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Assessment of Phytochemical Constituents and Antibiogram Activities of Vegetables Sold in Owerri, IMO State Nigeria

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Abstract

Phytochemical and antibiogram activities of vegetables sold at Relief Market Owerri were investigated. Ugu leaves (*Telfairia Occidentalis)*, Scent leaves (*Ocimum gratissimum*), Water leaves (*Talinum fruticosum*), and Oha leaves*, (Pterocarpus mildraedii)* were analysed. The vegetable samples were aseptically collected, blended, serially diluted, cultured and incubated. Standard microbiological and 16S rRNA gene sequencing method were used in the isolation and identification of bacteria. Soxhlex apparatus was used to extract active ingredients from vegetables. Methanol and hot water extracts of the vegetable samples were used against identified isolates. The Total heterophilic bacteria count ranged from 2.7-3.8 x 10^5 cfu/g; Total Coliform count ranged from 1.1-2.3 x 10^5 cfu/g; and Total *Salmonella Shigella* count ranged from 2.5-3.4 x 10^5 cfu/g. Five bacteria isolates *Escherichia coli, Salmonella typhi, Shigella dysenteriae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were identified while the tannin, alkaloids, flavonoids, saponin and steroids were extracted from the vegetables. Percentage growth inhibition of bacteria by methanol extract was greater than hot water extract. Percentage growth inhibition of bacteria by methanol and hot water extract of scent leaves at 200mg/ml gave the highest inhibition followed by oha leaves, ugu leaves, while water leaves gave the least growth inhibition. Vegetables should be thoroughly washed and cooked before consumption.

Keywords: Antibiogram, Phytochemicals, Soxhlex Apparatus and Vegetables

Introduction

Vegetable has been discovered and used since prehistoric ages because they are able to synthesize many chemical compounds for defense against bacterial diseases (Lichterman, 2004) [5]. The medicinal value of plants lies in the bioactive phytochemicals like alkaloids, tannin, saponin flavonoids and saponin present in the plants. There is a global shift to produce drugs from plants to ensure safety to health and better economy of a nation (Davis, 1963) [4].

Materials and Methods

Four fresh green leafy vegetables Ugu leaves (*Telfairia Occidentalis)*, Scent leaves (*Ocimum gratissimum*),Water leaves (*Talinum fruticosum*) Oha leaves*, (Pterocarpus mildraedii)* were purchased from market in Owerri, Imo state, Nigeria. Samples were collected in sterile sample containers, labelled properly and were sent to the laboratory for microbiological analysis. Isolation, morphological and biochemical identification of bacteria. The media used for isolation were nutrient agar and macConkey agar. Streaking plate method was used for isolation. Vegetable samples were serially diluted and cultured at 37 ℃ for 24 hours. The bacterial isolates were identified using colonial morphology and biochemical tests (Cheesbrough, 2006) Molecular identification.

The deoxyribonucleic acid (DNA) was extracted using Zymo research bacterial DNA mini prep extraction kit. The qualitative estimation of genomic DNA was done using agarose gel electrophoresis. The extracted DNA was amplified using Polymerase chain reaction amplification protocol. Sequencing protocol. PCR products were cleaned using ExoSAP Protocol. Fragments were sequenced using the Nimagen brilliant dye terminator cycle sequencing kit according to manufacturer's instructions (Platt *et al*., 2007) [10]. The sequenced data were subjected to Basic Local Alignment Search Tool Nucleotide (BLASTn) to identify

corresponding organisms from National center for bioinformatics information (NCBI) as described by (Altschul *et al*., 1990) [2]. The organic solvents used were Methanol and Hot water. Methanol Extraction. Fifty grams (50g) of samples of each leaves were used. Using a soxhlex apparatus, the active ingredients of the ground particles were extracted. Hot water Extraction. Fifty grams (50g) of samples of each leaves were used. Samples were soaked in hot water for 24 hours; the active ingredients of the ground particles were filtered.

Bacterial susceptibility testing. Extract-Nutrient Agar (NA) mixtures were prepared by mixing extract with molten NA before the agar solidified. Discs of the bacteria grown on NA (3 mm in diameter) were cut with cork borer and placed in the Centre of Petri plates containing different concentrations of the extracts. Controls were Petri plates prepared with NA but without the addition of extracts. Replicates of each treatment were incubated at 37^0C for 24hours. Radial growth was measured with a metric rule in extract-treated and controls and expressed as percentage. (Okorondu *et al.*, 2012) [8]. Minimum inhibitory concentration (MIC). Aliquot of the extract (400mg) was dissolved in 2 ml of sterile distilled water to obtain concentration of 200mg/ml. One milliliter (1ml) of each concentration of extract was transferred to a sterile Petri plate and 10 ml of cooled Nutrient agar were poured into the plates, swirled to mix and allowed to solidify. This was incubated at 37^0C for 24hours and observed for bacterial growth. The lowest concentration of the extract that inhibited the growth of bacteria was recorded as the minimum inhibitory concentration. Plates without the test extracts were used as control (Okorondu *et al*., 2012) [8]. Determination of Photochemical constituents was done according to (Trease and Evans, 1989) [11].

Results and Discussion

The Total heterophilic bacteria count ranges from 2.7-3.8 x 10^5 cfu/g; Total Coliform count ranges from 1.1-2.3 x 10^5 cfu/g; and Total *Salmonella Shigella* count ranges from 2.5-3.4 x 10^5 cfu/g. Table 1: Shows the Molecular characterization of identified isolates using the 16S rRNA sequence. Bacteria isolated were *Escherichia coli, Salmonella typhi, Shigella dysenteriae, Pseudomonas aeruginosa* and *Staphylococcus aureus*. Table 2: Qualitative analysis of phytochemical constituents (Phytochemical screening of Vegetables extracts). Table 3: Percentage growth inhibition of bacteria by methanol and hot water extract of Scent leaves, Oha leaves, Ugu leaves and water leaves.

Table 1: Molecular sequence 16S rRNA identity of various bacteria

S. No.	Biochemical Isolates	Percentage (%)	NCBI Match
1	*Escherichia coli*	99	*Escherichia coli* M75029
2	*Salmonella* spp	99	*Salmonella typhi* L21912
3	*Shigella* spp	100	*Shigella dysenteriae* LR739008
4	*Pseudomonas* spp	96	*Pseudomonas aeruginosa* WE 41437
5	*Staphylococcus aureus*	97	*Staphylococcus aureus* MH401415

Table 2: Qualitative analysis of phytochemical constituents (Phytochemical screening of Vegetables extracts)

Plant extracts	Tannin	Phytochemicals Alkaloid	Flavonoid	Saponin	Steroid
Ugu leaves (*Telfairia Occidentalis)*	+	+	+	+	+
Scent leaves (*Ocimum gratissimum*)	+	+	+	+	+
Water leaves (*Talinum fruticosum*)	+	+	+	+	+
Oha leaves, *(Pterocarpus mildraedii)*	+	+	+	+	+

Key: + Presence of Phytochemical

Table 3: Percentage growth inhibition of bacteria by methanol and hot water extract of Scent leaves, Oha leaves, Ugu leaves and water leaves

Isolates	Scent leaves 200mg/ml	Oha leaves 200mg/ml	Ugu leaves 200mg/ml	Water leaves 200mg/ml
Control	0.00	0.00	0.00	0.00
Methanol				
Escherichia coli	11.00 ± 1.000	6.00 ± 0.577	5.00 ± 0.577	3.00 ± 1.000
Salmonella typhi	13.00 ± 1.527	7.00 ± 0.577	4.00 ± 0.577	2.00 ± 0.577
Shigella dysenteriae	5.00 ± 1.000	4.00 ± 0.577	3.00 ± 0.577	2.00 ± 0.577
Pseudomonas aeruginosa	13.00 ± 1.000	6.00 ± 1.000	2.00 ± 0.577	0.00 ± 0.000
Staphylococcus aureus	3.00 ± 0.577	3.00 ± 0.577	2.00 ± 1.000	3.00 ± 0.577
Hot water				
Escherichia coli	5.00 ± 1.000	3.00 ± 0.577	3.00 ± 1.000	2.00 ± 0.577
Salmonella typhi	7.00 ± 1.000	2.00 ± 0.577	3.00 ± 1.000	2.00 ± 0.577
Shigella dysenteriae	2.00 ± 0.577	2.00 ± 0.577	2.00 ± 0.577	1.00 ± 0.577
Pseudomonas aeruginosa	6.00 ± 0.577	1.00 ± 0.577	2.00 ± 0.577	0.00 ± 0.000
Staphylococcus aureus	1.00 ± 0.577	1.00 ± 0.577	1.00 ± 0.577	0.00 ± 0.000

Vegetables from this study contain high bacterial load. Five bacteria isolates identified, were *Escherichia coli*, *Salmonella typhi*, *Shigella dysenteriae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* According to (Ahn, 2017) [1] observed the presence of *Escherichia coli* in scent leaves, oha leaves ugu leaves also, its presence indicates recent contamination by faecal matter. The study of (Nnam *et al*., 2013) [6] and (Oladosu-Ajayi *et al*., 2017) [9] observed that that scent leave (*Ocimum gratissimum*) and other local herbs contain both nutritive and phytochemical composition.

It was reported that the health benefits of vegetables such as scent leaves, ugu leaves, oha leaves and other plants lie on their phytochemical compositions (Okorondu *et al*., 2010b) [7].

Conclusion

From this study, vegetables contain various bacteria. Vegetables also contain phytochemicals and have various antimicrobial activities. Therefore, vegetables should be properly washed and cooked before consumption. They should also be used regularly at home and clinical setting because of their therapeutic potentials.

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