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PHYSICOCHEMICAL PROPERTIES, FATTY ACID COMPOSITION AND ACUTE ORAL TOXICITY OF COLD-PRESSED SEED OIL FROM *GIVOTIA STIPULARIS* RADCL.-SM., AN ENDEMIC SPECIES OF MADAGASCAR

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ABSTRACT

Givotia stipularis Radcl.-Sm. is an endemic Malagasy plant whose seeds constitute a potential source of vegetable oil. The present study aimed to characterize the physicochemical properties and fatty acid composition of cold-pressed seed oil and to perform a preliminary assessment of its acute oral toxicity.

Oil was extracted by mechanical cold pressing and characterized through physicochemical analyses, mineral determination, thin-layer chromatography (TLC) and gas chromatography (GC). Acute oral toxicity was evaluated in albino mice receiving single doses of 400, 800 and 1500 mg/kg body weight.

The extraction yield reached 18.42%. The oil exhibited a density of 0.7709 g/cm^3, iodine value of 132.17 g I_2/100 g, saponification value of 173.54 mg KOH/g, refractive index of 1.478 and peroxide value of 8.52 meq O_2/kg. Gas chromatographic analysis revealed that linoleic acid (36.25%), linolenic acid (27.65%) and oleic acid (24.31%) were the major fatty acids identified, representing approximately 88.21% of the total fatty acid composition. Mineral analysis showed predominance of potassium, phosphorus and magnesium. Thin-layer chromatography suggested the presence of fatty acids, sterols, tocopherols and carotenoid-like compounds. No mortality was observed in mice during the 24-hour observation period following administration of the tested doses.

These results demonstrate that *G. stipularis* seed oil is characterized by a high proportion of unsaturated fatty acids and a low acute oral toxicity under the experimental conditions employed. Further investigations on oxidative stability,

long-term safety and biological activities are required before considering nutritional, pharmaceutical or cosmetic applications.

Keywords: *Givotia Stipularis*; Seed Oil; Fatty Acids; Physicochemical Properties; Gas Chromatography; Acute Toxicity; Madagascar.

1 INTRODUCTION

Vegetable oils constitute an essential component of human nutrition and are widely utilized in the food, pharmaceutical, cosmetic, and industrial sectors. Their nutritional quality and functional properties are primarily determined by their physicochemical characteristics and fatty acid composition. In particular, oils rich in unsaturated fatty acids have attracted considerable attention because of their beneficial effects on cardiovascular health, lipid metabolism, and overall nutritional value [1,2].

In addition to their nutritional importance, plant-derived oils represent valuable renewable resources for the development of functional foods, nutraceuticals, and health-related products. Consequently, the characterization of underutilized oil-bearing plant species has become an important research area, particularly in biodiversity-rich regions where numerous indigenous species remain insufficiently investigated [3,4].

Madagascar is recognized as one of the world's major biodiversity hotspots, characterized by exceptional levels of endemism and unique plant genetic resources [5]. Among the endemic species found on the island, *Givotia stipularis* Radcl.-Sm. (Euphorbiaceae) is a tree species distributed mainly in the northern and northwestern regions of Madagascar. Its seeds are traditionally consumed by local populations, and the extracted oil is occasionally used for culinary purposes [6]. Despite its potential economic and nutritional value, this species has received limited scientific attention compared with other edible oil-bearing plants [6,7].

Recent investigations on *G. stipularis* seeds revealed high lipid (66.26 g/100 g) and protein (17.50 g/100 g) contents, together with appreciable amounts of essential minerals and bioactive phytochemicals, including leucoanthocyanins, triterpenes and unsaturated sterols, highlighting their potential for nutritional and industrial valorization [8]. However, information concerning the oil itself remains scarce. In particular, comprehensive data on its physicochemical quality, fatty acid profile, mineral composition, chromatographic characteristics, and toxicological safety are still lacking. Such information is essential for assessing its suitability for nutritional, pharmaceutical, and commercial applications

Therefore, the present study aimed to characterize cold-pressed seed oil obtained from *Givotia stipularis* through determination of its physicochemical properties, mineral composition, thin-layer chromatographic profile, and fatty acid composition by gas chromatography. Furthermore, an acute oral toxicity assessment was conducted in albino mice to provide preliminary information regarding its safety. The results are expected to contribute to the scientific documentation, sustainable utilization, and potential valorization of this endemic Malagasy species.

2 MATERIALS AND METHODS

2.1 Plant material

Mature fruits of *Givotia stipularis* Radcl.-Sm. were collected from the Ampandrana Forest located in Ambatosia Rural Commune, Bealanana District, Sofia Region, northwestern Madagascar (14°42′48.4″ S; 48°34′52.1″ E) (Figure 1). Botanical identification was performed by a botanist from the National Herbarium of Tsimbazaza, Antananarivo, Madagascar. After harvesting, seeds were separated from the fruits, cleaned and stored under dry conditions until use.

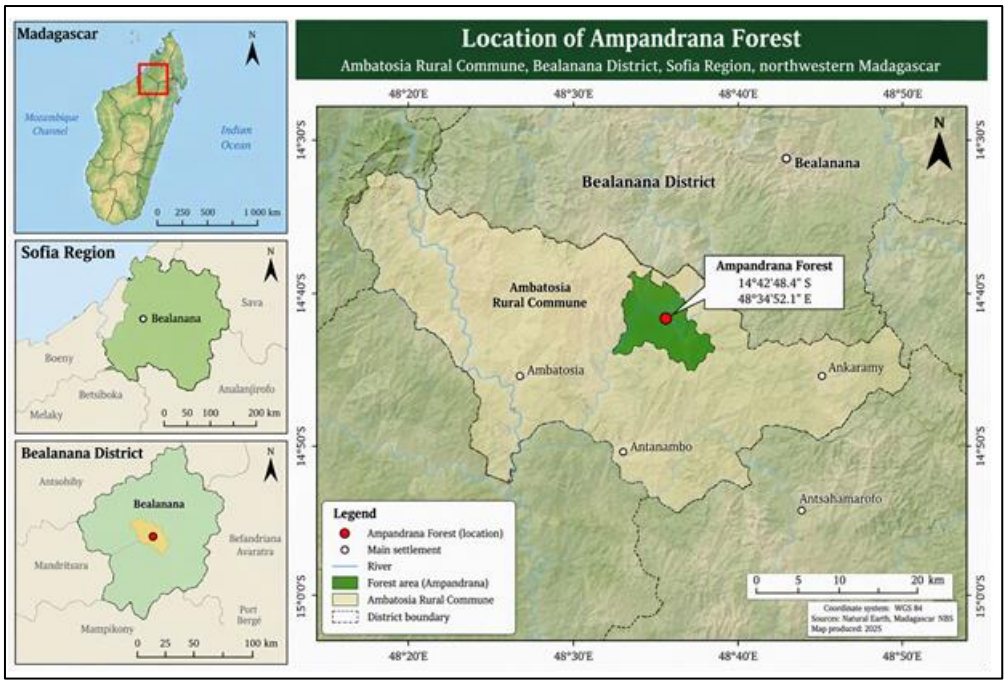


Figure 1: Geographical location of the Ampandrana Forest, northwestern Madagascar

2.2 Oil extraction and organoleptic evaluation

Seed oil was extracted by cold mechanical pressing using a screw press (Figure 2) at a temperature below 27°C. No solvent was used during the extraction process. Extraction yield was calculated as the percentage of oil recovered relative to the initial seed mass.

Organoleptic properties, including colour, odour, appearance and consistency, were assessed immediately after extraction.



Figure 2: Mechanical extraction of *Givotia stipularis* seed oil using a manual press.

2.3 Physicochemical characterization

The physicochemical properties of the oil were determined according to standard AFNOR methods [9]. Relative density, refractive index, acid value, peroxide value, iodine value and saponification value were measured following established analytical procedures for vegetable oils [9].

2.4 Proximate composition and mineral analysis

Moisture, crude ash, crude fat, protein and carbohydrate contents were determined according to AOAC standard methods [9,10]. Metabolizable energy was calculated using the Atwater conversion factors described by Greenfield and Southgate [11].

Mineral elements including calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), potassium (K), sodium (Na) and phosphorus (P) were quantified after mineralization of the samples. Calcium, magnesium, iron, zinc, manganese, copper, sodium and potassium were determined by atomic absorption spectrophotometry, whereas phosphorus was determined by colorimetric spectrophotometry according to AOAC procedures [9].

Nutritional indices including Ca/P ratio, Na/K ratio and potential renal acid load (PRAL) were calculated according to previously described methods [12].

2.5 Thin-layer chromatography (TLC)

Thin-layer chromatography was performed on silica gel plates using a mobile phase consisting of hexane, ethyl acetate and acetic acid (90:7.5:1, v/v/v). After migration, chromatograms were visualized under ultraviolet light (254 nm) and iodine vapour. Retention factor (Rf) values were determined for each spot.

2.6 Gas chromatographic analysis of fatty acids

Fatty acid composition was determined by gas chromatography after conversion of fatty acids into their methyl esters (FAMEs). Oil samples were first saponified and subsequently methylated prior to chromatographic analysis.

The methyl esters were analyzed using an OV-17 capillary column coupled to a flame ionization detector (FID). Fatty acids were identified according to their retention times, and their relative percentages were calculated by area normalization.

2.7 Acute oral toxicity study

Acute oral toxicity was evaluated following OECD Guideline 423 [13]. Twelve healthy albino mice (average body weight: 25 g) were randomly divided into four groups of three animals each.

Three groups received a single oral administration of *G. stipularis* seed oil at doses of 400, 800 and 1500 mg/kg body weight, respectively. The control group received an equivalent volume of edible oil.

Animals were fasted for 24 h before treatment and monitored for behavioural changes, signs of toxicity and mortality during the 24-h observation period following administration

3 RESULTS

3.1 Oil extraction yield and organoleptic characteristics

Cold mechanical pressing of *Givotia stipularis* seeds yielded 18.42% oil (Table 1). The extracted oil was pale yellow, clear and viscous, with a characteristic mild odour.

Table 1: Cold-press extraction yield of *Givotia stipularis* seed oil

Parameter	Result
Initial seed mass (g)	100
Oil extraction yield (%)	18.42

3.2 Physicochemical characteristics

The physicochemical characteristics of *G. stipularis* seed oil are summarized in Table 2. The oil exhibited a density of 0.7709 g/cm^3, an iodine value of 132.17 g I_2/100 g oil, a saponification value of 173.54 mg KOH/g oil, a refractive index of 1.478, an acid value of 0.002 meq H^+/g oil and a peroxide value of 8.52 meq O_2/kg oil.

Table 2: Physicochemical characteristics of *Givotia stipularis* seed oil

Parameter	Result	Unit
Density	0.7709	g/cm^3
Iodine value	132.17	g I_2/100 g
Saponification value	173.54	mg KOH/g
Refractive index	1.478	–
Acid value	0.002	meq H^+/g oil
Peroxide value	8.52	meq O_2/kg oil
pH	8.18	–

3.3 Proximate composition and energy value

The proximate composition of *G. stipularis* seed oil is presented in Table 3. Lipids represented the major component (93.22 g/100 g), followed by carbohydrates (4.84 g/100 g). Moisture, ash and protein were detected in lower amounts. The metabolizable energy value was 858.62 kcal/100 g.

Table 3: Proximate composition and energy value of *Givotia stipularis* seed oil

Parameter	Content per 100 g
Moisture	0.30 g
Crude ash	1.57 g
Lipids	93.22 g
Protein	0.07 g
Total carbohydrates	4.84 g
Metabolizable energy	858.62 kcal

3.4 Mineral composition

Table 4: Reported mineral composition and nutritional indices of *Givotia stipularis* seed oil

Parameter	Result per 100 g
Calcium (Ca)	59.91 mg
Magnesium (Mg)	134.15 mg
Iron (Fe)	4.31 mg
Zinc (Zn)	0.73 mg
Manganese (Mn)	0.61 mg
Copper (Cu)	0.67 mg
Phosphorus (P)	278.13 mg
Sodium (Na)	11.77 mg
Potassium (K)	290.23 mg
Ca/P ratio	0.21
Na/K ratio	0.04
Potential renal acid load (PRAL)	−0.036 mEq/100 g

Potassium (290.23 mg/100 g), phosphorus (278.13 mg/100 g) and magnesium (134.15 mg/100 g) were the predominant minerals detected in the oil. The Na/K ratio and PRAL values were 0.04 and −0.036 mEq/100 g, respectively.

3.5 Thin-layer chromatography

TLC analysis revealed five spots with Rf values ranging from 0.10 to 0.97. The observed spots were tentatively assigned to fatty acids, alcohols, sterol/tocopherol-like compounds, carotenoids and hydrocarbons.

Table 5: TLC spots observed in *Givotia stipularis* seed oil.

Spot	Rf value	Proposed assignment
1	0.10	Free fatty acids
2	0.19	Alcohols
3	0.32	Possible unsaturated sterols or tocopherols
4	0.77	Carotenoids
5	0.97	Possible trace alkanes

3.6 Fatty acid composition

The chromatographic profile is shown in Figure 3.

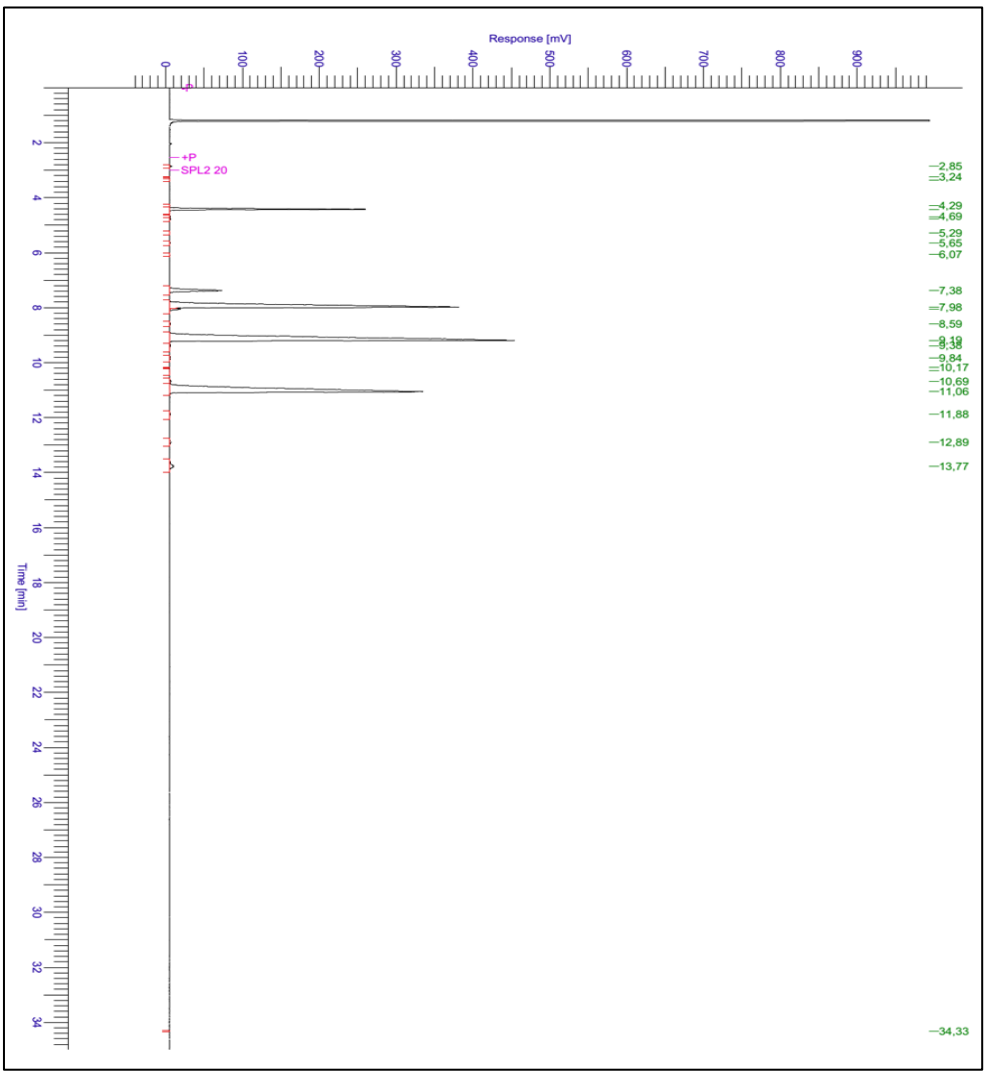


Figure 3: Gas chromatogram of fatty acid methyl esters obtained from *Givotia stipularis* seed oil.

Seven fatty acids were identified (Table 6). Linoleic acid (36.25%), α-linolenic acid (27.65%) and oleic acid (24.31%) were the major components. Unsaturated fatty acids accounted for approximately 88.21% of the total fatty acid composition.

Table 6: Fatty acid composition of *Givotia stipularis* seed oil.

Fatty acid	Symbol	Retention time (min)	Area (%)
Palmitic acid	C16:0	4.421	6.85
Stearic acid	C18:0	7.376	3.29
Oleic acid	C18:1 n-9	7.980	24.31
Trans-oleic acid	C18:1 n-7	8.053	0.44
Linoleic acid	C18:2 n-6	9.189	36.25
α-Linolenic acid	C18:3 n-3	11.055	27.65
Arachidic acid	C20:0	13.771	0.49

3.7 Acute oral toxicity

No mortality was observed in mice treated with 400, 800 or 1500 mg/kg body weight during the 24-h observation period. Transient behavioural changes were observed immediately after administration but disappeared within the observation period.

4 DISCUSSION

4.1 Extraction yield and physicochemical properties

Cold mechanical pressing of *Givotia stipularis* seeds yielded 18.42% oil (Table 1). This result confirms that the species possesses an exploitable lipid fraction and supports its potential as an unconventional oil-bearing resource. The extraction yield obtained in the present study was lower than the total lipid content previously reported for *G. stipularis* seed powder (66.26%), which may be attributed to the lower extraction efficiency of mechanical pressing compared with solvent-based methods [8].

Comparable oil yields have been reported for several underutilized tropical seed species. For example, oil yields ranging from 15 to 25% have been described for some traditionally exploited oilseeds, whereas higher values have been reported for sesame and other commercial oil crops [4,14]. Variations in oil yield may result from genetic factors, environmental conditions, seed maturity and extraction techniques.

The oil obtained was clear, viscous and pale yellow, with a mild characteristic odour. Similar organoleptic characteristics have been reported for many cold-pressed vegetable oils rich in unsaturated fatty acids [1]. The absence of unusual coloration or rancid odour suggests acceptable initial quality of the freshly extracted oil.

4.2 Physicochemical characteristics of the oil

The iodine value reached 132.17 g I_2/100 g oil, indicating a high level of unsaturation. This value was higher than those commonly reported for *Jatropha curcas* oil (92–105 g I_2/100 g oil) and sesame oil (103–117 g I_2/100 g oil) [15,16]. The elevated iodine value is consistent with the predominance of unsaturated fatty acids observed in the chromatographic analysis.

The saponification value was 173.54 mg KOH/g oil. This result is close to values commonly reported for several vegetable oils and suggests the predominance of long-chain fatty acids [15]. Saponification values are frequently used to evaluate potential industrial applications of oils, particularly in soap and cosmetic manufacturing.

The peroxide value was 8.52 meq O_2/kg oil, remaining below the maximum limits generally accepted for crude edible oils. Similar peroxide values have been reported in freshly extracted cold-pressed oils and indicate limited primary lipid oxidation [2]. This observation suggests satisfactory preservation of oil quality during extraction and storage.

The refractive index of 1.478 was within the range generally reported for highly unsaturated vegetable oils [1]. Since refractive index tends to increase with the degree of unsaturation, this result further supports the fatty acid profile obtained by gas chromatography.

Taken together, these physicochemical parameters indicate that *G. stipularis* oil belongs to the group of highly unsaturated vegetable oils and may possess interesting nutritional and technological properties.

4.3 Proximate composition and energy value

The predominance of lipids (>93%) is expected for a vegetable oil and is comparable to values generally reported for edible seed oils, which is consistent with the nature of vegetable oils. Minor amounts of moisture (0.30 g/100 g), crude ash (1.57 g/100 g), protein (0.07 g/100 g), and total carbohydrates (4.84 g/100 g) were also detected. The metabolizable energy value was estimated at 858.62 kcal/100 g.

The high lipid content and corresponding energy value are comparable to those reported for other edible seed oils, which generally contain more than 90% lipids and constitute concentrated energy sources [5,14]. The low moisture content is advantageous for storage stability because it may reduce the risk of hydrolytic deterioration and microbial growth. However, the presence of ash and trace non-lipid constituents suggests that small amounts of suspended materials may remain after extraction.

4.4 Mineral composition

The predominance of potassium (290.23 mg/100 g), phosphorus (278.13 mg/100 g) and magnesium (134.15 mg/100 g) observed in *G. stipularis* oil is consistent with mineral patterns reported for several tropical oilseed species, followed by calcium (59.91 mg/100 g). Lower amounts of iron, sodium, zinc, copper, and manganese were also recorded.

A similar predominance of potassium and phosphorus has been reported in several tropical oilseed species, including *Myrianthus arboreus* and *Sesamum indicum* [5,14]. Potassium is known to play an important role in electrolyte balance and cellular functions, whereas phosphorus contributes to energy metabolism and structural components of biological systems.

The Na/K ratio was 0.04, considerably lower than the value of 1 generally considered acceptable for a balanced mineral profile [17]. Low Na/K ratios have frequently been associated with favourable dietary mineral balance. Likewise, the PRAL value (-0.036 mEq/100 g) suggested a slight alkalizing potential.

Nevertheless, mineral concentrations in edible oils should be interpreted with caution because residual seed particles, suspended matter, extraction conditions, and analytical procedures may influence the measured values. Therefore, these results should be regarded as preliminary indicators of mineral composition rather than definitive nutritional claims.

4.5 Thin-layer chromatographic analysis

The TLC profile suggests the presence of a diverse unsaponifiable fraction including sterol-like compounds, tocopherols and carotenoid pigments.

Based on their migration behavior and comparison with values commonly reported in the literature, the detected compounds were tentatively assigned to free fatty acids, alcohols, sterol- or tocopherol-like compounds, carotenoid pigments, and trace hydrocarbons.

The presence of sterol-like compounds, tocopherols, and carotenoids is of particular interest because these minor constituents are frequently associated with antioxidant activity and oxidative stability in vegetable oils [1,2]. Similar TLC profiles have been reported for several edible plant oils containing complex mixtures of lipidic and unsaponifiable constituents.

However, identification based solely on Rf values should be considered preliminary. Definitive characterization would require more selective analytical techniques such as gas chromatography-mass spectrometry (GC-MS) or high-performance liquid chromatography (HPLC). Nevertheless, the TLC profile provides a useful preliminary chemical fingerprint and indicates that *G. stipularis* seed oil contains a chemically diverse range of lipid-associated compounds.

4.6 Fatty acid composition

The gas chromatogram of fatty acid methyl esters obtained from *Givotia stipularis* seed oil is presented in Figure 3. The chromatographic profile exhibited several well-resolved peaks, indicating effective separation of the fatty acid methyl esters under the analytical conditions employed. The major peaks corresponded to linoleic acid, α-linolenic acid, and oleic acid, whereas palmitic, stearic, and arachidic acids were detected in lower proportions.

The predominance of linoleic acid (36.25%), α-linolenic acid (27.65%) and oleic acid (24.31%) confirms that G. stipularis oil belongs to the group of highly unsaturated vegetable oils.

Linoleic acid (36.25%) was the predominant fatty acid, followed by α-linolenic acid (27.65%) and oleic acid (24.31%). Together, these three unsaturated fatty acids accounted for approximately 88.21% of the total fatty acid composition, indicating a highly unsaturated oil.

The predominance of linoleic acid observed in the present study is comparable to that reported for sunflower oil, where linoleic acid generally ranges from 35% to 75%, depending on the cultivar and growing conditions [18]. Similarly, sesame oil typically contains 35–50% linoleic acid and 30–45% oleic acid [14]. The linoleic acid content of *G. stipularis* oil therefore falls within the range reported for nutritionally valuable edible oils.

A particularly noteworthy feature of *G. stipularis* seed oil is its high α-linolenic acid content (27.65%). This value is substantially higher than those commonly reported for sesame oil, peanut oil, and *Jatropha curcas* oil, which contain only low concentrations of omega-3 fatty acids [14,15]. In contrast, the α-linolenic acid level found in *G. stipularis* oil approaches those reported for flaxseed oil and certain chia-derived oils, which are recognized as important plant sources of omega-3 fatty acids [18].

The oleic acid content (24.31%) was lower than that of olive oil, in which oleic acid commonly exceeds 70%, but was comparable to values reported for several tropical oilseed species [1]. Oleic acid contributes to nutritional quality and improves oxidative stability compared with highly polyunsaturated oils.

The saturated fatty acid fraction was relatively low, comprising palmitic acid (6.85%), stearic acid (3.29%), and arachidic acid (0.49%), corresponding to only 10.63% of total fatty acids. This proportion is markedly lower than that reported for palm oil, where saturated fatty acids may exceed 45% of total fatty acids [18]. The low level of saturated fatty acids observed in *G. stipularis* oil may therefore represent a favourable nutritional characteristic.

The calculated unsaturated-to-saturated fatty acid ratio was approximately 8.4, reflecting the strong predominance of unsaturated fatty acids. Similar ratios have been associated with vegetable oils considered nutritionally desirable because of their balanced contribution of omega-3, omega-6, and omega-9 fatty acids [18].

From a nutritional perspective, the simultaneous presence of substantial amounts of linoleic acid (omega-6), α-linolenic acid (omega-3), and oleic acid (omega-9) distinguishes *G. stipularis* oil from many conventional tropical seed oils. This composition suggests potential value as a source of essential fatty acids. However, the elevated degree of unsaturation, supported by the iodine value of 132.17 g I_2/100 g oil, may increase susceptibility to oxidative deterioration. Additional studies on oxidative stability, storage behaviour, antioxidant composition, and shelf life are therefore warranted.

Overall, the fatty acid profile obtained in the present study demonstrates that *G. stipularis* seed oil possesses an unusual combination of high linoleic acid and α-linolenic acid contents together with a low proportion of saturated fatty acids. These characteristics compare favourably with those of several commercially important vegetable oils and highlight the potential nutritional and technological interest of this underutilized Malagasy oilseed species.

4.7 Acute oral toxicity

The absence of mortality at doses up to 1500 mg/kg body weight suggests a low acute toxicity of *G. stipularis* seed oil under the experimental conditions employed. Although transient behavioural changes such as agitation and reduced locomotor activity were observed shortly after administration, animals recovered rapidly and subsequently exhibited normal behaviour.

These findings suggest low acute toxicity under the experimental conditions employed. Similar observations have been reported for several edible and medicinal plant-derived products evaluated according to OECD Guideline 423 [13].

However, the limited number of animals and the relatively short observation period do not allow accurate determination of the LD50 value. Therefore, additional toxicological studies including subacute, chronic, biochemical and histopathological evaluations are required to establish the long-term safety of the oil.

The absence of mortality at the investigated doses nevertheless provides encouraging preliminary evidence supporting further studies aimed at the valorization of this endemic Malagasy oil resource.

5 CONCLUSION

The present study provides the first detailed characterization of cold-pressed seed oil from *Givotia stipularis* Radcl.-Sm., an endemic Malagasy species. The oil exhibited favourable physicochemical characteristics and was distinguished by a high proportion of unsaturated fatty acids, particularly linoleic acid (36.25%), α-linolenic acid (27.65%) and oleic acid (24.31%), which together accounted for more than 88% of the total fatty acid composition. The mineral profile and TLC analysis further suggested the presence of nutritionally and technologically valuable constituents.

Acute oral toxicity assessment revealed no mortality at doses up to 1500 mg/kg body weight, indicating low acute toxicity under the experimental conditions employed. These findings support the potential valorization of *G. stipularis* seed oil as a novel source of unsaturated fatty acids for nutritional, cosmetic or pharmaceutical applications.

Nevertheless, additional investigations are required to confirm the fatty acid profile using certified standards, evaluate oxidative stability during storage, characterize minor bioactive compounds and assess long-term safety through subacute and chronic toxicity studies.

These findings support the potential valorization of *G. stipularis* seed oil as a novel source of essential fatty acids, particularly omega-3 and omega-6 fatty acids, for nutritional, cosmetic or pharmaceutical applications.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper

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