Revista Eletrônica ACERVO CIENTÍFICO
ISSN 2595-7899

Chemical composition, antioxidant potential and acute toxicity of aqueous extracts of leaves and bark of *Inga vera* Willd

Composição química, potencial antioxidante e toxicidade aguda de extratos aquosos de folhas e cascas de *Inga vera* Willd

Composición química, potencial antioxidante y toxicidad aguda de extractos acuosos de hojas y corteza de *Inga vera* Willd

Élica Renata Soares da Silva[1], Anahy Arruda Burigato[1], Lavinia Almeida Müller[1], Camila Rogério Damasceno[1], Brenadara Caldas de Oliveira[1], Jacenir Vieira da Silva[1], Sidney Mariano dos Santos[2], Gustavo Roberto Villas Boas[3], Claudia Andrea Lima Cardoso[2], Silvia Aparecida Oesterreich[4].

ABSTRACT

Objective: To characterize the chemical profile and evaluate the antioxidant potential and acute toxicity of aqueous extracts of *Inga vera* obtained from leaves and trunk bark. **Methods:** The phytochemical characterization involved the quantification of phenolics, tannins, and flavonoids. The antioxidant potential was determined using the DPPH assay. Acute toxicity was evaluated in female rats at a dose of 2,000 mg kg^{-1} via oral route, monitoring clinical signs, body weight, and absolute and relative organ weights for 14 days. **Results:** Both extracts exhibited high contents of phenolic compounds and tannins, with a higher flavonoid content found in the leaf extract. The extracts demonstrated relevant antioxidant activity in the DPPH assay. In the acute toxicity assay, there was neither mortality nor evident clinical signs of systemic toxicity, although a reduction in water intake was observed in the group treated with the bark extract. **Conclusion:** The findings of this study demonstrate that under the evaluated experimental conditions, the aqueous extracts of *I. vera* exhibited antioxidant activity and an absence of apparent acute toxicity, providing preliminary evidence of safety for the traditional use of these preparations.

Keywords: Medicinal plants, Phytochemistry, Antioxidants, Acute toxicity.

RESUMO

Objetivo: Caracterizar o perfil químico e avaliar o potencial antioxidante e a toxicidade aguda de extratos aquosos de *Inga vera* obtidos de folhas e cascas do tronco. **Métodos:** A caracterização fitoquímica envolveu a quantificação de fenólicos, taninos e flavonoides. O potencial antioxidante foi determinado pelo ensaio com DPPH. A toxicidade aguda foi avaliada em ratas na dose de 2.000 mg kg^{-1} por via oral, monitorando sinais clínicos, peso corporal e pesos absolutos e relativos dos órgãos por 14 dias. **Resultados:** Ambos os extratos

[1] Faculdades Ciências da Saúde, Universidade Federal da Grande Dourados, Dourados – MS.
[2] Centro de Estudos em Recursos Naturais, Universidade Estadual Mato Grosso do Sul, Dourados – MS.
[3] Grupo de Pesquisa em Desenvolvimento de Produtos Farmacêuticos (P&DProFar), Centro de Ciências Biológicas e da Saúde, Barreiras – BA.
[4] Centro Pedagógico de Três Lagoas, Curso de Medicina, Três Lagoas – MS.

SUBMETIDO EM: 6/2026 | **ACEITO EM: 6/2026** | **PUBLICADO EM: 7/2026**

apresentaram elevado teor de compostos fenólicos e taninos, com maior teor de flavonoides no extrato das folhas. Os extratos demonstraram atividade antioxidante relevante no ensaio DPPH. No ensaio de toxicidade aguda, não houve mortalidade nem sinais clínicos evidentes de toxicidade sistêmica, embora tenha sido observada redução da ingestão hídrica no grupo tratado com o extrato da casca. **Conclusão:** Nas condições experimentais avaliadas, os extratos aquosos de *I. vera* apresentaram atividade antioxidante e ausência de toxicidade aguda aparente, fornecendo evidência preliminar de segurança para o uso tradicional dessas preparações.

Palavras-chave: Plantas medicinais, Fitoquímica, Antioxidantes, Toxicidade aguda.

RESUMEN

Objetivo: Caracterizar el perfil químico y evaluar el potencial antioxidante y la toxicidad aguda de extractos acuosos de *Inga vera* obtenidos de hojas y corteza del tronco. **Métodos:** La caracterización fitoquímica involucró la cuantificación de fenólicos, taninos y flavonoides. El potencial antioxidante se determinó mediante el ensayo con DPPH. La toxicidad aguda se evaluó en ratas hembra a una dosis de 2000 mg kg^{-1} por vía oral, monitoreando se los signos clínicos, el peso corporal y los pesos absolutos y relativos de los órganos durante 14 días. **Resultados:** Ambos extractos presentaron un elevado contenido de compuestos fenólicos y taninos, con un mayor contenido de flavonoides en el extracto de las hojas. Los extractos demostraron una actividad antioxidante relevante en el ensayo DPPH. En el ensayo de toxicidad aguda, no hubo mortalidad ni signos clínicos evidentes de toxicidad sistémica, aunque se observó una reducción en la ingesta hídrica en el grupo tratado con el extracto de la corteza. **Conclusión:** Los hallazgos de este estudio demuestran que bajo las condiciones experimentales evaluadas, los extractos acuosos de *I. vera* presentaron actividad antioxidante y ausencia de toxicidad aguda aparente, proporcionando evidencia preliminar de seguridad para el uso tradicional de estas preparaciones.

Palabras clave: Plantas medicinales, Fitoquímica, Antioxidantes, Toxicidad aguda.

INTRODUCTION

Species of the *Inga* genus belonging to the Fabaceae family are widely distributed across the tropical regions of the Americas, extending from Mexico through much of South America. In Brazil, these species occur across diverse biomes, most notably within the Cerrado (FERNANDES JM, et al., 2022). Several *Inga* species are highly recognized for their traditional medicinal uses and therapeutic potential, alongside their ecological importance in reforestation and riparian recovery initiatives (LIMA NM, et al., 2018). Within this genus, *Inga vera* Willd. stands out prominently. Beyond its valuable environmental role, it is frequently employed in traditional medicine for wound healing, particularly through the consumption of teas prepared as aqueous infusions or decoctions from its trunk bark (RODRIGUES VEG and CARVALHO DA DE, 2001).

Current literature indicates that *I. vera* extracts possess significant biological activities, including gastroprotective, antifungal, and selective cytotoxic effects against tumor cells, as well as larvicidal and bacteriostatic properties (NESELLO LAN, et al., 2017). However, despite these promising findings, some studies report a lack of antimicrobial activity in hydroethanolic leaf extracts against pathogens such as *Staphylococcus aureus* and *Candida* species (GONÇALVES TPR, et al., 2018). This heterogeneity in results highlights the need for investigations that evaluate preparations consistent with traditional consumption. While aqueous infusions and decoctions are culturally rooted practices, their chemical and biological profiles remain under-investigated. Characterizing their phytochemical composition and functional properties alongside toxicity assessments is essential to elucidate the mechanisms underlying its popular use and to provide a scientific basis for pharmacological safety.

Compounding this problem, toxicological data regarding the genus *Inga* remain scarce and are mostly limited to in vitro assays, plant models, or alternative experimental systems. For instance, different fractions of *I. laurina* leaves induced DNA damage in genotoxicity assays evaluated by the comet test, demonstrating a

genotoxic potential that was strictly fraction and concentration dependent (FALCOSKI TOR, et al., 2021). Similarly, aqueous leaf extracts of *I. laurina*, *I. edulis*, *I. pilosula*, and *I. macrophylla* evidenced clear genotoxic and cytotoxic effects when tested against the *Allium cepa* plant model (NEVES LP, et al., 2022). Conversely, studies focused on flavonoids isolated from the leaves of *I. laurina* showed no inherent genotoxicity and instead suggested a promising chemoprotective action (FURTADO FB, 2014). Regarding alternative animal models, a trypsin inhibitor isolated from the seeds of *I. cylindrica* caused no observable toxic damage even at high concentrations when subjected to in vivo toxicity assays using *Galleria mellonella* (PEREIRA APR, et al., 2024). Furthermore, preliminary toxicity screening of aqueous extracts from *I. laurina* and *I. vera* against the *Artemia salina* model demonstrated a lack of acute toxicity even at elevated concentrations (SILVA ERS, et al., 2026). This divergence in the literature emphasizes the severe deficit of comprehensive safety information regarding *I. vera* and underscores the absolute importance of expanding investigations into mammalian models.

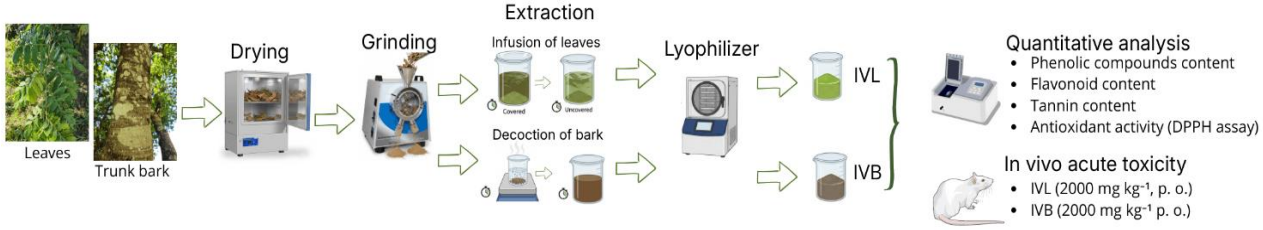
The justification for this study rests on the necessity to bridge these empirical and methodological gaps through a coordinated evaluation of *I. vera* leaf and bark aqueous extracts. Investigating these traditional preparations is justified by the requirement to map their phytochemical profiles, thereby identifying the primary secondary metabolites responsible for their reported therapeutic efficacy. Concurrently, exploring their antioxidant potential is essential to clarify the mechanisms by which these extracts mitigate oxidative stress, which is a common driver of inflammatory and gastric pathologies. Finally, because these aqueous preparations are frequently consumed by the population without standardized dosage guidelines, establishing a definitive in vivo toxicological profile in vertebrate systems is vital to substantiate public safety and support the rational transformation of traditional knowledge into validated phytotherapies.

Although previous studies have described the biological activities and chemical constituents of species belonging to the genus *Inga*, evidence regarding the phytochemical profile, antioxidant activity, and especially the toxicological safety of aqueous preparations obtained via traditional methods of use remains scarce. In this context, the present study aimed to characterize the chemical profile, determine the in vitro antioxidant potential, and evaluate the in vivo acute toxicity of aqueous extracts from the leaves and bark of *I. vera* to provide scientific validation for its traditional medicinal use. It was hypothesized that the aqueous extracts of this species would exhibit significant antioxidant activity and low acute toxicity due to their phenolic content and widespread ethnopharmacological use.

METHODS

Leaves and trunk barks of *I. vera* were collected in Dourados, Mato Grosso do Sul, Brazil, identified, and deposited in the herbarium of Universidade Federal da Grande Dourados (UFGD) (accession number 8133). Material access was registered in SISGEN (National System for the Management of Genetic Heritage and Associated Traditional Knowledge) under number A739836. The materials were processed and characterized according to the experimental design shown in **Figure 1**. Following cleaning, material was dried in a forced-air oven (TECNAL, Brazil) at 60°C to constant weight and ground using a blade mill (FORTINOX, Brazil). Extracts were prepared by infusion of leaves and decoction of bark at 10% (w/v). Leaves were immersed in distilled water at approximately 100°C for 10 minutes in a covered vessel, followed by 20 minutes uncovered. Bark decoction consisted of boiling in distilled water for 10 minutes, followed by 20 minutes rest for cooling. After filtration, both extracts were frozen and lyophilized (Christ, Germany), designated as IVL (*I. vera* leaves) and IVB (*I. vera* bark), respectively.

Figure 1 - Experimental design flowchart for the preparation and analysis of *I. vera* extracts.



Source: Silva ÉRS, et al., 2026.

For the analysis of total phenolic, flavonoid and tannin contents, the extract sample at a concentration of 1 mg mL^{-1} (m/v) was used, with all assays performed in triplicate. The phenolic compounds content was determined using the Folin Ciocalteu colorimetric method adapted from Djeridane et al. (2006). A 0.1 mL aliquot of the sample was combined with 0.5 mL of diluted Folin Ciocalteu reagent (1:10 ratio, v/v) and 1 mL of distilled water.

After 1 minute of reaction, 1.5 mL of a 20% sodium carbonate solution was added, and the mixture was incubated for 2 hours in the dark. Absorbance was measured at 760 nm (BEL Photonics, Brazil) and expressed as mg gallic acid equivalents per g extract (mg GAE g^{-1}). Flavonoid content was quantified using the colorimetric method of Djeridane A, et al. (2006) with aluminum chloride. One milliliter of sample was combined with 1 mL of 2% aluminum chloride solution in methanol and incubated for 15 minutes at room temperature. Absorbance was measured at 430 nm and expressed as mg rutin equivalents per g extract (mg RE g^{-1}). Tannin content was determined according to Pansera MR, et al. (2003) using Folin-Denis reagent. To 0.5 mL of sample, 0.5 mL of Folin-Denis reagent was added, incubated for 3 minutes, and then 0.5 mL of 8% sodium carbonate solution was added followed by 2-hour incubation. Absorbance was recorded at 725 nm and results expressed as mg tannic acid equivalents per g extract (mg TAE g^{-1}).

Antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical method according to Kumaran A and Joel Karunakaran R (2006). Two milliliters of methanolic DPPH solution were added to 100 µL of extract (20 to 1,000 µg mL^{-1}, m/v), protected from light, and incubated at 25 ± 1°C for 30 minutes. Absorbance was measured at 517 nm with distilled water as control. Results were expressed as mean inhibitory concentration (IC_{50}) (SANCHEZ-GONZALES G, et al., 2019).

Acute toxicity was evaluated in female Wistar rats (*Rattus norvegicus*) following OECD Guideline No. 425 (Up and Down procedure) (OECD, 2022). The protocol was approved by the Animal Ethics Committee of UFGD (Code No. 17/2022). Animals were acclimated for a minimum of 15 days in the facility, housed in polypropylene cages (410 × 340 × 175 mm) under 12-hour light/dark cycle at 20–24°C with ad libitum access to food and water. Three experimental groups were established per treatment (n = 5): Control (1 mL distilled water, p. o.), IVL (2,000 mg kg^{-1} of body weight, p. o.), and IVB (2,000 mg kg^{-1} of body weight, p. o.). Initially, in each group, one female was treated and observed for 4 hours; subsequently, the remaining four females were treated following the same protocol. Control animals received water under identical conditions. Following acute administration, animals were observed daily for 14 days with recordings of water and food consumption, individual body weight, and clinical signs of toxicity. At the end of the observation period, animals were euthanized with inhaled anesthetic (isoflurane), and internal organs (liver, kidneys, spleen, heart, lungs, ovaries, and uterus) were removed, rinsed, weighed, and macroscopically examined.

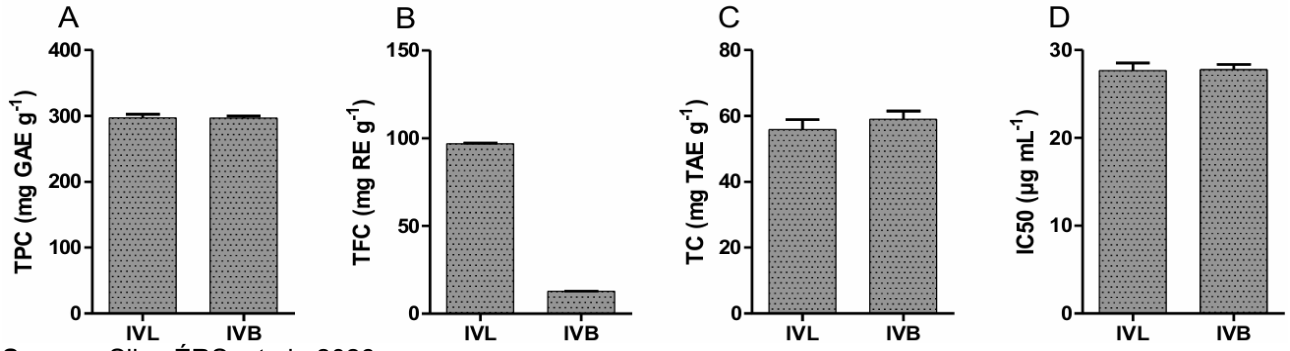
Regarding the statistical analyses, data distribution was previously evaluated for normality. The analyses were performed using STATISTICA software (version 7.0), employing independent samples t tests to compare the treated groups against the control group. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Analysis of secondary metabolite content and antioxidant potential revealed considerable similarities between IVL and IVB (**Figure 2**). Both extracts exhibited comparable phenolic compounds content (297.24 ± 5.34 mg GAE g^{-1} for IVL and 296.8 ± 3.33 mg GAE g^{-1} for IVB, **Figure 2A**) and tannin content (55.92 ± 2.95 mg TAE g^{-1} for IVL and 59.03 ± 2.47 mg TAE g^{-1} for IVB, **Figure 2C**). In contrast, flavonoid content differed markedly between extracts, with IVL yielding 96.99 ± 0.31 mg RE g^{-1} compared to only 12.71 ± 0.07 mg RE g^{-1} for IVB (**Figure 2B**).

Antioxidant potential, assessed by DPPH radical inhibition IC_{50}, was similarly equivalent between extracts, with values of 27.68 ± 0.84 µg mL^{-1} for IVL and 27.79 ± 0.56 µg mL^{-1} for IVB (**Figure 2D**). The quantitative analysis of secondary metabolites revealed that the aqueous extracts from the leaves and bark of *I. vera* exhibited high contents of phenolic compounds and tannins, with a notable emphasis on the higher flavonoid content found in the leaf extract.

Figure 2 - Phytochemical composition and antioxidant activity of IVL and IVB extracts. (A) Total phenolic content (TPC), (B) Total flavonoid content (TFC), (C) Tannin content (TC) and (D) DPPH radical scavenging activity. Values are presented as mean ± standard deviation.



Source: Silva ÉRS, et al., 2026.

In the acute toxicity assay, a single oral administration of 2,000 mg kg⁻¹ of either extract produced no mortality or clinical signs of toxicity throughout the 14-day observation period. Daily Hippocratic screening revealed no behavioral, physical, or physiological alterations in treated animals. Statistical evaluation of physiological parameters showed that IVL treatment induced no significant changes in body weight, food intake, or water consumption when compared to control group. For IVB, no significant differences were observed in body weight or food intake; however, a significant reduction in water consumption was detected relative to the control group ($p < 0.01$) (**Table 1**).

Table 1 - Body weight, food intake and water consumption of female rats orally treated with *I. vera* extracts.

Parameter	Control	IVL (2,000 mg kg⁻¹)	IVB (2,000 mg kg⁻¹)
Initial weight (g)	208.40 ± 4.49	203.80 ± 3.97	203.60 ± 4.87
Final weight (g)	226.40 ± 5.89	226.00 ± 2.90	224.00 ± 5.58
Body weight gain (g)	9.49 ± 0.89	9.09 ± 1.10	10.00 ± 1.11
Body weight gain (%)	4.45 ± 0.37	4.39 ± 0.60	4.80 ± 0.52
Food intake (g/day)	85.71 ± 3.76	81.93 ± 2.64	73.50 ± 5.36
Water intake (mL/day)	235.71 ± 12.78	210.00 ± 11.63	184.29 ± 14.10**

Note: Values are expressed as mean ± SEM, n = 5 animals/group. **p < 0.01 compared to the control group. Student's t-test for acute toxicity.
Source: Silva ÉRS, et al., 2026.

Macroscopic examination of organs revealed no evidence of toxicity. Likewise, analysis of absolute and relative organ weights (kidneys, heart, spleen, liver, lungs, ovaries, and uterus) showed no significant differences compared to the control group, further supporting the absence of relevant toxic effects in target organs following acute administration of both extracts (**Table 2**).

Table 2 - Absolute and relative organ weights (g/100 g of body weight) of female rats orally treated with *I. vera* extracts.

Parameter	Control	IVL (2,000 mg kg⁻¹)	IVB (2,000 mg kg⁻¹)
Kidneys (g)	0.82 ± 0.03	0.79 ± 0.02	0.76 ± 0.03
Kidneys (g/100 g)	0.36 ± 0.02	0.35 ± 0.01	0.34± 0.02
Heart (g)	0.70 ± 0.01	0.69 ± 0.02	0.64± 0.03
Heart (g/100 g)	0.31 ± 0.01	0.31 ± 0.01	0.28 ± 0.01
Spleen (g)	0.54 ± 0.02	0.53 ± 0.03	0.61± 0.03
Spleen (g/100 g)	0.24 ± 0.01	0.24 ± 0.01	0.27 ± 0.01
Liver (g)	8.63 ± 0.35	8.56 ± 0.62	8.60 ± 0.45
Liver (g/100 g)	3.81 ± 0.10	3.78 ± 0.23	3.84 ± 0.15
Lungs (g)	1.13 ± 0.03	1.24 ± 0.05	1.32 ± 0.10
Lungs (g/100 g)	0.50 ± 0.01	0.55 ± 0.02	0.59 ± 0.05
Ovaries (g)	0.17 ± 0.01	0.17 ± 0.02	0.18 ± 0.01
Ovaries (g/100 g)	0.07 ± 0.00	0.08 ± 0.01	0.08 ± 0.01
Uterus (g)	0.63 ± 0.05	0.76 ± 0.19	0.59 ± 0.10
Uterus (g/100 g)	0.28 ± 0.02	0.34 ± 0.08	0.26 ± 0.04

Note: Values are expressed as mean ± SEM, n = 5 animals/group.
Source: Silva ÉRS, et al., 2026.

DISCUSSION

The traditional use of medicinal plants represents a well-established practice in ethnopharmacology, providing a robust foundation for investigating bioactive compounds with therapeutic potential. Species of the *Inga* genus are widely utilized in folk medicine to treat various pathological conditions, including gastric disorders and inflammatory processes (RODRIGUES VEG and CARVALHO DA DE, 2001). This traditional knowledge holds high clinical relevance because preparations such as infusions, decoctions, and macerations of leaves, barks, stems, and roots are routinely employed across South America for their wound healing, antiseptic, antidiarrheal, anti-inflammatory, and astringent properties (KOROLKOVAS A, et al., 2010). Specifically, decoctions of the bark or stem are indicated for oral infections, wounds, ulcers, diarrhea, laryngitis, and rheumatism, whereas leaf and root teas are preferred for gastrointestinal distress and hepatic disorders (KOSZEWICZ M, et al., 2021). Scientific validation of these practices requires an integrated approach that correlates phytochemical characterization with biological activity and toxicological safety.

In the present study, the chemical composition of *I. vera* aqueous extracts revealed a high prevalence of phenolic compounds, flavonoids, and tannins. These findings align with literature establishing that *Inga* species are rich in these specific classes of secondary metabolites (LIMA NM et al., 2018). Similar profiles have been reported across the genus, including flavonoids in the bark of *I. jinicuil* (GALLEGOS-GARCÍA AJ, et al., 2022), the fruits of *I. marginata* (FLORES DCB, et al., 2023), the trunk wood of *I. alba* (GARCIA MG, et al., 2021), and the leaves of *I. fendleriana* and *I. edulis* (COSTA KC, et al., 2023; PINTO L DE PF, et al., 2018). Furthermore, tannins and phenolics have been documented in the fruits and seeds of *I. edulis* and *I. laurina* (LIMA NM, et al., 2022; DE MOURA MARTINS C, et al., 2020). The presence of these metabolites in the evaluated *I. vera* aqueous extracts confirm that traditional preparation methods effectively extract key bioactive constituents.

These phytochemical classes are widely recognized for their health promoting effects, particularly their role as free radical scavengers and modulators of oxidative stress, which protects cells against reactive oxygen species (OLUWOLE O, et al., 2022). Phenolic compounds, including tannins and flavonoids, possess a high capacity for antioxidant activities. Tannins, which are classic plant polyphenols, contribute significantly to this defense system and are intimately related to antioxidant efficacy (PANSERA MR, et al., 2003). The antioxidant efficiency of flavonoids is directly governed by their chemical structure, particularly the number and position of hydroxyl groups, the degree of molecular conjugation, and the capacity for metal chelation, all of which optimize the neutralization of free radicals. Flavonoids, characterized by the C6–C3–C6 skeleton, exhibit significant antioxidant and anti-inflammatory activities, reducing the risk of chronic diseases by neutralizing free radicals and regulating cellular pathways sensitive to oxidative damage (CHEN Y, et al., 2023).

In this study, the evaluated aqueous extracts demonstrated a strong capacity to reduce the DPPH radical, a mechanism based on the reduction of solution absorbance at 517 nm by antioxidant compounds (KUMARAN A and JOEL KARUNAKARAN R, 2006). This significant antioxidant capacity in *I. vera* leaves and bark extracts agrees with previous studies reporting similar potency in ethanolic extracts (LIMA CS, et al., 2015). It also mirrors the broader antioxidant trends observed in the genus, where leaves, barks, fruits, and seeds show positive outcomes heavily influenced by the extraction method and assay type. For instance, ultrasound extraction optimized the antioxidant performance of *I. laurina* (RIGHI DCBF, et al., 2025), while extracts of *I. feuilleei*, *I. marginata*, *I. stipularis*, *I. semialata*, *I. heteroptera*, and *I. verna* subspecies *affinis* demonstrated prominent radical scavenging capabilities (BOTELHO A DE S, et al., 2023; GUILLÉN-SÁNCHEZ JS, et al., 2024; MAZIERO M, et al., 2020). Extensive literature on *I. edulis* further validates that leaves and barks concentrate the highest antioxidant potential within the genus, despite notable activity in fruits and seeds (FERRO A et al., 2022). Therefore, the strong correlation between the identified polyphenols and the elevated antioxidant activity reinforces the therapeutic baseline of *I. vera* aqueous extracts.

The intersection of high antioxidant capacity and rich polyphenol content provides a mechanistic explanation for the traditional therapeutic claims of the species. A critical point of comparison is the work of Nesello LAN, et al. (2017), which demonstrated the potent gastroprotective effect of methanolic extracts from *I. vera* pulp and seeds at a dose of 250 mg per kg via the oral route in rodent ulcer models induced by ethanol

with hydrochloric acid or indomethacin. Although the present study evaluated aqueous leaf and bark extracts and did not directly employ in vivo gastric lesion models, the substantial flavonoid content in the leaves and the consistent phenolic and tannin levels across both tissues point toward a promising therapeutic overlap. To fully bridge the current findings with established gastroprotective evidence, certain methodological differences must be noted for future exploration, specifically regarding the solvent type (methanolic versus aqueous), the selected plant organ (fruits versus leaves or bark), and the specific biological endpoints (in vivo gastroprotection versus in vitro antioxidant metrics). Nonetheless, the antioxidant mechanisms identified here directly support gastroprotective and tissue repair pathways, as oxidative stress is a major driver of mucosal ulceration.

The high content of phenolic compounds observed in both extracts, associated with the antioxidant activity detected in the DPPH assay, suggests that these metabolites contribute significantly to the biological potential of the species. However, the lack of a more detailed chemical identification limits the attribution of this activity to specific compounds. Furthermore, the traditional application of *I. vera* for healing wounds and oral ulcers (RODRIGUES VEG and CARVALHO DA DE, 2001) is strongly supported by these results. Aqueous extracts rich in phenolics and flavonoids stimulate fibroblast proliferation and the deposition of collagen types I and III, which are essential steps for tissue regeneration (MARILYN S. C-M, et al., 2023). This is consistent with other species like *I. edulis*, whose leaf extracts exhibit high polyphenolic content and photostability under ultraviolet radiation, with myricetin derivatives serving as key photopreventive markers (ALVES G DE AD, et al., 2020). These cumulative data confirm that the genus *Inga* acts as a predictable source of bioactive flavonoids, justifying the ongoing isolation of specific therapeutic markers from *I. vera* aqueous extracts.

The translation of traditional knowledge into safe phytotherapies requires strict toxicological evaluation. In this study, we explored the acute safety profile of *I. vera* aqueous extracts through vertebrate model. The complete absence of mortality at the limit dose of 2,000 mg kg^{-1} allows the classification of these aqueous extracts under Category 5 of the Globally Harmonized System, indicating an estimated LD_{50} greater than 2,000 mg kg^{-1} (UNECE, 2020). Behavioral and clinical monitoring using Hippocratic screening parameters (MALONE MH and ROBICHAUD RC, 1962) revealed no systemic alterations. The conscious state and normal exploration within the cages demonstrated preserved behavioral integrity. Motor system coordination and neurological responses were unaffected, as evidenced by a healthy sensitivity to touch, normal auricular and corneal reflexes, and the complete absence of tremors, convulsions, straub tail, sedation, or anesthesia. Furthermore, autonomous nervous system evaluations confirmed the lack of toxic signs such as lacrimation, cyanosis, ptosis, salivation, or piloerection.

These clinical observations are fully congruent with the postmortem macroscopic metrics presented in **Table 2** which confirm that the internal organs suffered no structural or morphological alterations, maintaining a healthy state. Organ weights and food consumption showed no significant differences relative to the control group. A single localized variation was recorded: a significant decrease in water intake within the *I. vera* bark group. While high concentrations of tannins can alter the palatability of solutions due to their characteristic astringency, the quantitative tannin levels did not differ drastically between the extracts to conclusively establish a single causal link. Crucially, this transient reduction in water consumption produced no secondary repercussions on animal weight gain, behavioral health, or final organ integrity, confirming it as an isolated finding devoid of toxicological significance. The reduction in water intake observed in the group treated with the bark extract may be related to the astringency of phenolic compounds, especially tannins. However, this interpretation should be considered with caution, as the absence of complementary biochemical analyses precludes completely ruling out subtle physiological effects.

Preliminary in vitro evidence indicates low cytotoxicity across the genus (TAUFNER K, 2020). Because these aqueous extracts are consumed conventionally by the population without professional guidance on dosage, this study provides the first foundational in vivo evidence supporting the acute safety profile of *I. vera* aqueous extracts. This establishes a secure baseline for future chronic toxicological and clinical investigations, ensuring that the pharmacological potential of this species can be exploited safely. Although the acute administration of the extracts at the dose of 2,000 mg kg^{-1} caused neither mortality nor evident systemic clinical

alterations, these findings must be interpreted with caution, since they are restricted to an acute exposure model. Thus, the results indicate preliminary safety under the evaluated conditions but do not allow inferences regarding prolonged use, thereby requiring subchronic and chronic toxicity studies.

CONCLUSION

The aqueous extracts of leaves and bark of *I. vera* showed high content of phenolic compounds, in vitro antioxidant activity, and did not show apparent signs of acute toxicity in Wistar rats at the dose of 2,000 mg kg^{-1}, under the evaluated experimental conditions. These findings provide preliminary evidence of safety for the traditional use of these preparations, but do not allow inferences about prolonged use, requiring repeated dose toxicity studies and specific pharmacological investigations to expand the understanding of their therapeutic potential.

ACKNOWLEDGEMENTS AND FUNDING

The authors acknowledge the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (Finance Code 001) for the doctoral and postdoctoral scholarships and the Rede BioDiversa Centro-Oeste (Project CAPES No. 88887.964845/2024 00 and Process No. 88887.129820/2025-00; and FUNDECT Process No. 83/034.923/2024). We also thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the research grant awarded to CALC (Process No. 306065/2025-8).

REFERENCES

1. ALVES GDAD, et al. Obtainment of an enriched fraction of *Inga edulis*: identification using UPLC-DAD-MS/MS and photochemopreventive screening. *Preparative Biochemistry & Biotechnology*, 2020; 50(1): 28–36.
2. BOTELHO ADS, et al. First report of phenolic compounds isolated from *Inga stipularis* DC. (Fabaceae) leaves. *Natural Product Research*, 2023; 37(14): 2442–2445.
3. CHEN Y, et al. Dandelion (*Taraxacum mongolicum*) Extract Alleviated H_2O_2-Induced Oxidative Damage: The Underlying Mechanism Revealed by Metabolomics and Lipidomics. *Foods*, 2023; 12(17).
4. COSTA KC, et al. Photochemoprevention of topical formulation containing purified fraction of *Inga edulis* leaves extract. *Photochemical & Photobiological Sciences*, 2023; 22(9): 2105–2120.
5. DJERIDANE A, et al. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry*, 2006; 97(4): 654–660.
6. FALCOSKI TOR, et al. Genotoxicity, cytotoxicity and chemical profile from *Inga laurina* (Fabaceae). *Natural Product Research*, 2021; 35(4): 676–680.
7. FERNANDES JM, et al. Taxonomia de *Inga obtusata* (Leguminosae): uma espécie quase desconhecida nos estados de Mato Grosso e Rondônia, Brasil. *Scientific Electronic Archives*, 2022; 15(2).
8. FERRO A, et al. Active Compounds From *Inga edulis* Martius Seeds Against Multiple Myeloma. *Natural Product Communications*, 2022; 17(11).
9. FLORES DCB, et al. Extraction of antioxidant and antimicrobial compounds from *Inga marginata* Willd bark and pulp using different extraction techniques and phytochemical characterization. *Innovative Food Science & Emerging Technologies*, 2023; 83: 103244.
10. FURTADO FB. Estudo químico, análise do óleo essencial e atividades biológicas de *Inga laurina* (Sw.) Willd. 2014.
11. GALLEGOS-GARCÍA AJ, et al. Preliminary Phytochemical Profile and Bioactivity of *Inga jinicuil* Schltdl. & Cham. ex G. Don. *Plants*, 2022; 11(6): 794.
12. GARCIA MG, et al. Isolation of New Compounds from *Andira parviflora* and *Inga alba* Wood Residues Using LC-DAD-SPE/NMR. *Chemistry of Natural Compounds*, 2021; 57(2): 300–305.
13. GONÇALVES TPR, et al. Potencial antifúngico e antibacteriano de extratos vegetais da região de Divinópolis/MG. *Revista Ibero-Americana de Ciências Ambientais*, 2018; 9(3): 25–37.

14. GUILLÉN-SÁNCHEZ JS, et al. Andean Fabaceae Species with Pharmacological Potential: Exploration of Antioxidant, Anticarcinogenic, and Antimicrobial Properties. *Agriculture*, 2024; 14(12): 2337.
15. KOROLKOVAS A, et al. *Farmacopeia Brasileira*. 2010.
16. KOSZEWICZ M, et al. Dysbiosis is one of the risk factors for stroke and cognitive impairment and potential target for treatment. *Pharmacological Research*, 2021; 164.
17. KUMARAN A, JOEL KARUNAKARAN R. Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus*. *Food Chemistry*, 2006; 97(1): 109–114.
18. LIMA CS, et al. Mycorrhizal Fungi (AMF) increase the content of biomolecules in leaves of *Inga vera* Willd. seedlings. *Symbiosis*, 2015; 65(3): 117–123.
19. LIMA NM, et al. Quimiodiversidade, bioatividade e quimiossistemática do gênero *Inga* (Fabaceae): uma breve revisão. *Revista Virtual de Química*, 2018; 10(3): 459–473.
20. LIMA NM, et al. Metabolic Profiling of *Inga* Species with Antitumor Activity. *Molecules*, 2022; 27(15): 4695.
21. MALONE MH, ROBICHAUD RC. A Hippocratic screen for pure or crude drug materials. *Lloydia*, 1962; 25(4): 320–332.
22. MARILYN SCM, et al. Wound Healing Properties of Natural Products: Mechanisms of Action. *Molecules*, 2023; 28(2): 598.
23. MAZIERO M, et al. Phytochemical study, an evaluation of the antioxidant potential and the antimicrobial activity of *Inga semialata* (Vell.) C. Mart. hydroalcohol extract. *Natural Product Research*, 2020; 34(1): 192–196.
24. MOURA MARTINS C, et al. Antifungal and cytotoxicity activities and new proanthocyanidins isolated from the barks of *Inga laurina* (Sw.) Willd. *Phytochemistry Letters*, 2020; 40: 109–120.
25. NESELLO LAN, et al. Screening of Wild Fruit Trees With Gastroprotective Activity in Different Experimental Models. *Arquivos de Gastroenterologia*, 2017; 54(2): 135–138.
26. NEVES LP, et al. Evaluation of the cell cycle behavior of *Allium cepa* L. using the cytotoxic, genotoxic and antiproliferative potential of infusions of the dried leaves of four species of the genus *Inga* Mill (Fabaceae). *Brazilian Journal of Development*, 2022; 8(1): 5032–5046.
27. OECD. Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure. *OECD Guidelines for the Testing of Chemicals*, 2022.
28. OLUWOLE O, et al. Role of phenolic acid, tannins, stilbenes, lignans and flavonoids in human health – a review. *International Journal of Food Science and Technology*, 2022; 57(10): 6326–6335.
29. PANSERA MR, et al. Análise de taninos totais em plantas aromáticas e medicinais cultivadas no Nordeste do Rio Grande do Sul. *Revista Brasileira de Farmacognosia*, 2003; 13(1): 17–22.
30. PEREIRA APR, et al. Antimicrobial and antibiofilm activities of *Inga cylindrica* trypsin inhibitor. *Brazilian Journal of Biology*, 2024; 84.
31. PINTO LDPF, et al. Understanding perceptions and beliefs about different types of fermented milks through the application of projective techniques: A case study using Haire's shopping list and free word association. *Journal of Sensory Studies*, 2018; 33(3).
32. RIGHI DCBF, et al. Green technologies applied to extraction processes and analysis of polyphenolic compounds from *Inga laurina* Willd. *Food Chemistry*, 2025; 471: 142827.
33. RODRIGUES VEG, CARVALHO DA. Levantamento etnobotânico de plantas medicinais no domínio do cerrado na região do Alto Rio Grande, Minas Gerais. *Ciência e Agrotecnologia*, 2001; 25(1): 102–123.
34. SANCHEZ-GONZALES G, et al. Compuestos fenólicos y actividad antioxidante de los extractos de la hoja de chirimoya (*Annona cherimola* Mill). *Revista Colombiana de Química*, 2019; 48(2): 21–26.
35. SILVA ERS, et al. Tea made from the leaves and stems of *Inga laurina* and *Inga vera*: Chemical composition, toxicity, antimicrobial and photoprotective potential. *Acta Scientiarum*, 2026; 1.
36. TAUFNER K. Fermentação secundária de Kombucha com extrato de *Inga edulis*: efeitos do tempo de fermentação e da concentração do extrato na qualidade do produto. 2020.
37. UNECE. Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (Rev. 2). *Globally Harmonized System of Classification and Labelling of Chemicals (GHS)*, 2020.