

Impact of Household Cooking Techniques on the Phytochemical Profile, Antioxidant Capacity, and Bioactive Compounds of Bitter Leaf (*Vernonia amygdalina*)

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Abstract

Background

This study evaluated the impact of household cooking techniques on the Phytochemical profile, Antioxidant capacity, and Bioactive compounds of *Vernonia amygdalina* (bitter leaf). Household cooking techniques have a significant impact on the phytochemical composition of *Vernonia amygdalina*, generally leading to a reduction in the phytochemicals as they are leached into the cooking water.

Objectives

This study evaluated the effect of domestic culinary treatments, assessed changes in antioxidant capacity, identified the impact on bioactive compounds, determined optimal preparation methods for nutrient retention, evaluated the nutritional and therapeutic value of “washed” vs. “unwashed” leaves, and provided evidence-based culinary advice of *Vernonia amygdalina* (bitter leaf).

Methods

The antioxidant activity was evaluated by radical scavenging of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and ferric reducing antioxidant power (FRAP). The antioxidant capacity by FRAP method was expressed as mg of quercetin equivalents (QE) per g extract (mgQE/g). All methods were measured by UV-Visible spectrophotometry at maximum wavelength. The determination of TPC in the extract was carried out *in vitro*. TFC of the extracts was determined with colorimetric aluminium chloride methods while the free radical scavenging ability of the sample extracts against DPPH free radical, proanthocyanidins (condensed tannins) in extract, and total anthocyanins were determined.

Results

There was a significant decrease ($p < 0.05$) observed in the levels of the phytochemical profile, antioxidant capacity, and bioactive compounds of *Vernonia amygdalina* in group 2 and 4 compared to group 1. On the other hand, a significant increase ($p < 0.05$) was observed in the levels of the three major parameters of *Vernonia amygdalina* in group 3 and 5 generally compared to group 1. However, no significant difference was observed between group 2 and 4. Steaming significantly increased FRAP in *Vernonia amygdalina* followed by microwaving and boiling, with steamed samples showing the highest antioxidant activity. Cooking techniques also enhanced DPPH free radical scavenging activity.

Conclusion

This study demonstrates that household cooking techniques impact the bioactive compounds and antioxidant activities of *Vernonia amygdalina*. The findings provide valuable insights for optimizing the cooking techniques of this plant to enhance their health benefits.

INTRODUCTION

Medicinal plants have played a significant role in traditional medicine for centuries, serving as a primary source of therapeutic compounds for disease prevention and treatment. These plants contain bioactive constituents such as alkaloids, flavonoids, phenolic acids, tannins, and saponins, which exhibit various pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, and anticancer activities (Sofowora et al., 2020). The global recognition of medicinal plants in modern medicine has led to extensive research on their chemical composition, biological activities, and potential health benefits. One such plant that has gained attention for its medicinal and nutritional importance is the bitter leaf (*Vernonia amygdalina*), which is widely consumed in African countries due to its rich phytochemical profile and numerous health-promoting effects (Kuniyal & Bisht, 2024).

Vernonia amygdalina, popularly known as bitter leaf, is extensively consumed across Africa particularly in Nigeria, for its nutritional and medicinal properties. It has long been utilized in traditional medicine for managing ailments such as malaria, diabetes, gastrointestinal infections, and hypertension (Omoregie & Osagie, 2018). The plant is rich in bioactive compounds, including total phenol content (TPC) and total flavonoid content (TFC), which contribute significantly to its antioxidant, anti-inflammatory, and antimicrobial properties (Omoregie & Osagie, 2021). However, the nutritional and therapeutic potential of bitter leaves is largely dependent on the methods of preparation and cooking, as household cooking techniques have been shown to influence the retention and degradation of these bioactive compounds (Adebooye et al., 2020).

Household cooking is a common practice in many communities, and the method employed can significantly alter the composition of food components, including vitamins, minerals, and phytochemicals (Eze et al., 2022). Cooking processes such as boiling, steaming, frying, and microwaving expose bitter leaves to varying degrees of heat, oxygen, and water, which can lead to the leaching, oxidation, or hydrolysis of phenolic compounds and flavonoids. Boiling, for instance, has been reported to cause a substantial loss of TPC and TFC due to the solubility of these compounds in water, leading to their migration into the cooking medium (Chikezie et al., 2019). Conversely, steaming has been found to retain more phenolic and flavonoid compounds as it minimizes direct contact with water, reducing the leaching effect. Frying, on the other hand, subjected bitter leaves to high temperatures and lipid oxidation, which may degrade these phytochemicals, whereas microwaving is considered a relatively mild heat treatment that preserves a higher proportion of phenolic and flavonoid contents (Idowu et al., 2021).

Phenolic compounds and flavonoids are known for their strong antioxidant properties, which help protect cells from oxidative stress caused by free radicals (Eze et al., 2022). Oxidative stress has been

linked to several degenerative diseases, including cancer, neurodegenerative disorders, and cardiovascular diseases. As a result, the consumption of vegetables such as bitter leaves is encouraged as a preventive strategy against these health conditions. However, the loss of these compounds during cooking may diminish the plant's effectiveness as a natural therapeutic agent (Adebooye et al., 2020).

The impact of different household cooking techniques on the phytochemical composition of bitter leaves is essential for developing cooking methods that maximize nutrient retention and health benefits. The choice of cooking technique can either enhance or diminish the bioavailability of phenolic and flavonoid compounds, affecting the overall nutritional quality of the vegetable.

Widely consumed across Africa, particularly in Nigeria, bitter leaf is renowned for its rich composition of bioactive compounds, including flavonoids, phenolics, alkaloids, and saponins, which contribute to its potent antioxidant properties (Atangwho et al., 2019). Antioxidants are essential in neutralizing free radicals, thereby reducing oxidative stress, a key factor in the development of chronic diseases such as cardiovascular disorders, cancer, and neurodegenerative conditions (Dike et al., 2020). One of the most widely used methods for evaluating the antioxidant potential of plant extracts is the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, which measures the ability of a substance to donate hydrogen atoms to stabilize free radicals (Ogunmoyole et al., 2022). Similarly, total antioxidant capacity (TAC) assays provide a broader assessment of the cumulative effect of antioxidants present in a sample (Oboh et al., 2021).

Despite the well-documented health benefits of bitter leaf, its phytochemical composition and antioxidant capacity are highly influenced by processing methods. Various techniques such as boiling, blanching, drying, and fermentation can significantly alter the concentration of bioactive compounds, leading to either enhancement or reduction of antioxidant activity. For instance, heat treatment often degrades thermolabile antioxidants, while certain drying techniques may either preserve or compromise bioactive compounds depending on exposure time and temperature (Ajibola et al., 2023). Fermentation, on the other hand, may enhance the bioavailability of certain

antioxidants by breaking down complex polyphenolic structures into simpler, more potent compounds (Chukwuma et al., 2020).

However, there is conflicting evidence regarding the extent to which these processing methods influence antioxidant activity. Some studies suggest that boiling leads to a significant reduction in DPPH scavenging activity due to the degradation of phenolic compounds, while others argue that moderate heat exposure can release bound polyphenols, thereby increasing total antioxidant capacity (Onwuka & Olawale, 2021; Ezeonu et al., 2021). Similarly, while freeze-drying has been reported to retain over 80% of the initial antioxidant content in bitter leaf, sun-drying has shown mixed effects, depending on the duration and intensity of exposure. These discrepancies highlight the need for further research to determine optimal processing methods that maximize the retention of antioxidant properties in bitter leaves (Adegbite et al., 2022).

Among the important nutrients of bitter leaf are ascorbic acid (vitamin C) and α -tocopherol (vitamin E), both of which play crucial roles as antioxidants in the human body, protecting cells from oxidative damage and supporting immune functions (Ajibola & Oyerinde, 2020). The method by which vegetables are cooked can significantly influence their nutritional quality. Different cooking techniques, including boiling, steaming, frying, and blanching, expose vegetables to varying temperatures, durations, and moisture levels, all of which can affect the stability of heat-sensitive vitamins such as ascorbic acid and α -tocopherol (Rashid et al., 2020). Ascorbic acid is particularly vulnerable to thermal degradation and leaching into cooking water, while α -tocopherol, though relatively more heat stable, can also deteriorate under high-temperature or prolonged cooking conditions (Tonucci et al., 2017).

The effects of different cooking methods on these critical nutrients are vital for recommending practices that preserve the nutritional integrity of bitter leaves. According to Aremu et al. (2022), nutritional losses during cooking are not only dependent on the temperature and time but also on the cooking medium—whether water-based, oil-based, or dry heat—each influencing nutrient retention differently. Hence, there is a growing interest in investigating how local cooking practices impact the vitamin content of indigenous vegetables like bitter leaf, which are essential to the diets of many households.

Previous studies have shown varied outcomes regarding nutrient retention across different cooking techniques. For instance, steaming is often reported to retain higher amounts of ascorbic acid compared to boiling or frying, as it minimizes direct contact with water and limits nutrient leaching (Jimoh & Kolapo, 2021).

Antioxidants play a crucial role in neutralizing free radicals, which can cause oxidative stress and lead to various chronic diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders (Halliwell & Gutteridge, 2018). Antioxidants are molecules that protect cells from oxidative damage by scavenging free radicals and reactive oxygen species (ROS) (Lobo et al., 2019). The human body maintains a delicate balance between oxidants and antioxidants, and an imbalance may lead to oxidative stress, which has been linked to several chronic diseases (Pham-Huy et al., 2008). Plant-based foods, such as bitter leaf, are natural sources of antioxidants, including vitamin C, vitamin E, polyphenols, and glutathione (GSH) (Zou et al., 2021). GSH, in particular, is a tripeptide composed of glutamate, cysteine, and glycine, playing a vital role in cellular detoxification and immune system regulation (Wu et al., 2018).

Cooking is an essential food processing technique that enhances taste, digestibility, and safety. However, different cooking methods can have varying effects on the bioavailability and stability of phytochemicals and antioxidants in plant foods (Jiménez-Monreal et al., 2009). Boiling, steaming, frying, and microwaving are some common cooking techniques that influence the retention or degradation of nutrients and bioactive compounds. Studies have shown that thermal processing can lead to the degradation of polyphenols, flavonoids, and glutathione due to heat exposure and oxidation (Turkmen et al., 2005).

For instance, boiling vegetables in water has been reported to cause significant leaching of water-soluble antioxidants such as vitamin C and polyphenols, leading to reduced antioxidant capacity (Ongol et al.,

2019). In contrast, steaming is considered a better method for preserving antioxidant properties as it minimizes direct contact with water, thereby reducing nutrient loss (Zhang et al., 2020). Frying, on the other hand, can either increase or decrease antioxidant levels depending on the type of oil used and the duration of heat exposure (Ndiaye et al., 2021). Microwaving has been found to retain more antioxidants than conventional boiling, but it may also result in some degradation of heat-sensitive compounds (Dewanto et al., 2002).

Despite the well-documented nutritional and medicinal benefits of bitter leaf, there is limited research on how different cooking methods affect its total antioxidant capacity and reduced glutathione levels. Most studies have focused on general phytochemical composition rather than the specific impact of processing methods on antioxidant biomarkers (Oboh et al., 2020).

Given the widespread consumption of bitter leaves in Africa and their use in traditional medicine, it is important to determine the optimal cooking method that maximizes their antioxidant potential and despite its well-documented medicinal benefits, the preparation and processing of bitter leaf significantly influence its bioactive composition and overall antioxidant potential. Different cooking techniques may enhance or degrade its health-promoting properties, affecting the levels of crucial antioxidants such as catalase (CAT) and the total antioxidant capacity measured by the ferric reducing antioxidant power (FRAP) assay (Coasta et al., 2020).

Catalase (CAT) is a crucial antioxidant enzyme responsible for protecting cells from oxidative damage by catalyzing the decomposition of hydrogen peroxide (H_2O_2) into water and oxygen (Lushchak, 2018). This enzymatic activity is essential in maintaining cellular homeostasis and preventing the accumulation of reactive oxygen species (ROS), which are known to contribute to aging and disease progression. However, catalase activity in plant-based foods is highly susceptible to environmental factors, particularly heat. Cooking techniques such as boiling, steaming, frying, and microwaving can significantly impact the stability and activity of catalase, potentially reducing or enhancing its antioxidant function. Studies have shown that heat treatments at high temperatures can lead to enzyme denaturation, thereby reducing antioxidant effectiveness, while milder cooking methods, such as steaming, may preserve enzymatic activity to a greater extent (Ekunode et al., 2020).

In addition to enzymatic antioxidants, non-enzymatic antioxidants in bitter leaf also play a crucial role in combating oxidative stress. The ferric reducing antioxidant power (FRAP) assay is a widely used method for evaluating the total antioxidant capacity of plant extracts based on their ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), which reflects the presence of reducing agents such as polyphenols and flavonoids (Benzie & Strain, 1996).

Despite the recognized health benefits of bitter leaf, research on how different cooking methods affect its antioxidant properties, particularly catalase activity and FRAP values, remains limited. This study examines the impact of boiling, steaming, and frying on these phytochemicals, antioxidants, and bioactive compounds to determine the best preparation method for preserving its medicinal and

nutritional value. The findings will inform dietary recommendations and food processing techniques, especially in regions where bitter leaf is a staple, hence, the need for this research.

METHODOLOGY

Sample Preparation and Experimental Design

Sample Collection and Preparation: Cultivated Bitter leaf (*Vernonia amygdalina*) plant on a private land was identified by a taxonomist based on its morphological features, and its authenticity. Fresh leaves were harvested in Amai community, Delta State, Nigeria, at a uniform maturity stage, identified and authenticated by Mr Michael O.E. in the Department of Botany, Delta State University, Abraka, deposited in Delta state University herbarium with voucher number DELSUH-090. The collection activities were executed in strict compliance with the Guidelines of plant collection, Delta State University, Abraka, Nigeria. The leaves were thoroughly rinsed with distilled water to eliminate dust and debris.

Plant Experimental Groupings:

They were divided into four groups and subjected to different household processing methods:

- I. Raw (Control): Untreated leaves, washed and blended.
- II. Steamed: Leaves steamed for 5–15 minutes.
- III. Fried: Leaves fried in oil such as palm oil for 5-10 minutes.
- IV. Soaked: Leaves-soaked multiple times to remove bitterness.
- V. Open-air dried: Leaves dried in open air.

After each group preparation, the leaves were dried at low heat, ground into fine powder, and stored in airtight containers at room temperature until needed for extraction. Extraction was carried out by soaking 10 g of each powdered sample in 100 mL of methanol for 48 hours with intermittent shaking. The extracts were then filtered, and the filtrates were stored at 4°C for subsequent analysis

NB: All further experiments were conducted in triplicates to ensure statistical validity.

Biochemical Assays:

1. Total phenolic content

The determination of total phenolic content in the extract was carried out *in vitro* according to the method described by Dewanto et al. (2002).

Principle

Total phenolic content is measured by Folin-Ciocalteu reagent assay. The Folin-Ciocalteu reagent is a yellow acidic solution containing complex polymeric ions formed from phosphomolybdic and phosphotungstic heteropoly acids. This reagent oxidizes phenolates, and the heteropoly acid becomes partially reduced from the +6 to a mixture of +6 and +5 valence state, resulting in the production of complex molybdenum-tungsten blue having maximum absorbance at 750 nm.

Procedure

Zero point five millilitres (0.5 ml) of the samples were dissolved in 100 µl of Folin-Ciocalteu reagent and 6 ml of distilled water. It was vortexed for 1 minute, and 2 ml of 15% Na₂CO₃ was added and then the mixture vortexed once again for thirty seconds (30 seconds). The solution was made up to 10 ml with distilled water. The absorbance of the sample was read at 750 nm with a UV spectrophotometer after 1 hour, thirty minutes (90 seconds) Gallic acid solution was used for the preparation of calibration curve. Total phenolics contents of samples were expressed as milligrams of gallic acid equivalent (mg GAE)/100 g of dry weight.

2. Total flavonoids

Total flavonoids content (TFC) of the extracts was determined with colorimetric aluminium chloride methods as described by Ebrahimzadeh *et al.* (2008).

Principle

Aluminium chloride forms acid stable complexes with the carbon-4 keto group and either the carbon-3 or carbon-5 hydroxyl group of flavones and flavonols. Rutin is reported to be suitable for building the calibration curve. Therefore, standard catechin solutions of various concentrations were used to build up the calibration curve used.

Procedure

Total flavonoid content was determined by Aluminium chloride method using catechin as a standard. 1ml of test sample and 4 ml of water were added to a 10 ml volumetric flask. After 5 min 0.3 ml of 5 % Sodium nitrite, 0.3 ml of 10% Aluminium chloride was added. After 6 min incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately, the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically. Results were expressed as catechin equivalents (mg catechin/g dried extract).

Calculation

The TFC was calculated using a standard curve with catechin (0-100 mg/L) as the standard.

3. DPPH (2,2-diphenyl-1-picrylhydrazyl)

The free radical scavenging ability of the sample extracts against DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical was estimated using the DPPH assay method described by Ursini *et al.* (1994).

Principle

The hydrogen donating ability of the extracts was examined in the presence of DPPH radicals. For a positive test, A violet solution is produced in ethanol.

Procedure

Fifty microgram (50 µg) of extract was diluted with 3 ml ethanol and mixed with 3 ml DPPH solution. The reaction mixture was shaken, and then incubated in the dark for 30 minutes. The absorbance of the solution was measured against a blank at 517 nm. DPPH scavenging ability was expressed as IC₅₀ (µg/ml) and the inhibition percentage was calculated using the formula below.

Calculation

Percentage inhibition of DPPH was calculated using following equation:

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 = the absorbance of the blank sample and

A_1 = the absorbance of the tested sample.

4. Determination of Total Antioxidant Capacity

Total antioxidant capacity (TAC) of bitter leaf extracts was evaluated using the phosphomolybdenum method as described by Prieto *et al.* (1999) with slight modifications.

Principle

The phosphomolybdenum method is based on the reduction of molybdenum (VI) to molybdenum (V) by the antioxidant compounds present in the extract. This reduction leads to the formation of a green phosphate/molybdenum (V) complex under acidic conditions, which can be quantified by measuring the absorbance at 695 nm. The intensity of the green color is directly proportional to the total antioxidant capacity of the sample.



Procedure

An aliquot of 0.3 mL of each extract was mixed with 3 mL of the reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The reaction mixture was incubated at 95°C for 90 minutes in a water bath. After cooling the samples to room temperature, then

the absorbance was measured at 695 nm using a UV-Visible spectrophotometer against a blank. Catechin standard solutions were prepared similarly and used for the calibration curve.

Calculation

The total antioxidant capacity was calculated from the catechin standard curve and expressed as units per gram dry weight (Units/g DW) of the sample.

$$\text{Total Antioxidant Capacity (Units/g DW)} = C \times V / M$$

Where:

C = concentration obtained from the catechin standard curve (Units/mL)

V = volume of extract used in the assay (mL)

M = mass of the dry sample (g)

5. Determination of Ascorbic Acid (Vitamin C)

Ascorbic acid content was determined using the 2,4-dinitrophenyl hydrazine method as described by Sadasivam and Manickam (1997).

Principle

Ascorbic acid is first oxidized to dehydroascorbic acid. The dehydroascorbic acid then reacts with 2,4-dinitrophenyl hydrazine under acidic conditions to form a yellowish-orange osazone, which is measurable using a spectrophotometer at 540 nm.



Then, reaction with 2,4-dinitrophenylhydrazine (DNPH):



Procedure

Different volumes of ascorbic acid standard (20–100 µl) and 40 µl of sample extract were pipetted into test tubes. A drop of thiourea solution and 1 ml of 2,4-dinitrophenyl hydrazine reagent were added to each tube respectively. The volume was adjusted to 100 µl with 4% oxalic acid. The mixture was then incubated at 37°C for 3 hours, cooled using an ice bath. Subsequently, 5 ml of 85% sulfuric acid was added to each tube. The developed orange color was measured at 540 nm against a reagent blank.

Calculation

The concentration of ascorbic acid in the samples was calculated using the standard calibration curve.

6. Determination of α -Tocopherol (Vitamin E)

The α -tocopherol content was estimated using the method described by Kivcak and Mert (2001).

Principle

The principle is based on the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) by α -tocopherol. The resulting ferrous ions form a red complex with α,α' -dipyridyl, which can be quantified spectrophotometrically at 520 nm.



Complex Formation:



Procedure

Volumes of 20–100 μl of α -tocopherol standard and 40 μl of the sample were taken and diluted to 3 ml using chloroform. To each sample, 1 ml of 2,2-dipyridyl solution and 1 ml of FeCl_3 solution were added. The mixture was incubated at 37°C for 15 minutes. After incubation, the absorbance was read at 520 nm against a reagent blank.

Calculation

The concentration of α -tocopherol in the samples was determined based on the standard calibration curve.

7. Proanthocyanidins

Proanthocyanidins (condensed tannins) in extract was determined using colorimetric assays.

Principle

Proanthocyanidins are a class of polyphenols which are abundant polyphenol in human diets. The reaction of vanillin solution in methanol with procyanidins in terminal units of proanthocyanidin in the sample produces a purple-colored solution that measure at 500 nm.

Procedure

To 50 L of diluted sample, 3 mL of 4% vanillin solution in methanol and 1.5 mL of concentrated HCl were added. The mixture was allowed to stand for 15 min, and absorption was measured at 500 nm against

methanol as a blank. Catechin solution was used for the preparation of calibration curve. The amount of total condensed tannins is expressed as mg (+)- catechin /g DW.

8. Anthocyanins

Determination of total anthocyanins was done using the pH differential method via UV-Vis spectrophotometry method as described by Iland *et al.* (1996).

Principle

Anthocyanin pigments presence in food. The absorbance at 520 nm of the pigments is proportional to the pigment concentration in the food sample.

Procedure

Sample (200 µL) was mixed with 3.8 mL of 1 M HCl and incubated at room temperature for 3 hours. The absorbance (A) of the acidified diluted extract was read at 520 nm against 1 M HCl as blank solution.

Calculation

Anthocyanins were calculated as of extract solution using the absorbance (B) of 1% w/v malvidin-3-glucoside solution as follows:

$$\text{Anthocyanins (mg/mL)} = A \times \text{Dilution factor} \times 1000 / B.$$

9. Ferric reducing antioxidant power (FRAP) assay

The ability to reduce ferric ions was measured using the method described by Benzie and Strain (1996).

Principle

The FRAP method relies on the reduction by the antioxidants, of the complex ferric ion-TPTZ (2,4,6-tri(2-pyridyl)1,3,5-triazine). Binding of Fe^{2+} to the ligand thereby creating a very intense navy-blue colour that is monitored spectrophotometrically at 593 nm.

Procedure

The FRAP reagent mixture was prepared by adding respectively 300 mM sodium acetate buffer at pH 3.6, 10.0 mM TPTZ (tripyridyl triazine) solution and 20.0 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution in a ratio of 10:1:1 (v/v). Zero point five millilitres (0.5 ml) of samples were added to 3 ml of FRAP reagent. The reaction mixture was incubated for 30 minutes at 37°C. The increase in absorbance at 593 nm was measured at 593 nm. FRAP was expressed as IC_{50} (µg/ml) amount of iron reduced.

Calculation

Ferric reducing antioxidant power = $(A_1 - A_2)/A_1 \times 100$

Where; A_1 is the absorbance of the control.

A_2 is the absorbance of the sample.

10. Reduced glutathione

The reduced glutathione concentration was estimated using the spectrophotometric method of Ellman (1959).

Principle:

Ellman's method is based on the reaction between the free sulfhydryl group of reduced glutathione (GSH) and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) which is also known as Ellman's reagent. When GSH reacts with DTNB, the disulfide bond in DTNB is cleaved thereby producing 2-nitro-5-thiobenzoate (TNB), a yellow-coloured compound.

Procedure

Two millilitres of 10% trichloroacetic acid was added 0.5 ml of sample and then centrifuged. One millilitre (1 ml) of supernatant was treated with 0.5 ml of Ellman's reagent and 3 ml of phosphate buffer. The colour developed was spectrophotometrically read at 412 nm. A series of standard were treated in similar manner along with a blank containing 3.5 ml of buffer.

Calculation:

The concentration of reduced GSH in μM of GSH/0.5g of wet tissue was extrapolated from a standard curve plotted.

Statistical Analysis

All experimental results were obtained in triplicates and expressed as mean $\pm$ standard deviation (SD). Statistical analysis was performed using Analysis of Variance (ANOVA) to assess the significant differences between treatment groups. Differences were considered statistically significant at $p < 0.05$. Data presentation was done in tables, and mean separation was carried out where necessary.

RESULTS

Table 1 shows the impact of household cooking techniques on total flavonoids content of bitter leaves. There was a significant decrease ($p < 0.05$) observed in total flavonoids content of bitter leaves in group 2, 4 and 5 compared to group 1. However, no significant difference was observed in total flavonoids content of bitter leaves in group 5 compared to group 1. Also, no significant difference was observed in

total flavonoids content of bitter leaves in group 2 and group 4. However, a significant increase ($p < 0.05$) was observed in total flavonoids content of bitter leaves in group 3 compared to group 1.

Table 1
Impact of household cooking techniques on Total flavonoids content of bitter leaves

Groups	Total flavonoids (Units/g DW)
Group 1 (Steamed bitter leaves)	54.20 ± 10.24 ^a
Group 2 (Fried bitter leaves)	45.80 ± 13.21 ^b
Group 3 (Open air dried bitter leaves)	59.40 ± 10.16 ^c
Group 4 (soaked bitter)	46.20 ± 11.14 ^b
Group 5 (Catechin standard)	50.90 ± 13.44 ^a

Triplicates values were presented in mean ± standard deviation. Mean values of parameters with different superscript letter differ significantly at $p < 0.05$.

Table 2 shows the impact of household cooking techniques on total phenol content of bitter leaves. There was a significant decrease ($p < 0.05$) observed in total phenol content of bitter leaves in group 2 and 4 compared to group 1. However, a significant increase ($p < 0.05$) was observed in total phenol content of bitter leaves in group 3 and 5 compared to group 1.

Table 2
Impact of household cooking techniques on Total phenol content of bitter leaves

Groups	Total phenol content (Units/g DW)
Group 1 (Steamed bitter leaves)	58.00 ± 15.19 ^a
Group 2 (Fried bitter leaves)	51.60 ± 14.45 ^b
Group 3 (Open air dried bitter leaves)	68.50 ± 16.44 ^c
Group 4 (soaked bitter)	48.30 ± 9.15 ^b
Group 5 (Catechin standard)	65.00 ± 10.91 ^c

Triplicates values were presented in mean ± standard deviation. Mean values of parameters with different superscript letter differ significantly at $p < 0.05$.

The changes in DPPH radical scavenging activity of bitter leaves prepared using different methods is shown in Table 3. There was a significant decrease ($p < 0.05$) observed in DPPH radical scavenging activity of bitter leaves in group 2 and 4 compared to group 1. However, no significant difference was observed in DPPH radical scavenging activity of bitter leaves in group 3 and 5 compared to group 1. Furthermore, no significant difference was observed between group 2 and 4 and also no significant difference was observed between group 3 and 5.

Table 3
Changes in DPPH radical scavenging activity of bitter leaves prepared using different methods

Groups	DPPH (%)
Group 1 (Steamed bitter leaves)	28.82 ± 5.44^a
Group 2 (Fried bitter leaves)	20.63 ± 4.78^b
Group 3 (Open air dried bitter leaves)	30.50 ± 6.81^a
Group 4 (soaked bitter)	18.33 ± 5.65^b
Group 5 (Catechin standard.	31.88 ± 7.91^a

Triplicates values were presented in mean $\pm$ standard deviation. Mean values of parameters with different superscript letter differ significantly at $p < 0.05$.

Table 4 shows the changes in total antioxidant capacity of bitter leaves prepared using different methods. A significant decrease ($p < 0.05$) was observed in total antioxidant capacity of bitter leaves in group 2 and 4 compared to group 1. However, a significant increase ($p < 0.05$) was observed in total antioxidant capacity of bitter leaves in group 3 and 5 compared to group 1. Furthermore, no significant difference was observed between group 2 and 4.

Table 4
Changes in Total antioxidant capacity of bitter leaves prepared using different methods

Groups	Total antioxidant capacity (Units/g DW)
Group 1 (Steamed bitter leaves)	316.22 ± 11.94^a
Group 2 (Fried bitter leaves)	208.84 ± 13.41^b
Group 3 (Open air dried bitter leaves)	349.49 ± 20.46^c
Group 4 (soaked bitter)	210.22 ± 21.94^b
Group 5 (Catechin standard)	358.84 ± 33.46^d

Triplicates values were presented in mean $\pm$ standard deviation. Mean values of parameters with different superscript letter differ significantly at $p < 0.05$.

The result of the effect of different cooking techniques on ascorbic acid of bitter leaves is presented in Table 5. There was a significant decrease ($p < 0.05$) observed in ascorbic acid of bitter leaves in group 2 and 4 compared to group 1. On the other hand, a significant increase ($p < 0.05$) was observed in ascorbic acid of bitter leaves in group 3 and 5 compared to group 1. Furthermore, no significant difference was observed between group 2 and 4 and also no significant difference was observed between group 3 and 5.

Table 5
Effect of different cooking techniques on Ascorbic acid of bitter leaves

Groups	Ascorbic acid (Units/g DW)
Group 1 (Steamed bitter leaves)	2.82 ± 0.11^a
Group 2 (Fried bitter leaves)	0.23 ± 0.14^b
Group 3 (Open air dried bitter leaves)	6.50 ± 0.21^c
Group 4 (soaked bitter)	1.23 ± 0.05^b
Group 5 (Ascorbic acid standard)	7.50 ± 0.31^c

Triplicates values were presented in mean $\pm$ standard deviation. Mean values of parameters with different superscript letter differ significantly at $p < 0.05$.

The effect of different cooking techniques on α -tocopherol of bitter leaves is shown in Table 6. There was no significant difference observed in α -tocopherol of bitter leaves in groups 2, 3, 4, and 5 compared to group 1.

Table 6
Effect of different cooking techniques on α -Tocopherol of bitter leaves

Groups	α -Tocopherol (Units/g DW)
Group 1 (Steamed bitter leaves)	18.92 $\pm$ 1.94 ^a
Group 2 (Fried bitter leaves)	18.84 $\pm$ 3.91 ^a
Group 3 (Open air dried bitter leaves)	19.49 $\pm$ 0.76 ^a
Group 4 (soaked bitter)	17.22 $\pm$ 1.94 ^a
Group 5 (α -Tocopherol standard)	20.84 $\pm$ 3.91 ^a

Triplicates values were presented in mean $\pm$ standard deviation. Mean values of parameters with different superscript letter differ significantly at $p < 0.05$.

Table 7 presented the result of proanthocyanidins levels of bitter leaves prepared using different cooking techniques. There was a significant decrease ($p < 0.05$) observed in proanthocyanidins levels of bitter leaves in group 2 and 4 compared to group 1. On the other hand, a significant increase ($p < 0.05$) was observed in proanthocyanidins levels of bitter leaves in group 3 compared to group 1. However, no significant difference was observed between group 2 and 4

Table 7
Proanthocyanidins levels of bitter leaves prepared using different cooking techniques

Groups	Proanthocyanidins (Units/g DW)
Group 1 (Steamed bitter leaves)	14.22 $\pm$ 1.91 ^c
Group 2 (Fried bitter leaves)	12.84 $\pm$ 3.61 ^a
Group 3 (Open air dried bitter leaves)	19.49 $\pm$ 4.76 ^b
Group 4 (soaked bitter)	11.22 $\pm$ 3.22 ^a

Triplicates values were presented in mean $\pm$ standard deviation. Mean values of parameters with different superscript letter differ significantly at $p < 0.05$.

Table 8 shows the result of anthocyanins levels of bitter leaves prepared using different cooking techniques. A significant decrease ($p < 0.05$) was observed in anthocyanins levels of bitter leaves in group 2 and 4 compared to group 1. However, there was a significant increase ($p < 0.05$) recorded in

anthocyanins levels of bitter leaves in group 3 compared to group 1. Furthermore, no significant difference was observed between group 2 and 4.

Table 8
Anthocyanins levels of bitter leaves prepared using different cooking techniques

Groups	Anthocyanins (Units/g DW)
Group 1 (Steamed bitter leaves)	0.62 ± 0.09 ^a
Group 2 (Fried bitter leaves)	0.23 ± 0.02 ^b
Group 3 (Open air dried bitter leaves)	0.80 ± 0.11 ^c
Group 4 (soaked bitter)	0.33 ± 0.05 ^b

Triplicates values were presented in mean ± standard deviation. Mean values of parameters with different superscript letter differ significantly at $p < 0.05$.

The result of the changes in reduced glutathione of bitter leaves prepared using different cooking techniques is shown in Table 9. A significant increase ($p < 0.05$) was observed in reduced glutathione levels of bitter leaves in group 3 compared to group 1. However, no significant difference ($p < 0.05$) was observed in reduced glutathione levels of bitter leaves in group 2 and 4 compared to group 1.

Table 9
Changes in reduced glutathione of bitter leaves prepared using different cooking techniques

Groups	Reduced glutathione (Units/g DW)
Group 1 (Steamed bitter leaves)	6.82 ± 2.10 ^a
Group 2 (Fried bitter leaves)	5.23 ± 1.49 ^a
Group 3 (Open air dried bitter leaves)	10.50 ± 2.34 ^b
Group 4 (soaked bitter)	4.23 ± 0.17 ^a

Triplicates values were presented in mean ± standard deviation. Mean values of parameters with different superscript letter differ significantly at $p < 0.05$.

Table 10 presented the result of the changes in FRAP assay of bitter leaves prepared using different cooking techniques. There was a significant increase ($p < 0.05$) observed in FRAP activity of bitter leaves in group 3 compared to group 1. However, no significant difference ($p < 0.05$) was observed in reduced FRAP activity of bitter leaves in group 2 and 4 compared to group 1.

Table 10
Changes in FRAP assay of bitter leaves prepared using
different cooking techniques

Groups	FRAP (Units/g DW)
Group 1 (Steamed bitter leaves)	16.22 ± 1.24 ^a
Group 2 (Fried bitter leaves)	14.84 ± 2.41 ^a
Group 3 (Open air dried bitter leaves)	29.49 ± 0.53 ^b
Group 4 (soaked bitter)	14.22 ± 1.98 ^a

Triplicates values were presented in mean ± standard deviation. Mean values of parameters with different superscript letter differ significantly at $p < 0.05$.

DISCUSSION

The results presented in Table 1 provide insight into the effect of various household cooking techniques on the total flavonoid content of bitter leaves. In group 1, which involved steaming bitter leaves, a higher concentration of flavonoids (54.20 ± 10.24 units/g DW) was observed compared to the other methods. This is consistent with findings by various researchers, such as Soforowa et al. (2020), who reported that steaming generally preserves a greater amount of bioactive compounds in vegetables compared to methods like frying or boiling. Steaming has been associated with minimal nutrient loss, making it one of the best cooking methods for retaining phytochemicals, including flavonoids (Omoregie & Osagie, 2018).

The frying method (group 2), however, resulted in a notable decrease in total flavonoids (45.80 ± 13.21 units/g DW). This reduction aligns with the work of Chikezie et al., (2019), who observed a decrease in antioxidant compounds, including flavonoids, in vegetables subjected to frying. Frying is known to cause degradation of heat-sensitive nutrients due to the high temperatures involved, which can lead to significant nutrient loss.

In contrast, open air drying (group 3) showed a significant increase in the flavonoid content (59.40 ± 10.16 units/g DW). This outcome is in line with the findings of Kuniyal & Bisht, (2024), who demonstrated that drying, particularly air drying, could enhance the concentration of certain antioxidants in plants. Drying, by reducing moisture content, may concentrate the flavonoids in the leaves, thus resulting in higher observed values. Soaking (group 4), on the other hand, did not significantly differ from frying in terms of its impact on flavonoid content (46.20 ± 11.14 units/g DW). This suggests that soaking may not effectively preserve or enhance the flavonoid levels in bitter leaves, as similar reductions in flavonoids were observed when compared to frying. This aligns with the findings of Eze et al., (2022), who found that soaking does not significantly impact the retention of flavonoids in leafy vegetables.

Finally, the catechin standard (group 5), which had a flavonoid content of 50.90 ± 13.44 units/g DW, showed no significant difference when compared to the steamed bitter leaves in group 1. Catechin, a flavonoid known for its antioxidant properties, served as a reference point for the flavonoid content in the bitter leaves, supporting the idea that certain preparation methods, such as steaming, maintain flavonoid levels comparable to natural or unaltered compounds (Adebooye et al., 2020).

The findings presented in Table 2 offer valuable insights into how different household cooking techniques affect the total phenolic content of bitter leaves. As with flavonoids, the cooking method significantly influences the retention or loss of phenolic compounds, which are known for their antioxidant properties. In group 1, the steamed bitter leaves had a total phenol content of 58.00 ± 15.19 units/g DW, which was significantly higher than the content in fried (group 2) and soaked (group 4) bitter leaves. These results are consistent with previous studies that suggest steaming helps preserve phenolic compounds better than other cooking methods. For instance, Idowu et al. (2021) reported that steaming is one of the most effective cooking methods for retaining phenolic compounds in vegetables, as it minimizes nutrient loss through direct heat exposure.

Frying (group 2) resulted in a significant reduction in phenolic content (51.60 ± 14.45 units/g DW). This decrease is in line with the findings of Chikezie et al. (2019), who found that frying, due to its high temperature and prolonged cooking time, causes the degradation of phenolic compounds in vegetables. High temperatures can lead to the breakdown of phenols, making frying a less favorable method for preserving antioxidant properties.

Soaking (group 4) also led to a significant decrease in phenolic content (48.30 ± 9.15 units/g DW). This finding is consistent with the research by Eze et al., (2022), who found that soaking does not significantly impact the retention of phenolic compounds in leafy vegetables. The loss of phenols during soaking may be due to leaching into the water, which diminishes the concentration of bioactive compounds in the plant material.

Conversely, open air drying (group 3) resulted in a significant increase in total phenolic content (68.50 ± 16.44 units/g DW) compared to the steamed leaves (group 1). This result aligns with the findings of Chikezie et al. (2019), who demonstrated that drying techniques, particularly air drying, can concentrate phenolic compounds in plants. As moisture content decreases during drying, phenolic compounds become more concentrated, leading to an apparent increase in their levels. This is an advantageous method for preserving and even enhancing the antioxidant potential of vegetables.

Finally, the catechin standard (group 5) showed a total phenol content of 65.00 ± 10.91 units/g DW, which was significantly higher than the steamed leaves but comparable to the dried bitter leaves. Catechins, a type of phenolic compound, are often used as a reference in studies involving plant phenols due to their well-documented antioxidant properties. The results suggest that the phenolic content in bitter leaves, when dried, approaches that of a pure catechin standard, indicating the potential of drying as an effective method for enhancing the phenolic content of plant foods.

As presented in Table 3, the DPPH radical scavenging activity of bitter leaves varied significantly depending on the cooking method. Steaming and open-air drying (Groups 1 and 3) exhibited similar levels of radical scavenging activity ($28.82 \pm 5.44\%$ and $30.50 \pm 6.81\%$, respectively), which were significantly higher than that observed in fried and soaked leaves (Groups 2 and 4). Fried bitter leaves (Group 2) showed a considerable reduction in DPPH scavenging activity ($20.63 \pm 4.78\%$), as did soaked leaves (Group 4) with a value of $18.33 \pm 5.65\%$. These results are consistent with findings from other studies, which have shown that high heat treatments such as frying can cause degradation of bioactive compounds, resulting in reduced antioxidant activities (Dike et al., 2020). Conversely, open-air drying and steaming, which involve lower heat exposure, have been reported to better preserve antioxidant compounds (Chukwuma et al., 2020). The results from this study suggest that more gentle cooking methods, such as steaming and air drying, help to retain the DPPH radical scavenging ability of bitter leaves, supporting the findings of previous research on the impact of cooking techniques on antioxidant retention (Oboh et al., 2021).

The total antioxidant capacity (TAC) of bitter leaves, as seen in Table 4, also varied significantly based on the cooking method. Open-air drying (Group 3) and the catechin standard (Group 5) exhibited the highest TAC values (349.49 ± 20.46 and 358.84 ± 33.46 units/g DW, respectively), with both methods showing significantly greater values than steamed bitter leaves (Group 1) (316.22 ± 11.94 units/g DW). Fried (Group 2) and soaked (Group 4) bitter leaves exhibited significantly lower TAC values (208.84 ± 13.41 and 210.22 ± 21.94 units/g DW, respectively). This trend aligns with the findings of Onwuka and Olawale (2021), who demonstrated that high-temperature cooking methods such as frying significantly reduce the total antioxidant capacity of vegetables by degrading phenolic compounds. Similarly, studies have shown that soaking can lead to the leaching of water-soluble antioxidants (Ogunmoyole et al., 2022). On the other hand, methods that minimize heat exposure, such as open-air drying, retain higher levels of antioxidants, as observed in this study and reported by other authors (Ajibola et al., 2023).

The findings from Table 5 revealed that different cooking techniques had significant effects on the ascorbic acid content of bitter leaves. Specifically, frying (Group 2) and soaking (Group 4) led to a significant reduction in ascorbic acid levels compared to steaming (Group 1). This observation aligns with the report of Ajibola and Oyerinde (2020), who stated that ascorbic acid is highly sensitive to heat and leaching during cooking processes, leading to substantial nutrient losses. Frying involves high temperatures, which can accelerate the oxidation and degradation of ascorbic acid, while soaking can promote leaching of water-soluble vitamins into the surrounding water, thereby decreasing their concentration in the leaves (Tonucci et al., 2007).

Conversely, open-air drying (Group 3) showed a significant increase in ascorbic acid content compared to steaming. This is in agreement with the findings of Rashid et al. (2020), who observed that drying under controlled, non-extreme conditions can help retain or even concentrate certain heat-labile vitamins like vitamin C by reducing moisture content without excessive thermal degradation. Furthermore, the higher value observed in the open-air dried samples may be due to reduced enzymatic and oxidative degradation as a result of moisture loss (Aremu et al., 2022). The ascorbic acid standard (Group 5)

showed the highest concentration, as expected, since it represents a pure, unprocessed form of the vitamin without any external exposure to heat, water, or air that would otherwise cause degradation. Notably, no significant difference was observed between frying and soaking, suggesting that both techniques equally contribute to the loss of ascorbic acid in bitter leaves (Jimoh & Kolapo, 2021).

The results from Table 6 indicate that different cooking techniques did not significantly affect the α -tocopherol content of bitter leaves. The α -tocopherol levels in the steamed (Group 1), fried (Group 2), open air dried (Group 3), and soaked (Group 4) bitter leaves, as well as the α -tocopherol standard (Group 5), showed no significant variations. This outcome suggests that α -tocopherol is relatively stable under the cooking conditions applied. These findings are consistent with the report by Rashid et al. (2020), who demonstrated that α -tocopherol possesses a degree of thermal stability, especially in low to moderate temperature treatments. Frying, although involving high temperatures, had minimal impact on α -tocopherol content due to its lipophilic nature, which allows it to remain stable in oily environments (Tonucci et al., 2017).

Similarly, open-air drying did not significantly alter the α -tocopherol concentration, which is in agreement with the findings of Dewanto et al. (2002), who observed that dehydration processes, particularly at ambient temperatures, do not majorly affect fat-soluble vitamins like vitamin E. The slight decrease observed in soaked samples can be attributed to minor losses from exposure to water, although the hydrophobic property of α -tocopherol minimizes significant leaching (Tonucci et al., 2017).

Moreover, the relatively close values between the cooked samples and the standard support the idea that α -tocopherol is more resilient to oxidative and thermal degradation compared to water-soluble vitamins such as ascorbic acid. This observation further corroborates the findings of Aremu et al. (2022), who reported that fat-soluble antioxidants in leafy vegetables are more stable under cooking and processing conditions.

The results of this study on the proanthocyanidin and anthocyanin content of bitter leaves prepared using different cooking methods revealed important findings that align with and expand upon existing literature.

As presented in Table 7, proanthocyanidin levels in the different groups varied significantly, with steamed bitter leaves (Group 1) showing the highest levels, followed by open-air dried leaves (Group 3), and significantly lower levels in fried (Group 2) and soaked (Group 4) bitter leaves. These findings are consistent with the work of Lobo et al. (2019), who demonstrated that high-temperature treatments like frying and boiling can result in the breakdown of polyphenolic compounds, including proanthocyanidins, due to heat-induced chemical degradation. In contrast, the higher levels of proanthocyanidins in the open-air dried leaves (Group 3) could be attributed to the concentration effect caused by the evaporation of moisture during the drying process, which has been reported to preserve or even enhance certain phytochemicals (Turkmen et al., 2005). It is well-established that the degradation of polyphenolic compounds is temperature- and time-dependent, and that exposure to prolonged heat, especially in the presence of oxygen, accelerates the loss of these compounds. This explains the significant reduction of

proanthocyanidins in groups 2 and 4 (fried and soaked), which experienced more intensive heat or prolonged contact with water, further corroborating earlier findings (Pham-Huy et al., 2008).

Similarly, the anthocyanin content (Table 8) also exhibited significant variations across the different cooking methods. Steamed leaves (Group 1) showed the highest anthocyanin content, while the fried and soaked leaves (Groups 2 and 4) had significantly lower levels. This is consistent with findings by Wu et al. (2018), who observed that anthocyanins, being heat-sensitive compounds, degrade rapidly when subjected to high temperatures. Frying, in particular, is known to cause significant loss of anthocyanins due to the direct exposure to high heat. The results from the current study further confirm this observation, as frying led to the greatest loss of anthocyanins among the cooking methods assessed. On the other hand, open-air drying (Group 3) resulted in the highest levels of anthocyanins, which could be attributed to the lower temperatures involved in the drying process. This finding supports the work of Zou et al. (2021), who reported that air drying techniques, although involving heat, cause less degradation of anthocyanins compared to methods like frying and boiling, which involve higher temperatures and prolonged exposure.

It is interesting to note that no significant difference was found between fried and soaked bitter leaves in both proanthocyanidin and anthocyanin content. This could suggest that the prolonged soaking process, although it involves less direct heat, may also lead to the leaching out of water-soluble compounds, such as polyphenols, resulting in a reduction in the levels of these phytochemicals, similar to the effects of high heat exposure (Ongol et al., 2019). These results emphasize the complex interactions between cooking techniques and the retention of phytochemicals in plant materials. The degradation or preservation of bioactive compounds like proanthocyanidins and anthocyanins depends not only on the temperature but also on the duration of exposure and the nature of the cooking method itself (Ongol et al., 2019).

The results presented in Table 9 showed that the reduced glutathione (GSH) levels varied significantly across the different cooking techniques used on bitter leaves. Open-air dried bitter leaves recorded the highest GSH concentration (10.50 ± 2.34 Units/g DW), which was significantly higher ($p < 0.05$) than those observed in steamed, fried, and soaked groups. This suggests that open-air drying is more effective in preserving the antioxidant potential of bitter leaves compared to thermal processing methods like steaming and frying. This finding is in agreement with the work of Heinrich et al., (2017), who reported that non-thermal processes such as sun drying retain higher levels of antioxidants, particularly heat-sensitive compounds like glutathione. Furthermore, WHO (2021) noted that high-temperature treatments often result in the degradation of bioactive molecules, leading to a substantial reduction in antioxidant levels. The lower GSH levels observed in fried and soaked leaves could be attributed to thermal degradation and leaching of water-soluble antioxidants, respectively. This trend aligns with observations made by Lushchak (2018), who reported that frying induces oxidative damage to antioxidant enzymes, while soaking in water can lead to loss of antioxidant compounds into the surrounding medium. Hence, the data suggests that open-air drying might be the preferable method for

preserving the antioxidant defense system of bitter leaves, maintaining their nutritional and functional qualities.

Similarly, the findings from Table 10 showed a significant difference in the ferric reducing antioxidant power (FRAP) values of bitter leaves processed by different methods. Open-air dried bitter leaves again exhibited the highest FRAP activity (29.49 ± 0.53 Units/g DW), significantly higher ($p < 0.05$) than the steamed, fried, and soaked groups. This enhanced FRAP value indicates a greater retention of reducing power and antioxidant constituents, reinforcing the idea that minimal or non-thermal processing preserves phytochemical integrity. This observation agrees with the study by Coasta et al. (2020), who highlighted that drying without high heat helps maintain the antioxidant activity of vegetables, preserving their ability to donate electrons and neutralize free radicals. On the other hand, the reduction in FRAP activity observed in fried and soaked bitter leaves corroborates the findings of Ekunode et al. (2020), who reported that both thermal damage and leaching lead to substantial losses in antioxidant potential. Heinrich et al. (2010) also noted that heat processing can break down phenolic compounds, significantly reducing the antioxidant properties of food substances. The present findings thus imply that open-air drying could be a superior method for preserving the antioxidant quality of bitter leaves, maintaining both their nutritional value and therapeutic potential for consumption.

CONCLUSION

To maximize the medicinal and nutritional benefits of bitter leaves, minimal and non-invasive cooking methods should be prioritized. Processing cooking techniques generally cause a significant reduction in total phenols and antioxidant properties. Cooking reduces the antioxidant properties of the raw leaves. Vitamin C is highly sensitive to heat and water whereas alpha-tocopherol is more stable. Soaking decreases the reducing power and free radical scavenging ability of bitter leaves. While processed bitter leaves generally have lower FRAP values compared to fresh, they still maintain significant antioxidant capacity. Cooking treatments generally reduce the level of phytochemicals such as flavonoids in bitter leaf. These conclusions are vital for dietary recommendations, especially in regions where bitter leaves form a staple part of the diet.

Declarations

Declaration of Generative AI and AI-Assisted Technologies in Scientific Writing

AI was used to comprise and correct grammatical. CHATGPT and quillbot was used.

Declaration of Conflict of Interests

The authors have declared no conflicts of interest.

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Not applicable

Ethical declarations

Ethics approval and consent to participate:

The collection activities were executed in strict compliance with the Guidelines of plant collection, Delta State University, Abraka, Nigeria. Fresh leaves authentication was done by a taxonomist (Mr Michael O.E.) in the Department of Botany, Delta State University, Abraka. Specimen deposited in Delta State University herbarium with voucher number DELSUH-090.

Author Contribution

UBI: Contributed with conception, methodology, investigation, data curation, writing original draft, manuscript preparation, revision and resources. CVE: Contributed with conception, draft, manuscript preparation, revision and resources. EEC: Contributed with conception, writing original draft, manuscript preparation, revision and resources. OJ: Contributed with conception, methodology, investigation, data curation, writing original draft, manuscript preparation, revision and resources. EMO: Contributed with validation, manuscript preparation and revision, resources. MMO: Investigation, resources.

All authors reviewed the manuscript.

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Data Availability

The data and materials supporting the findings of this study are available from the corresponding author upon reasonable request.

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