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Effects of drying and Pre-cooking treatment on the nutrient and phytochemical contents of bitter leaf (*Vernonia amygdalina*)

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Abstract
This study investigated the effects of various drying methods and pre-cooking treatments on the nutrient and phytochemical contents of bitter leaf (*Vernonia amygdalina*), a widely consumed leafy vegetable in sub-Saharan Africa known for its nutritional and medicinal properties. Fresh bitter leaf samples were subjected to three drying methods (oven-drying, freeze-drying, and sun-drying) and a pre-cooking treatment (washing). The proximate composition, antinutrient content, phytochemical profile, and mineral content were analyzed for each treatment. Results showed that drying methods significantly affected the proximate composition of bitter leaf. Freeze-drying was most effective in moisture reduction (7.50%) and preserving crude fiber (14.11%), while oven-drying yielded the highest protein (17.25%) and fat (11.34%) content. Sun-drying resulted in the highest ash (14.23%) and carbohydrate (49.26%) content. The pre-cooking washing treatment generally reduced both antinutrients and beneficial phytochemicals. Saponins decreased by 63.7%, phytates by 53.4%, and phenols by 65.5%. However, alkaloid content unexpectedly increased by 28.6%. Mineral content analysis revealed that washing had varying effects on different minerals. While some minerals showed slight decreases, calcium and iron content increased significantly (10% and 28% respectively), suggesting enhanced bioavailability of certain minerals after washing.

Keywords: Bitter leaf, drying methods, pre-cooking treatment, phytochemicals, nutritional value

Introduction

Vernonia amygdalina, commonly known as bitter leaf, is a prominent leafy vegetable widely consumed across sub-Saharan Africa, particularly in West Africa. Its popularity stems not only from its culinary uses but also from its recognized medicinal properties (Yeap *et al*., 2020) [24]. The plant has been traditionally used to treat various ailments, including malaria, diabetes, and gastrointestinal disorders, owing to its rich phytochemical profile and nutritional composition (Oyeyemi *et al*., 2018) [19]. Recent scientific investigations have corroborated many of the traditional uses of bitter leaf, revealing its potential as a functional food and nutraceutical. Studies have shown that V. amygdalina contains a wide array of bioactive compounds, including flavonoids, saponins, alkaloids, and tannins, which contribute to its antioxidant, anti-inflammatory, and antimicrobial properties (Alara *et al*., 2021) [4]. Furthermore, the leaf is a good source of essential minerals and vitamins, making it a valuable contributor to nutritional security in regions where it is commonly consumed (Akpabio *et al*., 2021) [2].

However, the bioavailability and concentration of these beneficial compounds can be significantly affected by various processing methods employed in its preparation for consumption or preservation. Drying and pre-cooking treatments, in particular, are common practices used to extend the shelf life of bitter leaf and improve its palatability. These processes can have profound effects on the nutrient and phytochemical contents of the vegetable, potentially altering its nutritional value and health-promoting properties (Iweala *et al*., 2020) [14]. Drying methods, such as sun-drying, oven-drying, and freeze-drying, have been shown to impact the nutritional and phytochemical profiles of leafy vegetables differently. For instance, Ogbonna *et al*. (2023) [17] reported that while all drying methods generally led to a concentration of nutrients due to moisture loss, freeze-drying was most effective in preserving heat-sensitive compounds like vitamins and certain phytochemicals. Conversely, sun-drying, despite being the most economical method, often resulted in significant losses of bioactive compounds due to prolonged exposure to light and air.

Pre-cooking treatments, including washing and blanching, are often employed to reduce the bitterness of V. amygdalina and improve its organoleptic properties. However, these processes can also lead to losses of water-soluble nutrients and phytochemicals. Ilelaboye *et al*. (2022) [13] observed that while washing and blanching effectively reduced antinutrients like oxalates and phytates in bitter leaf, they also led to significant reductions in flavonoids and phenolic compounds, which are key contributors to the vegetable's antioxidant properties. The complex interplay between processing methods and the retention of nutrients and phytochemicals in bitter leaf underscores the need for a comprehensive study to optimize these processes. As noted by Okoduwa *et al*. (2022) [18], there is a delicate balance to be struck between reducing antinutrients, preserving beneficial compounds, and enhancing the bioavailability of nutrients in processed vegetables.

Furthermore, the growing interest in functional foods and natural remedies in the global market highlights the importance of understanding how processing methods affect the nutritional and medicinal value of traditional vegetables like bitter leaf. This knowledge is crucial not only for preserving the health benefits of V. amygdalina but also for potentially enhancing its utility as a functional food ingredient or nutraceutical (Yeap *et al*., 2020) [24]. In light of these considerations, this study aims to investigate the effects of various drying methods (sun-drying, oven-drying, and freeze-drying) and pre-cooking treatments (washing and

blanching) on the nutrient and phytochemical contents of bitter leaf (*Vernonia amygdalina*). By elucidating the impact of these processing methods on key nutritional components and bioactive compounds, this research seeks to provide valuable insights for optimizing the preparation and preservation of bitter leaf, thereby maximizing its nutritional and health-promoting potential.

Materials and methods

1. Plant material collection and preparation

Fresh bitter leaf (*Vernonia amygdalina*) samples were collected from a local farm in Ogbomoso. The leaves were authenticated by a botanist at Ladoke Akintola University of Technology. The collection and preparation methods were adapted from Alara *et al*. (2021) [4]. Fresh leaves were carefully sorted to remove damaged ones, thoroughly washed with running water to remove dirt and contaminants, and then divided into portions for different treatments.

2. Pre-cooking treatments

2.1. Washing treatment

One portion of the leaves was subjected to a washing treatment as described by Ilelaboye *et al*. (2022) [13]. Leaves were washed in distilled water at room temperature (25°C) for 5 minutes, then gently squeezed to remove excess water.

2.2. Blanching treatment

Another portion was blanched using the method described by Ogbonna *et al*. (2023) [17]. Leaves were immersed in hot water (95°C) for 3 minutes, then immediately cooled in an ice bath for 1 minute to stop the cooking process.

3. Drying methods

3.1. Sun drying

Sun drying was carried out according to the method described by Akpabio *et al*. (2021) [2]. Leaves were spread on clean, dry trays and exposed to direct sunlight for 5 days, until the leaves became crisp.

3.2. Oven drying

Oven drying was performed using the method described by Iweala *et al*. (2020) [14]. Leaves were spread on oven trays and dried at 50°C for 24 hours in a convection oven.

3.3. Freeze drying

Freeze drying was conducted according to method described by Yeap *et al*. (2020) [24]. Leaves were frozen at -80°C for 24 hours, then lyophilized using a freeze dryer for 48 hours.

4. Sample preparation for analysis

Dried samples were ground into a fine powder using a laboratory mill and sieved through a 0.5 mm mesh. The powdered samples were stored in airtight containers at 4°C until further analysis.

5. Quantitative phytochemical screening

5.1. Determination of alkaloid

Alkaloid content was determined according to the method described by Sánchez-Recillas (2022) [21]. 5g of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid which was dried and weighed.

5.2. Determination of tannin

The method described by Fraga-Corral (2020) [11] was adopted. About 200mg of finely ground sample was weighed into a 50ml sample bottle. 10ml of 70% aqueous acetone was added and properly covered. The bottles were put in an ice bath shaker for 2 hours at 30°C. Each solution was then centrifuged and the supernatant stored in ice. 0.2ml of each solution was pipetted into test tubes and 0.8ml of distilled water was added. Standard tannic acid solutions were prepared from a 0.5mg/ml stock and the solution made up to 1ml with distilled water.

Folin reagent (0.5ml) was added to both the sample and standard followed by 2.5ml of 20% Na_2CO_3. The solutions were then vortexed and allowed to incubate for 40minutes at room temperature after which absorbance was read at 725nm against a reagent blank concentration of the samples from a standard tannic acid curve.

5.3. Determination of saponin

The method used by Netala (2015) [15] was adopted. The samples were ground and 20g of each were put into a conical flask and 100cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90°C. The concentrate was transferred into a 250ml reparatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated.

N-Butanol (60ml) was added. The combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight.

5.4. Determination of oxalate

1g of the sample was weighed into 100ml conical flask. 75ml of $1.5NH_2SO_4$ was added and the solution was carefully stirred intermittently with a magnetic stirrer for about 1 hour and then filtered using Whatman No. 1 filter paper.

25ml of sample filtrate (extract) was collected and titrated hot (80 - 90°C) against $0.1NKMnO_4$ solution to the point when a faint pink colour appeared that persisted for at least 30 seconds (Barakoti, L., & Bains, K., 2023) [7].

5.5. Determination of phytate

The method described by Cúneo, F (2020) [8] was employed. 4g of finely ground samples were soaked in 100cm^3 2% HCl for 3 hours and then filtered. 25cm^3 of the filtrate was placed in a 100cm^3 conical flask and 5cm^3 of 0.03% NH_4SCN solution was then added as indicator. 50cm^3 of distilled water was then added to give it the proper acidity. This was titrated with ferric chloride solution which contained about 0.005mg of Fe per cm^3 of $FeCl_3$ used, the

equivalent was obtained and for this, the phytate content in mg/100g was calculated
Iron equivalent = titre value x 1.95
Phytic acid = titre value x 1.95 x 1.19 x 3.55mg/phytic acid

$$\% \text{ Phytic acid} = \frac{\propto \times 8.24}{1000} \times \frac{100}{weight\ of\ sample}$$

Where $\propto$ = titre value

5.6. Determination of Total Phenol
The method described by Alves *et al*. (2020) [5] was adopted. The fat free sample was boiled with 50ml of ether for the extraction of the phenolic component for 15 minutes. 5ml of the extract was pipetted into a 50ml flask, then 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated amylalcohol were also added. The samples were made up to mark and left to react for 30minutes for colour development. This was measured at 505nm.

5.7. Determination of Total Flavonoid Content
For the determination of total flavonoid, a modified spectrophotometric method described by Panche *et al*., (2016) [20] with $NaNO_2$ was used. Dry extract of phenolics was dissolved in 5ml of methanol. For the determination, 0.4ml of the sample solution was pipetted into a 10ml volumetric flask and diluted with distilled water. Subsequently, 1.2ml of 0.2mol/L H_2SO_4, 1.2ml of 3mol/L $NaNO_2$ and 1.2ml of 10% NaOH were added to the solution. After refilling with distilled water to mark and thorough agitation, the reaction mixture was left standing for 15minutes and then measured on the spectrophotometer at 395nm against the blank.

6. Determination of mineral composition
Standard method obtained from AOAC (2014) was used. The minerals were analysed from solution dilution by first dry ashing. About 1.5g of the sample was placed in a dish and heated gently on a Bunsen burner in a fume cupboard until the charred mass had ceased to emit smoke and it was transferred to muffle furnace at 550°C. Heating was continued until all the carbon was burnt. The dish and ash was transferred to a desiccator to cool after which 0.1M HNO_3 solution (10mls) was added to the crucible to break up the ash. It was then filtered through acid washed No. 43 Whatman filter paper into 100ml with the same dilute acid solution.
Atomic spectrophotometer was used for the analysis of the following metals: calcium, iron, sodium. The instrument was switched on and lamp for each metal was fixed. Hollow cathode lamps and air acetylene flame were used to analyze all metals. The standard for each metal were aspirated into the flame as well as the samples and their respective concentration in mg/L were read respectively while the absorbance of the standard was noted.

Result and discussion
1. Proximate Composition
The results of Proximate Nutrient Composition of Fresh, Oven-Dried, Freeze-Dried and Sun-Dried Samples of Bitter Leaf is shown in Table 1. The fresh bitter leaf samples had the highest moisture content (72.13%), which is expected for fresh vegetables. This is similar to findings by Ejoh *et al*. (2007) [10], who reported moisture content of 70.3% in fresh bitter leaf. The drying treatments significantly reduced moisture content, with freeze-drying being most effective (7.50%), followed by sun-drying (8.50%) and oven-drying (9.55%). This reduction in moisture content is crucial for extending shelf life by inhibiting microbial growth and enzymatic reactions. Ash content, indicative of mineral content, increased substantially after drying. Sun-dried samples had the highest ash content (14.23%), followed by freeze-dried (11.48%) and oven-dried (11.21%) samples. This increase is due to the concentration effect as moisture is removed. Sodamade *et al*. (2013) [22] reported similar trends in ash content increase after drying in their study on *Vernonia amygdalina* leaves. Freeze-dried samples showed the highest crude fibre content (14.11%), significantly higher than fresh samples (1.52%). Oven-dried (8.07%) and sun-dried (8.83%) samples also showed increased fibre content. This increase is partly due to the concentration effect and possibly some structural changes during drying. Adeboye *et al*. (2018) [1] similarly observed an increase in crude fibre content after drying bitter leaf. Oven-dried samples had the highest protein content (17.25%), followed by freeze-dried (11.60%) and sun-dried (9.45%) samples. All dried samples showed higher protein content than fresh samples (7.32%), again due to the concentration effect. However, the variation among drying methods suggests that different drying techniques may affect protein differently, possibly due to denaturation or other chemical changes. Fat content increased in all dried samples compared to fresh (3.38%), with oven-dried samples showing the highest fat content (11.34%), followed by freeze-dried (10.56%) and sun-dried (9.74%) samples. This increase is primarily due to the concentration effect of removing moisture. Yeap *et al*. (2015) [23] reported similar increases in fat content after drying in their study on *Vernonia amygdalina*.
Carbohydrate content increased significantly in all dried samples compared to fresh (13.44%). Sun-dried samples had the highest carbohydrate content (49.26%), followed by freeze-dried (44.76%) and oven-dried (42.59%) samples. This increase is partly due to the concentration effect and possibly some conversion of other compounds to simpler sugars during the drying process. These results demonstrate that drying methods significantly affect the nutritional composition of bitter leaf. Each drying method has its advantages: Freeze-drying best preserved crude fibre and resulted in the lowest moisture content. Oven-drying yielded the highest protein and fat content. Sun-drying resulted in the highest ash (mineral) and carbohydrate content.

Table 1: Proximate Nutrient Composition of Fresh, Oven-Dried, Freeze-Dried and Sun-Dried Samples of Bitter Leaf (*Vernonia amygdalina*)

Treatments	Moisture Content (g %)	Ash Content (g %)	Crude Fibre (g %)	Crude Protein (g %)	Fat (g %)	Carbohydrates (g %)
Fresh	72.13±0.83	2.23±0.13	1.52±0.12	7.32±1.15	3.38±0.46	13.44±1.77
Oven-dried	9.55±0.07	11.21±0.26	8.07±0.72	17.25±1.16	11.34±0.05	42.59±0.68
Freeze-dried	7.50±0.18	11.48±0.03	14.11±0.40	11.60±1.10	10.56±0.33	44.76±0.96
Sun-dried	8.50±0.37	14.23±0.59	8.83±0.75	9.45±0.0	9.74±0.35	49.26±0.56

2. Effects of Precooking Treatment on some Antinutrient and Phytochemical Contents of Bitter Leaf *(Vernonia amygdalina)*.

Table 2 shows the comparison between fresh and washed bitter leaves for various compounds. Saponin, decreased from 6.14 mg/100g (fresh) to 2.23 mg/100g (washed), a 63.7% reduction. The significant reduction in saponins aligns with findings by Ilelaboye *et al*. (2022) [13]. They reported that various processing methods, including washing, significantly reduced saponin content in bitter leaf, which can improve palatability and reduce potential negative health effects associated with high saponin intake. Alkaloids increased from 0.35 mg/100g to 0.45 mg/100g, a 28.6% increase. The slight increase in alkaloid content is interesting and somewhat unexpected. This could be due to the release of bound alkaloids during the washing process or variability in the samples. Further research might be needed to explain this observation. Flavonoids, decreased from 62.70 mg/100g to 48.10 mg/100g, a 23.3% reduction. Phenol, decreased from 3.10 mg/100g to 1.07 mg/100g, a 65.5% reduction. Flavonoids and Phenols, the reduction in these beneficial compounds is consistent with research by Ogbonna *et al*. (2023) [17]. They found that processing methods generally reduced bioactive compounds like flavonoids and phenols, but noted that this reduction might be offset by improved bioavailability of remaining compounds. Phytate, decreased from 59.91 mg/g to 27.90 mg/g, a 53.4% reduction. Oxalate decreased from 5.23 mg/g to 4.17 mg/g, a 20.3% reduction. The substantial reduction in phytates and moderate reduction in oxalates corroborate findings by Akpabio *et al*. (2021) [2]. They observed that processing methods, including washing, effectively reduced these antinutrients, which can interfere with mineral absorption. Tannin, decreased from 3.65 mg/100g to 2.09 mg/100g, a 42.7% reduction. The decrease in tannin content is in line with research by Iweala *et al*. (2020) [14]. They noted that processing methods generally reduced tannin content, which can improve the nutritional value of bitter leaf by reducing its astringency and potential interference with protein digestion. The overall trend shows that washing, as a precooking treatment, generally reduces both antinutrients and beneficial phytochemicals in bitter leaf. This has both positive and negative implications. The positive implication is that, reduction in antinutrients like phytates, oxalates, and tannins can improve mineral bioavailability and digestibility. Also, decreased saponin content can improve palatability and reduce potential negative effects of high saponin intake. The negative implication is that, reduction in beneficial compounds like flavonoids and phenols might decrease some health-promoting properties of bitter leaf. Recent research by Okoduwa *et al*. (2022) [18] emphasizes the importance of optimizing processing methods to balance the reduction of antinutrients with the retention of beneficial phytochemicals.

Table 2: Effects of Precooking Treatment on some Antinutrient and Phytochemical Contents of Bitter Leaf (*Vernonia amygdalina*)

Samples	Replicates	Saponin mg/100g	Alkaloids mg/100g	Flavonoids mg/100g	Phenol mg/100g	Phytate mg/g	Oxalate mg/g	Tannin mg/100g
Fresh	I	6.50	0.45	64.00	3.60	68.01	6.22	4.70
	II	5.78	0.25	61.40	2.60	51.80	4.23	2.60
	Mean ± SD	6.14	0.35	62.70	3.10	59.91	5.23	3.65
Washed	I	2.00	0.80	49.00	1.09	27.22	4.23	1.99
	II	2.46	0.10	47.20	1.05	28.58	4.10	2.19
	Mean ± SD	2.23	0.45	48.10	1.07	27.90	4.17	2.09

3. Effect of Pre-Cooking Treatment on Mineral Content (ppm) of Bitter Leaf

Table 3 shows the mineral content (in ppm) for fresh and washed bitter leaves. These results show that washing as a pre-cooking treatment has varying effects on different minerals in bitter leaf. Sodium (Na), decreased from 736.20 to 707.03 ppm (3.96% reduction), Manganese (Mn), decreased from 33.95 to 31.05 ppm (8.54% reduction), Copper (Cu), decreased from 9.7 to 9.32 ppm (3.92% reduction). The slight decreases in these minerals are consistent with findings by Iweala *et al*. (2020) [14]. They reported that washing and other processing methods could lead to some mineral losses, particularly for water-soluble minerals. Calcium (Ca) increased from 60588.33 to 66647.78 ppm (10% increase), Iron (Fe), increased from 119.3 to 152.7 ppm (28% increase). The significant increases in calcium (10%) and iron (28%) are particularly noteworthy. This aligns with findings by Ilelaboye *et al*. (2021) [12], who reported that some processing methods could enhance the bioavailability of certain minerals in vegetables. The increase might be due to the release of these minerals from bound forms during washing. Zinc (Zn), increased from 65.96 to 72.45 ppm (9.84% increase). The 9.84% increase in zinc content is consistent with research by Akpabio *et al*. (2022) [3]. They found that certain processing methods could increase the extractability and bioavailability of some minerals, including zinc, in leafy vegetables. Potassium (K), increased from 23328.5 to 23647.15 ppm (1.37% increase). Phosphorus (P), increased from 2831.98 to 2930.60 ppm (3.48% increase). The small increases in potassium and phosphorus content are interesting. Ogbonna *et al*. (2023) [17] suggested that mild processing methods like washing might not significantly affect these minerals, but could potentially increase their detectability in analysis. The general retention and even increase of most minerals after washing is a positive finding. This aligns with research by Okoduwa *et al*. (2022) [18], which emphasized that mild processing methods like washing can often preserve or even enhance the mineral content of leafy vegetables.

Table 3: Effect of Pre-Cooking Treatment on Mineral Content (ppm) of Bitter Leaf (*Vernonia amygdalina*)

Sample	Replicates	Na	K	Ca	Mn	Cu	Zn	Fe	P
Fresh	I	759.00	24050.00	62462.20	35.00	10.00	68.00	123.00	2919.50
	II	713.40	22607.00	58714.45	32.90	9.40	63.92	115.60	2744.45
	Mean ± SD	736.20	23328.50	60588.33	33.95	9.70	65.96	119.30	2831.98
Washed	I	730.95	24446.80	68901.55	30.00	9.63	74.90	157.90	3029.70
	II	683.10	22847.50	64394.00	32.10	9.00	70.00	147.5	2831.50
	Mean ± SD	707.03	23647.15	66647.78	31.05	9.32	72.45	152.7	2930.60

Conclusion

This study provides valuable insights into the effects of various drying methods and pre-cooking treatments on the nutritional and phytochemical profile of bitter leaf (*Vernonia amygdalina*). The findings demonstrate that processing techniques significantly influence the composition of this important leafy vegetable, with implications for its nutritional value, health benefits, and potential applications in food and nutraceutical industries. All drying methods effectively reduced moisture content, crucial for extending shelf life. Notably, freeze-drying was most effective in moisture reduction while best preserving crude fiber content. Oven-drying resulted in the highest protein and fat content, while sun-drying yielded the highest ash (mineral) and carbohydrate content. These findings suggest that the choice of drying method should be based on the desired nutritional outcome and intended use of the processed bitter leaf. These findings have important implications for both traditional culinary practices and potential industrial applications of bitter leaf. For culinary use, the results suggest that a combination of washing and appropriate drying methods could optimize the nutritional value and palatability of bitter leaf. In industrial applications, such as in the production of functional foods or nutraceuticals, the choice of processing method could be tailored to enhance specific nutritional or phytochemical profiles. However, it is important to note that while drying and pre-cooking treatments can offer benefits in terms of preservation and reduction of antinutrients, they may also lead to losses in some beneficial compounds. Future research should focus on optimizing processing methods to strike a balance between reducing antinutrients and preserving beneficial phytochemicals.

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