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Manifold Antioxidant Potentials of Riverine Bitter Leaf (*Vernonia Amygdalina*) Accession in Nigeria.

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Abstract
An analysis was done at the Food Profiling Biotechnology Laboratory, National Root Crops Research Institute (NRCRI) Umudike, Umuahia, Nigeria, to evaluate the proximate, minerals, vitamins and phytochemical compositions of Riverine accession of bitter leaf. The results showed that it contained comparatively high levels of Zinc(0.48mg),Vitamins A(0.29mg),Vitamin C(8.54mg) and Vitamin E(0.32mg) which are dietary antioxidants .Its high total phenol (74.63 µgGAE/ml), total flavonoids (81.39 µgRE/mg) and DPPH test(58.75 µg/mg) showed presence of phytochemical antioxidants. The primary characteristic of these antioxidants is their ability to entrap free radicals such as peroxide, hydroperoxide or lipid peroxyl and inhibit the oxidative mechanism that then will lead to degenerative diseases like cancer. Riverine bitter leaf high content of these compounds shows its potential role against free radical mediated diseases. The total phenol is greater than that for the medicinal plants, Ziziphus mauritiana L., Tamarindus indica L. and Capsicum spp. This indicates that the plant might be a better source of antioxidant than the widely recognized medicinal plants especially as it needs no maceration in water before use.

Key words: *Antioxidants, DPPH test (2,2-diphenyl-1-picrylhydrazyl),Riverine, total flavonoids and total phenol.*

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I. Introduction.

Vernonia amygdalina is a perennial shrub from Asteraceae family and also commonly called 'Bitter Leaf' because of the bitter taste of its leaves. The leaves are green in coloration with a characteristic odor and bitter taste (Akpaso et al., 2011). There are many cultivars based on environmental conditions and features like level of bitterness, size and color of leaves. The Riverine bitter leaf accession is slightly bitter, it can be consumed raw or cooked like any other vegetable and are naturally found in Riverine communities in High Rainfall areas of Nigeria. Bitter leaf is cultivated in Nigeria mainly for its nutritional value in human diet because of the presence of vitamins and mineral salts which are useful for the maintenance of health, prevention and treatment of various diseases. Bitter leaf have gained interest as rich sources of pro-healthy compounds, displaying high nutritive and biological value. They are a good source of vitamins, minerals, organic acids, fiber (Luo et al., 2017).**Antioxidants** are substances that can inhibit or slow damage to cells caused by free radicles, unstable molecules that the body produces as a reaction to environmental and other pressures The primary characteristic that an antioxidant has is its ability to entrap free radicals such as peroxide, hydro peroxide or lipid peroxyl and inhibit the oxidative mechanism that then will lead to degenerative diseases, although oxidation reactions are crucial for life (Wu et al., 2011).Plants are the main sources of Natural and Dietary antioxidants. Dietary antioxidants are in the form of Vitamins A, C and E and minerals like Selenium, zinc and copper. Main plants natural antioxidants are secondary metabolites of polyphenols and carotenoids groups. Plant polyphenols are secondary metabolites characterized by one or more hydroxyl groups binding to one or more aromatic rings [Liu *et al.*,2008]. Several thousand polyphenol molecules have been identified in higher plants, including edible ones. Plant polyphenols are divided into two major groups, flavonoids and non-flavonoids. Flavonoids can be divided into flavanols, flavonols, anthocyanidins, flavones, flavanones, and chalcones. Non-flavonoids include stilbene, phenolic acids, saponin, and tannins [Liu *et al.*,2008]Several studies carried out on *Vernonia amygdalina* had suggested that it contains high levels of the different natural and dietary antioxidants bioactive compounds mentioned above(Quasie

et al., 2016;).The aim of this project was to run an analysis to ascertain the vitamins, minerals and phytochemical manifold sources of antioxidants in the riverine bitter leaf accession and to determine the relationships between these compounds through correlation.

II. Materials and Methods

2.1*Sample collection and Extraction.* Fresh leaves of riverine bitter leaf were identified and collected from a farm at Rumuodomaya, Rivers State. The samples were sent to Food Profiling Biotechnology Laboratory, National Root Crops Research Institute (NRCRI) Umudike, Umuahia for analysis based on standard protocols. The leaves were dried at room temperature and afterwards,
grinded, sieved, and kept in desiccators for further analysis.1.0 g powdered sample in triplicate was mixed thoroughly with 10 mL of distilled water and centrifuged at 6000 rpm for 15 min. Supernatants were pooled in clean flasks. The whole process was repeated thrice to extract maximum phytochemicals soluble in water and supernatants were pooled.

2:.2 For proximate analysis, the dry matter, moisture, ash, crude fat, crude protein and crude fibre contents were determined in powdered *Vernonia amygdalina* leaves using the standard methods of the Association of Official Analytical Chemists [AOAC,2005] while carbohydrate content was calculated based on the net difference between the other nutrients and the total percentage composition. The Mineral contents in leaves were determined using an atomic absorption spectrophotometer (Buck Scientific Model 205,) using standard protocols (AOAC, 2006). A 0.5g amount of crushed composite sample was digested in concentrated nitric acid and perchloric acid at 115ºC for about 1h to generate the digest solution. An aliquot of the digest solution was used for determination of calcium, magnesium, sodium, copper, zinc, iron, and phosphorus contents. High-performance liquid chromatography (HPLC) methods for the determination of water- and fat-soluble vitamins in foods using the standard methods of the Association of Official Analytical Chemists [AOAC,2005].
2.3.Phytochemical screening was performed using standard procedures [Ayoola et.,al,2008].All the data collected were analyzed using Genstat 12 edition and means were separated using Least significance difference (L.S.D) at 0.05 level of significance.
*2.4. Polyphenolics Estimation. (a)*Total phenolics content (TPC) was assessed as described by [Chen *et al.*,2015]. In short, 5 mL Folin-Ciocalteu reagent (10-folds) and 4 mL sodium carbonate (7.5%) were added in 1.0 mL of each fruit extract (in triplicate). This blend was kept for 90 min at room temperature before measuring the absorbance at 760 nm.The TPC was presented as mg gallic acid equivalent/100 g fresh weight of sample (mg GAE/100g, FW).
(b)Total flavonoids content (TFC) was estimated as explained formerly [Chen *et al.*,2017]. Concisely, 5 mL of each extract (in triplicate) and 0.3 mL of sodium nitrite (5%) were blended thoroughly for 5 minutes and 0.3 mL of aluminum trichloride (10%) was mixed. This mixture was kept for 6 min at room temperature and 2 mL of sodium hydroxide was added to stop the reaction. After dilution (up to 10 mL) with distilled water absorbance was measured at 510 nm. TFC was
expressed as mg rutin equivalent per 100 g of fresh sample (mg RtE/100 g).
(c)2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging assays were used to determine free radical scavenging activity in the studied samples using the method of Aiyegoro and Okoh [2010).
Statistical Analysis. Data were presented as mean ± SD for all triplicate analysis and means difference was calculated by Tukey's multiple comparison test. The *P*-values less than0.05 were considered statistically significant. The SPSS 21.0
Version was used for the analysis.

III. Results and Discussion

The leaves of Riverine Bitter leaf Accession are very low in Fat(2%) which is advantageous health wise as they could not result in obesity if regularly consumed. Usunobun and Okolie (2016) reported 4% for Upland accession. This accession low Fibre(3%) eliminates the need for maceration and prolonged washing(Table 1) .Its crude protein(29.47%) is higher than upland cultivar(22.8%)as recorded by Usunobun and Okolie(2016) and Telferia leaves(22.97%)as reported by Omimakinde *et.,al*(2018).The carbohydrate content(35.9%)also compared favourably with that of other common leafy vegetables (Otitoju *et.,al 2014).*

Table1: Proximate Analysis on the of Riverine Bitter leaf (*Vernonia amygdalina)* Accession.

Proximate Composition	Values
Moisture Content(%)	**20.0567±0.04509**
Crude Protein(%)	**29.4733±0.02517**
Crude Fibre(%)	**3.0567±0.01528**
Fat (%)	**2.0833±0.01528**

Ash Mg/100g	**9.4467±0.02517**
Carbohydrate, Mg/100g	**35.9467±0.0057**
Energy Value. K/cal	**280.3067±0.03215**

The Riverine bitter leaf contains the following minerals; sodium (180.36mg/100g), magnesium(162.54mg/100g),phosphorus(27.87mg/100g),potassium (949.35mg/100mg), iron(1.13mg/100g) and zinc (0.48mg/100g) as shown on table 2.This makes it a very good source of minerals. *V.amygdalina* is rich in zinc (Georgewill and Georgewill,2010).Zinc is an essential micronutrient for human health since it cannot be produced or stored in excess in the body. This result affirms reports of Ezekiel *et al.,* (2015) that observed minerals content in *Vernonia amygdalina* in the trend; K > Na > Ca > Mg > Fe >Zn > Cu >Mn.

The Riverine bitter leaf is rich in Vitamin A, Vitamin C, Vitamin E, Vitamin B_1, and Vitamin B_2 and hence, is effective in the treatment of a variety of common ailments as postulated by (Adenuga *et. al.,*2010). Plants are the main sources of Natural and Dietary antioxidants. Dietary antioxidants are in the form of Vitamins A, C and E and minerals like Selenium, zinc and copper. Riverine bitter leaf high content of these compounds shows its potential role against free radical mediated diseases. The high content of Ascorbic acid of this accession indicates the leaves might be a source of vitamin C(Table 2). Although there are variations in bitter leaf vitamins composition, this finding is in line with that of (Nwaoguikpe, 2010) that bitter leaf is rich in Antioxidant Vitamins A, C and E.

Table2: Minerals and Vitamins Analysis on the of Riverine Bitter leaf (*Vernonia amygdalina)* Accession.

Minerals Composition	**Values(mg/100g)**	Vitamins	**Values(mg/100g)**
Calcium	70.51±**0.01**	**A**	**0.29±0.02**
Sodium	180.36±**0.02**	**B1**	**0.16±0.01**
Magnesium	162.54±**0.03**	**B2**	**0.19±0.01**
Phosphorus	27.87±**0.02**	**B3**	**0.14±0.01**
Potassium	949.35±**0.03**	**C**	**8.54±0.03**
Iron	1.13±**0.01**	**E**	**0.32±0.01**
Zinc	0.48±**0.01**		

The Riverine bitter leaf contains high concentrations of the tested phytochemicals and this is in accord with Udochukwu *et. al.,* (2015) and Areghore *et. al.,* (1997) reports(Table 3). Also, in a study conducted by Usunobun and Ngozi (2016),they found that saponins, tannins, alkaloids and flavonoids, triterpenoids, steroids and cardiac glycosides were high in *V. amygdalina* which served as a great source of pharmacologically active phytochemicals and effective as supplements in human and animal nutrition. Polyphenols in the form of flavonoids and phenols are bioactive antioxidants that are known to demonstrate two major mechanisms of action (either through direct scavenging of free radicals or interfering some enzyme activities such as with nitric oxide synthase activity or xanthine oxidase activity) (Quasie *et al.,* 2016).

Table 3:Phytochemical Analysis on the of Riverine Bitter leaf (*Vernoniaamygdalina)* Accession.

Proximate Composition	Values
Flavonoids	3.13±0.02
Alkaloids	1.17±0.01
Saponin	0.20±0.01
Tannins	0.41±0.01
Phenol	2.16±0.01
Phytate	103.87±0.15
Oxalate	0.86±0.01
Cyanogenic glycosides	37.10±0.05
Anthraquinone,	0.07±0.00
Steroids.	0.00±0.00

Riverine bitter leaf accession has high total phenol (74.63µgGAE/ml), total flavonoids(81.39µgRE/mg) and DPPH(58.75µg/mg)(Table 4). The primary characteristic of these antioxidants is their ability to entrap free radicals such as peroxide, hydroperoxide or lipid peroxyl and inhibit the oxidative mechanism that then will lead to degenerative disease (Wu et al., 2011). Plants with antioxidant phytochemicals are beneficial for health as they can protect against free radicals and retard progress of some chronic diseases (Khan *et. al.*, 2016).

Table 4:Phytochemical Antioxidants Analysis of the of Riverine Bitter leaf (*Vernonia amygdalina)* Accession.

Total phenol (µgGAE/ml)	Total Flavonoid (µgRE/mg)	DPPA(µg/ml)
74.63±0.03	**81.39±0.02**	**58.75±0.04**

The majority of these types of plants are a rich source of phenolic substances and possess anti-cancerous, antiviral, and antibacterial properties (Manach *et. al.*, 2004). There was more total phenol in the leaf extract of Riverine bitter leaf(74.63µgGAE/ml) than for the medicinal plants, *Ziziphus mauritiana L.* and *Tamarindus indica L.* (Lamien-Meda *et. al.*, 2008) and pepper, *Capsicum spp.* (Okunlola *et. al.*, 2017). This indicates that the plant might be a better source of antioxidant than the widely recognized medicinal plants.

Correlation of Phytochemicals and Minerals compositions of Riverine Bitter leaf (*Vernonia amygdalina)*Accession. The phytochemicals correlated positively among themselves at 0.05 level of significance except for Saponin,Anthroquinons and Steriods(Table 5).Also all the minerals had positive relationships with each other except Zinc and Sodium.

Correlation of Phytochemicals and Vitamins compositions of Riverine Bitter leaf (*Vernonia amygdalina)* Accession.

Correlation analysis between phytochemicals and Vitamins showed that the phytochemicals correlated positively at 0.01 and 0.05 level of significance more among themselves than with the Vitamins. But generally, phytochemicals and vitamins that function together correlated more positively eg. Flavonoids and Phenols with vitamins A,C and E which serve as antioxidants .Also Vitamins B2 and C correlated positively with most of the phytochemicals(Table 6).

IV. Conclusion.

The results showed that Riverine bitter leaf accession contained comparatively high levels of dietary antioxidants (Zinc, Vitamins A, Vitamin C and Vitamin E) and phytochemical antioxidants(total phenol and total flavonoids).Riverine bitter leaf high content of these compounds shows its potential role against free radical mediated diseases. The total phenol is greater than that for the medicinal plants, *Ziziphus mauritiana L.*,*Tamarindus indica L.*and *Capsicum spp.* This indicates that the plant might be a better source of antioxidant than the widely recognized medicinal plants.The phytochemicals correlated positively among themselves at 0.05 level of significance except for Saponin,Anthroquinons and Steriods.Also all the minerals had positive relationships with each other except Zinc and Sodium. Correlation analysis between phytochemicals and Vitamins showed that the phytochemicals correlated positively at 0.01 and 0.05 level of significance more among themselves than with the Vitamins. Crude extracts of fruits and vegetable possess powerful antioxidant and anticancer effects, which are mainly accredited to the additive and synergetic effects of phytochemicals [Liu *et. al.*,2002], such as vitamins, minerals, and polyphenols that provide protection to cellular system against oxidative impairment and consequently reduce the oxidative stress [Liu *et al.*,2002]. Pharmacogenetics have suggested that the active constituents should not be purified because in pure or isolated form they may be less active and not perform in the same way as the compounds in whole foods. [Aliyu *et. al.*,2008].This is a great advantage on Riverine bitter leaf accession since it does not need processing before consumption.

Conflict of Interest

The authors report no conflict of interest.

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Magnesium	162.54±**0.03**	**B2**	**0.19±0.01**
Phosphorus	27.87±**0.02**	**B3**	**0.14±0.01**
Potassium	949.35±**0.03**	**C**	**8.54±0.03**
Iron	1.13±**0.01**	**E**	**0.32±0.01**
Zinc	0.48±**0.01**		

Table 3:PhytoEchemical Analysis on the of Riverine Bitter leaf (*Vernoniaamygdalina)* Accession.

Proximate Composition	Values
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Alkaloids	1.17±0.01
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Tannins	0.41±0.01
Phenol	2.16±0.01

Phytate	103.87±0.15
Oxalate	0.86±0.01
Cyanogenic glycosides	37.10±0.05
Anthraquinone,	0.07±0.00
Steroids.	0.00±0.00

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