

Effect of *jambu mawar* [*Syzygium jambos* (L.) Alston] leaves extract on natural microbial populations in food

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Abstract

Raw food materials can be contaminated with foodborne pathogens and foodborne spoilage. Washing raw food material with water can reduce microbial loading in raw food material prior to the process, but not eliminate it. Due to food safety issues, synthetic food sanitisers are currently being avoided. Natural food sanitisers such as plant extracts are now widely researched and may be used. *Jambu mawar* [*Syzygium jambos* (L.) Alston] leaves extract has been reported for its medicinal uses and treatment of a variety of illnesses. The current study aimed to evaluate the effectiveness of *jambu mawar* leaves extract in order to determine its suitability as a natural food sanitiser. There were three different concentrations (0.05%, 0.50% and 5.00%) of *jambu mawar* leaves extract solutions used to treat shrimp and cherry tomatoes with immersion method at different exposure times (5 mins and 15 mins). Moreover, the treated samples were stored at different temperatures (25±2°C, 4±2°C, and -18±2°C) for 23 days. The results showed that washing with tap water for 5 mins and 15 mins, was not able to reduce the population of total plate count (TPC), *Vibrio parahaemolyticus*, *Staphylococcus aureus* and *Klebsiella pneumoniae* in food samples. In contrast, washing with 0.05% for 5 mins can significantly reduce the microorganism population in food samples. If the concentration is increased to 5.00% the microbial population in the food samples can be removed completely. Correspondingly, the extract can also inhibit or kill the microbial population in raw food materials at different temperatures. It can be concluded that *S. jambos* (L.) Alston leaves extract can reduce or eliminate the microbial population in raw food materials. These results suggested that this extract might be developed as a natural food sanitiser.

1. Introduction

In the last few decades, swift globalization of food production and commerce has maximized the potential possibility of food contamination. Formerly, the outbreak incidents of foodborne diseases once occurred within a small community, but now may happen in global proportions. Many of those foodborne diseases have been imputed to contamination of food, food products and drinking water (Hassanain *et al.*, 2013). In particular, developing countries are more influenced by foodborne infections because of the existence of a broad spectrum of diseases.

In many cases, raw food from animal sources contains pathogens. During slaughtering and butchering, the meat may become contaminated with these pathogens. Milk also can be a vector of antibiotic-

resistant pathogens of animal origins. Furthermore, fish and seafood can be produced in smaller cuts by a local fishmonger with the potentiality of contamination (FDA, 2006). Raw fruits and vegetables are particularly worrisome. Washing practice can reduce contamination but not eliminate it completely, so there's not a lot that consumers can do to protect themselves. There have been several recent outbreaks of fresh fruits and vegetables that have been processed under adverse sanitary conditions (Pönka *et al.*, 2009). The use of unclean water may contaminate many boxes of fruits and vegetables. Vegetables can be contaminated by using fresh manure as fertilizer (CDC, 2005).

The real global challenge for the food industry is to address consumer concerns about food safety, quality and preservation. This involves restricting the use of

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synthetic chemical additives (Negi, 2012). A wide range of plant products was evaluated for the feasibility of using natural antimicrobials and antioxidants to maintain and enhance the overall quality of food products, including meat and meat by-products (Shah *et al.*, 2014).

Syzygium jambos (L.) Alston is a species in the genus *Syzygium* belonging to the family Myrtaceae. These species were cultivated for both edible fruit and medicinal purposes. In the food industry, antimicrobial activities of *S. jambos* (L.) Alston leaves extract may use as a natural preservative or sanitiser. Over the past decade, plants generally considered Generally Recognized as Safe (GRAS) for humans have been linked to food consumption (Negi, 2012). In the absence of complementary biological aspects, the discovery of novel medicinal plants/-extracts/-constituents is more of pure phytochemistry (Meyer *et al.*, 1982). Therefore, plant extracts for food applications need to be declared biologically safe before humans can consume them. This study aimed to assess the effectiveness of *S. jambos* (L.) Alston leaves extract in order to determine its suitability as a natural sanitiser and the possibility to be applied in the food industry as a natural antimicrobial agent.

2. Materials and methods

2.1 Raw food materials

Raw food samples, shrimp and cherry tomatoes were purchased from the whole fruits market, Serdang, Selangor. A sample of shrimp was from *Penaeus vannamei* species (*udang kertas*). All raw samples were stored in cooler containers (2°C to 5°C) between the time of purchase and the commencement of the experiments. As the shrimp and cherry tomatoes were of different shapes in nature and could not be cubed, they were only weighed with about 10 g each for treatment.

2.2 Preparation of *Syzygium jambos* (L.) Alston leaves extract for treatment solution

Three different concentrations of *S. jambo* (L.) Alston leaves were prepared using deionized water

(DIW) (Braun Medical Industries Sdn. Bhd. Penang, Malaysia). Initially, 5 g of crude extract was dissolved in 50 mL of 10% DMSO, resulting in a concentration of 10% (100 mg/mL). Afterwards, it was further diluted to 5% by taking out 25 mL of 10% and diluted in 25 mL of DIW. The subsequent concentration of 0.5% (5 mg/mL) and 0.05% (0.5 mg/mL) was prepared by taking 4 mL (5% concentration) and 4 mL (0.5% concentration) in 36 mL of DIW in two separate universal vials, respectively (Yusoff *et al.*, 2015). Based on this dilution process, three types of solutions with different concentrations (0.05%, 0.50% and 5.00%) were prepared for a treatment analysis.

2.3 Preparation of selective media

There were six types of agars were used including the Plate count agar (PCA), Potato dextrose agar (PDA), *Pseudomonas* agar, Eosin methylene blue agar (EMB), Mannitol salt agar and Thiosulfate citrate bile salts sucrose agar (TCBS). The formulations for each agar were in accordance with the formulation found on the media bottle (Table 1). Selective agars were chosen based on their ability to grow and distinguish specific microorganisms from others for easier and faster detection.

2.4 Washing treatment of raw food materials with *Syzygium jambos* (L.) Alston leaves extract solutions

Washing treatment of raw food materials with *S. jambos* (L.) Alston leaves extract solutions were performed according to the method as reported by Yusoff *et al.* (2015) with slight modification. Each of the food samples was immersed in 40 mL of tap water, distilled water, and *S. jambos* (L.) Alston leaves treatment solutions (0.05%, 0.50% and 5%) at different time exposure (5 mins and 15 mins).

2.5 Effect of *Syzygium jambos* (L.) Alston leaves extract washing treatment at different storage temperatures

For storage study, samples were soaked in the different washing treatment solutions and stored at

Table 1. List of media

Types of Media	Instruction for Preparation	Brands of Media
Potato dextrose agar (PDA)	Suspend 39 g of media powder in 1 L of distilled water. Autoclave (121°C, 15 mins)	Difco™, USA
Plate count agar (PCA)	Suspend 17.5 g of media powder in 1 L of distilled water. Autoclave (121°C, 15 mins)	Oxoid, England
<i>Pseudomonas</i> agar	Suspend 38.0 g of media powder in 1 L of distilled water. Autoclave (121°C, 15 mins)	Difco™, USA
Eosin- methylene blue (EMB) agar	Suspend 37.4 g of media powder in 1 L of distilled water. Autoclave (121°C, 15 mins)	Difco™, USA
Mannitol salt agar (MSA)	Suspend 111 g of media powder in 1 L of distilled water. Autoclave (121°C, 15 mins)	Biokar diagnostic, France
Thiosulfate-citrate-bile-salts-sucrose (TCBS) agar	Suspend 88.0 g of media powder in 1 L of distilled water. Boil to dissolve the medium completely	Oxoid, England

different temperatures and duration as follow; cherry tomato samples were stored at room ($25\pm 2^\circ\text{C}$) and refrigerator (4°C) temperature for up to 23 days, and the shrimps were store in refrigerator (4°C) and freezer (-18°C) temperature for 23 days. Random samples were taken at daily intervals to be analysed. Microbiological analysis was carried out and the results were compared to untreated samples.

2.6 Microbiology analysis

The treated samples were collected randomly and then diluted using the stomacher bag (BAGLIGHT, BagSystem, Interscience, France) which contained 9 mL of phosphate saline buffer solution. The mixture was homogenized using the stomacher machine (BagMixer 400-P Interscience, France) at 250 rpm for 2 min. After the homogenization process, 1 mL of the mixture was serially diluted with 9 mL of phosphate saline buffer solution to make up for dilutions; 10^{-2} , 10^{-3} and 10^{-4} . All universal bottles containing different dilutions were vortexes first before were spread onto the selective agar separately. The agar plates were then incubated overnight at 37°C for bacteria growth and 35°C for fungi growth and the results were analysed by identifying and counting the presence of colonies from each plate. Untreated samples were used as a control. All

experiments were conducted at room temperature ($25\pm 2^\circ\text{C}$).

2.7 Statistical analysis

All experiments were carried out three times with three replications each ($n = 3\times 3$). The application of MINITAB software was used to analyze the data for the analysis of variance (ANOVA). The significance difference ($P < 0.05$) between the treatments was analyzed by using Turkey's test. Results were interpreted as means \pm standard deviation (SD) of replicate analysis.

3. Results and discussion

3.1 Effect of exposure times of *Syzygium jambos* (L.) Alston leaves extract solutions at different concentrations on microbial population in raw food samples

The findings of this study demonstrated the sanitizing effect of *S. jambos* (L.) Alston leaves extract on raw food samples, including shrimp and cherry tomato. Tables 2 and 3 illustrate the survivability of bacterial populations in food samples after being treated with different washing solutions including tap water and different concentrations of *S. jambos* (L.) Alston leaves extract (0.05%, 0.50% and 5%) at different soaking times (5 mins and 15 mins). Currently, the commercial

Table 2. Effects of different concentrations and exposure times of *S. jambos* (L.) Alston leaves extract on natural microbial in shrimp (\log_{10} CFU/mL).

Sample	Shrimp							
	TPC		<i>V. parahaemolyticus</i>		<i>S. aureus</i>		<i>K. pneumoniae</i>	
ET/Treatment	5 mins	15 mins	5 mins	15 mins	5 mins	15 mins	5 mins	15 mins
Control	6.31 \pm 0.02 ^{Ab}	6.39 \pm 0.12 ^{Ab}	5.96 \pm 0.16 ^{Ba}	6.04 \pm 0.19 ^{Aa}	6.19 \pm 0.06 ^{Aa}	6.02 \pm 0.09 ^{Aa}	5.95 \pm 0.07 ^{Bb}	5.90 \pm 0.08 ^{Bb}
Tap water	7.43 \pm 0.00 ^{Aa}	7.53 \pm 0.03 ^{Aa}	5.96 \pm 0.16 ^{Ca}	6.61 \pm 0.06 ^{Ba}	6.91 \pm 0.06 ^{Ba}	6.71 \pm 0.05 ^{Ba}	6.79 \pm 0.04 ^{Ba}	6.65 \pm 0.03 ^{Ba}
0.05%	6.43 \pm 0.15 ^{Ab}	1.76 \pm 2.44 ^{Bc}	0 \pm 0 ^{Cb}	0 \pm 0 ^{Cb}	0 \pm 0 ^{Cb}	0 \pm 0 ^{Cb}	0 \pm 0 ^{Cc}	0 \pm 0 ^{Cc}
0.50%	4.30 \pm 0.00 ^{Ac}	1.76 \pm 2.44 ^{Bc}	0 \pm 0 ^{Cb}	0 \pm 0 ^{Cb}	0 \pm 0 ^{Cb}	0 \pm 0 ^{Cb}	0 \pm 0 ^{Cc}	0 \pm 0 ^{Cc}
5.00%	3.52 \pm 0.00 ^{Ad}	0 \pm 0 ^{Bd}	0 \pm 0 ^{Bb}	0 \pm 0 ^{Bb}	0 \pm 0 ^{Bb}	0 \pm 0 ^{Bb}	0 \pm 0 ^{Bc}	0 \pm 0 ^{Bc}

ET: Exposure Time. Values are presented as mean \pm SD. Values with different lowercase alphabets within the same column are significantly different ($P < 0.05$) while values with different uppercase alphabets within the same row are significantly different ($P < 0.05$).

Table 3. Effects of different concentrations and exposure times of *S. jambos* (L.) Alston leaves extract on natural microbial in cherry tomato (\log_{10} CFU/mL).

Sample	Cherry tomato							
	Yeast and mould		<i>Pseudomonas</i> spp.		<i>S. aureus</i>		<i>K. pneumoniae</i>	
ET/Treatment	5 mins	15 mins	5 mins	15 mins	5 mins	15 mins	5 mins	15 mins
Control	6.26 \pm 0.09 ^{Ab}	5.31 \pm 0.06 ^{Bb}	5.91 \pm 0.18 ^{Bb}	5.90 \pm 0.08 ^{Bb}	6.13 \pm 0.07 ^{Aa}	5.92 \pm 0.11 ^{Bb}	5.87 \pm 0.12 ^{Bb}	6.34 \pm 0.66 ^{Aa}
Tap water	7.52 \pm 0.02 ^{Aa}	7.37 \pm 0.03 ^{Aa}	6.86 \pm 0.03 ^{Ba}	6.85 \pm 0.03 ^{Ba}	6.84 \pm 0.04 ^{Ba}	6.84 \pm 0.04 ^{Ba}	6.34 \pm 0.66 ^{Ba}	6.34 \pm 0.66 ^{Ba}
0.05%	5.60 \pm 0.02 ^{Ac}	3.67 \pm 0.21 ^{Bc}	0 \pm 0 ^{Cc}	0 \pm 0 ^{Cc}	0 \pm 0 ^{Cb}	0 \pm 0 ^{Cc}	0 \pm 0 ^{Cc}	0 \pm 0 ^{Cb}
0.50%	4.21 \pm 0.54 ^{Ad}	1.76 \pm 0.44 ^{Bd}	0 \pm 0 ^{Cc}	0 \pm 0 ^{Cc}	0 \pm 0 ^{Cb}	0 \pm 0 ^{Cc}	0 \pm 0 ^{Cc}	0 \pm 0 ^{Cb}
5.00%	1.76 \pm 2.44 ^{Ac}	0 \pm 0 ^{Bc}	0 \pm 0 ^{Bc}	0 \pm 0 ^{Bc}	0 \pm 0 ^{Bb}	0 \pm 0 ^{Bc}	0 \pm 0 ^{Bc}	0 \pm 0 ^{Bb}

ET: Exposure Time. Values are presented as mean \pm SD. Values with different lowercase alphabets within the same column are significantly different ($P < 0.05$) while values with different uppercase alphabets within the same row are significantly different ($P < 0.05$).

natural sanitiser was not available in the market. Additionally, most food sanitisers are targeting the food contact surfaces, such as utensils, sinks, cutting boards and so on. The food sanitisers that are most frequently applied in the food industry or food services to clean the food contact surface are chlorine, iodophore and quaternary ammonium compound (Strohbehn *et al.*, 2013). On the other hand, there are a number of chemical sanitisers that apply only to wash fruit and vegetable on farms, such as chlorine, peracetic acid, sodium hypochlorite, hydrogen peroxide and chlorine dioxide (Gil *et al.*, 2009). Consequently, tap water was used as a normal sanitiser in industry and at home. The effectiveness of the extract as a sanitiser has been compared to tap water and the effectiveness of the extract was measured by the observation of the ability of the extract to decrease the microbial population up to 3 log CFU/mL.

In this study, the contact time between the sanitiser agents (extracts) and the food samples was carried out over 5 and 15 mins. Based on Pérez-Gregorio *et al.* (2011), a 5 min exposure time was the common condition in the washing treatment. Additionally, peracetic acid showed the capacity to reduce *Escherichia coli* strains for 5, 15 and 25 mins of exposure time. Moreover, the study carried out by Chen *et al.* (2015), selected 5, 10, 15, 30, 45 and 60 mins of exposure time to determine the capacity of edible vinegar, salty solution and sodium bicarbonate solution in microbial population reduction on cowpea fruits. Furthermore, Zhang and Faber (1996) reported that a 10 mins exposure of shredded lettuce to chlorine oxide resulted in a maximum reduction of 1.1 log CFU/mL of *L. monocytogenes*. As a result, 5 mins was selected as the shortest exposure period and 15 mins for maximum exposure.

In the shrimp sample (Table 2), four types of bacteria were presented, representing the total plate count (TPC), *V. parahaemolyticus*, *S. aureus* and *K. pneumoniae* were detected in shrimp samples. In this study, the number of TPC was reduced significantly starting at 0.05% treatment for 5 mins of exposure time, which was from $6.41 \pm 0.0 \log_{10}$ CFU/mL to $4.65 \pm 0.34 \log_{10}$ CFU/mL. At the maximum concentration (5%), TPC had been reduced completely from $6.35 \pm 0.10 \log_{10}$ CFU/mL to $0 \pm 0 \log_{10}$ CFU/mL. The population of *V. parahaemolyticus*, *S. aureus* and *K. pneumoniae* also started to reduce significantly at treatment of 0.05% during 5 mins of exposure time. On the other hand, *V. parahaemolyticus*, *S. aureus* and *K. pneumoniae* were not detected after exposure started at 0.50% treatment in 5 mins exposure time. Overall, all bacteria populations including TPC showed the highest reduction at 5% treatment for 5 min time exposure. At 5% solution

concentration and 5 min exposure time, the population of TPC, *V. parahaemolyticus*, *S. aureus* and *K. pneumoniae* were not detected at this condition. This means *S. jambo* (L.) Alston leaves extract had significantly effective against natural microflora in shrimp starting at 0.05% for 5 mins exposure.

In addition to raw foods, fresh foods include fruits and vegetables that can harbour a variety of microorganisms that originated primarily from the environment in which they were raised. Microorganisms will continue to grow during post-harvest, food handling and processing and will cause food spoilage if appropriate decontamination methods are not applied (Khadka *et al.*, 2017). The growth and survival of these microorganisms with extended time particularly during the storage period will spoil the food and cause foodborne illness when consumed by people outside. According to Chang and Fang (2007), the survivability of *E. coli* O157:H7 and *S. Typhimurium* in shredded lettuce is within 10-12 days has been a potential health risk for consumers.

Tomato is highly perishable and susceptible to microbial spoilage because of its high water content and its relatively soft texture. These features of tomato lead it to deterioration during transit and storage where it might undergo conditions of humidity and high temperature. The presence of certain pathogenic microorganisms of public health significance makes it a potential health hazard for consumers (Ogundipe *et al.*, 2012). In this study, for cherry tomato sample, yeast and mould, *S. aureus*, *K. pneumoniae* and *Pseudomonas* spp. had begun to reduce significantly post-treatment to 0.05% for 5 mins. The results are shown in Table 3. Yeast and mould, *S. aureus*, *K. pneumoniae* and *Pseudomonas* spp. were undetected at 0.05% to 5 min of soaking time. In the meantime, the highest reduction in yeast and mould was observed at a 5% concentration of *S. jambos* (L.) Alston leaves extract with an exposure time of 15 mins were 6.75 ± 0.07 to $0 \pm 00 \log_{10}$ CFU/mL.

In this study, tap water treatment refers to the current washing method used by the household. Some researchers have indicated that tap water can reduce the overall bacterial count by approximately 2-3 \log_{10} CFU/mL (Beuchat *et al.*, 1998; Ukuku *et al.*, 2004). However, in this study, there was no reduction with tap water treatment. Brackett (1992) reported that applying tap water for washing is not able to completely eliminate bacterial populations on food materials. Moreover, there are limitations to the application of tap water in washing food materials due to the presence of chlorine residues in treated tap water. Chlorine residues have become a food safety concern because of their potential to produce carcinogenic compounds such as trihalomethanes,

haloacetic acids, haloketones and chloropicrin when they react with organic matter. Besides, applying tap water alone for washing will sometimes enhance the growth of foodborne pathogens (Klaiber *et al.*, 2005). As stated by Gil *et al.* (2009), re-using of processing water as a sanitiser will make the tap water another source of cross-contamination.

In addition, the microflora in food samples that were immersed in DIW (0.00% extract) showed no significant differences even after exposure at different times (Yusoff *et al.*, 2015). The number of surviving bacteria was decreased with increased *S. jambos* (L.) Alston leaves extract concentrations. The results were similar to Abadias *et al.* (2011) who also demonstrated that the reduction in microbial populations was increased as the concentration of sanitiser and washing time increased. However, some of them reported differently, which stated that the soaking time of the sanitiser did not influence the reduction in the number of bacterial populations in raw food materials. A study conducted by Tornuk *et al.* (2011) showed this situation where the thyme sanitiser, which was tested at the same concentration but with a different exposure time, did not give a significant reduction in bacterial populations in apple fruit.

Based on the finding of the bacterial reduction in treated food samples, it was noticed that the reduction of microbial populations was proportional to the increase of *S. jambos* (L.) Alston leaves extract concentration and soaking time. The relative influence in terms of microbial inactivation was Tap water < 0.05% < 0.50% < 5%. Generally, *S. jambos* (L.) Alston leaves extract had significantly antimicrobial activity against natural microflora in tested food samples starting at 0.50% for 5 mins exposure. Higher concentrations of *S. jambos* (L.) Alston leaves extract with a longer soaking time is more powerful to inhibit the growth of microorganisms. However, the effect of a higher concentration of extract on colour and texture needs to investigate because consumer preferences on types of food are categorized in various aspects including food nutrition value, food safety, food appearance and physical characteristics (Lazarova and Krystallis, 2010).

The Food Drug Administration (FDA) recommended that the common storage temperature should be -18°C for frozen products, while 4°C for refrigerated products. In this study, two temperatures were employed to compare the efficiency of *S. jambos* (L.) Alston leaves extract as an antimicrobial agent and is not affected by low temperature. The study was conducted for 23 days of storage at -18°C and 4°C, respectively. Although 0.05% of the extract and 5 minutes of exposure began to show a

significant difference in microbial reduction, however, during storage, microbial could be proliferative. Consequently, it is important to eliminate the microbial in the food to the minimum level. Thus, the 5% concentration of the extract and 15 mins of exposure time were chosen in the storage study due to the lower microbial reduction.

3.2 Effect of different concentrations and storage temperatures of *Syzygium jambos* (L.) Alston leaves extract on microbial population in raw food samples

The potential contamination of shrimp by foodborne pathogens depends primarily on the conditions of transportation, handling and processing. Frozen shrimp are frequently contaminated after catching. The handling of raw materials has an effect on the bacteriological quality of frozen shrimp. Inadequately frozen and inappropriate storage of shrimp at higher temperatures increases the growth of microorganisms that are responsible for microbiological changes (Rahman *et al.*, 2012). Furthermore, the storage temperature at which shrimp are exposed can promote the spread of pathogenic bacteria in humans (Gill and Badoni, 2005). Frozen shrimp is normally inspected prior to shipment based on physical and sensorial characteristics followed by microbiological characteristics. Certain stages are very critical for microbial quality in shrimp, such as immersion scalding and irradiation (Al-Dughaym and Altabari, 2010). Thus, controlling microbial contamination of shrimp during catching, processing, storage, handling and preparation is a major challenge.

Figure 1 (a and b) show the total plate count (TPC) and *V. parahaemolyticus* population in treated and untreated shrimp samples during storage for 23 days at 4°C. The TPC for control samples demonstrated the highest increase from 6.31±0.02 to 7.44±0.02 log₁₀ CFU/mL compared to the treated samples. The treated samples with 0.5% and 5% showed a reduction where the readings for the last day of storage were 4.301±0 and 3.5229±0 log₁₀ CFU/mL, respectively.

The initial count was 6.31±0.02 log₁₀ CFU/mL, then on the 23rd day, the population increased to 7.44±0.02 log₁₀ CFU/mL for untreated samples, however in treated samples, the population showed a lower decrease with reduced to 4.301±0 and 3.5229±0 log₁₀ CFU/mL for 0.5% and 5%, respectively. Besides that, the treated samples also showed a reduction in bacteria compared to control samples for *V. parahaemolyticus*. The population of *V. parahaemolyticus* in shrimp increased from 5.96±0.164 to 6.69±0.06 log₁₀ CFU/mL for control samples while for the treated samples with 0.5% and 5%, *V. parahaemolyticus* was not detected on the last day of storage.

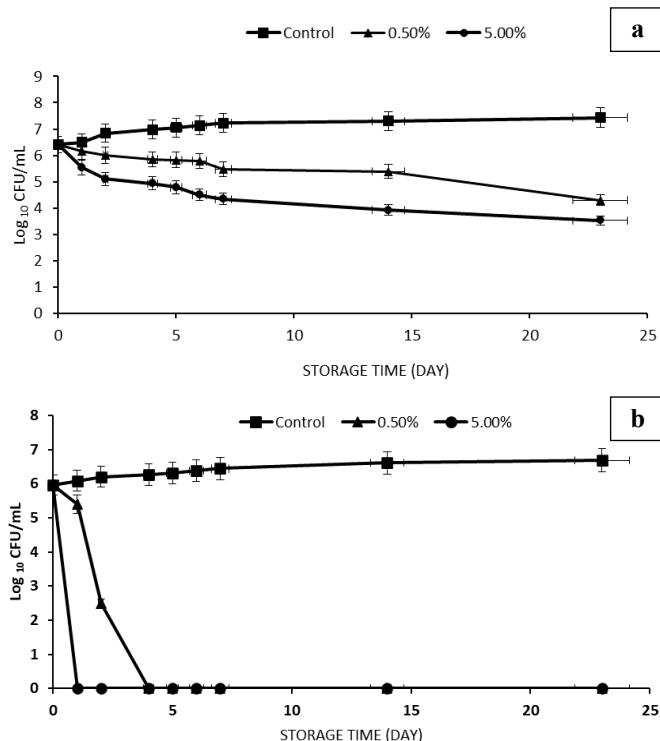


Figure 1. Representative the (a) total plate count and (b) *Vibrio parahaemolyticus* counts on the shrimp exposed to different concentrations of *S. jambos* (L.) Alston leaves extract during storage at 4°C for 23 days.

Figure 2 (a and b) show the changes in the microbial population in untreated and treated shrimps during storage at -18°C for 23 days. The total plate count of untreated shrimp that was stored at -18°C for 23 days increased from 6.41 ± 0.05 to 7.55 ± 0.01 log₁₀ CFU/mL on the last day of storage. Moreover, the treated samples show decreases with 3.67 ± 0.21 log₁₀ CFU/mL for 0.5% while the bacterial population was not detected for 5% extract on the last day of storage. Additionally, *V. parahaemolyticus* was detected in untreated samples up to the 23rd day of storage where it increased from 5.97 ± 0.06 to 6.59 ± 0.06 log₁₀ CFU/mL. However, 0.5% and 5% of extract gave the same effect on the reduction where *V. parahaemolyticus* was not detected on the last day of storage during the freezing condition. This showed *V. parahaemolyticus* was not found in the treated samples with 0.5% and 5% on the 5th and 1st day of storage, respectively. These results demonstrate that, *S. jambos* (L.) Alston leaves extract had an antimicrobial

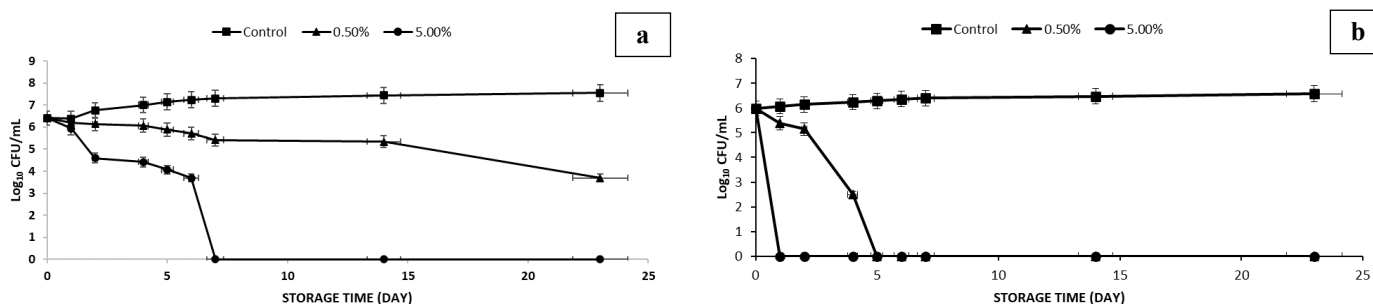


Figure 2. Representative the (a) total plate count and (b) *Vibrio parahaemolyticus* counts on the shrimp exposed to different concentrations of *S. jambos* (L.) Alston leaves extract during storage at -18°C for 23 days.

effect against foodborne pathogens on shrimp during storage.

For cherry tomato, the changes of yeast and mould and *Pseudomonas* spp. the population had been observed along the storage time at room temperature as shown in Figure 3 (a and b). Yeast and mould were observed to increase for control samples. However, 0.5% and 5% extract showed a decrease of yeast and mould in the food samples with 4.21 ± 0.54 and 1.76 ± 2.44 log₁₀ CFU/mL, respectively. Based on the Figure 3 (a and b), yeast and mould count in untreated samples were increased gradually from 6.26 ± 0.09 to 7.38 ± 0.02 log₁₀ CFU/mL. *Pseudomonas* spp. also had been increased until the end of storage time for the untreated samples while it was not detected in treated samples. However, the application of *S. jambos* (L.) Alston leaves extract as a sanitiser helps to slow down the growth rate of *Pseudomonas* spp. *Pseudomonas* spp. was not detected in treated samples started from the 6th and 1st day with 0.5% and 5% of the extract until the end of storage time. However, the application of *S. jambos* (L.) Alston leaves extract as a sanitiser helps to eliminate the growth rate of *Pseudomonas* spp.

Figure 4 (a and b) show the growth of yeast and mould and *Pseudomonas* spp. on cherry tomato during storage at 4°C for 23 days. Furthermore, from the observation, even though the population of yeast and mould had been increased in untreated samples, the treated samples showed a sharp reduction after the 7th day with 5% of the extract and this proved that *S. jambos* (L.) Alston leaves extract could inhibit yeast and mould growth. *Pseudomonas* spp. in control samples had been observed slightly increment until the last day of storage. Nevertheless, *Pseudomonas* spp. was not detected on the end day of storage for both treated samples with 0.5% and 5% of the extract. However, the population reduction was greatly in treated samples than in untreated samples. This finding showed that treatment with extract help to extend the shelf life of cherry tomato.

Microorganisms without doubt are responsible for the spoilage of shrimp and these microorganisms include bacteria and fungi, both heterotrophic and pathogenic

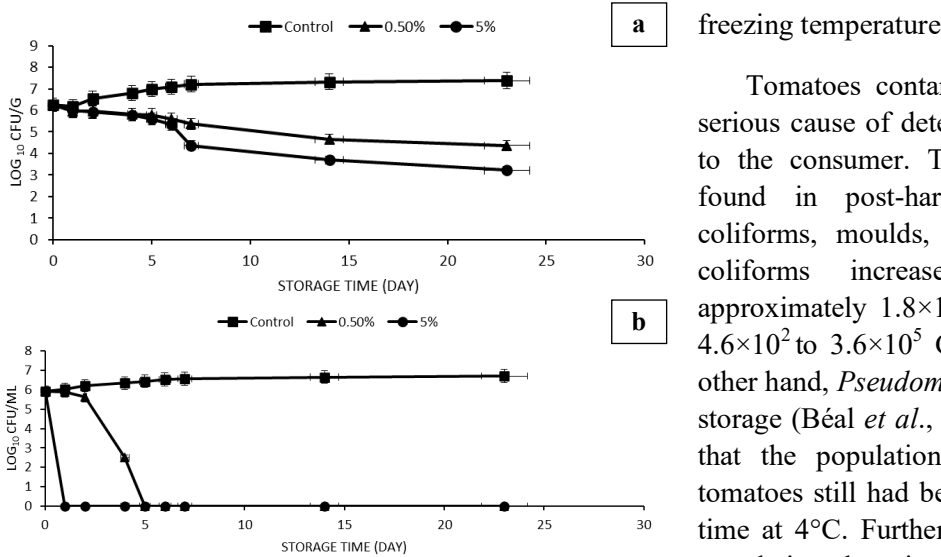


Figure 3. Representative the (a) yeast and mould count and (b) *Pseudomonas* spp. counts on cherry tomato exposed to different concentrations of *S. jambos* (L.) Alston leaves extract during storage at a of $25\pm 2^{\circ}\text{C}$ for 23 days.

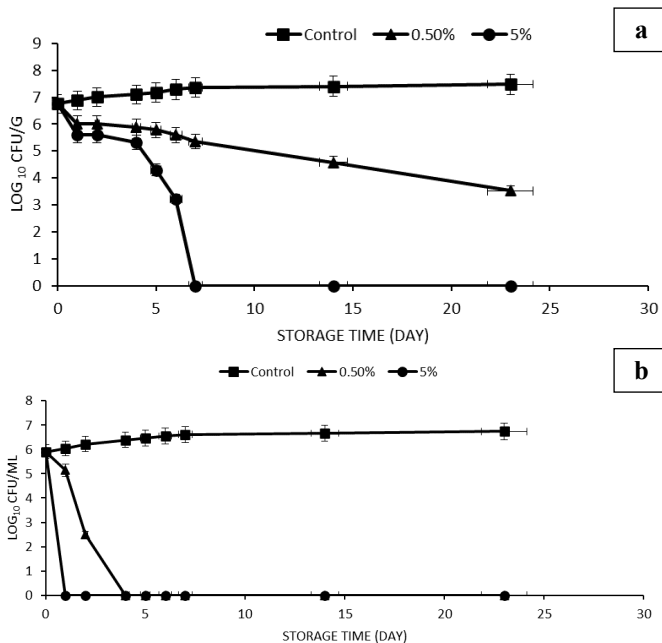


Figure 4. Representative the (a) yeast and mould count and (b) *Pseudomonas* spp. counts on cherry tomato exposed to different concentrations of *S. jambos* (L.) Alston leaves extract during storage at 4°C for 23 days.

forms. Moulds are belonging to fungi, which grow rapidly on food when kept in moist conditions. These organisms take advantage of the moist condition available and through the assistance of enzymes, which they possess, they weaken and penetrate the protective outer layer of the shrimp and lead to spoilage (Gómez *et al.*, 2013). *Vibrio parahaemolyticus* in shrimp samples exhibited a reduction during storage in the chiller and freezer temperature. According to Geiges (1996), *Vibrio* spp. was known to be sensitive to freezing. *V. vulnificus* in oysters, was reduced to 2-5 log during freezing. Moreover, *V. parahaemolyticus* in oyster homogenate also showed a reduction to 5 log after was kept at

freezing temperature

Tomatoes contamination with microbial may be a serious cause of deterioration and poses a health hazard to the consumer. The most common microorganisms found in post-harvested tomatoes are mesophyll, coliforms, moulds, and yeast. At 25°C , the rate of coliforms increased from 2.2×10^5 CFU/g to approximately 1.8×10^8 CFU/g and that of mould from 4.6×10^2 to 3.6×10^5 CFU/g (Vinha *et al.*, 2013). On the other hand, *Pseudomonas* is known to be resistant to cold storage (Béal *et al.*, 2001). The current study also found that the population of *Pseudomonas* spp. in cherry tomatoes still had been detected until the end of storage time at 4°C . Furthermore, the room temperature of the population has increased as a result of conditions favourable to its survival.

Findings by Gómez *et al.* (2013), a strawberry jam which was treated with pomegranate extract achieved a mean reduction of 0.71, 0.52, and 0.58 log units for aerobic mesophylls, lactose acid bacteria, yeasts, and moulds, respectively, while after being treated by a combination of pomegranate and lemon extract, the reductions were 1.52, 1.34, and 1.38 log units during storage at room temperature. This showed that the extract had antimicrobial properties and help extend food's shelf life during storage.

Refrigeration is employed to control the rate of certain chemical and enzymatic reactions as well as the rate of growth of food microorganisms (Srivastava and Kumar, 2002). The food spoilage process was slowed down as molecular motion was slow, and this retards the growth of bacteria that causes food to spoil (Whitman *et al.*, 2005). On the other hand, freezing causes mechanical damage to the cell walls and membranes due to the formation of intracellular crystals (Geiges, 1996), which leads microorganisms to die or injured. However, perishable food will deteriorate, even at refrigerator temperature, due to microorganisms, enzymes and oxidation (Jay, 2000).

Besides that, the thawing process also affected the microbial count where bacteria are able to reproduce again at this stage (Archer, 2004). However, the precaution had been taken seriously include thaw in sterile conditions and food was processed as soon as possible after thawing. Meanwhile, psychrotrophic bacteria may cause the spoilage of food materials during storage at cold temperatures. However, this type of bacteria does not determine in this analysis. This is because this assay aimed to ensure the effectiveness of *S. jambos* (L.) Alston leaves extract against selected foodborne pathogens and fungi. Hence, this leaves extract was effective in controlling the survival of

foodborne pathogens and spoilage microorganisms in food materials during storage.

4. Conclusion

The reduction in the microbial count is strongly correlated with the various concentrations of *S. jambos* (L.) Alston leaves extract and storage treatment time. Overall, a significant reduction in microbial loading was observed when the shrimp and cherry tomato samples were treated with a minimum concentration of 0.05% (v/v). A 5.00% (v/v) extract concentration appears to be the ideal antimicrobial concentration as it resulted in a complete reduction or 3 log₁₀ CFU/mL in microbial load. Extract at a concentration of 0.50% (v/v) resulted in a reduction of microbial load of at least 3 log₁₀ CFU/mL. These results suggested that the *S. jambos* (L.) Alston leaves extract can be developed as a natural sanitiser.

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