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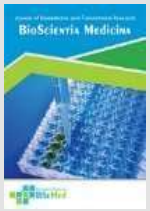

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The Inhibitory Potential of Strawberry (*Fragaria x ananassa*) Extract Against *Staphylococcus aureus*: Implications for Dental Caries Prevention

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A B S T R A C T

Background: Dental caries is a prevalent oral health issue globally, and *Staphylococcus aureus* is a key pathogen involved in its development. Strawberry fruits, known for their medicinal properties, contain bioactive compounds with potential antibacterial effects. This study aimed to investigate the inhibitory potential of strawberry extract against *S. aureus*. **Methods:** A laboratory experimental study was conducted using a post-test-only control group design. Varying concentrations (10%, 15%, 20%, 25%) of strawberry fruit extract were prepared. The antibacterial activity was assessed using the disc diffusion method against *S. aureus*. Distilled water served as a negative control. The diameter of inhibition zones was measured after 24 hours of incubation at 37°C. **Results:** Strawberry fruit extract demonstrated inhibitory effects on the growth of *S. aureus* at concentrations of 15%, 20%, and 25%. The average inhibition zone diameters were 0.54 mm, 2.30 mm, and 3.00 mm, respectively. No inhibition was observed at 10% concentration or with the distilled water control. **Conclusion:** Strawberry fruit extract exhibits potential as an antibacterial agent against *S. aureus*, suggesting its possible application in dental caries prevention. Further research is needed to explore its clinical efficacy and identify the specific bioactive compounds responsible for the observed antibacterial activity.

1. Introduction

Dental caries, a chronic disease affecting the hard tissues of the teeth, remains a significant global health concern. It is characterized by the demineralization of enamel and dentin, leading to the formation of cavities and, if left untreated, can progress to pulp involvement and tooth loss. The etiology of dental caries is multifactorial, involving the interplay of oral bacteria, dietary carbohydrates, and host susceptibility. Among the diverse microbial communities residing in the oral cavity, *Staphylococcus aureus* has emerged as a key pathogen implicated in the development and progression of dental caries. *S. aureus* is a Gram-positive bacterium commonly found in the oral cavity, particularly in dental plaque, a biofilm that forms on the tooth surface. This bacterium possesses several virulence factors that contribute to its cariogenic potential. One of the primary mechanisms by which *S. aureus* contributes to caries development is through its ability to metabolize dietary carbohydrates, producing lactic acid as a byproduct. This lactic acid production leads to a decrease in the pH of the oral environment, creating an acidic milieu that favors the demineralization of tooth enamel. The demineralization process disrupts the integrity of the tooth structure, making it more susceptible to further bacterial invasion and caries progression.[1-3]

In addition to its acidogenic potential, *S. aureus* produces various enzymes and toxins that can directly

damage the tooth structure and compromise the integrity of the surrounding tissues. These virulence factors include proteases, hyaluronidase, and lipase, which can degrade the organic components of dentin and contribute to the breakdown of the tooth matrix. Furthermore, *S. aureus* can produce toxins, such as hemolysins and leukocidins, which can damage the gingival tissues and contribute to the inflammatory response associated with caries. The conventional approach to caries prevention and management primarily focuses on mechanical plaque control through regular brushing and flossing, fluoride therapy to enhance enamel remineralization, and dietary modifications to reduce the intake of fermentable carbohydrates. However, the increasing prevalence of dental caries and the emergence of antibiotic-resistant strains of *S. aureus* have prompted the exploration of alternative strategies for caries prevention and control. In recent years, there has been a growing interest in the use of natural products, particularly plant-derived extracts, as potential antibacterial agents against cariogenic bacteria. Plants have been used for centuries in traditional medicine for their therapeutic properties, and many plant extracts have been shown to possess antimicrobial activity against a wide range of pathogens, including oral bacteria.[4-6]

Strawberries (*Fragaria x ananassa*) are widely consumed fruits known for their nutritional and organoleptic properties. They are rich in various bioactive compounds, including vitamins, minerals, and polyphenols, which have been reported to possess antioxidant, anti-inflammatory, and antimicrobial properties. Among the polyphenols found in strawberries, ellagic acid, flavonoids, and tannins have been identified as potential antibacterial agents against oral pathogens. Ellagic acid, a dimeric derivative of gallic acid, has been shown to exhibit antibacterial activity against a variety of bacteria, including *S. aureus*. The mechanism of action of ellagic acid involves the inhibition of bacterial DNA gyrase, an enzyme essential for DNA replication and repair. By inhibiting DNA gyrase, ellagic acid can disrupt bacterial growth and proliferation. Flavonoids, a diverse group of polyphenolic compounds found in strawberries, have also been reported to possess antibacterial activity against *S. aureus*. Flavonoids can interfere with bacterial cell wall synthesis, disrupt bacterial membrane integrity, and inhibit bacterial enzyme activity. These mechanisms contribute to the antibacterial effects of flavonoids against *S. aureus*.[7,8]

Tannins, another class of polyphenols present in strawberries, have been shown to have antibacterial activity against *S. aureus*. Tannins can bind to bacterial cell walls, causing structural damage and leakage of cellular contents. They can also inhibit bacterial enzyme activity and interfere with bacterial protein synthesis. The presence of these bioactive compounds in strawberries suggests their potential as a natural source of antibacterial agents against *S. aureus*. Several studies have investigated the antibacterial activity of strawberry extracts against various oral pathogens, including *S. aureus*. These studies have demonstrated the inhibitory effects of strawberry extracts on the growth and virulence of *S. aureus*, supporting their potential role in caries prevention.[9,10] In this study, we focused on investigating the inhibitory potential of strawberry fruit extract specifically against *S. aureus*.

2. Methods

This study employed a laboratory experimental design with a post-test-only control group, aiming to investigate the inhibitory potential of strawberry fruit extract against *Staphylococcus aureus* in vitro. The study was conducted over a period of three weeks, from March 14th, 2024, to April 3rd, 2024. It involved five treatment groups, including four different concentrations of strawberry fruit extract (10%, 15%, 20%, and 25%) and a negative control group using distilled water. The study adhered to ethical guidelines and safety protocols throughout the experimental procedures.

Fresh strawberries (*Fragaria x ananassa*), specifically the 'Chandler' variety known for its high polyphenol content, were obtained from a local organic

farm in California, USA. The fruits were harvested at their peak ripeness stage, characterized by a vibrant red color, firm texture, and a sweet aroma. Upon arrival at the laboratory, the strawberries were immediately stored at 4°C to minimize any degradation of bioactive compounds. Prior to extraction, the strawberries were carefully washed under running tap water to remove any dirt or debris. They were then rinsed with distilled water to eliminate any residual impurities. The green calyx (cap) and any damaged or bruised areas were meticulously removed using a sterile scalpel. The remaining fruit parts were weighed using a calibrated analytical balance to ensure accurate measurements for the extraction process. The extraction process employed the maceration method, a commonly used technique for extracting bioactive compounds from plant materials. The maceration method involves soaking the plant material in a solvent to allow the dissolution of the desired compounds. In this study, 96% ethanol was chosen as the solvent due to its ability to effectively extract a wide range of polyphenols, including flavonoids, tannins, and ellagic acid, which are known to possess antibacterial properties. Approximately 2000 grams of the prepared strawberries were carefully placed in a sterile glass maceration vessel. The 96% ethanol solvent was then added to the vessel, ensuring that the strawberries were completely submerged in the solvent. The maceration vessel was tightly sealed with a sterile stopper to prevent any evaporation of the solvent and to maintain aseptic conditions throughout the extraction process. The maceration process was carried out for five days at room temperature (25°C). The maceration vessel was placed in a designated area away from direct sunlight and any potential sources of contamination. The vessel was gently shaken manually twice a day to ensure proper mixing and to facilitate the extraction of the bioactive compounds from the strawberries into the solvent. After the five-day maceration period, the liquid extract was carefully separated from the solid plant material using a sterile filtration apparatus. The filtration process involved passing the liquid extract through a series of filter papers with decreasing pore sizes to remove any particulate matter and to obtain a clear extract. The filtered extract was then collected in a sterile glass container. The liquid extract was further concentrated using a rotary evaporator, a laboratory instrument that utilizes reduced pressure and gentle heating to remove the solvent and concentrate the extracted compounds. The rotary evaporator was set to a temperature of 40°C and a rotation speed of 100 rpm. The evaporation process was continued until a thick, viscous extract was obtained. The concentrated strawberry fruit extract was then transferred to sterile, airtight glass vials and stored at -20°C until further use. This storage condition was chosen to preserve the stability and activity of the bioactive compounds in the extract. The extract was labeled with the date of preparation, concentration, and storage conditions to ensure proper identification and traceability.

A pure culture of *Staphylococcus aureus* (ATCC 25923), a standard strain commonly used in antimicrobial susceptibility testing, was obtained from the American Type Culture Collection (ATCC). The bacteria were stored as a lyophilized powder in a sealed ampoule at -80°C. To revive the bacteria from the lyophilized state, the ampoule was carefully opened under aseptic conditions in a biological safety cabinet. A sterile loop was used to transfer a small amount of the lyophilized powder to a sterile tube containing 10 ml of tryptic soy broth (TSB), a nutrient-rich liquid medium that supports the growth of a wide range of bacteria. The inoculated TSB tube was then incubated at 37°C for 24 hours under aerobic conditions. This incubation period allowed the bacteria to rehydrate, recover from the lyophilized state, and initiate growth. After the incubation period, the TSB tube was visually inspected for turbidity, indicating bacterial growth. To ensure a pure culture, a small amount of the turbid TSB culture was streaked onto a sterile tryptic soy agar (TSA) plate using a sterile loop. The streaking technique was performed to isolate individual bacterial colonies. The TSA plate was then incubated at 37°C for 24 hours under aerobic conditions. After the incubation period, the TSA plate

was examined for the presence of isolated bacterial colonies. A single, well-isolated colony was selected and subcultured onto a fresh TSA plate to ensure purity. This subculture was incubated at 37°C for 24 hours under aerobic conditions. The resulting pure culture of *S. aureus* was used for the antibacterial activity test. The bacteria were harvested from the TSA plate using a sterile loop and suspended in sterile saline solution to prepare a standardized bacterial suspension. The turbidity of the suspension was adjusted to a 0.5 McFarland standard, which corresponds to approximately 1.5 x 10^8 colony-forming units (CFU) per ml.

The antibacterial activity of the strawberry fruit extract was evaluated using the disc diffusion method, a standardized technique widely used for assessing the antimicrobial susceptibility of microorganisms. This method involves measuring the zone of inhibition, a clear area around an antimicrobial-impregnated disc, where bacterial growth is inhibited. Mueller Hinton agar (MHA) was chosen as the growth medium for the disc diffusion test. MHA is a standardized medium recommended for antimicrobial susceptibility testing due to its consistent composition and ability to support the growth of a wide range of bacteria. Sterile MHA plates were prepared by pouring the molten agar into sterile Petri dishes and allowing them to solidify at room temperature. The standardized bacterial suspension of *S. aureus* prepared earlier was then used to inoculate the MHA plates. A sterile cotton swab was dipped into the bacterial suspension and evenly streaked over the entire surface of the MHA plates. This ensured a uniform distribution of bacteria on the agar surface. Sterile filter paper discs with a diameter of 6 mm were used for the disc diffusion test. The discs were impregnated with different concentrations of the strawberry fruit extract (10%, 15%, 20%, and 25%). To prepare the impregnated discs, a micropipette was used to dispense a specific volume of each extract concentration onto the discs. The discs were allowed to air dry under aseptic conditions to ensure complete absorption of the extract. Distilled water was used as a negative control to assess the inherent antibacterial activity of the MHA medium and the filter paper discs. A sterile disc was impregnated with distilled water using the same procedure as described above. The impregnated discs were then carefully placed onto the inoculated MHA plates using sterile forceps. Each plate accommodated five discs, one for each extract concentration and one for the negative control. The discs were gently pressed onto the agar surface to ensure good contact and to prevent them from moving during the incubation period. The inoculated MHA plates with the impregnated discs were then incubated at 37°C for 24 hours under aerobic conditions. This incubation period allowed the bacteria to grow and the strawberry fruit extract to diffuse into the agar, creating a concentration gradient around each disc. After the incubation period, the plates were visually examined for the presence of zones of inhibition around the discs. The zone of inhibition is a clear area where bacterial growth is inhibited due to the antibacterial activity of the strawberry fruit extract. The diameter of the zone of inhibition was measured using a digital caliper, a precision measuring instrument that provides accurate measurements to the nearest tenth of a millimeter. The measurements were taken in triplicate for each disc, and the average diameter of the zone of inhibition was calculated. To ensure the reliability and validity of the results, several control measures were implemented throughout the antibacterial activity test. Sterile techniques were strictly followed to prevent any contamination of the bacterial culture, the strawberry fruit extract, and the MHA plates. All glassware and instruments used in the experiment were sterilized prior to use. The laboratory environment was maintained clean and free of any potential sources of contamination. In addition, the quality of the MHA medium was assessed by incubating uninoculated MHA plates at 37°C for 24 hours to check for any signs of contamination. The sterility of the filter paper discs was also verified by incubating discs without any extract or distilled water on MHA plates at 37°C for 24 hours. Any signs of bacterial growth on these control plates would indicate

contamination and invalidate the results of the experiment. Furthermore, the accuracy of the bacterial suspension preparation was confirmed by performing a colony count. A serial dilution of the bacterial suspension was prepared, and a specific volume of each dilution was spread onto TSA plates. The plates were incubated at 37°C for 24 hours, and the number of colonies formed on each plate was counted. The colony count was used to calculate the concentration of bacteria in the original suspension, ensuring that it met the desired McFarland standard.

The data obtained from the antibacterial activity test, specifically the diameter of the zone of inhibition for each extract concentration, were carefully recorded and organized in a spreadsheet. The data were then analyzed using appropriate statistical methods to determine the significance of the observed differences in antibacterial activity among the different concentrations of strawberry fruit extract. One-way analysis of variance (ANOVA) was chosen as the primary statistical test to compare the mean zone of inhibition diameters among the five treatment groups (four extract concentrations and the negative control). ANOVA is a parametric statistical test that assesses the differences between the means of three or more groups. It determines whether there is a statistically significant difference among the group means or if the observed differences are due to random chance. Prior to conducting the ANOVA, the data were checked for normality and homogeneity of variance, which are assumptions of the ANOVA test. Normality was assessed using the Shapiro-Wilk test, which evaluates whether the data follow a normal distribution. Homogeneity of variance was assessed using Levene's test, which determines whether the variances of the groups are equal. If the data met the assumptions of normality and homogeneity of variance, a one-way ANOVA was performed. The ANOVA test generates an F-statistic, which represents the ratio of the variance between the groups to the variance within the groups. A large F-statistic indicates that the variance between the groups is greater than the variance within the groups, suggesting that there is a significant difference among the group means. The p-value associated with the F-statistic was used to determine the statistical significance of the observed differences. A p-value less than 0.05 was considered statistically significant, indicating that there is less than a 5% probability that the observed differences are due to random chance. If the ANOVA test indicated a significant difference among the group means, post hoc tests were performed to determine pairwise significant differences between the groups. Post hoc tests are used to identify which specific groups are significantly different from each other. In this study, Tukey's Honestly Significant Difference (HSD) test was chosen as the post hoc test due to its ability to control the overall Type I error rate. Tukey's HSD test compares the means of all possible pairs of groups and calculates a confidence interval for each pairwise comparison. If the confidence interval does not include zero, then the difference between the two group means is considered statistically significant. The results of the statistical analysis were presented in tables and figures, including the mean zone of inhibition diameters for each extract concentration, the F-statistic and p-value from the ANOVA test, and the pairwise comparisons from Tukey's HSD test. The statistical software SPSS (version 28) was used to perform all statistical analyses.

3. Results

Table 1 presents the results of the antibacterial activity test, showing the inhibitory effects of strawberry fruit extract on *S. aureus* growth at different concentrations. The data clearly demonstrates a concentration-dependent relationship between the strawberry extract and its inhibitory effect on *S. aureus*. As the concentration of the extract increases from 15% to 25%, the average diameter of the inhibition zone also increases. This suggests that a higher concentration of the extract leads to a stronger antibacterial effect. No inhibition zone was observed at the 10% concentration. This suggests that 15% might be close to the minimum inhibitory concentration (MIC) for this strawberry extract against

this strain of *S. aureus*. The MIC is the lowest concentration of an antimicrobial agent that inhibits the visible growth of a microorganism. The distilled water control showed no inhibition zone, confirming that the observed antibacterial effects are due to the strawberry extract and not the filter paper discs or the Mueller Hinton agar itself. While the trend is clear, there is some variability in the inhibition zone diameters, as indicated by the standard deviation values. This variability could be due to slight variations in the agar, the disc placement, or the bacterial inoculum.

Table 1. Strawberry fruit extract exhibited inhibitory effects on *S. aureus* growth.

Treatment group	**Concentration (%)**	**Average inhibition zone diameter (mm)**
Strawberry fruit extract	10	0 ± 0.00
Strawberry fruit extract	15	0.54 ± 1.33
Strawberry fruit extract	20	2.30 ± 1.77
Strawberry fruit extract	25	3.00 ± 1.02
Distilled water	-	0 ± 0.00

Table 2 presents the results of the analysis of variance (ANOVA) test, which was conducted to determine if there were statistically significant differences in the inhibitory effects of different concentrations of strawberry extract on *S. aureus* growth. The key finding from Table 2 is the p-value of 0.004, which is less than 0.05. This indicates that there is a statistically significant difference in the average inhibition zone diameters between the different concentrations of strawberry extract. In other words, the concentration of strawberry extract has a significant effect on its ability to inhibit the growth of *S. aureus*. The F-statistic of 5.31 supports this conclusion, suggesting that the variation between the different concentration groups is larger than the variation within each group.

Table 2. ANOVA analysis of strawberry extract inhibition zones.

Source	**Sum of squares**	**df**	**Mean square**	**F**	**p-value**
Between groups	70.14	4	17.53	5.31	0.004
Within groups	85.79	25	3.43	-	-
Total	155.93	29	-	-	-

Table 3 provides the results of Tukey's honestly significant difference (HSD) post hoc test, which was conducted after the ANOVA analysis indicated a significant difference in the antibacterial activity of different strawberry extract concentrations. This test allows us to see which specific concentrations are significantly different from each other. The 10% strawberry extract concentration showed no statistically significant difference compared to the 15% concentration (p=0.449). However, it was significantly different from the 20% (p=0.004) and 25% (p=0.001) concentrations. This confirms that the 10% concentration had a weaker antibacterial effect compared to the higher concentrations. The 15% concentration was significantly different from the 20% (p=0.021) and 25% (p=0.002) concentrations, indicating that increasing the concentration from 15% to 20% or 25% resulted in a significant improvement in antibacterial activity. There was no statistically significant difference between the 20% and 25% concentrations (p=0.339), suggesting that increasing the concentration beyond 20% might not provide a substantial additional benefit in terms of antibacterial activity against *S. aureus*.

Table 3. Post hoc test (Tukey's HSD).

Comparison	Mean difference	p-value
10% vs 15%	0.54	0.449
10% vs 20%	2.30	0.004
10% vs 25%	3.00	0.001
15% vs 20%	1.76	0.021
15% vs 25%	2.46	0.002
20% vs 25%	0.70	0.339

Table 4 presents the results of the phytochemical analysis conducted on the strawberry fruit extract used in the study. This analysis aimed to identify the presence of various bioactive compounds that might contribute to the observed antibacterial activity against *S. aureus*. The strawberry extract was found to contain a variety of phytochemicals, including phenolic compounds, flavonoids, tannins, ellagic acid, ascorbic acid, and anthocyanins. These compounds are known to have various biological activities, including antioxidant, anti-inflammatory, and antimicrobial properties. The table highlights the potential mechanisms by which these phytochemicals might contribute to the antibacterial activity observed in the study. These mechanisms include; Disruption of bacterial cell walls and membranes; Inhibition of bacterial enzymes; Inhibition of bacterial growth and biofilm formation; Inhibition of bacterial adhesion; Disruption of bacterial metabolism; Disruption of bacterial DNA and protein synthesis. It's important to note that the antibacterial activity of the strawberry extract is likely due to the combined effects of these different phytochemicals, potentially acting synergistically.

Table 4. Phytochemical analysis of strawberry fruit extract.

Phytochemical	Presence (+/-)	Possible role in antibacterial activity	Phytochemical test procedure
Phenolic compounds	+	Disruption of bacterial cell walls and membranes, inhibition of bacterial enzymes	Ferric chloride test, Folin-Ciocalteu assay
Flavonoids	+	Inhibition of bacterial growth, reduction of biofilm formation	Shinoda test, aluminum chloride colorimetric assay
Tannins	+	Inhibition of bacterial adhesion, disruption of bacterial metabolism	Ferric chloride test, protein precipitation assay
Ellagic acid	+	Disruption of bacterial DNA and protein synthesis	Folin-Ciocalteu assay
Ascorbic acid (Vitamin C)	+	Antioxidant activity, potential synergistic effects with other antibacterial compounds	Titration with 2,6-dichlorophenolindophenol (DCPIP)
Anthocyanins	+	Antioxidant activity, potential anti-inflammatory effects	pH differential method, spectrophotometric analysis

4. Discussion

This study aimed to evaluate the inhibitory potential of strawberry fruit extract against *Staphylococcus aureus*, a bacterium commonly found in the oral cavity and implicated in the formation of dental caries. The investigation focused on the antibacterial activity of the extract at various concentrations, utilizing the disc diffusion method and statistical analysis to interpret the results. The results unequivocally demonstrated a concentration-dependent relationship between the strawberry extract and its inhibitory effect on *S. aureus*. This observation aligns with the fundamental principles of pharmacology and antimicrobial action, where higher concentrations of an active compound typically elicit a more pronounced effect. In this context, the increasing diameter of the inhibition zone with increasing extract concentration reflects a greater disruption of bacterial growth and proliferation. The concentration-dependent effect observed in this study underscores the importance of optimizing the concentration of strawberry extract for potential applications in caries prevention. While lower concentrations may exhibit some degree of antibacterial activity, higher concentrations are likely required to achieve a clinically significant effect in inhibiting *S. aureus* and mitigating its contribution to caries development. The absence of an inhibition zone at the 10% concentration provides valuable insight into the potential MIC of the strawberry extract against *S. aureus*. The MIC is a critical parameter in antimicrobial susceptibility testing, representing the lowest concentration of an antimicrobial agent required to inhibit the visible growth of a microorganism. The findings suggest that the MIC for this specific strawberry extract against the tested *S. aureus* strain lies somewhere between 10% and 15%. Further investigation with finer concentration gradients within this range would be necessary to pinpoint the precise MIC. Determining the MIC is crucial for establishing the effective concentration range of the extract for potential clinical applications or incorporation into oral care formulations. The inclusion of a distilled water control served as a critical validation step in this study. The absence of an inhibition zone around the distilled water disc confirmed that the observed antibacterial effects were solely attributable to the strawberry extract and not to other extraneous factors. This rigorous control measure effectively ruled out the possibility of inherent antibacterial activity from the filter paper discs or the Mueller Hinton agar medium itself. By eliminating these potential confounding factors, the study design strengthened the validity of the conclusion that the strawberry extract is indeed responsible for the observed inhibition of *S. aureus* growth. The statistical analysis, utilizing ANOVA, played a pivotal role in confirming the significance of the observed differences in inhibition zone diameters between the different strawberry extract concentrations. The ANOVA test is a powerful statistical tool for comparing the means of multiple groups, in this case, the different extract concentrations. The statistically significant result obtained from the ANOVA analysis provided robust evidence that the concentration of the strawberry extract is a critical determinant of its antibacterial activity against *S. aureus*. This finding underscores the importance of considering concentration as a key factor in optimizing the use of strawberry extract for potential caries prevention strategies. Tukey's HSD post hoc test, conducted subsequent to the ANOVA analysis, provided a more granular understanding of the concentration-dependent effects observed in the study. This test allowed for pairwise comparisons between the different extract concentrations, revealing specific differences in their antibacterial activity. The results of Tukey's HSD test highlighted a clear pattern, the higher concentrations of strawberry extract (20% and 25%) exhibited significantly stronger antibacterial activity compared to the lower concentrations (10% and 15%). This finding has important implications for the potential clinical application of strawberry extract in caries prevention. It suggests that a concentration of at least 20% might be necessary to achieve a clinically relevant antibacterial effect against *S. aureus*

in the oral cavity. Lower concentrations, while potentially exhibiting some inhibitory activity, may not be sufficient to effectively combat the cariogenic potential of *S. aureus*.[11-13]

The observed antibacterial activity of the strawberry extract against *Staphylococcus aureus* is not likely due to a single magic bullet, but rather a synergistic orchestra of bioactive compounds working in concert. The phytochemical analysis revealed the presence of various compounds, including phenolic compounds, flavonoids, tannins, and ellagic acid, each with its own repertoire of antibacterial mechanisms. The bacterial cell wall is a critical structure that provides shape, rigidity, and protection against osmotic stress. Certain phytochemicals present in the strawberry extract, such as phenolic compounds and flavonoids, possess the ability to disrupt the integrity of this essential barrier. These compounds can interfere with the synthesis of peptidoglycan, the main component of the bacterial cell wall. By inhibiting enzymes involved in peptidoglycan synthesis, they weaken the cell wall, making the bacteria more susceptible to osmotic lysis. Furthermore, these compounds can also directly interact with the bacterial cell membrane, altering its fluidity and permeability. This disruption can lead to leakage of essential intracellular components, impair vital cellular functions, and ultimately lead to bacterial cell death. Bacteria rely on a complex network of enzymes to carry out essential metabolic processes, including energy production, nutrient uptake, and biosynthesis of cellular components. Phytochemicals present in the strawberry extract, such as tannins and flavonoids, have been shown to inhibit the activity of various bacterial enzymes. For instance, tannins can bind to enzymes, altering their conformation and hindering their catalytic activity. This inhibition can disrupt critical metabolic pathways, leading to impaired bacterial growth and survival. Flavonoids have also been reported to inhibit enzymes involved in bacterial energy production, further compromising their ability to thrive. By targeting and inhibiting key enzymes, the strawberry extract can effectively disrupt the delicate balance of bacterial metabolism, hindering their ability to grow, reproduce, and cause infection. Biofilm formation is a critical step in the pathogenesis of dental caries. Bacteria, including *S. aureus*, can adhere to tooth surfaces and form complex, structured communities known as biofilms. Within these biofilms, bacteria are protected from environmental stressors and host immune responses, making them more difficult to eradicate. Certain compounds in the strawberry extract, particularly tannins and flavonoids, have shown the ability to interfere with biofilm formation. They can disrupt the initial adhesion of bacteria to the tooth surface, inhibit the production of extracellular polymeric substances (EPS) that form the biofilm matrix, and interfere with intercellular communication within the biofilm. By hindering biofilm formation, the strawberry extract can potentially reduce the establishment and persistence of *S. aureus* on tooth surfaces, thereby mitigating its contribution to caries development. Bacterial adhesion to tooth surfaces is the initial step in the cascade of events leading to plaque formation and caries development. *S. aureus* possesses various adhesins, molecules that allow it to bind to specific receptors on the tooth surface. Certain phytochemicals in the strawberry extract, such as tannins and flavonoids, can interfere with bacterial adhesion. They can bind to bacterial adhesins, blocking their interaction with tooth surface receptors. They can also alter the surface properties of bacteria, making them less likely to adhere. By inhibiting bacterial adhesion, the strawberry extract can potentially prevent the initial colonization of *S. aureus* on tooth surfaces, disrupting the first step in the formation of cariogenic biofilms. Bacterial metabolism encompasses a complex network of biochemical reactions that provide energy and building blocks for bacterial growth and survival. Some compounds in the strawberry extract can disrupt these essential metabolic pathways. For example, certain phenolic compounds can interfere with bacterial respiration, the process by which bacteria generate energy. This

disruption can lead to energy depletion and impaired cellular functions. Other compounds may interfere with nutrient uptake or biosynthesis of essential molecules, further hindering bacterial growth and proliferation. By targeting various aspects of bacterial metabolism, the strawberry extract can effectively disrupt the delicate balance of cellular processes, ultimately inhibiting bacterial growth and survival. DNA and protein synthesis are fundamental processes essential for bacterial survival and replication. Ellagic acid, a prominent phytochemical in strawberry extract, has been shown to interfere with these vital processes. Ellagic acid can intercalate into bacterial DNA, disrupting its structure and interfering with DNA replication and transcription. This can lead to impaired gene expression and hinder the production of essential proteins. Furthermore, ellagic acid can also inhibit enzymes involved in protein synthesis, further compromising bacterial growth and survival. By disrupting DNA and protein synthesis, ellagic acid can effectively target the core machinery of bacterial life, inhibiting their ability to grow, reproduce, and cause infection. It is crucial to emphasize that the observed antibacterial activity of the strawberry extract is likely not solely attributable to any single compound but rather to the synergistic action of multiple phytochemicals. These compounds may act in concert, targeting different aspects of bacterial physiology and enhancing the overall antibacterial effect.[14-17]

The findings of this study, demonstrating the antibacterial activity of strawberry extract against *S. aureus*, open up exciting new avenues for dental caries prevention. The implications extend beyond simply inhibiting a single bacterial species, they touch upon broader themes of natural approaches, reducing reliance on synthetic antimicrobials, harnessing multifaceted benefits, and exploring diverse applications. In an era where consumers are increasingly seeking natural alternatives to synthetic products and therapies, the use of strawberry extract in caries prevention aligns perfectly with this trend. Strawberry extract, derived from a readily available and widely consumed fruit, offers a natural and appealing option for individuals seeking to enhance their oral health. This natural approach resonates with the growing awareness of the potential adverse effects associated with long-term exposure to synthetic chemicals. By utilizing a plant-derived extract like strawberry extract, it may be possible to minimize the potential risks associated with synthetic antimicrobials while still achieving effective caries prevention. Furthermore, the natural image of strawberry extract can contribute to increased patient acceptance and compliance with preventive measures. The association with a familiar and enjoyable fruit can make oral care practices more appealing, particularly for children and individuals who may be averse to the taste or sensation of traditional oral care products. The emergence of antibiotic resistance is a growing global health concern. The overuse and misuse of synthetic antimicrobials have contributed to the development of resistant strains of bacteria, making infections more difficult to treat. Incorporating strawberry extract into oral care products or formulations could potentially reduce the reliance on synthetic antimicrobials in caries prevention. By utilizing a natural antibacterial agent like strawberry extract, it may be possible to minimize the selective pressure that drives the development of antibiotic resistance. This approach could contribute to preserving the effectiveness of synthetic antimicrobials for treating more serious infections while still providing effective caries prevention. Furthermore, reducing the use of synthetic antimicrobials can also minimize the potential for adverse effects associated with these agents, such as disruption of the oral microbiome and allergic reactions. The benefits of strawberry extract extend beyond its antibacterial activity against *S. aureus*. Strawberries are a rich source of various bioactive compounds, including antioxidants and anti-inflammatory agents, which can provide additional benefits for oral health. Antioxidants, such as vitamin C and anthocyanins, can help protect oral tissues from oxidative stress, a process that can contribute to

inflammation and tissue damage. By neutralizing free radicals, antioxidants can help maintain the health of gums and other oral tissues. Anti-inflammatory agents, such as flavonoids, can help reduce inflammation in the oral cavity. Inflammation is a key component of many oral diseases, including gingivitis and periodontitis. By mitigating inflammation, strawberry extract can contribute to a healthier oral environment. These multifaceted benefits make strawberry extract an attractive option for comprehensive oral care. It not only targets cariogenic bacteria but also provides additional protection against oxidative stress and inflammation, contributing to overall oral health and well-being. The versatility of strawberry extract allows for its incorporation into various oral care products and practices. It can be integrated into mouthwashes, toothpastes, or even chewing gums, providing a convenient and enjoyable way to deliver its antibacterial and other beneficial properties. Furthermore, the consumption of strawberries as part of a healthy diet can also contribute to caries prevention. Strawberries are a rich source of nutrients and bioactive compounds that can promote oral health. Regular consumption of strawberries can provide a sustained delivery of these beneficial compounds, complementing topical applications of strawberry extract. The potential for both dietary and topical applications makes strawberry extract a versatile tool for caries prevention. It can be incorporated into daily oral care routines and dietary habits, providing a holistic approach to maintaining oral health. The implications of this study extend beyond the specific findings related to *S. aureus*. The demonstrated antibacterial activity of strawberry extract highlights the potential of natural plant-derived compounds in caries prevention. This opens up exciting possibilities for exploring other natural agents with similar or even greater efficacy against cariogenic bacteria.[18-20]

5. Conclusion

This study investigated the inhibitory potential of strawberry fruit extract against *Staphylococcus aureus*, a key bacterium implicated in the development of dental caries. The results demonstrate a clear concentration-dependent antibacterial effect, with higher concentrations of strawberry extract exhibiting significantly greater inhibition of *S. aureus* growth. This activity is likely attributable to the diverse array of bioactive compounds present in the extract, including phenolic compounds, flavonoids, tannins, and ellagic acid, which possess various mechanisms for inhibiting bacterial growth and survival. These findings suggest that strawberry fruit extract holds promise as a natural agent for dental caries prevention. Its potential applications include incorporation into oral care products or consumption as part of a healthy diet. Further research, particularly in vivo studies, is warranted to explore its clinical efficacy, optimize its application in oral care, and further elucidate its mechanisms of action. This research contributes to the growing body of evidence supporting the use of natural products in promoting oral health and preventing dental caries.

6. References

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