Evaluation of the Antimicrobial Activities of Telfairia Occidentalis on *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa*

***Ayisa, Timothy Terna[1], Adamu, B. B.[2], & Umar, Z.[3]**
[1]Department of Biological science,
The Federal Polytechnic Bida,
Nigeria
[2]National Biotechnology Research Agency,
FCT Abuja, Nigeria
[3]Department of Biological science,
Nigeria Defence Academy Kaduna,
Nigeria
*ayisatimothy@gmail.com, bubabinta@gmail.com,
umarz@gmail.com

Abstract
Telfairia occidentalis are medicinal plants which are widely used as sources of extracts with strong antibacterial and antioxidant properties. In this study the leaves extract of Telfairia occidentalis was tested for its antibacterial activity against some human pathogenic bacteria (Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa). The antimicrobial activity test was carried using agar plug hole technique. Plant materials were dried and extracted with 90% of ethanol and water. The results obtained showed that both aqueous and ethanolic extracts of T. occidentalis possess antimicrobial properties against the pathogens. Minimum inhibitory concentration of the extracts of these plants showed that the antimicrobial activity was more pronounced at higher concentration (25mg/ml). However intake of this plant should be encouraged as it has protective potential against some infections. Phytochemically, Telfairia occidentalis contains tannins, terpenoids, saponins and flavonoids. It invariably means that constituents of this plant extract may serve as a source of industrial drugs in the treatment of some infections.

Key words: *Antibacterial, antimicrobial, aqueous, pathogens, concentration*

1.0 Introduction

During the last century, the practice of herbalism became main stream throughout the world. In spite of great advances observed in modern medicine, plant still makes an important contribution to health care. This is due to the recognition of the value of traditional medicine system, particularly of Asian origin, and the identification of medicinal plants from indigenous pharmacopoeias, which have significant healing power. Medicinal plants are distributed worldwide, but they are most abundant in tropical countries (Calixto, 2000; Lewis, 2001). In Brazil alone, about 80000 species of higher plants were described which offer enormous prospect for discovering new compounds with therapeutic properties (Nakaruma *et al.*, 1999). The world health organization (WHO) defines medicinal plant as any plant which is one or more of its origin contains substance that can be used for the synthesis of useful drugs (WHO, 1977). Medicinal plants contain biologically active chemical substances such as saponins, tannins, essential oils flavanoids, alkaloids and other chemical compounds (Grubben and Denton, 2004), which have curative properties composition are found as secondary plant metabolites in one or more of these plants (Kayode and Kayode, 2011).

Moreover, a lot of work has been done to show the antimicrobial properties of the plant (*Telfairia occidentalis*) on some selected pathogens.

Telfairia occidentalis, is otherwise known as fluted pumpkin. It is a vegetable plant that belong to the family Cucurbitaceae. It is a crop of commercial importance grown in West Africa (Nigeria, Ghana, and Sierria leone), being the major producers (Esejin *et al.*, 2005).
Over the years, plant materials and their extracts have been used in the treatment and prevention of diseases and infections of microorganism origins but some of these extracts produced adverse allergies on their consumers.

This research work therefore seeks to examine the antimicrobial activities of both aqueous and ethanolic extracts of the plant (*Telfairia occidentalis*) and determine of MIC and MBC of the plant extract. There is little knowledge on antimicrobial activities of *Telfairia occidentalis* against commonly implicated pathogens isolated from clinical specimens. The Okoruwa and Ekhaise (2010) observed that the extract of these plants showed antimicrobial activities against isolates of *staphylococcus aureus, E. coli, P. aeruginosa.* and *Proteus mirabilis.* This study will evaluate comparatively the antimicrobial effects of ethanolic and aqueous extract of *Telfairia occidentalis* on *E. coli, Klesiella pneumoniae* and *Pseudomonas aerugniosa.*

2.0 Materials and method

2.1 Collection and processing of plant materials

Telfairia occidentalis leaves were identified and collected from Bida modern market (Gwadabe market) Niger state. The fresh leaves were thoroughly washed with clean tap water and spread on a clean sack and was allowed to dry completely for about four weeks at room temperature before using for the study. After drying, it was grinded to powder using a sterile electric blender. This was done to enhance the penetration of the extracting solvent into the plant cell thus facilitating extraction of the active ingredient from the plant.

2.2 Percolation and extraction of active ingredient

The 25 g each of the dried plant material was dissolved in 250 ml of 95% ethanol and 250 ml of distilled water in 500ml reagent bottle and was plugged with cotton wool and aluminum foil paper. This was stirred to mix properly before allowing it to percolate for 5days. Following extraction, a clear filtrate was obtained by filtering through whatman No1 filter paper. This filtrate was then placed in a steam water bath (GallenKamp 4B633D) at 70-80^{0}c to allow the alcohol and distilled water to evaporate. These concentrates now referred to as crude extract, were collected and stored in the refrigerator in a screw capped bottle.

2.3 Preparation of pure culture

Stock culture of *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa* on agar plate were obtained from the laboratory of Federal Medical Center Bida, Niger state sub-cultured on nutrient broth until it was required for use.

2.4 Media preparation

15g of nutrient agar (NA) were dissolved into 450ml of distilled water in a conical flask and allowed to dissolve for 10minutes, this was heated in a Bunsen burner (until when about to boil) so as to dissolve the particles completely, it was then plugged with cotton wool and sterilized in the autoclave at 121^{0}c bath set at 50^{0}c to keep the media in molten state before being used.

2.5 Determination of antimicrobial activity of crude plant extract on test organisms using agar plug hole method

The 0.1ml of 24hours broth culture of each the test organism were mixed separated with 15ml of the molten nutrient agar (obtained in 3.3 above) in a sterile test tube , this was mixed by rolling between the palms before pouring into sterile petri dishes to handen.

Holes were bored using no 4 cork bore. Sterile molten agar were placed drop wise on each of the holes in order to seal the base to prevent seepage of the extract. Subsequently, 0.1 ml of each of the different concentration of the two plant extracts (50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml) were placed in each of the holes. These plates were incubated at 37^0c for 24-48hours with the lids upper most. Zones of inhibition (mm) as exhibited by zones of clearing around the bored holes were measured using pairs of divider (Oyeleke *et al.,* 2008).

2.6 Minimum inhibitory concentration (mic)

The minimum inhibitory concentration was determined using tube diffusion method as described by Mohammed *et al.,* (2008) in which 0.5 g (500 mg) of the plant extract was added to 1ml of the solvent and 9ml of distilled water (500 mg/10ml) and 1ml of these extracts (50 mg/ml) was measured into sterile nutrient broth test tubes containing 1ml each and making the concentration of 25 mg/ml , 12.5 mg/ml, 6.25 mg/ml, 3.13 mg/ml and 1.57 mg/ml was used. The test tube were serially seeded with a loopful of test organisms and incubated for 24hours at 37^0c. control containing the same extract concentration in sterile nutrient broth without test organism were incubated at 37^0c for 24hours. After incubation period, the lowest concentration which showed no turbidity (No growth of organism) is regarded as the minimum inhibitory concentration.

2.7 Determination of minimum bactericidal concentration (mbc)

The minimum Bactericidal concentration of the plant extract was determined by sub culturing from those without growth and transferred to agar plates as described by Oyeleke *et al.,* (2008). The lowest concentrations that yield no growth on the culture agar plate after incubation for 24hours at 37^0c was regarded as the minimum bactericidal concentration.

3.0 Results

Tables 1-4 shows the inhibiting activity of plant extracts on test organism at 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml respectively. The broth extracts of *T. occidentalis* at 50 mg/ml on the overall showed greater antimicrobial activity on all the test organisms (*E. coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) and also showed no zone of inhibition at 6.25 mg/ml of broth aqueous extracts on the test organism while at 6.25 mg/ml of both ethanolic extract on the test organisms the inhibitory zones is low

Table 5-6 shows the minimum inhibitory concentration from 25 mg/ml – 1.57 mg/ml for the test organism. Both ethanolic and aqueous extract of the plant produces MIC at 6.25 mg/ml in the *Klebsiella pneumoniae, E. coli* and *Pseudomonas aeruginosa* (aqueous and ethanolic extract of the same plant) has MIC at 12.5 mg/ml. Also, the ethanolic and aqueous extract of the two (2) plants has MIC at 6.25 mg/ml on *E. coli.*

Table 9-12 shows the minimum bactericidal concentration (MBC) from 25 mg/ml – 1.57 mg/ml for the test organism. Both ethanolic and aqueous extracts of *T. triangulare* has MBC at 12.5 mg/ml for all the test organism (*E. coli, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae*) and the ethanolic and aqueous extract of *T. occidentalis* has MBC at 6.25 mg/ml for *E. coli, Klebsiella pneumoniae* only, while the aqueous extract of *T. occidentalis* has MBC at 12.5 mg/ml on *E. coli* and *P. aeruginosa.*.

Table 1: Zones of inhibition table (mm) of hot water extract of *T. occidentalis*

Organism	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml
Pseudomonas aeruginosa	8.0	4.0	0.0	0.0
Klebsiella pneumoniae	14.0	8.0	5.0	0.0
E. coli	15.0	8.0	2.0	0.0

Table 2: Zone of inhibition table (mm) of ethanolic extract of *T. occidentalis*

Organism	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml
Pseudomonas aeruginosa	16.0	11.0	6.0	2.0
Klebsiella pneumonia	20.0	17.0	9.0	4.0
E.coli	23.0	19.0	12.0	5.0

Table 3: Minimum inhibitory concentration of ethanol extract of *T. occidentalis*

Organism	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.13 mg/ml	1.57 mg/ml
E. coli	-	-	-	-	+
Pseudomonas aeruginosa	-	-	+	+	+
Klebsiella Pneumonia	-	-	-	+	+

Table 4: Minimum inhibitory concentration of hot water extract of *T. occidentalis*

Organism	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.13 mg/ml	1.57 mg/ml
E.coli	-	-	-	+	+
Pseudomonas aeruginosa	-	-	+	+	+
Klebsiella pneumonia	-	-	-	+	+

Keys: + = Turbid
- = Not turbid

Table 5: Minimum bactericidal concentration (MBC) of ethanolic extract of *T. occidentalis*

Organism	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.13 mg/ml	1.57 mg/ml
E.coli	-	-	-	+	+
Pseudomonas aeruginosa	-	+	+	+	+
Klebsiella pneumonia	-	-	-	+	+

Table 6: Minimum bactericidal concentration (MBC) of hot water extract of *T. occidentalis*

Organism mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.13 mg/ml	1.57
E.coli	-	-	+	+	+
Pseudomonas aeruginosa	-	-	+	+	+
Klebsiella pneumonia	-	-	-	+	+

Keys: + = Growth when sub-cultured
- = No growth when sub-cultured

3.2 Discussion

Naturally occurring substances of plant origin have been reported to inhibit the growth of microorganism. Plant extract have been used in folk and even modern medical practices for the treatment of different ailments, most of which are due to microbial activities (Irobi, 1992). Bacterial infections seem especially controllable due to good hygiene and the availability of effective antibacterial drugs. The development of resistance to antibiotic is an almost inevitable consequence of their application. The speed of resistance depends on the respective class of antibiotic and their product use (Ekhaise and Okoruwa, 2011).

The results obtained and presented in this study showed that both aqueous and ethanolic extracts of *T. occidentalis* possess antimicrobacterial activities against common pathogens thus confirming the use of the plant in the treatment of common infections (Table 5-10). This observed antibacterial effects of both ethanolic and aqueous extracts of *T. occidentalis* on the bacteria isolates used through invitro appears interesting and promising. This is an indication that the plant extracts may indeed be effective in the management of common infections, supporting its ethno medical importance. This finding is in agreement with (Aluyi *et al*., 2003, and Ewze *et al*., 2004) who found out that the various extracts of these plants inhibited the growth of some isolates. Moreso, in the minimum inhibitory concentration, antimicrobial activity was more pronounced at higher concentration of *T. occidentalis* (Table1, 2, 3 & 4).
Phytochemically, *Telfairia occidentalis* contains tannins, terpenoids, saponins and flavonoids (Gill, 2010 and Adeniyi, 2010). These components have been known to show medicinal activities as well as physiological activities (Sofowora, 1993; Nweze *et al.* 2004). The plants studied here can be seen as potential sources of useful drugs.

Conclusion

Results obtained from this study has shown that ethanolic and aqueous extracts of the plants *T. occidentalis* in varying concentrations had antimicrobial activities on almost all the test organisms that were used. This means that constituents of this plant extract may serve as a source of industrial drugs in the treatment of some infections.

Acknowlegments

I want to appreciate my research team for ensuring the success of this work, I also want to say thank you to my head of department for allowing us carry out the research work in the laboratory God bless you all.

References

Aluyi, A.S.H., Ekhaise, O.F. and Iruhuegbae, B.O. (2003). Antimicrobial properties of palisortahirsute (Thumb) K. Schum. *Nigeria Journal of Applied Sciences*. **21**:96-100.

Adeniyi S.A., Orjiekwe, C.L., Ehiagbonare, J.E. and Arimah, B.D. (2010). Preliminary analysis and insecticidal activity of ethanolic extracts of four tropical plants (*Vernonia amygolalina, Sida acuta, Ocimum gratissimum, and Telfairia occidentalis*) on beans weevil (*Acanthscelide sobectetus*) *International Journal of Physical Sciences,* 5(6):753-762.

Calixto, I.B. (2000). Efficacy, safety, and quality control, marketing regulatory guidelines for herbal (phytotherapeutic agents). *Brag. J. Med. Biol. Res.* **33**:179-189.

Donald, R.B., Cristobal, M. (2006). Anti-oxidants as activities of flavonoids. *Journal of Agriculture,* 52:125-757.

Ekhaise, F.O., Okoruwa, P. (2001). Antibacterial activity of aleo vera (aleo beabadensis) extracts on staphylococcus aureus. *Topical Journal of Environment Science and Health.* **4**:28-3.

Esegin, O.A., Igboasoiya, A.A., Oforah, E., Mbagwu, H., Umoh, E. and Ekpe, J.F. (2005). Studies of the effect of alcohol extract of *Telfairia occidentalis* on alloxan-induced diabetic rats, *Global Journal of Applied Sci*. **11**:85-87.

Grubben, G.J.H, Denton, O.A. (2004). Plant resources of tropical Africa II. pp.668. http://www.trafic.org/medicalplants

Irobi, O.N. (1992). Activities of chromolena odorata (Compositae) leaf extracts against pseudomonas aeruginosa and streptococcus faecalis. *Journal of Ethnopharmacology*. **37**:81-85.

Kayode, A.A.A, Kayode, O.T. (2011). Some medicinal values of Telfeira occidentalis : A Review. *American Journal of Biochemistry and molecular Biology*, **1**:30-38.

Lewis, E.M. (2001). Should be concerned about herbal remedies? *J. Ethnopharmacol.* 75-141.

Nakaruma, C.V., Nakaruma, T.U., Bando, E., Melo, A.F.N., Coretz, D.A.G. Diaz and Fillio, B.P. (1999). Antimicrobial activity of *Talinum triangulare* essential oil. *Men. Inst. Oswardo Cruz.* **94**:675-678.

Nweze, E.I., Okafor, J.I. and Njoku, O. (2004). Antimicrobial activities of methanolic extracts of Trema guniesnsis (Schum and Thorn) and Morinda Lucida being used in Nigeria *herbal and Biotechnology* **2**(1):39-46.

Okokon, J.E., Ekpo, A.J. and Esegun, O.A. (2007). Antiplasmodid activity of ethanolic root extract of telfaira occidentalis. *Res . J. parasitol*. **2**:94-98.

Oyeleke, S.B., Manga, B.S (2008). Essentials of laboratory practical in microbiology. Tobest publisher, minna. Pp. 20-70.

Sofowora, A. (1993). Medical plant and traditional medicine in Africa (2nd Edition). Spectrum Books limited, Ibadan, Nigeria. 1-153.

World health organisation (WHO), (1977). Resolution, promotion and development of training and research in tradition medicine, *WHO documented NO*. **30**:49-49.