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Original Article

Assessment of bio-active compounds in Ikom and Riverine bitter leaf (*Vernonia amygdalina* Delile) cultivars in Southeastern Nigeria

OPEN ACCESS

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A B S T R A C T

A comprehensive analysis was done to investigate the proximate, mineral, vitamin and phytochemical compositions of Ikom and Riverine cultivars of bitter leaf. The study was carried out using analytical procedures outlined by the association of official analytical chemists. The Riverine bitter leaf contained more moisture content (8.8%), crude fibre (8.5%), fat (2.8%) and ash (8.6%), while Ikom cultivar had more crude protein (27%), carbohydrate (51mg/100g) and dry matter content (94mg/100g). The Riverine bitter leaf contained more minerals; sodium (0.42%), phosphorus (27.87mg/100g), potassium (1.31%), iron (61.8mg/100g) and zinc (7.34mg/100g). While both cultivars had equal concentrates of calcium (3.8%) and magnesium (1.5%). The Ikom cultivar had more of Nitrogen (4.47%) and phosphorus (0.56%) This showed that they are good sources of minerals. The presence of more ash in riverine bitter leaf is a confirmation of the presence of more mineral elements. The riverine cultivar was relatively higher in all tested vitamins other than thiamine. The Riverine cultivar contained higher values of Flavonoids (4.29mg/100g), tannins (1.38mg/100g) and oxalates (0.086mg/100g) than the Ikom cultivar which had higher values of Alkaloids (3.61mg/100g), saponins (5.75mg/100g) and phenols (0.48mg/100g). The low alkaloids (mainly quinine) accounts for the slight bitter taste of the riverine cultivar. Generally, the two cultivars were rich in useful dietary nutrients, phytochemicals and antioxidants. It is recommended that more research be done to develop ways of multiplying the Riverine bitter leaf in rain forest and derived Savannah zones.

K E Y W O R D S : *Antioxidants, Cultivars, Dietary, Ikom, Phytochemicals, Proximate*

INTRODUCTION

Vernonia amygdalina is a perennial shrub from Asteraceae family and also commonly called 'Bitter Leaf' because of the bitter taste of its leaves. Bitter leaf is cultivated in Nigeria mainly for its nutritional value in human diet because of the presence of vitamins, phytochemicals and mineral salts which are useful for the maintenance of health, prevention and treatment of various diseases (Adebayo *et al*,.2014).The leafy part of bitter leaf contributes greatly to the nutritional requirement for human health and to food security since it

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contains enough concentrations of proximate composition (Usunomena & Ngozi, 2016) Phytochemicals are natural occurring bioactive compounds known for their health benefits (Luo *et al.*, 2017; Farombi & Owoeye, 2011).They are majorly responsible for the color, flavor and aroma of fruits and notably vegetables. Several studies carried out on *Vernonia amygdalina* had suggested that it contains different bio active compounds including; flavonoids, saponins, alkaloids, tannins, phenolics, terpenes, steroidal glycosides, triterpenoids, and several types of sesquiterpene lactones (Quasie *et al.*, 2016; Luo *et al.* 2017; Okolie, *et al*., 2022). It is the most cultivated and prominent species of the genus Vernonia that is made up of about 1,000 species of shrub (Toyang & Verpoorte, 2013; Egharevba *et al.*, 2014 Jibrin *et al*., 2024). The leaves are green in coloration with a characteristic odor and bitter taste (Nath, *et al*., 2017).

There are many cultivars based on environmental conditions and features like level of bitterness, size and color of leaves. This herb has been domesticated in many parts of West Africa, but grows freely in tropical Africa and also well distributed in Asia (Oseni & Babatunde, 2016). Specifically, it is used to prepare the popular Nigerian bitter leaf soup, "Onugbo" and as spice in the Cameroon dish called "Ndole" (Jibrin *et al*., 2024).

Other popular use of *Vernonia amygdalina* in Africa includes traditional treatment of diseases such as malaria, infertility, diabetes, gastrointestinal problems and sexually transmitted diseases (Luo, *et al.*, 2017). The bitterness is suspected to be due to factors such as the presence of alkaloids, saponins, tannins and glycosides which have been shown by various authors to be present in bitter leaf. These bioactive compounds made them possess different pharmacological properties like antimicrobial, antimalarial, antithrombotic, antioxidant, anti-diabetic, laxative, hypoglycemic, anthelmintic, anti-inflammatory, cathartic, anticancer, antifertility, anti-fungi, antibacterial, and among others (Udochukwu *et al.*, 2015; Alara *et al.*, 2017). The bitter taste had been associated with the presence of saponins, alkaloids, tannins, and glycosides. The type and quantity of phytochemicals in this plant is affected by the environment.The aim of this project is to run a comprehensive analysis to ascertain the phytochemicals and nutritional components of two cultivars of bitter leaf which will be of greatly influence consumers choice and post harvest processing approaches. One of the cultivars is the Riverine variety that can be consumed raw or cooked without processing while the other is the Ikom accession which is common in forest boarders of Cameroon and Cross River State of Nigeria.

MATERIALS AND METHODS

Collection, identification and preparation of plant materials.

Owing to the influence of environmental factors on plants nutritional and phytochemical components, the two cultivars of *Vernonia amygdalina (*bitter leaf) were planted and collected from the Training and Research Farm of Nnamdi Azikiwe University, Awka. The fresh leaves were identified, separated from the stalk, washed and air-dried at room temperature (24^{o}C) and then pulverized, crushed into fine powder and weighed.

Materials used in this experiment

Ikom bitter leaf cultivar

This cultivar is commonly found in the mountain and forest edges of Cameroon and Cross Rivers State of Nigeria. It is closely related to *V. calvoana* and has a very vigorous growth habit It must be washed severally before use. The leaves are wide and petiolate in shape with bitter taste and dark green color. The bark is densely pubescent at the young stage, as the bark gets mature it turns brown in color.

Riverine bitter leaf cultivar

This is common in the Riverine areas of South-South Nigeria. It is slightly bitter and so can be sliced and consumed raw or slightly cooked like any other vegetable and are naturally found in Riverine communities in High Rainfall areas of Nigeria. The leaves are petiolate in shape with slight bitter taste and are light green in color. The bark is densely pubescent at young stage, as the bark gets mature it turns light grey in color.It generally needs no maceration and washing.

Handling of the Samples

Moisture, ash, crude fiber, crude protein (% N x 6.25), and ether extract (EE) were determined for proximate compositions using the standard procedures outlined by the Association of Official Analytical Chemists (AOAC, 2010). By using the difference, the total carbohydrate content was calculated. The calorific values (KJ/100g) were calculated by summing up (% proteins x 2.44) + (% carbohydrates x 3.57) + (% lipids x 8.37) for each sample. Each parameter in the samples was calculated in triplicate, reported as in percentage, and its standard deviation was computed and displayed as indicated in the Tables. All the reagents used were of analytical grade.

Phytochemical analysis

The phytochemical compositions of the leafy vegetable extracts were screened to identify and quantify for the presence of tannins, saponins, alkaloids, flavonoids and phenol, using standard analytical procedures reported by Chukwuma & Ejikeme (2016) with slight modifications.

Data Analysis

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.

RESULTS AND DISCUSSION

Proximate Analysis on the two cultivars of Bitter leaf (*Vernonia amygdalina)*.

The Ikom cultivar had significantly higher crude protein (27%), carbohydrate (51%), and dry matter (94%) compared to the Riverine cultivar, which had higher moisture content (8.8%), ash (8.6%), crude fiber (8.5%), and fat (2.8%) (Table 1).The proximate values were in line with the findings of Okolie *et al*., (2021), .Also the quantitative proximate evaluation of the leaf extract by (Ali *et al.,* 2020) showed that it incorporates carbohydrates (37%), proteins (28.2%), fats (5.5%), crude fiber (11.6%), moisture content (8.4%), and ash content (9.3%) (Ali *et al*., 2020).Since food that provide more than 12% of their caloric value from proteins are very good sources of protein, the two vegetables can be classified as very rich in proteins and can serve as substitutes for protein.The Ikom cultivar is richer in energy related components.The Riverine bitter leaf contained more ash (8.5%) and therefore suggests a high deposit of mineral elements in the leaves (Antia,*et al*,.2006).The Ikom cultivar had a relatively high content of carbohydrate. Thus the high carbohydrate content contributes the highest Kilo joules to the energy value in *Vernonia amygdalina*. The high concentration value of protein, dry matter, crude fiber and ash was also investigated by Olumide *et al*., (2019).

Table 1: **Proximate Analysis on the two cultivars of Bitter leaf (*Vernonia amygdalina)*.**

Cultivars	MC (%)	CP (%)	CF (%)	FAT (%)	ASH Mg/100g	CHO Mg/100g	DM Mg/100g
Ikom	5.07	27.10	6.713	1.960	6.993	51.41	94.613
Riverine	8.8	23.58	8.513	2.833	8.620	47.88	91.25
$LSD_{0.05}$	0.926	2.045	0.3579	0.477	0.0925	1.009	0.0953

MC-Moisture Content, CP- Crude Protein, CF- Crude Fibre, CHO- Carbohydrate,DM-Dry Matter.

Phytochemical analysis on the two cultivars of bitter leaf (Vernonia amygdalina)

Table 2 showed that the Riverine cultivar had low alkaloids(1.33%) while that of the Ikom cultivar was significantly higher(3.6%),it also had lower saponins(3.69%) and phenols(0.093%), while the Ikom cultivar had lower flavonoids (3.396%), tannins (1.1147%) and oxalates (0.06767%).This is In accordance with the Imohiosen *et al*.. (2021) findings that bitter leaf has 139 mg/g of alkaloids, 180 mg/g of flavonoids, 60 mg/g of saponin, 2.3 mg/g of oxalate, and 167 mg/g of phytate. A similar study reported 305 mg/g flavonoids, 104 mg/g phytate, 6 mg/g saponin, 1.7 mg/mL tannin, and 20 mg/ mL alkaloids (Olumide *et al*., 2019).

Table 2: Phytochemical analysis on the two cultivars of bitter leaf (*Vernonia amygdalina)*

Cultivars	Flavonoids (%)	Alkaloids (%)	Saponin (%)	Tannins (%)	Phenols Mg/100g	Oxalate Mg/100g
Ikom	3.296	3.607	5.75	1.1147	0.478	0.067
Riverine	4.293	1.334	3.692	1.3837	0.093	0.086
$LSD_{0.05}$	0.133	0.016	0.169	0.0130	0.0045	0.0029

Minerals composition analysis of the two cultivars of bitter leaf (*V. amygdalina)*

Table3 showed that the Riverine cultivar had a significantly higher K(1.3%),Na(0.4%),Zn(7.3mg/100g) and Fe(61.8mg/100g).While the two cultivars had similar values of Ca3.8%) and Mg(1.5%),the Ikom cultivar had significantly higher values of N(4%) and P(0.56%).The high concentration values of minerals (sodium, potassium,calcium, magnesium, zinc, and iron), and ash in the leaves of the plant presented it as excellent source of food.This was in accordance with the findings of Usunomena & Ngozi (2016); Olusola & Olaifa, (2018).This result also 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.

Table 3: Minerals composition analysis of the two cultivars of bitter leaf (*V. amygdalina)*

Cultivars	CA %	NA %	MG %	P %	K %	FE Mg/100g	ZN Mg/100g
Ikom	3.845	0.309	1.503	0.5603	1.025	36.53	4.978
Riverine	3.75	0.4233	1.563	0.349	1.308	61.80	7.34
$LSD_{0.05}$	0.338	0.042	0.123	0.006	0.0691	8.31	0.1019

CA: Calcium, NA: Sodium, MG: Magnesium, P: Phosphorus, K: Potassium, FE: Iron, ZN: Zinc

Vitamins composition analysis on two cultivars of bitter leaf(*V. Amygdalina)*

The Riverine cultivar significantly had very high ascorbic acid(13.56mg/100g) as compared with that of Ikom cultivar(6.8mg/100g), high riboflavin(0.37mg/100g), naicin(0.175mg/100g),betacarotene (123.51mg/100g) and retinol(0.116mg/100g).Ikom cultivar had a higher thiamine(0.194mg/100g). Table 4.This is in accordance with Dafam *et al.*,(2020),that the leaf contains vitamins like vitamin A, vitamin C (ascorbic acid),vitamin E, vitamin B1, vitamin B2, niacin.This result was consistent with the study by Okolie *et al.*, (2021),which found similar concentrations of these vitamins in a related research.

Table 4: Vitamins composition analysis on two cultivars of bitter leaf(V. Amygdalina)

Cultivars	VIT A Mg/100g	VIT A1 Mg/100g	VIT B1 Mg/100g	VIT B2 Mg/100g	VIT B3 Mg/100g	VIT C Mg/100g	VIT E Mg/100g
Ikom	101.78	0.0292	0.194	0.206	0.132	6.766	0.12
Riverine	123.51	0.1167	0.164	0.367	0.175	13.563	0.32
$LSD_{0.05}$	2.851	0.0246	0.00453	0.1024	0.0435	0.1803	0.03

VIT: Vitamin; VITB1(Thiamine), VIT.B2(Riboflavin),VIT.B3(Naicin),VIT.C(Ascorbic acid),VIT. A1(Retinol),VIT. A(Beta carotene),VIT. E (Tocopherol).

CONCLUSION AND RECOMMENDATION

The Riverine bitter leaf contained more ash, crude fibre, and fat.since it contained more ash, it had more of potassium,sodium,zinc and iron while the two cultivars had the same values of calcium and magnesium.It was also relatively higher in all tested vitamins other than thiamine, but was low in alkaloids which accounts for its slight bitter taste. The Ikom cultivar had more carbohydrates and crude protein. Generally the two cultivars were rich in useful phytochemicals (flavonoides, alkaloids and phenols) which have anti-oxidant properties.It is recommended that more research be done to develop ways of vegetative multiplication of the Riverine bitter leaf cultivar in rain forest and derived Savannah zones. And also, to create more awareness of its usefulness to the public through seminars and public media especially its antioxidants activities.

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Authors contributions.

Authors OAU and HO managed data collection, interpretation of data and writing of manuscript, POO sourced the cultivars and review of manuscripts . PIE did type setting and cultivars logistics. CCO and EAO managed the literature searches and data analysis,development of methodology and data analysis. All authors read and approved the final manuscript.

Ethics Committee Approval

Not Applicable

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