PROXIMATE AND PHYTOCHEMICAL ANALYSIS OF AFRAMOMUM DANIELLI GROWN UNDER MATURE RUBBER PLANTATION: NUTRITIONAL AND **THERAPEUTIC**

Eseosa Osazuwa*1, Oghenetega Sunday1, Chioma Okwu-Abolo1, Ikhazuagbe Hilary Ifijen1, Omoigberale Jude1

¹Research outreach department, Rubber Research institute of Nigeria, Iyanomo, Edo State, Nigeria.

Article History: Received May 2025; Revised June 2025; Accepted June 2025; Published online July 2025

*Corresponding Author: Dr. Eseosa Osazuwa (Dreseosa.osazuwa@gmail.com; ORCID: http://orcid.org/0000-0003-0765-7147 **Tel**: +234 815 273 7239)

Article Information

Copyright: © 2025 Osazuwa et al. This open-access article is distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Citation: Osazuwa, E., Sunday, O., Okwu-Abolo, C., Ifijen, I. H., & Jude, O. (2025). Proximate and phytochemical analysis of Aframomum danielli grown under mature rubber plantation: Nutritional and therapeutic. Journal of Chemistry and Allied Sciences, 1(1), 15-

DOI:

https://doi.org/10.60787/jcas.vol1no1.29

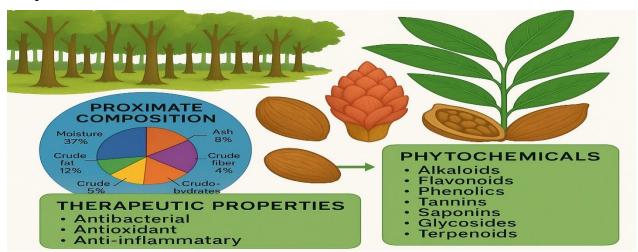
The Official Publication of the Tropical Research and Allied Network (TRANet), Department of Chemistry, Federal University of Technology, Minna

Abstract

This study evaluates the proximate and phytochemical composition of Aframomum danielli seeds cultivated under a mature rubber (Hevea brasiliensis) plantation in Edo State, Nigeria, highlighting its nutritional and therapeutic potential. Mature fruits were harvested, air-dried, and subjected to methanol extraction via Soxhlet apparatus. Proximate analysis revealed a nutrient-rich profile dominated by carbohydrates (55.67%), proteins (13.30%), fats (7.78%), fiber (3.64%), ash (5.77%), and moderate moisture content (13.84%), positioning A. danielli as a promising energy and nutrient source. Phytochemical profiling using GC-MS identified key bioactive compounds, including catechin (3.36 ppm), ellagic acid (2.37 ppm), luteolin (1.61 ppm), and quercetin (0.64 ppm), known for potent antioxidant, anti-inflammatory, neuroprotective, and anticancer activities. The presence of additional flavonoids, phenolic acids, stilbenes, and other bioactives underlines the plant's broad pharmacological relevance. The unique agroecological conditions of the rubber plantation likely influence secondary metabolite biosynthesis, enhancing therapeutic compound diversity. Collectively, these findings scientifically substantiate the ethnomedicinal uses of A. danielli and its development as a functional food, nutraceutical, and phytopharmaceutical resource. Further in vivo and clinical investigations are recommended to validate safety and efficacy.

Keywords: Aframomum danielli, proximate composition, phytochemicals, therapeutic potential, rubber agroecology

Graphical Abstract



1.0 INTRODUCTION

Aframomum danielli (Hook.f.) K. Schum, a member of the Zingiberaceae familywqqqqq [1], is indigenous to West and Central Africa and has long been employed in traditional medicine and as a natural food preservative. Its seeds and fruits are rich in essential oils and phenolic compounds, which are responsible for its antimicrobial, antioxidant, and antiinflammatory properties [2]. Prior studies have demonstrated that the plant effectively inhibits lipid oxidation and microbial proliferation, underscoring its value in food preservation applications [3, 4]. Despite its widespread ethnomedicinal use, there remains limited scientific documentation on the detailed nutritional and phytochemical profiles of A. danielli, when cultivated under distinct particularly agroecological systems such as mature rubber plantations.

Environmental variables such as soil quality, shading, and microclimatic conditions are known to influence the biochemical composition of plants [5]. A. danielli is typically found in shaded plantation environments, such as cocoa groves and riverine zones [5, 6]. When grown under mature Hevea brasiliensis (rubber) plantations, the plant is exposed to a distinctive microenvironment marked by reduced direct sunlight, increased organic matter from leaf litter, and altered soil nutrient availability. Such growing conditions have been shown to stimulate the production of secondary metabolites-including flavonoids and phenolic acids—that enhance the plant's antioxidant, antimicrobial, and anti-inflammatory activities [7]. However, no comprehensive investigation has been conducted to evaluate the specific impact of rubber agroforestry systems on the nutritional and phytochemical attributes of A. danielli.

This study aims to fill this knowledge gap by conducting a comprehensive assessment of the proximate and phytochemical composition of *A. danielli* cultivated under mature *Hevea brasiliensis* plantations, using methanol-based extraction methods. Given the plant's gradual decline in the wild, its cultivation is increasingly recommended on cocoa farms, oil palm plantations, and fallow lands to support conservation and sustainable utilization efforts [6]. Furthermore, the global surge in interest in natural compounds as sources of nutraceuticals and pharmaceuticals highlights the importance of characterizing plants with potential health benefits.

Studies on related species, such as *Aframomum melegueta* and *Aframomum zambesiacum*, have already demonstrated significant pharmacological potential, particularly due to their high flavonoid content [7, 8]. Therefore, elucidating the

phytochemical and nutritional profiles of *A. danielli* grown under rubber plantations may reveal new opportunities for its use in the health, wellness, and food industries. The aim of this study is to identify the key bioactive and nutritional constituents of *A. danielli* that contribute to its medicinal value and to evaluate its suitability for incorporation into functional foods, pharmaceuticals, and cosmetic formulations.

2.0 MATERIALS AND METHODS

2.1 Study Location

The study was conducted at the Rubber Research Institute of Nigeria (RRIN), Iyanomo, located in Edo State, Nigeria. The research site is characterized by a mature *Hevea brasiliensis* (rubber) plantation that has been established for over 20 years, offering a unique agroecological environment with limited direct sunlight and rich organic matter from decomposed rubber leaf litter.

2.2 Sample Harvesting and Preparation

Mature fruits of *Aframomum danielli* were carefully harvested from beneath the rubber canopy at RRIN. The fruit pods were manually split open, and the seeds were extracted, ensuring minimal mechanical damage. These seeds were air-dried at ambient temperature to a constant weight to prevent microbial growth and enzymatic degradation. Once fully dried, the seeds were pulverized using a laboratory grinder into fine powder and stored in airtight containers under cool, dry conditions to prevent moisture absorption prior to extraction.

2.3 Extraction Procedure

Methanol extraction of the seed powder was performed using the Soxhlet extraction method as described in standard protocols [9]. Precisely 10 grams of the powdered seed sample were loaded into a thimble and placed in the extraction chamber of a Soxhlet apparatus. Three hundred milliliters (300 mL) of methanol served as the solvent. The extraction was carried out for four hours at a constant temperature of to ensure exhaustive recovery phytochemicals. After extraction, the methanol solvent was removed under reduced pressure using a rotary evaporator, leaving behind a concentrated methanol extract. The extract was subsequently transferred to sterile vials and stored at 4 °C until further phytochemical analysis.

2.4 Proximate Composition Analysis

The proximate composition of the *A. danielli* seed powder was analyzed to determine the following nutritional parameters: moisture, ash, crude fiber, crude fat, crude protein, and carbohydrate content. All analyses were carried out using standard procedures

recommended by the Association of Official Analytical Chemists (AOAC) [10].

2.4.1 Moisture Content:

The moisture content was determined by drying a known weight of the sample in a pre-washed and oven-dried petri dish. Approximately 2 g of the sample was weighed into the dish, and the initial combined weight was recorded. The petri dish and sample were then placed in a hot air oven at 105 °C for 2 hours, after which the weight was measured. The drying process was repeated for an additional hour until a constant weight was achieved, ensuring complete moisture removal. This step was continued until no further weight change was observed, confirming a steady-state condition.

2.0 MATERIALS AND METHODS

2.1 Study Location

The study was conducted at the Rubber Research Institute of Nigeria (RRIN), Iyanomo, located in Edo State, Nigeria. The research site is characterized by a mature *Hevea brasiliensis* (rubber) plantation that has been established for over 20 years, offering a unique agroecological environment with limited direct sunlight and rich organic matter from decomposed rubber leaf litter.

2.2 Sample Harvesting and Preparation

Mature fruits of *Aframomum danielli* were carefully harvested from beneath the rubber canopy at RRIN. The fruit pods were manually split open, and the seeds were extracted, ensuring minimal mechanical damage. These seeds were air-dried at ambient temperature to a constant weight to prevent microbial growth and enzymatic degradation. Once fully dried, the seeds were pulverized using a laboratory grinder into fine powder and stored in airtight containers under cool, dry conditions to prevent moisture absorption prior to extraction.

2.3 Extraction Procedure

Methanol extraction of the seed powder was performed using the Soxhlet extraction method as described in standard protocols [9]. Precisely 10 grams of the powdered seed sample were loaded into a thimble and placed in the extraction chamber of a Soxhlet apparatus. Three hundred milliliters (300 mL) of methanol served as the solvent. The extraction was carried out for four hours at a constant temperature of ensure exhaustive 60 °C to recovery phytochemicals. After extraction, the methanol solvent was removed under reduced pressure using a rotary evaporator, leaving behind a concentrated methanol extract. The extract was subsequently

transferred to sterile vials and stored at 4 °C until further phytochemical analysis.

2.4 Proximate Composition Analysis

The proximate composition of the *A. danielli* seed powder was analyzed to determine the following nutritional parameters: moisture, ash, crude fiber, crude fat, crude protein, and carbohydrate content. All analyses were carried out using standard procedures recommended by the Association of Official Analytical Chemists (AOAC) [10].

2.4.1 Moisture Content

The moisture content (equation 1) was determined by drying a known weight of the sample in a pre-washed and oven-dried petri dish. Approximately 2 g of the sample was weighed into the dish, and the initial combined weight was recorded. The petri dish and sample were then placed in a hot air oven at 105 °C for 2 hours, after which the weight was measured. The drying process was repeated for an additional hour until a constant weight was achieved, ensuring complete moisture removal. This step was continued until no further weight change was observed, confirming a steady-state condition.

% Moisture Content =
$$\frac{W_1-W_2}{Weight \ of \ Sample} \ x \ 100 \ (1)$$

Where,

 W_1 = weight of petri dish and sample before drying W_2 = weigh of petri dish and sample after drying.

2.4.2 Ash Content

Ash content (equation 2) was determined by incinerating approximately 2 grams of the sample in a pre-weighed, clean, and dry platinum crucible using a muffle furnace at 550 °C for 3 hours. After combustion, the crucible containing the inorganic residue was cooled in a desiccator and then reweighed to obtain the final ash weight. This procedure yields the inorganic residue, known as ash, that remains after all organic components have been burnt off. However, it is important to note that the composition of the ash may not exactly reflect the true mineral content of the sample, as some volatile minerals may be lost during the high-temperature incineration. The sample was cooled in a desiccator after burning and weighed.

% Ash Content =
$$\frac{W_3 - W_1}{W_2 - W_1} \times 100$$
 (2)

Where,

 W_1 = weight of empty platinum crucible

 W_2 = weight of platinum crucible and sample before burning

 W_3 = weight of platinum and ash.

2.4.3 Crude Fiber

Crude fiber content (equation 3) was evaluated by subjecting approximately 2 grams of the sample defatted with petroleum ether if the fat content exceeded 10%—to a sequential digestion process involving both acid and alkaline treatments. The sample was first boiled under reflux for 30 minutes with 200 mL of a solution containing 1.25 g of H₂SO₄ per 100 mL, then filtered through linen and washed with boiling water until the filtrate was free of acid. The residue was subsequently transferred to a beaker and boiled again for 30 minutes with 200 mL of a solution containing 1.25 g of carbonate-free NaOH per 100 mL. After this alkaline digestion, the final residue was filtered through a pre-prepared Gooch crucible containing a thin, compact pad of washed and ignited asbestos. The crucible was then dried in an electric oven and weighed, followed by incineration of the residue, cooling, and final weighing to determine the crude fiber content. The loss in weight after incineration x 100 is the percentage of crude

% Crude Fibre =
$$\frac{\text{weight of fibre}}{\text{Weight of sample}} \times 100$$
 (3)

2.4.4 Crude Fat

Fat content was determined using the Soxhlet extraction method, which involves the continuous extraction of the sample with a non-polar organic solvent, typically petroleum ether, for approximately six hours. The procedure began with drying 250 mL clean boiling flasks in an oven at 105-110 °C for about 30 minutes, followed by cooling in a desiccator and recording their weights. Each flask was then filled with about 300 mL of petroleum ether (boiling point 40–60 °C), and the sample was placed in an extraction thimble, lightly plugged with cotton wool. The Soxhlet apparatus was assembled, and the system was allowed to reflux, facilitating the repeated washing of the sample by the solvent. After the extraction, the thimble was carefully removed, and the petroleum ether was recovered for reuse. The flasks, once nearly free of solvent, were further dried at 105-110 °C for one hour, cooled in a desiccator, and reweighed to determine the amount of fat extracted from the sample.

% Fat =
$$\frac{\text{Weight of flask + oil - Weight of flask}}{\text{Weight of sample}} x 100$$
 (4)

2.4.5 Crude Protein

Protein content was determined using the Kjeldahl method, which involves estimating the nitrogen content of the sample and converting it to protein using a factor of 6.25 (equation 5 and 6). The method

is based on the digestion of the sample with hot concentrated sulfuric acid in the presence of a metallic catalyst, which reduces organic nitrogen to ammonia. The ammonia is retained in the solution as ammonium sulfate, and upon alkalinization, it is released as free ammonia during distillation, then trapped in dilute acid and quantified by titration. In the procedure, exactly 0.5 g of the sample was carefully weighed into a 30 mL Kjeldahl flask to prevent the sample from adhering to the flask walls. The flask was stoppered, shaken, and then 0.5 g of Kjeldahl catalyst mixture was added. The mixture was heated cautiously in a digestion rack until a clear solution formed, which was then left to stand and cool for 30 minutes. After cooling, the digest was diluted to 100 mL with distilled water to prevent caking. A 5 mL aliquot of this solution was transferred to the Kjeldahl distillation apparatus, followed by the addition of 5 mL of 40% sodium hydroxide. A 100 mL receiver flask containing 5 mL of 2% boric acid and an indicator mixture (five drops of bromocresol blue and one drop of methylene blue) was positioned under the condenser such that the tip was submerged approximately 20 cm into the solution. Distillation commenced immediately, and once about 50 drops had collected in the receiver, the solution was titrated with 0.01 N hydrochloric acid until a pink endpoint was observed, indicating the amount of nitrogen, and thereby protein, in the sample.

% Nitrogen = Titre value
$$\times 0.01 \times 14 \times 4$$
 (5)

% Protein = % Nitrogen x
$$6.25$$
 (6)

2.4.6 Carbohydrate Content

Carbohydrate content (equation 7) was calculated using the differential method by subtracting the total percentages of moisture, ash, crude fiber, fat, and protein from 100%. This indirect approach provides an estimate of the carbohydrate fraction remaining in the sample, which includes both digestible carbohydrates and, in some cases, minor components such as organic acids and soluble fibers [11].

2.5 Phytochemical Analysis

The phytochemical composition of the methanol extract was analyzed using Gas Chromatography—Mass Spectrometry (GC-MS). The analysis was carried out on an Agilent 6890 GC system equipped with a flame ionization detector (FID) and fitted with a 15-meter MXT-1 capillary column. The sample extract was injected in split mode, and the temperature programming, flow rate of carrier gas (helium), and

detector settings were optimized for volatile compound resolution. Compounds were identified based on their retention times and spectral data by comparison with known standards and the National Institute of Standards and Technology (NIST) library database.

Quantification of phytochemical constituents was performed by integrating the area under each peak and comparing it to standard calibration curves for major compound classes (e.g., phenols, terpenoids, and alkaloids).

0.2g of extract was weighed and transferred in a test tube and 15ml ethanol and 10ml of 50%m/v potassium hydroxide was added. The test tube was allowed to react in a water bath at 60°c for 3hrsmins. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successfully with 20ml of ethanol, 10ml of cold water, 10ml of hot water and 3ml of hexane, which was all transferred to the funnel. These extracts were combined and washed three times with 10ml of 10%v/v ethanol aqueous solution. The ethanol solvent was evaporated. The sample was solubilized in 1000ul of pyridine of which 200ul was transferred to a vial for analysis [12].

3.0 RESULTS AND DISCUSSION

3.1 Proximate Composition

The proximate analysis (Table 1) of A. danielli methanol extract indicated that carbohydrates constituted the majority of the extract (55.665%), followed by significant amounts of protein (13.300%), fat (7.784%), and fiber (3.639%). The moisture content was moderate (13.843%), and ash content, representing the mineral composition, was 5.769%. These values are comparable to those reported by (4) for Aframomum melegueta, which similarly showed high carbohydrate and protein content. The high carbohydrate content suggests that A. danielli could serve as an energy-rich food source, while its moderate protein and fat levels enhance its nutritional profile, making it suitable for incorporation into diets.

Table 1. Proximate Composition of methanol extract *Aframomum danielli* showing the percentage of key nutrients

key nutrients	Percentage (%)	
Carbohydrates	55.665	
Proteins	13.300	
Moisture	13.843	
Fat	7.784	
Ash	5.769	
Fibre	3.639	

3.2 Phytochemical Composition

3.2.1 Nutritional and Functional Significance of Proximate Components

The proximate composition of the methanol extract of Aframomum danielli grown under a mature rubber plantation reveals a rich nutritional profile, underscoring its potential as both a food and therapeutic resource. Table 1 shows the proximate composition of methanol extract Aframomum danielli showing the percentage of key nutrients. Carbohydrates represented the most abundant macronutrient, comprising 55.67% of the extract. This high carbohydrate content suggests that A. danielli may serve as a significant energy source, particularly in dietary formulations where caloric intake is essential. Carbohydrates are not only vital for metabolic energy but also play roles in gut health and immune modulation when complex polysaccharides or fiber-like components are present.

Proteins accounted for 13.30%, indicating a notable presence of nitrogenous compounds, which could contribute to both structural and functional health benefits. Plant-derived proteins, although sometimes limited in certain amino acids, provide essential components for tissue repair and enzyme production. In the context of nutraceutical development, this protein content may support claims for use in dietary supplements targeted at muscle maintenance and general wellness. Moreover, the protein profile may contribute indirectly to the observed therapeutic effects, particularly if bioactive peptides are present.

Moisture content was determined to be 13.84%, a moderate level that suggests the material was adequately dried for preservation but still retains enough inherent water to influence texture and solubility. While a lower moisture content is often desirable to extend shelf life and reduce microbial spoilage, this value still falls within acceptable limits for dried botanical materials. It also hints at the potential for aqueous-based phytochemical extraction methods in future work, complementing methanolic extraction.

Fat content, recorded at 7.78%, reflects the presence of essential lipids and possibly lipid-soluble bioactives such as phytosterols and fat-soluble vitamins. While the fat content is not exceedingly high, it is substantial enough to support the absorption of lipophilic phytochemicals like resveratrol and luteolin. Notably, resveratrol is known for its cardioprotective and anti-aging properties, though it was not detected in measurable quantities in this particular sample [16]. Nonetheless, the presence of dietary fats can enhance the bioavailability of similar compounds.

The ash content of 5.77% signifies a considerable mineral load, pointing to the presence of essential micronutrients such as calcium, potassium, magnesium, and trace elements. This aligns with the ethnopharmacological use of *A. danielli*, where its mineral-rich profile may support metabolic processes and contribute to its traditional use in managing ailments. Minerals are integral to enzyme function, bone health, and cellular signaling, thus reinforcing the plant's potential nutraceutical value.

Crude fiber constituted 3.64% of the composition. Although modest, this fiber content contributes to digestive health by promoting bowel movement regularity and possibly reducing cholesterol levels. Fiber can also serve as a prebiotic, supporting beneficial gut microbiota, which in turn can influence systemic health. Furthermore, fiber may modulate the release of bioactives in the gastrointestinal tract, affecting their absorption kinetics.

These proximate components collectively provide a solid foundation for *A. danielli*'s functional food applications, particularly when viewed alongside its phytochemical profile. The GC-MS analysis of the methanol extract identified bioactive compounds such as quercetin, luteolin, and ellagic acid. Quercetin, though not detected in significant quantities in this sample, is a known anti-inflammatory agent that supports cardiovascular health by improving endothelial function [14]. Luteolin, which possesses neuroprotective potential, may synergize with the extract's nutrient profile to support cognitive health [15]. Most notably, ellagic acid was detected at 2.373 ppm, confirming the plant's antioxidant capacity and reinforcing its therapeutic promise [17].

Catechin, present at 3.361 ppm, further amplifies the extract's antioxidant profile. Although kaempferol and resveratrol were not detected in measurable quantities, their documented health benefits [13, 16] suggest that A. danielli may still harbor related compounds with similar functionalities. The interplay between the proximate and phytochemical constituents supports the plant's role in managing oxidative stress, inflammation, and possibly chronic diseases. Therefore, the nutritional composition of A. danielli, enriched with bioactive phytochemicals, substantiates its potential in food, pharmaceutical, and nutraceutical industries, especially as natural product interest continues to rise.

3.2.2 Bioactive Phytochemicals Identified by GC-MS Analysis

The phytochemical landscape of *Aframomum danielli*, as elucidated through Gas Chromatography-Mass Spectrometry (GC-MS), reveals (Table 2) a rich and diverse spectrum of bioactive secondary metabolites

predominantly belonging to the flavonoid and phenolic acid classes, with minor but pharmacologically relevant contributions from stilbenes and other specialized plant compounds. These constituents collectively confer profound biological potential and lend support to the ethnomedicinal applications of the plant, while also reflecting its nutritional and therapeutic versatility.

Among the phytochemicals identified, flavonoids dominate in both diversity and relative abundance. The highest concentration among all compounds detected was catechin (3.361 ppm), a flavonoid wellknown for its potent antioxidant properties. Catechin and its isomer epicatechin (1.763 ppm) are crucial dietary antioxidants found in green tea, cocoa, and some fruits. Their significant presence in A. danielli supports the proposition that this plant can combat oxidative stress-related conditions such cardiovascular disease, neurodegenerative disorders, and metabolic syndromes. The free radical scavenging ability of catechin, particularly through its hydroxyl functional groups, enables it to modulate intracellular signaling pathways, inhibit lipid peroxidation, and stabilize cellular membranes. These biochemical capabilities are not only beneficial at the cellular level but also influence systemic inflammation, vascular tone, and endothelial function, which are pivotal in preventing chronic degenerative diseases.

Closely following in prominence is ellagic acid, present at 2.373 ppm. This phenolic acid is widely regarded for its anticancer, anti-inflammatory, hepatoprotective, and antioxidant properties [18-19]. Its mechanism of action includes inhibition of carcinogen activation, induction of apoptosis in malignant cells, and modulation of inflammatory gene expression. Furthermore, ellagic acid has been shown to upregulate the Nrf2 signaling pathway, which regulates the expression of antioxidant response elements (ARE) in detoxification enzymes. The appreciable level of ellagic acid in *A. danielli* indicates its potential as a nutraceutical or functional food ingredient that could assist in chemoprevention and liver health maintenance.

A particularly notable finding is the presence of quercetin (0.637 ppm), a well-characterized flavonoid with a wide array of pharmacological activities. Quercetin exhibits anti-inflammatory, antihypertensive, antiviral, and vasoprotective effects [20-21]. Its presence in *A. danielli*, albeit in moderate amounts, contributes significantly to its therapeutic arsenal. Quercetin inhibits enzymes such as lipoxygenase and cyclooxygenase, reducing the synthesis of pro-inflammatory mediators. Moreover, it enhances endothelial nitric oxide synthase (eNOS) activity, improving vascular dilation and reducing

blood pressure. These cardiovascular benefits, when coupled with the antioxidant properties of catechin and ellagic acid, position *A. danielli* as a potential

botanical adjunct in managing hypertension, atherosclerosis, and metabolic syndrome [22].

Table 2. Phytochemical Composition identified in *Aframomum danielli* based on their major classes, retention time, area, amount, and compound class, illustrates the quantities of key phytochemicals present, including catechin (3.361 ppm), ellagic acid (2.373 ppm), quercetin (0.637 ppm), and luteolin (1.610 ppm).

Phytochemical Class	Phytochemicals Identified	Retention Time (min)	Area (pA*s)	Amount (ppm)
Flavonoids	Kaempferol	3.092	255.11549	0.00000
	Catechin	4.683	13.13251	3.36171
	Quercetin	5.749	4.68698	0.63746
	Luteolin	6.490	73.74344	1.61063
	Naringenin	8.742	5.41858	0.10952
	Apigenin	9.875	36.94077	1.97533
	Hesperidin	10.391	16.91737	1.95900
	Isorhamnetin	10.674	8.08912	0.00000
	Epicatechin	11.737	17.61545	1.76279
	Daidzein	11.912	15.35179	-
	Genistein	12.181	2.84417	-
	Myricetin	23.302	-	-
	Gallocatechin	17.260	-	-
	Butein	28.232	-	-
Phenolic Acids	Ellagic Acid	7.578	2.93829	2.37314
	Vanillic Acid	9.013	12.28830	2.34200
	Ferulic Acid	31.656	-	-
	Sinapinic Acid	-	173.47112	-
Stilbenes	Resveratrol	15.428	644.34863	-
Other Compounds	Lunamarin	16.296	14.08099	0.14929
	Baicalin	20.424	-	-
	Nobeletin	22.178	-	-
	Tangeretin	21.223	-	-

Luteolin (1.611 ppm), another key flavonoid identified, possesses neuroprotective and antiinflammatory activity. Luteolin has been shown to inhibit microglial activation and reduce proinflammatory cytokine expression in the brain, thereby protecting against neurodegeneration [23-24]. Its contribution to *A. danielli's* phytochemical profile underlines the possibility of formulating this plant for cognitive health and neurotherapeutic interventions. This is particularly relevant given the increasing incidence of age-related cognitive decline and Alzheimer's disease, for which natural polyphenols have become a focal point of preventive strategies [25].

Apigenin (1.975 ppm) and hesperidin (1.959 ppm), though slightly lower in concentration, reinforce the anti-inflammatory and anticancer spectrum of the extract. Apigenin has been reported to inhibit cell

cycle progression and induce apoptosis in various cancer cell lines, while hesperidin exhibits angioprotective and antiallergic properties. These flavonoids act not only through ROS scavenging but also via epigenetic modulation and suppression of inflammatory transcription factors such as NF-κB and STAT3. Their co-occurrence with other antioxidants in *A. danielli* suggests a synergistic pharmacological profile that could enhance bioefficacy compared to isolated compounds [26-27].

Other flavonoids such as naringenin (0.110 ppm), isorhamnetin, and kaempferol were detected either in trace amounts or at levels below the detection limit, though their spectral peaks were recorded. Kaempferol, despite being undetected in measurable quantity, is of high pharmacological interest. It is a well-studied compound with antioxidant, anti-inflammatory, and anticancer properties [28-29].

Even trace levels of kaempferol, when combined with other polyphenols, may exert synergistic effects through molecular crosstalk within the antioxidant defense network. The detection of isorhamnetin, a methylated metabolite of quercetin, though not quantifiable, is also significant as it contributes to the anti-inflammatory and antidiabetic potential of the extract [30].

The phytochemical profile also revealed the presence of resveratrol, a stilbene with a large spectral area (644.34863 pA*s) but unquantifiable in ppm. Resveratrol is a prominent compound in red wine and grapes and is associated with cardioprotective, antiaging, and anticancer effects [31]. It activates sirtuin enzymes (particularly SIRT1), enhances mitochondrial function, and improves metabolic flexibility. The spectral presence of resveratrol in A. danielli suggests that even if present in microconcentrations, its biological especially when considered alongside flavonoids and phenolic acids—may be substantial due to additive or synergistic interactions [32].

Interestingly, the extract also included lunamarin (0.149 ppm), a lesser-known compound with potential antimicrobial and antiparasitic activity. Though not as widely studied as the major flavonoids, the presence of lunamarin broadens the therapeutic scope of the extract, suggesting possible roles in infectious disease control. Compounds such as baicalin, myricetin, butein, gallocatechin, nobeletin, and tangeretin were detected spectrally but not quantified, indicating either low extractability under the conditions used or limitations in the GC-MS method sensitivity. Nonetheless, these compounds are recognized for their anticancer, anti-allergic, anti-inflammatory, and immunomodulatory properties, and their potential contribution to the overall bioactivity of A. danielli should not be underestimated [33-36].

The inclusion of vanillic acid (2.342 ppm), alongside ellagic acid, as a major phenolic acid further supports the extract's antioxidant potency. Vanillic acid is known for its anti-lipid peroxidation effect, making it useful in protecting lipoproteins and membrane lipids from oxidative damage. It also exhibits anti-ulcerogenic and antimicrobial activity, which aligns with *A. danielli's* traditional use in gastrointestinal health management. The detection of sinapinic acid (173.47112 area units), although unquantified, hints at another layer of antioxidative potential, as this compound has been implicated in neuroprotection and anti-inflammatory pathways.

3.2.3 Therapeutic Implications and Future Research Directions

In totality, the phytochemical fingerprint of A. danielli

is a vivid reflection of its polyphenol-rich nature, shaped by both its genetic makeup and environmental influences—particularly the unique microclimatic and edaphic conditions of the mature rubber plantation where it was grown. These agroecological factors likely contribute to the modulation of secondary metabolite biosynthesis, influencing both yield and compound diversity. This has important implications for phytopharmaceutical standardization and biofortification strategies aimed at maximizing therapeutic efficacy through controlled cultivation.

convergence of multiple phytochemicals in Aframomum danielli, including antioxidant flavonoids, anti-inflammatory phenolic acids, and protective stilbenes, clearly underscores its enormous potential as a nutraceutical, functional food, and phytomedicinal candidate. These findings also validate the traditional uses of the plant in folk medicine and provide a scientific basis for its development into therapeutic agents targeting oxidative stress, inflammation, cancer, cardiovascular disease, and neurodegeneration. Future studies should bioavailability, pharmacokinetics, synergistic interactions, and in vivo validation of these phytochemicals to fully harness the therapeutic capabilities of A. danielli in clinical contexts. The results of this study indicate that Aframomum danielli is a nutritionally and medicinally valuable plant. The methanol extract contains high levels of carbohydrates and proteins, along with a diverse range of bioactive phytochemicals with antioxidant, antiinflammatory, and potential anticancer properties.

4.0 CONCLUSION

The comprehensive proximate and phytochemical analyses of Aframomum danielli grown under mature rubber plantation conditions reveal its dual value as a nutritious and medicinally potent plant. The high carbohydrate and protein contents underscore its potential as a valuable dietary resource, while the diverse array of bioactive phytochemicals particularly flavonoids and phenolic acids such as catechin, ellagic acid, luteolin, and quercetinhighlight its significant antioxidant, antiinflammatory, neuroprotective, and anticancer properties. These findings validate the traditional ethnomedicinal uses of A. danielli and position it as a promising candidate for functional nutraceutical, and phytopharmaceutical applications. Moreover, the unique agroecological environment of the mature rubber plantation appears to influence its secondary metabolite profile, suggesting that cultivation conditions can be optimized to enhance therapeutic compound yield. To fully harness the health benefits and commercial potential of Aframomum danielli, further in vivo studies, clinical

trials, and bioavailability assessments are essential. Ultimately, this study contributes valuable scientific evidence supporting the integration of *A. danielli* into food and pharmaceutical industries aimed at natural, plant-based health solutions.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability Statement:

All data supporting this study are available upon request from the corresponding author.

Authors' Contribution

Eseosa Osazuwa, Oghenetega Sunday and Chioma Okwu-Abolo contributed to conceptualization, methodology, and original draft writing. Eseosa Osazuwa oversaw supervision and funding acquisition, while Ikhazuagbe Hilary Ifijen and Omoigberale Jude contributed in writing review and editing.

Authors' Declaration

The authors certify that this research is original, has not been published previously, and is not under consideration by any other journal. We assume full responsibility for the integrity of the data and the accuracy of the reported findings and will accept all liability for any claims about the content

Ethical Declarations Human/Animal Studies

The authors declare that no human/animal was used for the studies

Acknowledgments

The author wishes to express sincere appreciation to the Management of the Rubber Research Institute of Nigeria (RRIN), Iyanomo, Edo State, for providing institutional support and access to experimental fields under mature rubber plantations, which were vital to the success of this study. The contributions of colleagues in the Farming Systems Research and Extension Programme are gratefully acknowledged for their field assistance, constructive input, and consistent encouragement.

REFERENCES

- [1] Harris, D. J., & Wortley, A. H. (2018). Monograph of Aframomum (Zingiberaceae). *Systematic Botany Monographs*, 104, 1-204.
- [2] Adefegha, S. A., & Oboh, G. (2012). Acetylcholinesterase (AChE) inhibitory activity, antioxidant properties and phenolic composition of two Aframomum

- [3] Adegoke, G. O., Gbadamosi, R., Evwoerhurhoma, F., Uzo Peters, P. I., Falade, K., Itiola, O., Moody, O., & Skura, B. (2002). Protection of maize (Zea mays) and soybeans (Glycine max) using Aframomum danielli. *European Food Research and Technology*, 214(5), 408-411. https://doi.org/10.1007/s00217-001-0476-8
- [4] Abioye, A. O., Adegoke, G. O., & Bolarinwa, I. F. (2014). Evaluation of the preservative effect of Aframomum danielli spice in oils. *African Journal of Biotechnology*, *13*(2), 223-228.
- [5] Adegoke, G., Evwiehurhoma, F. O., & Afolabi, M. O. (2016). Essential oils in food preservation, flavor, and safety. In *Essential oils in food preservation, flavor and safety* (pp. 163-171). Academic Press.
- [6] Ifijen, I. H., Mamza, A. U., Fasina, K. A., Omoruyi, J. I., & Ikhuoria, E. U. (2019). Phytochemical analysis of Guiera senegalensis J.F. Gmel extract and its anti-plasmodial properties on Wister albino mice via oral route. *International Journal of Pharmacology, Phytochemistry and Ethnomedicine,* 13, 35-44. https://doi.org/10.18052/www.scipress.com/IJPP
- 44. https://doi.org/10.18052/www.scipress.com/IJPPE.13.35
- [7] Sarpong, A., & Abugre, S. (2020). The potential of domesticating grains of paradise (Aframomum melegueta), a non-timber forest product in off-reserve tree farms. *Journal of Sustainable Forestry, 41*(2), 159-
- 172. https://doi.org/10.1080/10549811.2020.1845743
 [8] Ifijen, I., & Nkwor, A. (2020). Selected underexploited plant oils in Nigeria: A correlative study of their properties. *Tanzania Journal of Science*, 46(3), 817-827.
- [9] Adegoke, G. O., Komolafe, G. O., & Falade, K. O. (2014). Effect of Aframomum melegueta and Aframomum zambesiacum on the shelf life of stored food products. *Journal of Food Science*, 79(5), 742-748. https://doi.org/10.1111/1750-3841.12483
- [10] Maliki, M., & Ifijen, I. H. (2020). Extraction and characterization of rubber seed oil. *International Journal of Scientific Engineering and Science*, 4(6), 24-27. http://ijses.com/
- [11] Olunkwa, U. E., Iheanacho, K. M. E., Igwe, C. U., Nwaogu, L. A., & Iheanacho, J. N. (2023). Bioactive component analysis of aqueous seed extract of Aframomum melegueta. *GSC Biological and Pharmaceutical Sciences*, 25(2), 249-272. https://doi.org/10.30574/gscbps.2023.25.2.0458 [12] Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Springer. https://doi.org/10.1007/978-94-009-5570-7
- [13] Dilebo, T., Feyissa, T., Asfaw, Z., & Zewdu, A. (2023). Analysis of proximate composition, mineral contents, and anti-nutritional factors of enset (Ensete

- ventricosum) landraces commonly used for amicho preparation in Hadiya Zone, Southern Ethiopia: Implications for food security and mineral bioavailability. *Journal of Agriculture and Food Research*, 14, 100771.
- [14] BeMiller, J. N. (2010). Carbohydrate analysis. In *Food analysis* (pp. 147-177). Springer.
- [15] Maliki, M., Ikhuoria, E. U., & Ifijen, I. H. (2020). Extraction and physiochemical characterization of oils obtained from selected under-utilized oil-bearing seeds in Nigeria. *ChemSearch Journal*, 11(1), 110-117. http://www.ajol.info/index.php/csj
- [16] Akuru, U. B., & Amadi, B. A. (2018). Phytochemicals and antioxidant properties of some selected medicinal plants. *Journal of Pharmacognosy and Phytochemistry*, 7(5), 283-285.
- [17] Calderón-Montano, J. M., Burgos-Morón, E., Pérez-Guerrero, C., & López-Lázaro, M. (2011). A review on the dietary flavonoid kaempferol. *Mini-Reviews in Medicinal Chemistry, 11*(4), 298-344. https://doi.org/10.2174/138955711795305335
- [18] Boots, A. W., Haenen, G. R., & Bast, A. (2008). Health effects of quercetin: From antioxidant to nutraceutical. *European Journal of Pharmacology*, 585(2-3), 325-
- 337. https://doi.org/10.1016/j.ejphar.2008.03.008
- [19] Kwon, Y. (2017). Luteolin as a potential preventive and therapeutic candidate for Alzheimer's disease. *Experimental Gerontology*, 95, 39-43. https://doi.org/10.1016/j.exger.2017.05.014
- [20] Baur, J. A., & Sinclair, D. A. (2006). Therapeutic potential of resveratrol: The in vivo evidence. *Nature Reviews Drug Discovery*, 5(6), 493-506. https://doi.org/10.1038/nrd2060
- [21] Chauhan, A., Yadav, M., Chauhan, R., Basniwal, R. K., Pathak, V. M., Ranjan, A., & Hussain, A. (2024). Exploring the potential of ellagic acid in gastrointestinal cancer prevention: Recent advances and future directions. *Oncology and Therapy, 12*(4), 685-699. https://doi.org/10.1007/s40487-024-00296-1
- [22] Belhouala, K., Korkmaz, C., Küçükaydın, M., Küçükaydın, S., Duru, M., & Benarba, B. (2024). Eco-Friendly Species Evernia prunastri (L.) Ach.: Phenolic Profile, Antioxidant, Anti-inflammatory, and Anticancer Properties. *ACS Omega*, *9*, 45719-45732. https://doi.org/10.1021/acsomega.3c10407
- [23] Ifijen, I. H., Odiachi, I. J., Maliki, M., & others. (2020). Investigation of the anti-malaria potency and chemical constituents of the bark extracts of Ficus elastica in Plasmodium berghei infected mice. *Chemistry Africa*, 3(4), 1045-1051. https://doi.org/10.1007/s42250-020-00163-2
- [24] Amrati, F., Mssillou, I., Boukhira, S., Bichara, M., Abdali, Y., De Azevedo, R., Mohamed, C., Slighoua, M., Conte, R., Kiokias, S., Pontes, G., &

- Bousta, D. (2024). Phenolic Composition of Crataegus monogyna Jacq. Extract and Its Anti-Inflammatory, Hepatoprotective, and Antileukemia Effects. *Pharmaceuticals*, 17(6), 786. https://doi.org/10.3390/ph17060786
- [25] Carrillo-Martinez, E., Flores-Hernández, F., Salazar-Montes, A., Nario-Chaidez, H., & Hernández-Ortega, L. (2024). Quercetin, a Flavonoid with Great Pharmacological Capacity. *Molecules*, 29(5).
- 1000. https://doi.org/10.3390/molecules29051000
- [26] Dedov, D., & Usoltseva, O. (2023). Flavonoids quercetin, dihydroquercetin (taxifolin): antioxidant and anti-ischemic effects, possibility of application in cardiology. *Cardiology*, 34(6), 7-
- 14. https://doi.org/10.29296/25877305-2023-06-07
- [27] Kania-Dobrowolska, M., & Baraniak, J. (2022). Dandelion (Taraxacum officinale L.) as a Source of Biologically Active Compounds Supporting the Therapy of Co-Existing Diseases in Metabolic Syndrome. *Foods,* 11(18),
- 2858. https://doi.org/10.3390/foods11182858
- [28] Nabavi, S., Braidy, N., Gortzi, O., Sobarzo-Sánchez, E., Daglia, M., Skalicka-Woźniak, K., & Nabavi, S. (2015). Luteolin as an anti-inflammatory and neuroprotective agent: A brief review. *Brain Research Bulletin*, 119, 1-11 https://doi.org/10.1016/j.brainresbull.2015.09.00
- 11. <u>https://doi.org/10.1016/j.brainresbull.2015.09.00</u> <u>2</u>
- [29] Rehfeldt, S., Silva, J., Alves, C., Pintéus, S., Pedrosa, R., Laufer, S., & Goettert, M. (2022). Neuroprotective Effect of Luteolin-7-O-Glucoside against 6-OHDA-Induced Damage in Undifferentiated and RA-Differentiated SH-SY5Y Cells. *International Journal of Molecular Sciences*, 23(6), 2914. https://doi.org/10.3390/ijms23062914
- [30] Khan, A., Jahan, S., Imtiyaz, Z., Alshahrani, S., Makeen, H., Alshehri, B., Arafah, A., & Rehman, M. (2020). Neuroprotection: Targeting Multiple Pathways by Naturally Occurring Phytochemicals. *Biomedicines*, 8(8), 284. https://doi.org/10.3390/biomedicines8080284
- [31] Cannataro, R., Fazio, A., La Torre, C., Caroleo, M., & Cione, E. (2021). Polyphenols in the Mediterranean Diet: From Dietary Sources to microRNA Modulation. *Antioxidants*, 10(3).
- 328. https://doi.org/10.3390/antiox10030328
 [32] Alajmi, M., Rehman, M., Hussain, A., & Rather, G. (2018). Pharmacoinformatics approach for the identification of Polo-like kinase-1 inhibitors from
- natural sources as anti-cancer agents. *International Journal of Biological Macromolecules*, 116, 173-181. https://doi.org/10.1016/j.ijbiomac.2018.05.023
 [331] Shahbaz M Alsagaby S Nacem H
- [33] Shahbaz, M., Alsagaby, S., Naeem, H., Abdulmonem, A., Hussain, M., Abdelgawad, M., El-Ghorab, A., Ghoneim, M., El-Sherbiny, M., Atoki, A.,

- & Awuchi, C. (2023). Anticancer, antioxidant, ameliorative and therapeutic properties of kaempferol. *International Journal of Food Properties*, 26(1), 1140-1166. https://doi.org/10.1080/10942912.2023.220504
- [34] Hussain, M., Altamimi, A., Afzal, M., Almalki, W., Kazmi, I., Alzarea, S., Gupta, G., Shahwan, M., Kukreti, N., Wong, L., Kumarasamy, V., & Subramaniyan, V. (2024). Kaempferol: Paving the path for advanced treatments in aging-related diseases. *Experimental Gerontology*, 188, 112389. https://doi.org/10.1016/j.exger.2024.112389
- [35] Speisky, H., Arias-Santé, M., & Fuentes, J. (2023). Oxidation of Quercetin and Kaempferol Markedly Amplifies Their Antioxidant, Cytoprotective, and Anti-Inflammatory Properties. Antioxidants, *12*(1), 155. https://doi.org/10.3390/antiox12010155 [36] Pannu, N., & Bhatnagar, A. (2019). Resveratrol: from enhanced biosynthesis and bioavailability to multitargeting chronic diseases. Biomedicine & Pharmacotherapy, 109. 2237-

2251. https://doi.org/10.1016/j.biopha.2018.11.075