Antimicrobial and Antioxidant Activities of Psidium Guajava Leaves Extract in Some Dairy Products: a mini review

Nahed Gomaa Kasem 1, Hend Ali Elshebrawy 1, Waleed Emad Mokbel 2, Heba Hany Alagwany 2, Asmaa Elsayed Elgharaz 2, Wafaa Ragab 3

1 Department of Food Hygiene, Safety and Technology, Faculty of Veterinary Medicine, Mansoura University

2 Senior students of Food Safety and Hygiene Program

3 Department of Bacteriology, Immunology and Mycology, Faculty of Veterinary Medicine, Mansoura University

Corresponding author: Nahed Gomaa Kasem (email: nahedkasem92@gmail.com)

Abstract

The dairy industry serves as a vital component of the global food system, offering a diverse array of products that cater to various tastes and dietary preferences. However, ensuring the safety, quality, and nutritional integrity of dairy items remains a significant challenge for producers. In response to this challenge, there has been a notable shift towards exploring natural alternatives to traditional preservatives and additives. Psidium guajava leaves extract has emerged as a promising candidate, thanks to its remarkable antimicrobial and antioxidant properties, positioning it as a natural ingredient with the potential to enhance the safety and quality of dairy products. By incorporating guava leaf extract into dairy formulations, producers have the opportunity to extend shelf life, improve microbial safety, and elevate the nutritional profile of their products, catering to the preferences of health-conscious consumers. This review delves into the existing literature on guava leaf extract, its chemical composition, and the best method to extract components to obtain good antioxidant and antimicrobial properties. We will discuss different studies which examined antioxidant and antimicrobial properties of psidium guajava leaves extract and its applications in dairy products (labneh, ghee and yoghurt), aiming to shed light on the benefits and opportunities presented by this natural ingredient in dairy products.

Key words: Psidium Guajava Leaves, dairy products, guava leaf extract, food safety

Introduction

The dairy industry is constantly evolving to meet the changing needs and preferences of consumers while ensuring product safety, quality, and nutritional value. In this dynamic landscape, the integration of natural additives has emerged as a promising strategy to enhance dairy products. One such natural additive that has garnered attention is Psidium guajava leaves extract, known for its potent antimicrobial and antioxidant properties [1].

Psidium guajava, commonly known as guava, is a tropical fruit tree whose leaves have long been recognized for their various health benefits. Extracts from guava leaves have been shown to possess antimicrobial properties, making them effective in inhibiting the growth of foodborne pathogens [2]. This natural preservative potential of guava leaf extract presents a sustainable and eco-friendly solution for dairy producers looking to enhance the safety and shelf life of their products [3].

In addition to its antimicrobial properties, guava leaf extract is also rich in antioxidants and other bioactive compounds that have been linked to various health- promoting properties including anti-inflammatory and anti-diabetic effects [4].

The utilization of guava leaf extract in dairy products represents a convergence of traditional dairy processing techniques with innovative natural ingredients [5]. This integration not only enhances the safety and health benefits of dairy products but also presents an opportunity for producers to differentiate their offerings in a competitive market [6].

As the dairy industry continues to innovate and adapt to changing consumer trends, the incorporation of natural additives like guava leaf extract holds promise for driving future product development and consumer satisfaction [7]. By exploring the potential of guava leaf extract in dairy products, producers can tap into the benefits of this natural ingredient to create products that are not only safe and nutritious but also align with the evolving needs of today's discerning consumers [8].

Literature review

1- Microbes' effects on dairy products and milk borne pathogens

The list of bacteria which can be responsible for milk-borne diseases is long and it includes Brucella spp, Campylobacter jejuni, Bacillus cereus, Shiga toxin-producing E. coli (E. coli O157:H7), Coxiella burnetii, Listeria monocytogenes, Mycobacterium tuberculosis, Mycobacterium bovis,

Mycobacterium avium subspecies paratuberculosis, Salmonella spp, Yersinia enterocolitica, and certain strains of Staphylococcus aureus which are capable of producing highly heat-stable toxins [9].

In 2023, the Centers for Disease Control and Prevention (CDC) stated that the presence of various bacteria in milk poses significant health risks if consumed without proper treatment or pasteurization. Among the common milk-borne bacteria are Salmonella spp., known for causing salmonellosis characterized by symptoms like diarrhea, fever, vomiting, and abdominal cramps [10]. In 2024, the CDC reported that certain strains of Escherichia coli (E. coli), particularly E. coli O157:H7, can lead to severe foodborne illnesses such as hemolytic uremic syndrome (HUS) when ingested through contaminated milk [11].

The Food and Drug Administration (FDA) stated that Listeria monocytogenes is another pathogenic bacterium found in milk that causes listeriosis. The latter is a serious infection with high morbidity and mortality rates, especially among vulnerable populations. FDA mentioned that Staphylococcus aureus, which commonly present in the environment and human skin, can produce heat-stable enterotoxins in milk, resulting in food poisoning symptoms like nausea, vomiting, abdominal cramps, and diarrhea. FDA also mentioned that Bacillus cereus, a spore-forming bacterium, can contaminate milk and dairy products, leading to gastrointestinal illnesses such as diarrheal syndrome and emetic syndrome, often known as "Fried Rice Syndrome" [12].

2- Methods of Dairy products preservation and its disadvantages

Dhanashekar et al. [9] examined various methods employed in the preservation of dairy products, aiming at extending shelf life and ensuring quality and safety. Among these methods, pasteurization stands out as a commonly utilized technique. It involves heating milk to a specific temperature for a set period to eradicate pathogenic bacteria and reduce microbial load while retaining its nutritional integrity. This process has significantly contributed to enhancing the safety of dairy items, mitigating the risks associated with raw milk consumption.

Tamime and Robinson [13] mentioned that drying or dehydration plays a significant role in preserving dairy products by removing moisture, thus inhibiting microbial growth and enzymatic activity. Products like powdered milk, condensed milk, and powdered cheese undergo drying methods, ensuring convenience and extended shelf life for storage and transportation. They noted that fermentation emerges as a traditional yet effective preservation technique in dairy proces3sing. Particularly prominent in the production of yogurt, kefir, and cheese, fermentation involves the

introduction of beneficial bacteria or cultures to milk. These microorganisms metabolize lactose into lactic acid, creating an acidic environment that inhibits the growth of harmful bacteria. Fermented dairy products not only boast prolonged shelf life but also offer distinct flavors, textures, and health benefits due to the presence of probiotics.

Another prevalent preservation method is ultra-high temperature (UHT) treatment. This involves subjecting milk to high temperatures for a brief duration, effectively sterilizing it and enabling extended storage at room temperature without refrigeration. UHT treatment facilitates prolonged shelf life for dairy products, making them viable for distribution in regions with limited access to refrigeration facilities [14].

In 2017, Amit et al. [15] emphasized that refrigeration serves as a fundamental method for dairy product preservation. Cold temperatures retard microbial growth and enzymatic reactions, thereby preserving the freshness and safety of dairy items such as milk, cheese, and butter until consumption. While these preservation methods are indispensable for extending shelf life and ensuring safety, they are not without drawbacks.

Mehra et al. [16] discussed potential disadvantages of preservation methods, including alterations in flavor and nutritional content due to high-heat treatments like pasteurization and UHT. Additionally, preservation methods such as pasteurization and UHT treatment may inadvertently eliminate beneficial microorganisms, diminishing potential health benefits associated with probiotics and other functional components.

3- Natural inhibitor and preservatives

Natural inhibitors offer potential advantages over conventional preservation methods. These inhibitors, such as antimicrobial compounds found in certain plants or controlled microbial fermentation may effectively preserve dairy products while minimizing alterations to flavor and nutritional content. Additionally, they have the potential to maintain beneficial microorganisms, reduce energy consumption, and offer cost-effective solutions for dairy product preservation [16].

Kaptan and Sivri [17] categorized the secondary components abundant in aromatic and medicinal plants, which included alkaloids, glycosides, flavonoids, tannins, phenols, colorants, and resins, known for their preservative and antioxidant properties. These compounds, particularly phenolics, exhibit antioxidant activities, metal chelation abilities, and antimicrobial effects against foodborne

pathogens and spoilage microorganisms. Moreover, they hold potential in managing chronic diseases like diabetes, cardiovascular conditions, and cancer.

Wahba et al. [18] demonstrated the efficacy of plant extracts, such as cinnamon, garlic, lemongrass, and rosemary, in reducing L. monocytogenes in processed cheeses. Natural preservatives are increasingly favored for their perceived health benefits.

In a study, Abd El-Aziz et al. [19] reported that plant-based chemicals, including diallyl sulphides in Allium species, terpenoids like carvone and limonene in spearmint essential oil, eugenol in clove oil, and thymol in thyme oil, are recognized for their antibacterial actions. These compounds, such as gingerols, gingerdiols, and shogaols found in ginger, further contribute to antibacterial activity.

Furthermore, Behrad et al. [20] found that aqueous licorice and cinnamon extracts exhibited significant inhibitory effects on Helicobacter pylori formation when added to yogurt.

Also, Shan et al. [21] reported that the inhibitory effects of extracts from cinnamon sticks, pomegranate peels, and oregano on the growth of S. aureus, L. monocytogenes, and S. enterica in cheese. They particularly highlighted clove extract's potential as an organic food preservative.

Tayel et al. [22] further supported the antimicrobial properties of green pepper and cayenne extracts in reducing S. aureus in Egyptian Kareish cheese.

Many reports [23] [24] have demonstrated the antibacterial action of essential oils from oregano, thyme, salvia, basil, and black cumin against L. monocytogenes in various cheese types. These additives (particularly the clove essential oil) extended the shelf life of paneer cheese.

4- Therapeutic Potential of Psidium guajava: Antimicrobial, Antioxidant, and Medicinal Attributes

Pandey and Rizvi [25] have focused in their investigation on Psidium guajava, particularly emphasizing its antimicrobial and antioxidant effects as derived from its leaves extract. Guava, renowned for its tropical origins and delectable fruits, carries additional significance due to its medicinal attributes. A pivotal constituent contributing to its therapeutic potential is the extract obtained from its leaves. Extensive research indicates that Psidium guajava leaves possess robust antimicrobial properties, effectively hindering the proliferation of diverse microorganisms encompassing bacteria, fungi, and viruses. Moreover, the extract derived from these leaves exhibits antioxidant capabilities attributed to bioactive compounds like flavonoids, phenolics, and tannins.

These compounds serve as scavengers of free radicals, thereby counteracting detrimental molecules within the body and safeguarding against oxidative stress—a precursor to numerous ailments including cancer, cardiovascular diseases, and neurodegenerative disorders. They found that Psidium guajava leaves extract was effective against both Gram-positive and Gram-negative bacteria, including Staphylococcus aureus and Escherichia coli. Moreover, it exhibits effectiveness against fungal infections such as Candida albicans, thus positioning Psidium guajava leaves extract as a prospective avenue for the development of novel antimicrobial agents to combat infectious diseases.

Bunu et al. [26] underscored the substantial antioxidant properties inherent in Psidium guajava leaves extract. These properties stem from the presence of various phytochemicals like polyphenols, flavonoids, and vitamin C, which serve as potent free radical scavengers. By neutralizing harmful reactive oxygen species in the body, the extract safeguards cells from oxidative damage, thereby mitigating oxidative stress-related ailments such as cancer, cardiovascular diseases, and neurological disorders. Furthermore, the extract exhibits promising anti-aging effects by diminishing the formation of wrinkles and fine lines on the skin. These cumulative antioxidant benefits position Psidium guajava leaves extract as a promising natural remedy for enhancing overall health and wellbeing.

5- Chemical composition of Guava leaves

Guava leaves are a rich source of various health-promoting micro- and macronutrients as well as bioactive compounds. They contain 82.47% moisture, 3.64% ash, 0.62% fat, 18.53% protein, 12.74% carbohydrates, 103 mg ascorbic acid, and 1717 mg gallic acid equivalents (GAE)/g total phenolic compounds. The overall proximate profile of GLs is presented in Table 1 [27].

Table 1: Nutritional profile of guava leaves [27].

Compounds	Content/Composition
Elements and ascorbic acid	
Potassium	1.11%
Phosphorus	0.23%
Nitrogen	1.02%
Ascorbic acid	142.55 mg/100 g
Carbohydrates/phenols/sulfates	
Fucose	1.44%
Rhamnose	3.88%
Arabinose	22.6%
Galactose	29.41%
Glucose	33.79%

Mannose	0.59%
Xylose	7.71%
Phenol	15.28%
Sulfate	18.58%
Carbohydrate	48.13%
Sulfate polysaccharide	66.71%
Protein	
Association of Official Analytical Chemists method	22.98 ± 0.036% [dry weight basis]

GLs are a rich source of essential oils (Table 2). The major constituent of GL essential oil includes 1,8-cineole and trans-caryophyllene [28]. Other secondary metabolites present in GLs include phenolic acids. Phenolic compounds serve as key bioactive compounds which provide antioxidant and hypoglycemic properties to GLs (figure 1) [27].

Table 2: Essential oil components of guava leaves [27].

Compounds	**Content/Composition**
Essential oil components	
α-Pinene	1.53%
Benzaldehyde	0.83%
p-cymene	0.52%
Limonene	54.7%
1,8-Cineole	32.14%
β-*cis*-Ocimene	0.28%
γ-Terpinene	0.38%
α-Terpineol	1.79%
β-Caryophyllene	2.91%
α-Humulene	0.77%
Total identified constituents	95.85%

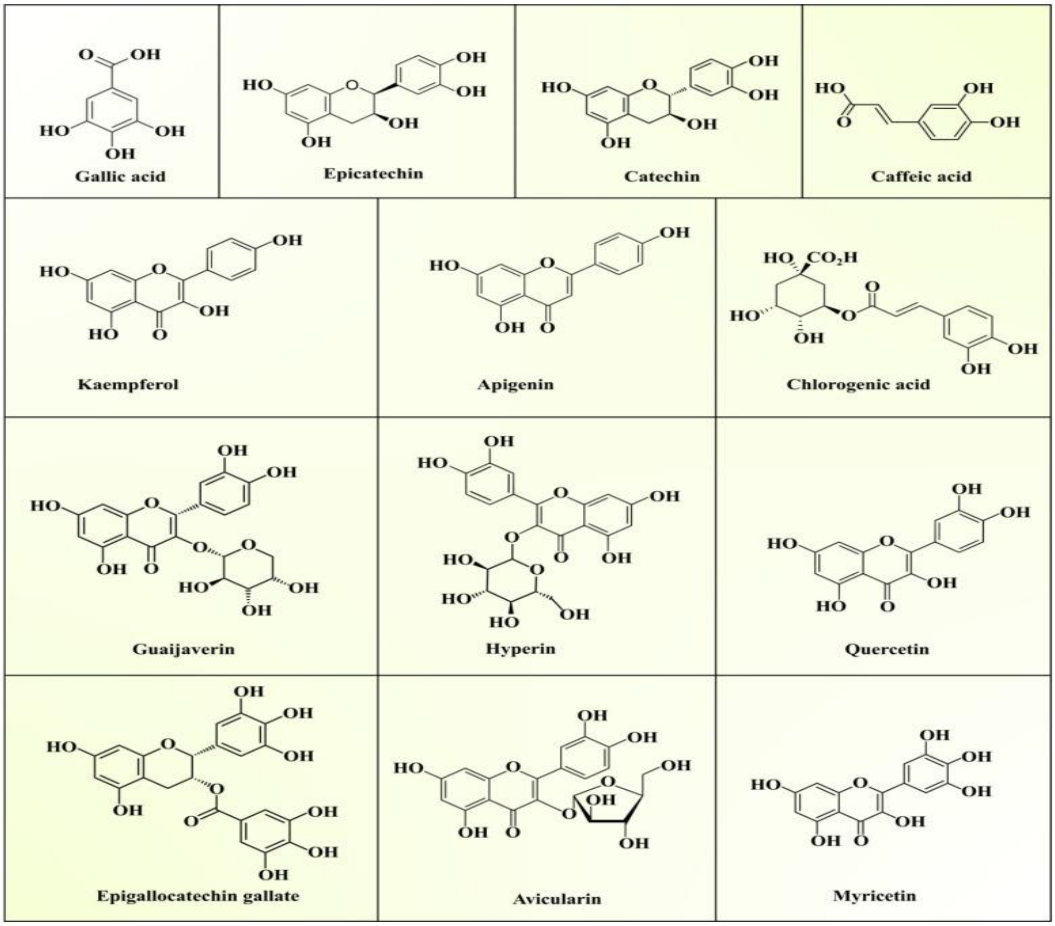

Figure 1: Phenolic compounds present in guava leaf extracts [27].

6- Antimicrobial Activity of Guava Leaves extract

Mickymaray [29] investigated the antimicrobial properties of guava leaves extract stem from a diverse array of bioactive compounds sourced from plants. These compounds operate through multiple mechanisms, including impeding microbial cell wall growth and integrity, biofilm formation, DNA replication, transcription, ATP synthesis, bacterial toxin suppression, and reactive oxygen species (ROS) generation.

Similarly, Soliman et al. [30] reported that essential oils derived from GLs exhibit robust antibacterial efficacy against various pathogens. They also reported that encompassing Pseudomonas aeruginosa, Escherichia coli, Streptococcus faecalis, Staphylococcusaureus, and Bacillus subtilis, along with demonstrated antiproliferative and antioxidant properties.

Bose and Chatterjee [31] found that biogenic silver nanoparticles synthesized using GL extract display antibacterial efficacy against pseudomonas aeruginosa due to strong antiradical activity against DPPH and ABTS radicals.

Another study stated that the presence of phenolic acids, flavonoids, terpenoids, glycosides, and saponins in both aqueous and organic extracts of guava leaves correlates positively with their antibacterial activity. Notably, water-soluble tannins within GLs act as bacteriostatic agents by impeding extracellular enzymes, oxidative phosphorylation, and substrate withholding, exhibiting efficacy against antibiotic-resistant Staphylococcus aureus strains [32].

Gonçalves et al. [33] investigated the effect of GL extracts against diarrhea-causing bacteria, including Staphylococcus aureus, Salmonella spp., and Escherichia coli. The extracts were obtained using hexane, ethyl acetate, and methanol. Staphylococcus aureus strains exhibited greater inhibition by the extracts, with the methanol extract demonstrating the highest bacterial inhibition. No statistically significant differences were observed among the tested extract concentrations and their effects. The essential oil extract displayed inhibitory activity against S. aureus and Salmonella spp. Moreover, the methanol extract showed more pronounced bacterial growth inhibition compared to hexane and ethyl acetate. The antimicrobial effect of guava leaf extracts was more pronounced on S. aureus than on E. coli and Salmonella spp. The authors also noted that guava leaf essential oil exhibited greater inhibition of S. aureus growth compared to methanol, hexane, and ethyl acetate extracts.

7- Antioxidant Activity of Guava Leaves Extract

In the study by Aktumsek et al. [34], the antioxidant properties of guava leaf extracts were evaluated through in vitro assays. The results indicated a positive correlation between phenolic compound levels and antioxidant capacity, with the water extract exhibiting the highest phenolic compound content and consequently the greatest antioxidant capacity. Moreover, the antioxidant activity increased with extract concentration, and the hydroethanolic extracts outperformed the water extracts, particularly the 50% hydroethanolic extract, which displayed the highest antioxidant activity.

The antioxidant properties of guava leaves are attributed to phenolic compounds like ferulic acid, gallic acid, and ellagic acid, along with flavonoids such as quercetin and kaempferol. GL extracts have shown moderate antioxidant activity, as demonstrated by a DPPH assay, with essential oils

exhibiting an IC50 value of ~460.37 ± 1.33 µg/mL. Additionally, GL extracts have been found effective in scavenging peroxyl radicals and reducing oxidation of linoleic acid. Studies have established a direct correlation between the phenolic content of GL extract, its antioxidant efficacy, and its ability to scavenge free radicals. GL polysaccharides have demonstrated protective effects against oxidative stress- induced damage by preventing the production of ROS and lipid peroxidation. Furthermore, GL extracts have been proposed as functional food additives to prevent oxidation in fresh pork sausages. Fermentation of GLs with bacterial and yeast strains has been shown to enhance the antioxidant capacity of soluble polyphenols in guava leaf extracts [27].

8- Some studies applied guava leaves extract on dairy products

El-Gazzar et al. [35] investigated the fortification of Labneh with guava leaves extract to create a functional product with natural antioxidant properties. Their research explored the effects of varying concentrations of guava extract on Labneh's chemical composition, antioxidant activity, and sensory attributes during storage. The results revealed elevated acidity and total solids, alongside alterations in texture parameters following the addition of guava extract. Moreover, higher concentrations of guava extract correlated with increased antioxidant activity. Microbiological analysis demonstrated the absence of molds, yeasts, or coliform bacteria, while organoleptic evaluation indicated enhanced quality with guava extract supplementation. Overall, the incorporation of guava extract influenced both the chemical composition and antioxidant activity of Labneh, indicating potential advantages in terms of nutritional value and shelf life.

Aditya and Divya [36] investigated the incorporation of natural antioxidants from guava leaves to enhance the shelf life of ghee. The antioxidant compounds extracted from guava leaves were found to have high potential in inhibiting lipid oxidation and enhancing the shelf life of ghee. The study also optimized the levels of alcoholic and aqueous extracts to improve the sensory and physico-chemical parameters of the ghee. Additionally, the results showed that the extracts effectively reduced the formation of peroxides, free fatty acids, and thiobarbituric acid in the ghee, indicating their potential as natural preservatives. Furthermore, the radical scavenging activity of the ghee incorporated with the extracts was found to be significant, demonstrating their ability to inhibit autooxidation. Overall, the study provided valuable insights into the use of natural antioxidants from guava leaves to improve the shelf life and quality of ghee, with potential applications in the food industry. These findings contribute to the development of natural and safe alternatives to synthetic antioxidants for food preservation.

In a recent study by Hassan et al., the addition of guava extracts to flavored yogurt showed promising results in terms of enhancing its antioxidant, antimicrobial, and sensory properties. The study found that guava extracts from seeds, green and red leaves, and juice exhibited significant antioxidant activity, as demonstrated by assays such as DPPH and ABTS, with this activity showing enhancement during cold storage. Additionally, these extracts showed antibacterial activity against tested microorganisms, indicating their potential for extending the shelf-life of yogurt. Furthermore, the supplementation of yogurt with guava extracts notably enhanced its sensory characteristics without adversely affecting its acidity, pH, or other physicochemical properties. The addition of guava extracts also increased the total solids and protein content of yogurt samples. Importantly, yogurt fortified with guava extracts demonstrated protection against spoilage and harmful bacteria, while also promoting the growth and viability of lactic acid bacteria (LAB) [37].

Conclusion

It can be concluded that the utilization of Psidium guajava leaves extract as a natural preservative and functional ingredient in dairy products offers a promising avenue for enhancing product safety, quality, and nutritional value. The potent antimicrobial and antioxidant properties of guava leaf extract provide a sustainable and natural solution to the challenges faced by dairy producers in preserving their products and meeting the evolving demands of consumers. By leveraging the bioactive components of guava leaf extract, producers can not only improve the microbial safety and shelf life of dairy items but also enhance their overall quality and appeal.

Thus, the integration of natural additives like guava leaves extract in dairy products presents an opportunity to enhance both the safety and health benefits of these products. By combining traditional dairy processing techniques with innovative natural ingredients, the industry can continue to provide diverse, nutritious, and safe dairy products that cater to the evolving needs and preferences of consumers globally.

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