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Micromorphological and Nutritional Attributes of two Varieties of *Vernonia amagdalina* Del. Domesticated in Delta State.

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Abstract: *This study examined the potential of two Vernonia amygdalina varieties (bitter and non-bitter) as sources of nutrients and medicinal compounds. Micromorphological analysis revealed variations in stomata size (bitter: 1.57±0.20 µm; non-bitter: 1.49±0.42 µm), index (bitter: 1.20±0.60%; non-bitter: 2.30±0.21%), and trichome index (bitter: 0.55±0.36%; non-bitter: 0.90±0.19%) between the varieties, while stomata type and anticlinal wall patterns were similar. Both varieties were rich in minerals, with bitter varieties containing higher levels of sodium (7.27±0.43 mg/g), potassium (17.07±0.45 mg/g), phosphorus (3.15±0.41 mg/g), iron (0.65±0.02 mg/g), zinc (0.15±0.03 mg/g), and copper (0.16±0.04 mg/g) compared to non-bitter varieties. Proximate composition analysis showed higher levels of crude fiber (1.76±0.50%), crude protein (21.07±0.30%), and carbohydrate (23.33±0.82%) in non-bitter varieties, while bitter varieties had higher levels of moisture (50.01±0.40%), crude ash (7.95±0.56%), and crude fat (2.15±0.20%). Phytochemical analysis revealed higher levels of tannin (0.13±0.85%), saponin (0.08±0.02%), and flavonoid (0.22±0.09%) in non-bitter varieties, while alkaloid levels (1.26±0.27%) were higher in bitter varieties. These findings suggest that both Vernonia amygdalina varieties are valuable sources of nutrients and phytochemicals, with potential applications in food and medicine.*

Keywords: *Micromorphological, nutritional characteristics, Vernonia amygdalina, Agbor, Ika South, Delta State,*

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1.0 Introduction

Vernonia amygdalina is a perennial shrub that belongs to the family Asteraceae and grows in tropical Africa (Ijeh and Ejike, 2011; Ojiako and Nwajo, 2005). It is identified by various local names in Nigeria, such as "Ewuro" in Yoruba language, "Onugbu" in Igbo language, "Oriwo" in Bini language, "Ityuna" in Tiv, "Chusar doki or fate fate" in Hausa, while it is known as "Etidot" in Cross River State of Nigeria. It is characterized by flaky, rough bark with gray or brown colour, and can attain a height of about 23 feet (Echem and Kabari, 2013). The herb is an indigenous African plant that is widely distributed throughout most of sub-Saharan Africa and Asia (Echem and Kabari, 2013), and is widely cultivated in regions such as Yemen, Brazil, South Uganda, Ethiopia, Kenya, and Tanzania (Bhattacharjee *et al*.,2013. Ecologically, this species requires direct sunlight and prefers a humid environment, and may grow on any kind of soil, especially humus-rich soils. It can thrive in regions such as the edges of forests, the areas surrounding rivers and lakes, woodlands, and grasslands usually characterized by 2,800 m of elevation and

750–2000 mm of annual rainfall (Ofori *et al*., 2013).

It is commonly called a bitter leaf due to its bitter taste, which is usually regulated by boiling or by soaking the leaves in several changes of water. The presence of phytochemicals such as alkaloids, saponins, tannins, and glycosides contributes to the bitter taste in this species. It is also known to contain other biologically active compounds such as terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones and anthraquinone, edotides and sesquiterpenes (Yeap *et al*., 2010; Danladi *et al*., 2018; Cimanga *et al*., 2010). This important species has been reported to contain both health and industrial benefits. The leaves of bitter leaf are often used as a green vegetable or as a spice in preparation for delicious bitter-leaf soup in Nigeria. This aqueous extract of species has been used in the treatment of various health conditions such as emesis, nausea, diabetes, loss of appetite, dysentery and other gastrointestinal tract problems to sexually transmitted diseases and diabetes mellitus (Igile *et al*., 1995; Danladi *et al*., 2018). The organic fraction extracts of the bitter leaf have been shown to possess antimicrobial and antiparasitic activities such as antihelmintic, antimalarial, laxative/purgative, enema, expectorant, worm expeller and fertility inducer in subfertile women (Akinpelu, 1999; Hladik *et al*., 2005). Some of these and other benefits have been verified experimentally and documented by various workers, thus providing scientific evidence to support many of these claimed health benefits, however, varietal differences (Non-bitter and bitter varieties) in some nutritional and morphological attributes have not been assessed in the study area, hence, the purpose of this study.

2.0 Materials and Methods

2.1 Study area

Agbor, Ika South Local Government Area, Delta State, Nigeria is located at coordinates of 6° 15' 50.7312" N and 6° 12' 6.7788" E, and is an important agricultural spot of Delta State. The main occupational activities of Agbor people are Trading, Business ventures and large-scale food and cash crop farming. Some of the major staple and cash food crops produced in large scales in Agbor are cassava, varieties of yams, palm oils, mangoes, oranges, plantains, bananas, guavas, etc. Delta State is situated in the tropics and therefore experiences a fluctuating climate, ranging from the humid tropical in the south to the sub-humid in the northeast. The lessening of humidity towards the north is accompanied by an increasingly mar ked dry season. The average rainfall is about 266.5mm in the coastal areas and 1905mm in the extreme north. Temperature increases from the south to the north. In Warri, located in the south, for example, the average daily temperature is 30°C, while the temperature in Asaba in the northeastern area is 44°C. There are three types of soil in Delta State. These consist of alluvial soil on the marine deposits along the coast; alluvial and hydromorphic soils on marine and lacustrine deposits found in the area closest to the Niger and Benin rivers; and the feral soils on loose sandy sediments in the dry land areas of the north and northeast. The feral soils are usually yellowish. The vegetation varies from the mangrove swamp along the coast to the evergreen forest in the middle, and the savannah in the northeast. (Niger Delta Budget, 2024); Niger Delta People, 2023).

2.2 *Collection of plant samples*

Leaves samples of Non-bitter and bitter varieties of *Vernonia amagdalina* were collected from mature trees of the test plant in Agbor, Ika South Local Government Area, Delta State. Plant samples were identified by a taxonomic expert in the Department of Biological Sciences, University of Delta, Agbor, Delta State, Nigeria.

2.3 Sample preparation and micro-morphological analysis

The standard method of Metcalfe and Chalk (1978) was used for the determination of quantitative (stomatal distribution, stomatal index, stomatal length and width, epidermal cell length and width, trichome index, trichome length and width) and qualitative (stomatal and trichome type) characteristics of epidermal peels in leaves of the two *V. amagdalina* varieties.

2.4 Mineral analysis in Leaves of Vernonia amagdalina (Non-bitter and bitter varieties)

Leaves of *Vernonia amagdalina* (Non-bitter and bitter varieties) were first shadow-dried for 3 days, and macerated into small components. They were then oven-dried at 100^0C for 4 hours. They were crushed and ground to powder, then 2 grams each of the powders were weighed into Porcelain crucibles and placed in a muffle furnace and the furnace was set at 450^0C to ash. The ashing was done for 6 hours. The resulting ash was acid-digested in 15ml of concentrated HNO_3 (Trioxonitric acid) on a hot plate. 20 ml of distilled water was then added to the crucible to dilute the acid in the crucible. The mixture was then filtered in a 100ml volumetric flask and made up to the mark with distilled water. All the samples were then ready for AAS analysis. The AAS machine was then set up and the various elements were analysed at the specific wavelengths, lamps, currents, and gas mixtures (A.O.A.C., 1999).

2.5. Phytochemical and proximate analysis of Leaves of Vernonia amagdalina (Non-bitter and bitter varieties)

Phytochemical (alkaloid, tannin, flavonoid, and saponin) and proximate (carbohydrate, fat, protein, fibre, ash and moisture) composition in leaves of both varieties of *V. amagdalina* were analyzed using standard procedures (A.O.A.C., 1999).

3.0 Results and Discussion

3.1 *Micromorphological characteristics of Vernonia amagdalina* (non-bitter and bitter varieties)

The micromorphological characteristics of *V. amagdalina* (Non bitter and bitter varieties) are presented in Table 1. There were marked variations ($P > 0.05$) in leave quantitative micromorphological features (stomata length, stomata width, stomata index, and trichome index) between the two varieties of the test plant, while the stomata type and anticlinal wall shared similar characteristics (Table 1). These observations agree with the fact these parameters such as epidermal cell shapes and anticlinal wall patterns are important taxonomic tools for species identification in various plant families (Adedeji *et al*., 2007). Studies on quantitative and qualitative consideration of epidermal cell shapes and anticlinal wall patterns have been reported as taxonomic tools with similar results to this work (Aworinde *et al.,* 2013; Asuzu, 2020; Okanume *et al.*, 2022).

Table 1: Micromorphological characteristics of *Vernonia amagdalina* (Non bitter and bitter varieties)

Parameters		Non-bitter variety	Bitter variety
Stomata length (μm)	Adaxial	1.49±0.42	1.57±0.20
	Abaxial	1.53±0.33	1.42±0.56
Stomata width (μm)	Adaxial	0.69±0.03	0.75±0.05
	Abaxial	0.74±0.06.	0.62±0.02
Stomata index (%)	Adaxial	2.30±0.21	1.20±0.60
	Abaxial	2.63±0.15	1.18±0.20
Trichome Index (%)	Adaxial	0.90±0.19	0.55±0.36
	Abaxial	1.27±0.25	1.40±0.22
Stomata type	Adaxial	Anomocytic	Anomocytic

	Abaxial	Anomocytic	Anomocytic
Anticlinal wall	Adaxial	Straight	Straight
	Abaxial	Straight	Straight

Mean ± standard error from 3 replicates

3.2 Mineral elements of Vernonia amagdalina (Non bitter and bitter varieties)

The Mineral elements in the leaves of *Vernonia amagdalina* (non bitter and bitter varieties) are presented in Table 2. The calcium and magnesium contents in non-bitter varieties were comparatively higher ($P > 0.05$) than that of bitter varieties. The sodium, potassium, phosphorus, iron, zinc, and copper contents in non-bitter varieties were comparatively lower ($P > 0.05$) than that of bitter varieties (Table 2). Studies on micronutrients, macronutrients, and minerals of *V. amagdalina* have shown variations in nutrient concentrations between fresh and dried leaves such that magnesium, copper, and lead were found to be high in fresh leaves and calcium and iron were high in dried leaves (Garba and Oviosa, 2019). The high concentration value of minerals such as sodium, potassium, calcium, magnesium, zinc, and iron in the leaves of the plant presented it as an excellent source of food (Okeke *et al*., 2015; Olumide *et al*., 2019; Degu*et al*., 2024). The contents of mineral elements in leaves of *V. amagdalina*, as shown in this study, are consistent with that reported in previous studies (Okolie *et al*., 2021; Usunobun and Okolie, 2015). These results indicate that this species is a rich source of important nutrients, although the concentrations of nutrients vary between the two varieties. These variations may be attributed to differences in soil type, environmental conditions, geographic locations and genetic composition (Etukudo *et al*., 2015; Okolie *et al*., 2021; Olowoveve *et al*., 2022).

Table 2: Mineral elements in Leaves of *Vernonia amagdalina* (Non bitter and bitter varieties)

Mineral elements (mg/g)	Non bitter variety	Bitter variety
Calcium	22.10±0.61	20.17±0.22
Magnesium	12.07±0.58	10.23±0.14
Sodium	6.16±0.17	7.27±0.43
Potassium	14.25±0.61	17.07±0.45
Phosphorus	2.96±0.12	3.15±0.41
Iron	0.50±0.05	0.65±0.02
Zinc	0.14±0.06	0.15±0.03
Copper	0.12±0.08	0.16±0.04

Mean ± standard error from 3 replicates

3.3 Proximate composition of Vernonia amagdalina (Non bitter and bitter varieties)

The Proximate composition in leaves of *V. amagdalina* (non-bitter and bitter varieties) are presented in Table 3. The crude fibre, crude protein and carbohydrate contents in non-bitter variety were relatively higher ($P > 0.05$) than that of the bitter variety. The moisture, crude ash and crude fats contents in non-bitter variety were comparatively lower ($P > 0.05$) than that of the bitter variety (Table 3). Various studies have confirmed the rich contents of protein (including essential amino acids), moisture, carbohydrates, ash, and fat in leaves of *V. amagdalina* (Etta *et al*., 2017;

Olumide *et al*., 2019; Omoyeni *et al*., 2015). The rich proximate composition in leaves of this species proves effective for its contribution to nutrient requirements for human health and to food security (Usumomena and Ngozi, 2016; Ali *et al*., 2020). Thus, this shows the leaves of this species is an excellent source of food for the teaming population (Usumomena and Ngozi, 2016; Olumide *et al*., 2019).

Table 3: Proximate composition in Leaves of *Vernonia amagdalina* (Non bitter and bitter varieties)

Proximate (%)	*N*on bitter variety	Bitter variety
Moisture	45.58±0.22	50.01 ± 0.40
Crude fibre	1.76 ± 0.50	1.22 ± 0.72
Crude ash	7.06± 0.43	7.95 ± 0.56
Crude fat	1.20± 0.63	2.15 ± 0.20
Crude protein	21.07±0.30	17.66 ± 0.60
Carbohydrate	23.33± 0.82	21.01 ± 0.40

Mean ± Standard error from three replicates

3.4 *Phytochemical composition of Vernonia amagdalina* (Non bitter and bitter varieties)

The phytochemical composition in leaves of *V. amagdalina* (non-bitter and bitter varieties) are presented in Table 4. The tannin, saponin, and flavonoid contents in non-bitter variety were relatively higher ($P > 0.05$) than that of bitter variety. The alkaloid contents in non-b bitter variety were comparatively lower ($P > 0.05$) than that of the bitter variety (Table 4). Several studies have revealed the presence of phytochemicals such as alkaloids, glycosides, sesquiterpene lactones, steroids, flavonoids, proanthocyanidins, tannins, terpenoids, phenylpropanoids, resins, lignans, furocoumarins, naphthodianthrones in leaves of *V. amagdalina* (Senthilkumar *et al*., 2018; Tian *et al*., 2023). In similar studies, the presence of flavonoids, alkaloids, saponins, tannins, triterpenoids, steroids, and cardiac glycosides has been reported (Usunomena and Ngozi, 2016). The phytochemicals found in this study make this species a bittering agent (spice) and an antimicrobial agent in beer production as well as the use of the leaves to prepare bitter leaf soup, as an appetizer and as a digestive tonic (Nursuhailli *et al*., 2019). Again, these phytochemicals have shown active biological activities, showing the plant's potential as a medicine, especially their antimicrobial and antioxidant effects, as well as free radical scavengers and strong anticancer, prevention and therapy of several diseases activities (Ugwu *et al.*, 2013; Cheng *et al*., 2002)

Table 4: Phytochemicals in Leaves of *Vernonia amagdalina* (Non bitter and bitter varieties)

Phtochemicals (%)	Non bitter variety	Bitter variety
Alkaloid	1.20±0.23	1.26±0.27
Tannin	0.13±0.85	0.12±0.45
Saponin	0.08±0.02	0.06±0.03
Flavonoid	0.22±0.09	0.19±0.06

Mean ± standard error from 3 replicates

4.0 Conclusion

This study has examined the micromorphological and nutritional characteristics of the bitter and non-bitter varieties of *Vernonia amygdalina* cultivated in Delta State, Nigeria. Significant differences were observed between the two varieties in terms of stomatal dimensions, trichome index, proximate composition, mineral content, and phytochemical profile. The non-bitter variety showed superior nutritional value, with higher crude protein, fibre, and carbohydrate levels, while the bitter variety exhibited higher moisture, ash, and crude fat contents. Mineral analysis revealed that the non-bitter variety contained more calcium and magnesium, while the bitter variety was richer in sodium, potassium, phosphorus, and trace elements such as iron and zinc. Additionally, the bitter variety had a higher concentration of alkaloids, which may contribute to its medicinal properties.

In conclusion, both the bitter and non-bitter varieties of *Vernonia amygdalina* offer significant nutritional and medicinal value, with notable differences in their composition. The non-bitter variety is more nutritionally dense, while the bitter variety contains higher concentrations of certain minerals and phytochemicals. These findings highlight the potential of both varieties for use as food supplements and in pharmaceutical applications, depending on the specific nutritional or medicinal needs.

It is recommended that further research be conducted to better understand the genetic and environmental factors influencing these variations. The promotion of *Vernonia amygdalina* cultivation and utilization in both food and pharmaceutical sectors should also be encouraged to leverage its full potential. Additionally, public awareness campaigns should be developed to educate communities on the health benefits of both the bitter and non-bitter varieties of this valuable plant.

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Compliance with Ethical Standards Declaration

Ethical Approval

Not Applicable

Competing interests

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Availability of data and materials

Data would be made available on request.

Authors' contributions

Thelma Konyeme collected the plant samples, processed them for analysis and proof read the manuscript, while Anthony Ukpene prepared the manuscript and data analysis.