prepared preferably during the Munpani kalam (margazhi, Thai-Mid January to Mid-February) and collection of the raw materials will be done in month karthigai (Mid November to mid-December). Finished product will be stored in a mud vessel and sealed properly and buried under earth for one *mandalam* -48 days (Bhoomi Pudam)[6].

It has got unique therapeutic values against skin lacerations, burns, contusions, and diabetic wounds. A case report signifies that Maha Megarajanga Thailam can be effectively used as an adjunctive treatment in diabetic foot ulcer along with the management of appropriate antidiabetic strategy[7]. From GC-MS analysis, it is evident that specific molecules like Squalene, methylsulfanyl, Caryophyllene, Vaccenic acid, and Ricinoleic acid in addition to fatty esters and fatty acids are accountable for the healing property. Further, compounds such as Eugenol, Squalene present improves medication absorption from skin and act against bacteria, fungi, angiogenesis, tumours respectively. Ricinoleic acid is the most abundant compound found in MMRT and its esters are primarily used in modern science as emulsion stabilizers and skin conditioners[8].

## Methodology

In the present study, agar well diffusion assay and broth dilution assays were followed to assess the antibacterial activity of the experimental samples MT and MMRT. The following bacterial cultures were chosen for the study: *Pseudomonas aeruginosa*- ATCC 27853TM; *Escherichia coli* - ATCC 25922TM; *Staphylococcus aureus*- ATCC 25923 TM; MRSA (*Methicillin-resistant Staphylococcus aureus*) - isolated from blood culture. Their storage and standardisation were carried out as per NABL norms.

### Agar well diffusion method:

Before proceeding to the agar well diffusion study, the test organisms were sub-cultured onto fresh plates of Mueller-Hinton agar (Hi Media laboratories, India) for 24 h at 37 °C. Colonies from these plates were suspended in Mueller-Hinton broth to a turbidity matching 0.5 McFarland standard ( $1.5 \times 10^8$  CFU/mL) and were incubated appropriately as specified for each organism for a period of 18–24 h (9).

Agar well diffusion assay was carried out(10). In brief, 100 µL of standardized inoculum of each test bacterium was spread onto sterile Muller-Hinton Agar. A 9 mm sterile polystyrene tip was used to make the well. Each well was filled with the defined volumes of MMRT (25, 50 and 100 µL, 130 µl, 150 µl) and then incubated at 37 C for 24 h. Triplicates were prepared for each sample. The experimental sample having antimicrobial activity inhibits microbial growth and formed the clear zones around the well. The zone of inhibition was measured in millimetres (11). The percentage activities of MMRT were calculated against standard drug (mupirocin) which were considered 100%.

### Broth dilution method:

To assess the antibacterial activity using the broth dilution method, experimental samples were emulsified with Tween 80. Tween 80 (0.05%) used as a control sample. Three different volumes of MMRT (100 µL, 130 µl, 150 µl) were chosen for the study. The aliquot of the emulsion was indented with nutrient broth and the final volume of the culture broth was kept at 1.5 ml. To each tube, 0.5ml of test bacterial culture (overnight grown) was indented and incubated at 37 °C for 12 hours. The test tubes were then subjected to serial dilution to the final concentration of  $10^{-7}$  and plating was done and followed by incubation, the number of colonies shown growth in each dilution was calculated accordingly.(12)

## Results and Discussion

*Aureus* and MRSA strains were found to be susceptible to Maha Megarajanga thailam though in different concentrations. MMRT shows that bacterial growth is effectively inhibited at 150 µL concentration. Formation of zone of inhibition started at a concentration of 100µL of MMRT. Table 1 illustrates the size of zone of inhibition formed by MMRT in varying concentrations. The highest antibacterial activity was recorded against *S. aureus* and MRSA at a concentration of 150 µL. The least activity was recorded against *Pseudomonas* & *E. coli*.(Table 1).

### Table 1: Antimicrobial properties of MMRT- using Agar well diffusion method

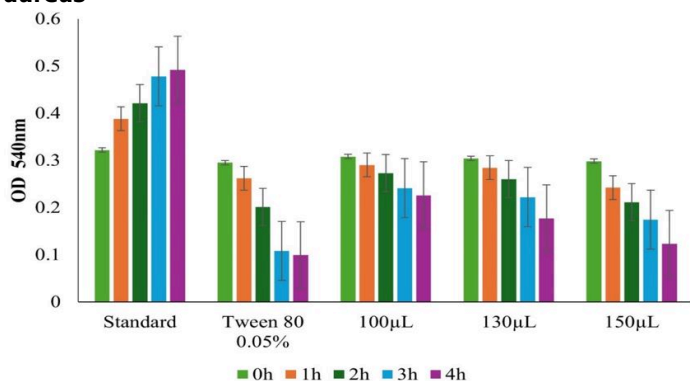
There was a significant variation in zone of inhibition with respect to Gram-negative and Gram-positive organisms. *E. coli* (ATCC 25922TM) showed moderate sensitivity of  $9.8 \pm 0.1$  mm towards MMRT samples when compared to the standard drug Mupirocin (5µg). The zone of inhibition of the standard was found to be  $22 \pm 2$  mm irrespective of increasing concentration. Zone of inhibition of *P. aeruginosa* (ATCC 27853 TM) was measured as  $9.4 \pm 0.2$  mm indicating that it has only mild to moderate sensitivity towards MMRT.

However, with respect to *Staphylococcus aureus*- ATCC 25923 TM, the size of zone of inhibition increases with an increase in the concentration of MMRT and the organism was sensitive to MMRT. The results obtained at higher concentration of 150 µg (20 ± 0.2 mm) was on par with the standard drug employed. It has been evidenced that MMRT sample inhibits the growth of the clinical strain MRSA effectively, also it was highly sensitive to MMRT at a higher concentration of 150 µg.

Followed by the assessment of growth inhibition by the well diffusion method, further experiments on the minimum inhibitory concentration of MMRT with respect to the chosen bacterial strains were carried out by following the broth dilution method.

Only two bacterial strains showing sensitivity in the form of clear zone towards MMRT have been taken for the MIC study. Figure a depicts the results on MIC for *Staphylococcus aureus* ATCC 25923 TM with respect to MMRT sample. Since Tween 80 at 0.05% has been used as a solvent to dissolve the oil samples, experiments with Tween 80 alone served as a positive control for the present experiment. The culture without any test samples showed an increase in growth OD measured up to 4 hours, whereas the cell density decreased with the addition of Tween 20. At the 0<sup>th</sup> hour, the absorbance at 540 nm was measured as 0.3 ± 0.02, and it decreases to 0.1 ± 0.01 within 4 hours of exposure to Tween 80.

**Figure 1: MIC of MMRT with respect to *Staphylococcus aureus***

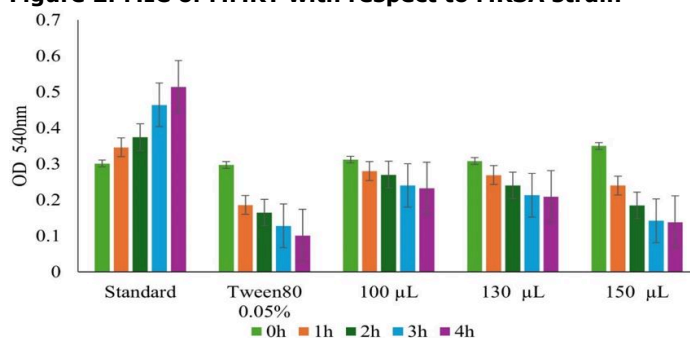


\*Standard -Microbes alone

MMRT sample displayed about a 65% reduction in cell density at 150 µg concentration within four hours of incubation suggesting the bactericidal property of MMRT towards the strain *S. aureus*.

With respect to MRSA (clinical strain), similar to the observations made with the bacterial strain *S. aureus*, about 50-60% reduction in cell density was exhibited by MMRT at a higher concentration of 150µg. The observations were well corroborated with the zone of inhibition exhibited by the samples ( Table 1 and Figure b).The highest MIC of MMRT was recorded in the first hour ; then gradually decreases till 4th hour. This action is exhibited against *S. aureus* as well as MRSA. In the first hour the MIC of the study and standard drug (Tween 80) is nearly equivalent to a 150 µL concentration. (Figure a and b).

**Figure 2: MIC of MMRT with respect to MRSA strain**



\*Standard-Microbes alone

Studies on essential oils revealed the same observation(13). A study on thyme oil by (14) revealed that the addition of Tween 80 has decreased the effectiveness of the antibacterial compounds against *S. aureus* and *Salmonella typhimurium*. It is believed that Tween 80 could reduce the binding between active compounds and bacterial proteins by making them both more hydrophilic. It is because of the indirect effects of Tween 80 on hydrophobic antimicrobials dispersed in solution (15).

Based on the results it is evident that Maha Megarajanga thailam has potent antimicrobial activity against *Staphylococcus aureus* and MRSA. The antimicrobial activity of Castor oil (*Ricinus communis* seed extract) which is the base oil for MMRT, inhibited the growth of *E. coli*, *S. aureus* and *Pseudomonas* spp. and it showed more effectiveness in inhibiting *S aureus* and the MIC determined as 9µg mL<sup>-1</sup> (16).

In contrast, MMRT did not demonstrate any inhibition against *E. coli* and *Pseudomonas* spp., despite the major ingredient in the formulation and the presence of tannins and phenols in MMRT which have got strong antimicrobial properties. The potent antimicrobial activity of castor oil would be the presence of lectin protein such as ricin in the castor bean (17).

Tannins are polyphenols that inhibit the growth of various pathogens and can inhibit Gram-positive and Gram-negative bacterial growth and most of the tannins have a bacteriostatic rather than bactericidal impact (18).

A study done by on Clove (*Syzygium aromaticum*) essential oil showed in vitro inhibitory and bactericidal effects against *S. aureus*, and *E. coli*(19). The presence of Eugenol in MMRT may also be contributed to the reason for the bactericidal effect. Squalene, a water-soluble cationic steroid destroys Gram-negative and Gram-positive bacteria, fungi and tumors. It has been studied for its effectiveness in the treatment of lung and ovarian cancer (20).

Further, MMRT products showed the presence of fatty acids and their esters. Fatty acids such as oleic acid and their ethyl and methyl esters were tested against various oral pathogens such as *Streptococcus mutans* and *Candida albicans* and exhibited strong antimicrobial activity against these oral microorganisms, demonstrating some specificity for individual microbial species (21).Thus, the present study on antimicrobial efficacy of MMRT support the claim of aseptic maintenance on wound environment.

## Conclusion

In this era of increase in transmission of communicable diseases and anti-microbial resistant strains it is becoming increasingly difficult to combat the same. Several Siddha formulations are known to possess potent anti-microbial activity and can be implemented in clinical practice to combat the current scenario efficiently. Studies have suggested that some forms of essential oils could have potent ability against highly resistant bacterial species such as MRSA(22). Implementing the usage of Siddha oil based formulation Maha Megarajanga Thailam in the treatment of Staphylococcal and MRSA infections would be highly effective. It inhibits bacterial growth and has been proven to be effective in diabetic wounds and skin infection. Further clinical studies have to be carried out to establish the same.

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