Research Article

Antimicrobial activity of mulberry (*Morus alba*) leave extract against foodborne pathogens in fermented fish (Pla-som)

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Abstract

Acute diarrhea and food poisoning are caused by the consumption of food or water contaminated with pathogenic microorganisms. These conditions remain major public health problems and are responsible for numerous deaths worldwide each year. Pla-som is a one kind of fermented fish that often contaminated with pathogens from the production process occurred both in raw materials and environments might harmful to the consumers. Pla-som is a traditional fermented fish product that is often contaminated with pathogens during the production process, originating from raw materials and the surrounding environment, which may pose health risks to consumers. This research aimed to evaluate the efficacy of mulberry (*Morus alba*) leave extract from MonPai cultivar in inhibiting the growth of foodborne pathogenic bacteria such as *Bacillus cereus, Escherichia coli, Staphylococcus aureus,* and *Salmonella* spp. in pla-som. The results revealed that ethanolic extract, aqueous extract, and powdered leaves of the MonPai cultivar at a concentration of 100 µg/g exhibited antibacterial activity. The most pronounced inhibitory effect was observed against *S. aureus* in 100 g of pla-som, with statistically significant differences ($P < 0.05$). The colony counts were 213 ± 80.83, 300 ± 95.40, and 340 ± 70.00 CFU/g, respectively. The inhibition percentages were 52.67%, 33.33%, and 24.44%, respectively. In summary, incorporating mulberry leaf extracts into fermented fish products prior to consumption may help inhibit pathogenic bacteria in the gastrointestinal tract and reduce the risk of diarrhea and food poisoning associated with the consumption of raw fermented fish.

Keywords: Mulberry leave, Antibacterial, Fermented fish, Food safety, *Staphylococcus aureus*

Introduction

The contamination of food and water with pathogenic microorganisms at any point in the food chain, from production to consumption, is a significant contributor to diarrheal diseases. These illnesses account for 20–40% of all food and waterborne diseases, with up to 80% of cases attributed to the use of contaminated water and inadequate hygiene practices during processing and washing [1]. This issue is particularly critical in the production of fermented foods, where cleanliness and safety rely heavily on ingredient quality, sanitary preparation practices, and proper fermentation techniques [2]. Reports have identified cases of bacterial infections associated with the consumption of fermented foods. Pathogens commonly linked to these infections include *Bacillus cereus*, *Escherichia coli*, *Salmonella* spp., *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Vibrio cholerae*, *Listeria monocytogenes*, *Aeromonas* spp., *Klebsiella* spp., *Campylobacter* spp., and *Shigella* spp. [3]. Addressing these microbial risks is essential to ensure the safety and quality of fermented foods, as they are a significant part of the global diet and cultural heritage.

In northeastern Thailand, fermented meat products such as naem, pla-som, and pla-ra are traditional delicacies but are often at risk of contamination with pathogenic microorganisms. This contamination originates from raw meat and spreads throughout the production process, from raw materials to the final consumers. The cleanliness and safety of these fermented foods depend on the quality of raw materials, hygienic preparation practices, proper cooking, and adherence to appropriate fermentation times [4].

To ensure food safety, the Bureau of Quality and Safety of Food, Ministry of Public Health, has established guidelines for acceptable levels of pathogenic bacterial contamination in fermented fish products. These include *E. coli* at less than 3 MPN/g, *S. aureus* at less than 100 CFU/g, *B. cereus* at less than 1,000 CFU/g, *Clostridium perfringens* at less than 1,000 CFU/g, and the absence of *Salmonella* spp. in 25 g of food [5]. Ensuring compliance with these standards is critical to minimizing the risks of foodborne illnesses associated with fermented foods.

Chemical preservatives are commonly added during the food production process to prevent contamination by pathogenic microorganisms and to inhibit food spoilage. While these chemicals effectively reduce the incidence of foodborne diseases, their repeated or excessive use can result in residues in food and across the food chain. Furthermore, environmental microorganisms and pathogenic bacteria may develop resistance to these chemicals, leading to adverse effects on human health. As a result, the development and use of natural preservatives that are safe for both human health and the environment have gained significant attention.

Globally, plant extracts are frequently used in foods to inhibit the growth of pathogenic bacteria such as *Salmonella*, *Listeria monocytogenes*, and *S. aureus* [6-8]. Recent studies highlight the antimicrobial properties of *Morus alba* Linn. leaf extracts, which have been shown to inhibit the growth of various microorganisms, including *S. aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *C. krusei*, *C. tropicalis*, and *Aspergillus flavus* [9]. Additionally, specific bioactive compounds extracted from various parts of *M. alba* demonstrate antimicrobial activity. For instance, polysaccharides from *M. alba* leaves can inhibit the growth of *Bacillus subtilis*, *E. coli*, and *S. aureus* [10]. Oxyresveratrol from the branches of *M. alba* inhibits *S. aureus* [11], and 1-deoxynojirimycin from *M.*

alba leaves inhibits *S. aureus*, *Aspergillus niger*, and *Penicillium* spp. [12]. These findings underscore the potential of *M. alba* extracts as natural antimicrobial agents for use in food preservation.

Abdeldaiem et al. [13] investigated the impact of mulberry leaf extract on the quality of minced beef and found that incorporating mulberry leaves enhanced the meat's quality, demonstrating its potential as a natural preservative for meat products. Abdel-Khalek and Mattar [14] explored the biological activities of mulberry by-products and their potential as natural food additives and nutraceuticals. Their study revealed that mulberry by-products are rich in phenolic compounds (5.97 mg/g DW) and flavonoids (5.61 mg/g DW), exhibiting strong antibacterial properties. Moreover, the ethanolic and water crude extracts of MonPai mulberry cultivar showed the best inhibitory effects, with minimum inhibitory concentrations (MIC) that could inhibit the growth of *S. aureus* and *B. cereus*. The minimum bactericidal concentrations (MBC) for *S. aureus* and *B. cereus* were in the range of 25.0-50.0 mg/ml and 3.1-12.5 mg/ml, respectively. When crude extracts from MonPai mulberry cultivar at a concentration of 50.0 mg/ml were used in the post-fermentation process of pla-ra for 28 days, they effectively reduced the overall bacteria count and *B. cereus*, while moderately inhibiting the growth of *S. aureus* [15]. The study showed a significant reduction in the overall bacterial count and *B. cereus* and moderate inhibition of *S. aureus*. These findings highlight the potential of mulberry leaf extracts as natural antimicrobial agents for use in food preservation and safety.

With the previous studies, we aimed to study the efficacy of leaf extracts from MonPai cultivar in inhibiting the growth of foodborne pathogenic bacteria such as *B. cereus*, *E. coli*, *S. aureus*, and *Salmonella* spp. in pla-som. The findings from this study will be applied to develop standard community product formulas for pla-som to prevent and reduce the risk of diseases caused by pathogenic bacteria in fermented foods. In addition, *M. alba* extracts might be included as an ingredient in the production of safer food in the future, which this approach could offer a healthier alternative to synthetic additives, aligning with growing consumer demand for natural food ingredients.

Objectives

To evaluate the efficacy of mulberry (*Morus alba*) leave extract from MonPai cultivar in inhibiting the growth of foodborne pathogenic bacteria, including *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* spp., in pla-som.

Methodology

Mulberry Leaves Collection

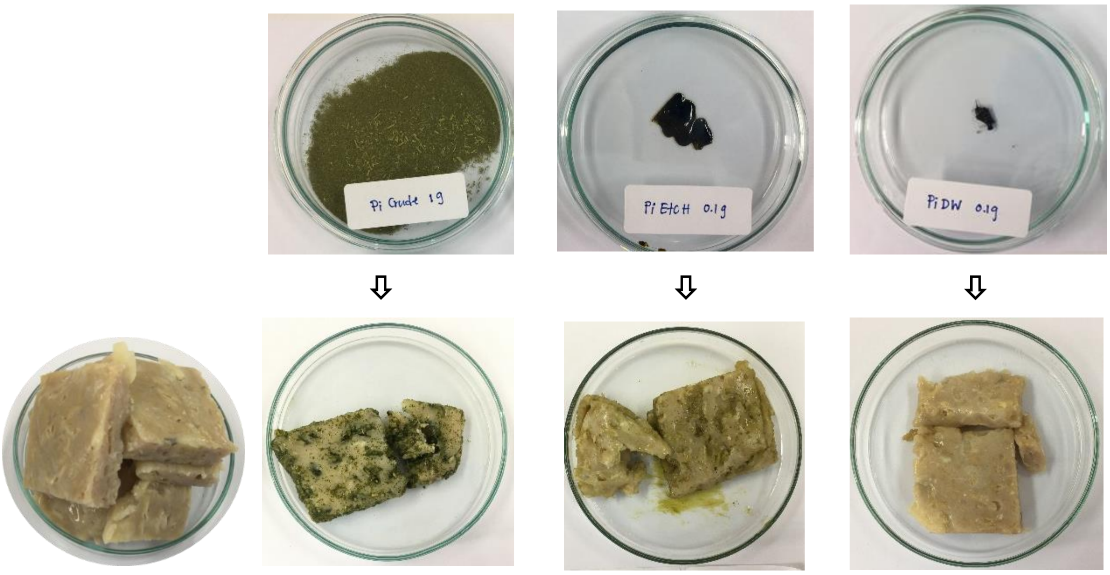
MonPai mulberry cultivar was selected based on the study by Tianyam et al. [15] which found that MonPai mulberry cultivar exhibited the best antimicrobial properties. The leaves were collected from the Chalermphrakiat Sericulture Station in Khon Kaen Province, aged between 1 year to 1 year and 5 months, during November 2020 to February 2021, between 09.00 and 10.00 AM. Tender leaves, particularly 1-3 leaves from the top of each plant, were randomly collected, constituting 15% of the total 150 mulberry trees, equivalent to 23 trees by random selection. Prior to analysis, the leaves were washed twice thoroughly with tap water, dried in a hot air oven at 50ºC for 3 hours, ground by using blender, and then vacuum-sealed and stored at -20ºC until further experimental used [16].

Mulberry Leaf Extraction

The ground leaves were extracted with water and 95% ethanol following the protocol. For ethanolic extraction, the grounded leaves were soaked in 95% ethanol at a ratio of 1:6 (w/v), fully submerged, and left for 7 days at room temperature. During the extraction process, the extraction bottle was shaken for 15 minutes each day to influence the extraction yield. Afterward, the solution was filtered using No. 1 filter paper, and the solvent part was collected and evaporated using a rotary vacuum evaporator at 40-50ºC. The extract was then freeze-dried until it turned into a powder then weighed and stored at 4ºC [17]. In case of water extraction, the ground leaves were soaked in hot water (60 ºC) for 2 hours at a ratio of 1:5 (w/v). The solution was then filtered to collect the solvent part, freeze-dried until became powder then weighed and stored at 4ºC [15].

Fermented fish (pla-som) preparation

After testing the antioxidant and bacterial inhibition properties of mulberry leaf extracts, the most effective bacterial inhibitory extract cultivar was tested on pla-som in independent three replicated experiments. The experimental procedure included fermenting pla-som according to the ingredient composition: 500 grams of grounded fish, 10% salt, 10% garlic, 10% sticky rice, and 2% monosodium glutamate (MSG), mixed them together and incubated at room temperature for 3 days. Then, pla-som were randomly selected and divided into three fractions, each weighing 100 g. The first fraction was mixed with 1% (w/w) of powdered mulberry leaf, the second fraction was mixed with 0.1 % (w/w) of ethanolic mulberry leaf extract, and the third fraction was mixed with 0.1 % (w/w) of water mulberry leaf extract (Figure 1). Pla-som with powdered and two kind of extracts were incubated for 1 hr then inhibitory effect of on the growth of pathogenic bacteria were tested.



(A) **(B)** **(C)** **(D)**

Figure 1 Additive of each fraction mulberry leave extracted in pla-som.

(A) control pla-som. (B) pla-som added with 1% (w/w) of powdered mulberry leaf. (C) pla-som added with 0.1 % (w/w) of ethanolic mulberry leaf extract. (D) pla-som added with 0.1 % (w/w) of water mulberry leaf extract.

Pathogenic Microbial Contaminated investigation

The contaminated of total and pathogenic bacteria in pla-som, which are *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Salmonella* spp., were investigated by using Bacteriological Analysis Manual; BAM [18]. In brief, weigh 25 grams of each group of samples. Then the samples were transferred into a sterile plastic bag. Totally of 225 ml of Butterfield's phosphate buffered dilution water (BPB) was poured and mixed the samples well as 300 rpm stomacher for 2 minutes (this sample was diluted to 1:10).

Analysis of *Escherichia coli*

Step 1: presumptive phase is the detection of coliform bacteria by pipetting 1 ml of 1:10 diluted sample into 9 ml of the lauryl tryptose broth culture (LSB) medium (Himedia, India) containing the gas trap tube and made 10 folds dilution which started from 1:100 to 1:10,000 for each three replications. All of them were incubated at 35°C for 24 ± 2 hr then selected only gas production tube to confirm in step 2.

Step 2: confirmation phase for fecal coliform or *E. coli* confirmation by transferred 1 loop from gas production LSB tubes into *Escherichia coli* (EC) broth (Himedia, India) culture medium containing tube to tube gas trap. Then incubated at 44.5°C for 24 ± 2 hr. Only acids and gases production tubes were confirmed with step 3. In cases of no gases and acids production, they were incubated until 48 ± 2 hr and reported the results as Most Probable Number (MPN) of *E. coil* by compared with the MPN table.

Step 3: complete phase examination for *E. coli* by using a loop to transferred positive EC broth culture tube to streak on the eosin methylene blue (EMB) agar (Himedia, India) culture medium. The medium agar plates were incubated at 35°C for 18-24 hr. The positive colonies were examined the black color at the middle of the colony which both produced or not produced metallic sheen. The suspected colonies were transferred into the nutrient broth and incubated at 35°C for 18-24 hr. Totally 1 loop was transferred into methyl red and voges-proskauer (MR-VP) medium (Himedia, India), sulfur-indole-motility (SIM) medium (Himedia, India), and simmon citrate agar (Himedia, India) for exploring the MR-VP test, indole generation, and citrate use as a carbon source testing, respectively.

Analysis of *Staphylococcus aureus*

A total of 0.1 ml of the diluted sample (1:10) was transferred onto 2 separated baird-parker (BP) agar (Difco, USA) plates then spread evenly until the agar surface dried. The agar plates were incubated at 35-37°C for 45-48 hr. Only the colonies with gray to black round shape with convex as a size 2-3 mm had been counted. To confirmed by biochemical techniques, at less 5 positive colonies were transferred into brain heart infusion (BHI) broth (Himedia, India) and incubated at 35-37°C for 18-24 hr. The coagulase plasma (rabbit) (Himedia, India) with EDTA 0.5 ml was added and mixed virtuously then incubated at 35-37°C. A clotted formation in tube was observed regularly within 6 hr.

Analysis of *Bacillus cereus*

Approximately, 0.1 ml of the diluted sample (1:10) was transferred onto 2 separated mannitol-egg yolk-polymyxin B (MYP) agar (Difco, USA) plates then spread evenly until the agar surface dried. The agar plates were incubated at 30°C for 16-24 hr. A plate with 15-150 colonies growth was selected and counted by characterized only pink with turbid zones colony. Therefore, if the colonies were not classified, incubated more 24 hr might be helpful. Around 5 positive colonies were transferred onto nutrient agar (NA) slant (Himedia, India) and incubated at 30°C for 24 hr. then confirmed by biochemical techniques which were nitrate reducing test, acetyl methyl carbinol generation test, and lecithin degradation test.

Analysis of *Salmonella* spp.

Totally, 1 ml of diluted sample (1:10) was transferred into 10 ml of selenite cystine broth (Himedia, India) and incubated at 37°C for 18 ± 2 hr., then streaked onto xylose lysine deoxycholate (XLD) agar (Himedia, India) and *Salmonella Shigella* (SS) agar (Himedia, India). The agar plates were incubated at 35°C for 24 hr. For XLD agar, the positive colonies were characterized as a pink color with or without a black dot at the center of colony. Whereas, SS agar must be a clear colony forming and presented a black dot at the center of colony, reflected from H_2S production. The positive colonies from both agar types were confirmed by cultured in triple sugar iron (TSI) agar (Himedia, India), lysine decarboxylase (LDC) medium (Himedia, India), urea medium (Himedia, India), and simmon citrate medium (Himedia, India).

Statistics analysis

The results were present mean±standard deviation (S.D.) of three replicated experiments. The comparison the differences of antibacterial activity between control and extraction method of mulberry in pla-som by using one-way ANOVA and Duncan' mutiple range test. A P-value as 0.05 was required for statistical significance. Data was analyzed using SPSS Software for Windows, Version 23.

Results and Discussion

Extract yield in mulberry leaf

After extraction using a rotary evaporator, the yields from the ground mulberry leaves were 4.72% for the ethanolic extract and 15.56% for the water extract. The higher yield obtained from water extraction is likely attributable to the high polarity of water, which facilitates the dissolution of a wide range of water-soluble constituents, including phenolic compounds, sugars, proteins, and organic acids [19]. However, previous studies conducted by our research group demonstrated that ethanol is a more efficient solvent for extracting bioactive compounds, yielding higher total phenolic and flavonoid contents than water extraction [15]. Phenolic and flavonoid compounds, classified as polar phytochemicals, are known to exhibit antibacterial activity by disrupting bacterial cell walls or inhibiting essential bacterial functions [20, 21], flavonoids, acting as antioxidants, might reduce oxidative stress in bacteria, potentially allowing certain bacteria, such as gut microbiota in animals, to proliferate [22].

The effect of mulberry leaf extracts on inhibiting of pathogenic bacteria growth in pla-som

The mulberry leaf extracts demonstrated the ability to inhibit the growth of pathogenic bacteria in pla-som. The overall bacterial contamination and *S. aureus* counts were reduced, whereas *E. coli* and *B. cereus* counts increased Notably, *Salmonella* spp. were detected in all samples. The total microbial contamination in the control sample (pla-som without extract) was $7.17 \pm 2.32 \times 10^7$ colony-forming units per gram (CFU/g). In contrast, pla-som treated with ethanolic extract, water extract, and powdered mulberry leaves exhibited significant reductions in total microbial counts to $2.02 \pm 0.67 \times 10^7$, $2.09 \pm 0.96 \times 10^7$, and $4.07 \pm 1.78 \times 10^7$ CFU/g, respectively. Although the aqueous extract showed a higher extraction yield, its antimicrobial efficacy was lower than that of the ethanolic extract. This suggests that a higher extraction yield does not necessarily correspond to greater biological activity, as it may reflect the extraction of a larger amount of total soluble constituents rather than a higher concentration of specific bioactive compounds responsible for antimicrobial effects. The inhibitory effects of ethanolic and water extracts on the total microbial counts were statistically significant ($P<0.05$) (Table 1).

Table 1 The comparison of effect of mulberry leaf extraction method on the total and pathogenic bacteria in pla-som.

Extraction method of Mulberry leaf	Total bacteria** (CFU/g)	*E. coli*ns (MPN/g)	*S. aureus*** (CFU/g)	*B. cereus*ns (CFU/g)	*Salmonella* spp. in 25 g ns
Day0	$5.73\pm0.91x10^5$	466±238	3200±1802	0±0.00	detected
Day3*					
Control	$7.17\pm2.32x10^{7b}$	553 ± 487.58^a	450 ± 26.46^b	1870 ± 1630^{ab}	detected
Powdered	$4.07\pm1.78x10^{7a}$	913 ± 1011.20^a	340 ± 70.00^{ab}	5500 ± 4920^b	detected
Ethanolic	$2.02\pm0.67x10^{7a}$	1133 ± 1150.36^a	213 ± 80.83^a	100 ± 170^a	detected
Water	$2.09\pm0.96x10^{7a}$	1433 ± 2482.61^a	300 ± 95.40^a	0 ± 0.00^a	detected

Remark: * The comparison was performed only in Day3, ** Different superscript lowercase letters in the same row indicate a statistically significant difference (P<0.05), ns = non-significance difference at P>0.05

The contamination of *E. coli* in pla-som treated with powdered mulberry leaves, ethanolic extracts, and water extracts increased to 913±1011.20, 1133±1150.36, and 1433±2482.61 MPN/g, respectively (Table 1). The effect of mulberry leaf extracts on inhibiting *E. coli* growth was not statistically significant (P>0.05).

E. coli is a gram-negative bacterium with a complex cell wall structure that includes lipopolysaccharides (LPS) in the outer membrane [23]. The LPS layer enhances its resistance to antimicrobial compounds. While mulberry leaf extracts have demonstrated effectiveness against gram-positive bacteria, their interaction with gram-negative bacteria, particularly *E. coli*, revealed limited inhibitory effects. The complex cell wall structure of gram-negative bacteria, including an outer membrane which supports that gram-negative bacteria possess a robust outer membrane that can serve as a barrier against antibacterial agents [24], including those present in mulberry leaf extracts.

Interestingly, the observed increase in *E. coli* contamination might be attributed to the flavonoid antioxidants present in mulberry leaf extracts. These antioxidants could potentially reduce oxidative stress in *E. coli*, thereby strengthening the bacterial cells and enabling them to better withstand adverse conditions. This is consistent with the findings of Wang et al. [22] who reported an increase in *E. coli* populations in the ruminal and abomasal digesta of calves following the addition of mulberry leaf flavonoids (MLF).

However, the precise mechanism underlying the relationship between antioxidants and the proliferation of *E. coli* remains unclear. Further research is necessary to elucidate the interaction between flavonoid antioxidants and the growth of *E. coli*, particularly in the context of probiotics and other beneficial microorganisms.

The contamination of *S. aureus* in the control sample was 450±26.46 CFU/g. After incubating pla-som with powdered mulberry leaf and two types of extracts for 1 hour, a decrease in *S. aureus* growth was observed. The counts were 213±80.83 CFU/g for the ethanolic extract, 300±95.40 CFU/g for the water extract, and 340±70.00 CFU/g for the powdered mulberry leaf, with inhibition activities of 52.67%, 33.33%, and 24.44%, respectively. These results showed statistical significance (P< 0.05) (Table 1). While both water and ethanolic extracts effectively reduced

S. aureus counts, they were unable to lower the contamination to below the acceptable limit of 100 CFU/g, as defined by the Bureau of Quality and Safety of Food [5]. This limitation may be attributed to the short reaction time between the mulberry leaf extracts and the bacteria in pla-som. Future studies should consider extending the reaction time to enhance the antimicrobial efficacy of mulberry leaf extracts against *S. aureus*. This could help achieve a more substantial reduction in bacterial contamination, potentially meeting regulatory safety standards.

The contamination of *B. cereus* in the control sample was 1870±1630 CFU/g CFU/g. The antibacterial activity of the water and ethanolic extracts of mulberry leaves in pla-som demonstrated the most significant reduction. Both water and ethanolic extracts successfully reduced *B. cereus* contamination to below the acceptable levels of pathogenic bacteria in fermented fish (< 1,000 CFU/g) [5]. However, pla-som mixed with powdered mulberry leaves did not significantly reduce the *B. cereus* count (Table 1). The antibacterial activity of mulberry leaf extracts on inhibiting *B. cereus* growth after adding ethanolic and water extracts in pla-som was not statistically significant ($P>0.05$).

The detection of *Salmonella* spp. in both control and treated samples suggested that the current approach using mulberry leaf extracts may have limited efficacy against this pathogen under the conditions tested. This finding is consistent with previous reports indicating that plant-derived flavonoids often exhibit stronger antimicrobial activity against gram-positive bacteria than gram-negative bacteria [25]. The reported of Thabit et al. [26] revealed the antimicrobial activities of plant extracts, particularly flavonoids extracted using methanol. It was found that flavonoid aglycones exhibited greater antimicrobial activity compared to their glycosidic counterparts. Among these compounds, quercetin and its analogs were identified as particularly effective, acting through the inhibition of DNA gyrase, an enzyme crucial for bacterial DNA replication. However, the effectiveness of these extracts was observed to be greater against gram-positive than gram-negative bacteria. Therefore, further investigation is warranted to evaluate alternative extraction methods, higher concentrations, or combination treatments to improve efficacy against Salmonella spp. and other gram-negative foodborne pathogens.

The efficacy of mulberry leaf extracts in inhibiting the growth of pathogenic bacteria in pla-som was observed. The mulberry leaf extracts showed inhibitory effects against all tested bacteria, including *S. aureus* and *B. cereus* in pla-som. The most effective inhibition of total bacterial counts in pla-som was achieved using mulberry leaf extracts with ethanol and water as solvents, respectively. The best inhibition against *S. aureus* was observed with mulberry leaf extracts using ethanol and water solvents, as well as the crude fraction. Regarding the inhibition of *B. cereus* in pla-som, the mulberry leaf extracts with ethanol and water solvents successfully inhibited bacterial growth, though the reduction in bacterial counts was not statistically significant at the 0.05 level. Abdeldaiem et al. [13] revealed that the application of mulberry leaf extract in minced beef significantly contributes to food safety and preservation. This study demonstrated the efficacy of methanolic mulberry leaf extract in reducing bacterial counts, including *S. aureus*, *B. cereus*, *E. faecalis*, and *E. coli*. It highlights the potential of natural plant extracts as effective antimicrobial agents in meat products. Mulberry leaf extracts are effective in inhibiting the growth of the gram-positive pathogenic bacteria *S. aureus* and *B. cereus*, consistent with previous studies [15], which found that mulberry leaf extracts inhibit gram-positive bacteria. These extracts contain high levels of phenolic and flavonoid compounds from mulberry leaves. Among these, derivatives of quercetin, kaempferol, and rutin are prominent. Quercetin derivatives, in particular, are effective in inhibiting the growth of *S. aureus*. The antimicrobial properties of flavonoids are closely related to their structure, with phenolic and flavonoid compounds contributing to their antimicrobial activity [26]. However, the precise mechanism of how these compounds inhibit gram-positive bacterial growth remains unclear. Future studies should investigate the effects of these compounds on bacteria, particularly focusing on disrupting the cell membrane or interfering with essential metabolic pathways [27].

The particle size of powdered and extracted mulberry leaves significantly impacts bacterial inhibition. Smaller particles, due to their larger surface area, interact more effectively with bacterial cell walls, resulting in enhanced bacterial inhibition. Additionally, smaller-sized mulberry leaf extracts not only have higher antioxidant levels but also provide more effective bacterial growth inhibition [28, 29]. This study also demonstrated that using water and ethanol for extraction—both of which evaporate easily—along with incorporating mulberry leaf powder into pla-som, is practical for food applications.

Summary

The study demonstrated that leaf extracts of MonPai mulberry cultivar effectively inhibit the growth of pathogenic bacteria in pla-som, a traditional fermented fish from northeastern Thailand. Extracts from the MonPai mulberry cultivar, including ethanolic, water, and powdered forms, significantly reduced *S. aureus* and *B. cereus* counts ($P<0.05$). Future research should explore extended fermentation times to enhance bacterial reduction and ensure compliance with food safety standards. Mulberry leaf extracts have the potential to improve food safety and reduce reliance on synthetic preservatives.

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