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Research Article **BOTANY**

Carbohydrates and Plant Extracts as Natural Alternatives for Preventing *Staphylococcus aureus* Biofilm Formation

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Abstract

Staphylococcus aureus is a common bacterial pathogen that can cause various infections, including biofilm-related infections that are difficult to treat. In this study, we investigated the effects of different carbohydrates and plant extracts on the biofilm formation of three standard strains of *S. aureus* obtained from different sources. We found that the adherence and biofilm formation of the strains were affected by their source, age, and culture conditions. Additionally, we found that *Nigella sativa* extract and certain monosaccharides, disaccharides, and polysaccharides were effective in reducing biofilm formation, with some degree of strain-specificity. Furthermore, we found that starch had a concentration-dependent effect on the biofilm formation of *S. aureus* strains. Finally, we investigated the acidogenicity of different carbohydrates in LB broth and found that they were less acidogenic than the control group, but with no significant differences between the strains. Our findings suggest that plant extracts and certain carbohydrates may have the potential as natural alternatives for preventing and treating biofilm-related infections caused by *S. aureus*.

Introduction

Staphylococcus aureus (*S. aureus*) is a typical human colonizer and pathogen that causes invasive illnesses like endocarditis, osteomyelitis, and pneumonia in addition to soft tissue and skin infections **(Allison *et al.*, 2010)**. Infections associated with indwelling catheters, wounds, orthopedic, cardio-vascular, and implant devices are frequently caused by *S. epidermidis* and *S. aureus* **(Kim *et al.*, 2017)**. Bacterial biofilm is a complex community of microorganisms that adhere to a surface and produce extracellular polymeric substances. Biofilm formation is a common strategy for bacterial survival and adaptation in various environments **(Jahid, and Ha, 2017)**. However, biofilm can also cause serious problems in many fields, such as medicine, industry, and ecology. For example, *S. aureus* is a notorious biofilm-forming pathogen that can cause chronic infections in humans and animals **(Oliveira *et al.*, 2021)**. Biofilm confers resistance to antibiotics and hosts immune responses, making it difficult to eradicate **(Götz, 2002; Chmielewski, *et al.*, 2003; Xiang, *et al.*, 2017)**.

Sugars can influence the formation and structure of biofilms **(Qu, *et al.*, 2020)**. Sugars also are involved in both the recognition and the communication of bacterial cells, which can affect the inflammatory response and the immune system **(Chen *et al.*, 2021; Kabir *et al.*, 2021; Lv *et al.*, 2022)**. Biofilms are influenced by various factors in the environment, such as the availability and type of sugars, as well as the presence of drugs **(Khangholi and Jamalli, 2016)**. Therefore, investigating the proper role sugars which might affect bacterial infections and biofilms is essential for developing new strategies to prevent and treat these diseases. Finding effective ways to prevent or disrupt biofilm formation is also a major challenge and a high priority for this work.

One of the promising approaches to combat biofilm is to use active polysaccharides as biocontrol agents. Active polysaccharides are natural or synthetic polymers that have biological activities, such as antimicrobial, anti-inflammatory, and anti-biofilm properties. They can interfere with different stages of biofilm development, such as initial attachment, maturation, or detachment **(Elkady *et al.*, 2021)**. They can also modulate the expression of genes involved in biofilm formation or enhance the susceptibility of biofilm to antibiotics or host defenses **(Elkady *et al.*, 2021)**. Active polysaccharides have several advantages over conventional antimicrobial agents, such as low toxicity, high biocompatibility, biodegradability, and functional diversity **(Zhao *et al.*, 2021)**. Therefore, active polysaccharides are emerging as potential alternatives or adjuncts to conventional therapies for biofilm-related infections.

Another way to combat biofilm formation is by using medicinal plants. Medicinal plants have been used for centuries as natural remedies for various diseases and infections **(Chitme *et al*., 2003)**. Some of these plants have antimicrobial properties that can inhibit the growth and attachment of bacteria on surfaces. For example, *garlic*, *ginger*, *turmeric*, and *cinnamon* have been shown to have anti-biofilm activity against different pathogens **(Abdullahi *et al*., 2020).** *Eucalyptus globulus*, also known as Tasmanian blue gum, contains bioactive compounds such as *eucalyptol*, *alpha-pinene*, and *cineole*, which have been shown to have antibacterial effects against *Staphylococcus* strains, including *Staphylococcus aureus* **(Ou *et al*., 2022)**. *Eucalyptus globulus* has been traditionally used to treat respiratory infections **(Yoon *et al*., 2021)**. *Nigella sativa*, also known as *black seed*, contains thymoquinone, which has been shown to have antibacterial activity **(Khan *et al*., 2021)**. *Nigella sativa* has been traditionally used for various purposes, including treating inflammation and boosting the immune system **(Shabir, 2021)**. *Fenugreek*, or *Trigonella foenum-graecum*, contains substances such saponins, flavonoids, and alkaloids that have been shown to have antibacterial properties on a variety of bacterial strains. *Fenugreek* has been traditionally used to treat diabetes and inflammation. These plants can be used as extracts, oils, or powders to treat biofilm-related infections or to prevent them from occurring **(Makhija, and Dandiya, 2021)**.

This study aimed to investigate the effect of different carbohydrates and plant extracts on the biofilm formation of *S. aureus* strains obtained from different sources. The study also aimed to evaluate the acidogenicity of different carbohydrates in the presence of *S. aureus*. Our findings demonstrated that various sugars and plant extracts have different effects on the biofilm formation of different *Staphylococcus* aureus strains. Some of them showed significant antibiofilm activity, while others had no effect or even enhanced the biofilm formation. The results suggest that sugars and plant extracts can be potential sources of novel bioactive molecules that can inhibit or disrupt bacterial biofilms. Further studies are needed to elucidate the mechanisms of action and the optimal conditions for the application of these natural compounds.

Material and Methods

Bacterial Strains and Growth Conditions

In this study, three different *Staphylococcal* strains, namely *S. aureus* ATCC25923, *S. aureus* ATCC6538, and *S. aureus* NCTC65711 were used. The microbes were obtained from the El Nasr pharmacy company in Cairo. The three *Staphylococcal* strains were cultivated on

Luria and Burrous (LB) agar. The LB agar was prepared by dissolving 10.0 g/L NaCl, 10.0 g Tryptone, and 5.0 g Yeast extract in 1000 mL of distilled water, according to the recipe by Luria and Burrous (1957). The LB agar was sterilized by autoclaving at 121°C for 15 minutes and then cooled to 45-50°C. Each *Staphylococcal* strain was streaked onto separate LB agar plates using a sterile loop. The plates were then incubated at 37°C for 24 hours for bacterial growth. This procedure was performed to obtain refreshed bacterial growth.

Biofilm formation assay

A 96-well microplate with a flat bottom was used to test biofilm formation by the three *Staphylococcal* strains, following the method by **Oliveira, *et al*., (2016).** The bacterial strains were cultured onto LB agar plates and incubated for 48 hours at 37°C. The bacteria were then collected by sterile distilled water and centrifuged at 6,000 xg for 5 minutes at 4°C and suspended in 2 mL of LB broth. The densities of all suspensions were adjusted to an optical density at 600 nanometers (OD_{600}) of 0.2 by diluting them in an LB medium. Next, 200 µL of each bacterial suspension was added into separate wells of a 96-well Corning microplate with a flat bottom. A negative control, consisting of 200 µL of LB medium, was also introduced. The plates were then incubated for 24 hours at 37°C while being shaken at 100 rpm. After incubation, the attached bacterial cells were rinsed three times with phosphate-buffered saline (PBS), and then 200 µL of 0.1% crystal violet (CV) solution in water (Sigma-Aldrich, Germany) was added to each well. The plates were incubated for 20 minutes for staining, and excess CV was removed. The cells were then rinsed twice with PBS (200 µL each). To dissolve the CV, 200 µL of 99% ethanol was added to each well. The absorbance of the CV at 492 nm was measured using an ELX 800 microplate reader (Bio-Tek Instruments).

Prepared plant extracts

Samples of fresh leaves and aerial portions of *Eucalyptus globulus, Nigella sativa*, and *Trigonella foenum* were collected from various locations at Tanta during the spring of 2020. The plant material was dried at room temperature and ground in an electric blender. Infusions were prepared by adding 100 grams of the ground plant material to 500 ml of 70% ethanol and leaving the mixture at room temperature for 24 hours. The mixture was then filtered and allowed to sit overnight before being dried by evaporation, following the protocol described by **Al-Bakri *et al*., (2010).**

Comparing biofilm formation of fresh versus overnight cultures of *Staphylococcus* strains:

The growth characteristics of fresh cultures and overnight cultures of *Staphylococcus* strains were evaluated by preparing two

sets of LB broth medium; one set was inoculated with a fresh culture of the *Staphylococcus* strains, while the other set was inoculated with an overnight culture of the same strains. The bacterial cultures were then incubated at 37°C for 24 h **(Lee *et al*., 2017).** The biofilm formation assay was then performed using the remaining procedures described previously.

Evaluation of organic compounds and plant extracts as anti-biofilm agents.

Carbohydrates including monosaccharides (fructose, glucose, and xylose), disaccharides (maltose, sucrose, and lactose), polysaccharides (starch and cellulose), and plant extract substances were evaluated for their antibiofilm properties against bacterial strains. To experiment, 5 mL of fresh bacterial culture for each strain was centrifuged and the bacterial cells were suspended in 1 mL of LB broth medium with 1% of various plant extracts and sugars for each type. A control sample was included that did not receive any treatment. The density of all suspensions was adjusted to an OD_{600} of 0.2 by diluting them in an LB medium. The biofilm formation assay was then performed using the remaining procedures described previously.

Effect of starch concentration on biofilm formation of different bacterial strains

Antibiofilm properties of different concentrations (0.5, 1, 1.5, and 2g) of starch were tested. One milliliter of bacterial culture was mixed with 200 µl of each starch concentration. The suspensions were diluted with LB medium to an OD600 of 0.2. 96-well microplates with a flat bottom were inoculated with 200 µl of the treated bacterial strains. Negative control was established by adding 200 µl of LB medium. The plates were incubated, and biofilm formation was measured using previously described methods.

Assessing acidogenicity of bacterial strains in LB broth medium with various sugars

Five mL of fresh bacterial culture for each strain was centrifuged. The bacterial cells were suspended in 1 mL of LB broth medium with 1% of various sugars for each type. A control sample was included that did not receive any treatment. 2 µl phenol red was added as a pH indicator to each tube to monitor changes in acidity over time. The tubes were then incubated for 24 hours at 37 °C while being shaken at 100 rpm. The tubes should be incubated in the absence of light to avoid interference with the pH indicator. The acidogenicity of each tube was assessed by comparing the final pH of each tube to the starting pH using a pH meter (Benchtop pH Meter, PH-B200E/PH-B200EM, Bioevopeak inc., USA).

Statistical analysis:

Data entries were performed using the Excel program (MS, Office, 2023, USA). Data were analyzed using the statistical

package of social science (SPSS v. 26, USA). The quantitative data were expressed as means and standard deviations (SDs). A two-way analysis of variance (ANOVA) procedure test was used to compare the continuous variables of different groups of data at *p* less than 0.05.

Results

Age of *Staphylococcal* strains affects biofilm formation abilities:

This study first aimed to compare the attachment and biofilm formation of three standard *Staphylococcal* strains obtained from different sources: *S. aureus* ATCC25923, *S. aureus* ATCC6538, and *S. aureus* NCTC6571. The strains were cultured in fresh and overnight media and their biofilm formation was assessed by crystal violet staining. The results showed that *S. aureus* NCTC6571 had the highest adherence and biofilm formation in overnight culture. In contrast, fresh culture had higher biofilm formation than overnight culture for *S. aureus* ATCC6538, and *S. aureus* NCTC6571. These findings suggest that the source, age, and culture conditions of the *Staphylococcal* strains affect their attachment and biofilm formation abilities (**Fig. 1**).

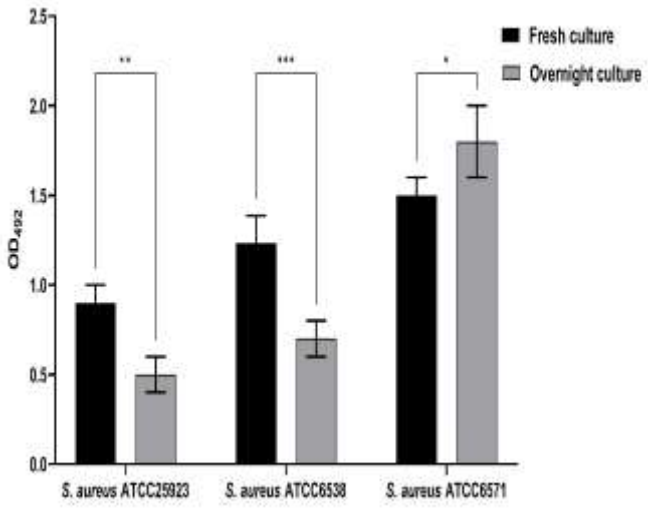

Fig. 1: Effect of fresh and overnight culture on biofilm formation of different *Staphylococcal* strains. Asterisks (*) represent a significant difference between the selected groups using two-way ANOVA at $p<0.05$.

The extracts of *Eucalyptus globules, Nigella sativa,* and *Trigonella foenum* showed a variable effect on the biofilm formation of selected *Staphylococcus* strains.

Eucalyptus globules, *Nigella sativa*, and *Trigonella foenum* are three medicinal plants that have been used for various purposes, such as respiratory infections, diabetes, and inflammation. They contain different bioactive compounds that may have antibacterial effects against *Staphylococcus* strains. This study was conducted to evaluate the effects of different extracts from these plants on the biofilm formation of the selected *Staphylococcus* strains. The results showed that all extracts reduced the biofilm formation of the bacteria, but with different degrees of efficacy. Among the three plants, *Nigella sativa* had the most significant effect on the biofilm formation

of *S. aureus* NCTC6571. The data suggested that *Nigella sativa* may have the potential as a natural alternative for preventing and treating *Staphylococcal* infections (**Fig. 2**).

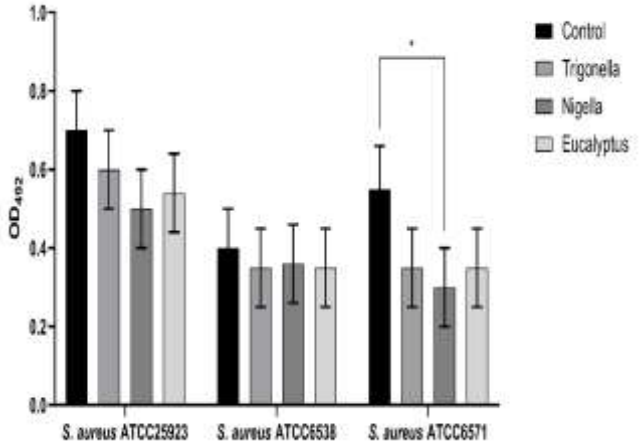

Fig. (2): Effect of different plant extracts on biofilm formation of the three selected *Staphylococcus* strains. Asterisks (*) represent a significant difference between the selected groups using two-way ANOVA at $p<0.05$.

Different monosaccharides showed a promising effect on the biofilm formation of the selected *Staphylococcus* strains.

The effect of different monosaccharides on the anti-biofilm formation of *Staphylococcus* strains was investigated in this study. The results showed that glucose, fructose, and xylose significantly reduced the biofilm formation of *S. aureus* ATCC25923, while only glucose and fructose had a similar effect on *S. aureus* NCTC 6571. However, none of the monosaccharides affected the biofilm formation of *S. aureus* ATCC6538, suggesting that different *S. aureus* strains may have different mechanisms of biofilm regulation and resistance to monosaccharides (**Fig. 3**).

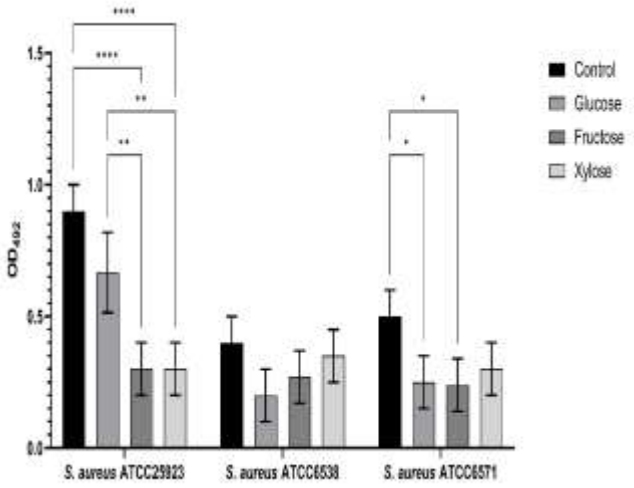

Fig. (3): Effect of different monosaccharides on biofilm formation of the three selected *Staphylococcus* strains. Asterisks (*) represent a significant difference between the selected groups using two-way ANOVA at $p<0.05$.

Different disaccharides showed a different effect on the biofilm formation of the selected *Staphylococcus* strains.

We measured the adhesion and biofilm formation of the three *Staphylococcus* strains in the presence or absence of the three different disaccharides: sucrose, maltose, and lactose. The results showed that sucrose, maltose, and lactose significantly reduced the biofilm formation of *S. aureus* ATCC25923 compared to the control group. Similarly, sucrose and maltose decreased the biofilm formation of *S. aureus* NCTC 6571, while lactose had no significant effect. However, none of the disaccharides affected the biofilm formation of *S. aureus* ATCC6538. These findings suggest that different disaccharides have different effects on the anti-biofilm formation of *Staphylococcus*

strains and that sucrose and maltose are more effective than lactose in inhibiting these processes (**Fig. 4**).

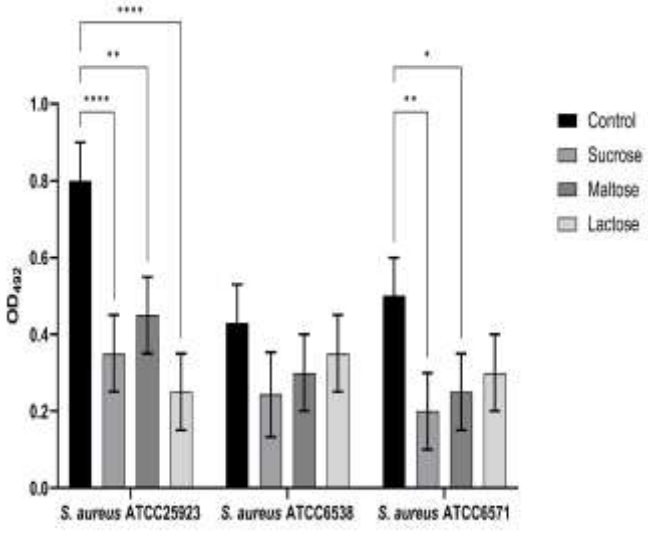

Fig. (4): Effect of different disaccharides on biofilm formation of the three selected *Staphylococcus* strains. Asterisks (*) represent a significant difference between the selected groups using two-way ANOVA at $p<0.05$.

The effect of different polysaccharides on the biofilm formation of the three strains of *Staphylococcus aureus*

We then investigated the effect of different polysaccharides on the biofilm formation of three strains of *Staphylococcus aureus*. We used starch, cellulose, and a control group without polysaccharides. The results showed that starch and cellulose significantly reduced the biofilm formation of *S. aureus* ATCC25923 compared to the control group. Similarly, only starch decreased the biofilm formation of *S. aureus* NCTC 6571, while cellulose had no significant effect. However, none of the polysaccharides affected the biofilm formation of *S. aureus* ATCC6538. These findings suggest that polysaccharides may have strain-specific effects on the biofilm formation of *S. aureus* and may be useful for preventing or treating biofilm-related infections (**Fig. 5**).

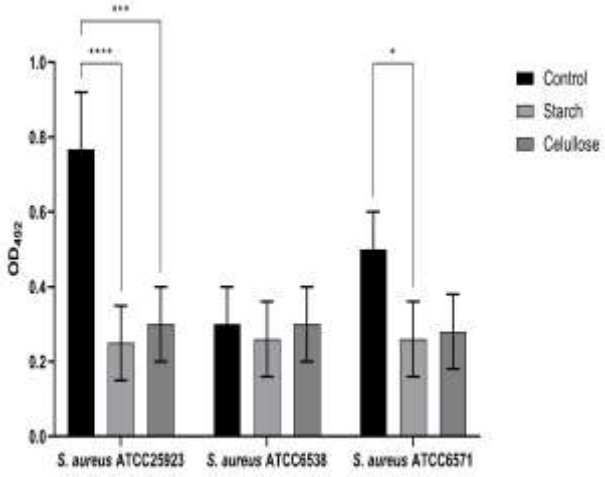

Fig. (5): Effect of different polysaccharides on biofilm formation of the three selected *Staphylococcus* strains. Asterisks (*) represent a significant difference between the selected groups using two-way ANOVA at $p<0.05$.

The starch reduced the biofilm formation of the *Staphylococcus* strains in a concentration-dependent manner

One of the objectives of this study was to evaluate the effect of different concentrations of starch on the biofilm formation of *Staphylococcus* strains. Starch is a polysaccharide that can interfere with bacterial attachment to surfaces and prevent biofilm formation. The results showed that the best-affected concentration of starch was 2gm for the three selected strains. However, the other concentrations of starch (0.5, 1, and 1.5gm) had a significant effect only on *S. aureus* ATCC25923. These findings suggest that starch has a concentration-

dependent effect on the biofilm formation of *Staphylococcus* strains and that 2gm is the optimal concentration for inhibiting these processes (**Fig. 6**).

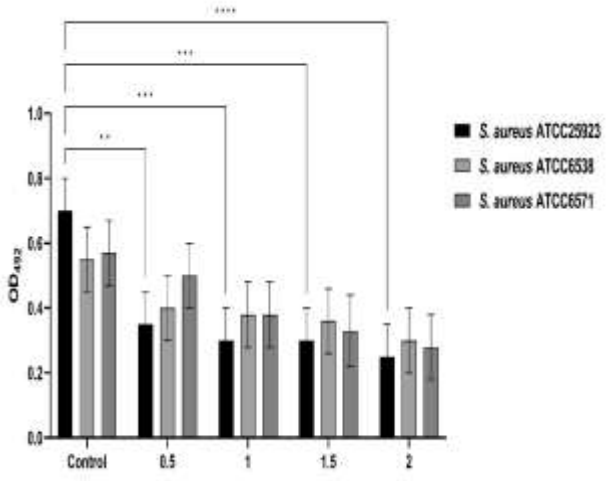

Fig. (6): Effect of different starch concentrations on biofilm formation of the three selected Staphylococcus strains. Asterisks (*) represent a significant difference between the selected groups using two-way ANOVA at $p<0.05$.

Acidogenicity of different *Staphylococcus aureus* during biofilm formation

We measured the pH reduction in LB broth containing different types of carbohydrates after 24 hours of incubation with *Staphylococcus* strains. The results suggest that the four types of carbohydrates (glucose, sucrose, lactose, and starch) were less acidogenic than the control group (LB broth without any added carbohydrates). However, the differences in pH reduction were non-significant. It's also no significant differences have been recorded between different strains of *Staphylococcus*. Therefore, further studies may be needed to investigate the acidogenicity of different carbohydrates in various strains of *Staphylococcus* and to determine whether any significant differences exist **(Table 1)**.

Table (1): Acidogenicity of different *Staphylococcus aureus* during biofilm formation

Types of Sugar	*S. aureus* ATCC25923	*S. aureus* ATCC6538	*S. aureus* NCTC6571
Control	5.3±0.87	4.6±0.65	4.6±1.2
Glucose	6.43±0.8	6.5±0.75	6.5±0.8
Sucrose	5.7±0.15	6.4±0.61	6.3±1.02
Starch	5.7±1.2	6.3±1.3	5.7±1.25

The mean value plus standard deviations from three independent analyses are shown. Two-way ANOVA at $p<0.05$ between the selected groups has been done.

Discussion:

In this study, we compared the adherence and biofilm formation of three standard *Staphylococcal* strains obtained from different sources: *S. aureus* ATCC25923, *S. aureus* ATCC6538, and *S. aureus* NCTC6571. We found that these strains exhibited different behaviors depending on the culture conditions and the source of isolation. Our results showed that *S. aureus* NCTC6571 had the highest biofilm formation in overnight culture, which is consistent with previous studies that reported this strain as a strong biofilm producer (**Sharma *et al.*, 2019; Fazeli *et al.*, 2020; Tsai *et al.*, 2020; Lahiri *et al.*, 2021)**. In addition, this strain was isolated from a human wound infection and may have acquired specific adaptations to survive and persist in the host environment. In contrast, *S. aureus* ATCC25923 and *S.*

aureus ATCC6538 had lower biofilm formation in overnight culture than in fresh culture. Probably because these strains were isolated from human blood and nasal swabs, respectively, and may have different metabolic requirements and stress responses than *S. aureus* NCTC6571. These findings might be due to the age and nutrient availability of the culture medium, which would affect the expression of biofilm-related genes and proteins in these strains (**Abadi *et al.*, 2021; Liu *et al.*, 2021; Wang *et al.*, 2021; Yang *et al.*, 2021; Bhattacharya *et al.*, 2022).**

We then investigated the antibacterial effects of three medicinal plants, *Eucalyptus globules, Nigella sativa*, and *Trigonella foenum*, on the adhesion and biofilm formation of *Staphylococcus* strains. Our results showed that all plant extracts inhibited bacterial biofilm formation, but *Nigella sativa* was the most effective against *S. aureus* NCTC6571. This suggests that *Nigella sativa* contains bioactive compounds that can interfere with the quorum sensing and virulence factors of *S. aureus*, which are responsible for its biofilm formation and pathogenicity. Our findings are consistent with previous studies that reported the antibacterial activity of some medicinal plants against various *Staphylococcus* strains. A study by **Ehsanollah *et al.*, (2009)** evaluated the anti-invasive activities of an oil-based di-herbal extract against methicillin-resistant S*taphylococcus aureus* (MRSA). The di-herbal extract was prepared from two herbs, namely *Echinacea purpurea* and *Rumex acetosa*, using a Soxhlet apparatus. The oil-based di-herbal extract was found to be effective in inhibiting the invasion of MRSA in vitro. The study suggests that the di-herbal extract could potentially be used as a natural remedy to prevent and treat MRSA infections. To the best of our knowledge, this is the first study to demonstrate the effect of *Nigella sativa* on the biofilm formation of *S. aureus* NCTC6571. Therefore, our study contributes to the understanding of the mechanisms and potential applications of *Nigella sativa* as a natural alternative for preventing and treating *Staphylococcal* infections.

The present study demonstrated the effect of different monosaccharides on the anti-biofilm formation of *Staphylococcus* strains. The results revealed that the monosaccharides glucose, fructose, and xylose had a significant inhibitory effect on the biofilm formation of *S. aureus* ATCC25923, a methicillin-sensitive strain. However, only glucose and fructose showed a similar effect on *S. aureus* NCTC 6571, a methicillin-resistant strain. Moreover, none of the monosaccharides had any effect on *S. aureus* ATCC6538, a biofilm-producing strain. These results indicate that the susceptibility of different *S. aureus* strains to monosaccharides may

depend on their biofilm formation ability and their resistance to antibiotics. The study also investigated the effect of three disaccharides: sucrose, maltose, and lactose on the biofilm formation of the three *Staphylococcus* strains. The results showed that all three disaccharides reduced the biofilm formation of *S. aureus* ATCC25923 compared to the control group. Similarly, sucrose and maltose decreased the biofilm formation of *S. aureus* NCTC 6571, while lactose had no significant effect. However, none of the disaccharides had any effect on *S. aureus* ATCC6538. These results suggest that different monosaccharides and disaccharides have different mechanisms of action on the anti-biofilm formation of *Staphylococcus* strains and that glucose, fructose, sucrose, and maltose are more potent than xylose, and lactose in inhibiting these processes. The mechanisms underlying these differences are not clear but may involve variations in various proteins related to biofilm formation, and quorum sensing **(Chen *et al.*, 2021; Muhammad *et al.*, 2021; Alreshidi *et al.*, 2022).**

Our results showed that starch and cellulose significantly reduced the biofilm formation of *S. aureus* ATCC25923 compared to the control group. Similarly, only starch decreased the biofilm formation of *S. aureus* NCTC 6571, while cellulose had no significant effect. These findings are consistent with some previous studies that reported the inhibitory effects of polysaccharides on bacterial biofilms **(Zuo *et al.*, 2021; Al-Masaudi *et al.*, 2022; Ashraf *et al.*, 2022; Li *et al.*, 2022).** These differences in the effects of polysaccharides on biofilm formation may depend on various factors, such as the type and concentration of polysaccharides, the bacterial strain and species, and the environmental conditions**.**

We tested four concentrations of starch (0.5, 1, 1.5 and 2gm) on the three *Staphylococcus* strains. The results showed that the best-affected concentration of starch was 2gm for the three selected strains. However, the other concentrations of starch (0.5, 1 and 1.5gm) had a significant effect only on *S. aureus* ATCC25923. These findings suggest that starch has a concentration-dependent effect on the biofilm formation of *Staphylococcus* strains and that 2gm is the optimal concentration for inhibiting these processes. The mechanism by which starch inhibits the anti-adhesion and biofilm formation of *Staphylococcus* strains is not clear and requires further investigation. Starch may affect the expression or function of bacterial adherence, such as protein A, fibronectin-binding proteins, or intercellular sticky molecules **(Moon *et al.*, 2020)**. It is also possible that starch affects the synthesis or composition of extracellular polymeric substances (EPS),

which are essential for biofilm stability and protection. Moreover, starch may modulate the quorum sensing system of *Staphylococcus* strains, which regulates the biofilm formation and virulence factors.

We hypothesized that the addition of carbohydrates would increase the acid production by *Staphylococcus* and lower the pH of the medium. We also hypothesized that there would be differences in acidogenicity among different strains of *Staphylococcus* and different types of carbohydrates. To test these hypotheses, we measured the pH of LB broth containing glucose, sucrose, lactose, starch, or no carbohydrates after 24 hours of incubation with four *Staphylococcus* strains. The results showed that all four types of carbohydrates were less acidogenic than the control group, as indicated by the higher pH values. However, the differences in pH increase were not statistically significant among the carbohydrate groups or the *Staphylococcus* strains. This suggests that the acid production by *Staphylococcus* was not influenced by the type of carbohydrate or by the strain of bacteria. These findings are inconsistent with our hypotheses and with some previous studies that reported significant differences in acidogenicity among different carbohydrates and among different *Staphylococcus* strains **(Harada *et al*., 2021; Salminen *et al*., 2021; Cao et al., 2022).** There are several possible explanations for the lack of significant differences in our study. One is that the concentration of carbohydrates used in our study was too low to elicit a significant response from *Staphylococcus*. Another is that the incubation time of 24 hours was not enough to detect any differences in acid production. A third is that the LB broth used as the medium was already acidic and buffered the effect of carbohydrate fermentation. A fourth is that there were other factors affecting the pH reduction, such as bacterial growth, oxygen consumption, or other metabolic products. Therefore, further studies are needed to investigate the acidogenicity of different carbohydrates in various strains of *Staphylococcus* and to determine whether any significant differences exist. Future studies should use higher concentrations of carbohydrates, longer incubation times, different media, and more sensitive methods to measure pH and other parameters. These studies may provide more insight into the metabolic diversity and adaptation of *Staphylococcus* to different environmental conditions.

Our study provides new insights into the variability and plasticity of *Staphylococcal* biofilm formation under different conditions. We demonstrated that the source, age, and culture conditions of the *Staphylococcal* strains affect their biofilm formation abilities, which may have implications for their pathogenicity and

antibiotic resistance. Moreover, the results suggest that monosaccharides and disaccharides may be used as natural agents to inhibit the biofilm formation of *Staphylococcus* strains, especially those that are sensitive to methicillin. Other implications of this study are that starch could be used as a natural anti-biofilm agent against *Staphylococcus* infections. Starch is cheap, abundant, biodegradable, and non-toxic to humans and animals. Starch could be applied as a coating on medical devices or implants to prevent bacterial colonization and infection. Starch could also be incorporated into wound dressings or topical formulations to enhance wound healing and prevent biofilm-related complications. However, more studies are needed to confirm the efficacy and safety of starch in vivo and to optimize its formulation and delivery.

Conclusion

In conclusion, this study demonstrates that the source, age, and culture conditions of *Staphylococcal* strains can affect their biofilm formation ability. Moreover, extracts from *Eucalyptus globules, Nigella sativa*, and *Trigonella foenum*, as well as different types of monosaccharides, disaccharides, and polysaccharides, can reduce the biofilm formation of *Staphylococcus* strains, with varying degrees of efficacy. Starch was found to have a concentration-dependent effect on the anti-adhesion and biofilm formation of *Staphylococcus* strains, with 2gm being the optimal concentration for inhibiting these processes. Finally, the study found that different types of carbohydrates can induce acidification in LB broth containing *Staphylococcus* strains, but further research is needed to investigate the acidogenicity of different carbohydrates in various strains of *Staphylococcus*. Overall, these findings suggest that natural products and carbohydrates may be useful in preventing and treating Staphylococcal infections and could serve as potential alternatives to conventional antibiotics.

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الكربوهيدرات والمستخلصات النباتية كبدائل طبيعية لمنع تكون الأغشية الحيوية للمكورات العنقودية الذهبية

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المكورات العنقودية الذهبية هي أحد مسببات الأمراض البكتيرية الشائعة التي يمكن أن تسبب عدوى مختلفة، بما في ذلك الالتهابات المرتبطة بالأغشية الحيوية التي يصعب علاجها. في هذه الدراسة، درسنا تأثيرات الكربوهيدرات المختلفة والمستخلصات النباتية على الالتصاق وتكوين الأغشية الحيوية لثلاث سلالات من المكورات العنقودية الذهبية. تم الحصول عليها من مصادر مختلفة. وجدنا أن الالتصاق وتشكيل الأغشية الحيوية للسلالات يتأثران بمصدرها وعمرها وظروفها البيئية. بالإضافة إلى ذلك، وجدنا أن مستخلص حبة البركة وبعض السكريات الأحادية، والسكريات الثنائية، والسكريات العديدة كانت فعالة في الحد من تكوين الأغشية الحيوية، مع درجة معينة من خصوصية الإجهاد. علاوة على ذلك، وجدنا أن النشا له تأثير يعتمد على التركيز وعلى مقاومة الالتصاق وتشكيل الأغشية الحيوية لسلالات المكورات العنقودية الذهبية. أخيرًا، تم فحص الحموضة للكربوهيدرات المختلفة في الوسط الغذائي LB ووجدنا أنها أقل حمضية من المجموعة الضابطة، ولكن مع عدم وجود فروق ذات دلالة إحصائية بين السلالات. تشير النتائج التي توصلنا إليها إلى أن المستخلصات النباتية وبعض الكربوهيدرات قد يكون لها القدرة على أن تكون بدائل طبيعية للوقاية من العدوى المرتبطة بالأغشية الحيوية التي تسببها بكتيريا المكورات العنقودية الذهبية وعلاجها.