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Targeting Methicillin Resistant *S. aureus*(MRSA) –Screening Mango Seed Kernel, Guava Leafand ε-PolyLysineon clinical isolates of MRSAfor Anti-biofilm activity- a pilot study.

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Abstract:
Background:*The biofilm of S. aureus is a major contributor for multidrug resistance and currently there are no drugs in use specifically targeting the biofilm. Inhibiting biofilms could potentially improve transport of drugs across the membrane/cell wall of the bacterium thereby enabling them to act on the intended targets in the bacteria. There is an urgent need for the development of new drugs or design alternate strategies for treating MRSA/MDR S. aureus infections.We aimed to screen the effect of some natural products and a pure compound whose effect on biofilms of S. aureus has not yet been reported. This study also attempted to re-purpose an existing food preservative for its anti-biofilm potential against clinical isolates of S. aureus.*
Materials and Methods:*The present study investigates the anti-biofilm properties of Mango Seed Kernel Extract (MSKE), Guava Leaf Extract (GLE) and ε-Polylysine (ε-PL)by the crystal violet assay on cultures ofS. aureus and MRSA by exposing them to ethanolic extracts of the Mango Seed Kernel Extract (MSKE), Guava Leaf Extract (GLE) and ε-Poly lysine (ε-PL)at sub-MIC level.The biofilm assay was done in 96 well microtitre plates. The morphology and structure of biofilmswere examined by Scanning Electron Microscopy (SEM).Biofilms were quantitated by spectrophotometry by crystal violet assay. The crude extract of the natural products were analysed by GC-MS.*
Results:*All the three compounds were able to inhibit the biofilm formation.* The extent of *inhibition of biofilm was as follows:ε-PL (64.8%), MSKE (47.5%) and GLE (42.2%) on MRSA and that on MSSA isolates was, ε-PL (72.2%), MSKE (57.3%) and GLE (44.8%). The SEM examination revealed that biofilm matrix was disrupted after exposure to these drug candidates.Through GC-MS analysis, seven major bioactive compounds were identified in MSK and GL extracts.*
Conclusion:*We conclude that the MSKE, GLE and ε-PL have good antibiofilm potential and they could be further investigated and developed as adjunct drugs to eliminate development of MDRin S. aureus and to treat MDR in general and MRSA in particular by targeting biofilms.*
Key Word:*MSKE; GLE; ε-PL;anti-biofilm activity; GC-MS analysis.*

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I. Introduction

Methicillin Resistant *Staphylococcus aureus*(MRSA) is a well-known multidrug resistant bacteria in the hospital settings. It is a challenge that clinicians are currently facing due to the restricted range of antibiotics available for treat infections caused by MRSA[1]. It is the most commonly identified drug resistant pathogen in many parts of the world, with MRSA being implicated in 50% of all nosocomial infections[2].The MRSA infections have the highest annual cost to the US health system costing $USD 2.2 billion per year[3].Furthermore, invasive MRSA infections caused over 19000 deaths in the United States in 2017[4]. The success of MRSA can be largely attributed to the ability of biofilm formation as one of the resistance mechanisms, making them more difficult to treat than their planktonic counterparts[5].Biofilm is a complex aggregate of microorganisms in which cells are embedded in a self-produced matrix of extracellular polymeric substance. *S. aureus* forms biofilms in clinical settings most often on the surface of catheters and implanted medical devices.The infections associated with biofilm may worsen the clinical disease and result in the replacement of implanted medical devices[6].This has necessitated the design and development of new therapeutic alternatives[7].

Medicinal plants are important sources of bioactive molecules for antimicrobial and anti-biofilm activity. One of the most significant benefits of these molecules of plant origin is, the reduced number of potential toxic effects, as well as the fact that they have activityagainst drug resistant strains[8,9]. *Psidium guajava* is a tropical and subtropical plant with antibacterial and antifungal properties. Extracts from various parts of *P. guajava* are commonly used to treat epilepsy, diarrhea, stomach ache, wound-healing, constipation and diabetes[10].*Mangifera indica* is a well-known plant for its medicinal properties.Its seeds contain phenols and tannins which haveantimicrobial activity[11]. In south India, MSKE has been used to treat diarrhea[12].The amino acid polymer, ε-PL, has broad-spectrum antibacterial activity and has been widely used as a preservative in food industries. It is a cationic peptide and consists of 25-35 residues of L-lysine produced by the filamentous actinomycetes[13].

Several studies have focused on development of small molecules or plant compounds to inhibit biofilm and other virulence factors of *S. aureus*[14,15,16].Considering the antimicrobial properties of MSKE, GLE and ε-PL, we attempted to investigate antibiofilm potential of these compounds against *S. aureus*. The aim of this study is to look into new compounds for combating microbial drug resistance conferred through biofilms. The findings of this study may provide newer leads for treating MRSA infections successfully.

II. Material and Methods

Bacterial Cultures, Chemicals and Compounds

The study was done on 152isolates of *S. aureus* from various clinical specimens (Blood, body fluids, pus, skin, tissue, sputum and other body sites) obtained from the Department of Microbiology of Gleneagles Global Hospital at Hyderabad, Bangalore and Chennai, AIIMS-New Delhi, and LV Prasad Eye Hospital Bhubaneshwar India. Reference strains, MRSA (ATCC 43300) and MSSA (ATCC 29213, ATCC 25923) were kindly provided by the Infection Biology Lab, Department of Animal Biology, School of Life Sciences, University of Hyderabad, and Hyderabad, India.

Mango Seed Kernels (*Mangifera indica*) and Guava leaves (*Psidium guajava*) were collected from Fruit Research Station Sangareddy, Telengana.Mannitol Salt Agar (MSA), Muller Hinton Agar (MHA), Muller Hinton Broth (MHB) and Tryptic Soy Broth (TSB) were purchased from HiMedia. Epsilon Polylysine (Cat,No.FP-155) was procured from Bimal Pharma Private Limited, Dimethyl sulphoxide (DMSO), Crystal Violet (Sigma Aldrich), Glacial Acetic Acid and Methanol (Merck), Ethanol (Hayman Group Limited), were used in this study.

Culture isolation on Mannitol Salt Agar (MSA)

The clinical isolates were first inoculated in Tryptic Soy Broth (TSB) for enrichment and further streaked on Mannitol Salt Agar (MSA) which is a selective media for *S. aureus*[17]. The inoculated plates were incubated at 37°C for 24 hrs.

The isolates confirmed as *S. aureus* on MSA were further screened using the Cefoxitin (30 µg) and Oxacillin (1 µg) on MHA plates for methicillin resistanceas recommended by CLSI[18]. Antibiotic sensitivity to methicillin and determination of MIC of the plant extracts were carried out as described [19].

Screening for Biofilm production by Micro titer plate Method

Our objective was to investigate the *in vitro* biofilm forming potential of *S. aureus* isolated from the clinical samples. Biofilm quantification was performed in 96 well microtiter plates. In brief, single colony of *S. aureus* was isolated from a fresh MHA plate and inoculated in 5 mL of MH broth. Inoculated broth was incubated at 37°C for 18 h and diluted to 10^8 cells /mL (0.5 McFarland) was reached. A sterile, round bottomed, 96-well microtiter plate was filled with 200 µL of this diluted culture in triplicates and incubated at 37°C for 24 hours. After incubation the contents of each well were carefullydecanted by gentle tapping and washed with 200 µL of PBS (Phosphate Buffer Saline) pH 7.3 to remove non-adherent bacteria. The adherent bacteria were fixed by 200 µL per well of 99% methanol, after 15 minutes plates were emptied and left to dry. The wells were stained with 200 µL of 0.1% of crystal violet, excess stain was washed gently and the plates were kept for drying. The biofilm was re-solubilized with 33% (vol/vol) glacial acetic acid and the OD of each well was measured at 610 nm in an ELISA reader. The reading was performed twice: once before addition of glacial acetic acid and then after addition of glacial acetic acid. Triplicate negative controls were prepared with only sterile MHB exactly as described for the test isolates. A positive control ATCC 43300 was also used.Interpretation of biofilm production was performed as per the criteria described by Stepanovic*et al*., and the isolates were categorized as non-producers, weak, moderate and strong producers[20].

Non producers ($OD \leq OD_{NC}$), Weak ($OD_{NC} < OD \leq 2\times OD_{NC}$), Moderate ($2\times OD_{NC} < OD \leq 4\times OD_{NC}$), or Strong ($OD > 4\times OD_{NC}$) biofilm producers.

Anti-biofilm activity of Mango Seed Kernel Extract (MSKE), Guava Leaf Extract (GLE) and ε-Polylysine (ε-PL) against *S. aureus*

The anti-biofilm effect of the compounds was assessed both qualitatively by Scanning Electron Microscopy (SEM)and quantitatively by Microtiter plate (MTP)based crystal violet staining method. To examine the anti-biofilm activity of the compounds (MSKE, GLE and ε-PL), *S. aureus* were cultured in the presence of sub-MIC concentrations (10 µg/mL) of the individual compounds and their effect on biofilms was evaluated qualitatively under SEM for morphological and structural changes and for their effect on synthesis of biofilms by MTP assay[21,22]. Sterile 96-well plates were filled with 198 µL of MHB containing sub-MIC concentrations of the drug compounds (triplicates). Then 2 µL of the 0.5 McFarland of appropriate cultures were added to each well. The microtiter plates were incubated for 24 hours under static conditions. After incubation the contents of each well were removed by inversion and gentle tapping. The wells were washed with 200 µL of PBS (Phosphate Buffer Saline) pH 7.3 to remove free floating bacteria. The adherent bacteria were fixed by 200 µL of 99% methanol per well, after 15 minutes plates were emptied and left to dry at room temperature. Then the wells were stained with 200 µL of 0.1% of crystal violet and the excess stain was washed gently and kept the plates for drying. The bound crystal violet from the biofilm was extracted with 33% (v/v) glacial acetic acid and the absorbance of each well was measured at 610 nm by using an ELISA reader.

Percentage of biofilm inhibition = 100-[(OD_{610} of the treated wells / OD_{610} of the untreated wells) X 100]

Scanning Electron Microscopy studies

Glass coverslips (approximately 10 mm X 10 mm) were kept at the bottom of each well in a 6 well micro titer plate.Four mL of *S. aureus*culture (10^8 CFU/mL)containing sub-MIC concentration (10 µg/mL)of each drug compoundwere added into the individual wells while the control cultures received an equal amount of DMSO. The plates were incubated at 37°C for 24 hours to allow the formation of biofilm on the coverslips.

The cultures were processed for SEM as described[23].Briefly, the samples were fixed overnight at 4°C in cold 2.5% (v/v) Glutaraldehyde containing 0.2 M Sodium Cacodylate Buffer (SCB pH=7.2). The coverslips were washed three times with 0.1M SCB buffer with 30 minutes interval to remove the excessive fixative and dehydrated by replacing the buffer with increasing concentration (30%, 50%, 70%, 80%, 90% and 100%) of ethanol (Ethyl alcohol 100%:Hayman Group Ltd., UK F204325). All the coverslips were removed from the ethanol and air dried under high vacuum (10^{-7}Torr) at the room temperature (25°C) for one day. All dried samples were mounted on aluminum stub (SPI supplies division of Structure Probe INC, USA no. 05072 –AB) with double sided adhesive tape and coated with ionic gold ($300A^0$) in sputter coating unit Model : E-1010 *Hitachi Japan*) at high vacuum. The processed samples were examined under scanning electron microscope (SEM) (S3400N*HitachiJapan)* at 15 kV and documentedat different magnifications.

Gas Chromatography-Mass Spectroscopy (GC-MS) analysis of MSKE & GLE extract

The GC-MS analysis was performed in Shimadzu, Gas Chromatography-Mass Spectroscopy GC-MS 2010QP plus. ZB-5 capillary column (30 m x 0.25mm x 0.25um) composed of 5 %Phenyl-95% Dimethylpolysiloxane was used for the identification of metabolites. Helium was used as carrier gas with a consistent flow rate of 1.10 mL/min and an injection volume of 1 µL was employed. Ion source temperature was 200^{o}C. The Oven temperature was programmed from 100^{o}C (isothermal for 4 min), with an increase of 4^{o}C/min, to 280^{o}C, then hold it for 12.95 min. Mass spectra were taken at 70eV; a scan interval of 10 spectra/s and fragments from 50 to 500 Da. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The relative content of each component was calculated by comparing its average peak area to the total area.

Interpretation of GC-MS was done using National Institute Standard and Technology (NIST) data base. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST and Wiley library. The molecular weight, name and structure of the components from the test material were determined.

III. Result

Growth in Mannitol Salt Agar (MSA) medium

Single, well isolated, colony from the MHA plate was streaked onto (Mannitol Salt Agar)MSA plates. Mannitol Salt Agar is widely used as a selective and differential growth medium containing high salt (7.5-10% NaCl) that mainly allows gram positive pathogens such as *Staphylococci* to grow which ferment mannitol and changes phenol red to yellow due to the production of acid. Non-fermenting isolates produce pink or red colonies without any change in medium colour.

Biofilm quantification by using Microtiter plate assay

Biofilm formation in clinical isolates of *S. aureus* (n=152) was estimated by using the Microtiter plate method. The isolates which are able to produce exo-polysaccharide (biofilm component) and bind to the surface of microtiterplate were the ones that form high biofilm. Fixation of the bacterial biofilm was done by chilled methanol. Crystal violet is a basic dye which binds non-specifically to the negatively charged surface molecules in the extracellular matrix such as polysaccharides and eDNA. Biofilms were quantitated by releasing the crystal violet from the stained biofilms with 33% Glacial Acetic Acid (GAA) and measuring the optical density at 610 nm (570nm) using an ELISA reader. Based on the quantity of biofilm produced, 92 isolates (n=97) of MRSA (94.8%) and 47isolates (n=55) of MSSA (85.4%) were grouped as strong biofilm producers (Fig. 1) and 5 isolates (n=97)of MRSA (5.1%) and 8 isolates(n=55) of MSSA (14.5%) were moderate biofilm producers. Notably the ability to form biofilm was higher among MRSA isolates when compared to MSSA isolates (94.8% vs. 85.4%).

Fig. 1. Biofilm assay by micro titer plate for *S. aureus* isolates. MRSA rows A-C; B4 (A_{610}:1.19), 2428 (A_{610}:2.28), 5013 (A_{610}:1.50) and MSSA rows G-H; 2180 (A_{610}:1.00), 25923 (A_{610}:1.16) and Control (A_{610}:0.21)**.**

Effect of MSKE, GLE & PL on the biofilm formation

The anti-biofilm effect of the compounds was evaluated qualitatively by Scanning Electron Microscopyand quantitatively by crystal violet staining method. Strong anti-biofilm activity of compounds was observed at sub-MIC levels (10 µg/mL) against both drug sensitive and resistant isolates. Sub-MIC concentration of the test compounds were selected sothat biofilm inhibition would not be secondary to growth inhibition. The MSKE, GLE andε-PL inhibited the biofilm formation in both MRSA & MSSA isolates. The extent of inhibition on MRSA isolates (Fig.2) was:ε-PL (64.8% ± 4.8), MSKE (47.5% ±10) and GLE (42.2% ±13) and the extent of inhibition on MSSAisolates was:ε-PL (72.2% ± 7.2), MSKE (57.3% ±14.9) and GLE (44.8%±17.3).

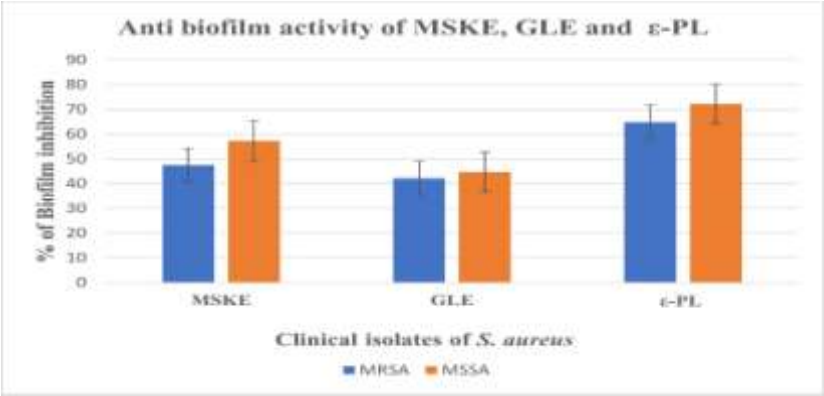

Fig. 2- Biofilm inhibitory effect of the MSKE, GLE and ε-PL on *S. aureus* (MRSA and MSSA) isolates. Biofilm inhibition (A_{610}) was quantified in the presence of sub-MIC of the compounds and the mean inhibition (error bars represent S.D of three separate experiments) of the compounds when represented in a decreasing order is:ε-PL> MSKE>GLE

Effect of drug compounds on biofilm integrity and structure by Scanning Electron Microscopy

Further, the impact of compounds on integrity and morphology of MRSA biofilm was studied using SEM.Bacterial cultures (both treated and un-treated) were seeded on coverslips for 24 hours. Micrographs obtained by SEM are shown in (Fig. 3). Control Biofilm of MRSA are shown in micrograph A andB, while C, D and E correspond to the biofilm treated with sub-MIC concentrations (10 µg/mL) of MSKE, GLE andε-PL respectively.

An abundant biofilm formation (multilayer clustered growth of bacterial biofilm) and the presence of an extracellular matrix in MRSA isolate was observed in the control (without treatment) group Figure3 A and B. Figure3 C, D andE corresponds to the biofilm of MRSA treated with MSKE, GLE andε-PL respectively which showed isolated, fewer cells and a significant decrease in the extracellular matrix of biofilmcompared untreated control group, indicating that the compounds have a good anti-biofilm activity. The bacteria appeared as a monolayer of dispersed cells scattered on the surface in the drug treated group

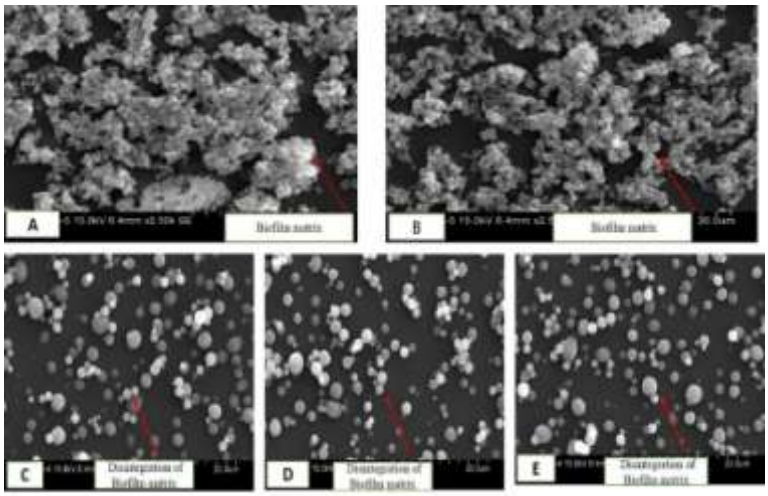

Fig. 3- Micrographs of MRSA biofilm on coverslips obtained by SEM. Biofilms were formed by type strain *S. aureus*43300 on coverslips under different conditions and viewed under SEM Model: S3400N under 10000 x magnification at resolution 20 µm. A) Untreated Biofilm B) Control Biofilm, exposed to DMSO (drug solvent control): Biofilm exposed to C) MSKE (10 µg/mL) D) GLE (10 µg/mL) and E) ε-PL (10 µg/mL).

Partial Characterization of natural products by Gas Chromatography–Mass Spectrometry:

a) Mango Seed Kernel Extract:

In comparison to reference molecules in NIST library a total 41 peaks were identified in GC-MS chromatogram of MSK (Fig. 4). By comparing their mass-spectral database in NIST library, these phytochemical compounds were identified and characterized as listed in Table 1. The MSKE contains a mixture of components with known antimicrobial and antioxidant properties. There were 7 major compounds identified from the chromatogram. The most abundant compound was 4-Methyl-1-decene (9.14%) followed by Methoxyacetic acid, 2-ethylhexyl ester (8.78%), n-Pentadecane (7.95%), 3,7-Dimethyldecane (7.30%), n-Heptadecane (6.11%), 2,3-Dimethylnonadecane (5.47%) and 2,6,10,14-Tetramethyloctadecane (4.33%). The rest of the compounds were considered as minor as they constituted less than 4% of the total.

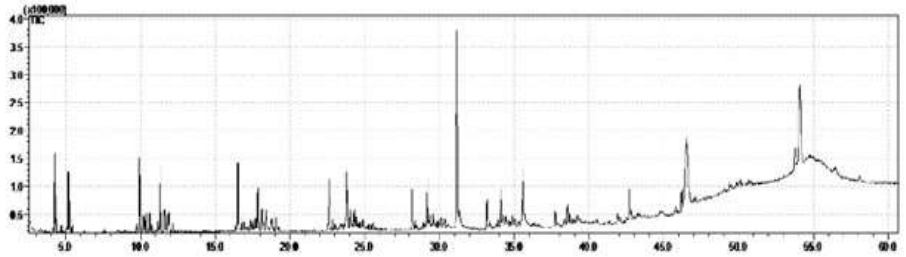

Fig. 4- GC-MS chromatogram of ethanolic extract of MSK. 7 major compounds were identified from the chromatogram. 4-Methyl-1-decene (9.14%), Methoxy acetic acid, 2-ethylhexyl ester (8.78%), n-Penta decane

(7.95%), 3,7-Dimethyl decane (7.30%), n-Heptadecane (6.11%), 2,3-Dimethyl nonadecane (5.47%) and 2,6,10,14-Tetramethyl octadecane (4.33%).

Table 1: The major bioactive compounds identified in MSKE by GC-MS.

S. No	RT	Compound name	Molecular formula	M.W	Peak area %	Reported biological activity	Ref
1	4.26	4-Methyl-1-decene	$C_{11}H_{22}$	154	9.14	Antioxidant activity	[24]
2	9.93	Methoxy acetic acid, 2-ethylhexyl ester	$C_{11}H_{22}O_3$	202	8.78	-	-
3	16.5	n-Penta decane	$C_{15}H_{32}$	212	7.95	Anti-microbial and antioxidant activity	[25]
4	5.15	3,7- Dimethyl decane	$C_{12}H_{26}$	170	7.30	Anti-microbial activity	[26]
5	22.6	Heptadecane	$C_{17}H_{36}$	240	6.11	Anti-microbial activity & antioxidant	[27, 28]
6	29.1	2, 3-Dimethylnonadecane	$C_{21}H_{44}$	296	5.47	-	-
7	28.1	2,6,10,14-Tetramethyloctadecane	$C_{22}H_{46}$	310	4.33	-	[29]

b) Guava leaf extract

The chemical composition and characterization of secondary metabolites present in the GLE was studied in detail by GC-MS analysis, and the mass spectra of active chemical constituents were identified from NIST library.

The GC-MS analysis showed the presence of 44 compounds as shown in (Fig. 5). The predominant compounds have antioxidant, antimicrobial and anti-allergic activitities. The6 major components were identified as 4-Methyl-1-decene (7.63%), 4-Dimethyldodecane (7.48%), 2-Methyl-n-tridecane (6.90%), Oxallic acid, 6-ethyloct-3-yl isobutyl ester (6.72%), Isohexyl neo-pentyl ester (6.47%), 2,2,3,3,5,6,6–Heptamethylheptane (6.11%), n-Heptadecane (5.17%) (Table 2). Other compounds were identified as minor compounds due to their relative content being less than 4% of the total.

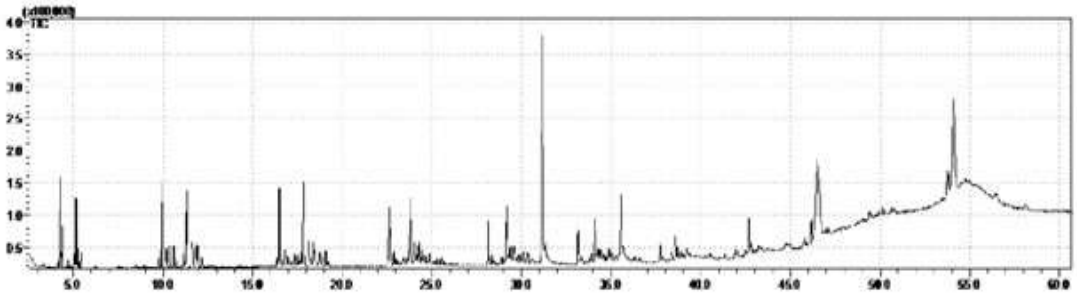

Fig. 5- GC-MS chromatogram of ethanolic extract of GLE. 6 major components were identified as 4-Methyl-1-decene (7.63%), 4-Dimethyldodecane (7.48%), 2-Methyl-n-tridecane (6.90%), Oxallic acid, 6-ethyloct-3-yl isobutyl ester (6.72%), Isohexyl neo-pentyl ester (6.47%), 2,2,3,3,5,6,6–Hepta methyl heptane (6.11%), n-Heptadecane (5.17%).

Table 2: The major bioactive compounds identified in GLE by GC-MS

S. No	RT	Compound name	Molecular formula	M.W	Peak area %	Reported biological activity	Ref
1	4.26	4-Methyl-1-decene	$C_{11}H_{22}$	154	7.63	Antioxidant activity	[24]
2	9.94	2,4- Dimethyldodecane	$C_{14}H_{30}$	198	7.48	Anti-microbial and antioxidant activity	[30]
3	23.8	Oxallic acid, 6-ethyloct-3-yl isobutyl ester	$C_{16}H_{30}O_4$	286	6.72	Antioxidant and anticancer activity	[31,32]
4	16.5	Oxalic acid, isohexyl neopentyl ester	$C_{13}H_{24}O_4$	244	6.47	-	[31]
5	5.16	2,2,3,3,5,6,6-Heptamethylheptane	$C_{14}H_{30}$	198	6.11	Anti-allergic activity	[28]
6	22.6	Heptadecane	$C_{17}H_{36}$	240	5.17	Anti-microbial activity and antioxidant	[27,28]

IV. Discussion

Biofilm infections are difficult to treat and therefore, it is clinically important to know if a particular isolate is capable of forming biofilms since biofilm is a strong marker for MDR and the antibiotic treatment strategy has to be changed accordingly. Unfortunately current antimicrobial therapies do not include any anti-biofilm drugs. All the *S. aureus* isolates in our study were positive for biofilm production. Higher biofilm

production was detected more in MRSA isolates than in MSSA isolates in our study (94.8%) compared to MSSA isolates (14.5%). Sharvari and Chitra 2012 also reported strong biofilm production in 72.3% of MRSA and 30.3% of MSSA[33]. In another study, Eiichi *et al*., 2004 found a very high percentage (95.4%) of biofilm production in MRSA isolates[34].It will be interesting to investigate if the MRSA/MDR is a cause or effect of biofilm phenotype of the bacterium

Despite advancements in understanding the biofilm composition, architecture and dynamics, little is known about the mechanism of action of anti-biofilm compounds which could suppress or disrupt these biofilms. In fact, no anti-biofilm drugs have sofar beenapproved for clinical use though several groups are investigating this possibility. Such compounds could potentiallybe used to i) treat bacteria resistant to conventional antibiotics ii) as an adjunct to the conventional antibiotics to prevent development of resistance to the antibiotics iii) as anti-virulence drugs to reduce the virulence of the bacteria and the extent of tissue damage.Interestingly, there are no reports of resistance to any known anti-biofilm or anti-virulence compound. Such compounds could be attractive and alternate candidate drugs to treat MRSA or to reduce development of MRSA. Chances of resistance development to such drugs is less compared to those antibiotics which target the normal bio-synthetic mechanisms of the bacteria as antibiofilm drugs pose no threat to the survival of the microbe.

In our study, the activities of MSKE, GLE and ε-PL were tested against the *S. aureus* biofilm. The ability of antibacterial compounds to inhibit formation/destruction of biofilms hold promise for reducing colonization of surfaces and cells. Some plant phenolic components have been found to inhibit biofilms by inhibiting the initial adhesion of bacteria and hinder the accessibility of nutrients which are necessary for adhesion and cell growth. Plant derived compounds at sub-MIC cause changes in the transcription of biofilm associated genes which are critical for biofilm formation thereby contributing anti-biofilm activity[35].

The Anti-biofilm effect of the compounds was evaluated qualitatively by SEM and quantitatively by crystal violet staining methods at sub-MICof the compounds (10 µg/mL). All the three compounds were able to inhibit the biofilm formation in the clinical isolates even at sub-MIC compared to the untreated controls. ε-PL showed higher anti-biofilm activity compared to MSKE and GLE.

The SEM was used to see the effect of exposure to these candidate drugs on the size, architecture, morphology and integrity of biofilms. In the untreated cells, most of the areas were covered with biofilm comprising clusters of bacteria. Dis-aggregation of the bacteriawas observed in the cultures treated with MSKE, GLE and ε-PL. Based on crystal violet staining and comparison of the absorbance values between the treated and untreated cells, percentage reduction in biofilm was used as a measurement for anti-biofilm activity. All the compounds exhibited remarkable inhibition of biofilm formation (>40%). One or more of the compounds present in MSKE and GLE may be responsible for the anti-biofilm activities. A study by Razak and Rahsim reported that ethanolic and acetone extracts of *Psidium guajava* blocks the adhesion of *Streptococcus mutans*[36]. Recent studies showed, isolated natural compounds from plants like *Camellia sinesis*, *Curcuma longa*, *Cinnamomum glaucescens* and *Ocimum sanctum* had antibiofilm activity against various pathogens[37]. Our results were similar to the ultra-structural changes observed in the MRSA isolates treated with *Quercus infectoria* extract, tannic acid[38].

It is most probable that Quorum Quenching effects of the extracts of MSK was due to more than one bioactive compound since a crude extract was used in our study. Mango Seed Kernel, which is usually discarded as waste, is indeed an invaluable part of the plant with immense potential for further development of quorum quenchers and anti-virulent drugs for treating *S. aureus* infections in general and MRSA in particular.

With respect to GLE, Guava leaves have several compounds such as coumarins, flavonoids, tri-terpenes, essential oils and ellagitannins which are known to have antimicrobial properties. The predominant compounds have the property of antimicrobial, antioxidant and anti-allergic properties.

S. aureus is a common bacteria wide-spread among the human population with many being asymptomatic carriers. This has enabled a rapid spread of MRSA among the society and among the hospitalized patients, causing life threatening infections in all parts of the world. Natural or synthetic compounds may be used to design and develop drugs which could be used as adjunct to the antibiotics to prevent development of multidrug resistance and also to treat the MRSA infections.

V. Conclusion

ε-PL, which caused cells to collapse, had the highest anti-biofilm activities followed by MSKE and GLE.ε-PL as the most promising compound for further development as resistance breakers to sensitize MRSA to antibiotics because it is already approved and in use as food preservative.

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