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IN-VITRO ANTIBACTERIAL EFFECT OF MUPIROCIN IN COMBINATION WITH THREE ESSENTIAL OILS AGAINST *STAPHYLOCOCCUS AUREUS*

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Keywords:

Mupirocin, MRSA, Essential oils, Resistance

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ABSTRACT: Mupirocin is a topical antibiotic which is very effective against staphylococci, streptococci and Methicillin-Resistant *Staphylococcus aureus* (MRSA) infection. Because of its high effectiveness against MRSA infection, it is frequently used in clinical practice for decolonization of MRSA. Due to the increased use of mupirocin, increased resistance rates have been reported. Essential oil is a natural antimicrobial agent which kills the bacteria without promoting the acquisition of resistance. Hence, the combination of mupirocin with essential oils would be an ideal solution for preventing mupirocin resistance. Therefore, in this study we have investigated the antibacterial effect of mupirocin in combination with 3 essential oils (cinnamon, eugenol, and eucalyptus). Zone of inhibition test using disk diffusion method and Minimum Inhibitory Concentration (MIC) test using broth dilution method was carried out to assess the antibacterial activity against *S. aureus*. In this study, we have checked the type of interaction (synergistic, antagonistic, indifference, additive effect). From the results, it was observed that all the essential oil showed potential synergistic antibacterial effect against *S. aureus*. There was no antagonistic effect was observed in any of the 3 combination.

INTRODUCTION: Mupirocin (pseudomonic acid A) is a topical antibiotic originally isolated from *Pseudomonas fluorescens*. İt is primarily effective against gram-positive staphylococci, streptococci and methicillin-resistant *Staphylococcus aureus* (MRSA). It has a unique mechanism of action, which is selective binding to bacterial isoleucyl-tRNA synthetase, which halts the incorporation of isoleucine into bacterial proteins [1].

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This topical antibiotic is widely used for treating skin and soft tissue infection [2]. It is often used in clinical practice for the decolonization of MRSA. Many articles reported this increased use has been associated with high mupirocin resistance rates [2-4].

To combat the mupirocin resistance, the combination of mupirocin with natural anti-microbial agent essential oils would be an ideal solution. Plant essential oils have been used for many years as a natural antimicrobial agent for treating any type of pathogens including bacteria, fungi, and virus. İn addition, there is evidence that they effectively kill bacteria without promoting the acquisition of resistance [5]. Therefore in this study, we have investigated the antibacterial effect of mupirocin in combination with 3 essential oils

cinnamon, eugenol and eucalyptus oil against *S. aureus*.

MATERIALS AND METHODS:

Materials:

Bacterial Strain: *Staphylococcus aureus* culture was collected from the Department of Microbiology, PSG Hospital.

Antimicrobials: Mupirocin was collected as a gift sample from Sai Meera pharmaceuticals, Chennai. Cinnamon oil (Nice chemicals limited, Mumbai), Eugenol (Lobachemie, Mumbai), Eucalyptus (Yarrow chemicals limited, Mumbai) were purchased. Mupirocin and essential oils were dissolved in dimethyl sulphoxide and were diluted in appropriate medium. The stock solution of tween 80 in sterile water (5%) was used for homogenization of essential oils

Zone of Inhibition Test: Antimicrobial activity of mupirocin and 3 essential oils were carried out against clinical *S. aureus* strain by using the disk diffusion method. A sterile cotton swab was dipped into the bacterial suspension and swabbed over the surface of the agar plates. 10 µl of 3 essential oil and 20 µg of mupirocin was added in separate sterile disk and dried. The disk containing essential oils and mupirocin were placed in petri dish.

Amoxicillin (10 µg) was used as the positive control. Then, the bacterial Petri plates were incubated at 37 ºC for 24 h. The sensitivity of test organism to each antimicrobial was indicated by clear zone of inhibition around the disk and the diameter of the zone of inhibition was measured [6].

Determination of MIC against Planktonic *S. aureus*: Clinical *S. aureus* stock culture was sub-cultured onto bacterial agar plates and incubated overnight at 37 ºC. The next day, three to four discrete bacterial colonies with similar morphology were inoculated into sterile Mueller Hinton broth (MHB) and incubated overnight at 37 ºC. The bacterial suspension was adjusted to 0.5 McFarland Standard. The determination of MICs for mupirocin, cinnamon, eugenol, and eucalyptus were carried out using a twofold microdilution method, according to Clinical and Laboratory Standards Institute guidelines. Concentrations of twofold serially-diluted solutions in Mueller-hinton broth used for determining MIC for mupirocin were in a range from 0.0625 µg/ml - 32 µg/ml, concentrations of cinnamon, eugenol, and eucalyptus were in ranges of 0.1953 mg/ml - 100 mg/ml [7-9].

Determination of MIC of Mupirocin – Essential Oil Combinations: Determination of MIC of the combination of mupirocin and essential oils were carried out using checkboard synergy assay in the 96 well plates. Concentrations used for determining MIC for mupirocin were in ranges from 0.0625-4 µg/ml, concentrations of cinnamon, eugenol, and eucalyptus were in ranges of 0.1953 µg/ml-100 mg/ml. Essential oils were diluted along the ordinate and mupirocin concentration was decreased along the abscissa. Cultures of *S. aureus* in the broth were used as positive control and broth without *S. aureus* was used as negative control. Plates were incubated for 24 h at 37 ºC in aerobic condition. At the end of the incubation, 20 µl of resazurin (0.02%) solution was added. The viable bacterial cells change resazurin sodium blue colour to pink color. All assays were performed in triplicate.

The results were interpreted using Fractional Inhibitory Concentration Index (FICI). $\sum$ FIC = FIC A + FIC B, where FIC A is the MIC of drug A in the combination / MIC of drug A alone, and FIC B is the MIC of drug B in combination / MIC of drug B alone. The combination is considered to be synergistic when FIC is ≤ 0.5, additive when it is above 0.5, indifferent when it is more than 1 and less than 4, antagonistic when it is more than 4 [8].

Statistical Analysis: All experiments were performed in triplicates. Statistical analysis was performed using Prism software (version 5). Statistical significance was calculated using ANOVA (non-parametric) and the value of $P<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION:

Zone of Inhibition Test: Zone of inhibition test was carried out for determining the antibacterial effect of mupirocin, cinnamon, eugenol and eucalyptus oil using disk diffusion method. The measured zones were mentioned in **Table 1**.

A statistically significant difference between the antibacterial efficacy of mupirocin *vs.* cinnamon, eugenol, eucalyptus and cinnamon *vs.* eugenol,

cinnamon *vs*. eucalyptus, eugenol *vs.* eucalyptus were analyzed by one way ANOVA test using prism software (version 5). The results revealed that mupirocin has significant (P<0.001) increased antibacterial activity against *S. aureus* than essential oils.

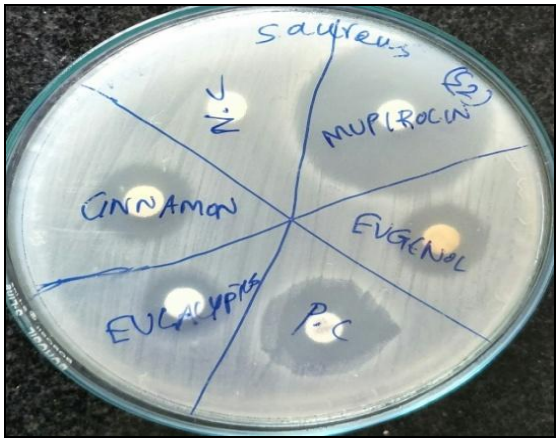

FIG. 1: ZONE OF INHIBITION

Among the essential oils, cinnamon and eugenol have significant (P<0.001) increased activity than eucalyptus.

TABLE 1: ZONE OF INHIBITION

Antimicrobial agents	Zone diameter (mm)
Mupirocin	50
Cinnamon	17
Eugenol	16
Eucalyptus	12
Positive control(Amp)	21

MIC of Antimicrobial Agents Alone: MIC was carried out using the broth microdilution method. All the experiments were performed in triplicates. MIC for the individual antimicrobial agents mentioned in **Table 2**. Statistical significance difference between mupirocin and essential oils were determined by one way ANOVA test using prism software (version 5).

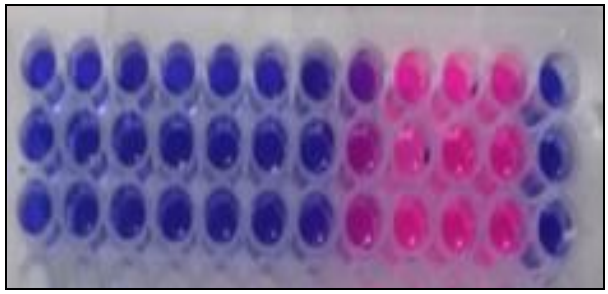

FIG. 2: MIC OF MUPIROCIN

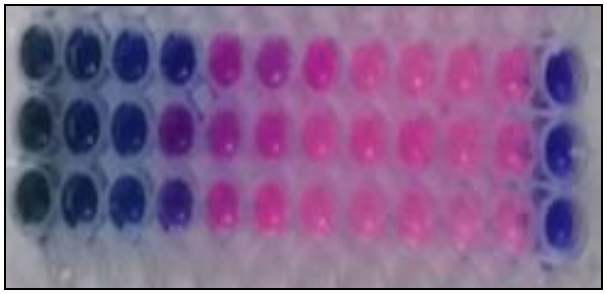

FIG. 3: MIC OF EUGENOL

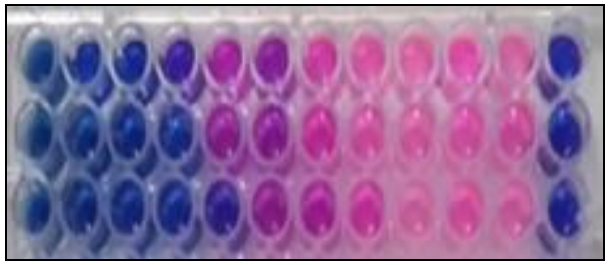

FIG. 4: MIC OF CINNAMON

FIG. 5: MIC OF EUCALYPTUS

TABLE 2: MIC OF ANTIMICROBIAL AGENTS ALONE

Antimicrobials	MIC
Mupirocin	0.5 µg/ml
Cinnamon oil	12.5 mg/ml
Eugenol	16.66 mg/ml
Eucalyptus oil	50 mg/ml

There is no significant (P<0.001) difference in antibacterial activity of eugenol and cinnamon oil against *S. aureus*. Eucalyptus oil has significantly (P<0.001) reduced antibacterial activity against *S. aureus* than mupirocin and 2 essential oils.

Determination of MIC of Mupirocin – Essential Oils Combinations: MIC of mupirocin – essential oils combinations were carried out using checkboard synergy assay.

TABLE 3: MIC OF MUPIROCIN – ESSENTIAL COMBINATIONS

Antimicrobials	MIC of mupirocin (µg/ml)	MIC of essential oil (mg/ml)	FICI	Effect
Mupirocin + cinnamon	0.0625	3.125	0.375	Synergistic
Mupirocin + eugenol	0.0625	3.125	0.312	Synergistic
Mupirocin + Eucalyptus	0.0625	6.25	0.25	Synergistic

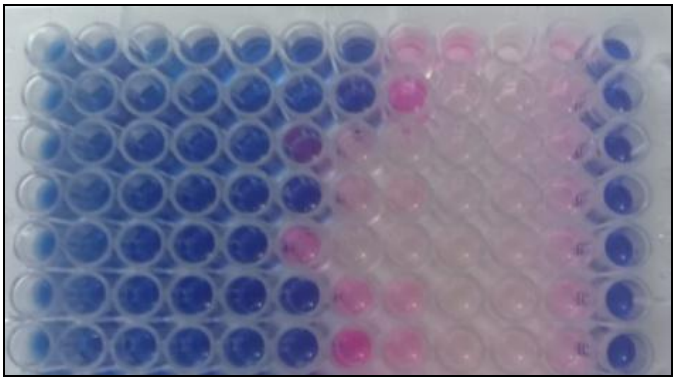

FIG. 6: MUPIROCIN + CINNAMON

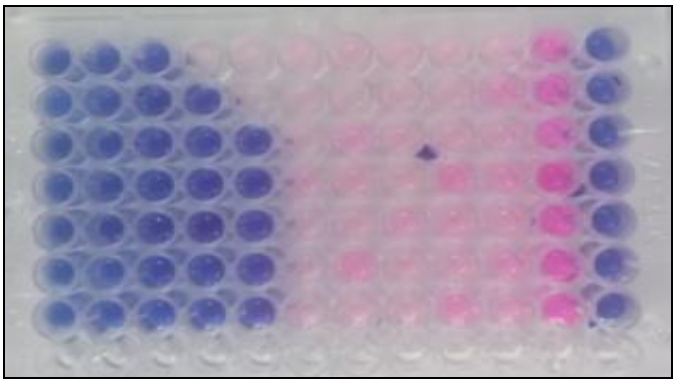

FIG. 7: MUPIROCIN + EUCALYPTUS

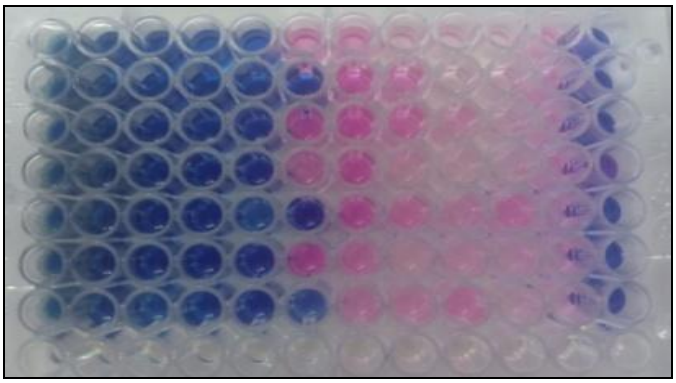

FIG. 8: MUPIROCIN + EUGENOL

On the basis of calculated FICI index **Table 3**, all of the tested combinations showed better synergistic effect against planktonic *S. aureus*.

This is the first investigation carried out with combination of mupirocin with cinnamon oil, eucalyptus oil, and eugenol showing potential synergistic activity. A similar study was carried out by Domagoj kifen *et al*., (2016) [11] with combination of mupirocin and 3 monoterpenes where the combination did not show synergistic effect against planktonic *S. aureus*.

CONCLUSION: Study outcomes revealed that low concentration of mupirocin and high concentration of essential oils produced better synergistic antibacterial effect against planktonic *S. aureus* when compared to individual components alone. Further studies should be carried out with mupirocin in combination with the anyone of the tested 3 essential oils against mupirocin resistant *S. aureus* strain.

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CONFLICTS OF INTEREST: Nil

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