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ANTIMICROBIAL AND ANTI-BIOFILM POTENTIAL OF *Physalis philadelphica* LEAF EXTRACT AGAINST *Staphlococcocu aureus*

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ABSTRACT

Plants and their components play a important role in human and animal health, as well as in microbial control. With the rise of drug-resistant organisms, there is an urgent need to find new, effective antimicrobial drugs. Medicinal plants have become a primary source for natural treatments of bacterial diseases. These plants provide a variety of drugs, with plant extracts being substances that carry active properties. *'Physalis philadelphica'* is used as a medicinal plants in Pakistan. This study aimed to investigate the antimicrobial activity of '' *Physalis philadelphica* '' leaf extracts against our pathogenic bacteria i-e *Staphylococcus aureus* and to determine biofilm control potential of plant extract against *Staphylococcus aureus* . Growth assay was carried out for quantification of biofilm in microtiter plates. At a lower concentration (50 mg/ml) plant show 15.71%,at 600 mg/ml show 96.14% reduction in Growth. Well Diffusion assay was carried which indicate that at 600 mg/ml concentration the zone of inhibition was about 1.9 mm and in positive cintrl we get 3.00 mm zone ,For Disc diffusion assay we obseved 2.99 mm positive contriol ,zero in negative control ans at 50 mg/ml we get 0.6 mm and at 600 mg/ml having 1.2 mm zone of inhibition.Crystal vio/let asssay we observed that in control group zero% in growth reduction and at 50 and 600 mg/ml we get 1% and 68.38 %growth reduction. In Petri dish biofilm microscopic examination of biofilm revealed a progressive decreased in biofilm density with incresaing concentration of extract Protein and Polysaccharide Production Estimation ,for protein we observed at 50 mg/ml 3.97 % and at 600 mg/ml 81.95 % reduction in Growth ,for that at 50 mg/ml and 600 mg/ml we get 2.05 % and 55.94 % reduction in growth was observed .These findings highlight the potential of *Physalis philadelphica* as effective agents against *S. aureus* infections, especially those involving biofilms. *Physalis philadelphica* used increased their efficacy, emphasizing their potential as antimicrobial and anti-biofilm agents against *Staphylococcus aureus.*

INTRODUCTION

The antimicrobial activity of plant extract are of great interest due to increase in demand for more natural food and medicinal products, and also many microbes have been able to build up a resistance to current antibiotics (Ali Syed et al., 2024; Ashley, 2000; Rehman et al., 2023). Finding antimicrobial compounds is becoming increasingly important during the antibiotic resistance (AR) period. Due to the rise in demand for herbal remedies as an alternative to conventional therapies, there has been a tendency in recent decades to look for these components in natural sources, such as plants (Laraib et al., 2023; Perez Zamora, Torres, & Nuñez, 2018; Rehman et al., 2023). A plant extract is a substance with active, beneficial properties that is extracted from plant tissues, usually using a solvent, for a specific purpose. Plant extracts are used in various fields, such as food (for antioxidants and texturizers), processing aids, chemical replacements, pharmaceuticals (as therapeutic agents), and cosmetics (for beauty and wellness). It's important to note that an extract begins with raw plant material, which is then processed for different uses (Muhammad, Ahmad, Basit, Khan, et al., 2024; Muhammad, Ahmad, Basit, Mohamed, et al., 2024; Rios & Recio, 2005).

According to World Health Organization (WHO) medicinal plants are the source to obtain a variety of drugs. About 80% in developing countries use traditional medicine, derived from medicinal plants (Basit et al., 2024; Selvamohan, Ramadas, & Rejibeula, 2012). It has been found that plant extract with known antimicrobial properties can be of great significance in therapeutic treatment. In the last few years, a number of studies have been conducted in different countries to prove such efficiency ((Junaid Ahmad & Ahmad, 2023; Khan et al., 2023; Nascimento, Locatelli, Freitas, & Silva, 2000). *Staphylococcus aureus*, a Gram-positive bacterium, causes a different of infections ((Prayoga, Wahyono, Fatimah, Purbandini, & Wibowo, 2024). Few strains are resistant to commonly used antibiotics, including those previously effective against S. aureus infections. Due to its biofilm-forming nature, the bacterium shows high resistance to different antibiotics ,necessitating the exploration of new approaches to address infections (Javed et al., 2023; Periasamy et al., 2012)

The formation of biofilms has significant challenges in various fields, including medicine, agriculture, industry, microbiology, and health. Traditional antimicrobial agents often face limitations such as resistance development and adverse effects on host tissues and cells.in the pursuit of novel and effective solutions, natural compounds have gained attention for their diverse bioactive beneficial properties. (Munir et al., 2023; Shah et al., 2023; Zhao et al., 2020). Hospital acquired infections is the vital issue owing to the various microbes (Raoofi et al., 2023). Various bacteria are involved in these infections. e.g. *Klebsiella pneumonia*, *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Proteus mirabilis and Streptococcus species*. Most of the biofilm related infections are due to the catheters and implanted devices (Junaid Ahmad, Amin, Mustafa, Subhan, & Qaiser; Aziz et al., 2022; Bryers & Ratner, 2004)

Staphylococcus aureus, a Gram-positive bacterium, causes a different of infections few strains are resistant to commonly used antibiotics, including those previously effective against *S. aureus* infections(Munawar et al., 2021; URREHMAN, NAILA, & JUNAID AHMAD). Due to its biofilm-forming nature, the bacterium shows high resistance to different antibiotics, necessitating the exploration of new t approaches to address infections. Bacteria are involved in these infections(Abdullah, 2022; Aziz et al., 2022). *Staphylococcus aureus*, a Gram-positive

bacterium, causes a different of infections including skin infections, pneumonia, bloodstream infections, and food poisoning, often leading to serious complications if untreated. Few strains are resistant to commonly used antibiotics, including those previously effective against *S. aureus* infections. Due to its biofilm forming nature, the bacterium shows high resistance to different antibiotics, necessitating the exploration of new t approaches to address infections (Periasamy et al., 2012) (J Ahmad & Pervez, 2021; Hayat et al., 2022).

Explaining the exact mechanisms of biofilm resistance to antibiotics sets challenges, but bacterial cells within biofilms show greater resistance compared to planktonic cells (J Ahmad & Pervez, 2021; Tuon et al., 2023). Various studies exhibit that biofilm resistance to antibiotics operates via diverse mechanisms and involves various elements, setting it apart from free-floating bacteria. The resistance in biofilms can be categorized into four stages: deactivation of functional molecules, altering the site of action, reducing the approach and absorption of medicine to the action site, and employing efflux pump systems. This resistance is reflected inherently and genetically driven, involving processes such as restricted interference, enzyme-based deactivation, diverse and altered activity, prolonged growth and development, presence of non-dividing bacterial cells. Also it changes in the biofilm cover layer (membrane). (Hogan & Kolter, 2002; Poole, 2002; Robina et al., 2021).

Various biological approaches exist for controlling biofilm, including the prevention of attachment through bacterial cell pili, which promote the binding of bacteria to cells and tissues (Dodson et al., 2001; Roberts et al., 1994). Employ synthetic chemical pilicides to halt the development and synthesis of pili, thereby disrupting the formation of biofilms (Åberg et al., 2007; Pinkner et al., 2006). Utilizing enzymes to break down the external bimolecular membrane of biofilms leads to the release of bacterial cells, making them more susceptible to targeting and elimination by immune cells and medications (Xavier, Picioreanu, Rani, van Loosdrecht, & Stewart, 2005). It proved that bacteriophage can control the biofilms (Vinodkumar, Kalsurmath, & Neelagund, 2008). The antimicrobial potential of nanoparticles in controlling biofilm formation has been investigated, with researchers striving to enhance their effectiveness against biofilms.

The tomatillo is a member of the genus *Physalis*, erected by Carl Linnaeus in 1753. Jean-Baptiste de Lamarck described the tomatillo under the name *Physalis philadelphica* in 1784 (BÜKÜN, UYGUR, UYGUR, TÜRKMEN, & DÜZENLİ, 2002) However, *Physalis philadelphica* is the most important species economically.(Simpson, Montes-Hernandez, Gutierrez-Campos, Assad-Garcia, & Herrera-Estrella, 1995) .Tomatillo or *Physalis ixocarpa* or *Physalis philadelphica),* also known as the Mexican husk tomato, is a plant of the nightshade family bearing small ,spherical and green or green-purple fruit (Morton, 1987).,The tomatillo genus name Physalis is from New Latin *Physalis*, coined by Linnaeus from Greek word in which Physalis means ‘’Bladder, wind instrument’ ’In general, tomatillo plants are tolerant to many different soil conditions. However, they do best in well-drained, sandy, fertile soil conditions with a pH between 5.5 and 7.3.(Masabni, Sun, Niu, & Del Valle, 2016) .

This plant Physalis philadelphica can grow up to fifteen to sixty centimeter. The leaves are acute (Montes Hernández & Aguirre Rivera, 1994).

MATERIALS AND METHODS

2.1 Study location for research

The research was carried out in the Microbiology Department at Hazara University, located in Mansehra, Khyber Pakhtunkhwa, Pakistan.

2.2 Chemicals, apparatus and accessories

Methanol, Ethanol, Acetic acid, Crystal violet, Nutrient broth. Aluminum foil, microtiter plates, Eppendorf tube, flask 1000/500 ml Funnels, beakers, test tubes, Vortex meter, PBS Double beam Spectrophotometer, Cuvettes, Saline Water, Glycerol, Spirit lamp, Shaking Incubator, laminar flow hood, Slides, Bacteria *Staphylococcus aureus*, Microscope were used in this study.

2.3 Pant Sample collection and extract preparation

The selected plant *Physalis Philadelphica* will be collected from Gulibagh Baffa District Mansehra and will be identified and authenticated with the help of a botanist of Hazara university and also with the help of Google App "Plant Identifier."

2.4 Plant material

2.4.1 Protocol for Plant Extraction Using Maceration Method (Ethanol)

(a) Collection and Preparation of Plant Material

The leaves were carefully taken off the plant stems and then leaves were first washed with tap water to remove dirt and debris. After that they were then rinsed with distilled water to get rid of any remaining impurities.

(b) Drying

The washed leaves were spread out on clean trays and air-dried at room temperature in a well-ventilated area, away from direct sunlight, for 7–10 days until completely dry. Alternatively, drying can be performed in a hot air oven at 40°C to speed up the process without damaging bioactive compounds.

(c) Grinding

The dried leaves were ground using a mechanical grinder to obtain a fine, uniform powder The powdered material was sieved to remove coarse particles, ensuring homogeneity.

(d) Preparation of Ethanolic Extract

A measured quantity of the powdered leaves (e.g., 100 g) was mixed with ethanol in a 1:5 ratios (e.g., 25 g powder + 125 mL ethanol). The mixture was transferred to flask covered with aluminum foil to prevent photo degradation of sensitive compounds. The flask was placed on a shaker at room temperature for 48 hours to facilitate extraction.

(e) Filtration

The liquid was then passed through Whatman filter paper No. 1 to obtain a clear extract.

Figure 2.1 Maceration Method of *Physalis philadelphica*

(f) Concentration of Extract

The extract was poured into a glass beaker or petri dish and dried in an oven at 50°-65 C until all the solvent evaporate. Then crude extract was scraped and collected in centrifuge.

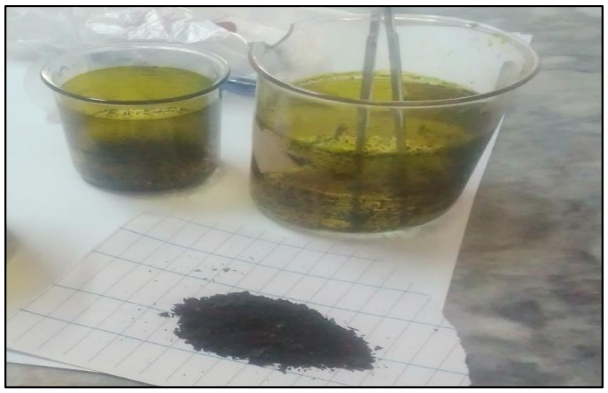


Figure 2.2 Crude extract of *Physalis philadelphica*

2.5 Bacteria, Media and Culturing

2.5.1 Media preparation

Nutrient broth is a general-purpose growth medium used for cultivation of bacteria so 2.6 gram of nutrient broth after weight the analytical balance, dissolved in 200 ml distilled water and stir until the solids are completely dissolved. Then put on hot plate for a while and finally Autoclaved at 121 temp.

2.5.2 Stock Culture

For making Bacterial stock, we take 50 microliter Glycerol put in centrifuge tube and then also 50 microliter *Staphylococcus aureus* put in centrifuge tube, mixed well and put in shaking incubator.

2.5.3 Preparation of Mother Stock and Sub Stock Solution

Mother Stock Solution (1 g / ml)

Preparation: Weight 1 g of *Physalis philadelphica* leaf extract and dissolve it in 19 ml Distilled water. Total volume should not exceed 20 mL.

Stock Solution

Use formula V1=C2×V2/C1

For this we take 7 Falcon Tube and labelled them as 50 ml ,100 ml, 200 ml, 300 ml ,400 ml, 500 ml, 600 ml. Then Take 20 ml Distilled water put in all tubes and then from 50 ml labeled Falcon tube removed 50 microliters distilled Water and same procedure follow with all Falcon tubes. In 50 ml Falcon tube add 50 microliter mother stock extract and same methodology is applied in all seven tubes.

2.6 Antimicrobial assay against planktonic bacterial growth

2.6.1 Bacteria culturing

Grow 100 µL bacterial cells from stock into 10 ml nutrient broth in two different tubes (50 ml tube), under shaking conditions of 200 rpm, temperature 30 C in shaker incubator for 24hrs. After 24 hours, All the cultures will be centrifuged at 4500 rpm for 15 minutes. Discard the supernatant and add fresh media to the pellets and mix it by vertexing. Measure the OD at 600 nm by diluting, using UV Spectrophotometry. Use media as a blank. Finally, we get OD in Triplets and then find Average and as an end results, we get 1.93 OD. Then calculate the volume from bacterial stock using formula given below The final concentration in the well of microtiter plate must be 0.01 OD. Calculate this by V1=C2V2/C1

V1=?

C1= 1.93 OD in stock culture

C2= 0.01 OD (your target OD in microtiter plate)

V2= final volume in microtiter plate which is 1 ml

V1= 66 microliter.

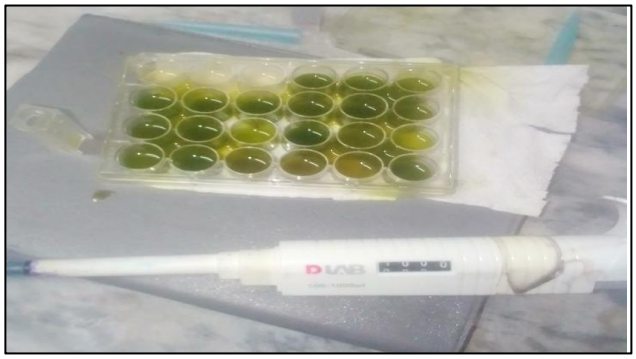


Figure 2.3 Growth assay on microtiter plate

2.6.2 Effect of extract on bacterial growth in microtiter plate

Microtiter plate will be labelled for each concentration of extract and first three wells labelled for control (without extract). Wells of all plates will be labeled. In control 50 microliter sterilized water, 66 microliter bacteria ,884 microliter Nutrient broth media put in first three wells. Desired bacterial cultures (0.01 OD) were added to microtiter plate. Then in Treated wells add 66 microliter of bacteria in triplicate wells. Add .50 microliter extract in all Treated wells. Then add 884 microliter nutrient media to all wells to complete total 1 ml. For Blank (untreated or control) 50 ul sterilized distilled water will be added instead of. Plates will be incubated in shaking incubator at 30 C, 120 rpm, 24 hrs.

The bacterial suspension from the wells was placed into a cuvette, and the optical density (OD) was measured at 600 nm. The media was used as a blank before measuring the OD of the sample.

2.6.3 Well diffusion assay

First of all, for this I inoculated 100 microliters bacterial strain of *Staphylococcus aureus* from glycerol into 10 ml nutrient broth in 50 ml Falcon tube under sterilized conditions and incubate at 37 temperatures for 24 hrs. and measure the optical density at 600 nm using a spectroscopy and adjust the bacterial culture to an OD of 0.1 by diluting with sterilized media. Then prepared nutrient agar (2.6 g) in 200 ml distilled water, after Autoclave poured in four sterilized petri plates and allowed it to solidify. Then using a sterilized swab, dip it into bacterial culture add gently spread the *Staphylococcus aureus* bacteria across surface of nutrient agar plate to form a lawn culture. allow the plate for dry for 15 minutes to prevent flooding the wells when adding plant extract. For well creation in agar plates for this using a sterilized small glass tube and created (6-8 mm) wells in the agar surface. typically, four well can be made on each plate, depending on the plate size. Then using a sterilized pipette carefully add 50 microliters of each concentration of plant extract into wells. Add 50 microliter of sterilized water (no plant extract) to one of wells as a negative control. This ensure that any observed antibacterial effect is due to the plant extract also add antibiotic solution to one well as appositive control to compare the antimicrobial effects of plant extract. Incubate the plate at 37 C for 18 /24 hrs. Then check zone of inhibition, then observed result and measured the diameter of zone of inhibition in mm using a scale. Results are in the next chapter.

2.6.4 Disc diffusion assay

Nutrient Agar Preparation

First of all, 2.6 g Nutrient Agar measure with the help of analytical balanced, then poured in 200 ml distilled water after Hot plate then put in Autoclave for sterilization.

Bacteria Culturing: Cool the Agar then 100 microliter *Staphylococcus aureus* then put in 200 ml of nutrient agar, mixed well. Then media pour in four sterilized petri plates, allow the agar to solidify at room temperature and all work was done under laminar flow hood.

Procedure

In this procedure we used various concentration of Sub Stock Solution for this as we take four petri plates and in first plate we labeled the petri plate with concentration of 50 and 100 ,2nd plate labeled with 200 and 300 concentration ,3rd plate labeled with 400 and 500 concentration and 4th plate labeled with only 600 concentration of plant extract. And in each plate we also take positive Control (Antibiotic 'Imipenem') and Negative Control (Only used 30 microliter Distilled water).Then placed 5 to 6 sterilized filter paper on the petri plate for absorbing the plant extract .Then add 30 microliter of plant extract to the petri plates means from 50 labeled Falcon tube take 30 microliter plant extract and put on first Petri plates which was already labelled with 50 and same procedure were follow with further three petri plates .Then incubate all plates at 37 temp for 24 hours. Results are in the next chapter

2.7 Biofilm Control Assays

2.7.1 Microtiter Plate and Crystal Violet Assay

Biofilm Control Assay in **Microtiter Plate**

Microtitre plate will be labelled for each concentration of extract and first three wells Will be labelled for control (without extract). In Control 50 microliter sterilized water, 66 microliter bacteria, 884 microliter Nutritent broth media put in first three wells. Add 0.01 OD cells of each bacterial 66 microliter isolate in triplicate wells. Wells of all plates will be labelled. Add 50 microliter extract in all Treated wells. Then add 884 microliter Nutrient Media to all wells to complete total 1

ml. For Blank (untreated or control) 50 ul Sterilized distilled water will be added. Plates will be incubated in shaking incubator at 30 C, 120 rpm, 24 hrs.

Crystal violent assay for biofilm analysis

Same plates from above experiment without bacteria suspension will be used for biofilm analysis.

After incubation of plates, from above experiment, Plates will be gently washed with saline or PBS or sterilized distilled water. Then water will be removed, plates will be air dried.1 ml of 99.9% ethanol will be added to each will and will be incubated at room temperature for 10 minutes. Liquid will be poured off and plates will be air dried and 1 ml of crystal violet dye (0.1% W/V) will be added to each will well and it will be left for 15 minutes. Tap water will be used for removing excessive stain and will be air dried. Dissolve attached dye: The dye bound to cells will be re-dissolve in acetic acid (33% v/v), 1 ml will be added to each will. Liquid from each will be transferred to Cuvette and OD will be determined at 595 nm. Results are in the next chapter.

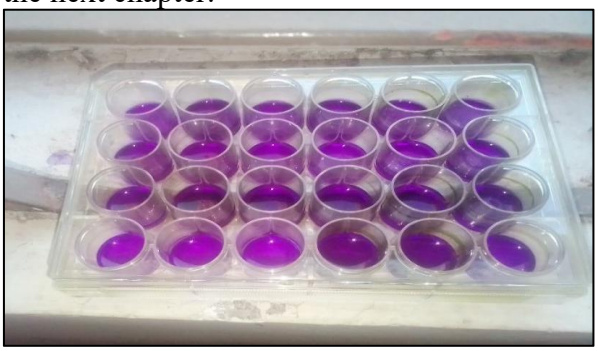


Figure2.4 Crystal violet Assay for Biofilm Analysis

2.7.2 Petri Dish biofilm assay

100 microliter of *Staphylococcus aureus* put in 10 ml of Nutritent Broth, then mixed well and placed in shaking incubator at 120 rpm for 24 hrs. After 24 hrs removed the Supernatant and in Pellets add fresh Nutritent Broth Media , find OD Using C1C2=V1V2 then with the help of formula finally we get V1=8 microliter bacteria, then in 7th petri plates we pour media and one sterilized glass slides, add desired bacterial volume kept for 24 hrs. after 24 hrs. removed media from petri plates and take all slides and labelled them and washed with saline water then apply 1 ml of Crystal Violet on all slides and then wash with distilled water and observed under light Microscope at 40× objective lens. Slides were stained with 0.1% crystal violet stain and then visualized. Results are in the next Chapter.

2.7.2.1 Extraction of Extracellular polymeric substances (EPS)

After 24 hours, the EPS was extracted from the slide surfaces. A cell scraper was used to gently remove the biofilm, which was then dissolved in 10 ml of sterilized water. After that sample was vortexed for 30 seconds in tubes. Distilled water was used as a negative control. The mixture was then centrifuged at 1000 rpm for 15 minutes. After centrifugation, the supernatant, containing the EPS (Extracellular Polymeric Substances), was transferred to a separate tube for further analysis.

2.7.2.2 Protein (EPS) Quantification by Bradford assay

Dissolve 500 mg Coomassie Brilliant Blue G-250 Reagent in 25 ml 95 % ethanol add 50 ml 85 % (w/v) phosphoric acid. Dilute to 500 ml when the dye has completely dissolved, and filter through Whatman #1 paper just before used.

Prepare the Coomassie Reagent solution by gently inverting the bottle several times before use (avoid shaking). Take the required amount of reagent in an aluminum foil-wrapped tube and let it reach room temperature. Pipette 1.0 ml of the unknown sample into a labeled 2 ml Eppendorf tube. Add 1.0 ml of the Coomassie reagent to each tube and mix by inverting the tube a few times. Allow the samples to incubate for 10 minutes at room temperature. Set the spectrophotometer to 595 nm, zero it with a cuvette filled with the negative control, and then measure the absorbance of all the

samples. The results can be found in the next chapter.

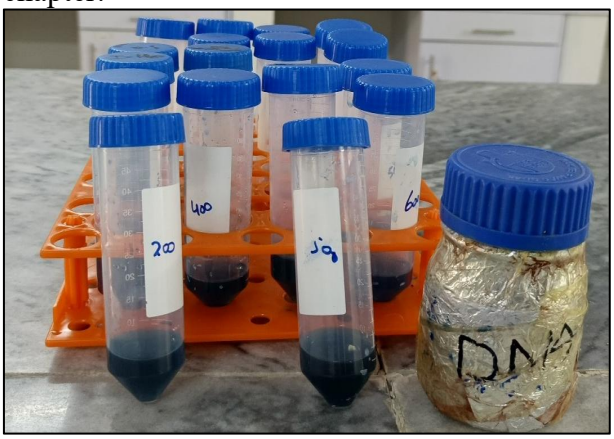


Figure 2.5 Protein Production Estimation for Biofilm Analysis

2.7.2.3 Exopolysaccharide (EPS) Quantification

Transfer 1 mL of the polysaccharide sample into a properly labeled glass tube. Add 0.5 mL of 5% phenol solution, followed by 2.5 mL of concentrated H2SO4 (carried out in a fume hood, and handle the chemicals with care). Mix the solution thoroughly and allow it to react for 10 minutes. After the reaction, transfer 1 mL of the sample to a quartz cuvette and measure the absorbance using a UV spectrometer at 492 nm. Auto-zero the spectrometer using the negative control (blank: distilled water or saline with 0.5 mL of 5% phenol and 2.5 mL of concentrated H2SO4). Subsequently, the absorbance of all the samples will be measured at 492 nm (Dubois et al., 1956). Note down OD for samples.

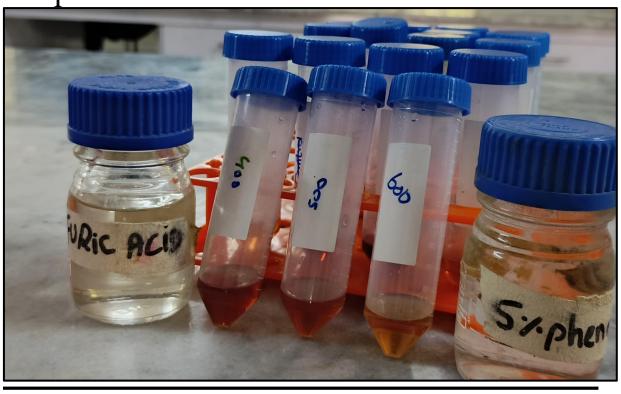


Figure 2.6 Polysaccharide Production for Biofilm Analysis

RESULTS AND DISCUSSION

3.1 Effect of Physalis Philadelphia on Growth of *Staphylococcus aureus*

The Figure 3.1 shows that the growth measured at Optical Density 600 nm against various concentrations of *Physalis philadelphica*. There are one sets of blue bars for various concentration. The x-axis is labeled for concentrations (mg/ml) with values ranging from Control, 1.712 ,1.443 ,0.433 ,0.247 ,0.132 ,0.113 ,0.100 ,0.066. The y-axis is labeled "Growth Optical Density 600 nm" with values ranging from 0 to 2. The antibacterial effect of *Physalis philadelphica* extract against *Staphylococcus aureus* was evaluated using optical density (OD) measurements at 600 nm. The Average OD of the control (untreated culture) was recorded at 1.712, indicating robust bacterial growth. Treatment with 50 mg/mL of extract reduced the OD to 1.443, suggesting moderate inhibition. A significant decrease in OD to 0.066 was observed at the highest concentration of 600 mg/mL, indicating strong antibacterial activity and almost complete inhibition of bacterial growth.These results demonstrate a clear dose-response relationship, where increasing concentrations of *Physalis philadelphica* leaf extract led to enhanced inhibition of *S. aureus* growth. The data strongly support the extract's potential as an effective antimicrobial agent.

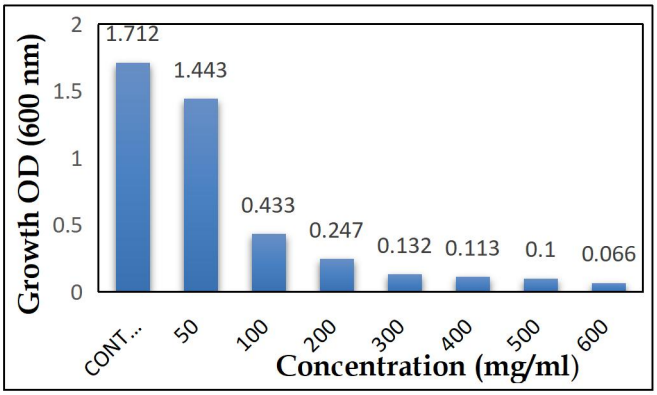


Figure 3.1 Effect of *Physalis Philadelphia* leaf extract on planktonic Growth of *Staphylococcus aureus.*

Table 3.1 Effect of various concentrations of *Physalis philadelphica* on growth reduction

Conc. mg/ml	Avergage	% Reduction
Control	1.712	0%
50	1.443	15.71%
100	0.433	74.70%
200	0.247	85.56%
300	0.132	92.29%
400	0.113	93.40%
500	0.10	94.16%
600	0.066	96.14%

The first column shows the concentration of *Physalis philadelphica* in milligrams per milliliter (mg/ml). The second column shows the Average and third column show the percentage of growth reduction by*Physalis philadelphica*. The first row shows the control group, which did not receive any treatment. The table suggests that *Physalis philadelphica* have growth reduction effect,.The growth reduction effect increased with the concentration of *Physalis philadelphica*.At 50 mg/ml , the growth reduction was 15.71 % There is a sharp increase in growth reduction between 500 and 600 mg/ml. effect is increased with the concentration, reaching the highest value of 94.16 %and 96.14 %. In one of the studies by other researchers it was observed that *Physalis philadelphic*a have antibacterial effect against *Staphlococcus aureus.*

3.2 Antibacterial activity of *Physalis philadelphica* using Well Diffusion Assay against *Staphylococcus aureus*

When we performed antimicrobial activity of *Physalis philadelphica* using Well Diffusion Assay against *Staphylococcus aureus* so as a result we get at 600 concentrations 1.99 mm of Zone of inhibition and in Positive Control we get 3.00 mm and in Negative Control we get Zero zone of inhibition which are shown On below figure

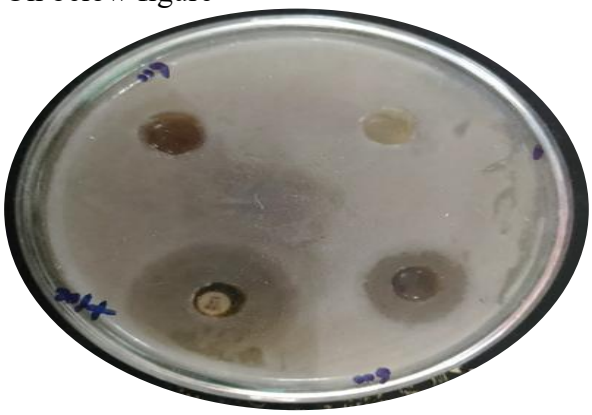


Figure 3.2 Shows the effect of various concentrations of *Physalis philadelphica* on Well diffusion (Growth Assay) at 600 mg/ml concentration

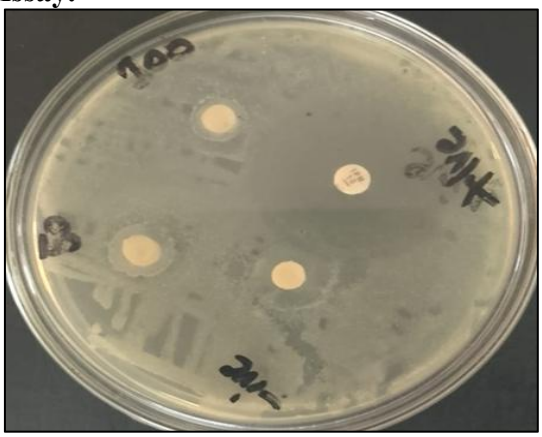
Table 3.2 shows the effect of various concentrations of *Physalis philadelphica* on Growth Assay.

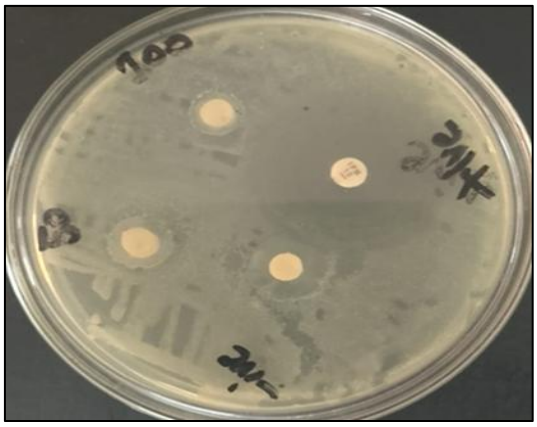
Sample	Zone of inhibition (mm)
Control (negative)	0
Control (positive)	3
600 mg/ml	1.9

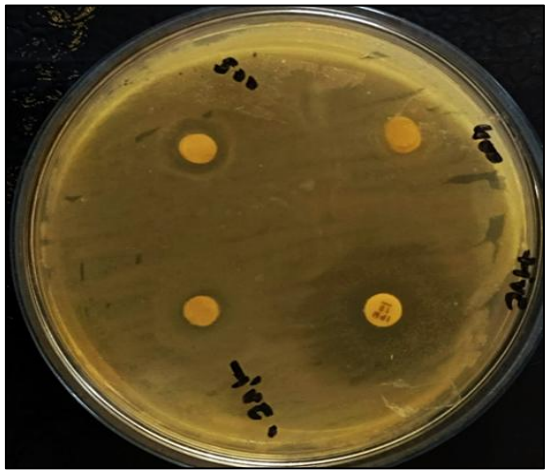
The first column represents the concentration of the *Physalis philadelphica* in milligrams per milliliter (mg/ml). The second and third columns represent the zone of inhibition of *Physalis philadelphica,* respectively. The table shows that at the concentration 600 of *Physalis philadelphica* the zone of inhibition was observed about 1.9 mm and in Negative control we get Zero Zone of inhibition and in Positive control we get 3.00 mm. The results support the objectives of the research, demonstrating the concentration-dependent antimicrobial and anti-biofilm activities of *Physalis philadelphica* against *Staphylococcus aureus.* And plant extract inhibits *Staph aureus* but less effectively than the standard antibiotic (Positive Control). The plant extract inhibits *Staphylococcus aureus,* but less effectively than the standard antibiotic (Positive Control). The smaller zone

(1.9 mm) means the extract has partial antimicrobial potential, but not very strong at this concentration**.**

3.3 Zone of inhibition of Leaf extract *(Physalis philadelphica)* against *staphylococcus aureus* using Disc Diffusion Assay.







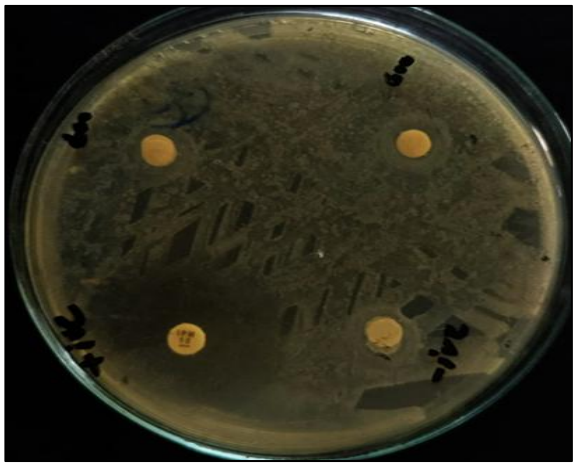


Figure 3.3 Antibacterial activity of *Physalis philadelphica* against planktonic *Staphylococcus aureus* Using Disc Diffusion Assay.

Table 3.3 Effect of *Physalis philadelphica* at various concentration against *Staphylococcus aureus.*

Sample	Zone of inhibition
Control (negative)	0
Control(positive)	2.9mm
50mg/ml	0.6
100mg/ml	0.7
200mg/ml	0.8
300mg/ml	0.8
600mg/ml	1.2

The table show that when The Disc diffusion assay was performed to evaluate the antimicrobial activity of *Physalis philadelphica* extract against *Staphlococcus aureus* in which Negative Control showed zero mm zone which conformed that there is no inherent inhibitory effect from the solvent or Disc.The Positive Control (Imipenem) produced 2.9 mm Zone ,indicationg strong antimicrobial activity.The plant extract at 50-300 mg/ml showed mild inhibition (0.6 mm ,0.7 mm and 0.8 mm)zone ,suggesting weak to moderate antimicrobial activity at lower concentration.at highest concentration (600 mg/ml) ,the zone increased to 1.2 mm,reflecting enhanced antibacterial activity but still less effective than the standard drug.

3.4 Biofilm assay followed by Crystal Violet assay

Various amounts of *Physalis philadelphica* plant extract were tested to check its impact on biofilm formation.

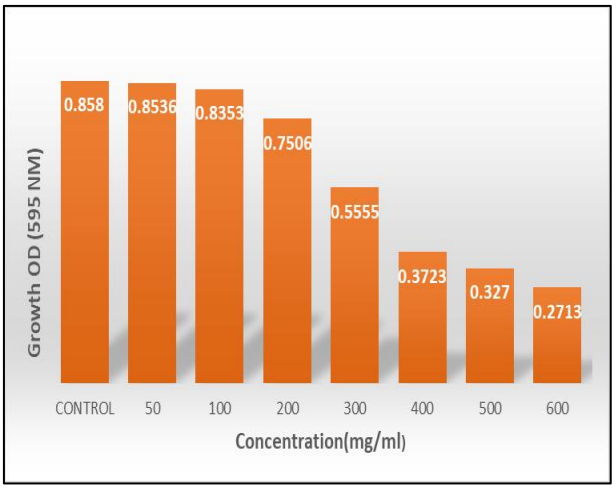


Figure 3.5 Effect of various concentrations of plant extract of *Physalis philadelphica* on biofilm formation by *Staphylococcus aureus* followed by Crystal violet assay.

The Figure 3.5 shows that the growth measured at Optical Density 595 nm against various concentrations of *Physalis philadelphica*. There are one set of bars: Yellow for various concentration. The x-axis is labeled for concentrations (mg/ml)" with values ranging from Control, 0.858, Treated are
0.8536 ,0.8353 ,0.7506 ,0.5555 ,0.3723 ,0.327, 0.2713. The y-axis is labeled "Growth Optical Density 595 nm" with values ranging from 0 to 2. The effect of *Physalis philadelphica* extract on biofilm formation by *Staphylococcus aureus* was assessed using the Crystal Violet assay, with optical density (OD) readings taken at 595 nm. The untreated control sample showed an OD of 0.858, representing full biofilm formation. Upon treatment with 50 mg/mL, the OD slightly reduced to 0.853, indicating minimal inhibition at lower concentrations. However, a significant reduction in OD to 0.2713 was recorded at 600 mg/mL, suggesting a strong inhibitory effect on biofilm development at higher concentrations. Therefore, this result supports the potential use of *Physalis philadelphica* extract in anti-biofilm strategies, especially against biofilm associated infections.

Table 3.5 Effect of various concentrations of plant extract of *Physalis philadelphica* formation by *Staphylococcus aureus* followed by Crystal violet assay.

mg/ml	Average	Reduction %
Control	0.858	0.00%
50	0.8536	1%
100	0.8353	3.00%
200	0.7506	12.52%
300	0.5555	35.25%
400	0.3723	56.61%
500	0.327	61.89%
600	0.2713	68.38%

The table 3.5 shows the effect of various concentrations of *Physalis philadelphica* on growth reduction. The first column shows the concentration of *Physalis philadelphica* in milligrams per milliliter (mg/ml). The second column shows the Average and third column show the percentage of % growth reduction by *Physalis philadelphica*. The first row shows the control group, which did not receive any treatment. The table suggests that they have growth reduction effect, the growth reduction effect increased with the concentration of *Physalis philadelphica*. At 50 mg/ml, the growth reduction was 1% There is a sharp increase in growth reduction between 500 and 600 mg/ml. effect is increased with the concentration, reaching the highest value of 61.89 %and 68.38 %. In one of the studies by other researchers it was observed that *Physalis philadelphica* have antibacterial effect against *Staphylococcus aureus* and effectively inhibit biofilm formation.

3.5 Petri Dish Biofilm Assay (Microscopy results)

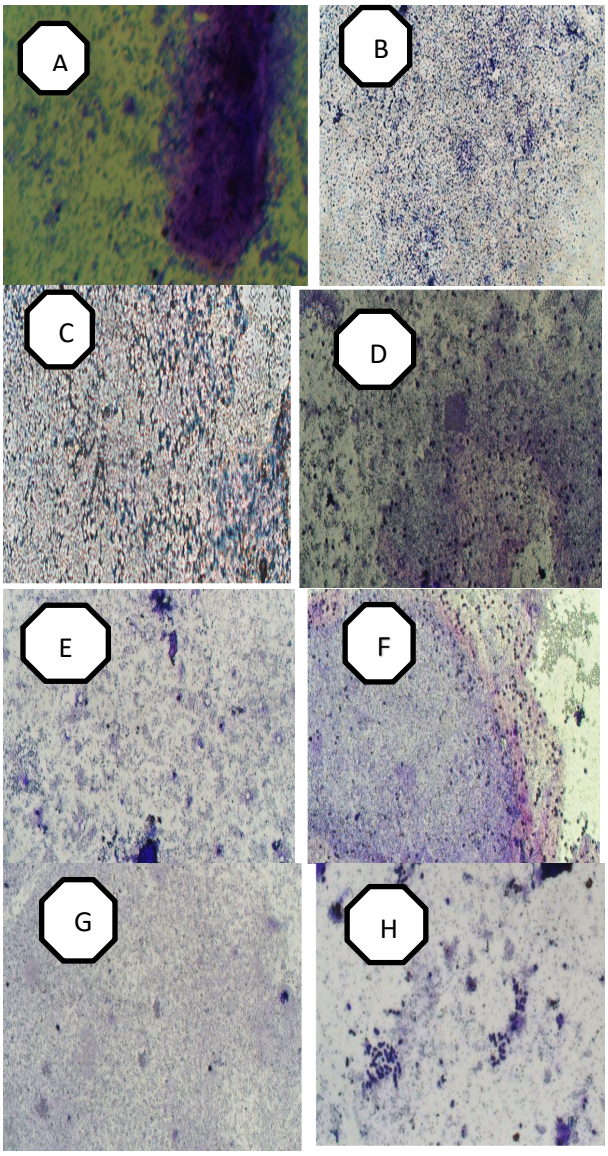


Figure3.6 The microscopic Visualization of the effect of different concentration of *Physalis philadelphica* on Biofilm (A) Control ,(B) 50 mg/ml (C) 100 mg /ml (D) 200 mg/ml (E) 300 mg/ml (F) 400 mg/ml (G) 500 mg/ml (H) 600 mg/ml

When we performed Petri dish biofilm assay for biofilm growth. A Petri dish biofilm assay was conducted to visually assess the effect of *Physalis philadelphica* extract on *Staphylococcus aureus* biofilm formation. The extract was tested at various concentrations Such as 50, 100, 200, 300, 400, 500, and 600 mg/mL. Microscopic examination of biofilm on the surface of the Petri dishes revealed a progressive decrease in biofilm density with increasing concentrations of the extract. At 50 mg/mL, thick and dense biofilm formation was observed, similar in appearance to the control. As the concentration increased, a noticeable reduction in the thickness and coverage of the biofilm was observed. These results confirm a dose-dependent anti-biofilm activity of *Physalis philadelphica* extract against *S. aureus.* The gradual reduction in biofilm observed microscopically from 50 to 600 mg/mL suggests that the extract interferes with the stages of biofilm development such as initial adhesion, maturation, or matrix production. Such findings are significant, as biofilm-associated infections are notoriously resistant to antibiotics. The ability of this plant extract to suppress biofilm formation supports its potential application in developing alternative antimicrobial or anti-biofilm therapies.

3.6 Effect of various concentration of plant extract on extracellular protein production of *staphylococcus aureus*

The Figure 3.7 shows that the growth measured at Optical Density 595 nm against various concentrations of *Physalis philadelphica*. There are one sets of bars: Green for various concentration. The x-axis is labeled for concentrations (mg/ml) with values ranging from Control, 0.119 ,0.095 ,0.065 ,0.038 etc. The y-axis is labeled for Protein (mg\ml) the quantification of EPS associated proteins in *Staphylococcus aureus* biofilms was assessed to determine the impact of *Physalis philadelphica* extract. Protein content was measured using spectrophotometry at 595 nm. The control group (no extract. treatment) showed an OD of 0.119, indicating a high level of protein in the biofilm matrix Treatment with 50 mg/mL of the extract resulted in a slight decrease in

OD to 0.113, suggesting minimal reduction in EPS protein production. However, at 600 mg/mL, the OD dropped significantly to 0.021, indicating a substantial decrease in EPS protein content. As EPS proteins contribute to the mechanical stability and antibiotic resistance of biofilms, this extract's ability to reduce EPS levels highlights its potential as an anti-biofilm agent, useful in treating persistent *S. aureus* infections.

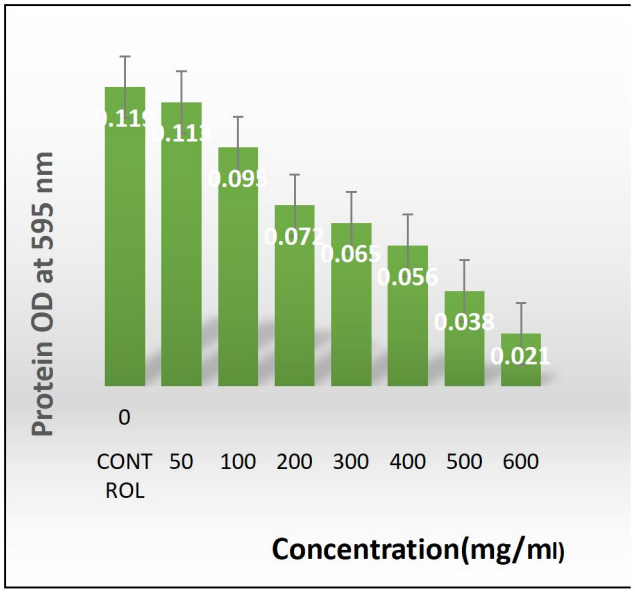


Figure 3.7 Effect of various concentration(50-600 mg/ml) of plant extract on extracellular protein production of *staphylococcus aureus.*

Table 3.7 Effect of various concentration of plant extract on extracellular protein production of *Staphylococcus aureus.*

Conc. mg/ml	AVERAGE	REDUCTION (%)
Control	0.118	0%
50	0.113	3.97%
100	0.095	19.49%
200	0.072	38.98%
300	0.065	44.66%
400	0.056	52.54%
500	0.038	67.54%
600	0.021	81.95%

The table 3.7 shows the effect of various concentrations of *Physalis philadelphica* on growth reduction. The first column shows the concentration of *Physalis philadelphica* in milligrams per milliliter (mg/ml). The second column shows the Average and third column show the percentage of growth reduction by *Physalis philadelphica.* The first row shows the control group, which did not receive any treatment. The table suggests that they have growth reduction effect. The growth reduction effect increased with the concentration of *Physalis philadelphica*. At 50 mg/ml, the growth reduction was 3.97 % There is a sharp increase in growth reduction between 500 and 600 mg/ml. effect is increased with the concentration, reaching the highest value of 67.54 %and 81.95 %. So it was observed that *Physalis philadelphica* leaf extract significantly reduces the extracellular protein production of *staph aureus*, with maximum suppression at 600mg/ml. Since extracellular protein are vital for bacterial virulence, this reduction implies that extract could effectively weaken *S aureus* pathogenicity. The results support the potential role of this plant in disrupting virulence factor expression in resistant bacterial strain.

3.7 Effect of various concentration of plant leaf extract on extracellular polysaccharide production of *staphylococcus aureus*

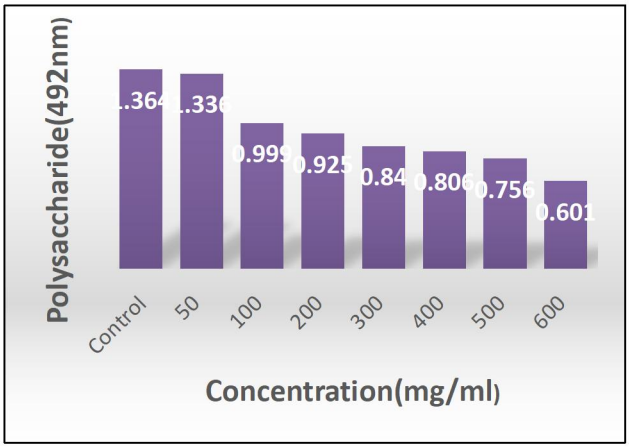


Figure 3.8 Effect of various concentration of plant extract on Polysaccharide production of staphylococcus aureus

The Figure 3.8 shows that the growth measured at Optical Density 492 nm against various concentrations of *Physalis philadelphica*. There are one sets of bars: Purple for various concentration. The x-axis is labeled for concentrations (mg/ml) with values ranging from Control, 1.364 ,0.999 ,0.84 ,0.756 and 0.0601 etc. The y-axis is labeled for Polysaccharide (mg\ml). The effect of *Physalis philadelphica* extract on the extracellular polysaccharide (EPS) content in *Staphylococcus aureus* biofilms was measured by recording optical density at 492 nm. The control group (untreated) showed an OD of 1.364, indicating a high level of EPS polysaccharide production, essential for stable biofilm formation. At a concentration of 50 mg/mL, the OD slightly decreased to 1.336, showing only a minor reduction in EPS production. However, a significant decline in OD to 0.601 was observed at 600 mg/mL, indicating a marked reduction in EPS polysaccharide levels within the biofilm matrix. Polysaccharides are critical components of the biofilm matrix, contributing to surface attachment, stability, and resistance to external stress. The observed reduction suggests that the plant extract disrupts biofilm formation by targeting matrix components, making it a promising candidate for anti-biofilm applications.

Table 3.8 Effect of various concentration of plant extract on Polysaccharide production of *Staphylococcus aureus*.

Column	AVERAGE	REDUCTMION %
Control	1.364	0%
50	1.336	2.05%
100	0.999	26.75%
200	0.925	32.19%
300	0.84	38.41%
400	0.806	40.91%
500	0.756	44.58%
600	0.601	55.94%

The table 3.8 shows the impact of plant (*Physalis philadelphica*) extract on growth reduction. The first column shows the concentration of *Physalis philadelphica* in milligrams per milliliter (mg/ml). The second column shows the Average and third column show the percentage of % growth reduction by *Physalis philadelphica*. The first row shows the control group, which did not receive any treatment. The table suggests that they have growth reduction effect. The growth reduction effect increased with the concentration of *Physalis philadelphica*. At 50mg/ml, the growth reduction was 2.05 % There is a sharp increase in growth reduction between 500 and 600 mg/ml. effect is increased with the concentration, reaching the highest value of44.58 %and 55.94 %. So it was observed that *Physalis philadelphica* extract significantly reduces the extracellular protein production of *Staph aureus*, with maximum suppression at 600 mg/ml resulted more than 50 % reduction highlight the extract therapeutic potential for impairing biofilm associated virulence mechanism in *S aureus.* Since extracellular protein are vital for bacterial virulence, this reduction implies that extract could effectively weaken *S aureus* pathogenicity .The results support the potential role of this plant in disrupting virulence factor expression in resistant bacterial strain.

4.1 CONCLUSION

This study demonstrates that *Physalis philadelphica* leaf extract has notable antibacterial and anti-biofilm activity against *Staphylococcus aureus*. The extract effectively inhibited bacterial growth and reduced biofilm formation in a concentration-dependent manner. It also interfered with essential biofilm components like proteins and polysaccharides. These results highlight the potential of *P. philadelphica* as a promising natural antimicrobial agent,

particularly for infections involving biofilm-forming bacteria.

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