Eugenol-Mediated Inhibition of Biofilm Formed by S. aureus: a Potent Organism for Pediatric Digestive System Diseases

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Abstract

*Ocimum tenuiflorum* (KT) is a common ethnobotanical plant of Southeast Asia. The ethnic communities of these regions use the various parts of the plants, especially the leaves, for the treatment of various ailments like cold and flu, chronic infections, and surface ailments. The leaves of these plants are consumed to act as immune boosters in the body. With this ethnical background, we performed the antimicrobial and antibiofilm potential of the methanolic extract of *Ocimum tenuiflorum* against biofilm formed by *S. aureus* biofilm. The biofilm formed by *S. aureus* is a potent cause for the development of gastrointestinal (GI)-associated chronic infection. The extract from the KT leaf was analyzed using UV spectroscopy and HPLC to confirm the presence of the active ingredients present within the extract. The HPLC and GC–MS studies revealed the presence of eugenol and linalool in a greater proportion having the maximum drug-like properties. It was observed that KT showed maximum inhibition of biofilms, proteins, and carbohydrates being present with the extracellular polymeric substance (EPS). Interestingly, the maximum inhibition to the quorum sensing (QS) and the genomic DNA, RNA content was reduced by eugenol and linalool in comparison to the plant extract. The studies were supported by in silico interaction between eugenol and linalool with the QS proteins of *S. aureus*. The studies were further confirmed with microscopic studies SEM and FCM. The IR studies also confirmed much reduction in biofilm when treated with eugenol, linalool, and KT with respect to the untreated sample.

Keywords Eugenol · *Ocimum tenuiflorum* · *Staphylococcus aureus* · Biofilm
Introduction

Microbial biofilms are a complex association of sessile microorganisms attached to biotic or abiotic substrata with the help of extracellular polymeric substances (EPS) [1] forming a matrix and develop as a defense mechanism during extremely stressed conditions such as insufficient nutrient supply, extreme temperature, salt concentrations, or pH variations. The reversible or irreversible binding to the solid surface with the help of EPS enables the microbes to spread their pathogenicity and adapts against an antagonistic environment. EPS comprises mostly water, extracellular DNA, RNA, proteins, lipids and is responsible for the efficient supply of nutrients like glucose, amino acids, and small molecules for the survival of biofilm-associated microbes. The genetic diversity and intercommunity interaction within the biofilm vary widely due to these stressed conditions forcing the indwelling microbes to undergo a cell number density-dependent sensing capabilities, also known as quorum sensing (QS), that helps in the survival of the microorganisms within the biofilm niche by modulating the biofilm formation via up/downregulating cascade of genes. QS or cell–cell communication is performed by small bacterial signaling molecules known as autoinducers (AI) that detect the neighboring population density and trigger specific bacterial genes controlling factors such as sporulation, antibiotic production, competence, virulence, motility, and biofilm formation [2]. Though the signal transduction mechanisms, the chemical nature of the signaling molecules, and the target genes associated with various bacterial QS circuits differ widely in bacteria, the ability of cell–cell communication, and coordinated gene expression is a common factor in the bacterial society [3].

In the context of the medical world, biofilms of single and multiple species are widely found on the surface of varied medical implants such as prosthetic heart valves, urinary catheters, and dental implants. [4]. EPS matrix provides a protective barrier to the penetrating antibiotics and bacterial drugs which can act only on the planktonic (free-floating cells) form of bacterial cells, whereas the sessile (immobilized) cells develop resistance toward antibiotics and keep on propagating inside the biofilm, [5] thus making the eradication of biofilm-forming bacteria more difficult and hence are of serious concern. Therefore, the conventional treatment strategies of medicines [6] required a novel approach with better pharmacological development. This involves the discovery of new antimicrobial agents [7], such as traditional, ethnic, and plant-derived natural products, having improved antimicrobial efficacy due to their secondary metabolites and possessing biofilm-disrupting properties. Several pieces of research have indicated toward plant bioactive compounds having potent antibiofilm activity against varied bacterial infections [8, 9]. Bioactive compounds from plants are relatively safe to use as they do not harm the host tissues while acting the biofilm cells and also provide extra nutritional constituents enhancing the physiological and cellular activities in host organisms consuming them [10].

*Staphylococcus aureus* is a pathogenic and nosocomial agent that is responsible for the development of gastrointestinal (GI)-associated chronic infections and needs to be controlled properly [11]. The present study is aimed at the use of the ethanolic extract of *Ocimum tenuiflorum* (KT) leaves and its bioactive compounds as a potent antibacterial and antibiofilm agent against *S. aureus*-mediated GI tract infections.
Materials and Methods

Microorganism and Biofilm Formation

Overnight culture of *Staphylococcus aureus* ATCC 0352 bacterial strain was cultivated in 50 ml of Luria–Bertani broth (pH 7, 37 °C). Biofilm formation by *S. aureus* was analyzed by using the microplate assay method [12] by incubating the plates at a temperature of 35 °C for a period of 72 h [13].

Collection of Plants

*Ocimum tenuiflorum* (KT) was collected from local areas and stored at room temperature.

Extraction of Plant Extract

The leaves of KT were pulverized in 95% ethanol with a mortar-pestle and kept for 24 h, following which, the extract was filtered and kept at 4 °C for further use [14].

HPLC Analysis of Bioactive Molecules

Crude KT extract was fed into the HPLC column for analytical HPLC analysis, followed by the identification of the highest peaks in the chromatogram. The compound showing the highest peak was isolated through preparative HPLC analysis and tested for antibacterial activities.

GC–MS Analysis

The chromatographic analysis of the ethanolic extract of the KT leaves was done using GC model Trace GC Ultra, MS model PolarisQ (Thermo Fisher Scientific). The data obtained were searched using the NIST (National Institute Standard and Technology) Library and compared by using Wiley Spectral Libraries search program [15].

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Ethanolic extracts of KT leaves were centrifuged at 10,000 rpm for 10 min, and the supernatant was used as an antibiofilm agent. The concentration of the crude solution of tetracycline (500 mg dissolved in 10 ml of deionized water) was adjusted by the serial dilution method for control experiments. The MIC was determined [16] by the disk diffusion technique and MBC was determined by the broth dilution method by dissolving 2,3,5-triphenyl tetrazolium salt (TTC).

Minimum Biofilm Eradication Concentration Analysis (MBEC)

Liquid cultures (100 µl of LB broth) of *S. aureus* were grown in 96-well polystyrene flat-bottom tissue culture microplates for a period of 72 h, followed by discarding the broth and
washing the cells with 0.1% normal saline (w/v). This was followed by the addition of the ethanolic extract of KT and tetracyclin, keeping one well blank as control. The concentration of viable bacterial cells was measured based on the ability of the living cells to convert yellow tetrazolium salt to purple formazan product. The percentage inhibition was calculated using the equation [17]:

\[
1 - \frac{A_{570 \text{ of the test}}}{A_{570 \text{ of non-treated control}}} \times 100
\]

**Spectroscopic Measurements of N-Acyl-Homoserine Lactone Concentration (AHL) and Quorum Sensing Assay**

A total of 5 ml of Luria–Bertani broth (HiMedia) were inoculated with *Staphylococcus aureus* ATCC 0352 overnight at 37 °C for 24 h. From this cultured broth, 2 ml of bacterial culture was transferred for centrifugation at 10,000 rpm for 15 min. The supernatant was collected and filtered using a membrane filter of diameter 0.2 μm to remove any traces of cell debris. The filtrate was treated with ethyl acetate with the mild shaking condition and was subsequently allowed to stand for 5 min. This resulted in a separation of the upper and lower immiscible layers. This method of liquid–liquid extraction (LLE) helps the upper layer to be separated off and dried in the oven at 40 °C. A total of 40 μl of LLE sample [18] was inoculated into wells of 96-well polystyrene flat-bottom tissue culture microplates which were previously supplemented with 50 μl of a 1:1 mixture of hydroxylamine (2 M):NaOH (3.5 M). This was followed by the addition of an equal amount of 1:1 mixture of ferric chloride (10% in 4 M HCl):95% ethanol. A dark brown color was shown from all of the samples containing lactone compounds. Optical density (OD) was measured at 520 nm. Precaution must be taken in maintaining a steady acidic pH for monitoring AHL. This is due to the inactivation of AHL under alkaline conditions by pH-dependent lactonolysis. During the lactonolysis, the homoserine lactone ring is hydrolyzed to open a ring form corresponding to N-acylhomoserine.

**Isolation and Purification of Extracellular Polymeric Substance (EPS)**

EPS matrix comprises proteins, polysaccharides, nucleic acids, multivalent cations, lipids, and inorganic particles [19] and forms about 50–90% of the total biofilm mass [20].

**Carbohydrate Estimation**

Treatment with plant extracts results in the disruption of EPS matrix elements. The bactericidal effects of KT extracts were tested on the amount of carbohydrate present in bacteria after treating with KT extracts and control. A total of 100 μl of EPS sample pretreated with KT extract was diluted with 900 μl of distilled water in a test tube. This was followed by 5 ml of 98% concentrated H₂SO₄ and 1 ml of phenol. The reaction mixture was kept undisturbed until the colorless solution turned yellow in color. The blank was set with H₂SO₄ and phenol. The OD was measured at 490 nm [21].
Protein Estimation

A total of 100 µl of EPS sample pretreated with KT extract was diluted with 900 µl of distilled water in a test tube. This was followed by adding 5 ml of 0.1 mM copper sulfate resulting in a blue solution. The test tubes were incubated for 10 min in a water bath at 60 °C followed by the addition of 500 µl Folin’s reagent and incubated for 1 h at 37 °C. This results in the transformation of the turbid sky blue color into a transparent dark blue–colored solution. The OD was measured at 700 nm. The blank was set with copper sulfate Folin solution without the sample.

Nucleic Acid Estimation

A total of 200 µl of EPS sample pretreated with KT extract was put in the test tubes followed by the addition of 600 µl of 99.9% w/v ethanol and incubated for 2 h at 37 °C. The OD was measured at 260 nm. The blank was set with ethanol [22].

Determination of the Viability Count of the Biofilm-Forming Cells

The viability of bacterial cells is best measured by monitoring the bacterial growth kinetics in the presence and absence of antimicrobials. For monitoring the viability of biofilm-forming S. aureus cells, liquid cultures of S. aureus in LB broth having 0.1% chitin flakes (w/v) were incubated for a period of 72 h. This was followed by discarding the media and washing it with 0.1% (w/v) normal saline so as to eliminate the planktonic groups of cells. The sessile cells within the biofilm were treated with KT extracts and the growth was determined by a UV–visible spectrophotometer at 590 nm at intervals of time.

Determination of Biofilm Formation by Scanning Electron Microscopy (SEM)

S. aureus biofilms were grown on chitin flakes submerged in LB broth for a period of 72 h in test tubes. This was followed by washing with 0.9% NaCl to remove planktonic cells and subsequently challenged with the KT extract for 2 h. Single chitin was recovered from the test tube and kept in 2.5% glutaraldehyde for 20 min followed by dehydration with ethanol overnight. The dried biofilms were coated with gold and visualized under a scanning electron microscope (model Zeiss EVO MA 10) [23].

Protein Structure Preparation and Molecular Docking

X-ray crystallographic structures of S. aureus quorum sensing protein (PDB ID- 3TIP) having a good resolution of about 1.5–2 Å was obtained by the Protein Data Bank (PDB). Any co-crystallized ligand attached to the above protein was removed from the binding site, and this modified protein structure was further used for molecular docking interaction studies with AutoDock 4.2 software. Bioactive compounds from Ocimum tenuiflorum were found from the PubChem database after it has been detected in GC–MS chromatogram profiles (Fig. 1). Ligands were obtained in SDF format and then converted into PDB format using Open Babel for its easy recognition in the AutoDock software. AutoDockTools, Gasteiger charges, and solvation terms were assigned to the modified protein to compute its bulk and
surface properties. The genetic algorithm (GA) method was used to compute the cluster of probable conformations of the ligand at the protein binding site. Standard docking settings were applied and the energetically most favorable binding poses (lowest docked energy) are taken to obtain the best conformation [24, 25].

CABS-Flex Analysis

It is an efficient modeling procedure for fast simulations of protein structure flexibility. It is based on a well-established coarse-grained protein modeling tool that generates protein dynamics at highly reduced (3 orders of magnitude) computational cost, although with some decrease in resolution. A consensus view of protein near-native dynamics is obtained from 10-ns MD simulations (all-atom, explicit water, for all protein metafolds using the four most popular force fields). The docked output file is loaded in the CABS-flex 2.0 online server (http://biocomp.chem.uw.edu.pl/CABSflex2) that helps predict the interaction of bioactive compounds of KT with the biofilm-forming proteins of *S. aureus* [26].

LIGPLOT Analysis

The docked protein–ligand complex is used for the identification of the nearest amino acid residues that closely interact with the ligand. 2-D representations of protein–ligand complexes from standard PDB file input are uploaded in LIGPLOT, and it generates images closely associated with residues of the bacterial QS protein [27].

Statistical Analysis

All the experimental outcomes obtained were performed in triplicate analysis and depicted as mean ± SE (standard deviation).

Chemicals

All chemicals used were obtained from Merck, Germany, and HiMedia, India. The chemicals were of analytical grade and used without any further purification.
Results and Discussions

GC–MS Analysis of the Ethanolic Extract of Ocimum tenuiflorum (KT)

A gas chromatographic analysis of the ethanolic extracts of KT was performed, and the mass chromatogram of the unknown bioactive compounds was identified by comparing with the chromatogram of known compounds already stored in the NIST library. The compound name, molecular weight, molecular formula, and the peak area of the test sample were determined. Various bioactive compounds of KT identified from the GC–MS chromatogram are listed in Table 1. The major components found were gallic acid, p-coumaric acid, cinnamic acid, catechol, caffeeic acid, 3,4-dimethoxycinnamic acid, luteolin, diosmetin, kaempferol, apigenin, rosmarinic acid, genistein, eucalyptol, camphor, and eugenol [28]. The presence of various compounds eluted at different retention times was detected once the large compound fragments into small compounds resulting in characteristic peaks at different m/z ratios which can be further compared with the standard NIST library. Among these detected bioactive compounds, few possess the potential to act as future drug candidate molecules which can be further tested for their biological activities in vitro and in vivo.

Quorum Sensing Assay

Quorum sensing (QS) is a cell population density-dependent signaling mechanism developed by microorganisms during biofilm formation and maturation. Bacterial cells striving within the EPS matrix of biofilm can efficiently sense the neighboring cells by a series of small signaling molecules such as acyl-homoserine lactones (AHL), small peptides, and (AI) regulated by complex genetic machinery. The formation of biofilm is directly proportional to the AHL secretion by the microorganisms. Thus, quantification of AHL serves an essential purpose for the detection of the QS mechanism. Figure 2 shows that the QS is drastically reduced in comparison to control (S. aureus) at OD at 520 nm which is 0.823 ± 0.073 to 0.382 ± 0.031 when treated with the KT extract and 0.513 ± 0.026 when treated with tetracycline. We detected a significant decrease in QS activity in the presence of ethanolic extracts of KT as compared to tetracycline treated and control sample indicating the anti-quorum sensing and antibiofilm efficacy of KT [29].

Effect of Ethanolic Extract of Ocimum tenuiflorum upon Various Components of EPS

A biofilm comprises sessile microbial colonies embedded in a slimy layer of exopolymeric substances known as EPS which consists majorly of water, carbohydrate, proteins, lipids, and nucleic acids. The presence and absence of the challenge of the ethanolic extract of KT were estimated upon various EPS components. It was observed that components of EPS viz. protein, carbohydrates, and nucleic acids were largely reduced by the challenge of the ethanolic extract of KT. The carbohydrate content within the EPS of S. aureus biofilm was affected maximum by the ethanolic extract of KT for a time challenge of 2 h showing 56.2% with respect to control (0.168 ± 0.023 to 0.072 ± 0.011 mM). The protein and nucleic acid contents of S. aureus EPS matrix were reduced by the ethanolic extract of KT for a time challenge of 2 h showing 21.8% and 44.3% reduction with respect to control (0.138 ± 0.013 to 0.108 ± 0.011 mM) and (0.183 ± 0.016 to 0.102 ± 0.041 mM), respectively (Fig. 3). The reduction in protein, carbohydrate, or nucleic acid content indicates the
| R/T (min) | Name of the Compound | Percentage Abundance | Molecular Formula | MW (g/mol) | 3D Structure |
|----------|-----------------------|----------------------|-------------------|------------|--------------|
| 4.4      | Eugenol               | 0.31                 | C10H12O2          | 164.2      | ![Eugenol](image) |
| 4.69     | Thymol                | 13.67                | C10H14O           | 150.22     | ![Thymol](image) |
| 5.06     | Caffeic Acid          | 12.83                | C9H8O4            | 180.16     | ![Caffeic Acid](image) |
| 7        | Gallic Acid           | 0.61                 | C6H2(OH)3COOH     | 170.12     | ![Gallic Acid](image) |
| 7.5      | Cinnamic Acid         | 6.05                 | C9H8O2            | 148.16     | ![Cinnamic Acid](image) |
| 7.78     | P-Coumaric Acid       | 7.7                  | C9H8O3            | 164.16     | ![P-Coumaric Acid](image) |
| 8.94     | Rosmarinic Acid       | 2.07                 | C18H16O8          | 360.3      | ![Rosmarinic Acid](image) |
| 9.33     | Ursolic Acid          | 7.4                  | C30H48O3          | 456.7      | ![Ursolic Acid](image) |
| 9.78     | Oleanolic Acid        | 6.09                 | C30H48O3          | 456.7      | ![Oleanolic Acid](image) |
| 10.43    | Linalool              | 20.48                | C10H18O           | 154.25     | ![Linalool](image) |
| Compound       | Amount | Molecular Formula | Molecular Weight |
|----------------|--------|-------------------|------------------|
| β-Caryophyllene| 0.17   | C10H14O           | 150.22           |
| Carvacrol      | 1.49   | C10H14O2          | 110.11           |
| Catechol       | 11.22  | C6H6O2            |                  |
| Luteolin       | 0.68   | C15H10O6          | 286.24           |
| Diosmetin      | 0.05   | C16H12O6          | 300.26           |
| Kaempferol     | 0.43   | C15H10O6          | 286.24           |
| Apigenin       | 0.48   | C15H10O5          | 270.24           |
| Rosmarinic Acid| 0.34   | C18H16O8          | 360.3            |
| Genistein      | 1.39   | C15H10O5          | 270.24           |
| Eucalyptol     | 0.07   | C10H18O           | 154.25           |
The disintegration of the EPS matrix in comparison to the stable and intact EPS matrix of the control sets [30]. All the data were mean ± SE. The data were statistically significant.

**Minimum Biofilm Eradication Concentration (MBEC) Determination Assay**

MBEC assay was performed to calculate the potency of KT in the successful eradication of the *S. aureus* biofilm. It provides information regarding the minimum dosage required for the clearance of the sessile bacterial colony formed on various biotic/abiotic surfaces.
From Fig. 4, it can be concluded that *S. aureus* was eradicated by 73.81 ± 8.97% in the challenge of *Ocimum tenuiflorum* (KT) at an inhibitory concentration of and 51.45 ± 5.13% in the presence of the antibiotic tetracycline.

**Viability Count of the Biofilm Cells in the Presence or Absence of the KT**

The viability count of the *S. aureus* cells was found to decrease growth (log CFU/ml) for a time challenge of 0 to 48 h with a maximum effect by *Ocimum tenuiflorum* (KT) from 6.18 ± 0.014 to 6.02 ± 0.02 in comparison to control (6.21 ± 0.03 to 6.61 ± 0.02) and tetracycline (6.19 ± 0.005 to 6.056 ± 0.018). It was also observed that the removal of the challenge of KT extract led to a negligible revival from log CFU/ml 6.22 ± 0.015 to 6.029 ± 0.024 in comparison to the control that showed a continuous growth from log CFU/ml 6.18 ± 0.03 to 6.58 ± 0.013 and log CFU/ml 6.198 ± 0.005 to 6.086 ± 0.018 on removing
the challenge of standard antibiotic (Fig. 5a and 6b). The revival studies were performed after allowing the cultures to grow for 24 h after removing the challenge of 0 to 48 h. The data were statistically significant.

**Microscopic Studies of Biofilm**

The effect of the time challenge of KT extract upon the *S. aureus* biofilm topology was observed by scanning electron microscopy. The control sample (Fig. 6b) comprises well-developed and mature biofilm cells adhered to the chitin surface, whereas a dispersed and partially distorted biofilm assembly was observed on the KT extract-treated samples (Fig. 6c). This was further supported by fewer bacterial colonies in the biofilm treated with KT extract. The SEM micrograph image and QS assay suggest that the ethanolic extract of *Ocimum tenuiflorum* directly affects the biofilm development and maturation by influencing the flagellum-driven motility which is necessary for attachment to the substratum via swimming and swarming motion [31].

**Docking Between Biofilm-Forming Protein (3TIP) of *S. aureus* with Potent Bioactive Compounds from *Ocimum tenuiflorum* (KT)**

The bioactive molecules identified from the GC–MS spectrograph of the ethanolic extract of KT were used for understanding the mechanism of binding with the biofilm-forming protein of *S. aureus* (3TIP) with the help of molecular docking. In silico docking analysis helps predict the binding sites and major interacting amino acid residues with the phytocompounds along with the binding interaction energies [32]. This analysis helps predict the best docked poses based on minimum binding energies along with the tentative interaction viz. H bonds, electrostatic interactions, or pi-pi interactions taking place between the amino acid residues and the phytocompounds, enabling the prediction of few putative lead molecules that can be looked for unsolid future drug compounds. Binding energy is released when a drug molecule is associated with a target, which causes the lowering of the overall energy of the complex. This release of binding energy compensates for any transformation of the ligand from its energy minimum to its bound conformation with the protein. Therefore, the greater the energy released (i.e., the greater the negative value), on the binding of a ligand to the protein, the greater is the propensity of the ligand to associate with that protein. From the docking results obtained, it is observed that rosmarinic acid, ursolic acid, and oleanolic acid show the highest binding energy (ΔG°) with the *Staphylococcus aureus* as compared to other bioactive
compounds present (Table 2). The docked results were further analyzed with the LIGPLOT software for identifying the key residues in the protein and the CABs-flex server for performing the 20-ns MD simulation.

From the LIGPLOT analysis, various amino acids that are interacting with the biofilm-forming bacterial proteins (3TIP) are observed [33]. These amino acids interact with the proteins via different types of bonds like hydrophobic bonds, hydrogen bonds, pi-pi stacking bonds, and salt bridges. Also, from the online server called PLIP (Protein–Ligand Interaction Profiler), the distances of the bioactive compounds from their binding pockets are obtained. These distances determine the type of bond each amino acid residue has with the bioactive compounds (ligands). Upon comparison of the results obtained from both the LIGPLOT software and PLIP server, it can be concluded that results obtained from them are almost identical and that both the software and server have given accurate results. From the results obtained, it can be seen that rosmarinic acid and linalool have the highest number of bonds, meaning that they have the highest amount of stability while interacting with the biofilm-forming proteins of *Staphylococcus aureus* (Table 3).

From the CABS flex analysis (Table 4), it is observed that almost all the bioactive compounds show significant variations in their interaction with the biofilm-forming proteins of *Staphylococcus aureus* (3TIP). From these variations, the interaction of the biofilm-forming proteins with and without their ligands (bioactive compounds) is observed. Most significant variations are observed in *Staphylococcus aureus* (3TIP).

| Table 2 Docking studies between the phytocompounds of KT and biofilm protein of *S. aureus* (3TIP) |
|---------------------------------|---------------------------------|-----------------|-----------------|-----------------|
| BIOACTIVE COMPOUND | PHYTOCHEMICALS | BIOFILM FORMING PROTEIN | AUTODOCK RESULTS | ENERGY VALUE (ΔG°) Kcal/mol |
| ESSENTIAL OILS | EUGENOL | 3TIP (Staphylococcus aureus) | | |
| | THYMOL | 3TIP (Staphylococcus aureus) | | |
| TERPENOIDS | OLEANOLIC ACID | 3TIP (Staphylococcus aureus) | | |
| | URSOLIC ACID | 3TIP (Staphylococcus aureus) | | |
| | LINALOOL | 3TIP (Staphylococcus aureus) | | |
| | β-CARYOPHYLLENE | 3TIP (Staphylococcus aureus) | | |
| | CARVACROL | 3TIP (Staphylococcus aureus) | | |
| OTHER PHENOLIC COMPOUNDS | ROSMARINIC ACID | 3TIP (Staphylococcus aureus) | | |

From the CABS flex analysis (Table 4), it is observed that almost all the bioactive compounds show significant variations in their interaction with the biofilm-forming proteins of *Staphylococcus aureus* (3TIP). From these variations, the interaction of the biofilm-forming proteins with and without their ligands (bioactive compounds) is observed. Most significant variations are observed in *Staphylococcus aureus* (3TIP).
Table 3 LIGPLOT analysis depicting the major interacting amino acid residues

| SL. NO. | BIOACTIVE COMPOUND   | PHYTOCHEMICAL | BIOFILM FORMING PROTEIN | LIGPLOT | HYDROPHOBIC BOND | HYDROGEN BOND |
|---------|----------------------|--------------|-------------------------|---------|------------------|---------------|
| 1.      | ESSENTIAL OILS       | EUGENOL      | 3TIP (Staphylococcus aureus) | ![Image](1) | LYS513 (3.95) PRO549 (3.97) | THR516 (2.54) THR516 (3.01) |
|         | THYMOL               |              | 3TIP (Staphylococcus aureus) | ![Image](2) | PRO594 (3.41) LEU596 (3.44) | LEU596 (2.74) SER606 (2.90) |
| 2.      | TERPENOIDS           | OLEANOLIC ACID | 3TIP (Staphylococcus aureus) | ![Image](3) | ARG538 (3.23) LYS525 (2.88) | - |
|         | URSOLIC ACID         |              | 3TIP (Staphylococcus aureus) | ![Image](4) | ARG538 (3.03) VAL541 (3.37) | - |
|         | LINALOOL             |              | 3TIP (Staphylococcus aureus) | ![Image](5) | PRO562 (3.40) GLU564 (2.84) | GLU564 (2.84) LYS565 (3.23) |
|         | Β-CARYOPHYLLENE      |              | 3TIP (Staphylococcus aureus) | ![Image](6) | PRO549 (3.17) LEU596 (3.50) | - |
|         | CARVACROL            |              | 3TIP (Staphylococcus aureus) | ![Image](7) | LYS551 (3.87) PRO594 (3.62) | GLY552 (2.72) GLY (2.03) THR (2.46) |
| 3.      | OTHER PHENOLIC COMPOUNDS | ROSMARINIC ACID | 3TIP (Staphylococcus aureus) | ![Image](8) | ARG538 (3.75) PRO539 (3.34) | VAL541 (3.97) |
Table 4  CABS-flex analysis showing the major alterations in the interacting residues in the presence of phytocompounds

| SL. NO. | BIOACTIVE COMPOUNDS | PHYTOCHEMICAL | BIOFILM FORMING PROTEIN | ROOT MEAN SQUARE FUNCTION |
|---------|----------------------|---------------|-------------------------|---------------------------|
| 1.      | ESSENTIAL OILS       | EUGENOL       | 3TIP (Staphylococcus aureus) | [Graph with EUGENOL and WITHOUT EUGENOL] |
|         |                      |               |                         |                           |
| 2.      | TERPENOIDS           | OLEANOLIC ACID| 3TIP (Staphylococcus aureus) | [Graph with OLEANOLIC ACID and WITHOUT OLEANOLIC ACID] |
|         |                      |               |                         |                           |
|         |                      | URSOLIC ACID  | 3TIP (Staphylococcus aureus) | [Graph with URSOLIC ACID and WITHOUT URSOLIC ACID] |

Conclusion

Biofilms are formed by sessile microorganisms adhered to biotic/abiotic surfaces and are well protected within a self-secreted exopolymeric matrix consisting of proteins, carbohydrates, lipids, nucleic acids, small signaling molecules, etc. Biofilm formation is a defense strategy developed by the microbial cells under stress situations such as adverse environments, host immune responses, or the presence of antimicrobials that
lead to enhanced immune responses of the microbial cells persisting within the biofilm matrix. This matrix is also responsible for improved resistance toward the antimicrobial agents leading to the development of the phenomenon known as antimicrobial resistance (AMR). This has led to a surge in the development of resistance toward a wide range of antibiotics. Thus, alternative treatment strategies are an urgent requirement for fighting the ever-increasing problems of AMR. One plausible mechanism involves biofilm reduction with phytochemicals of herbal plants of ethnobotanical origin. In the present work, we have investigated the antibiofilm and antimicrobial effects of ethanolic extract of the whole plant of *Ocimum tenuiflorum* (KT), which is known to contain a vivid range of bioactive compounds such as essential oils, flavonoids, and terpenes against *Staphylococcus aureus* ATCC 0352 biofilm responsible for the development of gastrointestinal (GI)-associated chronic infections in comparison to the common antibiotic tetracycline. KT consists of bioactive compounds such as eugenol, apigenin, ursolic acid, carvacrol, linalool, caryophyllene, and carvicol, which brings a subsequent reduction
in the sessile cell population density as depicted by the slow rate of bacterial revival after withdrawal of antimicrobial stress of plant extracts. KT extracts are also successful in diminishing the bacterial QS mechanism by affecting the secretion of signaling molecules like AHLs. The molecular docking of S. aureus biofilm protein with bioactive compounds showed a good binding propensity. However, future studies relating to biofilm eradication ability, mechanism of action, and in vivo potencies in controlling bacterial propagation are required to elucidate the potential usefulness of these herbal compounds as drug molecules.

**Author Contribution** All authors contributed equally.

**Data Availability** Not applicable.

**Declarations**

**Ethics Approval** Not applicable.

**Consent to Participate** All authors have their consent to participate.

**Consent for Publication** All authors have their consent to publish their work.

**Competing Interests** The authors declare no competing interests.

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