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Comparative Antimicrobial Analyses of the Leaf and Stem Extracts of *Telfairia occidentalis*.

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ABSTRACT

This research evaluated the comparative antimicrobial analyses of the leaf and stem extracts of *Telfairia occidentalis*. The Bacterial isolates used in this work were two Gram-positive and two Gram-negative. *Staphylococcus aureus* and *Bacillus subtilis* for Gram-Positive Bacteria, *Escherichia coli* and *Samonella typhi* for Gram-negative bacteria. Nutrient Agar was the media used, and it was prepared according to the manufacturer directives. Microbial resistance to several antibiotics is becoming a source of challenge and concern to public health. In view of the increasing rate of antimicrobial drug resistance ravaging not only the African continent, but the world at large, alternative, effective and affordable substitutes is essential if bacterial infections are to be properly controlled. The result of this study supports the use of the leaf and stem parts of *Telfairia occidentalis* for medicinal purposes. The extent of sensitivity of the test organisms to the extracts of plant parts was assessed. Results showed the antimicrobial activity of *Telfairia occidentalis* leaf and stem extracts using different extracting solvents. The results revealed that the ethanol extract of the leaf of *Telfairia occidentalis* was most effective against the test organisms than the aqueous extracting solvent. Using ethanol as the extracting solvent, *Bacillus subtilis* showed the highest susceptibility (9.17 ± 2.02) to the leaf extract, while *Salmonella typhi* showed the least susceptibility (4.67 ± 1.04). Using aqueous extract, *Escherichia coli* showed the highest susceptibility (5.83 ± 3.40) to the leaf extract, while *Bacillus subtilis* showed the least susceptibility (3.33 ± 2.08). The results also revealed that the ethanol extract of the stem of *Telfairia occidentalis* was most effective against the test organisms than the aqueous extracting solvent. Using ethanol as the extracting solvent, *Escherichia coli* showed the highest susceptibility (6.83 ± 6.11) to the stem extract, while *Bacillus subtilis* showed the least susceptibility (5.16 ± 5.03). However, using aqueous extract, *Bacillus subtilis* showed the highest susceptibility (2.17 ± 1.60) to the leaf extract, while *Escherichia coli* showed the least susceptibility (1.5 ± 1.5). This is in agreement with the observations of [28], who concluded that the stronger extraction capacity of ethanol could have been responsible for the higher antimicrobial activity. *Telfairia occidentalis* leaf and stem are very rich source of bioactive compounds and the intake of these plants chemicals have a protective potential against some tropical disease in the use of leaf in folk medicine in Nigeria.

Keywords: Antimicrobial, *Telfairia occidentalis*, medicinal, and bioactive compounds

INTRODUCTION

The practice of traditional medicine is as old as the origin of man [1,2,3]. The use of plants in traditional medicine referred to as herbalism or botanical medicine falls outside the mainstream of the Western or Orthodox medicine [4]. It has been estimated that about two third of the world's population (mainly in the developing countries) rely on traditional medicine as their primary form of health care [5]. The use of traditional medicine in the treatment and management of diseases in the African continent cannot fade away and this could be attributed to the socio-cultural, socio-economic, lack of basic health care and qualified personnel [6]. *Telfairia occidentals* is a vigorous perennial vine, growing to 10m or more in length. The stems have branching tendrils and the leaves are divided into 3-5 leaflet. The fruits are pale green, 3-10kg in weight, strongly ribbed at maturity and up

to 25cm in diameter. The seeds are 3-5cm in diameter. *Telfairia occidentalis* known as fluted pumpkin occurs in the forest zone of West and Central Africa; they are found more in Benin, Nigeria and Cameroon. It is a well-known vegetable all over Nigeria. It was found first in South-east Nigeria and was distributed by the Igbos', who have cultivated this crop for a very long time. It is possible that fluted pumpkin was originally wild throughout its current range, but that wild plants have been harvested to local extinction and are now replaced by cultivation forms [7,8,9,10,11]. The antibacterial activity of the leaf of *Telfairia occidentalis* (fluted pumpkins) against selected intestinal pathogens has been investigated using the agar diffusion technique [12,13]. The extract showed a higher antibacterial activity against *E. coli, S.faecalis* and *S. typhi*. MIC was 0.5, 5.0 and 500mg/ml for *E. coli, S. Typhi* and, *S. faecalis*, respectively [14, 15]. Similarly, the ethanolic leaf extract had a higher inhibitory effect on some of the commonly encountered Entero bacterioceae in Nigeria. The aqueous extracts had a higher inhibition of the growth. The crude extract inhibited the growth of 93.1% of the tested microorganisms [16]. The extracts of the plant caused concentration dependent paralysis and death of the worms, with the aqueous extracts showing higher worm inhibitory and destructive activities compared to the methanol extracts [17]. The primary aim of this study is to comparatively analyze the antimicrobial properties of the leaf and stem extracts of Ugu (*Telfairia occidentalis*).

MATERIALS AND METHODS

Collection of samples

Fresh leaves and stem of ugu, fluted pumpkin (*Telfairia occidentalis)* were bought from Ihiagwa market Imo state and taken to the laboratory for analysis.The leaves and stem were oven dried at 40^0C for 48 hours. The leaves were reduced to powder form using an electric blender. The ground leaves and stem were later packed in a clean polythene bag and kept in the laboratory until when it was needed.

Test Organism

The Bacterial isolates used in this work were two Gram-positive and two Gram-negative. *Staphylococcus aureus* and *Bacillus subtilis* for Gram-Positive Bacteria, *Escherichia coli* and *Samonella typhi* for Gram-negative bacteria. All stains are Clinical isolates obtained from New concepts Analytical Laboratory and Environmental Services Limited Obinze, Owerri Imo state and identified by Mr Chidebere a microbiologist in the Laboratory.

MATERIALS

Nutrient Agar was the media used, and it was prepared according to the manufacturer directives. Autoclave, hot air oven, incubator, refrigerator weighing balance, stem borer, inoculating wire loop, beakers, conical flask, petri dish, soxhlet extractor, distilled water, Whitmans (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 used.

METHODS

Sterilization of Materials

All the glass wares used for the experiment were sterilized using the laboratory autoclave at a temperature of 121°C for 15 minutes at 15 lb psi. Wire loop was sterilized over burning flame and allowed till its red-hot, while glass spreader was sterilized by dipping into 70 % ethanol and passing over Bunsen flame, The media used in this study include: Nutrient agar which was prepared according to manufacturer's instructions and sterilized using the autoclave at a temperature of 121° C at 15 lb psi for 15minutes and was allowed to cool to a temperature of 45° C. Exactly 20 milliliters of the cooled, prepared media each was poured into sterile petri-dishes. The plates were allowed to cool and set for inoculation [18].

Extraction of Active Ingredients in the Leaves

Ethanol Extraction

Twenty grams (20g) of each of the ground leaves was extracted using soxhlet extraction method. For soxhlet extraction, 20g of each of the sample was placed in the thimble which was placed in the sample holder of the extraction chamber of the soxhlet extraction apparatus. The set up was completed and the condenser (containing the water in-let and out-let hoses) as well as the heating mantle was put in place. The quantity of ethanol in the flat bottom flask used was 200m1. Then the heating mantle was connected to the mains to begin the extraction. The process lasted 1 hour after which the extracts were recovered from the ethanol mixture using the Rotary Vacuum Evaporator.

Plate 1: Image of the setup of a Soxhlet Extractor

Aqueous Extractions

Cold Water Extract

20g of finely pulverized leaves and stem of *Telfaira occidentalis* was weighed, poured into an empty Beaker, 100 ml of distilled water was also added to the contents in the bottle after measuring, and then allowed to stand for 24 hours without disturbance. After 24 hours, I then decant into another clean empty beaker using a filter paper so as to obtain the extract. The extract was heated in a water bath at 40^0C so as to evaporate the solvent and then stored in a sample collection container until when needed.

Antimicrobial Sensitivity Screening

The modified [19] agar well diffusion method was employed to determine the antimicrobial activities of the ethanoic and aqueous extract of the leaves and stem of fluted pumpkin. Different concentration of the extracts 500mg/l, 750mg/l and 1000mg/l was prepared. 0.1 ml of the standardized 24 hour culture of the tested organisms in nutrient broth was spread unto sterile prepared nutrient agar plate, these when then allowed to set. With the aid of a sterile cork borer, wells of about 5mm in diameter were made on the plate. 0.05ml of each concentration of the extracts was dispensed into the wells and then allowed to stand for about 15minutes for pre diffusion of the extracts to occur, these were then incubated at 37^0c for 24 hours. At the end of the period, the inhibition zone formed in the agar was evaluated in mm [20]. The diameter of the zones of inhibition in the plates were measured by calculating the difference between the cork borer (5mm) and the diameter of inhibition [21] A commercial antibiotic was prepared as a positive control by dissolving 250mg of the drug chloramphenicol into10ml of distilled water.

Minimum inhibition concentration

The minimum inhibition concentration (MIC) which is defined as the lowest concentration of the antimicrobial ingredient or agent that is bacteriostatic(prevents visible growth of bacterial) was determined by measuring 5ml of the nutrient broth into empty sterile tubes, one milliliter of the different concentrations of extracts was then added and 0.5ml of the test organisms was also added. This was incubated for 24hours at 37^0c. The tubes were then observed for visible growth with the help of a spectrophotometer (5ml of broth: 1ml of extract). The tubes with the least concentration of the extract that showed no growth (-ve) was determined as MIC.

Minimum Bacterial Concentration

Minimum Bacterial Concentration (MCB) which is defined as the lowest concentration of the antimicrobial ingredient or agent that is bactericidial (will kill a particular bacterial) was determined by sub-culturing The negative tubes form MIC which were pour plated on nutrient agar and incubated for 24 hours at 37^0c. The tube with the least concentration of the extract that showed no growth at the concentration was reported as the minimum bacterial and concentration (MBC) on plate.

RESULTS

Table 1: Antimicrobial Sensitivity of Leaf Extracts of *Telifeira occidentalis* on Selected Standard Micro-organism using Ethanol

S/N	Microorganism	500mg/l		750mg/l		1000mg/l		Control
1	*Echerichia coli*	5	4	9	9	12	13	17
2	*Salmonella typhi*	3	4	5	5	5	6	11
3	*Staphylococcus aureus*	4	3	7	6	8	8	20
4	*Bacillus subtilis*	7	7	10	9	11	11	11

Table 2: Antimicrobial Sensitivity of Leaf Extracts of *Telifeira occidentalis* on Selected Standard Micro-organism using Water

S/N	Microorganism	500mg/l		750mg/l		1000mg/l		Control
1	*Echerichia coli*	2	2	7	7	9	8	17
2	*Salmonella typhi*	2	1	5	4	9	10	11
3	*Staphylococcus aureus*	1	2	5	6	8	7	20
4	*Bacillus subtilis*	1	1	4	4	5	5	11

Table 3: Antimicrobial Sensitivity of Stem Extracts of *Telifeira occidentalis* on Selected Standard Micro-organism using Ethanol

S/N	Microorganism	500mg/l		750mg/l		1000mg/l		Control
1	*Echerichia coli*	2	1	5	6	14	13	17
2	*Salmonella typhi*	1	-	5	4	10	11	11
3	*Staphylococcus aureus*	2	3	4	5	10	11	20
4	*Bacillus subtilis*	1	-	5	4	11	10	11

Table 4: Antimicrobial Sensitivity of Stem Extracts of *Telifeira occidentalis* on Selected Standard Microorganism using Water

S/N	Microorganism	500mg/l		750mg/l		1000mg/l		Control
1	*Echerichia coli*	-	-	1	2	3	3	17
2	*Salmonella typhi*	-	1	2	2	2	3	11
3	*Staphylococcus aureus*	-	-	1	2	4	4	20
4	*Bacillus subtilis*	1	1	1	2	4	4	11

Table 5: Mean and Standard Deviation of Antimicrobial Sensitivity of Leaf and Stem Extracts of *Telifeira occidentalis* on Selected Standard Microorganism using Ethanol and Water

Test Organisms	Leaf Extract		Stem Extract	
	Ethanol	Water	Ethanol	Water
Echerichia coli	8.67 ± 4.01	5.83 ± 3.40	6.83 ± 6.11	1.5 ± 1.5
Salmonella typhi	4.67 ± 1.04	5.17 ± 4.04	5.17 ± 5.03	1.67 ± 1.04
Staphylococcus aureus	6.0 ± 2.29	4.83 ± 3.06	5.83 ± 4.16	1.83 ± 2.02
Bacillus subtilis	9.17 ± 2.02	3.33 ± 2.08	5.16 ± 5.03	2.17 ± 1.60

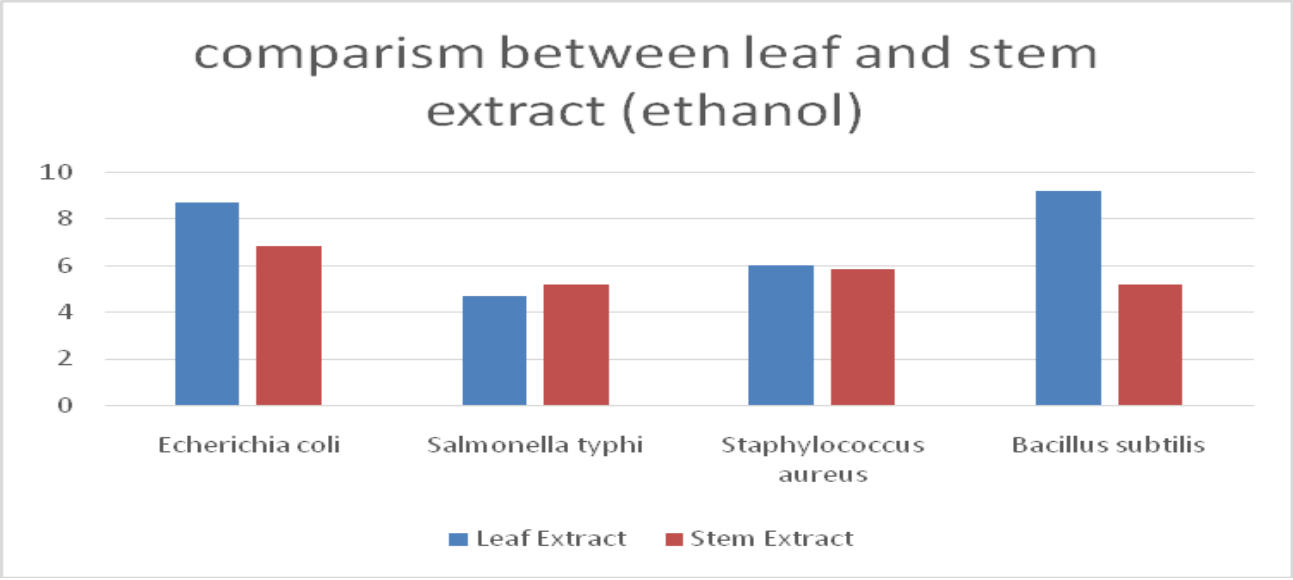

Figure 1: bar chart of the leaf and stem extract (ethanol)

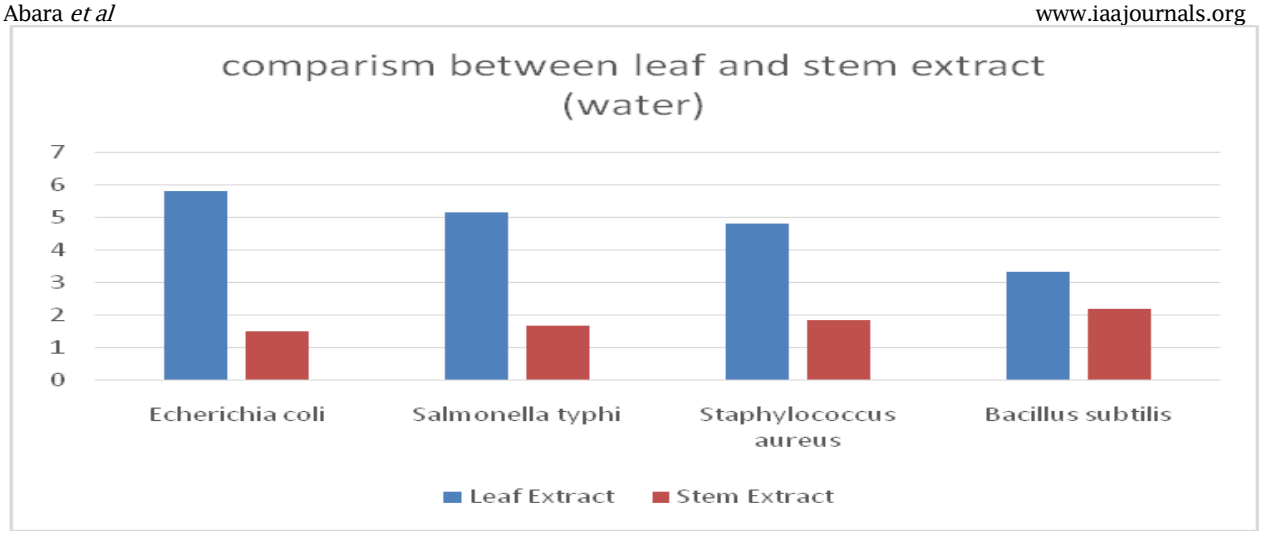

Figure 2: bar chart of the leaf and stem extract (water)

Figure 1 and Figure 2 above, shows the different zones of inihition from the leaf and stem extracts of *T.occidentalis.* Comparing their ethanoic and aqueous extracts on the test organisms of the leaf and stem extract we observe that the leaf extracts have higher zones of inhibition than the stem extracts.

Table 6: MIC and MCB on Leaf Extracts of *Telifeira occidentalis* on Standard Micro-Organisms using Ethanol

S/N	Microorganism	500mg/l	750mg/l	1000mg/l	MIC	MBC
1	*Echerichia coli*	++	+	-	≥1000mg/l	>100mg/l
2	*Salmonella typhi*	+++	++	++	>1000mg/l	>1000mg/l
3	*Staphylococcus auerus*	+++	++	++	>1000mg/l	>1000mg/l
4	*Bacillus subtilis*	++	+	+	>1000mg/l	>1000mg/l

Key: +++ = very turbid; ++ = moderate; += turbid; - = clear

Table 7: MIC and MCB on Leaf Extracts of *Telifeira occidentalis* on Standard Micro-Organisms using Water

S/N	Microorganism	500mg/l	750mg/l	1000mg/l	MIC	MCB
1	*Echerichia coli*	+++	++	+	>1000mg/l	>100mg/l
2	*Salmonella typhi*	+++	++	+	>1000mg/l	>1000mg/l
3	*Staphylococcus auerus*	+++	++	++	>1000mg/l	>1000mg/l
4	*Bacillus subtilis*	+++	++	+	>1000mg/l	>1000mg/l

Key: +++ = very turbid; ++ = moderate; += turbid, - = clear

Table 8: MIC and MCB on Stem Extracts of *Telifeira occidentalis* on Standard Micro-Organisms using Ethanol

S/N	Microorganism	500mg/l	750mg/l	1000mg/l	MIC	MCB
1	*Echerichia coli*	+++	++	-	≥1000mg/l	>100mg/l
2	*Salmonella typhi*	+++	++	++	>1000mg/l	>1000mg/l
3	*Staphylococcus auerus*	+++	++	++	>1000mg/l	>1000mg/l
4	*Bacillus subtilis*	+++	++	++	>1000mg/l	>1000mg/l

Table 9: MIC and MCB on Stem Extracts of *Telifeira occidentalis* on Standard Micro-Organisms using Water

S/N	Microorganism	500mg/l	750mg/l	1000mg/l	MIC	MBC
1	*Echerichia coli*	+++	+++	++ +	>1000mg/l	>100mg/l
2	*Salmonella typhi*	+++	++	++	>1000mg/l	>1000mg/l
3	*Staphylococcus auerus*	+++	++	++	>1000mg/l	>1000mg/l
4	*Bacillus subtilis*	+++	++	++	>1000mg/l	>1000mg/l

Key: +++= very turbid, ++= moderate, +=turbid, - = clear

Table 10: MIC and MCB on Leaf and Stem Extracts of *Telifeira occidentalis* on Standard Micro-Organisms using Ethanol

Microorganism	Leaf Extract			Stem Extract		
	500mg/l	750mg/l	1000mg/l	500mg/l	750mg/l	1000mg/l
Echerichia coli	++	+	-	+++	++	-
Salmonella typhi	+++	++	++	+++	++	++
Staphylococcus auerus	+++	++	++	+++	++	++
Bacillus subtilis	++	+	+	+++	++	++

Table 11: MIC and MCB on Leaf and Stem Extracts of *Telifeira occidentalis* on Standard Microorganisms using Water

Microorganism	Leaf Extract			Stem Extract		
	500mg/l	750mg/l	1000mg/l	500mg/l	750mg/l	1000mg/l
Echerichia coli	+++	++	+	+++	+++	+++
Salmonella typhi	+++	++	+	+++	+++	+++
Staphylococcus auerus	+++	++	++	+++	+++	+++
Bacillus subtilis	+++	++	+	+++	+++	+++

Key: +++ = very turbid; ++ = moderate; + = turbid, - = clear

DISCUSSION

In Nigeria, the consumption of the leaf of *Telfairia occidentalis* as a leafy vegetable in diet or as an infusion in medical preparation is being promoted in view of the various medicinal properties such as anti-microbial, anti-anemic, anti-diabetic and a purgative leafy vegetables. Epidemiological studies have shown that consumption of fruits and vegetables is associated with reduced risk of chronic diseases [22].

Microbial resistance to several antibiotics is becoming a source of challenge and concern to public health. In view of the increasing rate of antimicrobial drug resistance ravaging not only the African continent, but the world at large, alternative, effective and affordable substitutes are essential if bacterial infections are to be properly controlled [23].

The results of this study supports the use of the leaf and stem parts of *Telfairia occidentalis* for medicinal purposes. The phytochemical constituents could be responsible for their antimicrobial activity [24]. Different plant parts contain a complex of chemicals with unique biological activity [25], which is thought to be due to toxins and secondary metabolites, which act as attractants or deterrents [26]. Over the years, these bioactive principles have been exploited in traditional medical practice for the treatment of various ailments [27].

The extent of sensitivity of the test organisms to the extracts of plant parts was assessed. Results showed the antimicrobial activity of *Telfairia occidentalis* leaf and stem extracts using different extracting solvents. The results revealed that the ethanol extract of the leaf of *Telfairia occidentalis* was most effective against the test organisms than the aqueous extracting solvent. Using ethanol as the extracting solvent, *Bacillus subtilis* showed the highest susceptibility (9.17 ± 2.02) to the leaf extract, while *Salmonella typhi* showed the least susceptibility (4.67 ± 1.04). Using aqueous extract, *Escherichia coli* showed the highest susceptibility (5.83 ± 3.40) to the leaf extract, while *Bacillus subtilis* showed the least susceptibility (3.33 ± 2.08). The results also revealed that the ethanol extract of the stem of *Telfairia occidentalis* was most effective against the test organisms than the aqueous extracting solvent. Using ethanol as the extracting solvent, *Escherichia coli* showed the highest susceptibility (6.83 ± 6.11) to the stem extract, while *Bacillus subtilis* showed the least susceptibility (5.16 ± 5.03). However, using aqueous extract, *Bacillus subtilis* showed the highest susceptibility (2.17 ± 1.60) to the leaf extract, while *Escherichia coli* showed the least susceptibility (1.5 ± 1.5). This is in agreement with the observations of [28], who concluded that the stronger extraction capacity of ethanol could have been responsible for the higher antimicrobial activity.

Comparatively, the leaf extract of *Telfairia occidentalis* proved to be more effective against test organisms than the stem extract. Using ethanol and aqueous as extracting solvents, the leaf showed more antimicrobial activity than the stem (Table 5). Ethanol extract of the leaf had the

highest susceptibility value of 9.17 ± 2.02, while the stem had the highest susceptibility value of 6.83 ± 6.11. Aqueous extract of the leaf had the highest susceptibility value of 5.83 ± 3.40, while the stem had the highest susceptibility value of 2.17 ± 1.60. Ethanolic extracts were observed to be more effective than the aqueous extracts, this suggests that water was not able to dissolve all the principal compounds present in *Telfairia occidentalis* as did the ethanolic extracts. The ethanolic extracts were more effective in all the test bacteria. This agrees with the report of [29] on garlic who attributed this to the fact that ethanol is an organic solvent and will dissolve organic compounds better, hence liberate the active compounds (phytochemicals) needed for antibacterial activity. The difference in the bacterial toxicity between the extraction medium may also be as a result of the different susceptibility of each of the test pathogenic bacteria to different concentrations of the extract. This also agrees with the report of some workers [30,31,32,33,34]. The subjection of the 4 test organism to MIC and MBC showed very turbid to 500mg/l concentration of ethanolic and aqueous extraction solvent of the leaf and stem of *Telfairia occidentalis*. There was however no growth of *E. coli* in 1000mg/l ethanolic extract of both stem and leaf extract of Telfairia occidentalis (Table 10). This is to say that the higher the ethanolic concentration, the greater the inhibition to these bacteria. There was no inhibition of growth of the test organisms in all the aqueous extract of the stem of *Telfairia occidentalis* (Table 11). Comparatively, ethanolic and aqueous extracts of the leaf of *Telfairia occidentalis* showed more inhibition to the growth of test organisms than the stem.

CONCLUSION AND RECOMMENDATION

Telfairia occidentalis leaf and stem are very rich source of bioactive compounds and the intake of these plants chemicals have a protective potential against some tropical disease in the use of leaf in folk medicine in Nigeria. The antimicrobial screening of the *T. occidentalis* leaf and stem extracts have shown that the leaf has more effective antimicrobial compounds than the stem. Differential antimicrobial activity of the extracts against different bacteria was due to the presence of different active phyto-compounds which made the test organisms to be susceptible. The study has also shown that ethanol is an organic solvent and will dissolve organic compounds better, hence liberate the active compounds (phytochemicals) needed for antibacterial activity. In the antibacterial activities, it's been found out that the ethanolic extract can inhibit the growth of *E. coli and Bacillus subtilis*. Therefore the ethanolic extract has higher inhibitory effect/higher antibacterial properties than the aqueous extract. Therefore there is need to include *Telfairia occidentalis* leaf in our daily intake so as to get all the possible health benefits from the consumption of the leaf of *T. occidentalis.*

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