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# Nutritional and Phytochemical Characteristics of *Givotia stipularis* Seeds, Endemic to Madagascar

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***Abstract*—***The seeds of Givotia stipularis, a plant endemic to Madagascar and belonging to the Euphorbiaceae family, are traditionally consumed in the northwestern region of the island. This study aims to address the lack of scientific data on its seeds. Accordingly, nutritional analysis, phytochemical screening, antioxidant activity evaluation, and toxicological assessment were conducted. The nutritional analysis revealed a high lipid content (66.26 g/100 g), substantial protein levels (17.50 g/100 g), and a high energy value (704.86 kcal/100 g). These seeds are also distinguished by their richness in essential minerals, notably potassium (547.89 mg/100 g), phosphorus (478.49 mg/100 g), magnesium (244.80 mg/100 g), and calcium (142.18 mg/100 g). Phytochemical screening highlighted the presence of leucoanthocyanins, sterols, triterpenes, and alkaloids. The antioxidant activity of ethanolic extracts, evaluated using the DPPH assay, demonstrated a progressive inhibition of free radicals reaching approximately 70% at the highest concentration, compared to nearly complete inhibition (≈100%) observed with vitamin C. Although the antioxidant activity is moderate, it remains significant and suggests a synergistic effect among the identified bioactive compounds, particularly leucoanthocyanins and triterpenes, despite the absence of classical flavonoids and polyphenols. Finally, the acute toxicity test confirmed the safety of the seeds. These findings highlight the nutritional and antioxidant potential of Givotia stipularis, paving the way for its valorization in natural formulations targeting oxidative stress and its integration into local dietary strategies.*

***Keywords*—***Givotia stipularis, Euphorbiaceae, Endemic Species, Seeds, Nutritional Analysis, Phytochemical Screening, Antioxidant Activity, DPPH Assay, Toxicological Evaluation, Safety Profile.*

## I. Introduction

Trees and forests play a fundamental role in global food security by providing a continuous source of food throughout the year, particularly during periods of staple food shortages (FAO, 1985). As an old Kashmiri proverb reminds us: “Food will last as long as forests endure.” Indeed, forest ecosystems offer a wide diversity of edible resources, including fruits, nuts, leaves, seeds, grains, tubers, and roots.

Globally, approximately 17% of the population depends directly on forests for their food supply (UN, 2017). This dependence varies according to geographical context: in the forested areas of northern Thailand, 60% of consumed foods come directly from the forest (Hoskins, 1988); whereas in Java, a densely populated region with limited forest cover, 60% of foods are derived from home gardens where cultivated trees play a central role (Widagda, 1981). In Madagascar, forests are crucial to rural food systems. According to the Food System Profile of Madagascar published by FAO, CIRAD, and the European Union in 2021, non-timber forest products (NTFPs) represent an important source of food and income for rural communities, particularly during lean seasons or agricultural crises. This dependence is further exacerbated by chronic food insecurity affecting more than 40% of the Malagasy population.

Due to its geographic isolation in the heart of the Indian Ocean, Madagascar is recognized as a biodiversity hotspot, harboring between 14,000 and 19,000 plant species, of which 83% are endemic (Madagascar Plant Specialist Group, 2021). Among this exceptional flora is *Givotia stipularis*, a species of the Euphorbiaceae family found in the northern, western, and northwestern forests of the island.

The seeds of *Givotia stipularis* are traditionally consumed in northwestern Madagascar, often prepared with cassava leaves, a highly appreciated local dish. The oil extracted from these seeds is also used for frying, highlighting their importance in regional culinary practices. However, despite this ancestral use, no scientific study has yet documented the nutritional properties, phytochemical composition, antioxidant activity, or toxicity of this plant.

In light of this gap, it is essential to conduct an in-depth investigation to valorize this endemic resource. Therefore, the present study aims to perform a nutritional analysis, phytochemical screening, antioxidant activity evaluation, and toxicological assessment of *Givotia stipularis* seeds collected from the Ampandrana forest, located in the Sofia Region in northwestern Madagascar. The objective is to establish a solid scientific basis to support their dietary and functional use, and to contribute to a better recognition of their potential in local food security strategies.

## II. Materials And Methods

### *2.1 Plant Material*

The plant material used in this study consisted of *Givotia stipularis* seeds, extracted from drupaceous fruits (Fig. 1), collected in the Ampandrana forest, located in the Rural Commune of Ambatosia, Bealanana District (geographical coordinates: 14°42′48.4"S; 48°34′52.1"E), in the Sofia Region, northwestern Madagascar. Botanical identification of the plant was carried out by Mrs. Hanta Razafindraibe, Botanist at the National Herbarium Center of Tsimbazaza,

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Antananarivo (Madagascar). In the region, *Givotia stipularis* is known under two vernacular names: “Harakasaka” and “Farafatse.”

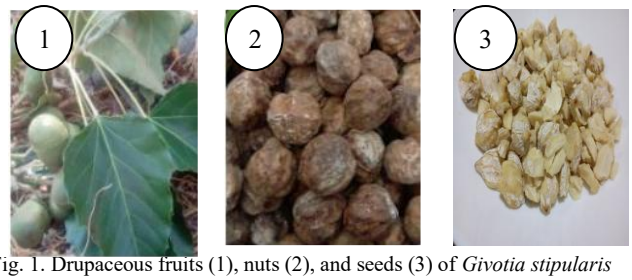

Fig. 1. Drupaceous fruits (1), nuts (2), and seeds (3) of *Givotia stipularis*

After harvesting, the nuts were carefully cleaned and then shade-dried for 48 hours in order to preserve their physicochemical, nutritional, and phytochemical properties. They were subsequently stored in airtight containers, protected from moisture, light, and any source of contamination, until further processing.

*2.2 Seed Processing into Powder*

The process of transforming seeds into powder followed the steps of dehulling, drying, grinding, sieving, stabilization, and packaging. Dehulling was performed to remove the external shell of the seeds. The seeds were then air-dried in a dry and well-ventilated environment until superficial moisture was reduced. Grinding was subsequently carried out using a knife mill, with the seeds introduced in small quantities to minimize system overheating. The resulting powder was sieved using a 500 µm mesh to standardize particle size; oversized particles were returned to the mill for regrinding. The powder was then stabilized by resting in a cool, dry place to eliminate residual moisture. Finally, it was packaged in airtight containers and stored protected from light, humidity, and heat.

*2.3 Determination of Moisture and Dry Matter Content of the Produced Powder*

The moisture and dry matter contents of the powder were determined using the gravimetric method described by AOAC (1989). For this purpose, 5 g of powder were placed in an empty capsule previously weighed with precision. The capsule containing the sample was weighed and then placed in an oven set at 103 °C for 4 hours. After drying, the capsule was transferred to a desiccator to cool for 1 hour before being weighed again. The analysis was performed in duplicate to ensure the reliability of the results. The moisture content (% *MC*) and dry matter content (% *DM*) of the sample were calculated using equations (1) and (2) in accordance with the aforementioned method:

$$\%MC = \frac{m_1 - m_2}{m_1 - m_0} \times 100 \qquad (1)$$

$$\%DM = 100 - \%MC \qquad (2)$$

Where $m_0$ is the mass of the empty capsule (g); $m_1$ is the mass of the capsule containing the sample before drying (g) and $m_2$ is the mass of the capsule containing the sample after drying (g)

*2.4 Determination of Crude Ash Content*

The crude ash content (% CA) was determined according to the method described by AOAC (2005). In this procedure, 5 g of *Givotia stipularis* powder were placed into an empty incineration crucible previously weighed with precision. The crucible containing the sample was then placed in a furnace and heated at 550 °C until complete incineration, characterized by the formation of ash with a white, light gray, or reddish color. After incineration, the crucible was transferred to a desiccator to cool to room temperature, and subsequently weighed again to determine the mass of the residual ash.

The crude ash content, expressed on a fresh matter basis, was calculated using equation (3), in accordance with the AOAC (2005) method:

$$\%CA = \frac{m_1 - m_0}{m.t} \times 100 \qquad (3)$$

Where $m_1$ is the mass of the crucible + sample after incineration (g); $m_0$ is the mass of the empty crucible (g) and $m.t$ is the mass of the test portion (g).

*2.5 Determination of Lipid Content: Crude Fat*

The crude fat content was determined according to the official AOAC 963.15 method (AOAC, 2000), based on acid hydrolysis followed by solvent extraction using the Soxhlet procedure. The principle consists of hot acid hydrolysis of the sample with hydrochloric acid, allowing the release of bound lipids. After filtration, rinsing, and drying of the residue, extraction was performed using hexane, a non-polar organic solvent. The lipid residue obtained was then dried and weighed to calculate the crude fat content gravimetrically.

Specifically, 5 g of *Givotia stipularis* powder were placed in a conical flask, to which 40 ml of 3 N hydrochloric acid were added. The mixture was heated for one hour using a heating mantle, then filtered through filter paper. The retained residue was rinsed with distilled water and dried at room temperature until complete desiccation. The filter paper containing the residue was placed in a cartridge, inserted into a Soxhlet extractor. An empty flask ($W_0$) was weighed, filled with 100 ml of hexane, then connected to the extractor and heated for six hours. At the end of the extraction, the solvent was removed using an evaporator. The flask containing the extracted lipids was dried at 103 °C for 30 minutes, cooled in a desiccator, and then weighed again ($W_1$).

The lipid content, expressed on a fresh matter basis (% *CF*), was calculated using equation (4) in accordance with the official AOAC 963.15 method:

$$CF\% = \frac{W_1 - W_0}{M} \times 100 \qquad (4)$$

Where $W_1$ is the weight of the flask containing the dried lipid residue after desiccation; $W_0$ is the weight of the empty flask before extraction and $M$ is the mass of the sample used (in grams)

*2.6 Determination of Protein Content*

Protein content was determined using the Kjeldahl method, in accordance with the recommendations of BIPEA (1976). This method is based on the conversion of organic nitrogen into ammonium ions ($NH_4^+$) through acid mineralization with

concentrated sulfuric acid, in the presence of a catalyst composed of potassium sulfate ($K_2SO_4$) and copper sulfate ($CuSO_4$). The analytical protocol consists of three successive steps: mineralization, distillation, and titration.

In the first step, mineralization, 1 g of *Givotia stipularis* powder was introduced into a flask containing 20 ml of concentrated sulfuric acid ($H_2SO_4$) and 1 g of catalyst composed of copper sulfate ($CuSO_4$) and potassium sulfate ($K_2SO_4$). The mixture was hermetically sealed and heated at 350 °C for 4 hours, allowing the conversion of organic nitrogen into ammonium ions, visible as a white precipitate.

The second step, distillation, involved pouring 25 ml of boric acid containing a few drops of color indicator into a beaker, followed by the addition of the ammonium precipitate, 20 ml of distilled water, and 50 ml of sodium hydroxide (NaOH). This mixture was placed in a distillation apparatus for 10 minutes, during which the solution changed color from pink to green.

The third step, titration, was carried out using a burette containing a sulfuric acid solution, which was gradually added to the distillate until the appearance of a persistent pink coloration, indicating the equivalence point. The volume of sulfuric acid ($H_2SO_4$) required to produce this color change was recorded.

The protein content, expressed on a fresh matter basis, was calculated using equation (5), as described by AFNOR (1988):

$$\%N = \frac{V \times 14 \times 100 \times 0.001 \times n}{s.w} \quad (5)$$

Wher $V$ is the volume of titrant solution (hydrochloric or sulfuric acid) used (ml); 14 the atomic mass of nitrogen (g/atom); 100 the conversion factor to obtain a percentage; 0.001 the conversion of milliliters into liters in the calculation of moles; $n$ the normality of the titrant solution (0.107 N); and $p.e$ the mass in grams of the test sample.

Hence, the protein content (% $P$) on a fresh matter basis was calculated using equation (6), in accordance with the Kjeldahl method described by AFNOR (2002).

$$\%P = \%N \times 6.25 \quad (6)$$

Where %N represents the nitrogen content in the sample, and 6.25 is the average conversion factor used to estimate protein content (corresponding to 100 ÷ 16).

### *2.7 Functional Nutritional Indices*

To evaluate the metabolic impact of the powder derived from *Givotia stipularis* seeds, several nutritional indices were calculated based on its chemical composition.

• *Calcium/Phosphorus Ratio (Ca/P)*

This ratio plays a crucial role in bone health. An excess of phosphorus (low ratio) may limit calcium absorption and compromise bone tissue mineralization. The ratio is calculated according to equation (7) (Houdji *et al*., 2018):

$$Ca/\mathrm{P} = \frac{\%Ca}{\%P} \quad (7)$$

where % $Ca$ is the calcium content (mg/100 g) and % $P$ is the phosphorus content (mg/100 g).

• *Sodium/Potassium Ratio (Na/K)*

This ratio is an indicator of electrolyte balance and cardiovascular health. A ratio below 1 is recommended to reduce the risk of hypertension, often associated with diets high in sodium and low in potassium. It is calculated as follows (Houdji *et al.,* 2018):

$$Na/K = \frac{\%Na}{\%K} \quad (8)$$

where % $Na$ is the sodium content (mg/100 g) and % $K$ is the potassium content (mg/100 g).

• *PRAL Index (Potential Renal Acid Load)*

The PRAL index estimates the effect of a food on the body's acid–base balance. A positive PRAL value indicates an acid load, often linked to high intakes of protein and phosphorus, which may strain the kidneys and affect bone health. Conversely, a negative PRAL value reflects an alkalinizing effect.

The calculation takes into account the contents of protein, phosphorus, potassium, magnesium, and calcium, and is expressed in milliequivalents (mEq/100 g) (Remer & Manz, 1995; Pamplona-Roger, 2016):

$$\begin{aligned}\mathrm{PRAL(mEq/100g)} = {} & (0.49 \times \%P) + (0.037 \times \%P) \\ & -(0.021 \times \%K) - (0.026 \times \%Mg) - (0.013 \times \%Ca)\end{aligned} \quad (9)$$

where the first % $P$ refers to crude protein content, the second %P to phosphorus content, % $K$ to potassium, % $Mg$ to magnesium, and % $Ca$ to calcium.

### *2.8 Determination of Carbohydrate Content*

The total carbohydrate content was estimated indirectly by difference, by subtracting the sum of the contents of water or moisture (% $MC$), proteins (% $P$), lipids (% $CF$), and ash (% $CA$) from 100% (Mercier & Tollier, 1984; AFNOR, 1988). Thus, the total carbohydrate content (% $C$) of the sample, based on fresh matter, was calculated using the following equation (10):

$$\%C = 100 - (\%P + \%CF + \%CA + \%MC) \quad (10)$$

With %$C$ is the Total carbohydrate content; %$P$ is the Protein content; %$CF$ is the Lipid content; %$CA$ is the Ash content; and %$MC$ is the Moisture content.

### *2.9 Calculation of Metabolizable Energy Value*

The metabolizable energy value was determined according to the recommendations of Greenfield and Southgate (1992), by applying standard energy coefficients to the main macronutrients: 1 g of carbohydrates provides 4 kcal, 1 g of lipids provides 9 kcal, and 1 g of proteins provides 4 kcal. To ensure the reliability of the results, two trials were conducted, and the mean of the two values was taken as the final result. The energy value was expressed in kilocalories per 100 g of product and was calculated using the following equation (11):

$$ME = (\%P \times 4) + (\%CF \times 9) + (\%C \times 4) \quad (11)$$

With % $P$: Protein content; 4: Energy provided by 1 gram of protein, expressed in kilocalories. % $CF$: Lipid content; 9: Energy provided by 1 gram of lipids, expressed in kilocalories. % $C$: Carbohydrate content; 4: Energy provided by 1 gram of carbohydrates, expressed in kilocalories

### *2.10 Determination of Mineral Elements*

Mineral contents were determined according to the method described by AOAC (1990). The crude ash, obtained in

accordance with the procedure for crude ash determination, was dissolved in an acidic medium consisting of 5 ml of nitric acid and distilled water. The prepared solution was boiled for 15 minutes on a hot plate at approximately 95 °C. After cooling, it was filtered using filter paper. The resulting filtrate was then diluted to a final volume of 100 ml with distilled water. Mineral elements were quantified by atomic absorption spectrophotometry, except for phosphorus, which was determined by colorimetry.

• *Determination of Iron, Calcium, Copper, Manganese, Zinc, Potassium, Sodium, and Magnesium*

- *Principle of Atomic Absorption Spectrophotometry*

Atomic absorption spectrophotometry is a widely used analytical technique for the detection and quantification of metallic elements at trace levels. It is based on the absorption of electromagnetic radiation by free atoms in a sample. In general, the spectrometer emits a light beam that passes through the solution to be analyzed and records the absorption spectrum at wavelengths specific to each element. These wavelengths are characteristic of the electronic transitions of the atoms, and the intensity of absorption is proportional to their concentration. The sample was heated at high temperature in a flame, causing its dissociation into atoms and ions, which absorb or emit radiation in the visible or ultraviolet range.

- *Sample Calibration*

Calibration of the instrument is a crucial step to ensure the accuracy and reliability of measurements. For each element analyzed (Na, Fe, K, Mg, Ca, Cu, Zn, Mn), standard solutions of known concentrations were prepared. These solutions were introduced into the atomic absorption spectrophotometer to establish a calibration curve. The concentrations of the analyzed samples must fall within the range defined by this curve to guarantee correct and reproducible readings.

- *Reagents Used*

The reagents required for the analysis included: Nitric acid ($HNO_3$), used for dissolving the ash; Lanthanum, employed as a buffer agent for the determination of calcium and magnesium; and Cesium, used as a buffer agent for the determination of sodium and potassium.

- *Operating Procedure*

The analysis began with the activation of the atomic absorption spectrophotometer, with air and gas flow adjusted to generate a stable flame. A hollow cathode lamp specific to each element was then activated to emit the corresponding resonance line. The prepared solutions were introduced into the instrument, where the suspended minerals were nebulized into a fine mist and subsequently atomized in the flame. The atoms formed absorbed the emitted radiation, and the measured optical density was used to determine their concentration. The values displayed on the instrument screen were recorded for the calculation of mineral element contents.

• *Determination of Phosphorus by Colorimetry (UV/VIS Spectrophotometry)*

Ultraviolet-visible absorption spectrophotometry is a widely used analytical technique for the quantification of mineral and organic substances. It is based on the measurement of the absorbance of a solution, i.e., the amount of light absorbed by molecules after reaction with a coloring reagent. The decrease in transparency of the solution is proportional to the concentration of the constituent analyzed, in accordance with the Beer–Lambert law.

In the case of phosphorus, the determination is based on a colorimetric reaction using the vanadium molybdate reagent (Varado Molybdic), which produces a yellow coloration. The intensity of this coloration, measured with a UV/VIS spectrophotometer, is directly proportional to the phosphorus content of the sample. Chemical substances absorb a beam of radiation emitted by a light source, and a photoelectric cell measures the intensity of the transmitted light beam (absorbance or optical density) according to the Beer–Lambert law, illustrated by the following formula:

$$OD = \sigma \times L \times C \qquad (12)$$

Where $OD$ is the optical density or absorbance, $\sigma$ is the molar extinction coefficient of a given solute, $L$ is the path length of the solution (in cm), and $C$ is the concentration of the solute or element to be determined.

- *Operating Procedure*

A series of phosphorus standards, with quantities not exceeding 40 µg, was prepared from a stock solution. For each analysis, 5 ml of the sample and 5 ml of the coloring reagent were taken, mixed, and shaken. The mixture was then left to stand for 10 minutes to allow complete color development. Optical density measurements were performed at 430 nm using a UV/VIS spectrophotometer. A calibration curve was constructed from the standard solutions, enabling accurate estimation of the phosphorus concentration in the analyzed samples. The values obtained were used for the final calculation.

- *Calculation Method*

The phosphorus content, as well as that of other mineral elements such as iron, calcium, copper, manganese, zinc, potassium, sodium, and magnesium, was determined from the ash derived from plant material. Concentrations were calculated based on the measured absorbances and the corresponding calibration curves. The formula used was as follows:

$$\%ME = \frac{C \times 10^{-3} \times di \times V}{m_{t.p}} \times 100 \qquad (13)$$

With % $ME$ is the mineral element content; $C$ is the Concentration of the solution in mg/g (value displayed on the spectrophotometer screen); $10^{-3}$ is the conversion factor*;* $di$ is the Dilution factor; $V$ is the Final volume of the sample in milliliters and $m_{t.p}$is the Mass of the test portion in grams.

*2.11 Phytochemical Screening*

To identify the secondary metabolites, present in the hydroalcoholic extract (80%) of *Givotia stipularis* seed powder, phytochemical screening was carried out according to the method described by Fong *et al*. (1977). This approach relies on the use of specific reagents that induce the formation of complexes, either insoluble (precipitates) or soluble, characterized by distinctive coloration.

Thus, 20 g of *Givotia stipularis* seed powder were mixed with 133 ml of hydroalcoholic solution, and the mixture was macerated at 70 °C for 1 h, with agitation every 15 minutes. The resulting macerate was filtered using cotton, yielding 112 ml. For each test, the ratio of 20 g of powder producing 112 ml of alcoholic extract was used.

- *Alkaloid Test*:

In a crystallizer, the equivalent of 2.5 g of *Givotia stipularis* seed powder was evaporated on a water bath. The residue obtained was dissolved in 15 ml of 5% hydrochloric acid and agitated again on the water bath for 3–5 minutes. The solution was filtered with cotton and equally distributed into four test tubes labeled T1, T2, T3, and T4 (control). Five drops of Wagner's, Mayer's, and Dragendorff's reagents were added respectively to tubes T1, T2, and T3. The appearance of turbidity, flocculation, or precipitation indicated the presence of alkaloids.

- *Flavonoid and Leucoanthocyanin Screening*

In a crystallizer, the equivalent of 3 g of powder was evaporated on a water bath and cooled to room temperature. Depigmentation was then performed by triturating the residue with 15 ml of n-hexane, followed by filtration; this operation was repeated until complete removal of pigments. The depigmented residue was dissolved in 30 ml of 80% ethanol, filtered with cotton, and equally distributed into five test tubes labeled T1–T5, with T5 serving as control.

For the Wilstater (cyanidin) test, the filtrate in T1 was treated with 0.5 ml of concentrated hydrochloric acid and four magnesium turnings, and the color change was observed after 10 minutes. In T2, the same procedure was carried out with the addition of 1 ml ethanol and 1 ml isoamyl alcohol, and the coloration of the upper phase was noted.

The Bate-Smith test was performed in T3 by adding 0.5 ml concentrated HCl, followed by heating in a water bath for 30 minutes and cooling, with observation of the color change.

For anthocyanin screening, the filtrate in T4 was simply treated with 0.5 ml concentrated HCl and left to stand, with any color modification recorded.

- *Steroid and Triterpene Test*

In a crystallizer, the equivalent of 10 g of plant powder was evaporated on a water bath. The residue obtained was depigmented with petroleum ether, then treated with 10 ml chloroform ($CHCl_3$). The mixture was agitated for 5–10 minutes, dried with anhydrous sodium sulfate ($Na_2SO_4$), filtered, and equally distributed into three test tubes labeled T1, T2, and T3 (control).

For the Libermann–Burchard test (triterpene detection), three drops of acetic anhydride were added to tube T1, followed by gentle agitation. One drop of concentrated sulfuric acid ($H_2SO_4$) was then incorporated, and the mixture was left to stand for one hour. A color change indicated a positive test.

For the Salkowski test (unsaturated sterol detection), tube T2 was inclined at 45°, and 2 ml concentrated $H_2SO_4$ were added. The mixture was gently agitated, and any color modification was observed as evidence of sterols.

- *Tannin and Polyphenol Test*

In a crystallizer, the equivalent of 10 g of plant powder was evaporated on a water bath. The residue obtained was dissolved in 25 ml of hot distilled water and carefully agitated. After filtration, the filtrate was equally distributed into four test tubes labeled T1–T4, each previously treated with 4 drops of 10% NaCl solution. In the first three tubes, 5 drops of 1% gelatin (T1), 5 drops of salted gelatin (T2), and 5 drops of ferric chloride ($FeCl_3$) in methanolic solution (T3) were respectively added, while T4 was kept as control. At the end of the manipulations, the appearance of precipitates and their coloration were carefully observed and recorded.

### *2.12 Evaluation of Antioxidant Activity*

The antioxidant activity of the ethanolic extract of *Givotia stipularis* seed powder was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, as described by Yamaguchi et al. (1998). This method is based on the ability of antiradical compounds to reduce the free radical DPPH ($C_{18}H_{12}N_5O_6$), a stable violet-colored radical with a maximum absorbance at 517 nm. In the presence of antioxidants, DPPH captures a hydrogen atom, is converted into 2,2-diphenylhydrazine ($DPPH_2$), and its color gradually shifts to yellow.

For the assay, 1 ml of each extract was mixed with 1 ml of DPPH solution (0.1 mM in ethanol). After homogenization using a vortex, the mixtures were incubated at room temperature (25 °C), protected from light, for 30 minutes. Absorbance was then measured at 517 nm, compared to a control consisting of 1 ml of DPPH solution and 1 ml of ethanol. The absorbance values obtained were used to calculate the percentage of DPPH radical inhibition, which is directly proportional to the antiradical power of the sample.

The percentage inhibition of free radicals (% IP) was determined according to equation (14):

$$\%\,IP = \left[1 - \left(At_{30} / At_0\right)\right] \times 100 \quad (14)$$

Where %*IP* is the inhibition Percentage; $At_0$ is the absorbance of the control (containing no antioxidant) after 30 minutes; and $At_{30}$ is the absorbance of the extracts after 30 minutes.

The $IC_{50}$ value (50% inhibitory concentration) corresponds to the concentration of the tested sample required to reduce DPPH radical activity by 50%, determined graphically by interpolation from the dose-response curve (Samarth *et al.*, 2008).

### *2.13 Acute Toxicity Test*

The primary objective of the acute toxicity test is to determine whether the plant extract under study can cause cessation of vital functions in mice following the administration of a high dose. In the context of our study, we sought to verify whether the seeds of *Givotia stipularis* are suitable for consumption, which justified the implementation of a toxicity test.

According to the toxicological classification proposed by the United Nations (UN) and the Organisation for Economic Co-operation and Development (OECD) in 2013, a pharmacodynamic substance is divided into five categories based on its oral $LD_{50}$: Category 1 ($LD_{50} \leq 5$ mg/kg) corresponds to extremely toxic substances; Category 2 ($LD_{50} >$

5 – ≤ 50 mg/kg) to highly toxic substances; Category 3 ($LD_{50}$ > 50 – ≤ 300 mg/kg) to toxic substances; Category 4 ($LD_{50}$ > 300 – ≤ 2000 mg/kg) to slightly toxic substances; and Category 5 ($LD_{50}$ > 2000 – ≤ 5000 mg/kg) includes weakly toxic or tolerable substances, often considered non-hazardous in the case of single exposure. The dose of 5000 mg/kg constitutes the upper threshold in acute toxicity tests, and if no toxic effects or mortality are observed at this dose, the substance is generally considered non-toxic or very slightly toxic according to the official UN document on GHS (Globally Harmonized System of Classification and Labelling of Chemicals).

For this experiment, we followed the method described in the OECD Guidelines for Testing of Chemicals on acute toxicity (OECD 423, Acute Oral Toxicity – Acute Toxic Class Method). Albino mice with an average weight of 25 g were used and divided into four groups: three groups received different doses of *Givotia stipularis* seed powder extract, while the fourth group, serving as control, was treated with distilled water. The animals were fasted for 24 hours prior to administration, which was performed orally (gavage). Clinical signs were observed during the 24 hours following administration. Three doses were tested: 400 mg/kg, 800 mg/kg, and 5000 mg/kg, the latter being administered only to groups three and four.

## III. Results and Discussion

Nutritional analyses of *Givotia stipularis* seed powder reveal a remarkable composition, highlighting its potential as both a nutritional and medicinal source. Table 1 presents the main parameters evaluated.

TABLE I: Nutritional Information of *Givotia stipularis* Seed Powder

| Parameters (Unit) | Nutritional Value per 100 g of Sample |
|---|---|
| Moisture (g) | 3.61 |
| Ash (g) | 3 |
| Lipids (g) | 66.26 |
| Protein (g) | 17.50 |
| Carbohydrates (g) | 9.63 |
| Metabolizable Energy Value (Kcal) | 704.86 |
| Calcium (mg) | 142.18 |
| Magnesium (mg) | 244.80 |
| Iron (mg) | 2.88 |
| Zinc (mg) | 1.25 |
| Manganese (mg) | 1.11 |
| Copper (mg) | 1.19 |
| Phosphorus (mg) | 478.49 |
| Sodium (mg) | 21.93 |
| Potassium (mg) | 547.89 |
| Ca/P ratio | 0.30 |
| Na/K ratio | 0.05 |
| PRAL index (mEq/100 g) | 6.56 |

According to the results presented in the table, the moisture content of the seed powder is low (3.61 g/100 g), which favors preservation and limits the risk of microbial growth. Similar values have been reported for *Sesamum indicum* seeds (3–6%) (Akinoso *et al*., 2010). Consequently, the dry matter content is high (96.39 g/100 g), confirming the nutritional density of the product.

The crude ash content (3.00 g/100 g) reflects a richness in mineral elements. This value is comparable to that observed in sesame seeds (3.5–4.5%) (FAO, 2017), suggesting a noteworthy contribution of micronutrients.

Furthermore, the lipid content is particularly high (66.26 g/100 g), conferring a substantial metabolizable energy value of 704.86 kcal/100 g. This lipid richness indicates that the seed could serve as an interesting source of edible or industrial oil. However, such a high lipid level may limit its direct use in human diets, as it increases energy density and may contribute to excessive caloric intake if consumed in large quantities. This value is comparable to that of *Sesamum indicum* seeds (57–65%) cultivated in West Africa (Koné *et al*., 2021), but exceeds that of *Jatropha curcas* (≈ 47%) (Kpoviessi *et al*., 2004). This positions *Givotia stipularis* among the oilseeds richest in lipids, making it a potential candidate for vegetable oil production.

In addition, the protein content (17.50 g/100 g) is significant and enhances the nutritional value of the seed, particularly in plant-based diets. However, this value remains lower than that of soybean (35–40%) (Friedman & Brandon, 2001). This indicates that the seed can contribute to protein intake but cannot be considered a primary protein source. Its protein level is closer to that observed in nuts and almonds (15–20%) (USDA, 2020), making it valuable as a complementary component in a diversified diet.

Regarding carbohydrates, the low content (9.63 g/100 g) is typical of oilseeds, confirming that energy is derived mainly from lipids. Nutritionally, this characteristic is favorable for low-carbohydrate diets but limits the intake of fiber and complex sugars. Similar values have been reported for sunflower seeds (≈ 10%) (FAO, 2017).

The seed also exhibits a high calcium content (142.18 mg/100 g), higher than that of peanuts (≈ 50 mg/100 g) (USDA, 2020), but the Ca/P ratio is low (0.30). Such an imbalance may reduce calcium absorption efficiency and compromise bone health (Bonjour, 2011). Magnesium is also abundant (244.80 mg/100 g), comparable to sesame (≈ 350 mg/100 g) (FAO, 2017). Phosphorus is very high (478.49 mg/100 g), influencing the Ca/P ratio. Potassium (547.89 mg/100 g) is much higher than sodium (21.93 mg/100 g), resulting in a Na/K ratio (0.05) well below 1, which is considered protective against hypertension (Chia *et al*., 2024). Thus, despite an unfavorable Ca/P ratio, the seed presents an overall mineral profile beneficial for cardiovascular and metabolic health.

Additionally, the Ca/P ratio of 0.30 is lower than the ideal ratio (>1) recommended for optimal calcium absorption (Bonjour, 2011). In contrast, the Na/K ratio of 0.05, well below 1, is considered protective against hypertension (Chia *et al*., 2024).

Finally, the PRAL index is positive (6.56 mEq/100 g), indicating a moderate acid load. This suggests that regular consumption of these seeds could contribute to body acidification, particularly if not balanced by alkalinizing foods such as fruits and vegetables. This result is consistent with the observations of Remer & Manz (1995), who showed that foods rich in protein and phosphorus tend to increase renal

acid load. From a nutritional perspective, this implies that the seed should be integrated into a balanced diet to avoid acid–base imbalance.

These findings suggest that *Givotia stipularis* could be valued not only for its traditional uses but also as an alternative source of nutrients in regions where it is available. Following the nutritional results that highlighted the mineral and protein richness of *Givotia stipularis* seeds, phytochemical analysis provides further insight into their functional and therapeutic potential. Phytochemical screening of the seed powder revealed a strong presence of leucoanthocyanins (+++), unsaturated sterols (+++), triterpenes (+++), and alkaloids (+++), while flavonoids, tannins, polyphenols, and steroids were absent or not detected (Table 2).

TABLE II: Phytochemical Results of *Givotia stipularis* Seed Powder

| Familles Chimiques | Résultats |
|---|---|
| Leucoanthocyanins | +++ |
| Flavonoids | - |
| Tannins | - |
| Polyphenols | - |
| Steroids | - |
| Unsaturated Sterols | +++ |
| Triterpenes | +++ |
| Alkaloids | +++ |

The abundant presence of leucoanthocyanins, precursors of anthocyanins, suggests an interesting antioxidant potential (Sadowska-Bartosz & Bartosz, 2024; Farias *et al*., 2023), despite the absence of conventional polyphenols. Triterpenes and unsaturated sterols are well known for their anti-inflammatory, hypocholesterolemic, and anticancer effects (Mantiniotou *et al*., 2025; Sut & Dall'Acqua, 2023; Jeong & Bae, 2024), which confer added value to *Givotia stipularis* in the prevention of metabolic diseases. Alkaloids, also abundantly represented, are associated with antimicrobial, analgesic, and immunostimulatory properties (Casciaro *et al*., 2020; Thawabteh *et al*., 2024; Cushnie *et al*., 2014).

In comparison, the seeds of *Lagenaria siceraria*, traditionally used in pharmacopoeia in Niger, exhibit a different phytochemical composition: they contain flavonoids, tannins, saponosides, and heterosides, but lack leucoanthocyanins, catechols, and cyanidins depending on the harvesting region (Sabiou *et al*., 2019). This distinction highlights the specificity of *Givotia stipularis*, particularly its richness in leucoanthocyanins and triterpenes, which are rarely observed in conventional edible seeds.

Thus, *Givotia stipularis* stands out not only for its nutritional density but also for its unique phytochemical profile, which could justify its use in the formulation of dietary supplements with antioxidant and immunomodulatory purposes. This phytochemical richness suggests a potential biological activity, particularly antioxidant, that warrants verification through appropriate experimental studies.

To evaluate the antioxidant activity of our extracts, we employed the DPPH free radical scavenging assay, following the protocol adapted from Yamaguchi *et al*. (1998). This test measures the ability of extracts to reduce free radicals. The DPPH radical, characterized by an intense purple color, turns pale yellow when neutralized by antioxidant substances. This color change, as well as the intensity of the initial hue, depends on the nature, concentration, and potency of the radical-scavenging compound (Ebrahimzadeh *et al*., 2010). DPPH contains a hydrogenated free radical, which gives it a characteristic absorption at 517 nm. In the presence of radical scavengers, the purple color of the solution rapidly diminishes, reflecting the reduction of DPPH to a non-radical form by hydrogen-donating antioxidants (AH).

Absorbance measurements were performed by spectrophotometry at 517 nm. Based on the values obtained, inhibition percentages were calculated, and the curves shown in Figure 2 were plotted, illustrating the variation in inhibition percentage as a function of extract concentration.

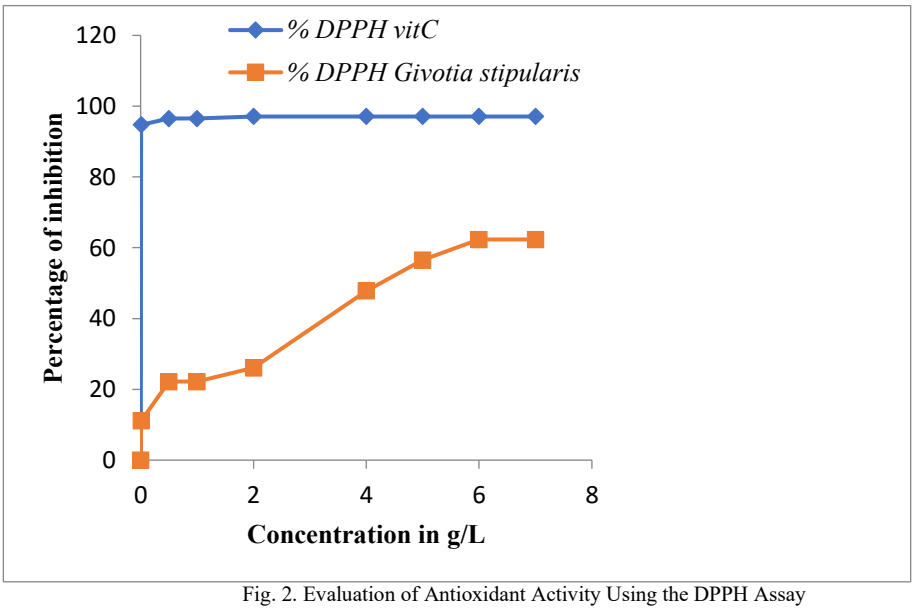


Fig. 2. Evaluation of Antioxidant Activity Using the DPPH Assay

The obtained graph shows a progressive inhibition of the DPPH radical as a function of concentration, reaching approximately 70% at the highest concentration, while vitamin C, used as a positive control, exhibited nearly complete inhibition (≈100%) at all tested concentrations (Fig. 2).

This moderate but significant antioxidant activity suggests the presence of bioactive compounds capable of scavenging free radicals, such as leucoanthocyanins, triterpenes, unsaturated sterols, and alkaloids, identified in the phytochemical analysis. Although conventional flavonoids and polyphenols are absent, leucoanthocyanins may exert a comparable antioxidant role, as suggested by Ouahiba *et al*. (2020) in their study on *Myrtus communis* and *Rhamnus alaternus*, where extracts rich in polyphenols and flavonoids demonstrated DPPH inhibition above 85%.

In comparison, Hassi and Azzi (2023) evaluated the antioxidant activity of *Ocimum basilicum* seeds and observed a maximum inhibition of 78% at 1 g/L, attributed to their high flavonoid and tannin content. Although *Givotia stipularis* does not contain these compounds, its antioxidant efficacy remains competitive, likely due to the synergistic action of leucoanthocyanins and triterpenes.

These findings confirm that *Givotia stipularis* possesses noteworthy antioxidant potential, albeit slightly lower than extracts rich in flavonoids. Its unique profile could be exploited in natural formulations targeting oxidative stress, particularly as a complement to stronger sources such as vitamin C or *Myrtus communis* extracts.

The evaluation of acute toxicity of *Givotia stipularis* seeds was conducted by oral administration of high doses to mice. Behavioral observations revealed initial agitation (scratching, hyperactivity), followed by a phase of rest and transient fatigue, before returning to normal within two hours. After 24 hours, no severe clinical signs or mortality were observed, and the animals displayed behavior comparable to the control group. These results suggest that *Givotia stipularis* seeds do not exhibit apparent acute toxicity at the tested dose, in accordance with the safety criteria defined by the United Nations Globally Harmonized System (GHS), which considers a substance administered at a single dose of 5000 mg/kg without lethal effect as slightly toxic or non-toxic (United Nations, 2013).

This tolerance is comparable to that observed for aqueous extracts of *Vernonia colorata* and *Crescentia cujete*, two plants commonly used in Ivorian pharmacopoeia. Studies have shown that doses up to 6000 mg/kg for *Vernonia colorata* and 7500 mg/kg for *Crescentia cujete* did not induce mortality or significant behavioral alterations in mice, confirming their safety profile in acute toxicity (Stéphane, 2023). Conversely, certain seeds such as *Ricinus communis* (castor bean) or *Abrus precatorius* (jequirity) are recognized for their extreme toxicity, with $LD_{50}$ values below 20 mg/kg, capable of causing death after ingestion of a single seed (ANSES, 2021).

Thus, the results obtained for *Givotia stipularis* reinforce the hypothesis of a favorable toxicological profile, supporting its potential for valorization in phytotherapy or nutritional formulations. Nevertheless, further investigations addressing subacute, chronic, genotoxic, and histopathological effects are essential to confirm its long-term safety.

## IV. Conclusion

The seeds of *Givotia stipularis* exhibit a remarkable nutritional profile, characterized by high energy density, a significant protein content, and a richness in essential minerals such as magnesium, phosphorus, and potassium. These findings highlight their potential as a complementary food resource, while also emphasizing the importance of balanced consumption due to the unfavorable Ca/P ratio and moderate acid load.

From a phytochemical perspective, the species is distinguished by the abundant presence of leucoanthocyanins, triterpenes, unsaturated sterols, and alkaloids, which confer functional and therapeutic potential to the seeds. The antioxidant activity assessed by the DPPH assay, although moderate compared to vitamin C, demonstrates a significant radical-scavenging capacity, suggesting a synergistic effect among the identified compounds and reinforcing the relevance of this resource in combating oxidative stress.

Finally, the acute toxicity evaluation at a single dose of 5000 mg/kg revealed neither mortality nor severe clinical effects, confirming a safety profile consistent with the criteria of the Globally Harmonized System. These results strengthen the interest in *Givotia stipularis* as a nutritional and nutraceutical resource, highlighting its immediate safety and valorization potential. They also pave the way for future studies aimed at deepening the understanding of its bioactive constituents and exploring their bioavailability, with the goal of optimizing its use in innovative nutritional and phytotherapeutic formulations.

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*Conflicts of Interest*

The authors declare that they have no conflicts of interest related to this article.

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