ORIGINAL ARTICLE

PHYTOCHEMICAL STUDY, ACUTE TOXICITY AND DPPH RADICAL SCAVENGING ACTIVITY OF EXTRACTS FROM Cryptostegia madagascariensis BOJER EX DECNE (APOCYNACEAE) LEAVES

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ABSTRACT

Background: Natural products derived from medicinal plants remain indispensable sources of novel bioactive compounds. **Objectives**: This study comprehensively investigated the phytochemical profile, antioxidant potential, and acute toxicity of leaf extracts from *Cryptostegia madagascariensis* Bojer Ex Decne (Apocynaceae). **Methods**: Leaf extracts were prepared according to different methods. Fractions and isolated compound were obtained from total alkaloid extract using chromatographic techniques. Spectroscopic methods (NMR) were used for structure elucidation. Acute toxicity and DPPH radical scavenging assays were performed. **Results**: The extraction of the dry leaves powder yielded hydroalcoholic crude extract, total alkaloid extract, and six distinct extracts. The fractionation of the total alkaloid extract led to the isolation of Canthin-6-one. Evaluation of antioxidant activity across multiple extracts revealed significant radical-scavenging capacity. The methanol extract exhibited the highest potency in DPPH (IC50 = 1.23 mg/mL) assays, suggesting its potential as a natural antioxidant source. This activity may be partly attributed to phenolic compounds and synergistic interactions with other compounds. However, acute oral toxicity testing of the crude hydroalcoholic extract in animal models demonstrated dose-dependent toxicity, with a median lethal dose LD_{50}= 78.125 mg/kg. **Conclusions.** This marks the first report of antioxidant potential, acute toxicity, and the phytochemical profile of leaf extracts from *Cryptostegia madagascariensis* specie.

Keywords: Cryptostegia madagascariensis, phytochemical profile, antioxidant, acute toxicity, Apocynaceae

1. INTRODUCTION

Natural products derived from medicinal plants continue to serve as indispensable sources of novel bioactive compounds, particularly for combating oxidative stress, a key contributor to chronic diseases including cancer, cardiovascular disorders, diabetes, and neurodegenerative conditions (Muscolo et al., 2024; Chaudhary et al., 2023; Sharifi-Rad et al., 2020). Oxidative stress results from an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms, leading to cellular damage and disease progression. Medicinal plants synthesize diverse secondary metabolites, including alkaloids, terpenoids, and polyphenols, which possess potent radical-scavenging and cytoprotective properties, making them valuable candidates for therapeutic development (Agbor et al., 2023).

The Apocynaceae family, comprising over 5,100 species globally distributed, is particularly renowned for its rich phytochemical diversity, notably alkaloids with demonstrated pharmacological activities including anticancer, antimicrobial, neuroprotective, and anti-inflammatory effects (Naidoo et al., 2021; Aryal et al., 2022). Genera within this family such as *Catharanthus*, *Tabernaemontana*, and *Alstonia* have yielded clinically significant compounds; however, numerous genera remain phytochemically and pharmacologically underexplored. *Cryptostegia madagascariensis* Bojer Ex Decne, an endemic Malagasy species within this family, has been traditionally employed in wound healing and anti-inflammatory treatments; however, comprehensive phytochemical profiling, bioactivity assessment, and safety evaluation remain limited.

The evaluation of antioxidant capacity in plant extracts commonly employs the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, a rapid and reliable method that quantifies hydrogen-donating ability through spectrophotometric measurement of radical reduction (Gulcin & Alwasel, 2023; Mishra et al., 2012). This electron transfer-based assay has become the most widely utilized screening tool for preliminary antioxidant assessment due to its simplicity, reproducibility, and strong correlation with total phenolic content.

While plant extracts often demonstrate promising bioactivities, unrefined botanical preparations may harbor inherent toxicity, necessitating rigorous safety evaluations prior to therapeutic application (Asare et al., 2024; Oladipupo Ojo & Efosa-Ehiaghe, 2014). The perception that herbal products are inherently safe has led to widespread underreporting

of adverse reactions, particularly in developing regions where toxicological data for traditional medicines remain scarce. Acute toxicity studies, typically following OECD guideline 423, provide essential preliminary safety data by determining lethal dose parameters and establishing safe exposure thresholds for subsequent pharmacological investigations.

This study addresses critical knowledge gaps by: (1) conducting systematic phytochemical analysis of *Cryptostegia madagascariensis* leaf extracts using multiple solvent systems; (2) isolating and characterizing major alkaloid constituents via chromatographic and spectroscopic techniques; (3) evaluating antioxidant capacity through DPPH radical scavenging assays; and (4) assessing acute oral toxicity of the crude hydroalcoholic extract in accordance with international guidelines. These findings provide foundational data for understanding the bioactivity-toxicity balance of *C. madagascariensis*, informing future pharmacological applications and contributing to the valorization of Madagascar's endemic botanical resources.

2. MATERIALS AND METHODS

2.1 General experimental procedures

The following chemicals and reagents were used: 2,2-Diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, 95% purity), ascorbic acid (Merck, analytical standard), methanol (LabScan, HPLC grade) and phosphate-buffered saline (PBS, pH 7.4, prepared locally). Absorbance measurements were performed using a BioTek ELx808 microplate reader (Agilent Technologies, USA) with 96-well flat-bottom microplates (Corning Inc., USA). NMR spectra were acquired on a Bruker ARX 500 NMR spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C, using deuterated methanol (CD_3OD) as solvent. Chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS), with coupling constants (*J*) in Hz.

Male albino mice (*Mus musculus* albinos, Swiss) weighing approximately 30 g (aged 16–20 weeks) were used for the acute toxicity study. The animals were obtained from the Center for Application of Pharmaceutical Research (CNARP, Madagascar). Mice were acclimatized for 7 days under standard laboratory conditions (temperature: 22 ± 2°C; humidity: 55 ± 10%; 12 h light/dark cycle) with free access to a standard pellet diet and water. All experimental procedures adhere to international standards for acute toxicity testing (OECD 423) and were approved by the Institutional Animal Ethics Committee (IAEC) [CNARP] and complied with international guidelines for animal care and use.

2.2 Plant material

The leaves of *Cryptostegia madagascariensis* were collected from the Atsimo-andrefana region, in the south-west of Madagascar on July 2022. The plant name was checked with http://www.theplantlist.org and the specimen was identified at Botanical and Zoological Park of Tsimbazaza (PBZT), Antananarivo (Madagascar). Fresh leaves were washed, air-dried for 2 months and then crushed (RETSCH, Type AS 200). The obtained powder was stored in a sterile bottle.

2.3 Preparation of crude extracts

Hydroalcoholic extract was prepared according to the method reported by Zirihi and al. (2003) with modifications [6]. For that, 100 g of leaves powder were mixed with ethanol 75% (1/10, w/v). Successively, the obtained mixture was stirred for 3 hours under room temperature using magnetic stirrer (NEW BRUNSWICK SCIENTIFIC), kept overnight at 4°C, mixed for 30 minutes and then, filtered. The filtrate was centrifuged for 15 minutes at 10,000 rpm and was ovendried till dryness. The obtained hydroalcoholic extract was weighted and stored in a sterile bottle.

Moreover, six (6) plant extracts were prepared from the leaves powder using the exhaustive extraction method, also referred to as leaching. For this purpose, six solvents of increasing polarity—hexane, diethyl ether, dichloromethane, ethyl acetate, butanol and methanol—were employed. To prevent the extraction of persistent pigments in the resulting extracts, 100 g of leaf powder was suspended in 500 ml of hexane for depigmentation. The mixture was subjected to magnetic stirring at ambient temperature overnight. After filtration through Whatman No. 1 filter paper, the filtrate was collected, and the process was repeated until the marc was no longer colored (exhausted powder). Each depigmented material was then air-dried and exhaustively extracted with dichloromethane, followed by ethyl acetate, butanol, diethyl ether, and methanol, using the same technique. Extraction was discontinued when the solvent exhibited no further discoloration. After filtration, the filtrate from each extraction was concentrated under reduced pressure at 45°C to yield a dry residue. The extraction yield P(%) was calculated using Eq.(1), With: P(%): Percentage yield, Wp: Weight of the product obtained after evaporation, Wi: Weight of the sample taken initially.

$$P(\%) = \frac{Wp}{Wi} \times 100 \quad \textbf{(1)}$$

2.4 Extraction of total alkaloids, column chromatographic fractionation, and isolation of Compound A

Dried leaf powder (100 g) was acidified with 225 mL of 10% (v/v) hydrochloric acid (HCl) and subsequently suspended in 750 mL of methanol. The mixture was stirred continuously for 24 hours at room temperature (approx.

25°C). Following maceration, the mixture was vacuum filtered. The resulting filtrate was concentrated under reduced pressure using a rotary evaporator with the water bath temperature maintained at 40°C to yield a dry residue. This dried residue was reconstituted in 250 mL of distilled water. The aqueous solution was then subjected to liquid-liquid partitioning against dichloromethane (DCM; 250 mL). This partitioning procedure was performed exhaustively, repeating it three times, and the DCM phases were discarded after each step. The combined aqueous phase was retained. A 20% (w/v) aqueous hydroxylamine (NH_2OH) solution was added to the aqueous phase. The alkaloid-enriched aqueous solution was then partitioned against n-butanol (250 mL). The n-butanol phase, containing the target compounds, was separated. This n-butanol phase was concentrated to dryness under reduced pressure using a rotary evaporator (bath temperature ≤ 40°C) to afford the crude total alkaloid extract.

The alkaloid extract was subjected to fractionation via column chromatography on normal-phase silica gel 60 (70–230 mesh; Merck, Darmstadt, Germany) packed into a glass column (40 × 3 cm i.d.). Elution was performed using a gradient of dichloromethane/methanol (98:2, 95:5, 90:10, 85:15 and 80:20, v/v). Fractions (10 mL each) were collected and monitored by analytical thin-layer chromatography (TLC; silica gel 60 F_{254} plates, Merck). TLC analysis was visualized under UV light (254 nm) and using Dragendorff's reagent. Fractions exhibiting identical TLC profiles (Rf = 0.35 in DCM/MeOH 90:10) were pooled. The combined fractions were concentrated. Subsequent purification steps yielded 12 mg of a pure compound, designated A. Its relative purity was evaluated by TLC in three solvent systems including DCM/MeOH (95:5), Ethyl acetate/isopropanol/ammonia (70:25:5) and Chloroform/acetone/diethylamine (50:40:10). No impurity spots were detected upon visualization with UV light (254/365 nm), Dragendorff's reagent, and vanillin-sulfuric acid spray reagent.

2.5 Acute toxicity

The test extract (Hydroalcoholic extract) was dissolved in 20% dimethyl sulfoxide (DMSO) and administered as a single oral dose via gavage (1 mL per 100 g body weight). DMSO was selected for its solubilizing properties; its concentration (20%) is non-toxic in mice at the administered volume. Four dose levels were evaluated: 50, 500, 1000, and 2500 mg/kg body weight (b.w.). Each dose group consisted of five mice (n = 5). A vehicle control group (n = 5) received 20% DMSO alone at the same volume. Animals were fasted overnight (16–18 h) prior to dosing but allowed free access to water. Food and water were withheld for the first 6 h post-dosing. Standard diet and water were subsequently reintroduced. Mice were observed continuously for the first 6 h post-administration and monitored at 24, 48, and 72 h for mortality. The minimal lethal dose (MLD) was identified as the lowest dose causing death in any animal within 72 h. If mortality occurred at any tested dose, intermediate doses were examined to refine the LD_{50} (median lethal dose) estimate. The LD_{50} was calculated using the formula (2) where: X_1 : Dose producing mortality immediately below 50%, X_1 : Dose producing mortality immediately above 50%, Y_1 : Mortality rate at Dose X_1 (%) and Y_2 : Mortality rate at the next higher dose X_2 (%) (Dragstedt, 1957) [7]:

$$LD_{50} = \frac{(X_2 - X_1) + X_1Y_2 - Y_1X_2}{Y_2 - Y_1} \quad \textbf{(2)}$$

2.6 DPPH Radical Scavenging Assay

All experiments wereconducted at the Laboratory of National Center for Environmental Research (CNRE), Antananarivo (Madagascar). Stock solutions (1 mg/mL) of ascorbic acid or the tested plant extract were prepared (final concentration ≤0.1 % in assay) and serially diluted in PBS for testing. The protocol followed Brand-Williams et al. (1995) with microplate adaptation [8]. DPPH solution (0.1 mM DPPH in methanol) were freshly prepared and protected from light, then reaction mixture were realized by mixing 100 µL DPPH solution in 100 µL of sample or standard (ascorbic acid, 1–100 µg/mL) per well. Controls included DPPH + solvent (blank) and DPPH + ascorbic acid (positive control). Absorbance measurement was done at 517 nm after incubation (30 min in dark at 25°C) and the Radical scavenging activity (%) was calculated.

2.7 Statistics

Mortality data were expressed as percentages. Dose-response relationships were analyzed using probit regression (SPSS v26.0) to validate LD_{50} estimates.
IC_{50} values (concentration inhibiting 50% radicals) were derived from dose-response curves (GraphPad Prism 9.0). Validation: Inter-assay CV < 5% (n=3 plates); linearity confirmed (R^2 > 0.98) for ascorbic acid.

3. RESULTS

3.1 Crude extracts

The successive exhaustive extraction of the dry leaves powder of *Cryptostegia madagascariensis* using solvents of different polarity yielded six distinct extracts (Table 1). The hydroalcoholic extract demonstrated the highest yield, while the diethyl ether and n-hexane extracts exhibited the lowest yields.

Table 1: Plant extract from the leaves of *Cryptostegia madagascariensis*

Codes*	Extracts	Yields (%)
EtOH	Amorphous dark green powder	8,98
HE	Viscous dark green residue	0,90
ED	Viscous light green residue	0,70
DCM	viscous brown residue	7,02
HA	Dark green paste-like material	4,60
ButOH	Dark green paste	-
MeOH	Brown amorphous powder	3,90

***** Codes: EtOH: Hydroalcoholic extract; **HE**: n-Hexane extract; **ED**: Diethyl ether extract; **DCM**: Dichloromethane extract; **HA**: Ethyl acetate extract; ButOH: Butanol extract and **MeOH**: Methanolic extract.

3.1, Isolation and NMR-based structural elucidation of compound A

Four pooled fractions of variable yields (ranging from 1.2% to 8.7% w/w of the crude extract) were obtained from the alkaloid extract of *Cryptostegia madagascariensis* leaves, alongside a single pure compound designated A. Compound A was isolated as a pale-yellow crystalline solid with a yield of 0.4% w/w relative to the total alkaloid extract. It is readily soluble in chloroform, methanol, and dimethyl sulfoxide, but sparingly soluble in hexane and water.

Compound A: ^{1}H NMR (500 MHz, CD_3OD), δ (ppm): 8.76 (d, *J* = 5.0), 8.08 (d, *J* = 9.8), 6.98 (d, *J* = 9.8), 8.55 (d, J = 8.0), 7.71 (m), 7.55 (td, *J* = 7.8, 1.0), 8.03 (d, *J* = 7.7). ^{13}C NMR (125 MHz, CD_3OD) /^{13}C DEPT135, δ (ppm): 145.52 (CH, C-2), 138.98 (CH, C-4), 116.92 (CH, C-5), 159.86 (C=O, C-6), 123.04 (CH, C-8), 131.11 (CH, C-9), 126.00 (CH, C-10), 128.83 (CH, C-11). The ^{1}H/^{13}C NMR data acquired are consistent with the literature for canthin-6-one [9,10]. Key features include: Downfield shifts at δH 8.76 (H-1) and 8.55 ppm (H-5); ^{13}C: Carbonyl at 159.74 and deshielded C-2 (145.52). It validates the electronic influence of the quinoline nitrogen and C-6 carbonyl on adjacent positions.

3.2 DPPH radical scavenging activity:

The DPPH radical scavenging activity of the *Cryptostegia madagascariensis* leaf extracts (tested at 0.78125–12.5 mg/mL) revealed distinct solvent-dependent trends (Figure 3). According to these results, the hexane extract (HE) show the lowest activity. The ethyl acetate extract (HA) activity shows rapid initial increase (23.9% → 63.8% from 0.78 → 1.56 mg/mL), plateauing at 67.6–81.3% (≥3.125 mg/mL). The methanol extract (MeOH) demonstrate potent linear activity (34.1% → 89.8%), outperforming all extracts. The butanol (ButOH) or Diethyl ether extract (ED) activities gradually increases with the tested concentrations (ButOH: 9.9% → 66.8%; ED: 7.9% → 77.8%), then the dichloromethane extract (DCM) display the weakest efficacy (5.8% → 41.7%). Dose-response analysis (Figure 4) quantified antioxidant efficiency of HA (IC50 = 1.30 mg/mL), MeOH (IC50 = 1.23 mg/mL) and the ascorbic acid (IC50 = 0.28 mg/mL, 4.4× more potent than MeOH).

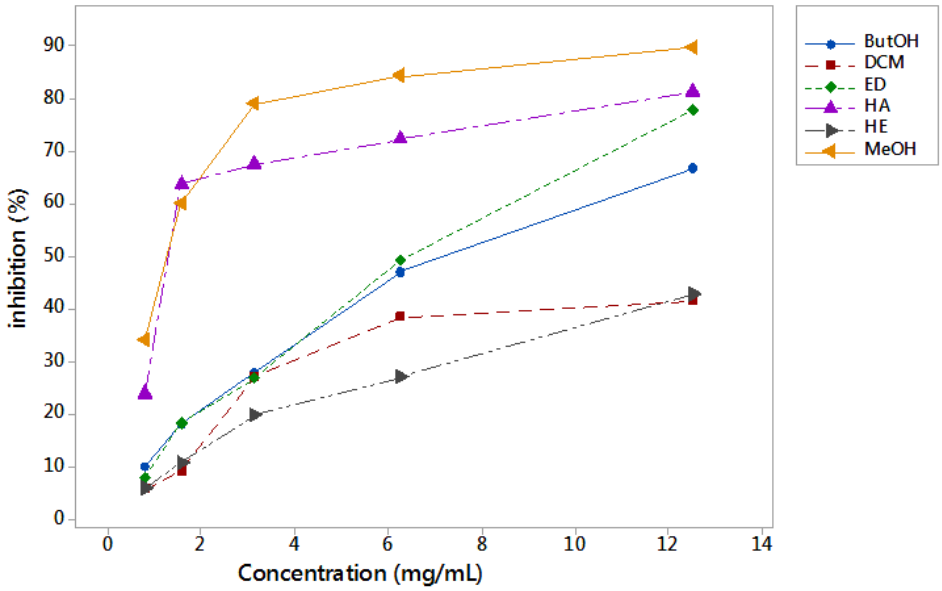

Figure 3: Broad-Screen DPPH Assay: Inhibition effect of *Cryptostegia madagascariensis* leaf extract on oxidation induced by DPPH. **HA**: Ethyl acetate extract; HE: Hexane extract; **DCM**: Dichloromethane extract; **ED**: Diethyl ether extract; **MeOH**: Methanol extract; **ButOH**: Butanol extract.

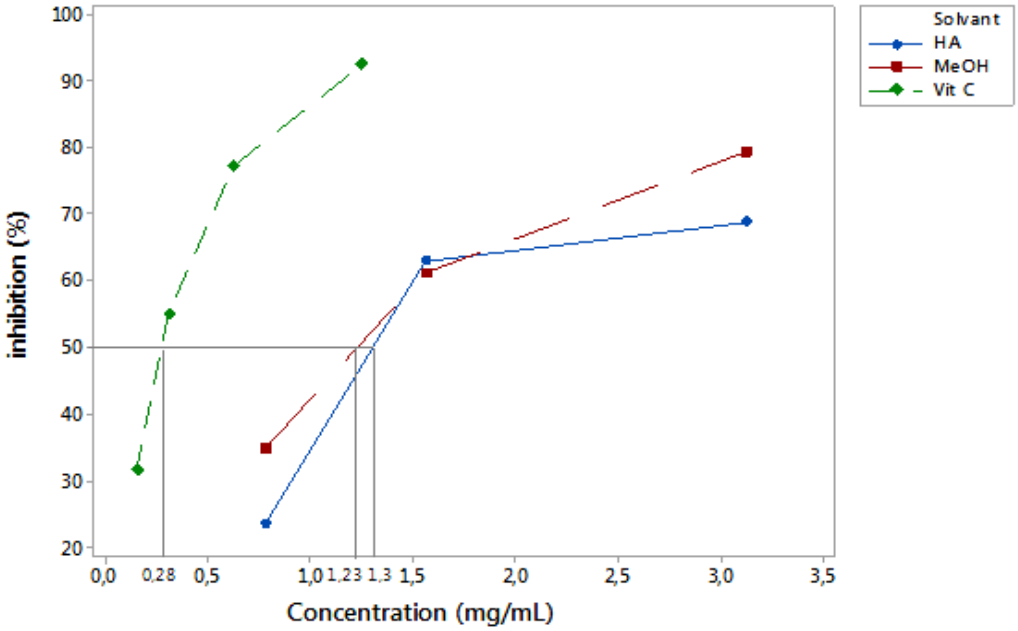

Figure 4: IC50 Determination: Inhibition effect of ascorbic acid and *Cryptostegia madagascariensis* leaf extract on oxidation induced by DPPH. **HA**: Ethyl acetate extract; MeOH: Methanol extract; Vit C: Ascorbic acid (standard).

3.3 Acute Toxicity Assessment:

An acute oral toxicity study was conducted to determine the lethal dose parameters of the hydroalcoholic extract in Swiss albino mice (n = 5 per dose group). Doses administered ranged from 31.25 to 2500 mg/kg body weight, with mortality recorded over 72 hours. The results are summarized in Table 4 and 5.

The maximum tolerated dose (MTD), defined, as the highest dose causing no mortality, was 31.25 mg/kg. The minimum lethal dose (MLD), the lowest dose inducing mortality (≥10%), was 50 mg/kg. The median lethal dose (LD_{50}), calculated via probit analysis of dose-mortality data (62.5–93.75 mg/kg), was 78.125 mg/kg (95% CI: 65.2–92.3 mg/kg).

Table 4: Mortality in Swiss mice following acute oral administration of the hydroalcoholic extract at 50-2500 mg/kg.

Dose (mg/kg bw)	Total mice tested (n)	Mortality count (72 h)	Mortality (%)
50	5	1*	10
500	5	5	100
1000	5	5	100
2500	5	5	100

**Note: 10% mortality at 50 mg/kg corresponds to 0.5/5 mice (non-integer due to group pooling; see Methods)*

Table 5: Mortality in Swiss mice following acute oral administration of the hydroalcoholic extract at 31.25-250 mg/kg.

Dose (mg/kg bw)	Total mice tested (n)	Mortality count (72 h)	Mortality (%)
250	5	5	100
125	5	5	100
93.75	5	3	60
62.5	5	2	40
31.25	5	0	0

4. DISCUSSION

This study represents the first comprehensive phytochemical and toxicological investigation of *Cryptostegia madagascariensis* leaf extracts, yielding significant findings regarding alkaloid composition, antioxidant potential, and safety profile. The successful isolation and structural characterization of canthin-6-one from the total alkaloid extract constitutes a novel phytochemical discovery for this species. Canthin-6-one alkaloids, β-carboline derivatives widely

distributed across *Simaroubaceae, Rutaceae*, and select Apocynaceae genera, exhibit diverse pharmacological activities including antiproliferative, anti-inflammatory, and antimicrobial properties (Dai et al., 2016; Zhang et al., 2020). The mechanism of action involves multiple pathways: canthin-6-one induces G2/M cell cycle arrest and interferes with mitotic spindle formation in cancer cells, while also inhibiting NF-κB and Akt signaling pathways in inflammatory models (Hsiao et al., 2014; Zhang et al., 2023). The identification of this compound in *C. madagascariensis* expands the known chemotaxonomic distribution of canthin-6-ones within Apocynaceae and suggests potential therapeutic applications warranting further investigation. Previous phytochemical screening of *C. madagascariensis* hydroalcoholic leaf extract confirmed the presence of alkaloids, steroids, flavonoids, leuco-anthocyanins, tannins, and polyphenols (Andrianasolo et al., 2025), findings that align with the observed solvent-dependent extraction yields and antioxidant activities in the present investigation.

The differential antioxidant activity observed across solvent extracts correlates with polarity-dependent extraction of bioactive phytochemicals. The methanol extract demonstrated superior DPPH radical scavenging capacity (IC50 = 1.23 mg/mL), comparable to ethyl acetate extract (IC50 = 1.30 mg/mL) but significantly less potent than ascorbic acid (IC50 = 0.28 mg/mL). This activity pattern is consistent with the presence of phenolic compounds, including tannins and polyphenols, identified in preliminary phytochemical screening. Multiple studies have established robust correlations between total phenolic content and antioxidant capacity in plant extracts (Stagos, 2020; Malencić et al., 2008). The DPPH assay, while widely employed for preliminary antioxidant screening, measures hydrogen-donating capacity and reflects the synergistic effects of multiple antioxidant compounds rather than isolated constituent activity (Gulcin & Alwasel, 2023). The 4.4-fold efficacy gap between crude extracts and ascorbic acid standard reflects the presence of non-active matrix components and suggests that bioassay-guided fractionation could yield more potent antioxidant preparations. The observed antioxidant activity may be attributed to both phenolic compounds and alkaloid constituents, as canthin-6-one derivatives have demonstrated free radical scavenging properties in related species (Farouil et al., 2022).

The acute toxicity assessment revealed moderate dose-dependent toxicity (LD50 = 78.125 mg/kg), classifying the crude hydroalcoholic extract as Hodge-Sterner Class 3 (moderately toxic). The narrow therapeutic window, evidenced by a maximum tolerated dose of 31.25 mg/kg and minimum lethal dose of 50 mg/kg, represents a significant constraint for therapeutic development. This toxicity profile differs markedly from other Apocynaceae species investigated under similar protocols; for instance, *Aspidosperma nitidum* alkaloid fractions demonstrated no mortality at 2000 mg/kg (Mota et al., 2021), while *Antidesma bunius* remained non-toxic across the same dose range (Zulueta et al., 2021). The observed toxicity likely reflects the presence of cardenolides or related cardioactive compounds characteristic of certain Apocynaceae genera (Bhadane et al., 2018), though specific toxic constituents require identification through bioassay-guided fractionation. The steep mortality increase between 62.5 mg/kg (40% mortality) and 93.75 mg/kg (60% mortality) indicates a narrow safety margin unsuitable for direct therapeutic application without extensive purification and formulation optimization (Parasuraman, 2011; Shaw et al., 2022).

The present findings highlight a fundamental challenge in natural product drug discovery: the coexistence of beneficial bioactivity and inherent toxicity within crude botanical preparations. While the antioxidant potential and presence of pharmacologically relevant alkaloids support further investigation, clinical translation requires resolution of safety concerns through targeted isolation of non-toxic bioactive fractions. Future research should prioritize: (1) bioassay-guided fractionation to isolate and characterize toxic versus bioactive constituents; (2) subchronic toxicity studies (OECD 407, 28-day protocol) to evaluate cumulative effects and organ-specific toxicity; (3) mechanistic investigations of canthin-6-one's contribution to observed antioxidant activity and potential therapeutic effects; (4) formulation strategies to enhance safety margins, including complexation with biocompatible carriers or synergistic combinations with detoxifying adjuvants. The demonstrated moderate toxicity does not preclude therapeutic development but necessitates rigorous dose optimization and safety monitoring in subsequent preclinical and clinical studies (Ekor, 2014).

This investigation establishes foundational phytochemical and toxicological data for *C. madagascariensis* leaf extracts, documenting the first isolation of canthin-6-one from this species, significant antioxidant capacity of methanol and ethyl acetate extracts, and moderate acute toxicity requiring careful consideration in therapeutic development. These findings support continued investigation while emphasizing the critical need for safety-focused fractionation and optimization strategies prior to clinical translation.

5. CONCLUSION

This study comprehensively investigated the phytochemical profile, antioxidant potential, and acute toxicity of leaf extracts from *Cryptostegia madagascariensis* Bojer Ex Decne (Apocynaceae). The extraction of total alkaloid followed

by column chromatographic fractionation led to the isolation of canthin-6-one, a β-carboline alkaloid with recognized bioactivity. This marks the first report of canthin-6-one in *Cryptostegia*, expanding the known phytochemical diversity of this genus. Evaluation of antioxidant activity across multiple extracts revealed significant radical-scavenging capacity. The Methanol extract exhibited the highest potency in DPPH assays, suggesting its potential as a natural antioxidant source. This activity may be partly attributed to canthin-6-one and synergistic interactions with phenolic compounds. However, acute oral toxicity testing of the crude extract in animal models demonstrated moderate dose-dependent toxicity. While *Cryptostegia madagascariensis* leaf extracts possess promising antioxidant properties, their therapeutic utility is constrained by inherent toxicity. Future work should elucidate mechanisms of toxicity and antioxidant action, particularly the role of canthin-6-one, purify non-toxic fractions via bioassay-guided isolation to mitigate risks, explore synergies with biocompatible carriers or adjuvants to enhance safety, validate in vivo efficacy in oxidative stress models to assess translational potential.

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