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Effect of Brine Concentration on Bacteria Isolated from Leafy Vegetables (*Talinum triangulare, Telfairia occidentalis* and *Vernonia amygdalina*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

The effect of brine on bacteria isolated from leafy vegetables was evaluated. Fresh waterleaf (*Talinum triangulare*), Pumpkin leaves (*Telfairia occidentalis*) and Bitter leaves (*Vernonia amygdalina*) including cooking salt were bought from vendors in Mile 3 market, Port Harcourt, Rivers State. Brine concentrations of 1, 2, 3, 4 and 5% were prepared by dissolving appropriate grams of salt in distilled water. Nine millilitres of the respective concentrations were transferred into clean test tubes, labelled, stoppered with foil and autoclaved at 121℃ for 15 minutes at 15psi. Sterile distilled water served as a control. The test isolates were standardized based on 0.5McFarland and 1mL each was introduced into different brine concentrations. The standard plate count was used to monitor brine effects on isolates and this was done hourly for six hours.

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Inoculated plates were incubated at 37℃ for 24 hours. After incubation, enumerated colonies were used to deduce isolate mortality. The total heterotrophic bacterial (THB) load of bitter, Pumpkin and water leaves were $3.49\pm0.4\times10^6$, $3.25\pm0.4\times10^6$ and $1.99\pm0.2\times10^6$ CFU/g, respectively. The staphylococcal counts for bitter, Pumpkin and water leaves were $1.65\pm0.3\times10^4$, $3.13\pm0.5\times10^4$ and $1.55\pm0.4\times10^4$ CFU/g, respectively. Total coliform counts for bitter, Pumpkin and water leaves were $1.52\pm0.8\times10^5$, $2.85\pm0.1\times10^5$ and $1.75\pm0.6\times10^5$ CFU/g, respectively. Staphylococcal counts of pumpkin leaves were significantly ($P\leq0.05$) higher than those obtained for bitter leaf and water leaf. There was no significant difference($P>0.05$) in the THB and Coliform counts of all samples. *E. coli* was predominant in Pumpkin and water leaves while *Staphylococcus* sp was predominant in bitter leaves. The LC_{50} values for *E. coli, Klebsiella, Staphylococcus,* and *Bacillus* sp were; 5.39, 3.88, 1.62, and -0.41mg/ml, respectively. The LC_{50} showed that the brine was very lethal on *Bacillus* sp and *Staphylococcus* sp. High brine concentration is recommended to achieve reduced bacterial load.

Keywords: Brine; leafy vegetables; bacterial isolates.

1. INTRODUCTION

Due to continuous demands, lack of jobs and lifestyle changes over the past decades, vegetable farming activities have increased in urban and peri-urban areas, however, urbanization and increasing population size has led to the scarcity of land and water with most farmers having access to smaller land size for farming as observed in other West African countries [1]. In a bid to supply nutrients and water to farmlands, farmers site their farms close to various water sources such as pipes, wells, streams, and drains for irrigation [1]. More so, fertilizers of inorganic and organic origins (poultry and cow manure) are applied in the farms and the most used organic fertilizers in vegetable farms are those of poultry origin since they are very cheap and readily available [2]. Harvesting of leafy green vegetables into containers or sacks is done by hand with or without knives before they are transported to the market centres and other retail outlets under non-refrigerated conditions by market women or middlemen. Before these vegetables are presented for sale, they are most times washed with water to rid them of dirt [3].

Despite the nutritional importance of fresh vegetables, the danger of microbiological contaminants in vegetables is of public concern since it can serve as a route of microbial infection [4]; this worry is compounded by the way these vegetables are for the most part eaten raw (not cooked) and washing may not ensure the cleaning, so any occupant microorganism effectively enter the nutritious trench. Subsequently, these public concerns are justifiably informed based on detailed cases regarding various foodborne disease episodes brought about by the utilization of vegetables contaminated by microorganisms like *Listeria monocytogenes, Escherichia coli* O157:H7, and *Salmonella* spp [5,6]; (Maffei et al. 2013). Different evaluations of occurrences of foodborne disease flare-ups have brought about diseases, hospitalizations, death, and even food reviews in certain nations, especially the US [6-8]. In a previous study, it was reported that increased consumption of fresh leafy vegetables has been associated with an increasing number of foodborne outbreaks in the U.S., Canada, and European countries [9] and the majority of such outbreaks are caused by bacteria (*S. enterica* and *E. coli*) or viruses (Hepatitis A and Norwalk virus) which can be transmitted through the faecal-oral route [10,11]. Microbiological contamination of vegetables can occur directly or indirectly through (i) contact with soil, dust, or water, and (ii) punctures and open cuts of tissues of vegetables; thus, contaminations of vegetables may occur internally or externally during cultivation, harvest, packaging, storage, transporting and marketing [12].

Brining or salting is the oldest and cheapest way of preserving vegetables, meat, fish and other foods while maintaining a fair amount of their nutritional value [13]. Salt absorbs much of the water in the vegetables and makes it difficult for microorganisms to survive. In brine preservation of vegetables, the preserving effect is obtained by the combined action of the salt and the acid produced by fermentation. Four different ways of salting or brining vegetables have been identified by Fraser [13]: They comprise i) weak brine (5-15% by weight); ii) strong brine (15-25% by weight); iii) a weak brine (5-15% by weight) plus vinegar and iv) a strong brine (15-25% by weight) plus vinegar. Salt concentration has a significant

influence on controlling pathogen growth in foods and also plays a critical role to ensure food safety [14]. A study demonstrated that high salt concentrations result in higher osmotic pressures that alter metabolism in microorganisms thereby restricting their growth in fermented foods [15]. Therefore, low brine concentration has the potential to increase food spoilage rates and the presence of pathogens. Thus, this study was carried out to investigate the toxicity of brine concentrations on bacterial isolates of leafy vegetables.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Fresh leafy vegetables which include waterleaf (*Talinum triangulare*), Pumpkin leaves (*Telfairia occidentalis*) and Bitter leaves (*Vernonia amygdalina*) were bought from different vendors in Mile III market, Port Harcourt City Local Government Area, Rivers State. The samples were wrapped in foil and transported to the Microbiology Laboratory, at Rivers State University for immediate analysis. The salt used in this study was also bought from the Mile III market.

2.2 Processing of Samples

The samples were processed by transferring 10g of each leaf sample into a separate 250 ml conical flask containing 90 ml sterile normal saline. The flasks were swirled gently to dislodge the microflora in the leaves (Amadi et al. 2014). After which, a ten-fold serial dilution was carried out by transferring 1ml from the original stock (10^{-1}) to test tubes containing 9ml sterile saline. This was repeated serially to obtain 10^{-6} dilution.

2.3 Isolation of Total Heterotrophic Bacteria

The total heterotrophic bacterial load of the leave was determined by transferring aliquots (0.1 ml) of 10^{-3} and 10^{-4} dilutions using a sterile 1mL pipette into freshly prepared pre-dried nutrient agar (NA) plates in duplicates. The plates were evenly spread with a flame-sterilized bent glass rod, inverted and incubated at 37 ℃ for 24 hours. After incubation, distinct colonies on the plates were subcultured on pre-dried NA plates by picking the colony with a flamed inoculating loop. Plates were later incubated at 37 ℃ for 24 hours.

2.4 Isolation of Total Coliform

The total coliform and faecal coliform loads of the leaves were determined by transferring aliquot (0.1 ml) of 10^{-1} and 10^{-2} dilutions into freshly prepared pre-dried Eosin Methylene Blue (EMB) agar plates in duplicates with the aid of a sterile 1mL pipette. The plates were evenly spread with a bent glass rod, inverted and incubated at 37℃ for 24 hours for total coliform while the faecal coliform plates were incubated at 44℃ for 24 hours [16]. After incubation, distinct colonies on the plates were subcultured on pre-dried NA plates by picking the colony with a flamed inoculating loop. Plates were later incubated at 37 ℃ for 24 hours.

2.5 Isolation of Total *Staphylococcus*

The total staphylococci in the leaves were determined by transferring aliquot (0.1 ml) of 10^{-2} dilution using a pipette into freshly prepared pre-dried Mannitol Salt Agar (MSA) plates in duplicates. The plates were evenly spread with a flame-sterilized bent glass rod, inverted and incubated at 37 ℃ for 24 hours [16]. After incubation, distinct colonies on the plates were subcultured on pre-dried NA plates by picking the colony with a flamed inoculating loop. Plates were later incubated at 37 ℃ for 24 hours.

2.6 Identification of Bacterial Isolates

The bacterial isolates were identified based on their morphology and biochemical characteristics. Morphological characteristics employed were colonial morphology (colour, shape, size and texture of the colonies) and microscopic appearance (which include the gram reaction, cell shape and arrangements of the cells) under an oil emersion light microscope. Biochemical tests adopted include; catalase test, citrate utilization, oxidase, Methyl-Red, Voges Proskauer, coagulase, indole and sugar fermentation tests.

2.7 Preparation of Brine Concentration

The brine concentrations used were 1, 2, 3, 4 and 5%. The brine concentration was prepared according to the methods [17] with slight modifications. This was done by dissolving 1 g, 2 g, 3 g, 4 g and 5g of normal cooking salt into well labelled 100ml conical flasks containing 9, 8, 7, 6 and 5ml distilled water, respectively [18]. After which, 9 ml of the respective concentrations were

transferred into clean test tubes. Test tubes were well labelled according to the concentration of brine they contained and were stoppered with cotton wool. These were later sterilized by autoclaving at 121℃ for 15 minutes at 15psi. After sterilization, test tubes were arranged in racks according to their concentrations and used to test the various isolates. The control which is the 0% had no salt but only sterile distilled water.

2.8 Preparation and Determination of Inoculum Size

A colony of twenty-four hours old cultures was transferred into 9ml sterile peptone broth. This was later incubated at 37℃ for eighteen hours. After incubation, 10-fold serial dilution was carried out by transferring 1ml from the eighteen hours old broth culture into test tubes containing sterile 9ml peptone broth. The serial dilution was carried out until the dilution of 10^{-6} was obtained. This was done for all the isolates. The different dilutions were compared with the 0.5 McFarland standard and aliquots from dilutions that matched the 0.5McFarland standard were transferred into pre-dried nutrient agar plates. This was spread evenly using a sterile bent glass rod, and incubated at 37℃ for 24 hours. This was done to determine the size of the initial inoculum.

2.9 Effect of Brine Concentration on Isolates

The effect of different brine concentrations on the isolates was determined by the plate count method [18]. In this method, 1mL of each of the standardized bacterial inoculum was transferred into the different brine concentrations and incubated. The effect of the brine concentration on the test isolates was monitored hourly for six hours by inoculating aliquots from the set-up into pre-dried nutrient agar plates in duplicates. Inoculated plates were evenly spread using a sterile bent glass rod and were incubated for 24 hours at 37℃. Counts from plates that showed growth were recorded and used in determining the % survival, %mortality and lethal concentration of brine on the isolate.

The formula used for the calculation were those adopted by Williams and Dilosi [18] which state as follows;

$$LC_{50} = LC_{100} - \frac{\sum \text{Concentration Difference} \times \%\text{Mean Mortality}}{\%\ Control} \quad \text{(Eqn. 1)}$$

$$\%\ \text{Survival} = \frac{\text{LogConcentration of Toxicants} \times 100}{Log\ concentration\ of\ control} \quad \text{(Eqn. 2)}$$

$$\%\ \text{Mortality} = 100 - \%\log survival \quad \text{(Eqn. 3)}$$

2.10 Statistical Analysis

The bacterial counts obtained were spread on an Excel worksheet (Microsoft Excel 2016). The mean and standard deviations, % survival and mortality were all calculated using SPSS (v25). The Analysis of variance was carried out on the means and where there were significant differences, the Duncan Multiple Range test was employed to separate the means.

3. RESULTS

The results for the total heterotrophic bacterial counts (THB), staphylococcal counts (SC), and coliform counts (TCC) of the vegetables are presented in Table 1. The results showed that the THB of bitter leaves, Pumpkin leaves and water leaves were $3.49\pm0.4\times10^6$, $3.25\pm0.4\times10^6$ and $1.99\pm0.2\times10^6$ CFU/g, respectively. Results for the staphylococcal counts for bitter, Pumpkin and water leaves were $1.65\pm0.3\times10^4$, $3.13\pm0.5\times10^4$ and $1.55\pm0.4\times10^4$ CFU/g, respectively. The total coliform counts for bitter leaves, Pumpkin leaves and water leaves were $1.52\pm0.8\times10^5$, $2.85\pm0.1\times10^5$ and $1.75\pm0.6\times10^5$ CFU/g.

The cultural characteristics of the bacterial isolates showed that twenty-eight bacterial isolates belonging to *Bacillus, Staphylococcus, E. coli, Klebsiella, Proteus* and *Enterobacter* sp were isolated from the vegetables. The distribution of the bacterial isolates showed that they were not evenly distributed across the vegetable samples. That is, not all the isolates were isolated from a particular vegetable. This is illustrated in Fig. 1. For instance, *Proteus* sp which was not isolated from Pumpkin and Bitter leaves was isolated from water leaves. Also, *Klebsiella* sp was isolated in both water leaf and Pumpkin leaves but was not isolated in bitter leaves. However, *Bacillus* sp, *Staphylococcus* sp and *E. coli* were all isolated from the vegetables. The percentage occurrence showed that *E. coli* was the most dominant bacterial isolate in water leaves and Pumpkin leaves while *Staphylococcus* sp was the most dominant bacterial isolate in bitter leaves. The percentage

occurrence of bacterial isolates in Pumpkin leaves was: *Bacillus* sp (28%), *Staphylococcus* sp (8%), *E. coli* (40%), *Klebsiella* sp (12%), *Proteus* sp (4%) and *Enterobacter* sp (8%). The percentage frequency of occurrence of bacterial isolates in water leaves is *Bacillus* sp (29.4%), *Staphylococcus* sp (23.5%), *E. coli* (35.3%), *Klebsiella* sp (5.9%), *Proteus* sp (0%) and *Enterobacter* sp (5.9%). While the percentage frequency of occurrence of bacterial isolates in bitter leaves is: *Bacillus* sp (30%), *Staphylococcus* sp (50%), *E. coli* (10%), *Klebsiella* sp (0%), *Proteus* sp (0%) and *Enterobacter* sp (10%).

The result of the percentage survival and mortality of brine concentrations at different hours for *E. coli, Klebsiella* sp, *Staphylococcus* sp and *Bacillus* sp is presented in Tables 2, 3, 4, and 5, respectively. In Table 2, the results showed that there was no mortality of *E. coli* at 0 to 4% brine concentration in the first hour. This was also seen in the 0 to 1% brine concentration after the one-hour interval while mortality was recorded in 2 to 5% brine concentrations after the one-hour interval. More so, it was observed that *E. coli* thrived in 1% salt concentrations in all the hours (i.e., from 1-6 hours) but declined in population as the concentration increased as well as extended time. The *Klebsiella* isolates, like the *E. coli,* showed similar responses to the brine concentrations in the first hour for the 0-3% concentrations in which there was no cell death but an increased population (Table 3). The highest mortality of 31.1 was recorded in the 4% concentration at the 5th-hour mark. The response of *Staphylococcus* sp to the different brine concentrations showed that at 1% concentration from the first hour to the 5th hour, there was a decline in the population (Table 4). The highest mortality was observed in the 3% brine concentration in the 4th hour. The effect of brine on *Bacillus* sp as illustrated in Table 5 showed that the *Bacillus* isolates increased in population from what was recorded in the 0% concentration in the first hour for the 1-5% concentrations.

Table 1. Variation of microbial population in the various leafy vegetables examined

Samples	THB (×10^6)	SC (×10^4)	TCC (×10^5)
Bitter leaf	3.49±0.4 ^{a}	1.65±0.3^a	1.52±0.8 ^{a}
Pumpkin leaf	3.25±0.4 ^{a}	3.13±0.5^b	2.85±0.1 ^{a}
Water leaf	1.99±0.2 ^{a}	1.55±0.4^a	1.75±0.6 ^{a}

**Means with same superscript are not significantly different (P> 0.05)*
Key: THB: total heterotrophic bacteria; SC: staphylococci count; TCC: total coliform counts

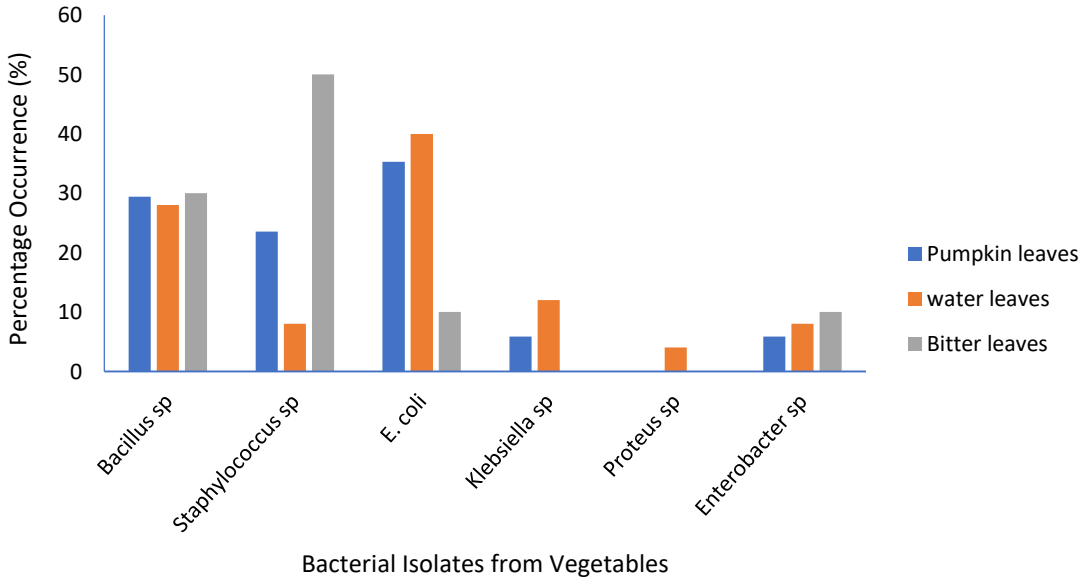

Fig. 1. Percentage occurrence of bacterial Isolates from the vegetables

The effects of brine on the different bacterial isolates after six hours are presented in Table 6. The results showed that all the different brine concentrations had an impact on the growth of the bacterial isolates. The result showed that the response/ mortality of the bacterial isolates to the different concentrations varied with increased brine concentration. Statistically, there was no significant difference (P≥0.05) in the brine concentration on the isolates at 0, 1, and 2%. While at 3, 4, and 5%, there was a significant difference (P≤0.05) in the response of the bacterial isolates. The mortality rate of *Bacillus* sp to the different brine concentrations is given as 100.0±0.00, 93.58±23.93, 81.80±26.24, 69.26±23.80, 75.40±25.41 and 77.37±20.92 CFU/ml for 0, 1, 2, 3, 4, and 5%, respectively. The mortality of *E. coli* to the brine concentration is given as 100.00±0.00, 105.92±2.23, 101.58±04.69, 100.97±7.75, 101.15±17.11 and 96.50±15.85CFU/ml for 0, 1, 2, 3, 4, and 5%, respectively. The response of *Klebsiella* sp to the brine concentration is given as 100.0± 0.00, 102.28±8.18, 95.28±7.02, 92.37±18.41, 82.51±10.19 and 105.31±13.34 CFU/ml for 0, 1, 2, 3, 4, and 5%, respectively. The response of *Staphylococcus* sp to the brine concentration is given as 100.0±0.00, 95.97±12.68, 86.32± 20.50, 80.12±13.86, 85.30±6.77 and 84.65± 19.27 CFU/ml for 0, 1, 2, 3, 4, and 5%, respectively.

The result of the LC50 is presented in Table 7. The result showed that the LC50 values were not very high. The LC50 values for *E. coli, Klebsiella, Staphylococcus,* and *Bacillus* sp were recorded as 5.39, 3.88, 1.62, and -0.41mg/ml, respectively.

Table 2. Percentage of Survival and Mortality of Brine on *E. coli*

Time (Hour)		Concentrations (%)					
		0	**1**	**2**	**3**	**4**	**5**
1	%S	100	106.5	108.9	102.1	104.1	92.4
	%M	0	-6.5	-8.9	-2.1	-4.1	7.6
2	%S	100	104.1	97.5	94.1	97.1	87.3
	%M	0	-4.1	2.5	5.9	2.9	12.7
3	%S	100	108.4	105.1	94.1	94.3	97.5
	%M	0	-8.4	-5.1	5.9	5.7	2.5
4	%S	100	105.5	101.8	96.2	133.0	127.7
	%M	0	-5.1	-1.8	3.8	-33	-27.7
5	%S	100	102.8	96.9	105.7	82.4	88.1
	%M	0	-2.8	3.1	-5.7	17.6	11.9
6	%S	100	108.2	99.3	113.6	96.0	86.0
	%M	0	-8.2	0.7	-13.6	4	14.0

Keys: % S: percentage survival; % M: percentage mortality

Table 3. Percentage survival and mortality of brine on *Klebsiella* sp

Time (Hour)		Concentrations (%)					
		0	**1**	**2**	**3**	**4**	**5**
1	%S	100	110.7	101.4	102.1	95.5	95.9
	%M	0	-10.7	-1.4	-2.1	4.5	4.1
2	%S	100	110.5	92.9	101.2	91.7	100.8
	%M	0	-10.5	7.1	-1.2	8.3	-0.8
3	%S	100	98.2	91.7	119.3	85.7	85.7
	%M	0	1.8	8.3	-19.3	14.3	14.3
4	%S	100	102.5	95.4	82.8	75.8	112.8
	%M	0	-2.5	4.6	17.2	24.2	-12.8
5	%S	100	102.9	104.9	68.9	68.9	117.8
	%M	0	-2.9	-4.9	31.1	31.1	-17.8
6	%S	100	88.9	85.4	79.9	77.5	118.9
	%M	0	11.1	14.6	20.1	22.5	-18.9

Keys: % S: percentage survival; % M: percentage mortality

Table 4. Percentage survival and mortality of brine on *Staphylococcus* sp.

Time (Hour)		Concentrations (%)					
		0	1	2	3	4	5
1	%S	100	98.4	124.3	92.8	83.6	67.5
	%M	0	1.6	-24	7.2	16.4	32.5
2	%S	100	85.0	86.7	87.5	77.7	67.5
	%M	0	15	13.3	12.5	22.3	32.5
3	%S	100	90.8	85.5	78.4	89.9	87.5
	%M	0	9.2	14.5	21.6	10.1	12.5
4	%S	100	91.7	68.9	54.1	81.5	87.5
	%M	0	8.3	31.1	45.9	18.5	12.5
5	%S	100	89.6	67.5	79.7	82.6	78.4
	%M	0	10.4	32.5	20.3	17.4	21.6
6	%S	100	120.3	85.0	88.2	96.5	119.5
	%M	0	-20.3	15	11.8	3.5	-19.5

Keys: % S: percentage survival; % M: percentage mortality

Table 5. Percentage survival and mortality of brine on *Bacillus* sp.

Time (Hour)		Concentrations (%)					
		0	1	2	3	4	5
1	%S	100	113.9	111.4	114.6	116.6	116.6
	%M	0	-13.9	-11.4	-14.6	-16.6	-16.6
2	%S	100	112.1	115.2	68.0	96.0	70.8
	%M	0	-12.1	-15.2	32	4	29.2
3	%S	100	71.4	71.4	45.0	64.5	68.8
	%M	0	28.6	28.6	55	35.5	31.2
4	%S	100	56.3	48.8	59.59	56.3	58.0
	%M	0	43.7	51.2	40.4	43.7	42
5	%S	100	101.5	77.6	59.59	51.8	83.6
	%M	0	-1.5	22.4	40.4	48.2	16.4
6	%S	100	106.3	66.4	68.8	67.2	66.4
	%M	0	-6.3	33.6	31.2	32.8	33.6

Keys: % S: percentage survival; % M: percentage mortality

4. DISCUSSION

Vegetables play a remarkable role in human nutrition and health since they supply dietary fibre, phytochemicals, vitamins, and minerals [19]. The effects of different concentration of brine on bacterial isolates from leafy vegetables were investigated. The microbial result showed that the bitter leaves had the highest total heterotrophic bacterial counts while the Pumpkin leaf had the highest staphylococcal and coliform counts. Waterleaf had the least total heterotrophic bacterial counts and staphylococcal counts. However, it had the second highest counts which were higher than those recorded for bitter leaf. Despite the variations in the counts, there were no significant differences ($P > 0.05$) in the total heterotrophic bacterial counts and total coliform counts of the various leaves. There was a significant difference ($P < 0.05$) in the staphylococcal counts as the counts in the Pumpkin leaves were higher than those recorded for the bitter leaves and water leaves which were not significantly different from each other. The total heterotrophic bacterial counts in all leafy vegetables were more than the counts recorded for *Staphylococcus* and coliforms. This observation could be attributed to the inhibitory effects in the special medium used in cultivating *Staphylococcus* sp, and coliforms while the medium used in enumerating the heterotrophic bacteria in the leafy vegetables is non-selective. Thus, it allows for the growth of diverse microbial genera. This is well documented (Tom et al. 2011); Prescott et al. [16]. The high coliform counts recorded in the Pumpkin and water leaves could be attributed to the use of poultry droppings in treating the soils to enhance the fertility of the soil thereby increasing high yield. The total heterotrophic bacterial counts in this current study are lower than the range of 8.30 to 9.20 log CFU/g reported by Quansah et al. [3] of leafy vegetables grown and sold in Accra. The microbial loads of leafy vegetables in this current study are generally high and agreed with previous work [20,3,21].

Table 6. The mean percentage survival (%) of bacterial isolates after six hours

	Brine Concentrations					
Isolates	**0%**	**1%**	**2%**	**3%**	**4%**	**5%**
Bacillus	100.0±0.00[a]	93.58±23.93[a]	81.80±26.24[a]	69.26±23.80[a]	75.40±25.41[a]	77.37±20.92[a]
E. coli	100.00±0.00[a]	105.92±2.23[a]	101.58±04.69[a]	100.97±7.75[b]	101.15±17.11[b]	96.50±15.85[ab]
Klebsiella sp	100.0±0.00[a]	102.28±8.18[a]	95.28±7.02[a]	92.37±18.41[b]	82.51±10.19[ab]	105.31±13.34[ab]
Staphylococcus sp	100.0±0.00[a]	95.97±12.68[a]	86.32±20.50[a]	80.12±13.86[ab]	85.30±6.77[ab]	84.65±19.27[b]

Table 7. Lethal concentration (LC_{50}) of brine on the isolates

Isolates	LC_{50} (%)
E. coli	5.39
*Klebsiella*spp	3.88
Staphylococcus spp	1.62
Bacillus spp	-0.41

The presence of these bacterial contaminants on leafy vegetables could be due to contamination from the environment or from the field and handling processes of sellers. More so, the presence of *E. coli* in leafy vegetables could as a result of contamination with faecal material from animals. *E. coli* is known as indicator organisms and their presence is an indication of faecal contamination [16]. Yafetto et al. [21] in their study isolated *Enterobacter* spp., *Escherichia coli, Klebsiella* spp., *Serratia marcescens,* and *Staphylococcus* sp. Thus, except for *Serratia marcescens* which was not isolated in this current study, all other isolates were similar to our findings. Furthermore, the bacterial isolates from this study have been reported by previous studies [22,3]. Unsanitary postharvest practices, unhygienic postharvest handling practices, and improper storage conditions have been reported to be the major cause of poor microbial quality [3]. Vegetables transported to the markets are stored under non-refrigeration temperatures in sacks or boxes kept in rooms or the open at the market centres. Some vegetables are washed to remove dirt before being displayed at the market for sale using water which is not changed regularly. This practice is known to promote cross-contamination and microbial growth in vegetables [3].

The effect of the brine concentration on the bacterial isolates after six hours showed that the concentration changes influenced the growth of the isolates. The bacterial load decreased as the concentration increased but fluctuated with respect to concentration. This was observed in all isolates. For *E. coli* sp and *Staphylococcus* sp, the populations declined with increased brine concentration while the population of *Bacillus* sp and *Klebsiella* sp which declined gradually to the increased concentration increased at 5% brine concentration. More so, none of the brine concentrations completely inhibited the growth of the bacterial isolates. This agreed with Omotoyinbo [23] who also reported that higher NaCl concentration affected the optimal growth of *S. aureus* and *E. coli.* The growth of bacteria and the increase in population in an environment with sufficient nutrients and the decline in population due to limited nutrient or inhibitory substances in the environment is well understood [17]. In this study, *Bacillus* sp, *E. coli, Klebsiella* sp, and *Staphylococcus* sp at 1 hour at 1% were multiplying and this could be that the growth environment was still suitable for microbial proliferation. As the concentration increased and the incubation period of cells was extended to 6 hours, shock-related stress caused by the brine concentrations could have caused an accumulation of waste metabolites and toxins in the cultures which could have resulted in morphological damage and plausible death of cells. This agreed with Hajmeer et al. [17] who observed the effects of brine on *E. coli* and *S. aureus* for six and twelve hours. The decrease in bacterial growth as a result of increasing salt concentrations was also reported by Abdulkarim et al. (2009), who further suggested that the observed effect could be due to the hyperosmotic effect on the bacteria which led to osmotic shock on the organisms thereby causing growth suppression. The LC_{50} showed that the brine concentration has a bactericidal effect on *Bacillus* sp and *Staphylococcus* sp which had the least LC_{50} values, and therefore, means that the brine was more effective on *Bacillus* and *Staphylococcus* sp. Thus, this finding contradicts previous reports which have shown that *Bacillus* and *Staphylococcus* sp could tolerate higher salt concentrations [16].

5. CONCLUSION

As the demand for vegetables increases, techniques in agricultural practices have improved to meet high demands. Many subsistence farmers use cheap substances to enhance soil fertility thereby increasing the yield of their crops. Such acts result in the contamination of the crops with faecal matter which could cause spoilage and diseases [24,25].

The different brine concentration on the bacterial isolates despite having intermediate inhibitory effects was not enough to completely inhibit the bacterial growth and multiplication.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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