Bacteriological Load Analysis of *Moringa oleifera* Lam. Leaves Consumed in Guinea Savannah Vegetation Zones of Nigeria

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**Abstract**

Green leafy vegetables are consumed fresh or dried by human beings, for they are good sources of food rich in nutritional quality including beta-carotene, minerals, fibers and essential oils which play significant physiological role in human body including stimulating enzymes, reducing diseases such as diabetes, cancer and destroying bacteria such as *Salmonella* species, *Escherichia coli* and *Staphylococcus aureus*. The aim of this study is to evaluate the bacteriological load in *Moringa oleifera* Lam. leaves consumed in guinea savannah vegetation zones of Nigeria, via: Abuja (Gwagwalada market), in Southern guinea savannah, Katsina (Daura market), in Northern guinea savannah and Sokoto (Central market), in Sudan guinea savannah. Three (3) fresh and dried *Moringa oleifera* Lam. leafy samples each of 50 grams were randomly collected per market location for analysis of total viable cells (cfu/mL) using standard procedures of analyses. The bacterial load in each sample was determined in triplicates and analyzed with SPSS Version 16. Bacterial isolates were classified on the basis of cultural morphology, Gram reaction and Biochemical tests.

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Results showed that there was bacterial growth on Nutrient, Mannitol and MacConkey media. Sabouraud dextrose, Brilliant green and Salmonella-Shigella media recorded no growth in all the leave extracts analyzed. This could be ascribed to the selective nature of the Sabouraud dextrose, Brilliant green and Salmonella-Shigella media, and suggested that fungi/yeast, Salmonella species and Salmonella-Shigella species were not among the bacterial contaminants or that the active ingredient component-Pterygospermin, in Moringa oleifera leaves extract inhibited the growth of micro-organisms in the leaves extract. The bacterial load in the dried leave extracts increased by 86.70 – 88.96% compared with the fresh leave extracts. The highest viable cell count (12.2 x 10^4 ±6.95 cfu/mL) was recorded by Katsina dried leave, west of the market; while the lowest microbial load (1.0 x 10^4 ±0.68 cfu/mL) was reported by Katsina and Sokoto fresh leave extracts. The study recorded two pathogenic bacteria from all the locations, with Staphylococcus aureus being more dominating, followed by Escherichia coli. These are indicator organisms for poor hygienic conditions and suggests health hazards. Consumers and vegetable vendors should be educated on proper hygienic handling, transportation and storage of vegetables to avoid bacteriological food spoilage and other related health issues.

Keywords: Moringa oleifera leaves; Nutritional quality; Bacteriological load; Vegetables.

1. Introduction

Green leafy vegetables are rich in Vitamins, mineral elements, phytochemical compounds and antioxidants that the body needs to function effectively [1,2]. Vegetables are good source of food because of their riches in nutritional quality which include beta-carotene, ascorbic acid, minerals, fibers and essential oils which plays significant physiological role in human body as an antioxidant, stimulating enzymes, destroying bacteria and reducing diseases such as diabetes, Cardiac infarction and cancer. The phytochemical compounds in green leafy vegetables possess antimicrobial properties and it include alkaloids, anthraquiones, flavonoids, phenols, tannins, terpenoids and saponins [3]. Internal system of antioxidants exists in human body to get rid of excessive free radicals from metabolism, but exogenous/natural antioxidant which green leafy vegetables can provide is needed [4]. The vegetables, including Moringa leaves, either fresh or dried, are available, accessible, and affordable at the least costs to every household, including the rich and the poor [1,2]. Moringa oleifera Lam. leaves is consumed worldwide because of its nutritional quality including macro and micro nutrients, for medicinal purposes and industrial uses in water effluent treatments [5, 6, 7]. Different parts of Moringa oleifera Lam. plant are sources of proteins, vitamins, minerals and phytochemical compounds which exhibits pharmacological and biotechnological potentials. On the other hand, the leaves, flowers, pods and seeds of the plant are considered essential food source of high nutritional quality in developing countries such as Nigeria. Moringa oleifera Lam. leaves can be eaten cooked or fresh and can be stored dried for long period unrefrigerated without loss in nutrient quality. Reference [1] has documented that room/shade drying is the best processing method that preserves the nutritional quality of Moringa oleifera Lam. leaves. Moringa oleifera plant (Moringa or drumstick) is native to sub-Himalaya region of Northwest India. It is widely distributed throughout Africa, Southeast Asia, the Caribbean Islands and South America [8]. Health workers now treat malnutrition in small children and pregnant and nursing women with M. oleifera leave powder because of its nutrients. The iron content of the leave is very high and the powder is prescribed for the treatment of anemia in the Philippines [1,2,5,6,7,9]. M. oleifera leaves contain phenolics and flavonoids compounds which exhibits various biological
activity including antioxidants, anticarcinogenic, immunomodulatory, antidiabetes and the regulation of thyroid status [10]. Also, *Moringa oleifera* leaves is often the only source of protein, vitamins and minerals to the less privileged in the society and the leaves are used in the control of hypertension because the Na/K ratio content of the leaves is low [11, 12]. Sun drying is a traditional method of preservation of agricultural produce including vegetables such as *Moringa* leaves, grains, seeds and fruits in Africa [13]. Although, this practice is carried out under poor unhygienic conditions, it confers on agricultural produce storage stability, reduces losses, makes food available for consumption during scarcity, inhibits the growth of food spoilage microorganisms including bacteria, viruses, fungi, and parasites [1, 14]. The process is slow and takes much time to achieve the required food safety limit of food contaminants by World Health Organization [15]. Besides, the process is carried out in an opened poor unhygienic condition which enhances the increase of microbial contamination from the environment, human and animal activities [16, 17]. Among the drying methods: Room, Sun, Solar, Oven, Lyophilization, Commercial food dehydrator; [1], reported that room/shade drying is the best processing method that preserves the nutritional quality of *Moringa oleifera Lam.* leaves. Thus, [18, 19], reported that the microbial counts and pathogens isolated from commercially and conventionally produced fresh and dried fruits and vegetables are higher than the international accepted limits (10³ cfu g⁻¹ for fungi and 10¹ cfu g⁻¹ for bacteria). Similarly, [17, 20] have isolated pathogenic strains of *Salmonella* and *Escherichia coli* from home dried food products. Green leafy vegetables, either fresh or dried, are examples of few original processed food that carry high risk of contamination with pathogenic bacteria such as members of gram negative including *Escherichia coli*, *Salmonella species*, *Pseudomonas aeruginosa* and members of gram positive such as *Staphylococcus aureus*, and *Bacillus cereus* from the soil, human and animal excreta, water, harvest and processing procedures [21, 22, 23]. Thus, microbial loads in food stuffs are a measure of the degree of food contamination by microorganisms and related contaminants and this has been demonstrated by many researchers including [24], who reported mean log total bacteria count of 18.5; yeast and mould of 12.9 in wet cabbage; [25], documented microbial critical points of Saffron from farm in Iran, using two methods of sampling: hands and forceps picking. They recorded microbial mean of 2 X 10⁷±1.1X10⁵ for samples picked by forceps and 4.66 X10²±5X10¹ for samples picked by bear hands; [26], assessed microbial quality of 36 fresh vegetables from several regions of Ropar, Punjab, India. The major contaminants recorded include yeast and mould, and *E.coli*, in Cauliflower, pea, cabbage and potato. They further reported that the Microbial loads found in low economic area was significantly higher than the one recorded in high economic area; [27], investigated the bacteriological load of 5 fresh vegetables: Potato, Tomato, Cauliflower, Cucumber and Spinach, from Mandi at Dehradun, in India. They reported total viable cells found as follows: Cucumber: 5.8 x 10⁶ CFU/ml; Potato: 5.0 x 10⁶ CFU/ml; Tomato: 4.2 x 10⁸ CFU/ml; Cauliflower: 4.0 x 10⁸ CFU/ml and Spinach: 3.8 x 10⁸ CFU/ml. Organisms identified on basis of morphology, gram stains and negative stains, were *Entrobacter aerogenes*, *Serretia entomophila*, *Bacillus cereus*, *Listeria monocytogene*, *Proteus vulgaris and Micrococcus*; [28], enumerated microbial load in vegetables irrigated with sewage water in village Banur, in Patiala district, Punjab, India. They documented microbial load of mean values (MPN/100g) ranging from 353 x 10² in tomato (of organism *Lycopersicon esculentum var. esculentum*) to 605 x 10² in Radish (of organism *Raphanus sativus*). *Moringa oleifera* plant is abundant all over Nigeria, and the products serve multipurpose values to meet recent human challenges which include malnutrition, diseases, hunger, portable water, and employment [1, 2]. Traditional home drying of fruits and vegetables is practiced in guinea savannah vegetation zone of Nigeria, where drying processing of
vegetables is carried out under poor hygienic and sanitary practices due to lack of awareness, education, food safety and legislation. This lack of knowledge of good hygiene and sanitary processing of vegetables, creates high potential risk of microbial contamination and enhances easy transmission of pathogenic microorganisms to humans. However, the leaves of *Moringa oleifera* Lam are widely consumed in Nigeria, but the Bacteriological Load in *Moringa oleifera* Lam. leaves Consumed in Guinea Savannah Zones of Nigeria, via: Abuja (Gwagwalada market), in Southern guinea savannah, Katsina (Daura market), in Northern guinea savannah and Sokoto (Central market), in Sudan guinea savannah, has hardly been documented, and that is why this study was undertaken.

2. Materials and Methods

2.1 Sampling

Locally processed fresh and powdered leaves of *Moringa oleifera* were randomly collected from three locations in Guinea Savanna Vegetation Zones via: Abuja (Gwagwalada market) in Southern Guinea Savanna; Katsina (Daura market) in Northern Guinea Savanna, and Sokoto (Central market) in Sudan Guinea Savanna of Nigeria. Three fresh and powdered leave samples of approximately 50 g per market location (East, West and North) were collected aseptically into a sterile polythene zip bags for analysis.

2.2 Analytical Methods

Reference [18,29,30,31] methods were adopted for the microbial load analysis of the samples. Each parameter was determined in triplicates and their mean values recorded.

2.3. Microbial Load Analysis

Procedures of [18,29,30,31], were adopted for the enumeration, isolation and characterization of bacteria and fungi. Analysis of each analyte was done in triplicates and their means recorded.

2.4 Preparation of Media

Mannitol agar (selective for isolation of *Staphylococcus aureus*); Nutrient agar (general purpose, used for total bacteria count); MacConkey agar (Differential and selective, used for total coli form count); Sabouraud Dextrose agar (Selective, used for total fungi count); Brilliant green agar (selective for isolation of *Salmonella* species) and *Salmonella-Shigella* agar (*Salmonella-Shigella species*). All the media were prepared according to the manufacturers instruction and were autoclaved (XY-280A Model) at 121°C for 15 minutes under 1.6Kgcm² pressure.

2.5 Pre-treatment of Samples

Fresh leaves were the only pre-treated samples, since the other samples were in powdery form. The healthy fresh leaves were thoroughly washed 5 times with sterile distilled water (autoclaved) in a sterile stainless 60 cm
basin (surface sterilized with 70% alcohol), in order to remove extraneous substances on the leaves. Thereafter, the leaves were collected in a sterile plastic sieve (surface sterilized with 70% alcohol) to drain the water, then ready for analysis.

2.5.1 Sample Preparation

Each leave sample (fresh and powdered) was analyzed in triplicates. Twenty grams sample was weighed using aeAdam analytical balance, model N17250, and suspended in 80 ml of sterile 0.1% (w/v) peptone water (Oxoid, Cambridge, UK) in a 500 ml sterile plastic beaker and homogenized for two minutes on a vortex mixer. A four tenfold serial dilution was carried out on the supernatant of each sample in triplicates. A 10 ml of water sample was mixed with 90 ml of peptone water using vortex mixer. A serial dilution of $10^{-1}$ to $10^{-5}$ of each sample was pour-plated in triplicates on each specific medium.

2.6 Bacterial Count

One milliliter of the small volumes of the most diluted ($10^{-3}$ and $10^{-4}$) of each dilution of each sample, was pipetted separately into different sterile petri dishes containing 20 L of sterile molten medium of various specific media used for pour plating. The setup was mixed together by swirling and allowed to solidify. Thereafter, inoculated plates were incubated in an incubator (UK, Gallenkamp 340 model) at 37°C for 24 hours for total aerobic count, total coliform, Salmonella spp, Salmonella-Shigella spp and at 25°C for 5 days for Fungi/yeast. Plates with colonies between 20 to 300 were counted with the colony counter. Mannitol agar was used for the count of Staphylococcus aureus; Nutrient agar was used for total bacteria count; MacConkey agar was used for total coliform count; Sabouraud Dextrose agar was used for total fungi count; Brilliant green agar was used for Salmonella species count and Salmonella-Shigella agar was used for the count of Salmonella-Shigella species [18, 31].

2.6.1 Total Plate Count of Bacteria (CFU/mL)

Microbial load of each sample was determined as CFU/mL and was calculated from the expression:

$$\text{CFU/mL} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume of inoculums}}$$

2.6.2 Characterization and Identification of Isolates

The isolates were classified on the basis of cultural morphology, Gram reaction and Biochemical tests and matched against standard microbial cultures [18,30,31,33].

2.7 Statistical Analysis

The research experimental design was a factorial experiment fitted into a complete randomized design. Four treatments, three locations and two factors of three replicates each were involved. The data were subjected to statistical analyses to evaluate the differences between microbial loads of the samples. The data were analyzed
with the SPSS, version 16 for windows in a general linear model. The mean separation of data analyzed was done with the Duncan Multiple Range Test $P < 0.05$. The results were expressed as mean and standard error of means. The difference was considered significant at $P < 0.05$.

3. Results and Discussion

3.1 Microbial Load Analysis

Microbial load of food measures the degree of food contamination by micro-organisms and related contaminants. The mean values of microbial load analysis of *Moringa oleifera* Lam. leaves from three different locations in guinea savannah of Nigeria, is presented in Table 1. Bacterial growths were recorded in all the Nutrient, Mannitol and MacConkey agar media of leave extracts analyzed (Table 1). Nutrient media is a general-purpose media that allows the growth of many bacteria. Mannitol media is a selective media that allows the growth of *Staphylococcus species* and MacConkey media is a differential and selective media that allows the growth of coli form species. Sabouraud dextrose, Brilliant green and Salmonella- Shigella media recorded no growth in all the leave extracts analyzed (Table 1). This could be ascribed to the selective nature of the Sabouraud dextrose, Brilliant green and Salmonella- Shigella media, and suggested that fungi/yeast, Salmonella species and Salmonella-Shigella species were not among the bacterial contaminants or that the active ingredient component-Pterygospermin, in *Moringa oleifera* leaves extract inhibited the growth of micro-organisms in the leaves extract. However, numerous bacterial growths were observed in all the Nutrient, Mannitol and MacConkey media of leave extracts (Table 1). Mean bacterial count in nutrient agar varied between $2.6 \times 10^4 \pm 0.22$ to $3.8 \times 10^4 \pm 2.17$ cfu/mL fresh leave extracts and $10.6 \times 10^4 \pm 6.04$ to $12.2 \times 10^4 \pm 6.95$ cfu/mL dried leave extracts (Table 1). Similarly, bacterial mean count in mannitol agar ranged from $2.0 \times 10^4 \pm 1.14$ to $2.6 \times 10^4 \pm 1.48$ cfu/mL fresh leave extracts and $7.6 \times 10^4 \pm 4.33$ to $9.7 \times 10^4 \pm 5.53$ cfu/mL dried leave extracts (Table 1). Also, mean bacterial count recorded in MacConkey agar ranged between $1.0 \times 10^4 \pm 0.68$ to $1.2 \times 10^4 \pm 0.68$ cfu/mL fresh leave extracts and $1.6 \times 10^4 \pm 0.91$ to $3.4 \times 10^4 \pm 1.94$ cfu/mL dried leave extracts (Table 1). In all the incubated samples, the highest microbial load ($12.2 \times 10^4 \pm 6.95$ cfu/mL) was recorded in Katsina dried leave, west of the market; while the lowest microbial load ($1.0 \times 10^4 \pm 0.68$ cfu/mL) was reported by Katsina and Sokoto fresh leave extracts (Table 1). The study recorded two pathogenic bacteria from all the locations, with *Staphylococcus species* being more dominating followed by coli form species (Table 1). These are indicator organisms for poor hygienic conditions including improper handling, unhygienic transportation and improper storage. However, the bacterial counts in nutrients, Mannitol and MacConkey media of the study are higher than the international stipulated limits of $10^7$ cfu/mL in fresh and dried leafy vegetables [15, 34]. This is a suggestive of poor hygiene and sanitary conditions during processing. However, there is distinct statistical significance ($P<0.05$) among the recorded mean values of microbial loads of the analyzed leave samples when compared with the microbial load of fresh leave samples. It is observed that the results of the study agreed with the works of [24], who reported mean log total bacteria count of 18.5, in wet cabbage; [25], documented microbial mean of $2 \times 10^3 \pm 1.1 \times 10^3$ for samples picked by
Table 1: Mean count Microbial Loads(cfu/mL) of *Moringa oleifera* leaves at various locations

<table>
<thead>
<tr>
<th>Locations.</th>
<th>Samples.</th>
<th>Total Mean Colony Count on Nutrient agar.</th>
<th>Total Mean Colony Count on Mannitol agar.</th>
<th>Total Mean Colony Count on MacConkey agar.</th>
<th>Total Mean Colony count on Sabouraud dextrose agar.</th>
<th>Total Mean Colony Count on Brilliant agar.</th>
<th>Total Mean Colony Count on Salmonella-Shigella agar.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh leaves extract</td>
<td>3.8 x 10^4 ± 2.17a</td>
<td>2.6 x 10^4 ± 1.48a</td>
<td>1.2 x 10^4 ± 0.68a</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td>Abuja, Gwagwalada market.</td>
<td>Dried leaves extract, East of Market.</td>
<td>10.8 x 10^4 ± 6.16b</td>
<td>9.2 x 10^4 ± 9.24d</td>
<td>1.6 x 10^4 ± 0.91b</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td></td>
<td>Dried leaves extract, West of market.</td>
<td>11.0 x 10^4 ± 6.3c</td>
<td>8.3 x 10^4 ± 4.73c</td>
<td>2.7 x 10^4 ± 1.54c</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td></td>
<td>Dried leaves extract, North of Market.</td>
<td>10.6 x 10^4 ± 6.04b</td>
<td>7.6 x 10^4 ± 4.33b</td>
<td>3.4 x 10^4 ± 1.94d</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
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</tr>
<tr>
<td>Katsina, Daura Market.</td>
<td>Fresh leaves extract</td>
<td>2.6 x 10^4 ± 0.22a</td>
<td>2.0 x 10^4 ± 1.14a</td>
<td>1.0 x 10^4 ± 0.68a</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td></td>
<td>Dried leaves extract, East of Market.</td>
<td>11.3 x 10^4 ± 6.44c</td>
<td>8.9 x 10^4 ± 5.07b</td>
<td>2.8 x 10^4 ± 1.6b</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td></td>
<td>Dried leaves extract, West of Market.</td>
<td>12.2 x 10^4 ± 6.96d</td>
<td>9.6 x 10^4 ± 5.47c</td>
<td>3.2 x 10^4 ± 1.82c</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td></td>
<td>Dried leaves extract, North of Market.</td>
<td>10.9 x 10^4 ± 6.21b</td>
<td>8.8 x 10^4 ± 5.02b</td>
<td>2.1 x 10^4 ± 1.2b</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td>Sokoto, Central Market.</td>
<td>Fresh leaves extract</td>
<td>3.0 x 10^4 ± 1.71a</td>
<td>2.1 x 10^4 ± 1.2a</td>
<td>1.0 x 10^4 ± 0.68a</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td></td>
<td>Dried leaves extract, East of Market.</td>
<td>11.3 x 10^4 ± 6.44c</td>
<td>9.7 x 10^4 ± 5.53c</td>
<td>2.3 x 10^4 ± 1.31c</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td></td>
<td>Dried leaves extract, West of Market.</td>
<td>10.8 x 10^4 ± 6.16b</td>
<td>8.9 x 10^4 ± 5.53b</td>
<td>1.6 x 10^4 ± 0.91b</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td></td>
<td>Dried leaves extract, North of Market.</td>
<td>11.1 x 10^4 ± 6.33c</td>
<td>9.6 x 10^4 ± 5.47c</td>
<td>2.0 x 10^4 ± 1.14c</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
</tr>
</tbody>
</table>

Key: a-d Means in the same column but with different superscripts differ significantly (P< 0.05).

±SEM = Standard Error Mean. Values are means of three (3) determinations.
forceps and 4.66 x 10^2 ± 5 x 10^3 for samples picked by bear hands from the analysis of microbial critical points of saffron from farm in Iran, using two methods of sampling [26, 27], recorded bacteriological load in 5 fresh vegetables from Dehradun, India; Cucumber: 5.8 x 10^8 cfu/mL; Tomato: 4.2 x 10^8 cfu/mL; Spinach: 3.8 x 10^8 cfu/mL; Cauliflower: 4.0 x 10^8 cfu/mL. The Cultural Morphology, Gram reaction and Biochemical characteristics of bacteria isolates in *Moringa oleifera* leave extracts at various locations investigated are as presented in Table 2. Nutrient, Mannitol and MacConkey agars recorded bacterial colony in all leave extracts (Table 1). Sabouraud dextrose, Brilliant green and Salmonella-Shigella media recorded no growth in all the leaf extracts analyzed (Table 1). All the bacteria colony growth in nutrient agar showed mixed cultural morphology of creamy large, smooth surface, circle and pasty, slightly opalescent and amber in color, large colony, thick, greyish white, moist, opaque/translucent discs (Table 2). Bacteria colony growth in MacConkey agar showed cultural morphology of dry, donut shaped and dark pink, surrounded with dark pink area of precipitated bile salts (Table 2). Bacteria colony growth in Mannitol agar showed cultural morphology of yellow colonies with yellow zones (Table 2). Bacterial colony was gram stained in each of the media that recorded colony growth. Bacteria colony growth in nutrient agar showed mixed results of dark purple, clustered cocci and pink color bacilli, under the microscope, indicating gram positive cocci in clusters and gram-negative bacilli (Table 2). The bacterial colony tested positive to catalase, Methyl red, citrate, and negative to Oxidase, Voges Proskauer, biochemical tests confirming bacteria colony in nutrient media to be *Staphylococcus* species and coli form species (Table 2). Bacteria colony growth in MacConkey agar was pink colored bacilli, under the microscope, indicating gram negative bacilli (Table 2). The bacteria colony tested positive to catalase, Methyl red and negative to Oxidase and Voges Proskauer, biochemical tests confirming bacteria colony in MacConkey media *Escherichia coli* (Table 2). Bacteria colony growth in Mannitol agar was dark purple, clustered cocci, under the microscope, indicating gram positive cocci in clusters (Table 2). The bacteria colony in Mannitol agar tested positive to catalase, citrate, coagulase, Methyl red, Voges Proskauer and negative to Indole and Oxidase, biochemical tests confirming bacteria colony *Staphylococcus aureus* (Table 2). In conclusion, the bacteriological load of *Moringa oleifera* Lam. leaves consumed in the studied areas are *Escherichia coli* and *Staphylococcus aureus*.
### Table 2: Cultural Morphology, Gram Reaction and Biochemical Characteristics of Bacteria Isolates in Moringa oleifera leave Extracts at Various Locations

<table>
<thead>
<tr>
<th>Locations</th>
<th>Samples</th>
<th>Growth on Nutrient Agar</th>
<th>Growth on Mannitol Agar</th>
<th>Growth on MacConkey Agar</th>
<th>Growth on Sabouraud Agar</th>
<th>Growth on Brillant Green Agar</th>
<th>Growth on Salmonella Shigella Agar</th>
<th>Cultural Characteristics</th>
<th>Gram Reaction</th>
<th>Biochemical Tests</th>
<th>Bacteria Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abuja</td>
<td>Fresh leaves</td>
<td>Creamy large, Yellow Colonies, Dry, donut shape, Dark pink, Precipitated bile salt</td>
<td>No Growth</td>
<td>No Growth</td>
<td>Creamy large, Smooth surface, Amber, Greyish white, opaque discs</td>
<td>Yellow Colonies, Yellow zones, Dry, donut shape, Dark pink, Precipitated bile salt</td>
<td>Positive</td>
<td>+/+</td>
<td>+/-</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>Gwagwalada market</td>
<td>Extracts</td>
<td>Yellow Colonies, Smooth surface, Yellow zones</td>
<td>No Growth</td>
<td>No Growth</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Dried leave extracts, East of Market.</th>
<th>Creamy large, Yellow Colonies, Smooth surface, Yellow zones, Dark pink, Greyish white, opaque discs</th>
<th>Yellow Colonies, Yellow zones, Dry, donut shape, Dark pink, Precipitated bile salt</th>
<th>Yellow Colonies, Yellow zones, Dry, donut shape, Dark pink, Precipitated bile salt</th>
<th>Positive cocci in clusters and negative bacilli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried leave extracts, West of Market.</td>
<td>Creamy large, Yellow Colonies, Smooth surface, Yellow zones, Dark pink, Greyish white, opaque discs</td>
<td>Yellow Colonies, Yellow zones, Dry, donut shape, Dark pink, Precipitated bile salt</td>
<td>Yellow Colonies, Yellow zones, Dry, donut shape, Dark pink, Precipitated bile salt</td>
<td>Positive cocci in clusters and negative bacilli</td>
</tr>
</tbody>
</table>

*Staphylococcus aureus* and *Escherichia coli*
Dried leaves yield extracts, these extracts are used for the growth of bacteria. No growth was observed in the absence of bile salt. Positive results were obtained with Staphylococcus aureus and Escherichia coli, while negative results were obtained with other bacteria. The colonies were yellow in color, with a dry, donut shape. The zones around the colonies were yellow. The surface was smooth and the color was amber with a greyish-white hue. The discs were opaque, white, and bile salt precipitate was observed.
<table>
<thead>
<tr>
<th>Dried leave extracts, East of Market.</th>
<th>Creamy large, Smooth shape, Dark pink, Precipitated bile salt</th>
<th>Precipitate d bile salt</th>
<th>Positive</th>
<th>+/+</th>
<th>+/-</th>
<th>+/-</th>
<th>+/-</th>
<th>+</th>
<th>+</th>
<th>_</th>
<th>Staphylococcus aureus and Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Katsina Daura Market Fresh leaves extracts</td>
<td>Creamy large, Smooth shape, Dark pink, Precipitated bile salt</td>
<td>Precipitate d bile salt</td>
<td>Positive</td>
<td>+/+</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>_</td>
<td>Staphylococcus aureus and Escherichia coli</td>
</tr>
</tbody>
</table>

Dried leave extracts, East of Market.

Creamy large, Smooth shape, Dark pink, Precipitated bile salt.

Precipitated bile salt.

Positive

+/

+/-

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+/-

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Staphylococcus aureus and Escherichia coli

Katsina Daura Market Fresh leaves extracts.

Creamy large, Smooth shape, Dark pink, Precipitated bile salt.

Precipitated bile salt.

Positive

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+/-

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Staphylococcus aureus and Escherichia coli

Dried leave extracts, East of Market.

Creamy large, Smooth shape, Dark pink, Precipitated bile salt.

Precipitated bile salt.

Positive

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+/-

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Staphylococcus aureus and Escherichia coli

Katsina Daura Market Fresh leaves extracts.

Creamy large, Smooth shape, Dark pink, Precipitated bile salt.

Precipitated bile salt.

Positive

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Staphylococcus aureus and Escherichia coli

Dried leave extracts, East of Market.

Creamy large, Smooth shape, Dark pink, Precipitated bile salt.

Precipitated bile salt.

Positive

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Staphylococcus aureus and Escherichia coli

Katsina Daura Market Fresh leaves extracts.

Creamy large, Smooth shape, Dark pink, Precipitated bile salt.

Precipitated bile salt.

Positive

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+/-

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+/-

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Staphylococcus aureus and Escherichia coli

Dried leave extracts, East of Market.

Creamy large, Smooth shape, Dark pink, Precipitated bile salt.

Precipitated bile salt.

Positive

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+/-

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+/-

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+

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Staphylococcus aureus and Escherichia coli

Katsina Daura Market Fresh leaves extracts.

Creamy large, Smooth shape, Dark pink, Precipitated bile salt.

Precipitated bile salt.
| Dried leave Extracts, West of Market. | Creamy | Yellow Colonies, Dry, donut shape, | No Growth | No Growth | Creamy | Positive | +/+ | +/- | +/+ | +/- | + | + | _  |
|--------------------------------------|--------|----------------------------------|------------|------------|--------|----------|-----|-----|-----|-----|   |   | Staphylococcus aureus and Escherichia coli |
| white, opaque discs                  | large, | Yellow Colonies, Dry, donut shape, |            |            | large, |         |     |     |     |     |   |   |                                          |
|                                      | Smooth | surface, Yellow zones             |            |            | Smooth |         |     |     |     |     |   |   |                                          |
|                                      | Amber, | Amber, Greyish white,              |            |            | Amber, |         |     |     |     |     |   |   |                                          |
|                                      | Greyish| opaque discs                       |            |            | Greyish|         |     |     |     |     |   |   |                                          |

white, opaque discs, Yellow Colonies, Yellow zones, Dry, donut shape, Dark pink, Precipitated bile salt

No Growth

Positive cocc in clusters and Negative bacilli
<table>
<thead>
<tr>
<th>Dried leave extracts, North of Market.</th>
<th>Creamy large, yellow colonies, dry, donut shape, dark pink, precipitated bile salt</th>
<th>No growth</th>
<th>No growth</th>
<th>Creamy large, smooth surface, amber, greyish white, opaque discs, yellow colonies, yellow zones, dry, donut shape, dark pink, precipitated bile salt</th>
<th>Positive (+/+), (+/-), (+/+), (+/-), +, +, -</th>
<th>Staphylococcus aureus and Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sokoto Central Market</td>
<td>Creamy large, yellow colonies, dry, donut shape, dark pink, precipitated bile salt</td>
<td>No growth</td>
<td>No growth</td>
<td>Creamy large, smooth surface, amber, greyish white, opaque discs, yellow colonies, yellow zones, dry, donut shape, dark pink, precipitated bile salt</td>
<td>Positive (+/+), (+/-), (+/+), (+/-), +, +, -</td>
<td>Staphylococcus aureus and Escherichia coli</td>
</tr>
</tbody>
</table>

99
<table>
<thead>
<tr>
<th>Condition</th>
<th>Comment</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amber, Greyish white, opaque discs</td>
<td>Pink, Precipitated bile salt</td>
<td>Negative bacilli</td>
</tr>
<tr>
<td>Yellow Colonies, Yellow zones, Dry, donut shape, Dark pink, Precipitated bile salt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creamy large, Smooth surface, Amber, Greyish white, opaque discs</td>
<td>Yellow Colonies, Yellow zones</td>
<td>Positive cocci in clusters</td>
</tr>
<tr>
<td></td>
<td>Dry, donut shape, Amber, Greyish white, Precipitated bile salt</td>
<td>Negative bacilli</td>
</tr>
</tbody>
</table>

*Staphylococcus aureus* and *Escherichia coli*
<table>
<thead>
<tr>
<th>Creamy Large, Yellow Colonies</th>
<th>Yellow Zones, Dry, Donut Shape, Dark Pink, Precipitated Bile Salt</th>
<th>Creamy Large, Yellow Colonies, Yellow Zones, Dry, Donut Shape, Dark Pink, Precipitated Bile Salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smooth Surface, Amber, Greyish White, Opaque Discs</td>
<td>Smooth Surface, Amber, Greyish White, Precipitated Bile Salt</td>
<td>Smooth Surface, Amber, Greyish White, Precipitated Bile Salt</td>
</tr>
<tr>
<td></td>
<td>No Growth</td>
<td>No Growth</td>
</tr>
<tr>
<td></td>
<td>Positive Cocci in Clusters and Negative Bacilli</td>
<td>Positive Cocci in Clusters and Negative Bacilli</td>
</tr>
</tbody>
</table>

*Staphylococcus aureus and Escherichia coli*
Smooth surface, Amber, Greyish white, opaque discs

Yellow shape, Dark pink, Precipitated bile salt

Yellow Colonies, Yellow zones, Dry, donut shape, Dark pink, Precipitated bile salt

Smooth clusters and

Key: + = Positive; - = Negative
4. Conclusion

The results of this investigation revealed that the bacteriological load of *Moringa oleifera* Lam. leaves consumed in the studied areas are *Escherichia coli* and *Staphylococcus aureus*. This poses potential public health hazard to consumers as the samples fell short of meeting international food safety standard. Vegetable consumers and Vendors should be educated on proper hygienic handling, transportation and storage of vegetables to avoid bacteriological food spoilage and other related health issues. However, results from this study would be valuable for further risk assessment of the impact on human health of consuming agricultural produce, especially home dried seeds, fruits, grains and vegetables such as *Moringa oleifera* Lam. leaves. Among the bacteria pathogens isolated, *Staphylococcus aureus*, was the dominant bacteria. Bacteria contamination may be due to improper handling, storage and poor hygienic conditions.

5. Recommendations

Incidences of food borne diseases and infections caused by contaminated fresh/dried vegetables by microorganisms can be avoided by applying proper hygiene and sanitary practices. Traditional drying of fruits and vegetables including *Moringa oleifera* Lam. produce should be carried out under good hygienic conditions to avoid microbial contamination including enteric pathogens such as *Escherichia coli*, *Salmonella* and *Shigella* species. Vegetables such as Carrots, Tomatoes, Radishes, Cabbage, Cucumber, Lettuce, which are frequently consumed raw without proper processing must be thoroughly washed 3 to 4 times with clean water to remove extraneous materials, thereafter, are soaked in 0.85% Sodium Chloride solution for 5 to 10 minutes to eliminate pathogenic microorganisms. Then, are rinsed thoroughly in clean water for 3 to 4 times before consuming. Government policies to embrace measures that are focused on monitoring and evaluating food safety, good hygiene and sanitary practices through education, training and re-training programmes for food vendors and consumers in relation to food processing from farm to table.

6. Conflict of Interest

Authors declare no conflict of interest.

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