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# Microbiological and Proximate Evaluation of *Tagelus adansonii*, Bosc, 1801 (Mollusca: Bivalvia, Solecurtidae) in Mangrove Swamps of Iko Estuary, Southeast, Nigeria

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ABSTRACT

*Tagelus adansonii* has served as man's food around the world from time immemorial. However, the aquatic ecosystem in which they live is constantly polluted. Microbial and proximate compositions and energy value of *T. adansonii* were evaluated as indices for food safety and biomarker of pollution. Standard microbiological techniques and standard methods of AOAC were employed. Results showed that *Bacillus subtilis*, *Micrococcus species*, *Proteus species*, *Klebsiella species*, *Staphylococcus aureus*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Enterobacter sp*, *Escherichia coli*, *Bacillus cereus*, and *Chromatium species* were the probable bacteria while *Rhizopus stolonifer*, *Aspergillus niger*, *Penicillium species*, *Candida tropicalis*, *Fusarium species* and *Aspergillus flavus* were the probable fungi isolated from the sample. Total Heterotrophic Bacterial Count (THBC), TVC, TCC, TSC and TFC in fresh sample were  $2.01 \pm 0.14 \times 10^5$ ,  $2.77 \pm 0.27 \times 10^5$ ,  $2.79 \pm 0.81 \times 10^5$ ,  $6.08 \pm 0.21 \times 10^5$ , and  $2.08 \pm 0.21 \times 10^4$  cfu g<sup>-1</sup> respectively and concentrated mostly in the gut. The mean crude protein, moisture, carbohydrate, ash, lipid and crude fibre contents of the soft tissues were  $60.92 \pm 2.38$ ,  $40.75 \pm 1.85$ ,  $26.58 \pm 2.91$ ,  $5.99 \pm 0.43$ ,  $5.56 \pm 0.51$  and  $4.13 \pm 0.10\%$  respectively, while the energy or caloric value was  $397.65 \pm 11.97$ . Proper monitoring and surveillance should be adopted by Government to check pollution of the aquatic environments and proper processing should be adopted before consumption for good public health.

## 1. Introduction

Shellfish and finfish are the most important sources of animal protein in the diet because of their good quality

and quantity of protein<sup>[1]</sup>. Anthony *et al.*<sup>[2]</sup>, reported that shellfishes contain crude protein and fat higher than 20 and less than 5 percent respectively. They are relatively

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good sources of calcium, phosphorus, magnesium and potassium; and are also a major source of income for coastal towns and villages<sup>[3]</sup>. Tagelus is a genus of marine bivalve molluscs belonging to the family Solecurtidae, in the order Cardiida, an order of bivalves belonging to the class Bivalvia. In previous centuries, Bivalvia referred to as the Lamellibranchiata and Pelecypoda, is a class of marine and freshwater molluscs that have laterally compressed bodies enclosed by a shell consisting of two hinged parts. Adanson's tagelus is a short razor clam commonly known as knife clam<sup>[4]</sup>. It has wide distribution across eastern Atlantic<sup>[5]</sup>.

Due to geometric rise in human population, the aquatic ecosystems have been grossly polluted. The primary sources of nutrient pollution include fertilizer from agriculture farmlands, animal manure, sewage treatment plant discharge, detergents, storm water runoff, cars and power plants, failing septic tanks and pet waste, with considerable regional variation in the relative importance of each<sup>[6]</sup>. Nutrient pollution is of great environmental concern globally. It is a matter of concern due to excessive amount of nitrogen and phosphorus in water and air. Marine pollution has become one of the biggest threats due to industrialization and agricultural activities. It is hazardous not only for the water kingdom but for human beings directly or indirectly. In many forms, marine pollution alters the physical, chemical, and biological characteristics of the ocean and coastal areas, negatively impacting the health of biodiversity and ecosystems<sup>[7]</sup>, and may increase susceptibility to other stressors, including disease.

For the past decades, marine mollusks have received special attention and become a natural resource of economic importance<sup>[8]</sup>. Apart from being valued as food and their ornamental importance, marine mollusks from polluted waters have been widely implicated in the outbreak of foodborne illnesses such as typhoid fever, hepatitis and other similar intestinal disorders in various parts of the world<sup>[9,10]</sup>.

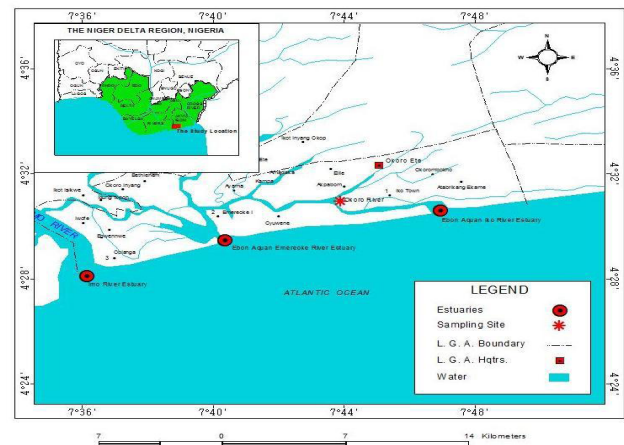
Microbiological and proximate analyses of seafood have great importance in food safety and quality assurance since, they are major constituents of living matter<sup>[11,12]</sup>. It is very useful in nutrient content compartmentalization and thus often affords a great deal of attention to consumers due to its potential benefits. It can also serve as biomarkers of aquatic pollution. This research therefore seeks to establish baseline data which will be useful in food safety, quality assurance and future monitoring of aquatic pollution.

## 2. Materials and Methods

### 2.1 Study Area

Live specimens of *T. adansonii* were obtained from the

intertidal zone of the Iko Estuary, a brackish water habitat characterized by tides, mangroves, several species of fin and shellfish (Figure 1).



**Figure 1.** Location of the study area on the map of Niger Delta Region, Nigeria

### 2.2 Sampling Procedure and Processing

Samples were collected monthly from February 2019 to January 2020 in Iko Lagoon in Nigeria. This receives its catches from the Qua Iboe and Imo river estuaries. A total of five hundred and thirty (530) live specimens of *T. adansonii* were handpicked from the sediments in randomly sampled areas and washed with water from the creek and then transported in a sterile ice-packed coolers containing mud collected from the tidal mudflat to the Microbiology Laboratory University of Uyo, Uyo, Nigeria for analysis. Prior to analysis, sample was washed with a brush and water to remove all debris on the shells, spread on laboratory table and allowed to air-dry at room temperature. Selection of the healthy-looking clams was done through visual examination. A total of one hundred and fifty (150) samples each were selected for gut, shell and soft body analyses. Thereafter, extraction of the samples' soft body was done using a sterile scalpel. After each sampling date, samples' flesh weighing 10 g were homogenized in a blender with 90 mL of sterile distilled water, corresponding to a  $10^{-1}$  dilution<sup>[13]</sup>. A sterile surgical blade was used to obtain samples' guts through dissection then pulverized using a sterile mortar. The clam shell was also pulverized separately and then all the specimens were used for analysis. Part of the samples were weighed prior to laboratory analysis of its proximate composition<sup>[14]</sup>.

### 2.3 Microbiological Analysis

#### 2.3.1 Serial Dilution of the Homogenate

Prior to culture, serial dilution of the homogenate was

conducted according to the method of Fawole and Oso<sup>[15]</sup>. The samples were first blended using Laboratory Blender (Lab Blender 400 series, UK). Next was the weighing of ten (10) grams of samples and homogenization in 90 mL of sterile distilled water. This was shaken vigorously to dislodge bacteria and other microbes attached to it. Sterile pipette was then used to prepare tenfold dilution of the homogenates after which the aliquot (1 mL) was serially transferred to other test tubes containing 9 mL of distilled water up to 10<sup>-6</sup>. Molten agar was prepared, and one (1) mL of the diluents of 10<sup>-4</sup> was aseptically dispensed into the Petri dishes containing 15 mL of the agar. Samples culture was done in triplicates. All plates were incubated at 37 °C for 24 hours. In order to obtain pure colonies, subculture of the samples was carried out. Biochemical test of the isolates was carried out confirmed by microscopic examination prior to characterization and identification. Nutrient Agar (Oxoid, USA), Thiosulphate citrate bile-salt agar (Oxoid, USA), Eosin Methylene Blue (Oxoid, USA), Mannitol salt agar ((Difco Laboratories, Detroit, Mich), and Sabourad Dextrose Agar (Difco Laboratories, Detroit, Mich) were used for the enumeration of Total Heterotrophic Bacteria Count (THBC), Total Vibrio Count (TVC), Total Coliform Count (TCC), Total Staphylococcal Count (TSC) and Total Fungal Count (TFC) respectively. Nutrient agar slants in McCartney bottles was used to maintain the isolates and preserved in a refrigerator at 4 °C for further analysis. A sterile inoculation needle was used to pick up morphologically different colonies and aseptically transferred to sterile nutrient slant for further characterization. Purity check and characterization was done following the standard characterization key<sup>[16]</sup> partitioned into Gram reaction, catalase, coagulase, motility, starch hydrolase, citrate, urease, MR, VP, Kovac's oxidase, oxidation/fermentation (O/F) test, indole, mannitol, glucose, sucrose and lactose tests.

## 2.4 Experimental Procedure

Completely Randomized Design (CRD) with four (4) treatments and three (3) replicates was used. Total Heterotrophic Bacteria Count, TVC, TCC, TSC and TFC stood for treatments 1, 2, 3, 4 and 5 respectively. Each quarter year sampling represented a replicate; the one year sampling represented three replicates.

## 2.5 Analysis of Proximate Composition

### 2.5.1 Moisture Content Determination

Weighing bottle was washed, oven-dried at 80% for five (5) minutes, cooled and weighed and recorded as beaker weight (a). Two (2) grammes of the sample was weighed into the weighing bottle, now bottle weight plus sample recorded (b). The weighing bottle with the sample was

then oven-dried at 105°C for 24 hours. The bottle allowed to cool in a desiccator to room temperature, weighed with a minimum exposure to atmosphere. This was repeated till constant weigh is obtained (c). Percentage moisture was therefore calculated thus:

$$\text{Percentage Moisture (\% wet weight)} = \frac{b-c}{b-a} \times 100$$

The dried sample was ground with grinding machine into powder form often necessary to pass through sieve of particular mesh size and then stored at low temperature in dry air-tight container.

### 2.5.2 Preparation of Sample for Subsequent Analysis

After taking part of the fresh sample for moisture content determination, the remaining sample was to be dried to a constant weight before subsequent analysis. Low temperature (50-60 °C) was employed to reduce any possible effect of high temperature on the protein (and probably other nutrient) in the food sample, such effects include Protein denaturation, Loss of vitamins, decomposition of anions. However, the oven dried material was ground in a mortar into a powdered form, often necessary to pass through a sieve of a particular mesh size and then stored at a low temperature in dry air-tight container specifically having a plastic cover.

The following parameters were determined from the ground samples; Ash, crude fibre, lipids, crude protein, fat, etc.

### 2.5.3 Ash and Organic Matter Determination

This was done by ignition of crucibles with lid in a muffle furnace at 105 °C for an hour, and transferred to a desiccator to cool and the weight was recorded as weight of empty crucible (a). Next, 1 – 5 grammes of dry sample, finely pulverized, was transferred into the pre-weighed crucible and the weight of the crucible plus sample (b) was recorded. Then, crucible plus sample were charred on a heater or Bunsen flame in a fume cupboard, to drive off most of the smoke (until smoking ceased), then transferred to a muffle furnace heated at (500-600 °C) to burn off all the organic matter; then left for 2 hours. The crucible was taken out, when cool, covered and placed in a desiccator and weighed (c). Percentage ash was determined as follows:

$$\text{Percentage Ash} = \frac{c - a}{b - a} \times 100$$

The portion of sample which burnt off is organic matters. Therefore the percentage organic matters were determined thus:

$$\text{Percentage organic matters} = 100 - \% \text{Ash.}$$

### 2.5.4 Crude Fiber Estimation

To estimate crude fibre, 2 grammes of sample was

defatted with petroleum ether for 2 hours, boiled under reflux for 30 minutes with 200 mL of a solution containing 1.25% of H<sub>2</sub>SO<sub>4</sub> for 100 mL solution. Residue was obtained by filtering the solution through linen fixed on a fluted funnel washed with boiling water until the washing was no longer acidic. This was transferred to a beaker and boiled for another 30 minutes with 200 mL of a solution containing 1.20 g of NaOH for 100 mL. The final residue was filtered and washed with boiling water several times until it is base (NaOH) free. The residue was finally washed twice with ethanol, and qualitatively transferred into a pre-weight crucible, oven dried at 105°C (W<sub>1</sub>). This was incinerated in a furnace at 550 °C for 2 hours (W<sub>2</sub>). It was then cooled in a desiccator and weighed. Loss in weight after incineration was also taken.

$$\text{Percentage crude fibre} = \frac{W_2 - W_1}{\text{Weight of original sample taken}} \times 100$$

### 2.5.5 Ether Extract Determination

Two grammes of the sample was weighed into a thoroughly-washed and oven-dried extractor thimble and plugged lightly with cotton wool. Next, 150 mL of petroleum ether (boiling point 60 – 80 °C) was formed into a 250 mL round bottom flask. The Soxhlet extractor was fitted into the round bottom flask on a heated mantle. The Soxhlet apparatus was assembled and allowed to reflux for about 4 hours and the extract was poured into a dried pre-weighed beaker (W<sub>1</sub>) and the thimble was rinsed with a little quantity of the ether back to the beaker. The beaker was oven-heated to drive off the excess solvent. The beaker was cooled in a desiccator and weighed (W<sub>2</sub>). Crude fat was estimated thus:

$$\text{Percentage Ether Extract} = \frac{W_2 - W_1}{\text{weight of sample}} \times 100$$

### 2.5.6 Crude Protein Determination

The crude protein content was determined by Kjeldahl method. One gramme of the sample was put in a standard 250 mL Kjeldahl flask containing 1.5 g of CuSO<sub>4</sub> and 1.5 g of Na<sub>2</sub>SO<sub>4</sub> as catalyst and 5 mL concentrated H<sub>2</sub>SO<sub>4</sub>. The Kjeldahl flask (digestion) was heated gently to prevent frothing for some hours until clear bluish solution was obtained, allowed to cool and quantitatively transferred to 100 mL standard flask and made up to the mark with distilled water. Twenty (20) milligrams of the digest were pipetted into a semi micro Kjeldahl distillation apparatus and treated with equal volume of 40% NaOH solution. The ammonia evolved was steamed, distilled into a 100 mL conical flask containing 100 mL solution of saturated boric to which 2 drops of Tashirus indicators (double indicator) have been added. The tip of the condenser was immersed into the boric

acid double inductor and then the distillation continued until about 2/3 of the original volume obtained. The tip of the condenser was rinsed with a few millimeters of distilled water in the distillate which was then titrated with 0.1 M. HCL until a purple-pink end point was observed (Sample titre, T<sub>1</sub>). The blank determination was also carried out in similar manner except for the omission of the sample (Blank titer, T<sub>1</sub>). The crude protein was obtained by multiplying the % nitrogen content by a factor (6.25). Percentage crude protein is estimated thus:

$$\text{Percentage crude protein} = \frac{T_1 - T_2 \times 0.1 \times 0.04 \times 20 \times 100}{\text{weight of sample}} \times 6.25/10$$

Note: Most protein contain about 16% Nitrogen; that is 16 mg. Nitrogen equals 100 mg protein, therefore one milligram of nitrogen equals 100/16 equals to 6.25 mg of protein. The Nitrogen value is therefore multiplied by 6.25 to get the weight of protein.

### 2.5.7 Estimation of Nitrogen Free

Nitrogen free Extract (NFE) was determined as the difference obtained after subtracting total organic Nitrogen (protein) lipid, ash and fibre from the total dry matter thus:

$$\text{Percentage Nitrogen Free Extract} = 100 \% - (\% EE + \% CP + \% Ash + \% CF)$$

### 2.5.8 Estimation of Calorific Value (Energy)

The Atwater system [17] was used in the determination of the total calorific value of the sample by employing the 4-9-4 method. This system applies energy conversion factors to the macronutrients carbohydrate, fat, protein and fiber.

## 2.6 Statistical Analysis

Data on microbial and fungal loads on the body, gut and shell were log transformed (log<sub>10</sub>) for the purpose of statistical analysis while those on proximate composition were used directly. One – way analysis of variance (ANOVA) was used to test the variation in mean values and significance accepted at P≤0.05 level. Where significant difference existed, *post hoc* test was performed using Duncan Multiple Range Test (DMRT). The IBM SPSS statistics version 20 computer program for Windows was used.

## 3. Results

### 3.1 Characteristics of Bacterial Isolates

The cultural, morphological, structural and biochemical characteristics of bacterial isolates from *T. adansonii* are presented in Table 1.

The isolates were *Bacillus subtilis*, *Micrococcus sp*,

**Table 1.** Cultural, Morphological, Structural and Biochemical Characteristic of Bacterial isolate from *Tagelus adansonii* caught in Mangrove Swamps of Iko Estuary, Southeast, Nigeria

Bacteria shape	Gram reaction	Catalase	Coagulase	Motility	Starch hydrolase	Citrate	Urease	MR	VP	Form spore	H <sub>2</sub> S	Hae	Glucose	Maltose	Lactose	Fructose	Sucrose	Galactose	Mannitol	Probable organism
Rod	+	+	-	+	+	+	-	-	+	+	+	-	AG	A	-	A	-	A	-	B. subtilis
Cocci in pair	+	+	-	-	+	+	+	+	-	-	-	-	-	A	+-	A	-	A	-	Micrococcus sp
Rod	+	+	-	+	+	+	+	+	-	-	-	-	AG	A	AG	AG	AG	AG	-	<i>Proteus sp.</i>
Rod	-	+	-	+	-	+	+	+	-	+	+	-	AG	AG	AG	-	AG	AG	-	<i>Klebsiella sp.</i>
Cocci in cluster	+	+	+	-	-	-	-	-	+	-	-	B	A	A	-	AG	A	A	AG	Staphylococcus aureus
Cocci	-	+	-	+	+	-	-	-	+	-	-	-	A	A	-	A	AG	A	-	Vibrio cholera
Rod	-	+	-	-	+	+	-	-	+	+	-	-	A	A	AG	A	-	A	-	Vibrio parahaemolyticus
Rod	-	+	-	-	+	-	-	-	-	-	+	-	AG	AG	AG	AG	AG	AG	-	Enterobacter sp.
Rod	-	+	-	+	-	-	-	+	-	-	-	-	A	AG	-	-	-	AG	-	Escherichia coli
Rod	+	+	-	+	+	-	-	-	+	-	+	-	AG	A	AG	A	-	-	AG	Bacillus cereus
Rod	+	-	-	+	+	+	-	-	+	+	+	-	AG	AG	+	-	A	AG	-	Chromatium sp.



*Proteus sp, Klebsella sp, Staphylococcus aureus, Vibrio cholera, Vibrio parahaemolyticus, Enterobacter sp, Escherichia coli, Bacillus cereus and Chromatium sp.*

### 3.2 Characteristics of Fungal Isolates

The type of fungal pigmentation, soma, special vegetative structure, asexual spore, special reproductive structure, conidial head, vesicles shape and nature of hyphae identify were used to isolate *Rhizopus stolonifer, Aspergillus niger, Penicillium sp, Candida tropicalis, Fusarium sp, and Aspergillus flavus* as probable fungi found in *T. adansonii*. This is shown in Table 2.

Table 3 shows the microbial loads of the sample. Mean THBC of  $2.01 \times 10^5$  cfu g<sup>-1</sup> with the highest ( $2.83 \times 10^5$  cfu g<sup>-1</sup>) in the gut and the least ( $1.60 \times 10^5$  cfu g<sup>-1</sup>) seen in flesh. Mean THBC was significantly higher (P<0.05) in the gut than in other body organs. Mean TVC was  $2.77 \times 10$  cfu g<sup>-1</sup> with the highest ( $2.90 \times 10$  cfu g<sup>-1</sup>) in flesh and least

( $2.68 \times 10$  cfu g<sup>-1</sup>) in gut. However, there was no significant difference (P<0.05) in the mean TVC among different organs. Mean TCC was  $2.79 \times 10$  cfu g<sup>-1</sup> with the highest ( $2.88 \times 10$  cfu g<sup>-1</sup>) in gut and least ( $2.70 \times 10$  cfu g<sup>-1</sup>) in clam flesh. No significant difference (P<0.05) in the mean TCC among different organs.

Mean TSC was  $6.08 \times 10$  cfu g<sup>-1</sup> with the highest ( $6.63 \times 10$  cfu g<sup>-1</sup>) in fresh and no staphylococcal count on the shell. Total staphylococcal count (TSC) was significantly higher (p<0.05) in the flesh. Mean TFC was  $2.08 \times 10^4$  cfu g<sup>-1</sup> with highest ( $2.30 \times 10^4$  cfu g<sup>-1</sup>) in clam gut and least ( $1.72 \times 10^4$  cfu g<sup>-1</sup>) in shell. Total fungal count (TFC) was significantly higher (p<0.05) in the gut and flesh than the shell.

Proximate Composition of knife clam is presented in Table 4. The moisture content of the mollusc ranged from 38.27% to 43.14% with  $40.75 \pm 1.85\%$  being the mean. Ash ranged 5.12 – 6.23% and the mean was  $5.99 \pm 0.42\%$ . The

**Table 2.** Cultural, Morphological, Structural and Biochemical Characteristic of Fungal isolates from *Tagelus adansonii* from caught in Mangrove Swamps of Iko Estuary, Southeast, Nigeria.

Pigmentation	Type of Soma	Nature of hyphae	Special vegetable structure	Asexual spore	Special reproductive structure	Conidial head	Vesicles shape	Probable organism
White becoming greyish	Filamentous	Coenocytic	Stolon rhizoids	Ovoid sporangiospores	Tall sporangiophores in group, black brown sporangia	-	-	Rhizopus stolonifer
Black colony	Filamentous	Septate	Foot cell	Globose conidia	Smooth wall erects conidiophores	Globose	Globose	Aspergillus niger
Dark green colony	Filamentous	Septate	Broom like appearance	Globose conidia produce in long columnar	Erect conidiophores terminating in whorls of phialides	-	-	Penicillium sp
Creamy white colony	Pseudo-hyphae	Septate	Apothelium	Blastoconidia	Conidia	Radiate	Globose	Candida tropicalis
pink	Filamentous	Septate	-	Micro conidia	Short branch conidiophores	-	-	Fusarium sp
Yellow	Filamentous	Septate	Foot cell	Globose conidia	Phialides born directly on the vesicle sclerotia	Radiate	Sub-globose	Aspergillus flavus

**Table 3.** Microbial load (cfu g<sup>-1</sup>) of *Tagelus adansonii* samples caught in Mangrove Swamps of Iko Estuary, Southeast, Nigeria

Anatomical sites	THBC (10 <sup>5</sup> cfu g <sup>-1</sup> )	TVC (10 <sup>1</sup> cfu g <sup>-1</sup> )	TCC (10 <sup>1</sup> cfu g <sup>-1</sup> )	TSC (10 <sup>1</sup> cfu g <sup>-1</sup> )	TFC (10 <sup>4</sup> cfu g <sup>-1</sup> )
Gut	2.82±3.1 <sup>c</sup>	2.68±2.3 <sup>a</sup>	2.88±3.5 <sup>a</sup>	5.53±2.6 <sup>a</sup>	2.30±2.5 <sup>bc</sup>
Flesh	1.60±1.7 <sup>a</sup>	2.90±2.3 <sup>a</sup>	2.70±3.6 <sup>a</sup>	6.63±3.1 <sup>c</sup>	2.21±5.4 <sup>c</sup>
Shell	1.61±1.3 <sup>a</sup>	2.75±2.8 <sup>a</sup>	2.78±4.0 <sup>a</sup>	-	1.72±2.2 <sup>a</sup>
MEAN	2.01±0.14 <sup>b</sup>	2.77±0.27 <sup>a</sup>	2.79±0.18 <sup>a</sup>	6.08±0.21 <sup>b</sup>	2.08±2.10 <sup>b</sup>

Values are mean ± SD. Mean±sd in the same column with different alphabets are significantly different (p<0.05). THBC = Total Heterotrophic Bacterial count, TVC= Total Vibrio Count TCC = Total Coliform Count, TSC = Total Staphylococcal Count, TFC = Total fungal count.

fibre content ranged from 3.96 – 4.21% while the mean was 4.13±0.10%. The protein content ranged from 56.45 – 63.31% and mean was 60.92±2.38%. Fat content ranged 4.99 – 6.03% while mean was 5.56±0.51%. Carbohydrate content was within the range of 21.26 – 29.46% and had 26.58±2.91% as the mean, while the caloric value was ranged from 384.97 – 410.39 kcal and had 397.65±11.97 kcal as the mean.

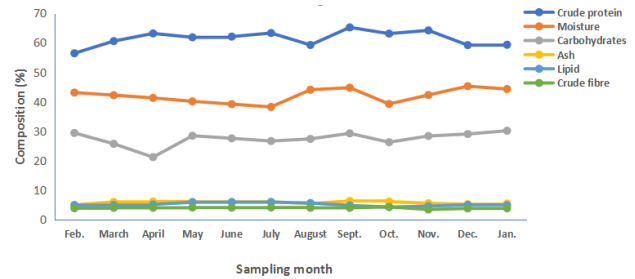
**Table 4.** Mean proximate composition of flesh of *Tagelus adansonii* caught in Mangrove Swamps of Iko Estuary, Southeast, Nigeria

Parameters	Composition (%)
Moisture	40.75±1.85
Ash	5.99±0.42
Crude fibre	4.13±0.10
Crude protein	60.92±2.38
Ether extract	5.56±0.51
Nitrogen free extract	26.58±2.91
Caloric value (Kcal)	397.65±11.97

Values are means ± standard deviation of triplicate samples.

### 3.3 Monthly Variation in the Proximate Composition

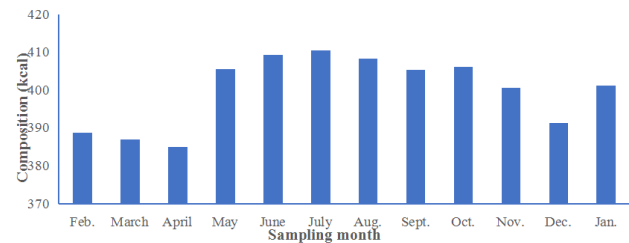
The monthly variation in the proximate composition of *T. adansonii* is presented in Figure 2. The highest crude protein content was recorded in September while the least was seen in February. However, there was no significant difference ( $P>0.05$ ) in the monthly composition of crude protein in this clam. The highest moisture content was recorded on September while the least was seen in July. However, there was no significant difference ( $P>0.05$ ) in the monthly composition of moisture in this clam. The highest carbohydrate content was recorded in January while the least was seen in April but was not significantly different ( $P>0.05$ ). Ash content showed the highest content in September and the least in February and were not significantly different ( $P>0.05$ ). Lipid content showed the highest value in July while the least was seen in October. However, there was no significant difference ( $P>0.05$ ) in the monthly composition of lipid in this clam. Crude fibre content of *T. adansonii* showed the highest value in October while the least was seen in November and was not significantly different ( $P>0.05$ ).



**Figure 2.** Monthly variations in the proximate compositions of *Tagelus adansonii* caught in Iko Estuary Southeast Nigeria

### 3.4 Monthly Variation in Caloric Value

The monthly variation in the caloric value of *T. adansonii* is presented in Figure 3. The highest caloric value was recorded in July while the least was seen in April. However, caloric values were significantly higher ( $P<0.05$ ) in June and July.



**Figure 3.** Monthly variation in caloric value of *Tagelus adansonii* caught from Iko Estuary Southeast, Nigeria

## 4. Discussion

*Tagelus adansonii* caught in Iko region of the Niger delta estuaries accumulate and concentrate microbial pathogens and those that are GRAS (Generally Regarded as Safe) present in the water. In all, eleven (11) bacterial species and six (6) fungal species were isolated. *Bacillus subtilis* isolated from the sample was an indication that not only pathogenic microbes but useful ones were also isolated. For instance the isolation of *B. subtilis* was an indication that commercially useful bacteria (GRAS) was presented in the samples. According to Martinez <sup>[17]</sup>, *B. subtilis* is important in biotechnology for enzyme production. In food industry, it is used in the production of animal feed additives, sweeteners, fermented products and flavour enhancers. The production of household detergent, vitamins and antibiotics is also accomplished using *B. subtilis*. Above all, it is used in the development of vaccines and in the development of sporicides. *Micrococcus species* are very useful in cheese

production. It helps in the ripening cheeses made from farm animals' milk; halotolerant *M. Species* is a major component of micro flora of some cheeses and help in desired body, texture and flavour of soft cheese. Although not considered a pathogen, *M. Species* has generally been regarded as opportunistic pathogen since some strains such as *M. luteus* has been implicated as the causative agent of septic arthritis, recurrent bacteraemia etc. and pneumonia in patient with acute leukaemia<sup>[18]</sup>.

Some of the isolates may have been derived from external sources during handling and as such, the clams become transient carriers of such microbes. For instance, isolation of *Proteus Spp.* may mean that the sample was not prepared hygienically so that it entered from the environment and when consumed by humans it can cause osteomyelitis<sup>[19]</sup>. However, in this study most isolates from the clam gut and homogenized flesh may be accounted for mainly by the filter feeding effect of the clam<sup>[20]</sup>. *Klebsiella Spp* can cause diseases and spoilage therefore its isolation from *T. adansonii* mean that if eaten raw or not well processed it can cause UTIs, wound and respiratory infection as well as serious pneumonia in humans<sup>[21]</sup>. *Staphylococcus aureus* is pathogenic and has been isolated in the sample. This means that consumption of this sample by human can result in the type of food poisoning known as SFP (staphylococcal food poisoning) and late on-set infection in neonates<sup>[22]</sup>. *Vibrio cholera* and *Vibrio parahaemolyticus* were also isolated from *T. adansonii* and according to Ababouch *et al.*,<sup>[23]</sup> they both cause gastrointestinal disease. It can hereby be deduced that eating of raw or unprocessed *T. adansonii* from this study area can result in outbreak of cholera *V. parahaemolyticus* infection. According to Powel *et al.*<sup>[24]</sup> *V. parahaemolyticus* has been responsible for several recent seafood-associated outbreaks and therefore considered an emerging bacterial pathogen in Europe.

The isolation of *Enterobacter Spp.* from the sample is an indication that consumption of unprocessed clam from this study area can result in enterobacter infections. Such infection include but not restricted to UTIs, bacteraemia, endocarditis, osteomyelitis etc. *Escherichia coli* isolation is an indicator for pathogenic organisms. The isolation of *E. coli* from *T. adansonii* in this study area is an indication of fecal contamination by man and his livestock. This is of public health significance as these organisms have generally been agent of gastroenteritis in humans<sup>[25,27]</sup>. For instance *E. coli* have been reported to be responsible

for foodborne illnesses and deaths<sup>[28]</sup> through poor processing and handling of foods or farm animals. *Bacillus cereus* is known to cause cutaneous disease. Its isolation from this sample is an indication that eating of unprocessed clam from this water body may result in skin infection as well as pneumonia, meningitis and necrotizing fasciitis<sup>[29]</sup>. *Chromatium Spp* was also isolated from the sample. It is not pathogenic, rather it is used in photosynthetic sulfur oxidation which is an indication that it entered the clam body via the water body.

The six (6) species of pathogenic fungi isolated from *T. adansonii* in this study area include: *Rhizopus stolonifer*, *Aspergillus niger*, *Penicillium Spp*, *Candida tropicalis*, *Fusarium Spp* and *Aspergillus flavus*. This shows that consumption of unprocessed clam from this study area can result in mucormycosis, rot, food spoilage, candidiasis, opportunistic mycosis and production of aflatoxins which can be of serious health implication to human and his livestock. According to Zhai, *et al.*<sup>[30]</sup> *C. tropicalis* can cause fatal digestive system disease and septicemia. *Aspergillus Spp* has been associated with outbreaks of seafood diseases known as nosocomial aspergillosis which is a serious threat for severely immune-compromised patients<sup>[31]</sup>. The level of mean THBC reported in this study was higher than the range reported by Udoh *et al.*<sup>[32]</sup> for *G. paradoxa* in Cross River Estuary and Volta Lake while TCC fell within the range<sup>[32]</sup>. Total heterotrophic bacteria and coliform may be directly influenced by many anthropogenic activities and rainfall. Run-off from rain might carry raw sewage from the surrounding villages and leachate from waste sites in the catchment area into the freshwater; the clams being filter feeders are able to accumulate the isolates in their tissues to level twice that in the surrounding waters<sup>[33]</sup>. This is in agreement with Antai<sup>[34]</sup> who reported that the high microbial load in the sample is a clear indication that clam serves as a medium through which microbes multiplied rapidly. Udoh, *et al.*<sup>[32]</sup> added that these species of microbes are demonstrating strong resistance to novel. Generally, microbial load was higher in the gut than other parts confirming the fact that clam should be degutted before processing for consumption. *Tagelus adansonii* are protein rich food and therefore serves as a suitable substrate in supporting growth of different types of bacteria and fungi; the microbial growth in these fresh seafood will encourage food spoilage and



seafood poisoning. On the other hand estuaries or rivers are constantly polluted with faecal matter from riverine dwellers. This finding agrees with the work of [35] that shellfish and coastal waters contaminated by human pathogens could be sources of shellfish-borne or water-borne outbreaks. In fact, shellfish can accumulate and concentrate microbial pathogens present in waters by their filter-feeding activities. The risk of disease from these agents varies by pathogen, dose, host and characteristics of the seafood matrix. The extent to which a microbial hazard is likely to be present in seafood and give rise to a public health and safety risk depends on numerous factors, including the biology of the particular seafood species, its growth environment and the specific activities along its production and processing supply chain [36]. This gives relevant information regarding the food safety and sanitary conditions of *T. adansonii* and the coastal water [37]. The results obtained from this study also showed that *T. adansonii* appears in the river between the months of February – January.

The moisture content of  $40.75 \pm 1.85$  obtained from the mollusk was lower than the range of 66.17 – 99.20% reported on snails, *Pila globosa* and Whelk found in Europe, Asia and Africa [38]. Comparison with other molluscs of economic importance shows that the moisture content was lower than that of oyster, clam, rough and smooth periwinkle and whelk which recorded 73.37, 73.72, 84.80, 80.22 and 60.97 percent respectively [1]. Moisture content of 79.60% – 81.20% had earlier been reported for periwinkle [38]. These variations could be due to the effect of environment as reported by Osibona *et al.* [39]. The ash content of  $5.99 \pm 0.42$  is similar to 5.84% observed by Obande *et al.* [40] for fresh water snail (*Pila ampullacea*) from River Benue, Nigeria and smaller compared to 6.85% for rough periwinkle to 14.02% for whelk [1]. This value can be attributed to the fact that aquatic mollusk absorbs more minerals from the water as rivers serve as effluent to some industries whose chemical waste discharge into the water body may increase the absolute minerals in the water. The crude fibre content of  $4.13 \pm 0.10\%$  was higher than the range observed by Eneji *et al.* [41] of 0.50-1.50% for land and water snails. It is also high as compared to other shellfishes [42]. The protein content of  $60.92 \pm 2.38$  of *T. adansonii* is higher than that of *Pila ampullacea* (10.40%), fresh water fishes and the giant land snail *Archchatina maginata* [40]. It is also far higher than 9.97% to 13.96% recorded for other aquatic

molluscs [1]. On the other hand, the value is similar to that ( $61.9 \pm 4.3\%$ ) reported by Rodríguez *et al.* [43] for mussels *T. peruvianus* from the Gulf of Nicoya, Puntarenas, Costa Rica. However, it is still comparable to values obtained in other livestock [44]. Bender [45] has responded that the amino acids in the protein of aquatic molluscs could be used to compliment the cereal sources of protein making good their relative deficiency of lysine. The result had shown that molluscs constitute a rich source of protein which according to Egonmwan [46], are of high biological value. Protein is the major structural component of cells and is responsible for the building and repair of body tissues. Thus, with increased consumption, the serious problem of protein deficiency can be mitigated. The Ether extract of  $5.56 \pm 0.51\%$  is higher than 0.09% in *P. ampullacea* [40] and other species of aquatic molluscs [1]. It is high as compared to other species of animals except mullet and octopus [41]. This could be related to location and origin of the mollusc [47]. Judith and Jenny [42] indicated that consumption of molluscs in large proportion reduced the risk of hypercholesterolemia which is capable of causing cardiovascular disease, due to its high omega-3 fatty acid content. The NFE value of  $26.58 \pm 2.91$  is higher than 0.92%, 0.72%, 0.55%, 0.26%, and 0.93% for oyster, clam, rough periwinkle, smooth periwinkle and whelk respectively reported by Kiin-kabari *et al.* [1] and 7.66% reported earlier by Obande *et al.*, [40] for the Fresh water snail (*Pila ampullacea*) from River Benue. High carbohydrate content indicates that high consumption of molluscs can be supplemented with low energy-rich foods to balance the energy-protein intake requirement. This is further supported with high caloric values ( $397.65 \pm 11.97$  kcal) recorded for this species of molluscs.

## 5. Conclusions

These findings showed that Knife clam contains considerable number of pathogenic microorganisms which are major sources of water borne diseases and death. It also contains considerable amount of protein and other nutrients, and high caloric value. From the foregoing, it has been established that the aquatic ecosystem is under threats of pollution and so clams as well as other aquatic food organisms must be processed properly before consumption. Considering the importance of shellfishes as primary protein source, proper monitoring and surveillance should be adopted by Government to check pollution of the aquatic environments.

## Authors' Contributions

Imefon Udo conceived the work, designed the experiment, supervised the work and prepared the manuscript. Dora Udoh carried out all microbiological analyses and also read and approved the manuscript. Otobong Isang did the sampling, carried out the proximate analysis and also approved the manuscript.

## Conflict of Interest

The authors have declared no conflict of interest for this work.

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