Antinociceptive and Antioxidant Activities of the Methanolic Extract of *Telfairia occidentalis* Seeds

**Abstract**

**Context:** The seeds of *Telfairia occidentalis* have been known to possess different biological properties and are used in traditional medicine in Africa and Asia to treat many ailments. The plant is particularly noted traditionally for its healing properties and is usually consumed in the form of herbal decoctions/concoctions as a blood tonic, to treat sudden attacks of convulsions, pain, malaria and anaemia. **Aims:** In the present study, various phytochemical and pharmacological studies were done on the methanolic extract of the seeds of *Telfairia occidentalis* to evaluate its antitoxic and antinociceptive properties to substantiate its traditional use. **Methods:** Phytochemical screening of the extract was done according to standard procedures. Antioxidant potential was ascertained using 2,2-diphenyl-1-picryl hydrazyl (DPPH) scavenging activity, total phenolic content and total flavonoid content assays. Analgesic activity was analyzed using formalin induced paw licking test in albino rats at 100, 200 and 400 mg extract per kg body weight. **Statistical Analysis Used:** All results extrapolated from the experiments were expressed as mean ± SEM. Data obtained was analyzed statistically using ANOVA (one-way) followed by Dennett’s posthoc test. **Results:** Phytochemicals present in the extract were alkaloids, flavonoids, saponins, terpenoids, steroid and anthraquinones. The extract significantly inhibited DPPH scavenging activity with percentage inhibition of 147.3%. The methanolic seed extract of *T. occidentalis* significantly reduced (P < 0.05) formalin induced paw licking in both neurogenic and inflammatory phases of formalin induced paw licking test, with 35.59 and 78.51% inhibition at 400 mg/kg, in albino rats in a dose dependent manner. **Conclusions:** The seed extract in this study significantly reduced formalin induced hind paw licking, and could be used as an analgesic for treatment of pain and also showed marked antioxidant potential.

**Keywords:** Analgesic, fluted pumpkin, free radicals, nociception, pain, reactive oxygen species, secondary metabolites, ugu

**Introduction**

Plants are used to treat various kinds of diseases such as diabetes, hypertension, pain, worm infection amongst others. Information about the treatment/cure of diseases with the use of plants has been passed amongst generation which is the basis for “tradomedicine”. In the past, people attributed tradomedical healing to supernatural forces because of lack of knowledge of the constituents of the plants used but the isolation of active compounds such as morphine, quinine and alkaloids in 1816 brought about understanding of the use of plants in bringing about healing.

Nociception is the neural processes of encoding and processing noxious stimuli. This activity is initiated by nociceptors (pain receptors) which detects mechanical, thermal or chemical changes, above a set threshold. A nociceptor, once stimulated, transmits a signal along the spinal cord to the brain. Pain is primarily associated with tissue damage or described in terms of tissue damage or both. It forms part of most diseases and it is usually the major factor that alerts the patient to seek medical attention. Pain affects health and well-being of individuals having negative impacts on relationships, cognitive abilities and working abilities.

*Telfairia occidentalis*, commonly known as fluted pumpkin, fluted gourd and locally referred to as ugu, is a tropical vine grown in West Africa as a leaf vegetable and for its edible seeds. The plant is drought-tolerant, dioecious perennial that is usually grown trellised. *T. occidentalis* is a member of the Cucurbitaceae family. The fluted gourd grows in many nations of Africa and Asia. It is a member of the Cucurbitaceae family.
West Africa, but is mainly cultivated in Nigeria (indigenous to southern Nigeria), used primarily in soups and herbal medicines. The fluted gourd is noted traditionally for its healing properties and used as a blood tonic administered to the weak or ill by the Ibo tribe of Nigeria. It is also used to treat sudden attack of convulsion, pain, malaria and anaemia. Although the fruit is inedible, the seeds produced by the gourd are high in protein and fat, and can, therefore, contribute to a well-balanced diet. The leaves of this plant are known for analgesic and anti-inflammatory properties. This study aims to scientifically validate the antinociceptive property of the seed of Telfairia occidentalis as well as establish its antioxidant activities.

Methods

All reagents and chemicals used in this study were of analytical grade and include methanol (CH₃OH), ammonia (NH₃), sulphuric acid (H₂SO₄), chloroform (CHCl₃), acetic acid (CH₃COOH), Folin-Ciocaltu reagent, sodium carbonate (Na₂CO₃), gallic acid, Methanol, 1,1-diphenyl-2-picrylhydrazyl, trichloroacetic acid (TCA), ferric chloride (FeCl₃), diclofenac Na and ascorbic acid.

Sample collection

Telfairia occidentalis seeds were bought from a local market in Ado-Ekiti, Ekiti State, Nigeria. The seeds were thoroughly dried under the sun. The dried seeds were shelled and cracked open to remove the inner portion (mesocarp). The mesocarp was ground into a fine powder.

Collection of animals

Both male and female white albino rats (Ratus albus) were purchased and were fed with feeds and water ad libitum.

Sample preparation (methanolic extraction)

The fine powder of Telfairia occidentalis was macerated in 70% methanol. The liquid filtrates were concentrated and evaporated to dryness in vacuum at 40°C. It was covered and left for 24 h in a dark cupboard and thereafter placed in water bath to produce a sticky gummy mass of extract, which was used for further analysis.

Phytochemical screening

Phytochemical analysis of secondary metabolites such as alkaloids, flavonoids, saponins, coumarins, anthraquinones, terpenoids, steroid and sterols were carried out according to the common phytochemical methods described by Harborne and Trease and Evans. Three methods were used to test for flavonoids. First, dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. To this, H₂SO₄ (1 ml) was added. A yellow colouration that disappeared on standing indicated the presence of flavonoids. In the second method, a few drops of 1% aluminium solution was added to a portion of the filtrate. A yellow colouration indicated the presence of flavonoids. In the third method, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration indicated the presence of flavonoids.

Test for saponins

Distilled water (5 ml) was added to 0.5 g of extract in a test tube. The solution was shaken vigorously and observed for a stable and persistent froth. The froth so formed was mixed with 3 drops of olive oil and shaken vigorously. Formation of an emulsion indicated a positive result.

Test for alkaloids

0.5 g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. 5 ml of the filtrate was added to 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer’s reagent was added to one portion and Dragendorf’s reagent to the other. The formation of a cream (with Mayer’s reagent) or reddish brown precipitate (with Dragendorf’s reagent) was regarded as positive for the presence of alkaloids.

Test for cardiac glycosides (Keller–Kiliani test)

To 0.5 g of extract in a test tube, 5 ml of water and 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was followed by the careful addition of 1 ml of concentrated H₂SO₄ along the wall of the tube. A brown ring at the interface indicated the presence of a deoxy sugar characteristic of cardenolides.

Test for anthraquinones

0.5 g of the extract was boiled with 10 ml of H₂SO₄ and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipetted into another test tube and 1 ml of dilute ammonia was added. A yellow coloured solution is an indication of a positive result.

Test for terpenoids (Salkowski test)

2 ml of chloroform was added to 0.5 g of the extract. Concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicated the presence of terpenoids.

Test for flavonoids (Fehling’s test)

The methanolic extract (0.5 g in 5 ml of water) was added to boiling Fehling’s solution (A and B) in a test tube. A brick red colour solution indicated the presence of reducing sugars.
Test for tannins
About 0.5 g of the extract was boiled in 10 ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Antioxidant activities
1,1-Diphenyl-2-picryl hydrazyl radical scavenging activity
DPPH radical-scavenging activity was measured according to the method of Shimada et al.[14] with slight modification. In that, 1 ml of 0.1 mM freshly prepared DPPH solution in methanol was added to 1 ml of each sample (0.04–0.2 mg/ml), the mixture was shaken vigorously and left in the dark at room temperature for 30 min. The absorbance of the resultant solution was measured at 517 nm, using ascorbic acid as the standard. All determinations were performed in duplicate. The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation.[15]

\[ \text{Scavenging activity} \% = \left( \frac{A_b - (A - A_0)}{A_b} \right) \times 100\% \]

Where \( A_0 \) is the value of DPPH without sample; \( A \) is the value of sample and DPPH; \( A_b \) is the value of sample without DPPH.

Total phenolic content
The total phenol content of the extract was determined by the method of Vermerris and Nicholson.[16] In this, 0.1 ml of the extract (10 mg/ml) was mixed with 2 ml of freshly prepared sodium carbonate (2%). After 5 min, 100 µl of Folin-Ciocalteu reagent (1 N) was added to the mixture and incubated for 30 min at room temperature. Absorbance was taken against blank at 750 nm. Gallic acid was used as the standard at varying concentrations (0.04, 0.08, 0.12, 0.16, 0.2 mg). The result was expressed as mg gallic acid equivalent per gram of dry extract (mg GAE/g).

\[ T = \frac{CV}{M} \]

Where \( T \) = Total phenolic content, \( C \) = Concentration of gallic acid, \( V \) = volume of extract, and \( M \) = mass of the pure plant.

Total flavonoid
Total flavonoid content was measured with the aluminium chloride colorimetric assay as modified by Patel et al.[17] In this, 1 ml aliquot of extract and 1 ml standard quercetin solution (0.04, 0.08, 0.12, 0.16, 0.2 mg/ml) was placed into test tubes and 4 ml of distilled water and 0.3 ml of 5% sodium nitrite solution was added into each tube. After 5 min, 0.3 ml of 10% aluminium chloride was added. At the 6th minute, 2 ml of 1 M sodium hydroxide was added. Finally, the volume was made up to 10 ml with distilled water and mixed vigorously following which an orange yellowish colour developed. The absorbance was measured at 510 nm. Blank contained distilled water in place of extract or standard. Quercetin was used as standard. The samples were performed in triplicates. The data of total flavonoids were expressed as mg of quercetin equivalents/100 g of dry mass.

Formalin induced paw licking test
The procedure was similar to that described by Hunskaar and Hole[18] and Okokon and Nwafor.[19] Adult albino rats (23–27 g) of both sex randomised into five groups of 3 rats each were used for the experiment. The animals were fasted for 24 h before administration (p.o) but allowed access to water. The animals in Group I (negative control) received 10 ml/kg of normal saline, Group 2 received 100 mg/kg of diclofenac Na (positive control) while Groups 3-5 received 450, 900 and 1350 mg/kg doses of the seed extract, 30 min before pain was initiated with buffered formalin administration. A 20 µl of 2.5% formalin solution (0.9% formaldehyde) made up in phosphate buffered saline (PBS) of pH 7.2 (PBS concentration: NaCl, 100 mM, and phosphate buffer, 25 mM) was injected subcutaneously under the surface of the right hind paw of each animal. The number of times spent licking the injected paw was noted down and considered as indication of pain. The first of the nociceptive response normally peaks 5 minutes after injection and the second phase 15-30 min after formalin injection, representing the neurogenic and inflammatory pain responses, respectively. The responses were measured for 5 min after formalin injection (neurogenic response) and 15–45 min after formalin injection (inflammatory response). The experiment was conducted under conditions of no disturbance that may affect animals’ response. Percentage inhibition was determined by comparing the results of extract with control group using the following formula;

\[ \text{Percentage inhibition} = \frac{(A - T)}{A} \times 100\% \]

Where \( A \) is the average number of paw licking of control and \( T \) is the average number of paw licking of test group.

Statistical analysis
All results extrapolated from the experiments was expressed as mean ± SEM. Data obtained was analyzed statistically using ANOVA (one-way) followed by Dennett’s posthoc test.

Results
Phytochemical screening
The phytochemical analysis of the methanolic extract of \textit{T. occidentalis} showed the presence of different groups of secondary metabolites such as alkaloids, flavonoids, saponins, terpenoids, steroid and anthraquinones which are of medicinal importance and are presented in Table 1.

1,1-Diphenyl-2-picryl hydrazyl radical scavenging
The result of DPPH scavenging activity [Figure 1] in this study indicated that the plant was potentially active.
Methanolic extract showed percentage inhibition of 147.3% as compared to ascorbic acid which showed 147.0 percentage inhibition at the highest concentration of 0.20 mg/ml.

**Total phenolic content and total flavonoid content**

The total flavonoid and total phenolic contents of the extracts were 24.785 ± 0.185 mg QE/g of dry plant material and 82.142 ± 0.527 mg GAE/g respectively.

**Antinociceptive activity**

The methanolic seed extract of *T. occidentalis* significantly decreased formalin induced paw licking in both neurogenic and inflammatory phases of formalin induced paw licking test in albino rats in a dose dependent manner as shown in Table 2. The lowest paw licking activity was observed with the extract at dose of 400 mg/kg body weight in both phases of pain.

**Discussion**

The seed of *Telfairia occidentalis* which is used by the *Ibibios* of Niger Delta of Nigeria for nutritive purposes in the making of soup was evaluated for both antioxidant and antinociceptive activity in rodent. Phytochemicals synthesized by medicinal plants contribute greatly to their medicinal value. For instance, saponins generally have antidiabetic, cytotoxic and insecticidal activities.[20]

In this study, phytochemicals such as alkaloids, tannins, phenolics, saponins, flavonoids and anthraquinones were found to be present in the methanolic seed extract of *T. occidentalis*.

Various reactive oxygen species (ROS) encompass a spectrum of diverse chemical species including superoxide anions, hydrogen peroxide, hydroxyl radicals, nitric oxide, peroxynitrite, and others. These oxidants can play a variety of roles in both animals and plants *in vivo*. For instance, some ROS are involved in cellular signalling, cell growth regulation, specific cellular physiology, and energy production.[21] However, the oxidation of lipids, DNA, protein, and carbohydrate by toxic ROS can also often cause DNA mutation, damage target cells or tissues, and result in cellular senescence and death. Knowledge and application of potential antioxidant activities in reducing oxidative stresses *in vivo* have prompted many investigators to search for potent natural antioxidants from various plant sources.[22,23]

In this study, we characterized not only the antioxidant activities but also the analgesic effect for the methanolic extracts of the seeds of *T. occidentalis*. In DPPH radical scavenging assay, *T. occidentalis* methanolic extracts showed significant activity in a concentration dependent manner. The antioxidant potential is mainly due to the presence of phenolic compounds which was confirmed present in the phytochemical screening results. Phenolic compounds have been reported to be associated with antioxidant activity and that they play an important role in stabilizing lipid peroxidation.[24] These phytochemical compounds are known to provide support for bioactive properties of plants, and thus they are responsible for the antioxidant properties.

**Table 1: Phytochemical screening on methanolic extract of *Telfairia occidentalis***

| Phytochemicals | Methanolic extract |
|----------------|--------------------|
| Alkaloids      | Present            |
| Tannins        | Present            |
| Phenolics      | Present            |
| Glycosides     | Absent             |
| Saponins       | Present            |
| Flavonoids     | Present            |
| Steroids       | Absent             |
| Anthraquinones | Present            |
| Terpenoids     | Absent             |

**Table 2: Antinociceptive effect of methanolic extract of *Telfairia occidentalis* seeds in formalin induced paw licking test**

| Group        | Dose (mg/kg) | Early phase response | Late phase response |
|--------------|--------------|----------------------|---------------------|
|              |              | Number of paw licking| Percentage of inhibition | Number of paw licking | Percentage of inhibition |
| A (negative control) | Vehicle | 19.670±0.880 | - | 52.670±1.760 | - |
| B (drug)     | 100          | 17.330±0.880 | 11.90 | 15.000±1.000 | 71.52b |
| C (extract)  | 100          | 16.970±0.330 | 13.73ab | 18.670±2.030 | 64.55b |
| D (extract)  | 200          | 14.330±0.880 | 27.15a | 15.590±4.260 | 70.40b |
| E (extract)  | 400          | 12.670±1.450 | 35.59b | 11.320±1.200 | 78.51b |

Results were expressed as mean±SEM and analysed by ANOVA (one-way) followed by Diment’s test. Significant difference *P<0.01 and b*P<0.05 compared with control group (n=3), *abStatistically nonsignificant values. SEM: Standard error of mean.
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Conflicts of interest
There are no conflicts of interest.

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