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Sanitary quality of food ingredients displayed for sale in the local markets in rivers state, Nigeria

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Abstract
Food ingredient made from various plant are very important for every average Nigerian home. The study investigated the sanitary quality of Egusi, Achi, Offor, Cameroon pepper and red pepper sold in markets. The analysis was done using standard microbiological methods for microbial isolation and characterization. The total heterotrophic bacteria bacterial count done on samples studied revealed counts ranging from $4.1x10^5$ to $6.4x10^6$, $1.03x10^5$ to $1.02x10^7$, $5.4x10^5$ to $7.2x10^6$, $3.2x10^4$ to$6.4x10^6$, and $4.3x10^4$ to $5.0x10^6$ cfu/g for Achi, Egusi, Offor, grinded pepper and Cameroon pepper respectively. Total coliform count ranging from $1.0x14x10^3$ to $8.2x10^4$ in all the samples studied, Total Staphylococcus count ranged from $3.35x10^3$ to $6.4x10^5$ cfu/g. Fungal counts ranged from $3.6x10^3$ to $6.8x10^4$ cfu/g. Six bacterial genera were isolated in this study including *Bacillus* sp. (25.5%), *Micrococcus* sp. (23.5%), *Staphylococcus* sp. (23.5%), *Proteus* sp. (13.8%), *Enterobacter* sp. (11.8%) and *Enterococcus* sp. (1.9%) while the fungal species isolated includes *Saccharomyces* sp., *Aspergillus niger*, *Candida* sp., *Penicillium* sp and *Rhizopus* sp. Antimicrobial susceptibility testing showed that all Gram Positive Isolates (*Bacillus*, *Staphylococcus* and *Micrococcus* sp were resistant to Ceftazidime, Erythromycin, Augmentin and Cloxacillin while the Grams negative (*Proteus*, *Enterococcus* and *Enterobacter)* showed resistance against Cefixime and cefuroxime. Proper handling and storage of food ingredient is recommended.

Keywords: Food ingredient, sanitary quality, microorganism, antibiogram

Introduction
Plants are a cheap and reliable source of food. Certain nutrients like proteins and vitamins, which are important to make a balanced meal or diet, are cheaper to get from plants than from animals [1, 2]. Owing to the heritage of Africa and the fact that resources are very scarce in developing countries, cheaper options of meal and are often preferred by a majority of the population in these countries. Wild legumes are among the plants which have been commonly used in the developing world as a cheap and reliable source of nutrition. Notable among these nutritious plants are egusi (*Colocynthis citrillus L*) *Achi* (*Brachystogia euryc oma*), Offor, (Diaterium microcarpum) and pepper (Capsinum annum).
From the earliest times, man has derived food, shelter, medicine and decoration from plants. In most of the ancient civilization, one can find some references to plants [3]. In Nigeria, dietary pattern varies and is influenced by the vegetation belt. For example, in the northern parts of Nigeria, cereals dominate, while in the south, legumes, nuts, seeds and starchy roots or tubers are the main food components [4]. Among the legumes used in soups (mainly for emulsification and stabilization of soups) are *Brachystegia eurycoma* (achi), Detarium microcapum (ofor). Each of the soup thickeners differ in species from the others and so have their individual characteristic flavours, which they impart to soups. Often, choice depends on individuals, but *Brachystegia eurycoma* and Detarium microcarpum are favorite soup thickeners in South Eastern Nigeria [4].
Soups made from Egusi on the other hand are among the most popular delicacies in Nigeria. Melon seeds contain a fairly high amount of unsaturated fatty acid, linoleic acid (suggesting a possible hypocholesteronic effect [2]. Thickening, usually improves the taste, but most important is the nutritional value of foods. In fact, every time the soup is thickened, its nutritive value is determined by the ingredients added to it [3]. Thickening agents are often used as food additives and in cosmetics and personal hygiene products.

Egusi melon is mainly a type of watermelon which many botanists think is not easily distinguishable, especially in appearance. In west Africa, where egusi melon is popular, its seed kernels are common components of daily meals and a major soup ingredient [5, 6]. Egusi melon seed kernels have been reported to be a rich source of protein, essential nutrients, and oil [7]. The oil is edible and potentially useful for producing biofuel for automobiles the propagation of all varieties of melon, including egusi melon, is carried out using the seeds.

(Brachystogia. Eurycoma) Achi is an economic tree that belongs to the family Caesalpiniaceae. It is a dicotyledonous legume that grows in the swaps or rain forests and well drained soil of South-Eastern Nigeria and Western Cameroun. It is a huge tree which has twisted and spreading branches with a bark that often exudes a buttery gum [8]. B. eurycoma is called Achi in Igbo, Ekalado or Eku in Yoruba, Okweri in Edo, Akpakpa or Taura in Hausa, Apaupan in Ijaw, and Odukpa in Ibibio [9]. The seed flour, which is a good source of carbohydrate and fiber, is used as flavoring and thickening agents for soups in Eastern Nigeria [2, 10]. The seeds are used in folkloric medicine to maintain body temperature, soften stool, and protect against colon and rectal cancer [2]. A range of proximate, phytochemical, and pharmacological screening has been carried out in different parts of the plant following anecdotal account of its nutritious and medicinal value by local residents and traditional medical practitioners, respectively, in the localities where the plant predominately grows. Phytochemical screening has shown that B. eurycoma contains diverse bioactive compounds including flavonoids, phenolic compounds, alkaloids, saponins, and tannins. Nutritious compounds present in the plant include carbohydrate, proteins, lipids, and minerals [2].

Chilli pepper, a fruit and a spice, is used in small quantities to add flavour and taste to food. Commonly, chilli is dried and ground to use as a spice but is also used fresh in salads and curries. Chilli is thought to increase energy expenditure, so that over a long period of time (combined with less energy intake than energy expenditure) it may reduce body weight and obesity, Chilli is one of the richest sources of antioxidant vitamin C. The active ingredient of chilli, capsaicin, is also a potent antioxidant.

The most important concerns are those ascribed to physiological harm and contamination with spoilage microorganisms [12]. Food ingredients such as Egusi, Achi, Offor and Pepper are easily perishable and requires to be used almost immediately after processing. However, in markets, these food condiments are grinded and kept in the markets sometimes for days without being sold out. Their constant exposure may encourage microorganisms to drop on them and proliferate. Traders with the mind of not running at loss still sell them after grinding for days to weeks. These may lead to potential public health risk. This study is aimed at determining the levels of contamination of grinded Egusi, Red Pepper, Achi, Offor and Cameroon pepper.

Materials and methods

A total of 45 samples grinded and exposed in the market (Egusi, Offor, Achi, red pepper and cameroon pepper). Samples were sourced from three markets within Obio-Akpor Local Government Area, of Rivers State. Each representative samples were grinded hygienically with a clean blender and properly packaged to avoid exposure and this was used as control and taken to the Microbiology laboratory for analysis in sterilized bags.

Isolation of Microorganisms

Twenty five grams (25g) of each sample (Egusi, offor, achi, red pepper and Cameroon pepper) were added into 225 mL of peptone water, swirled and allowed to stay for about 3hours on a laboratory shaker, serial dilution was 10^{-4}. 0.1 ml of each last two prepared dilutions were transferred into sterile petri plates containing solid Plate count agar, MacConkey agar and Mannitol salt agar for bacteria and potato dextrose agar for fungi. The plates were incubated at 37ºC for 24 hours and 28ºC for 72hours for bacteria and fungi respectively. The number of colonies obtained was counted and multiply by inverse of the dilution factor to obtained the number of colony forming unit per milliliter (CFU/g) of the sample [25]. Discrete colonies on the different media were randomly selected based on their morphology and were sub cultured and incubated at 37ºC for 24 hours (h) to obtain pure colonies. Isolates were identified based on their morphological and cultural characteristics on growth media. Identification materials, reagents and protocols according to [22] were used to identify discrete colonies from the media of sub-cultured isolates. The identities of the isolates were confirmed using biochemical tests [22].

The cultural characteristics of each fungi isolates were identified according to their colour, shape and the cell morphology was done based on mycelia, hyphae, septate, spore formation using lactophenol blue. A piece of the mycelium from the Petri plates was mounted on a clean grease free slide using a sterile wire loop and covered with a cover slip, after which a drop of lactophenol cotton blue was added and examined with the microscope [22].

Antimicrobial Sensitivity Testing

Sensitivity Testing: Antibiotic sensitivity patterns of all the confirmed isolates were performed by standard disk diffusion method according to Kirby-Bauer on Mueller-Hinton agar (Titan, Biotech Ltd, Indian) following the procedures recommended by CLSI (2012). Eight commonly used antibiotics (µg/disc) viz. amoxicillin-clavulanate or Augmentin (AUG), gentamycin (GEN), nitrofurantoin (NIT), cefuroxime (CRX), ofloxacin (OFL), cefixime (CXM), ciprofloxacin (CPR), ceftazidime (CAZ), Abtek, (UK) for Grams negative and Gram's positive bacteria were tested. From an overnight culture of all isolates, 0.5 MacFarland turbidity standards bacterial culture was prepared in sterile saline, from which 0.1mL was inoculated onto Mueller Hinton agar, after which antibiotic discs were carefully and aseptically placed on the surface of the agar. The plates were incubated at 37ºC for 24h. Zone of inhibition was measured in millimeter a ruler.

Results

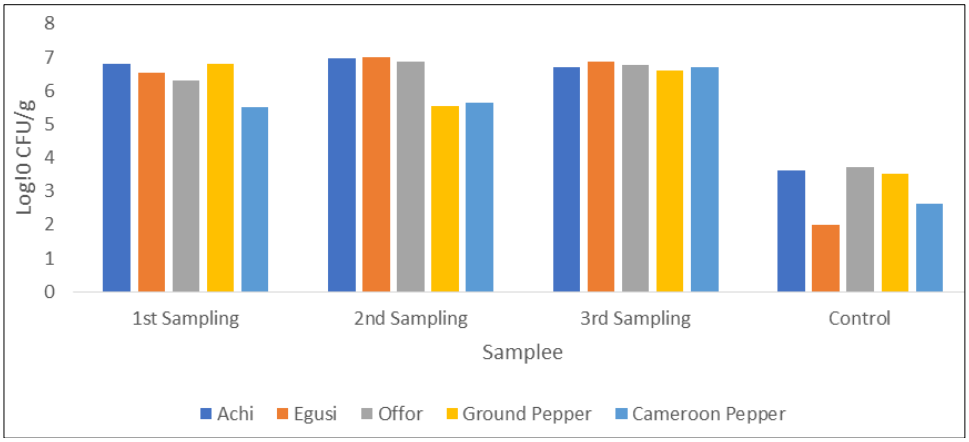

Fig 1: Mean Total Heterotrophic Count of Samples Obtained from Different Food Ingredients

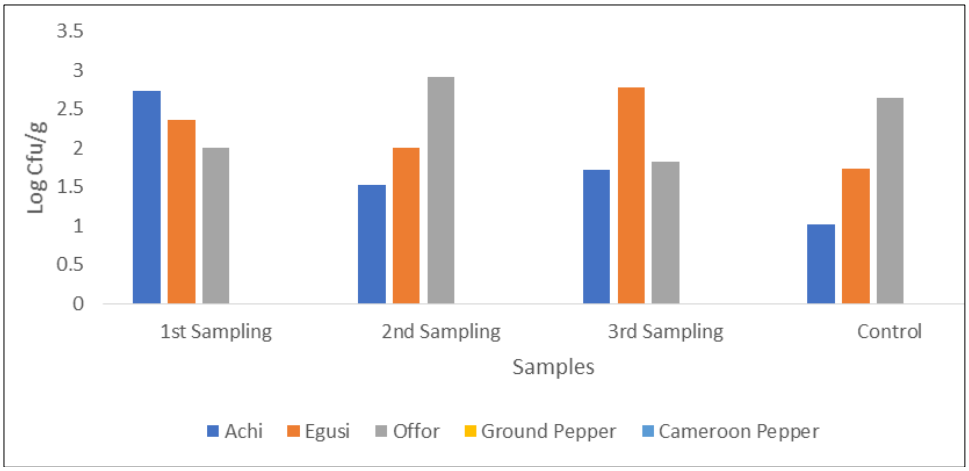

Fig 2: Mean Total Coliform Count of Samples Obtained From Different Food Ingredients

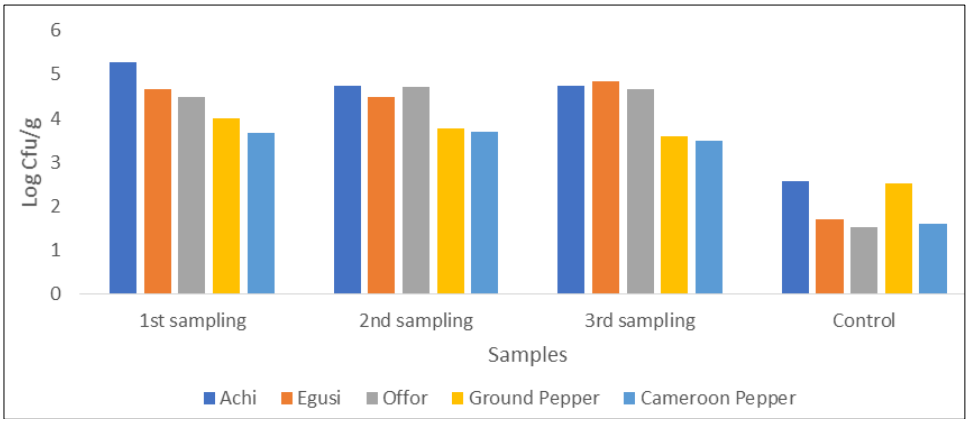

Fig 3: Mean Total Staphylococcus Count of Samples Obtained from Different Food Ingredient

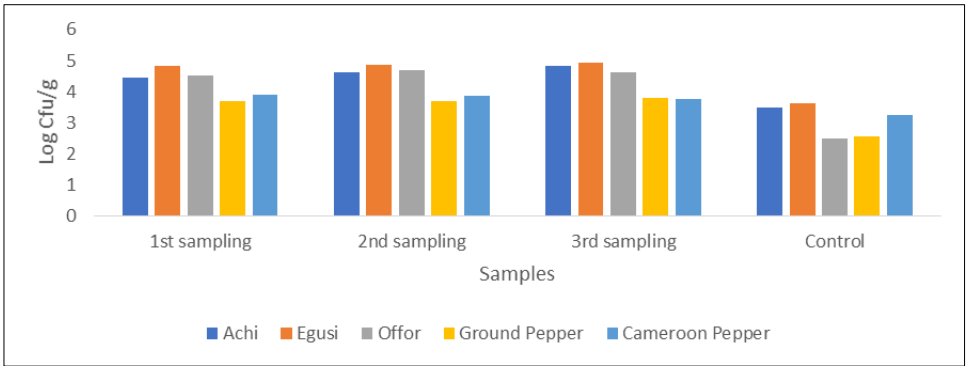

Fig 4: Mean Total Fungi Count of Samples Obtained from Different Food Ingredient

Table 1: Antimicrobial Susceptibility Pattern of Gram Positive Bacteria in millimeter (mm)

S/N	Antibiotics	*Bacillus* spp.	*Staphylococcus* spp.	*Micrococcus* spp.
1	OFL	8.5	26	24
2	AUG	R	R	R
3	CRX	18	14	15
4	CAZ	R	R	R
5	GEN	22	19	19
6	CTR	20	14	12
7	ERY	R	R	R
8	CXM	R	R	R

amoxicillin-clavulanate or Augmentin (AUG), gentamycin (GEN), nitrofurantoin (NIT), cefuroxime (CRX), ofloxacin (OFL), cefixime (CXM), ciprofloxacin (CPR), ceftazidime (CAZ),

Table 2: Antimicrobial Susceptibility Pattern of Gram-Negative Bacteria in millimeter (mm)

S/No	Antibiotic	Proteus spp.	Enterococcus spp.	Enterobacter spp.
1	CRX	R	R	16
2	GEN	12	12	18
3	CXM	R	R	R
4	OFL	12	12	25
5	AUG	R	R	08
6	NIT	R	R	15
7	CPR	10	10	24
8	CAZ	15	12	32

amoxicillin-clavulanate or Augmentin (AUG), gentamycin (GEN), nitrofurantoin (NIT), cefuroxime (CRX), ofloxacin (OFL), cefixime (CXM), ciprofloxacin (CPR), ceftazidime (CAZ),

Table 3: Frequency of Bacterial Isolates from Samples

Isolates	Frequency
Bacillus sp.	13 (25.5)
Micrococcus sp.	12 (23.5)
Staphylococcus sp.	12 (23.5)
Proteus sp.	7 (13.8)
Enterobacter sp.	6 (11.8)
Enterococcus sp.	1 (1.9)
Total	51(100)

Discussion

Microbial quality of Food ingredients displayed for sales

Food safety is a primary concern for everyone, especially in our country Nigeria where it forms part of the government 7-point agenda (3). Biochemical changes in foods when perceived undesirable are mediated by microorganisms that degrade them leading to their spoilage. Because food especially those of plant origin are excellent sources of nutrient, microorganisms find them favorable for their proliferation. The microbial population of food is an important indicator of food safety and quality. High microbial count in food poses a danger to the health of consumers, and their presence may also contribute to decomposition and depletion of nutrients.

This study investigated the sanitary quality of some Food ingredients which includes Egusi, Achi, Offor, grinded pepper, and Cameroon Pepper sold in market places around Port Harcourt. Figure 1 presents the total heterotrophic bacterial count done on all the Food ingredients samples ranged from 4.3x104 to .2x106 cfu/g. The THBC for all control samples were lower indicating that exposure in market places increases the contamination levels of samples studied.

The THBC counts observed in this study is however higher than the counts of 2.0x104 to 7.0x104 cfu/g reported by Tsado *et al*., [12] in Minna, Niger State. Our study is however in concomitance with the range published by Salari *et al*., [13] reported THBC of various food ingredients studied to be 1.0x102 to 4.0x106 cfu/g, This is comparable to the report of Salari [13] reported THBC of various samples studied to be 1.0x102 to 4.0x106 cfu/g from melon seeds. Filiz [14]. reported total heterotrophic bacteria the average numbers of 8.1 × 106 cfu/g in powdered red pepper. Elmali *et al*. [15] examined 15 powdered red peppers and found counts of 2.7 × 106 cfu/g. A study conducted by Winfred *et al*., [16] on Egusi powder sold in different markets had Total bacteria count ranged from Log 10 3.2 -4.4 cfu/g for the different markets in Ghana.

Figure 2 shows high levels of coliform contamination ranging from 1.4x103 to 8.2x104 cfu/g for all the samples. This is comparable to the report of Salari *et al*., [13] who reported higher counts of 1.9x102 to 3.25x106 cfu/g. Elmali *et al*. [15] found the average number of 1.3 × 104 cfu/g of coliform in powdered red pepper. Filiz [14] reported the average number of 1.7 × 102 cfu/g of Coliforms in powdered red pepper. Schwab *et al*. [17] found very low level of Coliforms and E. coli (3-19 cfu/g) in powdered red pepper samples.

Staphylococcus count was higher ranging from 3.35x103 to 6.4x105 cfu/g for all samples studied as shown in Figure 3. The high Staphylococcus count could be as result of frequent contacts of food stuffs with the skin during storage and restorage after sales daily.

Staphylococcus is considered the third most important cause of disease in the world amongst the reported food-borne illness [19, 20]. Staphylococcal food poisoning is a persistent cause of gastroenteritis worldwide, especially in developed countries [21].

All the control samples had lower counts compared to the grinded samples sourced from the markets for all microbial parameters analyzed. This could be attributed to the fact that they have not been exposed and also not stored over time. Sanitary measures were taken during the grinding of the control samples. Our counts recorded for both samples sourced from the market and the controls are however high when compared to microbiological standards and acceptable

range for food stuffs which is (1.0x102Cfu/g). The implication is that the samples are not fit for consumption as they harbor microorganisms above required standards. Consequently, these samples are not eaten raw and there are all chances that the pathogens will be denatured or killed during cooking or pasteurization. Fungal counts ranged from 3.6x103 to 6.8x104 cfu/g for all the samples as shown in Figure 4. This implies that most of the samples supported the proliferation of fungal species.

Six bacterial genera were isolated in this study as shown in Table. *Bacillus* sp. (25.5%), *Micrococcus* sp. (23.5%), *Staphylococcus* sp. (23.5%), *Proteus* sp. (13.8%), *Enterobacter* sp. (11.8%) and *Enterococcus* sp. (1.9%) while the fungal species isolated includes Saccharomyces sp. (24.5%), *Aspergillus niger* (19.%), *Candida* sp (7.5%), *Penicillium* sp (32.5%), and *Rhizopus* sp (16.5%), Most of the organisms isolated has the potentials of causing public health concerns as Enterobacter and Enterococcus are capable of causing illnesses of public health concerns such as gastroenteritis and diarrhea. The high microbial contamination obtained from this study may be ascribed to improper handling of grinded food ingredient and poorsanitary practices, which indicate inefficient process controls. Milling methods, residue build-up in milling machines, and storage practices may constitute a significant source of microbiological contamination which may negatively affect the safety and health of the consumer. Evidently the vendors store the powders in flexible pouches, wooden or plastic boxes, and plastic bowls/buckets. This practice may easily expose the food product to microbial contamination and adversely compromise its safety. Most of the sellers indicated that a batch of these grinded food ingredient lasts a maximum of a week or even more, but this heavily depends on patronage. The ingredients, according to the women, is usually kept in HDPE bowls/buckets from which measured portions are scooped and sold to consumers. The powder is sold in unbranded, clear, and flexible polyethylene or polypropylene bags and secured with a knot. This practice is likely to increase the risk of rancidity occurring in the powder whiles in storage since it is often exposed to air and moisture.

The result of the antibiogram conducted on the Gram-Positive Isolates (Bacillus, Staphylococcus and Micrococcus spp.) revealed resistance to Ceftazidime, Erythromycin, Augmentin and Cloxacillin This agrees with other findings. [22, 23]. The Gram-negative isolates (Proteus, Enterococcus and Enterobacter) showed resistance against Cefiixidime and cefuroxime. The antimicrobial susceptibility pattern observed for Enterococcus sp agrees with Shareef and Shara [24] who reported similar patterns from clinical samples. The resistance to the antibiotics tested may be attributed to the production of beta-lactamase, an enzyme that inactivates β-lactam rings in β-lactam antibiotics and closely related antibiotics. Resistance in antibiotic pose risk as their ineffectiveness can result in mortality.

Conclusion

These Food ingredients were found to be potential vectors in the transmission of opportunistic pathogenic microorganisms. This study confirms the occurrence of different pathogens in samples of food ingredients and concludes that these pose potential public health risk if not adequately processed before consumption. If these food ingredients are processed and handled carefully it would minimize the possibility of contamination and ensure that the product is of good quality and is safe for consumption.

Recommendations

1. Proper and hygienic storage food condiments in market places
2. Buyers should not be allowed to touch food stiffs before buying
3. Traders should blend amount of product they can sell off within the shortest time to avoid over storage.

Competing interests disclaimer:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

References

1. Bhat R, Karim AA. Exploring the nutritional potential of wild and underutilized legumes. Comprehensive reviews in food Science and food safety. Compr Rev Food Sci F. 2009;8(4):305-31.
2. Ighodaro I, Agbonlahor O. Expanding the insights into the usefulness of *Brachystegia eurycoma* Harms: A review of its nutritional and medicinal values. Journal of Intercultural Ethnopharmacology. 2018;7(1):1-13.
3. Ikechukwu OI, Emmanuel IA. Analysis of four seeds used as soup thickeners in the south eastern part of Nigeria. International Conference on Chemistry and Chemical Engineering, 2010, 426-430.
4. Friday OU, Chioma CO, Emeka EJI, Ijeoma K. Effect of Processing Methods on Nutritive and Antinutritive Properties of Seeds of Branchiostegal eurycoma and Detarium microcarpum from Nigeria. Pakistan Journal of Nutrition. 2009;8(4):316-320.
5. Ann PI, Moses O, Kabuo NO, *et al* Comparative evaluation of proximate compositions, functional and physicochemical properties of raw melon seeds of five members of cucurbitaceae family. Am J Food Sci Nutr. 2016;3(1):8-17.
6. Solomon OG, Taiwo OA. A Review on Food Uses and the Prospect of Egusi Melon for Biodiesel Production. BioEnergy Research. 2020. https://doi.org/10.1007/s12155-020-10145-4
7. Olubi O, Felix-Minnaar JV, Jideani VA. Physicochemical and fatty acid profile of egusi oil from supercritical carbon dioxide extraction. Heliyon. 2019;5(1):e01083.
8. Olajide O, Udo ES, Out DO. Diversity and population of timber tree species producing valuable non-timber products in two tropical rainforests in Cross River State, 0Nigeria. J Agric Soc Sci. 2008, Pak, 4:65-8.
9. Bafor EE, Chiekwe O, Ofeimun J, Amaechina F, Ayinde B. *Brachystegia eurycoma* harms (Fabaceae) stem bark extract modulates gastrointestinal motility in animal models. Afr J Biomed Res. 2017;20(3):309-16.
10. Uhuegbu FO, Onwuchekwa CC, Iweala EE, Kanu I. Effect of processing methods on nutritive and antinutritive properties of seeds of *Brachystegia*

eurycoma and detarium microcarpum from Nigeria. Pak J Nutr. 2009;8(4):316-20.
11. Okoli RI, Turay AA, Mensah JK. The phytochemical analysis and antibacterial effects of stem bark extracts of *Brachystegia eurycoma* harms. Int J Herb Pharmacol Res, 2015;4(2):10-6.
12. Tsado EK, Ekpa D, Salaudeen MT, Adesina OA, Yusuf ST, Izuegbu LN. Microbial status of dried pepper (*capsicum* spp.), tomato (*Lycopersicum esculentus*), and roselle (*Hibiscus sabdariffa*) marketed in Minna, Niger State, Nigeria. Direct Res. J Agric. Food Sci. 2017;6(1):13-18.
13. Salari R, HabibiNajafi MB, Boroushaki MT, Mortazavi SA, Fathi NM. Assessment of the Microbiological Quality and Mycotoxin Contamination of Iranian Red Pepper Spice. J Agr. Sci. Tech. 2012;14:1511-1521.
14. Filiz N. Microbial flora of some ground spices consumed in Bursa. Journal of the Faculty of Veterinary Medicine of Uludag University. 2001;20:103-107.
15. Elmali M, Yaman H. Microbiological quality of some spices sold in the markets of Bitlis district. Journal of Faculty of Veterinary Medicine, University of Erciyes. 2005;2(1):9-14.
16. Winifred Arthur, Jemima Ofori, Peter Addo, Nelson Amey, Nii Korley Kortei, Paa Toah Akonor. Chemical, Microbial Quality, and Risk Assessment due to Toxic Metal Contamination of Egusi (*Citrullus colocynthis* L.) Powder Sold in Selected Ghanaian Markets. International Journal of Food Science, 2020, 8.
17. Schwab AH, Harpested SA, Lanier JM, Wentz B, Duran AP, Barnard RJ, *et al*. Microbiological quality of some spices and herbs in retail markets. Applied and Environmental Microbiology. 1982;44(3):627-630.
18. Ananou S, Maqueda M, Martinez-Bueno M, Galvez A, Valdivia E. Bactericidal synergism through enterocin AS-48 and chemical preservatives against *Staphylocoocus aureus*. Letters in Applied Microbiology. 2007;45(1):19-23.
19. Zhang S, Iandolo JJ, Stewart GC. The enterotoxin D plasmid of Staphylococcus aureus encodes a second enterotoxin determinant (sej). FEMS Microbiology letters. 1998;168(2):227-233.
20. Vora P, Senecal A, Schaffner DW. Survival of Staphylococcus aureus ATCC 13565 in intermediate moisture foods is highly variable. Risk Analysis. 2003;23(1):229-236.
21. Adesoji AT, Onuh JP, Bagu J, Itohan SA. Prevalence and antibiogram study of Staphylococcus aureus isolated from clinical and selected drinking water of Dutsin-Ma, Katsina state, Nigeria. Afr Health Sci. 2019;19(1):1385-1392. doi:10.4314/ahs.v19i1.11
22. Taddesse Z, Tiruneh M, Gizachew MS. aureus and it's Antimicrobial Susceptibility Pattern in Patients, Nasal carage of Health Personnel, and objects at Dessie referral hospital, Northern Ethiopia. Global Journal of Medical research: Microbiology and Pathology. 2014;14(2):29-35.
23. Shareef HA, Shara NA. Antimicrobial susceptibility of enterococcus isolated from clinical sources in Kirkuk Provency. Haitham Journal for pure and applied Science. 2018;2:125-132.
24. Cheesbrough M. District laboratory \practice in tropical countries, E. C.B.S 2nd ed. Cambridge University Press. 2002, p. 256-267.