PHYTOCHEMICAL SCREENING, ANTI-NUTRITIONAL AND MINERALCOMPOSITION OF *Telfairia Occidetallis* (FLUTED PUMPKIN) AND *Cleome Rutidosperma*(FRINGE SPIDER FLOWER)

ABSTRACT

The study was conducted to investigate phytochemicals, antinutrients and mineral compostions of *Telfeira Occidentalis* and *Cleome rutidospermas* leaves. The leaves were analyzed for phytochemicals using, standard methods. The High Performance Chromatography (HPLC) was used in the Quantitative analysis of Phytochemicals as well as the antinutrient contents while the Elemental Compositions was analysed using Atomic absorption spectrophotometer (AAS) (Buck Scientific). The antinutrient content analysed were as follows hydrocyanic acid (31.0±0.001 and 25.0±0.001), oxalate (570±0.004 and 740±0.003), phytic acid (7.50±0.002 and 9.20±0.005 mg/100g), for *T. Occidentallis* and *C. rutidosperma* respectively and the values were all within the NAFADAC/WHO tolerable limit. The Minerals Compositions was found to be, Mn (1.684±0.40 and 0.718±0.31mg/100g), Zn (1.740±0.10 and 1.570±0.31mg/100g), Fe (3.823±0.03 and 4.329±0.01 mg/100g), Na (2.572±0.42 and 2.659±0.80 mg/100g), Ca (74.405±13.60 and 29.677±13.50 mg/100g), Mg (35.277±10.05 and 12.438±10.4 mg/100g), Cu (0.049±0.03 and 0.044±0.01 mg/100g) for *T. Occidentallis* and *C. rutidosperma* respectively. The presences of some secondary metabolites like alkaloids, flavonoids, terpenoids, tannins, and cardiac glycosides and some essential minerals shows that the plants can be alternative sources of medicine. The results of the Antinutrients indicated that the samples are free of toxic substances which might cause ill health to the body. Though, the anti-nutrient contents found in both *T. occidentallis* and *C. rutidosperma* were low, it will still be safer if these leaves were boiled for about 5 to 15 minutes to reduce the anti-nutritional factors significantly.

Keywords: Phytochemical Screening, Anti-nutritional, Mineral Composition, *Telfairia Occidetallis, and Cleome Rutidosperma.*

Introduction

T. occidentallis (fluted pumpkin), is a tropical vine grown in West Africa as a leaf vegetable and for its edible seeds. It is dioecious and perennial commonly known as *"Ugwu"* in Igbo language and is a creeping vegetable that spread across the ground with lobed leaves and twisting tendril s[1]. *T. occidentallis* belongs to the family Cucurbitaceae and the young leaves of the plant are the main ingredients of Nigeria soup [2]. The leaves play important role in human and live stock nutrition as it is believe to be source of protein, carbohydrates, minerals and vitamins [3]. Fresh leaves of fluted pumpkin are used for the treatment of Anaemia, sudden attack of convulsion and malaria [4].

C. rutidosperma belongs to the *Capparidaceae* family [5]. It is a low-growing herb, up to 70 cm tall, found in waste grounds and grassy places with trifoliate leaves and small, violet-blue flowers, which turn pink as they age. According to Bidla *et al*.[6], it could be argued that the plant is native to West Africa, from Guinea to Nigeria, Zaire and Angola. However, it has become naturalized in various parts of tropical America as well as Southeast Asia [6]. *Cleome rutidosperma* has been well studied by different researchers. The analgesic, antipyretic, anti-inflammatory, anti-microbial, diuretic, laxative antioxidant and anti plasmodial activities of the plant have already been reported *Bose et al.*, [7]. *Cleome rutidosperma* is traditionally used in the treatment of paralysis, epilepsy, convulsions, spasm, earache, pain and skin disease [8]. *Cleome rutidosperma* is palatable to humans and is sometimes eaten as a cooked vegetable [9].

Plants have been one of the important sources of medicines since the dawn of civilization[10]. Recently the use of plants as medicine has been patronized more vigorously, and has therefore resulted in an increase in the amount of herbal products traded within and across countries[11]. A number of well-established and important drugs have their source from plants. Plants also serve as source of chemical intermediates needed for the production of some drugs. New medicinal compounds are derived from plant species that have been used as folk, traditional or native remedies for centuries [12]. In another development researches have also indicated that, vegetables supply most of the nutrients that are deficient in other food materials, these include the supply of minerals especially calcium and Iron [13]. They are also considered as acid neutralizers e.g. Okra, cucumber, Amaranthus, lettuce, and cabbage. They can be as well considered as the rich sources of vitamin A, B and C which help to lower susceptibility to infection, for instance Carrots, Sweet corn, Amaranthus and celosa provides Vitamin A, Bitter leaf, Water leaf, Salanum and celosa provide Vitamin B, Tomatos, Carrots, and Lettuce provide Vitamin *C* [14]. Above all, many more of plants and vegetables are yet to come into limelight because their uses in ethno medicine and many other forms have not been subjected to any scientific investigation[15].

The rate of vegetable consumption in Nigeria like rest of Africa countries has shown an indiscriminate pattern which is an indication that most people are not aware of anti-nutrients contents in most of plants. In most cases, green leafy vegetables despite their nutritional value are to be consumed with caution because of the presence of toxic anti-nutrients [16]. Anti-nutrients are natural compounds that interfere with the absorption of nutrients, hence are known to reduce nutrients availability to animals and humans [17].

Malnutrition can be tremendously reduced with an increased use of foods rich in energy, proteins, iron and vitamins most especially those from the rural environment. The lack of nutritional information and inadequate development of nutritionally improved products from local raw materials have direct bearing on nutrition. Much effort has been concentrated on seeds while leafy vegetables have to a large extent been ignored. Leaves are reportedly inexpensive and easy to cook. They are known as potential sources of minerals and vitamins[18]. They are rich especially in carotenoids as well as in iron, calcium, ascorbic acid, riboflavin and folic acid and appreciable amounts of other minerals [19]. Nigeria has abundance of leafy vegetables, most of which grow all year long. Most of the species are found in the rural areas and grow in the wild. Over the years, efforts have been made to domesticate these species and to study their nutritional qualities.

Among vegetables that highly consumed in Nigeria are *T .occidentallis and C. rutidosperm*a. Therefore, this study is to determine the phytochemical screening, anti-nutritional and mineral composition of *T. occidentallis* (fluted pumpkin) and *C. rutidosperma* (fringe spider flower) leaves consumed in Mubi metropolis of Adamawa state, north-eastern Nigeria.

Materials and Methods

Sample Collection

Fresh samples of *T. occidentallis and C. rutidosperma* were randomly collected in Mubi North Local Government Area, along River Yadzaram at Mallam Adamu farms in Mubi North, Adamawa State, Nigeria. Fresh leaves samples were collected from the farms into a labeled large size brown envelope in order to preserve its coloration and moisture content, then, was taken to the laboratory for analysis. The samples were identified in the Department of Biological sciences Adamawa State University, Mubi, Nigeria [10].

Sample Preparation

The collected fresh leaves samples of *T. occidentallis and C. rutidosperma* were taken to the laboratory and washed thoroughly with ordinary tap water to removed dirt, dust and other contaminants, and then they were further, washed with distilled water and were allowed to drip. About 5g of each of the leaves samples were analysed for moisture content then the remaining plants leaves samples were air-dried at room temperature. The dried plant leaves were crush, ground into fine powder using mortar and pestle in the laboratory and then homogenize using laboratory blender. The powdered samples were sieved using 90 micron sieve and stored in polyethylene air- tight containers for further processing [10]. The powdered samples were use for anti-Nutrient, mineral and phytochemicals analysis [20].

Sample Extraction:

About 20g of each dry powdered sample were subjected for soxhletation in 200cm^3 of petroleum ether. 20g of each powdered plants sample were weighed and placed into the thimble of soxhlet apparatus and then the extraction process was carried out with 200cm^3 of petroleum ether in round bottom flask at temperature 70^{0}C, the extract was collected in a round bottom flask, then evaporated with the aid of rotary evaporator at constant temperature of 60^{0}C with reduced pressure for 2hours [20].

Determination of Phytochemicals

Phytochemical analysis for the screening and identification of bioactive chemical constituents such as flavonoids, terpenoids, alkaloids, glycosides, steroids, saponins, osozone, and tannins of the leaves extracts were determined qualitatively and quantitatively using standard procedures as described by AOAC, [21]; Edeoga et al.[22] and Sofowora [23] with slight modification

Proximate Analysis: Proximate analysis (moisture, ash, protein, fat, fibre and CHO) were determined using standard method of AOAC. [21] and Okonwu et al. [24].

Anti-Nutritional Analysis

Determination of Antinutrient was carried out using High performance Liquid chromatography (HPLC) Buck scientific USA, BLC10/11 – model. HPLC equipped with UV 320nm detector, a (C-18), 5u, 150 x 4.6mm column and a mobile phase of 70:30 met: H_2O was used at a flow rate of 0.45 mL/minute and an ambient operating temperature. A 0.1mg of mixed standards were analysed in a similar manner for identification. Peak identification was conducted by comparing the retention times of authentic standards and those obtained from the samples. Concentrations were calculated using a four point calibration curve [21].

Elemental Analysis

Mineral analysis was carried out by method described by Imaga *et al.* [25]. About 2g of each plants sample were subjected to dry Ashing in a well clean porcelain crucible at 550^0C in a muffle furnace. The resultant ash was digested in $5cm^3$ of concentrated nitric acid, Hydrochloric acid, and water in the ratio 1:2:3 respectively, then it was heated, gently until brown fumes disappear. To the remaining materials in each crucible, $5cm^3$ of distilled water was added and heated until a colorless solution was obtained and the mineral solution in each crucible was transferred to $100cm^3$ volumetric flask through filtration with Whatman filter paper (No. 42) and the volume was filled to mark with distilled water. Then the filtered solution was loaded to an atomic absorption spectrophotometer bulk scientific 200A to determine Calcium, Iron, zinc, copper, and magnesium.

RESULTS AND DISCUSSION

Phytochemical screening

The results of the phytochemical screening of the leaves extracts of *T. Occidentallis* and *C. Rutidosperma* plants indicated the presence of tannins, alkaloid and flavonoids while terpenoids and cardiac glycosides are absent (Table 1 and Table 2). The result of the quantitative analysis showed higher concentration of tannins in *C. Rutidosperma than T.Occidentallis* while the alkaloid content is higher in *T. Occidentallis* than in *C. Rutidosperma*. This investigation indicated that both plants leaves have bioactive compounds which are found in medicinal plants. These metabolites are known to have varied pharmacological actions or applications in man and animals. The investigation showed that the concentration of the phytochemical constituents analysed were significantly higher in *C. rutidosperma*, than in *T. Occidentallis* ($p<0.05$), except alkaloids which was significantly higher in *T. occidentallis* than *C. rutidosperm*a. These results showed that the bioactive compounds in the plants leaves are more significantly observed in *C. rutidosperma* which indicated higher medicinal values than *T. Occidentallis*. This finding is in agreement with the studies by Oyeyemi *et al.* [26] and Odiaka and Schippers [27]. This result indicated that the medicinal values in *T. occidentallis* is less as compared to the studies according to Nwangwa *et al*. [28]; Chakraborty, and Roy [29].

Anti-nutrients Constituents

Anti-nutrients are also referred to as nutritional stress factors. These factors may either be in the form of synthetic or natural compounds and they impede nutrient absorption. The commonly occurring anti nutrients in plants includes; cyanide, Phytates, nitrates and nitrites, Phenollic compounds and oxalates among others. As much as green leafy vegetable contains various beneficial nutrients, it also has anti-nutritional and toxic substances, which impair nutrient uptake and absorption of nutrients [30]. The result of anti-nutrients as presented in Table 3, shows that the average values of the anti-nutrients are as follows hydrocyanic acids 31.00±0.001mg/100g for *T. occidentallis,* while 25.00 ±0.001mg/100g was recorded for *C. rutidosperma* plants. However, the hydrocyanic acids recorded in both plant leaves were within the 35.00mg/100g, tolerable limit by WHO. The oxalate value recorded for *T. occidentallis* was 570±0.004mg/100g while for *C. rutidosperma,* 740 ±0.003mg/100g was observed. The values of oxalate recorded in both plant leaves were within 2000mg/100g, the tolerable limit by WHO. The level of phytic acid recorded in *T. occidentallis* was 7.50±0.002mg/100g, while in *C. rutidosperma was* 9.20±0.005mg/100g. However, the content of phytic acid in both plants exceeded the 5mg/100g tolerable limit set by WHO/FAO [31]. The anti-nutrients recorded in the investigated leaves of *T. occidentallis* and *C. rutidosperma* were Hydrocyanic acids, oxalate and phytic acid. However, the values of these anti-nutrients recorded in this study are too small to be harmful for human consumption. Based on the findings of this research, the studied plant leaves were suitable for human consumption; since the amount of anti-nutrients in them is negligible. This finding is in agreement with the report of Odabasi *et al.* [32]. However, there is need to boil these vegetables for 5 to 15 minutes in order to reduce the anti-nutritional factors significantly.

Mineral Compositions

The results on mineral compositions as recorded in Table 4 showed that the plant leaves of, *C. rutidosperma* and *T. occidentallis*, are rich in minerals, when compared with other plants, such as legumes and tubers. From the result of the investigation carried out the mineral compositions recorded in the two plant leaves of *C. rutidosperma* and *T. occidentallis* are as follows Ca (29.677±13.50mg/100g and 74.405±13.60mg/100g), Mg (12.438±10.4mg/100g and 35.277±10.05mg/100g), Fe(3.823±0.03mg/100g and 4.329±0.01mg/100g), Na (2.659±0.80mg/100g and 2.572±0.42mg/100g), Zn (1.570±0.31mg/100g and 1.740±0.10mg/100g) and Cu (0.044±0.01mg/100g and 0.049±0.03mg/100g) for *C. rutidosperma* and *T. occidentallis* respectively. From the result of the investigation carried out calcium and magnesium are the most predominant elements in *T. occidentallis* and *C. rutidosperma*, however, their amount are higher in *T. occidentallis* than *C. rutidosperma*. According to Skulan *et al.* [32], calcium is an essential mineral for maintaining healthy bones – a factor in the development of numerous diseases such as osteoporosis, rheumatoid arthritis and others. Calcium is another substance that can be found from many vegetables and green leafy plants. The higher calcium content of the studied plant leaves implies that consuming any of these plants can cater for osteoporosis [34]. The higher level of calcium recorded in both plant leaves reaffirmed that *T. occidentallis* and *C. rutidosperma* as important source of calcium for human. Likewise, Harder *et al.* [35] expressed that calcium is heavily involved in bone manufacture. Therefore, shortage or lack of calcium can be responsible for many bone diseases, such as hydroxyapatite in molecular structure [35].

The results from this study showed high presence of magnesium in, *T. occidentallis* (35.277±10.05 mg/100g) as compared to (12.438±10.4 mg/100g) in *C. rutidosperm*a. This result shows that both the plant leaves are good sources of magnesium. Magnesium is a mineral that is important for normal bone structure in the body. Romani [36] expressed that a low magnesium levels in the body have been linked to diseases such as osteoporosis, high blood pressure, clogged arteries, hereditary heart disease, diabetes, and stroke. Report according to Ayuk and Gittoes [37], expressed that magnesium aids in the chemical reactions in the body, intestinal absorption, and also prevents heart diseases and high blood pressure.

The concentration of sodium in the plant leaves are 2.572± 0.42 mg/100g and 2.659±0.80 mg/100g for *T. occidentallis and C. rutidosperm*a respectively. The amount of sodium recorded in the studied plant leaves are very low compared to the recommended level by NAFDAC [38] (3000mg/100g). Sodium has an important role in maintenance of normal acid- base balance. An adult need about 3g per day of sodium but modern diatery habits take in 5 – 20per day [39].

Proximate compositions

Table 5 presents the results of the proximate compositions for *T. occidentallis* and *C. rutidosperma* plant leaves. The results were as follows protein (35.75 ± 0.07% and 12.46±0.01%); fat (was 9.67±0.03% and 4.73±0.02 %); fibre (7.31±0.31 %, and 16.33±0.02%); ash content (8.12±0.07 % and 5.27±0.03 %); moisture content (9.29±0.05 % and 9.15±0.01%) and CHO (29.86±0.29 % and 52.06±0.04 %) for *T. occidentallis* and *C. rutidosperma* plant leaves respectively. These results showed that both plants contain appreciable amount of protein which indicates further that they can both serve as essential ingredient for building and repairing of body tissues, regulation of body processes and formation of enzymes and hormones. The fibre content was higher in *Cleome rutidosperma* than for *Telfairia occidentallis*, this showed that they can help in keeping the digestive system healthy and functioning properly. Fibre aids and speeds up the excretion of waste and toxins from the body, preventing them from sitting in the intestine or bowel for too long [40]. The low percentage of fat contents in both plants could be an advantage in the diets of people based on age and body mass. That means that the low lipid content in these vegetables could be an advantage by helping uptake of water soluble vitamins. More so, Carbohydrate-rich *Cleome rutidosperma* could *increase* glucose metabolism leading to the production of pyruvate and energy. Pyruvate is known to be the preferred substrate essential for the activity and survival of sperm cells [41].

Table 1: Qualitative Test for Phytochemicals Constituents of *Telfairia occidentallis* and *Cleome rutidosperma* Plant leaves.

Phytochemicals	***T. occidentallis***	***C. rutidosperma***
Alkaloid	+	+
Flavonoids	+	+
Tarpenoids	–	–
Tannins	+	+
Cardiac glycosides	–	–

+ present, - absent

Table 2: Phytochemical constituents of *Telfairia occidentallis* and *Cleome rutidosperma* Plant leaves (mg/100g dry weight)

Phytochemicals	***T. occidentallis***	***C. rutidosperma***
Alkaloid	712.40±0.08	615.30±0.03
Flavonoids	232.34±0.03	312.52±0.06
Tarpenoids	10.44±0.02	13.10±0.03
Tannins	845.23±0.04	892.35±0.07
Cardiac glycosides	5.30±0.02	6.23±0.03

Results were presented as mean ± SD of triplicate determinations.

Table 3: Anti-nutrients Constituents of *Telfairia occidentallis* and *Cleome rutidosperma* Plant leaves (mg/100g dry weight).

Components	***T. occidentallis***	***C. rutidosperma***	**WHO/FAO (mg/100g)**
Hydrocyanic Acids	31.0±0.001	25.0±0.001	35
Oxalate	570±0.004	740±0.003	2000
Phytic acid	7.50±0.002	9.20±0.005	5

Results were presented as mean ± SD of triplicate determinations

Table 4: Mineral Composition for *T. occidentallis* and *C. rutidosperma* leaves (mg/100g dry weight)

Elements	*T. occidentallis*	*C. rutidosperma*	NAFDAC Standards (mg/100g)
Mn	1.684±0.40	0.718±0.31	2
Fe	4.329±0.01	3.823±0.03	500
Zn	1.740±0.10	1.570±0.31	500
Na	2.572±0.42	2.659±0.80	3000
Ca	74.405±13.60	29.677±13.50	3000
Mg	35.277±10.05	12.438±10.4	2000
Cu	0.049±0.03	0.044±0.01	500

Results were presented as mean ± SD of triplicate determinations.

Table 5: Proximate composition for *Telfairia occidentallis* and *Cleome rutidosperma* Plant leaves (%)

Components	*T. occidentallis*	*C. rutidosperma*
Protein	35.75±0.07	12.46±0.01
Fat	9.67±0.03	4.73±0.02
Fibre	7.31±0.31	16.33±0.02
Ash	8.12±0.07	5.27±0.03
Moisture	9.29±0.05	9.15±0.01
CHO	29.86±0.29	52.06±0.04

Results were presented as mean ± SD of triplicate determinations

Conclusion

Vegetables are very important part of our diets. This study has demonstrated that the two studied vegetables *Telfairia occidentallis* and *Cleome srutidosperma* contains some of the biologically active phytochemicals which include Alkaloid, flavonoids and Tannins. *Cleome rutidosperma* contains relatively higher phytochemicals than *Telfairia occidentallis.* The anti-nutrient composition for the plant leaves of *T. occidentallis* and *C. rutidosperma* were low compared to the WHO standard. More so, this study had shown that *T. occidentallis* contains higher mineral composition than *Cleome rutidosperma* , this showed that *T. occidentallis* is a good source of minerals which can serve as supplement to meet the daily requirement for minerals in human body. The data obtained in the present work will be useful in the synthesis of new drugs of pharmaceutical importance through our local plants. Aithough, the anti-nutrient contents found in both *Telfairia occidentallis* and *Cleome rutidosperma* were low, it will still be safer if these leaves were boiled for about 5 to 15 minutes to reduce the anti-nutritional factors significantly.

References

1. Horsfall, M. J. and Spiff, I. A. (2008). Equilibrium sorption study of AL, CO and Ag in aqeous solutions by fluted pumpkin waste biomass. *Acta chim* 52:174 – 181.

2. Saalu, L. *C.*, Kpela, T, Benebo, A. S., Oyewopo, A. O., Anifowope, E. O., Oguntola, J. A. (2010). The Dose-Dependent Testiculoprotective and Testiculotoxic Potentials of *Telfairia occidentalis Hook f.* Leaves Extract in Rat. *International Journal of Applied Research in Natural Products* 3 (3):27-38.

3. Fasuyi A. O. (2008). A nutritional potential of some tropical vegetable leaf meals: chemical characterization and functional properties. *African Journal of Biotechnology*, 5:49 – 53.

4. Ukwuoma, B. and Muanya, L. (2009). Fighting degenerative diseases with vegetable soup. Accessed at http/www.gaurdiannewsngr.com/natural-health.article 01 – 12 – 2017.

5. Bose, A., Smith, P.J. and Lategan *C.*A. (2010). Studies on *In Vitro* antiplasmodial activity of *Cleome rutidosperm*a. *Acta Polanica Pharm-Drug Research* 67:315-318.

6. Bidla, G., Titanji, V.P.K., Bolad, A., and Berzins, K. (2014). Antiplasmodial activity of seven plants used in African folk medicine. *Indian Journal of Pharmacology*;36(4):245-246
7. Bose, A., Saravanan, V.S., and Karunanidhi, N. (2014). Analgesic and locomotors activity of extracts of *Cleome rutidosperma* D *C. Indian Journal of Pharmacological Science*; 66:795-7.
8. Bose, A., Mondal, S., Gupta, J.K., and Ghosh, T. (2011). Studies on diuretic and laxative activity of ethanol extract and its fractions of *Cleome rutidosperma* aerial parts. *Pharmacology Magazine*, 2(7):178-82.

9. Bose, A., Gupta, J. K. and Ghosh, T. (2009). Antimicrobial Activity of Certain Extracts of *Cleome rutidosperm*a. *Indian Journal Nat and Prod*;2 (1):39-41.

10. Priscilla Alexander, Ismaila Yada Sudi and Martin Tizhe (2019). Phytochemical and Antimicrobial Studies of the Crude Extracts of the Leaves of Carica papaya Linn (Pawpaw) and Psidium guajava Linn (Guava) Microbiology Research Journal International 28(1): 1-7.

11. Suresh, K., Deepa, P., Harisaranraji, R. and Vaira A. (2008). Antimicrobial and Phytochemical Investigation of the Leaves of *Carica papaya* L., *Cynodon dactylon* (L) Pers., *Euphorbia hirta* L., *Melia azedarach* L. and *Psidium guajava* L. *Ethnobotanical Leaflets* 12**:** 184-191.

12. Okafor, J. *C.* (2009). Strategies for Conservation of the Genetic Resources of Medicinal Plants of Nigeria. In: Checklist of Medicinal Plants of Nigeria and their uses. Trinity-Biz Publishers, Abakpa-Enugu pp 9 – 12.

13. Adeyeye, E. I., and Omolayo, F. O. (2011). Chemical composition and functional properties of leaf protein concentrate of *Amaranthus hybridal* and *Telfairia occidentalis*. *Agricultural and Biological Journal of N. Am*. 2011;2 (3):499-513.

14. Alexander and Susuty (2015). Nutritional Potential and some elemental components of some Tropical leafy Vegetables consumed in Mubi Region of Adamawa State, Nigeria. International Journal of Technical Research and applications 3(5); 190-193

15. Ayoola, GA (2010). Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research.* **7** (3): 1019–1024.

16. Erukainure, O. L., Oke, O. V., Ajiboye, A. J., and Okafor, O. (2011). Nutritional Qualities and Phytochemical Constituents of *Clerodendrum voluble*, a tropical Non-Conventional Vegetable. *International Food Research Journal 18 (4): 1393-1399.*

17. Fasuyi, A. O. and Nonyerem, A. D. (2009). Biochemical, nutritional and haematological implications of *Telfairia occidentalis* leaf meal as protein supplement in broiler starter diets. *African Journal of Biotechnology 6 (8): 1055-1067.*

18. Ejoh, R. A., Djuikwo, V. N., Gouado, I. and Mbofung, *C.* M. (2007). Nutritional Components of Some Non-Conventional Leafy Vegetables Consumed in Cameroon. *Pakistan Journal of Nutrition* 6 (6): 712- 717.

19. Imaobong, I. U., Roland, U. E. and Efiok, J. U. (2013). Effect of Thermal Processing on Antinutrients in Common Edible Green Leafy Vegetables Grown in Ikot Abasi, Nigeria. *Pakistan Journal of Nutrition,* 12: 162-167.

20. Murthy, E. N., Pattanaik, *C.*, Reddy, *C.* and Sudhakar, V. S. (2010). Piscicidal Plants used by Gond Tribe of Kawal Wildlife Sanctuary, Andhra Pradesh, *India*, pp. 97–101.

21. Association of Analytical Chemists (AOAC) (2010). International Guidelines for Laboratories Performing Microbiological and Chemical Analyses of Food and Pharmaceutical, 24^{th} Edition. AOAC international Galtters burg MD, USA.

22. Edeoga HO, Okwu DE, Mbaebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology. 4(7):685-688.

23. Sofowora A. (1996). Research on medicinal plants and traditional medicine in Africa.Journal of Alternative and Complementary Medicine. 2(3):365–372.

24. Okonwu, K. and Enyinnaya, A. P. (2016). Comparative Phytochemical Studies and Proximate Analysis of Five Commonly Consumed Vegetables of Southern Nigeria. *Asian Journal of Biology 1 (2): 1-7.*

25. Imaga, N.A., Gbenle, G.O., Okochi, V.I., & Adenekan, S. (2010). Phytochemical and antioxidant nutrient constituents of Carica papaya and Parquetina nigrescens extracts. *Scientific Research and Essays,* 5(16), 2201 – 2205.

26. Oyeyemi, M.O., Leigh, O.O., Ajala, O.O., Badejo, A.O., & Emikpe, B.O. (2012). The Effects of “Ugu” *Telfairia occidentalis* Leaves on the Testis and Spermatozoa Characteristics in Male Albino Rat. *Folia Veterinaria*, 52 (2): 102 – 105.

27. Odiaka, N. I. and Schippers, R. R. (2014). *Telfairia occidentalis* Hook. f, in G. J. H. Grubben and O. A. Denton (Editors), *Plant Res. Trop. Afr. 2: Vegetables*, (PROTA Foundation, Netherlands/Backhuys Publishers: Leiden, Netherlands/CTA Wageningen, Netherlands, 522-527.

28. Nwangwa, E,K., Mordi, J., Ebeye, O.A. & Ojieh, A.E. (2012). Testicular regenerative effects induced by the extracts of *Telfairia occidentalis* in rats. *Caderno de Pesquisa, série Biologia*, 19: 27-3.

29. Chakraborty, A.K. and Roy, H.K. (2010). Evaluation of anti-arthritic activity of ethanolic extract of *Cleome rutidosperm*a. *Journal of Pharmaceutical Science and Technology*. 2 (10), 330 – 332. ISSN 0975±5772.

30. Jigna, P. and Sumitra, V. *C.* (2009). Photochemical Screening. *Turkey Journal of Biology,* 31:53 – 58.

31. WHO and FAO., 2004. Vitamin and Mineral Requirements in Human Nutrition. 2nd Edn., World Health Organization, Geneva, Switzerland, ISBN-13: 9789241546126, Pages: 341.

32. Odabasi, E, Turan, M., Aydin A, & Kutlu, M. (2008). Magnesium, Zinc, Copper, Manganese, and Selenium levels in Postmenopausal Women with Osteoporosis. Can magnesium play a key role in osteoporosis? *Annals of Academic Medicine Singapore*; 37(7):564 – 567.

33. Skulan, J., Depaolo, D. J. and Owens, T. L. (2009). Biological Control of Calcium Isotopic Abundances in the Global Calcium Cycle". *Geochimica et Cosmochimica Acta. 61 (12): 2505–10.*

34. Verla, A. W., Adowei, P., Briggs, A., Horsfall, M., and Spiff, A. I. (2014). Preliminary chemical Profile of *Telfairia occidentalis Hook. F* (Fluted pumpkin) Seed Shell. *Merit Research Journal of Environmental Science and Toxicology*, 2 (4): 064-070.

35. Harder, S., Feil, F., & Knoll, K. (2011). Novel Calcium Half-Sandwich Complexes for the Living and Stereo selective Polymerization of Styrene. *Angew. Chem. Int. Ed. 40: 4261–4264.*

36. Romani, A.P. (2013). Chapter 3. Magnesium in Health and Disease. In Astrid Sigel; Helmut Sigel; Roland K. O. Sigel. Interrelations between Essential Metal Ions and Human Diseases. *Metal Ions in Life Sciences. 13. Springer. pp. 49–79.*

37. Ayuk, J. and Gittoes, N.J. (2014). Contemporary view of the Clinical Relevance of Magnesium Homeostasis. *Annals of Clinical Biochemistry. 51 (2): 179–88.*

38. Alexander Priscilla (2016). Phytochemical screening and mineral composition of the leaves of Ocimum gratissimum (Scent Leaf). International Journal of Applied Sciences and Biotechnology;4(2): 161-165.

39. Lakshmi, S. P. & Bindu R. N. (2013). Proximate composition, mineral elements and anti-nutritional factors in *cleome viscosa* l., *cleome burmanni and cleome* W. & A. (CLEOMACEAE). *International Journal of Pharmacy and Pharmaceutical Sciences*, 7(14):20 – 23.

40. Gemede, H.F., & Ratta, N. (2014). Anti-nutritional factors in plant foods: potential health benefits and adverse effects. *Global Advanced Research Journal of Food Science and Technology*, 3(4):103-117.

41. National Agency for Food, drug, Administration and Control (NAFDAC) (2012). Guidelines for Mineral determination of Selected Fruits Samples, 2012 p.23−25