doi.org/10.36721/PJPS.2026.39.2.REG.14747.1

# Beyond antibiotics: Essential oils as novel biofilm inhibitors

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**Abstract**: **Background**: Multidrug and extensively antibiotic-resistant microorganisms, also known as "superbugs," are now becoming a global threat to mankind. Bacteria constitute a protective layer of extracellular polymeric substances (EPS) around their colonies, which produces persistent bacterial infection. Bacteria coordinate in aggregated biofilm with each other through a mechanism named quorum sensing. **Objective**: This study aimed to explore the potential of essential oils as novel agents for inhibiting bacterial biofilm formation, specifically through their quorum sensing inhibitory properties. **Methods**: The extracts and essential oils of three herbal plants, viz. lavender oil, peppermint oil and clove oil, were used for different biological, therapeutic, anti-quorum sensing and biofilm inhibition. Chemical fingerprints of essential oils and extracts were performed using GC-MS and HPLC. Hemolytic activity and Ames assay was performed to document toxicological profile of samples. Anti-quorum activity of essential oils and extracted bio-functional components was performed against sinusitis isolates and reporter strain. **Results**: According to toxicological analysis, samples were non-toxic. Ames assay expressed that the samples used were non mutagenic while, DNA damage protection assay exhibited that essential oils and water extracts protected the DNA damage. High antibacterial activity of lavender essential oil was 21.83 ± 1.60 mm. According to SEM studies, essential oils and extracts have shown the destruction in three-dimensional structure of biofilm matrix with shrinkage of bacterial cell. The potency of extracts on violacein production is studied and results bring about moderate to good inhibition. **Conclusion**: It is concluded that due to antibiotic resistance, medicinal plant extracts are an alternative for chronic infection treatment.

**Keywords**: Ames assay; Biofilm; DNA damage assay; Essential oil; Sinusitis

*Submitted on 16-06-2025 – Revised on 01-08-2025– Accepted on 26-08-2025*

## INTRODUCTION

Due to the rise of multidrug-resistant bacteria, controlling bacterial infections remains a difficult challenge in the twenty-first century (Mba and Nweze, 2021). The discovery of the first antibiotic, Penicillin, was seen as a watershed moment in the development of antibiotics. Owing to over use of antibiotics, microorganisms are getting resistance to antibiotics (Mancuso *et al*., 2021). Hence, researchers are focusing on alternative targets that reduce this antibiotic resistance. Certain bacteria use quorum sensing (QS), a unique cell communication mechanism, to promote the synthesis and release of their virulence characteristics. (Qin *et al*., 2022).

Sinusitis, also known as rhinosinusitis, is defined as an inflammation of the paranasal sinuses, which is caused by viral upper respiratory infection, autoimmune disorder, allergic infection and secondary bacterial infections (Volpe *et al*., 2023). Typically, isolated bacteria in patients with rhinosinusitis involve *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* (Drago *et al*., 2019). The onset and spread of drug resistance among sinusitis causing bacteria is a major hindrance in the treatment of sinus infections. Almost 8% sinusitis causing bacterial infections are biofilm based. Biofilm is defined as the unique growth pattern in the life cycle of microbes that gives a higher level of organization, advantages and specific properties to free living microbes during colonization. It is structured and aggregated communities of bacterial species encased in extracellular polymeric substances (EPS) (Flemming *et al*., 2023).

Production of virulence factors, biofilm formation, spore formation, drug resistance, luminescence, toxin production and cell motility in pathogenic bacteria are due to quorum sensing system (Atkinson *et al.,* 2009). Quorum sensing, also known as density sensing, is a cell to cell and cell to surface communication process. This particular signal-response based quorum sensing system is dependent on the production, release and uptake of molecules known as autoinducers (AIs). AIs are the starting point for triggering and synchronizing the expression of quorum sensing related behaviors or traits (Oliveira *et al*., 2023). Essential oils (EO) are the natural substances obtained from certain medicinal plants having specific fragrances. They show bactericidal, fungicidal, herbicidal, insecticidal, antioxidant and anti-inflammatory activities (Raveau *et al*., 2020). *Syzygium aromaticum* EO is of great importance. The main components present in the clove oil are eugenol, beta-caryophyllene and eugenyl acetate, of which eugenol is present up to 80% of the whole composition. Eugenol is majorly used as an insecticide, fungicide, antioxidant, anti-inflammatory and antiviral agent (Boughendjioua, 2018). Lavender EO contains several antimicrobial compounds, most of which belong to the alcohol group, including 1,8-cineole, linalool, terpinen-4-ol and α-terpineol. Among them, linalool demonstrated to be the strongest active ingredient against a wide range of microorganisms. *Mentha*

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*piperita* yields 0.1-1.0% EO composed primarily of menthone, menthofuran, menthol, menthyl acetate, 1,8-cineole and limonene. It also contains other pharmaceutically important ingredients like flavonoids, polymerized polyphenols, caffeic acid, tocopherols, choline and tannins (Moghaddam *et al*., 2013). These constituents of peppermint oil, especially menthol, have quorum sensing inhibition activities with no side effects, hence, can be used to combat sinusitis isolates. The aims of the current study are to evaluate the biological and toxicological properties of selected bio-functional components from selected medicinal plants and their effect on biofilm and quorum sensing.

## MATERIALS AND METHODS

### *Collection of samples*

Sinusitis samples were collected from the Ear nose and throat (ENT) Department of Allied Hospital, Faisalabad, Pakistan. Plant samples of clove, lavender and peppermint were purchased from the local market of Faisalabad, Pakistan, in crude form. Extracts were prepared through maceration (Distilled water, acetone, ethanol) and oils were extracted through a superheated steam distillation process.

### *Chemical fingerprints of plant samples*

For the characterization of bioactive compounds in EO, Gas Chromatography-Mass Spectrometry (GCMS-ISQ 7000, Thermo scientific) analysis was employed, following the methodology reported by (Niaz et al., 2023). Additionally, High-Performance Liquid Chromatography (HPLC LC-2010, Shimadzu) analysis was performed, as described by (Mizzi et al., 2020), to detect phenolic compounds and flavonoids in the plant extracts.

### *Toxicological analysis*

Hemolytic activity was used to analyze the cytotoxicity of the essential oils and extracts, a procedure adapted from **(**Ghosh *et al.*, 2018). According to this method, 3 mL of fresh blood is taken and added to the EDTA tube. After centrifugation, plasma was discarded and blood was washed with 5 mL chilled phosphate buffer saline (PBS) and centrifuged for five minutes. This washing process was repeated 3 times. After washing, the red blood cells (RBCs) were suspended in PBS. Then, 180 µL of these red blood cells suspended in PBS were mixed with 20 µL of the tested samples and incubated for 30 minutes. After incubation, this mixture was centrifuged at 1000 rpm for five minutes. Then, 100 µL of the supernatant was diluted with 900 µL of PBS saline. Then, 200 µL of this solution was pipetted into the 96 well plate and absorbance was measured at 576 nm. To assess the mutagenicity of samples, the Ames assay of extracts and essential oils was performed as reported by (Maron and Ames,1983). Chemicals which are required for mutagenicity assay were glucose, bromocresol purple, Davis Mangoli salts, L-histidine, Biotin, $NaN_3$ and $K_2Cr_2O_7$ added together in PBS to form reagent mixture. Then, in the test tube, add 5 µL bacterial strain (T-98 and TA-100) to 5 mL reagent mixture. Samples were added to an amount of 10 µL to each test tube, 200 µL of solution was taken in 96 well microtiter plate. Only reagent mixture is put in the 96 well plate for the blank plate. For the background plate, a reagent mixture and bacterial solution were used. For the standard plate reagent mixture, bacterial solution and standard ($NaN_3$ for TA-100 and $K_2Cr_2O_7$ for TA-98) were added. The plates were placed in an incubator at 37°C for four days, after which the color change was observed. Wells that remain purple are considered negative, while a change to yellow indicates a positive result. To confirm the compounds' mutagenic potential, the number of yellow wells must be at least twice that of the background plate. The DNA damage protection assay for the samples was conducted according to the procedure outlined by (Ruma *et al*., 2013). Calf thymus DNA (ct DNA) was used for the Assay. The preparation of the Fenton reagent was carried out by adding 4 µL of $H_20_2$ and 4 µL of ferric chloride solution. Reaction mixture was formed by mixing 10 µL of Calf thymus DNA and twenty-five microliters of the sample and then placed at 37 °C for five minutes, after the addition of the Fenton reagent and then incubated at 37 °C for 30 minutes. Agarose gel (1 %) electrophoresis was used to study DNA fragmentation in the reaction mixture.

### *Antibacterial activity of plant samples*

Antibacterial activity was evaluated by measuring the diameter of the growth inhibition zones in millimeters for the organisms and comparing them to the control (Shahid *et al*., 2021).

### *Biofilm inhibition assay*

Quantitatively, biofilm inhibition assay against sinusitis isolates was performed by using 96 well microtiter plate by using a microtiter plate reader (µQuant BioTek, USA) method according to the procedure as described by Shahid *et al.* (2021). A qualitative biofilm inhibition assay was performed by using phase contrast microscopy according to the procedure as explained by (Mergoni *et al*., 2021). Confirmation of biofilm inhibition by samples was assessed by scanning electron microscopy (FEI Quanta 400FEG ESEM/EDAX Genesis X4M, FEI Company, USA).

### *Quorum sensing inhibition by plant samples*

QS inhibition potential of essential oils and extracts was performed according to (Moradi *et al.*, 2020) method with minor modifications. *Chromobacterium pseudoviolaceum* after inoculation with Miller Luria Bertani Broth (LB) was incubated. Then, 500 µL of essential oil and 500 µL of diluted bacterial culture were added in all wells of microtiter plate. After incubation, 1 mL solution was taken from each well and centrifuged at 8000g for 5 minutes. Then supernatant was discarded and 1 mL DMSO (di methyl sulfoxide) was added into the pellet and vortexed to mix the pellet homogeneously. It was centrifuged again at 8000g for 5 minutes. After that, 200 µL of supernatant was added into 96 well microtiter plate and its absorbance were measured at 600 nm.

## RESULTS

### *Chemical fingerprints of plant samples*

Green spectra in fig. 1 represents the results of *Mentha piperita* EO. Major components in it were 2-Propanol, 1-(2-methoxy-1-methylethoxy), Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1. α.,2. β,5. α.), Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1. α.,2. β,5. α.)- and Carvone. Black spectra represent the result of *Syzygium aromaticum* EO. Major components present in oil were eugenol, Bicyclononane, 2-methylene-4,8,8-trimethyl-4-vinyl, Naphthalene, 2,2-dimethyl-1-oxa-2-sila-1,2-dihydro-, 9,12-Octadecadiynoic acid pyrrolidine, Benzene, 1-methyl-4-(1,2,2-trimethylcyclopentyl)-, Aristolene and Isopropyl palmitate. Red spectra represent the results of *Lavandula stoechas* EO. Components present in *Lavandula stoechas* EO with the highest concentration were Propane, 1,2-dimethoxy-, Linalyl acetate, Isobornyl acetate, Glycerol 1,2-diacetate, alpha. -Terpinyl acetate and Cyclopenta-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl. In table 1, phenolics and flavonoid content were measured through HPLC as given.

### *Toxicological analysis*

Hemolytic assay of *Mentha piperita* (MP) showed that samples range from nontoxic to slightly toxic, highest hemolysis was shown by ethanolic extract of MP. In the case of clove samples, the water extract had a maximum hemolytic activity of 8.3 ± 3.15, which was lower than that of the positive control. Lavender extracts were the least hemolytic among all samples. Fig. 2 contains the hemolytic assay results. The reverse mutation test on microbes was devised by Ames (Moron and Ames, 1983). Extracts and oils of medicinal plants had been subjected to a mutagenicity assay. When comparing with the background plate of both *Salmonella typhimurium* strains TA 98 and TA 100, all plant samples were non mutagenic. Fig. 3 represents the results of the Ames assay of plant samples. Agarose gel electrophoresis was performed to accomplish the DNA damage test. The results were studied by the gel doc system to evaluate the free radical DNA damage protection by the extract. Fig. 4 represents the results of different extracts of 3 medicinal plants. In lane labeled as L, contains 2kb DNA ladder, C-1 lane Calf thymus DNA showed an intact band which act as negative control. In C2 lane, $FeSO_4$ was used as positive control along with Calf thymus DNA. $FeSO_4$ produces Fenton reaction which results in the production of $OH^-$ which destroys the calf thymus DNA. This damage to Calf thymus DNA was seen by the development of a smear of damaged DNA in lane C2. DNA in lane labeled as 1,2,4,5 and 6 showed an intact band which protected the DNA where as in lane labeled as 3, 7, 8 and 9 there can be seen smear formation which showed that the sample in these lanes has not protected the Calf thymus DNA from hydroxyl free radical produced due to Fenton reaction. None of the plant extracts showed DNA damage. After all of these toxicological studies, it was clear that our samples are safe to use as medicine.

### *Antibacterial activity of plant samples*

Antibacterial activity of essential oils and extracts against sinusitis isolates (both gram + ve and gram -ve bacteria) was determined by agar well diffusion method. As essential oils and extracts have antibacterial activity against sinusitis isolates, they created a zone of inhibition around the wells. This zone of inhibition was measured by using a zone reader and then compared with the zone of inhibition of the positive control (ciprofloxacin). The results of antibacterial activity are presented in fig. 5.

### *Biofilm inhibition assay*

In this study, biofilm inhibiting potential of essential oils and leaf extracts (DW extract, acetone extract, ethanol extract) was checked against sinusitis isolates (both gram +ve and gram -ve bacteria) by using 96 well microtiter plate method. Ciprofloxacin was used as a positive control, while nutrient broth and bacteria were added into the negative control well. Optical density (OD) was measured at 600 nm by using a microtiter plate reader. Results of biofilm inhibition by EOs and extracts (distilled water extracts, acetone extracts, ethanol extracts) are given in table 2.

Phase contrast microscopy was performed for the confirmation of bacterial biofilm formation. In the case of biofilm of *E. coli,* on negative control slide fig. 6, most areas can be seen covered with biofilm (Colored areas). whereas on positive slide (Ciprofloxacin) colored areas were in minority. Essential oils have shown clear areas (less biofilm formation) as compared to ethanolic extracts, which have shown more biofilm with compacted aggregates of bacteria. Scanning electron microscopy of samples was done for confirmation of biofilm inhibition. When *K. pneumoniae* biofilm (Fig. 7) was treated with a standard antibiotic there was clear reduction in biofilm matrix formation and bacteria could be seen without biofilm. Essential oils and extracts have shown the destruction in three-dimensional structure of biofilm matrix with shrinkage of bacterial cell.

### *Quorum sensing inhibition by plant samples*

The potency of extracts on violacein development was studied and results bring about moderate to good inhibition as given in fig. 8. *C. pseudoviolaceum* used as strain to evaluate Quorum sensing inhibition (QSI) in gram -ve bacteria. In the present study, *E. coli* and *K. pneumonia* were gram -ve bacteria recovered from sinusitis patients. The results were signified as QS inhibition (positive) when *C. pseudoviolaceum* strain was unable to form violet pigment (violacein) and if violacein compound formed then no QSI takes place. The end results showed QSI with a value that was lower than the minimum inhibitory concentration (MICs) of the compound. At 0.625 and 0.313 mg/mL concentration of extracts, violacein was produced with small effect on growth of *C. pseudoviolaceum*.

**Table 1**: Quantification of phenolic and flavonoid contents of plant samples through HPLC (ppm).

| Sr. No | Phytochemical | Peppermint | Clove | Lavender |
|---|---|---|---|---|
| 1 | Quercetin | 0.55 ± 0.2 | 40.50 ± 0.16 | 5.37 ± 0.13 |
| 2 | Gallic acid | 33.05 ± 0.4 | 46.71 ± 0.2 | 2.48 ± 0.3 |
| 3 | Benzoic acid | 129.53 ± 0.03 | ND | 14.82 ± 0.9 |
| 4 | Syringic acid | 5.88 ± 0.01 | ND | 32.37 ± 0.12 |
| 5 | p-Coumaric acid | 2.37 ± 0.04 | ND | ND |
| 6 | m-Coumaric acid | 8.39 ± 0.12 | ND | 8.29 ± 0.12 |
| 7 | Cinamic acid | 10.69 ± 0.08 | 132.12 ± 0.12 | ND |
| 8 | Ferulic acid | 94.14 ± 0.17 | 53.58 ± 0.9 | ND |

All samples were analyzed in triplicate manner and results were expressed as mean ±SD, ND- Not detected.

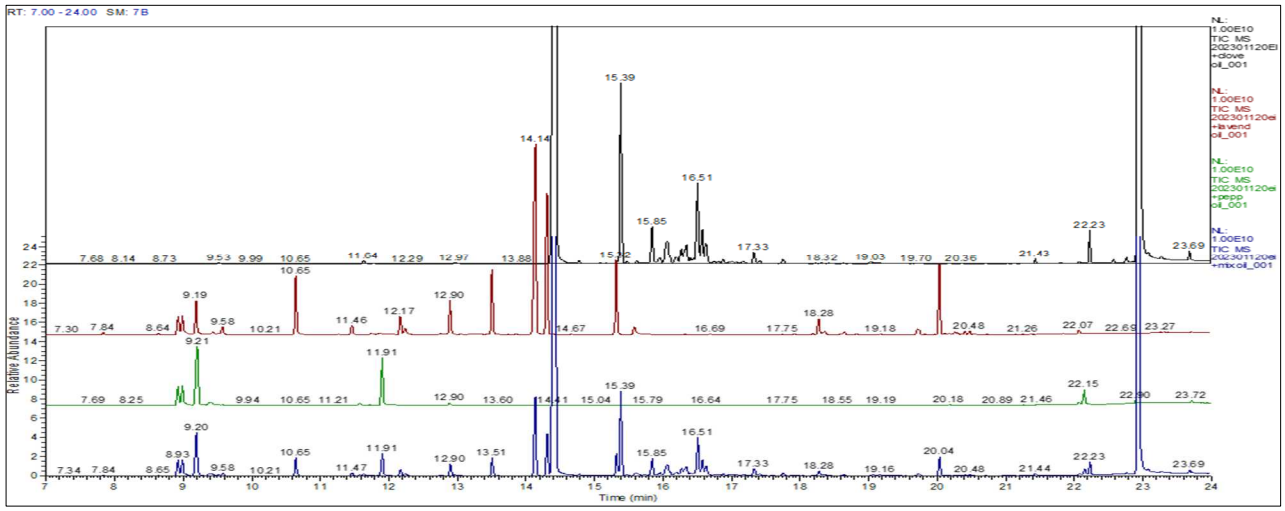


**Fig. 1**: GC-MS spectra of essential oils.
***** Green spectra represent *Mentha piperita* EO, Black spectra represent the result of *Syzygium aromaticum* EO and Red spectra of *Lavandula stoechas* EO.

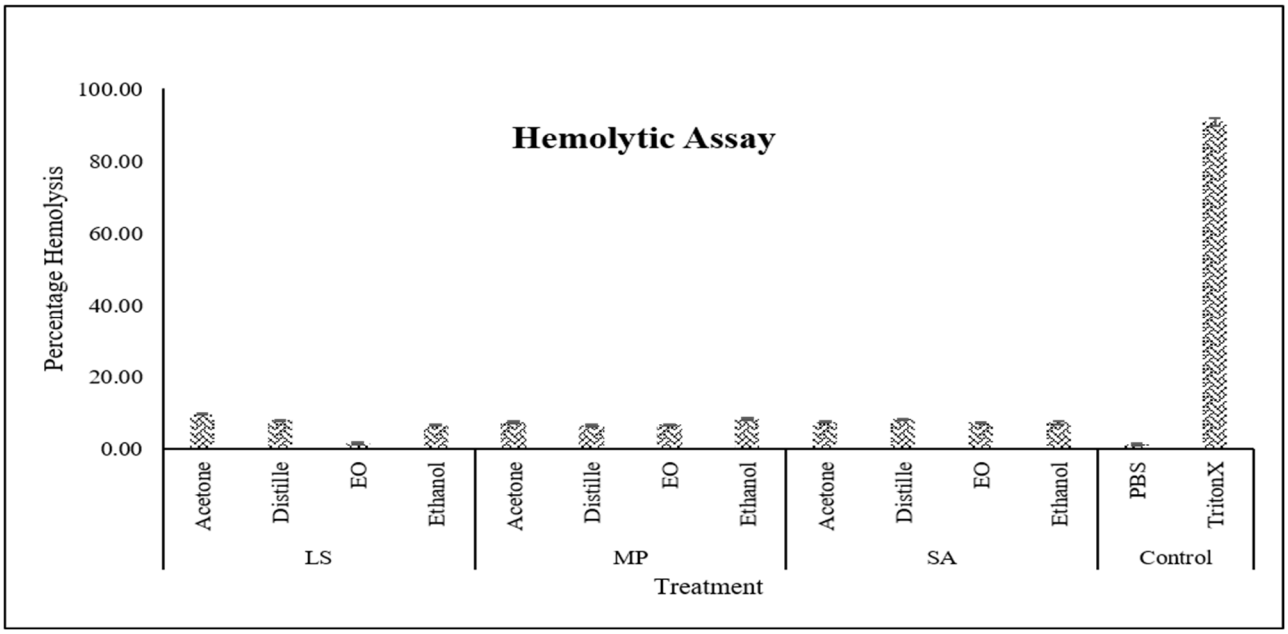


**Fig. 2**: Results of percentage hemolysis (%) for toxicological analysis

**Table 2**: Percentage biofilm inhibition (%) of selected sinusitis isolates by essential oils and extracts of plants.

| Sr | Strain Sample | *E. coli* | *S. aureus* | *P. mirabilis* | *K. pneumoniae* |
|---|---|---|---|---|---|
| | | Biofilm inhibition (%) Mean ± S. D | | | |
| 1 | MP water extract | 13.3± 0.3 | 7.9 ± 0.4 | 12.3 ± 0.3 | 36.8 ± 0.1 |
| 2 | MP acetone extract | 34.1 ± 1.5 | 14.4 ± 0.3 | 38.7 ± 0.3 | 33.1 ± 1.0 |
| 3 | MP ethanol extract | 12.7 ± 0.3 | 20.1 ± 0.1 | 36.2 ± 1.1 | 12 ± 1 |
| 4 | MP EO | 65.4 ± 0.3 | 53.9 ± 0.1 | 58.6 ± 1.2 | 42.2 ± 1.2 |
| 5 | SA water extract | 18.3 ± 2.0 | 30.9 ± 0.1 | 41.6 ± 1.0 | 12.3 ± 0.3 |
| 6 | SA acetone extract | 16.2 ± 1.9 | 16.4 ± 0.5 | 18.7 ± 0.2 | 7.4 ± 0.6 |
| 7 | SA ethanol extract | 8.1 ± 0.7 | 12.5 ± 0.8 | 8.6 ± 0.5 | 6.8 ± 0.2 |
| 8 | SA EO | 67.1 ± 1.2 | 55.2 ± 1.2 | 65.6 ± 0.9 | 35.8 ± 0.1 |
| 9 | LS water extract | 6.2 ± 0.4 | 25.3± 0.3 | 8.4 ± 0.3 | 17.4 ± 0.3 |
| 10 | LS acetone extract | 11.3 ± 0.4 | 19.3 ± 0.4 | 8.3 ± 0.4 | 31.1 ± 0.8 |
| 11 | LS ethanol extract | 4.2 ± 0.5 | 13.2 ± 0.6 | 3.1 ± 0.9 | 18.9 ± 0.2 |
| 12 | LS EO | 32.1 ± 2.8 | 61.3 ± 0.4 | 49.1 ± 0.6 | 34.6± 0.3 |
| 14 | Ciprofloxacin | 85± 0.4 | 89.5 ± 0.6 | 90.7 ± 0.4 | 90.2 ± 0.4 |
| 1 | MP water extract | 13.3± 0.3 | 7.9 ± 0.4 | 12.3 ± 0.3 | 36.8 ± 0.1 |

All samples were analyzed in a triplicate manner and results were expressed as mean ±SD

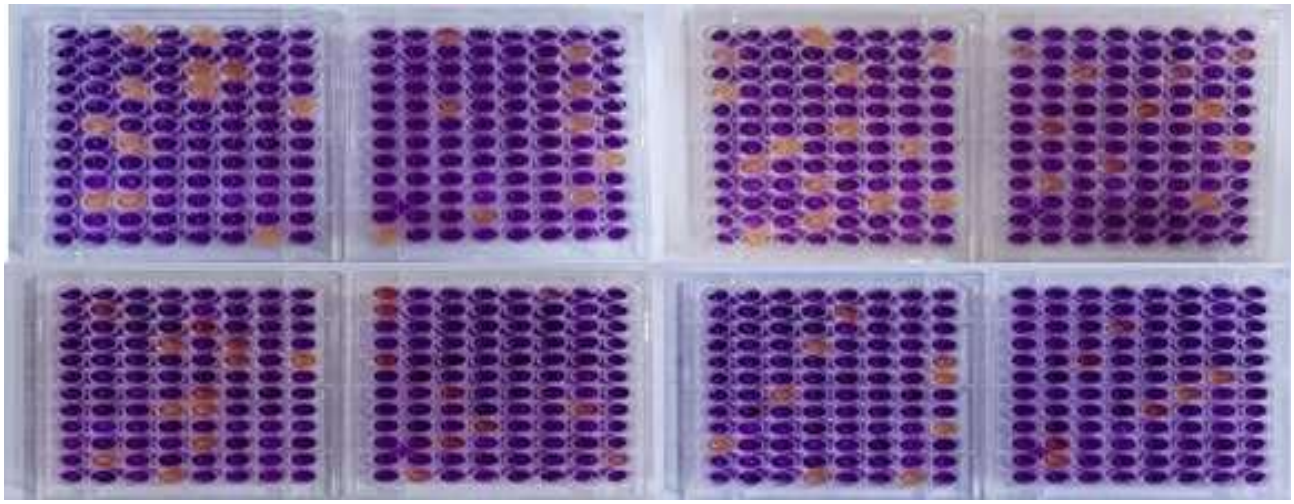

**Fig. 3**: Representative picture of Ames assay

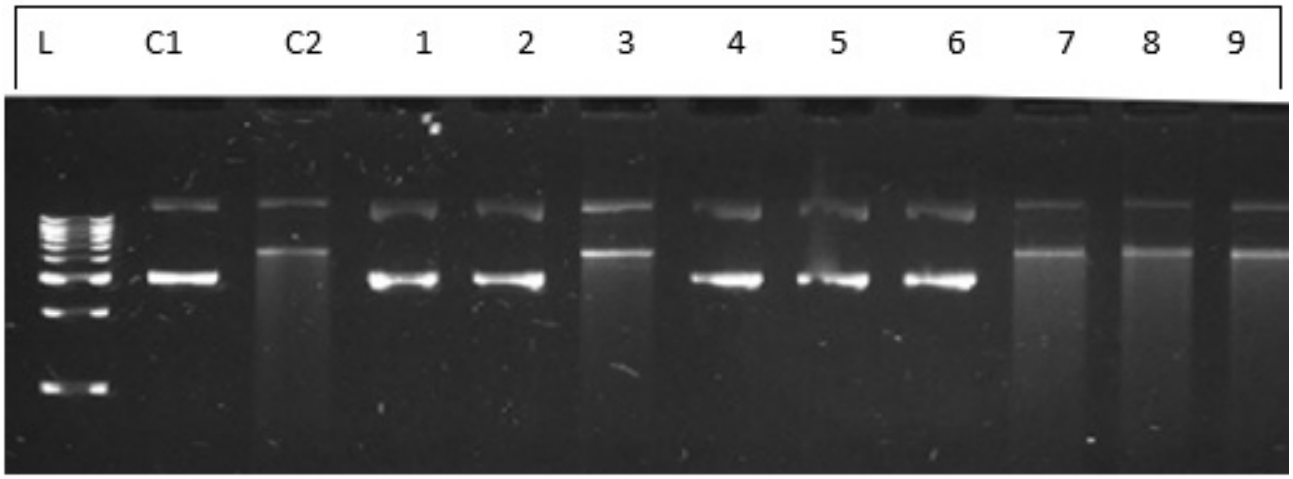


**Fig. 4**: Representative picture of the DNA damage protection assay of the samples

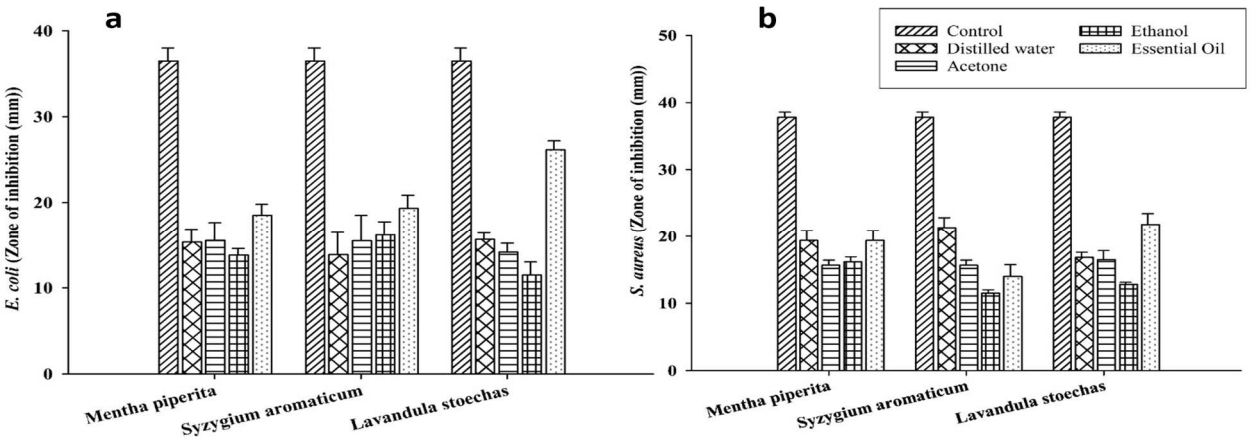


**Fig. 5**: Antibacterial activity of essential oils and extracts against sinusitis isolates.
(a) against *E. coli*, (b) against *S. aureus*

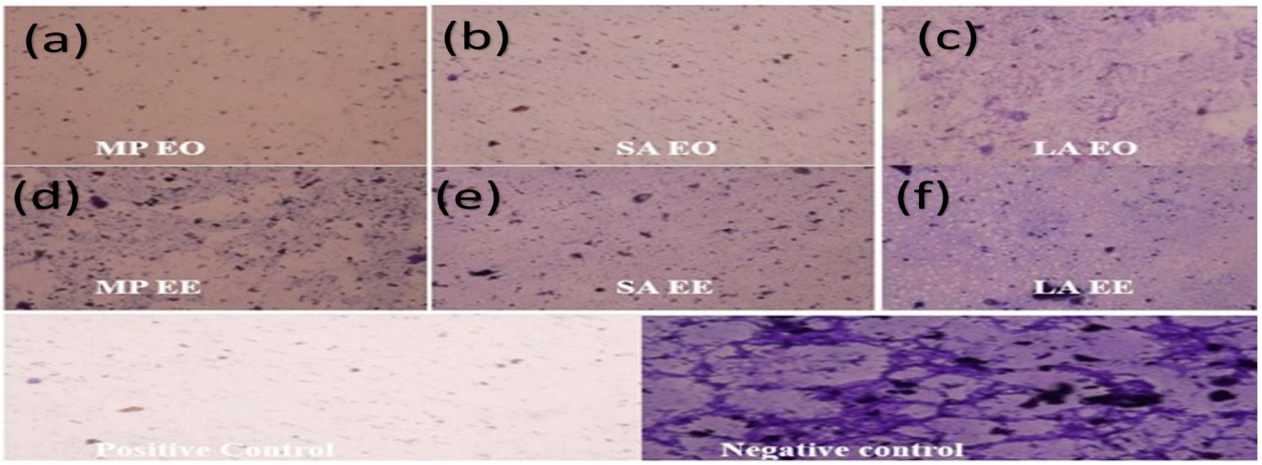


**Fig. 6**: Phase contrast micrograph of essential oils and ethanolic extracts against biofilm.
(a) *Mentha piperita* essential oil (MP EO), (b) *Syzygium aromaticum* essential oil (SA EO), (c) *Lavandula stoechas* essential oil (LA EO), (d) *Mentha piperita* ethanolic extract (MP EE), (e) *Syzygium aromaticum* ethanolic extract (SA EE), (f) *Lavandula stoechas* ethanolic extract (LA EE).

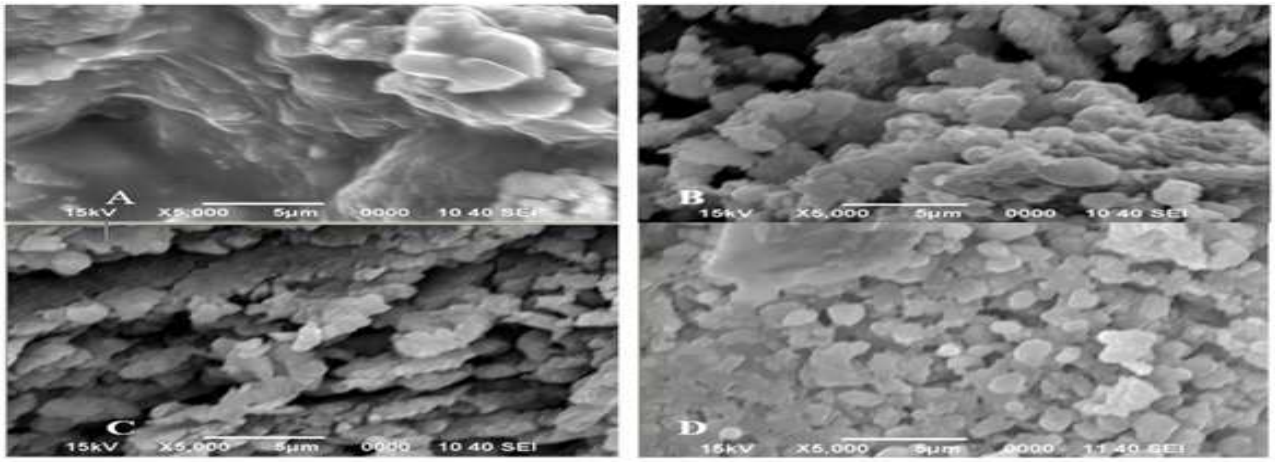


**Fig. 7**: Scanning electron microscopic pictures of biofilm.
(A) SEM image of native biofilm, (B) SEM image of biofilm treated by ciprofloxacin, (C) SEM image of biofilm treated by MP EE, (D) SEM image of biofilm treated by MP EO

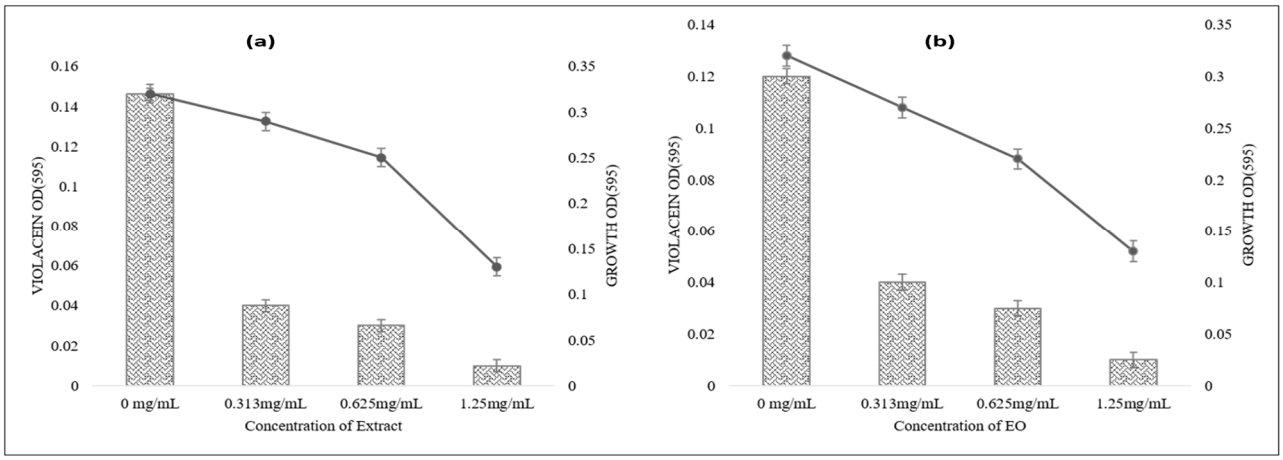


**Fig. 8**: Violacein formation by *C. pseudoviolaceum* when compared with bacterial growth in presence of (a) ethanolic extract and (b) essential oil.

## DISCUSSION

The chemical fingerprinting of the essential oils (EOs) via GC-MS provides crucial insights into their major constituents, which likely underpin their observed biological activities against multidrug-resistant bacteria and their biofilms. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of peppermint oil revealed that 2-Propanol, Cyclohexanol and Carvone are the major components present in it. Carvone is a well-known compound having antibacterial, anti-inflammatory and anti-cancer properties (Anjum and Raza, 2025). Eugenol was identified as the predominant compound in clove oil. Eugenol, a potent phenolic compound, is extensively documented for its strong antimicrobial, antioxidant and anti-inflammatory properties (Damasceno *et al*., 2024). Other identified components, such as Bicyclo, nonane, 2-methylene-4,8,8-trimethyl-4-vinyl and Naphthalene, 2,2-dimethyl-1-oxa-2-sila-1,2-dihydro-, may also play supportive roles or contribute to the oil's broad spectrum of activity. The *Lavandula stoechas* EO (lavender oil) analysis identified Propane, 1,2-dimethoxy-, Linalyl acetate, Isobornyl acetate, Glycerol 1,2-diacetate and alpha-terpinyl acetate as its most concentrated constituents. linalyl acetate is noted for its antimicrobial and anti-inflammatory effects (Wu *et al*., 2025). The presence of Linalyl acetate, alongside other terpenes and esters like Isobornyl acetate and alpha-terpinyl acetate, provides a plausible chemical basis for the high antibacterial activity observed for *Lavandula stoechas* EO.

The unknown samples were identified and quantified by matching the retention time of peak of HPLC chromatograph of samples with the standards. The compounds (08) which were identified in HPLC were gallic acid, benzoic acid, syringic acid, p-coumaric acid, m-coumaric acid, cinamin acid, ferulic acid and quercetin. The highest phenolic compound found in peppermint extract was benzoic acid, which was 129.53 ppm, and the lowest quantity was of Quercetin. (Yun *et al*., 2019) reported that essential oils in peppermint have shown good antioxidant activity. Studies confirm its significance in hypertension, inflammation, respiratory diseases and diabetes and improve digestion.

During the process of medicine development, safety of compound is a prime concern. Many compounds possess very good therapeutic potential but demonstrate toxic issues in case of cytotoxicity, which causes lysis of RBCs. Liberated hemoglobin produces problems for main organs like liver, heart and kidney (Rifkind *et al*., 2015). The DNA damage protection assay demonstrated that the samples exhibited no significant DNA damaging effects. DNA damage protection is due to antioxidant and free radical scavenging ability of extracts. Scavenging activity may be due to the presence of polyphenols in samples. This finding is critical as it indicates that these samples are non-genotoxic under the experimental conditions. This result is further supported by the negative outcomes of the Ames test, which also confirmed the non-mutagenic nature of the samples. (Fahmy *et al*., 2022) stated that essential oil of lavender reduced the mutant colonies of TA-98 strain.

Multidrug resistant microbes produce biofilm and quorum sensing for their survival. Samples in current study provided significant antibacterial activity. Our findings show essential oils, particularly *Lavandula stoecha*, exhibit significant antibacterial activity (21.83 ± 1.60 mm), directly contributing to biofilm inhibition. This disruption of the protective bacterial layer is critical for preventing chronic infections. Linalyl acetate disrupts the membrane due to linalool which is alcoholic in nature. These are lipophilic compounds and they can easily penetrate into bacterial membrane (Mączka *et al*., 2022). The anti-biofilm activity is linked to key chemical constituents like linalyl acetate in lavender oil (Ciocarlan *et al*., 2021) and eugenol in clove oil, both known antimicrobials. Menthol, which is primary component of peppermint oil, also targets the

bacterial cell membrane. Its antibacterial and anti-biofilm activity accounts for its ability to compromise the membrane's integrity. It can alter the membrane potential, inhibit enzyme activity within the cell and ultimately cause the leakage of intracellular materials. It inhibits the quorum sensing by interfering with the acyl homoserine lactones signaling mechanism (Hong *et al*., 2021). Eugenol's hydroxyl group is key to its action; it can bind with proteins in the bacterial cell, inhibiting essential enzymes (Ribeiro *et al*., 2024). Scanning electron microscopy provided qualitative evidence that essential oils disrupted the biofilm. Images of SEM showed there is a significant reduction of biofilm, and in bacterial cells, there are morphological changes. Samples disrupt the biofilm and are critical for preventing chronic infections. Beyond direct action, samples demonstrated inhibition of quorum sensing mechanism. Disruption of QS causes to disarm bacteria by preventing them to produce virulent factors (Lubis *et al*., 2024). It also reduces biofilm formation and antibacterial resistance production.

## CONCLUSION

In conclusion, all samples are safe and effective alternative choices for antibacterial treatment. These samples are potent multi-target drugs which have dual action, direct antibacterial activity and inhibition of quorum sensing action against superbugs.

***Acknowledgment***
The authors extend their appreciation to University of Agriculture, Faisalabad and Ear nose and throat (ENT) Department of Allied Hospital, Faisalabad, Pakistan, for supporting this work.

***Authors' contributions***
Conceptualization, Methodology, Software, Data curation, Writing- Original draft preparation, Visualization, Investigation: Muhammad Nauman Gulzar; critical revision and Supervision: Muhammad Shahid; Writing-Reviewing: Zahid Mushtaq, Muhammad Amir Aslam; Final Approval of the manuscript: All authors read and approved the final version of the manuscript.

***Funding***
There was no funding.

***Data availability statement***
The data that support the findings of this study are available from the corresponding author, Nauman Gulzar and Muhammad Shahid upon reasonable request.

***Ethical Approval***
Not applicable.

***Conflict of interest***
There is no conflict of interest among the authors, and the provided information is correct to the best of my knowledge, so all of the consequences based on the provided information will be the sole responsibility of the author.

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