Comparative Study of the Phytochemical Contents and Selected Micronutrient Contents of Vernonia amygdalina (Onugbu Leaf) and Pterocarpus mildbreadii (Oha Leaf) Brought from Igbariam, Anambra State

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Abstract:- The purpose of this study is conducting a comparative study on the nutritional compositions of bitterleaf (Vernonia amygdalina) and oha leaf (Pterocarpus mildbraedii), focusing on its phytochemicals and micronutrients so as to determine their nutritional value, the bioactive compounds present in them and relate them to their health benefits. The collected leaf samples of V. amygdalina and P. mildbreadii were divided into three parts each, for the phytochemicals, vitamin and mineral determination. The oils of the grounded leaf samples were extracted using soxhlet extraction method and the phytochemical contents of the leaves were analyzed using GC-FID. Vitamin A and Vitamin E absorbance were measured at mg/L using a UVspectrophotometer at different wavelength while Vitamin C was titrated and measured at mg/100ml. The samples were also digested for the mineral analysis and their filtrates were placed in an atomic absorption spectrophotometer and the absorbance of the different elements contained in the samples was measured at mg/L. The results indicated that bitter leaf was high in flavonoid, alkaloid and kaempferol having values of 15.877 ± 0.011 , 15.577 ± 0.006 and 13.890 ± 0.004 , respectively while the oha leaf was high in kaempferol, sapogenin and phenol having values of 16.873 ± 0.001 , 15.125 ± 0.001 and 14.779 ± 0.001 , respectively. The Vitamin A (mg/L), Vitamin E (mg/L) and Vitamin C (mg/100) values of bitter leaf and oha leaf were 18.579, 22.120,1174.493 and 20.323, 25.918, 1275.235 respectively. The mineral levels of V. amygdalina and P. mildbraedii revealed significant amounts of magnesium and potassium having higher values of 4.779mg/L, 3.265mg/L and 3.782mg/L, 2.712mg/L respectively. Iron and zinc were not present in the P. mildbreadii leaves while copper, iron and sodium were not present in the V. amygdalina leaves. The evaluation of the leaves of V. amygdalina and P. mildbreadii showed that the leaves had high phytochemical, mineral and vitamin contents. However, the difference between the two samples on their phytochemical and micronutrient composition had

significant variations at p<0.05. The result of this analysis will aid to inform the consumers on the appropraite usage of this leaves for optimal nutritional yield. It should also be recommended by food nutritionist as part of a dietary plan so as to help boost the immune system and support other body processes.

Keywords:- V. Amygdalina, P. Mildbreadii, Phytochemicals, Micronutrients, Soxhlet Extraction.

I. INTRODUCTION

The global importance of vegetables has necessitated a need for an increase in the demand of knowledge of their nutrients and chemical composition. Green leafy vegetables provide phytochemicals and micronutrients that are pertinent for growth and maintenance of good health. These compounds have a specific pharmacological effect on the human body (Liu and Wang, 2008). In recent years, there have been an increasing keenness on the nutritional composition and health-promoting properties of indigenous vegetables like bitter leaf and oha leaf. Studies have shown that these leafy greens are rich sources of phytonutrients and antioxidants, which play crucial roles in human health and preventing chronic diseases (Udensi and Anyika, 2012).

Anderson, (2004) defined phytochemicals as plantderived chemicals, which are beneficial to human health and disease prevention. However, the levels of these phytochemicals vary from plant to plant depending upon the variety and climate growing conditions (Rao, 2003).

Micronutrients are nutrients that are required by the body in small amounts for its optimal metabolic functions and growth. These include vitamins and minerals. Even though the body cannot produce them and because of its importance to the body the must be obtained from the different sources (diet) food. The content of vitamins and minerals for every food differs due to their phytochemical and antioxidant compositions, therefore consumption of Volume 9, Issue 10, October – 2024

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variety of food is important.

Vernonia amygdalina plant has a wide spectrum of uses in African traditional medicine as an antioxidant, antidiabetic clotting and anti microbial properties that has been used in the management and treatment of a number of health conditions. The specie's secondary metabolites, which include saponins, tannins, alkaloids and glycosides, are dietary components and these constituents contribute to the source of the bitter taste in this medicinal plant (Danladi *et al.*, 2018).

Pterocarpus mildbraedii are used as vegetables in the preparation of soup and is prized for its distinctive aroma and nutritional value. Research has shown the presence of various bioactive compounds in oha leaf, including phenolic acids, flavonoids, and vitamins, which contribute to its antioxidant and anti-inflammatory properties (Ogunlesi *et al.*, 2017). Okonwu *et al.*, (2018) reported that *Pterocarpus mildbreadii* leaves contain vitamin A and C.

Evaluating the nutritional composition of bitter leaf and Oha leaf can provide valuable insights into their potential health benefits and nutritional value. This information can be used to promote the consumption of these leafy vegetables, support their incorporation into food products, and improve strategies for improving their nutritional content through cultivation and processing practices.

II. MATERIALS AND METHODS

A. Materials

- Equipment used are Analytical balance (accuracy 0.1mg Ohaus), soxhlet apparatus, boiling flask, electric hot plate, rotatory evaporator, hot air oven, dessicator, separating funnel, beakers, laboratory heater, pasteur pipette, laboratory grinder, gas chromatographic machine equipped with flame ionization detector (GC-FID), filtration paper, Atomic absorption spectrometer, UVspectrophotometer.
- Chemicals and Reagents: All chemicals and reagents used for this study were of analytical grade except where otherwise stated.
- Samples Bitter leaf (Vernonia amygdalina) -Oha leaf (Pterocarpus mildbraedii)

B. Methods

> Sample Collection:

The samples used for this study were collected from the farm house of the department of Crop Science and Horticulture, Faculty of Agriculture, Chukwuemeka Odumegwu Ojukwu University, Igbariam. The leaves where collected in air ventilated medium to avoid spoilage. The samples were divided into three based on the analysis and they were used for the determination of their phytochemical and selected micronutrient contents.

> Preparation of Collected Samples for the Analysis:

The collected Oha leaves and bitter leaves were thoroughly washed with distilled water to remove any dirt, debris, and surface contaminants. The leaves were air-dried so as to remove excess moisture. After drying, the leaves were grounded into a fine powder using a laboratory-grade grinder. This was done to increase the surface area of the sample so as to facilitate the extraction process. The powdered samples were stored in an airtight container to protect them from moisture, light, and oxidation. They were properly labelled for easy identification.

Extraction of oil using Soxhlet Extraction Method for phytochemical Amalysis:

A flask of 250ml was filled with 100ml of n-hexane solvent. 20g of the grounded sample was weighed and inserted into the thimble of the soxhlet apparatus with cotton wool underneath to serve as filter. The apparatus was assembled on the boiling flask of the soxhlet apparatus and allowed to stand on electric hot plate at temperature of 60-75°C, and allow to reflux about 4 times for five repeated extractions. Extract from the flask was collected and emptied into rotatory evaporator at temperature of 40-60°C to separate the n-hexane solvent from the extracted oil. The extracted oil was collected and stored in a reagent bottle for phytochemical analysis with Gas Chromatography.

Procedure for the Phytochemical Analysis using GC-FID:

The analysis of Phytochemical was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector (FID). A RESTEK 15 meter MXT-1 column (15m x 250um x 0.15um) was used. The injector temperature was 280°C with splitless injection of 2ul of sample and a linear velocity of 30cms^{-1} , Helium 5.0 pas was the carrier gas with a flow rate of 40 mlmin⁻¹. The oven operated initially at 200°C, it was heated to 330° C at a rate of 3° C min⁻¹ and was kept at this temperature for 5min, the detector operated at a temperature of 320° C. Fatty acid profile components were determined by the ratio between the area and mass of internal standard and the area of the identified fatty acid profile components. The concentration of the fatty acid profile components expressed in ug/g.

C. Vitamin Determination

> Determination of Vitamin A

• This was Measured According to AOAC, 2010.

One gram (1g) of the sample was weighed into a beaker containing 30ml of absolute alcohol and 3ml of 50% KOH solution mixed and boiled gently for 30minutes under reflux. After washing with distilled water, vitamin A was extracted with 50ml of diethyl ether with the aid of separating funnel. The extract was evaporated to dryness at low temperature and then dissolved in 10ml of isopropyl alcohol. 1ml of standard vitamin A solution was prepared and that of the dissolved extract were transferred to separate curvettes and their respective absorbance were read in UV-spectrophotometer at 325nm with a reagent blank at zero.

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> Determination of Vitamin E

• This was measured according to AOAC, 2010.

1g of the sample was mixed with 10ml of 12% ethanoic sulphuric acid and boiled gently under reflux for 30mins. It was transferred to a separating funnel and treated with 3x30ml diethyl ether, the ether extract was transferred to a dessicator and dried for 30mins and later evaporated to dryness at room temperature. The dried extract dissolved in 10ml of pure ethanol. 1ml of the dissolved extract and equal volume of standard vitamin E were transferred to separate tubes. 5mls of absolute alcohol and 1ml of concentrated nitric acid solution were added, the mixtures were allowed to stand for 5mins and the absorbance was measured in a UV- spectrophotometer at 410nm with blank reagent at zero.

➢ Determination of Vitamin C

• This was measured according to AOAC, 2010.

Two grams (2g) of the sample was homogenized in 6% EDTA solution. The homogenate was filtered and used for analysis. 20ml of 30% KI solution was added to the homogenate followed by 100ml of distilled water. 1ml of 1% starch solution was added to it and it was titrated against 0.1M CUSO₄ solution. The end point was marked by a black coloration. A reagent blank was also titrated.

D. Mineral Determination

Digestion of Sample for Mineral Analysis

Two grams (2g) of the samples (oha and bitter leave) each were measured into a digestion flask containing 20ml of aqua regia (acid mixture, 65ml conc. HNO₃, 8ml perchloric acid and 2ml conc H_2SO_4). The mixture sample in the flask was heated on electric hot plate at temperature of $65^{\circ}C$ until a clear digest was obtained. The digest was unplug, and cool in a desicator. The digest was diluted with distilled water to the 100ml mark, filtered with whatman filter paper and the filtrate stored in a sample vial for mineral assay.

Working Principle of Atomic Absorption Spectrophotometer (AAS)

Atomic absorption spectrometer's working principle is built basically on the sample being aspirated into the flame and atomized when the AAS's light beam from the monochromator is directed through the flame onto the detector that measures the amount of light absorbed by the atomized element in the flame when ignited. Metal elements on their own have distinct characteristic wavelength on the composed source hollow cathode lamp of the element/metals to be analyzed. The amount of energy of the characteristic wavelength absorbed in the flame is directly proportional to the concentration of the element in the tested sample. The sample is aspirated into the oxidizing airacetylene flame. When the aqueous sample is aspirated, the sensitivity for 1% absorption is observed.

III. RESULTS

Phytochemical Composition of the leaves of V. amygdalina and Pterocarpus mildbraedii.

From the result, preview in table 1, the evaluation of the phytochemical content (%) of bitter leaf and oha leaf had significant differences in some parameters. Bitter leaf was high in flavonoid, alkaloid and kaempferol having values of 15.877±0.011, 15.577±0.006 and 13.890±0.004 respectively while the oha leaf was high in kaempferol, saponin and phenol having values of 16.873±0.001, 15.125±0.001 and 14.779±0.001 respectively. The phytochemical content (%) of V. amygdalina in a increasing order showed that flavonoid> alkaloid > kaempferol > tannin > phenol > oxalate > epicatechin > saponin > steroid > terpenoid > quercetin > phytate > anthocyanin while the phytochemical content (%) of Pterocarpus mildbreadi showed that kaempferol > saponin > phenol > flavonoid>alkaloid > epicatechin > oxalate > tannin > quercetin > phytate > anthocyanin > terpenoid > steroid.

Flavonoids have bactericidal, antioxidant, hepatoprotective, cognitive and antiviral properties. Also flavonoids and phenolic compounds synergistically work as potent water soluble anti inflammatory, anti cancer, antioxidants and free radical scavengers, which prevent oxidative cell damage (Okwu, 2004). More so phenolic compound reduce cardiovascular diseases and diabetes.

Saponins impact the immune system and possess cholesterol lowering potential that has been demonstrated in animal and human trials (Gūçlū and Mazza, 2007). The inhibit dental caries and platelet aggregation, they are also used in the treatment of high level of calcium in urine.

Tannins have astringent and bitter taste properties. Tannin was reported to possess anti diarrhoea ability (Amabeoku, 2009). They also have biological antioxidant activity and act as defence against oxidative stress. They have bactericidal, antioxidant, hepatoprotective and antiviral properties. The presence of tannins in these leaves indicates the high nutritional and medicinal features of these vegetables.

According to Ali, (2012), alkaloids are reported to have antitumor, antiviral, antihypertensive, antidepressant, antimicrobial and anti-inflammatory activities. Their presence in these leaves can attest to their use in the management of diseases like high blood pressure.

Phytate have some health benefits like anticancer, antioxidant, hypocholesterol and hypolipidemic effects. (Ali, 2012) reported that the level of phytate can be lowered by processing which is carried out for *V. amygdalina* by washing in several changes of water.

Terpenoids include several sesquiterpene lactones which may be associated with the ability of *V. amygdalina* to regulate blood sugar (Njan *et al.*, 2008). They exhibit anti-bacterial, antiviral, anti-oxidant and analgesic activity. They aid digestion and help in anti-cancer treatment. Some

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terpenoids have special aroma and can give food unique sensory qualities.

Steroids have anti-helminthic and anti-inflammatory properties. It also acts against gastrointestinal disorders (Quasie *et al.*, 2016). Steroidal compounds are of importance and interest in pharmacy due to their relationship with sex hormones.

Phenols protect plants from pathogens, they produce poisons that protect the plants. The presence of phenolic compounds in plants indicates that these plants may be antimicrobial agents. Phenols are used to eliminate bacteria and also used as poisons to burn up parasites (Sofowora, 1993).

Kaempferol has a wide range of pharmacological activities such as antioxidant, anti-inflammatory, anticancer, cardioprotective, neuroprotective, anti-allergic and anti-diabetic properties.

The anthocyanin content of these leaves were all low, this was expected since anthocyanin is associated with nongreen colored fruits and vegetables (Dewanto *et al.*, 2002). It may prevent inflammation and protect against type 2 diabetes, cancer and heart disease.

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Oxalate has positive roles in plants including regulating calcium homeostasis, photosynthesis, postharvest quality, plant protection and metal detoxification.

Epicatechins are known to have potent antioxidant properties, it can help inhibit production and activation of harmful bacteria while allowing beneficial bacteria to flourish. It has the capability to reduce inflammatory responses. It reduces blood pressure and diabetes level.

Quercetin scavenges free radicals which damage cell membranes, tamper with DNA and even cause cell death. It has protective functions against aging. It can help stabilize cells that release histamine in the body. It has antioxidant and anti-inflammatory effects that might reduce swelling, kill cancer cells, control blood sugar and help prevent heart disease.

Table 1: Phytochemical Contents of	Vernonia amvgdalina	(bitter leaf) and Pterocar	<i>pus mildbreadii</i> (oha leaf).

Components (%)	Components (%)Bitter leaf	
Kaempferol	13.890±0.004	16.873±0.001
Anthocyanin	1.660±0.161ª	2.257±0.001
Phenol (Total)	8.410±0.009	14.779±0.001
Oxalate	7.033±0.009	7.451±0.000
Saponin	5.805±0.003	15.125±0.001
Epicatechin	6.875±0.010	8.726±0.001
Flavonoid	15.877±0.011	9.656±0.000
Quercetin	2.902±0.002	3.378±0.000
Alkaloid	15.577±0.006	8.831±0.001
Tannin	9.927±0.001	5.354±0.001
Phytate	2.760±0.003	3.267±0.001
Terpenoid	3.920±0.022	2.214±0.000
Steroid	5.366±0.103ª	2.093±0.000

Values are mean \pm standard deviation of triplicate determination

Values with superscripts are significantly at variance at p<0.05

Vitamin Composition of the leaves of V. amygdalina and P. mildbreadii.

According to the results of the selected vitamins review in Table 2, the leaves of *V. amygdalina* and *P. mildbreadii* contains high amounts of Vitamin A, E and C. Vitamins are a type of micronutrient that cannot be synthesized and must be obtained primarily through food. Vitamins are essential for good health and must be taken into account when accessing nutrition security. From the results, two fat soluble vitamins (Vit. A and E) and one water soluble vitamin (Vit. C) were tested. The vitamin A values of *V. amygdalina* and *P. mildbreadii* were 18.579mg/L and 20.323mg/L respectively. The presence of vitamin A in both leaves makes them good for the maintenance of immune system, good vision and also serves as an antioxidant.

The vitamin E values of *V. amygdalina* and *P. mildbreadii* were 22.120mg/L and 25.918mg/L respectively. Vitamin E is an antioxidant which also helps to form red blood cells and widen blood vessels to keep blood from clotting inside them.

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The vitamin C values of V. amygdalina and P. mildbreadii were 1174.493mg/100 and 1275.235mg/100 respectively. Vitamin C is important for the maintenance of tissues, bones and teeth and helps in protection against scurvy. The vitamin values of V. amygdalina and P. mildbreadii were all high, however, P. mildbreadii was significantly higher.

Mineral Composition of the leaves of V. amygdalina and P. mildbreadii.

From the results of the selected minerals review in table 2, the leaves of *V. amygdalina* and *P. mildbreadii* contains some levels of mineral. Minerals are essential for

basic bodily functions including regulating heartbeat, muscle contractions, movement, and development. The mineral levels of *V. amygdalina* and *P. mildbreadii* revealed significant amounts of magnesium and potassium having higher values of 4.779mg/L, 3.265mg/Land 3.782mg/L, 2.712mg/L respectively. Iron and zinc were not present in the *P. mildbreadii* leaves while copper, iron and sodium were not present in the *V. amygdalina* leaves. The mineral elements of *V. amygdalina* in a decreasing order showed that zinc >manganese >selenium >calcium while the mineral elements of *P. mildbreadii* in a decreasing order showed that sodium >manganese >selenium >copper >calcium.

Parameters	Bitter Leaf	Oha Leaf
Vitamin A (mg/L)	18.579 ±0.07	20.323±0.045
Vitamin E (mg/L)	22.120 ±0.09	25. 918±0.089
Vitamin C (mg/100g)	1174.493	1275.235

Values are mean \pm standard deviation of triplicate determination. Values within the same row are statistically at variance at p<0.05

Parameters (mg/L)	Bitter Leaf	Oha Leaf
Calcium	0.073 ± 0.005	0.009 ± 0.004
Copper	0.000 ± 0.000	0.158 ± 0.004
Manganese	0.403 ± 0.011	$0.381 {\pm} 0.005$
Iron	0.000 ± 0.000	0.000 ± 0.000
Zinc	0.836 ± 0.010	0.000 ± 0.000
Magnesium	4.779 ± 0.010	3.782± 0.011
Sodium	0.000 ± 0.000	1.142 ± 0.010
Potassium	3.265 ± 0.010	2.712 ± 0.004
Selenium	0.209 ± 0.010	0.343 ± 0.010

Table 3: Evaluation Result of the Mineral Content (mg/L) in Bitterleaf and Oha Leaf.

Values are mean \pm standard deviation of triplicate determination Values within the same row are statistically at variance at p<0.05

IV. CONCLUSION

The usefulness of plant products to humans can never be over emphasized hence their nutritional and therapeutic needs. The micronutrient content of *Vernonia amygdalina* and *Pterocarpus mildbreadii* was determined using conventional analytical techniques. The unique nutritional values of *Vernonia amygdalina* (bitter leaf) and *Pterocarpus mildbreadii* (oha leaf) has been demonstrated to contribute to human health's benefit associated with their consumption. These leaves have potential health benefits against many diseases.

The findings of the quantitative phytochemical study on the leaves of *Vernonia amygdalina* and *Pterocarpus mildbraedii* indicated the presence of alkaloid, kaempferol, flavonoid, sapogenin, phenol, tannin, oxalate, epicatechin in high amounts and steroid, phytate, terpenoid, quercetin, kaempferol in low amounts.. These leaves are therapeutic and should be consumed to promote health. *V. amygdalina* is a multipurpose plant which possesses many uses, benefits and bioactivities. The high phytochemical contents found in *Pterocarpus mildbraedii* leaves helps to boost the immune system and can be utilized in pharmaceutical industries for the manufacturing of drugs used in the treatment of various diseases.

Micronutrients are necessary in small amounts to maintain overall healthy state. The evaluation of micronutrients on the leaves of *V. amygdalina* and *P. mildbreadii* showed that the leaves had high mineral and vitamin contents. However, the micronutrient values of oha leaves were higher than that of bitter leaf.

The result of this research will aid to encourage the society on the usage of this leaves due their nutritional value.

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