Journal of Complementary and Alternative Medical Research

Volume 21, Issue 3, Page 8-17, 2023; Article no.JOCAMR.97895
ISSN: 2456-6276

Phytochemical Screening and Evaluation of the Anti-Salmonella Activity of Leaf Extracts of *Telfairia occidentalis* Hook. F. (Cucurbitaceae) on *Salmonella typhii* Cell Culture

VL Tamanji [a], EAF Tembe [b], GA Mbong [c], H Bayaga [d], BN Njinkio [b], DJ Fonmboh [e], MV Tchadji [b], JO Moody [a], HK Gonsu [f] and CN Fokunang [b*]

[a] Department of Pharmacognosy, Pan African Institute of Life and Earth Sciences (PAULESI), University of Ibadan, Nigeria.
[b] Department of Pharmacotoxicology and Pharmacokinetics, Faculty of Medicine, University of Dschang, Cameroon.
[c] Biomedical Sciences, University of Yaoundé 1, Cameroon.
[d] Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Medicine and Biomedical Sciences, University of Yaoundé 1, Cameroon.
[e] Department of Nutrition, Food Science and Bioresource Technology in the College of Technology, University of Bamenda, Cameroon.
[f] Department of Microbiology, Heamatology, Parasitology and infectious Diseases, Faculty of Medicine and Biomedical Sciences, University of Yaoundé 1, Cameroon.

Authors' contributions

This work was carried out in collaboration among all authors. Authors TVL, FCN, MJO conceived the study and designed the methods. Authors TVL, HB, and NBN did the experimental and laboratory work, collected data and other materials. Author TVL drafted the manuscript. Authors TEAF, MJO and FCN edited and finalized the manuscript for publication. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JOCAMR/2023/v21i3437

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/97895

**Corresponding author: E-mail: charlesfokunang@yahoo.co.uk;*

Original Research Article

Received: 04/02/2023
Accepted: 07/04/2023
Published: 12/04/2023

ABSTRACT

Typhoid fever, a bacterial infection caused by Salmonella typhi whose treatment is by use of antibiotics. And the use of these antibiotics associated with drug resistance, high cost of medication and less accessibility to rural populations remains an issue of concern. The aim of this research was to indentify bioactive metabolites of the leaf of *T. occidentalis* and determine the anti-salmonella typhi activity. 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 phyto-chemical constituents, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the leaf was 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. Due to the extraordinarily high concentration of extract needed to produce anti-salmonella activity, the study's findings imply that the leaf extract has negligible activity against Salmonella.

Keywords: Phytochemical characterization; antisalmonella; Telfairia occidentalis; cucurbitaceae; salmonella typhi.

1. 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]. Estimates have indicated that a minimum of 25% of modern drugs found on the pharmaceutical market today originate either directly or indirectly from plants. Salicylic acid, vincristine, paclitaxel, and artemisinin are only a few examples [2,3]. According to WHO, in developing countries, estimates show 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.

Telfairia occidentalis is a well-known and locally cultivated vegetable, which 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]. "Indigenous populations locally use the crop 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].

"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 typhii* 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 Enterobacterioceae in reported in Nigeria, namely *Escherichia coli* (4.0 nm), *Pseudomonads aeruginosa* (8.0 nm) and *Proteus sp* (4.0 nm), except for *Salmonella typhii* (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].

Aside from the anti-bacterial studies carried out on the plant, numerous studies have been carried out 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 *Telfiaria 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].

2. MATERIALS AND METHODS

2.1 Plant Collection

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.

2.2 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 mousse line 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.

2.3 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.

2.4 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.

2.5 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 $1x10^8$ CFU/ml” [44, 45].

2.6 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 24hours. After incubation, the plates were observed for microbial growth and diameters of inhibition were measured. The plate with the highest diameter was considered to have most anti-salmonella activity.

2.7 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.

2.8 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 24hours. 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 24hours confirms the MBC" as described by (Andrews, 2001). These were done in triplicate.

3. RESULTS

3.1 Yield of the Extraction

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

3.2 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. There was high presence of polyphenols for infusion, alkaloids for aqueous maceration and decoction, mucilage for infusion and flavonoids and coumarins for the decoction and infusion extraction.

3.3 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 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

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	-	-	-	-

3.4 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*. In contrast to the ethanolic extract, which had a minimum inhibitory concentration of 15.625 mg/ml, none of the three other extracts showed any growth above 62.5 mg/ml.

4. 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]. In this light, there is a global acceptance of herbal remedies which is on the rise [24, 25]. As a result of abundant usage and propensity for prolonged use of the fresh leaves of T. occidentalis in traditional medicine, especially, in West African countries, a phytochemical screening and the evaluation of the anti-salmonella effects of different leaf extracts of the plant were carried out in this study.

The yield of the extractions showed the decoction having the highest yield at 16.82% and the ethanol maceration having the lowest at 8.18%. This contradicted the works done by Akindele et al in 2018 [7] who have a yield of 15% for their ethanolic extract after carrying a double maceration to render the extraction exhaustive [27, 33]. This can be explained by the fact that the extraction was not exhaustive due to absence of double maceration. 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 of 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 [39] 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].

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

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

Isolates	Maceration MIC	Maceration MBC	Infusion MIC	Infusion MBC	Decoction MIC	Decoction MBC	Ethanol MIC	Ethanol 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

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. Given the fact that they worked with just two extracts; which include maceration with distilled water and ethanol maceration; this could explain their results showing a marked difference [31,50,51].

"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 %) were 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; αphellandrene; α-campholene aldehyde;terpinen4-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 dichloremethane fraction"[5, 14, 49]).

5. 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 extremely low 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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

The authors are grateful to technical support of the Bacteriology laboratory, Pr Gonsu Hortense, Mme Tchaffa Anicette and Miss Kila Clarisse T. Thanks go to the financial funding of the Project by the scholarship of the Pan African Institute of Life and Earth Sciences (PAULESI), University of Ibadan, Nigeria. Thanks to the technical and financial support of the laboratory for preclinical animal and pharmaco-toxicology research, University of Yaoundé 1, and the Ministry of Higher Education Research Modernization allowance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Abidemi J, Akindele J, Awulika OS, Usuwah BA. Antinociceptive and antiinflammatory activities of telfairia occidentalis hydroethanolic leaf extract (Cucurbitaceae). International Journal of Physical Sciences; 2015. Available:https://doi.org/10.1089/jmf.*2014.0146*
2. Adeniyi SA, Orjiekwe CL, Ehiagbonare JE, Arimah BD. Preliminary phytochemical analysis and insecticidal activity of ethanolic extracts of four tropical plants (Vernonia amygdalina, Sida acuta, Ocimum gratissimum and Telfaria occidentalis) against beans weevil (Acanthscelides obtectus). International Journal of Physical Sciences. 2010;*5*(6):753–792.
3. Aghaei S, Nikzad H, Taghizadeh M, Tameh AA, Taherian A, Moravveji A. Protective effect of pumpkin seed extract on sperm characteristics, biochemical parameters and epididymal histology in

adult male rats treated with Cyclophosphamide. Andrologia; 2014; 46(8):927-935.

4. Agwu EJ, Odo GE, Uwagbae M, Onuawueke CC. Acute toxicity of aqueous extract of the root of *Telfairia occidentalis* on *Clarias gariepinus* fingerlings. International Journal of Agriculture, Forestry and Fisheries. 2016;4(1):1-6.
5. Akang EN, Oremosu AA, Osinubi AA, Dosumu OO, Kusemiju TO, Adelakun SA, Umaru ML. Histomorphometric studies of the effects of *Telfairia occidentalis* on alcohol-induced gonado toxicity in male rats. Toxicology Reports. 2015;2:968–975.
6. Akindele AJ, Oladimeji-Salami JA, Usuwah BA. Antinociceptive and anti-inflammatory activities of *Telfairia occidentalis* hydroethanolic leaf extract (Cucurbitaceae). Journal of Medicinal Food, 2015;18:1157–63.
7. Akindele A, Oladimeji-Salami J, Oyetola R, Osiagwu D. Sub-Chronic Toxicity of the Hydroethanolic Leaf Extract of Telfairia occidentalis Hook. f. (Cucurbitaceae) in Male Rats. *Medicines*, 2018;*5*(1):4. Available: https://doi.org/10.3390/medicines5010004
8. Akoroda MO. Ethnobotany of *Telfairia occidentalis* (cucurbitaceae) among Igbos of Nigeria. Economic Botany, 1990;44(1):29–39. Available: https://doi.org/10.1007/BF0286064
9. Alada A. The haematological effect of *Telfairia occidentalis* Diet preparation; 2000;3:185–186.
10. Andrews JM. Determination of minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy. 2001;48(1):5–16. Available: https://doi.org/10.1093/jac/48.suppl_1.5
11. Aneja KR. Experiments in microbiology, plant pathology and biotechnology. (IVEd.) New Age International (P) Limited, Publishers. New Delhi. 2007;145-156.
12. Badifu GI O, Ogunsua AO. Chemical composition of kernels from some species of Cucurbitaceae grown in Nigeria. Plant Foods for Human Nutrition. 1991;41(1):35–44. Available: https://doi.org/10.1007/BF02196380
13. Dobgima JF et al. An overview of methods of extraction, isolation and characterization of natural medicinal plant products in improved traditional medicine research asian journal of research in medical and pharmaceutical sciences; 2020. Article no. AJRIMPS.61961 ISSN: 2457-0745
14. Edeoga HO, Okwu DE, Mbaebie1 BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol 2005;4:685-688.
15. Enujiugha VN, Oluwole TF, Talabi JY, Okunlola AI. Selected bioactive components in fluted pumpkin (*Telfairia occidentalis*) and Amaranth (*Amaranthus caudatus*) leaves. American Journal of Experimental Agriculture; 4(9):996-1006.
16. Egharevba EM. Phytochemical analysis, proximate and mineral composition and in vitro antioxidant activities in *telfairia occidentalis* aqueous leaf extract; 2014. Available:https://www.researchgate.net/publication/279529603
17. Enujiugha VN, Oluwole TF, Talabi JY, Okunlola AI. Selected bioactive components in fluted pumpkin (*Telfairia occidentalis*) and Amaranth (*Amaranthus caudatus*) leaves. American Journal of Experimental Agriculture. 2014;4(9):996-1006.
18. Eseyin OA, Akaninyene DA, Paul TS, Attih E, Emmanuel E, Ekarika J, Zubaid A, Sattar MA, Ashfaq A, Afzal S, Ukeme A. Phytochemical analysis and antioxidant activity of the seed of *Telfairia occidentalis* Hook (Cucurbitaceae). Journal Natural Product Research, 2017;32(4):444-447.
19. Eseyin, OA, Ebong P, Ekpo A, Igboasoiyi A, Oforah E. Hypoglycemic effect of the seed extract of *Telfairia occidentalis* in rat. Pakistan Journal of Biological Sciences. 2007;10(3):498–501. Available: https://doi.org/10.3923/pjbs.2007.498.501
20. Eseyin OA, Sattar MA, Rathore HA. A review of the pharmacological and biological activities of the aerial parts of *Telfairia occidentalis* Hook.f. (Cucurbitaceae).Tropical Journal of Pharmaceutical Research. 2014;13(10):1761–1769. Available:https://doi.org/10.4314/tjpr.v13i10.28
21. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environmental Health Perspectives, 2001;109(1):69–75. Available:https://doi.org/10.1289/EHP.01109S169

22. Fokunang CN. Qui a peur de la medicine traditionnelle? Esquisse d'une lecture geopolitique de la question. In: La Pandémie de la COVID-19 : Réalités et avenir de la pharmacopee et la medecine traditionnelle camerounaise (Ndi FC, Mbacham WF eds).2021;182.
23. Imosemi IO. Review of the toxicity, medicinal benefits, pharmacological actions and morphological effects of T*elfairia occidentalis* Hook. F.Eur. J. Pharmaceut. Med Res. 2018;5(7):22-32.
24. Imosemi IO, Adekanmbi AJ, Atiba FA. *Telfairia occidentalis* leaf extract protects the cerebellar cortex against cisplatin-induced oxidative damage in Wistar rat. Archives of Basic and Applied Medicine. 2017;5(2):119-124.
25. Inoue H, Yamazaki S, Shimizu M, Uozaki H, Goto T, Ohnishi S, Koike K. Liver injury induced by the Japanese herbal drug Kamishoyosan. Gastroenterology and Hepatology;2011;7(10):692-695.
26. Kpu KA, Annih MG, Ambang AL, Agwah ED, Agwah ED. Influence of tillage systems and sowing dates on the incidence of leaf spot disease in *Telfairia occidentalis* caused by Phoma sorghina in Cameroon; 2022.
27. Krause J, Tobi G. Discovery, development, and regulation of natural products. in using old solutions to new problems - natural drug discovery in the 21st century. InTech; 2013.
Available: https://doi.org/10.5772/56424
28. Kuete V, Efferth T. Cameroonian medicinal plants: Pharmacology and derived natural products. Frontiers in Pharmacology. 2010;1:123-129.
Available:
https://doi.org/10.3389/fphar.2010.00123.
29. Leonard BS, Karen LL, Bruce RB, Thomas FK, Jay HH. Complementary and alternative medicine in chronic liver disease. Hepatology (Baltimore, Md.), 2001;34(3):595–603.
Available:https://doi.org/10.1053/JHEP.2001.27445
30. Maduka SO, Ugwu CE, Onwudinjo OJ. The effect of aqueous leaf extract of *Telfairia occidentalis* (Cucurbitaceae) on gentamycin-induced renal damage. Journal of Basic and Clinical Physiology and Pharmacology. 2016;28(1):11-17.
31. Njoku RC, Abarikwu SO, Uwakwe AA, Mgbudom-Okah CJ. Telfairia occidentalis-supplemented diet induces changes in sperm parameters and testosterone level in rats. Andrologia. 2018;50(7): 13044.
DOI: 10.1111/and.13044
32. Nwidu LL, Oboma YIJ. Telfairia occidentalis (Cucurbitaceae) pulp extract mitigates rifampicin-isoniazide induced hepatotoxicity in an in vivo rat model of oxidative stress. Integr Med. 2019;17(1): 46-56.
DOI: 10.1016/j.joim.2018.11.008
33. Nwozo S, Adaramoye O, Ajaiyeoba E. Anti-diabetic and hypolipidemic studies of teliairia occidentalis on alloxan induced diabetic rabbits. Nigerian Journal of Natural Products and Medicine. 2005;8(1).
Available:
https://doi.org/10.4314/NJNPM.V811.11814
34. Oboh G, Nwanna EE, Elusiyan CA. Antioxidant and antimicrobial properties of *telfairia occidentalis* (fluted pumpkin) leaf extracts. Journal of Pharmacology and Toxicology. 2006;1(2):167–175.
Available:https://doi.org/10.3923/jpt.2006.167.175
35. Odebiyi A. Sofowora AE. Phytochemical screening of Nigeria medicinal plant part III Lloydia. 1978;4:234-246.
36. Ogunka-Nnoka CU, Amagbe R, Amadi BA, Amadi PU. Biochemical effects of *Telfairia occidentalis* leaf extracts against copper-induced oxidative stress and histopathological abnormalities. Journal of Advances in Medical and Pharmaceutical Sciences. 2017;12(2):1-15.
37. Okokon JE, Ekpo AJ, Eseyin OA. Evaluation of in vivo antimalarial activities of ethanolic leaf and seed extracts of *Telfairia occidentalis*; 2009.
38. Oladele JO, Anyim JC, Oyeleke OM, Olowookere BD, Bamigboye MO, Oladele OT, Oladiji AT. *Telfairia occidentalis* mitigates dextran sodium sulfate-induced ulcerative colitis in rats via suppression of oxidative stress, lipid peroxidation, and inflammation. J Food Biochem. 2021;45(9):13873.
DOI: 10.1111/jfbc.13873
39. Oladele JO, Olowookere MOB, Oyeleke BD, Olaleye J. Identification of Bioactive Chemical Constituents presents in the Aqueous extract of Telfairia occidentalis and its in vitro Antioxidant activities. Nat Ayurvedic Med. 2020; 4(2):000237.
Available:https://doi.org/10.23880/jonam.16000237

40. Oladele JO, Oyewole OI, Bello OK, Oladele OT. Hepatoprotective effect of aqueous extract of *Telfairia occidentalis* on cadmium chloride induced oxidative stress and hepatotoxicity in rats. Journal of Drug Design and Medicinal Chemistry. 2017;3(3):32-36.
41. Olaniyan MF, Adeleke A.. Short Communication: A study of the effect of pumpkin (ugu *Telfaira occidentals*) milk and raw egg mixture in the treatment of anaemic pregnant women in a rural area. African Journal of Traditional, Complementary and Alternative Medicines. 2005;2(3).
Available:https://doi.org/10.4314/AJTCAM.V2I3.31126
42. Osuntoki AA, Sanusi AO. Telfairia occidentalis stabilizes human erythrocyte membranes. Nig Q J Hosp Med. 2007;17(3):116-9.
DOI: 10.4314/nqjhm.v17i3.12557
43. Owoeye O, Gabriel MO. Protective effects of aqueous extract of *Telfairia occidentalis* on mercury-induced histological and oxidative changes in the rat hippocampus and cerebellum. African Journal of Biomedical Research. 2016;19(3):241-247.
44. Owumi SE, Olugbami JO, Akinnifesi AO, Odunola OA.J Complement Integr Med; 2021.
DOI: 10.1515/jcim-2021-0151
45. Oyewole O, Abalaka M. Antimicrobial activities of *Telfairia occidentalis* (fluted pumpkins) leaf extract against selected intestinal pathogens. International Journal Of Health Science. 2012;*2*(2):1–4.
Available:https://doi.org/10.5923/j.health.20120202.01
46. Pirro S, Obembe O. The complete genome sequence of *Telfairia occidentalis*, the African fluted pumkin. Biodivers Genomes. 2022:10.56179/001c.40155.
DOI: 10.56179/001c.40155
47. Sakpa LC, Onovughakpo-Sakpa OE, Okhimamhe AF. Profertility effects of aqueous leaf extract of *Telfairia occidentalis* in adult male Wistar rats. Journal of Experimental and Clinical Anatomy. 2015;14(2):88-94.
48. Salman TM, Alagbonsi IA, Feyitimi AA, Ajayi PO. Telfairia occidentalis Hook F associated haematopoitic effect is mediated by cytokines but independent of testosterone. A preliminary report J Ethnopharmacol. 2018;216:157-161.
DOI: 10.1016/j.jep.2018.01.018
49. Salman TM, Alagbonsi IA, Sulaiman SO. Counter-regulatory responses to *Telfairia occidentalis*-induced hypoglycaemia. Metabol Open. 2020;5(8):100065.
DOI: 10.1016/j.metop.2020.100065
50. Salman TM, Iyanda MA, Alli-Oluwafuyi AM, Sulaiman SO, Alagbonsi AI. Telfairia *occidentalis* stimulates hepatic glycolysis and pyruvate production via insulin-dependent and insulin independent mechanism. Metabol Open. 2021;14(10): 100092.
DOI: 10.1016/j.metop.2021.100092
51. Tamanji VL, Tembe-Fokunang EAF, Mbong GA, Koffi GY, Agbor MA, Njinkio BN, Akinali ET, Moody JO, Fokunang CN. An overview of ethnobotanical, medicinal and pharmacological potential of *Telfairia occidentalis* Hook. F Cucurbitaceae in Cameroon. European Journal of Pharmaceutical and Medical Research. 202;18(11):142-159

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/97895