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Research Article

Antibacterial Activity of Toothpaste Containing Sunflower Seed Extract against Staphylococcus aureus In Vitro

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Abstract

The increasing prevalence of oral bacterial infections and growing concerns regarding the long-term use of synthetic antibacterial agents have encouraged the exploration of natural alternatives in oral care products. This study aimed to evaluate the antibacterial effectiveness of toothpaste containing sunflower seed extract (Helianthus annuus Linn.) against Staphylococcus aureus ATCC® 29213 in vitro. Sunflower seeds were extracted using maceration, followed by phytochemical screening to identify bioactive secondary metabolites. The extract was formulated into toothpaste at concentrations of 2.5%, 5%, and 10%. A commercial antibacterial toothpaste (Siwak-F) was used as a positive control, while toothpaste base without antibacterial agents served as a negative control. Physicochemical characteristics of the formulations, including organoleptic properties, homogeneity, pH, spreadability, and viscosity, were evaluated to ensure formulation quality. Antibacterial activity was assessed using the agar well diffusion method on Mueller–Hinton Agar, and inhibition zone diameters were measured after 24 hours of incubation at 37°C. Statistical analysis was performed using the Kruskal–Wallis test followed by the Mann–Whitney post hoc test. The results demonstrated a concentration-dependent increase in antibacterial activity. Toothpaste containing 10% sunflower seed extract produced the largest inhibition zone and exhibited antibacterial activity comparable to the positive control, with no statistically significant difference. Lower concentrations (2.5% and 5%) also showed significant antibacterial effects compared to the negative control. These findings indicate that sunflower seed extract-based toothpaste has strong potential as a natural antibacterial dentifrice and may serve as a safer alternative to synthetic antibacterial agents in oral healthcare.

Keywords: Sunflower seed extract; Herbal toothpaste; Antibacterial activity; Staphylococcus aureus; In vitro study; Natural dentifrice

INTRODUCTION

Oral and dental health remains a major public health concern in dentistry, particularly due to the high prevalence of periodontal diseases. Periodontal disease is a chronic inflammatory condition characterized by progressive destruction of the supporting tissues of the teeth, including gingiva, periodontal ligament, and alveolar bone. This condition arises from a complex interaction between pathogenic microorganisms in the oral cavity and an abnormal host immune response, leading to tissue breakdown and tooth instability (Arnawati et al., 2024). Gingivitis and periodontitis are the most common clinical manifestations of periodontal disease and continue to pose significant challenges in preventive and curative dental care (Adnyasari et al., 2023).

Epidemiological data highlight the magnitude of this problem, particularly in developing countries. The Indonesian Health Survey (SKI) in 2023 reported that 56.9% of the Indonesian population experienced oral and dental health problems, with periodontal disease identified as one of the most prevalent conditions (Kemenkes, 2023). Earlier data from the Basic Health Research Survey (RISKESDAS) showed an extremely high prevalence of periodontal disease, reaching 96.58% in 2007, placing it second only to dental caries (Rohmawati & Santik, 2019). Although a decline to 74.1% was reported in 2018, this prevalence remains alarmingly high and underscores the need for sustainable preventive strategies (Arnawati et al., 2024). These findings emphasize that periodontal disease continues to represent a significant burden on oral health systems and population well-being.

The primary etiological factor in periodontal disease is the accumulation of dental plaque. Dental plaque is a structured biofilm composed of diverse microorganisms embedded in an extracellular polymeric matrix that adheres firmly to tooth surfaces and other hard structures in the oral cavity (Hinrichs et al., 2024). Plaque can be classified into supragingival and subgingival types, both of which play critical roles in the initiation and progression of periodontal disease (Rohmawati & Santik, 2019). The persistence of plaque facilitates a pathogenic microbial ecosystem capable of inducing chronic inflammation, tissue destruction, and disease progression (Hinrichs et al., 2024).

Among the microorganisms associated with dental plaque, Staphylococcus aureus has attracted increasing attention due to its opportunistic pathogenic potential. Staphylococcus aureus is a Gram-positive, non-motile, non-spore-forming coccus that typically appears in grape-like clusters and possesses a complex cell wall structure containing polysaccharides and proteins that function as virulence-associated antigens (Campos et al., 2023; Nasution, 2014). Although not considered a primary periodontal pathogen, S. aureus has been identified as an opportunistic bacterium capable of colonizing the oral cavity and contributing to various oral infections, including root canal infections, periodontitis, and dental caries (Zaatout, 2021).

The role of Staphylococcus aureus in dental plaque formation is associated with its ability to adhere to tooth surfaces through adhesin-mediated binding to salivary pellicles. Following adhesion, the bacterium produces extracellular polysaccharides that promote biofilm formation and enhance co-aggregation with other oral microorganisms (Foster et al., 2014; Setiabudy et al., 2023). Additionally, S. aureus secretes enzymes and toxins that exacerbate gingival inflammation and disrupt microbial homeostasis within the oral cavity, leading to the formation of thicker, more resistant plaque and potentially worsening periodontal disease (Campos et al., 2023; Zaatout, 2021).

The oral cavity has been recognized as a potential reservoir for Staphylococcus aureus, which may contribute to recurrent infections and antimicrobial resistance (Campos et al., 2023). Several studies have confirmed its presence in periodontal tissues and dental plaque. Kim & Lee, (2015) reported the isolation of S. aureus strains from the oral cavities of patients with periodontal disease, while Cuesta et al., (2010) detected Staphylococcus species in both subgingival plaque and oral tissues of periodontal patients, with prevalences of 13.4% in periodontal lesions and 15.8% in the overall oral cavity. These findings indicate that S. aureus colonization may serve as an indicator of oral microbial imbalance and reinforce the importance of effective plaque control strategies (Yacoubi et al., 2015).

Mechanical plaque control through tooth brushing remains the most effective method for preventing plaque accumulation, particularly when combined with chemical agents delivered through toothpaste formulations (Dix-Cooper & Kosatsky, 2019). Although mouthwashes may provide adjunctive benefits, their effectiveness is generally inferior to that of regular tooth brushing using toothpaste (Prete et al., 2022). Conventional commercial toothpastes commonly contain chemical agents such as fluoride and triclosan, which have demonstrated efficacy in inhibiting plaque-forming bacteria and preventing dental caries (Weatherly & Gosse, 2017). However, prolonged use of these chemicals has been associated with adverse effects, including dental fluorosis, tooth discoloration, increased tooth sensitivity, and contact dermatitis (Islam et al., 2016) (Weatherly & Gosse, 2017). These concerns have driven growing interest in the development of herbal or natural toothpaste formulations with comparable antibacterial efficacy but fewer side effects.

Sunflower (Helianthus annuus Linn.) seeds represent a promising natural resource with documented antimicrobial properties. Sunflower is an annual plant belonging to the Asteraceae family, and its seeds contain high levels of vegetable oil, proteins, and various bioactive compounds (Guo et al., 2017). Previous studies have reported that sunflower seeds exhibit stronger antibacterial activity than other parts of the plant, attributed to their higher concentration of bioactive constituents (Amirul, 2020). Phytochemical analyses have identified the presence of phenolics, flavonoids, alkaloids, saponins, steroids, and tannins in sunflower seed extracts, all of which are known to exert antimicrobial effects through mechanisms such as cell membrane disruption, inhibition of protein synthesis, and interference with bacterial metabolism (Cushnie & Lamb, 2011).

Several studies have demonstrated the antibacterial activity of sunflower seed extracts against Gram-positive bacteria, including Staphylococcus aureus. Wulandari and Ariyani (2020) reported that sunflower seed oil nanogel formulations inhibited S. aureus growth at concentrations of 2.5%, 5%, and 7.5%. Similarly, Yulisma et al. (2022) found that topical cream formulations containing sunflower seed extract effectively suppressed S. aureus growth at concentrations ranging from 5% to 20%. These studies collectively support the antibacterial potential of sunflower seed-derived products; however, their applications have been largely limited to topical or pharmaceutical formulations rather than oral hygiene products.

From an agronomic and socioeconomic perspective, sunflower cultivation has considerable potential in Indonesia, particularly in North Sumatra, where sunflower varieties such as Russian Giant are actively cultivated in collaboration with local agricultural authorities (Admin limakoma, 2024). Beyond agricultural value, sunflower cultivation also contributes to tourism development in regions such as Padangsidimpuan (Marlina, 2018). The availability of locally sourced sunflower seeds enhances the feasibility and sustainability of developing sunflower-based health products, including herbal toothpaste formulations.

Despite extensive evidence supporting the antibacterial properties of sunflower seed extracts, no previous study has specifically evaluated sunflower seed extract formulated as a toothpaste for inhibiting the growth of Staphylococcus aureus. This gap highlights a lack of research integrating phytochemical potential with oral hygiene applications. The present study addresses this gap by investigating the antibacterial activity of toothpaste containing sunflower (Helianthus annuus Linn.) seed extract against Staphylococcus aureus using an in vitro experimental model.

The novelty of this study lies in the formulation and evaluation of sunflower seed extract as an active antibacterial ingredient in toothpaste, rather than as a topical or pharmaceutical preparation. This approach offers a novel contribution to the development of herbal dentifrices and provides scientific evidence supporting the use of sunflower seed extract as a natural alternative for plaque control.

Therefore, the objective of this study was to analyze the effectiveness of toothpaste containing sunflower (Helianthus annuus Linn.) seed extract at concentrations of 2.5%, 5%, and 10% in inhibiting the growth of Staphylococcus aureus in vitro. The findings of this study are expected to contribute theoretically to the advancement of knowledge in dental materials and oral microbiology, while practically supporting the development of safer, plant-based antibacterial toothpaste formulations for improving oral health.

METHODS

Study Design

This study employed an experimental laboratory design using a post-test only control group approach. Measurements were conducted exclusively after the completion of all treatments to evaluate the antibacterial activity of toothpaste formulations containing sunflower (Helianthus annuus Linn.) seed extract against

Staphylococcus aureus in vitro. This design was selected to ensure objective comparison of antibacterial effects among different treatment groups under controlled laboratory conditions.

Study Site and Period

The research was conducted between September and November at several specialized laboratories of Universitas Sumatera Utara (USU), Indonesia. Preparation of sunflower seed extract, formulation of toothpaste, and evaluation of physicochemical characteristics were performed at the Cosmeceutical Laboratory, Faculty of Pharmacy, USU. Phytochemical screening was conducted at the Natural Product Organic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences (FMIPA), USU. Microbiological culturing and antibacterial activity assays were carried out at the Microbiology Laboratory, FMIPA, USU.

Bacterial Strain and Sample Size

The bacterial population used in this study was Gram-positive Staphylococcus aureus. The sample consisted of a standardized reference strain, Staphylococcus aureus ATCC® 29213, cultured on Mueller–Hinton Agar (MHA).

Five experimental groups were established: toothpaste containing sunflower seed extract at concentrations of 2.5%, 5%, and 10%; a commercial toothpaste (Siwak-F) as a positive control; and a toothpaste base without antibacterial agents as a negative control. Sample size determination followed Federer's formula $(t-1)(r-1) \geq 15$, resulting in a minimum of five replications per group. Thus, a total of 25 experimental units were analyzed to minimize bias and ensure data reliability.

Plant Material and Preparation of Sunflower Seed Extract

Sunflower seeds were obtained purposively from a single cultivation area in Patumbak District, Deli Serdang Regency, North Sumatra, Indonesia. Only intact, non-moldy seeds were included. Plant identity was confirmed through botanical determination to ensure the material was Helianthus annuus Linn.

The seeds were washed with running water, drained, and dried in a drying cabinet until constant weight. The dried material (simplisia) was ground into powder and stored in airtight containers. Extraction was performed using maceration with 70% ethanol. Two hundred grams of powdered material were immersed in 2 L of solvent, stirred intermittently for the first 6 h, and left to stand for 18 h. The mixture was filtered, and the residue was re-macerated with 1 L of solvent. Combined filtrates were concentrated using a rotary evaporator at 40°C to obtain a viscous extract.

Phytochemical Screening

Qualitative phytochemical screening was conducted to identify secondary metabolites associated with antibacterial activity. The sunflower seed extract was tested for alkaloids, flavonoids, phenolics, saponins, terpenoids/steroids, and tannins using standard reagents. Screening confirmed the presence of all tested bioactive compounds, supporting the extract's potential antibacterial properties.

Formulation of Toothpaste

Toothpaste formulations were prepared with sunflower seed extract concentrations of 2.5%, 5%, and 10%. These concentrations were selected based on previous studies demonstrating antibacterial activity of sunflower seed-derived preparations against Staphylococcus aureus (Wulandari & Ariyani, 2020; Yulisma et al., 2022). A commercial toothpaste (Siwak-F) served as a positive control, while a formulation without active extract served as a negative control.

The base formulation consisted of calcium carbonate (abrasive), glycerin (humectant), sodium carboxymethyl cellulose (binder), sodium lauryl sulfate (surfactant), saccharin (sweetener), methyl paraben (preservative), and distilled water. CMC-Na was dispersed in hot water and allowed to swell before mixing with other components. The extract was dissolved in glycerin and incorporated gradually until a homogeneous paste was obtained. Final products were packed in labeled tubes.

Evaluation of Toothpaste Characteristics

Organoleptic properties (color, odor, taste, and consistency) were assessed visually. Homogeneity was examined by spreading a small amount of toothpaste on a glass slide. pH was measured using a calibrated pH meter, and values between 4.5 and 10.5 were considered acceptable. Spreadability was determined by measuring the diameter of paste under incremental loads, while viscosity was measured using a Brookfield viscometer at 60 rpm.

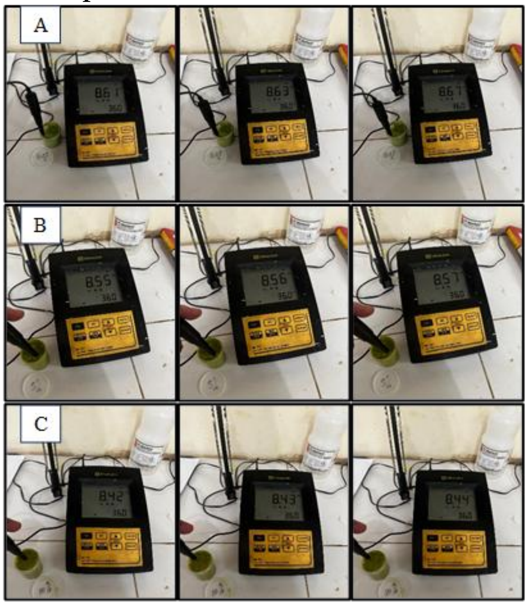

Figure 1. Physical appearance of toothpaste formulations containing sunflower seed extract at concentrations of 2.5%, 5%, and 10%.

Antibacterial Activity Assay

Antibacterial activity was evaluated using the agar well diffusion method, which is appropriate for semi-solid formulations such as toothpaste (Balouiri et al., 2016). All equipment and media were sterilized prior to use. Mueller–Hinton Agar was prepared, sterilized at 121°C for 15 min, poured into Petri dishes, and allowed to solidify.

A bacterial suspension equivalent to 0.5 McFarland standard (approximately 10^8 CFU/mL) was prepared from fresh cultures of Staphylococcus aureus ATCC® 29213. The suspension was evenly spread on MHA plates using sterile swabs. Wells were created using sterile tips, and 0.1 g of each toothpaste formulation was placed into the wells. Plates were incubated at 37°C for 24 h. Antibacterial activity was determined by measuring the diameter of inhibition zones using a digital caliper.

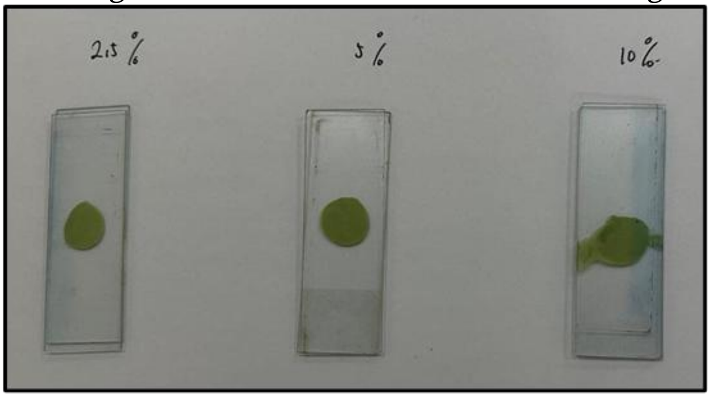

Figure 2. Agar well diffusion assay showing inhibition zones around toothpaste samples after 24 h incubation.

Data Analysis

Data were analyzed using SPSS software. Normality was assessed using the Shapiro–Wilk test, followed by homogeneity testing. If data were normally distributed and homogeneous, one-way ANOVA was applied, followed by LSD post hoc analysis. For non-normally distributed data, the Kruskal–Wallis test was used, followed by Mann–Whitney tests for pairwise comparisons. Statistical significance was set at $p < 0.05$.

Ethical Considerations

This study was approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Sumatera Utara, and conducted in accordance with applicable national research ethics guidelines.

RESULT AND DISSCUSSION

Phytochemical Screening of Sunflower Seed Extract

Phytochemical screening was conducted to identify secondary metabolites present in sunflower seed (Helianthus annuus Linn.) extract prior to antibacterial testing. The screening confirmed the presence of flavonoids, alkaloids, tannins, saponins, and terpenoids, indicating that the extract contains bioactive compounds potentially responsible for antibacterial activity. The detailed results of the phytochemical screening are presented in Table 1.

Table 1. Phytochemical Screening Results of Sunflower Seed Extract (Helianthus annuus Linn.)

Secondary Metabolites	Reagent Used	Result
Flavonoids	Mg(s) + HCl	Positive (+)
Alkaloids	Mayer and Dragendorff reagents	Positive (+)
Tannins	$FeCl_3$ 5%	Positive (+)
Saponins	Aquadest + ethanol 96% + HCl 2N	Positive (+)
Terpenoids	Liebermann–Burchard reagent	Positive (+)

Physicochemical Characteristics of Toothpaste Formulations

1. Organoleptic Properties

The organoleptic evaluation included assessment of form, color, aroma, and taste of toothpaste formulations containing sunflower seed extract at concentrations of 2.5%, 5%, and 10%. All formulations exhibited a semi-solid and homogeneous consistency. Color intensity increased with higher extract concentration, ranging from light green to dark green. A minty aroma and sweet taste were consistently observed at day 0 and day 7 of storage, indicating acceptable stability during short-term storage. The organoleptic evaluation results are shown in Table 2.

Table 2. Organoleptic Properties of Sunflower Seed Extract Toothpaste

Formulation	Storage Time	Form	Color	Aroma	Taste
F1 (2.5%)	Day 0	Semi-solid, homogeneous	Light green	Minty	Sweet
	Day 7	Semi-solid, homogeneous	Light green	Minty	Sweet
F2 (5%)	Day 0	Semi-solid, homogeneous	Green	Minty	Sweet
	Day 7	Semi-solid, homogeneous	Green	Minty	Sweet
F3 (10%)	Day 0	Semi-solid, homogeneous	Dark green	Minty	Sweet
	Day 7	Semi-solid, homogeneous	Dark green	Minty	Sweet

2. Homogeneity Test

Homogeneity testing demonstrated that all toothpaste formulations had uniform color distribution, smooth texture, and no visible lumps or coarse particles. These findings indicate that all formulations met quality requirements for homogeneity. The results are summarized in Table 3.

Table 3. Homogeneity Test Results of Sunflower Seed Extract Toothpaste

Formulation	Observation	Result
F1 (2.5%)	Uniform color, smooth texture, no lumps	Homogeneous
F2 (5%)	Uniform color, smooth texture, no lumps	Homogeneous
F3 (10%)	Uniform color, smooth texture, no lumps	Homogeneous

3. pH Measurement

The pH values of toothpaste formulations ranged from 8.43 to 8.63, indicating weak alkaline characteristics. All formulations were within the acceptable pH range for toothpaste products (4.5–10.5), suggesting suitability for oral use. The pH values are presented in Table 4.

Table 4. pH Values of Sunflower Seed Extract Toothpaste

Formulation	pH Value	Classification
F1 (2.5%)	8.63	Weak alkaline
F2 (5%)	8.56	Weak alkaline
F3 (10%)	8.43	Weak alkaline

4. Spreadability Test

Spreadability increased with both applied load and extract concentration. All formulations demonstrated spreadability values within the acceptable range for commercial toothpaste products (2.61–5.32 cm), indicating appropriate consistency for effective application. Detailed results are shown in Table 5.

Table 5. Spreadability of Sunflower Seed Extract Toothpaste

Formulation	Load (g)	Spreadability (cm)
F1 (2.5%)	50–250	2.8–3.8
F2 (5%)	50–250	3.3–4.3
F3 (10%)	50–250	3.5–4.5

5. Viscosity Test

Viscosity values ranged from 20,326 to 20,577 cPs for all formulations, classifying them as semi-solid. All formulations complied with standard toothpaste viscosity requirements, indicating adequate stability and ease of application. The viscosity results are presented in Table 6.

Table 6. Viscosity Values of Sunflower Seed Extract Toothpaste

Formulation	Viscosity (cPs)	Consistency
F1 (2.5%)	20,326	Semi-solid
F2 (5%)	20,329	Semi-solid
F3 (10%)	20,577	Semi-solid

Antibacterial Activity Against Staphylococcus aureus

The antibacterial activity of sunflower seed extract toothpaste was evaluated using the well diffusion method against Staphylococcus aureus ATCC® 29213. Clear inhibition zones were observed around wells containing toothpaste formulations with extract concentrations of 2.5%, 5%, and 10%, as well as the commercial toothpaste used as a positive control. No inhibition zone was observed in the negative control group. Representative inhibition zones are shown in Figure 5.

The mean inhibition zone diameters increased with increasing extract concentration. Statistical analysis using the Kruskal–Wallis test showed a significant difference among all treatment groups ($p < 0.05$), indicating concentration-dependent antibacterial activity. The inhibition zone diameters are presented in Table 7.

Table 7. Inhibition Zone Diameters Against Staphylococcus aureus

Group	Inhibition Zone (Mean ± SD, mm)	Inhibition Category
2.5%	14.54 ± 1.156	Strong
5%	18.46 ± 1.298	Strong
10%	22.74 ± 2.313	Very strong
Positive control (Siwak-F)	20.30 ± 1.035	Very strong
Negative control	0	–

Further analysis using the Mann–Whitney test demonstrated no significant difference between the 10% extract toothpaste and the positive control ($p > 0.05$). Significant differences were observed among the other treatment groups ($p < 0.05$). Detailed pairwise comparisons are shown in Table 8.

Table 8. Mann–Whitney Post Hoc Analysis

Comparison	p-value
2.5% vs 5%	0.009*
2.5% vs 10%	0.009*
2.5% vs Positive control	0.009*
5% vs 10%	0.009*
5% vs Positive control	0.028*
10% vs Positive control	0.076
All groups vs Negative control	0.005*

Significant at $p < 0.05$

Discussion

The present study demonstrates that toothpaste formulated with sunflower seed extract (Helianthus annuus Linn.) exhibits significant antibacterial activity against Staphylococcus aureus ATCC® 29213 in vitro. This finding is particularly relevant in the current context of oral health, where S. aureus has been increasingly recognized as a colonizer of the oral cavity and a contributory pathogen in periodontal disease and oral infections (Campos et al., 2023; Zaatout, 2021). The antibacterial effect observed in this study supports the growing interest in plant-based dentifrices as safer alternatives to conventional synthetic antibacterial agents, which have raised concerns regarding toxicity and antimicrobial resistance (Dix-Cooper & Kosatsky, 2019; Weatherly & Gosse, 2017).

The antibacterial activity of sunflower seed extract toothpaste can be attributed to the presence of multiple secondary metabolites identified in the phytochemical screening, including phenolic compounds, flavonoids, tannins, saponins, and terpenoids. Previous studies have reported that sunflower seeds are rich in phenolic compounds, particularly chlorogenic acid, which acts as a dominant bioactive constituent (Guo et al., 2017). Chlorogenic acid has been shown to disrupt bacterial cell wall integrity and membrane permeability, leading to leakage of intracellular components and inhibition of essential enzymatic functions. In addition, phenolic compounds can induce oxidative stress and interfere with macromolecular synthesis in bacterial cells, collectively resulting in growth inhibition.

Flavonoids represent the second major class of bioactive compounds in sunflower seeds and play a crucial role in antibacterial activity. Flavonoids exert their antibacterial effects through multiple mechanisms, including disruption of bacterial membrane permeability, protein denaturation, inhibition of metabolic enzymes, and interference with nucleic acid synthesis (Cushnie & Lamb, 2011). The multi-target mode of action of flavonoids is particularly advantageous, as it reduces the likelihood of bacterial resistance development compared to single-target synthetic antimicrobials. Cushnie and Lamb (2011) emphasized that flavonoids require a minimum effective concentration to induce significant membrane damage, which is consistent with the dose-dependent antibacterial activity observed in the present study.

The results showed a clear concentration-dependent increase in the diameter of inhibition zones against S. aureus. Toothpaste containing 2.5%, 5%, and 10% sunflower seed extract produced inhibition zones of 14.54 ± 1.156 mm, 18.46 ± 1.298 mm, and 22.74 ± 2.313 mm, respectively. This dose–response relationship indicates that higher concentrations of bioactive compounds enhance the antibacterial effect by increasing the intensity of interactions between active constituents and bacterial cell structures. Such findings are in accordance with fundamental antibacterial principles, where increased availability of active compounds amplifies membrane disruption and intracellular damage (Balouiri et al., 2016).

Interestingly, the 10% sunflower seed extract toothpaste produced a larger inhibition zone than the positive control (commercial Siwak-F toothpaste), although this difference was not statistically significant. This phenomenon may be explained by the non-specific and multi-target mechanisms of natural extracts, which contain a complex mixture of bioactive compounds acting simultaneously on multiple bacterial targets. In contrast, commercial antibacterial toothpastes often rely on specific active ingredients that act on defined molecular targets (Tong et al., 2015) (Foster et al., 2014). From the authors' perspective, this finding highlights the potential advantage of plant-based formulations in exerting broad-spectrum antibacterial pressure, particularly in in vitro conditions.

Comparisons with previous studies further contextualize the present findings. Wulandari and Ariyani (2020) reported that sunflower seed oil formulated as a nanogel produced an inhibition zone of approximately 18 mm against S. aureus at its highest concentration. The enhanced antibacterial activity in nanogel formulations is likely due to reduced particle size, which increases surface area and facilitates diffusion of active compounds into the agar medium. In contrast, Yulisma et al. (2022) observed smaller inhibition zones (6–7 mm) using sunflower seed oil cream formulations, even at higher concentrations. The high viscosity and oil-based nature of cream formulations may limit the release and diffusion of active compounds, thereby reducing antibacterial efficacy. These comparisons suggest that formulation type and delivery system play critical roles in determining antibacterial performance, in addition to the nature of the active ingredient.

Unlike the aforementioned studies that utilized sunflower seed oil, the present study employed sunflower seed extract, which contains a broader spectrum of bioactive compounds. This compositional diversity likely contributes to the stronger antibacterial activity observed. Moreover, the toothpaste formulation itself may enhance antibacterial efficacy. Surfactants and humectants commonly used in toothpaste bases can facilitate the release of active compounds and improve contact between the antibacterial agents and bacterial cells. This potential synergistic interaction between the sunflower seed extract and the toothpaste base may partly explain the superior inhibition observed at higher extract concentrations.

The positive control, Siwak-F toothpaste, demonstrated strong antibacterial activity against S. aureus, consistent with its formulation containing standardized herbal extracts and additional antibacterial agents commonly used in commercial dentifrices. Such formulations are designed to ensure consistent bioactive content and optimized antibacterial performance (Prete et al., 2022) (Saikia et al., 2024). The inclusion of fluoride, triclosan, or similar agents may further enhance antibacterial effects, making Siwak-F an appropriate and robust positive control for comparison.

The negative control, consisting of toothpaste base without antibacterial agents, exhibited slight inhibition zones. Similar findings have been reported in previous studies, where base components such as sorbitol, glycerin, sodium lauryl sulfate, and calcium carbonate exert mild physicochemical effects on bacterial growth. These effects may arise from changes in surface tension, osmotic pressure, or bacterial adhesion rather than true antibacterial activity. Importantly, the markedly smaller inhibition zones in the negative control confirm that the primary antibacterial effect observed in this study originates from the secondary metabolites present in the sunflower seed extract.

In addition to antibacterial efficacy, the physicochemical characteristics of the toothpaste formulations were evaluated to ensure product stability and suitability for oral use. All formulations exhibited acceptable organoleptic properties, homogeneity, pH, spreadability, and viscosity. The slightly alkaline pH values (8.43–8.63) fall within a safe range for oral application and may contribute modestly to antibacterial activity by creating a less favorable environment for bacterial growth (Nasution, 2014). However, pH is considered a supportive rather than a dominant factor in the observed antibacterial effects.

The spreadability and viscosity results indicate that all formulations possess appropriate rheological properties for toothpaste products. Adequate spreadability ensures even distribution of the toothpaste on tooth surfaces during brushing, which may enhance contact between the antibacterial agents and oral microorganisms. Slight increases in viscosity at higher extract concentrations are expected due to increased solid content and do not compromise product usability. From a formulation perspective, these findings suggest that sunflower seed extract can be incorporated into toothpaste without adversely affecting its physical quality.

Overall, the findings of this study indicate that sunflower seed extract-based toothpaste has strong potential as a natural antibacterial agent against Staphylococcus aureus. Given the increasing prevalence of periodontal disease and concerns regarding long-term use of synthetic antibacterial agents, the development of effective herbal dentifrices represents a promising strategy for improving oral health outcomes. From the authors' viewpoint, further studies are warranted to evaluate the in vivo efficacy, long-term safety, and potential synergistic effects of sunflower seed extract with other natural antibacterial agents in oral care formulations.

CONCLUSION

This study concludes that toothpaste formulated with sunflower seed extract (Helianthus annuus Linn.) exhibits significant antibacterial activity against Staphylococcus aureus ATCC® 29213 in vitro. A concentration-dependent increase in antibacterial efficacy was observed, with higher extract concentrations producing larger inhibition zones. Toothpaste containing 10% sunflower seed extract demonstrated the strongest antibacterial activity and generated a larger inhibition zone than the commercial antibacterial toothpaste used as the positive control; however, this difference was not statistically significant. Lower concentrations (2.5% and 5%) also showed statistically significant antibacterial effects compared to the negative control, indicating that even moderate concentrations of sunflower seed extract possess substantial antibacterial potential. These findings confirm that sunflower seed extract can be effectively incorporated into toothpaste formulations as a natural antibacterial agent without compromising physicochemical quality.

The results of this study support the potential development of sunflower seed extract-based toothpaste as an alternative herbal dentifrice, particularly in the context of growing concerns regarding long-term use of synthetic antibacterial agents in oral care products. The strong antibacterial activity observed, combined with acceptable physical characteristics of the formulations, suggests that sunflower seed extract may contribute to safer and more sustainable oral health strategies.

Future studies are recommended to expand the scope of investigation by evaluating the antibacterial activity of sunflower seed extract toothpaste against a broader range of oral pathogens, including both Gram-positive and Gram-negative bacteria. In addition, comprehensive toxicity and safety assessments are necessary to ensure its suitability for long-term human use. Further research exploring a wider range of extract concentrations and formulation optimizations may help identify the most effective and stable product composition. Finally, in vivo and clinical studies are essential to confirm the antibacterial efficacy, safety, and therapeutic potential of sunflower seed extract toothpaste under real oral conditions prior to clinical application.

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