

Phytochemical Screening and evaluation of the anti-salmonella activity of leaf extracts of *Telfairia occidentalis* Hook.f. (Cucurbitaceae) on *Salmonella typhi* cell culture.

ABSTRACT

Typhoid fever commonly called typhoid is a bacterial infection caused by *Salmonella typhi*. Treatment is by the use of antibiotics which presents other challenges such as drug resistance, high cost of medication and less accessibility to the rural population. The aim and objective of this research was to identify the bioactive metabolites of the leaf of *T. occidentalis*, determine the antimicrobial activities on *Salmonella typhi*. The antibacterial activity of the leaf extracts (aqueous maceration, decoction, infusion and ethanol maceration) of *Telfairia occidentalis* (Fluted pumpkin or gourd) on *Salmonella typhi*, was determined using the agar well diffusion technique to investigate its potential use as anti-bacterial agent. The phytochemical constituents, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the leaf were analyzed. The phytochemical analysis of the extracts showed the presence of saponins, flavonoids, tannins, phenolics and coumarin with the absence of phlobatanins and oxalates. The decoction of the leaves demonstrated the highest activity against *S. typhi* (15+/-2.7mm) at 1000mg/ml. MIC for aqueous maceration, infusion, and decoction was recorded at 62.5mg/ml and that of the ethanol maceration was at 15.625mg/ml. The result of this study suggests that the leaf extract has an insignificant activity on *Salmonella* due to the extremely high concentration of the extract required to achieve activity.

Keywords: Phytochemical characterization, antisalmonella, *Telfairia occidentalis*, Cucurbitaceae, *Salmonella typhi*,

INTRODUCTION

Natural products have always been a great source for new medicines. Although products from natural sources might not represent active ingredients in their final form, many commercial drugs have their origins in natural products [1]. It is estimated that at least 25% of modern medicines are derived, either directly or indirectly, from medicinal plants. Artemisinin, paclitaxel, vincristine, salicylic acid can be cited just to name a few [2, 3]. According to WHO, in developing countries, it is estimated that 80% of individuals use traditional medicine [4]. Drug discovery involves the identification of new chemical entities (NCEs) of potential therapeutic value, which can be obtained through isolation from natural sources, through chemical synthesis or a combination of both [5, 6]. These medicinal plants represent an alternative treatment in non-severe to severe cases of infectious diseases [7,8]. This is especially important as the pathogenic microbes are getting increasingly resistant against standard antibiotics.

Traditional Uses of *Telfairia occidentalis*

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Comment [EDTOA3]: Re-write. Author(s) need to justify insignificant activity on salmonella thyphi with p-value.

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The vegetable shows bioactive molecules such as oxalates, saponins, glycosides, flavonoids, alkaloids and resins [9, 10]. The toxicity of *T. occidentalis* has been associated to alkaloids and saponins present in the roots and leaves [11, 12], while the chemo preventive and protective effects, free radical scavenging activity of the plant have been associated to the presence of high number of flavonoids and phenolic compounds [13, 14]. The edible seeds are consumed boiled and eaten whole, or fermented and added to other cooked leafy vegetable and stew sources, or made into egusi puddings [15, 16]. The crop has been locally used by indigenous as a blood tonic, due to its high protein content. Flour produced from the seeds are used in Cameroon for high-protein breads. In addition, the shoots and leaves are consumed as vegetables. The *T. occidentalis* herbal cocktail is prepared for local oral administration to treat many common illnesses link to bacteria and parasitic infections in Gastro intestinal tracts [17, 18].

Antimicrobial activity

The antibacterial potential of the leaf of *Telfairia occidentalis* against selected intestinal pathogens has been reported using the method of agar diffusion technique [19, 20]. The extract showed an increase in antibacterial activity on *Escherichia. coli*, *Salmonella faecalis* and *Salmonella typhi* at minimum inhibitory concentration (MIC) of 0.5, 5.0 and 500 mg/ml for *E. coli*, *S. typhi* and *S. faecalis*, respectively [21, 22]. Furthermore, the ethanolic leaf extract had a higher inhibitory effect on some of the commonly encountered Enterobacteriaceae in reported in Nigeria, namely *Escherichia coli* (4.0 nm), *Pseudomonads aeruginosa* (8.0 nm) and *Proteus sp* (4.0 nm), except for *Salmonella typhi* (2.0 nm), with the aqueous extracts showing a higher inhibition of the mycelial growth. The crude extract inhibited the growth of 93.1 % of the tested microorganisms and showed synergistic effects at MIC/2 and MIC/5 with seven of the tested antibiotics on more than 70 % of the tested bacteria [23, 24]. The plant extracts showed a dose-dependent paralysis and death of the worms, with the aqueous extracts showing higher worm inhibitory and destructive activities when compared with the methanol extracts [10, 25, 26].

Previous research on *Telfairia occidentalis*

The plant *Telfairia occidentalis* Hook. f. (Cucurbitaceae), commonly referred to as “fluted gourd” and “fluted pumpkin,” is grown in West Africa as a leaf vegetable and for its edible seeds [7, 27]. It occurs in the forest zone of West and Central Africa, most frequently in Benin, Nigeria and Cameroon. It is a popular vegetable all over Nigeria. It is rare in Uganda and absent in the rest of East Africa [28, 29]. *Telfairia occidentalis* is a large perennial plant which climbs by means of bifid and tendrils which are usually coiled. The stem has five ridges often covered with multi-cellular hairs, especially when young. The leaves of the plant are compound, usually 3-5 foliate, with blades and petioles also covered with multicellular hairs. The fruits are marked by 10 conspicuous longitudinal ridges and are among the largest known (16-50 cm length, 9 cm diameter). The seeds which are embedded within a bright-yellow fibrous endocarp are large, non-endospermic and usually dark red in colour [22, 25, 30]. In Nigeria, the leaf is consumed in different parts of the country due to its nutritional and medicinal benefits. It has different

traditional names—among Igbos it is known as “Ugu”; “Iroko” in Yoruba; “Ubong” in Efik and “Umeke” in Edo [25]. It is also called okonghobong (NW /SW regions of Cameroon), ekobon (Ewondo); nkongbong (Nkongsamba); okonabong (Nsa). [15, 31]. In folkloric medicine, the fresh leaves are used in the treatment of anemia, a sudden attack of convulsion and malaria [32, 33, 34].

Previous studies had reported the anti-anemic by Alada [32], hepatoprotective (Nwozo *et al.*, [35], hypoglycemic (Eseyin *et al.*[36], antinociceptive and anti-inflammatory [37, 38] activities of extracts of *T. occidentalis*. The aims and objectives of this research was to identify the phytochemical components of the leaf of *T. occidentalis*, to determine the antimicrobial activities of the leaf extract of *T. occidentalis*, to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *T. occidentalis* on *Salmonella typhi*.

The medicinal potential of the leaf and seed essential oil of *Telfairia occidentalis* has been well documented. The plant has been shown to possess important antiinflammatory, anxiolytic, haematological, antiplasmodial antioxidant, antidiabetic, hepatoprotective, antimicrobial, testiculoprotective, anticancer, and sedative properties[3, 39]. Most of the activities validates the medicinal claims of tradi-practitioners, although clinical trials studies, using human subject, still requires future research. Many researchers have also attributed some of the medicinal potential of the plant to the high level of antioxidant components and phytochemicals. However, the presence of other phytochemicals such as saponins may also play valuable roles in the activities of the plant.

As researchers continue to focus their investigation on this plant, it is expected that more of the medicinal properties of the plant will be elucidated. However, not much has been done on the chemistry of the plant, especially the leaf. Therefore, researchers need to focus their investigation on the isolation and identification of bioactive components of the plant [6, 39]. It is worth noting that the identity of the components that are responsible for each of the identified medicinal properties of the plant needs to be elucidated and linked to structure activity relationship of the compounds. This may provide a “lead “to the discovery of novel drugs from the plants[40, 41]. The biosafety, biodiversity and conservation of the plant is very important for any sustainable crop production. The safety, efficacious preclinical studies done so far on *Telfairia occidentalis* *in vitro* and *in vivo*, in animal models coupled with toxicity studies prospects for galenic formulation and quality testing for the development of an improved traditional medicine from this plant of great pharmaceutical potential[8, 42].

MATERIAL AND METHOD

Plant collection

Comment [EDTOA6]: Introduction; Author(s) does not need subsections like **Traditional Uses of *Telfairia occidentalis***, anti-microbial activities, **Previous research on *Telfairia occidentalis***, but have paragraph for each section with good flow from paragraph to paragraph.

The fresh leaves of *T. occidentalis* were harvested from the Caramba – Nkolbisson neighbourhood in Yaoundé in February 2021. Fresh mature leaves were collected as well as other material (flowers, buds, tendrils) for identification by the National Herbarium. The plant samples were identified by a Botanist at the National Herbarium by comparison with the voucher specimen: Westphal botanic collection No 10147 registered at the National Herbarium as **No 42523/HNC**.

Preparation of plant extracts

The leaves of the plant were air-dried for 4 weeks in a shade, and ground to a fine powder before storing in an air-tight container to avoid the absorption of moisture. A quantity of 50 g was weighed and placed into 4 conical flasks. In flask 1, 500ml of distilled water was added to it and shaken thoroughly to homogenize then allowed to macerate. In flask 2, 500 ml of ethanol 95° was added and shaken and left to macerate. In flask 3, 500ml of distilled water was added, adequately shaken to homogenized and placed in a hot water bath to boil. In flask 4, distilled water heated to 100°C is poured on the powder, shaken to homogenize and allowed to infuse. Each flask is set aside after proper labeling for 72 hours while shaking or stirring from time to time. Later they were individually sieved using a clean mousseline cloth and then filtered using N° 1 Whatman filter paper. The filtrates were oven dried at 55°C. Each extract was separately preserved in sterile well sealed bottles until use.

Phytochemical screening of the extracts

Phytochemical screening of the extracts was carried out according to methods described by Odebiyi and Sofowora [34, 37]. For the purpose of our study, we carried out phytochemical tests for alkaloids, steroids, terpenoids, cardiac glycosides, saponins, tannins, total phenols, and flavonoids, mucilages, oxalates, quinones, resins, phlobotannins, betacyans,

Comment [EDTOA7]: The methods for these phytochemical tests need to be described in case of modification to [34, 37].

Microorganism and growth condition

The microorganisms used in this study constituted of clinical isolates of strains of *S.typhi*. The strains were obtained from the Microbiology/Bacteriology laboratory of the Yaoundé University Teaching Hospital Centre. The microorganisms obtained were tested for identity and purity using standard biochemical methods [43]. They were later sub-cultured and maintained on Nutrient agar (NA) in sterile cryotubes and stored at 4°C to be used later.

Preparation of microbial suspensions

Microorganisms were sub-cultured prior to each testing in Muller Hinton agar at 37°C for 24 hours. Stock bacterial inoculums suspensions were prepared in sterile normal saline. Three-four well isolated colonies were picked using the inoculating loop and suspended in sterile saline under aseptic conditions. Bacterial suspensions were adjusted visually to 0.5 McFarland turbidity standards 1×10^8 CFU/ml [44, 45].

Anti-microbial Susceptibility Test

Agar well diffusion method was used to test varying concentrations of the different plant extracts in order to deduce which of them had the highest anti-salmonella activity. Initially, varying concentrations were prepared from individual selected plant extracts. 5, 50, 100, 250, 500 mg/ml respectively. Mueller Hinton agar was prepared as recommended by the manufacturer, sterilized at 121°C and allowed to cool. Standard antimicrobial agent was used, Ciprofloxacin at 5 mg/ml. An inoculating loop was used to transfer a given quantity of microbial suspension onto the agar plates and uniformly seeded by streaking. Using a sterile cork borer or pipette tip a hole is created aseptically. The hole is henceforth filled with the varying concentration of extracts. The susceptibility plates were then placed in an incubator at 37°C for 24 hours. After incubation, the plates are observed for microbial growth and diameters of inhibition were measured. The plate with the highest diameter was considered to have most anti-salmonella activity.

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Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the plant extract was determined by serially diluting extract from 10^1 to 10^{10} . One millilitre (1ml) of each of the dilutions representing a known concentration of the extract was introduced into 1ml of sterile nutrient broth (Mueller Hinton broth) in a test tube. The mixture was then inoculated with 0.1ml of the test organisms previously standardized at 10^8 . It was then incubated at 37 °C for 24 hours. The least concentration of the plant extract in the test tube with no turbidity was taken as the MIC [3]. These tests were carried out in triplicate.

Determination of Minimum Bactericidal Concentration (MBC)

The plant extract was serially diluted from 10^1 to 10^{10} . One millilitre (1ml) of each of the dilutions representing a known concentration of the extract was introduced into 1ml of sterile nutrient broth in test tubes. The mixture was then inoculated with 0.1ml culture of the test organisms previously standardized to 10^8 . It was then incubated at 37 °C for 24 hours. The least concentration of plant extract in the test tube with no turbidity was taken as the MIC. Subsequently, tubes that indicated no turbidity were plated out on nutrient agar plates and absence of growth after incubation for 24 hours confirms the MBC as described by (Andrews, 2001). These were done in triplicate.

RESULTS

Yield of the extraction

After the extraction, the yield of each extract was calculated and the results were presented in the table 1 below. The highest yield was obtained for the decoction, followed by maceration with water. The lowest yield corresponded to the ethanol maceration.

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Table 1: Yield of the extraction

Type of extraction	Mass of powder (g)	Volume of solvent (ml)	Mass of extract obtained (g)	Yield (%)
Aqueous infusion	50	500	6.8	13.6
Aqueous maceration	50	500	8.24	16.48
Decoction	50	500	8.41	16.82
Ethanol maceration	50	500	4.09	8.18

Phytochemical characterization of extract

Table 2 shows the phytochemical components of extracts of *Telfairia occidentalis*. The result indicated the presence of saponins, alkaloids, tannins, phenolics, and absence of oxalates, phylobatanins, and anthraquinones.

Comment [EDTOA13]: The author(s) need to discuss the extent of each phytochemicals for each extraction method used.

Table 2: Phytochemical components of *Telfairia occidentalis*

Secondary Metabolites	Ethanol maceration	Aqueous maceration	decoction	Infusion
Polyphenols	+	+	++	+++
Alkaloids	++	+++	+++	-
Saponins	-	-	+	+
Mucilage	-	+	++	+++
Oxalates	-	-	-	-
Total tannin	+++	++	++	++
Gallic tannin	+	+	+	-
Catechic tannin	++	++	++	++

Flavonoids	++	++	+++	+++
Coumarins	+++	+++	+++	+++
Anthraquinone	-	-	-	-
Phylobatanins	-	-	-	-
Anthocyanin	++	++	++	++
Chalcones	++	++	++	++
Betacyanin	+	+	+	+
Terpenoids	-	++	++	++
Triterpenoid	+	+	+	+
Cardiac glycosides	+	-	-	-
Vitamin A	+	-	-	-
resins	-	-	-	-

Anti-microbial Susceptibility Test

The leaf extracts of *T. occidentalis* showed varying antimicrobial activities against strains of *Salmonella typhi*. The decoction of the leaves demonstrated the highest activity at 1000mg/ml against the microorganism as shown in Table 2.

Table 3 Diameters of Inhibition

Extracts	Maceration	Infusion	Decoction	Ethanol
Diameters at 5 mg/ml (mm)	-	-	-	-
Diameters at 50 mg/ml(mm)	-	-	-	-
Diameters at 250 mg/ml(mm)	-	-	-	-
Diameters at 500 mg/ml(mm)	-	-	-	-
Diameters at 1000 mg/ml(mm)	7±1.8	8±2.06	15±2.7	6±1.00

Minimum inhibitory concentration and minimum bactericidal concentration of *T. occidentalis* on *Salmonella typhi*

Table 4 shows the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of extracts of *T. occidentalis*. For 3 the extracts no growth was recorded above 62.5mg/ml whereas for the ethanolic extract minimum inhibitory concentration were recorded at 15.625mg/ml.

Comment [EDTOA14]: Recast

Table 4 MIC and MBC of the different extracts of *T. occidentalis*

Isolates	Maceration		Infusion		Decoction		Ethanol	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
St1	62.5	62.5	125	125	125	250	62.5	62.5
St2	62.5	62.5	62.5	125	250	250	15.625	62.5
St3	62.5	125	250	250	250	250	62.5	62.5
St4	125	125	125	250	250	250	125	250
St5	62.5	125	62.5	250	250	250	62.5	125
St6	250	250	250	250	250	250	15.625	125
St7	125	125	250	250	250	250	62.5	125
St8	125	125	125	125	62.5	125	125	125
St9	125	62.5	125	125	125	250	62.5	125

St = *Salmonella typhi*. No growth was recorded in the control tubes containing ciprofloxacin at 5mg/ml

DISCUSSION

Globally, a large number of medicinal plants and botanical drugs are being employed as major therapeutic agents or supplements for treatment of various human diseases [19, 42]. Subsequently with this comes an increase in the acceptance of herbal remedy which is gaining renounce worldwide (Inoue *et al.*, 2011; Imosemi *et al.*, 2017). As a result of abundant usage and propensity for prolonged use of the fresh leaves of *T. occidentalis* in traditional medicine, especially, in Cameroon and West Africa as a whole, a phytochemical screening and the evaluation of the anti-salmonella effects of different leaf extracts of the plant were carried out in this study.

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Comment [EDTOA16]: West Africa countries,

The yield of the extractions showed the decoction having the highest yield at 16.82% and the ethanolic maceration having the lowest at 8.18%. This contradicted the works done by Akindede and associates in 2018 who have a yield of 15% for their ethanolic extract after carrying a double maceration to render the

Comment [EDTOA17]: Akindede *et al* () with

extraction exhaustive [27, 33]. The phytochemical screening of the decoction, infusion, aqueous and ethanolic maceration of the leaves of *T. occidentalis* showed the presence of tannins, phenolics, coumarins, alkaloids and flavonoids. Saponins were present but to lesser degree and a total absence of phlobatanins, oxalates and anthraquinones. This corroborates the findings earlier researchers [22, 44], who found similar components in an aqueous maceration. Tannin a component found in excess in extracts of *T. occidentalis*, is a water-soluble polyphenol with anti-inflammatory and anti-microbial effect. It has both bactericidal as well as bacteriostatic effect against *Staph. aureus* and other micro-organisms [27]. Prior studies have shown that the presence of tannins in the extract provides the effect for its use as purgative [46, 47], anti-asthmatic, antitussives and anti-hay fever [48, 49]. A study on the identification of bioactive chemical constituents by Oladele *et al* (2020) showed the presence of aromatic CH bend, C-O stretch, phenol, aromatic ring stretch, alkenyl stretch, hydroxyl group (alcohol) in the extract by the Fourier Transform Infrared, FTIR Techniques. This result further corroborates the presence of phytochemicals in aqueous leaf extracts of *Telfairia occidentalis* [48]

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Comment [EDTOA19]: The author(s) need to justify the wide variation in ethanolic extraction.

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Comment [EDTOA22]: It should be replaced with "[39]".

Based on the antimicrobial susceptibility test, the highest activity was registered for the decoction of the leaf extract. This is contrary to other studies carried out by Oyewole and Abalaka [31], where the zones of inhibition of the plant were higher than the reference standard at 500mg/ml. Owing to the fact that they worked with just two extracts; maceration with distilled water and ethanol maceration, their results showed a marked difference [31, 50].

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Comment [EDTOA24]: Not understandable.

The reported presence of tannins, reducing sugars, glycosides, saponins and sterol and triterperoids in the root, and only tannins, flavonoids, alkaloids, saponins, steroids, anthraquinones, and reducing sugars are found in the stem and leaves [20, 35]. The long chain n-3-unsaturated fatty acid have been isolated from the leaf using an arginated silica gel column (8 cm, 0.5 mm diameter) eluted with n-hexane [3, 47]. Palmitoleic acid (16.62 %) and elaidic acid (0.85 %) are the most identified omega 9 fatty acid present in the leaf. GC-MS analysis of hexane and dichloromethane fractions of the seed have indicated that the seed contained compounds such as pentadecanoic acid, hexadecanoic acid; hexadecanoic acid, methyl ester; α -phellandrene; α -campholene aldehyde; terpinen-4-ol Octadecadienoic acid (Z)-, 2, 3-dihydroxypropyl ester, 16-octadecenoic acid methyl ester; 9, 12-octadecadienoyl chloride (Z,Z); 9-; Octadecanoic acid; hexadecanoic acid, 2,3-bis[(trimethylsilyl)oxy] propyl ester in the hexane fraction and 2,4-heptadien-6-ynal, (E,E); benzoic acid; dodecanoic acid; linoleic acid ethyl ester; trans- β -ocimene; borneol; stigmastan-3-ol, in the dichloromethane fraction [5, 14, 49]).

Comment [EDTOA25]: Replace with "were"

Our research led us to the conclusion that the anti-salmonella activity of *T. occidentalis* is very low with activity only possible at 1000mg/ml. This therefore implies that the plant extract has insignificant action on *Salmonella typhi*. This is similar to the finding of Oboh *et al*. (2006) [50, 51], who reported inhibitory

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effects of ethanolic extract of *T. occidentalis* on *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus sp.* but no inhibitory effect on *Salmonella typhi* [52]. On the other hand, the study carried out by Abalaka and Oyewole who determined that the ethanolic extract of *T. occidentalis* was tolerant to *S. typhi* at 5.0mg/ml. The minimum Inhibitory Concentration for *S.typhi* was found to be 5.0mg/ml [31, 53]

CONCLUSION

The phytochemical analysis of the extracts showed the presence of saponins, flavonoids, tannins, phenolics and coumarin with the absence of phlobatanins and oxalates. The study also showed that the decoction had the greatest activity against *Salmonella typhi*. The anti-salmonella activity was insignificant given that it was only observed as from concentrations of 1000 mg/ml which was relatively high. The use of *T. occidentalis* in the treatment of typhoid could possibly be attributed to the anti-anaemic potential of the plant as indicated by earlier researchers on the erythropoietic potential of the plant. The study confirmed the use of this plant in category 1 traditional medicine due to its easy accessibility, environmentally friendly nature to the local population.

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Comment [EDTOA27]: Is this a conclusion. Then, how many conclusion does the author(s) has? It should be deleted.

Comment [EDTOA28]: I think the word "insignificant" should be deleted since there is no statistical data to justify that.

Comment [EDTOA29]: Author(s) need not to state the earlier researches here, rather they should identify the anti-anaemic agent(s) in their phytochemical screening.

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