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Phytochemicals and Mineral Element Compositions of Bitter-Leaf (*Vernonia Amygdalina*) Young and Matured Leaves

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Abstract

This study was aimed to evaluate and compare phytochemical and mineral elements in young and mature leaves of *V. amygdalina*. The young leaves contained (0.281 g/100g) flavonoid, (0.397 g/100g) phenols, (0.064 g/100g) saponin, (1.4731 g/100g) alkaloids with the mature sample containing (0.371 g/100g) flavonoid, (0.642 g/100g) phenol, (0.072 g/100g) saponins and (1.621 g/100g) alkaloid. Magnesium, potassium, phosphorus, and calcium were the mineral elements determined in the young and matured leaves of *V. amygdalina*. The young leaf contained 105 (mg/kg) potassium, 113.15 (mg/kg) calcium, 134.65 (mg/kg) magnesium and 21.85 (mg/kg) phosphorus with the mature leaf containing 96.25 (mg/kg) potassium, 117.6 (mg/kg) calcium, 141.45 (mg/kg) magnesium and 19.75 (mg/kg) phosphorus. The mineral analysis of both leaves extracts showed that as the leaf matures the concentration of potassium, and phosphorus reduces, but the matured leaf contains more calcium and magnesium than the young leaf. Quantitative analysis of the extracts showed that the mature leaf contains more flavonoid, phenol, saponin and alkaloid than the mature leaf. From the findings of this study, it shows that though the leaves of both young and matured *V. amygdalina* has therapeutic properties and can be used in dietary supplements, the mature leaf has more concentration of phytochemical and mineral elements than the young leaf.

Keywords: Phytochemicals, Mineral; Element Compositions, Bitter-Leaf, *Vernonia Amydalina*, Young and Matured Leaves.

Introduction

It is well known that medicinal plants contain one or more of their organ components that can be employed therapeutically or as a starting point for the manufacture of valuable pharmaceuticals (Sofowora, 1996). The therapeutic value of several of these plants, some of which include *Allium sativum* (Garlic), *Azadirachta indica* (Dogonyaro), *Zingiber officinale* (Ginger), *Piper guineese* (Iyere) and *Vernonia amygdalina*, has been thoroughly investigated (Bitter leaf). According to reports, these plants have been utilized in traditional medicine to cure conditions like

gastrointestinal disorders, fever symptoms, and coughs (Ayoola *et al.,* 2006). Plants are effective at treating sickness, and because they serve as both food and medicine, they also have little or no negative side effects. For instance, in the treatment of hypertension, herbs are first used to reduce blood pressure, clear the arteries, slow and regulate heartbeat, enhance blood flow, and calm the mind (Mann *et al.,* 1983). These differ from the basic conventional medications that widen the arteries or veins till their maximum elastic point at which they may abruptly burst, triggering a vascular accident that could result in death or stroke (Kafaru, 1994).

A perennial shrub in the Asteraceae family, *Vernonia amygdalina* is also known by the names bitter leaf (English), Shuwaka (Hausa), Ewuro (Yoruba), Oriwo (Edo), and Olubu (Igbo) (Ghamba *et al.,* 2021). Tropical regions are home to the shrub *V. amygdalina*, which can reach a height of 10 m and has elliptic-shaped petiole leaves with a diameter of about 6 mm. Africa and has been domesticated in several regions of West Africa, including Nigeria, where it is used locally as a vegetable in soups and as an aperitif, febrifuge, and weaning food (Emmanuel *et al.,* 2020; Etim *et al.,* 2012; Abosi and Raseroka, 2003). According to pharmacological research, *V. amygdalina* has anthelmintic and antimalarial characteristics, antitumorigenic properties, analgesic and antipyretic activities, and hypoglycemic and hypolipidemic effects in experimental animals (Habtamu and Melaku, 2018; Izevbigie *et al.,* 2004). The reported naturally active constituents in *V. amygdalina* include flavonoids, anthraquinones, saponins, edotides, terpenes, coumarins, phenolic acids, xanthones, and lignans.

Their various pharmacological characteristics are also a result of these bioactive molecules. Considering that various researchers have worked on the phytochemicals and mineral constituents and the total effect of the entire leaf extract (mixture of both young and mature leaf) but have not compared the parameters between the young and old leaves of *V. amygdalina*, this study is designed to analyze young and mature leaves of *V. amygdalina* for phytochemical and mineral properties with the objective of comparing the composition of the young and mature leaves.

Materials and Methods

Sample Collection

Fresh leaves of young and matured of *V.amygdalina* were obtained from farm in Owo. The leaves were identified at the Department of Biological Sciences, Achievers University, Owo, Nigeria. Follin-ciocalteu's reagent, ethanol, sodium trioxocarbonate, sodium hydroxide, aluminium trichloride, Drangedoff's reagent, disodium sulfide, nitric acid, thio urea, bismuth nitrate pentahydrate, sodium carbonate, H_2SO_4, HCl, NaOH, ferric chloride, vanillin ethanol.

Extraction Procedure

10 grams of the freshly harvested young and mature leaves of *V.amygdalina* was air-dried and grinded with a blender. The extractions were carried out with sohxlet apparatus using 150 mL of ethanol and hexane. The extract was then filtered through a Whattman No.1 filter paper. The filter samples were designated as: EYV (Extract of young *V.amygdalina* leaf*)* and EMV(Extract of mature *V.amygdalina* leaf).

Qualitative Analysis

The freshly harvested leaves of young and mature *V. amygdalina* was air-dried and grinded with a mortal. 10 mL of ethanol and hexane each was mixed with 5 g of the samples in a conical flask. It was stirred and soaked for 10 mins then filtered. The phytochemical screening of *V. amygdalina* EYV and EMV was assessed for the existence of phytochemicals using the following standard method:

Phytochemical Screening (Mayer's Test)

- **Test for Alkaloid**

The plant extract was mixed in 1 % v/v HCl acid after which it was warmed and filtered. The filtrate was then treated with Mayer's reagent (mercuric chloride+potassium iodide in water). A yellow colouration precipitate specified the presence of alkaloid.

- **Test for Glucoside (Froth's Test for Saponic Glucoside)**

The plant extract was diluted and shaken for 15 minutes in a graduated cylinder. The presence of saponin was indicated by formation of 1 cm layer of foam.

- **Test for Flavonoid**

The plant extract with 2-3 days of sodium hydroxide solution. Acute yellow colour formation indicates the presence of flavonoid by the addition of some drops of sulphuric acid that changed to colourless.

- **Test for Phenol and Tannin**

The powdered plant sample was taken and dissolved in 20 mL of distilled water in a test tube, after which it was boiled and then filtered. 3-4 drops of 0.1 v/v ferric chloride were added to the filtered sample. A brownish green or blue colourshows that phenols and tannins are present.

Quantitative Analysis

Determination of Total Phenolic

The phenolic in extracts was estimated using Singleton *et al.* (1999*)* spectrophotometric method. All analysis was done in triplicate to obtain mean values of sample. The calibration curve of garlic acid was constructed in the range of 20-100 μg/mL.Lastly, phenolic concentration was expressed in Gallic acid equivalent terms(mg GA/g of dw).

Determination of Total Flavonoids

The total flavonoid content was measured according to Chang *et al.*(2002) aluminium trichloride colorimetric method. The extracts were diluted with ethanol till the concentration reached 1 mg/mL and a calibration curve was drawn using ethanol dissolved quercetin (20-100 μg/ml).2.0 mL diluted extracts/quercetin was mixed with aluminium trichloride (0.1 mL of 10% (W/V), and CH_3COOK (0.1 mL of 0.1 Mm) and whole solution was prepared in ethanol. The absorbance

was measured at 415 nm post 30 mins of incubation at 25 ℃ and total flavonoid content was represented as mg quercetin equivalent per gram dry weights of extracts(mg Q/g of dw).

Determination of Total Alkaloids

The preparation of exactly 200 mL of 10% acetic acid in ethanol was added to a portion of plant sample in a 250 mL beaker and allowed to stand for 4 hours. The extract was concentrated on a water bath to one-quater of the original volume followed by addition of 15 drops of concentrated ammonium hydroxide drop wise to the extract until the precipitation was complete immediately after filtration. After 3 hours of mixture sedimentation, the supernatant was discarded and the precipitates were washed with 20 mL of 0.1 M of ammonium hydroxide and then filtered using filter paper. The residue was dried in an oven and the percentage of alkaloid is represented mathematically as

$$\% \text{ Alkaloid} = \frac{\text{weight of alkaloid } x \text{ } 100}{\text{weight of sample}}$$

Determination of Total Saponins

The samples were grounded and 20 g of each was put into conical flask and 100 mL of 20 % aqueous ethanol was added. The samples was heated over a hot water bath for 4 hrs with continuous stirring at about 55℃. The mixture was filtered and the residue was extracted with another 200 mL 20% ethanol. The combined extracts was reduced to 40 mL over water bath at about 90 ℃. The concentrate was transferred into a 250 mL separating funnel and 20 mL of diethyl ether was added and shaken thoroughly. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 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 was calculated as a percentage

$$\% \text{ Saponin} = \frac{\text{weight of saponin } x100}{\text{weight of sample}}$$

Determination of Minerals

Standard Operation Procedure of Sample Preparation (Extraction/Digestion)

The accepted sample method of determination of metals /Heavy metals in food/plant sample involves complete digestion of sample in hot concentrated acid.

Results and Discussions

Qualitative and Quantitative Phytochemical Screening

The phytochemical screening (qualitative and quantitative) of both matured and young leaves of *V. amygdalina* are presented in table 1 and table 2 respectively.

Five preliminary phytochemical studies on the presence of flavonoid, alkaloid, phenol, saponin, and glycosides were conducted and the result of the screening carried out on ethanol crude

extract of the young and mature leaves of *V. amygdalina* revealed that these phytochemicals except glycosides are presence. The presence of these phytochemicals listed supports its uses for various medicinal purposes. The phytochemical screening of both matured and young leaves of *V. amygdalina* revealed that the leaves contained phytochemicals like tannins, alkaloids, phenols, saponin, and flavonoids.

Phytochemicals are naturally active plant's components in small quantities that are not recognised as nutrients but, nevertheless, add considerably to defending the body against deteriorative diseases (Loliger, 1991; Omale and Okafor, 2008). Phytochemical components present in the ethanoic extracts of both young and matured leaves of *V. amygdalina* in this study revealed the presence of flavonoids, alkaloids, saponins and phenols.

Alkaloids are the most efficient therapeutically significant plant substances commonly found to have antimicrobial properties due to their ability to intercalate DNA of the micro-organisms (Falzi *et al.,* 2008). Analgesic, bactericidal and antispasmodic effects have all being attributed to alkaloidal composition in plants. The presence of alkaloids may be attributed to the acclaimed antibacterial property of this plant (kasolo *et al.,* 2010).

Saponins are known to possess both anti-inflammatory and antibacterial activities(Soetan *et al.,* 2006). Studies have also reported the beneficial effects of saponins on level of cholesterol which also stimulate the immune system (Hassan *et al.,* 2012; Cheeke, 2010).

Phenols are strong antioxidants which inhibit oxidative loss to molecules like lipids, proteins and DNA that contribute to chronic ailments such as cardiovascular diseases and cancer. Phenols from plant sources may interfere with the various stages of cancer mechanism, possibly causing a decline in cancer threat (Hollman, 2001). The cardiac glycosides medicinally have the capacity to strengthen the potency and power of the heartbeat. It can improve the effectiveness of the heart and also stabilize the heart beats without stressing to the organ.

Tannins have shown anti-viral and antibacterial activity, its astringent properties help with quick healing of wounds and inflamed mucous membranes (Enzo, 2007). Flavonoids and tannins are noted for anti-diarrhea activity (Adisa *et al.,* 2010; Farquar, 1996).

Flavonoids have shown to have antifungal activity in vitro (Williams *et al.,* 2004). The effective antioxidant properties of flavonoids reveal their ability to mask lipid peroxyl radicals, superoxide anions and hydroxyl radicals; this may be the important function of flavonoids (Galeotti *et al.,* 2008). They also induce mechanisms that may kill cancer cells and inhibit tumour invasion. The flavonoids present may be responsible for the medicinal properties accorded the plant (Cheng *et al.,* 2006; Chen *et al.,* 2008; Saleem *et al.,*2005).

These chemical constituents discovered in the leaves of *V. amygdalina* may therefore serve as the active substances appropriately responsible for the claimed therapeutic indications recorded for this plant, and is also an indication of preliminary validation of the claims.

Table 1: Qualitative Phytochemical Screening of *V.Amygdalina*

S/N	Phytochemicals	Matured Leaf	Young Leaf
1.	Alkaloid	+	+
2.	Phenol	+	+
3.	Saponin	+	+
4.	Flavonoids	+	+
5.	Glycoside	-	-

– = Absent

+ = present

Table 2: Quantitative Phytochemical Screening of *V. Amygdalina*

S/N	Phytochemicals	Young Leaf (g/100g)	Matured Leaf (g/100g)
1.	Alkaloid	1.473 ± 0.02	1.621 ± 0.03
2.	Phenol	0.397 ± 0.03	0.642 ± 0.09
3.	Saponin	0.072 ± 0.02	0.064 ± 0.01
4.	Flavonoid	0.281 ± 0.02	0.371 ± 0.02

Table 2 shows the quantitative determination of the phytochemical constituent of young and mature *V. amygdalina* leaf crude extract. The values obtained for alkaloids ranged from 1.473 to 1.621. The higher value was obtained in the mature leaf (1.621 g/100g) while the lower value (1.473 g/100g) was obtained in the young leaf. The values obtained in this research is slightly lower than 2.04 that was reported by Singhal and Kulkarni (2007) and 4.60 reported by Ali *et al.* (2020) for leaves of *V. amygdalina* purchased from Sabon-Gari Market in Kano. The matured leaf having the higher value correlate with a report by Ojewuyi *et al.* (2014) where he checked for the phytochemical contents of young and mature leaves of *P. longifolia.* The differences in the results can be attributed to variation in the methods used, environmental factors, soil, harvest time. Alkaloids play an important metabolic roles and development in the system of living organisms. Alkaloids are known for their antimicrobial properties which accounted for its antimicrobial action (Kadiri and Olawoye, 2016).

The values obtained for phenol ranged from 0.397 to 0.642. The higher value was obtained in the mature leaf (0.642 g/100g) while the lower value (0.397 g/100g) was obtained in the young leaf. A relationship was observed between alkaloids and phenol, it was observed that the higher the alkaloids, the higher the phenol. As also found in alkaloid, the higher value was seen in the matured leaf. The value obtained in this research was slightly lower than the 0.76 g/100g that was obtained by Teye *et al.* (2019) for air dried *V. amygdalina* leaf, while a significant difference was observe in 1.64 g/100g obtained by Teye *et al.* (2019) for oven dried *V. amygdalina* leaf. Also, the result for both young and matured leaf in this research is higher than the 0.34 and 0.35 by Ojewuyi *et al.* (2014) for mature and young leaves of *P. longifolia* respectively. Phenolic compounds have been recorded to contain antioxidant property which inhibits oxidative damage of cell owing to the existent of free radical scavengers. Phenolic compounds reduce the danger of heart diseases and provide anti-inflammatory action due to their capacity to nullify or neutralize free radicals (Okechukwu *et al.,* 2013).

The data obtained for saponin ranged from 0.064 g/100g to 0.072 g/100g, saponin was found to be higher in mature leaf 0.072 g/100g than in young 0.064 g/100g. The results obtained for saponin content in this study for both young and mature leaf is low compared to 2.70 g/100g reported by Ali *et al.* (2020) and slightly higher than 0.21 g/100g reported by Okoli *et al.* (2021). Saponin is known to possess both anti-inflammatory and antimicrobial activities (Hassan *et al.,* 2012).

Mineral Composition

The mineral analysis result showed that both young and mature leaves of *V. amygdalina* contains calcium, phosphorus, potassium and magnesium. Both samples contain appreciable amount of calcium and moderate levels of phosphorus, potassium and magnesium.The Phosphorous values obtained in this study ranged from 19.75 mg/kg to 21.85 mg/kg with the higher value recorded in young leaf. These values are lower than 60 mg/kg reported by Ali *et al.* (2020) for leaves of *V. amygdalina* purchased from Sabon-Gari Market in Kano. The differences in the results can be attributed to variation in the methods used, environmental factors, soil, harvest time.The Potassium values obtained in this study ranged from 96.25 mg/kg to 105 mg/kg with the higher value recorded in young leaf. It was observed that the higher value of Potassium was found in young leaf as also found in phosphorus. These values are lower than 60 mg/kg as recorded by Ali *et al.* (2020) for leaves of *V. amygdalina* purchased from Sabon-Gari Market in Kano. Potassium is responsible for nerve action and some osmo-regulation in the body fluid (Odoemena and Ekanem, 2006).

Table 3: Mineral Compositions of *V. Amygdalina*

V. Amygdalina Leaf	**P (mg/kg)**	**K (mg/kg)**	**Ca (mg/kg)**	**Mg (mg/kg)**
Young leaf	21.85 ± 0.21	105 ±0.11	113.15 ± 1.06	134.65 ± 0.21
Matured leaf	19.75 ± 0.35	96.25 ± 1.06	117.6 ± 0.56	141.45 ± 0.77

The calcium values recorded in this research ranged from 113.15 mg/kg to 117.6 mg/kg with the higher value recorded in mature leaf. These recorded values obtained are moderately higher than the values reported by Singhal and Kulkarni (2007) where *O. gratissimum* had the highest value of calcium, while *M. oleifera* and*V. amygdalina*are not significantly different. Calcium is essential for strong teeth and bones. This shows that *V. amygdalina*can offer part of the daily calcium requirement in the body when consumed.

Magnesium value gotten in this research ranged from 134.65 mg/kg to 141.45 mg/kg. A significant difference was observed within the young leaf and mature leaf where the higher value was recorded in the mature leaf.

Analysis of mineral composition of *V. amygdalina* leaf in this study confirmed the presence of mineral (potassium, magnesium, calcium and phosphorous) elements. This justifies the vitality of the plant leaf nutritionally rich when consumed by animals or humans (Singhal and Kulkarni, 2007). Minerals play an important metabolic role in the body of animals, such activities include maintenance of acid balance in the body, production and activity of enzymes and so on. Presence of potassium in the extracellular body fluid is vital; it conducts several functions to the body

system such as regulation of osmotic pressure, conduction of nerve impulse and maintenance of acid-base balance (Teye *et al.,* 2019). Potassium is important in regulation of electrolyte, water and acid-base balance in the body, as well as responsible for nerve action and functioning of the muscles (Indrayan *et al.,* 2009). It also helps to maintain body weight. Calcium played a major role in the formation and development of bones and teeth, coagulation of blood, contraction of muscle, normal functioning of heart and nervous system. Calcium is needed in the composition of living systems; its presence in bones and provides the body with the vitalsupport and rigidity. Calcium is essential for bone and teeth formation and development, blood clotting and for normal functioning of the heart, nervous systems and muscles (Ibrahim *et al.,* 2001).

Presence of magnesium in the diet is essential for decreasing blood sugar as a result it improves the function of insulin (Ali *et al.,* 2020). Magnesium plays fundamental roles in most reactions involving phosphate transfer. It is believed to be essential in the structural stability of nucleic acids. It plays a significant role in the intestinal absorption of electrolyte in the body. Its deficiency in man includes severe diarrhoea and persistent migraines (Appel, 1999).

Conclusion

The results of the quantitative analysis of phytochemicals and mineral elements in both young and mature leaf of *V. amygdalina* shows the presence of phytochemicals and mineral elements in the two extract samples. The results of the mature leaves show higher concentration of the phytochemicals and mineral elements than the young leaves though not at a significant difference. However, the results still indicate that the concentration of the bioactive components increase with age. Also base on the results from several studies on *V. amygdalina*, this plant has reportedly been used in traditional treatment of ailments due to the presence of these bioactive compounds hence, this research recommends that more mature leaves of *V. amygdalina* will be a good source of these bioactive compounds for traditional and pharmaceutical uses.

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