Formulation and antianaemic evaluation of the tablets containing *Telfairia occidentalis* aqueous leaf extract

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**INTRODUCTION**

Herbal medicine is fast becoming a mainstay in the health system as about 80 % of the populace in developing countries rely on herbal medicines for the treatment of several diseases and for their regular health needs (WHO, 2011; Chintamunnee and Mahomoodally, 2012; Ekor, 2014). Medicinal plants have found use in diverse diseases in addition to being used as starting materials for the development of some conventional therapeutic agents (Owolabi et al., 2007). The advent of ethnobotany has also heightened the use of herbal medicines by improving collaborations between traditional practitioners and scientists in the area of plant research (Sharma and Kuma, 2013).

Anaemia is a challenging public health disease in tropical countries like Nigeria due to factors such as poor nutrition, high prevalence of parasitic infestations (malaria) and others like human immunodeficiency virus (HIV)  decrease haemoglobin. Anaemia affects people of all ages but is particularly threatening to children and women of child-bearing age (WHO/UNICEF, 2004). Plant remedies and dietary traditions play an effective role in diminishing the suffering due to anaemia. The potential role of medicinal plants as hematinic agents is supported by the ethnobotanical surveys and traditional medicines of different cultures (Akoroda, 1990).

The plant *Telfairia occidentalis* also known as fluted pumpkin belongs to the family; Cucurbitaceae. In Nigeria, it is popularly called *Ugu* (Igbo), *Aworoko* or *Eweroko* (Yoruba), *Umeke* (Edo) and *Ikong* (Efik and Ibibio). It is mainly grown for its highly nutritious leaves and tender shoots which is used for making soups and sauces (Akoroda, 1990). The leaves which contain fats, minerals, vitamins, proteins, iron and phosphorus are traditionally prescribed for nursing mothers, and in the treatment of diabetes and anemia (Okochi et al., 2003; Salman et al., 2008; Kayode and

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**KEYWORDS:** *Telfairia occidentalis*; excipients; tablets; antianaemic
Kayode, 2011; Nwauzoma and Dappa, 2013; Eseyin et al., 2014). Several studies have also reported the hematinic properties of the aqueous leaf extract of *Telfairia occidentalis* in animal models as well as in human erythrocytes (Ajayi et al., 2000; Dina et al., 2000; Osuntoki and Sanusi, 2007; Odede et al., 2010; Lawal et al., 2015; Ohadoma, 2016).

A major concern for patients in the use of herbal medications is the issue of its presentation and acceptability (this implies a formulation that is appealing such that it can encourage patient’s compliance). However, the scientist is not only concerned about the presentation and acceptability but also about stability of the product. This makes the development of plant extracts into simple, convenient, elegant and cost effective dosage forms like tablets, a requisite for patient compliance and therapy. Tablets are composed of the active ingredients and other excipients like binders which hold the ingredients of the tablets together and impart mechanical strength to the tablet. Appropriate choice of a binder is therefore a major determinant in the physical stability of a drug and its rate of release from the formulation.

The aim of this study is to investigate the effect of different binders (polyvinyl pyrollidone, corn starch and gelatin) on the physicochemical properties of the dried aqueous leaf extract of *Telfairia occidentalis* (TOLE) and to evaluate the anti-anaemic properties of the tablet formulations.

**MATERIALS AND METHODS**

**Materials**

Polyvinyl pyrollidone (Aldrich Chemicals, Inc. USA), Gelatin (Aldrich chemicals Ltd, Germany), Corn starch (BDH chemicals Ltd, UK), Lactose (BDH chemicals Ltd, UK), Magnesium stearate (BDH chemicals Ltd, UK), Dried aqueous leaf extract of *Telfairia occidentalis* (TOLE) prepared in the laboratory of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria.

**Methods**

**Collection and identification of Telfairia occidentalis leaves and preparation of Telfairia occidentalis extract**

Fresh leaves of *Telfairia occidentalis* were obtained from the local market at Karmo, Abuja and identified in the herbarium of the Institute by Mallam Muazam and given a voucher number NIPRD/H/7084. 500g weight of washed and chopped fresh leaves were blended with 500 mL of distilled water (ratio 1:1) using a blender (Qlick, Japan). The wet mass was filtered and the filtrate was air-dried for 24 h. The resulting dried aqueous extract (TOLE) was sieved through a 250 µm sieve mesh, packaged in air-tight container and kept in a desiccator until further use.

**Evaluation of Telfairia occidentalis extract**

**Organoleptic properties** (color, odor and texture) of the dried powdered extract (TOLE) were assessed using sensory organs.

**Flow rate**

The time taken for the powdered extract (5 g) to flow through a funnel was noted and the flow rate (g/sec) was computed as the ratio of the granule weight to the time taken.

**Angle of repose**

Powdered extract: TOLE (5 g) was poured into a funnel whose orifice had been plugged, the height and diameter of the powder heap formed after the orifice of the funnel had been opened were measured and used in computing the angle of repose (A) as;

\[ A = \tan^{-1}\left(\frac{\text{height of heap}}{\text{radius of heap}}\right) \]  

**Bulk and tapped densities**

The bulk volume was noted as the volume occupied by the granules in the cylinder after automated tapping and the tapped volume as the volume occupied by the granules by the wet granulation method of massing. The equations below were used to compute the bulk (Bd; g/mL) and tapped densities (Td; g/mL);

\[ Bd = \frac{\text{weight of granules}}{\text{bulk volume}} \]  
\[ Td = \frac{\text{weight of granules}}{\text{tapped volume}} \]  

**Hausner ratio (HR) and Carr’s compressibility Index (CI)**

These were calculated from data obtained from the bulk densities (Bd) and tapped densities (Td);

\[ HR = \frac{Td}{Bd} \]  
\[ CI = \frac{Td - Bd}{Td \times 100} \]

**Moisture content**

A quantity of the powdered extract (1 g) was placed into the Ohaus moisture content analyzer and percentage of moisture was obtained automatically.

**Preparation of dried aqueous extract of Telfairia occidentalis (TOLE) granules**

The binders employed in this study: polyvinyl pyrollidone (PVP), corn starch (CS) and gelatin (GEL) were used at a concentration of 5 %w/v to prepare granules by the wet granulation method of massing and screening. A batch size of 30 tablets with target weight of 300 mg was prepared. Appropriate quantities the extract (TOLE), diluent (lactose) and...
disintegrant (corn starch) were geometrically mixed in a mortar and wet-massed with the binder solution according to the composition in Table I. The damp mass was screened through a sieve (1.7 mm mesh size) and dried in the oven (60 °C) for 20 min after which the granules were further screened using a sieve of 1 mm mesh size and dried again in the oven (60 °C) for 20 min. The dried granules were stored in air-tight containers and kept in a desiccator until further use. All the batches were prepared similarly according to the composition in Table I.

**Evaluation of Telfairia occidentalis extract (TOLE) granules**

The flow parameters; angle of repose, bulk density and tapped density of the prepared granules were evaluated as already described for the powdered extracts (TOLE).

**Preparation of Telfairia occidentalis extract (TOLE) tablets**

The granules were lubricated (Table 1) and compressed in the Manesty tabling machine (Shanghai, China) using the 10 mm punch and die set. The tablets were kept for 24 h after ejection to allow for elastic recovery before tablet analysis were carried out.

**Table 1. Composition for preparation of TOLE granules.**

| Ingredients (g/Batch) | FP | FC | FG |
|-----------------------|----|----|----|
| TOLE                  | 6.00 | 6.00 | 6.00 |
| Lactose               | 1.96 | 1.96 | 1.96 |
| Polyvinyl pyrrolidone (PVP) | 0.45 | - | - |
| Corn starch (CS)      | - | 0.45 | - |
| Gelatin (GEL)         | - | 0.45 | - |
| Corn starch           | 0.45 | 0.45 | 0.45 |
| Talc                  | 0.09 | 0.09 | 0.09 |
| Magnesium stearate    | 0.05 | 0.05 | 0.05 |
| Total                 | 9.00 | 9.00 | 9.00 |

TOLE = Dried aqueous extract of Telfairia occidentalis leaves, FP = formulations containing polyvinyl pyrrolidone (PVP) as binder, FC = formulations containing corn starch (CS) as binder, FG = formulations containing gelatin (GEL) as binder.

**Evaluation of Telfairia occidentalis extract (TOLE) tablets**

**Uniformity of weight**

Ten (10) tablets randomly selected from each batch were weighed on the digital weighing balance (Metler Toledo, ME303E); the average and standard deviation were calculated.

**Thickness and diameter**

The diameter and thickness of ten (10) tablets randomly selected from each batch were determined using the Mitutuyo micrometer screw gauge.

**Hardness**

The hardness (Kg/cm²) of 5 tablets was determined using the Monsanto hardness tester and the mean was calculated.

**Friability**

Five (5) tablets were collectively weighed, transferred into the Erweka Friabilator and set to rotate at 25 rmp for 4 min. After this, the tablets were dusted and re-weighed; friability, F (%) was calculated as the percentage loss in weight of the tablets;

\[
F = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100
\]

**Tensile strength**

This was computed from data obtained from the thickness (T), diameter (D) and crushing strength (F; N/m²) of the tablets.

\[
TS = \frac{2F}{\pi DT}
\]

**Disintegration time**

The Erweka Disintegration tester was used; six (6) tablets were placed in each of the disintegration tester compartment containing distilled water thermostated at 37 ± 0.5 °C. The time taken for the particles to pass through the mesh of the disintegration compartment was recorded and the average was calculated.

**Anti-anaemic study of TOLE formulations**

**Experimental animals**

This study was carried out following approval of the National Institute for Pharmaceutical Research and Development (NIPRD) ethical committee (NIPRD/05.03.05-6). Adult mice (25 - 30 g) of both sexes were obtained from the Animal facility of the Institute. The animals were kept in propylene cages in the Department of Pharmacology and Toxicology, NIPRD and maintained under standard laboratory conditions. The mice were divided into 5 groups consisting of 5 animals each and quarantined for two (2) weeks before the experiment commenced but allowed food and water ad libitum.

**Induction of anemia**

Anemia was induced in 4 of the groups (B, C, D and E as stated below) by administering 4 mg/kg phenylhydrazine (Lobal Chemie, India) intraperitoneally for 3 days according to the method of Sanni et al., (2005). On the fourth day, the tails of the mice were nipped and blood was collected into crayon bottles containing EDTA for hematological studies (Musyoka et al., 2016).

**Treatment of animals**

Mice in Group A received only distilled water per oral (p.o.) and served as negative control, Group B was left untreated, Group C received 100 mg/kg of dried aqueous Telfairia occidentalis extract (TOLE) after

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suspension in distilled water, Group D received 100 mg/kg of FP per oral; the tablet was crushed and a preparation of 10 mg/kg was made in distilled water then a dose of 100 mg/kg was orally administered. Group E received 0.2 mg/kg ferrous sulphate per oral and served as the positive control; the tablet was crushed and dissolved in distilled water to obtain a concentration of 2 mg/mL according to the method of Chauhan et al., (2015). Oral administration was done using a gavage needle.

The animals were treated for 7 days after induction of anemia and blood samples were collected on day 8. Hematological parameters such as red blood cells (RBC), haemoglobin (Hb), and white blood cells (WBC) were determined using the Wincom YNH7021 3-Diff haematology analyser.

Statistical analysis
Data were expressed as mean ± standard error of mean (SEM). Analysis was done using two way ANOVA on Prism 6.02 and comparison of means was done using Dunnett’s post hoc test.

RESULTS AND DISCUSSION

Organoleptic and flow properties of *Telfairia occidentalis* extract

The extract (TOLE) was found to be dark green in color, with a characteristic odor and smooth texture (Table 2).

| Parameter          | TOLE            |
|--------------------|-----------------|
| Color              | Dark green      |
| Odor               | Characteristic  |
| Texture            | Smooth          |
| Flow rate          | 4.46 ± 0.49     |
| Angle of repose (°) | 28.02 ± 0.22   |
| Bulk density (g/mL)| 0.50 ± 0.01    |
| Tapped density (g/mL) | 0.57 ± 0.00 |
| Carr’s Index       | 11.30 ± 1.08    |
| Hausner ratio      | 1.13 ± 0.01     |
| Angle of repose (°) | 28.02 ± 0.22   |
| Moisture content   | 12.61 ± 0.59    |

TOLE = Dried aqueous extract of *Telfairia occidentalis* leaves.

Flow characteristics of TOLE determined by angle of repose and powder density are also presented in Table 2. Angle of repose measure the ability of a material to flow freely and values < 30° are an indication of excellent flow while those between 31 and 35, and those > 40° are indicative of good and poor flow respectively (Mohammadi and Harnby, 1997). The flow rate was 4.46 ± 0.49 g/sec and angle of repose was 28.02 ± 0.22; indicating the extract possess good flow properties (Mohammadi and Harnby, 1997). Compressibility of TOLE as measured by Carr’s index (11.30 % ± 1.08) showed good propensity of the extract to reduce in volume under pressure while also being free-flowing with Hausner ratio of 1.13 ± 0.01 (Panda et al., 2008). Moisture content of the dried aqueous plant extract was found to be 12.61 % ± 0.59; this was determined to ascertain the appropriate means of packaging and storage of the extract before being used in any formulation. The presence of excessive moisture can influence the flow, mechanical properties and chemical constituents of the extract, it can also lead to microbial growth in the tablet formulations especially during storage (Adane et al., 2006; Emeje et al., 2008).

**Flow properties of Telfairia occidentalis granules**

The flow properties of the granules as shown in Table 3 portrays that all the granules exhibited good flow properties. All the granules had angle of repose between 31.07 and 31.76 indicating that they possess good flow.

| Ingredients (g/Batch) | FP   | FC   | FG   |
|-----------------------|------|------|------|
| Angle of Repose (°)   | 31.76 ± 0.34 | 31.53 ± 2.73 | 31.07 ± 0.66 |
| Bulk density (g/mL)   | 0.37 ± 0.01  | 0.42 ± 0.01  | 0.42 ± 0.18  |
| Tapped density (g/mL) | 0.41 ± 0.01  | 0.48 ± 0.00  | 0.50 ± 0.00  |
| Carr’s index (%)      | 10.35 ± 1.84 | 11.04 ± 2.17 | 14.89 ± 3.54 |
| Hausner ratio         | 1.12 ± 0.02  | 1.13 ± 0.03  | 1.18 ± 0.05  |

FP = formulations containing polyvinyl pyrollidone (PVP) as binder, FC = formulations containing corn starch (CS) as binder, FG = formulations containing gelatin (GEL) as binder.

Bulk and tapped densities are indirect measurements of material flow and determines the volume of material that would fill the die during compression (Santomaso et al., 2003). Granules prepared with CS (FC) and GEL (FG) had similar bulk and tapped densities (0.42 g/m/L) which is significantly different (p < 0.05) from those of FP (0.37 g/m/L). Carr’s compressibility index (CI) is a parameter that assesses the ability of a material to deform under pressure while Hausner ratio (HR) measures the cohesiveness of a powdered material by determining the degree of densification of that material. CI for all the granules was found to be between 10.35 and 14.89 % indicating that they have excellent flow while HR was between 1.12 and 1.18; these values are within the stated limits for free flowing materials (Panda et al., 2008). This shows that the granules possess good flow irrespective of the binders used in the formulation.

**Effect of binder type on properties of Telfairia occidentalis tablets**

Table 4 shows that all the tablets were dark green in color due to the color of the dried aqueous leaf extract.

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of Telfairia occidentalis (TOLE), the tablets were also found to have smooth texture.

The results show that average weight of the tablets is between 291 and 305 mg (Table 4). The deviation of the average tablet was found to be within the specifications for tablets with target weight of ≥ 250 mg (BP, 2002). This shows that all the binders produced tablets of uniform weight; this is important because lack of uniformity in weight would lead to inconsistencies in bioavailability of the active ingredient (TOLE).

Table thickness and diameter are essential factors to consider in product packaging as tablets with dimensions other than specified would reduce the quantity specified for a given packaging container. Tablet diameter across the batches was found to be similar (10.04 - 10.13 mm). Tablet thickness of FC and FG were similar (3.98 and 3.79 mm respectively) while that of FP (4.13) was significantly different at p < 0.05. However, all tablets irrespective of the binder used, were found to be within specified requirements of ± 5 % of the average diameter and thickness (Chaturvedi et al., 2017).

Table 4. Organoleptic and mechanical properties of tablets containing TOLE

| Parameter/Batch | FP | FC | FG |
|-----------------|----|----|----|
| Color           | Dark green | Dark green | Dark green |
| Texture         | Smooth | Smooth | Smooth |
| Tablet weight (mg) | 296.04 ± 5.38 | 304.79 ± 2.47 | 291.32 ± 1.16 |
| Diameter (mm)   | 10.12 ± 0.07 | 10.04 ± 0.07 | 10.13 ± 0.02 |
| Thickness (mm)  | 4.13 ± 0.12 | 3.98 ± 0.03 | 3.79 ± 0.02 |
| Hardness (Kgf)  | 4.40 ± 0.86 | 5.18 ± 0.53 | 5.22 ± 0.45 |
| Friability (%)  | 0.34 | 0.2 | 0.00 |
| Tensile strength (Kgf/cm²) | 0.087 | 0.083 | 0.086 |
| Disintegration (min) | 28.34 ± 0.23 | > 60 | > 60 |

FP = formulations containing polyvinyl pyrrolidone (PVP) as binder, FC = formulations containing corn starch (CS) as binder, FG = formulations containing gelatin (GEL) as binder.

Although PVP possess film-forming ability, its inclusion produced tablets with appreciably lower strength than those prepared with GEL or CS. Tablets prepared with starch produced strong tablets which were of comparable strength with those prepared with GEL. Harder tablets produced with GEL could be attributed to higher spreading coefficient of GEL solution, better interlocking between particles and stronger intermolecular forces during granulation cumulating to creation of stronger solid bridges. All the binders however produced tablets with hardness within the stated acceptable criteria. Tablet hardness is directly related to friability which assesses the ability of a tablet to withstand abrasion, chipping and fracture during packaging, shipping or handling (Itilola et al., 2006). Compact tablets have good resistance to deformation and percentage weight loss of ≤ 1 %w/w is considered as an acceptable limit (Banker and Anderson, 1986) for tablet friability. All the formulated tablets had friability values between 0.00 and 0.34 % in the order FG>FC>FP which corroborates the results of tablet hardness already discussed.

Tensile strength is another parameter that is used to assess the quality of a tablet; high values are indicative of strong mechanical strength and high quality (Alebiowu and Adeyemi, 2009). The tensile strength of the tablets were between 0.067 and 0.086 Kg/cm² with tablets prepared with GEL having the highest value which is similar to FC and significantly different from those prepared with PVP (p<0.05). Results of the mechanical strength of the tablets show that GEL possessed greater magnitude of cohesive strength than PVP thus, imparted better densification and compaction on TOLE. The study by Ebere et al., (2013) also reported similar trend in tablet formulations of Moringa oleifera leaf. However, all the binders employed produced robust and compact tablets.

Disintegration test gives information on the ability of the tablets to break up within specified time (USP, 2003). Disintegration time for the tablets were between 28.34 and > 60 min in the order FG>FC>FP. A positive correlation exists between tablet mechanical strength and disintegration time; tablets that were strong, compact, and resistant to fracture were able to withstand breakup leading to the long disintegration times observed. Tablets prepared with PVP had the least disintegration time which is within the 30 min limit specified for such preparations (USP, 2014) and was significantly different (p<0.05) from those containing GEL. This shows incorporation of PVP would ensure faster breakup leading to faster dissolution and bioavailability of the extract.

Anti-anaemic study

Literature reveals that the leaves of Telfairia

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**Table 5. Effect of TOLE and its tablet formulation on haematological indices in mice**

| Post treatment | Haemoglobin | WBC | RBC |
|----------------|-------------|-----|-----|
|                | Pre treatment | % Recovery | Pre treatment | % Recovery | Pre treatment | % Recovery | Pre treatment | % Recovery |
| **Group A**    | 8.83         | 8.51 | -   | 260.83 | 252.17 | -   | 5.48 |
|                | ± 0.76       | ± 0.44# | ± 16.41 | ± 15.11 | ± 0.68 |
| **Group B**    | 4.64         | 6.17 | 37  | 206.80 | 213.25 | 12  | 15.54 |
|                | ± 0.30       | ± 0.25 | ± 11.90 | ± 12.89 | ± 2.85 |
| **Group C**    | 5.32         | 7.33 | 57  | 230.00 | 242.83 | 42  | 16.72 |
|                | ± 0.81       | ± 0.49*# | ± 14.22 | ± 13.61 | ± 1.90 |
| **Group D**    | 5.08         | 6.63 | 41  | 225.67 | 217.20 | 24  | 12.04 |
|                | ± 0.76       | ± 0.55 | ± 17.87 | ± 11.36 | ± 1.14 |
| **Group E**    | 5.41         | 7.58 | 63  | 237.33 | 249.60 | 52  | 21.68 |
|                | ± 0.17       | ± 0.70*# | ± 8.66 | ± 18.03 | ± 1.64 |

*n=5: Data are expressed as Mean ± SEM; A multiple t-test was used to compare differences in mean between pre and post-treatment statistical significance was determined by the Holms-Sidak method; *p < 0.05 is significant when compared to pre-treatment. A One-way ANOVA was used to compare between groups followed by Dunnett’s post hoc test; # p < 0.05 is significant when compared to untreated anaemic Group B.

Extrapolation of the human dose from the tested animal dose according to the Allometric scaling (Nair and Jacob, 2016) is shown below:

Human Equivalent doses (HED) in mg/kg = (Animal dose mg/kg) × (Animal; mouse Km / Human Km).

HED = (100) x (3/37) = 8.11 mg/kg

Therefore, the potential extrapolated dose for a 70 kg adult would be 8.11 mg/kg x 70 kg = 567.60 mg. It is important however to note that other factors may also come into play when determining the HED.

**CONCLUSION**

This study has shown the possibility of developing robust tablet formulations of the dried aqueous leaf extract of *Telfairia occidentalis* (TOLE). Polyvinyl pyrrollidone (PVP), corn starch (CS) or gelatin (GEL) are capable of being used as wet binders in the formulation of compact *Telfairia occidentalis* tablets. However, based on disintegration time, polyvinyl pyrrollidone (PVP) is considered the most suitable. *In vivo* anti-anaemic study showed that the dried aqueous leaf extract of *Telfairia occidentalis* retained its anti-anaemic property in the tablet formulation and can therefore be developed as an oral anti-anaemic supplement.

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