

Comparative Study for Phytochemical Analysis of Dry Bitter Leaf (*Vernoniaamygdalina Delile*) and Sweet Bitter leaf (*Vernoniahymenolepis*) leave for their Nutritional and Medicinal Benefits.

Imohiosen Ojeaga^{1*}, Elisha Akuki¹ and Samaila Danladi²

¹Department of Science Laboratory Technology, Federal Polytechnic Bali

²Department of Agricultural Technology, Federal Polytechnic Bali

Abstract

Green leafy vegetables together with other components of the plant world are the major source of some phytochemicals (secondary metabolites) that are used by the pharmaceutical and food industries. The focus of this study was to conduct the phytochemical analysis and mineral/elemental composition of Bitter Leaf and Sweet Bitterleaf in Bali as probable justification of their nutritional and medicinal applications. The plant were identified using pertinent taxonomic literature by Garjila (2016). Standard procedures by Trease and Evans (2009), Association of Official Analytical Chemist, Official method of Analysis (AOAC, 2003), Pearson (1976), Lucas and Markakes (1975) and Harbone (1973) were used to analyse the sample for phytochemical compositions. The result of the study shows that Bitter leaf comprise of 13.86% alkaloid, 18.00% flavonoid, 6.00% saponin, 2.34 mg/g oxalate and 16.73% phytate. Sweet Bitterleaf have 8.00% alkaloid, 9.43% flavonoid, 5.77% saponin, 3.60 mg/g oxalate, 40.67% phytate. In conclusion, the two (2) vegetables: Bitter Leaf (*Vernoniaamygdalina Delile*) and Sweet Bitterleaf (*Vernoniahymenolepis*) contain varied and appreciable amount of phytochemical constituents (alkaloid, tannins, flavonoids, saponins and protein). Thus, the vegetables may be use as phytochemical supplements and also useful in the management of various ailment and disorders in human.

Keywords: Phytochemical, Secondary metabolites, Vegetables

Introduction

Most vegetables are commonly used as medicinal plant for the treatment of some diseases. Leafy vegetables contain little fat but high in protein and dietary fibre and they are rich sources of minerals (calcium, potassium and magnesium) and vitamins and high in phytochemicals [1]. Vegetables comprise bioactive compounds which protect the body from nutritional deficiency diseases [2] and free radicals that cause oxidative damage to cells. Vegetables are important foods both from economic and nutritional stand points. Their nutritive significance is the richness in minerals which is very essential in the maintenance of human health [3]. Thus, they are important constituent of healthy diet, if consumed daily in appropriate amounts, could help to prevent major diseases such as cardiovascular diseases and certain cancers [4]. Phytochemicals are chemical compounds produced by plants; generally, to make them thrive or to fight off competitors, predators, or pathogens. Some phytochemicals have been used as poison, and others as traditional medicines. Additionally, these compound have antioxidants, anti-cancer, anti-inflammatory and pain relieving properties. Some examples of phytochemicals are saponins, flavonoids, terpenoids, alkaloids, coumarins, salicintexol e.t.c. [5]. Similarly, Phytochemicals could be grouped into primary and secondary metabolites. Primary metabolites are the classes of food which are protein, carbohydrate, fats and oils, vitamins. Secondary metabolites are often found in disposable parts of plants. These include roots, stems, barks, leaves, flowers, fruits and seeds. Common phytochemicals (secondary metabolites) found in plants include alkaloids, flavonoids, steroids, quinines, tannins, phlobatannins, saponins, terpenoids, cardiac glycosides, sugar etc. [5]. Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism. Thus, play an important role in plant defense against herbivory and other interspecies defences. Human use secondary metabolites as medicines, flavoring and recreational drug [6]. The aim of this study was to determine and

compare the secondary metabolites (alkaloid, flavonoid, saponin, oxalate and phytate) in dry Bitter Leaf (*Vernoniaamygdalina Delile*) and Sweet Bitter leaf (*Vernoniahymenolepis*) leaves for their nutritional and medicinal benefits when consumed.

Literature Reviews

Bitter Leaf (*Vernoniaamygdalina Delile*)

Description of Bitter Leaf (*Vernoniaamygdalina Delile*)

The plant *Vernoniaamygdalina* also known as bitter leaf in English, Shuwaka in Hausa,

Ewuro in Yoruba, Onugbo in Igbo belong to a family asteraceae. It is found in all the geo-political zones of Nigeria. It's a shrub or small tree with a height up to 10m tall, much branched, trunk up to 40 cm in diameter, bark grey to brown smooth, becoming fissured, young branches densely pubescent [7] (Figures 1 & 2).

Taxonomy/Classification of Bitter Leaf (*Vernoniaamygdalina Delile*)

Kingdom: Plantae

Family: Asteraceae

***Corresponding author:** Imohiosen Ojeaga, Department of Science Laboratory Technology, Federal Polytechnic Bali, Tel: 07033187782; E-mail: ojeaga2003@yahoo.com

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Figure 1: Picture of *Vernonia amygdalina Delile*.

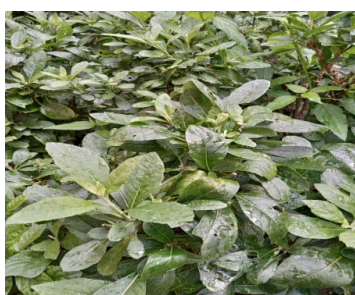


Figure 2: Picture of Sweet Bitterleaf *Vernonia hymenolepis*.

Genus: Vernonia

Species: amygdalina

Botanical name: *Vernonia amygdalina Delile*

Vernacular name: Shuwaka (Hausa)

Nutritional Value and Uses Bitter Leaf (*Vernonia amygdalina Delile*)

The nutritional composition of *Vernonia amygdalina* leaves per 100 g edible portion is water 82.6 g, energy 218 kJ (52 kcal), protein 5.2 g, fat 0.4 g, carbohydrate 10.0 g, fiber 1.5 g, Ca 145 mg. This composition is in line with other dark green leafy vegetables. The bitterness is caused by sesquiterpene. Lactones (e.g. vernoin, vernolepin and vernomydin) and steroid glucosides (vernoinosides). Aqueous extracts of *vernonia amygdalina* leaves exhibit cytostatic action to retard the growth of human breast cancer cells [7].

Bitter leaf is a highly appreciated vegetable in West and Central Africa and can be consumed in various dishes. Additionally, Bitter leaf is commonly used in traditional medicine. Leaf decoctions are used to treat Fever, malaria, diarrhea, dysentery, hepatitis and cough, as laxative and as a fertility inducer. They are also used as a medicine for scabies, headache and stomach-ache. Root extracts are also used as treatment against malaria and gastrointestinal disorders [7].

Sweet Bitter leaf (*Vernonia hymenolepis*)

Description of Sweet Bitter leaf (*Vernonia hymenolepis*)

The plant *Vernonia hymenolepis* also known as sweet bitter leaf in English, belongs to a family asteraceae. It occurs locally wild in mountainous and high plateau regions of West, Central, East and southern Africa. Its cultivation is only known from Nigeria and

Cameroon. *Vernonia hymenolepis* is a perennial herb, shrub or small tree up to 12 m tall; young branches densely tomentose [7].

Taxonomy/Classification of Sweet Bitter leaf (*Vernonia hymenolepis*)

Kingdom: Plantae

Family: Asteraceae

Genus: Vernonia

Species: hymenolepis

Botanical name: *Vernonia hymenolepis*

Vernacular name: Shuwakamaizaki (Hausa)

Nutritional Value and Uses of Sweet Bitter leaf (*Vernonia hymenolepis*)

The nutritional composition of *Vernonia hymenolepis* leaves is comparable to that of bitter leaf (*Vernonia amygdalina*). It is less bitter than other vernonia species. They also contain Beta-carotene: high in leaves; vitamin E: medium; folic acid: medium; ascorbic acid: high; calcium: medium; iron: high; protein: 3.8%. Leaves contain sesquiterpene lactones and steroid glucosides that show antiparasitic and platelet anti-aggregating properties [7]. The leaves of *Vernonia hymenolepis* are consumed fresh and in dry form as a garnish, potherb or salad. Additionally, *Vernonia hymenolepis* is used medicinally as a cure for pneumonia, and a hot leaf placed on a wound is said to stop bleeding. Juice from crushed leaves is used to treat diarrhoea in babies and jaundice. In Kenya a root decoction is used as a purgative and to treat abdominal pains. Dry branches and stems serve as fuel [7].

Phytochemicals

World Health Organization (WHO) define medicinal plant as any plant which, one or more of its organs, contains constituents that can be used for therapeutic purposes, or which are precursors for chemopharmaceutical semi synthesis. Such plant parts including leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds, employed in the control or treatment of a disease condition and therefore contains chemical constituents that are medically active. These non-nutrient plant chemical compounds or bioactive constituents are often referred to as phytochemicals [8-10].

Some Classes of phytochemicals (Secondary Metabolites)

Alkaloids

Alkaloids are basic substances, which contain one or more nitrogen atom(s) in a ring and because of their toxic effects and dramatic physiological activities; they are widely employed in medicine. Alkaloids have marked effect on the central nervous system; examples include cocaine, caffeine, nicotine, belladonna and cocaine. The antimalarial drug, quinine, obtained from the bark of cinchona plant is another example of an alkaloid. The solutions of alkaloids are intensely bitter. Alkaloids have pharmacological applications as anesthetics and CNS stimulants (Madziga *et al.*, 2010; Trease and Evans, 2009).

Flavonoids

Flavonoids also collectively known as vitamin P and citrin (found to be eriodictyol), are a class of plant secondary metabolites or yellow pigments having a structure to that of flavones. Flavonoids (specifically

flavonoids such as the catechins) are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plant. Flavonols, the origin bio-flavonoids such as quercetin, are also found ubiquitously, but in lesser quantities [11-14].

Saponins

According to [15] saponins are recognized by their ability to produce a soapy lather when shaken with water. They are widely distributed in nature and reported to be present in 500 genera of plants. Steroidal saponins are widely distributed in nature and exhibit various biological activities. The aglycone of steroidal saponins is usually a spirostanol or its modification. They are found in oats, peppers, aubergine, tomato seed, alliums, asparagus, yam, fenugreek, yucca and ginseng. In Alkaloids saponins, aglycone carry N atom as a bridge between two rings e.g. solanidine. Saponins are extremely poisonous, as they cause hemolysis of blood and are known to cause cattle poisoning [14]. Saponins possess a bitter and acrid taste, besides causing irritation to mucous membranes and are also necessary for activity of cardiac glycosides [16].

Phenolics

Phenolics also known as phenols or polyphenolics or polyphenol extracts are chemical components that occur ubiquitously as natural colour pigments responsible for the colour of fruits of plants. Plant phenolics are mostly synthesized from phenylalanine via the action of phenylalanine ammonia lyase (PAL). They are very significant to plants and have multiple functions. The most vital role may be in plant defence against pathogens and herbivore predators, and thus are applied in the control of human pathogenic infections [17]. Phenolics basically represent a host of natural antioxidants, used as nutraceuticals, and found in apples, green-tea, and red-wine for their enormous ability to combat cancer and are also thought to prevent heart ailments to an appreciable degree and sometimes are anti-inflammatory agents. Other examples include flavones, rutin, naringin, hesperidin and chlorogenic [14].

Materials and Methods

Sample Collection, Identification and Preparation

The young leaves of the two plants (Bitter Leaf and Sweet Bitterleaf) were bought at Daniya and behind rest house resident, Bali, Bali Local Government Area, Taraba State, Nigeria in the month of October, 2020. The plants were identified using pertinent taxonomic literature by Garjila [7].

The leaves of the two plants were rinsed separately with clean flowing water from the laboratory tap to remove all the foreign materials that was attached to them. The leaves were detached from the stem by hand, the leaves were then dried on the laboratory table of Science Laboratory Technology Department, Federal Polytechnic Bali and each labelled according to the sample number. After drying at room temperature the leaves were milled using pestle and mortar and the powder obtained each were transferred into a clean sample container with same label and were ready for used for the analysis. The phytochemical analysis were carried out in Science Laboratory Technology Department, Federal Polytechnic Bali.

Note: S/No = Sample Reference Number

New Family Name (Old Family Name)

Asteraceae (Composite)

Quantitative Phytochemical Analysis

Alkaloid Determination

Apparatus/Reagents: Weighing balance, Hot Plate, Conical flask, Filter paper, Cren, Acetic acid, Ethanol, Conc. Ammonium hydroxide, 1% Ammonium hydroxide

Procedure

5 g of finely ground powdered sample was weighed in to a conical flask, 50 ml of 10% acetic acid in ethanol was added (10% n acetic acid in ethanol is 10ml of acetic acid into 90 ml of ethanol). It was allowed to stand for 24hours at 28°C and filtered, the filtrate was heated in to a hot plate to one quarter of its original volume by evaporation and the remaining concentrated was treated with drop wise addition of 5 ml (one ammonium hydroxide solution until all the alkaloid was precipitated. A filter paper (W_2) was weighed, the precipitate was filtered through the pre-weighed filter paper and the filter paper was washed while still inside the funnel with 1% ammonia solution and was dried inside the oven at 30°C. It was allowed to cool and the Alkaloid content was calculated as $\frac{W_3 - W_2}{W_1}$

$$\% \text{ Alkaloid} = \frac{W_3 - W_2}{W_1} \times 100 \text{ [18,19].}$$

Flavonoid Determination

Apparatus/Reagents

Weighing balance, Hot plate, Oven, Filter paper, 100 ml Conical flask, 2M HCl

Procedure

2g of sample (W_1) was weighed into conical flask and 50 ml of 2M HCl was added, then boiled for 30minutes and was allowed to cool and filtered. 5 ml of the extract was pipette into another flask and 5ml of ethyl acetate was added starting with a drop to obtain a precipitate. A filter was weighed (W_2) and the precipitate was filtered through the pre-weighed paper and dried in the oven at 30°C and was allowed to cool. The dried filter paper was weighed as (W_3).

$$\% \text{ flavonoid} = \frac{W_3 - W_2}{W_1} \times 100 \text{ [19].}$$

Where W_1 = Weight of sample taken

W_2 = Weight of Pre-weighed filter paper before oven-drying

W_3 = Weight of filter paper after oven-drying

Saponin Determination

Apparatus/Reagent

N-Hexane, Methanol, Hot plate, Fridge, Acetone, Oven, Filter papers

Procedure

1.0 g of the sample (W_1) was weighed into a flask and was De-fatted by adding 30ml of n-hexane, 30ml of methanol was added to the residue and then filtered, for the third time, 30 ml of methanol was added to the residue and then filtered. The concentrate/filtrate was heated to one quarter (1/3) and 100 ml of cold acetone was added. It was then, placed in the fridge for 50minutes and the empty filter paper was weighed as (W_3) and then dried in the oven at 30-40°C. It was allowed to cool and the filter paper was re-weighed after drying (W_3)

$$\% \text{ Saponin} = \frac{W_3 - W_2}{W_1} \times 100 \text{ [18,19].}$$

Where

W_1 =Weight of sample

W_2 =Weight of empty filter paper

W_3 =Weight of filter + residue after drying

Determination of Oxalate

Apparatus/Reagent

Weighing Balance, Water bath, Filter paper, 100ml conical flask, Burette & Pipette, 1.5M H_2SO_4 , 0.1M $KMnO_4$.

Procedure

1.0g of sample (W_1) was weighed. 75 ml of 1.5M H_2SO_4 was added to the sample inside the 100 ml conical flask and was stirred for 1 hour using a magnetic stirrer. The filtrate was filtered using Whatman filter paper and a pipette was used to transfer 25 ml of the extract into another conical flask. 25 ml of the extract when HOT was titrated against 0.1M $KMnO_4$ solution to a faint pink coloured point, the titre values were recorded and the value of oxalate was calculated using the formular below:

$$\text{Oxalate} = (\text{Titre value} \times 0.9004) \text{ mg/g [18].}$$

Determination of Phytate

Apparatus/Reagent

Weighing balance, 100 ml conical flask, filter paper, burette & pipette, 2% HCl, 0.3% Ammonium Thiocyanate (NH_4SCN), 0.005 M ferric chloride ($FeCl_3$).

Procedure

1.0 g of the powdered sample was weighed into a conical flask. 25 ml of 2% HCl was added and soaked for 3hrs. It was then filter through a Whatman filter paper. 25 ml of the extract/filtrate was transferred into a conical flask, 5 ml of 0.3% Ammonium Thiocyanate solution was added and then 53.5 ml of distilled H_2O added. The solution was titrated against 0.005M standard ferric chloride ($FeCl_3$) solution until a reddish brown persists for 5 minutes, the titre values were recorded and % of phytic acid was calculated using the formular below:

$$\% \text{Phytic acid} = \frac{8.24t \times 100}{1000 \times \text{wt of sample}} [19].$$

Results and Discussions

Mean of triplicate determinations \pm standard deviation (SD)

Discussion

Table 1 shows the alkaloid, flavonoid, saponin, oxalate and phytate content in the dry plant samples. The alkaloid content of the dry Bitter Leaf and Sweet Bitterleaf leaves were 13.86% and 8.00% respectively. The amount of alkaloid in both plants is significant, though it is higher in Bitter Leaf compared to Sweet Bitterleaf. Alkaloids occurred to be extremely important for human beings for ages, besides they are secondary metabolites, what could suggest that they are useless. Alkaloids showed strong biological effects on animal and human organisms in very small doses. Alkaloids are present not only in

S/No	Common Name	Scientific Name	Family	Hausa Name
1	Bitter Leaf	<i>Vernonia amygdalina</i>	Asteraceae	Cuwaka
2	Sweet Bitterleaf	<i>Vernonia hymenolepis</i>	Asteraceae	Cuwakamaizaki

Table 1: Identification of Plant Samples.

human daily life in food and drinks but also as stimulant drugs. They showed anti-inflammatory, anticancer, analgesics, local anesthetic and pain relief, neuropharmacologic, antimicrobial, antifungal, and many other activities. Alkaloids are useful as diet ingredients, supplements, and pharmaceuticals, in medicine and in other applications in human life. Alkaloids are also important compounds in organic synthesis for searching new semisynthetic and synthetic compounds with possibly better biological activity than parent compounds [20]. The concentration of flavonoids in Bitter Leaf is higher than that of Sweet Bitterleaf. Both leaves show a reasonable value of 18.00 % and 9.43 % respectively. The amount of flavonoids in food (vegetables) varies, depending on the species. Flavonoids are present in significant amounts in many vegetables; natural antioxidants and flavonoids have been reported as two of the most important micronutrients, which can be used in industry to reduce the use of synthetic compounds on foods and improve health in humans due their potential to decrease several diseases. These bioactive compounds can be used to prolong shelf-life and preserve many foods due to their antimicrobial and antioxidant properties. In addition, the decrease in consumption of vegetables has been associated with several diseases so, it is important to consider the recommended daily intake of these compounds to promote their functions on the organism. Flavonoids attract attention due to their ability to reduce the incidence of many diseases. Its recommended intake or supplementation of flavonoids with a combination of another antioxidants, vitamins and minerals can reduce incidence of cancer, cardiovascular mortality and ischemic vascular disease. According to many studies, in flavonoid-rich foods the daily intake of these compounds can be range between 50 and 800 mg/day, mainly with the consumption of vegetables [21]. The flavonoid contents in the vegetables under study were between the ranges of 50 and 800 mg/day as reported by [21]. Saponins are promoted commercially as dietary supplements and are used in traditional medicine, there is no high-quality clinical evidence that they have any beneficial effect on human health [22]. Quillaja is toxic when consumed in large amounts, involving possible liver damage, gastric pain, diarrhea, or other adverse effects [23]. The saponin content of the Bitter Leaf and Sweet Bitterleaf were 6.00 and 5.77% respectively. Both leaves also show a considerable value of saponin with Bitter Leaf have the higher amount. Similarly, Table 2 shows the concentration of oxalate in dry Bitter Leaf and Sweet Bitterleaf were 2.34 mg/g and 3.60 mg/g respectively. This show that the amount of oxalate in Sweet Bitterleaf is higher compared to bitterleaf and are both within the recommended daily oxalate ingestion of not more than 100 mg/day as reported by [24]. Oxalic acid is an organic compound found in many plants including leafy green vegetables. The body can produce oxalate on its own or obtain it from food. Vitamin C can also be converted into oxalate when metabolized. One of the main health concerns about oxalate is that it can bind to minerals in the gut and prevent the body from absorbing them [25]. Oxalate can bind to minerals to form compounds, including calcium oxalate and iron oxalate. This mostly occurs in the colon, but can also take place in the kidneys and other parts of the urinary tract. The consumption of high-oxalate foods is more likely to pose health problems in those who have an unbalanced diet or those with intestinal malfunction. Thus, a

Parameter	Bitter Leaf	Sweet Bitterleaf
Alkaloid (%)	13.86 \pm 0.02	8.00 \pm 0.02
Flavonoid (%)	18.00 \pm 0.02	9.43 \pm 0.01
Saponin (%)	6.00 \pm 0.05	5.77 \pm 0.04
Oxalate (mg/g)	2.34 \pm 0.01	3.60 \pm 0.02
Phytate (%)	16.73 \pm 0.02	40.67 \pm 0.03

Table 2: The result of quantitative phytochemical analysis of dry Bitter leaf and Sweet Bitterleaf leave.

diet high in oxalate and low in essential minerals, such as calcium and iron, is not recommended. Calcium oxalate stone patients should be encouraged to avoid these oxalate-rich foods and herbs, because the consumption of even small amounts may exceed the recommended daily oxalate ingestion of no more than 100 mg [24]. The phytate content of the samples varies from 16.73 % – 40.67 % bitterleaf have the least value whereas Sweet Bitterleaf the higher value. The estimated intake of phytic acid (PA) is 2100 mg/day respectively as reported by [26] which is slightly above the values of vegetable under study. Moreso, the PA in foods is expected to increase due to climate change, and may further deplete some essential minerals in rice grain. As such, micronutrient deficiencies are also expected to increase in the developing countries. For this reason, there is need for people from this region (developing countries) to improve on their dietary pattern in addition to nutrition education.

Conclusion

Leafy vegetables are foods from plant leaves with different uses and are good source of phytochemical (bioactive components) that are involved in pharmacodynamics activity. Thus, they are vital sources of supplementary diets of several bioproducts essential for the biochemical functions in human, provide health benefits and are important for the prevention of illnesses. Eating the recommended amount of vegetables each day can reduce the risk of chronic disease.

This study revealed that Bitter Leaf (*Vernoniaamygdalina Delile*) and Sweet Bitterleaf (*Vernoniahymenolepis*) contain varies and considerable amount of secondary metabolites (alkaloid, flavonoid, saponin, oxalate and phytate) in their leaves. The phytochemical constituents in the vegetables have a favorable effect on the human body because they regulate metabolism in general, stimulate the muscular and skeletal systems, internal glands, and enzymatic activity. Thus, the vegetables may be use as supplements and also useful in the management of various aliment and disorders in human.

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