

HPLC Analysis and Nutritional Comparison of Three Indigenous Plants (*Parquetina nigrescens*, *Vernonia amygdalina*, and *Piper guineense*) for Enhanced Dietary Decision in Ekiti State Nigeria

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Abstract

Parquetina nigrescens, Bitter Leaf (*Vernonia amygdalina*), and African Black Pepper (*Piper guineense*) are among leafy vegetables that play a crucial role in diverse cultural diets, providing rich nutritional content and potential medicinal benefits. This study focuses on assessing and comparing the proximate composition of these three widely consumed leafy vegetables in Ekiti State. Employing standard laboratory methods, the study aims to analyse in percentage the Moisture Content, Ash Content, Crude Protein, Crude Fiber, Crude Fat, and Carbohydrate for each vegetable. Also, elucidation of bioactive compound in *Parquetina nigrescens* leaf extract using High Performance Liquid Chromatography was studied. The obtained results reveal distinctive compositions, with *P. nigrescens* exhibiting 13.543±0.001, 1.820±0.004, 2.189±0.001, 14.245±0.001, 7.438±0.001, and 60.765±0.001, bitter leaves showing 16.593±0.001, 1.944±0, 1.750±0.001, 13.658±0.001, 7.743±0.001, and 58.312±0.001, and African black pepper displaying 12.183±0.001, 4.035±0.001, 1.838±0.001, 20.993±0.001, 7.617±0.001, and 53.334±0.001 accordingly. HPLC analysis detected alpha linolenic, beta-sitosterol, camphor, kaemferol and additional compounds. The findings provide valuable insights into the nutritional profiles and antioxidant potential of these leafy vegetables, contributing to an understanding of their potential health benefits and aiding consumers in making informed dietary choices. This analysis enhances the knowledge base surrounding the dietary contributions of these leafy greens and promotes their incorporation into balanced and health-conscious diets

Keywords: *Parquetina nigrescens*; Bitter leaves; African black pepper; Leafy vegetables; Nutritional value

1 Introduction

The nutritional content of leafy vegetables is of significant importance due to their role in human diets and overall health. In Nigeria, leafy vegetables are staples and play a crucial role in traditional diets. They are vegetables whose young shoots, leaves and flowers are edible (Orech *et al.*, 2005). They have excellent nutritional value and can be used for medicinal benefits (Popkin *et al.*, 2001). Their concentration of functional compounds varies according to the climate season, their growth phase and their existence in particular plant part (Elias *et al.*, 2012). These green leafy vegetables are mostly rich in essential minerals, vitamins and dietary fibre and have therapeutic properties. With the wake of the novel coronavirus disease (COVID-19) pandemic, there is need to boost our immune system to prevent from the rate of disease incidence (Padhee and Joanna Kane-Potaka, 2020). Commonly consumed Leafy vegetables in Nigeria include waterleaf (*Talinum fruticosum*), fluted pumpkin (*Telfaira occidentalis*), bitter leaf (*Vernonia amygdalina*), jute mallow (*Corchorus olitorius*), and among others. Leafy vegetables display varied sensory attributes and may be bitter, aromatic, or bland to taste tasteless (Ishiekwene, 2019). In particular, African leafy vegetables have gained attention for their potential health-promoting properties. Africa black pepper, bitter leaf, and *Parquetina nigrescens* are three such

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vegetables that hold cultural and nutritional significance in various African communities. *Parquetina nigrescens* (Afzel.) Bullock (*Periplocaceae*) is a shrub that is found in West African countries especially Nigeria and Ghana (Irvine, 1961; Agbor and Odetola, 2005). The plant flourishes in equatorial climate and it is commonly used in different parts of West and East Africa for the treatment of several ailments which includes diarrhoea, gonorrhoea, menstrual disorders, insanity, intestinal worm infections, skin lesions and erectile dysfunction (Sofowora, 1993; Iwu, 1993; Odetola *et al.*, 2006). Reports obtained from traditional medical practitioners in Ilorin, Nigeria show that the leaves of this plant are also extracted with water and the extract is taken orally for the treatment of fever and pain especially headache. Also, Bitter leaf, scientifically known as *V. amygdalina* is one of the most famous plants found in Africa and Asia. It is the most cultivated species of the genus *Vernonia* that is about 1,000 species of shrub (Njan *et al.*, 2008). *V. amygdalina* has been the most prominent species in the family of Asteraceae that had been studied in Africa (Ankit *et al.*, 2010). Normally, *V. amygdalina* does not produce seeds but its cultivation is usually done by stem planting and mostly grow in tropical areas. This plant is found majorly along the drainage, commercial plantation or forest (Yeap *et al.*, 2010). More so, African black pepper (*P.guineense*) is a spice plant from the family *Piperaceae* and from genus piper. It is a West African spice plant commonly called Ashanti pepper (Katzer, 2015). It is known as Uziza in Igbo and Iyere in Yoruba. Other common names are Benin pepper, Guinea pepper and false cubeb (Katzer, 2015). Spices generally are parts of various plants cultivated for their aromatic pungent or otherwise desirable substances. They consist of rhizomes, bulbs, flower bud, fruit, seed, and leaves. They usually are categorized into tiny wild fruits, nuts, herbs, and leafy vegetables. The fruits of *P.guineense* occur in clusters, small, reddish or reddish brown when ripe and black when dry (Okwute, 1992). This study delves into the proximate composition of *Parquetina nigrescens*, bitter leaf, and African black pepper (uziza) found in Ekiti state, examining their moisture content, protein, fat, carbohydrate, fiber, and ash content. However, comprehensive information on their micronutrient composition and proximate analysis is limited.



Figure 1 *Parquetina nigrescens* leaves



Figure 2 *Vernonia amygdalina* (Yeap *et al.*, 2010)



Figure 3 African black pepper (*P. guineense*) leaves and unripe seeds

2 Materials and Methodology

Fresh samples of *Parquetina nigrescens*, bitter leaf (*Vernonia amygdalina*) and African black pepper (*Piper guineense*) were collected from the Federal Polytechnic farm land. Care was taken to ensure the samples were representative of their respective varieties

2.1 Sample Preparation

The collected samples were thoroughly washed, cleaned, and air-dried to remove excess moisture. They were then chopped into smaller pieces and were then analyzed for their moisture content, protein content, fat content, carbohydrate content, dietary fiber, and ash content.

2.2 Moisture Content

2.2.1 Procedure

A clean and well labeled dish that has been oven dried (W1) was weighed and 5 g of the sample added into the dish and weigh (W2). The dish and content were transferred to the thermo setting oven at about 105°C for about 24 hours. The dish was transferred from oven to desiccator, cool for about one hour and weighed. It was repeated (i.e. repeat step 4) to constant, weigh W3. Then % moisture content was calculated.

$$\% \text{ moisture} = \frac{\text{loss in weight}}{\text{Weight of sample before drying}} \times 100$$

$$W2 - W3 \times 100$$

$$W2 - W1$$

$$\% \text{ Total solid or } \% \text{ Dry matter} =$$

$$W3 - W1 \times 100$$

$$W3 - W1$$

2.3 Total Ash

2.3.1 Procedure

The silica dish or crucible was placed in muffle furnace for about 15 minutes at 350°C. The dish or crucible was removed, cooled in desiccators for about one hour or cool room temperature, weigh crucible W1. Two grams (2 g) of the sample was then added into the crucible, the quantity will depend on texture and source of sample) and weigh content (w2). The sample should be pre-dried if sample is wet or fresh plant. The crucible was placed inside the muffle furnace, and

slowly increase the temperature from 200°C-450°C this is to avoid incomplete ashing. It was ash (sample) until it become whitish in color. It was removed from furnace to desiccators and allows cooling to room temperature, and the content reweighed (W3).

2.3.2 Calculation

$$\% \text{ Ash} = \frac{W_3}{W_2} \times 100$$

$$\% \text{ organic matter} = 100 - \% \text{ Ash.}$$

2.4 Determination of percentage oil/lipid content

Two grams (2 g) of the sample was weighed into a filter paper and wrapped; the filter paper was placed inside the inner part of the Soxhlet extractor. The apparatus was then fitted to a round bottom flask, which contained 200 mL of hexane solvent. It was then attached to a reflux condenser. The set-up was clamped and heated in a water bath such that extraction is considered completed by the extracting solution becoming clear. The solvent was distilled off in the distillation set. The oil was then poured into a bottle and left for some times for the remaining solvent to evaporate. The oil was then weighed and the percentage oil content determined using the following expression (Ani *et al.*, 2012).

$$\text{Percentage lipid yield} = \frac{W_{oil}}{W_{sample}} \times 100$$

$$W_{sample}$$

Key:

- W_{oil} = weight of the oil
- W_{sample} = Weight of sample

2.5 Crude Fibre

2.5.1 Procedure

The experiment of crude fiber was carried out using the standard method as described by AOAC (2005). Two gram of fat free sample previously extracted with petroleum ether and it was boiled with 200 ml of H₂SO₄ for 30 minutes with the help of bumping chips. Thereafter, the mixture was filtered through a muslin cloth and then washed with boiling water until the residue was free from acid. The residue was then boiled with 200 ml NaOH solution for 20 minutes. Again, the mixture was filtered through a muslin cloth. The residue was then transferred to a pre weighed ashing dish. Thereafter, it was dried for 2 hours at 130 °C, cooled in a desiccator and then weighed. The dry dishes containing the samples were then ignited for 30 minutes at 600 °C. Finally, the sample was cooled in a desiccator and then weighed again.

$$\text{Crude fiber (\%)} = \frac{\text{Weight of the residue} - \text{weight of the Ash}}{\text{Weight of the sample taken}} \times 100$$

2.5.2 Determination of percentage protein

Two gram (2 g) of the grinded the sample was transferred to a Kjeldahl digestion flask and 8 g of the catalyst (96 % anhydrous Na₂SO₄, 3.5 % CuSO₄•5H₂O, 0.5 % Selenium dioxide) were added. 20 mL of conc. H₂SO₄ were added in an inclined position and shaken occasionally 11 times for 2hrs. The liquid formed was cooled and washed into the distil flask with distilled water. 50 mL of boric acid (2 %) solution and screened methyl red indicator were added to the receiving flask. The distillation apparatus was collected with the delivery tube deeping below the boric acid solution. The diluted digest was made alkaline by the addition of 50 % NaOH solution. About 50 mL of the distillate was collected and titrated with 0.1 M H₂SO₄. A blank was also titrated under the same condition.

$$\% \text{ nitrogen} = \frac{V \times 0.1 \text{ M H}_2\text{SO}_4}{10} \times 0.28$$

$$\text{Protein content (\%)} = \% \text{ nitrogen} \times 6.25$$

Where V 0.1 M H₂SO₄ represent volume of H₂SO₄

2.5.3 Characterisation of Aqueous Extract of *Parquetina nigrescens*

The characterization of the extract was carried out using the method of Zaib (2015). The plant extract of 1000 mg was used in a

HPLC system that was arranged with a photodiode array detector having UV visible detector at a wavelength of 280 nm along with pump shim pack column was used. Two mobile phases, a mixture of H₂O, CH₃COOH and acetonitrile of two mobile phases were used with a flow speed of 1mL/min as gradient. The standard peak was used as reference by comparison in determination of the result (Zafar *et al.*, 2020).

2.6 Statistical Analysis

All experiments were performed in triplicate and the results or values are expressed as mean; standard deviation denoted by error bars.

3 Results

HPLC characterisation result of the aqueous extract of *Paquetina nigrescens* leaves revealed fourteen bioactive constituents having veraquensin, Kaemferol and beta-sitosterol on the highest level (Fig. 4)

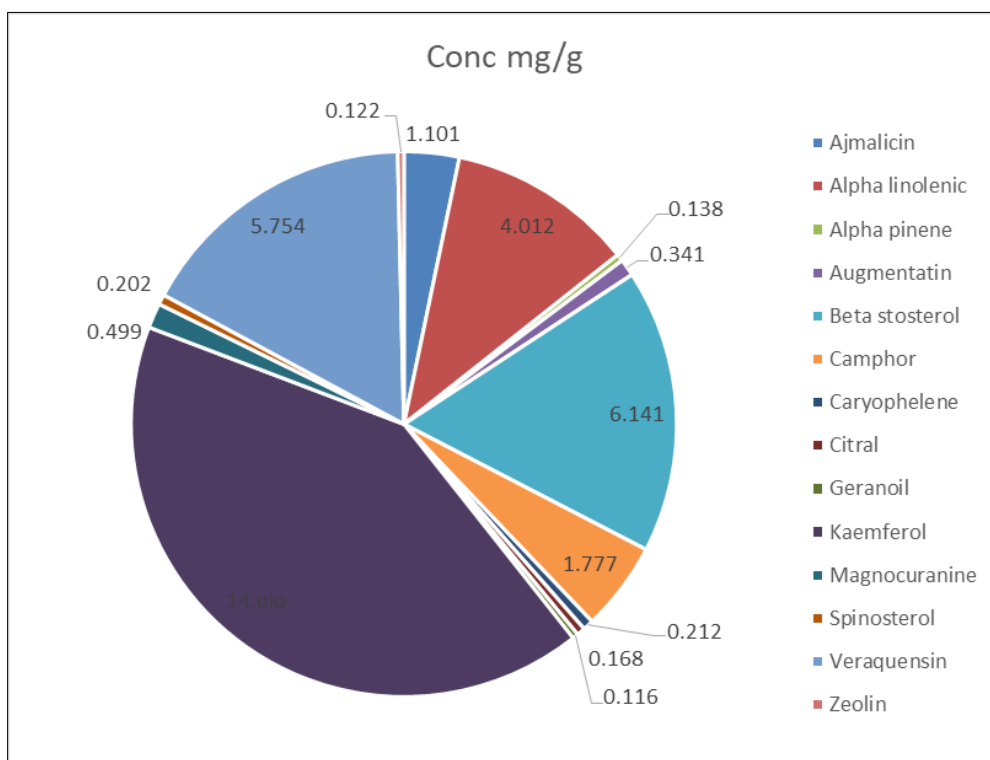


Figure 4 HPLC constituents of aqueous extract of *Paquetina nigrescens* leaves

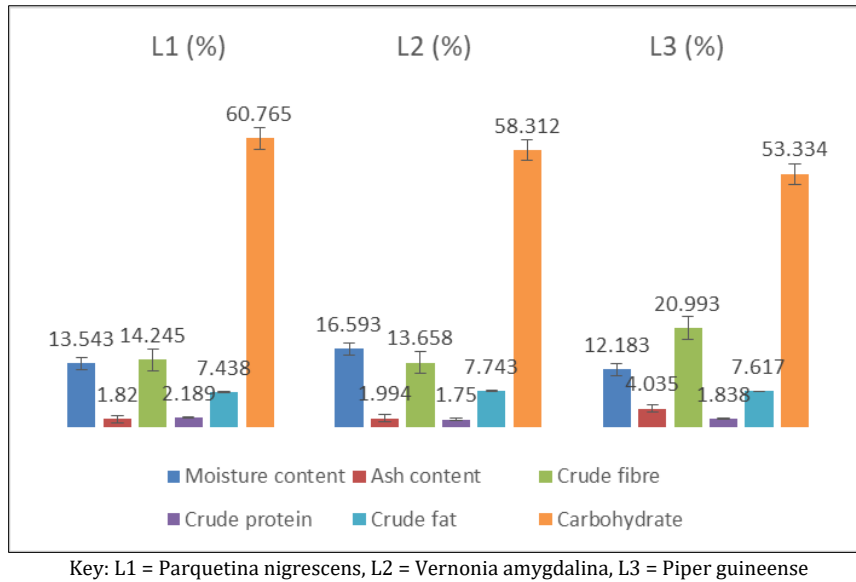


Figure 5 Percentage (%) Proximate composition of the three leafy Vegetables

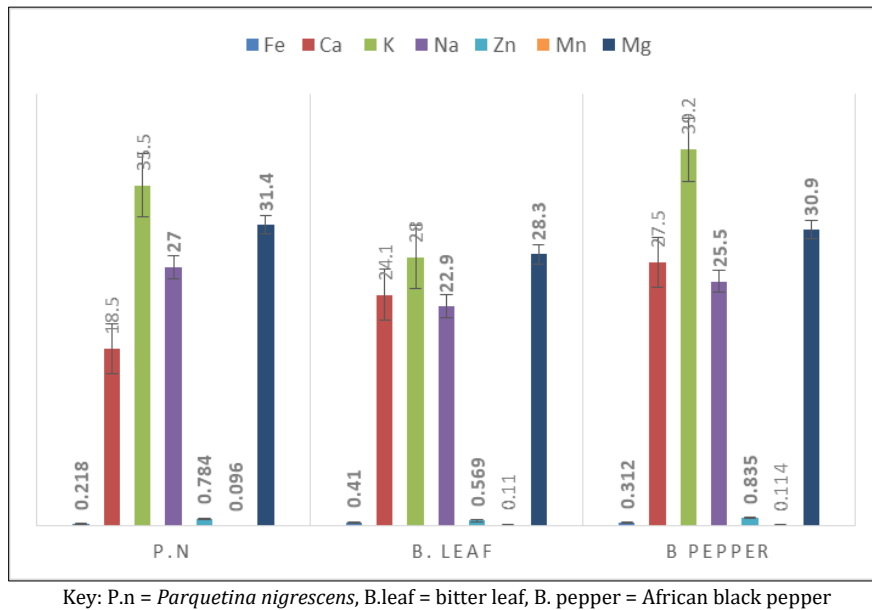


Figure 6 The Mineral composition of the selected three leafy Vegetables (mg/kg)

4 Discussion

HPLC result showed a lot of bioactive constituents in the extracts such as alpha linolenic, ajmalicin, caryophellene, veraquensin, beta sitosterol and kaempferol. β -Sitosterol for instance is a natural phytosterol similar to cholesterol but of plant origin. These active constituents identified from this plant have been over time reported to have antidiabetic potential (Ponnulakshmi *et al.*, 2019). Thus, *P.nigrescens* may have a range of medicinal related potentials attributable to the presence of its different quality secondary metabolites embedded in it The proximate analysis results from the study highlight the diverse nutritional profiles of the selected leafy vegetables: *Parquetina nigrescens*, bitter leaves, and African black pepper. These findings have important implications for dietary planning and nutritional recommendations.

Parquetina nigrescens stands out as a particularly protein-rich option among the three vegetables, aligning with the fact that most dietary proteins consumed by humans are plant-based (Fennema *et al.*, 1996; Odukoya, 2015). Incorporating this vegetable into diets can be beneficial for those seeking to increase their protein intake, especially vegetarians and vegans, contributing to muscle maintenance and overall satiety.

All three vegetables have low lipid content, making them suitable for low-fat diets that promote heart health and weight management. The fat content ranged from 7.438 ± 0.001 in *Parquetina nigrescens* to 7.743 ± 0.001 in bitter leaf, with African black pepper at 7.617 ± 0.001 . Despite the slight variation, bitter leaf has the highest lipid content and is noted for its potential medicinal properties, such as supporting digestive health and managing blood sugar levels. Its bitterness, due to compounds like sesquiterpenoids, contributes to these health benefits.

The three vegetables also demonstrated appreciable fiber content, with African black pepper showing the highest percentage. Dietary fiber supports digestion, helps regulate blood sugar levels, and promotes a feeling of fullness (Emebu and Anyika, 2011). High fiber content aids digestive health by promoting regular bowel movements and supporting gut microbiota (Asaolu *et al.*, 2012).

The ash content, representing the mineral composition, underscores the potential of these leafy vegetables to contribute essential minerals to the diet. African black pepper has the highest ash content at 4.035 ± 0.001 , compared to 1.820 ± 0.004 for *Parquetina nigrescens* and 1.994 ± 0.00 for bitter leaf. These minerals, such as calcium, magnesium, and potassium, are crucial for bone health, nerve function, and overall well-being (Idris *et al.*, 2011). Additionally, African black pepper (*Uziza*) leaves, known for their peppery flavor, also contain antioxidant compounds that protect cells from oxidative stress (Etim *et al.*, 2013).

The moisture content of 13.543 ± 0.001 in *Parquetina nigrescens*, 16.593 ± 0.001 in bitter leaf, and 12.183 ± 0.001 in African black pepper suggests their role in maintaining hydration and providing refreshing properties. The carbohydrate content varied significantly, with 60.765 ± 0.001 in *Parquetina nigrescens*, 58.312 ± 0.001 in bitter leaf, and 53.334 ± 0.001 in African black pepper. This variability indicates that leafy vegetables can serve as important sources of dietary carbohydrates, meeting Recommended Dietary Allowance (RDA) values (F.N.D., 2002). These results are comparable to those reported for other leafy vegetables (Hussain *et al.*, 2009; Faruq *et al.*, 2002), emphasizing their essential role in nutrition.

Individuals monitoring their carbohydrate intake, such as those managing diabetes, can make informed choices based on these findings. It is important to consider that the nutritional composition of leafy vegetables can be influenced by factors such as cultivation methods, soil quality, and environmental conditions. These results underscore the importance of incorporating a variety of leafy vegetables into the diet to meet daily nutritional requirements and promote overall health.

5 Conclusion

The macronutrients conducted on the three varieties of leafy vegetables have shed light on their moisture, protein, lipid, carbohydrate, and ash content as well as the mineral compositions. This research contributes to the existing body of knowledge regarding the nutritional composition of commonly consumed leafy vegetables. This analysis not only underscores the health benefits of leafy vegetables but also highlights their cultural importance in Ekiti state.

Recommendation

Considering the observation made of the three vegetable plants studied (*P.nigrescens*, *V. amydalina* and *P.guineense*) I wish to recommend that more consumption of these plants should be encouraged owing to its appreciable amount of macronutrients content observed from the study. Fiber in leafy vegetables supports digestion, helps regulate blood sugar levels, and promotes a feeling of fullness. Thus, local use of these plant studied due to its dietary fiber should be promoted because dietary fibre helps in preventing of cardio vascular disease and lower cholesterol level in the living system.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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