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REVIEW

Theaflavins Applications to Ameliorate Implant Failure and Eradicate Microbial Infections and Foodborne Pathogens: A Comprehensive Review

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ABSTRACT

Theaflavins, powerful antioxidants found in black tea (*Camellia sinensis*), have garnered increasing interest for their promising therapeutic potential. Experimental studies have contributed to enlightening about the advantages of theaflavins, including their antioxidant, anti-inflammatory, anticancer, antiosteoporosis, and antimicrobial properties. Theaflavin and its derivatives, particularly theaflavin-3,3′-digallate, have been particularly noted for their enhanced action in different areas. These compounds have found an important role as alternatives or adjuvants in the pharmaceutical sector, food industry, and in the improvement of health conditions. This review focuses on the antioxidant and anti-inflammatory aspects of theaflavins, particularly their potential in addressing peri-implant osteolysis. We explore mechanisms and pathways involved in this therapeutic action. Furthermore, we cover some of the relevant studies on the antimicrobial action of theaflavins in both the health and food sectors. Specifically, we explore the use of theaflavins for the treatment of dental infections, where these compounds have shown particular efficacy against several bacterial strains and their antimicrobial application in food matrices. Given the low solubility and stability of theaflavins in physiological conditions, we emphasize the benefits of the development of biocompatible and biodegradable nanoformulations to enhance the stability, bioavailability, and efficacy of these polyphenols, to promote their broader research and application. Given the potential demonstrated so far by in vitro and in vivo studies, the application of theaflavins stands as a promising alternative to enhance the existing strategies and fight prosthetic failure and antimicrobial resistance in the health and food sectors.

1 | Introduction

Black and green teas, derived from *Camellia sinensis* (*C. sinensis*, classified by (L.) O. Kuntze), are highly popular beverages, specifically in Asia (Seow et al. 2020). Both teas present a wide variety of bioactive compounds, with polyphenols being particularly notable. In green tea, polyphenols such as epigallocatechin, epicatechin-3-gallate epigallocatechin-3-gallate, and epicatechin, gallocatechin gallate, and gallocatechins are found in abundance (Luz et al. 2023). Black tea also presents a high concentration of polyphenols (approximately 30%–40%), with theaflavins being the predominant type. Theaflavins including

Abbreviations: EPS, extracellular polymeric substance; ERK, extracellular signal-regulated kinase; GSH, intracellular reduced glutathione; HO1, hemeoxygenase 1; MDA, erythrocyte malondialdehyde; MIC, minimum inhibitory concentration; MMPs, matrix metalloproteinases; MRSA, methicillin-resistant *Staphylococcus aureus*; NQO1, NADPH:quinine oxidoreductase 1; NRF2, nuclear factor erythroid 2-related factor 2; PMRS, plasma membrane redox system; RANKL, nuclear factor kappa-B ligand; ROS, reactive oxygen species; SOD2, superoxide dismutase-2.

theaflavin-3-3′-digallate, theaflavin-3-gallate, and theaflavin-3′-gallate represent 2%–6% of the dry weight of *C. sinensis*. These theaflavins are the result of catechins' oxidation and aggregation during the tea fermentation process in the presence of certain enzymes (Ito and Yanase 2022; Luo and Jiang 2021).

These polyphenols have attracted significant attention for a variety of therapeutic applications derived from their antioxidant, antimicrobial, and anti-inflammatory activities (Salimikia et al. 2023; Shan et al. 2021). Figure 1 depicts the protective effects of theaflavin and its derivatives on different health conditions and their applications in the food sector. The biological activities of these compounds are specific to their chemical structure.

For instance, the galloyl group was identified as the key factor for the antioxidant and anticancer activity of these polyphenols (Tan et al. 2019). The effects of consuming these compounds have been extensively reported to include lowering serum cholesterol levels (Maron et al. 2003), suppressing postprandial hypertriacylglycerolemia (Kobayashi et al. 2009), and modulating postprandial hyperglycemia (Chen et al. 2020) thereby aiding in the prevention of lifestyle-related diseases. Yet, the concentration of theaflavins obtained directly from the production of *C. sinensis* is still low to allow a broader study of the effect of these compounds' effects or their medical application for specific health conditions. Consequently, many extraction and synthesis methods involving chemical oxidizing reagents or enzyme production have been explored (Sinija, Mishra, and Bal 2007; Takemoto et al. 2017).

The concentration of theaflavins has enabled the study of their pharmacological properties in a dose-dependent manner and the elucidation of possible mechanisms of action. Due to the variety of promising results obtained so far, several reviews have addressed the advantageous applications of theaflavins and encouraged their wider exploration (Shan et al. 2021; Takemoto and Takemoto 2018). Despite this, theaflavins still present low bioavailability due to the characteristics such as high molecular weight and large polar surface area, which hinder their wider application in the clinical setting. However, recent advances in drug formulation might be the key to safely delivering these compounds and allow to enhance their efficacy with minimal toxicity and associated side effects. In the present review, we aim to discuss the antioxidant, anti-inflammatory, and antimicrobial actions of theaflavins, with particular emphasis on their therapeutic applications in prosthetic implantation, dental diseases, and potential use to guarantee food safety. This brief overview will explore the in vitro and in vivo experimental data available in the field. As a secondary goal, we also explore recent advances in theaflavin formulations, aiming to broaden the knowledge and research of these potential phyto-therapeutic agents and additives.

2 | Antioxidant Activity

Theaflavins as major antioxidants in *C. sinensis* have been reported for their capability to inhibit free radical generation (Miller et al. 1996), pro-oxidative enzyme activities (Yang et al. 2008), and for chelating transition metal ions, ultimately preventing lipid peroxidation both in vitro and in vivo (Miller et al. 1996). Yet, the antioxidant capacity of theaflavin and its respective derivatives has been shown to differ according to the

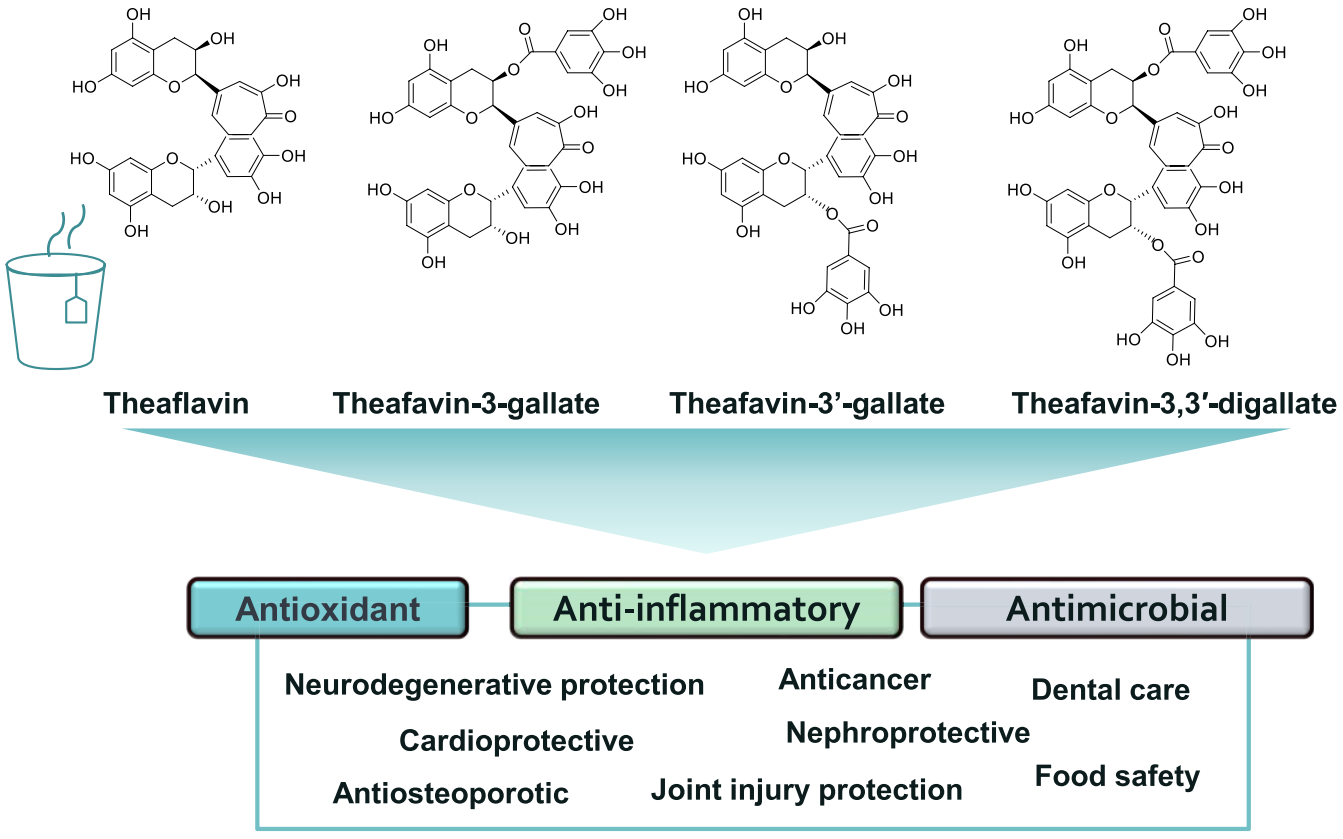

FIGURE 1 | Schematic illustration of theaflavin and its derivatives, including their studied therapeutic actions and protective effects.

chemical structure, consistent with the number and arrangement of phenolic hydroxyl groups (Miller et al. 1996). According to the ABTS assays, the antioxidant activity follows the sequence of reactivity as theaflavin 3,3′-digallate > theaflavin 3-monogallate ≈ theaflavin 3′-monogallate > theaflavin.

Although the scavenging abilities of these four compounds for reactive oxygen species (ROS) (including hydrogen peroxide (H_2O_2), hydroxyl radical (OH), oxygen singlet ($^1O_2$), and superoxide radicals) are still not fully disclosed, they appear to present selectivity (Wu et al. 2011). While theaflavin-3,3′-digallate scavenges mainly H_2O_2 and OH, theaflavin is only effective in neutralizing superoxide radicals. On the other hand, theaflavin-3-gallate and theaflavin-3′-gallate present a similar potency to inhibit OH and the superoxide radical (Wu et al. 2011).

ROS can be harmful when in excessive concentrations because they can accelerate membrane damage, DNA base oxidation, DNA strand break, and chromosome aberrations (AbdulSalam, Thowfeik, and Merino 2016). Consequently, these species can play significant roles in the development of several diseases (Brieger et al. 2012). Supplementation with either vitamin C or E is considered a promising therapeutic strategy for reducing the impact of ROS on health conditions (Jomova et al. 2023). Nonetheless, theaflavins from *C. sinensis* exhibit superior free radical scavenging abilities compared to vitamin C due to their greater number of hydroxyl groups, making them potential protective supplements. This benefit also derives from the fact that theaflavin and its respective derivatives inhibit free radical-induced DNA oxidative damage, which is associated with numerous diseases (Pham-Huy, He, and Pham-Huy 2008).

The preventive effects of these compounds on OH-induced DNA damage align with their selectivity to scavenge this radical. This protective effect has also been evaluated in erythrocytes by assessing oxidative stress biomarkers, during stress-induced conditions (Fatima et al. 2013). Biomarkers such as erythrocyte malondialdehyde (MDA), intracellular reduced glutathione (GSH), and plasma membrane redox system (PMRS), were monitored in vitro. Under stress conditions, these biomarkers significantly increased above basal levels. The presence of theaflavin in the incubation medium protected cells from stress, leading to increases in MDA and GSH levels at a concentration of 1 μm, while PMRS activity increased at a concentration of 10 μm (Fatima et al. 2013). Due to the impact of ROS in different cells and organs in the human body, theaflavin and its derivatives have been reported to exhibit antioxidant properties and offer valuable protection in the prevention of neurodegenerative disorders (Grelle et al. 2011; Zhang et al. 2016), cardiovascular conditions (Stangl et al. 2007), kidney injury (Li et al. 2021), cancer (Gao et al. 2019), among other conditions. Derivatives such as theaflavin-3,3-digallate can exhibit cell-specific properties, displaying different mechanisms of action according to the health ROS-mediated condition. For example, in cancer cells, this theaflavin derivative can induce ROS efflux, whereas in normal GN46 fibroblast cells, it increases GSH levels to provide an antioxidant effect (Schuck et al. 2008). The positive effect of theaflavins in oxidative stress can be particularly helpful in injury and inflammatory situations, such as those that occur in response to implants and infectious conditions (Anderson 1988; Spooner and Yilmaz 2011). The impact of theaflavin and its derivatives on the inhibition of ROS production, DNA damage, and the upregulation of antioxidant enzymes in injury cases has been verified in different cell lines, including hematopoietic cells (Han et al. 2017). Exposure to ionizing radiation can lead to genetic instability and even result in bone marrow failure. Despite this, strategies to mitigate induced injury remain scarce. Theaflavins have been associated with a decline in ROS levels and DNA damage, primarily by activating the nuclear factor erythroid 2-related factor 2 (NRF2) pathway (Figure 2). Theaflavins promote the NRF2 transportation into the nucleus, where it binds to the antioxidant response elements (ARE), leading to the transcriptional activation of antioxidant enzymes, such as hemeoxygenase 1 (HO1), NADPH: quinine oxidoreductase 1 (NQO1) and superoxide dismutase-2 (SOD2). This mechanism may not only prevent oxidative stress damage but also inhibit genomic instability and malignancies (Han et al. 2017). The activation of the NRF2 pathway is also crucial for scavenging ROS and reducing their accumulation in chondrocytes (Teng et al. 2022). Notably, the same enzymes, HO1 and SOD2, are expressed in these cells. By using this mechanism, theaflavin-3,3-digallate is capable of protecting articular cartilage from inflammatory processes. Since inflammation and ROS are interconnected, anti-inflammatory and antioxidant actions are integral to achieving successful therapeutic outcomes. The depletion of ROS from these cells, mediated by antioxidant enzymes, leads to iNOS or COX2 protein expression, which in turn reduces inflammatory reactions by downregulating the NF-κB pathway. Consequently, in vivo tests have shown that theaflavin-3,3-digallate can protect cartilage from degradation and alleviate osteoarthritis in rats, when infused into the knee joint, at 4 mm (Teng et al. 2022). These findings are supported by other studies highlighting the in vivo benefits of theaflavins, such as theaflavin-3,3-digallate, to treat osteoporosis, and bone diseases related to excessive osteoclastogenesis by inhibiting the intracellular ROS levels (Ai et al. 2020; Wenshu Liu and Li 2019). Theaflavin and its derivatives have demonstrated promising antioxidant properties with strong protective effects on biochemical and metabolic processes. Beyond researching their benefits, it is crucial to establish a therapeutic concentration that can be incorporated into a delivery system and to identify any potential side effects that might arise. This would represent a significant step forward in the journey toward making theaflavins widely used as phyto-therapeutics and prescribed by clinicians as a complementary therapy for a wide range of health conditions.

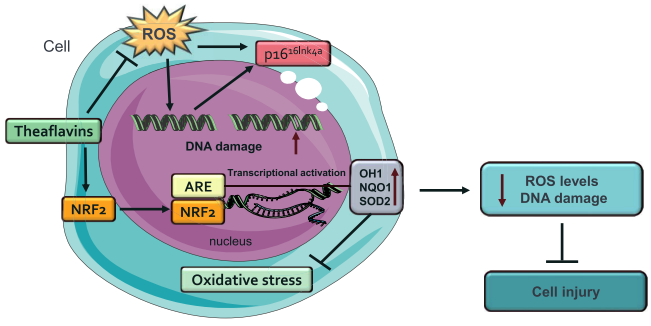

FIGURE 2 | Inhibition of ROS production and DNA damage by theaflavins through the activation of the NRF2 pathway (adapted from (Han et al. 2017)). The image was made with a servier medical art cell and adapted by the authors according to servier under the CC-BY 3.0 license (at https://smart.servier.com).

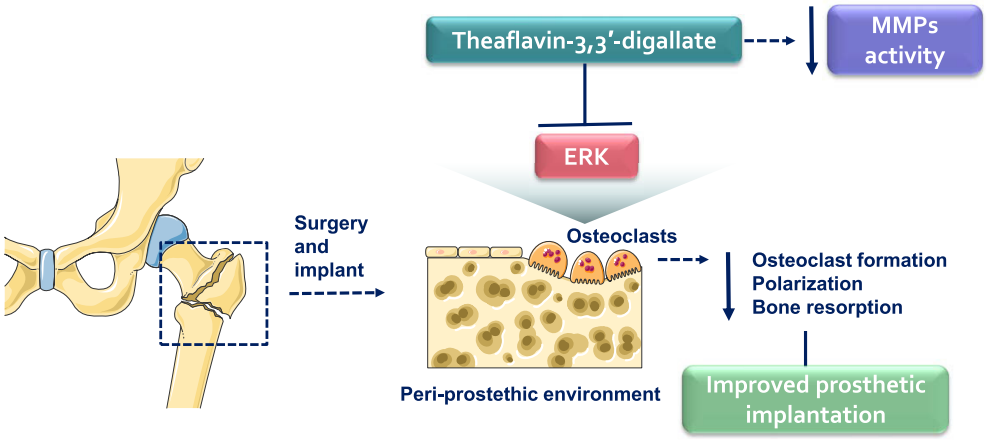

FIGURE 3 | Schematic summary of the mechanism of action of theaflavin-3,3′-digallate in inhibiting osteoclast formation and osteoclastic bone resorption, which can prevent implant failure. Image made with a servier medical art cell and adapted by the authors according to servier under the CC-BY 3.0 license (athttps://smart.servier.com/).

3 | Health Benefits and Its Applications

3.1 | Impact of Theaflavins in Prosthetic Failure

Joint arthroplasty is commonly employed to replace a damaged joint or as therapy for end-stage joint diseases (Brumat et al. 2022; Khattak et al. 2023). However, peri-implant osteolysis can lead to aseptic loosening, which is a major cause of prosthesis failure. Additionally, peri-implant osteolysis can be caused by activation of receptor activator of nuclear factor kappa-B ligand (RANKL)-induced osteoclastic bone resorption (Connors et al. 2022). According to in vitro and in vivo results, theaflavin-3,3′-digallate suppressed osteoclast formation, polarization, and osteoclastic bone resorption, induced by titanium particles by targeting the RANKL-induced extracellular signal-regulated kinase (ERK) signaling pathway (Hu et al. 2017). After treatment with 1 μm of theaflavin-3,3′-digallate, the bone resorption area can be decreased to 25.9%.

In this process, matrix metalloproteinases (MMPs) play a crucial role, as they can degrade a wide variety of extracellular matrix components (Mengshol, Mix, and Brinckerhoff 2002). Specifically, MMP-9 is essential in initiating osteoclastic resorption by removing the collagenous layer from the bone surface prior to demineralization, and it is highly produced in osteoclasts (Logar et al. 2007). Theaflavin-3,3′-digallate can decrease the activity of MMP-9 and MMP-2, thereby inhibiting the formation and differentiation of osteoclasts more effectively than epigallocatechin-3-gallate (Oka et al. 2012). The inflammatory microenvironment surrounding implants and in numerous bone diseases decreases osteoblast activation and inhibits bone formation, leading to implant failure. However, theaflavin-3,3′-digallate has been shown to reduce the release of proinflammatory cytokines and increase the expression of osteogenic markers in vivo, ultimately increasing bone mass (Ge et al. 2021). Thus, theaflavins represent promising options to tackle osteolysis and potentially improve prosthetic implantation (mechanism depicted in Figure 3).

Despite the promising therapeutic effects of theaflavins on prosthetic outcomes evidenced so far, they may not represent a standalone treatment option. However, exploring the use of theaflavins as a phyto-supplement during the recovery phase post-implant could be essential for ensuring patient success and well-being.

3.2 | Dental Infections

Several investigations have demonstrated that polyphenols from *C. sinensis* can inhibit the growth of pathogenic microorganisms (Wugao Liu et al. 2020; Zhang et al. 2021). Cariogenic bacteria or acidogenic (acid-forming) bacteria include Lactobacilli, such as *Lactobacillus Beijerinck*, *Streptococcus mutans*, *Streptococcus sobrinus*, and *Actinomyces* (Niu et al. 2020; Park et al. 2023; Rozen et al. 2001) can form biofilm and destroy and demineralize dental enamel by converting starch and glucose into acids (Augustinho do Nascimento et al. 2021). To prevent dental caries (damage of enamel and dentine) is essential to reduce cariogenic bacteria and remove biofilm plaque (Liu et al. 2022). Bacteria can form and develop dental biofilm in five stages: attachment, proliferation, early dispersal, mature, and dispersal—as depicted in Figure 4 (Sedarat and Taylor-Robinson 2022).

The application of antibiotics, antiseptic agents, and fluoride can help reduce cariogenic bacteria and biofilm formation. However, prolonged use of fluoride and chlorhexidine can cause dental fluorosis and oral erosion, respectively (Cui et al. 2023). Therefore, finding biocompatible and biodegradable antibacterial compounds is crucial for dental health. Polyphenols from *C. sinensis* can inhibit bacterial growth, adhesion, biofilm formation, and acid production of cariogenic bacteria (Goto et al. 2023; Li et al. 2019). Additionally, these natural compounds can enhance the acid resistance of tooth hard tissues against acid produced by cariogenic bacteria (Kurek-Górecka et al. 2022). Theaflavins exhibit strong antibacterial action against *S. mutans*, *S. sobrinus*, and *Actinomyces viscosus*. When used at 40%, they demonstrate minimum inhibitory concentration (MIC) values of 4 mg/mL for *S. mutans* and 2 mg/mL for the remaining strains, (Cui et al. 2023). As illustrated in Figure 5, at 1/16 of these MIC values, theaflavins reduced the cariogenic biofilm formation, and could potentially serve as anticaries drugs (Cui et al. 2023).

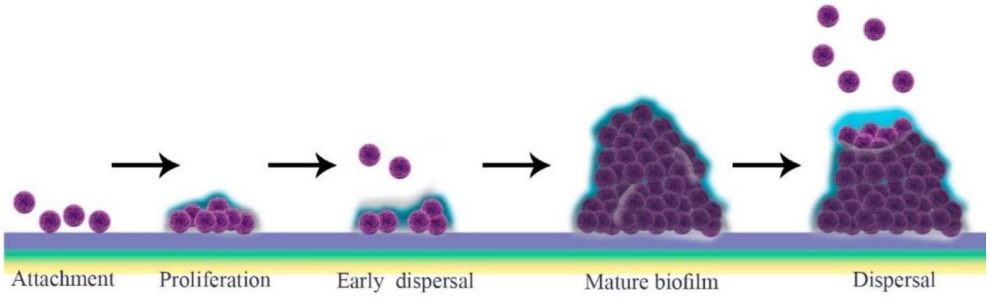

FIGURE 4 | Schematic image for the development of bacterial biofilm in five steps (adapted from (Sedarat and Taylor-Robinson 2022)).

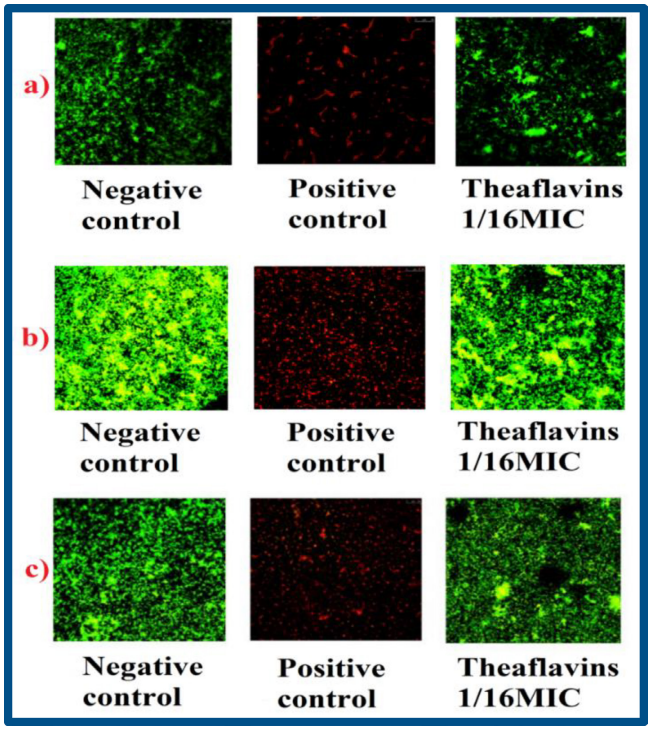

FIGURE 5 | Reduction of the biofilm formation by *S. mutans* (a), *S. sobrinus* (b), and *A. viscosus* (c) by theaflavins at a concentration of 1/16MIC (adapted and modified from (Cui et al. 2023)).

Interestingly, a significant reduction in oral pathogens, such as *Selenomonas, Prevotella*, and *Atopobium*, and an increase in oral-health-related bacteria, such as *Rothia* and *Streptococcus*, were observed in groups using toothpaste containing theaflavins. Theaflavin-containing toothpaste also reduced the synthesis of extracellular polymeric substances (EPS) by bacteria (Kong, Zhang, Xia, et al. 2021). Mature biofilms of *S. mutans* are highly resistant to antibiotics owing to their abundant EPS. Theaflavins have been shown to inhibit *S. mutans* in vitro and impact its acid production (Wang et al. 2019). Furthermore, theaflavin-3,3′-digallate can suppress biofilm formation at sub-inhibitory concentrations by reducing the biofilm matrix. The mechanism involves decreased gene expression of glucosyltransferases and extracellular DNA (Kong, Xia, Su, et al. 2021). Consequently, biofilm formation of *S. mutans* UA159 under a concentration of 15.6 μg/mL of theaflavin-3,3′-digallate shown to be less dense, with fewer connections between bacterial cells, as shown microscopically in Figure 6 (Wang et al. 2019). Concentrations of theaflavin-3,3′-digallate 10×MIC allowed to inactivate all bacterial cells in *S. mutans* biofilm, after 24 h, but did not show a significant bactericidal effect for treatments of 5 min. This finding suggests that the inactivation efficacy of theaflavin-3,3′-digallate against bacteria might rely on high concentrations and be time-dependent.

The combination of the antimicrobial and anti-inflammatory actions of theaflavins has been observed in the treatment of periodontal diseases caused by infectious agents such as *Porphyromonas gingivalis* (Ben Lagha and Grenier 2017). Theaflavins can inhibit the expression of genes encoding the major virulence factors of *P. gingivalis* in a dose-dependent manner. Additionally, these compounds can reduce the bacterium's adherence to gingival keratinocytes. Simultaneously, theaflavins decrease the secretion of pro-inflammatory factors, reduce NF-κB pathway stimulation, and inhibit gelatin degradation by MMP-9 (Ben Lagha and Grenier 2017). Current studies underscore the promising multifunctional therapeutic potential of theaflavins for preventing and treating dental infections, including associated inflammatory conditions (Kong et al. 2015; Lombardo Bedran et al. 2015). These beneficial therapeutic actions might be valuable not only as supplements but also actively incorporated into orthodontic products, such as toothpaste or mouthwash, to promote healthier oral hygiene.

4 | Antimicrobial Application of Theaflavins in the Food Industry

Inactivation and growth inhibition of pathogenic microorganisms, particularly, in biofilm form, are critical for food safety and public health (Rather et al. 2021). Theaflavin polyphenols offer potential applications in this area due to their antimicrobial and antioxidant properties (Ullah et al. 2022). However, the antibacterial effects of theaflavins can vary depending on environmental conditions and food matrices or additives. Factors such as salinity, pH, temperature, and the presence of substances like lecithin, sucrose, and skimmed milk powder in food matrices can impact their antibacterial efficacy (Wang et al. 2023). While higher salt concentrations in the media tend to enhance the antimicrobial action of theaflavins against *Staphylococcus aureus* and *Salmonella paratyphi B*, this activity significantly decreases in the presence of skimmed milk powder concentrations ranging from 20–120 g/L.

Furthermore, food additives such as lecithin and sucrose have been found to enhance the selective antimicrobial action against *Staphylococcus aureus* (Wang et al. 2023). Few reports address the antibacterial mechanisms of polyphenols such as

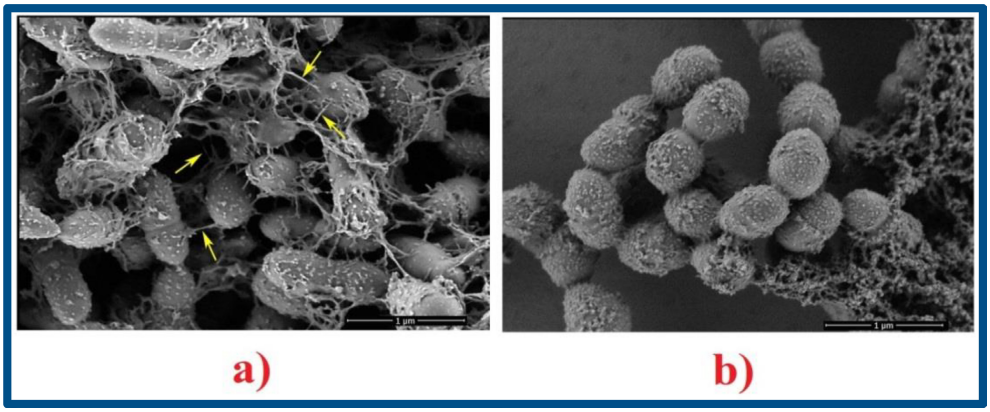

FIGURE 6 | FE-SEM images of (a) bacterial biofilm of *S. mutans* UA159 in the control group (ethanol 0.031%) and (b) reduction of EPS and bacterial dense upon the effect of theaflavin-3,3′-digallate (1/4×MIC) group at a magnification of 30,000× (yellow arrows demonstrate nanofibers connecting bacteria; extracted and modified from (Wang et al. 2019)).

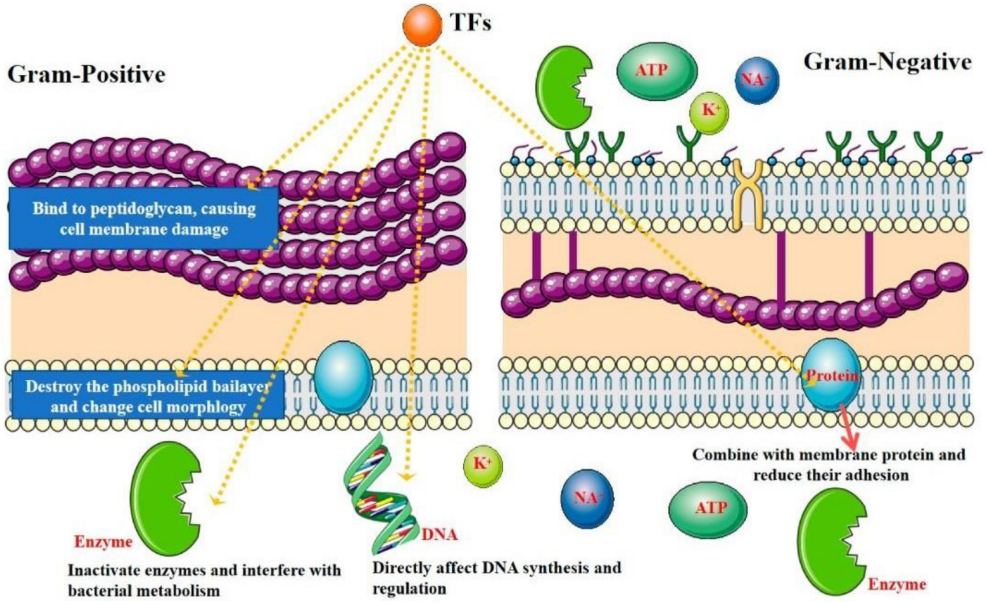

FIGURE 7 | Proposed mechanisms of bacterial inactivation using theaflavins strategy (extracted and modified from (Wang et al. 2023)).

theaflavins (Guan et al. 2022; Noor Mohammadi et al. 2019). Most studies focus on catechins (monomers of theaflavins) and suggest that these compounds can attach to the bacterial cell envelope or generate ROS, such as H_2O_2, which has bactericidal action (Arakawa et al. 2004; Nakayama et al. 2011).

Figure 7 shows probable antibacterial targets of theaflavins toward Gram-positive and Gram-negative bacteria (Wang et al. 2023).

Bacterial spores can lead to food spoilage and severe human diseases, such as *botulism*, *tetanus*, and *anthrax*. These diseases are caused by *Clostridium botulinum*, *Clostridium tetani*, and *Bacillus anthracis*, respectively (Koopman et al. 2022). Therefore, the successful inhibition and eradication of bacterial spores is crucial, particularly in the food industry where insufficiently heat-processed foods are a concern. Studies made with *Clostridium perfringens* suggested that theaflavin-3,3′-digallate can be a potential natural antimicrobial food additive (Noor Mohammadi et al. 2019). The mechanisms of action of this theaflavin derivative were explored showing that at high concentrations (500 mg/L), it could reduce biosynthetic and metabolic activities leading to bacterial cell death. Likewise, theaflavin-3,3′-digallate significantly decreased the viable spores of *Bacillus cereus* and *Bacillus subtilis* compared to the control group after 1 h incubation, when used at a concentration of 625 μg/mL (Yussof et al. 2022). The inactivation or growth inhibition of bacterial strains can be attributed to the ability of theaflavin to bind to the bacterial surface and affect gene expression, resulting in a slower germination process. Interestingly, according to the results of quartz-crystal microbalance analysis, there was a remarkable affinity of theaflavin derivatives with the membrane phosphatidyl glycerol bilayers of these bacteria. As a result, the antibacterial activity of theaflavin derivatives was significant against this strain at a concentration of 50 μmol/L (Sato et al. 2022).

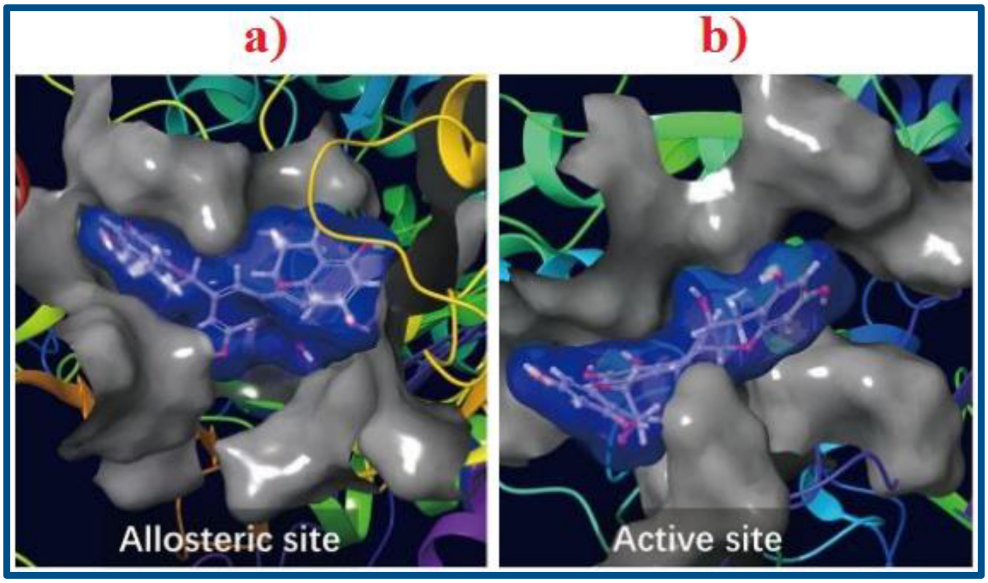

FIGURE 8 | Molecular docking representation of allosteric site bind of theaflavin to PBP2a as the main mechanism to boost β-lactam antibiotics to eradicate MRSA (extracted and modified from (Guan et al. 2022)).

Although out of the scope of this section, it should be highlighted that the antimicrobial action of theaflavin and its derivatives also plays an important role in the eradication of antibiotic-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) (Vanamala et al. 2021). The resistance of these bacteria is mainly attributed to the expression of the penicillin-binding protein 2a (PBP2a) enzyme that has a low affinity to β-lactam antibiotics, such as penicillin, and inactivate these antibiotics (Fergestad et al. 2020). Synergistic effects can be led by combination therapy of theaflavins with conventional antibiotics, particularly β-lactam antibiotics such as penicillin. Theaflavin can open the active site of PBP2a by interacting with the allosteric site of this enzyme, resulting in enhanced drug activity in vivo (Figure 8) (Guan et al. 2022).

Viral contamination of food is also of great concern as it facilitates the spread of numerous infectious diseases (Petrović and D'Agostino 2016). Studies with theaflavins have shown that these compounds can also exhibit activity against different viruses, such as HSV-1, HIV1, and influenza virus (de Oliveira et al. 2015). Theaflavins appear to provide a protective effect against the virulence of the influenza virus by influencing viral particle infectivity (Zu et al. 2012). Moreover, the incorporation of theaflavins as food additives can play an important role in the prevention of the spreading of fungi species such as *Aspergillus fumigatus*, *Candida albicans*, *Fusarium sp.*, and *Aspergillus niger* (Siddiqui et al. 2016; Xu et al. 2022).

Theaflavins exhibit antibacterial, antifungal, and antiviral activity, significantly impacting microbial load and spread in various foods. However, their application as adjuvants in food preservation is limited due to the insufficient exploration of complex matrices. Further research is needed to determine the optimal concentrations of theaflavins and to ensure they are not cytotoxic. By doing so, it would be possible to regulate theaflavins as food additives and expand their broader application.

5 | Theaflavin Formulations and Their Impact on Therapeutic Efficacy

Theaflavins exhibit low water solubility, primarily due to their polyphenolic structure. This solubility limitation negatively impacts the bioavailability and formulation of theaflavins, thereby restricting their clinical application (Qu et al. 2021). Therefore, advances in theaflavin formulations using biocompatible organic and inorganic nanomaterials are crucial to overcoming these challenges (Adefegha et al. 2022). Despite the fact that theaflavins have been successfully incorporated in nanoemulsions and nanoparticles with positive impact on the final therapeutic action, most studies focus on the polyphenolic tea content rather than being theaflavin-specific.

The encapsulation of tea polyphenols in nanoemulsions prepared by the ultrasound-assisted method has shown significant bioactivity and storage stability up to 5 weeks (Li et al. 2023). The system displayed good biocompatibility with human hepatoma cells Hep-G2 cells, in concentrations of polyphenols up to 0.4 mg/mL. However, although the nanoemulsion with tea polyphenols demonstrated an effect on bacterial cell morphology and damage, the effect of the nanoemulsion without the tea compounds was not tested. Therefore, synergistic effects cannot be conclusively determined. Nanogels based on lysozyme and carboxymethyl cellulose have also been used to encapsulate and protect tea polyphenols (Liu et al. 2019). These nanoformulations enable the encapsulation of tea polyphenols with an 89% efficiency and can provide a sustained release of these compounds. Incorporating tea polyphenols in nanoformulations resulted in a stronger antitumor activity on HepG2 cells compared to tea polyphenols alone. This finding clearly highlights the benefits of formulating tea compounds for their future application. Nanoformulation can be also beneficial in improving the pharmacokinetics and bioavailability of tea polyphenols. This has been particularly demonstrated using gelatin nanoparticles (Kulandaivelu and Mandal 2017). A comparative in vivo pharmacokinetic study

using a rabbit model demonstrated that when encapsulated in gelatin nanoparticles tea polyphenols are delivered in a sustained release profile and achieved higher plasma concentrations than the polyphenols alone (15.73 vs. 27.42 μg/mL, respectively) after administration of a single oral dose of 50 mg/kg.

These studies have facilitated the research of theaflavin-specific nanosystems. One of the main advantages of encapsulating theaflavins is the possibility to extend their stability and shelf-life, consequently maintaining efficacy (Yang et al. 2023). Zein protein has shown to be capable of stabilizing theaflavins in nanoformulations for more than 30 days at 4°C, with an encapsulation efficiency maintained above 70%. The use of this type of formulation enables to enhance theaflavins solubility and provides a controlled release of these compounds in gastrointestinal mimetic conditions. (Yang et al. 2023). The therapeutic efficacy of theaflavins can also be improved in nanoformulations by conjugating theaflavins; theaflavin is conjugated with gold nanoparticles (AuNPs), and its possible to enhance the apoptotic ability in comparison to the bare theaflavin in PA-1 cells (Maity et al. 2019). Yet, the lack of in vivo studies represents a drawback toward the possibility to further explore theaflavins in the treatment of diseases such the ovarian cancer cells.

The use of nanoliposomes has been shown promising to help improve theaflavins stability during in vitro digestion under both mild alkaline pH and simulated intestinal fluid conditions (Ding et al. 2018). This possibility can be promising to expand the application of theaflavin and its derivatives as functional ingredients in the food industry. However, both research on theaflavin formulations applied to the health sector and their nanoformulation for the food sector are still limited and require more comprehensive studies.

6 | Materials and Methods

This review was conducted following a comprehensive literature search using PubMed, Web of Science, Google Scholar, and Scopus. Keywords such as "theaflavins," "theaflavin derivatives," "antioxidant," "antioxidant activity," "prosthetic failure," "implant," "osteolysis," "dental infections," "cariogenic bacteria," "biofilm," "anti-inflammatory," "antimicrobial," "food matrices," "food industry," "bacterial spores," "nanoformulations," and related terms were used. Boolean operators (AND, OR) were employed to combine these terms during the search. References cited in relevant articles were also examined to ensure comprehensive coverage of all publications related to the topic. The identified articles were reviewed by the authors and included in the review if deemed relevant to each section. The review primarily focuses on experimental studies, with the literature from 2016 to 2024 being the main focus, as this range encompasses the most relevant and recent studies on the topic. Research articles outside this range were included only if they provided significant insights or were the first to report a particular discovery. The exclusion criteria were applied as follows: studies based on case reports, conference abstracts, editorial comments, articles with insufficiently detailed methodology or data, and those with unreliable results were excluded.

7 | Conclusions and Future Directions

Theaflavin and its derivatives display antioxidant activity, which can be beneficial in scavenging different ROS. Their antioxidant properties are particularly relevant for treating different health conditions, including their ability to treat osteoclast-related osteolytic diseases. Based on the current studies, these compounds stand as promising adjuvants for improving the success of implants by reducing the inflammatory response in the surrounding tissue.

The anti-inflammatory action of these polyphenols can be combined with their remarkable antimicrobial potential against different pathogens, proving effective on both Gram-negative and Gram-positive bacteria. Due to their broad spectrum of action, theaflavins have demonstrated a beneficial effect in the treatment of dental infections. In the future, incorporating these compounds into nanomaterials such as nanoparticles or nanocomposites can be seen as a promising strategy to increase the biocompatibility and bioavailability of theaflavins and deliver these compounds to their therapeutic target. Since theaflavins can exert their effect and have a protective action on human cells, their application in healthcare products, such as toothpastes and antiseptic agents, may be preferred to avoid hard excipients that induce unwanted side effects.

Likewise, theaflavins can help inactivate or inhibit the growth of bacterial strains, viruses, and fungi that compromise food safety and can act as vectors of disease spreading. The application of theaflavins as food additives can extend the shelf-life of food products by providing both antimicrobial and antioxidant protection. Therefore, these polyphenols are critical compounds to decrease microbial contamination and increase product quality in the health and food sectors.

Due to the low water solubility and stability displayed by theaflavins, their formulation is majorly affected and scarcely explored. The development of novel nanoformulations can represent a step forward to maximize the therapeutic potential of theaflavins, making them more effective in various applications, including the treatment of cancer, inflammation, and microbial infections. The progress of their research will enable the design of not only simple delivery systems for sustained release but also enable the dual delivery of theaflavins with other therapeutic agents, such as anti-cancer drugs or antibiotics, potentially leading to synergistic effects and improved treatment outcomes. The research and application of these formulations in the food sector can even enable the development of functional foods with enhanced antioxidant, anti-inflammatory, and antimicrobial properties. Despite the need for long-term safety data from clinical trials on the use of theaflavins in the food and health sectors, the experimental data gathered so far shows a promising future for these compounds. Theaflavins are not only appealing to consumers but also offer numerous health benefits.

Author Contributions

Mehran Alavi: conceptualization, investigation, methodology, validation, visualization, writing – original draft, writing – review and editing. **Sónia N. Pedro:** writing – original draft, writing – review and

editing. **Mara G. Freire:** writing – original draft, writing – review and editing. **Morahem Ashengroph:** project administration, resources, supervision, validation, visualization, writing – review and editing. **Haroon Khan:** writing – review and editing.

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Conflicts of Interest

M.A., M.G.F., S.N.P., H.K., and M.As. declare that there are no known conflicts of interest associated with this publication and there has been no prominent financial support for this work that could have influenced its outcome.

Data Availability Statement

The authors have nothing to report.

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