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RESEARCH ARTICLE

A REVIEW ON EFFECT OF QUERCETIN AND KAEMPFEROL ON BIOFILM FORMING BACTERIA

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ABSTRACT

Quercetin and Kaempferol, flavonoids isolated from the medicinal plant *Psidium guajava Linn.*(Guava) studied for their activity against common pathogenic biofilm forming bacteria. Guava leaves are traditionally used for various common illness and are rich in phytochemical content, including different antioxidants. Biofilm-forming bacteria present a major clinical challenge due to their protective Extracellular Polymeric Substance (EPS) matrix and the presence of antibiotic-tolerant dormant cells, which lead to chronic, resilient infections. Quercetin and Kaempferol offer a promising, non-antibiotic strategy by specifically targeting crucial stages of biofilm development. Kaempferol mainly functions by inhibiting the initial bacterial attachment and adhesion to surfaces by downregulating surface protein production (e.g.,*Staphylococcus aureus*). Quercetin reduce the overall production of EPS and altering its monosaccharide composition due to which weak biofilm structural integrity. Quercetin demonstrates molecular efficacy by suppressing the expression of key genes related to virulence, stress response, and quorum sensing (e.g.,*Salmonella Typhimurium*). This mechanismuse forblocking adhesion and disrupting matrix maturation and signalingsuggests that Psidium guajava leaves extracts rich in these flavonoids are highly favorable candidates for developing novel therapies to combat persistent, antibiotic-tolerant infections in healthcare and food safety.

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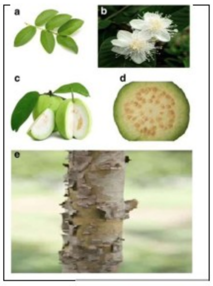
INTRODUCTION

Guava (*Psidium guajava Linn*) is a tropical tree abundantly grown for fruit: it belongs to the Myrtaceae family. The family includes many different species that produce fruit in large number. Guava is commonly known in English, jambubatu in Malaya, pichi,posh and enandi are the common famous name in America and Mexico (Sumra Naseer, *et al*.,2018).*Psidium guajavaLinn.* leaves have been traditionally used to treat various health issues like wounds, diarrhea, coughs and dengue fever. The extract from these leaves is known to be a great antioxidant (Batubara, *et al*., 2017).The guava plant is a well-known and powerful medicinal plant that shows great promise for developing new pharmacological (drug-related) uses (Eman Butt, *et al.,*2025). Guava is shrub like tree that can grown up to 10 m tall and has a wide spread network of branches curved, which display opposite leaves with small petioles. (Sumra Naseer, *et al*.,2018) axonomical classification of *Psidium guajava* is given below- The leaves of *Psidium guajava L.* sometimes contain numerousphytochemicallike phenoliccompound such asferulic acid, gallic acid and flavonoids such as catechins, quercetin and kaempferol. These flavonoids are known to have antioxidant, antimicrobial, andanti-inflammatory activity (Batubara, *et al*., 2017). The bark of guava is thin and has green-coloured spots containing huge number of antimicrobial and antibacterial compound (Sumra Naseer, *et al*.,2018). *Psidium guajava L.* frequently employed in numerous parts of the world for the cure of a lot of sickness like diarrhea, reducing fever, dysentery, hypertention. Guava skin extract can control level of diabetes after 21 days treatment(Eman Butt, *et al.,*2025). Biofilms are microbial communities that stick firmly to surfaces, whether they are living (biotic) or non-living (abiotic). These settled, complex structures are encased in a slimy matrix they make themselves, called extracellular polymeric substances (EPSs). This protective slime is a mix of stuff like sugars (polysaccharides) and proteins. Biofilms are widely Present in the environment (AreumHan,*et al*.,2023). Biofilm forming microorganisms include*Bacillus cereus, Escherichia coli, Listeria monocytogenes, Pseudomonas spp., Staphylococcus aureus, Streptococcus spp., and Vibrio parahaemolyticus,* can form biofilm. Thesemicroorganism can be planktonic or free- living and frequently organize themselves into a consortium of microbes (Kaustubh *et al*., 2015). Antibiotic tolerance in biofilms isattributable to dormant cells, which develop because gradients of nutrients and oxygen from top to bottom decrease bacterial metabolic activity and increase the cells' doubling times.Contributing to the survival of these structures are conventional mechanisms like chromosomal β-lactamase, upregulated efflux pumps, and mutations in antibiotic target molecules. Biofilm growth is associated with both increased mutations and mechanisms regulated by quorum sensing(Kaustubh *et al*.,2015). Biofilms are characterized by gradients of nutrients and oxygen from top to bottom, resulting in reduced bacterial metabolic activity and longer cell doubling times.These conditions produce dormant cells responsible for their tolerance to antibiotics. Their survival is also due to several mechanisms: an increased level of mutations, quorum-sensing regulation, and conventional resistance, including chromosomal β-lactamase, upregulated efflux pumps, and mutations in antibiotic target molecules(Batubara, *et al*., 2017).

Rank	Scientific Name and Common Name
Kingdom	Plantae – Plants
Division	Magnoliophyta
Class	Magnoliopsida – Dicotyledons
Subclass	Rosidae
Order	Myrtales
Family	Myrtaceae
Sub – family	Myrtoideae
Genus	Psidium
Species	*Psidium guajava Linn* – Guajava



(a) Leaves (b) Flowers (c) Fruit

(d) Seed in the fruit (e) Bark

While traditional strategies involve early aggressive antibiotic prophylaxis or therapy for prevention and chronic suppressive therapy for treatment, a promising new strategy targets the biofilm itself. This approach includes the use of enzymes DNase and alginate lyase to dissolve the matrix and quorum-sensing inhibitors to increase the biofilm's susceptibility to antibiotics. This is necessary because the characteristic top-to-bottom nutrient and oxygen gradients—which decrease metabolic activity and lengthen doubling times, leading to antibiotic-tolerant dormant cellsalong with elevated mutations, quorum-sensing mechanisms, and conventional resistance like β-lactamase and efflux pumps, make these structures highly resilient(Kaustubh *et al*.,2015). Effectiveness of Quercetin on biofilm forming bacteria quercetin reduced intercellular substances produce by bacteria. Quercetin treatment resulted in the cells becoming less hydrophobic, shown by cell surface hydrophobicityassay. This reduction in cell surface hydrophobicity is suggest at least a partial mechanism for the observed inhibition of biofilm formation (Yongqi Mu *et al.,*2021). Reduced oxidative stress and inhibit biofilm formation by removing reactive oxygen species accumulated in bacterial cell (Yu Kyung Kim, *et al*.,2022). Quercetin reduced the overall production of Extracellular Polymeric Substances (EPS). It also significantly altered the monosaccharide composition of the EPS: galactosamine (GalN) was absent and glucose (Glu) appeared, while galacturonic acid (GalA) was reduced, and galactose (Gal) was increased(Yongqi Mu *et al.,*2021). Kaempferol inhibits Microorganism biofilm formation effectively, showing up to 80% inhibition at concentrations as low as 64 µg/ml. This anti-biofilm effect is independent of its ability to kill or slow the growth of planktonic free-moving bacteria, as its MIC (Minimum Inhibitory Concentration) is high it doesn't alter the growth curve of planktonic cells(Di Ming *et al*.,2017).

REVIEW OF LITERATURE

Areum Han & Sun – Young Lee (2023) studied onAnoverview of various methods for in vitro biofilm formationin-virto biofilm formation method is critical for research to choose sutible method the review explore that practical applications of biofilm investigation, showing how different assays are used to characterize and study these persistent microbial communities. Batubara,H Suparto& N S Wulandari (2017) studied on The Best Extraction Technique for Kaempferol and Quercetin Isolation from Guava Leaves (Psidium guajava) concluded that direct method of sonication is the best extraction technique for isolating kaempferol and quercetin from leaves of guava, contain respective contents of 0.02% and 2.15%. This method was preferred for its short extraction time, low impurities which is desirable for anticancer potential. Christina Wilson, Rachel Lukowicz, Stefan Merchant, Helena Valquier-Flynn, Jeniffer Caballero, Jasmin Sandoval, Macduff Okuom, Christopher Huber, Tessa Durham Brooks, Erin Wilson, Barbara Clement, Christopher D Wentworth & Andrea E Holmes (2017) studied on Quantitative and qualitative assessment methods for biofilm growth itcontain biofilm assessment excels by systematic arranging and comparing various techniques. It helps through methods, from classic quantification using Crystal Violet staining and ATP bioluminescence, to advanced characterization with instruments like Scanning Electron Microscopy (SEM)helps easily select the most proper assay based on their research goals and available equipment. Di Ming, Dacheng Wang, Fengjiao Cao, Hua Xiang, Dan Mu, Junjie Cao, Bangbang Li, Ling Zhong, Xiaoyun Dong, Xiaobo Zhong, Lin Wang &Tiedong Wang (2017) studied on kaempferol inhibit the primary attachment phase of biofilm formation in staphylococcus aureus conclude that kaempferolprevents *S. aureus* biofilm formation. Its mechanism involves reducing the production of S. aureus surface proteins like ClfA, ClfB, FnBPA, and FnBPB by down-regulating key genes, which are essential for bacteria to adhere to surfaces and also inhibits the SrtA enzyme, its main effect appears to be through this gene repression, suggesting kaempferol is a potential new anti-biofilm compound for use on medical devices. Eman Butt, Ammar B. Altemimi , Aqsa Younas, Masood Sadiq Butt, Maryam Jalal, Maham Bhatty, Gholamreza Abdi &Rana Muhammad Aadil (2025) studied onGuava (*Psidium guajava*): A brief overview of its therapeutic and health potentialconcludes that guava (P. guajava) is a highly nutritious fruit contain a large number of therapeutic properties, which is consistent with its traditional value. Pre-clinical evidence supporting its role as an anti-inflammatory, antioxidant, and antidiabetic agent and ensure its safety for long-term health applications. GebreselemaGebreyohannes , Andrew Nyerere, Christine Bii, Desta Berhe &Sbhatu (2019) studied on Challenges of intervention, treatment, and antibiotic resistance of biofilm-forming microorganisms conclude that infections caused by biofilms are highly resistant to present antimicrobial agents forms a major issue in healthcare. Because of biofilms are resistance to antibiotic there is an urgent need to discover good treatment method. Strategies that include isolating quorum quenching compounds, dispersing formed biofilms, and combining antibiotics with quorum quenching compounds. Hera Nirwati, Kian Sinanjung, Fahrina Fahrunissa, Fernando Wijaya, SarastiaNapitupulu, Vania P Hati, Mohamad S Hakim, Andreanita Meliala, Abu T Aman, TitikNuryastuti (2019) studied onBiofilm formation and antibiotic resistance of Klebsiella pneumoniae isolated from clinical samples in a tertiary care hospital, Klaten, Indonesiaconcludes that most Klebsiella pneumoniae isolates from clinical samples in the tertiary care hospital studied demonstrated resistance to a wide range of antibiotics and were strong biofilm producers. This emphasizes the critical need for proper diagnosis, infection control, and treatment strategies, particularly for respiratory tract infections, due to the combination of high antibiotic resistance and the bacteria's ability to form protective biofilms.

Kaustubh D. Sonkusale and vidya S. Tale (2015) studied on Isolation and Characterization of Biofilm Forming Bacteria from Oral Microflora that the oral cavity of healthy individuals a diverse population of bacteria capable of forming biofilms, including Gram-positive cocci like *Streptococcus species* and Gram-negative coccobacillus. The biofilm formation capacity observed across the isolates, ranging from strong to weak under both static and rotary conditions, highlights the complexity and variability of these protective architectures. This strongly suggests that effective anti-biofilm strategies must account for the heterogeneity of oral microbiota and the protective layer formed by the exopolysaccharide matrix. Pantu Kumar Roy,Min Gyu Song &Shin Young Park (2022)studied on Impact of Quercetin against Salmonella Typhimurium Biofilm Formation on Food–Contact Surfaces and Molecular Mechanism Pattern the antibiofilm activity of the quercetin against Salmonella Typhimurium on common food-contact surfaces Quercetin inhibition of biofilm formation by physically disturbing cell-to-cell connections, inducing cell lysis, and inhibiting bacterial motility. Alsothe compound was found to effective to suppress the expression of virulence (avrA, hilA), stress response (rpoS), and quorum-sensing (luxS) genes. These conclude that plant-derived quercetin is a natural compound for use as an antibiofilm agent to improve food safety and hygiene. Sumar Naseer, Shabbir Hussian, Naureen Naeem, Muhammad Pervaiz & Rahman (2018) studied on The phytochemistry and medicinal value of *Psidium guajava* (guava)concludes that Psidium guajava (guava) is a highly valuable tropical plant with large number of medicinal uses. Its leaves and fruit are rich sources of essential vitamins, minerals, and bioactive compounds like antioxidants (e.g., quercetin and keamoferol) and polyphenols. Its application for various ailments, demonstrating antidiarrheal, anti-inflammatory, antimicrobial, antidiabetic, and antioxidant properties, suggest potential for therapeutic development and health promotion. Yan Zeng, Anna Nikikova1, Hossam Abdelsalam1, Jiyao Li, &Jin Xiao1 (2019) studied on Activity of Quercetin and Kaemferol against Streptococcus mutans Biofilm concluded that flavonoid Quercetin inhibits Streptococcus mutans biofilm formation by reducing the production of insoluble and soluble glucans. This results in a less dense, less viable, and less acidic biofilm structure. Yongqui Mu, Hong zeng&weichen (2021) studied on Quercetin Inhibits Biofilm Formation by Decreasing the Production of EPS and Altering the Composition of EPS in *Staphylococcus epidermidis* this concluded that quercetin does biofilm inhibiting activity by decreasing polysaccharides intracellular adhesion production and decrease cell surface water taking capacity make it hydrophobic. Also decrease production of extracellular polymeric substance and change composition of extracellular polymeric substance. Yu Kyung Kim, Pantu Kumar Roy, Md Ashrafudoulla, Shamsun Nahar, Sazzad Hossen Toushik Md Iqbal Hossain, Md Furkanur Rahaman Mizan, Si Hong Park & Sang-Do Ha (2022) studied on Antibiofilm effects of quercetin against Salmonella enterica biofilm formation and virulence, stress response, and quorum-sensing gene expressionit concluded that quercetin compound found in plants controlling Salmonella in food processing. It inhibited the bacteria from forming protective biofilms on surfaces like plastic, rubber gloves, and chicken skin with an inhibitory effect of up to 2.61log CFU/cm it worked by disarming the bacteria stopping their movement and suddenlyturning down the mechanism of genes responsible for virulence, stress response, and quorum sensing.

DISCUSSION

The extensive use of Guava plant leaves (*Psidium guajava Linn.*) as a medicinal agent is scientifically supported by its rich phytochemical profile, particularly its abundance of the flavonoids Quercetin and Kaempferol. This is highly significant given the challenge of biofilm infections, which exhibit high antibiotic tolerance due to dormant cells and the protective Extracellular Polymeric Substance (EPS) matrix. Quercetin inhibits biofilm maturation by altering the Extracellular polymeric substance (EPS) and suppressing genes responsible for stress response and virulence and Kaempferol is use for blocking the essential initial bacterial attachment. The reviewed literature strongly indicates that these flavonoids act as potent anti-biofilm agents. Specifically, they work by targeting the initial microbial adhesion phase and by reducing or altering the composition of the EPS and the compounds demonstrate molecular efficacy by downregulating genes associated with quorum sensing and virulence, thereby offering a mechanism-based alternative to conventional antibiotics.

CONCLUSION

The reviewconclude that Psidium guajava plant leaves are a powerful natural source for developing anti-biofilm therapies. The bioactive compounds mainly the flavonoids Quercetin and Kaempferolpresent in the leaves demonstrate significant efficacy against antibiotic-resistant of biofilm-forming bacteria at concentrations that inhibit their growth. This action against crucial stages of biofilm formation suggests that guava leaf extracts are highly favorable candidates for creating novel, non-antibiotic treatments to combat persistent infections in both clinical and food safety contexts in the future.

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