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Original Research Article

Evaluation of antimicrobial activities of fluted pumpkin leaf extract (*Telfairia occidentalis)* against selected pathogenic bacteria using standard drug

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Keywords

Bacterial count, minimal inhibitory concentration (MIC)

A B S T R A C T

The antimicrobial effect of *Telfairia occidentalis* against pathogenic bacteria was investigated. The method used includes disc diffusion method and dilution technique. The fluted pumpkin extract was tested against *Staphylococcus aureus* and two *Escherichia coli* isolates. The leaf extract produced 1.0mm zone of inhibition against *Staphylococcus aureus*, and also produced 2.00mm zone of inhibition against *Escherichia coli* isolated from frozen chicken. The leaf extract produced no inhibitory activity on *E. coli* isolated from hospital laboratory but at 8ug/ml, it inhibited *Staphylococcus aureus*, while 2ug/ml, 4ug/ml and 6ug/ml produce no inhibitory activity. The tested material (leaf extract) was bactericidal on *Staphylococcus aureus* at 8ug/ml.

Introduction

Bacteria constitute a large domain of prokaryotic microorganisms typically ranging from 0.15 to 4 micrometers in length. Bacteria have a number of shapes, ranging from spheres to rods and spirals. They were among the first life forms to appear on earth and are present in most habitats. Bacteria inhabit soil, water, acidic springs, radioactive waste and deep portions of earth crust. (Fredrickson *et al.,* 2004). Bacterial also live in symbiotic and parasitic relationship with plants and animals. There are typically million bacterial cells in a gram of soil and a million bacterial cells in a milliliter of fresh water. There are approximately $5x10^{30}$ bacteria on earth. (Whitman *et al.,* 1998) forming a biomass which exceeds that of all plants and animals (Michael, 2010). According to reports, they are extremely adaptable to conditions and survive wherever they are. (Choi Charles, 2013). The most common fatal bacterial disease are respiratory infections with tuberculosis alone killing about 2 million people a year, mostly in sub-Saharan Africa (Sears, 2005; Alada, 2000; Baynes, 1991; Fasuyi and Nonyerem, 2007). In developed countries, antibiotics are used to treat

bacterial infections and are also used in farming, making antibiotic resistance growing problems. Bacteria infections are major cause of death especially for and elderly people (Wassenaar and Blaser, 1999). Treatment of an infection with pathogenic bacteria involves the use of antibiotics drugs which have been superficially formulated to kill bacteria. Some bacteria have developed antibiotic resistance, which means that they may not respond to many common antibiotics (Fish, 2002).

Fluted pumpkin has been found to be medicinally useful, apart from the nutritional, agricultural and industrial importance of it (Justus, 2012). *Telfairia occidentalis* (fluted pumpkin) commonly called "ugu" is a vegetable which belongs to the family *Cucurbitaceae.* It is a crop of commercial importance grown in West Africa (Nigeria, Ghana and Sierra Leone), being the major producers (Eseyin *et al*, 2005). It is called "ugu" in Igbo land, "iroko" in Yoruba land and "umeke" in Edo. Fluted pumpkin cultivated majorly for its leaves and eaten as potherbs (potherb is any plant used to add flavour in cooking) and the seeds can be eaten whole by boiling (Nwanna, 2008). According to the World Health Organization (WHO) a medicinal plant is any plant which in one or more of its organs contains substances that can be used for the synthesis of useful drugs.

Medicinal plants contain biologically active chemical compounds such as saponins, tannins essential oils flavonoids, alkaloids and other chemical compounds (Grubben and Denton, 2004) which have curative properties. These complex chemical compounds of different compositions are found as secondary metabolites in one or more of these plants (Kayode and Kayode, 2011).

Fluted pumpkin is a green leafy vegetable, rich in dietary properties such as calcium, iron potassium and some levels of folic acid and manganese. The leaves also contain high levels of vitamin A and K and vitamins C, B_2 and E. The leaves have great antioxidant capacities to help in restoring damaged cells and skins. Fluted pumpkin leave protects hearts and liver from harmful toxins and painkillers such as paracetamols. It reduces the risk of heart diseases because it contains a lot of natural anti-inflammatory. It contains lots of phytonutrients which is said to reduce the risk of breast cancer and stomach cancer. Researchers and studies have also indicated that fluted pumpkin possess antibacterial, erythropeotic (erythropoesis, process of red blood cells production), anticholestrollemic (prevents the buildup of cholesterol) and antidiabetics activities. Fluted pumpkin treated diabetes mellitus lowering glucose levels in the blood (Justus, 2012). This research was undertaken to investigate if the tested material has antimicrobial activity, determine the strength of the tested material on the isolates and to obtain the concentration of the plant extract using a standard drug (antibiotics) and to determine the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC).

Materials and Methods

Fresh leaves of fluted pumpkin (*Telfairia occiedentalis*) were purchased from Orie Emene market, in Emene, Enugu State, Nigeria and brought to Microbiology laboratory of Godfrey Okoye University Enugu. The leaves were air-dried at room temperature over a period of one week. The leaves were reduced to powder using an electric blender. The ground leaves were later packed in a clean polythene bag and kept in the laboratory until when it was

used. The test organisms used were *Staphylococcus aureus* and *Escherichia coli.*

Nutrient Agar, MacConkey, agar, nutrient broth, methanol and DMSD (Dimethyl Sulphodioxide) were the media and chemical reagent. Autoclave, hot air oven, incubator, refrigerator weighing balance, inoculating wire loop, beakers, conical flask, distilled water, Whitman's (Noll) filter paper, muslin cloth, knife, dropper pipette, cotton wool, aluminum foil, spatula, electric blender, Bunsen burner, bijoux bottles, funnel, test tubes were the instruments applied.

The ground leaves (100G) were weighed out into a conical flask using a weighing balance. The ground leaves were extracted using 500ml of methanol for 72 hours with constant agitation (Silver *et al.* 1997) after which it was filtered using filter paper. The filtrate was evaporated to dryness using a steam water bath at 100°C (Silver *et al.,* 1997). The extract was then stored inside the refrigerator at 4°C.

The cultural characters of the test organism were studied by inoculating the organisms into different media. The media used were prepared according to manufacturers guide. They include 28g for nutrient agar, 13g for nutrient broth, 52g for MacConkey agar, and 15g for peptone water per little of distilled water. The media were sterilized using an autoclave at the temperature of 121°C for 15 minutes. The media were allowed to cool and were poured into sterile disposable Petri dishes. After solidification, the organisms were inoculated by streaking under aseptically condition and were incubated for 24 hours at 37°C using an incubator. The morphological characters such as colony and colour were observed.

A smear of the test organisms were made on different clean slides and were allowed to air dried for few seconds. The smears were flooded with crystal violet and were allowed to stand for 30 seconds. The stains were washed off with distilled water and were allowed to drain off. The stains were covered with Lugoes iodine and allowed to stand for 30 seconds before washing off with distilled water. The stains were flooded with acetone and washed off immediately. The smears were later counterstained with safranin and let to stand for 1 minute. The stains were washed off with distilled and allowed to air-dry. They were examined under oil immersion (100x). Catalase test was carried out in which a small amount of the organisms were smeared in different slides, a drop of hydrogen peroxide were added on each slide and were observed for vigorous bubbling. The coagulase test was done to differentiate strains of S*taphylococcus* species. Test tubes containing 0–1ml of plasma were labeled. The organisms were inoculated differently in the plasma tubes, capped and stored in a water bath at 37^0C for several hours. The bottles were checked fro clumping solidification or semisolid. The Indole test was used to differentiate gram-negative rods especially *Escherichia coli* that breakdown the amino acid tryptophan with the release of indole. The organism was cultured in test tubes containing 3ml of sterile peptone water and was incubated at 37°C for 48 hours. After the incubation 0.5ml of Kovac's indole reagent was added to the tubes, stirred and were allowed to stand for 10mins. A red colour in the surface layer was observed. After 72 hours, the filtrate evaporated to dryness using steam water bath remaining the extract. The methanol extract was then standardized using dimethyl sulphodioxide (DMSO). The extract was standardized by dissolving 1g of the extract in 5ml of 40% DMSO. The standardize extracts were stored in the refrigerator until when used.

The extract was tested for antibacterial activities using disc diffusion method. The test material was tested against *Staphylococcus aureus* and *Escherichia coli.*

Sterile nutrient agar plates were streaked with the test organism 0.5mm sterile paper disc soaked inside the extract from two hours were placed on the agar surface with different concentrations of standard drug (Table 3 and 4). On the agar surface were four disc papers containing the standard drug and one disc paper containing the extract in the middle. The plates were incubated for 24 hours at 37°C and the results were observed.

The minimum inhibitory concentration of the plant extract was determined by serial dilution method. Nutrient broth used was prepared according to manufacturers guide and 1ml of the sterile nutrient was transferred into four separate tubes labeled according to the concentration of the extracts (2, 4, 6, 8ug/ml) a loop full of the test organisms were inoculated into the tubes, they were then incubated at 37°C for 24 hours. The concentration of the plant extract, which was 8ug/ml with no turbidity, was recorded as the MIC. The extract without the microorganism served as the control. The MBC was determined by plating out tube with no turbidity on nutrient agar plates and absence of growth after incubation for 24 hours confirms the MBC.

Results and Discussion

The confirmatory results of the tested organisms are presented in Table 1. While the zones of Inhibition produced using the methanol extract are presented in Table 2. This showed that the plant extract has antimicrobial properties on the tested organisms. This result agrees with earlier research carried on the same plant by other researchers like Oboh *et al.,* (2006); Glud *et al.,* (2013); Akoroda (1990); Aderibigbe *et al.,* (1999) and Koraceri (2004). Most of their result showed a higher antimicrobial activity against the following organisms with their zones of inhibition: *E. coli* (0.58mm at 500mg/ml) *Streptococcus faecalis* (1.10mm at 5.0mg) and *Salmonella typhi* (0.70mm at 50mg/ml). Figure 1, the bar chart shows that the extract produced a higher zone of inhibition on *E. coli* strain isolated from frozen stock (bird) but has low effect on *Staphylococcus aureus.*

The tested material (absolute) that produced a zone of inhibition of 2.0mm has a concentration of 0.007ug/ml. Table 5 shows the result of the minimal inhibitory concentration of the leaf extract. The result of the minimal inhibitory showed that at the concentration of 8ug/ml, the leaf extract was able to inhibit *Staphylococcus aureus*. At 4ug/ml, 2ug/ml, 6ug/ml produce no inhibitory activity on *Escherichia coli* strain 2 (hospital lab isolate), the reason may be due to exposure to antibiotics.

The fluted pumpkin leaf extract is bactericidal on S*taphylococcus aureus* only. Therefore, the idea of producing various synthetic drugs was gotten from plant. Today, about 25% of drugs are produced using plants but many are not used as an antimicrobial agent. As the drug resistant microorganisms are on a rapid increase, new and effective drugs are needed for the health sector. The results showed that the leaf extract of *Telfairia occidentallis* was able to inhibit pathogenic microorganisms both gram-positive and gram negative bacteria. If well modified, the plant leaf may serve as an agent for antimicrobial drug whose activity will produce little or no side effect to the body system.

Table.1 Confirmatory result for the test organisms

Characteristics	***Staphylococcus* aureus**	***Escherichia coli***
Colours of colony	Light cream	Pink
Shapes	Cocci	Rod
Gram reaction	+	_
Nutrient agar	+	+
Mac Conkey agar	+	+
Catalase test	+	N/A
Indole test	_	+
Coagulase test	+	N/A

+ = Positive; - = Negative; N/A = Not Applicable

Table.2 Zones of inhibition produced using the methanol extract

Bacterial culture	**Leaf extract**	**Diameters of zones of inhibition(mm)**
E. Coli	MD	2.0
Staphylococcus aureus	MD	1.0

Table.3 Concentrations of the standard drugs used on *Escherichia coli*

Standard drug concentration (ug/ml)	**Zones of inhibition (mm)**
0.0063	3.0
0.0031	2.9
0.0016	2.7
0.00078	2.5
Tested material	2.0

Table.4 Concentrations of the standard drugs used on *Staphylococcus aureus*

Standard drug concentration (ug/ml)	**Zones of inhibition (mm)**
0.0063	3.5
0.0031	2.5
0.0016	2.0
0.00078	1.7
Tested material	1.0

Fig.1 Graph for derivation of tested material concentration using the standard drug- ciprofloxacin (500mg)

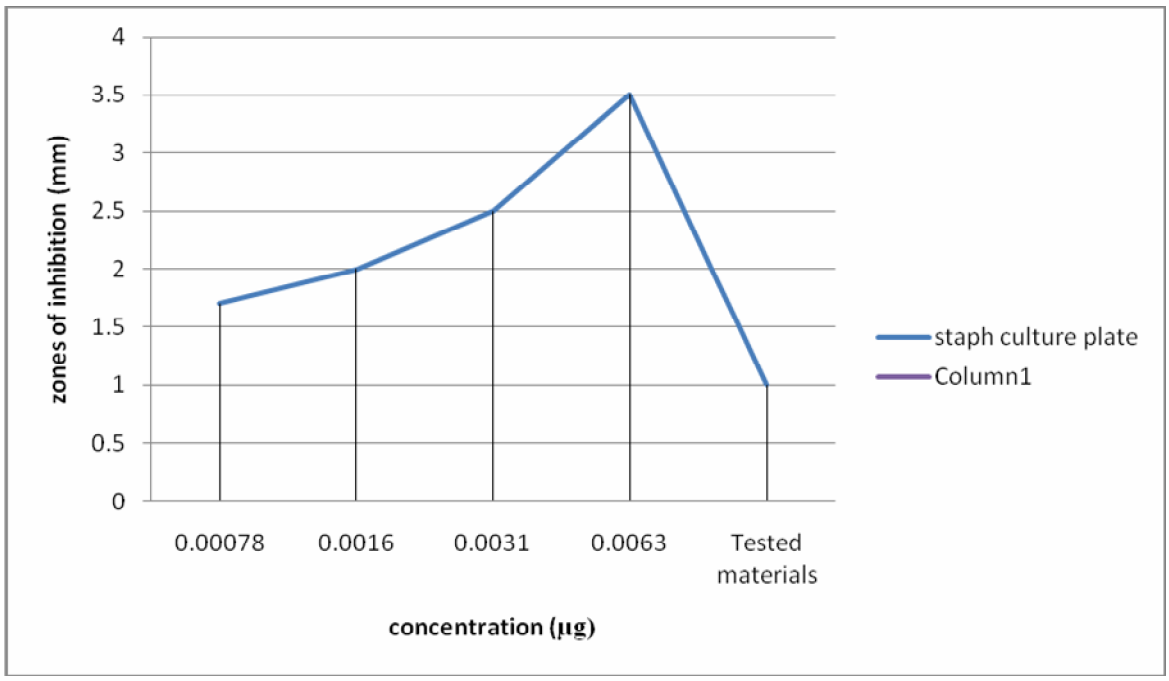

Table.5 Result of minimum inhibitory concentration

ORGANISMS	CONCENTRATION OF THE LEAF EXTRACT (ug/ml) 2ug/ml, 4ug/ml 6ug/ml, 8ug/ml			
	2ug/ml	4ug/ml	6ug/ml	8ug/ml
S. aureus	-	-	-	+
E. coli	-	-	-	-

+ = Growth; - = No Growth

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