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Comparative Study of the Phytochemical and Nutritional Composition of Bitter Leaf (*Vernonia Amygdalina*) and Bitter Gourds Leaf (*Momordica Charantia*)

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Abstract*: In this study, the phytochemicals and nutritional makeup of bitter and bitter gourd leaves are compared. Numerous conventional procedures were used to determine qualitative and quantitative phytochemical analyses, as well as proximate analyses. During the qualitative phytochemical evaluation, alkaloids, flavanoids, saponin, phenol, and tannin were discovered in the plants tested in this study. According to a quantitative study, bitter leaf has the largest concentration of alkaloids and flavanoids. According to the findings of this study, bitter leaf had nutritional compositions of 31.1±2.04, 8.1±1.38, 12.2±1.7, 6.96±2.29, 91.6±3.42, and 38.07±5.2 for crude protein, crude fat, ash content, moisture content, dry matter, and carbohydrate, respectively. In addition, protein, crude fat, ash content, moisture content, dry matter, and carbohydrates were found in bitter gourd leaves with nutrition compositions of 2.13±0.4, 0.61±0.23, 0.89±0.11, 91.9±1.76, 83.7±5.78, and 7.40±0.62, respectively. Aside from being a good source of pharmacologically active photochemicals, medicinal plants can also be used as food supplements for humans and animals.*

Keywords: Phytochemicals, Bitter leaf, bitter gourd leaf, Nutritional composition

1. INTRODUCTION

Plants produce a variety of chemical substances for biological purposes, such as protection against insects, fungi, and herbivorous mammals. So far, at least 12,000 such compounds have been isolated, accounting for less than 10% of total chemical compounds in plants. These compounds mediate their effects on the human body through processes that are similar to those already well understood for chemical compounds in conventional drugs. Thus, herbal medicines work similarly to conventional drugs. This allows herbal medicines to have therapeutic pharmacology, but it also means that they have the same potential for causing undesirable side effects as conventional pharmaceutical (Tapsell *et al*., 2006). Furthermore, plant material contains a number of chemicals that can have unfavorable effects; these can be decreased through processing.

Plants have been used as medicines since before written history. Ethnobotany, or the study of traditional human usage of plants, is a proven method for discovering new medicines. In 2001, researchers discovered 122 chemicals utilized in contemporary medicine that were obtained from traditional plant sources; 80 percent of the plant's active ingredients were discovered to be derived from traditional plant sources (Fabricant and Farnsworth, 2001). Aspirin, digoxin, quinine, and opium are just a few of the medications currently available to doctors that are derived from plants that have a long history of usage as herbal remedies. Herbs are widely used to treat sickness in non-industrialized countries.

In 2012, the global export value of medicinal plants was more than US $2.2 billion (MedicinalPlants.Traffic.org.). Throughout history, human beings have relied on nature for their basic needs, such as medicine, shelters, food, scents, clothing, tastes, fertilizers, and modes of transportation. Medicinal plants continue to play a significant role in the health-care system for huge segments of the world's population, particularly in poorer countries where herbal medicine has a long history of use. In both developed and emerging countries, the development and acknowledgement of the medical and financial benefits of these plants is increasing (WHO, 1998).

Medicinal plants include a wide range of natural antioxidants and are used to cure a variety of diseases all over the world. Antimicrobial (Sharafati *et al*., 2011), anti-cancer (Shirzad *et al*., 2012), anti-diabetic (Kazemi *et al*., 2010), anti-atherosclerosis (Khosravi *et al*., 2011), immunomodulatory (Shirzad *et al*., 2009), and even reno-protection or hepatoprotective benefits are just few of these capabilities. There has recently been a lot of interest in finding natural antioxidants from plant sources because of the

beneficial effects of antioxidants, especially natural antioxidants, in the treatment and prevention of diseases. The majority of medicinal plants have significant antioxidant properties, according to studies.

Tracing the evolution of medicinal plants in phytotherapy is akin to tracing the evolution of humanity. The discovery of certain plants' curative properties must have been instinctive. Plants must have been investigated as food sources first. A link with some plant properties must have been established as a result of food ingestion (Mendonça-Filho, 2006). For thousands of years, medicinal plants have been used to treat a variety of diseases. From ancient times, terrestrial plants have been used as medicines in Egypt, China, India, and Greece, and an impressive number of modern drugs have been developed from them. The Sumerians and Akkaidians left the first written records on medicinal plant uses around 2600 BC, describing the well-established medicinal uses of laurel, caraway, and thyme.

Until the nineteenth century, medicinal plants were the primary source of products used to maintain health. Friedrich Wohler, a German chemist, accidentally synthesized urea while attempting to make ammonium cyanide from silver cyanide and ammonium chloride in 1828. According to Mendonça-Filho (2006), this was the world's first organic synthesis, ushering in the era of synthetic compounds.

Plants contain a variety of chemical compounds that are necessary for biological functions such as defense against insects, fungi, and herbivorous mammals, as well as treatment of various infectious diseases in humans. Aspirin, digoxin, quinine, and opium are just a few of the medications currently available to doctors that are derived from plants that have a long history of usage as herbal remedies. Herbs are widely used to treat sickness in non-industrialized countries.

Medicinal plants contain a wide range of natural antioxidants and phytochemicals that are used to treat diseases all over the world (Rafieian and Baradaran, 2013). Some of these properties include antimicrobial (Sharafati *et al*., 2011), anti-cancer (Shirzad *et al*., 2012), anti-diabetic (Kazemi *et al*., 2010), anti-atherosclerosis (Khosravi *et al*., 2019).

Vernonia amygdalina is used as a therapeutic agent in traditional medicine, and it has a significant impact on the health of individuals and communities (Ojiako *et al*., 2006). Its medicinal value is derived from phytochemicals, which have specific physiological effects in the human body. Alkaloids, tannins, flavonoids, and phenolic compounds are among the plant chemicals. Every part of vernonia amygdalina contains pharmacologically useful active components such as anthraquinones, steroids, and cardiac glycosides. The leaves are green and have a distinct odor and bitter flavor. In ethnomedicine, the roots and leaves are used to cure fever, hiccups, renal issues, vomiting, intestinal disease, and stomach discomfort as antibacterial, active cancer, anti-parasitic, and anti-malarial agents (Ebong *et al*., 2011).

In order to determine their comparative analysis, this study profiled and quantified the nutritional, phytochemical composition, and antibacterial properties of an aqueous extract of bitter, scent, neem, and bitter gourd leaves, as well as discussed the bioactivities of the most abundant of the detected compounds. Traditional medicine made from this plant isn't commonly used, and there's a lack of awareness among the general public. This is due to the fact that the general populace continues to take synthetic medicines despite being aware of their side effects. The majority of the plant's photochemical compounds contain chemotherapeutic agents that can assist in safeguarding or avoiding diseases like diabetes and cancer. The leaves of these herb species have been the subject of the majority of research. The study's main goal is to assess a comparative analysis of bitter and bitter gourd leaves based on nutritional and phytochemical parameters.

2. METHODOLOGY

2.1 Sample collection and preparation

Fresh bitter and bitter gourd leaves were collected from parent trees on farms in Ilesha, Osun State, and transported to the laboratory, where they were washed and cut to eliminate dirt.

2.2 Nutritional Composition

Proximate nutrient composition analysis was performed on the freshly manufactured samples, with crude protein, crude fat, ash content, moisture content, dry matter, and carbohydrate being the components examined. The Association of Official Analytical Chemists (AOAC, 2002) had already outlined how to do this.

2.2.1 Crude Protein

The micro-Kjedahl method was used to accomplish this. Using copper sulphate as a catalyst, the nitrogen component of the protein in 5 g of the sample was transformed into ammonium sulphate by digestion with concentrated hydrogen tetraoxosulphate (VI) acid. The ammonia was collected in a boric acid double indicator solution, and nitrogen was measured using a normal hydrochloric acid titration until the end point was achieved. After that, a factor of 6.25 was used to calculate the amount of crude protein.

2.2.2 Crude Fat

5 g of plant materials, petroleum ether, and a soxhlet extractor device were used to extract crude fat from the sample. The crude fat in the samples was calculated using the weight of the fat obtained after evaporating the petroleum ether from the extract, and this was stated as a percentage.

2.2.3 Ash Content
To remove organic components, five grams of the material were put in a crucible and heated to 550°C. After cooling and weighing the crucible and its contents, the ash was calculated as a percentage of the original dry weight of the samples.

2.2.4 Moisture
The ground sample was weighed exactly 5 g each and oven dried at a constant temperature of 70°C. After cold weighing, the amount of moisture in the sample was reported as a loss in weight.

2.2.5 Dry Matter
The fibre content of samples was determined using five grams of defatted samples extracted by acid digestion, filtration, and base digestion. At 550°C, the resultant leftovers were eventually ignited. Fibre content was then represented as a proportion of initial weight loss after ashing

2.2.6 Carbohydrate
The difference between 100 and the total of crude protein, fat, ash, and fibre was then used to calculate the amount of carbohydrate in the sample.

2.3 Qualitative Analysis of the Phytochemicals

2.3.1 Test for Alkaloids
In a steam bath, around 0.5g of each extract was mixed with 5ml of 1% aqueous hydrochloric acid; 1ml of the filtrate was treated with a few drops of Mayer's reagent, and another 1ml portion was treated similarly with Draggendorff's reagent. As preliminary evidence for the presence of alkaloids in the extracts, turbidity or precipitation with either of these reagents was detected (Trease and Evans, 1989).

2.3.2 Test for Flavonoids
A portion of the aqueous filtrate of plant extract was treated with 5 ml of diluted ammonia solution, followed by concentrated sulphuric acid. The presence of flavonoids is indicated by a yellow hue.

2.3.3. Test for Saponins
In a water bath, about 2 g of powdered sample was cooked in 20 ml of distilled water, and the filtrate was mixed with 5 ml of distilled water and rapidly shaken for stable persistent foam. The foaming was combined with three drops of olive oil and vigorously shaken. Saponins are present in the emulsion formation.

2.3.4. Test for phenols by ferric chloride test
A few drops of a neutral 5% ferric chloride solution were added to 50 mg of extract dissolved in 5 ml of distilled water for each sample. The presence of phenolic compounds was indicated by a dark green color (Mace, 1963).

2.3.5 Test for Tannins
In a test tube, about 0.5 gram of dried powdered sample was cooked in 20 ml of water and then filtered before adding a few drops of 0.1 percent ferric chloride. The presence of tannins is indicated by the presence of a brownish green color.

2.4. Quantitative Analysis of the Phytochemicals
2.4.1. Estimation of Alkaloids
In a 250 mL beaker, 5 g of the sample was weighed. Then 200 mL of acetic acid in ethanol (10%) was added and left to stand for 4 hours. The extract was then filtered and concentrated to one-quarter of its original volume in a water bath. To produce precipitation, concentrated ammonium hydroxide was applied to the extract drop by drop. The entire solution was allowed to settle, and the precipitate was collected and filtered after being washed with diluted ammonium hydroxide. The residue is the dried and weighed alkaloid (Harborne and Baxter, 1983).
Formula = B - A × 100 / S
Where,
B = Weight of Whatman filter paper.
A = Weight of Whatman filter paper, after drying.

S = Sample weight.

2.4.2 Estimation of Total Flavonoids

The volume was made up to 100 ml with distilled water after 100 mg of tannic acid has been dissolved in a small amount of distilled water. By diluting the standard with distilled water, different concentrations of the standard were achieved (Chun, 2005). The solution's concentration was 100 mg/mL. At zero time, 0.5 ml of aqueous extract sample was diluted with 3.5 ml of distilled water. The tubes were filled with 0.3 mL of 5% sodium nitrate. After five minutes, all of the tubes received 0.3 mL of 10% aluminum chloride. 2 ml of 1 M sodium hydroxide was added to the mixture on the sixth minute. The contents of the reaction mixture were immediately diluted with 2.4 mL of distilled water and properly stirred. The mixture's absorbance was immediately measured at 510 nm in comparison to a prepared blank. Total flavonoids were measured in mg per 100g of edible part, using tannic acid as a reference ingredient.

2.4.3. Estimation of Saponins

A conical flask containing 100 ml of 20% aqueous ethanol was filled with 20 g of sample. At roughly 55°C, the sample was heated for four hours in a hot water bath with constant stirring (Obadoni and Ochuko, 2001). The residue was re-extracted with another 200 mL of 20% ethanol after the mixture was filtered. Over a water bath at roughly 90°C, the combined extract was reduced to 40 mL. The concentrated solution was poured into a 250 mL separator funnel along with 20 mL of diethyl ether and rapidly shaken.
The aqueous layer was kept, while the ether layer was discarded, and the purification procedure was repeated. After that, 60 milliliters of n-butanol extract were added. The extracted n-butanol will be rinsed twice with 10 mL of aqueous sodium chloride each time. In a water bath, the residual solution was heated. The sample was dried in the oven to a constant weight after evaporation. The percentage of saponins was computed.
Formula = B – A × 100 / S
Where,
B = Weight of Whatmann filter paper.
A = Weight of Whatmann filter paper with sample.
S = Sample weight.

2.4.4. Estimation of Phenols

In the test tubes, 0.5 mL of freshly prepared was taken. All of the tubes received 8 mL of distilled water. Folin's Ciocalteau reagent (0.5 mL) was also added to each tube (Malick and Singh, 1980). All of the tubes were kept in B.O.D for a 10-minute incubation period at 40°C. The sodium carbonate solution was then added to each test tube at a volume of 1 mL. After that, the tubes were put in the dark for one hour to incubate. At 660 nm, the color formed was spectrophotometrically read. Tannic acid was used to draw the standard curve. In a Shimadzu UV-1650 spectrophotometer, the O.D. was read at 660 nm for different amounts of tannic acid. The standard curve was used to compute the sample concentrations.

2.4.5 Estimation of Tannins

100 mg of tannic acid was dissolved in 100 ml of distilled water. 5 ml of stock solution was diluted to 100 ml with distilled water. 1 ml containing 50 µg tannic acid (Robert, 1971).
Extraction of Tannin: 0.5 gm of the powdered material was weighed and transferred to a 250 ml conical flask and 75 ml water was added. The flask was heat gently and boiled for 30 min centrifuge at 2,000 rpm for 20 min and the supernatant was collected in 100 ml volumetric flask and make up the volume. 1 ml of the sample extract was transferred to 100 ml volumetric flask containing 75 ml water. 5 ml of folin denis reagent, 10 ml of sodium carbonate solution were added and diluted to 100 ml with water. Shake well. The absorbance was read at 700 nm after 30 min. If absorbance is greater than 0.7 make a 1 + 4 dilution of the sample. A blank was prepared with water instead of the sample. A standard graph was prepared by using 100 mg tannic acid.
The tannins content of the sample was calculated as tannic acid equivalents from the standard graph.

2.5 Statistical analysis

Data are expressed as mean ± standard deviation (SD) of triplicate

3. RESULT AND DISCUSSION

3.1. RESULT

3.1.1 Qualitative Phytochemical analysis of Bitter and Bitter gourds leaves

Five different phytochemicals such as Alkaloids, flavanoids, Saponin, Phenol and Tannin were examined in bitter and bitter gourd leaves. Bitter leaf had saponin in large quantity (+++), alkaloids, flavanoid and tannin in moderate level (++) while phenol is absent

(-) (Table 1). Bitter gourd leaf contains alkaloids, flavanoids and phenol in slight quantity (+) while saponin and tannin are not detected (Table 1).

3.1.2 Quantitative Photochemical property of Bitter and bitter gourd leaves

Figure 4.1 shows Quantitative Phytochemical properties of bitter and bitter gourd leaves. For alkaloids, bitter leaves had higher concentration (6.57±0.28), follow by bitter gourd leaf (4.04±0.04) (Figure 1). The highest quantity of flavanoid was found in bitter leaf (3.07±012), follow bitter gourd leaf (2.99±0.07) (Figure 1). The quantity of saponin is very high in bitter leaf (4.41±0.03). However, no quantity was recorded in bitter gourd leaf for saponin (Figure 1). Bitter gourd leaf had highest quantity of phenol (2.19±0.03) (Figure 1). The quantities of phenol present in bitter leaf (0.05±0.01) are very low (Figure 1). Bitter leaf recorded highest quantity (3.15±0.61) of tannin, while no quantity was recorded for bitter gourd leaf (Figure 1).

Table 1: Qualitative Phytochemical analysis of the extract of bitter leaf and Bitter gourd leaf (*Momordica charantia*) (mg/100 g)

S/N	Phytochemicals	Bitter Leaf	Bitter Gourd leaf
1	Alkaloids	++	+
2	Flavanoid	++	+
3	Saponin	+++	-
4	Phenol	-	+
5	Tannin	++	-

Key: (-) = Not detected; (+) = Slightly detected; (++)= Moderately present; (+++)= Largely present

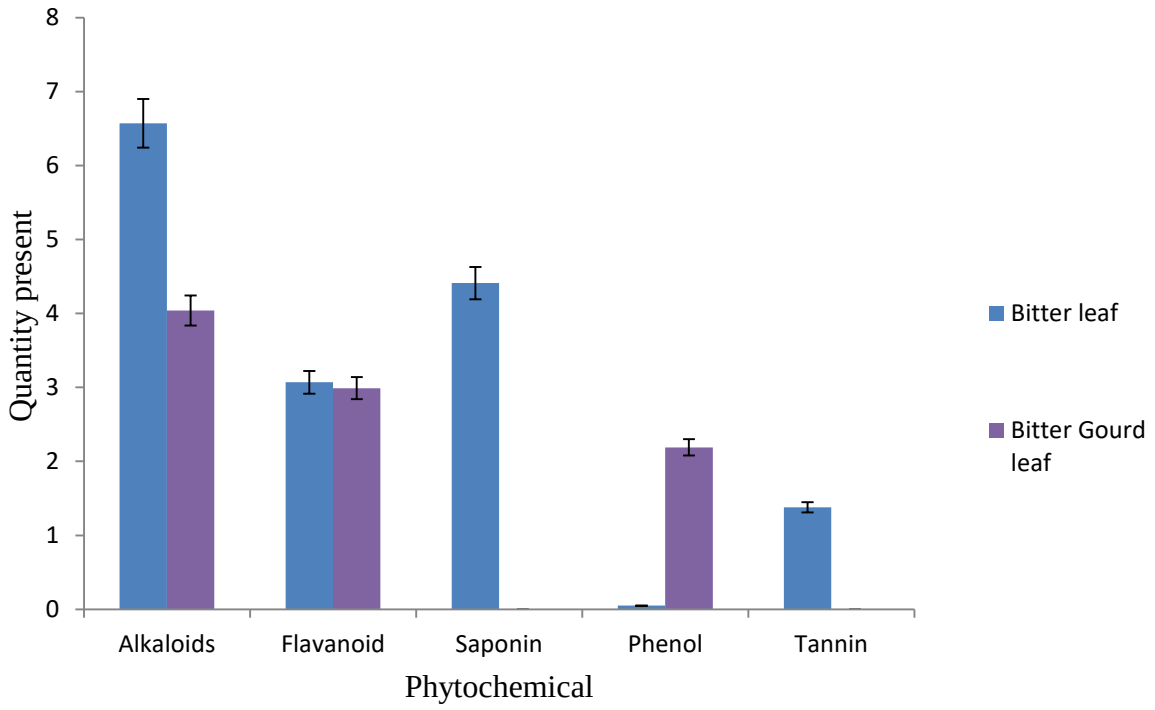

Figure 1: Quantitative Photochemical properties of bitter and bitter gourd leaf leaves

3.1.3 Nutritional composition of Bitter and bitter gourd leaves

Nutrients examined in above study include crude protein, crude fat, ash content, moisture content, dry matter and carbohydrate. Nutritional constituents of bitter leaf and bitter gourd leaf are shown in Table 2.

The results in this study show that bitter had nutritional composition of 31.1±2.04, 8.1±1.38, 12.2±1.79, 6.96±2.29, 91.6±3.42 and 38.07±5.2 for crude protein, crude fat, ash content, moisture content, dry matter and carbohydrate respectively (Table 2). Bitter gourd leaf had protein, crude fat, ash content, moisture content, dry matter and carbohydrate as nutrition composition with 2.13±0.4, 0.61±0.23, 0.89±0.11, 91.9±1.76, 83.7±5.78 and 7.40±0.62 respectively (Table 2).

The highest crude protein, crude fat and ash content was recorded by bitter leaf (31.1±2.04, 8.1±1.38 and 12.2±1.79) while bitter gourd leaf had the least quantities (2.13±0.5, 0.61±0.23 and 0.89±0.11) (Table 4.2). The moisture content recorded in this study is

highest in bitter gourd leaf (91.9±1.76) while least quantity was observed in bitter leaf (6.96±2.29). The quantity of dry matter is very high in bitter leaf (91.6±3.42). The carbohydrate constituent of bitter leaf and bitter gourd leaf are 38.07±5.2 and 7.40±0.63 respectively (Table 2).

Table 2: Nutritional composition of Bitter and Bitter gourd leaves

S/N	Phytochemical	Bitter leaf	Bitter Gourd leaf
1	Crude protein	31.1±2.04	2.13±0.04
2	Crude fat	8.1±1.38	0.61±0.23
3	Ash content	12.2±1.79	0.89±0.11
4	Moisture content	6.96±2.29	91.9±1.76
5	Dry matter	91.6±3.42	83.7±5.78
6	Carbohydrate	38.07±5.2	7.40±0.62

3.2 Discussion

Alkaloids, flavanoids, saponin, phenol, and tannin were found in the plants studied. Bitter leaf has the highest concentration of alkaloids and flavanoids, according to the quantitative study. The plants studied in this study are employed as medicinal agents in traditional medicine and have an essential role in the health of individuals and communities (Ojiako *et al*., 2006). The therapeutic efficacy of these plants is derived from phytochemical elements that have specific physiological effects in the human body. Alkaloids, tannins, flavonoids, saponin, and phenolic compounds are among the plant chemicals. Every section of the plant includes a complex active component that is pharmacologically beneficial (Georgewill *et al*., 2010). The leaves are green and have a distinct odor and bitter flavor. In ethnomedicine, the roots and leaves are used to cure fever, hiccups, renal issues, vomiting, intestinal disease, and stomach discomfort as antibacterial, active cancer, anti-parasitic, and anti-malarial agents (Ebong *et al*., 2011). According to the nutritional composition of the plants, the bitter leaf had the highest crude protein, crude fat, and ash content.

4. CONCLUSION

The presence of phytochemicals such as alkaloids, flavanoids, saponins, phenol, and tannin in various amounts is revealed by qualitative and quantitative phytochemical analysis of *Vernonia amygdalina* and bitter gourd leaf. *Vernonia amygdalina* has the most alkaloids and flavanoids, outnumbering other plants including fragrance, neem, and bitter gourd leaves.

Crude protein, crude fat, ash content, moisture content, dry matter, and carbohydrate were found in various amounts in *Vernonia amygdalina* and bitter gourd leaf nutritional composition analyses. In comparison to bitter gourd leaf, bitter leaf had the highest quantity of crude protein, crude fat, and ash. The usage of *V. amygdalina* is strongly advised. Protein found in higher amounts in *V. amygdalina* is used to improve macromolecule penetration through cell membranes, indicating the importance of V. amygdalina use. Bitter gourd leaf and scent leaf have higher moisture and carbohydrate content, respectively. Because of their photochemical, therapeutic, and culinary properties, these plants are recommended for use.

Plants have played a significant influence in medicine. They are a key source of most medications used to treat human and plant illnesses. The plants employed in this study were discovered to contain a key component needed to fight illness in humans. Plants should be regarded in traditional medicine for treating many ailments, based on the current revelations in the above work and the identification of key phytochemical substances.

This research also shows that they can be used as supplements in human and animal diet, in addition to being a good source of pharmacologically active phytochemicals. As a result, the plants should be considered for use in the locations mentioned.

This research reveals that bitter and bitter gourd leaves are high in phytochemical elements, and that using them for animal and human health is a smart idea. Free radical scavenging compounds such as tannins, vitamins, alkaloids, phenolic acids, flavonoids, and other metabolites are abundant in bitter and bitter gourd and fragrance leaves, and are useful in antioxidant activities. It is important to note that these plant elements can be genetically modified to increase yield and improve quality.

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