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*Full Length Research Paper*

# Prevalence of *Escherichia coli* O157:H7 in some animal products sold within Sokoto Metropolis, Nigeria

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***Escherichia coli* O157:H7 is among the most pathogenic of all known foodborne pathogens. It causes severe diarrhoea with apparently low infective dose (< 10 cells). This study aimed to determine the prevalence of *E. coli* O157:H7 in foods of animal sources sold in Sokoto Metropolis, Sokoto, Nigeria. A total of 175 samples were collected from different locations within Sokoto metropolis. Culture and biochemical characterisation revealed *E. coli* with an overall detection rate of 50.9% (89/175) with percentages of isolation rates of 30% (12/40), 75% (30/40), 43.6% (24/55) and 57.5% (23/40) for fresh milk, fermented milk, egg and raw meat respectively. Further characterization of the isolated *E. coli* on Sorbitol MarcConkey (SMAC) agar yielded *E. coli* O157:H7 strain with a positive detection rate of 31.4% (55/175) comprising 22.5% (9/40), 50.0% (20/40), 18.2% (10/55) and 40.0% (16/40) for fresh milk, fermented milk, egg and raw meat respectively. Molecular identification of shiga-toxin 1 (Stx I) and shiga-toxin 2 (Stx II) genes in the *E. coli* O157:H7 isolates by polymerase chain reaction (PCR) yielded 10 amplicons of Stx 1 genes and 6 amplicons of Stx II genes. The study confirmed the presence of toxigenic *E. coli* O157:H7 in animal products sold within Sokoto metropolis. The application of Hazard Analysis Critical Control Point (HACCP) protocol in the production processes is recommended to identify probable sources of microbial contaminants and to appropriately prevent contamination. The public should be enlightened on the zoonotic potential of this foodborne pathogen and the role of good hygiene practices in food safety.**

**Keywords:** *Escherichia coli* O157:H7, Shiga-like toxin, Sorbitol MacConkey agar.

## INTRODUCTION

*Escherichia coli* are Gram-negative, facultative anaerobic bacteria that belong to the family Enterobacteriaceae. The bacterium is typically rod-shaped and about 2 µm long and 0.5 µm in diameter. Some strains possess flagella which enable the bacterium to move (Xia et al.,

2010). The *E. coli* strains causing enteric diseases are categorized by their symptoms, virulence-factors, and the pathomechanisms that led to their categorization into various pathotypes such as enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), verotoxin-

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producing *E. coli* (VTEC), enteroaggregative *E. coli* (EAaggEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC) and the diffusely adherent *E. coli* (DAEC). *E. coli* strains responsible for urogenital infections (UPEC), and sepsis or meningitis of the neonates (MAEC) belong to the group causing extra-intestinal outbreaks. These pathogens are transmitted to humans through consumption of contaminated foods, such as raw or undercooked vegetables, meat, milk and their products (Castro et al., 2017). The most important common property of the pathogens is the possession of virulence-factors that are encoded on a variety of mobile genetic elements such as on plasmids, bacteriophages, transposons and pathogenicity islands. The harboured adhesins and toxins enable the colonisation of the intestinal mucosa, differently from the non-pathogenic members of the normal intestinal flora, thus their ability to cause a wide range of enteric infections (Schaeffer, 2012).

The first reported case of *E. coli* O157:H7 haemorrhagic colitis was in 1990. Since then, many sporadic cases of bloody diarrhoea have been reported in many areas of South Africa. Effer et al. (2001) reported verotoxigenic *E. coli* from South Africa in 1992, a large outbreak of bloody diarrhoea caused by *E. coli* O157:H7 infections occurred in Swaziland, South Africa. About 40,912 patients were suspected to be infected. The attack rate was 42% among 778 residents screened. Female gender and consumption of beef and untreated water were significant risks for the illness. *E. coli* O157:H7 was recovered from seven affected foci in Swaziland and South Africa, 27 out of 31 patients and environmental isolates had undistinguished pulsed field gel electrophoresis patterns. Cattle deaths also occurred due to verotoxigenic *E. coli*. Drought carriage of *E. coli* O157:H7 by cattle and heavy rains with contamination of surface water appear to be important factors contributing to verotoxigenic outbreaks. Molecular techniques were also used for studying the epidemiology of diarrhea infections due to *E. coli* in Gauteng region of South Africa.

In Nigeria, Akinyemi et al. (1998), studied *E. coli* infections for over 12 months. A total of 852 stool samples from patients (both children and adults) with acute diarrhoeal diseases attending some public and some government recognised health institutions in Lagos metropolis were screened for diarrhoeagenic bacterial agents. Of all 83 isolates for *E. coli* group, 49 (59%) were EPEC, 17 (20.5%), ETEC, 10 (12.1%) EIEC and seven (8.4%) EHEC. The EPEC strains particularly serotype O55, were mostly encountered in children aged over five years. On the other hand, EIEC and ETEC strains were found mainly in adults while EHEC O157:H7 strains occurred in all age group studied (Akinyemi et al., 1998). Olorunshola et al. (2000) examined the prevalence of sorbitol non-fermenting *E. coli* O157:H7 (EHEC) in 100 patients with diarrhoea by stool culture on sorbitol macConkey agar in Lagos, Nigeria. The authors reported 6%

detection rate of *E. coli* O157:H7 and five of the six patients were children below five years of age and a teenager. This study was aimed at determining the prevalence of *E. coli* O157:H7 in milk, egg and meat sold within Sokoto Metropolis, Sokoto, Nigeria.

## MATERIALS AND METHODS

### Study area

The study was conducted in Sokoto metropolis. The State lies within the semi-arid region of north-western Nigeria between longitudes 4°8' E and 6°54' E and latitudes 12°N and 13°58' N. It covers a total land area of about 32,000 square km. The estimated population of the State as at 2016 is about 5 million (NBS, 2017) with an estimated animal population of 1.8 million cattle, 2.6 million sheep, 2.9 million goats, 48,000 camels and variable species of poultry (RIMS, 1991; MAHF, 2012).

### Sample collection

A total of 175 samples comprised up of 40 samples each of fresh milk, fermented milk and raw meat and 55 eggs from different outlets within Sokoto metropolis were collected. Fresh and fermented milk samples were aseptically collected in a sterile screw-capped bottle from identified dairy farms within the metropolis and central dairy outlet popularly known as 'Kasuwar Gawo' respectively. Similarly, raw meat and eggs samples were aseptically collected from different outlets into a sterile polythene bag and in a clean crate respectively. All the samples collected were immediately transported in an insulating flask with ice packs to the Veterinary Microbiology laboratory for analyses.

### Culture, isolation and identification

All the samples collected were pre-enriched in buffered peptone broth. A gram of meat samples (already cut into small portions with a sterile blade) and 1 ml of both fresh and fermented milk samples and egg wash samples were pre-enriched by inoculating into 9mls of buffered-peptone broth each in different test tubes, homogenized and incubated at 37°C for 24 h. After incubation, a loopful inoculum from peptone broth was streaked onto MacConkey and Eosin Methylene Blue (EMB) agar plates and incubated at 37°C for 24 h. Those pinkish colonies on MacConkey and greenish metallic sheen appearance on EMB agar were presumptively identified as *E. coli* and thus, selected and subcultured for further phenotypic and biochemical analysis; these include Gram staining, Indole, methyl red (MR), Voges Proskauer (VP) and Citrate tests. Those Gram negative isolates that are Indole and MR tests Positive with VP and Citrate tests negative were confirmed as *E. coli*. Confirmed *E. coli* isolates were further characterized on Sorbitol macConkey agar for identification of *E. coli* O157:H7 from *E. coli* non-O157:H7 strains. Smooth and colourless colonies (Non-sorbitol fermenters) were phenotypically identified as *E. coli* O157:H7 as illustrated elsewhere (Safarikova and Safarik, 2001; Atikson et al., 2012).

### DNA extraction

The genomic DNA of identified *E. coli* O157:H7 isolates were extracted using boiling method as described elsewhere (Junior et

**Table 1.** Information on oligonucleotide primers used for PCR in the study.

Primer name	Primer sequence	Expected amplicon size (bp)
stx I	F 5'ATAAATCGCCATTCGTTGACTAC3'	180
	R 3'AGAACGCCCACTGAGATCATC5'	
stx II	F 5'GGCACTGTCTGAAACTGCTCC3'	255
	R 3'TCGCCAGTTATCTGACATTCT5'	

Paton and Paton (1996).

**Table 2.** Isolation rates of *E. coli* in food samples of animal origin in Sokoto metropolis, Sokoto.

Type of sample	No. samples collected	No. (%) of samples positive for <i>E. coli</i>	No. (%) of samples Negative for <i>E. coli</i>
Fresh milk	40	12 (30.0%)	28 (70.0%)
Fermented milk	40	30 (75.0%)	10 (25.0%)
Egg	55	24 (43.6%)	31 (56.4%)
Raw meat	40	23 (57.5%)	17 (42.5%)
<b>Total</b>	<b>175</b>	<b>89 (50.9%)</b>	<b>86 (49.1%)</b>

al., 2016). Briefly, a loopful of the 18-24 h old *E. coli* O157:H7 isolates was suspended in 200 µl of molecular-grade water in a microcentrifuge tubes. The suspension was heated in a water bath at 96°C for 30 min and centrifuged at 1300 rpm for 2 min. The supernatant (DNA templates in solution) was used as DNA template in polymerase chain reaction (PCR) techniques.

#### Detection OF *E. coli* O157:H7 virulence genes (STX I & STX II)

A multiplex PCR was conducted using TopTaq™ Master Mix PCR kit (Biolabs®) using extracted genomic DNA of *E. coli* O157:H7 isolates. The PCR was conducted with 25 µl reaction mixture containing TopTaq™ Master Mix (12.5 µl), RNase-free water (7.5 µl), DNA template 200 ng (2.5 µl) and 1 µl of four-primer cocktail (0.25 µM) (nucleotide sequence in Table 1). The primers are amplifying Stx1 (180 bp) and Stx2 (255 bp) genes respectively as adopted (Paton and Paton, 1996). Amplification was conducted in Geneamp 9700 PCR system (Applied Biosystem). The reaction mixtures were subjected to cycling parameters of 35 cycles of 1 min of denaturation at 95°C, 2 min of annealing at 65°C for the first 10 cycles, decrementing to 60°C by cycle 15, and 1.5 min of elongation at 72°C, incrementing to 2.5 min from cycles 25 to 35. Template DNA of a confirmed *E. coli* O157:H7 and sterile molecular-grade water were used as positive and negative controls respectively. Before loading samples into agarose-gel wells, 2 µl of DNA ladder was mixed with 2 µl of loading dye and dispensed in the first well. Subsequent wells were loaded with 5 µl samples of the PCR product and analyzed using 1.5% agarose gel electrophoresis and viewed in a documentation system (Gel Doc™ XR+, Bio- Rad).

#### Data analysis

The data were presented in tables and charts. Descriptive statistics were used to display the distribution of shiga-toxin 1 and 2 genes in the *E. coli* isolates.

## RESULTS

Out of the total (n=175) samples collected; which comprises fresh milk (n=40), fermented milk (n=40), egg (n=55) and raw meat (n=40). The overall prevalence of *E. coli* was 50.9 (89/175) with percentages isolation rates of 30% (12/40), 75% (30/40), 43.6% (24/55) and 57.5% (23/40) for fresh milk, fermented milk, egg and raw meat respectively (Table 2).

Out of total 89 *E. coli* isolates, 55 were identified as *E. coli* O157:H7 with an overall detection rate of 31.4% (55/175) which comprises of 22.5% (9/40), 50.0% (20/40), 18.2% (10/55) and 40.0% (16/40) for fresh milk, fermented milk, egg and raw meat respectively (Figure 1). DNA templates of the fifty-five *E. coli* O157:H7 isolates (previously isolated from food samples) were subjected to a polymerase chain reaction in search of shiga-toxin1 (*Stx I*) and shiga-toxin 2 (*Stx II*) genes which indicate Shiga-like toxin-producing *E. coli* O157:H7. The result of this study yielded 10 amplicons of *Stx I* genes and 6 amplicons of *Stx II* genes (Figure 2). The overall prevalence of Shiga-like toxin-producing *E. coli* O157:H7 stood at 5.7% (10/175) which comprises of 2.3% (4/175) those harbouring *Stx I* gene alone and 3.4% (6/175) harbouring *Stx I* and II together. This indicated that the remaining 94.3% (165/175) are non-toxicogenic *E. coli* O157:H7 (Table 3). Out of the nine *E. coli* O157:H7 isolates obtained from fresh milk samples, only one isolate is positive (and both *stx I* and *stx II* genes were amplified). Similarly, out of twenty *E. coli* O157:H7 isolates obtained from fermented milk samples, three showed positives for *Stx I* among which one (lane 5,

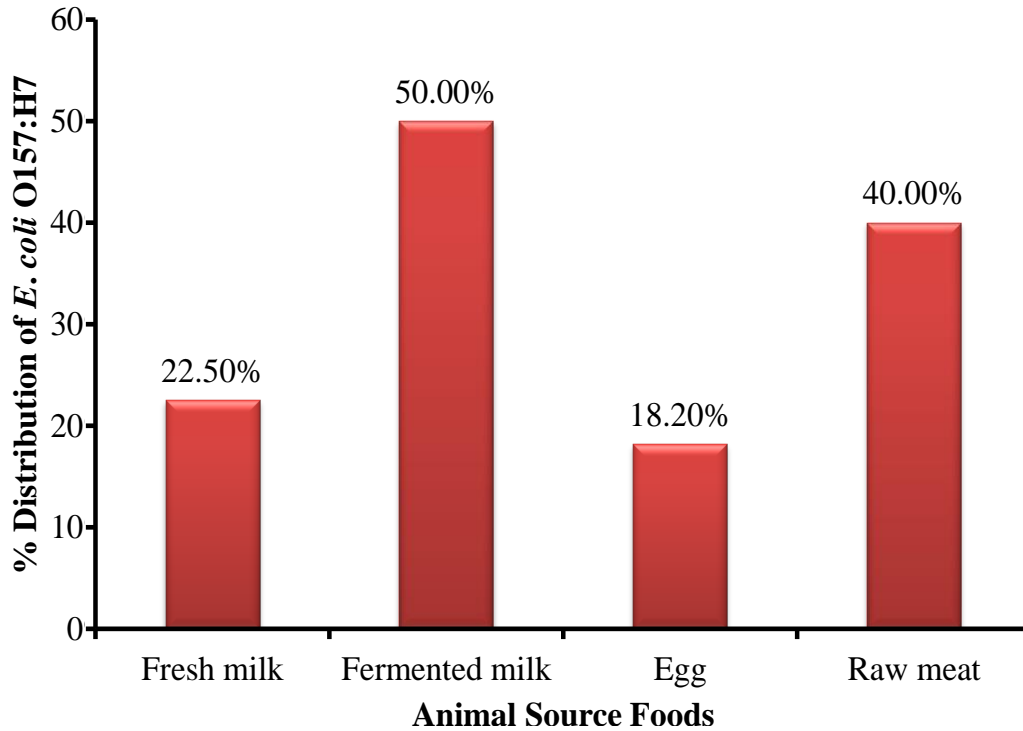


Figure 1. Distribution of *E. coli* O157:H7 in animal source foods sampled in Sokoto metropolis, Sokoto.

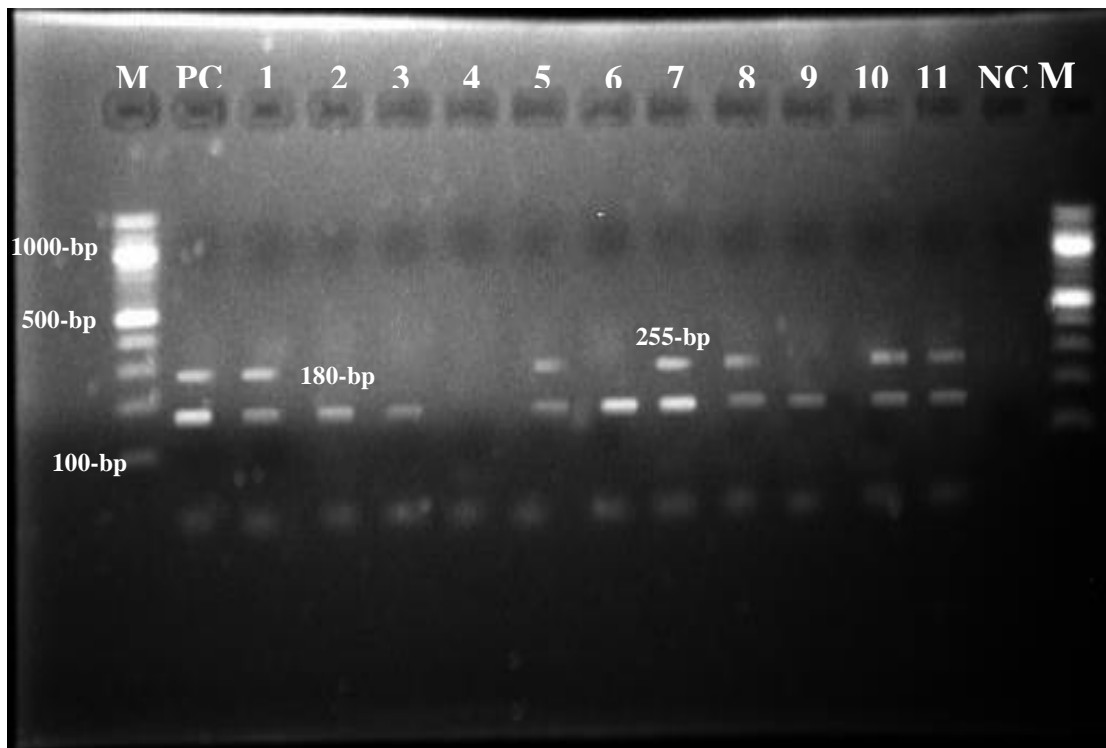


Figure 2. Multiplex PCR result of *stx* I (180 bp) and *stx* II (255bp) of *E. coli* O157:H7 isolated from foods of animal origin in Sokoto metropolis. M=100 bp DNA Ladder, PC=positive control, NC=negative control, Lane 1= an *E. coli* O157:H7 from fresh milk samples, Lane 2 to 5= *Escherichia coli* O157:H7 from fermented milk samples, Lane 6 = an *E. coli* O157:H7 from meat samples and Lane 7 to 11= *Escherichia coli* O157:H7 from egg samples

**Table 3.** Distribution of shiga-toxin I and II (*stx* I and *stx* II) genes in *E. coli* O157:H7 isolated from food of animal sources in Sokoto Metropolis.

Source of <i>E. coli</i> O157:H7	No. (%) positive for <i>Stx</i> I gene alone	No. (%) positive for both <i>Stx</i> I and II genes	No. (%) negative for both <i>Stx</i> I and II genes	Total
Fresh milk	0 (0.0%)	1 (2.5%)	39 (97.5)	40
Fermented milk	2 (5.0%)	1 (2.5%)	37(92.5)	40
Egg	1 (1.8%)	4 (7.3%)	50(90.9)	55
Raw meat	1 (2.5%)	0(0.0%)	39(97.5)	40
<b>Total</b>	<b>4 (2.3%)</b>	<b>6 (3.4%)</b>	<b>165(94.3)</b>	<b>175</b>

Figure 2) showed both amplification for *Stx*I and *Stx* II genes. Of the ten (10) *E. coli* O157:H7 isolates obtained from egg samples, five showed positive for *Stx*I gene among which four showed positive for both *Stx* I and *Stx*II genes. Lastly, out of the sixteen *E. coli* O157:H7 isolates gotten from meat samples, only one showed the presence of *Stx*I gene with no amplification in *Stx* II gene (Figure 2).

## DISCUSSION

*E. coli*, especially Shiga-toxin producing strains, are an important cause of diarrhoea and gastrointestinal illness in humans and animals especially young. Some of which are life-threatening such as haemolytic-uremic syndrome (an important cause of acute renal failure in children with morbidity and mortality in adults) and haemorrhagic colitis and thrombotic thrombocytopenic purpura (Al-Zogibi et al., 2015). Fresh and fermented milk is known to be widely consumed in both rural and urban areas in the study area. This might be due to its affordability and availability. Fermented milk is obtained from fresh milk that had undergone series of processing before finally converted to fermented milk, however, several cross contaminations do occur during collection (unclean hands of worker, unhygienic condition of utensils, and unclean water used for washing the utensils), handling, processing, transportation and marketing, therefore, exposes human population at risk of getting *E. coli* infection. Of economic importance, however, is the occurrence of pathogenic strains of *E. coli* O157:H7 in milk, meat and egg samples analysed in this study, which could be hazardous to consumers. The prevalence of *E. coli* O157:H7 in milk and milk products was found to vary between 1.0 and 11.0% (Reuben et al., 2002; Yakubu et al., 2018). The survival of this pathogen in low pH milk derivatives has also been documented in the various literatures (Reuben et al., 2002).

The method of handling, transporting and marketing of the raw meat and eggs are unhygienic. Similarly, the raw meat and eggs fall on an easy prey to bacterial contamination because of the high ambient temperature of Sokoto state. Such condition could pose favorable

environment for bacterial contamination of the product. Raw beef, vegetables and milk products have been described as the principal vehicle of *E. coli* O157:H7 transmission to humans (Reuben et al., 2002; Castro et al., 2017; IFSAC, 2019). Global testing of beef had shown *E. coli* O157:H7 prevalence that ranges between 0.1 and 54.0% (Chapman et al., 2001; Hussein and Bollinger, 2005) and had been isolated from retail meat samples in many developing countries such as Thailand (Vuddhakul et al., 2000), South Africa (Mukhufhi et al., 2004), Saudi Arabia (Al-Humam, 2019) and in Algeria (Chahed et al., 2006), with prevalence that ranges between 4 and 9%.

## CONCLUSION AND RECOMMENDATIONS

The results obtained from this study confirmed the presence of *E. coli* O157:H7 in some food items of animal origin (milk, eggs and meat) traded for human consumption in Sokoto metropolis. The presence of both *Stx* I or *Stx* I and *Stx* II affirmed the virulence of the *E. coli* O157:H7 strains isolated in this study. The prevalence of *Stx* I (10 amplicons) was found to be more as compared to *Stx* II (6 amplicons) which is a peculiar characteristic of the genes when amplified. The milk, eggs and meat could be contaminated with these pathogens along the production line, during storage or in the course of transportation. This situation highlights a serious concern and threat to public health as naïve and less immunocompetent hosts (young, elderly and immunodeficient individuals) may fall prey to this pathogens. The research findings pointed at the need for total overhaul of the existing methods of milk production in the study area. This may include the application of hazard analysis critical control point (HACCP) to guide the identification of probable sources of contaminations in order to ascertain, mitigate and outline prevention measures. Primary health workers could be trained on hazard analysis critical control to ensure improvement in food hygiene for the upliftment of health standard of individuals. Engaging Healthcare workers, Veterinary extension officers and environmental health workers in a “One-health” approach should be encouraged to monitor

the progress in identifying and preventing microbial contamination of the products. Health workers and the general public need to be enlighten on the zoonotic potentials of this organism and importance of strict hygiene practices in controlling its transmission.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# **Bacteriological load analysis of *Moringa oleifera* Lam. leaves consumed in Guinea Savannah vegetation zones of Nigeria**

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The aim of this study was to evaluate the bacteriological load in *Moringa oleifera* Lam. leaves consumed in Guinea Savannah vegetation zones of Nigeria, via: Abuja (Gwagwalada market), Southern Guinea Savannah; Katsina (Daura market), Northern Guinea Savannah and Sokoto (Central market), Sudan Guinea Savannah. Three fresh and dried *M. oleifera* Lam. leafy samples each of 50 g 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. Results showed bacterial growth on Nutrient, Mannitol and MacConkey media. Sabouraud dextrose, Brilliant green and *Salmonella-Shigella* media recorded no growth in all the leaf 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* spp. and *Salmonella-Shigella* species were not among the bacterial contaminants or that the active ingredient component-Pterygosperrin, in *M. oleifera* leaves extract inhibited the growth of micro-organisms in the leaves extract. The study recorded two pathogenic bacteria from all the locations, with *S. aureus* being more dominating, followed by *Escherichia coli* and these organisms suggest 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.

**Key words:** *Moringa oleifera* leaves, nutritional quality, bacteriological load, vegetables.

## **INTRODUCTION**

Vegetables are good source of food because of their riches in nutritional quality which include beta- carotene,

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ascorbic acid, minerals, fibers and essential oils which play significant physiological role in human body as an antioxidant, stimulating enzymes, destroying bacteria and reducing diseases such as heart and cancer. The phytochemical compounds in green leafy vegetables possess antimicrobial properties and include alkaloids, anthraquinones, flavonoids, phenols, tannins, terpenoids and saponins (Paulsamy and Jeeshua, 2011). 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 (Yanishlieva et al., 2006).

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 (Osuagwu et al., 2014; Monica et al., 2015). *Moringa oleifera* Lam. leaf is consumed worldwide because of its nutritional quality including macro and micro nutrients, for medicinal purposes and industrial uses in water effluent treatments (Joshi and Mehta, 2010; Moyo et al., 2011; Xiaopin et al., 2011). Different parts of *M. oleifera* Lam. plant are sources of proteins, vitamins, minerals and phytochemical compounds which exhibit 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. *M. oleifera* Lam. leaves can be eaten cooked or fresh and can be stored dried for long period unrefrigerated without loss in nutrient quality. Osuagwu et al. (2014), documented that room/shade drying is the best processing method that preserves the nutritional quality of *M. oleifera* Lam. leaves.

*M. 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 (Miracle Tree, 2014). Health workers now treat malnutrition in small children and pregnant and nursing women with *M. oleifera* leaf powder because of its nutrients. The iron content of the leaf is very high and the powder is prescribed for the treatment of anemia in the Philippines (Dhaka et al., 2011; Joshi and Mehta, 2010; Moyo et al., 2011; Xiaopin et al., 2011; Osuagwu et al., 2014; Monica et al., 2015). *M. oleifera* leaves contain phenolics and flavonoids compounds which exhibits various biological activity including antioxidants, anticarcinogenic, immunomodulatory, antidiabetes and the regulation of thyroid status (Jung, 2014). Also, *M. 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 (Fahey, 2005; Kasolo et al., 2010).

Sun drying is a traditional method of preservation of agricultural produce including vegetables such as *Moringa*

leaves, grains, seeds and fruits in Africa (Wilhelm et al., 2004). 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 (Osuagwu et al., 2014; Karam et al., 2016). The process is slow and takes much time to achieve the required food safety limit of food contaminants by World Health Organization (Food Safety Programme, 2002). 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 (Vivas et al., 2010; Beuchat et al., 2013). Among the drying methods: room, sun, solar, oven, lyophilization, commercial food dehydrator, Osuagwu et al. (2014), reported that room/shade drying is the best processing method that preserves the nutritional quality of *M. oleifera* Lam. leaves. Thus, Witthuhn et al. (2005) and Barkari-Golan and Paster (2011), reported that the microbial cells count and pathogens isolated from commercially and conventionally produced fresh and dried fruits and vegetables are higher than the international accepted limits ( $10^3$  CFUg<sup>-1</sup> for fungi and  $10^1$  CFUg<sup>-1</sup> for bacteria). Similarly, Beuchat et al. (2013) and Finn et al. (2013) have isolated pathogenic strains of *Salmonella* and *E. 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 (Braga et al., 2005; Pandey and Singh, 2011; Sapkota et al., 2012). 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 Bhila et al. (2010), reported mean log total bacteria count of 18.5; yeast and mould of 12.9 in wet cabbage; Khazaei et al. (2011), documented microbial critical points of Saffron from farm in Iran, using two methods of sampling: hands and forceps picking. Recorded microbial mean of  $2 \times 10^2 \pm 1.1 \times 10^3$  for samples picked by forceps and  $4.66 \times 10^2 \pm 5 \times 10^3$  for samples picked by bear hands; Kumar et al. (2013), 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; Pinky and Nishant (2015) 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 count found as follows: cucumber:  $5.8 \times 10^8$  CFU/ml; potato:  $5.0 \times 10^8$  CFU/ml; tomato:  $4.2 \times 10^8$  CFU/ml; cauliflower:  $4.0 \times 10^8$  CFU/ml; and spinach:  $3.8 \times 10^8$  CFU/ml. Organisms identified on the basis of morphology, Gram stains and negative stains, were *Enterobacter aerogenes*, *Serratia entomophila*, *B. cereus*, *Listeria monocytogene*, *Proteus vulgaris* and *Micrococcus*; Singla and Kamboj (2017), 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/100 g) ranging from  $353 \times 10^2$  in tomato (of organism *Lycopersicon esculentum var. esculentum*) to  $605 \times 10^2$  in Radish (of organism *Raphanus sativus*).

*M. 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 (Osuagwu et al., 2014; Monica et al., 2015). 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 *M. oleifera* Lam are widely consumed in Nigeria, but the bacteriological load in *M. 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.

## MATERIALS AND METHODS

### Sampling

Locally processed fresh and powdered leaves of *M. 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 leaf samples of approximately 50 g per market location (East, West and North) were collected aseptically into a sterile polythene zip bags for analysis.

### Analytical methods

APHA (2001), Witthuhn et al. (2005); Gupta et al. (2010) and Ntuli et al. (2013) methods were adopted for the microbial load analysis of the samples. Each parameter was determined in triplicates and

their mean values recorded.

### Microbial load analysis

Procedures of APHA (2001), Witthuhn et al. (2005), Gupta et al. (2010) and Ntuli et al. (2013), were adopted for the enumeration, isolation and characterization of bacteria and fungi. Analysis of each analyte was done in triplicates and their means recorded.

### Preparation of media

Mannitol agar (selective for isolation of *S. 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 manufacturer's instruction and were autoclaved (XY-280A Model) at 121°C for 15 min under 1.6 kg.cm<sup>2</sup> pressure.

### 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 for 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.

### Sample preparation

Each leaf 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 2 min 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.

### 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 mL 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 h 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 and 300 were counted with the colony counter. Mannitol agar was used for the count of *S. aureus*; Nutrient agar was used for total bacteria count; MacConkey agar was used for total coli form count; Sabouraud Dextrose agar was used for total fungi count; Brilliant green agar was used for *Salmonella* spp. count and *Salmonella-Shigella* agar



**Table 1.** Mean count microbial loads (cfu/mL) of *Moringa oleifera* leaves at various locations.

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

<sup>a-d</sup>Means in the same column but with different superscripts differ significantly ( $P < 0.05$ ).  $\pm$ SEM = Standard Error Mean. Values are means of three (3) determinations.

was used for the count of *Salmonella-Shigella* spp. (Witthuhn et al., 2005; Ntuli et al., 2013).

#### Total plate count of bacteria (CFU/mL)

Microbial load of each sample was determined as CFU/mL and was calculated from the expression: CFU/ml = Number of colonies  $\times$  Dilution factor / Volume of inoculums (Prescott et al., 2002).

#### Characterization and identification of isolates

The isolates were classified on the basis of cultural morphology (opaque/translucent discs, donut shape and dark pink, creamy large smooth surface, yellow colonies with yellow zones, thick greyish white, opalescent and amber in color), Gram reaction and Biochemical tests and matched against standard microbial cultures (Witthuhn et al., 2005; Cheesebrough, 2005; Gupta et al., 2010; Ntuli et al., 2013).

#### 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 analysis 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 mean. The difference was considered significant at  $P < 0.05$ .

## RESULTS AND DISCUSSION

#### 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 *M. 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* spp. and *Salmonella-Shigella*

spp. were not among the bacterial contaminants or that the active ingredient component-Pterygospermin, in *M. oleifera* leaves extract inhibited the growth of microorganisms 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  $1.0 \times 10^4 \pm 0.68$  and  $1.2 \times 10^4 \pm 0.68$  cfu/mL fresh leave extract and  $1.6 \times 10^4 \pm 0.91$  to  $3.4 \times 10^4 \pm 1.94$  cfu/mL dried leave extract (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* spp. being more dominating followed by coli form species (Table 1). These are indicator organisms for poor hygienic conditions. However, the bacterial counts in nutrients, Mannitol and MacConkey media of the study are higher than the international stipulated limits of  $10^1$  cfu/mL in fresh and dried leafy vegetables (Food Safety Programme, 2002; FAO/WHO, 2014). 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 Bhila et al. (2010), who reported mean log total bacteria count of 18.5, in wet cabbage; Khazei et al. (2011), documented microbial mean of  $2 \times 10^2 \pm 1.1 \times 10^3$  for samples picked by forceps and  $4.66 \times 10^2 \pm 5 \times 10^3$  for samples picked by bear hands from the analysis of microbial critical points of saffron from farm in Iran, using two methods of sampling; Kumar et al. (2013) and Pinky and Nishant (2015), recorded bacteriological load in 5 fresh vegetables from Dehradun, India; Cucumber:  $5.8 \times 10^8$  cfu/mL; Tomato:  $4.2 \times 10^8$  cfu/mL; Spinach:  $3.8 \times 10^8$  cfu/mL; Cauliflower:  $4.0 \times 10^8$  cfu/mL.

The cultural morphology, Gram reaction and biochemical characteristics of bacteria isolates in *M. oleifera* leave extracts at various locations investigated are 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 leave 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, and 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* spp. 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 *E. 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, and biochemical tests confirming bacteria colony *S. aureus* (Table 2). In conclusion, the bacteriological load of *M. oleifera* Lam. leaves consumed in the studied areas are *E. coli* and *S. aureus*.

## Conclusion

The results of this investigation reveal that the bacteriological load of *M. oleifera* Lam. leaves consumed in the studied areas are *E. coli* and *S. 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 *M. oleifera* Lam. leaves. Among the bacteria pathogens isolated, *S. aureus*, was the dominant bacteria. Bacteria contamination may be due to improper handling, storage and poor hygienic conditions.

**Table 2.** Cultural morphology, Gram reaction and biochemical characteristics of bacteria isolates in *Moringa oleifera* leave extracts at various locations.

Location	Samples	Growth on Nutrient agar	Growth on Mannitol agar	Growth on MacConkey agar	Growth on Sabouraud agar	Growth on Brilliant Green agar	Growth on Salmonella-Shigella agar	Cultural characteristics	Gram reaction
Abuja Gwagwalada market	Fresh leaves Extracts	Creamy large, Smooth surface, Amber, Greyish white, opaque discs	Yellow Colonies, Yellow zones	Dry, donut shape, Dark pink, Precipitated bile salt	No Growth	No Growth	No Growth	Creamy large, Smooth surface, Amber, Greyish white, opaque discs, Yellow Colonies, Yellow zones, Dry, donut shape, Dark pink, Precipitated bile salt	Positive cocci in clusters and Negative bacilli
	Dried leave extracts, East of Market.	Creamy large, Smooth surface, Amber, Greyish white, opaque discs	Yellow Colonies, Yellow zones	Dry, donut shape, Dark pink, Precipitated bile salt	No Growth	No Growth	No Growth	Creamy large, Smooth surface, Amber, Greyish white, opaque discs, Yellow Colonies, Yellow zones, Dry, donut shape, Dark pink, Precipitated bile salt	Positive cocci in clusters and Negative bacilli
	Dried leave Extracts, West of Market.	large, Smooth surface, Amber, Greyish white, opaque discs	Yellow Colonies, Yellow zones	Dry, donut shape, Dark pink, Precipitated bile salt	No Growth	No Growth	No Growth	Creamy large, Smooth surface, Amber, Greyish white, opaque discs, Yellow Colonies, Yellow zones, Dry, donut shape, Dark pink, Precipitated bile salt	Positive cocci in clusters and Negative bacilli
	Dried leave Extracts, North of Market.	Creamy large, Smooth surface, Amber, Greyish white, opaque discs	Yellow Colonies, Yellow zones	Dry, donut shape, Dark pink, Precipitated bile salt	No Growth	No Growth	No Growth	Creamy large, Smooth surface, Amber, Greyish white, opaque discs, Yellow Colonies, Yellow zones, Dry, donut shape, Dark pink, Precipitated bile salt	Positive cocci in clusters and Negative bacilli
Katsina Daura Market	Fresh leaves Extracts	Creamy large, Smooth surface, Amber, Greyish white, opaque discs	Yellow Colonies, Yellow zones	Dry, donut shape, Dark pink, Precipitated bile salt	No Growth	No Growth	No Growth	Creamy large, Smooth surface, Amber, Greyish white, opaque discs, Yellow Colonies, Yellow zones, Dry, donut shape, Dark pink, Precipitated bile salt	Positive cocci in clusters and Negative bacilli
	Dried leave extracts, East of Market.	Creamy large, Smooth surface, Amber, Greyish white, opaque discs	Yellow Colonies, Yellow zones	Dry, donut shape, Dark pink, Precipitated bile salt	No Growth	No Growth	No Growth	Creamy large, Smooth surface, Amber, Greyish white, opaque discs, Yellow Colonies, Yellow zones, Dry, donut shape, Dark pink, Precipitated bile salt	Positive cocci in clusters and Negative bacilli

Table 2. Contd.

Location	Samples	Growth on Nutrient agar	Growth on Mannitol agar	Growth on MacConkey agar	Growth on Sabouraud agar	Growth on Brilliant Green agar	Growth on Salmonella-Shigella agar	Cultural characteristics	Gram reaction
	Dried leave Extracts, West of Market.	Creamy large, Smooth surface, Amber, Greyish white, opaque discs	Yellow Colonies, Yellow zones	Dry, donut shape, Dark pink, Precipitated bile salt	No Growth	No Growth	No Growth	Creamy large, Smooth surface, Amber, Greyish white, opaque discs, Yellow Colonies, Yellow zones, Dry, donut shape, Dark pink, Precipitated bile salt	Positive cocci in clusters and Negative bacilli
	Dried leave Extracts, North of Market.	Creamy large, Smooth surface, Amber, Greyish white, opaque discs	Yellow Colonies, Yellow zones	Dry, donut shape, Dark pink, Precipitated bile salt	No Growth	No Growth	No Growth	Creamy large, Smooth surface, Amber, Greyish white, opaque discs, Yellow Colonies, Yellow zones, Dry, donut shape, Dark pink, Precipitated bile salt	Positive cocci in clusters and Negative bacilli
		Creamy large, Smooth surface, Amber, Greyish white, opaque discs	Yellow Colonies, Yellow zones	Dry, donut shape, Dark pink, Precipitated bile salt	No Growth	No Growth	No Growth	Creamy large, Smooth surface, Amber, Greyish white, opaque discs, Yellow Colonies, Yellow zones, Dry, donut shape, Dark pink, Precipitated bile salt	Positive cocci in clusters and Negative bacilli
Sokoto Central Market		Creamy large, Smooth surface, Amber, Greyish white, opaque discs	Yellow Colonies, Yellow zones	Dry, donut shape, Dark pink, Precipitated bile salt	No Growth	No Growth	No Growth	Creamy large, Smooth surface, Amber, Greyish white, opaque discs, Yellow Colonies, Yellow zones, Dry, donut shape, Dark pink, Precipitated bile salt	Positive cocci in clusters and Negative bacilli
		Creamy large, Smooth surface, Amber, Greyish white, opaque discs	Yellow Colonies, Yellow zones	Dry, donut shape, Dark pink, Precipitated bile salt	No Growth	No Growth	No Growth	Creamy large, Smooth surface, Amber, Greyish white, opaque discs, Yellow Colonies, Yellow zones, Dry, donut shape, Dark pink, Precipitated bile salt	Positive cocci in clusters and Negative bacilli
		Creamy large, Smooth surface, Amber, Greyish white, opaque discs	Yellow Colonies, Yellow zones	Dry, donut shape, Dark pink, Precipitated bile salt	No Growth	No Growth	No Growth	Creamy large, Smooth surface, Amber, Greyish white, opaque discs, Yellow Colonies, Yellow zones, Dry, donut shape, Dark pink, Precipitated bile salt	Positive cocci in clusters and Negative bacilli
		Creamy large, Smooth surface, Amber, Greyish white, opaque discs	Yellow Colonies, Yellow zones	Dry, donut shape, Dark pink, Precipitated bile salt	No Growth	No Growth	No Growth	Creamy large, Smooth surface, Amber, Greyish white, opaque discs, Yellow Colonies, Yellow zones, Dry, donut shape, Dark pink, Precipitated bile salt	Positive cocci in clusters and Negative bacilli

Table 2. Contd.

Location	Samples	Growth on Nutrient agar	Growth on Mannitol agar	Growth on MacConkey agar	Growth on Sabouraud agar	Growth on Brilliant Green agar	Growth on <i>Salmonella</i> - <i>Shigella</i> agar	Cultural characteristics	Gram reaction
		Biochemical tests							Bacteria Isolates
		Catalase	oxidase	Methyl red	Voges Proskauer	Citrate	Coagulase	Indole	
Abuja Gwagwalad a market	Fresh leaves extracts	+/+	+/-	+/+	+/-	+	+	-	<i>Staphylococcus aureus</i> and <i>Escherichia coli</i>
	Dried leave extracts, East of Market.	+/+	+/-	+/+	+/-	+	+	-	<i>Staphylococcus aureus</i> and <i>Escherichia coli</i>
	Dried leave Extracts, West of Market.	+/+	+/-	+/+	+/-	+	+	-	<i>Staphylococcus aureus</i> and <i>Escherichia coli</i>
	Dried leave Extracts, North of Market.	+/+	+/-	+/+	+/-	+	+	-	<i>Staphylococcus aureus</i> and <i>Escherichia coli</i>
Katsina Daura Market	Fresh leaves Extracts	+/+	+/-	+/+	+/-	+	+	-	<i>Staphylococcus aureus</i> and <i>Escherichia coli</i>
	Dried leave extracts, East of Market.	+/+	+/-	+/+	+/-	+	+	-	<i>Staphylococcus aureus</i> and <i>Escherichia coli</i>
	Dried leave Extracts, West of Market.	+/+	+/-	+/+	+/-	+	+	-	<i>Staphylococcus aureus</i> and <i>Escherichia coli</i>
	Dried leave Extracts, North of Market.	+/+	+/-	+/+	+/-	+	+	-	<i>Staphylococcus aureus</i> and <i>Escherichia coli</i>
Sokoto Central Market		+/+	+/-	+/+	+/-	+	+	-	<i>Staphylococcus aureus</i> and <i>Escherichia coli</i>
		+/+	+/-	+/+	+/-	+	+	-	<i>Staphylococcus aureus</i> and <i>Escherichia coli</i>
		+/+	+/-	+/+	+/-	+	+	-	<i>Staphylococcus aureus</i> and <i>Escherichia coli</i>
		+/+	+/-	+/+	+/-	+	+	-	<i>Staphylococcus aureus</i> and <i>Escherichia coli</i>

+ = Positive; - = Negative.

## RECOMMENDATIONS

Incidences of food borne diseases and infections caused by contaminated fresh/dried vegetables by micro-organisms can be avoided by applying proper hygiene and sanitary practices. Traditional drying of fruits and vegetables including *M. oleifera* Lam. produce should be carried out under good hygienic conditions to avoid microbial contamination including enteric pathogens such as *E. coli*, *Salmonella* and *Shigella* spp. Vegetables such as carrots, tomatoes, radishes, cabbage, cucumber, and 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 min to eliminate pathogenic microorganisms. Then, are rinsed thoroughly in clean water for 3 to 4 times before consumption. 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.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interest

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