JASEM ISSN 1119-8362

Full-text Available Online at www.ajol.info and www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage. *Dec.*, 2015
Vol. 19 (4) 727 - 734

Quality Assessment and Proximate Analysis of *Amaranthus hybridus, Celosia argentea* and *Talinum triangulare* obtained from open Markets in Benin City, Nigeria.

[*1]AKINNIBOSUN, FI; ADEOLA, MO

Department of Microbiology, Faculty of Life Sciences, University of Benin, P. M. B 1154, Benin City, Edo State, Nigeria.
[*]Corresponding author e-mail: fakinnibosun@yahoo.co.uk

ABSTRACT: The aim of this research is to determine the quality and proximate composition of *Amaranthus hybridus*, *Celosia argentea*, and *Talinum triangulare* obtained from open markets in Benin City, Nigeria. Microbiological and proximate analysis were carried out using standard methods. Results of the proximate analysis revealed the presence of proteins (12.86 ± 0.19 %; 9.28 ± 0.07 % and 2.82 ± 0.08 %), carbohydrates (9.06 ± 4.19 %; 8.28 ± 0.48 % and 12.22 ± 0.53 %), fibre (10.38 ± 0.25 %; 8.45 ± 0.13 % and 3.55 ± 0.17 %), lipids (0.23 ± 0.13 %; 1.44 ± 0.28 % and 1.06 ± 0.28 %), moisture (62.38 ± 4.05 %; 62.98 ± 0.38 % and 79.65 ± 0.56 %) and ash (mineral content) (5.10 ± 0.14 %; 9.58 ± 0.05% and 0.70 ± 0.12 %) in the green leaf, soko and water leaf respectively, needed for normal functioning of the body. However, microbial contaminants were observed which included bacterial and fungal species. The bacteria isolated were: *Staphylococcus aureus*, *Bacillus subtillis*, *Enterobacter* sp, *Klebsiella* sp, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Escherichia coli*, while the fungal species isolated were: *Rhizopus stolonifer, Aspergillus niger, Fusarium solani, Aspergillus flavus, Mucor mucedo, Penicillium notatum,* and *Penicillium expansum.* The bacterial count, coliform count and fungal count of *A. hybridus* were: $1.19 \times 10^5 \pm 4.52 \times 10^4$ cfu/g, $3.30 \times 10^4 \pm 1.82 \times 10^4$ cfu/g and $6.50 \times 10^3 \pm 3.32 \times 10^3$ cfu/g respectively. *C. argentea* had $1.56 \times 10^5 \pm 7.00 \times 10^4$ cfu/g, $3.88 \times 10^4 \pm 2.56 \times 10^4$ cfu/g and $6.00 \times 10^3 \pm 3.74 \times 10^3$ cfu/g respectively, while *T. triangulare* had $1.99 \times 10^5 \pm 9.31 \times 10^4$ cfu/g, 4. 93 x $10^4 \pm 4.77 \times 104$ cfu/g and $4.78 \times 10^3 \pm 2.18 \times 10^4$ cfu/g respectively. Antibiotic susceptibility pattern of the bacterial isolates revealed multi-drug resistance and plasmid-mediated resistance genes among the isolates. Public enlightenment, proper washing and cooking with potable water are therefore recommended to avoid the menance of these multi-drug resistant microbial contaminants.

http://dx.doi.org/10.4314/jasem.v19i4.21

***KEYWORDS*:** *Amaranthus hybridus, Celosia argentea, Talinum triangulare,* Microbial analysis, Proximate composition

INTRODUCTION

Vegetables are the fresh and edible portions of herbaceous plants, which can be eaten raw or cooked Fayemi (1999); Dhellot (2006). They are valued mainly for their high carbohydrate, vitamin and mineral contents. Vegetables may be edible roots, stems, leaves, fruits or seed. Each group contributes to diet in its own way Robinson (1990). Ononugbu (2002) reported that vegetable fats and oil lower blood lipids thereby reducing the occurrence of disease associated with damage of coronary artery. Apart from the variety which they add to the menu (Mepha and Eboh, 2007; Subukola, 2007), they are valuable sources of nutrients especially in rural areas where they contributes substantially to protein, minerals, vitamins, fibers and other nutrients which are usually in short supply in daily diets (Mohammed and Sharif, 2011). *Amaranthus hybridus, Celosia argenta* and *Talinum triangulare* are popular edible vegetables in Nigeria.

A. hybridus belongs to the family Amaranthacae, local name: Tete-arowojeja (yoruba). The leaves are green, variable in shape and size (Iheanacho and Udebuani, 2009). In Nigeria, the leaves combined with condiments are used to prepare soup Mepha, (2007). These leaves boiled and mixed with a groundnut sauce are eaten as salad in West and Southern Africa Oliveria and DeCarvalho (1975); Martin and Telek, (1979).

C. argentea is an annual herbaceous vegetable of the family Amaranthaceae. In south-western Nigeria, it is known as *soko yòkòtò* in Yoruba or *farar álàyyafó* in Hausa. *C. argentea* is propagated by seeds. The seeds are extremely small and the leaves and stems are cooked into soups, sauces or stew with other ingredients (Grubben and Denton, 2004) and may be consumed with maize, rice, yam and cassava (Tindall, 1983). In Ethiopia, the seeds are used for

[*]Corresponding author e-mail: fakinnibosun@yahoo.co.uk

the treatment of diarrhea, dysentery, bleeding nose, disinfectant, inflammation, haematological, gynaecologic disorders and muscle troubles (Chweya and Eyzaguirre, 1999). Three types of Celosia argentea are cultivated in Nigeria, green broad-leaved cultivars called soko green and the broad-leaved cultivars with anthocyanin pigmentation of the leaf blades and part of the stem called soko pupa and cultivars with deep green narrow leaves with a hard texture and early flowering (Brenan, 1981; Grubben and Denton, 2004).
T. triangulare (water leaf) belongs to the family Portulaceae. It is a short -lived perennial herb, growing to 30-60cm in height. The leaf is greenish in colour with succulent stem agricultural sustainability. It is a herbaceous plant widely grown in tropical regions as a leaf vegetable (Akobundu and Agyakwa, 1998). It is consumed as a vegetable and constituent of a sauce in Nigeria.

Vegetable microbiota is very diverse and vegetable quality and safety depend on many factors, including soil, fertilizer, irrigation water, presence of animals on the field and good practices during handling and merchandizing. Leaf shapes can further contribute to the differences in the contamination levels of different vegetable varieties, hence, it is difficult to control contamination with pathogenic and food-spoilage microorganisms (Beuchat, 2002). Despite the health benefits, the risk of microbiological contamination on leafy greens is concerning. Many food borne illness outbreaks in numerous countries have been associated with consumption of contaminated fresh vegetables (Beuchat, 2002; FAO/WHO, 2008). The effectiveness of the antibiotics has greatly reduced due to the emergence of the antibiotic-resistant bacteria (Wasfy, 2000). The susceptibility of bacteria to antibiotics can be analysed by phenotypic and genotypic techniques.

Leafy vegetables occupy a very important place in the human diet, but unfortunately are sources of heavy metal accumulation. Some common vegetables are capable of accumulating high levels of heavy metals from contaminated and polluted soils (Cobb *et al*., 2000; Benson and Ebong, 2005).

This study was carried out to determine the microbial contamination, antibiotic susceptibility pattern of the microbial isolates, nutrient composition, heavy metal content and their correlation with the microbial load of *A. hybridus, C. argentea* and *T. triangulare* sold in open markets in Benin City. Nigeria.

MATERIALS AND METHODS

Sample collection: Three types of vegetables *A. hybridus* (Green), *C. argentea* (Soko), *T. triangulare* (water leaf) were randomly purchased from open markets in Benin City, Nigeria. All the samples were obtained fresh in sterile polythene bags and transported to the laboratory for analysis.

*Preparation of culture media***:** Nutrient agar and potato dextrose agar were used for the isolation of bacteria and fungi respectively. All media were prepared according to manufacturer's instruction.

*Isolation Procedures***:** Ten grams of the vegetable leaf samples were homogenized with 90 ml of distilled water to obtain stock suspension. Ten-fold serial dilution was then carried out. Aliquot of 0.1 ml of the 10^{-3} dilution was pour-plated in nutrient agar and potato dextrose agar plates. The Nutrient agar plates were incubated at 37 ^{0}C for 24 - 48 hrs until appreciable amount of growth was observed. The potato dextrose agar plates were incubated at 28 ± 2 ^{0}C for 72 hours. Pure culture of isolates obtained after sub-culturing was stored in the refrigerator at about 4 ^{0}C and later identified by biochemical tests and morphological characterization.

*Isolation and Enumeration of Bacteria***:** The bacteriological analysis of the samples were carried out according to the methods of Oyeleke and Manga (2008a). The bacterial isolates were identified and characterized using standard biochemical tests (Jolt *et al*., 1994; Cheesebrough, 2006). The tests carried out included colonial and morphological characterization, Gram stain, motility, catalase, methyl red, Voges-Proskauer, indole production, urease activity, citrate utilization, glucose, sucrose and lactose utilization tests.

Characterization and identification of Fungal isolates: The fungal species were identified and characterized based on their morphological characteristics, which included the colour of aerial hyphae, substrate mycelium, hyphae and conidial arrangement. Microscopic analysis using taxonomic guides and standard procedures was also carried out. (Gilman, 1944; Barnett and Hunter, 1972; Ellis, 1976; Domsch *et al*., 1980).

Antibiotic susceptibility test: This was carried out using the Kirby-Bauer disc diffusion method (Bauer *et al.*, 1966), according to National Committee for Clinical Laboratory Standards (NCCLS, 2002), and using antibiotics containing discs from Oxoid. 20ml of the Mueller-Hinton agar was prepared and poured into sterile plates. The agar medium was allowed to

solidify at room temperature on a flat bench. Isolates were streaked on the surface of the well dried agar plates. Gram-positive and Gram-negative multi-discs were used. The antibiotic discs were gently and firmly placed on the agar plates which were then left at room temperature for 1hr to allow diffusion of the antibiotics into the agar medium.. The plates were then incubated at 37 °C for 24 hrs. Zones of growth inhibition were then measured and the mean of triplicate results was taken to the nearest millimeter and recorded. Multidrug resistance was defined as resistance to ≥3 of the antimicrobial agents tested (Oteo *et al*., 2005). The antibiotics used for the isolates were: Amoxicillin (30µg), Chloramphenicol (30µg), Augmentin (30 µg), zinnacef (20 µg), rocephin (25 µg), ciprofloxacin (l0 µg), streptomycin (30 µg), erythromycin (l0 µg), gentamycin (I0 µg), septrin (30 µg), perfloxacin (l0 µg), ampiclox (30 µg), sparfloxacin (30 µg) and ofloxacin (30 µg)

*Proximate analysis***:** Proximate analysis of the samples was carried out according to the methods recommended by AOAC, 1999.

RESULTS AND DISCUSSION

The results of this study showed that microbial flora (Table 1) exists on the leaves of *A. hybridus*, *C. argentea* and *T. triangulare*. The bacterial count, coliform count and fungal count of *A. hybridus* were: $1.19 \times 10^5 \pm 4.52 \times 10^4$ cfu/g, $3.30 \times 10^4 \pm 1.82 \times 10^4$ cfu/g and $6.50 \times 10^3 \pm 3.32 \times 10^3$ cfu/g respectively. *C. argentea* had $1.56 \times 10^5 \pm 7.00 \times 10^4$ cfu/g, $3.88 \times 10^4 \pm 2.56 \times 10^4$ cfu/g and $6.00 \times 10^3 \pm 3.74 \times 10^3$ cfu/g respectively, while *T. triangulare* had $1.99 \times 10^5 \pm 9.31 \times 10^4$ cfu/g, $4.93 \times 10^4 \pm 4.77 \times 10^4$ cfu/g and $4.78 \times 10^3 \pm 2.18 \times 10^4$ cfu/g respectively. The bacteria isolated in this study were *S. aureus*, *B. subtilis*, *Enterobacter* sp., *Klebsiella*, *P. vulgaris*, *P. aeruginosa* and *E. coli*. (Table 2). This pattern of isolation corroborates an earlier report by Banwart, (1979). Similarly, the organisms were isolated from samples of *T. occidentalis* from a different (south-southern) region of Nigeria (Obieze *et al*. 2010). *S. aureus* is likely to have been introduced onto the leaves from the hands of the handlers which include the farmers and the sellers. As ascribed by (Madden, 1992), this could be from the point of harvesting to the packaging, sizing for sale, and also from the utensils and packaging bags used by the sellers. The presence of *E. coli* indicated the presence of recent human or animal faecal contaminants on the leaves. The isolated Enterobacteriaceae could have come from the soil and the contaminated water used in irrigation and washing of the vegetables (Okafo *et al*. 2003). The presence of *Enterobacter aerogenes* could probably be due to washing of these vegetables with water contaminated with faecal matter. *P. vulgaris* and *Klebsiella* sp have similarly been associated with various vegetables (Obieze *et al*., 2010).

The fungi isolated from the leafy vegetables were *R. stolonifer, A. niger, F. solani, A. flavus, M. mucedo, P. expansum, P. notatum* (Table 2). This result agrees with the report of Amaike and Keller (2011) who reported that these organisms could be linked to the spoilage of raw vegetables. *A. flavus* has also being implicated in causing aspergillosis that infects and contaminates pre-harvest and post-harvest fruits and vegetable crops with the carcinogenic secondary metabolite aflatoxin (Amaike and Keller, 2011). *R. stolonifer* has also been implicated in several human ailments (Schipper, 1984).

From this study, the degree of susceptibilities shown by the isolates to the antibiotics indicated the efficacy the antibiotics (Pelczar and Reid, 1998; Nkang *et al*., 2009). The susceptibility of a chemotherapeutic agent is usually expressed on the basis of the higher zones of inhibition (Nkang *et al.*, 2009; Nnela and Cox, 1998). The bacteria isolated were found to be resistant to a wide range of standard antibiotics (Tables 3 and 4). The results of this study showed that most of the isolates were resistant to multiple antibiotics, hence they were multi-drug resistant (Wasfy *et al.*, 2000; Akpomie and Akpan, 2013). Similar multidrug-resistant epiphytic bacteria have been reported in Finland (Österblad *et al*., 1999) and in the United Kingdom (Hamilton-Miller and Shah, 2001). The antibiotic-susceptibility of some of the bacteria isolated from the leafy vegetable after plasmid curing (Tables 5 and 6), indicated that the genes responsible for the antibiotic resistance were plasmid-mediated.

The results of the proximate composition of the vegetables which included moisture content, ash content, crude protein, crude lipid, crude fibre, available carbohydrate, and energy values (Table 7) showed that moisture content varies from the highest in water leaf (65.0 ± 0.56 %) to the lowest in Green leaf (62.38 ± 4.05 %%). This is within the reported range (0.83 to 90.30 %) for Nigeria green leafy vegetables (Akubugwo *et al*., 2007), but higher than those reported by Chionyedua *et al*. (2009). The results of the mean ash content revealed that the soko leaf had the highest (9.58 ± 0.05 %), and the lowest was water leaf (0.70 ± 0.12 %). The ash content of any sample is the measure of the mineral content of the food (Nnamani *et al*., 2009). This result agrees with the results of Taiga *et al*. (2008). The results of the mean fibre content showed that the leaf of *A.*

hybridus recorded the highest (10.38 ± 0.25 %) and water leaf recorded the lowest (3.55 ± 0.17 %). This is in agreement with those reported by Akachukwu and Fawusi, 1995. The results of the crude protein content revealed that it was the highest in the leaf of *A. hybridus* (12.86 ± 0.19 %), and the lowest in the leaf of *T. triangulare* (2.82 ± 0.08 %). The results of the crude protein agree with those of Taiga *et al*. (2008) and Ogle and Grivetti (1985). The results of the lipid content revealed that the leaves of Soko has the highest value (1.44 ± 0.28 %), while Green leaf has the lowest value (0.23 ± 0.13 %). This showd that vegetables are low in lipids. The results are similar to those reported by Okafor (1998) and Nnamani *et al*. (2009).

The heavy metal content and their correlation with the microbial load (Tables 8 and 9), revealed that there was significant correlation between lead and the bacterial count, while there was no significant correlation between the other heavy metals and microbial load (Ogbeibu, 2005).

Table 1: Microbial counts (cfu/g) of the vegetable samples

Vegetables	Bacterial counts ($\bar{X}$ ± SD)	Coliform counts ($\bar{X}$ ± SD)	Fungi counts ($\bar{X}$ ± SD)
Green Leaf	$1.19 \times 10^5 \pm 4.52 \times 10^{4a}$	$3.30 \times 10^4 \pm 1.82 \times 10^{4b}$	$6.50 \times 10^3 \pm 3.32 \times 10^{3a}$
Soko Leaf	$1.56 \times 10^5 \pm 7.00 \times 10^{4a}$	$3.88 \times 10^4 \pm 2.56 \times 10^{4b}$	$6.00 \times 10^3 \pm 3.74 \times 10^{3a}$
Water leaf	$1.99 \times 10^5 \pm 9.31 \times 10^{4a}$	$4.93 \times 10^4 \pm 4.77 \times 10^{4a}$	$4.78 \times 10^3 \pm 2.18 \times 10^{4b}$
Sign. diff.	P (0.35) > 0.05	P (0.78) > 0.05	P (0.74) > 0.05

Note:
P < 0.05 = significance difference
P > 0.05 = No significance difference
Values are mean ± Standard deviation
Mean values of the same column followed by different letter differ significantly.

Table 2: Microbial isolates from the samples

Bacterial Isolates	Fungal Isolates
S. aureus	*R. stolonofer*
B. subtilis	*A. niger*
E. aerogenes	*F. solani*
Klebsiella sp.	*Flavus*
P. vulgaris	*M. mucedo*
P. aeruginosa	*P. expansum*
E. coli	*P. notatum*

Table 3: Antibiotic susceptibility pattern of Gram-positive (Gram +ve) bacterial isolates before curing

Gram+ve	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
S. aureus	S	S	I	I	R	S	S	I	R	I
Subtilis	S	I	R	S	I	I	S	I	R	I

Key: R = Rocephin, Z = Zinnacef, PEF = Pefloxacin, CN = Gentamicin, AM = Amoxicillin, E = Erythromycin, APX = Ampiclox, CPX = Ciprofloxacin, S = Streptomycin, SXT = Septrin, R = Resistant, S = Susceptible, I = Intermediate**.**

Table 4: Antibiotic susceptibility pattern of Gram-negative (Gram -ve) bacterial isolates before curing

Gram –ve	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
E. aerogenes	R	R	S	S	R	S	S	S	S	R
***Klebsiella* sp**	R	R	S	S	R	S	S	S	S	R
P. vulgaris	R	S	S	S	S	S	S	S	S	R
P. aeruginosa	R	R	S	S	R	R	S	S	S	I
E. coli	R	R	S	S	S	R	S	S	S	R

Key: CH = Chloramphenicol, SP = Sparfloxacin, PEF = Pefloxacin, CN = Gentamicin, AM = Amoxicillin, AU = Augmentin, OFX = Ofloxacin, CPX = Ciprofloxacin, S = Streptomycin, SXT = Septrin, R = Resistant, S = Susceptible, I = Intermediate.

Table 5: Antibiotic susceptibility pattern of Gram-positive (Gram +ve) bacterial isolates after curing

Gram+ve	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
S. aureus	S	S	I	S	S	S	S	S	S	S
B. subtilis	S	I	R	S	S	S	S	S	R	S

Key: PEF = Pefloxacin, CN = Gentamicin, APX = Ampiclox, Z = Zinnacef, AM = Amoxicillin, R = Rocephin, CPX = Ciprofloxacin, S = Streptomycin, SXT = Septrin, E = Erythromycin, R = Resistant, S = Susceptible, I = Intermediate.

Table 6: Antibiotic susceptibility pattern of Gram-negative (Gram -ve) bacterial isolates after curing

Gram–ve	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
E. aerogenes	R	I	I	S	S	S	S	S	S	R
***Klebsiella* sp**	R	S	I	S	S	I	S	S	S	I
P. vulgaris	R	S	I	S	S	S	S	S	S	S
P. aeruginosa	I	I	S	S	S	S	S	S	S	S
E. coli	I	S	S	S	S	S	S	S	S	S

KEY: Key: CH = Chloramphenicol, SP = Sparfloxacin, PEF = Pefloxacin, CN = Gentamicin, AM = Amoxicillin, AU = Augmentin, OFX = Ofloxacin, CPX = Ciprofloxacin, S = Streptomycin, SXT = Septrin, R = Resistant, S = Susceptible, I = Intermediate.

Table 7: Proximate composition (%) of the samples**.**

Vegetable	Moisture	Protein	Crude fiber	Fat (Lipid)	Ash	CHO
Green leaf	62.38 ± 4.05^{b}	12.86 ± 0.19^{a}	10.38 ± 0.25^{a}	0.23 ± 0.13^{c}	5.10 ± 0.14^{b}	9.06 ± 4.19^{b}
Soko leaf	62.98 ± 0.38^{b}	9.28 ± 0.07^{b}	8.45 ± 0.13^{b}	1.44 ± 0.28^{a}	9.58 ± 0.05^{a}	8.28 ± 0.48^{c}
Water leaf	79.65 ± 0.56^{a}	2.82 ± 0.08^{c}	3.55 ± 0.17^{c}	1.06 ± 0.28^{b}	0.70 ± 0.12^{c}	12.22 ± 0.53^{a}
Sign. diff.	P (0.00) < 0.05	P (0.00) < 0.05	P (0.00) < 0.05	P (0.00) < 0.05	P (0.00) < 0.05	P (0.11) > 0.05

Note:
P < 0.05 = significance difference
P > 0.05 = No significance difference
Values are means ± standard error
Mean values of the same column followed by different letter differ significantly.
CHO = Cabohydrate

Table 8: Heavy metal concentration (mg/g) of the samples**.**

Vegetables	Zinc	Copper	Iron	Lead
Green leaf	0.190 ± 0.005^{a}	0.915 ± 0.011^{a}	4.127 ± 0.113^{a}	0.001 ± 0.001^{b}
Soko leaf	0.150 ± 0.002^{b}	0.836 ± 0.025^{b}	3.100 ± 0.149^{b}	0.000 ± 0.001^{b}
Water leaf	0.092 ± 0.004^{c}	0.469 ± 0.006^{c}	2.751 ± 0.199^{c}	0.002 ± 0.001^{a}
Sign. diff.	P (0.00) < 0.05	P (0.00) < 0.05	P (0.00) < 0.05	P (0.01) < 0.05

Note:
P < 0.05 = significance difference
P > 0.05 = No significance difference
Values are mean ± Standard deviation
Mean values of the same column followed by different letter differ significantly.

Table 9: Correlation between heavy metal content and microbial count

Heavy metal	Bacterial count	Coliform count	Fungal count
Zinc	-0.479	-0.243	0.254
Copper	-0.414	-0.242	0.242
Iron	-0.438	-0.253	0.207
Lead	0.619*	0.124	-0.320

Key: * = Correlation is significant at the 0.05 level (2- tailed).

*Conclusion***:** The data obtained from this study showed that these vegetables contain appreciable amount of proteins, fat, fibre, carbohydrate, calorific value, and sufficient amount of mineral elements needed for normal body functioning, maintenance of the body, and reproduction. It can therefore, be concluded that these vegetables can contribute significantly to the nutrients of man and animals and should be used as source of nutrients to supplement other major sources of nutrients. However, the results of this research has shown the high levels of microbial contaminants in the leafy vegetables sold in open markets in Benin City. Adequate care should be taken in processing these vegetables to destroy the microorganisms before they enter inside the human body. Farmers should use clean and potable water to irrigate their fields. In addition, vegetables should be properly washed in clean or iodized water before sending them to the markets for sale.

*Acknowledgements***:** The authors are grateful to the Department of Microbiology, Faculty of Life Sciences, University of Benin, P. M.B 1154, Benin City, Edo State, Nigeria for the provision of materials to do this work.

REFERENCES

Akachukwu, C.O., Fawusi M.O. (1995). Growth characteristics, yield and nutritive values of water leaf. *Discovery and Innovative* 7(2): 163-172.

Akobundu, I. O. and Agyakwa, C. W. (1998).A handbook of West African weeds. International Institute of Tropical Agriculture, Ibadan, Oyo State, Nigeria. 2nd Edition.

Akpomie, O. O. and Akpan, I. (2013). Multidrug resistance among bacteria isolated from some foods sold in restaurants in Abraka, Nigeria. *International Journal of Microbiology Research and Reviews* **2**(6): 97-102.

Akubugwo, I. E., Obasi, N. A., Chinyere, G. C. and Ugbogu, A. E. (2007). Nutritional and chemical value of *Amaranthus hybridus* leaves from Afrikpo. *Nigerian African Journal of Biotechnology* **6**(4): 2833-2839.

Amaike, S. I. and Keller, N. P. (2011). *Aspergillus flavus*. *Annual Review of Phytopathology* .**49**:107-233.

AOAC. (1999). Official methods of analysis. 21st Edition, Association of official analytical chemists. Washington D. C. USA.

Bannett, H. L. and Hunter, B. B. (1972). Illustrated genera of Imperfect Fungi, Burgress Publishing Company, Minneapolis, Minnesota. 241pp.

Banwart, G.J. (1979). Basic Food microbiology. West Connecticut.AVI publishing Co.inc. pp. 22-84.

Bauer, A.W. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical pathology* 45(4): 493 - 496.

Benson, N.U., and Ebong, G.A.(2005).Heavy metals in vegetables commonly grown in a tropical garden Utisol. *Journal of Sustainable and Tropical Agricultural Resources* **16**: 77 - 80.

Beuchat, L. R. and Ryu, J. (1997). Produce handling and processing practices. *Emerging Infectious Diseases* **3**(2): 459-465.

Brenan, J.P.(1981). The genus Amaranthus in Southern Africa. *Journal of South African Botany* **47**(3): 451 - 492.

Cheesbrough, M. (2006).District laboratory practice in tropical countries.Cambridge University Press 434pp.

Chionyedua, T.O., Anuoluwa, M.O. and Adedoja, D.W. (2009). The proximate and mineral composition of three leafy vegetables commonly consumed in Lagos, Nigeria. *African Journal of Biotechnology* **3**(6): 102 - 107.

Cobb, G. P., Sands ,K., Waters, M. Wixson ,B. G., Dorward, K.E. (2000). Accumulation of heavy metals by vegetables grown in mine wastes. Environmental Toxicology and Chemistry.19, 600-607.

Chweya, J.A. and Ezaguirre, P.B.(1999). The biodiversity of traditional leafy vegetables. International Plant Genetic Resources Institute. Rome 54pp.

Dhellot, J.R., Matouba, E., Maloumbi, M.G. Nzikou J.M., Dzondo, M.G., Linder, M.,

Parmenteir, M. and Desobry, S.(2006b). Extraction and Nutritional properties of *Solanum nigrum* L seed oil. *African Journal of Biotechnology*. 5: 987 - 991.

Domsch, K. H., Gams, W. and Anderson, T. H. (1980). Compendium of soil fungi, Academic press, A subsidiary of Harcourt Brace Jovanovich, publisher. 383pp

Ellis, M. B. (1976). Dermatacious Hyphomycetes, Commonwealth Mycological Institute, Kew, Surrey, UK. 507pp

Fayemi, P.O. (1999).Nigerian vegetables. Heinemann Educational Books, Nigeria Plc. Ibadan. pp 17 - 18.

Gilman, J. C. (1944). A manual of soil fungi, Revised 2nd edition, Oxford and IBH publishing Co

Grubben, G.J. and Denton, O.A. (2004). Plant Resources of Tropical Africa 2.Netherlands. 668pp.

Hamilton, M. J. (2001). Identity and antibiotic susceptibility of enterobacterial flora of salad vegetables. *International Journal Antimicrobial Agents* **18**: 81 – 83.

Iheanacho, K. and Ubebani, A. C. (2009). Nutritional composition of some leafy vegetable consumed in Imo- State, Nigeria. Journal of Applied Science and Environment Management, **13**(3): 35 - 38.

Jolt, J. G., Krieg, N. R., Sneath, P. H. A., Stanley, J. T. and Williams, S. T. (1994). Bergey's manual of systematic bacteriology. Williams & Wilkins Co. Baltimore, Maryland 9th edition 786 pp.

Madden, J. M. (1992). Microbial pathogens in fresh produce–the regulatory perspective. *Journal of Food Protection* **55**:821 – 823.

Martin, F.W. and Telek, K.L.(1979). Vegetables for the hot humid. Part 6: Amaranth Celosia. U.S. Department of Agriculture, New orleans, pp.156 - 163.

Mepha, H.D., Eboh L. and Banigbo D.E. (2007). Effects of processing treatment on the Nutritive Composition and consumer acceptance of some Nigerian edible leafy vegetables. African Journal of Food and Agricultural Nutritional Development.**7**(1): 1-18.

Mohammed, M. I. and Sharif, N. (2011). Mineral composition of some leafy vegetables consumed in Kano, Nigeria. Nigerian Journal of Basic and Applied Science **19**(2): 208 - 211.

National Committee for Clinical Laboratory Standards (NCCLS) (2002). Performance standards for antimicrobial susceptibility testing: twelfth informational supplement. NCCLS document M100-S12. PA, USA.

Nkang, A. O., Okonko, I. O., Mejeha, O. K., Adewale, O. G., Udeze, A. O., Fowotade, A.,

Fajobi, E. A., Adedeji, A. O. and Babalola, E. T. (2009). Assessment of antibiotics susceptibility profiles of some selected clinical isolates from laboratories in Nigeria**.** *Journal of Microbiology and Antimicrobials* **1**(2): 19 - 26.

Nnamani, C.V., Oselebe, H.O. and Agbatutu, A.(2009). Assessment of nutritional values of three underutilized indigenous leafy vegetables of Ebonyi State, Nigeria. *African Journal of Biotechnology* **8(**9**):** 2321 - 2324.

Nnela, K. S. and Cox, K. T. (1988). Potency deterioration of benzyl penicillin, and tetracycline. *Annual Review of Medical Microbiology* **121**(26): 166 - 172.

Obieze, K. O., Ogbuagu, C. N., Asikong, B. E., Onyido, A. E. and Ogolo, B. A. (2010). Bacteriological study of vegetables from markets of Calabar, Cross-River State, southeastern Nigeria. *International Journal of Public Health* **1**(1):10 - 14.

Ogbeibu, A. E. (2005). *Biostatistics: A practical approach to research and data handling.* Mindex press, Benin City, Nigeria, 264p.

Ogle, B.M. and Grivetti, L.E. (1985). Legacy of the chameleon: Edible wild plants in the kingdom of Swaziland, Southern Africa. A cultural, ecological nutritional study. Ecology of Food Nutrition 17: 41 - 64.

Okafo, C. N., Umoh, V. J. and Galadima, M. (2003). Occurrence of pathogens on vegetables

harvested from soils irrigated with contaminated streams. Science and Total Environment **311**: 49 – 56

Oliveria, J.S. and DeCarvalho, M.F. (1975). Nutritional value of some edible leaves in Mozambique. *Economic Botany* 29: 255 - 259.

Ononugbu,I.C.(2002). Lipids in human existence.1st Edition Ap Expess publishing Company. Nsukka Nigeria. 1 - 15.

Österblad, M., Pensala, O., Peterzéns, M., Heleniusc, H. and Huovinen, P. (1999).

Antimicrobial susceptibility of Enterobacteriaceae isolated from vegetables. *Journal of Antimicrobial Chemotherapy* **43**:503 – 509.

Oyeleke, S. B. and Manga, S. B. (2008).Essentials of laboratory practicals in Microbiology Tobest publisher, Minna. Nigeria pp. 36 -75.

Pelczar, M.J. and Reid, R.D. (1998). Activities of antimicrobial agents in Microbiology. 87(6): 74 - 83.

Robinson, D.S.(1990). Food Biochemistry and Nutritional value. Longman scientific and Technical publisher, New York, USA.

Schipper, M.A.(1984). A revision of the genus Rhizopus: The *Rhizopus stolonifer* group and *Rhizopus oryza*, studies in Mycology pp. 1 - 19.

Sobukola, O.P., Dairo, O.U, Odunewu, A.V., and Fafiolu B.O.(2007). Thin layer drying process of some leafy vegetables under open sun. *Food Science and Technology* 13(1):35 - 40.

Taiga, A., Suleiman, M.N., Aina, D.O., Sule, W.F. and Alege, G.O. (2008). Proximate analysis of some dry season vegetables in Anyigba, Kogi State, Nigeria. Africa Journal Biotechnology **7**(10): 1588 - 1590.

Tindall,H.D.(1983). Vegetables in the tropics. Macmillian Press Ltd, London. 533p.

Wasfy, M. O., Oyofo, B. A., David, J. C., Ismail, T. F., Elgendy, A. M. and Peniski, L. F.

(2000). Isolation and antibiotic susceptibility of *Salmonella*, *Shigella* and *Campylobacter* from acute enteric infections in Egypt. .*Journal on Environmental Sciences* **18**(11): 35-38.