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Antibacterial effects of kaempferol-loaded silver nanoparticles (AgNP-K) against *Streptococcus mutans* and *Escherichia coli*: Insights from Naqli and Aqli knowledge

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Abstract

Silver nanoparticles (AgNPs) have attracted significant attention as therapeutic agents due to their broad-spectrum antibacterial properties. From the Aqli perspective, they can be synthesized through green methods utilizing plant-derived bioactive compounds, such as kaempferol. Kaempferol, a natural flavonoid, possesses well-documented antimicrobial and antioxidant activities. From the Naqli perspective, the exploration of natural products for healing purposes resonates with Quranic guidance that emphasizes the benefits of plants and the wisdom within creation as sources of cure. This study integrates both perspectives by evaluating the antibacterial activity of silver nanoparticles incorporated with kaempferol (AgNP-K) against *Streptococcus mutans* (SM) and *Escherichia coli* (EC). Antibacterial efficacy was determined through disc diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays. The largest inhibition zones were observed at 1.25 mg/mL for SM (9.00 ± 0.06 mm) and 10 mg/mL for EC (7.17 ± 0.06 mm). The MIC and MBC values for both bacteria were 0.3125 mg/mL. AgNP-K exhibited enhanced antibacterial activity compared to AgNPs alone, suggesting a synergistic effect between silver and kaempferol. These findings demonstrate the potential of AgNP-K as an effective antimicrobial agent in combating biofilm-associated infections, while reflecting the harmony of Naqli and Aqli knowledge in advancing scientific discovery.

Keywords: Silver nanoparticles, Kaempferol, Antibacterial activity, *Streptococcus mutans*, *Escherichia coli*

1.0 Introduction

Silver nanoparticles have been known as potent antimicrobial agent, capable of combating bacterial infections both in vitro and in vivo. Their size ranges from 1nm to 100nm. They are commonly used in many applications such as drug delivery, wound dressings, food packaging and industrial areas. Silver nanoparticles have a major influence on these applications due to their larger capacity and surface area compared to bulk silver. These materials have unique electrical, optical, and catalytic capabilities, which have led to the development and

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manufacture of devices for targeted imaging delivery, diagnostics, and detection. Several approaches were used to create the silver nanoparticles. Silver nanoparticles are made using a variety of methods, including physical, chemical, and biological synthesis. Chemical procedures are advantageous because the equipment required is more convenient and straightforward than that employed in biological methods. However, synthesis generates a large number of harmful and toxic byproducts. Furthermore, the reducing substances employed in these procedures such as borohydride, 2-mercaptoethanol, citrate and thio-glycerol are hazardous. Aside from that, physical procedures demand a significant quantity of energy and a long time to complete the entire operation. Then, biological approaches use fungi, bacteria, yeast, and plant sources. It has been reported that microbe and plant-based nanoparticle production processes are safer, more cost-effective, and less hazardous to the environment than chemical synthesis (Almatroudi, 2020).

Kaempferol is a flavonoid that can be found in a wide range of plants that can bring numerous beneficial health advantages. Apart from presenting anticarcinogenic and anti-inflammatory potentials, the pure kaempferol compounds and extracts have been investigated as potential antibacterials. Kaempferol was found to be the most efficient flavonoid in inhibiting the bacterial DNA gyrase in *E. coli*, demonstrating a significant antibacterial mechanism. A study that has also been conducted demonstrated that kaempferol and silver nanoparticles work synergistically to combat *E. coli* (Pazli et al., 2024). Because of its low water solubility, the effectiveness of kaempferol is restricted. Drugs that were not very soluble in water, however, showed improved solubility through nanoformulation. For this reason, silver nanoparticles (AgNPs) were combined with kaempferol to improve their solubility and antibacterial activity (Pazli et al., 2024).

Green synthesis is a method of synthesizing materials that is environmentally friendly and sustainable. It is based on the principles of green chemistry, which aim to reduce or eliminate the use and generation of hazardous substances in chemical processes. Green synthesis methods use natural, renewable and non-toxic starting materials, and often involve the use of water as a solvent. The goal of green synthesis is to minimize the environmental impact of chemical synthesis while producing high-quality materials.

It has been reported that nanoparticles production methods based on microorganisms and plants are safe, economical and are relatively less harmful to the environment compared to the chemical synthesis. Moreover, microorganisms and plants are able to absorb and accumulate inorganic metallic ions from their surrounding environment. Biological production of silver nanoparticles mainly involves the use of microorganisms and plant sources.

The lowest concentration of an antibiotic that will stop a microorganism from growing visibly after an overnight incubation is known as a minimum inhibitory concentration (MIC), and the lowest concentration of an antibiotic that will stop an organism from growing after it has been subcultured onto media free of antibiotics is known as a minimum bactericidal concentration (MBC). MIC is typically determined using broth or agar dilution methods. Various antimicrobial agent concentrations are tested against a standardized bacterial inoculum. The MIC is defined as the lowest concentration that completely inhibits visible growth (Othman et al., 2019). After the MIC is determined, samples from the MIC wells are subcultured onto antibiotic-free media. The MBC is the lowest concentration that shows no visible bacterial growth after subculture. Therefore, this study aimed to determine the antibacterial activity of silver nanoparticles incorporated with kaempferol (AgNP-K) against *S. mutans* and *E. coli*.

2.0 Literature Review

The manufacture and use of silver nanoparticles in a variety of sectors, including biotechnology and nanomedicine, have drawn increasing attention in recent years (Naseer et al., 2022). Silver nanoparticles have garnered significant attention in the field of nanotechnology because of their distinctive characteristics and extensive range of applications (Ansari et al., 2016). Silver nanoparticles are highly suitable for use in catalysis, biosensors, electronics, pharmaceuticals, therapeutics, optics, and more due to their unique size-dependent optical and electrical properties, good stability, low toxicity, and biocompatibility (Ansari et al., 2016). The intrinsic antibacterial characteristics of silver have also drawn a lot of interest to the synthesis of silver nanoparticles, which might have potential uses in a variety of biotechnological processes such the generation of biohydrogen, biosensors, and biocatalysis (Kumar et al., 2023). The creation of silver nanoparticles has attracted attention in the world of medicine as well, with further study and development seeking to utilize the antibacterial qualities for novel treatment approaches. A range of physical-chemical

techniques have been used in the field of nanoparticle synthesis to create silver nanoparticles for a range of uses (Naseer et al., 2022).

The synthesis of silver nanoparticles has been intensively pursued by both nanotechnology and biotechnology, with a special emphasis on the employment of biological techniques, notably the utilization of medicinal plants in agriculture, for the biosynthesis of silver nanoparticles. Compared to chemical approaches, the employment of biological technologies has demonstrated superior effects, with promising results. Suryawanshi et al. (2018) highlight the possibility for large-scale manufacturing due to the scalability and cost-effectiveness of biological approaches for nanoparticle synthesis. Due to their great structural integrity, wide range of applications in several sectors, and different impacts, silver nanoparticles have become more popular in nanoscience and biomedicine.

Given their non-toxic and environmentally benign characteristics, green synthesis techniques that employ natural sources including fungus, bacteria, yeast, and plants have attracted increased interest. Plant extracts, which are abundant in bioactive substances that can serve as reducing and stabilizing agents for the creation of silver nanoparticles, are used in these green synthesis techniques. Kaempferol is one such plant extract that has been used in the production of silver nanoparticles. It has been demonstrated that the flavonoid kaempferol, which is present in many plants, possesses anti-inflammatory, antioxidant, and anticancer qualities.

There are several benefits to the environmentally friendly production of silver nanoparticles that employ kaempferol as a stabilizing and reducing agent. These consist of the possibility for sustainable manufacturing, the use of sustainable and non-toxic materials, and the removal of dangerous compounds. Additionally, kaempferol-synthesised silver nanoparticles have demonstrated minimal cytotoxicity and outstanding biocompatibility, rendering them appropriate for use in biomedical applications. For instance, kaempferol-synthesized silver nanoparticles have demonstrated encouraging outcomes in medication delivery systems. Drugs may be placed into these nanoparticles and directed towards certain bodily locations, enabling regulated release and enhanced therapeutic results. Additionally, silver nanoparticles made with kaempferol have antibacterial qualities that enable them to effectively combat a variety of infections. As a result, they might be used in wound healing, disinfection, and water purification applications. Overall, there is a lot of potential for use in biotechnology, environmental research, and medicine with the green production of silver nanoparticles that use kaempferol as a stabilizing and reducing agent. To sum up, the utilization of kaempferol as a reducing and stabilizing agent in the green synthesis of silver nanoparticles is a technology that has great promise due to its many benefits, including non-toxicity, eco-friendliness, scalability, and biocompatibility Moreover, kaempferol's antioxidant and antibacterial qualities make it an ideal option for the production of silver nanoparticles with improved performance. A developing subject with considerable promise for applications in medicine, biotechnology, and environmental research is the manufacture of silver nanoparticles employing plant extracts, especially kaempferol, as reducing and stabilizing agents.

Kaempferol is a flavonoid which gives out health benefits that may be found in a variety of plants. Plant-derived chemicals known as flavonoids do have a wide range of health-related benefits. Found in many different types of plants and herbs but was first discovered in Camelia sinensis (tea tree), it is one of the most well-known and researched flavonoids. Kaempferol is named after Engelbert Kaempfer, a German doctor, naturalist, and historian who lived in the 17th century and was instrumental in bringing medical knowledge from Japan to the West. It is famous for its anticarcinogenic and anti-inflammatory benefits, but kaempferol also produces antibacterial activity.

In light of emerging pathogen resistance and complex molecular interactions between various drug therapies, the development of drugs and treatment schemes based on these compounds is becoming increasingly important. Furthermore, many kaempferol-containing plants have been used for centuries in traditional systems all over the world to treat a variety of conditions. Some molecular mechanisms of kaempferol antimicrobial activity are well known due to its diverse sources and associated compounds, while others are still being investigated. The natural flavanol kaempferol (K), which comes from plant sources, has antimicrobial properties. Unfortunately, their huge size and water insolubility limit their antibacterial activity (Santhosh Kumar et al., 2021). Drugs that were not very soluble in water, however, showed improved solubility by nano formulation. It has been noted from earlier research that adding kaempferol to other metallic nanoparticles has increased their antibacterial activity. Therefore, the key properties of silver nanoparticles can be achieved through green synthesis and further enhanced with kaempferol to combat antibiotic-resistant bacteria, particularly *Streptococcus mutans* (*S. mutans*) and *Escherichia coli* (*E. coli*). Regarded as one of the main bacteria causing dental caries, or tooth decay, *Streptococcus mutans* (*S. mutans*) is a facultative anaerobic

coccus bacterium that is Gram-positive and lives in the human oral cavity. It is thought to be one of the main causative factors of dental caries, or tooth decay, in humans. The bacterium *S. mutans* is well-known for its capacity to generate acids from dietary carbohydrates, a process that can cause dental caries and demineralize tooth enamel. Infectious endocarditis is also linked to it.

S. mutans expresses specific extracellular enzymes that allow it to metabolize sucrose into lactic acid through the process of fermentation. Dental caries is caused by this, which also lowers the pH in the mouth and demineralised tooth enamel. It is best used in an oral environment with a pH of 5.5 and a temperature range of 18 to 40°C. In order to firmly adhere to the tooth enamel, *S. mutans* also generates glycans. Both the fermentation and respiration processes which require oxygen can be used by *S. mutans* to digest carbohydrates. This enables it to continue even in the event of variations in oxygen levels. In addition, it exhibits resistance to high acidity, hydrogen peroxide therapy, hyperosmotic stress and metal ions like copper that are frequently found in the dynamic oral cavity environment. The cariogenic potential of *S. mutans* is increased by these versatile virulence features. The capacity of *S. mutans* to produce adherent biofilms, or dental plaque, on tooth surfaces is a crucial aspect of its pathogenesis. In order for microcolonies to group together and get entangled in a sticky extracellular matrix that shields *S. mutans* cells, glucan polymers are generated. The bacteria within these biofilms develop more resistance to environmental changes and standard antibiotic treatments. This permits tooth decay and the creation of harmful acids to proceed further. *S. mutans* is a major pathogen that causes dental caries. It is well-known for its capacity to colonize the oral cavity, build biofilms on tooth surfaces, and ferment sugar to make acids. According to Yang et al. (2021) *S. mutans* is a cariogenic bacterium that is important in the development of dental plaque and tooth decay. Furthermore, it has been determined that *S. mutans* is a crucial microbiological biomarker in the deciduous molar plaque of children who have caries, demonstrating its strong correlation with the condition. This demonstrates the complex role *S. mutans* plays in the pathophysiology and causation of dental caries in humans. All things considered, dental plaque and tooth decay are mostly caused by the cariogenic bacterium *S. mutans*. In addition to this bacterium, another antibiotic-resistant species is *E. coli.*

E. coli is an enteric bacterium that belongs to the Enterobacteriaceae family, which is classified under the order Enterobacterales. *Escherichia coli* (*E. coli*) is a gram-negative bacillus that is normally found in the human intestine but can also cause intestinal and extraintestinal illness. There are hundreds of *E. coli* strains known to cause disease ranging from mild, self-limited gastroenteritis to renal failure and septic shock (Mueller & Tainter, 2023). *E. coli* appears as a straight, rod-shaped, non-sporing, acid-fast bacillus that exists in singles and pairs. Cells are typically rod-shaped, measuring 1-3 m 0.4-0.7 m (micrometer) in length, 0.35 m in width, and 0.6-0.7 m in volume. It is the most widely studied prokaryotic model organism in biotechnology and microbiology. It can survive in faeces, soil, and water for extended periods of time and is frequently used as a water contamination indicator organism. Under aerobic conditions, the bacterium multiplies rapidly in fresh faeces for 2-3 days, but its numbers gradually decline after that (Basavaraju & Gunashree, 2022). It can be found in the intestines of humans and warm-blooded animals. The majority of *E. coli* strains are not harmful. However, some strains, such as Shiga toxin-producing *E. coli* (STEC), can cause severe foodborne illness. *E. coli* infections cause approximately 265,000 illnesses and 100 deaths in the United States each year. The *E. coli* strain O157:H7, which belongs to the shiga toxin-producing group of *E. coli* bacteria (STEC), is responsible for approximately 40% of these infections.

It is primarily transmitted to humans through the consumption of contaminated foods such as raw or undercooked ground meat, raw milk, and contaminated raw vegetables and sprouts (World Health Organization, 2018). STEC is destroyed by thoroughly cooking foods until all parts reach 70 °C or higher. In terms of public health, *E. coli* O157:H7 is the most important STEC serotype. STEC produces toxins known as Shiga-toxins due to their similarity to *Shigella dysenteriae* toxins. STEC can grow in temperatures ranging from 7 °C to 50 °C, with 37 °C being the optimum. As it can give bad effects including abdominal cramps and diarrhoea. Not treating the symptoms may lead to a higher effect on health such as acute kidney failure. Under the right conditions, the bacterium can be easily and cheaply grown in a laboratory setting. It can be replicated in as little as 20 minutes and has been extensively researched for over 60 years. *E. coli* is the most studied prokaryotic model organism and an important species in biotechnology and microbiology, where it serves as the host organism for recombinant DNA and as an experimental workhorse for DNA manipulation and protein production.

3.0 Methodology

3.1 Synthesis AgNP-K

The synthesis of silver nanoparticles conjugated with kaempferol (AgNPs-K) was carried out using a green synthesis approach (Hairil et al., 2024). The reaction was performed at a volume ratio of 9:1, in which 900 mL of 1 mM aqueous silver nitrate solution (Nacalai Tesque, Japan) was added dropwise to 100 mL of 1 mM aqueous kaempferol solution under vigorous stirring for 30 minutes. Ultrapure water was used as the solvent for both silver nitrate and kaempferol. The resulting mixture was incubated in an oven (Memmert UF 110, Germany) at 60 °C for 168 hours. The reduction of silver nitrate to silver nanoparticles was confirmed by a distinct color change from colorless to reddish-brown. Finally, the solution was freeze-dried (Christ Alpha 1–4 LSC basic, Germany) to obtain the AgNPs-K powder.

3.2 Bacteria Culture

The *E. coli* strain (ATCC 8739) and *S. mutans* strain (ATCC 35668) were cultured in Mueller Hinton Broth (MHB) and incubated for 24 hours. The bacterial suspensions were then adjusted to a turbidity equivalent to the 0.5 McFarland standard

3.3 Disc diffusion assay (DDA)

The disc diffusion assay (DDA) was performed following the method described by Abd Ghafar et al. (2022). Sterile discs were prepared using Whatman No. 1 filter paper. For the assay, *S. mutans* and *E. coli* cultures were adjusted to 0.5 McFarland standard in Mueller Hinton Broth (MHB). Using a sterile cotton swab, 100 µL of the bacterial suspension was evenly spread onto Mueller Hinton Agar (MHA) plates. The prepared discs were then placed on the inoculated agar surface and impregnated with 10 µL of the test samples at concentrations of 10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.625 mg/mL, and 0.3125 mg/mL for AgNPs-K, kaempferol, and AgNPs alone. Penicillin and ampicillin served as the positive controls for *S. mutans* and *E. coli*, respectively. The plates were incubated at 37 °C for 24 hours; *E. coli* was cultured under aerobic conditions, while *S. mutans* was cultured under facultative anaerobic conditions. Following incubation, the inhibition zones were measured. To ensure reproducibility and minimize experimental error, all experiments were conducted in triplicate. Statistical analysis was carried out using the Student's *t*-test to compare the treatment groups with the control, and differences were considered significant at $P < 0.05$.

3.4 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assays were performed using sterile 96-well microplates, following the method described by Othman et al. (2019). As in the DDA, *S. mutans* and *E. coli* were cultured overnight in Mueller Hinton Broth (MHB) and adjusted to the 0.5 McFarland standard. The MIC test was conducted using the 96-well plate serial dilution technique as described by Mohamad Hanafiah et al. (2015). Briefly, 100 µL of the bacterial suspension was dispensed into each well, followed by the addition of 100 µL of test samples (AgNPs-K, AgNPs alone, or kaempferol alone) at serially diluted concentrations starting from 10 mg/mL. Penicillin and ampicillin served as positive controls for *S. mutans* and *E. coli*, respectively. The plates were incubated at 37 °C for 24 hours. Following incubation, 20 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) solution was added to each well. The sterile MTT solution was prepared by dissolving 10 mg of MTT in phosphate-buffered saline (PBS) and filtering through a sterile filter. MIC values were determined by observing color changes after 30–60 minutes of incubation, with the lowest concentration that inhibited visible bacterial growth recorded as the MIC. For MBC determination, aliquots from wells showing no visible growth were streaked onto fresh agar plates prior to MTT addition. All experiments were performed in triplicate to ensure reproducibility. Statistical analysis was carried out using the Student's *t*-test to compare the treatment groups with the control, and differences were considered significant at $P < 0.05$.

4.0 Results and Discussion

The results obtained from the disc diffusion assay involved measuring the inhibition zones around each disc treated with varying concentrations of AgNP-K, AgNP alone and kaempferol. Table 1 presents the inhibition zone results for AgNP-K, AgNP, kaempferol, and the positive control (penicillin) against *S. mutans*. The inhibition zones ranged from 6 mm to 9 mm, with slightly larger zones observed for *S. mutans* compared to *E. coli*. AgNP-K at concentrations of 10 mg/mL and 5 mg/mL exhibited the highest mean inhibition zones. In contrast, AgNP and kaempferol (both at 10 mg/mL) showed inhibition zones of 7.00 mm. Notably, AgNP-K demonstrated slightly greater antibacterial activity against *S. mutans* than penicillin, which produced zones between 8 mm and 9 mm, especially at the higher concentrations.

Table 1
Inhibition zone (mm) of AgNP-K, AgNP and kaempferol at different concentrations against *S. mutans.* Values are expressed as mean ± SD from three independent replicates. Penicilin (2U) served as the positive control against *S. mutans*. Larger inhibition zones represents stronger antibacterial activity.

Samples	Concentration samples (mg/ml)			
	Diameter zone (mm)			
	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml
AgNp-K	9±0.06	8±0.03	7±0.02	6±0.03
AgNp	7±0.05	7±0.04	6±0.04	6±0.04
Kaempferol	7±0.04	7±0.03	7±0.03	6±0.03
Penicillin (2U)	7±0.02			

As shown in Table 2, AgNP-K at 10 mg/mL exhibited the largest mean zone of inhibition (7.17 mm), followed by 2.5 mg/mL (6.83 mm) and 1.25 mg/mL (6.67 mm). Interestingly, AgNP-K at 5 mg/mL produced a consistent inhibition zone of 7 mm with zero standard deviation, indicating high reproducibility across replicates. However, AgNP-K displayed limited antibacterial activity against *E. coli* compared to the standard antibiotic ampicillin, which produced a substantially larger inhibition zone of 20 mm.

Overall, the results suggest that AgNP-K possesses stronger antibacterial activity against *S. mutans* than against *E. coli*. Although the inhibition zones for *E. coli* were modest and significantly smaller than those produced by ampicillin, AgNP-K showed comparable or slightly greater efficacy than penicillin against *S. mutans*, especially at lower concentrations (2.5 mg/mL and 1.25 mg/mL). Moreover, while the inhibition of *E. coli* was relatively consistent, the variation in standard deviations for *S. mutans* indicates greater variability in response

Table 2
Inhibition zone (mm) of AgNP-K, AgNp and kaempferol at different concentrations against *E. coli.* Values are expressed as mean ± SD from three independent replicates. Ampicilin (2U) served as the positive control against *E. coli*. Larger inhibition zones indicate stronger antibacterial effects.

Samples	Concentration samples (mg/ml)			
	Diameter zone (mm)			
	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml
AgNp-K	7.17±0.06	7±0.00	6.83±0.02	6±0.03
AgNp	7±0.05	7±0.04	6±0.04	6±0.04
Kaempferol	7±0.04	7±0.03	7±0.03	6±0.03
Ampicilin (2U)	20±0.03			

MIC values are determined by finding the lowest treatment concentration that inhibits the bacteria from growing after adding an MTT test to it to see any color changes. Prior to the addition of MTT, MBC was also carried out by streaking one well category to each agar plate. The MIC is determined as the lowest concentration where there is no visible growth where the wells are yellow instead of purple. From Table 3, the MIC for AgNP K-against *S. mutans* is at 0.3125 mg/ml. MIC results for AgNP-K against *E. coli* is 0.3125

mg/ml. The MBC values of AgNP-K against *S. mutans* and *E. coli* is 0.3125 mg/mL respectively.

Table 3
MIC and MBC values of AgNP-K, AgNp and kaempferol against *S. mutans* and *E. coli*

	MIC against *S. mutans* (mg/mL)	MBC against *S. mutans* (mg/mL)
AgNp-K	0.3125	0.3125
AgNp	5	10
Kaempferol	10	>10
	MIC against *E. coli* (mg/mL)	MBC against *E. coli* (mg/mL)
AgNp-K	0.3125	0.3125
AgNp	10	>10
Kaempferol	10	>10

The antimicrobial evaluation of AgNP-K, AgNP, and kaempferol against *S. mutans* and *E. coli* demonstrated that AgNP-K exhibits notable antibacterial activity, particularly against *S. mutans*. The disc diffusion assay revealed that AgNP-K produced larger inhibition zones compared to AgNP alone and kaempferol at all tested concentrations. Notably, AgNP-K at 10 mg/mL and 5 mg/mL exhibited the highest inhibition zones against *S. mutans* (9 mm and 8 mm, respectively), surpassing the inhibition zone of the standard antibiotic, penicillin (7 mm). This suggests that the conjugation of AgNP with kaempferol enhances its antibacterial potency, likely due to the synergistic effects between the silver nanoparticles and the flavonoid compound.

In contrast, the inhibition zones recorded against *E. coli* were relatively smaller. AgNP-K at 10 mg/mL produced a zone of 7.17 mm, which was significantly lower than that of the standard antibiotic, ampicillin (20 mm). This indicates that although AgNP-K exhibits antibacterial effects against *E. coli*, its efficacy is considerably weaker than conventional antibiotics. The consistency in the inhibition zone of 7 mm at 5 mg/mL (with no standard deviation) suggests a stable antibacterial performance at this concentration. The MIC and MBC assays further support the findings from the disc diffusion test. AgNP-K demonstrated the lowest MIC and MBC values against both *S. mutans* and *E. coli* (0.3125 mg/mL), indicating strong bacteriostatic and bactericidal activity. In comparison, AgNP and kaempferol alone required significantly higher concentrations to achieve similar effects. For instance, the MIC of kaempferol against *S. mutans* and *E. coli* was 10 mg/mL, while AgNP required 5 mg/mL and 10 mg/mL, respectively. This reinforces the notion that the combination of AgNP and kaempferol in AgNP-K enhances antimicrobial efficacy.

Interestingly, while AgNP-K performed consistently against *E. coli*, its activity against *S. mutans* showed greater variability, as indicated by the standard deviations in the inhibition zones. This variability may be attributed to differences in bacterial cell wall composition between Gram-positive and Gram-negative bacteria, with *S. mutans* (a Gram-positive bacterium) potentially being more susceptible to the combined mechanism of action of AgNP-K.

Overall, these results highlight the promising antibacterial potential of AgNP-K, especially against oral pathogens such as *S. mutans*. The enhanced activity at lower concentrations and improved efficacy compared to traditional antibiotics like penicillin suggests that AgNP-K could serve as a viable candidate for future therapeutic applications in oral healthcare. However, its reduced effectiveness against Gram-negative bacteria such as *E. coli* indicates a need for further formulation optimization or combination therapy to broaden its antimicrobial spectrum. A natural flavonoid, kaempferol, is present in numerous plants, demonstrates a strong antimicrobial activity. Its mode of action consists of breaking down bacterial cell membranes and blocking essential bacterial enzymes. Kaempferol engages with the lipid bilayer of bacterial membranes, enhancing permeability and resulting in the leakage of cellular components, which ultimately results in cell death (Chen et al., 2019).

Silver nanoparticles (AgNPs) are well-known for their antimicrobial abilities, mainly due to the production of reactive oxygen species (ROS) and the liberation of silver ions, which harm the cell membranes and disrupt cellular functions of the bacteria. The antimicrobial effectiveness of AgNPs is greatly improved when paired with kaempferol. Kaempferol enhances the infiltration of AgNPs into bacterial cells by destabilizing the bacterial membrane, which permits increased intracellular uptake of silver ions. This

combined effect also enhances ROS production, causing oxidative stress that surpasses bacterial defense systems and speeds up cell death compared to AgNPs alone (Zhang et al., 2021).

Research has shown that the pairing of AgNPs with kaempferol displays enhanced antimicrobial effectiveness when compared to AgNPs by themselves. For example, AgNPs by themselves might need greater concentrations to produce the same bactericidal effect as the combination of AgNP and kaempferol (Kumar et al., 2020). The combination's greater efficiency is attributed to the complementary mechanisms at function: AgNPs produce ROS and release silver ions, whereas kaempferol breaks bacterial membranes and inhibits enzymatic activity. This dual mechanism improves antibacterial efficacy while lowering the risk of bacterial resistance (Mohammed et al., 2023).

The findings from this research clearly show that the combination of silver nanoparticles and kaempferol (AgNpK) has antimicrobial effects against both *E. coli* and *S. mutans*. Notably, *S. mutans* appeared more sensitive to lower concentrations of the treatment, aligning with existing research that suggests Gram-positive bacteria like *S. mutans* are generally more vulnerable to silver nanoparticle-based treatments (Rai et al., 2014). This increased susceptibility is likely due to the structure of Gram-positive bacteria, which have a thick peptidoglycan layer that makes them more exposed to the damaging effects of nanoparticles. In contrast, *E. coli*, a Gram-negative bacterium, has an outer membrane made of lipopolysaccharides (LPS), which acts as a protective barrier, reducing the nanoparticles' ability to penetrate and inhibit bacterial growth.

A clear dose-dependent trend was observed in both bacteria, with higher concentrations (10 mg/mL) generally producing larger zones of inhibition. This makes sense, as higher concentrations of silver nanoparticles release more silver ions (Ag^+), which are known to enhance the production of reactive oxygen species (ROS). These ROS can disrupt bacterial membranes, interfere with essential cellular functions, and ultimately kill the bacteria (Durán et al., 2016). The fact that *S. mutans* responded well even at lower concentrations suggests that AgNpK could be particularly useful in oral health products, such as toothpaste or mouthwash, where lower concentrations may be more practical and safer for regular use.

Silver nanoparticles (AgNPs) and kaempferol together have demonstrated encouraging promise for improving antibacterial activity. The two drugs' complementary actions, which work together to enhance bacterial inhibition through membrane rupture, enzyme inhibition, and oxidative stress generation, are the source of this synergistic impact. As a natural flavonoid, kaempferol has known antibacterial properties, such as disrupting bacterial membranes and inhibiting key enzymes required for bacterial survival. When combined with silver nanoparticles, kaempferol likely amplifies the overall antibacterial effect through a synergistic impact. This synergy might involve improving the nanoparticles' ability to penetrate bacterial cells or boosting ROS production, leading to greater bacterial inhibition than either compound could achieve alone.

From the **aqli (rational/scientific)** standpoint, this study supports the potential of nanotechnology and natural products in addressing the growing challenge of antimicrobial resistance. The enhanced antibacterial activity of AgNP-K, especially against *Streptococcus mutans*, demonstrates the synergy between silver nanoparticles and kaempferol. The scientific findings are supported by quantitative assays (disc diffusion, MIC, MBC), confirming that even at low concentrations, AgNP-K can effectively inhibit bacterial growth, particularly Gram-positive oral pathogens. In parallel, the **naqli (Islamic revealed knowledge)** perspective emphasizes the importance of seeking treatment and preserving health as part of maqasid al-shariah (objectives of Islamic law), particularly the protection of life (ḥifẓ al-nafs).

Islam encourages the use of permissible and beneficial substances for healing, as illustrated in the hadith:

“Allah has not sent down a disease except that He has also sent down its cure.” *(Sahih al-Bukhari, Hadith 5678)*

This hadith encourages continuous effort in scientific exploration to discover new treatments. The use of kaempferol, a compound naturally found in plants, aligns with Islamic principles of utilizing **halal**, natural, and clean resources for medical purposes. Furthermore, the Quran also mentions the healing properties of natural elements:

"And your Lord taught the bee to build its cells in hills, on trees, and in (men's) habitations… There issues from within their bodies a drink of varying colors, wherein is healing for mankind." *(Surah An-Nahl, 16:68–69)*

This verse reflects the idea that nature holds healing potential created by Allah, and it is the responsibility of humans to explore and apply these resources wisely. The combination of modern nanotechnology with natural plant-derived compounds reflects a holistic approach, bridging scientific advancement with ethical and spiritual principles. Thus, the integration of naqli and aqli perspectives in this research supports not only the scientific significance of AgNP-K as an antimicrobial agent but also reinforces the Islamic worldview that encourages innovation, preservation of health, and ethical responsibility in scientific discovery.

5.0 Conclusion

The present study demonstrates that AgNP-K exhibits enhanced antibacterial activity compared to AgNP alone and kaempferol, particularly against *S. mutans*. The synergistic effect between silver nanoparticles and kaempferol highlights their potential as a novel antimicrobial agent, especially in addressing Gram-positive oral pathogens. From the **Aqli perspective**, this research advances scientific understanding by validating the role of nanotechnology and phytochemicals in improving antibacterial efficacy. From the **Naqli perspective**, the use of plant-derived compounds reflects the guidance of the Qur'an, which encourages humanity to explore the healing potential embedded within natural creations. The integration of these two domains underscores that scientific discovery and revealed knowledge are not contradictory but complementary, offering a more holistic foundation for innovation. These findings strengthen the concept that modern biomedical solutions can be enriched through insights drawn from both rational inquiry and divine wisdom. While the results are promising for dental and biomedical applications, further investigations on safety, biocompatibility, and underlying mechanisms are essential before clinical translation. Ultimately, this work contributes to bridging Naqli and Aqli knowledge in the pursuit of impactful, ethical, and holistic healthcare solutions.

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