

Nutritional Evaluation of Endemic Wild Yam (*Dioscorea Comorensis*) Tuber Flour Harvested in the Comoros

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DOI: <https://doi.org/10.51584/IJRIAS.2026.11070113>

Received: 24 July 2026; Accepted: 29 July 2026; Published: 08 August 2026

ABSTRACT

Wild edible yam species remain underutilized despite their recognized potential to enhance food security, dietary diversification, and biodiversity conservation. Among them, *Dioscorea comorensis*, an endemic and endangered species from the Comoros, has received little scientific attention, and its nutritional potential remains largely unexplored. This study provides the first comprehensive nutritional and phytochemical characterization of flour produced from its tubers. Tubers collected from Ouellah (Ngazidja Island) were processed into flour and analyzed using standardized AOAC methods. Proximate composition, mineral profile, phytochemical constituents, and nutritional indices (PRAL, Ca/P, and Na/K ratios) were determined using atomic absorption spectrophotometry, colorimetry, and qualitative phytochemical tests. The flour exhibited low moisture content (7.13%) and high dry matter (92.87%), with 6.37% protein, 83.37% carbohydrates, and a metabolizable energy value of 357.46 kcal/100 g. It was particularly rich in potassium (780.41 mg/100 g), sodium (599.41 mg/100 g), phosphorus (444.17 mg/100 g), and calcium (422.70 mg/100 g), while also containing appreciable amounts of magnesium, iron, zinc, manganese, and copper. Phytochemical screening confirmed the presence of several bioactive compounds. The negative PRAL value (−9.80 mEq/100 g) indicated an alkalizing effect, whereas the Ca/P (0.95) and Na/K (0.77) ratios suggested a favorable mineral balance. These findings demonstrate that *D. comorensis* flour is a promising nutrient-rich and functional food resource suitable for composite flour and breadmaking. This study establishes a scientific basis for the sustainable utilization, conservation, and valorization of this endemic species, supporting future strategies to strengthen food and nutritional security in the Comoros.

Keywords: *Dioscorea comorensis*, root and tuber crops, yam, endemic species, nutritional characterization

INTRODUCTION

Roots and tubers are essential components of food systems in tropical regions, contributing significantly to caloric intake, dietary diversification, and rural livelihoods [1,2]. Among these crops, yams (*Dioscorea* spp.) occupy a central place due to their wide geographical distribution, cultural importance, and high nutritional value. They are cultivated and consumed in more than 30 countries and contribute to the food security of nearly 300 million people worldwide, making them the third most important tropical tuber crop after cassava and sweet potato [3,4,5]. The genus *Dioscorea*, which includes approximately 600 described species, comprises nearly 400 taxonomically recognized species [6,7]. These range from highly domesticated crops such as *D. rotundata*, *D. cayenensis*, and *D. alata* to numerous wild or underutilized species with strong agronomic, nutritional, and genetic potential [8,9].

Wild yam species are increasingly recognized for their role in enriching diets, enhancing resilience to climate shocks, and serving as reservoirs of genetic diversity for breeding programs [10]. Their superior ecological adaptability and distinct biochemical profiles often make them valuable but underexploited food resources. However, despite their potential, wild yams remain insufficiently studied, particularly in island ecosystems where endemism levels are high and food systems depend on volcanic archipelagos located within the Madagascar and Indian Ocean Islands biodiversity hotspot [11]. This hotspot harbors a remarkable but under-documented diversity of edible tuberous plants. The geographic isolation of the archipelago, the mosaic of its habitats, and its unique biogeographic history has shaped a flora rich in endemic species [12, 13].

Despite this biological heritage, the country continues to face persistent food and nutritional challenges, linked to low agricultural productivity, soil degradation, and a strong dependence on imported staple foods [14, 15]. Local diets rely mainly on rice, cassava, green bananas, and a small number of cultivated tubers, while many indigenous edible plants remain marginalized or are only occasionally used [16,17].

Among the underutilized tuber species of the archipelago, *Dioscorea comorensis* (R. Knuth) emerges as one of the most important. This species is strictly endemic to the Comoros and holds significant ecological value; it is found mainly in residual forest fragments and humid montane habitats [7,13]. Its conservation status is concerning, as it is currently listed as “Endangered” on the IUCN Red List due to severe habitat fragmentation, anthropogenic pressures, and unsustainable practices [7,18]. Recent field assessments indicate that its populations are highly localized and confined to isolated forest remnants, leaving its ecological and nutritional roles insufficiently understood [19]. As with other wild *Dioscorea* species, the physiological adaptations of *D. comorensis* to volcanic soils and forest ecosystems may confer distinctive nutritional properties that have never been evaluated. To date, no published study has examined the physicochemical or nutritional characteristics of *D. comorensis*.

Existing literature, both regionally and globally, has largely focused on cultivated species such as *Dioscorea rotundata*, *Dioscorea alata*, and *Dioscorea dumetorum* [20, 21, 22]. This lack of documentation represents a major gap, particularly in the context of national strategies aimed at strengthening food security, valorizing local biodiversity, and promoting the use of neglected and underutilized species (NUS). The transformation of yam tubers into flour is a widely recognized approach to improve shelf life, reduce post-harvest losses, and facilitate transport and storage. Flour production also expands agro-food applications, notably in composite flours, porridges, and improved traditional formulations [23, 24, 25]. For *Dioscorea comorensis*, which is seasonal, localized, and harvested in small quantities, flour processing represents a strategic method to valorize an endemic resource while promoting its sustainable use and conservation. In this context, the objective of the present study is to characterize, for the first time, the nutritional composition of flour produced from *Dioscorea comorensis* tubers harvested in the Comoros. By analyzing its macronutrient profile, mineral composition, energy value, and selected nutritional indices, this study aims to evaluate the potential of this endemic species as an alternative food resource and to contribute to its valorization within local food systems. Beyond its nutritional profile, *Dioscorea comorensis* flour also presents technological potential for innovative agro-food applications, particularly in breadmaking and composite flour formulations, opening perspectives for dietary diversification and nutritional security in the Comoros.

MATERIALS AND METHODS

Sampling Area

The sampling site for *Dioscorea comorensis* tubers is indicated in **Figure 1**. It is located in Ouellah, a town in the Mitsamiouli region with approximately 5,480 inhabitants, situated southeast of Ouémani and northwest of Koua in the Union of the Comoros. The geographic coordinates are latitude 11.3903°S (11°23' South) and longitude 43.3319°E (43°19'55" East). Ouellah cultivates several species and varieties of root and tuber crops, including *Dioscorea comorensis*. **Figure 2**, illustrates the actual distribution of this species on Ngazidja Island (Grande Comore). The region benefits from a humid tropical climate, marked by two distinct seasons: a relatively cool dry season from April to November and a hot rainy season from December to March [14], [16]. The landscape of Ouellah forms a mosaic of habitats, including secondary forests, agricultural plots, and relic forest fragments, the latter constituting the last natural refuge of the species.

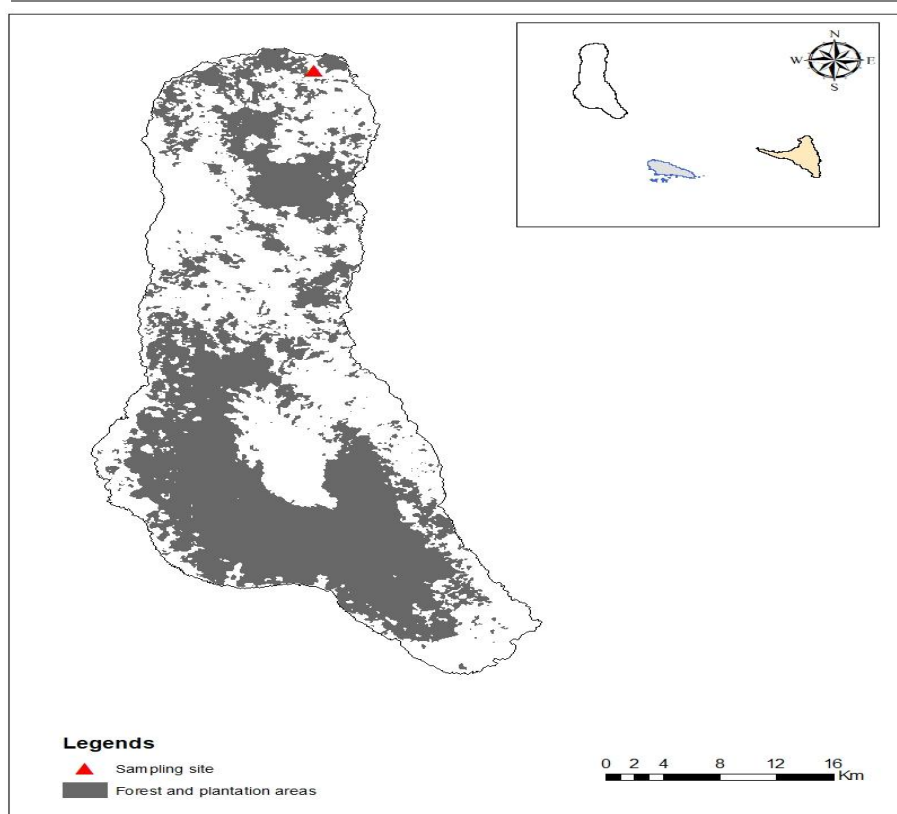


Figure 1: Location of the tuber collection area

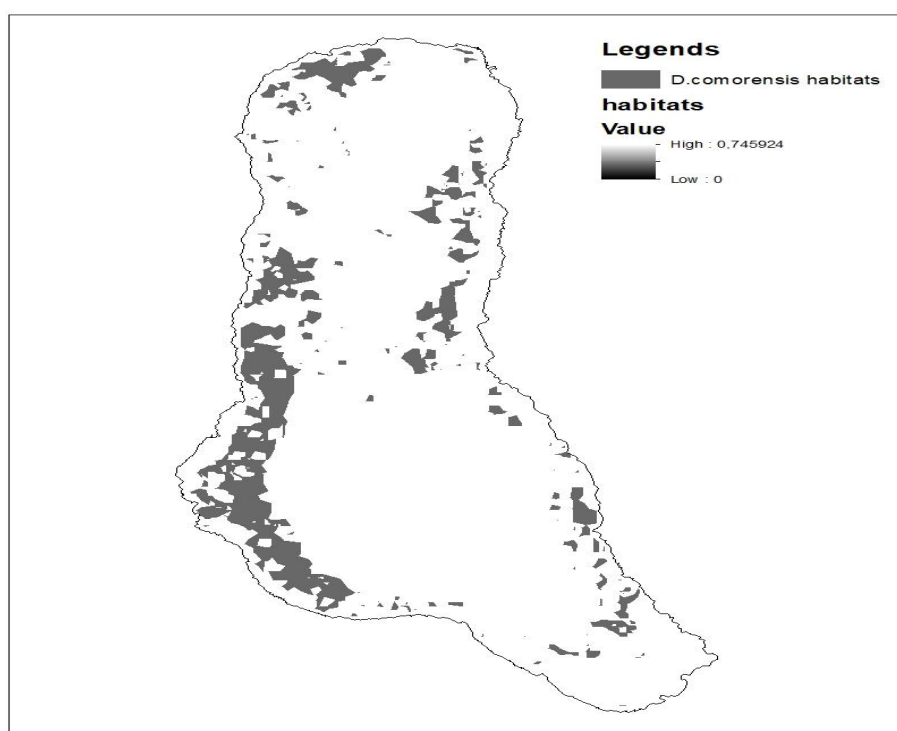


Figure 2: Distribution of *Dioscorea comorensis* on Ngazidja Island, Union of the Comoros

Plant Material

The plant material used in this study consisted of tubers of *Dioscorea comorensis* (**Figure 3**). These tubers were employed as raw material to produce flour, with the aim of valorizing this edible plant species available in the Comoros Islands.



Figure 3: Tubers of *Dioscorea comorensis*

Flour Production

Upon reception, the tubers were manually sorted to discard those showing alterations related to harvesting or transport. Healthy specimens were weighed using a Terrillon Tradition 500 electronic balance to evaluate the final yield. An initial washing was performed to remove soil and impurities, ensuring sanitary quality. Tubers were then trimmed to remove fibrous ends, peeled to retain only the usable cylindrical portion, and washed again to guarantee optimal cleanliness.

The cleaned tubers were sliced into thin pieces and subjected to maceration for 10–15 minutes in potable water supplied by Jiro sy Rano Malagasy (JIRAMA). This step limited oxidation and prevented enzymatic browning by reducing polyphenol oxidase activity. After maceration, slices were drained to remove excess water and facilitate drying. Drying was carried out in open air, on black plastic protected from dust with a mosquito net, for five days. This process yielded a product with moisture content below 12%, a critical threshold preventing microbial development and ensuring product stability prior to further processing.

The dried slices were manually ground using a mortar and sieved through a fine mesh (0.55 mm), producing a homogeneous flour free of lumps. The flour was then weighed, packaged in hermetically sealed dark glass jars, and stored in a dry place. This packaging ensured the preservation of hygienic, nutritional, and organoleptic qualities in accordance with international standards [23].

Physicochemical Analyses of the Samples

Determination of Moisture and Dry Matter Content

The moisture and dry matter content of *Dioscorea comorensis* flour was determined using the oven-drying method. Approximately 10 g of sample were weighed with an electronic balance, placed in clean capsules, and introduced into an oven at 103 °C for 2-3 hours. The operation was repeated until a constant weight was obtained, with mass loss corresponding to moisture content. After drying, capsules containing the samples were cooled for one hour in a desiccator and then weighed with a precision balance. The residual mass was used to calculate dry matter content, while the mass loss was used to determine moisture content.

$$\% \text{ H (FMB)} = \frac{M_2 - M_3}{M_2 - M_1} \quad \% \text{ MS (FMB)} = 100 \% - (\% \text{ H}) \quad [1]$$

Formulas used:

Where: **M1**: Mass (in grams) of the empty capsule; **M2**: Mass (in grams) of the capsule containing the sample to be analyzed; **M3**: Mass (in grams) of the capsule containing the sample after drying; **FMB**: On a fresh matter basis.

Determination of Crude Protein Content

The crude protein content of the yam flour was determined using the Kjeldahl method, applying a conversion factor of 6.25 to convert total nitrogen into protein. This method is based on the conversion of organic nitrogen into mineral nitrogen in the form of ammonium sulfate $[(\text{NH}_4)_2\text{SO}_4]$ through the oxidizing action of boiling sulfuric acid on the organic matter in the presence of a catalyst. The protein content, expressed in grams per 100 g of sample, was calculated using the following formula:

$$\% \text{ PrB (FMB)} = \frac{1,40 \times V \times N \times FC}{M} \quad [2]$$

Where: **FC**: Conversion factor from nitrogen to protein (6.25); **% CP**: Crude protein content, expressed in grams of fresh matter per 100 g of flour; **M**: Mass (in grams) of the flour sample analyzed in fresh state; **V**: Volume (in mL) of 0.1 N sulfuric acid solution added during titration; **N**: Normality of the sulfuric acid solution used; **FMB**: On a fresh matter basis.

Determination of Crude Fat Content

The crude fat content was determined according to the Soxhlet method [24], using hexane as the extraction solvent. This method relies on the insolubility of lipids in water and their high solubility in non-polar organic solvents. The crude fat content was expressed as a percentage using the following formula:

$$(\%) \text{ MGF (FMB)} = \frac{M_3 - M_1}{M_2} \times 100 \quad [3]$$

Where: **% CF**: Crude fat content, expressed as mass percentage of the sample; **M1**: Mass (in grams) of the empty flask before extraction; **M2**: Mass (in grams) of the sample to be analyzed; **M3**: Mass (in grams) of the flask containing the dry residue after drying; **FMB**: On a fresh matter basis.

Determination of Crude Ash Content

The gravimetric method was used to determine the crude ash content. The ashes obtained were used to assess the mineral content of the flour sample. The principle of crude ash determination consists of mineralizing the organic matter contained in *Dioscorea comorensis* flour at 550 °C using a muffle furnace. At this temperature, water and other volatile constituents are vaporized, while organic compounds are burned and converted into gaseous products and water. Mineral constituents remain in the residue in the form of oxides, sulfates, phosphates, and other compounds.

The remaining residue after incineration constitutes the crude ash of *Dioscorea comorensis* flour. The crude ash content (CA) of the flour was expressed in grams per 100 g of sample and calculated using the following formula:

$$\% \text{ CA (FMB)} = \frac{M_1 - M_2}{M_2} \quad [4]$$

Where: M1: Mass (in grams) of the empty crucible during incineration, M2: Mass (in grams) of the sample to be analyzed (test sample); FMB: On a fresh matter basis.

Determination of Total Carbohydrate Content

The total carbohydrate content (TC) of *Dioscorea comorensis* flour was determined using the difference method. This approach consists of subtracting from 100% the sum of the moisture, protein, crude fat, and crude ash contents of the sample [14]. The formula used on a fresh matter basis (FMB) is as follows:

$$\% \text{ TC (FMB)} = 100 - (\% \text{ H} + \% \text{ P} + \% \text{ L} + \% \text{ CB}) \quad [5]$$

Where: % H: Moisture content of the sample (g/100 g fresh matter); % CP: Protein content (g/100 g fresh matter); % CF: Crude fat content (g/100 g fresh matter); % CA: Crude ash content (g/100 g fresh matter). This method provides a global estimation of carbohydrates, including starches and soluble sugars, present in the analyzed sample. It also allows comparison of the energy and nutritional value of the flour with that of other local tubers and food products.

Determination of Metabolizable Energy

The metabolizable energy value of the flour was determined using Atwater's specific calorific coefficients, in accordance with FAO recommendations (2003). Metabolizable energy (ME) of a product is obtained by summing up the metabolizable energies of the main energy sources: lipids, carbohydrates, and proteins.

$$\text{ME (kcal/100 g)} = (\% \text{ CP} \times \text{SCCP}) + (\% \text{ CF} \times \text{SCCF}) + (\% \text{ TC} \times \text{SCC}) \quad [6]$$

Where: ME: Metabolizable energy, expressed in kcal per 100 g of food product; % CP: Crude protein content of the product; % CF: Crude fat content of the product; % TC: Total carbohydrate content of the product; SCCP: Specific calorific coefficients of Atwater, in kcal/g of protein; SCCF: Specific calorific coefficients of Atwater, in kcal/g of lipids; SCCT: Specific calorific coefficients of Atwater, in kcal/g of carbohydrates.

Determination of Mineral Content

The concentrations of minerals (K, Na, Ca, Cu, Mg, Fe, Zn, Mn) were determined by atomic absorption spectrophotometry, while phosphorus was measured by colorimetry after solubilization of the ashes in an acidic medium. The method used complies with the recommendations of the Inter-Institutes Committee, as described by Pinta [26].

Determination of Ca/P and Na/K Ratios

The Ca/P and Na/K ratios of *Dioscorea comorensis* flour were calculated from the mineral contents obtained by atomic absorption spectrophotometry and colorimetry, according to the method described by Pinta [26]. The ratios were calculated using the following formulas:

$$\text{Ca/P} = \frac{\% \text{ Ca}}{\% \text{ P}} \quad [7]$$

$$\text{Na/K} = \frac{\% \text{ Na}}{\% \text{ K}} \quad [8]$$

Where: % Ca: Calcium content (mg/100 g); % K: Potassium content (mg/100 g); % Na: Sodium content (mg/100 g); % P: Phosphorus content (mg/100 g).

Determination of the PRAL Index

The PRAL index (Potential Renal Acid Load) is used to estimate the acidifying or alkalinizing effect of a food on the acid–base balance of the blood. A positive value indicates an acidifying effect, while a negative.

$$\text{PRAL (mEq/100 g food)} = (0.49 \times \text{protein (g)}) + (0.037 \times \text{phosphorus (mg)}) - (0.021 \times \text{potassium (mg)}) - (0.026 \times \text{magnesium (mg)}) - (0.013 \times \text{calcium (mg)}) \quad [9]$$

value reflects an alkalinizing effect [27]. It is calculated from the protein and mineral composition using the following formula:

This index provides a practical tool for evaluating the alkalinizing or acidifying potential of foods within the framework of a balanced diet. These standardized analyses not only characterize the nutritional composition of *Dioscorea comorensis* flour but also assess its technological potential for innovative agri-food applications, such as breadmaking and integration into composite flours.

Phytochemical Screening

Phytochemical screening was performed on a hydroalcoholic extract (80% ethanol) prepared from *Dioscorea comorensis* flour to identify the major secondary metabolites present in the sample. Qualitative analyses were carried out according to the methods [28], based on specific colorimetric and precipitation reactions. Alkaloids were detected using Mayer's, Wagner's, and Dragendorff's reagents; flavonoids and leucoanthocyanins using the Wilstätter and Bate-Smith tests; triterpenes and sterols using the Liebermann–Burchard and Salkowski tests; while polyphenols, tannins, cyanogenic glycosides, anthraquinones, 2-deoxy sugars, coumarins, saponins, and polysaccharides were identified using their respective qualitative tests. The results were interpreted according to the intensity of the observed reactions and expressed on a semi-quantitative scale ranging from absent (–) to strongly present (+++).

Statistical Analysis

All analyses were performed in triplicate ($n = 3$), and the results are expressed as mean $\pm$ standard deviation (SD). Statistical calculations were performed using IBM SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA). Since the study aimed at the nutritional characterization of a single yam species without treatment comparisons, no inferential statistical tests were performed.

RESULTS

Moisture, Dry Matter, Macronutrients, Crude Ash, and Metabolizable Energy Content of *D. comorensis* Flour

Table 1. summarizes the proximate composition of the flour. Carbohydrates predominate, representing the main energy source, while proteins and lipids contribute modestly. The relatively low moisture content supports product stability.

Table 1. Proximate composition of *D. comorensis* (g/100 g DW)

Parameter	Mean $\pm$ Standard Deviation (SD)	95% CI	Min–Max
Moisture	7,13 $\pm$ 0,25	6,51 – 7,76	6,90 – 7,40
Dry Matter	92,87 $\pm$ 0,25	92,24 – 93,49	92,60 – 93,10
Protein	6,37 $\pm$ 0,15	5,99 – 6,75	6,20 – 6,50

Fat	0,45 ± 0,05	0,33 – 0,57	0,40 – 0,50
Ash	7,13 ± 0,21	6,62 – 7,65	6,90 – 7,30
Carbohydrates	83,37 ± 0,35	82,49 – 84,24	83,00 – 83,70
Energy (kcal/100 g)	357,5 ± 4,5	346,3 – 368,7	353 – 362

Note: Data are presented as mean ± standard deviation (SD), calculated from triplicate determinations (n = 3).

FMB: on a fresh matter basis, **(95% CI):** 95% Confidence Interval, **Min–Max:** Minimum – Maximum (Range)

Mineral Content, Ca/P and Na/K Ratios, and PRAL index of *Dioscorea comorensis* Flour

Table 2. summarizes the mineral composition of the flour. Macro elements predominate, with appreciable contributions from trace elements. The Ca/P and Na/K ratios remain below unity, while the negative PRAL index indicates a potential alkalinizing effect.

Table 2. Mineral composition and indices of *D. comorensis* (mg/100 g DW)

Parameter	Mean ± Standard Deviation (SD)	95% CI	Min–Max
Manganese	220,7 ± 5,2	207,8 – 233,7	215,5 – 225,9
Zinc	223,1 ± 4,9	210,8 – 235,4	218,2 – 228,1
Iron	252,3 ± 6,3	236,8 – 267,9	246 – 258,5
Magnesium	287,7 ± 7,6	268,9 – 306,5	280 – 295,1
Copper	332,2 ± 8,1	312,2 – 352,2	324 – 340,1
Calcium	422,7 ± 9,6	399,0 – 446,4	413 – 432,1
Phosphorus	429,7 ± 5,9	415,1 – 444,3	423 – 434
Sodium	599,5 ± 12,5	568,4 – 630,6	587 – 612
Potassium	780,5 ± 15,5	742,0 – 819,0	765 – 796
Ca/P ratio	0,95 ± 0,03	0,88 – 1,02	0,92 – 0,98
Na/K ratio	0,77 ± 0,02	0,72 – 0,82	0,75 – 0,79
PRAL index (mEq/100 g)	–9,80 ± 0,40	–10,79 – –8,81	–10,2 – –9,4

Note: Data are presented as mean ± standard deviation (SD), calculated from triplicate determinations (n = 3).

FMB: on a fresh matter basis, **(95% CI):** 95% Confidence Interval, **Min–Max:** Minimum – Maximum (Range).

Phytochemical Characteristics of *Dioscorea comorensis* Flour

Table 3. present the qualitative phytochemical composition of *Dioscorea comorensis* flour. The findings reveal a marked predominance of bioactive compounds, notably flavonoids, tannins, polyphenols, and polysaccharides, whereas major antinutritional metabolites such as alkaloids, saponins, and cyanogenic glycosides were

consistently absent. The reproducibility of the assays, confirmed through triplicate testing, strengthens the reliability of these observations.

Table 3. Qualitative phytochemical screening results of *Dioscorea comorensis* flour

Compound family	Presence/Absence	Reaction intensity (Mean $\pm$ SD, n=3)
Alkaloids	Absent	—
Flavonoids	Present	Strong coloration (± 0.2)
Anthocyanins	Present	Moderate (± 0.1)
Triterpenes	Present	Strong (± 0.3)
Unsaturated sterols	Present	Moderate (± 0.2)
Saponins	Absent	—
Polysaccharides	Present	Strong precipitation (± 0.2)
Cyanogenic glycosides	Absent	—
Tannins	Present	Strong precipitation (± 0.3)
Polyphenols	Present	Strong coloration (± 0.2)
Anthraquinones	Absent	—
2-deoxy sugars	Present	Distinct red-purple ring (± 0.1)
Coumarins	Absent	—

DISCUSSION

This study provides the first detailed characterization of the nutritional and phytochemical composition of flour derived from *Dioscorea comorensis*, a wild species endemic to the Comoros archipelago. The findings highlight nutritional value and confirm its potential contribution to food and nutritional security in island contexts where dependence on imported food remains high.

The proximate composition reveals a predominance of carbohydrates (83.37 g/100 g), positioning *D. comorensis* as a high-energy food comparable to cultivated species such as *D. rotundata* and *D. alata* [40,51–53]. The metabolizable energy (357.5 kcal/100 g) confirms its role as a staple food, while the moderate protein content (6.37 g/100 g) provides a relevant contribution in starch-based diets. These values are consistent with those reported for *D. rotundata* (6.0–7.0 g/100 g; 350–360 kcal/100 g) and *D. alata* (6.5–8.0 g/100 g; 355–365 kcal/100 g) [27,64]. The low-fat content (0.45 g/100 g) reduces susceptibility to oxidation, thereby enhancing sensory stability and shelf life. Although dietary fiber was not quantified in the present study, recent findings indicate that *Dioscorea comorensis* is exceptionally rich in this component, with reports of up to 19.6% [65]. This level is significantly higher than those typically found in other common yam species such as *Dioscorea rotundata*, *Dioscorea bulbifera*, and *Dioscorea cayenensis*, which generally range between 3% and 7%.

The substantial fiber content in this endemic species further confirms its high nutritional and functional value, as dietary fiber plays a critical role in gastrointestinal health, satiety, and the regulation of glycemic response. Such high fiber levels, combined with the energy-rich carbohydrate profile observed in our results, suggest that

Dioscorea. comorensis could be a powerful tool in dietary strategies to reduce the risk of cardiovascular diseases and improve digestive health within the Comorian population

The mineral profile is remarkable, with high concentrations of macronutrients (K, Na, P, Ca, Mg) and appreciable levels of micronutrients (Fe, Mn, Zn, Cu). These values, obtained by atomic absorption spectrophotometry in triplicate, exceed those generally reported for cultivated yam flours [14,57]. Soil conditions are characterized by high carbon and clay content [56], as well as access to deep saprolite horizons rich in essential cations [61,62], likely explain these concentrations. Although low standard deviations reinforce the reliability of the results, multi-site sampling remains necessary to confirm reproducibility.

Phytochemical screening revealed abundant polyphenols, flavonoids, tannins, triterpenes, and polysaccharides, consistent with previous findings in *Dioscorea* species [27,57,63]. These compounds confer antioxidant and functional properties, while the absence of alkaloids, saponins, and cyanogenic glycosides enhances food safety. Thus, the characterization of *D. comorensis* aligns with broader trends observed in yams, where bioactive fractions contribute to nutritional value and functional potential.

CONCLUSION

This study provides the first comprehensive nutritional and phytochemical characterization of *Dioscorea comorensis* flour, an endemic wild yam from the Comoros archipelago. The results demonstrate its high carbohydrate content and energy value, moderate protein contribution, and low-fat levels, positioning it as a stable, nutrient-dense food resource. The mineral profile is particularly remarkable, with elevated concentrations of both macro and trace elements, while phytochemical screening confirmed the presence of bioactive compounds such as flavonoids, tannins, polyphenols, and triterpenes, alongside the absence of potentially harmful metabolites. These findings highlight the dual nutritional and functional potential of *D. comorensis*, supporting its integration into composite flours and breadmaking. Beyond its dietary relevance, the valorization of this species contributes to biodiversity conservation and food security in island contexts where reliance on imported staples remains high. Future research should expand sampling across sites and seasons, and investigate amino acid profiles, mineral bioavailability, and functional properties, to consolidate its role as a sustainable food resource.

ACKNOWLEDGEMENTS

The authors of this study would like to thank the Director of the Laboratory of the National Center for Environmental Research in Antananarivo for his material support throughout the completion of this work. We also extend our gratitude to Dr. Ralambondrahety Rahanira for his technical assistance during our stay at the National Center for Environmental Research in Antananarivo.

Conflict of Interest Declaration

The authors declare that they have no known financial or non-financial conflicts of interest that could have influenced the results, interpretation, or discussion of this study.

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