European Journal of Nutrition & Food Safety

13(1): 29-39, 2021; Article no.EJNFS.64097
ISSN: 2347-5641

Evaluation and Comparison of the Antioxidant Activities and Nutritional Composition of *Cucurbita maxima* and *Vigna unguiculata* Leaf Extracts

K. K. Sha'a[1*]

[1]Department of Science Laboratory Technology, School of Science and Technology, Adamawa State Polytechnic, P.M.B. 2146, Yola, Nigeria.

Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/EJNFS/2021/v13i130343
Editor(s):
(1) Dr. Hudson Nyambaka, Kenyatta University, Kenya.
Reviewers:
(1) Goabaone Gaobotse, Botswana International University of Science and Technology, Botswana.
(2) Manoharan K. Pillai, National University of Lesotho, Lesotho.
(3) Virginia Coimbra Zuvanov, University of Minho, Portugal.
Complete Peer review History: http://www.sdiarticle4.com/review-history/64097

Original Research Article

Received 20 November 2020
Accepted 25 January 2021
Published 17 February 2021

ABSTRACT

Antioxidant activities and nutritional composition are essential ingredients normally considered in the choice of vegetables for human consumption. Leafy vegetables in particular, are regarded as protective foods in human diet due to their many health benefits. The aim of this research was to carry out the quantitative phytochemical screening, the antioxidant activities of extracts and determine the nutritional content of Cucurbita maxima and Vigna unguiculata leaves. Quantitative phytochemical screening were conducted using standard techniques. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays were used to determine antioxidant activities of these extracts. Nutritional composition was determined using standard procedures. Quantitative analysis revealed the phytochemicals in C. maxima and V. unguiculata as; saponins (1.03%, and 1.34%), tannins (3.49% and 2.60%), terpenoids (0.0% and 0.47%), flavonoids (2.81% and 4.11%), alkaloids (5.72% and 3.5%), phenols (4.02% and 3.83%) respectively. There was a significant (p=0.05) difference in radical scavenging activity of ethanol leaf extract of V. unguiculata comprared to C. maxima. In FRAP both plants' extract revealed a good antioxidant reducing power at 100mg/ml (range 0.40 to 0.5 absorbance) at 700nM. Antioxidant activities of extracts is attributed to their flavonoid and phenolic contents. Proximate analysis revealed the nutrients for C. maxima and V. unguiculata as; crude protein (11.58% and

**Corresponding author: Email: kkshaakiri50@gmail.com;*

14.83%), crude fat (0.47% and 0.61%), ash (4.11% and 3.72%), crude fiber (6.95 and 4.68%), moisture (1.03% and 1.38%), carbohydrate (75.86% and 74.78%) respectively. This shows that the leaves are a good source of energy to both humans and animals. Also, both vegetables revealed good percentages of proteins which can be used to compliment other sources of protein.

Keywords: Cucurbita maxima; Vigna unguiculata; extracts from leaves; DPPH radical scavenging assay; FRAP assay; phytochemical analysis.

1. INTRODUCTION

Phytochemicals also referred to as phytobiotics, are natural occurring bioactive compounds derived from whole plants or any parts of the plants [1]. They are non-nutritive compounds which play an important role in human and animal diseases prevention and control. Over 10,000 phytochemicals have been discovered and their chemical structures have been elucidated. The major groups of phytochemicals are identified as polyphenols, phenols, flavonoids, terpenoids, saponins, steroids, alkaloids, glycosides, tannins etc [2, 3]. In the prevention and control of diseases, phytochemicals act as antioxidants, anti-aging, antibacterial, anti-inflammatory, antiviral, antifungal and cholesterol reducing agents [4].

Often times, phytochemicals are identified in plant parts but quantities of such phytochemicals are not determined. There is a need for both qualitative and quantitate determination of these plant chemicals. This would provide a base-line information for formulation of diet for control and prevention of diet related diseases [5].

Cucurbita maxima (Pumpkin) is said to have originated from temperate regions of South America, but it has been now distributed all over the tropical and sub-tropical countries. The plant is a drought resistant, sensitive to frost and waterlogging [6]. It grows in home gardens, refuse heaps and termite mounds. *C. maxima* is an angiosperm belonging to the family Cucurbitaceae [7]. As a creeper, it is found mostly on the roofs of houses [8]. *C. maxima* represents one of the economically important species now cultivated and distributed all over the world, especially in India, China, Burma, Thailand and Vietnam [9]. The fruits (pulp), seeds and leafy shoots are eaten as vegetables [10]. In traditional medicine, it is known to exhibit many health benefits which include prevention of growth and reduction of size of prostrate, reduction of bladder and urethral pressure and alleviates diabetes [8]. *C. maxima* has also exhibited anthelmintic, antihypertensive, anticancer, antibacterial and anti-inflammatory properties [11].

Vigna unguiculata (cowpea) is an annual leguminous plant belonging to the Fabceae/Leguminoceae family. *C. unguiculata* originated from Sub-Saharan Africa [12]. It is cultivated and/or grown in semi-arid regions in Africa, Asia, Europe, Central and South Americas, and southern parts of United States of America [13]. *V. unguiculata* is a source of food for millions of people all over the world, particularly in Sub-Saharan Africa [14], due to its proteinous seeds.

V. unguiculata is a drought resistant and salinity tolerant legume. It's an excellent intercrop in tuber and cereal based faming system where it is reported to improve yield of component crops by 30% [15]. As a leuminous plant, the legume plays an important role in erosion control and in restoring soil fertility through/by symbiosis with nodular *Bradyrhizobia spp* [16]. As a leafy vegetable, it has been known to a good source of protein, containing as much as 34.9% in its edible leaves [15]. Again, because of its high protein content, edible leaves (pulse) is highly needed by animal rearers, for example herders for livestock consumption, and also recommended as supplementary protein feed for animals in low diets [17].

As a leafy vegetable, *V. unguiculata* is rated as superior to others because minerals such as calcium and iron in its leaves are not bound to phytic acid, and therefore are more bioavailable than those in seeds [18]. In addition, leaves are produced earlier and made available than seeds; protein output from leaves turn out to be much more than that of seeds [19, 20].

V. unguiculata leaves and husk have some appreciable amounts of phenolic compounds such as polyphenols, phenolic acids, flavonoids and tannins [21]. The biological activities of these phytochemicals in the legume include, antioxidant, anti-inflammatory, anticancer and antiatherogenic activities [22, 23].

In Nigeria, *V. unguiculata* seeds is a major food grain and is consumed by majority of the people. However, the leaves are consumed by only a negligible number of people across the nation, unlike in most part of sub-Saharan Africa where the leaves rank third or fourth in terms of quantity consumed [24]. The aim of this research is to determine the quantity of phytochemicals, antioxidant activities and nutritional content of *C. maxima* and *V. unguiculata* leaves in Yola, Adamawa State, Nigeria.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Samples

The leaves of *C. maxima* and *V. unguiculata* were collected in October, 2018 from a farm in Nyibango, Yola, Adamawa State, Nigeria. The plants were identified and authenticated by Associate Prof. D. F. Jatau of the Forest Resources Department, Modibbo Adama University of Technology, Yola, Nigeria. Voucher specimens were kept separately in the place. The leaves were air dried at room temperature. The dried samples were separately ground to powder using a mortar and pestle. The powdered samples was stored in air-tight containers until the time for extraction and phytochemical screening.

2.2 Preparation of Leaf Extracts and Preliminary Phytochemical Screening

The powdered leaves of *C. maxima* and *V. unguiculata* were extracted separately by cold maceration successively with petroleum ether, chloroform and ethanol for 48 hours each. Preliminary phytochemical screening was carried out using the method described by Harborne [25]. The leaf extracts were subjected to phytochemical analysis such as saponins, tannins, terpenoids, flavonoids, alkaloids, glycosides, steroids and phenols.

2.3 Quantitative Determination of Phytochemicals

The coarse powder of *C. maxima* and *V. unguiculata* were used separately for quantitative analysis.

Preparation of fat sample: 2 g of the sample were defatted with 100 mL of diethyl ether using a soxhlet apparatus for 2 hours [26].

The quantity of phenols was determined by spectrometric method as described by Edeoga et al. [26]. The fat free sample was boiled with 50 mL of ether for the extraction of the phenolic component for 15 min. 5 mL of the extract was pipetted into a 50 ml flask, then 10 mL of distilled water was added. 2 mL of ammonium hydroxide solution and 5 mL of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. This was measured at 505 nM.

2.3.1 Alkaloid determination using Harborne [27] method

5 g of the sample was weighed into a 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

2.3.2 Tannin determination by Van-Burden and Robinson method [28]

500 mg of the sample was weighed into a 50 mL plastic bottle. 50 mL of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 mL volumetric flask and made up to the mark. Then 5 mL of the filtered was pipetted out into a test tube and mixed with 2 mL of 0.1 M FeCl3 in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nM within 10 min.

2.3.3 Saponin determination

The method used was that of Obdoni and Ochuko [29]. 20 g of each were put into a conical flask and 100 mL of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 mL 20% ethanol. The combined extracts were reduced to 40 mL over water bath at about 90°C. The concentrate was transferred into a 250 mL separator funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 mL of n-butanol was added. The

combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

2.3.4 Flavonoid quantitative determination

Quantification of flavonoids was based on a procedure described by Amorim et al. [30], consisting of a spectrophotometric test at 420nm. Samples of 500mg of each plant extract were transferred to a 50mL Erlenmeyer flask. 25mL of methanol pure was added, and the mixture was then heated on a hot plate at 80◦C±5◦C for 30 minutes. The extract was finally filtered using filter paper and transferred to a 50mL volumetric flask. The precipitate was washed with 25mL of methanol and filtered again into the same flask, completing the volume of methanol. From this solution, 0.5mL were transferred to a 25mL volumetric flask. Into these flasks were added 0.6mL of glacial acetic acid, 10mL of a pyridine-methanol solution (2:8) and 2.5mL of a 5% methanolic solution of aluminum chloride. Distilled water was then added to fill the flask. After 30 minutes at rest at room temperature, absorbance readings were taken by spectrophotometry at 420 nM.

2.3.5 Terpenoids

100 mg of plant powder were taken and soaked in ethanol for 24 hour. The extract was filtered and the filtrate was extracted with petroleum ether using separating funnel. The ether extract was treated as total terpenoids.

2.4 *In vitro* Antioxidant Activity of Extracts

2.4.1 DPPH radical scavenging

The free radical scavenging activity of the extracts was measured *in vitro* by 2, 2diphenyl -1 – picryl- hydrazyl (DPPH) assay. About 0.3mM solution of DPPH in 100% ethanol was prepared and 10mL of this solution was added to 3ml of the fraction dissolved in methanol at different concentrations (20 – 100mg/mL). The mixture was then shaken and allowed to stand at room temperature for 30min and the absorbance was measured at 517nM using a spectrophotometer. The percentage scavenging activity at different concentrations was determined or calculated using the formula:

DPPH radical scavenging activity (%) = 100 x Absorbance of Test sample)/ Absorbance of Standard [31].

2.4.2 Ferric reducing antioxidant power (FRAP)

In ferric reducing antioxidant power assay, 1mL of test sample of ethanolic extract in different concentrations (20- 100mg/mL) were mixed with 1mL of 0.2M sodium sulphate buffer (pH 6.6) and1ml of 1% potassium ferricyanide in separate test tubes. The reaction mixtures were incubated in temperature controlled water bath at 50^0C for 20 minutes followed by addition of 1mL of hydrochloric acid. The mixture was then centrifuged for 10 minutes at room temperature. The supernatant obtained (1mL) was added with 1mL of deionized water and 200µl of 0.1% Fe^3Cl.The blank was prepared in the same manner as the sample except that 1% ferricyanide was replaced by distilled water. The absorbance of the reaction mixture was measured at 700nM. The reducing power was expressed as an increase in the A_{700} after blank subtraction [32].

2.5 Proximate Analysis of Experimental Plants

Proximate analysis refers to the determination of the major constituents of leave extract and it partitions nutrients into six components: moisture, ash, crude protein, ether or crude fat, crude fiber and carbohydrate. The moisture content was determined by the loss in weight that resulted from drying a known weight of sample to constant weight at 100^oC. The ash content was determined by ignition of a known weight of the food sample at 550^oC until all carbon was removed. The residue is ash and is taken to represent the inorganic contents of the food sample [33].

The protein content was calculated from the nitrogen content of the food sample, determined by a modification of technique originally devised by Kjeldahl. The crude fat was determined by subjecting the food sample to a continuous extraction with petroleum ether for a defined period. The residue after evaporation of the solvent is the crude fat. When the sum of the amounts of moisture, ash, crude protein, crude fat and crude fiber expressed in percentages is subtracted from 100, the difference is the amount of carbohydrate, referred to as nitrogen-free extractives (NFE).

2.6 Data Analysis

The data was analyzed using ANOVA and results expressed as mean and standard deviation. Where applicable, P. values less than 0.05 were considered as statistically significant.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis of Leaves of *Cucurbita maxima* and *Vigna unguiculata*

The quantitative estimation of phytochemicals in the two experimental plants is presented in Table 1. Alkaloids are the most abundant in *C. maxima* (5.72%), and flavonoids most abundant in *V. unguiculata* (4.11%). Both plants extracts revealed phenols in almost equal quantities (*C. maxima* 4.02%, *V. unguiculata* 3.83%). Phytochemicals are naturally occurring compounds in plant foods such as fruits, vegetables, whole grains, beans, nuts and seeds. In laboratory studies, many phytochemicals act as antioxidants, neutralizing free radicals and removing their power to create damage [1]. The result of this research revealed the presence of very important phytochemicals such as phenols, flavonoids, alkaloids, known to be used in preventing and treating numerous disease conditions, and contributing to the welfare of individuals [34]. This validates the use of these plants' leaves in the cure and prevention of diseases.

Both plant leaves contain phenols (*C. maxima* 4.02%; *V. unguiculata* 3.83%). Phenolic compounds are said to exert beneficial effects mainly by reducing risk factors that lead to cardiovascular disease and age related disease that involves anti- inflammation and anti-aging activities. [35]. Also, the consumption of vegetable containing phenolic compounds may limit the incidence of coronary heart diseases, such as atherosclerosis by acting as potent inhibitors of low density lipoproteins oxidation, considered to be a key mechanism in development of atherosclerosis [36].

Alkaloids are most abundant in ethanolic extracts of experimental plants (*C. maxima* 5.72%, *V. unguiculata* 3.55%). Plant derived alkaloids such as ones revealed in these plants possess potential therapeutic effects against many neurodegenerative diseases such as Parkinson's disease, epilepsy, schizophrenia, Alzheimer's disease and stroke [37]. Alkaloids also prevent or cure intestinal bowel disease such as intestinal inflammation, through therapeutic mechanisms which include, modulation of gut microbiota, restoration of epithelial barrier function, and regulation of colonic oxidative and inflammatory status [38].

Flavonoids have also been found in considerable amounts (*C. maxima* 2.81%; *V. unguiculata* 4.11%). Fruits and vegetables are known to be the main dietary sources of flavonoids for humans, along with tea and wine. A number of flavonoids have shown to have free-radical scavenging activity, coronary heart disease prevention, and anti-cancer activity, while other flavonoids exhibit potential for anti–human immunodeficiency virus function [39].

Tannins, though of a little concentration (*C. maxima* 1.03%; *V. unguiculata* 1.34%), are known to exhibit health and related benefits in humans and animals. They reduce oxidative stress and free radical damage to human cells [40]. In plants, tannins inhibit the activities of foodborne bacteria. By this action, tannins in fruits serve as natural defense against microbial action. Saponins and terpenoids also in relatively low quantities are said exhibit health benefits in humans.

3.2 Antioxidant Activities of *Cucurbita maxima* and *Vigna unguiculata* Ethanolic Leaf Extracts

Antioxidant activities of *C. maxima* and *V. unguiculata* ethanolic leaf extracts were determined using DPPH assay in different concentrations (20 to 100 mg/mL) by an *in vitro* model. Changes in the free radical scavenging ability of the extracts of the leaves of *C. maxima* and *V. unguiculata* on the basis of percentage inhibition is presented in Table 2. The percentage inhibition of DPPH of *V. unguiculata* extract at 100mg/ml was 82.70±0.56%, whereas that of C. maxima at 100mg/ml was 73.01±1.63%. There was a significant ($p=0.05$) difference in the radical scavenging activity of ethanolic leaf extract of *V. unguiculata* compared to *C. maxima* at 100mg/mL. Both plants extracts showed a good antioxidant activity expressed in a dose dependent pattern. The antioxidant property of *V. unguiculata* in this work tallies with Adjei-Fremal [41] in which they also found a correlation between phenolic content with antioxidant power. The antioxidant property of *C. maxima* extract at 100mg/mL agrees with [42] in which they reported a good antioxidant activity.

Similarly, the antioxidant activity revealed in this work is in agreement with Saha et al. [11]. Their work showed a good dose dependent free radical scavenging activity and a potent antioxidant free radical scavenging activity of aerial parts.

The Ferric reducing antioxidant power (FRAP) of leaf extracts is presented as Fig. 1. The extracts exhibited a linear increase in reducing power over the concentration range 20 – 100 mg/mL. It could also be observed that *C. maxima* ethanolic extract showed a slightly higher ferric reducing power than *V. unguiculata* at 100mg/mL.

In FRAP model, ethanol extract of *C. maxima* showed a good antioxidant reducing power of 0.43 (absorbance at 700 nm) at the concentration of 100 mg/ml relative to control having total reducing power of 0.47. (Absorbance at 700 nM). *V. unguiculata* also exhibited a good reducing power of 0.42 (absorbance at 700 nm) compared to control (Fig.1). The ferric antioxidant reducing of *C. maxima* is similar to that of *C. rutidosperma* reported in [43]. The reducing power serves an indicator of a compound's potential antioxidant activity [44].

3.3 Nutritional Composition of Leaf extracts *C. maxima* and *V. unguiculata*

Proximate analysis for determination of nutritional composition of the plants is presented as Table 3. Carbohydrates are most abundant substances in both plants (*C. maxima* 75.86%; *V. unguiculata* 74.78%). Both plants also showed proteins (*C. maxima* 11.58%; *V. unguiculata* 14.83%) as the second most abundant.

The proximate analysis of leaf extracts showed high percentages of carbohydrates in both plants (*C. maxima* 75.86%; *V. unguiculata* 74.78%). The carbohydrate composition of *C. maxima* recorded in this closely agrees with [8, 10]. This result indicated that the leaves are a good source of energy to both humans and animals. Moisture content is used as a measure of susceptibility to microbial action or contamination [45]. The relatively low concentration of moisture (*C. maxima* 1.035; *V. unguiculata* 1.38%) indicates that their dried leaves may not easily be susceptible to microbial spoilage when preserved. The low moisture content will drastically slow down the development of microorganisms and also hinder the hydrolysis of component material [46].

Proteins are a major food substance needed by the body. They are very vital to the body, next to water in abundance and are used as building blocks and in maintenance of body tissue, including development and repair [47]. This work revealed appreciable amounts of protein in both plant leaves (*C. maxima*, 11.58%; *V. unguiculata*, 14.83%). The appreciable amounts of proteins in this plants leaves shows that they can be used in the human diet to supplement or meet protein needs, and also reduce poverty and malnutrition among small holder farmers who cannot afford protein rich foods such as meat and fish [19]. The high amount of protein, especially in leaves of *V. unguiculata* justifies its use by most cattle rearers for their cattle.

The amount of ash is an indication of mineral elements they contain. This research showed averagely good amounts of ash in both plant leaves (*C. maxima*, 4.11%; *V. unguiculata*, 3.72%). The amounts of ash in both plants however, are relatively lower than the one of *Corchorus oliterius*- 11.18% [48]. Mineral elements play a vital role in nutritional development of humans and animals [49].

Table 1. Quantitative phytochemical analysis of leaf extracts

Phytochemicals	*Cucurbita maxima* (%)	*Vigna unguiculata* (%)
Saponins	1.03	1.34
Tannins	3.49	2.60
Terpenoids	-	0.47
Flavonoids	2.81	4.11
Alkaloids	5.72	3.55
Glycosides	-	-
Steroids	-	-
Phenols	4.02	3.83

Table 2. DPPH free radical scavenging of ethanolic leaf extracts

Plant Extract concentration (mg/ml)	*C. maxima* (Mean±SD) %	*V. unguiculata* (Mean±SD) %
20	37.10±1.02	43.37±0.55
40	47.45±0.61	52.53±0.36
60	61.30±1.46	62.44±0.25
80	65.23±0.28	67.13±1.67
100	73.01±1.63	82.70±0.56

Means ± SD of triplicate determinations

Table 3. Nutritional composition of leaf extracts *C. maxima* and *V. unguiculata*

Nutrients	*Cucurbita maxima* (%)	*Vigna unguiculata* (%)
Moisture	1.03	1.38
Ash	4.11	3.72
Crude Fat	0.47	0.61
Crude Fibre	6.95	4.68
Crude Protein	11.58	14.83
Carbohydrate	75.86	74.78

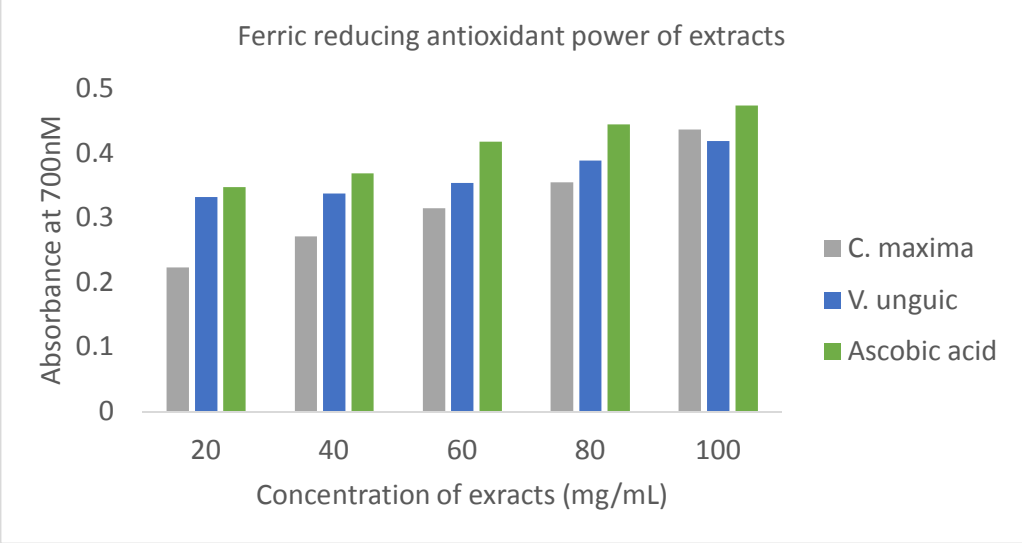

Fig. 1. The erric reducing antioxidant power (FRAP) of leaf extracts

Fats as part of plant products yield energy and also contain fat soluble vitamins and essential fatty acids. The relative low amounts of crude fats (*C. maxima* 0.47%; *V. unguiculata* 0.61%) found in this work shows that the leaves of both plants can be recommended as weight reducing diet and can be consumed in large quantities without the fear of cardiovascular disease or obesity [50]. Fiber is mainly found in fruits, leaves whole grains and legumes. It is best known to prevent or relieve constipation, helps to maintain healthy weight and also lowers the risk of diabetes, heart disease and colon cancer [51]. In this work, the two plant leaf extracts revealed a relatively good amount of fiber (*C. maxima*, 6.95%; *V. unguiculata*, 4.68%). This shows that the consumption of these plant leaves may aid digestion and absorption of water from the body, soften stool and in effect prevents constipation [52].

4. CONCLUSION

The quantitative phytochemical screening of extracts revealed the presence of saponins, tannins, flavonoids alkaloids and phenols in various quantities. This seemed to support their use in ethnomedicine; both as preventive and

curative remedies. The ethanolic extract of *C. maxima* and *V. unguiculata* exhibited a good antioxidant activity in both DPPH and FRAP assays. In both cases, the antioxidant activities of plants extracts is attributable to their phytochemical contents, especially their flavonoid and phenolic contents. The proximate analysis of leafy vegetables in question showed various concentrations of phytonutrients which is a reason for their being used widely. Leaf extracts showed high percentages of carbohydrates in both plants (*C. maxima* and *V. unguiculata)*. This indicates that the leaves are a good source of energy to both humans and animals. Also, both vegetables revealed a good percentage of proteins which can be used to compliment other sources of proteins especially in rural communities where animal proteins are sometimes lacking. The consumption of these vegetables should be encouraged, especially *V. unguiculata* since aerial parts are widely used as livestock feeds.

ACKNOWLEDGEMENT

I thank Mal. Ibrahim Ahmed Hayatu of Biochemistry Department, Modibbo Adama University of Technology, Yola, Nigeria for Technical assistance.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Collins K. What is the difference between an Antioxidant and a phytochemical? American Institute for Cancer Research, 2015
2. Zhang YJ, Gan RY, Li S, Zhou Y, Li AN, Zu DP, et al. Antioxidant phytochemicals for the prevention and treatment of chronic diseases: Review. Molecules. 2015; 20: 21138-21156. 23
3. Forni C, Facchiano F, Bartoli M, Pieretti S, Facchiano A, D'Arcangelo D, et al. Beneficial role of phytochemicals on oxidative stress and age-related diseases. BioMed Research International. 2019;2-16.
4. Asaduzzaman Md, Asao T. Phytochemicals and disease prevention. Introductory chapter: In Phytochemicals-Sources of Antioxidants and Role in Disease Prevention; 2018. Available:http://dx.doi.org/10.5772/intechopen.81877
5. Nwankwo RN, Buba D, Nwankwo JI. Nutrient and phytochemical determination of two under-utilized green leafy vegetables cassia tora and sesamum indicum with medicinal potentials in Mubi, Adamawa State of Nigeria. Greener Journal of Epidemiology and Public Health. 2015; 3(2):14-21.
6. Chigumira Ngwerume F, Grubben GJH. Cucurbita maxima Duchesne. [Internet] Record from PROTA4U. Grubben GJH & Denton OA (Editors). PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands; 2004.
7. Acquaah G. Horticulture: principles and practices. 3rd Edition.Upper Saddle River: New Jersey. Pearson Education Inc. 2004.
8. Duvey SD. Overview on Cucurbita maxima. Review Article. International Journal of Phytopharmacy. 2012;2(3):68-71.
9. Yenda B, Rao BV, Rao BG. *In Vitro* antioxidant activity studies on leaves of *Cucurbita maxima*. International Journal of Advanced Research in Science and Technology. 2015; 4(1): 241-24
10. Ojiako OA, Igwe CU. Nutritional and antinutritional compositions of cleome rutidosperma. Lagenaria Siceraria and Cucurbita Maxima Seeds from Nigeria. Journal of Medicinal Food. 2007;10(4): 735-738.
11. Saha P, Mazumder UK, Haldar PK. In vitro Antioxidant Activity of Cucurbita maxima aerial parts. Science Direct. 2011;1(1):42-48
12. Adjei-Fremah S, Jackai LEN, Worku M. Analysis of Phenolic Content and Antioxidant Properties of Selected Cowpea Varieties Tested in Bovine Peripheral Blood. American Journal of Animal and Veterinary Sciences. 2015;10(4): 235- 245.
13. Timko MP, Singh, BB. Cowpea, a Multifunctional Legume. Plant Genetics and Genomics: Crops and Models. 2008; 1: 227.
14. Singh BB, Ajeigbe HA, Tarawali SA, Fernandez-Rivera S, Abubakar M. Improving the production and utilization of cowpea as food and fodder. Field Crops Research. 2003; 84:169-177. DOI: 10.1016/S0378-4290(03)00148-5
15. Enyiukwu DN, Amadioha AC, and Ononuju CC. Nutritional Significance of Cowpea

Leaves for Human Consumption. Greener Trends in Food Science and Nutrition. 2018;1(1): 01-10.
16. Awurum AN, Enyiukwu DN (2013): Evaluation of the seed-dressing potentials of phytochemicals from Carica papaya and Piper guineense on the germination of cowpea (Vigna unguiculata L. Walp) seeds and incidence of the seed-borne fungi. Continental Journal of Agricultural Science. 2013;7(1):29-35.
17. Gwanzura T, Ngambi JW and D. Norris D. 2012; Nutrient composition and tannin contents of forage sorghum, cowpea, lablab and mucuna hays grown in Limpopo Province of South Africa. Asian Journal of Animal. Science. 2012;6:256-262.
DOI: 10.3923/ajas.2012
18. Bittenbender HC, Barrett RP, Indire-Lavusa BM. Beans and Cowpeas as leaf vegetables and grain legumes. Occasional Monograph Series 1, Bean/Cowpea C.R.S.P., Michigan State University, East Lansing, MI, USA; 1984.
19. Okonya JS, Maass BL. Protein and iron composition of cowpea leaves: an evaluation of six cowpea varieties grown in eastern Africa. African Journal of Food, Agriculture and Nutrition. 2014;14(5): 2129-2140.
20. Mamiro PS, Mbwaga AM, Mamiro DP, Mwanri AM, Kinabo JL Nutritional quality and utilization of local and improved cowpea varieties in some regions in Tanzania. African Journal of Food, Agriculture and Nutri. Devpt. 2011;11(1): 4490-4506.
21. Rochfort, S, Panozzo J. Phytochemicals for health, the role of pulses. Journal of Agriculture and Food Chemistry. 2007; 55: 7981-7994.
DOI: 10.1021/jf071704w
22. Ojwang LO, Banerjee N, Noratto GD, Angel-Morales G, Hachibamba T, Awika JM. et al. Polyphenolic extracts from cowpea (Vigna unguiculata) protect colonic myofibroblasts (CCD18Co cells) from lipopolysaccharide (LPS) induced inflammation-modulation of microRNA 126. Food Funct. 2015 6: 145-153.
DOI: 10.1039/C4FO00459K
23. Cui EJ, Song NY, Shrestha S, Chung IS Kim JY et al. Flavonoid glycosides from cowpea seeds (Vigna sinensis K.) inhibit LDL oxidation. Food Sci. Biotechnol. 2012; 21: 619-624.
DOI: 10.1007/s10068-012-0080-7
24. Ekesa BN, Walingo MK and Abukutsa-Onyango MO. Accesibility to and consumption of indigenous vegetables and fruits by rural households in Matungu division, western Kenya. African Journal of Food Agriculture, Nutrition and Development. 2009; 9(8): 1725-1738
25. Harborne JB. Phytochemical methods. Chapman and Hall, London, New York. 1984; 120.
26. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical Constituents of some Nigerian Medicinal plants. African Journal of Biotechnology. 2005; 4(7):685-688.
27. Harborne JB. Phytochemical Methods, London. Chapman and Hall, Ltd, 1973; 49-188.
28. Van-Burden TP, Robinson WC. Formarion of complexes between protein and Tannin acid. J. Agric. Food chem. 1981; 1:77
29. Obdoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria. Global Journal of Pure and Applied Sciences. 2001;8b:203-208
30. Amorim ELC, Nascimento JE, Monteiro JM, Peixoto Sobrinho TJS, Ara´ujo TAS and Albuquerque UP. A simple and accurate procedure for determination of tannin and flavonoid levels and some aplications in ethnobotany and ethnopharmacology, Functional Ecosystem sand Communities. 2008;2(1):88–94.
31. Dar P, Farman M, Dar A, Khan Z, Munir R, Rasheed A. and Waqas U. Evaluation of Antioxidant potential and comparative analysis of Antimicrobial activity of various extracts of Cucurbita pepo L. Leaves. Journal of Agricultural Science and Food Technology. 2017; 3(6):103-109.
32. AOAC. Official Methods of Analysis (18th edition) Association of Official Analytical, Chemists International, Maryland, USA; 2005.
33. Sutharsingh R, Kavimani S, Jayakar B, Uvarani M, Thangathirupathi A. Quantitative phytochemical estimation and antioxidant studies on aerial parts of Naravelia zeylanica DC. International Journal of Pharmaceutical Studies and Research. 2011; 2(2):52-56.
34. Cardozo LF, Pedruzzi LM, Stenvinkel P, et al. Nutritional strategies to modulate inflammation and oxidative stress pathways via activation of the master

antioxidant switch Nrf2. Biochimie. 2013; 95:1525–

35. Wang X. Ouyang Y, Liu J, Zhu M, Zhao G, Bao W, Hu FB. Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: Systematic review and dose-response meta-analysis of prospective cohort studies. British Medical Journal. 2014;349: g4490.
36. Aviram M, Dornfeld L, Rosenblat M, Volkova N, Kaplan M, Coleman R, et al. Pomegranate juice consumption reduces oxidative stress, atherogeni modifications to LDL, and platelet aggregation: Studies in humans and in atherosclerotic apolipoprotein E-deficient mice. American Journal of Clinical Nutrition. 2000;71: 1062–1076.
37. Hussain G, Rasul A, Anwar H, Aziz N, Razzaq A, Wei W et al. Role of Plant Derived Alkaloids and Their Mechanism in Neurodegenerative Disorders. International Journal of Biological Sciences. 2018; 14(3): 341-357.
DOI: 10.7150/ijbs.23247
38. Peng J, Zeng TT, Li X, Liang Y, Wang LJ, Huang YC, et al. Plant-derived alkaloids: the promising disease-modifying agents for inflammatory bowel disease. A Review Article. Frontiers. Pharmacology; 2019. Available:https://doi.org/10.3389/fphar.2019.00351
39. Yao LH, Jiang YM, Shi J, Tomas-Barberan FA, Datta N, Singanusong R, et al. Flavonoids in Food and Their Health Benefits. Plant Foods for Human Nutrition. 2004; 59:113–122, 2004
40. Ruggeri C. Five Benefits of Tannins in Wine & Other Food Sources. Available:https://draxe.com/nutrition/tannins-in-wine/ 2019
41. Adjei-Fremah S, Jackai LEN, Worku M. Analysis of Phenolic Content and Antioxidant Properties of Selected Cowpea Varieties Tested in Bovine Peripheral Blood. American Journal of Animal and Veterinary Sciences. 2015;10 (4): 235.245.
42. Indrianingsih AW, Rosyida VT, Apriyana W, Nur Hayati S, Nisa K, Darsih C et al. Comparisons of antioxidant activities of two varieties of pumpkin (Cucurbita moschata and Cucurbita maxima) extracts. 2nd International Conference on Natural Products and Bioresource Sciences; 2018.
IOP Conf. Series: IOP publishing.
DOI:10.1088/1755-1315/251/1/012021
43. Sha'a KK, Artimas SP. Antioxidant activities, nutritional composition and antinutritional properties of Two Leafy vegetables (Cleome rutidosperma and Cassia tora) consumed in Adamawa State, Nigeria. Asian Food Science Journal. 2020; 14(4): 18-27.
44. Elmastas M, Gulcin I, Isikdak O, Kufrevioglu OI, Ibaoglu K, Aboul-Enein HY. Radical scavenging activity and antioxidant capacity of Bay leaf extracts. Journal of Iran Chemical Society. 2006; 3: 258-266.
45. Uyoh EA, Ita EE, Nwofia GE. Evaluation of the chemical composition of tetrapleura tetrapter schum and thonn) tuap. Accessions from Cross River State, Nigeria. International Journal Medicinal and Aromatic Plants. 2013;3:386-394.
Available:http://www.openaccessscience.com
46. Ngaha NMI, Dahlan I, Massoma LD, Mandengue SH, Yusuf AA. Comparative Proximate Analysis of Leaves and bark of Alchornea cordifolia (Euphorbiaceae). Journal of Agriculture and Environmental Sciences. 2016;591: 84-90.
DOI: 10.15640/jaes.v5n1a21
47. Topness ES. Six Primary Functions of Proteins.
Available:https://healthyeating.sfgate.com/6-primary-functions-proteins-5372.html 2018.
48. Sha'a KK, Clarkson GP, Artimas SP. Phytochemical analysis, proximate composition and antinutritional factors of corchorus oliterius plant. International Journal of Biological and Chemical Sciences. 2019; 13(4): 2147-2157.
DOI:https://dx.doi.org/10.4314/ijbcs.vol13i4.21
49. Rahman MR, Shariff MA, Rahman MO, Uddin MS, ShafiqUllah AKM, Shameen MA. et al. Studies of Essential and Trace elements in some fruits and vegetables of southwestern Bangladesh by PIXE Technique. Pakistan Journal of Nutrition. 2014; 1392): 62-66.
50. Okon WI, Ogri AI, Igile GO, Atangwho IJ. Nutritional quality of raw and processed unripe *Carica papaya* fruit pulp and its contribution to dietary diversity and food security in some peasant communities in Nigeria. International Journal of Biological and Chemical Sciences. 2017;11(3): 1000-1011.

DOI: https://dx.doi/10.4314/ijbcs.v11i3.5

51. Shemishere UB, Taiwo JE, Erhunse N, Omoreghie ES. Comparative study on the proximate analysis and nutritional composition of musanga cercropioides and maesobotyra barteri leaves. Journal of Environmental Science and Management. 2018; 22(2): 287-291

52. Ayoola PB, Adeyeye A. Proximate analysis and nutrient evaluation of some Nigerian pawpaw seeds varieties. Science Focus. 2009; 14(4): 554-558.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/64097