Antifungal effect and phytochemical screening of *Telfairia occidentalis* (hook f.) Leaf extracts.

Umeoka Nkiru*

Department of Plant Science and Biotechnology (Botany), Faculty of Science, Imo State University, Owerri, Imo State, Nigeria

Abstract

Telfairia occidentalis **leaf is a very rich source of phytochemicals and the intake of these plant chemicals have a protective potential against some tropical disease in the use of leaf in folk medicine in Nigeria. The phytochemical screening of *Telfairia occidentalis* leaf extract shows that it contains Alkaloid 1.35%, Flavonoid 1.50%, Tannin 0.437%, Saponin 3.60%, Phytate 0.095%, Phenol 12.20%, HCN 0.0135% and the Vitamin contents are Vitamin A 16.61 mg/100 g, Vitamin C 64.13 mg/100 g, Riboflavin (B_2) 12.38 mg/100 g. The antifungal activity revealed that the leaf of the aqueous extract can be used to inhibit the growth of all the four organisms used *(Geotrichum candidum, Aspergillus niger, Fusarium oxysporum, Rhizopus stolonifer*). This result shows that the ethanolic extract have higher inhibitory effect than the aqueous extract. The result also shows that this leafy vegetable have high nutritional value and should be added to our dietary intake. This leafy vegetable also tends to bring down glucose level when taken in large quantity with small portion of carbohydrate food (for diabetic patient).**

Keywords: Antifungal effect, Phytochemical screening, *Telfairia occidentalis*, Leaf extract.

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Introduction

The constituents or chemical components of some plants determine their medicinal actions and nutritive values. These constituents affect the conditions and functions of the various organs in the human body, clear up residual symptoms or destroy the agents of the diseases in most cases by infective microorganisms [1]. World Health organization (W.H.O) noted that, a medicinal plant is any plant which in one way or more of its organ contains substances that can be used for the synthesis of useful drugs [2]. Biologically, medicinal plants contains active chemical substances such as Saponins, tannins, essential oils, Flavonoids, Alkaloids and other chemical compounds [3] which have curative properties. These complex chemical substances of different compositions are found as secondary plant metabolites in one or more of these plants [4].

Kayode and Kayode (2011) noted that *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 [4-6].

Telfairia occidentalis has been reported by many investigators to have medicinal attributes. The herbal preparation of the plant has been used in the treatment of anaemia, chronic fatigue and diabetes [4,7,8]. It is noted that the leaves contain essential oils, vitamins; root contains cucubitacine, sesquiterpene, lactose [9]. In the treatment of convulsion, the young leaves sliced and mixed with coconut water and salt stored in a bottle can be used traditionally [10]. *Telfairia* leaf extract is useful in the management of cholesterolemia, liver problems and impaired defense immune systems [11].

In soup and folk medicine preparation, *Telfairia occidentalis* is always used in the management of various diseases like diabetics, anemia and gastrointestinal disorder [12] as an inhibitory effects on some enterobacteria [13], whereas [1] reported *Telfairia occidentalis* anti-inflammatory [4]. The study was undertaken to determine the following objectives: (1) to determine the nutritional value of *Telfairia occidentalis*. (2) To identify the phytochemical components of the leaf of *Telfairia occidentalis*. (3) To determine if the leaf extracts of *Telfairia occidentalis* can have inhibitory effect on some of the common fungi. (4) to determine which of the leaf extracts (aqueous and ethanolic extract) have higher antifungal property.

Materials and Methods

The study was done at two Departmental Laboratories in Owerri, Imo State, Nigeria. The antioxidant activity was done for the S.A.A.T Departmental Laboratory at the Federal University of Technology Owerri and the antifungal activity was done at the Departmental Laboratory of Plant Science and Biotechnology in Imo State University Owerri. The *Telfairia occidentalis* (Ugu) leaves were collected from the University botanical garden. The four (4) pure organisms namely: *Geotrichum candidum, Aspergillus niger, Fusarium oxysporum* and *Rhizopus stolonifer* was taken from a stock culture of the Departmental laboratory that has been identified.

The medium, Potato Dextrose Agar (PDA) which is a semi-synthetic medium was used for the experiment and it was prepared following the manufacturer's directive. 39 g of

the PDA media was dissolved in one liter of sterile distilled water and sterilized by autoclaving at 121°C and 15 psl for 13 minutes as instructed by the manufacturer. 0.5 g of penicillin, an antibiotic was added to autoclaved the medium so as to inhibit any bacterial growth, and then it was shaken properly. It was allowed to cool to about 45°C and then sterilely dispensed into sterile petri-dishes. Slants were then prepared by dispensing the dissolved media into the McCartney bottles before autoclaving and then the bottles were slanted to cool. Sterility test was performed to the media by incubating them inoculated for 24 hours at 37°C as described by Cheesbrough [14]. Only media that passed the sterility test were used to multiple the cultures.

Preparation of plant extracts

Two methods of extraction were employed in this process, namely: Ethanol and Crude Water extraction.

Ethanol extraction: 50 g each of the dried milled plant material was weighed into the weighed beaker containing 300 mL of ethanol and stirred vigorously. The beaker was covered and allowed to stand for 24 hours after which it was filtered with Muslin cloth and filtrate placed on the water bath. Ethanol contained in the extract was evaporated with water bath to give pure crude extract of the plant. Reconstitution was done by addition of 100 mLs of distilled water.

The weight of the sample was calculated as follows: final weight of the beaker – initial weight of the beaker.

Water extraction: 50 g each of the dried milled plant material was weighed into the weighed beaker containing 300 mL of water and stirred vigorously. The beaker was covered and allowed to stand for 24 hours after which it was filtered with Muslin cloth and filtrate placed on the water bath. Water contained in the extract was evaporated with water bath to give pure crude extract of the plant. Reconstitution was done by addition of 100 mLs of distilled water.

The weight of the sample was calculated as follows: final weight of the beaker – initial weight of the beaker.

Quantitative determination of phytochemicals

(a) Determination of Tannin: the Follins-Dennis spectrophotometer method [15] was used.

(b) Determination of Flavonoid: the method used for the determination of Flavonoid is that of [16].

(c) Determination of Phenol: the Follins method [15] was used to determine the Phenol content.

(d) Determination of Phytate: the phytic acid content was determined by modification of the method [17].

(e) Determination of Saponin: the saponin content was determined according to the method described by [18].

(f) Determination of Hydrogen Cyanide (HCN): this was determined by the modified alkaline picrate colorimetric method [19].

(g) Determination of Vitamin A (Retinol): the vitamins in the leafy vegetables were determined by the official methods of the Association of Official Analytical Chemists [19].

In-vitro antifungal activity

Four (4) test fungi, *Geotrichum candidum* (Link V), *Aspergillus niger* (Van Tieghem), *Fuscarium oxysporum* (Mart.) and *Rhizopus stolonifer* (Penz.) were used in the experiment. Surface coating of Potato Dextrose Agar (PDA) medium with botanical extracts (1 mL and 2 mLs) at the concentration of 20 mg/mL in the method used to investigate the efficacy of the extracts from the two methods (ethanol and water). PDA medium was prepared by suspending 39 g of PDA in one liter distilled water and autoclaving at 121°C for 15 minutes. The medium was poured into sterilized petri dishes and allowed to solidify. One and 2 mLs (20 mg/mL) to each botanical extract preparation was separately spread thinly on the surface of the PDA in petri dishes. The extract was allowed to dry and coated medium inoculated centrally with discs (5 mm diameter) obtained from one-week-old cultures of the four (4) test fungi. Three replications were set to each treatment.

Controls were set up in which PDA with no botanical extract were inoculated with test fungi. The whole set-up was incubated at 28°C and measurement of growth as radius of a growing fungi colony was undertaken at intervals of twenty-four hours using a ruler [20].

Statistical analysis

The set up was arranged in Complete Randomized Design (CRD). The data collected were subjected to statistical analysis of variance (ANOVA) using SPSS 20.0 version (Statistical Package for the Social Science) and means were separated using Least Significance Difference (LSD) to determine levels of significance ($P \leq 0.05$) and expressed in tables and graphs.

Results

Result of the quantitative determination of the phytochemical

The result of the antioxidant analysis of *Telfairia occidentalis* leaves are shown in Table 1 which reveals the percentage mean value of the antioxidants. The fluted pumpkin leaves studied have alkanoid, flavonoid, tannin, saponins, phytate, phenol, Hydrogen cyanide, vitamin A, vitamin C and riboflavin with the following percentage mean value of 1.35%, 1.50%, 0.437%, 3.60%, 0.095%, 12.2%, 0.0135%, 16.61 mg/100 g, 11.00 mg/100 g, 64.13 mg/100g, 12.38 mg/100 g.

Values are percentage mean of triplicate data collected during the practical.

Table 1. *Results of the quantitative determination of the antioxidants.*

Phytochemicals/Vitamins	Percentage Composition
Alkaloid	1.35
Flavonoid	1.50
Tannin	0.437
Saponins	3.60
Phytate	0.095
Phenol	12.2
HCN	0.0135
Vit A	16.61
Vit C	64.13
Riboflavin (B_2)	12.38

In-vitro antifungal activity (cm)

The result of the *In-vitro* antifungal activity of *T. occidentalis* (Ugu) leaves ethanol and crude water extract at the concentration of one and 2 mLs (20 mg/mL) of each botanical extract for three (3) days reading on four (4) test fungi, *Geotrichum candidum* (Link.) *Aspergillus niger* (Van Tieghem), *Fusarium oxysporum* (Mart.) and *Rizopus stolonifer* (Penz) revealed that there were no significant difference at ($P \leq 0.05$) between *G. candidum* (3.10 ± 1.37^{ab}) and *A. niger* (2.70 ± 0.98^{ab}) *while F. oxysporum* (4.12 ± 0.43^{b}) and *R. stolonifer* (0.10 ± 0.00^{a}) were significantly different at ($P \leq 0.05$) in relation to the control for the treatment with 1 mL of *T. occidentalis* leave crude water extract.

For the treatment of 1 mL *T. occidentalis* (Ugu) leaves ethanol extract showed that there was no significant difference at ($P \leq 0.05$) among the four (4) organisms syudied as they recorded similar level of inhibition at the end of the three (3) days with high value of (2.80 ± 0.62^{a}) in *R. stolonifer* and low value of (1.44 ± 0.82^{a}) in *A. niger.* The result of the 2 mLs treatment with *T. occidentalis* (Ugu) leaves crude water extract showed that *R. stolonifer* (0.00 ± 0.00^{a}) were significantly different at ($P \leq 0.05$) while there were no difference at ($P \leq 0.05$) among *G. candidum* ($3.09 \pm 1.091.09^{ab}$), *A. niger* (2.86 ± 1.09^{ab}) and *F. oxysporum* (2.98 ± 1.27^{ab}).

The treatment with 2 mL *T. occidentalis* (Ugu) leaves ethanol extract revealed that *G. candidum* (0.27 ± 0.00^{ab}) and *F. oxysporum* (0.29 ± 0.13^{ab}) has no significant difference at ($P \leq 0.05$) and also there was no significant difference at ($P \leq 0.05$) between *A. niger* (0.00 ± 0.98^{ab}) and *R. stolonifer* (0.00 ± 0.00^{a}).

Discussion and Conclusion

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-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 [21].

In this study the phytochemical screening was typified by the phytochemical content, vitamins content and the antifungal activity as typified by its inhibition of the growth of some commonly encountered fungi of their extracts (aqueous and ethanolic) of *Telfairia occidentalis* leaf are highlighted below.

The result of the phytochemical screening and vitamins is shown in Table 1, it revealed that the extracts contains tannin, alkaloids, saponins, flavonoids, HCN, phytate, Vit A, Vit C and riboflavin. This finding agrees with earlier report of [22] which he said that the leaf of *Telfairia occidentalis* contains tannins, alkaloids and saponins. In that, vegetables are rich source of phytochemicals, this phytochemical have a protective and therapeutic effect essential to preventing diseases and maintaining a state of wellbeing, by stimulating enzymes in the liver that render some carcinogens harmless and help the body stimulate others [23]. These studies have shown that regular consumption of fruit and vegetable has been corrected with a reduced risk of developing chronic diseases.

The inhibitory properties of aqueous and ethanolic extracts of *Telfairia occidentalis* leaf against some commonly encountered fungi namely, *Geotrichum candidum, Aspergillus niger, Fusarium oxysporum* and *Rhizopus stolonifer* shown in Tables 2-5 revealed that there were no significant different at ($P \leq 0.05$) an inhibitory effect on *Fusarium oxysporum* and R. stolonifer but does not have *G. candidum* and *A. niger*. The ethanolic extract has no inhibitory effect in any of the four (4) organisms.

Conclusion

Telfairia occidentalis leaf is a very rich source of phytochemicals and the intake of these plants chemicals have a protective potential against some tropical disease in the use of leaf in

Table 2. *Mean dependent of organisms treatment with t. occidentalis crude water extract (1 mL).*

Organisms with their control	Mean of Dependent
G. candidum	3.1
A. niger	2.7
F. oxysporum	4.1
R. stolonifer	0.1
Control *G. candidum*	2.8
Control *A. niger*	2.9
Control *F. oxysporum*	3.9
Control *R. stolonifer*	2.3

Table 3. *Mean dependent of organisms treatment with t. occidentalis ethanol extract (1 mL).*

Organisms with their Control	Mean of Dependent
G. candidum	1.6
A. niger	1.4
F. oxysporum	1.9
R. stolonifer	2.8
Control *G. candidum*	3.0
Control *A. niger*	2.0
Control *F. oxysporum*	3.8
Control *R. stolonifer*	2.0

Table 4. *Mean dependent of organisms treatment with t. occidentalis crude water extract (2 mL).*

Organisms with their Control	Mean of Dependent
G. candidum	3.0
A.niger	2.8
F. oxysporum	2.9
R. stolonifer	0.00
Control *G. candidum*	3.1
Control *A. niger*	2.9
Control *F. oxysporum*	3.7
Control *R. stolonifer*	2.8

Table 5. *Mean dependent of organisms treatment with t. occidentalis ethanol extract (2 mL).*

Organisms with their Control	Mean of Dependent
G. candidum	0.2
A.niger	0.0
F. oxysporum	0.2
R. stolonifer	0.0
Control *G. candidum*	3.0
Control *A. niger*	2.0
Control *F. oxysporum*	3.8
Control *R. stolonifer*	2.0

folk medicine in Nigeria. The antioxidant screening of the *T. occidentalis* leaf extract has shown that the leaf has a high content of phytochemical such as alkaloids, phytic, tannins, flavonoids etc., and also contains Vitamins A, C and riboflavin. The phytochemicals appear to work alone and in combination, and perhaps in conduction with the vitamins and other nutrients in food to prevent halt or lessen disease [24]. In its antifungal activities, it's been found out that the aqueous extract can inhibit the growth of *R. stolonifer*, while the eyhanolic extract can inhibit the growth of all the four organisms. Therefore the ethanolic extract has higher inhibitory effect/higher antifungal 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 benefit from the consumption of the leaf of *T. occidentalis.*

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***Correspondence to:**

Umeoka Nkiru
Professor
Department of Plant Science and Biotechnology (Botany)
Faculty of Science
Imo State University
Owerri
Imo State
Nigeria
Tel: +234 8183026551
E-mail: umeokankiru@yahoo.co.uk