International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2192-2198www.ijprajournal.com ISSN: 2249-7781

Targeting multidrug resistant *A. baumannii* to evaluate anti-biofilm and anti-quorum sensing potential of some compounds

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Submitted: 15-02-2023 Accepted: 25-02-2023

ABSTRACT: The continuing search for new and novel antimicrobials for their potential as anti-biofilm and anti-quorum sensing agents has turned to many plant products possessing broad-spectrum anti-microbial activities. This potential has gained importance recently because the plant secondary metabolites exhibit anti-quorum sensing activity without interfering with the growth of the organism which thus would minimize the development of multidrug resistance. *A. baumannii* was the test organism in this study and we used the carbapenem resistant and sensitive clinical isolates. We have evaluated the anti-biofilm and anti-quorum sensing potential of two popular plant sources, namely mango seed kernels and guava leaves. Commonly used antimicrobial food preservative ε-Poly lysine, was also evaluated for its anti-quorum sensing potential.

The present study demonstrated that MSKE, GLE ethanolic extracts and ε-Poly lysine have strong potential as antibiofilm and anti- QS compounds which could be developed further as adjunct drugs for treating multi drug resistant *A. baumannii* and for co-therapy with other antibiotics to eliminate development of resistance.

KEYWORDS: Biofilms, Anti-biofilm agents, Anti-quorum sensing, acyl homoserine lactone, adjunct drug, *A. baumannii*.

I. INTRODUCTION

Acinetobacter baumannii is an obligate Gram-negative and aerobic coccobacillus, has emerged as the most important organism causing deadly bacterial infections. [1] This organism's resistance to almost all the current generation of antibiotics further decreases its treatment options in the hospital acquired infections. Antibiotic resistance can be transferred among these bacteria, leading to rapid spread of resistance by the day. [2]Many studies have been done to investigate the virulence factors of this bacterium; however, the most important virulence factors which contribute to the pathogenesis of *A. baumannii* disease are the ability to form biofilms, the presence of lipopolysaccharide in the cell wall, phospholipases, presence of siderophore mediated iron acquisition system, chaperone-usher pili assembly system, OmpA outer membrane proteins and penicillin-binding protein. [3] It is an established fact that, *A. baumannii* due to its biofilm forming ability, survives on artificial surfaces for a long period thereby, allowing it to persist in the hospital environment. [4] Biofilms provide bacteria with three main advantages, including trapping the essential elements and nutrients for bacterial usage, tolerating harsh conditions as well as the protection against the host's immune system and the opportunity to transfer antibiotic resistance genes. [5] Furthermore, the availability of fewer nutrients in deeper space within the biofilm and consequently a slower metabolism can prevent the bacteria from succumbing to an antibiotic and antimicrobial effects. [6] The process of biofilm formation in many bacteria is facilitated by flagella; however, for *A. baumannii*, pili seem to be involved in this process under the regulation of the CsuA/BABCDE chaperone-usher pilus system which is necessary for the initiation of biofilm formation on abiotic surfaces. It has been shown that inactivation of the *csuE* gene eliminates pilus production and biofilm formation.[7] Among the several factors, the biofilm-associated protein encoded by the *bap* gene plays an important role in intercellular adhesion, accumulation of bacterial cells, and establishment of biofilm. This is one of the cell surface-associated proteins and involved in biofilm maintenance and maturation. Mutation in the bap gene showed the inhibition in the biofilm formation by *A. baumannii*.[8] Currently, the need

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2192-2198www.ijprajournal.com ISSN: 2249-7781

for a novel therapeutic strategy to combat biofilm-associated infection of *A. baumannii* has gained more attention. Anti-biofilm agents attenuate adherence and virulence factors of pathogen, instead of affecting its growth and hence, the possibility of resistance development is much lower.[9] In addition, anti-biofilm therapy enhances the sensitivity of bacteria to antibiotics and to the host immune system. This indicates the importance of anti-biofilm therapy and the urgent need for the discovery of novel anti-biofilm agents. Previously, numerous anti-biofilm agents from various natural sources have been explored against *A. baumannii* biofilms. However, the present study is the first report on the anti-biofilm and anti-quorum sensing potential of Mango seed kernel extract (MSKE), guava leaf extract (GLE) and a synthetic compound, ε-Poly lysine on *A. baumannii* clinical isolates.

II. MATERIALS AND METHODS

Bacterial strains and culture conditions

Thirty clinical isolates of *Acinetobacter calcoaceticus-Acinetobacter baumanni*(ACB) complex were obtained from the Department of Clinical Microbiology Department of Gleneagles Global Hospitals, Hyderabad. The original sources of these isolates were: respiratory secretions, blood, wound swabs, sputum, body fluids including urine and aspirated fluids. *Acinetobacter baumannii* ATCC 19606 reference strain was obtained from Culture collections of Himedia Pvt. Ltd. These specimens were inoculated into Mueller Hinton Agar (MHA) and incubated overnight at 37 °C. Pure and isolated colonies of the cultures were diluted to 1.0 McFarland (A600nm = 0.2 - 0.4) and used for all the experiments. *Agrobacterium tumefaciens* NTL4 (pZLR4), a monitor strain was kindly given by Dr. Clay Fuqua, Indiana University, Indiana, USA was utilized to detect AHLs with long acyl chains.

Antimicrobial drug sensitivity testing by disc diffusion method

Antimicrobial sensitivity testing (AST) was performed on the *A. baumannii* isolates against two antibiotics – Imipenem (10µg) and Meropenem (10µg) (Himedia, Mumbai, India) as per BSAC guidelines (2015) by Kirby-Bauer disk diffusion method to confirm sensitivity to carbapenems.

Preparation of Mango seed kernel extract and Guava leaf extract

Mango seed kernel from a popular variety of mango namely Mallika (*Mangifera indica* sp.Mallika) and guava (*Psidium guajava*) leaves were obtained from Fruit Research Station, Sangareddy, Telangana. Ethanolic extracts of Mango seed kernels and Guava leaves were prepared as per the protocol as published earlier from our laboratory.[10] Briefly, the air-dried kernels and leaves were coarsely powdered and subjected to serial extraction with absolute alcohol in a Soxhlet apparatus and the distillate was collected at 80°C.[11] The extracts were filtered and dried in a rotary evaporator. A stock solution of 100mg/mL was prepared in dimethyl sulfoxide (DMSO, Cat. No. 41639, Sigma- Aldrich) and stored at -20ºC for further use.

Preparation of ε-Poly lysine solution

ε-Poly lysine (food grade, Cat. No. FP-155) was purchased from Bimal Pharma Pvt. Ltd., Mumbai. Sterile stock solutions of 100mg/mL were made in molecular biology grade water and stored at -20ºC for further use.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) of MSKE, GLE and ε-Poly lysine on Reference strain and clinical *A. baumannii* isolates were determined using Kirby-Bauer Disc Diffusion method.[12] Filter paper discs impregnated with different concentrations within a range of 10-100 µg mL^{-1} of the compounds, positioned on the *A. baumannii* bacterial lawn and incubated at 37°C for 24 h. The lowest concentration which showed the zone of growth inhibition around the discs was reported as the MIC of the compounds.

Determination of biofilm inhibition

The bacterial cells were allowed to adhere to the base of 96-well micro titre polystyrene plate and the biofilm formed was estimated by the method of Stepanovic etal.[13] with some modifications. Overnight culture of *A. baumannii* was used to inoculate 200 µL of MHB in 96-well plate without and with candidate drug compounds and incubated at 37 °C for 24 h. *A. baumannii* type strain ATCC 19606 was used as a positive control, while un-inoculated MHB media was used as a negative control. Streptomycin was used as reference antibiotic for demonstrating anti-biofilm effect. After incubation, the cell density was measured at 610 nm. The non-adherent cells were discarded, and the biofilms were washed using sterile phosphate buffered saline (PBS) to remove

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unbound cells followed by cold methanol fixation and air drying. The plate was then stained with 0.1% (w/v) crystal violet for 10 minutes and washed under running tap water to remove excess stain. The biofilm bound dye was extracted in 30% glacial acetic acid and its absorbance measured at 610 nm. Finally, the percentage of biofilm inhibition was calculated for both CRAB and CSAB isolates using following formula: % inhibition = Treated biofilm A_{610} – Untreated biofilm A_{610}/Untreated biofilm A_{610} X 100.

Direct demonstration of quorum quenching on clinical isolates of *A. baumannii* by candidate drugs.

The blue-green complex formed by AHL was estimated in a 96-well plate format as described previously with some modifications. [14,15,16] Single isolated colony of *Agrobacterium tumefaciens* monitor strain, NTL4pZLR4 was cultured overnight in 3mL of LB broth substituted with antibiotics streptomycin (50 µg/mL) and tetracycline (5 µg/mL). 180 µL of 1.0 McFarland NTL4pZLR4 culture was transferred to 96-well flat bottom polystyrene plate containing X-Gal of 40 µg/mL final concentration. 20 µL *A. baumannii* culture supernatant and 2 µL each of the candidate drugs (sub-MIC concentrations of 10 µg/µL) was added to each well in duplicates. Cultures unexposed to the drug candidates were used as control. The plate was incubated at 28ºC for 24 h. After 24 h, absorbance was measured at 610 nm and inhibition of AHL (Blue-green complex) was calculated for both CRAB and CSAB isolates with respect to the absorbance of the untreated control. The AHL inhibition was measured using the formula:

% inhibition = Treated culture OD – Untreated culture OD/Untreated culture OD X 100.

Scanning Electron Microscopy (SEM)

Biofilms from CRAB and CSAB isolates of *A. baumannii* were grown on glass coverslips by adding 4 mL of *A. baumannii* culture (1.0 McFarland) supplemented with sub-MIC concentration of the extracts (10 µg/mL) in a 6-well microtiter plate while the control cultures had an equal amount of DMSO. After 24 h of incubation at 37ºC, the cover slips were rinsed with distilled water to remove non-adherent cells and processed for scanning electron microscopy (SEM) examination. Samples were analyzed by SEM as described previously.[17] 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 thrice with 0.1M SCB buffer every 30 minutes to get rid of the excessive fixative and dehydrated with increasing concentration (30%, 50%, 70%, 80%, 90% and 100%) of ethanol. All the coverslips were removed from the ethanol and air dried under high vacuum (10^{-7}Torr) at room temperature (25°C) for a 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 Hitachi Japan) at 15KV, and high vacuum (10^{-7} Torr) and the pictures were taken at different magnifications.

GC-MS Analysis and identification of components

GC-MS analysis was carried out in Shimadzu, Gas Chromatography – Mass Spectroscopy GC-MS 2010QP plus. ZB-5 column capillary column (30m x 0.25mm x 0.25µm) of 5% – Phenyl – 95% Dimethyl poly-siloxane was used for the identification of metabolites in the candidate compound extracts. Helium was used as carrier gas with a consistent flow rate of 1.10 ml min^{-1} with sample injection volume was 1µl and ion source temperature was 200°C. The oven temperature was programmed from 100°C (isothermal of 4 min), with an increase of 4°C min^{-1}, to 280°C, then hold it for 12.95 minutes. Mass spectra were taken at 70eV: a scan interval of 10 spectra s^{-1} and fragments from 50 to 500 Da. The relative percentage of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST and Wiley library to ascertain the name, molecular weight, and structure of the components of the test materials.[18]

III. RESULTS

Antimicrobial Sensitivity Testing (AST) profile

The 30 clinical isolates were characterised for carbapenem sensitivity and grouped as Carbapenem Resistant (CRAB) (n=22) and Carbapenem Sensitive (CSAB) (n=08). The isolates were considered CRAB if the inhibition zone diameter was ≤13mm (Imipenem) and ≤12mm (Meropenem). CSAB isolates showed an

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inhibition zone of ≥ 25mm (Imipenem) and ≥ 20mm (Meropenem).

Impact of candidate drug compounds on Biofilm Formation by *A. baumannii*

The effect of the 3 candidate drug compounds was initially investigated on 30 clinical isolates of *A. baumannii* biofilm formation in 96-well polystyrene plates using a crystal violet assay. All compounds were tested at Sub-MIC (Figure 1). The percentage inhibition of biofilm by the compounds was categorized as weak (<20%), moderate (20-60%) and strong inhibition (>60%). These compounds were equally effective in inhibiting the biofilms of both Carbapenem resistant
and sensitive isolates. Of these three compounds, MSKE and ε-Poly lysine were the most effective biofilm inhibitors (Table 1) as they showed strong biofilm inhibition in more than 60% (both CRAB and CSAB) of *A. baumannii* isolates(Figure 2).

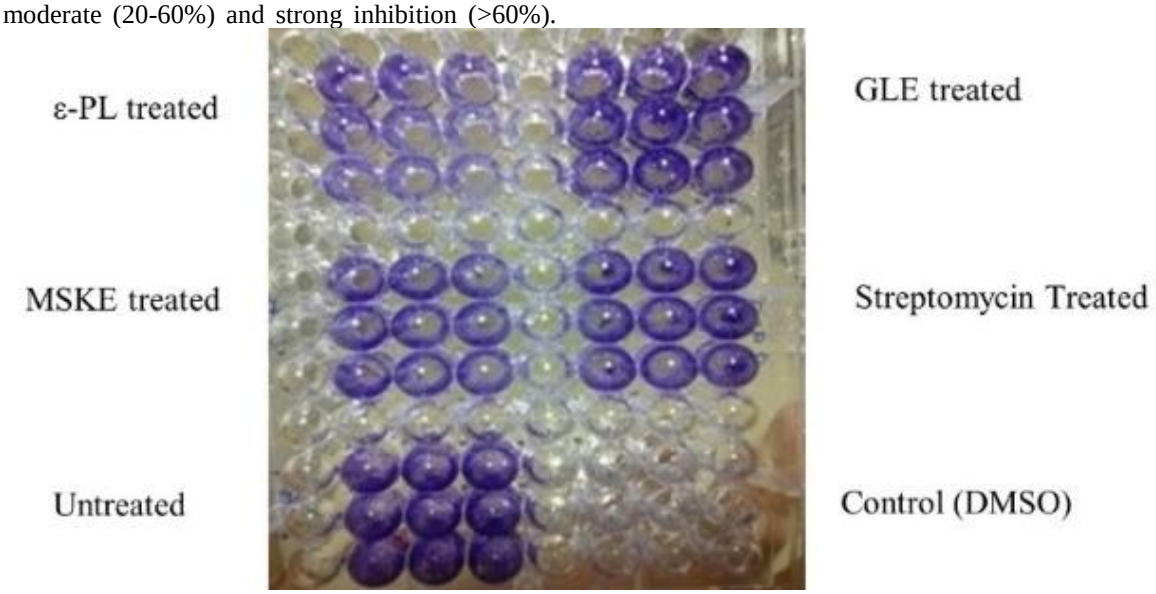

Figure 1: Inhibitory effect of compounds on biofilm formation by *A. baumannii* clinical isolates in crystal violet-stained microtiter plates. Biofilm formation by *A. baumannii* clinical isolates were quantified in the presence of MSKE, GLE, ε-Polylysine at 10 µg/mL for 24 h in 96-well plates vs untreated controls in triplicates. Streptomycin was used as a reference antibiotic.

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Table1: Categorisation of the test compounds based on the extent of Biofilm inhibition in clinical isolates of CRAB and CSAB *A. baumannii*.

Compound	Biofilm Inhibition, the number of positive isolates is presented as % total number ofCRAB isolates (n=22)/CSAB isolates (n=8)		
	Weak inhibition	Moderate inhibition	Strong inhibition
MSKE	4.5/0	31.8/37.5	63.6/62.5
GLE	45.4/62.5	54.5/25	0/12.5
ε-Poly lysine	0/0	36.3/25	63.6/75
Streptomycin	18.1/12.5	63.6/50	18.1/37.5

Weak Inhibition=<20% biofilm inhibition, Moderate inhibition=20-60% biofilm inhibition, Strong inhibition=>60% biofilm inhibition.

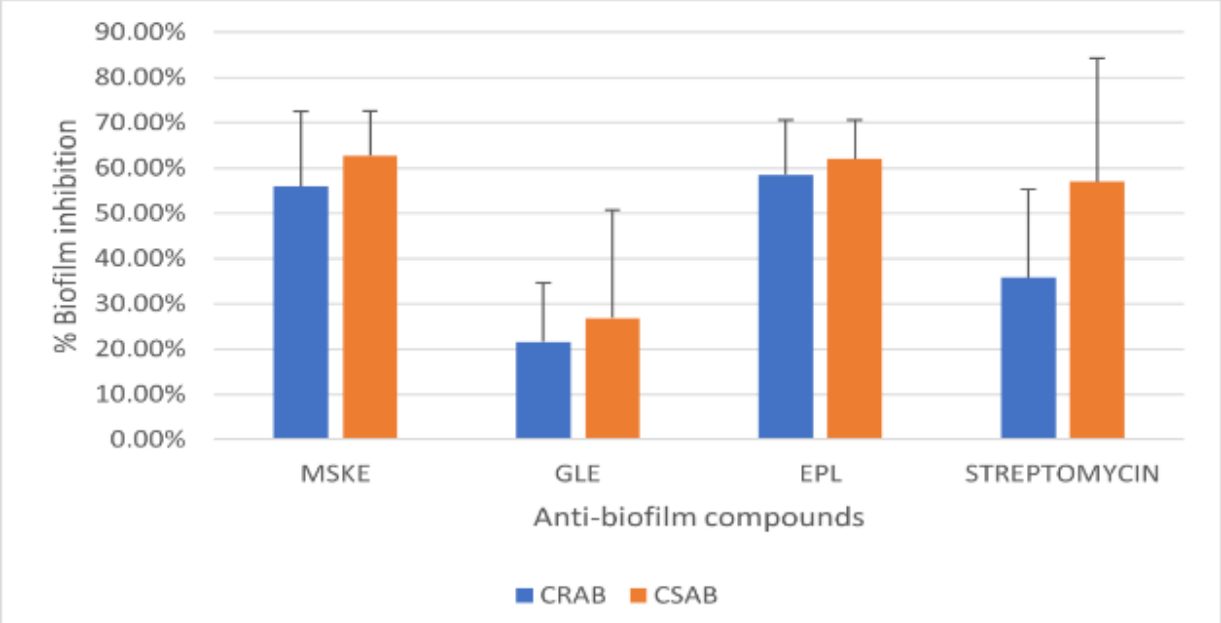

Figure 2: Graph representing inhibitory effect of compounds on biofilm formation by *A. baumannii* clinical isolates. Biofilm formation by *A. baumannii* clinical isolates were quantified in the presence of MSKE, GLE, ε-Polylysine at 10 µg/mL after 24 h in 96-well plates vs untreated controls. Streptomycin was used as reference antibiotic control to show the anti-biofilm effect. Error bars indicate standard deviation of the mean biofilm inhibition % values in triplicate.

Morphological and structural impact on the Biofilm by the drug candidates

SEM images revealed a reduced number of *A. baumannii* cells in all four test groups compared with the control, specifically in terms of biofilm biomass, thickness, integrity of the film and substrate coverage were approximately dramatically reduced compared to untreated controls. The untreated cells appeared to be regular spherical in shape with smooth surfaces and intact cell walls. The SEM images of the biofilm

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specimens after they were exposed to compounds revealed that *A. baumannii* cells exhibited membrane disruption and irregular cell morphology (Figure 3).

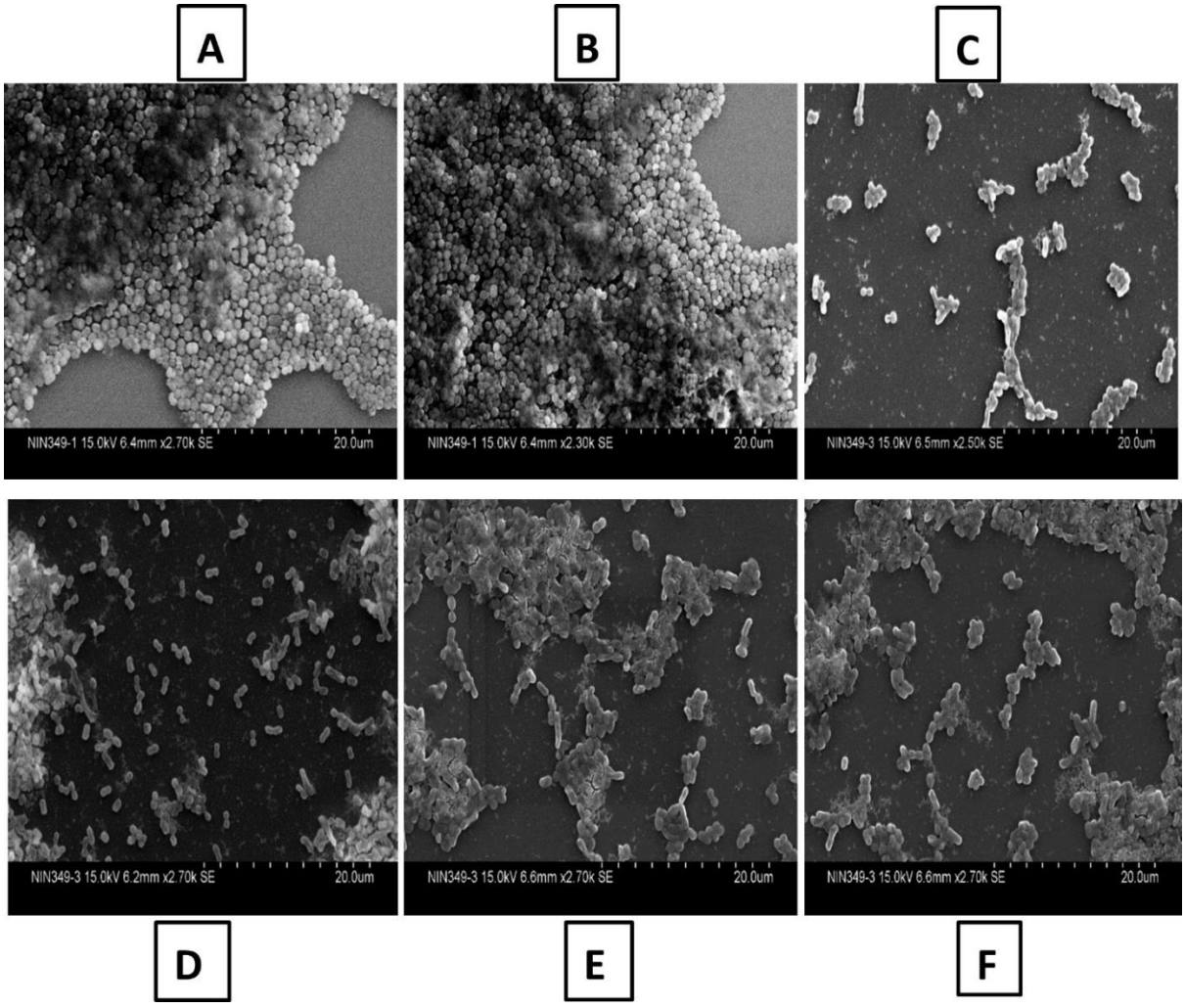

Figure 3: SEM images of 24-hour biofilm of *A. baumannii* isolates. A. control biofilm without any antimicrobial added; B. Control biofilm with DMSO added; C. Streptomycin added as positive control at sub-MIC concentrations of 10µg/mL; D. with MSKE (Mango Seed Kernel Extract) at sub-MIC concentrations of 10µg/mL; E. with GLE (Guava Leaf Extract) at sub-MIC concentrations of 10µg/mL; F. with ε-Polylysine at sub-MIC concentrations of 10µg/mL.

Phytochemical Analysis of bioactive compounds

Ten major compounds were identified in the kernel extract of *Mangifera indica* L. The GC-MS chromatogram is shown in Figure 4 and the corresponding compounds with their retention times, molecular formulae, molecular weights (MW) and concentrations (%) are shown in Table 2.

Ten major compounds were identified in the leaf extract of guava. The GC-MS chromatogram is shown in Figure 5 and the corresponding components with individual retention times, molecular formulae, molecular weights (MW) and relative proportion (%) of the total compound are shown in Table 3.

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Table 2:GC-MS analysis of MSKE.4-Methyl-1- decene followed by 2-ethylhexyl ester were the major compounds with highest % found in the MSKE extract.

S.No.	RT	Area	Compound Name	Molecular Weight	Molecular Formulae	Area%
1	RT:4.261	95276	4-Methyl-1- decene	154	C11H22	9.147266
2	RT:4.369	38295	4- Methyl decane	156	C11H24	3.676629
3	RT:5.153	76093	3,7- Dimethyl decane	170	C12H26	7.305543
4	RT:9.936	91457	2-ethylhexyl ester	202	C11H22O3	8.780611
5	RT:10.594	24876	2,6,11-Trimethyl do-decane	212	C15H32	2.388297
6	RT:16.511	82861	n-Penta decane	212	C15H32	7.955326
7	RT:18.394	32103	1-Iodo-2-methyl un- decane	296	C12H25I	3.082147
8	RT:22.636	63727	n-Heptadecane	240	C17H36	6.118307
9	RT:28.161	45135	2,6,10,14-Tetramethyl octa decane	310	C22H46	4.333325
10	RT:29.186	56999	2,3- Dimethyl nonadecane	296	C21H44	5.472365

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Table 3:GC-MS analysis of GLE. 4-Methyl-1- decene and 2,4-Dimethyldodecane were the highest found compounds in the extracts of GLE.

S.No	RT	Area	Compound Name	Molecular Weight	Molecular Formulae	Area%
1	RT:4.269	79433	4-Methyl-1- decene	154	C11H22	7.636944
2	RT:5.169	63648	2,2,3,3,5,6,6-Heptamethyl heptane	198	C14H30	6.119323
3	RT:5.286	30769	Hendecane	156	C11H24	2.958231
4	RT:9.944	77851	2,4-Dimethyl do-decane	198	C14H30	7.484845
5	RT:11.336	71825	2-Methyl-n-tri decane	198	C14H30	6.905486
6	RT:16.511	67326	Oxalic acid	244	C13H24O4	6.472938
7	RT:22.636	53815	n-Heptadecane	240	C17H36	5.173947
8	RT:23.803	69933	6-ethyloct-3-ylisobutyl ester	286	C16H30O4	6.723583
9	RT:28.161	41361	10-Methylnona decane	282	C20H42	3.97658
10	RT:34.078	35559	n-Docosane	310	C22H46	3.418757

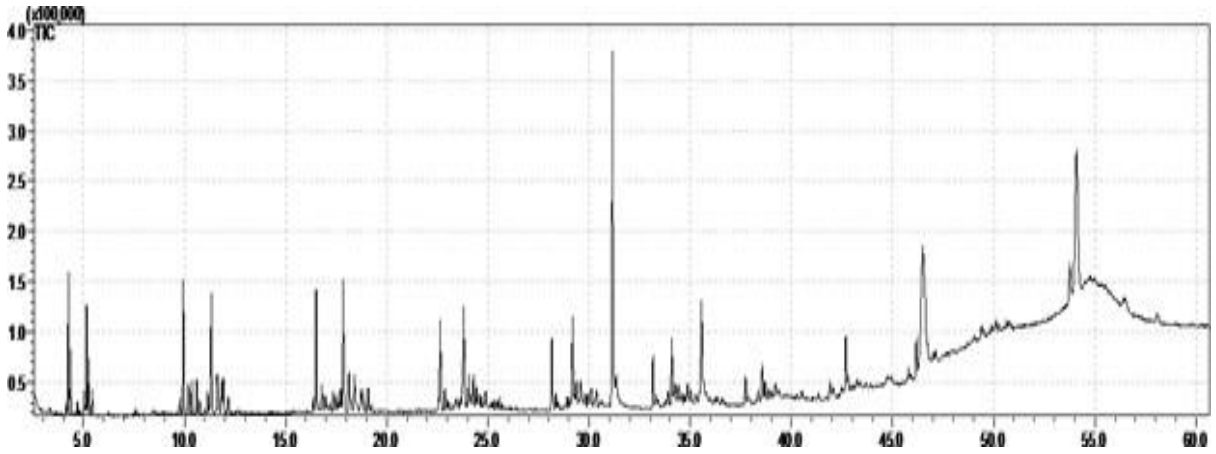

Figure 4:GC/MS Chromatogram of MSKE. Ten major compounds were identified in the kernel extract of *Mangifera indica L.* GC-MS analysis was carried out in Shimadzu, Gas Chromatography – Mass Spectroscopy GC-MS 2010QP plus.

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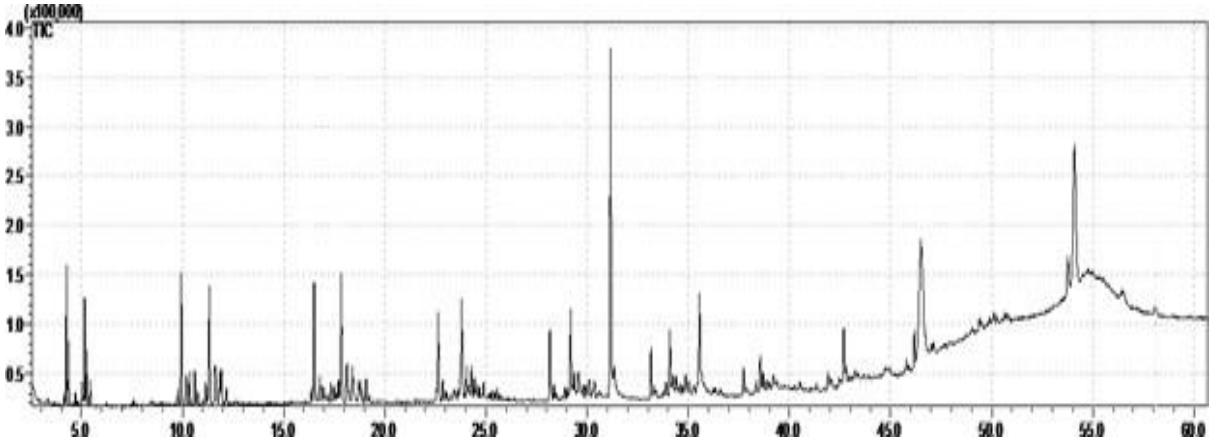

Figure 5:GC/MS Chromatogram of Guava Leaf Extract. Ten major compounds were identified in the leaves extract of *Psidium guajava.* GC-MS analysis was carried out in Shimadzu, Gas Chromatography – Mass Spectroscopy GC-MS 2010QP plus.

Quorum quenching effect of compounds on clinical isolates of *A. baumannii*

The anti-QS effect of all the four compounds was investigated on just 10 CRAB and 10 CSAB clinical isolates in 96-well polystyrene plates. *A. tumefaciens* monitor strain NTL4pZLR4, with 40µg mL^{-1} of X-gal and 20 µL *A. baumannii* culture supernatants was incubated with sub-MIC amounts (10µg mL^{-1}) of the compounds and allowed to grow at 28°C for 24 hours. Agrobacterium monitor strain utilizes the AHL diffused from the *A. baumannii* culture supernatants and forms a blue-green complex. Untreated (DMSO without candidate drugs) cultures were used as control. After 24 hours, absorbance was measured at 610 nm and inhibition of AHL production (Blue-green complex) was calculated with respect to the absorbance of the untreated control. The formation of Blue-green complex was inhibited by the test compounds and the decrease in absorbance was compared to that of the control untreated well. The inhibition was expressed as % (mean ±SD) for each drug on both CRAB and CSAB isolates (Table 4). The candidate drugs showed good anti-QS effect in carbapenem sensitive isolates than carbapenem resistant isolates. MSKE showed remarkable effect in decreasing the blue-green complex formation. The inhibition of blue-green complex production by MSKE was 47% in CRAB and 82% in CSAB isolates, while GLE showed 42% and 69% inhibition in CRAB and CSAB isolates respectively. Streptomycin and ε-Poly lysine both could inhibit the production of the blue-green complex by around 40% in CRAB and 80% in CSAB isolates without affecting the growth of A. tumefaciens monitor strain. This data shows the promising potential of these candidate drugs as anti-QS compounds.

Table 4: Quorum quenching effect of compounds on clinical isolates of *A. baumannii.* Data showing the percentage inhibition in production of blue-green complex in the presence of candidate compounds. Mean and SD are calculated for each data sets.

Compounds / **Drug Sensitivity**	**MSKE**	**GLE**	**Streptomycin**	**ε-Poly lysine**
CRAB	47±7%	42±0.08	43.8±7%	45±9%
CSAB	84±0.06%	69.7±4%	80.8±4%	81.4±3%

IV. DISCUSSION

The medicinal use of *P. guajava*and *M. indica*has already been reported in native system of medicines and its use continues even today in

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contemporary home remedies and personal care products. Reports show that *P. guajava* essential oil has capacity to inhibit the growth of both gram-positive and gram-negative bacterial strains.[19]Hence, we chose to look at its anti-biofilm effect on *A. baumannii* isolates. Of these three compounds, MSKE and ε-Poly lysine were the most effective biofilm inhibitors. They showed more than 60% inhibition of biofilm formation in maximum number of clinical isolates. A similar effect was observed by Angad and his co-workers at an MIC of 125 µg mL^{-1} on *P. aeruginosa* and *E. coli*. The antibiofilm test showed the ethanol extract inhibited the bacterial adhesion on glass tube which caused biofilm detachments when compared with control tubes.[20]Our study demonstrated that our candidate drugs were much more effective as biofilm inhibitors at a concentration several times lower (10 µg mL^{-1}) on *A. baumannii* compared to how *P. aeruginosa* and *E. coli* responded. The results obtained in this study are similar to those reported by several other authors: Adesina et al., reported that *M. indica*, *Psidium guajava* and *Ocimumgratissimum* leaf extracts prevented the *E. coli* biofilm formation on catheters.[21]

Many studies have showcased the potential of natural extracts as anti-QS compounds where they have shown effect on violacein pigment production in *Chromobacteriumviolaceum* or blue green colour complex in *Agrobacterium tumefaciens*.[22,23,24] This study was the first to demonstrate the direct effect of these compounds on AHL production by *A. baumannii* clinical isolates in a microtiter plate by using A. tumefaciens monitor strain NTL4pZLR4.

Our results were further confirmed by a Scanning Electron Microscopy analysis of the biofilms. The SEM images of the biofilms exposed to compounds (sub-MIC of 10µg mL^{-1}) shows poorly developed biofilms with membrane disruption and irregular cell morphology compared with untreated cells. *A. baumannii* biofilms treated with compounds showed fewer cells of different size scattered than the controls and the matrix was also scarcely visible. Presently, there is no report on the anti-biofilm potential of ε-Poly lysine against *A. baumannii*. However, the anti-biofilm activity of G3 dendrons of ε-Poly lysine was demonstrated against *P. aeruginosa*.[25]

Ten components were identified in the GLE and MSKE extracts by Gas chromatography Mass spectrometry. The major components reported were: 4-Methyl-1-decene (7.63%), 2,4-Dimethyl dodecane (7.48%), 4-Methyl-1-decene (9.14%) and 2-ethyl hexyl ester (8.78%). All these components were basically essential oils, volatile organic compounds, esters, hydrocarbons, fatty acids like oxalic acid. Many reports confirm the presence of these major components.[26,27,28] Identifying particularly the most active antimicrobial compounds of the essential oils is burdensome and requires extensive purification of the extracts because essential oils are complex mixtures of up to 30 different components.

V. CONCLUSION

The present study demonstrated that MSKE, GLE ethanolic extracts and ε-Poly lysine have strong potential as antibiofilm and anti- QS compounds against carbapenem-resistant *A. baumannii* strains. The active compounds of the crude extract, at sub-MIC, caused damage to the stability and integrity of its biofilm as well as its morphology. Theantibiofilm effect of these compounds could be exploited for anti-virulence therapy and for co-therapy with other antibiotics to eliminate development of resistance or as adjunct drugs for treating biofilm associated clinical problems caused by this pathogen.

REFERENCES

[1]. Hosseini, A; Nejadsattari, T; and Zargar, M.,2019, "In vitro Anti-Biofilm Activity of Curcumin Nanoparticles in *Acinetobacter baumannii*: A Culture-Based and Molecular Approach," Arch. Clin. Infect. Dis. 10:83263.

[2]. Howard, A; O'Donoghue, M; Feeney, A; and Sleator, RD., 2012,"*Acinetobacter baumannii*: an emerging opportunistic pathogen," Virulence. 1:243-50.

[3]. McConnell, MJ;Actis,L;andPachón, J., 2013,"*Acinetobacter baumannii*: human infections, factors contributing to pathogenesis and animal models," FEMS Microbiol Rev. 37:130-55.

[4]. Espinal, P; Martí,S;andVila, J.,2012, "Effect of biofilm formation on the survival of *Acinetobacter baumannii* on dry surfaces," J Hosp Infect. 80:56-60.

[5]. Zhang, K; Li, X; Yu, C; and Wang, Y., 2020, "Promising Therapeutic Strategies Against Microbial Biofilm Challenges," Front Cell Infect Microbiol. 10:359.

[6]. YeomJinki, S; Ji-Hyun, Y; Ji-Young, K;Jungmin, H; andGeum, S., 2013,"1H NMR-Based Metabolite Profiling of

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2192-2198www.ijprajournal.com ISSN: 2249-7781

Planktonic and Biofilm Cells in *Acinetobacter baumannii*,"PloS one. 2:1656.

[7]. Gaddy, JA;Actis, LA., 2009,"Regulation of *Acinetobacter baumannii* biofilm formation," Future Microbiol.4:273-8.

[8]. Eze, EC;Chenia,HY;andEl Zowalaty, ME., 2018, "*Acinetobacter baumannii* biofilms: effects of physicochemical factors, virulence, antibiotic resistance determinants, gene regulation, and future antimicrobial treatments," Infect Drug Resist. 11:2277-2299.

[9]. Beceiro, A; Tomás,M;andBou, G., 2013, "Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world," Clin Microbiol Rev. 26:185-230.

[10]. Saipriya, K; Swathi, CH;Sireesha, D; Archana, G; and Venkataraman, S., 2020, "Is Quorum sensing inhibition a potential target to combat multidrug resistant *Acinetobacter baumannii*? -A Proof of concept study," IOSR J Biotechnol Biochem.6:35-42.

[11]. Yoswathana,N;andEshtiaghi, M., 2014,"Extraction of fatty acid from mango seed kernel using supercritical carbon dioxide by response surface methodology," Asian J. Chem.26:3009-3012.

[12]. Cahit, A; andGulsen, S., 2006,"Antibacterial activity of crude methanolic extract and its fractions of aerial parts of *Anthemistinctoria*," Indian J. Biochem. Biophys. 42:395-7

[13]. Stepanovic, S;Vukovic, D;Dakic, I;Savic, B; andSvabic-Vlahovic, M., 2000,"A modified microtiter-plate test for quantification of *staphylococcal* biofilm formation," J Microbiol Methods.40:175-9.

[14]. Bernabè, G;Marzaro, G; Di Pietra, G; Otero, A;Bellato, M; andPauletto, A.,2022, "A novel phenolic derivative inhibits AHL-dependent quorum sensing signaling in *Pseudomonas aeruginosa*," Front. Pharmacol.13:996871.

[15]. Khalid, SJ; Ain, Q; Khan, SJ; Jalil, A; Siddiqui,MF;andAhmad, T.,2022,"Targeting Acyl Homoserine Lactones (AHLs) by the quorum quenching bacterial strains to control biofilm formation in *Pseudomonas aeruginosa*," Saudi J Biol Sci.29:1673-1682.

[16]. Bernier, SP; Beeston,AL;andSokol, PA.,2008, "Detection of N-acyl homoserine lactones using a traI-luxCDABE-based biosensor as a high-throughput screening tool," BMC Biotechnol. 30:8-59.

[17]. Karnovsky, M.J., 1965, "A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron-microscopy," J. Cell Biol. 27:137-138A.

[18]. Bhagya, H;Rameshwaran, P; Navya, P N; and Murthy, P., 2015,"In-vitro Mycological activity of essential oil from *Zingiber zerumbet* rhizomes," J. Essent. Oil Res.28: 1-8.

[19]. Nisha, K; Darshana, M; Madhu,G;andBhupendra, M., 2011,"GC-MS Analysis and anti-microbial activity of *Psidium guajava* (leaves) grown in Malva region of India," Int. J. Drug Dev. & Res.3:237-245.

[20]. Amgad, A; Martin, RP; Ismail, M; Abdel kareem, M; Ahmad, M; and Mohamed, E., 2012,"Antimicrobial and Antibiofilm Activity of Mango Seeds Extract," Adv Microbiol. 2:571-576.

[21]. Adesina, TD;Nwinyi,OC;andOlugbuyiro, JAO., 2015,"Prevention of bacterial biofilms formation on urinary catheter by selected plant extracts," Pak J Biol Sci.18:67-73.

[22]. Osuntokun,OT;andCristina, GM., 2019,"Bio isolation, chemical purification, identification, antimicrobial and synergistic efficacy of extracted essential oils from stem bark extract of *Spondiasmombin* (Linn)," Int J Mol Biol. 4:135-143.

[23]. Fantner, GE;Barbero, RJ;and Gray, DS., 2010,"Kinetics of antimicrobial peptide activity measured on individual bacterial cells using high-speed atomic force microscopy," Nat Nanotechnol. 5:280-285.

[24]. Osuntokun,OT;andOgunleye, AJ.,2017, "Prospects of essential oils in drug discovery," Adv Cytol Pathol. 2:17-19.

[25]. Issa, R; Meikle, S; James, S; and Cooper, I.,2015, "Poly (ε-lysine) dendrons as modulators of quorum sensing in

Pseudomonas aeruginosa," J Mater Sci Mater Med. 26:10.

[26]. Oyedeji, FO;Erazua,EA;andAdeleke, BB., 2018,"GC-Mass Spectroscopic chemical characterization and physico-chemical properties of oil from seed kernels of four cultivars of *Magnifera indica*," Eur. J Pure Appl. Chem. 5:7-17.

[27]. Al-Haidari, RA; Shaaban, MI; Ibrahim, SRM; and Mohamed, GA., 2016,"Anti-Quorum Sensing Activity of some Medicinal Plants,"Afr J Tradit Complement Altern Med. 13: 67-71.

[28]. Omwenga, EO; Hensel, A; Pereira, S;Shitandi, AA; andGoycoolea, FM., 2017,"Antiquorum sensing, antibiofilm formation and cytotoxicity activity of commonly used medicinal plants by inhabitants of Borabu sub-county, Nyamira County, Kenya,"PloS one. 12: e0185722.