

Antibacterial activity of *asam gelugur* (*Garcinia atroviridis* Griff) fruit extract against foodborne pathogens and its effect on microbial population in raw chicken

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Abstract

Foodborne illness has a major impact on public health and is a major concern worldwide. The awareness about foodborne pathogens has increased among consumers because of their significant impact on public health. Moreover, research on safe natural plant products possessing antimicrobial activities against foodborne pathogens is vital because many consumers prefer natural compounds to synthetic ones. This study aimed to determine the antibacterial activity of *Garcinia atroviridis* Griff against foodborne pathogens and to evaluate the effect of the fruit extract in the microbial population in raw chicken meat at different concentrations and temperatures during storage. The antibacterial activity of *G. atroviridis* Griff was assessed using disc diffusion assay (DDA), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The DDA results showed that the inhibition zone of the extract ranged from 6.50±0.50 to 11.00±0.00 mm. The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) values of the extract ranged from 3.13 to 25.00 mg/mL and 6.25 to 25.00 mg/mL, respectively. *G. atroviridis* Griff maintained its antibacterial activity after being treated with different temperatures (4°C, 25°C, 60°C and 80°C) and various pH ranges (pH 3.0, 7.0, and 9.0). A 5.00% concentration of extract can reduce the microbial population in the raw chicken and maintain its microbiological quality with longer exposure within 21 days in storage conditions at chiller and freezer temperature. The findings showed that the *asam gelugur* extract might be used as a natural alternative preservative for reducing the microbial population in raw chicken and maintaining its microbiological quality during storage prior to cooking.

1. Introduction

The microbiological safety of food is still a major concern to consumers, food industries and regulatory agencies throughout the world. Due to their health awareness, people are more concerned about food safety and the illness related to foodborne pathogens and despite the wide range of preservation techniques available, the contamination of foods and spoilage by microorganisms is still a problem that is not yet under control but the pathogens causing the illness to have been eliminated or controlled with the emergence of new ones (Altekruse *et al.*, 2016). The anti-microbial substances added to food could be either artificial that is added purposely to foods or naturally occurring and biologically derived substances (Gyawali *et al.*, 2014). To prevent the growth of micro-organisms in food,

traditional antimicrobials like propionic acid, lactic acid, benzoic acid, nitrite, and sulphites are used in food for the last few years but nowadays microorganisms have become resistant to many antimicrobials due to the increased use of drugs, which is decreasing efficiency of conventional medicines (Tayel *et al.*, 2013). Scientists are making novel approaches to the development of new antimicrobials from various sources including medicinal plants (Roberts *et al.*, 2009).

Thus, it has become necessary to discover new antimicrobial agents. One of the reasons to look for new antimicrobial agents is that people are getting suspicious about the chemical additives and their adverse effects on health and consumers are getting aware of the benefits of using natural antimicrobials over synthetic ones (Abdallah, 2011). The most common foodborne illness-

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causing bacteria are *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Salmonella enterica* serovar Typhimurium, *Listeria monocytogenes*, *Clostridium botulinum*, *Vibrio vulnificus*.

Asam gelugur or scientifically known as *Garcinia atroviridis* Griff is very famous among locals in Peninsular Malaysia, India, and Thailand. *Garcinia* belongs to the family Clusiaceae with more than 450 species, widely distributed over tropical Asia, Africa, Madagascar, Northeast Australia, and Polynesia (Lim, 2016). It is a medium-sized, perennial tree and the length of the tree can be up to 27 m with 70 cm girth, a long trunk which is smooth grey and drooping branches and a narrowly conical crown. The most utilised part is the fruit. It is large and globular in shape, yellowish-green to yellow. It is mainly used in cooking as a flavouring agent. 'Asam keping' is the local name given to the sundried slices of the fruits and are commercially available and are popularly used as a seasoning in curries, for dressing fish and sour relish (Corner, 1988).

Garcinia atroviridis Griff extract has been used in traditional ayurvedic medicine. The phytochemicals derived from the plants form the basis of traditional drug pharmacopoeia and serve as a proven source of therapeutic medicines (Jones and Kinghorn, 2012). The fruit part of the *G. atroviridis* Griff contains many organic acids such as citric acid, ascorbic acid, malic acid, tartaric acid, pentadecanoic acid and non-decanoic acid. The most interesting one above all is the hydroxy citric acid (HCA). It is also present in the rind of the *G. atroviridis* Griff fruit (Taher et al., 2016). This acid originated from *Garcinia cambogia* and has been recognized as a potential supplement agent for reducing weight and management.

As per the research done by Taher et al. (2017), *G. atroviridis* Griff plant parts possess a wide range of pharmacological activities such as antioxidant, antimicrobial, anti-allergic, anti-inflammatory, antihyperlipidemic or anti-hypercholesterolemic, anti-obesity, antithrombotic, cytotoxic activities. It includes many types of assays such as DPPH (1,1-diphenyl-2-picrylhydrazyl), thiobarbituric acid (TBA), ferric thiocyanate (FTC), ferric reducing antioxidant power (FRAP). All these methods mentioned above have different mechanisms for determining the antioxidant activity of plant extracts. Except for the fruit, all other parts of the plant exhibited strong antioxidant capacity with ranges of 64-90% for FTC and 87-93% for the TBA method.

According to a study conducted by Permana et al. (2001), *G. atroviridis* Griff plant parts possess antimicrobial properties. Antibacterial activity was

predominantly exhibited by the methanolic extracts of different parts of the *G. atroviridis* Griff with root extract showing the strongest inhibition at a minimum inhibitory dose of 15.6 µg/disc. Among all the plant parts, only the fruit extract exhibits the antifungal activity against *Cladosporium herbarium* in an assay was performed (Lim, 2016). According to Suwanmanee et al. (2014), the *G. atroviridis* Griff roots have anti-inflammatory activity in two cellular systems when used in the anti-inflammatory analysis of bioactive compounds. Research done by Lim (2016) reported that the anti-inflammatory activity of the *G. atroviridis* Griff fruit was determined through cyclooxygenase inhibitor screening assay.

This research aimed to determine the antibacterial activity of *G. atroviridis* Griff against foodborne pathogens namely *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella enterica* serovar Typhimurium. The antibacterial activity of *G. atroviridis* Griff extract was investigated by means of disc diffusion assay (DDA), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), to assess the antibacterial stability of the extract at different temperatures and pH and, to evaluate the effect of the fruit extract on the microbial population in raw chicken meat samples at different concentrations and temperatures during storage.

2. Materials and methods

2.1 Asam gelugur (*Garcinia atroviridis* Griff) and raw chicken samples

Asam gelugur (*G. atroviridis* Griff) fruit was obtained from Taman Pertanian, Malaysia (UPM). The sample was stored at room temperature prior to use. Fresh raw chicken sample was purchased from Wet Market, Seri Kembangan Serdang, Selangor. After purchasing the chicken sample, it was processed immediately at Food Safety and Quality Laboratory, Faculty of Food Science and Technology, Universiti Putra Malaysia, Selangor.

2.2 Asam gelugur (*Garcinia atroviridis* Griff) fruit extraction

Pre extraction is a very important step in the extraction process. The fruit was washed, cleaned and cut into small pieces. The fruit pieces were spread evenly on the tray and kept in the oven for drying at a temperature of 50±2°C for more than 24 hrs. Dried fruit pieces of *G. atroviridis* Griff were ground using the blender to make a fine powder. A 200 g of the fine powder of *G. atroviridis* Griff was soaked with 800 mL of 99.8% absolute ethanol (Sigma-Aldrich, Missouri, USA). It was kept in a water bath shaker for 24 hrs at the temperature of 30°C to steadily shake and mix the

sample while maintaining a constant temperature and speed.

The extraction of *G. atrovirdis* Griff was adopted from a study conducted by Rukayadi *et al.* (2008) with modifications. The resulting ethanolic extract from the pre-extraction step was filtered through sterile Whatman No. 2 filter paper. The filtrate obtained was evaporated under reduced pressure by an aspirator pump to which a Buchner funnel and flask were connected. The filtrate was concentrated by using a rotary evaporator at 55°C at a speed of 110 rpm until a gum-like viscous extract was obtained and it was transferred to a sterile bottle stored at 4°C prior to use.

2.3 Preparation of extract solution and chlorhexidine

In this study, the concentration of extract used was 10.0% which equals 100 mg/mL extract. For the preparation of 10% *G. atrovirdis* Griff extract, 10 mL of extract stock solution was diluted with 90 mL of sterile distilled water. The extract was kept in the refrigerator (4.0±2.0°C) prior to use. The positive control used in the study was chlorhexidine. 0.1% chlorhexidine was prepared by weighing 0.1 g of chlorhexidine powder (OXOID, United Kingdom) into 100.00 mL of the volumetric flask containing 100.00 mL sterile distilled water and to prevent the solution from light exposure, it was transferred accordingly into an opaque and dark container with a tightly closed cap and kept in the chiller for further use.

2.4 Preparation of susceptibility test discs

For the preparation of susceptibility test discs, a method used by Opinde *et al.* (2016) was followed with slight modifications. The 6 mm discs were prepared from the filter paper Whatman no.2, discs were punched using a paper punch and were sterilized prior to use.

2.5 Bacterial strain and inoculum preparation

The following pathogenic bacteria *E. coli* ATCC43895, *L. monocytogenes* ATCC19112, *S. enterica* ser. Typhimurium ATCC14028 and *S. aureus* ATCC2737 were used *in-vitro* antibacterial assay. All the bacterial strains were obtained from the American Type Culture Collection (Rockville, MD, USA). The bacterial strains were sub-cultured on Muller Hinton Agar (MHA) (Difco Becton Dickinson, Sparks, MD, USA), incubated at 37°C for 24 hrs, and maintained at 4°C in the refrigerator.

2.6 Antimicrobial activity of *Garcinia atrovirdis* Griff fruit extract against foodborne pathogens

The antimicrobial activity of *G. atrovirdis* Griff fruit was determined by means of disc diffusion assay,

minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

2.6.1 Disc diffusion assay

The antibacterial activity of *G. atrovirdis* Griff extract was determined by disc diffusion assay according to the method of Clinical Laboratory Standard Institute (2013). From the freshly prepared inoculum, a single colony of the bacteria was spread on MHA using a sterile cotton swab. The 6mm sterile filter discs were placed at equal distances between them on the MHA agar plates inoculated with a bacterial pathogen. For positive control, 0.1% CHX was used and for the negative control, dimethylsulfoxide (DMSO) was used. Susceptibility discs were impregnated with 10 µL of *G. atrovirdis* Griff fruit extract, for the positive control and the negative control discs, 10 µL of 0.1% CHX and 10% DMSO was infused on it respectively. The Petri plates were incubated at 37°C for 24 hrs to check the formation of the inhibition zone by the fruit extract against the test microbes. The experiment was carried out in duplicates and the diameter of the zones of inhibition formed was measured.

2.6.2 Minimal inhibitory concentration (MIC)

The minimum inhibitory concentration was determined by a two-fold broth microdilution method using sterile 96-well microtiter plate as reported in the guidelines of the Clinical and Laboratory Standard Institute (2013) with modifications. Sterile Mueller-Hinton broth (MHB) was used to prepare bacterial suspension and overnight culture of each bacterium was inoculated and diluted until a turbid suspension was formed. All the bacterial suspensions were adjusted to further dilutions with new broth until achieving a final concentration of 10⁶ CFU/mL by comparing with a 0.5 McFarland standard. An inoculum of 100 µL (0.5 McFarland standards) of the overnight clinical culture of each bacterium was added to the wells of the microtiter plate. A 100 µL of 100 mg/mL of *G. atrovirdis* Griff fruit extract was added to 100 µL of sterile MHB in the 12th well of the 96 well microplates and mixed well with a micropipette. A 100µL of this dilution was transferred subsequently to the wells two folding each dilution of the original extract. The wells in column 2 served as positive control which were filled with 100 µL of bacterial suspension (without *G. atrovirdis* Griff fruit extract). Column 1 served as the negative control filled with 100 µL of MHB only. The microdilution was performed at extract concentration between 50.00 mg/mL in column 12 to 0.09 mg/mL in column 3. The microtiter plate was incubated at 37°C for 24 hrs. After overnight incubation, the MIC value was determined by the lowest concentration of the extract that produced no visible

growth in wells.

2.6.3 Minimum bactericidal concentration

The minimum bactericidal concentration was determined by subculturing approximately 10 μ L of suspension from microtiter plates onto MHA plates. The plates were incubated at 37°C for 24 hrs. MBC was defined as the lowest concentration of the *G. atroviridis* Griff fruit extract where there was no bacterial growth occurred in the MHA plates.

2.7 Stability of *Garcinia atroviridis* Griff fruit extract at different pH and temperatures

A four sets of extract were prepared by pipetting 1.0 mL of 10% *G. atroviridis* Griff fruit extract solution into a 1.5 mL sterile Eppendorf tube. At different temperatures (4.0 \pm 2.0°C, 25.0 \pm 2.0°C, 60.0 \pm 2.0°C and 80.0 \pm 2.0°C) the extracts were incubated for 1 hr. The antibacterial activity of the extract was evaluated against foodborne pathogens using DDA after being exposed to different temperatures. The antibacterial stability of the extract was evaluated by measuring the zone of inhibition produced on the agar.

The stability of *G. atroviridis* Griff extract was determined by adjusting the pH in the range of 3, pH 7 and pH 9, using 0.1 M of hydrochloric acid (HCl, Merck, Darmstadt, Germany) or 0.1 M of sodium hydroxide (NaOH, Sigma Aldrich, United States). The extracts were then incubated for one hour. The antibacterial stability of the extract was evaluated by measuring the zone of inhibition produced on the agar.

2.8 Treatment of raw chicken samples by *Garcinia atroviridis* Griff fruit extract

2.8.1 Preparation of different concentrations of *Garcinia atroviridis* Griff fruit extract

The five different concentrations i.e., tap water, 0.00%, (Distilled water), 0.05%, 0.5% and 5% were used for the treatment of chicken samples. A concentration of 5.00% extract was prepared by adding 5.0 mL of the extract into 95.0 mL of 10% DMSO. 0.50% and 0.05% concentrations were made by adding 99.5 mL and 99.95 mL of 10% DMSO into 0.50 mL and 0.05 mL of extract accordingly. Distilled water (DW) was used for chicken stored at 0.00% *G. atroviridis* Griff fruit extract concentration.

2.8.2 Preparation of selective media for microorganism enumeration

Different media like Plate Count Agar for total plate count (TPC) (Oxoid Ltd, Hampshire), Mannitol Salt Agar (MSA) (Biokar Diagnostics) for *S. aureus*, Eosin Methylene Blue, levine (EMB) (CRITERION, Santa

Maria, USA) Agar for *E. coli*, Palcam agar (OXOID, United Kingdom) for *L. monocytogenes* and Xylose Lysine Deoxycholate (OXOID, United Kingdom) for *S. enterica* ser. Typhimurium (XLD) was prepared for the enumeration of microorganisms in chicken.

2.8.3 Treatment of chicken sample with *Garcinia atroviridis* Griff fruit extract

Chicken samples obtained from the wet market were cut into small pieces of 5 g and kept in universal sterile bottles. The samples were treated with different concentrations of *G. atroviridis* Griff fruit extract (tap water, 0.00% (DIW), 0.05%, 0.50% and 5.00%). The final volume of each bottle was 4.0 mL including different concentrations of *G. atroviridis* Griff fruit extract. The samples were then kept at three different temperatures: room temperature (25.0 \pm 2.0°C), refrigerator (4.0 \pm 2.0°C), freezer (-18.0 \pm 2.0°C) for 21 days. An hour prior to analysis, the frozen samples were thawed at 4.0 \pm 2.0°C. The sampling was done by taking out 2 g of treated chicken samples into 18 mL of sterile peptone water (Oxoid Ltd, Hampshire, UK) followed by shaking using. After the dilution of the chicken samples with the different plant extracts, serial dilution was done from each treatment series. 10 μ L was taken from each treatment was pipetted into 990 μ L of phosphate buffer saline (0.1%) to create 10⁻² and 10⁻⁴ dilutions. According to the requirement of the respective media used, the agar plates were incubated accordingly. After incubation at 37°C for 24 hrs, the logarithm numbers of colony-forming unit per grams (log₁₀ CFU/g) of samples were calculated

2.9 Statistical analysis

MINITAB (Version 17, Coventry, UK) statistical application software was used to analyse the data for the one-way analysis of variance (ANOVA). The significance difference (P < 0.05) was analysed by using Turkey's test. Results were interpreted as means \pm standard deviation (SD) of duplicate analysis.

3. Results and discussion

3.1 Yield of extraction of *Garcinia atroviridis* Griff

The maceration process reported by Rukayadi *et al.* (2008) with modifications was used for the ethanolic extract of dried and ground *G. atroviridis* Griff fruit. The results of the extraction process are shown in Table 1. Based on the macroscopic observation, the extracted crude oil had a blackish brown colour and was slightly viscous. The selection of solvent system largely affects the nature of the bioactive compound being targeted in *G. atroviridis* Griff fruit. Mahdi-Pour *et al.* (2012) mentioned that in addition to the extraction technique,

the extraction solvent is an important criterion that is mainly associated with the extraction yield and biological activity of the resulting extract.

Table 1. Total yield of *G. atroviridis* Griff fruit extract

Ethanollic extraction	Weight of <i>G. atroviridis</i> Griff Fruit (g)	Weight of <i>G. atroviridis</i> Griff extract (g)	Yield (%)
1	100	32.2	32.2
2	100	34	34
Mean±SD	100.00±0.00	33.10±0.09	33.10±0.09

However, in various studies, different percentages of yield extract were obtained. In a study conducted by Arya et al. (2012), the highest extraction yield with the maximum presence of phytoconstituents was obtained by the ethanolic extraction of *Psidium guajava* L. leaves and hydroalcohol extract in comparison with extraction using other solvents like chloroform, water, and petroleum ether. Non-polar compounds such as chloroform and water showed no active compounds and very few tannins were present. According to Al Mansoub et al. (2014), methanolic extracts of *G. atroviridis* Griff showed higher antioxidant activity than aqueous extracts, although higher anti-hyperlipidemic activity was observed in aqueous extracts. The powdered dried samples of *Moringa oleifera* macerated with 70% ethanol yielded the highest phenolic and flavonoid contents when compared to soxhlet extraction and percolation using the same solvents (Vongsak et al., 2013).

3.2 Disc diffusion assay

The antibacterial activity of *G. atroviridis* Griff fruit extract against, *S. aureus*, *L. monocytogenes*, *S. enterica* ser. Typhimurium and *E. coli* was demonstrated by disc diffusion assay. The presence of a clear inhibition zone surrounded by the disc was measured and compared to the positive control, chlorhexidine (CHX). The antibacterial activity of *G. atroviridis* Griff fruit and the average results of the diameter of the inhibition zones produced were presented in Table 2. However, the largest zone of inhibition was observed in Gram-positive bacteria, *S. aureus* (11.00±0.00 mm), while Gram-negative bacteria *E. coli* had a smaller inhibition zone (6.50±0.50 mm). It could be due to the higher

susceptibility of penetration of extract in Gram-positive bacteria due to the lack of outer membrane (Negi et al., 2008). Extract effectiveness upon the bacteria tested as indicated by the size of the diameter of the inhibition zone. The susceptibility of the bacteria towards the extract is determined due to the differences between cell wall compositions (Henie et al., 2009).

Similar results were observed in a study conducted by Yassen (2017) that showed an ethanolic extract of *N. sativa* L. with a control positive antibiotic at a concentration of 100 mg/mL had a lower inhibition zone than the control antibiotic against *S. aureus* bacteria. A study conducted by Al-Tememy (2013) resulted in a 10 mm inhibition zone for *E. coli*, 13 mm for *P. aeruginosa* and 15 mm for *S. aureus*, by using 100 µL of ethanolic *P. cubeba* L. extract on the tested disc. According to a study by Monero et al. (2006), the absence of an inhibition zone did not necessarily mean the extract is inactive. It could be due to the reason that it diffuses more slowly into the culture medium.

3.3 Minimum inhibitory concentration and minimum bactericidal concentration

A two-fold microdilution method was performed to determine the MIC and MBC values of *G. atroviridis* Griff fruit extract followed by sub-culturing the suspension from MIC wells. Both the test results were recorded and presented in Table 3. The results clearly show that *G. atroviridis* Griff fruit extract has bacteriostatic and bactericidal activities towards the pathogens tested with MIC values ranging from 6.25 to 12.50 mg/mL and MBC values from 6.25 to 25.00 mg/mL.

The extract was found to inhibit the growth of *S. aureus* at MIC value of 6.25 mg/mL and at MBC value of 6.25 mg/mL which if compared to the conventional antiseptic used for positive control has the same MIC and MBC value for *S. aureus*. The fruit extract had the same MICs and MBCs values for *L. monocytogenes* and *E. coli* 12.50 mg/mL and MBC 25.00 mg/mL. In this study, *G. atroviridis* Griff fruit extract showed effective antibacterial activities against all the tested pathogens including Gram-negative bacteria such as *E. coli* and *S. enterica* ser. Typhimurium.

Table 2. Inhibition zone of *G. atroviridis* Griff. fruit extract against foodborne pathogens

Bacteria Strains	Average inhibition zone (mm)	
	Control (Chlorhexidine)	<i>G. atroviridis</i> Griff fruit extract
<i>Staphylococcus aureus</i> ATCC29737	11.50±0.40 ^{Aa}	11.00±0.00 ^{Aa}
<i>Listeria monocytogenes</i> ATCC19112	13.00±0.50 ^{Ba}	9.50±1.50 ^{ABb}
<i>Salmonella enterica</i> serovar Typhimurium ATCC14028	9.00±0.40 ^{Ca}	9.00±1.00 ^{Ba}
<i>Escherichia coli</i> ATCC43895	10.00±0.50 ^{Ca}	6.50±0.50 ^{Cb}

Values are presented as mean±standard deviation. Values with different lowercase superscript within the same row and values with different uppercase superscript within the same column are significantly different (P<0.05).

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *G. atroviridis* Griff fruit extract against foodborne pathogens.

Bacteria Strains	<i>G. atroviridis</i> Griff fruit extract (10%)		Control (Chlorhexidine)	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
<i>Staphylococcus aureus</i> ATCC2737	6.25	6.25	6.25	6.25
<i>Listeria monocytogenes</i> ATCC19112	12.50	25.00	1.56	1.56
<i>Salmonella enterica</i> serovar Typhimurium ATCC14028	6.25	12.50	3.13	3.13
<i>Escherichia coli</i> ATCC43895	12.50	25.00	6.25	6.25

A study done by Prabuseenivasan *et al.* (2006) reported that for different Gram-positive and Gram-negative bacteria, the MIC's values were found in between the range of 3.2 and 12.8 mg/mL while *in vitro* these concentrations may be effective but when used on food products higher amounts will likely be needed. The inhibitory power of the natural antimicrobials changes a bit when applied to foods as compared to the inhibitory power demonstrated *in vitro* due to the various characteristics of foods like water activity, moisture, pH and other factors such as food composition (David *et al.*, 2013).

3.4 Stability of *Garcinia atroviridis* Griff fruit extract at different temperature conditions

The antibacterial stability of *G. atroviridis* Griff fruit extract was demonstrated using DDA after exposing the extract to various temperatures (4±2.0°C, 25±2.0°C, 60±2.0°C and 80±2.0°C) for one hour. The average diameter of the inhibition zone for all the bacteria tested is shown in Table 4. According to Table 4, the *G. atroviridis* Griff fruit extract exhibited antimicrobial activity against all bacteria tested at different

temperatures. The inhibition zone diameter produced varyingly ranging between, 6.01 to 10.25 mm, 7.71 to 11.90 mm, 7.30 to 11.35 mm, and 7.00 to 10.90 mm for 4°C, 26 °C, 60°C and 80°C accordingly. Among all the bacteria tested treated at different temperatures, the extract showed the highest inhibitory effect against *S. aureus* at 26 °C with an inhibition diameter of 11.90 mm and the least inhibitory activity was observed in *E. coli* at a temperature of 80°C with an inhibition zone of 7.00 mm. As compared to other temperatures, the maximum zone of inhibition was observed at room temperature (25°C).

Antimicrobial activity functions effectively at room temperature (Ahmed *et al.*, 2016). The increasing incubation temperature of the extract showed a significant difference against all the bacteria tested. At cooking temperature (80°C), the extract showed the least activity in *E. coli*, and this might be due to some loss of antibacterial compounds responsible for the antibacterial properties of the extract. However, with increasing temperatures, the antibacterial activity was reduced, and this might be due to the damage caused during heating (Duraira *et al.*, 2009), resulting in a loss of antimicrobial

Table 4. Effect on the antibacterial activity of *G. atroviridis* Griff fruit extract against pathogenic bacteria at different temperatures

Bacteria strains	Medium	Average diameter of inhibition zone (mm)			
		4°C	25°C	60°C	80°C
<i>Staphylococcus aureus</i> ATCC2737	<i>G. atroviridis</i> Griff fruit extract	10.25±0.35 ^{Aa}	11.90±0.00 ^{Aa}	11.35±0.45 ^{Aa}	10.90±0.00 ^{Aa}
	Control (Chlorhexidine)	12.63±1.50 ^{Aa}	12.50±0.53 ^{Aa}	12.30±0.88 ^{Aa}	11.80±0.40 ^{Aa}
<i>Listeria monocytogenes</i> ATCC19112	<i>G. atroviridis</i> Griff fruit extract	9.30±0.20 ^{Ab}	9.50±1.50 ^{Aab}	10.90±0.40 ^{Aa}	9.00±0.00 ^{Ab}
	Control (Chlorhexidine)	11.00±0.50 ^{Aa}	12.00±0.18 ^{Aa}	11.50±0.00 ^{Aa}	11.30±1.50 ^{Aa}
<i>Salmonella enterica</i> serovar Typhimurium ATCC14028	<i>G. atroviridis</i> Griff fruit extract	8.10±0.00 ^{Ab}	8.70±0.71 ^{Ab}	10.10±1.06 ^{Aa}	9.01±0.15 ^{Ab}
	Control (Chlorhexidine)	9.30±0.18 ^{Aa}	9.90±0.14 ^{Aa}	9.50±0.00 ^{Aa}	8.60±0.20 ^{Aa}
<i>Escherichia coli</i> ATCC43895	<i>G. atroviridis</i> Griff fruit extract	6.71±0.53 ^{Ba}	7.71 ± 0.34 ^{Aa}	7.30±0.21 ^{Aa}	7.00±0.18 ^{Aa}
	Control (Chlorhexidine)	10.30±0.80 ^{Aa}	11.50±0.35 ^{Aa}	11.00±0.00 ^{Aa}	10.90±0.20 ^{Aa}

Values are presented as mean±standard deviation. Values with different lowercase superscript within the same row and values with different uppercase superscript within the same column are significantly different (P<0.05).

activity in the extract. Hence the results of this experiment depict that the *G. atroviridis* Griff fruit extract was stable at different temperatures, meaning that the antibacterial compounds present in the fruit extract were still retained after exposure to high temperature making it possible to be applied in foods.

3.5 Stability of *Garcinia atroviridis* Griff extract at different pH

The antibacterial stability of *G. atroviridis* Griff fruit extract was examined by DDA at different pH treatments (pH 3, pH 7, and pH 9) after one hour of incubation, and the average diameter of the inhibition zone was calculated and presented in Table 5. The result (Table 5) showed that the inhibition zones produced against all the bacteria tested strains were in the range of 6.30 to 12.40 mm. The antimicrobial activity varied with the incubation of extract at different pHs. The average diameter of the inhibition zone of the extract against bacteria tested at pH 3 decreased from 12.90 to 11.80 mm for *S. aureus*, 10.76 to 10.00 mm for *L. monocytogenes*, and 9.90 to 9.00 mm for *S. enterica* ser. Typhimurium and 8.00 to 7.50 mm for *E. coli*. Greater antibacterial efficacy was demonstrated by *G. atroviridis* Griff fruit extract at neutral pH (7.00). The antibacterial activity of the fruit extract was still present after adjusting the pH to different conditions. Therefore, the extract remained stable even after adjusting the pH to highly acidic and alkaline. If the fruit extract is to be used in food applications, it must be stable under a variety of pH conditions. The ability of *G. atroviridis* Griff fruit to withstand different pH treatments allows it to be developed as a natural food preservative.

3.6 Effect of *Garcinia atroviridis* Griff fruit extract on the microbial population in raw chicken

Detection and enumeration of microorganisms in the food is the most important part of any food safety and quality. Detection of microorganisms in food depends on the incorporation of the food sample into a nutrient medium in which the microorganisms can multiply, thus providing visual confirmation of their growth. The microbial growth was monitored in the chicken samples mixed with different concentrations of fruit extract (0.00%, 0.05%, 0.50% 5.00%) at different temperatures (room temperature, refrigerator temperature and different storage conditions (0 hr, 4 hrs, 24 hrs, 3 days, 7 days, 14 days and 21 days).

3.6.1 Total plate count

Plate count agar is considered an ideal agar as it is quite rich in nutrients and free from selective supplements. The results of microbiological total plate count, upon storage of 21 days treated with different concentrations of extracts at room temperature are presented in Figure 1.

According to Figure 1, an increasing trend was observed in an untreated chicken sample. The initial microbial load at 0 min was 7.71 Log₁₀ CFU/g and increased to 9.22 Log₁₀ CFU/g to 8.35 Log₁₀ CFU/g upon 21 days of storage in case of tap water (TW) and 0.00% respectively. In the case of untreated samples, with the increased concentration of extract, the microbial population was reduced for up to 3 days. On the other hand, after the third day of the storage period, there was a rise in the microbial population, which could be attributed to the extract's loss of antibacterial

Table 5. Effect on the antibacterial activity of *G. atroviridis* Griff fruit extract against pathogenic bacteria at different pH

Bacteria strains	Medium	Average size of inhibition zone (mm)		
		pH 3	pH 7	pH 9
<i>Staphylococcus aureus</i> ATCC2737	<i>G. atroviridis</i> Griff fruit extract	11.25±0.35 ^{Aa}	12.40±0.00 ^{Aa}	10.00±0.00 ^{Aa}
	Control (Chlorhexidine)	11.90±0.28 ^{Aa}	12.90±0.50 ^{Aa}	11.20±0.18 ^{Aa}
<i>Listeria monocytogenes</i> ATCC19112	<i>G. atroviridis</i> Griff fruit extract	10.00±0.27 ^{Aa}	10.75±0.71 ^{Aa}	9.25±0.30 ^{Aa}
	Control (Chlorhexidine)	12.10±0.00 ^{Aa}	12.50±0.18 ^{Aa}	11.00±0.00 ^{Aa}
<i>Salmonella enterica</i> serovar Typhimurium ATCC14028	<i>G. atroviridis</i> Griff fruit extract	9.00±0.00 ^{Aa}	9.90±0.27 ^{Aa}	8.25±0.35 ^{Aa}
	Control (Chlorhexidine)	10.75±0.35 ^{Aa}	11.00±0.00 ^{Aa}	10.00±0.71 ^{Aa}
<i>Escherichia coli</i> ATCC43895	<i>G. atroviridis</i> Griff fruit extract	7.50±0.03 ^{Aa}	8.00±0.00 ^{Aa}	6.30±0.44 ^{Aa}
	Control (Chlorhexidine)	10.15±0.71 ^{Aa}	10.78±0.20 ^{Aa}	9.55±0.00 ^{Aa}

Values are presented as mean±standard deviation. Values with different lowercase superscript within the same row and values with different uppercase superscript within the same column are significantly different (P<0.05).

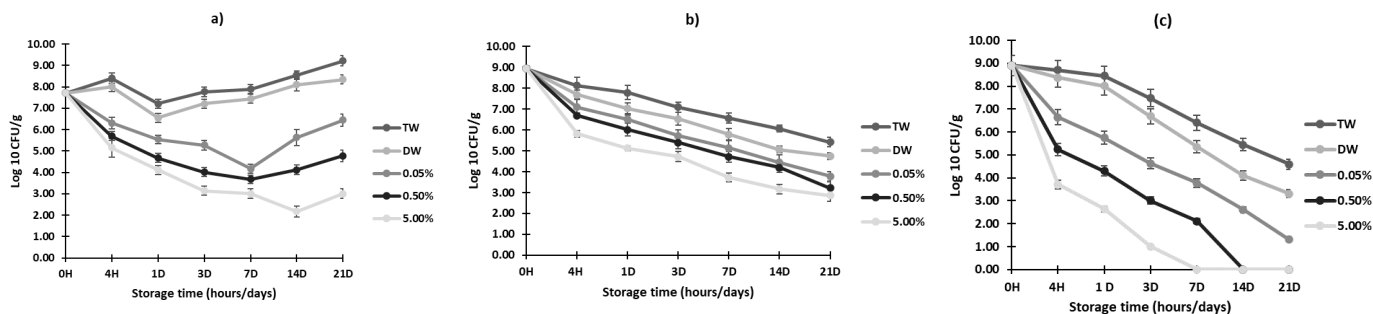


Figure 1. Total plate count upon storage of 21 days treated with different concentration of extracts and conditions: (a) room temperature (b) chiller temperature (c) freezer temperature

effectiveness. This observation might be due to the loss of some volatile compounds within the storage period (Guerra-Rosas *et al.*, 2017), hence favouring the growth of microorganisms.

Moreover, plate count agar is highly rich in nutrients making it favourable conditions for the bacteria to grow while as in the case of chiller and freezer, reduction in TPC values were observed starting from 0h up to 21 days of storage period. In Figure 2, the values of TPC were reduced from 8.93 Log₁₀ CFU/g to 5.34 Log₁₀ CFU/g and 4.74 Log₁₀ CFU/g in the chiller, as well as 4.59 Log₁₀ CFU/g and 3.13 Log₁₀ CFU/g in the freezer. This tremendous reduction in bacterial population might be due to the different storage temperatures as room temperature favours the growth of microorganisms while the other two storage conditions 4°C and -18°C reduce the microbial growth as biochemical and chemical reactions are minimised at low temperatures and microbiological activity is controlled during coagulation by limiting microbial growth and water activity (Lenovich, 2017). In the case of the chiller, the microbial population was reduced from 8.88 to 2.86 Log₁₀ CFU/g while a complete obstruction of microbial growth was recorded in the freezer upon the 7th day of storage with 5.00% of fruit extract concentration.

Similar results were obtained in a study conducted by Ann and Rukayadi (2019), the *P. cubeba* L. extract was able to reduce the TPC values of chicken stored under different storage conditions. Higher concentrations of extract at chiller and freezer temperature reduced the microbial population in beef quite significantly. In a

study by Sahu and Bala (2017) at lower temperatures, most biochemical processes are slowed down thereby reducing the spoilage process. Hence proving that storage at low temperature might increase the shelf life.

3.6.2 *Listeria monocytogenes*

The effect of *G. atroviridis* Griff extract on *L. monocytogenes* count at different conditions upon storage of 21 days is presented in Figure 2. All colonies of *L. monocytogenes* that appeared on Palcam agar were calculated and expressed in Log₁₀ CFU/g. In this study, the population of *L. monocytogenes* at room temperature was increased with the storage time in the untreated sample (tap water and distilled water). Chicken treated with 0.05% concentration showed a reduction of bacterial count value from 4.55 Log₁₀ CFU/g to 1.80 Log₁₀ CFU/g after seven days. At 24 hrs, with 5.00% of extract concentration, the growth of *L. monocytogenes* was inhibited. At different storage conditions and treatments, the growth of *L. monocytogenes* was reduced but at slower rates. While in the case of the chiller, the microbial population in the chicken sample showed decreasing trends even for untreated samples and the reason for this decreasing trend might be due to the chiller temperature as this temperature is not favourable for the growth of microorganisms.

In the case of room temperature with 5.00% of fruit extract, complete inhibition of *L. monocytogenes* was observed after 14 days while as in the case of chiller and freezer, total inhibition was achieved on the 7th day and 1st day respectively. Chicken treated with 0.05%

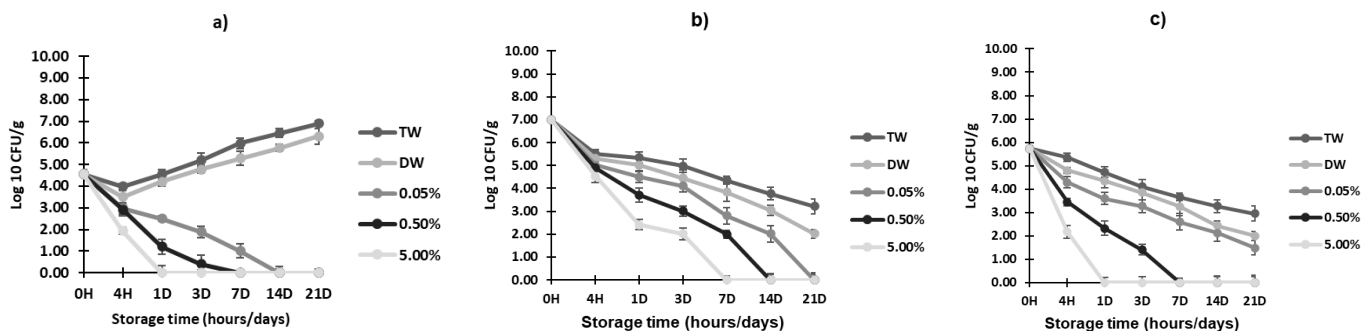


Figure 2. *Listeria monocytogenes* count upon storage of 21 days treated with different concentrations of extracts and conditions: (a) room temperature (b) chiller temperature (c) freezer temperature

concentration showed a reduction of bacterial count value from 4.90 log₁₀ CFU/g to 2.01 log₁₀ CFU/g after 14 days. Treatment with 0.50% showed total inhibition only after 14 days. The effect of *G. atroviridis* Griff extract on *L. monocytogenes* count in the freezer is presented in Figure 2. In the freezer, a decreased trend of growth was observed even for untreated samples starting at 4 hrs until 21 days of storage. A combination of freezing temperature with the application of extract in chicken managed to extend the product's shelf life. Treatment of chicken with the lowest concentration of *G. atroviridis* Griff fruit extract (0.05%) was enough to maintain the *L. monocytogenes* count at a safety level limit for more than a week. The population reached 1.489 Log₁₀ CFU/g after the 3rd to 21st day of storage, which was still below the limit. The results obtained in this study were consistent with the results obtained by Ben Lagha *et al.* (2020), who found that *L. monocytogenes* were inhibited with higher concentrations of extract for 10 days in a chiller and 7 days in a frozen temperature.

3.6.3 *Escherichia coli*

The effect of *G. atroviridis* Griff fruit extract on *E. coli* count was plotted and shown in Figure 3. The growth of the *E. coli* population was monitored at different temperatures and concentrations of extract up to the 21st day of storage. All the colonies formed on the Eosin Methylene Blue (EMB) agar were calculated and expressed in Log₁₀ CFU/g. Based on the *E. coli* graph, *G.*

atroviridis Griff fruit extract concentration showed increased trends after 24 hrs of storage in untreated samples. The *E. coli* count increased from 5.3 Log₁₀ CFU/g to 8.3 Log₁₀ CFU/g after 24 hrs of exposure. There was a fairly minor reduction in growth rate when treated with the lowest concentration of the extract (0.05%). According to Figure 4, the total inhibition of *E. coli* was seen on the 21st day of storage at the 5.00% concentration of extract, whereas in the chiller there was a decline in the microbial count but at a relatively low pace.

Upon the 14th day of storage chicken samples treated with 0.05% concentration showed a reduction of bacterial count value from 6.56 Log₁₀ CFU/g to 3.40 log₁₀ CFU/g while samples treated with 0.50% extract showed a reduction from 6.56 Log₁₀ CFU/g to 2.00 Log₁₀ CFU/g. From Figure 4, the antibacterial activity of *G. atroviridis* Griff fruit extract at 0.50% concentration extended the shelf life of raw chicken upon storage at freezer temperature for 14 days. With 0.50% fruit extract, the lowest value of microbial growth was achieved (2.01 Log₁₀ CFU/g) after 7 days of storage. On 3rd day of storage, no growth was observed at 5.00% fruit extract concentration. The most important factor in preventing spoilage in chicken is by controlling temperature (Fung *et al.*, 2008). The storage that advantageously preserves the nutritional and sensory value of foods is frozen storage. The survival rate of most bacteria is poor due to the damage caused by ice crystal formation and electrolytes (Tedeschi and De

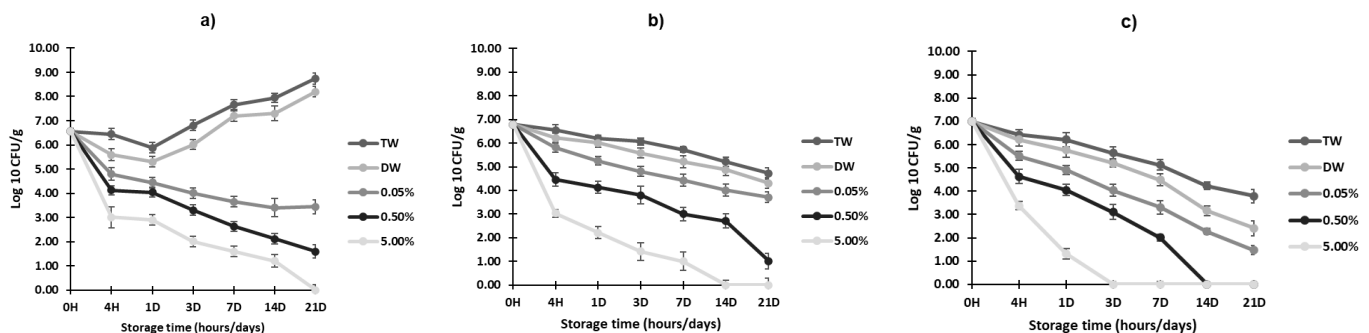


Figure 3. *Escherichia coli* count upon storage of 21 days treated with different concentrations of extracts and conditions: (a) room temperature (b) chiller temperature (c) freezer temperature

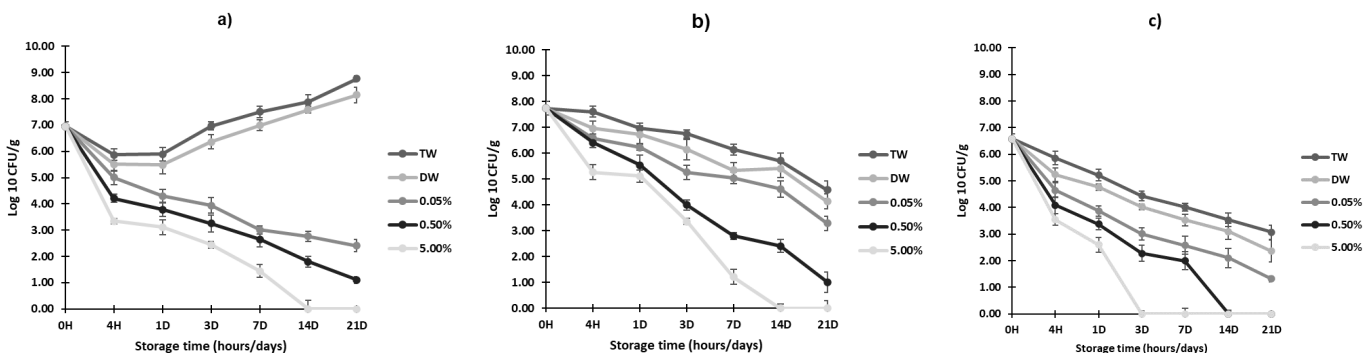


Figure 4. *S. enterica* ser. Typhimurium count upon storage of 21 days treated with different concentrations of extracts and conditions: (a) room temperature (b) chiller temperature (c) freezer temperature.

Paoli, 2011).

3.6.4 *Salmonella enterica* serovar Typhimurium

The effect of the *G. atroviridis* Griff extract was evaluated against *S. enterica* ser. Typhimurium up to 21 days of storage (Figure 4). Xylose Lysine Deoxycholate (XLD) agar was used for the enumeration of *S. enterica* ser. Typhimurium. Based on Figure 5, the shortest storage condition to completely obstruct the growth of *S. Typhimurium* was recorded when samples were treated with 5.00% extract concentration in the freezer while in the chiller, the obstruction was recorded at a slower growth. In the case of untreated samples in the chiller and freezer, the microbial count was reduced from 7.74 Log₁₀ CFU/g to 4.58 Log₁₀ CFU/g and 4.13 Log₁₀ CFU/g respectively and from 7.74 Log₁₀ CFU/g to 3.07 Log₁₀ CFU/g and 2.76 Log₁₀ CFU/g respectively. There was an overall bacterial growth reduction except for room temperature. The biochemical and chemical reaction stops at lower temperatures and the water activity is also restricted, hence the retardation in the growth of the micro-organisms.

On the contrary, the untreated samples at room temperature showed an increasing trend of growth and the highest concentration of growth was observed in tap water and the highest reduction was observed with 5.00% of fruit extract reducing the microbial growth from 6.95 to 0.00 Log₁₀ CFU/g. Hence proved that bacterial growth is directly proportional to the increasing concentration of the extract used. The higher the concentration of the extract, the lower the microbial growth and vice versa. The highest growth reduction of *S. enterica* ser. Typhimurium was observed when treated with a 5.00% extract concentration.

4. Conclusion

In conclusion, *G. atroviridis* Griff fruit extract was successfully extracted using ethanol as a solvent and the phytochemicals present in the fruit were retained. The results from antibacterial tests of the ethanolic *G. atroviridis* Griff fruit extract showed positive effectiveness against both Gram-positive (*S. aureus* ATCC 25923 and *L. monocytogenes* ATCC1 9112) and Gram-negative (*S. enterica* ser. Typhimurium ATCC 14028 and *Escherichia coli* ATCC 43895) bacteria. The extract was found to be stable at all temperatures and pH conditions based on the stability test conducted. The results showed that *G. atroviridis* Griff fruit extract has the potential to be developed as a natural food preservative since it could prolong the storage life and was stable under all different conditions. The application results of the bacterial enumeration in chicken resulted in a reduction of the bacterial count. Among all the

treatment groups, samples treated with 5.00% extract concentration showed a consistent reduction of bacterial count upon -18°C storage for 21 days.

Conflict of interest

The authors declared no conflict interest. The authors alone are responsible for the content of the paper.

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