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Evaluation of Antioxidant and Antinutritional Maturity Variability Stages in Vegetable Fern (*Diplazium esculentum*) Fronds Consumed as Food in Nigeria

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Abstract
This study evaluated the effect of maturity stages on the antinutritional and antioxidant activity of vegetable fern fronds using standard methods. Fresh tender and matured fronds of vegetable fern were obtained from Mkpat Enin Local Government Area, Akwa Ibom State, Nigeria. The plant was identified in the herbarium, Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo. The study adopted experimental research design to systematically analyze the impact of maturity on DPPH scavenging activity, reducing power (RP) activity, total phenolic and flavonoid content, phytochemical composition, and antinutritional factors in tender and matured fronds. Results showed that total phenolic content was significantly ($p < 0.05$) higher in matured fronds (91.7 mgGAE/g) than in tender fronds (29.6 mgGAE/g). Similarly, total flavonoid content was higher in matured (266 mgRE/g) than in tender fronds (75 mgRE/g). Antinutritional composition was higher in matured fronds, except for alkaloid and saponin, which were significantly ($p < 0.05$) higher in tender fronds. Phytochemicals (steroids, total anthraquinone, and cardiac glycoside) were not significantly affected by maturity or pretreatment. DPPH scavenging activity was significantly ($p < 0.05$) higher in matured fronds at 20, 40, and 100 µg/ml, except in hot water blanched samples, where tender fronds had higher activity. Sun-dried matured fronds exhibited the highest DPPH inhibition (66–85%) across all concentrations. Reducing power activity was higher in shade-dried and untreated tender fronds, while treatment variables did not significantly ($p < 0.05$) affect matured fronds. The IC50 for untreated tender fronds (29.5 µg/ml) was significantly ($p < 0.05$) higher than that of untreated matured fronds (15.0 µg/ml), indicating stronger antioxidant potential in matured fronds. From the findings of the study, it was recommended that matured fronds, rich in phenolics and flavonoids, offer superior antioxidant benefits, while tender fronds, high in alkaloids and saponins, are suitable for medicinal use. That public health campaigns should educate stakeholders, and future studies should explore phytochemical bioavailability for pharmaceutical and dietary applications.

Keywords: *Antioxidant, Antinutrient, Phytochemicals, Vegetable Fern, Fronds, Maturity Stages, Nigeria*

Introduction

The rising incidence of diet-related illnesses such as diabetes, obesity, and cardiovascular diseases has led to increased interest in nutrient-rich, bioactive vegetables with therapeutic properties. Vegetable fern (*Diplazium esculentum*), widely consumed in various parts of the

world, is recognized for its antioxidant capacity and medicinal benefits (Singh, Prasad & Sharma, 2022; Lobo, Patil, Phatak & Chandra, 2022). Antioxidants play a vital role in human health by neutralizing free radicals, slowing the aging process, and reducing inflammation and oxidative stress associated with chronic conditions such as cardiovascular diseases, arteriosclerosis, cancer, diabetes, cataracts, cognitive disorders, and neurodegenerative diseases (Eliassen, Hendrickson & Brinton, 2012; Chen, Wang & Zhang, 2022).

D. esculentum is a rich source of flavonoids, phenolic compounds, carotenoids, and ascorbic acid, which contribute to its strong antioxidant potential (Smriti, Manivannan & Venkata, 2018; Rahman, Hossain & Akhter, 2021). Among these, flavonoids exhibit neuroprotective effects against cytotoxic stress, potentially aiding in the treatment of neurodegenerative conditions like stroke and Alzheimer's disease (Someya, Yoshiki & Okubo, 2002). The antioxidant activity of flavonoids operates through scavenging or chelation, while phenolic compounds also contribute significantly due to their hydroxyl groups, which enhance free radical scavenging ability.

Phytic acid, another antioxidant present in vegetable fern, serves as a natural food preservative by enhancing nutritional value, extending shelf life, and preventing food discoloration (Dost & Tokul, 2005). Extracts from *D. esculentum* exhibit iron-chelating properties, lipid peroxidation activity, and scavenging effects against reactive oxygen species (ROS) and reactive nitrogen species (RNS), including hydroxyl radicals, superoxide, hydrogen peroxide, nitric oxide, and peroxynitrite (Roy et al., 2013a). However, these antioxidant properties are influenced by environmental factors such as soil composition, harvesting methods, and plant developmental stages (Lal, Choudhary & Yadav, 2020).

Despite its nutritional benefits, *D. esculentum* also contains antinutritional compounds, particularly saponins and tannins, which have pharmacological significance (Roy et al., 2013b). Saponins can disrupt cell membranes and are linked to haemolytic activity, while tannins, being heat-resistant, remain stable during boiling and may contribute to the plant's toxicity (Roy, Tamang, Dey & Chaudhuri, 2013a). The presence of these compounds raises concerns about potential adverse effects, particularly when consuming matured fronds.

The chemical composition of *D. esculentum*, especially its antioxidant and antinutritional properties, varies depending on the plant's maturity at harvest (Zhang, Li & Zhao, 2021). Tender fronds tend to be richer in vitamins and polyphenols, while matured fronds contain higher concentrations of secondary metabolites that enhance oxidative defense mechanisms (Eliassen, Hendrickson & Brinton, 2012; Jha, Singh & Kumar, 2023). Though tender fronds are often preferred due to their soft texture and mild flavor, matured fronds are associated with higher fiber content and possibly greater levels of bioactive compounds (Rathore, Mishra & Sen, 2023).

Research on leafy vegetables suggests that maturity significantly affects the concentration of beneficial and harmful compounds (Mondal, Nahar & Chowdhury, 2021). While tender fronds of *D. esculentum* are rich in antioxidants, matured fronds accumulate antinutritional factors such as oxalates and tannins, which can interfere with mineral absorption, particularly calcium and iron, leading to potential deficiencies if consumed in excess (Singh, Pandey & Verma, 2023; Umar, Bello & Aliyu, 2022).

Antinutritional compounds tend to increase as the fronds mature, reducing the bioavailability of essential minerals like calcium, iron, and zinc (Gupta & Sharma, 2020; Chakraborty, Das & Banerjee, 2021). These compounds can form complexes with proteins and minerals, inhibiting digestion and absorption, thus raising concerns about the long-term dietary effects of consuming matured fronds without proper processing (Ahmad Bello & Ojo, 2022).

Traditional cooking methods such as boiling, blanching, and fermentation can reduce these antinutritional factors, but their effectiveness across different maturity stages remains largely underexplored (Soetan, 2008).

The balance between the antioxidant potential and antinutritional content of *D. esculentum* is a critical research area with implications for nutritional science, food security, and public health (Rahman, Hossain & Akhter, 2021; Adebayo, Ogunleye & Abiodun, 2023; Ibrahim, Omoniyi & Adewale, 2023). In Nigeria, these edible ferns are an integral part of indigenous diets, particularly in rural communities where they serve as affordable sources of micronutrients and antioxidants. However, prior research has primarily focused on conventional vegetables such as spinach and amaranth, leaving the potential of edible ferns largely unexplored (Nahar, Hossain & Chowdhury, 2021).

Environmental factors such as soil composition, geographical location, and climatic variations influence the biochemical properties of *D. esculentum* at different maturity stages (Adeyemi & Aluko, 2023). In Nigeria, the fern is commonly subjected to treatments such as blanching, soaking, and drying to improve palatability, extend shelf life, and reduce bitterness and antinutrients. The variability in nutrient and antinutrient content across different growth stages presents a challenge in determining the optimal harvest stage to maximize health benefits while minimizing risks. Without empirical data, food scientists and consumers lack sufficient information to make informed decisions regarding the dietary applications of *D. esculentum*.

To fully harness the nutritional and medicinal benefits of *D. esculentum*, it is essential to evaluate its antioxidant and antinutritional profile across different maturity stages. This study aims to generate empirical evidence to guide food scientists, policymakers, and consumers on the optimal harvest stage for maximizing health benefits. The findings will contribute to sustainable nutrition strategies, food security policies, and dietary interventions, ensuring that indigenous vegetables like *D. esculentum* are effectively utilized for public health and economic development.

Objectives of the Study

1. The effect of stage of maturity on 2,2-diphenyl-1-Picrylhydrazyl (DPPH) scavenging activity of vegetable fern fronds.
2. The Reducing Power (RP) activity of tender/matured vegetable fern fronds and ascorbic acid.
3. The total phenolic and flavonoid content of vegetable fern fronds.
4. The effect of stage of maturity on the phytochemical composition of vegetable fern fronds.
5. The stage of maturity effect on the antinutritional factors in vegetable fern fronds.

Materials and Methods

1. Material Procurement, Preparation and Extract

This study adopted experimental research design. Fresh tender and matured fronds of vegetable fern were collected from Mkpat Enin Local Government Area, Akwa Ibom State, and identified at the University of Uyo Herbarium, (UUPH A10 (a). The fronds were cleaned, washed with distilled water, and cut into pieces. A total of 1.5kg of fresh fronds (tender and matured) was used, divided into five portions of 300g each. One portion served as the control sample, while the remaining portions were treated as outlined below.

i. **Oven dried**: 300g of vegetable fern fronds were oven-dried at 60°C for 48 hours using a Blast Air Oven (KX350A, Kenixin, China) and then stored in an airtight container.

ii. **Sun dried**: 300g of fronds were sun-dried at an ambient temperature of 35-41°C from 10:30 am to 5:30 pm daily for 5 days until constant weight was reached, then stored in an airtight container.
iii. **Hot water blanched**: 300g of fronds were blanched in hot water at 70°C for 2 minutes, then cooled with cold running water and drained.
iv. **Shade dried**: 300g of fronds were dried under shade with good ventilation, low humidity, and no direct sunlight exposure, similar to sun drying.

The fresh, blanched, and dried fronds were macerated with 70% ethanol at room temperature (26-33°C) for 72 hours with intermittent shaking. Afterward, the liquid extract was filtered using Whatman No. 1 filter paper, concentrated at 50°C using a rotary evaporator (RE–201D IL, China), and stored in a refrigerator at 4°C.

2. Determination of 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Activity

DPPH free radical scavenging of tender and matured fronds of vegetable fern and ascorbic acid (prepared in methanol) at concentrations of 20, 40, 60, 80, and 100 µg/ml was evaluated following Shekhar and Anju (2014). DPPH (1 ml of 0.04 g/L) was added to 3 ml of ascorbic acid and the vegetable fern extract at various concentrations, and stirred for 1 min. The mixtures were kept in the dark for 30 min, and absorbance (As) was measured at 517 nm using a UV spectrophotometer (L7 Double Beam, Shanghai, China). The assays were done in triplicate, and results were expressed as mean ± standard error. Lower absorbance indicated higher free radical activity. The percent DPPH scavenging effect was calculated using:

$$\text{DPPH scavenging effect (\%)} = \frac{Ao - As}{Ao} \times 100$$

Where: Ao = Absorbance of control reaction and As = Absorbance in test sample

3. Determination of Reducing Power (RP) Activity of Tender/Matured Vegetable Fern Fronds and Ascorbic Acid

The reducing power of pretreated tender and matured fronds of vegetable fern was determined using the method of Yen and Chen (1995). Extracts of vegetable fern at concentrations of 20, 40, 60, 80, and 100 µg/ml, along with 1 ml ascorbic acid (standard), were mixed with 1 ml of 0.2M sodium phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min, then 1 ml of 10% trichloroacetic acid was added. After shaking for 10 min, the upper layer (4 ml) was mixed with 4 ml deionized water and 1 ml of 0.1% ferric chloride, and absorbance was measured at 700 nm using a UV spectrophotometer (L7 Double Beam, Shanghai, China). Higher absorbance indicates higher reducing power. The assays were done in triplicate, with results expressed as mg/µg/ml of the standard solution used.

4. Determination of Total Phenolic and Flavonoid Content of Vegetable Fern Fronds

Total phenolic content of crude extracts of tender fronds of vegetable fern was determined using Folin-Ciocalteu reagent. A 0.5 ml extract was mixed with 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of 7% Na2CO3, shaken, and incubated at 40°C for 30 min. Absorbance was measured at 517 nm using an L7 Double Beam UV Spectrophotometer (Shanghai, China). A calibration curve was prepared with gallic acid (20–100 mg/ml). Total phenolic content was expressed as mg gallic acid equivalent per gram dry weight. The procedure was done in triplicates. Total flavonoid content was estimated using Zhishen et al. (1999). A 1 ml extract was mixed with 0.2 ml distilled water, followed by 0.15 ml of 5% sodium nitrite solution. After 5 min, 0.15 ml of

10% aluminium chloride solution was added, incubated for 6 min, then 2 ml of 4% sodium hydroxide solution was added and made up to 5 ml with distilled water. After 15 min, absorbance was measured at 510 nm using the UV spectrophotometer. A calibration curve was prepared with rutin (20–100 µg/ml). Total flavonoid content was expressed as mg rutin equivalent per gram extract on a dry weight basis.

5. Determination of Phytochemicals in Tender and Matured Fronds of Vegetable Fern

Total alkaloid content was determined following Madhu et al. (2016). Five grams of powdered plant material were mixed with 50 ml of 20% acetic acid in ethanol in a 250 ml conical flask, covered, and left for 4 h. The mixture was filtered, and the filtrate concentrated to 25% of the original volume on a water bath (Precisterm spectra, JP Selecta, Spain). Sodium hydroxide was added dropwise to the filtrate until precipitation occurred, then allowed to settle. The precipitate was washed with dilute ammonium hydroxide, filtered, dried, and weighed. Alkaloid content was determined using:

$$\% \text{ Alkaloid} = \frac{\text{weight of residue}}{\text{weight of the plant material}} \times 100$$

Saponin content was determined following Ejikeme, Ezeonu, and Eboatu (2014). Five grams of plant samples were added to 100 ml of 20% ethanol in a 250 ml conical flask, heated at 55°C for 4 h with continuous stirring on a water bath (Precisterm spectra, JP Selecta, Spain). The mixture was filtered, and extraction was repeated with the residue. The extracts were combined and concentrated to 40 ml over a water bath at 90°C. Diethyl ether (20 ml) was added in a separating funnel, agitated, and the aqueous layers were recovered. The purification was repeated twice. N-butanol (60 ml) was added, extracted twice with 10 ml of 5% sodium chloride, and the sodium chloride layer discarded. The remaining solutions were heated in a water bath for 30 minutes, transferred to a crucible, and dried in an oven (KX350A, Kenixin International Co. Ltd, China) to a constant weight. Saponin content was determined using:

$$\% \text{ Saponin} = \frac{\text{Weight of extract}}{\text{weight of sample}} \text{ X } 100$$

Steroid content was determined using Madhu et al. (2016). One ml of extract was added to a 10 ml volumetric flask, followed by 2 ml of 4N sulfuric acid, 2 ml of 0.5% iron (III) chloride, and 0.5 ml of 0.5% potassium hexacyanoferrate (III). The mixture was heated at 70°C for 30 min with continuous agitation, then made up to volume with distilled water. Absorbance was measured at 780 nm using a UV spectrophotometer (L7 Double Beam, Shanghai, China) against a reagent blank. Total anthraquinone content was determined using Naqishbandi, Jäer, and Al-Khateeb (2009). Ten grams of dried plant samples were mixed with 1 ml of 60% FeCl3 and 1 ml of 5N HCl, then refluxed with 50 ml of 80% ethanol for 30 min. The process was repeated 3 times, filtered, and the filtrates concentrated to dryness. The residue was dissolved in 100 ml distilled water and partitioned with chloroform. The chloroform fractions were dried and weighed. Anthraquinone content was calculated as follows:

$$\% \text{ Anthraquinone} = \frac{\text{weight of extract}}{\text{weight of plant sample}} \ x \ 100$$

Cardiac glycoside content was determined using Madhu et al. (2016). Five grams of plant sample were mixed with 100 ml of freshly prepared Baljet's reagent (95 ml of 1% picric acid + 5 ml of 10% NaOH). After 1 hour, the mixture was filtered, diluted with 20 ml distilled water, and absorbance measured at 495 nm using a spectrophotometer (L7 Double Beam, Shanghai, China). Cardiac glycoside content (mg/100g) was calculated as: Absorbance x 0.77, where 0.77 is the factor.

6. Determination of Antinutrients in Vegetable Fern

Tannin determination was performed as described by Iwuozor (2019). A 5g sample was mixed with 50ml distilled water and shaken for 30 minutes, stirring every 5 minutes. The mixture was then centrifuged to obtain the extracts. A 2.5ml extract was transferred into a 50ml volumetric flask, along with 2.5ml of standard tannic acid. Each flask received 1.0ml Folin-Denis reagent and 2.5ml saturated Na_2CO_3 solution. The mixtures were diluted to 50ml and incubated at room temperature for 90 minutes. Absorbance was measured at 250nm using a UV spectrophotometer (L7 Double Beam, Shanghai, China). Samples (5ml) were mixed, cured for 5 hours, filtered, and aliquots (250ml) were titrated with standard iron (III) chloride solution to a persistent brownish-yellow color. Tannic contents and phytate levels were calculated as:

$$\% \text{ Tannin} = \frac{\text{An}}{\text{As}} \text{ x C x } \frac{100}{\text{w}} \text{ x } \frac{\text{VF}}{\text{VA}}$$

Phytic acid = Titre value × 0.00195 × 1.9 × 100

Where: An = absorbance of the test samples; As = absorbance of standard solution; C = concentration of standard solution; w = weight of the samples used; VF = total volume of extracts; VA = volume of extracts analyzed

Oxalate quantification was performed following Ejikeme et al. (2014). A 2.50g sample was extracted three times using 20cm³ of 0.3M HCl at 50°C for 1 hour with constant stirring. For oxalate estimation, 1.0cm³ of 5M ammonium hydroxide and 5.0cm³ of the extract were mixed, followed by phenolphthalein, acetic acid, and 1.0cm³ of 5% calcium chloride. The mixture stood for 3 hours before centrifugation at 3000rpm for 15 minutes. The supernatant was discarded, and the precipitate washed three times with hot water. Afterward, 2.0cm³ of 3M tetraoxosulphate (VI) acid was added to dissolve the precipitate at 70°C. The solution was titrated with freshly prepared 0.01M potassium permanganate ($KMnO_4$) at room temperature until a persistent pink color appeared. The solution was heated at 70°C for 3 minutes, then titrated again. Trypsin determination was conducted using the method by Madhu et al. (2016). A 1g sample was dispersed in 50ml of 0.5M NaCl, stirred for 30 minutes, and centrifuged at 1500rpm for 5 minutes. The supernatant was filtered, and 2ml of standard trypsin solution was added to 10ml of the sample substrate. The absorbance was measured at 410nm.

$$\% \text{ oxalate composition g/100g} = \frac{W}{2.5} \times \frac{100}{1}$$

Where W = weight of oxalate in titre

ANALYSIS OF RESULTS

1. The Effect of Stage of Maturity on 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Scavenging Activity of Vegetable Fern Fronds.

Table 1: 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Scavenging Activity of Vegetable Fern Fronds

Samples	Percentage DPPH (%)					
	20(µg/ml)		40 (µg/ml)		60 (µg/ml)	
	Tender	Matured	Tender	Matured	Tender	Matured
FDE (Negative Control)	$43.12 \pm 0.06^{c\,B}$	$64.02 \pm 0.01^{c\,A}$	$57.09 \pm 0.03^{d\,B}$	$74.10 \pm 0.03^{c\,A}$	$69.10 \pm 0.03^{c\,A}$	$68.16 \pm 0.00^{c\,B}$
ODE	$36.27 \pm 0.03^{f\,B}$	$55.10 \pm 0.00^{d\,A}$	$40.07 \pm 0.03^{f\,B}$	$57.06 \pm 0.01^{d\,A}$	$41.14 \pm 0.01^{f\,B}$	$58.09 \pm 0.01^{e\,A}$
HBD	$51.01 \pm 0.03^{b\,A}$	$27.11 \pm 0.01^{f\,B}$	$58.10 \pm 0.03^{c\,A}$	$50.13 \pm 0.00^{f\,B}$	$70.05 \pm 0.03^{b\,A}$	$55.04 \pm 0.01^{f\,B}$
SDE	$40.42 \pm 0.06^{e\,B}$	$66.09 \pm 0.01^{b\,A}$	$42.13 \pm 0.06^{e\,B}$	$79.18 \pm 0.01^{b\,A}$	$46.06 \pm 0.01^{e\,B}$	$86.10 \pm 0.00^{b\,A}$
SHD	$41.19 \pm 0.01^{d\,B}$	$43.14 \pm 0.03^{e\,A}$	$61.12 \pm 0.03^{b\,A}$	$52.07 \pm 0.01^{e\,B}$	$62.17 \pm 0.00^{d\,A}$	$60.08 \pm 0.02^{d\,B}$
Ascorbic Acid (Positive)	$93.00 \pm 0.02^{a\,A}$	$93.00 \pm 0.00^{a\,B}$	$93.01 \pm 0.03^{a\,A}$	$93.00 \pm 0.00^{a\,A}$	$94.01 \pm 0.01^{a\,A}$	$94.00 \pm 0.01^{a\,A}$

Samples	Percentage DPPH (%)			
	80(µg/ml)		100(µg/ml)	
	Tender	Matured	Tender	Matured
FDE (Negative Control)	$77.05 \pm 0.06^{c\,B}$	$85.07 \pm 0.03^{c\,A}$	$82.11 \pm 0.06^{c\,B}$	$84.13 \pm 0.01^{c\,A}$
ODE	$43.07 \pm 0.03^{f\,B}$	$82.03 \pm 0.03^{d\,A}$	$44.16 \pm 0.03^{f\,B}$	$83.10 \pm 0.00^{d\,A}$
HBD	$83.13 \pm 0.03^{b\,A}$	$65.14 \pm 0.01^{f\,B}$	$83.10 \pm 0.03^{b\,A}$	$74.09 \pm 0.03^{f\,B}$
SDE	$47.09 \pm 0.03^{e\,B}$	$87.05 \pm 0.01^{b\,A}$	$47.08 \pm 0.01^{e\,B}$	$87.07 \pm 0.02^{b\,A}$
SHD	$68.15 \pm 0.01^{d\,B}$	$74.11 \pm 0.01^{e\,A}$	$73.13 \pm 0.03^{d\,B}$	$81.14 \pm 0.00^{e\,A}$
Ascorbic Acid (Positive Control)	$94.00 \pm 0.01^{a\,A}$	$94.00 \pm 0.00^{a\,A}$	$95.01 \pm 0.03^{a\,A}$	$95.00 \pm 0.00^{a\,A}$

Note: Values are Means ± SD of triplicate determination. Means in the same column with different superscript (lower case) are significantly ($p < 0.05$) different, while mean in the same row with different superscript (upper case) are significant ($p < 0.05$) different. FDE = Fresh vegetable fern fronds; ODE = Oven dried vegetable fern fronds; HBD = Hot water blanched vegetable fern fronds; SDE = Sun dried vegetable fern fronds; SHD = Shade dried vegetable fern fronds.

Table 1 shows that the stage of maturity significantly ($p<0.05$) affected the DPPH scavenging activity of vegetable fern fronds. Pretreatment significantly ($p<0.05$) influenced the percentage DPPH of tender fronds at 20, 40, 60, 80, and 100 µg/ml. At 20 µg/ml, hot water blanched fronds had the highest DPPH (51.01%), followed by fresh, shade dried (41.19%), and sun-dried (40.42%) fronds. Matured fronds generally had higher DPPH than tender ones, except for the hot water blanched sample. At 40 µg/ml, shade dried tender fronds had the highest DPPH (61.12%), followed by hot water blanched (58.10%), fresh (57.09%), and sun-dried (42.13%). Oven dried fronds had the lowest DPPH (40.07%). For matured fronds at 40 µg/ml, sun-dried had the highest DPPH (79.18%), followed by fresh (74.10%), oven dried (57.06%), shade dried (52.07%), and hot water blanched (50.13%). Generally, matured fronds showed higher DPPH than tender fronds, except for hot water blanched and shade dried samples.

At 60 µg/ml, pretreatment significantly ($p<0.05$) affected the DPPH of tender fronds. Hot water blanched fronds had the highest DPPH (70.05%), followed by negative control (69.10%), shade dried (62.17%), sun dried (46.06%), and oven dried (41.14%), in descending order. For matured fronds, sun dried had the highest DPPH (86.10%), followed by negative control (68.16%), shade dried (60.08%), oven dried (58.09%), and hot water blanched (55.04%). Except for oven dried and sun dried, tender fronds had higher DPPH than matured fronds.

At 80 µg/ml, hot water blanched tender fronds had the highest DPPH (83.13%), followed by negative control (77.05%), shade dried (68.15%), sun dried (47.09%), and oven dried (43.07%). For matured fronds, sun dried had the highest DPPH (87.05%), followed by negative control (82.03%), oven dried (82.03%), shade dried (74.11%), and hot water blanched (65.14%). Generally, matured fronds had higher DPPH, except for the sun dried sample where tender fronds had higher DPPH.

At 100 µg/ml, hot water blanched tender fronds had the highest DPPH (83.10%), followed by negative control (82.11%), shade dried (73.13%), sun dried (47.08%), and oven dried (44.16%). For matured fronds, sun dried had the highest DPPH (87.07%), followed by negative control (82.11%), oven dried (83.10%), shade dried (81.14%), and hot water blanched (74.09%). Except for hot water blanched, matured fronds had higher DPPH than tender fronds. Ascorbic acid (positive control) had significantly ($p<0.05$) higher DPPH than both tender and matured fronds at all concentrations.

Table 2: Effective concentration, IC_{50} (µg/ml) of vegetable fern fronds

Samples	**DPPH (IC_{50})[a] (µg/ml)**		**RP (IC_{50})[b] mg/µg/ml**	
	Tender	Matured	Tender	Matured
FDE (Negative Control)	$29.5^{b\,A}$	$15.0^{e\,B}$	$>100.0^{a\,A}$	$>100.0^{a\,A}$
ODE	$>100^{a\,A}$	$19.0^{c\,B}$	$>100.0^{a\,A}$	$>100.0^{a\,A}$
HBD	$20.0^{c\,B}$	$40.0^{a\,A}$	$>100.0^{a\,A}$	$>100.0^{a\,A}$
SDE	$>100^{a\,A}$	$15.8^{d\,B}$	$>100.0^{a\,A}$	$>100.0^{a\,A}$
SHD	$29.5^{b\,B}$	$36.0^{b\,A}$	$>100.0^{a\,A}$	$>100.0^{a\,A}$
Ascorbic Acid (Positive Control)	$10.0^{d\,A}$	$10.0^{f\,A}$	$12.0^{b\,A}$	$12.0^{b\,A}$

Note: Values in the same column with different superscript (lower case) are significantly ($p < 0.05$) different, while values in the same row with different superscript (upper case) are significantly ($p < 0.05$) different. IC_{50} [a]: Effective concentration needed to reduce DPPH by 50%; IC_{50} [b]: Effective concentration at which the absorbance is 0.5.

The IC50 values for tender and matured vegetable fern fronds, shown in Table 2, were derived from the DPPH inhibition graphs in Figures 1 and 2. The stage of maturity significantly affected the IC_{50}. For tender fronds, oven dried (>100) and sun dried (>100) samples had the highest IC_{50}, which were not significantly different. This was followed by fresh (29.5) and shade dried (29.5) samples, which were also not significantly different. Hot water blanched had the highest IC_{50} (40), followed by shade dried (36), oven dried (19), sun dried (15.8), and fresh (negative control) samples, which were significantly different. Generally, tender fronds had a higher IC_{50} than matured fronds, except for hot water blanched and shade dried samples, where matured fronds had a lower IC_{50}.

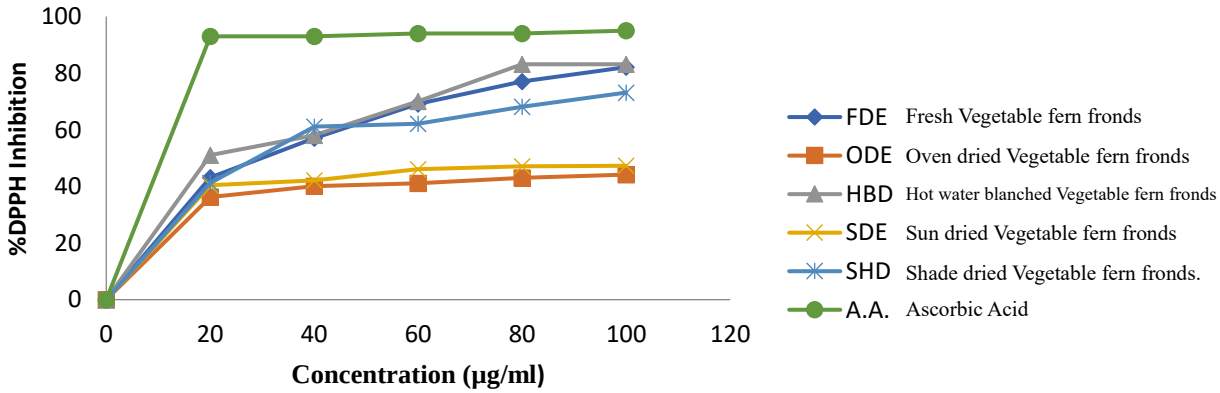

Figure 1: Free Radical Scavenging Activity (%) on DPPH Radicals against Concentration of Vegetable Fern Fronds and Ascorbic Acid.

Figure 1 shows the dose-dependent free radical scavenging activity (%) on DPPH radicals for vegetable fern fronds and ascorbic acid. Hot water blanched treatment exhibited the strongest DPPH inhibition, while oven dried samples showed the weakest activity from 20 to 100 µg/ml.

Between 45 and 66 µg/ml, there was no significant difference ($p > 0.05$) in the inhibition activity of hot water blanched and fresh (control) samples, both showing 55-70% DPPH inhibition.

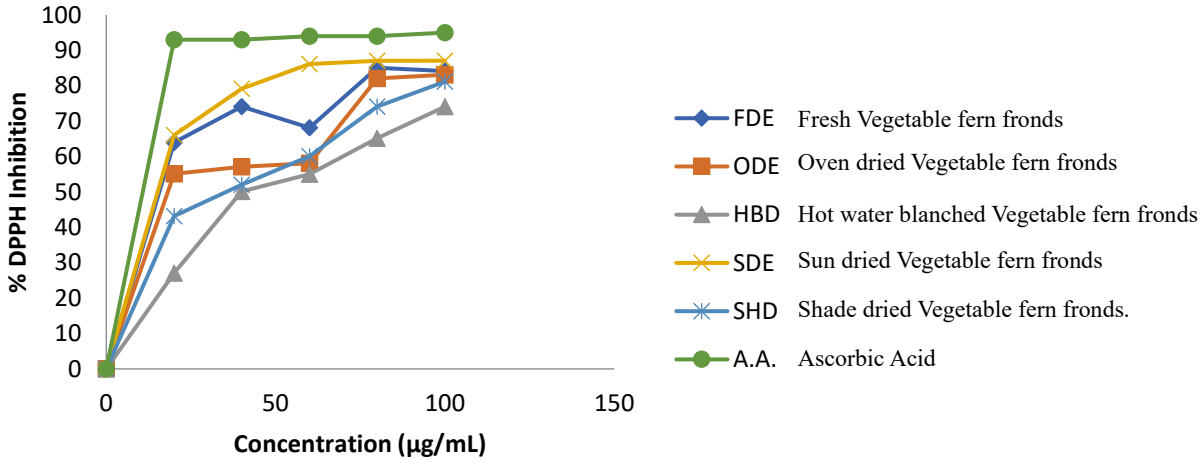

Figure 2: Free Radical Scavenging Activity (%) on DPPH Radicals against Concentration of Matured Vegetable Fern Fronds and Ascorbic Acid

Figure 2 shows a concentration-dependent relationship with a more linear pattern. Sun dried samples exhibited the highest DPPH inhibition (66-85%) from 20-100 µg/ml, followed by fresh (control) samples (64.02%), oven dried, and shade dried samples (43.14%). Hot water blanched samples had the lowest inhibition (27.11%). Significant variations ($p < 0.05$) were observed across treatments. Hot water blanched samples showed the weakest scavenging power (28-71%) from 20-100 µg/ml.

2. The Reducing Power (RP) activity of Tender/Matured Vegetable Fern Fronds and ascorbic acid.

Table 3: Reducing power (RP) activity of vegetable fern fronds

Samples	RP Values 20		40		60 µg/ml	
	Tender	Matured	Tender	Matured	Tender	Matured
FDE (Neg. Control)	$0.286 \pm 0.07^{c\,B}$	$0.341 \pm 0.03^{b\,A}$	$0.298 \pm 0.03^{c\,B}$	$0.366 \pm 0.03^{c\,A}$	$0.321 \pm 0.03^{c\,B}$	$0.405 \pm 0.09^{b\,A}$
ODE	$0.181 \pm 0.06^{d\,B}$	$0.251 \pm 0.03^{f\,A}$	$0.187 \pm 0.06^{e\,B}$	$0.282 \pm 0.07^{f\,A}$	$0.191 \pm 0.06^{e\,B}$	$0.328 \pm 0.03^{f\,A}$
SDE	$0.154 \pm 0.01^{e\,B}$	$0.333 \pm 0.01^{c\,A}$	$0.167 \pm 0.03^{f\,B}$	$0.373 \pm 0.03^{b\,A}$	$0.182 \pm 0.03^{f\,B}$	$0.378 \pm 0.03^{c\,A}$
HBD	$0.341 \pm 0.03^{b\,A}$	$0.319 \pm 0.01^{e\,B}$	$0.347 \pm 0.06^{b\,B}$	$0.357 \pm 0.03^{d\,A}$	$0.349 \pm 0.03^{b\,B}$	$0.365 \pm 0.03^{d\,A}$
SHD	$0.285 \pm 0.09^{c\,B}$	$0.329 \pm 0.03^{d\,A}$	$0.295 \pm 0.03^{d\,B}$	$0.332 \pm 0.01^{e\,A}$	$0.301 \pm 0.09^{d\,B}$	$0.341 \pm 0.03^{e\,A}$
Ascorbic Acid (positive control)	$0.771 \pm 0.03^{a\,A}$	$0.771 \pm 0.03^{a\,A}$	$1.066 \pm 0.01^{a\,A}$	$1.066 \pm 0.01^{a\,A}$	$1.255 \pm 0.02^{a\,A}$	$1.255 \pm 0.02^{a\,A}$

Samples	RP Values 80		100	
	Tender	Matured	Tender	Matured
FDE (Neg. Control)	$0.323 \pm 0.03^{c\,B}$	$0.434 \pm 0.07^{b\,A}$	$0.335 \pm 0.03^{c\,B}$	$0.438 \pm 0.03^{b\,A}$
ODE	$0.197 \pm 0.06^{d\,B}$	$0.329 \pm 0.01^{f\,A}$	$0.218 \pm 0.03^{e\,B}$	$0.330 \pm 0.03^{f\,A}$
SDE	$0.186 \pm 0.01^{e\,B}$	$0.405 \pm 0.01^{c\,A}$	$0.194 \pm 0.01^{f\,B}$	$0.425 \pm 0.02^{c\,A}$
HBD	$0.354 \pm 0.01^{b\,B}$	$0.379 \pm 0.06^{e\,A}$	$0.381 \pm 0.03^{b\,B}$	$0.393 \pm 0.06^{e\,A}$
SHD	$0.323 \pm 0.03^{c\,B}$	$0.387 \pm 0.07^{d\,A}$	$0.324 \pm 0.03^{d\,B}$	$0.400 \pm 0.03^{d\,A}$
Ascorbic Acid (positive control)	$1.393 \pm 0.02^{a\,A}$	$1.393 \pm 0.02^{a\,A}$	$1.755 \pm 0.01^{a\,A}$	$1.755 \pm 0.01^{a\,A}$

Note: Values are means ± SD of triplicate determination. Means in the same column with different superscript (lower case) are significantly ($p < 0.05$) different, while mean in the same row with different superscript (upper case) are significant ($p < 0.05$) different. FDE = Fresh vegetable fern fronds; ODE = Oven dried vegetable fern fronds; HBD = Hot water blanched vegetable fern fronds; SDE = Sun dried vegetable fern fronds; SHD = Shade dried vegetable fern fronds.

Table 3 shows that maturity stage significantly ($p < 0.05$) affected RP activity of vegetable fern fronds. At 20 µg/ml, hot water blanched tender fronds had the highest RP activity (0.341 mg/100ml), followed by fresh (control) and shade dried samples (0.285 mg/100ml). Oven dried (0.181 mg/100ml) and sun dried (0.154 mg/100ml) samples had significantly lower RP activity. For matured fronds, fresh (control) had the highest RP activity, followed by sun dried (0.333 mg/100ml), shade dried (0.329 mg/100ml), hot water blanched (0.319 mg/100ml), and oven dried (0.251 mg/100ml) samples. Generally, RP activity of matured fronds was higher than tender fronds, except in hot water blanched samples.

At 40 µg/ml, pretreatment significantly ($p < 0.05$) affected RP activity of tender vegetable fern fronds. Hot water blanched had the highest RP activity (0.341 mg/100ml), followed by fresh (0.298 mg/100ml), shade dried (0.295 mg/100ml), oven dried (0.187 mg/100ml), and sun dried (0.167 mg/100ml) samples. For matured fronds, sun dried had the highest RP activity (0.366 mg/100ml), followed by fresh (0.357 mg/100ml), hot water blanched (0.332 mg/100ml), shade dried (0.282 mg/100ml), and oven dried (0.251 mg/100ml) samples. RP activity of matured fronds was higher than tender fronds except in hot water blanched samples.

At 60 µg/ml, hot water blanched tender fronds had the highest RP activity (0.321 mg/100ml), followed by fresh (0.301 mg/100ml), shade dried (0.191 mg/100ml), and oven dried (0.182 mg/100ml) samples. For matured fronds, fresh had the highest RP activity (0.405 mg/100ml), followed by sun dried (0.378 mg/100ml), hot water blanched (0.365 mg/100ml), shade dried (0.341 mg/100ml), and oven dried (0.328 mg/100ml) samples. RP activity of matured fronds was consistently higher than tender fronds.

At 80 µg/ml, RP activity of tender vegetable fern fronds was significantly ($p < 0.05$) affected by pretreatment. Hot water blanched had the highest RP activity (0.381 mg/100ml), followed by fresh (0.323 mg/100ml) and shade dried (0.323 mg/100ml), which were not significantly different. Oven dried (0.197 mg/100ml) had significantly lower RP activity than sun dried. For matured fronds, fresh had the highest RP activity (0.405 mg/100ml), followed by sun dried (0.387 mg/100ml), shade dried (0.379 mg/100ml), hot water blanched (0.329 mg/100ml), and oven dried (0.329 mg/100ml) samples. RP activity of matured fronds was higher than tender fronds.

At 100 µg/ml, hot water blanched tender fronds had the highest RP activity (0.381 mg/100ml), followed by fresh (0.335 mg/100ml), shade dried (0.324 mg/100ml), oven dried (0.218 mg/100ml), and sun dried (0.194 mg/100ml) samples. For matured fronds, fresh had the highest RP activity (0.438 mg/100ml), followed by sun dried (0.425 mg/100ml), shade dried (0.400 mg/100ml), hot water blanched (0.393 mg/100ml), and oven dried (0.330 mg/100ml) samples. RP activity of matured fronds was consistently higher than tender fronds. The IC50 values for both tender and matured vegetable fern fronds were greater than 100 µg/ml, with no significant difference ($p < 0.05$) based on pretreatment or maturity stage.

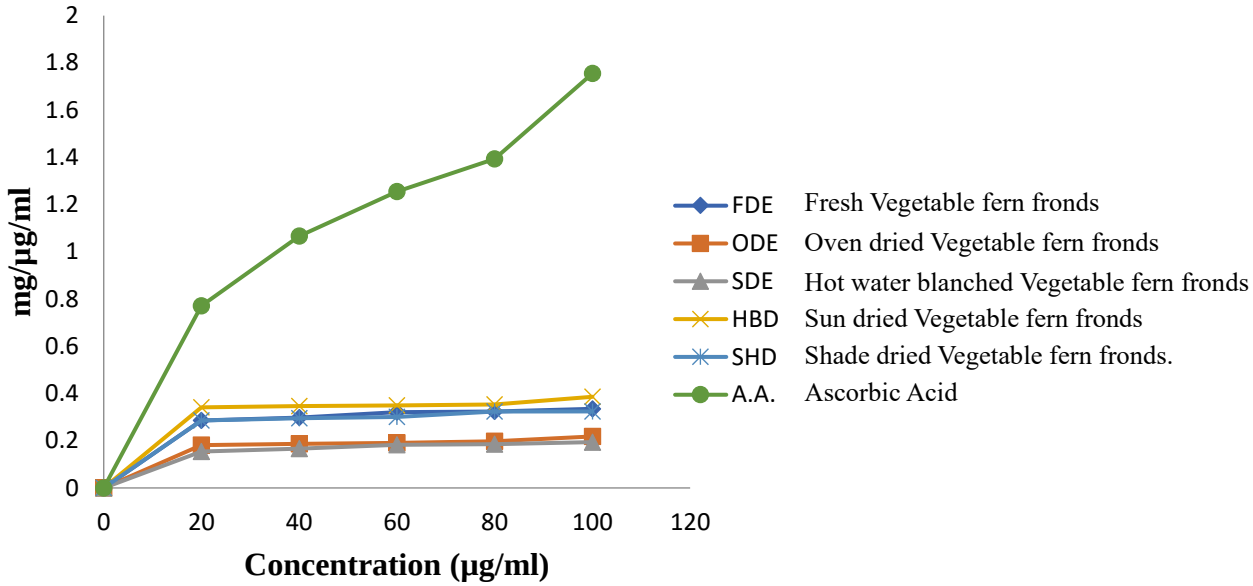

Figure 4: Reducing Power of tender vegetable fern fronds and ascorbic acid

From Figure 4, sun dried samples showed significant ($p < 0.05$) higher RP activity, with no significant differences between oven dried and hot water blanched samples, or between shade dried and fresh (control) samples. Shade/fresh samples had higher RP activity than oven/fresh dried samples (20 - 100 µg/ml). Overall, RP activity was low (0 - 0.3 unit) across treatments, with sun dried showing the highest value ($p < 0.05$) at 20 - 100 µg/ml.

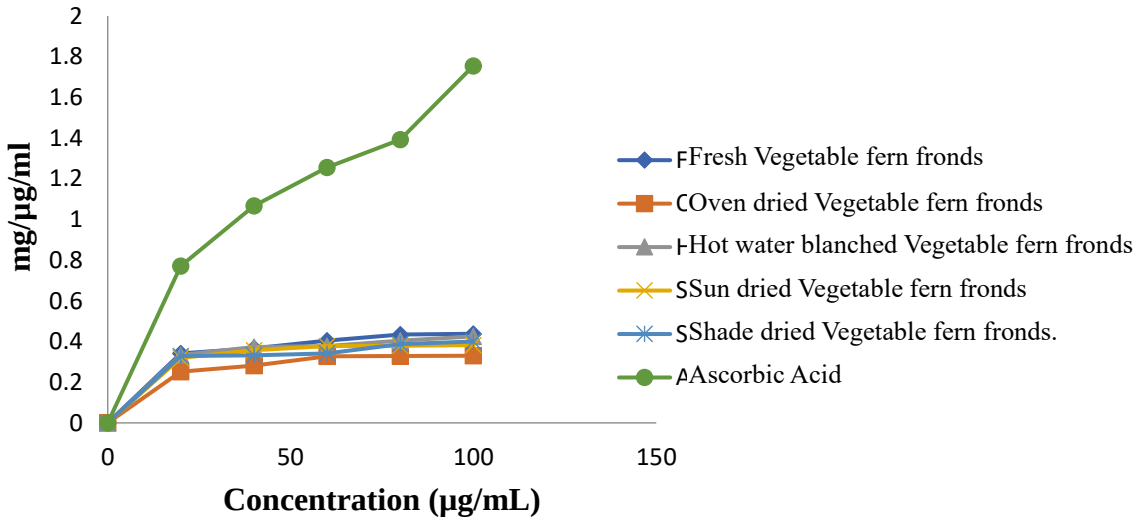

Figure 5: Reducing Power of Matured Vegetable Fern Fronds and Ascorbic Acid

From Figure 5, the lowest RP value was observed in the oven dried sample. Most treatment variables did not significantly ($p < 0.05$) affect the vegetables. Ascorbic acid, as the standard antioxidant reagent, exhibited the strongest RP scavenging power (0 - 100 µg/ml).

3. The Total Phenolic and Flavonoid Content of Vegetable Fern Fronds

Table 2: Total Phenolic and Flavonoid Contents of Vegetable Fern Fronds

Sample	Total Phenolic (mgGAE/g)		Total Flavonoid (mgRE/g)	
	Tender	Matured	Tender	Matured
FDE (Control)	$29.6 \pm 0.002^{a\,B}$	$91.7 \pm 0.001^{a\,A}$	$75.0 \pm 0.002^{a\,B}$	$266.0 \pm 0.002^{a\,A}$
ODE	$9.9 \pm 0.003^{d\,B}$	$52.0 \pm 0.003^{c\,A}$	$13.0 \pm 0.002^{e\,B}$	$120.0 \pm 0.001^{d\,A}$
HBD	$18.7 \pm 0.001^{c\,B}$	$74.4 \pm 0.002^{b\,A}$	$65.0 \pm 0.003^{b\,B}$	$132.0 \pm 0.003^{c\,A}$
SDE	$6.6 \pm 0.002^{e\,B}$	$37.9 \pm 0.001^{e\,A}$	$56.0 \pm 0.001^{d\,B}$	$89.0 \pm 0.001^{e\,A}$
SHD	$19.8 \pm 0.001^{b\,B}$	$41.4 \pm 0.003^{d\,A}$	$61.0 \pm 0.001^{c\,B}$	$144.0 \pm 0.001^{b\,A}$

Note: Values are means ± SD of triplicate determination. Means in the same column with different superscript (lower case) are significantly ($p < 0.05$) different, while mean in the same row with different superscript (upper case) are significant ($p < 0.05$) different. FDE = Fresh vegetable fern fronds; ODE = Oven dried vegetable fern fronds; HBD = Hot water blanched vegetable fern fronds; SDE = Sun dried vegetable fern fronds; SHD = Shade dried vegetable fern fronds.

The stage of maturity significantly ($p < 0.05$) affected the total phenolic and flavonoid contents of vegetable fern fronds. For tender vegetable fronds, the control (fresh) sample had the highest total phenolic content, followed by shade dried (19.8 mgGAE/g), hot water blanched (18.7 mgGAE/g), oven dried (9.9 mgGAE/g), and sun dried (6.6 mgGAE/g), with significant ($p < 0.05$) differences. For matured vegetable fronds, the control also had the highest total phenolic content, followed by hot water blanched (74.4 mgGAE/g), oven dried (52.0 mgGAE/g), shade dried (41.4 mgGAE/g), and sun dried (37.9 mgGAE/g). Total phenolic content was significantly ($p < 0.05$) higher in matured than in tender vegetable fronds, regardless of treatment.

The total flavonoid content of tender vegetable fern fronds was significantly ($p < 0.05$) affected by pretreatment, with the control (fresh) sample having the highest content. The values for hot water blanched, shade dried, sun dried, and oven dried were 65.0, 61.0, 56.0, and 13.0 mgRE/g, respectively, showing significant ($p < 0.05$) differences. For matured vegetable fronds, the control also had the highest flavonoid content, followed by shade dried (144.0 mgRE/g), hot water blanched (132.0 mgRE/g), oven dried (120.0 mgRE/g), and sun dried (89.0 mgRE/g), with significant ($p < 0.05$) differences. The total flavonoid content of matured fronds was significantly ($p < 0.05$) higher than that of tender fronds, regardless of treatment.

4. The effect of stage of maturity on the phytochemical composition of vegetable fern fronds.

Table 1: Effect of stage of maturity on the phytochemical composition of vegetable fern fronds.

Samples	Alkaloids (%)		Saponins (%)		Steroids (%)	
	Tender	Matured	Tender	Matured	Tender	Matured
FDE (Control)	$10.03 \pm 1.04^{a\,B}$	$10.95 \pm 0.96^{a\,A}$	$0.53 \pm 0.07^{a\,B}$	$0.61 \pm 0.02^{a\,A}$	$0.04 \pm 0.00^{a\,A}$	$0.06 \pm 0.00^{a\,A}$
ODE	$8.10 \pm 1.15^{c\,B}$	$9.71 \pm 0.87^{c\,A}$	$0.35 \pm 0.02^{c\,B}$	$0.44 \pm 0.05^{c\,A}$	$0.03 \pm 0.01^{a\,A}$	$0.04 \pm 0.00^{a\,A}$
HBD	$7.83 \pm 1.20^{d\,B}$	$8.37 \pm 0.69^{d\,A}$	$0.29 \pm 0.03^{d\,B}$	$0.35 \pm 0.01^{d\,A}$	$0.02 \pm 0.01^{a\,A}$	$0.03 \pm 0.00^{a\,A}$
SDE	$8.12 \pm 1.11^{c\,B}$	$9.74 \pm 0.84^{c\,A}$	$0.38 \pm 0.07^{c\,B}$	$0.48 \pm 0.04^{c\,A}$	$0.03 \pm 0.01^{a\,A}$	$0.04 \pm 0.00^{a\,A}$
SHD	$9.77 \pm 1.20^{b\,B}$	$10.04 \pm 0.11^{b\,A}$	$0.47 \pm 0.03^{b\,A}$	$0.52 \pm 0.01^{b\,A}$	$0.03 \pm 0.00^{a\,A}$	$0.05 \pm 0.00^{a\,A}$

Samples	Anthraquinone (%)		Cardiac Glycosides (mg/100g)	
	Tender	Matured	Tender	Matured
FDE (Control)	$0.04 \pm 0.01^{a\,A}$	$0.06 \pm 0.00^{a\,A}$	$0.03 \pm 0.01^{a\,A}$	$0.04 \pm 0.00^{a\,A}$
ODE	$0.03 \pm 0.01^{a\,A}$	$0.04 \pm 0.00^{a\,A}$	$0.02 \pm 0.00^{a\,A}$	$0.02 \pm 0.00^{a\,A}$
HBD	$0.03 \pm 0.00^{a\,A}$	$0.03 \pm 0.00^{a\,A}$	$0.01 \pm 0.02^{a\,A}$	$0.01 \pm 0.01^{a\,A}$
SDE	$0.04 \pm 0.00^{a\,A}$	$0.05 \pm 0.00^{a\,A}$	$0.02 \pm 0.01^{a\,A}$	$0.02 \pm 0.01^{a\,A}$
SHD	$0.04 \pm 0.00^{a\,A}$	$0.05 \pm 0.00^{a\,A}$	$0.03 \pm 0.00^{a\,A}$	$0.02 \pm 0.02^{a\,A}$

Note: Values are means ± SD of triplicate determination. Means in the same column with different superscript (lower case) are significantly ($p < 0.05$) different, while mean in the same row with different superscript (upper case)

are significant ($p < 0.05$) different. FDE = Fresh vegetable fern fronds; ODE = Oven dried vegetable fern fronds; HBD = Hot water blanched vegetable fern fronds; SDE = Sun dried vegetable fern fronds; SHD = Shade dried vegetable fern fronds.

Stage of maturity significantly ($p < 0.05$) affected phytochemical contents in vegetable fern fronds. For tender fronds, alkaloid content was significantly ($p < 0.05$) higher in the control (fresh) sample compared to pretreated samples. Alkaloid contents for shade dried, sun dried, oven dried, and hot water blanched were 9.77, 8.12, 8.10, and 7.83%, respectively. For matured fronds, the control also had the highest alkaloid content, followed by shade dried (10.04%), sun dried (9.74%), oven dried (9.71%), and hot water blanched (8.37%). Alkaloid contents of tender fronds were significantly ($p < 0.05$) higher than those of matured fronds. Saponin content was also significantly ($p < 0.05$) higher in the control for both tender and matured fronds compared to pretreated samples.

Saponin content in vegetable fern fronds was highest in shade dried samples, followed by sun dried and oven dried samples, which were not significantly ($p < 0.05$) different. Hot water blanched fronds had significantly ($p < 0.05$) lower saponin content. Saponin content in matured fronds was significantly ($p < 0.05$) higher than in tender fronds for both control and pretreated samples. Treatment and stage of maturity did not significantly affect steroid, total anthraquinone, and cardiac glycoside contents. The low saponin content in this study suggests it would not interfere with the absorption of vitamins A and E or lipids. This finding aligns with Oloyede et al. (2012), who found no detectable saponin content in a tropical fern. Saponins can impact taste and irritate the throat, affecting protein digestibility and potentially influencing renal stone formation (Loewus, Reddy & Sathes, 2001). Steroids are important for growth, protein and carbohydrate metabolism, and have anti-inflammatory properties (Nielson & Cox, 2005).

5. The Stage of Maturity Effect on the Antinutritional Factors in Vegetable Fern Fronds

Table 9: Effect of stage of maturity on the antinutritional factors in vegetable fern fronds

Samples	Tannins (%)		Phytate (%)		Oxalate (%)		Trypsin Inhibitor (%)	
	Tender	Matured	Tender	Matured	Tender	Matured	Tender	Matured
FDE (Control)	$12.13 \pm 0.03^{a\,B}$	$19.37 \pm 0.05^{a\,A}$	$0.25 \pm 0.07^{a\,B}$	$0.42 \pm 0.02^{a\,A}$	$0.65 \pm 0.01^{a\,B}$	$0.87 \pm 0.09^{a\,A}$	$0.15 \pm 0.00^{a\,A}$	$0.18 \pm 0.01^{a\,A}$
ODE	$12.11 \pm 0.04^{b\,B}$	$19.21 \pm 0.03^{b\,A}$	$0.13 \pm 0.08^{c\,B}$	$0.28 \pm 0.01^{c\,A}$	$0.44 \pm 0.01^{c\,B}$	$0.79 \pm 0.08^{c\,A}$	$0.13 \pm 0.01^{a\,A}$	$0.14 \pm 0.01^{a\,A}$
HBD	$8.35 \pm 0.07^{e\,B}$	$15.14 \pm 0.05^{d\,A}$	$0.04 \pm 0.02^{d\,B}$	$0.13 \pm 0.05^{d\,A}$	$0.20 \pm 0.03^{e\,B}$	$0.29 \pm 0.01^{e\,A}$	$0.11 \pm 0.02^{a\,A}$	$0.13 \pm 0.01^{a\,A}$
SDE	$10.72 \pm 0.03^{c\,B}$	$18.74 \pm 0.04^{c\,A}$	$0.10 \pm 0.09^{c\,B}$	$0.25 \pm 0.02^{c\,A}$	$0.38 \pm 0.01^{d\,B}$	$0.57 \pm 0.03^{d\,A}$	$0.13 \pm 0.00^{a\,A}$	$0.14 \pm 0.01^{a\,A}$
SHD	$10.57 \pm 0.04^{d\,B}$	$19.23 \pm 0.03^{b\,A}$	$0.19 \pm 0.02^{b\,B}$	$0.31 \pm 0.01^{b\,A}$	$0.51 \pm 0.01^{b\,B}$	$0.85 \pm 0.02^{b\,A}$	$0.14 \pm 0.02^{a\,A}$	$0.16 \pm 0.01^{a\,A}$

Note: Values are means ± SD of triplicate determination. Means in the same column with different superscript (lower case) are significantly ($p < 0.05$) different, while mean in the same row with different superscript (upper case) are significant ($p < 0.05$) different. FDE = Fresh vegetable fern fronds; ODE = Oven dried vegetable fern fronds; HBD = Hot water blanched vegetable fern fronds; SDE = Sun dried vegetable fern fronds; SHD = Shade dried vegetable fern fronds.

Tannin content in tender vegetable fern fronds was significantly ($p < 0.05$) affected by pretreatment. Fresh fronds had the highest tannin content (control, 19.37%), followed by shade dried (19.23%) and oven dried (19.21%) samples, which were not significantly different. Sun dried (18.74%) and hot water blanched samples had significantly lower tannin content. Matured fronds had higher tannin content than tender fronds, regardless of treatment.

Phytate content in both tender and matured vegetable fern fronds was also significantly ($p < 0.05$) affected by pretreatment. The control sample had the highest phytate content in both groups, with matured fronds having higher levels overall. For tender fronds, the order was: control (0.25%), shade dried (0.19%), oven dried (0.13%), sun dried (0.10%), and hot water blanched (0.04%). For matured fronds: control (0.42%), shade dried (0.31%), oven dried (0.28%), sun dried (0.25%), and hot water blanched (0.13%).

Oxalate content in both tender and matured vegetable fern fronds was significantly ($p < 0.05$) affected by treatment. The control (fresh fronds) had the highest oxalate content, followed by shade dried, oven dried, sun dried, and hot water blanched samples, which were not significantly different from each other. Generally, matured fronds had significantly higher oxalate content than tender fronds, irrespective of treatment.

Treatment and stage of maturity had no significant ($p > 0.05$) effect on trypsin inhibitor content. Oxalate inhibits calcium absorption by binding it as oxalate salt, which can cause kidney stones and bone pain. Tannins reduce nutrient digestibility by forming strong bonds with proteins and carbohydrates. Phytates can impair the absorption of iron and zinc, leading to deficiencies. Despite these antinutrients, their concentrations in this study were within safe limits. No established Recommended Daily Intake (RDI) exists for these antinutrients, though their potential health benefits are recognized (Dreosti, 2000).

Conclusion

This study highlights the impact of maturity stage on the antioxidant and antinutritional properties of vegetable fern fronds. Matured fronds, with higher phenolic and flavonoid contents, are rich in natural antioxidants that help prevent oxidative stress-related diseases. In contrast, tender fronds contain more alkaloids and saponins, offering distinct nutritional and medicinal benefits. Different pretreatment methods, such as shade drying, oven drying, and hot water blanching, significantly influenced the retention of bioactive compounds, affecting antioxidant activity. Differences in DPPH scavenging activity between tender and matured fronds further emphasize the role of processing in preserving beneficial properties.

The complexity of phytochemical and antinutritional changes in vegetable fern fronds highlights the importance of selecting appropriate processing techniques to maximize their health benefits. Consumers and food processors should consider both maturity stage and treatment methods. Given their high antioxidant potential, matured fronds are suitable for functional foods, while tender fronds may be valuable in medicinal applications. Future research should focus on optimizing processing techniques to enhance bioavailability while minimizing antinutrients, ultimately improving the nutritional quality of vegetable fern fronds for dietary and pharmaceutical applications.

Recommendations

1. Matured fronds, having higher phenolic and flavonoid contents, should be used for their superior antioxidant properties in preventing oxidative stress-related diseases.
2. Refining shade drying, oven drying, and hot water blanching techniques should be used to enhance bioavailability to minimizing nutrient loss.
3. Tender fronds, rich in alkaloids and saponins, should be used for medicinal applications, with education on the distinct benefits of both frond types.
4. Matured fronds should be incorporated into functional foods like nutraceuticals, antioxidant-rich beverages, and dietary supplements.
5. Further research should be carried out on the potential toxicity and safe dietary intake of alkaloids and saponins in tender fronds.
6. Sun-dried matured fronds, with the highest DPPH scavenging activity, should be used as a cost-effective antioxidant preservation method.
7. Public health campaigns and extension programs should be used educate stakeholders on the nutritional and health benefits of different frond maturity stages and processing methods.

8. Future studies should explore phytochemical bioavailability and applications in pharmaceutical, nutraceutical, and dietary interventions.

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