

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-Pterygospermin, 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; Xiaompin 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; Xiaompin 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⁻¹ for fungi and 10^1 CFUg⁻¹ 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: 5.8×10^8 CFU/ml; potato: 5.0×10^8 CFU/ml; tomato: 4.2×10^8 CFU/ml; cauliflower: 4.0×10^8 CFU/ml; and spinach: 3.8×10^8 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 353×10^2 in tomato (of organism *Lycopersicon esculentum var. esculentum*) to 605×10^2 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.cm² 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

^{a-d}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 leaf, 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 leaf 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 leaf samples when compared with the microbial load of fresh leaf 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: 5.8×10^8 cfu/mL; Tomato: 4.2×10^8 cfu/mL; Spinach: 3.8×10^8 cfu/mL; Cauliflower: 4.0×10^8 cfu/mL.

The cultural morphology, Gram reaction and biochemical characteristics of bacteria isolates in *M. oleifera* leaf extracts at various locations investigated are presented in Table 2. Nutrient, Mannitol and MacConkey agars recorded bacterial colony in all leaf 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, 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
Sokoto Central Market	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
		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 Gwagwalada 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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