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ORIGINAL RESEARCH ARTICLE

Analysis of Proximate, Mineral and Phytochemical Composition of Fresh and Dry *Vernonia amygdalina* (Bitter Leaf) in Bida Metropolis, Niger State

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ABSTRACT
Vernonia amygdalina is tropical shrub with high biomass yield which is used to prepare several dishes, it is also utilized as herb for health improvement. This study was carried out to determine, the proximate, mineral and phytochemicals composition of fresh and dry bitter leaf (*Vernonia amygdalina*) leaves in Bida metropolis. The *Vernonia amygdalina* leaf samples were evaluated using standard procedures. The result of the proximate analysis show that percentage dry matter and moisture content shows significant ($p<0.05$) difference between the samples. The percentage ash content and the crude fiber of both samples does not differ significantly ($p>0.05$). The mean percentage of crude protein, crude fat and carbohydrate were significantly ($p<0.05$) higher in the dry bitter leaf when compared with the values of the fresh bitter leaf. The mineral composition shows Sodium (0.07 ± 0.00 and 0.08 ± 0.00), potassium (0.04 ± 0.00 and 0.06 ± 0.00), calcium content (0.20 ± 0.03 and 0.47 ± 0.02), magnesium (1.33 ± 0.10 and 0.98 ± 0.00) and Phosphorus (2.11 ± 0.17 and 2.18 ± 3.41). The phytochemical analysis shows that flavonoid value was significantly ($p<0.05$) higher in the fresh bitter leaf sample than the dry bitter leave sample. The dry bitter leaf sample significantly ($p<0.05$) recorded high value in alkaloid, oxalate and cyanide when compares to the values obtain from the fresh bitter leaf sample. Both the dry and fresh better leaf sample did not differ significantly in term of phytate and tannin composition. According to this study, the leaves of *Vernonia amygdalina*, both fresh and dry, have varying and significant amounts of secondary metabolites (alkaloid, flavonoid, cyanide, oxalate, and phytate), mineral elements (sodium, potassium, calcium, magnesium, and Phosphorus), and proximate compositions (moisture, ash, crude fat, crude fibre, protein, and carbohydrate). The outcomes demonstrate the significant health benefits of the two leaves.

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Bitter leaf, proximate, mineral, phytochemical composition, Bida

INTRODUCTION

Numerous studies conducted in Africa have revealed that a large variety of native plants are important to the nutrition of the people living there (Appiah, 2018). According to Egedigwe (2010), plants are the most affordable and readily available sources of critical nutrients, providing the body with energy, protein, minerals, vitamins, and some hormone precursors. According to Ijeh (2011), medicinal plants have ingredients that can be used as medicine or for therapeutic purposes. Most tropical nations are endowed with a wide variety of foods essential for proper nutrition and physical growth. Regretfully, 789 million people are estimated to be malnourished in developing nations, with newborns and children living in rural areas being the most affected (FAO, 2015).

In Africa, there has been a recent surge in interest in using herbs for health benefits. Although they were among the first functional foods, herbs have largely been forgotten in the contemporary, westernised diet. The utilization of culinary herbs to improve food flavour and taste and as a source of dietary medicine has made them just as valuable today as they were in the past (Appiah, 2018). The main causes of these herbs' underutilization are ignorance of their nutritional qualities and the existence of certain phytochemicals. So many people consume vegetables for their flavours and tastes, rather than their nutritional value (Egedigwe, 2010).

Indigenous knowledge of people in any given location is derived from the traditional utilization of plant extracts as a source of herbal preparations to relieve several sicknesses or diseases. These preparations are based on

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rudimentary knowledge and experiences passed down from generation to generation, typically by oral tradition and practice (FAO, 2015). The majority of these herbal medicines are well-known to the elderly men and women of rural families as well as our traditional leaders. Almost every part of the plant is utilised to make regional medications (Adamu et al. 2021). This study focuses on the bioactive components and minerals found in the bitter leaf of Vernonia amygdalina.

Traditional medical practices worldwide employ various medicinal plants to treat diabetes (FAO, 2015). One example of such a plant is the shrub *Vernonia amygdalina*, whose morphology has been characterized and has a characteristic bitter flavour. Among many other things, the roots and leaves cure fever, hiccups, kidney issues, and stomach pain. Stem, bark, root, and leaf extracts are used as antimalarials, purgatives, and treatments for eczema (Ijeh, 2011).

The world is full of natural and unusual medicinal plants, which are gaining more interest than ever due to their potential for numerous advantages for society and all of humanity, both pharmacologically and in terms of flora. These plants' therapeutic qualities stem from their (bioactive) phytochemical components, which have specific physiological effects on humans (Pieroni and Andrea, 2005). Bitter leaf has several therapeutic uses and advantages for a healthy lifestyle. Due to its antibacterial and antifungal characteristics, the leaf can be used as a useful at-home treatment for various illnesses, including diarrhoea, dysentery, high blood pressure, and many others (Rious, 2015).

The bitter flavour of bitter leaf may not appeal to everyone, but the health advantages of bitter leaf, which are discussed in the sections below, will likely influence some people's opinions of the leaf (Pozderec et al., 2018). Flavonoids and other plant components with nutritional benefits that support general health can be found in bitter leaves. The most significant bioactive (phytochemical) components include tannins, terpenoids, alkaloids, flavonoids, essential oils, saponins, phenolic compounds, and many others. These organic substances were the basis for contemporary prescription medications (FAO, 2015). Okeke et al. (2015), Farombi and Owoeye (2011) and Toyang and Verpoorte (2013), amongst others, conducted studies on proximate and phytochemical composition of *Vernonia amygdalina* in Nigeria. However, there is very little to no information on the Proximate and phytochemical composition of *Vernonia amygdalina* in Bida metropolis, in Niger State Nigeria.

The objective of this study was to determine the differences in the phytochemical, mineral, and approximate contents in fresh and dry *Vernonia amygdalina* (bitter leaf) leaves in Bida Metropolis, Niger State and also provide information on the samples (dry and fresh) with the best nutritional value based on the research.

MATERIALS AND METHODS

Collection of plant samples and identification

Vernonia amygdalina Del. (Bitter leaf) plant part (leaves) was collected from the Dutch-ville garden opposite Federal Polytechnic Bida, small gate, according to Babagana *et al.*, 2021. After that, the plant samples were transported to the Laboratory of the Department of Biological Science, Federal Polytechnic Bida, Niger State, Nigeria for identification and analysis.

Preparation of the plant materials

The plant materials were prepared according to Usunobun and Okolie (2016).

The leaves were washed under running tap water, and dried at room temperature in the Laboratory for one week. Mortar, pestle, and Miller electric grinder were used to pulverize the plants' parts and sieved using a 2mm mesh sieve to obtain a fine powder and stored in airtight bottles protected from sunlight for Proximate, Mineral and Phytochemical analysis. The fresh bitter leaves were sorted, de-stalked and rinsed in water to remove dust and dirt, and were left to drained and used for further analysis (Proximate, Mineral and Phytochemical).

Extraction of the plant leaves

The plant leaves were extracted according to Usunobun and Okolie (2016).

Vernonia amygdalina leaves, both fresh and dried, were prepared for ethanol extraction by soaking 400g of the plant's leaves, both fresh and dried, in 1000 ml of 100% ethanol for 48 hours at room temperature. After that, the extract was filtered twice: once through cotton wool and once through Whatmann filter paper No. 42 (125 mm). The extracts were then concentrated using a freeze dryer, a rotary evaporator, and a water bath set at 40ºC to a tenth of its initial volume. At 4ºC, the dry residue was after that kept. For experimental examination, portions of the residue from the crude plant extracts were weighed and dissolved in distilled water.

Determination of proximate analysis

Using the standard procedures of the Association of Official Analytical Chemists (2002), the dry matter of both fresh and dry Vernonia amygdalina leaves was determined, along with the contents of moisture, ash, crude fat, crude protein (nitrogen x 6.25) and crude fibre. The carbohydrate content was computed using the net difference between the other nutrients and the total percentage composition.

Determination of Mineral Analysis

The digested samples were analysed for minerals using their different methods of analysis. A flame photometer (Jenway Ltd, Dunmow Essex UK) was used to determine calcium, magnesium, potassium and sodium contents in the samples (Mgbemena et al., 2019).

Determination of Phytochemical Analysis

The phytochemical screening of the extracts of both fresh and dry *Vernonia amygdalina* leaves for secondary metabolites such as tannins, alkaloids, flavonoids, phytate, oxalate and cyanide were carried out following the standard procedures according to the methods described by Abdullahi (2012).

Check for tannins.

In a test tube, roughly 0.5g of the extract was heated in 10 ml of water and filtered. After adding a few drops of 0.1% ferric chloride, the solution's colour was checked for blue-black or brownish-green.

Check for flavonoids

A small amount of the extract's aqueous filtrate was mixed with five millilitres of diluted ammonia. One millilitre of strong sulfuric acid was then added. A yellow colouring indicated the presence of flavonoids.

Check for alkaloids

After dissolving the extract in diluted HCl, it was filtered. Potassium mercuric iodide, often known as Mayer's reagent, was applied to the filtrates. When an ammonia-colored precipitate forms, alkaloids are present.

Check for Phytate Content

A 250 ml conical flask was filled with 2g of the material. The sample was soaked in 100ml of 2% concentrated HCl for three hours, and filter paper was utilised after that. 50 cm3 of the filtrate and 10 cm3 of distilled water were added to get the right acidity. The solution was titrated with a standard Iron II Chloride solution containing 0.00195g Iron/ml after 10 ml of 0.3% ammonium thiocyanate solution was added as directed. The endpoint was seen to be a brownish-yellow hue that lasted for 5 minutes (Lucas and Markakas, 2015).

Check for Oxalate

A 250cm3 volumetric flask containing 2g of the material, 190 ml of distilled water, and 6m HCl was added to the extract. Following a 4-hour water bath warming period, the mixture was centrifuged for five minutes at 2000 rpm to extract the digested sample. 250 cm3 of the supernatant was diluted. The brown precipitate was filtered and cleaned after the supernatant was reduced to 25 cm3 in three 50 cm3 aliquots. After that, drops of strong ammonia solution were added to the mixed solution and washings until the methyl red's pink colour turned yellow. After heating the mixture to 90 degrees Celsius in a water bath, the oxalate was precipitated using 5% CaCl2. The mixture was centrifuged after standing for an overnight period, and the precipitate was then cleaned with hot 25% H2SO4, diluted to 125 millilitres using distilled water, and titrated against 0.05 millimetres of KMnO4 (Sanchez-Alonso and Lachica, 2017).

Test for Cyanide:

We weighed two grammes of the extract sample and dissolved it in twenty millilitres of distilled water. After an overnight stay, the cyanide extraction was filtered. To see if it became brown, 1 millilitre of the sample filtrate, 4 millilitres of picric solution, and 15 minutes of boiling were added to a test tube. A UV Spectrophotometer was used to measure cyanide (Sanchez-Alonso and Lachica, 2017).

Statistical analysis

Using the two-way analysis of variance (ANOVA), the study's data were statistically analysed to ascertain their level of significance where the probability ($p<0.05$) is regarded as significant.

RESULTS

Proximate Analysis

Table 1 below shows the result of the proximate analysis of both fresh and dry bitter leaves. The mean dry matter is 19.85±0.06 in the fresh sample and 90.59±0.63 in the dry bitter leaf sample. Regarding moisture content, the fresh bitter leaf significantly ($p<0.05$) recorded higher than the dry bitter leaf sample. Both samples' ash content and crude fiber do not differ significantly ($p>0.05$). The mean crude protein, crude fat and carbohydrate were significantly ($p<0.05$) higher in the dry bitter leaf when compared with the values of the fresh bitter leaf

Table 1. Proximate Compositions of Fresh and Dry Bitter leaf (*Vernonia amygdalina).*

PARAMETERS	Fresh bitter leaf (*Vernonia amygdalina*) concentration (%)	Dry bitter leaf (*Vernonia amygdalina*) concentration (%)
Dry Matter	19.85 ± 0.06	90.59 ± 0.63
Moisture	80.14 ± 0.05	8.95 ± 1.04
Ash	6.05 ± 0.01	6.08 ± 0.01
Crude Fat	2.10 ± 0.05	5.97 ± 0.03
Crude Protein	2.42 ± 0.02	9.41 ± 0.02
Crude Fibre	3.99 ± 0.01	4.01 ± 0.01
Carbohydrate	5.26 ± 0.05	65.11 ± 0.63

Values are expressed as Mean ± standard deviation (SD): n = 3

Mineral composition

Figure 1 shows the mineral compositions of fresh and dry Bitter leaf (*Vernonia amygdalina).* The sodium content is 0.07 ± 0.00 and 0.08 ± 0.00, while the potassium content is 0.04 ± 0.00 and 0.06 ± 0.00, respectively, for both fresh and dry bitter leaf samples. In this study, the sodium and potassium content of both fresh and dry bitter leaves was low when compared to other minerals like calcium, magnesium and Phosphorus. Calcium content (0.20 ± 0.03 and 0.47 ± 0.02), Magnesium (1.33 ± 0.10 and 0.98 ± 0.00) and Phosphorus (2.11 ± 0.17 and 2.18 ± 3.41)

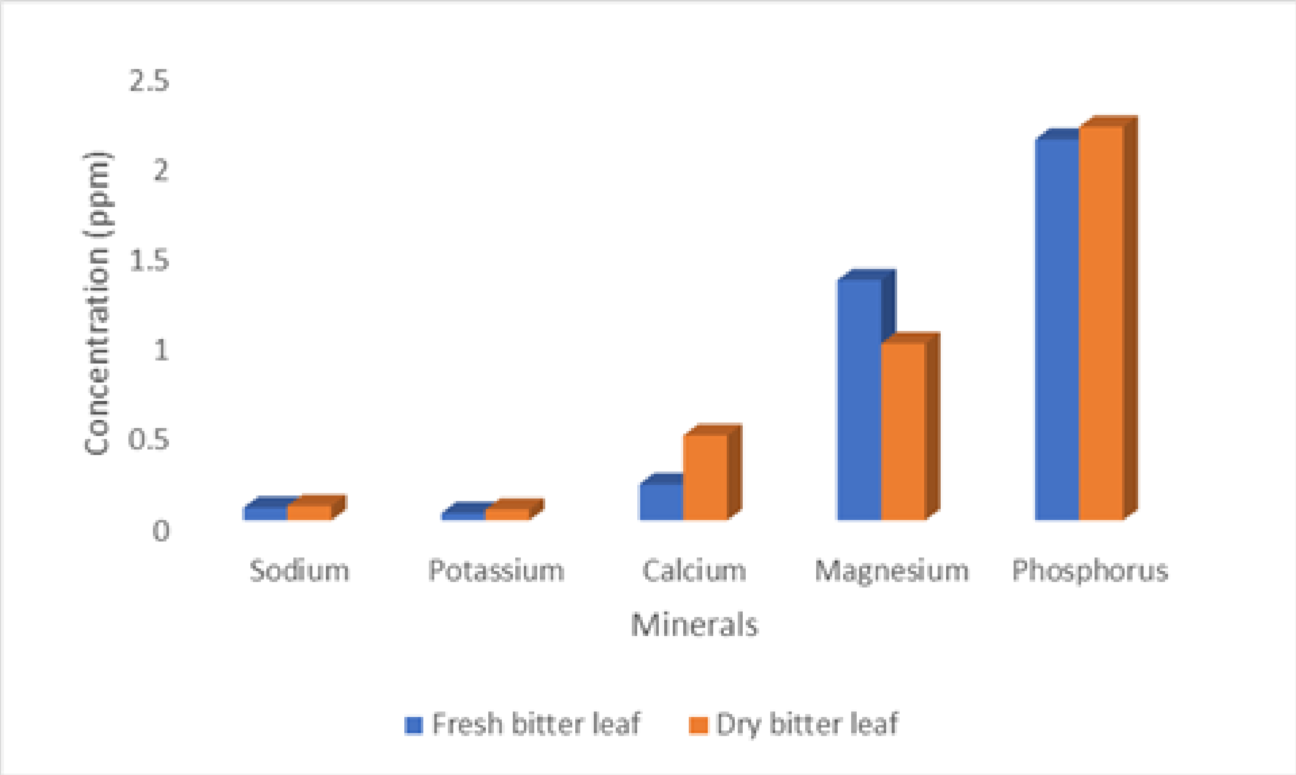

Figure 1: Mineral Compositions of Fresh and Dry Bitter leaf (*Vernonia amygdalina).*

Phytochemical composition

Table 2 shows the plant samples' Alkaloid, Flavonoid, Cyanide, Tannin, Oxalate and Phytate content. Flavonoid was significantly (p<0.05) higher in the fresh bitter leaf sample than the dry bitter leave sample. The dry bitter leaf sample significantly (p<0.05) recorded a high value in alkaloid, oxalate and cyanide compared to the values obtained from the fresh bitter leaf sample. The dry and fresh better leaf sample did not differ significantly in terms of phytate and tannin composition.

Table 2. Phytochemical Compositions of fresh and dry Bitter leaf (*Vernonia amygdalina).*

CONSTITUENTS	FRESH BITTER LEAF (*Vernonia amygdalina*) CONCENTRATION (%)	DRY BITTER LEAF (*Vernonia amygdalina*) CONCENTRATION (%)
Flavonoid	16.86 ± 0.19	4.50 ± 0.70
Alkaloid	3.96 ± 0.07	7. 12 ± 0.00
Phytate	1.35 ± 0.00	1.74 ± 0.00
Oxalate	14.78 ± 0.04	31.08 ± 0.35
Cyanide	1.55 ± 0.04	2.51 ± 2.26
Tannin	0.05 ± 0.10	0.05 ± 0.10

Values are expressed as Mean ± standard deviation (SD) : n = 3

DISCUSSION

The proximate composition of the leaves of *Vernonia amygdalina* varies between the dry and fresh bitter leaves. The dry matter contents of the fresh *Vernonia amygdalina* (19.85 ± 0.06) and dry *Vernonia amygdalina* (90.59 ± 0.63) differed substantially (p< 0.05). This could be because the fresh and dried contents of the Vernonia samples had different moisture contents. The study's air-dried *Vernonia amygdalina's* dry mater (90.59) is more than Moses et al., 2019's reported values of 87.4¬ and 89.82 for both air-dried and oven-dried leaves and lower than 90.68 and 92.10 reported by Usunobun and Okolie (2016) and Belewa et al., 2009. Given that older leaves have been found to contain more dry matter than younger ones, this could result from variations in the maturity of the leaves utilised (Moses et al., 2019). For fresh *Vernonia amygdalina* leaves, the proximate content decreased in the following order on average: moisture, energy, matter, ash, carbohydrates, crude fibre, protein, and fat; for dry *V. amygdalina* leaves, the sequence was dry matter, carbohydrates, proteins, moisture, ash, fat, crude fibre, and energy.

Vernonia amygdalina leaves have a greater percentage of protein than leafy vegetables, as stated by Oulai et al. (2014). Compared to fresh *Vernonia amygdalina* leaves, dried *Vernonia amygdalina* leaves are much richer in proteins and can be used as a protein substitute, especially for those who live in rural areas. This is because foods with a high percentage of their calories from proteins are considered excellent sources of protein. The veggies have less moisture than *Vernonia amygdalina* and *Amaranthus cruentus* L, reported by Onwordi et al. (2009) and certain green vegetables in Nigeria (Akindahunsi and Salawu, 2005). Compared to Dry *Vernonia amygdalina*, Fresh *Vernonia amygdalina* had a higher moisture content.

The time of year the plants were collected—the start of the rainy season, which is marked by low rates of water evaporation from plants—may have impacted the moisture content. While fresh *Vernonia amygdalina* has a high moisture level that indicates it cannot be stored because it will decay soon, dry *Vernonia amygdalina* has a low moisture content that suggests it can be preserved.

The carbohydrate contents of dried and fresh Vernonia amygdalina varied significantly as the dry leaf had a higher carbohydrate content of 65.11% compared to 5.26% of the fresh leaves. Compared to typical vegetables in southwest Nigeria, *Vernonia amygdalina* has a greater carbohydrate content (Akinwumi and Omotayo 2016). The dry *Vernonia amygdalina* leaf has a higher carbohydrate content than the fresh *Vernonia amygdalina* leaf, which indicates that the dry leaf is a better energy source than the fresh leaf. The carbohydrate content of dry *Vernonia amygdalina* reported in this research is similar to the report of Muhammad et al. (2020), who also reported a high amount of carbohydrate in *Vernonia amygdalina.* However, the carbohydrate content of the fresh *Vernonia amygdalina* reported in this research contrasts with the report of Muhammad et al. (2020) owing to its extremely low carbohydrate content.

Vernonia amygdalina fresh leaves had a lower density than dried leaves. Vegetables would not be excellent providers of minerals, as indicated by the ash content, which measures the plant's mineral concentration. Dry *Vernonia amygdalina's* crude fibre resembled *C. olitorius's* (Akindahunsi and Salawu, 2005). Compared to fresh *Vernonia amygdalina* leaves, the dry leaves had a greater crude fibre content. Of the two veggies, there was little crude fibre. These vegetables are low in lipids, as seen by their low crude fibre content. However, the low lipid content of these veggies is beneficial because excessive fat consumption in humans causes cancer, ageing, and cardiovascular illnesses. This result is similar to the report of Yeap et al. (2010), who reported crude fat content of 2 -15% crude fat content and Muhammad et al. (2020) reported crude fat content of 5.50 %.

Both the fresh and dried bitter leaf plants had different mineral concentrations. This may be explained by the sampling area's geographic location or the type of plant that was planted there. Phosphorus and magnesium were found in greater amounts in fresh *Vernonia amygdalina* leaves than in other mineral elements present in the leaf. Similarly, Phosphorus and magnesium were found in larger amounts in dry *Vernonia amygdalina* leaves than in other mineral elements. *Vernonia amygdalina* leaves contain magnesium and Phosphorus, which suggests that eating it could be a good way to get those elements. (Okunlola et al., 2018). The development and upkeep of bones, teeth, and muscles are linked to the combination of calcium and Phosphorus (Wilcox et al., 2015). Additionally, potassium is present, which aids in restoring the blood's proper pH and water balance (Mgbemena and Amako 2020). Teeth and bones that are strong need calcium. The element magnesium is present. High levels of magnesium may be related to their significance because they support the maintenance of a regular heart rhythm (Mgbemena and Amako 2020).

The presence of magnesium, Phosphorus, calcium and sodium is similar to the report of Muhamad et al. (2020), who also reported the presence of these major elements in *Vernonia amygdalina.*

Both plants contain a considerable amount of alkaloids. However the amount in the dry bitter leaves is more than in the fresh bitter leaves. Alkaloids have long been known to be vital to humans despite the fact that they are secondary metabolites, which may imply that they have no value. Even at very low dosages, alkaloids had significant biological impacts on human and animal creatures. Alkaloids are found in foods and beverages consumed by humans daily, as well as in stimulant medications. In addition to numerous other actions, they demonstrated anti-inflammatory, anticancer, analgesic, local anaesthetic and pain relief, neuropharmacological, antibacterial, and antifungal properties. Alkaloids have several human health uses, including medications, diet elements, and supplements. In the process of creating new synthetic and semisynthetic chemicals with potentially greater biological activity than parent compounds, alkaloids are also crucial substances in organic synthesis. The range of Alkanoid in this research (4.50% - 16.50%) in both dry and fresh *Vernonia amygdalina* is similar to that (4.60%) reported by Muhammad et al. (2020)

Fresh bitter leaf has a higher flavonoid content than dry bitter leaf. Flavonoids have been found in many vegetables, although their concentration varies depending on the species. Flavonoids and natural antioxidants are two crucial micronutrients that can be utilized industrially to reduce synthetic compounds on foods and improve human health because they can potentially reduce several diseases (Patel, 2008).

Because these bioactive chemicals have antioxidant and antibacterial qualities, they can be employed to preserve food and extend its shelf life. Furthermore, a decline in the consumption of vegetables has been linked to a number of disorders; therefore, it is crucial to consider the necessary daily intake of these compounds to support their physiological functioning. Numerous studies have shown that eating foods high in flavonoids can increase one's daily intake of these chemicals by 50–800 mg, mostly from vegetables. The vegetables under investigation have flavonoid concentrations ranging from 50 to 800 mg/day.

Commercial cyanides are marketed as dietary supplements and have been utilised in conventional medicine. Both leaves in this investigation exhibit a significant level of cyanide, with the dried bitter leaf exhibiting the highest level.

Comparably, the amounts of oxalate in fresh and dry bitter leaves were 14.78 ± 0.04% and 31.08 ± 0.35 %, respectively. A common organic component in many plants, especially leafy green vegetables, is oxalic acid. Oxalate is either naturally occurring in the body or is acquired by diet. When metabolized, vitamin C can also yield oxalate. Because oxalate can bind to minerals in the intestine and hinder the body from absorbing them, this is one of the main health issues associated with oxalate.

Tannin's use in treating diarrhoea and preventing the growth of bacteria is demonstrated by the presence of tannin in the sample. It can be used to tan animal skin since it has the ability to precipitate protein, which allows them to turn animal skin into leather. Tannin-containing plants are recognised to offer therapeutic benefits. This suggests that the plants may be utilised to treat specific illnesses (Agbaire, 2019).

The samples have a phytate concentration ranging from 1.35 ± 0.04% to 1.74 ± 0.05%; fresh bitter leaf has the lowest value, while dry bitter leaf has the highest value. According to Sebastian et al. (2020), the estimated daily consumption of phytic acid (PA) is 2100 mg, which is marginally higher than the values of the vegetables under investigation. Furthermore, it is anticipated that phytic acid in food may rise as a result of climate change, perhaps further depleting certain vital minerals. Micronutrient deficits are, therefore, predicted to rise in developing nations. Because of this, individuals in this region (developing countries) must enhance both their nutrition and eating habits.

The presence of Tanin, Saponins, Flavonoids, and Alkanoids established in this research is supported by Usunbun and Ngozi (2016), who reported the the same phytochemical elements in *Vernonia amygdalina*.

CONCLUSION

According to this study, the leaves of *Vernonia amygdalina*, both fresh and dry, have varying and significant amounts of secondary metabolites (alkaloid, flavonoid, cyanide, oxalate, and phytate), mineral elements (sodium, potassium, calcium, magnesium, and Phosphorus), and proximate compositions (moisture, ash, crude fat, crude fibre, protein, and carbohydrate). The phytochemical components found in vegetables benefit human health because they stimulate internal glands, the skeletal and muscular systems, and enzymatic activity. They also generally control metabolism. As a result, the veggies can be taken as supplements and utilised to treat various human illnesses and problems. The outcomes demonstrate the significant health benefits of the two leaves. However, the nutritional content of dried *Vernonia amygdalina* leaves is higher than that of fresh leaves.

Recommendations

The outcomes of this research demonstrated the significant health benefits of the two leaves. Therefore, additional examination, such as the veggies' approximate composition, mineral content, pollution level, and phytochemical content should be performed to precisely ascertain their nutritional and therapeutic qualities. Although *Vernonia amygdalina* leaves fluctuate significantly in composition when dried, they have a sufficient nutritional value. Dry *Vernonia amygdalina* is recommended for consumption because it provides minerals, protein, energy, and phytochemical elements for metabolic and pharmacodynamic activities essential for maintaining one's health.

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