ABSTRACT
Shell fishes constitute a vital source of food for humans due to its high nutritional values. Bacteriological and nutritional assessments of Galatea paradoxa treated with Citrus aurantifolia and NaCl were determined using bacteriological and analytical protocols. The results revealed a reduction from 4.945 to 2.301 Log CFU/g in Total Heterotrophic Bacterial Counts (THBC) in G. paradoxa treated with 10% NaCl for 5 mins. The G. paradoxa treated with 7.5% NaCl for 5 min had a reduction in Total Coliform Counts (TCC) ranging from 3.903 to 2.398 Log CFU/g, while Total Faecal Coliform Counts (TFC) in G. paradoxa treated with 5% and 10% for 10 min reduced by 99.99%. There was 53.46% THBC reduction in G. paradoxa treated with 10% C. aurantifolia for 5 min; THBC in G. paradoxa treated with 10% C. aurantifolia for 10 min reduced by 79.36%; THBC in G. paradoxa treated with 10% equal concentrations of NaCl and C. aurantifolia decreased by 99.99% within 10 min, while TCC in G. paradoxa treated with 7.5% equal concentrations of NaCl and C. aurantifolia within 10 min of exposure had 99.99% decrease. The predominant survived bacterial genera in treated samples were Bacillus, Vibrio and Micrococcus. There was insignificant difference (p ≥ 0.05) between the nutritional compositions of treated and untreated samples. This study showed that G. paradoxa could be treated with C. aurantifolia and NaCl so as to avert possible foodborne diseases associated with consumption of this aquatic food.

Keywords: Bacteriological, Citrus aurantifolia, NaCl, Galatea paradoxa, Nutritional.

INTRODUCTION
An increase in population worldwide has resulted in a substantial increase in consumption of aquatic food such as Galatea paradoxa (Sanya and Mohammed, 2006). Galatea paradoxa (Born 1778) are freshwater clams, bivalve and filter feeding mollusc that belong to order ‘Veneroidae’, superfamily ‘Tellinoidea’ and family ‘Donacidae’ (Moses, 1990). These aquatic animals, without vertebral column, have two hinged calcareous shells that aid its protection and are endemic in West African countries such as Ghana, Nigeria and Cameroun (Etim and Brey, 1994; Villalobs and Elguezabel, 2001). The high nutritional values of shellfishes have triggered its increased consumption (Ekanem and Adegoke, 1995; Simopoulos, 2003). Galatea paradoxa constitute a vital source of food for humans due to its high protein content, low cholesterol content, significant amounts of omega-3-fatty acids (Ekpenyong et al., 2013), vitamins, iron, potassium, zinc, copper, manganese and selenium (Davies and Jamabo, 2016).

The G. paradoxa are a suitable bio-indicator of environmental pollution (Chiesa et al., 2018; Okon et al., 2020). They can accumulate human pathogenic organisms from sewage contaminated waters and also accumulate toxins in its soft tissues through feeding on toxic phytoplankton (Gram et al., 2002). The ingestion of contaminated soft tissues of G. paradoxa by humans may result in food-borne related diseases (Hathal et al., 2005). The soft tissue of G. paradoxa is consumed after frying, smoking, roasting, steaming or cooking (King, 2000; Villalobs and Elguezabel, 2001) and G. paradoxa also serves as a means of livelihood some dwellers in parts of Southern Nigeria.

Sodium chloride (NaCl) is one of the most extensively used food additives as a preservative, enhancing the flavour and enzymatic activities responsible for organoleptic parameters (Cheng et al., 2003; Silva et al., 2003), and improving water adsorption which aids in inhibiting growth of spoilage and pathogenic organisms (Lawrence et al., 2003; Man, 2007). The reduction of water activity due to addition of salt and presence of ions exerts osmotic pressure effects on the microorganisms, thus, increase the shelf life of the processed food (Anbalagan et al., 2014). The inadequacy of NaCl as a sole preservative in ready-to-eat and other food products has necessitated its combination with other preservation processes such as addition of chemicals, drying and osmotic dehydration.

The Food Safety and Inspection Service of the United States Department of Agriculture has approved the use of lime (Citrus aurantifolia) juice as a natural antimicrobial agent having been recognized as safe for its application in food (Skrivanova et al., 2011). The juice of C. aurantifolia has been reported to reduce the growth of some bacteria in family Enterobacteriaceae and its antibacterial activities has attributed to its low pH that can penetrate the bacterial membranes (Davidson and Taylor, 2007; Bradley et al., 2011). However, there is a need to evaluate natural occurring organic compound that can be applied as post-harvest treatment to G. paradoxa. Consequently, this study determined the effect of citrus aurantifolia and NaCl singly and in combination on bacterial loads and nutritional quality of G. paradoxa.
MATERIALS AND METHODS

Collection of Samples
Freshly harvested *G. paradoxa* were obtained from two major markets (Itam and Akpan Andem markets) in Uyo using sterile wide-mouth plastic containers and were transported to the Microbiology Laboratory, University of Uyo. The *G. paradoxa* was identified and confirmed by a Fish Taxonomist in the Department of Fisheries and Aquaculture, University of Uyo. *Galatea paradoxa* were extensively washed with sterile distilled water and rinsed with normal saline to remove all extraneous materials before shocking. The edible part was aseptically removed as described by APHA (1998) and was transferred into sterile containers for bacteriological and nutritional analyses.

Bacteriological Analysis of Samples
Ten (10) grams of each fleshy blended parts of *G. paradoxa* was aseptically suspended into 90 ml sterile distilled water, vigorously shaken to dislodge adhered bacteria and ten-fold serial dilutions were made to obtain dilutions 10⁻¹ to 10⁻³. One (1) ml of aliquot was pour-plated in triplicate onto each plate of Nutrient Agar (NA), MacConkey Agar (MCA), Eosine Methylene Blue Agar (EMB) and the plates were aerobically incubated at 37°C for 24 hr. After incubation, colonies on plates were counted and multiplied by the dilution to obtain the Total Heterotrophic Bacterial Counts (THBC), Total Coliform Counts (TCC) and Total Faecal Coliform Counts (TFC), respectively. The discrete colonies were sub-cultured onto freshly prepared NA plates and aerobically incubated at 37°C for 24 hr. The pure cultures of isolates were streaked onto NA slant, incubated at 37°C and stored in a refrigerator at 4 °C for characterization and identification. All isolates were Gram stained and subjected to convectional biochemical tests (Holt et al., 1994).

Effect of *C. aurantifolia* and NaCl on the Bacterial Loads of *G. paradoxa*

The *C. aurantifolia* juice was extracted using the method of Ndelekwute and Enyenihi (2017). Fleshy part of *G. paradoxa* was suspended into sterile conical flasks containing varied concentrations (2.5 %, 5.0 %, 7.5 % and 10 %) of NaCl and *C. aurantifolia*, respectively. The contents of sterile conical flasks were allowed to stand for 5 and 10 min, respectively. Thereafter, 10g of each fleshy part was blended, separately suspended into 90 ml sterile distilled water, vigorously shaken to dislodge adhered bacteria and dilutions were made to obtain 10⁻¹ and 10⁻³. One (1) ml of the aliquot was pour-plated in triplicate onto each plate of NA, MCA, EMB and incubated aerobically at 37 °C for 24 hr. The same procedure was carried out for combination of NaCl and *C. aurantifolia* at ratio of 1:1 (vol/vol). The same procedures were carried on control (*G. paradoxa* untreated with NaCl and *C. aurantifolia*). After incubation, the bacterial counts were recorded and mean, standard deviations were appropriately calculated.

Proximate Analysis of *G. paradoxa* treated with NaCl and *Citrus aurantifolia*

The moisture, lipid and ash contents of fleshy part of *G. paradoxa* samples were carried out using the methods of AOAC (2005). The fibre and protein contents were obtained by Kjeldahl’s procedure and subsequently converted to crude protein by multiplying the values obtained with a protein conversion factor of 6.25. The energy content was calculated as follows: Energy Kcal 100g = (crude lipid x 8) + (crude protein x 2) + (CHO x 4), where CHO was carbohydrate contents of *G. paradoxa*. All determinations were done in triplicates and values obtained were expressed as mean ± standard deviation.

Statistical Analysis

The Statistical Package for Social Sciences (IBM SPSS Version 22.0) was used for data analysis. The significant difference (p ≤ 0.05) between the nutritional compositions of *G. paradoxa* treated with NaCl / *Citrus aurantifolia* and the untreated *G. paradoxa* were determined using one-way Analysis of Variance (ANOVA).

RESULTS

The logarithmic and percentage reductions of bacterial loads in *G. paradoxa* treated with NaCl are presented in Table 1. The results revealed a THBC reduction in *G. paradoxa* treated with 10% NaCl for 5 mins from 4.845 to 2.301 Log CFU/g, while THBC in *G. paradoxa* samples treated with 10% NaCl for 5 mins decreased from 4.845 to 1.146 Log CFU/g. The *G. paradoxa* treated with 7.5 % NaCl for 5 min had a TCC reduction ranging from 3.903 to 2.398 Log CFU/g; *G. paradoxa* treated with 7.5 % at 10 min had a reduction in TCC (3.903 to 1.041 Log CFU/g), while TFC in *G. paradoxa* treated with 5 % and 10 % for 10 min reduced by 99.99 % (Table 1).

The logarithmic and percentage reductions of bacterial loads in *G. paradoxa* treated with *C. aurantifolia* are presented in Table 2. The

---

**Fig 1: G. paradoxa**
results showed 53.46% THBC reduction in *G. paradoxa* treated with 10% *C. aurantifolia* for 5 min, while THBC in *G. paradoxa* treated with 10% *C. aurantifolia* for 10 min reduced by 79.36%. *G. paradoxa* treated with 7.5% *C. aurantifolia* for both 5 min and 10 min did not have TCC, respectively; while *G. paradoxa* treated with 7.5% *C. aurantifolia* for 5 min had no TFC (Table 2).

The logarithmic and percentage reductions of bacterial loads in *G. paradoxa* treated with combination of NaCl and *C. aurantifolia* are presented in Table 3. The THBC in *G. paradoxa* sample treated with equal concentrations (10%) of NaCl and *C. aurantifolia* decreased by 99.99% within 10 min of exposure, while TCC and TFC in *G. paradoxa* treated with equal concentrations (vol/vol) of 7.5% NaCl and *C. aurantifolia* within 10 min of exposure had 99.99% decrease (Table 3).

The trends of occurrence of bacterial isolates from *G. paradoxa* treated with NaCl and *C. aurantifolia* singly and in combination are shown in Table 4. The results revealed that *B. subtilis*, *S. aureus*, *M. varians*, *E. coli*, *K. pneumoniae*, *V. choleracea* and *P. aeruginosa* survived in *G. paradoxa* treated with 5% of NaCl or *C. aurantifolia* within 5 min of exposure. Although, *B. subtilis* were predominant in all the treated samples, but combination of 10% equal volume (vol/vol) of *C. aurantifolia* and NaCl killed the bacterial isolate.

The comparative analyses of proximate compositions of *G. paradoxa* treated with NaCl and *C. aurantifolia* singly and in combination are presented in Table 5. The highest moisture content of 67 ± 1% was obtained from sample C4 (control), followed by sample C5 (*G. paradoxa* treated with 10% *C. aurantifolia*) with 62 ± 1%, while the lowest moisture content of 50 ± 2% was obtained from sample C2 (*G. paradoxa* treated with equal volume of 10% NaCl and 10% *C. aurantifolia*). The ash content was highest in sample C5 with 3.950 ± 0.01% and lowest in sample C2, with 7.5 ± 0.03%. Samples C2 and C4 had the lowest fibre (0.023 ± 0.02%) and protein (32.10 ± 0.25%) content respectively, while the highest fibre (0.045 ± 0.01%) and protein (33.229 ± 0.10%) content was obtained in sample C4. The crude lipid was highest in sample C5 with 1.360 ± 0.02% and was lowest in sample C4 (1.290 ± 0.001%). The total carbohydrate was highest in sample C5 (62.700 ± 0.11%) and lowest in sample C4 (58.606 ± 0.20%). There was no statistically significant difference (p > 0.05) between the nutritional compositions of *G. paradoxa* treated with NaCl / *C. aurantifolia* and the untreated *G. paradoxa* (Table 5).

### Table 1. Logarithmic and Percentage Reductions of Bacterial Loads in *Galatea paradoxa* Treated with NaCl

| Exposure (mins) | Time | Microbial Group | Concentration (%) | Plate Counts (CFU/g) | Log Reduction (%) | Log reduction |
|----------------|------|-----------------|-------------------|----------------------|-------------------|---------------|
| 5              | 0    | THBC            | 0                 | 7.0 ± 0.4 x 10^6    | 4.845             | NA            |
| 2.5            | 2.5  | THBC            | 6.4 ± 0.7 x 10^4  | 4.806                | 0.80              | 0.04          |
| 5              | 5.0  | THBC            | 4.5 ± 0.1 x 10^3  | 3.653                | 24.60             | 1.19          |
| 7.5            | 7.5  | THBC            | 3.0 ± 0.7 x 10^2  | 2.447                | 48.88             | 2.37          |
| 10             | 10   | THBC            | 2.0 ± 0.3 x 10^2  | 2.301                | 52.51             | 2.54          |
| 7              | 7    | TCC             | 0                 | 8.0 ± 0.1 x 10^3    | 3.903             | NA            |
| 2.5            | 2.5  | TCC             | 3.9 ± 0.3 x 10^3  | 3.591                | 7.994             | 0.31          |
| 5              | 5.0  | TCC             | 5.6 ± 0.8 x 10^2  | 2.748                | 29.59             | 1.16          |
| 7.5            | 7.5  | TCC             | 2.5 ± 0.5 x 10^2  | 2.398                | 38.66             | 1.51          |
| 10             | 10   | TCC             | 1.5 ± 0.4 x 10^2  | 1.176                | 52.52             | 1.30          |
| 5              | 5    | TFC             | 0                 | 3.0 ± 0.7 x 10^4    | 2.477             | NA            |
| 2.5            | 2.5  | TFC             | 2.1 ± 0.2 x 10^2  | 2.322                | 6.258             | 0.16          |
| 5              | 5.0  | TFC             | 1.5 ± 0.4 x 10^2  | 1.176                | 52.52             | 1.30          |
| 7.5            | 7.5  | TFC             | NG                | NA                   | ≥ 99.99           | 2.48          |
| 10             | 10   | TFC             | NG                | NA                   | ≥ 99.99           | 2.48          |
| 10             | 10   | THBC            | 0                 | 7.0 ± 0.4 x 10^6    | 4.845             | NA            |
| 2.5            | 2.5  | THBC            | 5.2 ± 0.4 x 10^6  | 4.716                | 2.663             | 0.13          |
| 5              | 5.0  | THBC            | 3.9 ± 0.3 x 10^2  | 3.591                | 25.88             | 1.25          |
| 7.5            | 7.5  | THBC            | 2.1 ± 0.2 x 10^2  | 2.322                | 52.07             | 2.52          |
| 10             | 10   | THBC            | 1.4 ± 0.1 x 10^1  | 1.146                | 76.35             | 3.70          |
| 7              | 7    | TCC             | 0                 | 8.0 ± 0.1 x 10^1    | 3.903             | NA            |
| 2.5            | 2.5  | TCC             | 4.5 ± 0.1 x 10^2  | 2.653                | 32.03             | 1.25          |
| 5              | 5.0  | TCC             | 2.1 ± 0.2 x 10^2  | 2.322                | 40.50             | 1.58          |
| 7.5            | 7.5  | TCC             | 1.1 ± 0.0 x 10^1  | 1.041                | 73.33             | 2.86          |
| 10             | 10   | TCC             | NG                | NA                   | ≥ 99.99           | 3.90          |
| 5              | 5    | TFC             | 0                 | 3.0 ± 0.7 x 10^2    | 2.477             | NA            |
| 2.5            | 2.5  | TFC             | 1.0 ± 0.0 x 10^2  | 1.000                | 59.63             | 1.48          |
| 5              | 5.0  | TFC             | NG                | NA                   | ≥ 99.99           | 2.30          |
| 7.5            | 7.5  | TFC             | NG                | NA                   | ≥ 99.99           | 2.30          |
| 10             | 10   | TFC             | NG                | NA                   | ≥ 99.99           | 2.30          |

Keys: THBC: Total Heterotrophic Bacterial Counts; TCC: Total Coliform Counts; TFC: Total Faecal Coliform Counts; NG: No Growth; NA: Not Applicable CFU: Colony Forming Units; Log: Logarithmic.
Table 2  Logarithmic and Percentage Reductions of Bacterial Loads in *Galatea paradoxa* Treated with *Citrus aurantifolia*

| Exposure Time (mins) | Microbial Group | Concentration (%) | Plate Counts (CFU/g) | Log (CFU/g) | % Reduction | Log reduction |
|----------------------|-----------------|-------------------|----------------------|-------------|-------------|--------------|
| 0                    | THBC            | 7.0 ± 0.4 x 10^4  | 4.845                | NA          | NA          |              |
| 2.5                  | TCC             | 8.0 ± 0.1 x 10^3  | 3.903                | NA          | NA          |              |
| 5                    | TFC             | 3.0 ± 0.7 x 10^2  | 2.472                | NA          | NA          |              |
| 10                   | TCC             | 8.0 ± 0.1 x 10^3  | 3.903                | NA          | NA          |              |

Keys: THBC: Total Heterotrophic Bacterial Counts; TCC: Total Coliform Counts; TFC: Total Faecal Coliform Counts; NG: No Growth; NA: Not Applicable; CFU: Colony Forming Units; Log: Logarithmic.
Table 3: Logarithmic and Percentage Reductions of Bacterial Isolates in *Galatea paradoxa* Treated with NaCl and *Citrus aurantifolia*

| Exposure Time (mins) | Microbial Group | Concentration (%) | Plate Counts (CFU/g) | Log (CFU/g) | % Reduction | Log Reduction |
|----------------------|-----------------|-------------------|----------------------|-------------|-------------|--------------|
| 0                    | THBC            | 7.0 ± 0.3 x 10^4  | 4.845                | NA          | NA          |
| 2.5                  | TCC             | 8.0 ± 0.4 x 10^6  | 3.903                | NA          | NA          |
| 2.5                  | TFC             | 3.0 ± 0.7 x 10^4  | 2.477                | NA          | NA          |
| 5                    | THBC            | 1.8 ± 0.4 x 10^3  | 3.255                | 32.62       | 4.85        |
| 5.0                  | TCC             | 1.6 ± 0.6 x 10^2  | 2.204                | 54.51       | 4.85        |
| 7.5                  | TFC             | 1.0 ± 0.3 x 10^2  | 2.000                | 58.72       | 4.85        |
| 10                   | THBC            | 1.6 ± 0.4 x 10^3  | 3.903                | NA          | NA          |
| 5.0                  | TCC             | 1.0 ± 0.3 x 10^2  | 2.000                | NA          | NA          |
| 7.5                  | TFC             | 1.0 ± 0.3 x 10^2  | 2.000                | NA          | NA          |
| 10                   | TCC             | 1.0 ± 0.3 x 10^2  | 2.000                | NA          | NA          |
| 0                    | THBC            | 7.0 ± 0.3 x 10^4  | 4.845                | NA          | NA          |
| 2.5                  | TCC             | 8.0 ± 0.4 x 10^6  | 3.903                | NA          | NA          |
| 2.5                  | TFC             | 3.0 ± 0.7 x 10^4  | 2.477                | NA          | NA          |
| 5                    | THBC            | 1.8 ± 0.4 x 10^3  | 3.255                | 32.62       | 4.85        |
| 5.0                  | TCC             | 1.6 ± 0.6 x 10^2  | 2.204                | 54.51       | 4.85        |
| 7.5                  | TFC             | 1.0 ± 0.3 x 10^2  | 2.000                | 58.72       | 4.85        |
| 10                   | THBC            | 1.6 ± 0.4 x 10^3  | 3.903                | NA          | NA          |
| 5.0                  | TCC             | 1.0 ± 0.3 x 10^2  | 2.000                | NA          | NA          |
| 7.5                  | TFC             | 1.0 ± 0.3 x 10^2  | 2.000                | NA          | NA          |
| 10                   | TCC             | 1.0 ± 0.3 x 10^2  | 2.000                | NA          | NA          |

Keys: THBC: Total Heterotrophic Bacterial Counts; TCC: Total Coliform Counts; TFC: Total Faecal Coliform Counts; NG: No Growth; NA: Not Applicable; CFU: Colony Forming Unit; Log: Logarithmic.
Table 4: Occurrence of Bacterial Isolates in *G. paradoxa* treated with NaCl and *C. aurantifolia*

| Bacterial Isolates | UTD (5 mins) | A (10 mins) | B (5 mins) | A (10 mins) | A + B (5 mins) | A + B (10 mins) | Total |
|--------------------|--------------|-------------|------------|-------------|---------------|----------------|-------|
|                    | 0% | 5% | 10% | 5% | 10% | 5% | 10% | 5% | 10% | 5% | 10% | 5% | 10% |
| B. subtilis        | +  | +  | +  | +  | +  | +  | +  | +  | +  | -  | -  |    | 11  |
| S. aureus          | +  | +  | -  | +  | -  | +  | -  | +  | -  | -  | -  |    | 6   |
| M. varians         | +  | +  | +  | +  | -  | +  | -  | +  | -  | -  | -  |    | 7   |
| S. enterica        | +  | -  | -  | +  | -  | +  | -  | +  | -  | -  | -  |    | 3   |
| S. pyogenes        | +  | -  | -  | +  | -  | -  | -  | -  | -  | -  | -  |    | 2   |
| E. coli            | +  | -  | -  | +  | -  | +  | -  | -  | -  | -  | -  |    | 6   |
| K. pneumoniae      | +  | -  | -  | +  | -  | -  | -  | -  | -  | -  | -  |    | 4   |
| E. aerogenes       | +  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |    | 1   |
| V. cholerae        | +  | +  | -  | +  | -  | -  | -  | +  | -  | -  | -  |    | 7   |
| E. faecium         | +  | -  | -  | -  | -  | +  | -  | -  | -  | -  | -  |    | 3   |
| P. aeruginosa      | +  | +  | -  | +  | -  | -  | -  | -  | -  | -  | -  |    | 5   |
| Total              | 11 | 7  | 2  | 6  | 1  | 10 | 2  | 8  | 1  | 5  | 1  | 1  | 0   | 55  |

Keys: UTD: Untreated; A: NaCl; B: *Citrus aurantifolia*; A + B: Sodium Chloride + *Citrus aurantifolia*; +: Present; -: Absent.
Table 5: Proximate Compositions of *G. paradoxa* treated with NaCl and *Citrus aurantifolia*

| Sample | Treatment                  | Moisture Content | Ash Content | Fiber Content | Crude Lipid | Crude Protein | Total CHO | Calorie Value (kcal) |
|--------|----------------------------|------------------|-------------|---------------|-------------|---------------|-----------|----------------------|
| Ca     | NaCl                       | 61±2             | 3.800±0.01  | 0.040±0.01    | 1.360±0.002 | 32.100±0.25  | 62.700±0.11 | 391.468±0.001        |
| Cb     | *C. aurantifolia*          | 62±1             | 3.800±0.02  | 0.041±0.01    | 1.322±0.000 | 32.222±0.022 | 62.615±0.15 | 391.228±0.002        |
| Cc     | NaCl + *C. aurantifolia*   | 50±2             | 3.750±0.03  | 0.023±0.02    | 1.290±0.001 | 32.631±0.15  | 58.606±0.20 | 376.558±0.000        |
| Cd     | Control                    | 67±1             | 3.950±0.01  | 0.045±0.01    | 1.320±0.002 | 33.229±0.10  | 61.951±0.30 | 392.600±0.004        |

Each value represents the means of triplicate and standard deviation. CHO: Carbohydrate, ANOVA (p > 0.05)
**DISCUSSION**

Even though shellfishes, *G. paradoxa*, are substantially nutritious and have become an increasingly significant source of inexpensive proteins and other nutrients essential for maintenance of healthy body of a large section of world population, nevertheless, shellfishes harbour some pathogenic microorganisms attributable to poor hygienic conditions of the water bodies from where they are obtained (Adebayo-tayo et al., 2006; Oranusi et al. 2018).

In our study, a high THBC, TCC and FCC were obtained from *G. paradoxa* and these high bacterial loads substantiated the results of Ekanem and Adegoke (1995) and Oranusi et al. (2018) who discretely observed unacceptable bacterial loads in shell fishes. The high bacterial loads from *G. paradoxa* not treated with NaCl and *C. aurantifolia* in our study conform with the findings of Antai (1998) and Tonbarapagha et al. (2018) who obtained high microbial loads in shell fishes, but these findings contradicted the report of Udoh et al. (2017) who reported low bacterial loads from *G. paradoxa* in Cross River, Nigeria. Our findings showed that bacterial loads from *G. paradoxa* in Uyo, Akwa Ibom State, exceeded the acceptable limits for shell fishes as specified by FDA (1991) and ICMSF (2005). The high bacterial loads in the shell fishes, *G. paradoxa*, could be attributed to poor handling and processing in the markets (Odu et al., 2010; Akinjogunla et al., 2011).

In this study, logarithmic and percentage reductions in bacterial loads (THBC, TCC and TFC) in *G. paradoxa* treated with different concentrations of NaCl and *C. aurantifolia* singly or in combination for ≥ 10 mins were obtained. The decrease in logarithmic and percentage reductions of bacterial loads in *G. paradoxa* treated with different concentrations (5 to 10%) of NaCl agrees with Soyiri et al. (2008) and Anbalagan et al. (2014) who reported that NaCl concentrations between 7.5 and 10 % eliminated all pathogenic bacteria from shell fishes. A medium containing 10 % NaCl has been reported as unfavourable medium for proliferation of pathogenic micro-organisms (Onyeagba and Isu, 2006; Anbalagan et al. 2014). Similarly, studies have showed that NaCl removed water from food products by osmosis and as NaCl content in food increased its water content also decreased, thus, leading to plasmolysis of cell wall of pathogenic micro-organisms (Anbalagan et al. 2014; Orjimelukwe et al. 2017). This study revealed 79.36 % THBC reduction in *G. paradoxa* treated with 10% *C. aurantifolia* for 10 min. The reduction in bacterial loads to an acceptable level for human consumption in our study corroborated the findings of Rodrigues et al. (2000) and similarly agrees with the results of Mata et al. (1994) who reported extinction of some bacterial isolates in acidic medium containing lime (*C. aurantifolia*) juice.

The eleven bacterial genera obtained from *G. paradoxa* not treated with NaCl and *C. aurantifolia* were *Staphylococcus*, *Micrococcus*, *Salmonella*, *Streptococcus*, *Escherichia*, *Klebsiella*, *Bacillus*, *Enterobacter*, *Vibrio*, *Enterococcus* and *Pseudomonas*. The isolation of *S. pyogenes*, *V. cholerae*, *E. coli*, *S. enterica* and *S. aureus* in our study substantiated the reports of Udoh et al. (2017) who obtained these bacterial isolates from *G. paradoxa* in Cross River, Nigeria.

Our findings revealed 33.229 % protein content in untreated *G. paradoxa* (control) and this value was lower than 47.0 % obtained by Ehiogbor and Akse (2016) in Delta State, but our findings agrees with Ivon and Eyo (2018) who reported 32.10 % protein content in *G. paradoxa* from Calabar River, Nigeria. The moisture content in *G. paradoxa* was high in this study and this was in conformity with Zhu and Bai (2007). The high moisture content in shellfish has been attributed to the quantity of water absorbed into their cells from the external environment (Davies and Jamabo, 2016). A high ash content was discretely obtained from the untreated and *G. paradoxa* treated with NaCl and *C. aurantifolia*, and this concurs with results of Adebake and Odedeji (2010). The ash content greater than 0.5 % has been reported as an indication of good mineral content in food (Adebake and Odedeji, 2010).

**CONCLUSION**

This study showed that *G. paradoxa*, harbour some pathogenic bacteria of public interest and its treatment with equal concentration (vol/vol) of 10 % *C. aurantifolia* and NaCl singly or in combination for 10 min holding time will avert possible foodborne diseases associated with consumption of this aquatic food.

**REFERENCES**

Adebayo-tayo, B.C., Onilude, A. A., Ogunjobi, A. A. and Adeoye, D. O. (2006). Bacteriological and proximate analysis of periwinkles from two different creeks in Nigeria. *World Applied Science Journal*, 1(2): 87 - 91.

Adeleke, R. O. and Odedeji, J. O. (2010). Functional properties of wheat and sweet potato flour blends. *Pakistan Journal of Nutrition*, 9: 535 – 538.

Akinjogunla, O. J., Inyang, C. U. and Akinjogunla, V. F. (2011). Bacterial species associated with anatomical parts of fresh and smoked Bonga fish (*Ethmalosa fimbriata*). Prevalence of cephalosporins. *Research Journal of Microbiology*, 6(1):87-97.

Anbalagan, M., Ganesh Prabu, P., Krishnaveni, R. E. and Manivannan, S. (2014). Effect of sodium chloride (NaCl) on the bacterial load in chicken, mutton and beef meat samples in relation to meat spoilage. *International Journal of Research in Zoology*, 4 (1): 1 – 5.

Antai, S. P. (1998). Study of the microbial flora of Nigeria mussel species. *International Journal of Food Microbiology*, 6: 259 - 261.

AOAC (2005). *Official Method of Analysis* (18th ed). Association of Official Analytical Chemist International, Maryland, USA. 876p.

APHA (American Public Health Association). (1998). Standard methods for the examination of water and wastewater. 16th Edition, Washington D.C.

Bradley, E. M., Williams, J. B., Schilling, M. W., Coggin, P. C and Crist, C. (2011). Effects of sodium lactate and acetic acid preservatives on the quality and sensory characteristics of hot-
boned pork sausage patties. *Meat Science*, 88: 145 - 150.

Chiesa, L. M., Nobile, M., Malandra, R., Pessina, D. and Panseri, S. (2018). Food safety traits of mussels and clams: distribution of PCBs, PBDEs, OCPs, PAHs and PFAFs in sample from different areas using HRMS-Orbitrap and modified QuEChERS extraction followed by GC-MS. *Food Additives and Contaminants*, 35: 5.

Cheng, H. Y., Ye, R. C. and Chou, C. C. (2003). Increased acid tolerance of *Escherichia coli* O157:H7 by acid adaptation time and conditions of acid challenge. *Food Research International* 36: 49 - 56.

Davidson, P. M. and T. M. Taylor. (2007). Chapter 33. Chemical preservatives and natural antimicrobial compounds. In Doyle, M. P. and Larry R. Beuchat (eds.), *Food microbiology Fundamentals and Frontiers*, 3rd ed. ASM Press, Washington D.C. pp. 713 – 745.

Davies, I. C. and Jamabo, N. A. (2016). Determination of mineral contents of edible parts of shellfishes from Okpoka creeks in Rivers State, Nigeria. *International Journal of Fisheries and Aquaculture Research*, 2 (2): 10 - 18.

Ehigiator, F. A. R. and Akise, O. G. (2016). Proximate, amino acid and mineral composition of wild and cultivated fresh water clam (*Egeria radiate*). *Nigerian Journal of Agriculture Food and Environment*, 12 (2): 103 - 108.

Ekanem, E. O. and Adegoke, G. O. (1995). Bacteriological study of West African clam (*Egeriaraadiata Lamarch*) and their overlying waters. *Food Microbiology*, 12: 381 - 385.

Ekpenyong E, Williams I.O, Osakpa U. U. (2013). Variation in the proximate, energy and mineral compositions of different body parts of *Macrobrachium macrobrachion* (Prawn). *Journal of Food Research*, 2 (2): 150 – 156.

Etim, L and Brey, T. (1994). Growth, productivity and significance for fishery of the bivalve *Egeriaraadiata* (*Donacidae*) of live bivalve mollusces. pp. 1 - 19.

FDA, (1991). Sanitation of shellfish. growing areas considering and seafood safety (F.E. Ahmed), National Academic Press. Washington DC. *Food and Environment*, 12 (2): 103 - 108.

Gram, L., Ravn, L. and Rasch, M. 2002. Food spoilage interactions between food spoilage bacteria. *International Journal of Food Microbiology*, 78: 79-97.

Hatha, A. A. M., Christi, K.C., Singh, R. and Kumar, S. (2005). Bacteriology of the fresh water bivalve clam *Batissa violacea* (Kai) sold in the Suva Market. *The South Pacific Journal of National Science*, 23: 48 – 50.

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Stately, J. T. and Williams, S. T. (1994). *St. Bergey’s Manual of Determinative Bacteriology* (9th Edition). Baltimore, Williams and Wilkins. 787p.

ICMSF International commission on microbiological specificat ions for foods (2005). *Microorganisms in Foods*. 2nd ed, Kluwer Academic/ Plenum Pub. New York. pp. 84 – 86.

Ivon, E. A. and Eyo, V. O. (2018). Proximate composition and mineral contents of edible part of four species of shellfishes from the Calabar River, Nigeria. *Annual Research and Review in Biology*, 26 (1): 1 – 10.

King, R. P. (2000). Population structure, growth performance and mortality rates of the freshwater clam *G. paradoxa* Born 1778, in Nun River, Nigeria. *Archive of Fisheries and Marine Research*, 48(1):21 - 30.

Lawrence, T. E., Dikeman, M. E., Stephens, J. W., Obuz, E. and Davis, J. R. (2003). In Situ Investigation of the calcium-induced proteolytic and salting-in mechanisms causing tenderization in calcium-enhanced muscle. *Meat Science*, 66: 69 - 75.

Man, C. M. D. (2007). Technological functions of salt in food products. In Kilcast, D and Angus, F (eds), *Reducing salt in foods*. CRC Press LLC, Boca Raton Fl. pp. 157 - 173.

Mata, L., Vives, M. and Vicente, G. (1994). Extinction of *V. cholerae* in acidic substrata: contaminated fish marinated with lime juice (ceviche). *Revista de Biologia Tropical*, 42: 479 - 485.

Moses, S. B. (1990). Growth, biomass, mortality, production and potential yield of the West African clam, *Egeria radiata* (Lamarche) (*Lamellibranchia, Donacidae*) in the Cross-River State, Nigeria. *Hydrobiologia*, 196: 1–15.

Ndelekwe, E. K. and Enyehini, G. E. (2017). Lime juice as a source of organic acids for growth and apparent nutrient digestibility of broiler chickens. *Journal of Veterinary Medicine and Surgery*, 1: 1 – 5.

Odu, N. N., Obafemi, A. and Njoku, H. O. (2010). Comparative assessment of bacteriological quality and proximate composition of laboratory shucked and traditionally shucked tropical periwinkle (*Tympanotonus fuscatus*). *Scientia Africana*, 9 (1): 140 - 149.

Okon, M. U., Inyang, C. U. and Akinjogunla, O. J. (2020). Bacterial isolates from bivalve clams (*G paradoxa*, Born 1778): Occurrence, multi-drug resistance, location of antibiotic resistance marker and plasmid profiles. *South Asian Journal of Research in Microbiology*, 7(3): 35 – 46.

Orjimelukwe, P. C., Ekong, K. S. and Akachukwu, D (2017). Effect of different processing methods on the nutrient composition and sensory properties of *E. fimbrata*. *American Journal of Agricultural Science*, 4(5): 107 – 113.

Onyeagba, R. A. and Isu, N. R. (2006). *General Microbiology*, 2nd (ed) Crystal Publishers, Okigwe Nigeria, 600p.

Oranusi I., Effiong, E. D. and Duru, N. U. (2018). Comparative study of microbial, proximate and heavy metal compositions of some gastropods, bivalve and crustacean seafood. *African Journal of Clinical and Experimental
Logarithmic and Ok

Rodrigues, A., Sandstorm, A., Ca, T., Steinsland, H., Jensen, H. and Aaby, P. (2000). Protection from Cholera by adding lime juice to food - results from community and laboratory studies in Guinea-Bissau, West Africa. *Tropical Medicine and International Health*, 5: 418 - 422.

Samya, H. M. and Mohamed, S. Y. (2006). Proximate evaluation of some economical seafood as a human diet and as an alternative prospective valuable of fish meal. *Journal of Fisheries and Aquatic Science*. 11 (1): 12 - 27.

Silva, J. G., Morais, H. A. and Silvestre, M. P. C. (2003). Comparative study of the functional properties of bovine globin isolates and sodium caseinate. *Food Research International*, 36: 73 – 80.

Simopoulos, A. P. (2003). Importance of the ratio of omega-6/omega-3 essential fatty acids: evolutionary aspects. *World Review of Nutrition and Dietetics*, 92: 1 - 22.

Skrivanova, E., Molatova, M., Houf, K and Marounek, M. (2011). Inhibitory effect of organic acids on Arcobacters in culture and their use for control of Arcobacter butzleri on chicken skin. *International Journal Microbiology*, 144: 367 - 371.

Soyiri, I. N., Agbogh, H. K and Dongdem, Y. T. (2008). A pilot microbial assessment of beef sold in the Ashaiman Market, a suburb of Accra Ghana. *Journal of Applied Microbiology*. 8: 72 – 74.

Tonbarapagha, K., Douye, V. Z. and Deborah, A. (2018). “Assessing the hygienic status of processed fresh water clam (*Galatea paradoxa*) in Yenagoa Metropolis, Bayelsa State, Niger Delta, Nigeria.” *American Journal of Food Science and Technology*, 6 (5): 219 - 222.

Udoh, D. I., Udo, I. U. and Udoh, E. I. (2017). Microbiological analysis of the freshwater clam from Cross River, Nigeria. *Nigeria Journal of Agriculture, Food and Environment*, 13 (3): 59 – 64.

Villalobos, L. B. and Elguezabal, L. A. (2001). Microbiological quality of the bivalves *Pinctada imbricata* commercialized in Cumana, Venezuela. *Food Technology. Acta Cientifica Venezolana*, 52: 55 – 61.

Zhu, Q. and Bai, R. (2007). Comparison of biological characteristics between cultured and wild crab (*Eriocheir sinensis*) *Jiangsu Journal of Agricultural Science*, 23: 218 - 223.

©2020 This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 International license viewed via [https://creativecommons.org/licenses/by/4.0/](https://creativecommons.org/licenses/by/4.0/) which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is cited.