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Aristolochia Plant Used in Congolese Traditional Medicine: Ethnopharmacology and Chromatographic Analysis for Aristolochic Acids Identification and Quantification

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Abstract

The *Aristolochia* genus contains aristolochic acids (AAs), nephrotoxic and carcinogenic compounds found in many species. Although the use of *Aristolochia* has been restricted worldwide due to safety concerns, no information is currently available on the species occurring in the flora of the Democratic Republic of the Congo (DRC). This lack of information regarding occurrence, use, and chemical composition of *Aristolochia* species limits the evaluation of potential exposure risks associated with traditional medicinal practices. This study identified *Aristolochia* species reported in the DRC through a bibliographic survey and assessed the presence of AAs in *A. heppii*, a species native to Katanga. Microscopic examination of root powders and HPLC-DAD/MS analysis of hydroalcoholic and aqueous extracts were performed according to the European Pharmacopoeia on one authenticated sample and twelve commercial samples purchased in Lubumbashi. Microscopy confirmed diagnostic features consistent with *Aristolochia*. Mass spectrometry analysis identified AAs, with AA-I quantified in hydroalcoholic extracts at 566 ± 5 ppm to 3533 ± 32 ppm (0.057–0.353% *w*/*w*) and in the aqueous extract at 204.2 ± 0.4 ppm (0.020% *w*/*w*). These results demonstrate that traditional preparations of *A. heppii* may lead to exposure to AA-I and underline the need for risk assessment and regulatory oversight.

Keywords: *Aristolochia*; aristolochic acid; nephrotoxicity; carcinogenicity; hepatotoxicity; abortifacient; HPLC-DAD/MS; DR Congo

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1. Introduction

Plant species belonging to the genus *Aristolochia* (*Aristolochiaceae*) represent a major public health concern due to the presence of aristolochic acids (AAs), compounds known for their nephrotoxic, hepatotoxic, and carcinogenic effects [1–5]. Despite their well-documented toxicity, several *Aristolochia* species continue to be used in traditional medicine in different regions of the world, including Africa and America [6,7]. In addition to intentional medicinal use, unintentional exposure resulting from accidental substitution or misidentification of plant materials has also been reported [8]. Furthermore, beyond

their nephrotoxic and carcinogenic effects, AAs exhibit abortifacient activity, which has been widely documented in the literature [9,10]. According to the Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency (EMA), AA-I and AA-II are the most abundant and toxic AAs found in plant material [5].

Although the use of *Aristolochia* species represents a complex toxicological and regulatory issue, their use has been prohibited or strictly regulated in several countries, particularly within the European Union, where the presence of aristolochic acids in herbal products is limited to trace levels (<2 ppm) [11,12]. However, very limited information is currently available regarding the toxicity risks associated with the presence of AAs in *Aristolochia* species occurring in the flora of the Democratic Republic of Congo (DRC). A study carried out in Southern Katanga (2018) reported the medicinal use of *Aristolochia heppii* Merxm (Kapangapanga, in Luba language) for the treatment of diabetes, measles, syphilis, and dysmenorrhea [13]. More recently, a 2025 survey highlighted the widespread commercial availability of the same species, which is notably used for abdominal pain in children, hemorrhoids, prostatitis, and anemia [14]. Furthermore, this species is also used in other regions of Africa, including Zimbabwe for the treatment of diarrhea, and as an abortifacient in South Africa [7].

Liquid chromatography is the Pharmacopoeia reference method for the detection and quantification of aristolochic acids in plant materials. In other regions of the world, several studies reporting HPLC-based detection of aristolochic acids are available, particularly for Asian species such as *A. fangchi* [15] and *A. chilensis* [16]. In contrast, analytical data remain scarce for African species, with only limited information reported for African species, including *A. albida* [17].

In this context, important knowledge gaps remain regarding the diversity of *Aristolochia* species occurring in the flora of the DRC, their use in traditional medicine, and their AAs content.

Therefore, this study aims to *(i)* document the diversity of *Aristolochia* species occurring in the flora of the DRC; *(ii)* describe key diagnostic microscopic botanical features to enable accurate species identification and reduce the risk of accidental substitution; and *(iii)* investigate the phytochemical profile and determine AA-I and AA-II content in *A. heppii* using high-performance liquid chromatography coupled with diode-array detection and mass spectrometry (HPLC-DAD/MS), a species widely used in traditional medicine in the Katanga region.

2. Materials and Methods

2.1. Solvents and Reference Compounds

Acetonitrile (HPLC grade), methanol (HPLC grade), and formic acid (HPLC grade) were purchased from VWR Chemicals, Belgium. The AA-I and AA-II reference mixture (Y0001175) was obtained from the European Pharmacopoeia (EDQM), Strasbourg, France.

2.2. Inventory of Aristolochia Present in DRC Flora

Species of *Aristolochia* occurring in the flora of the DRC were compiled based on data from Plants of the World Online (POWO), maintained by the Royal Botanic Gardens, Kew; the Meise Botanic Garden Herbarium, which holds the world's largest collection of specimens from the DRC [18]; and available publications on *Aristolochia* in Africa, Madagascar, and adjacent islands [19]. In the Kew database, searches were performed using the keyword combination "*Aristolochia*" and "location: Zaire" (According to the TDWG botanical country codes applied by Kew, this should be "*location: Congo-Kinshasa*", but this location is not implemented in the database) [20].

2.3. Aristolochia Used in Traditional Medicine in Lubumbashi

A bibliographic search was conducted using Google Scholar and PubMed to identify *Aristolochia* species used in traditional medicine in Katanga, DRC. A total of 784 records were retrieved through database searching. After removal of duplicate records and studies not meeting the inclusion criteria (i.e., relation with medicinal use), 226 records were screened.

195 records were excluded for the following reasons: focus on cannabis trade, integration of traditional medicine, ecological studies or forest inventories, medicinal pluralism surveys, or studies conducted outside the defined study area. These studies did not provide species-level ethnobotanical data relevant to the objectives of the present study. Consequently, 31 ethnobotanical studies conducted in Katanga were included in the review. The bibliographic selection process is presented in Figure 1.

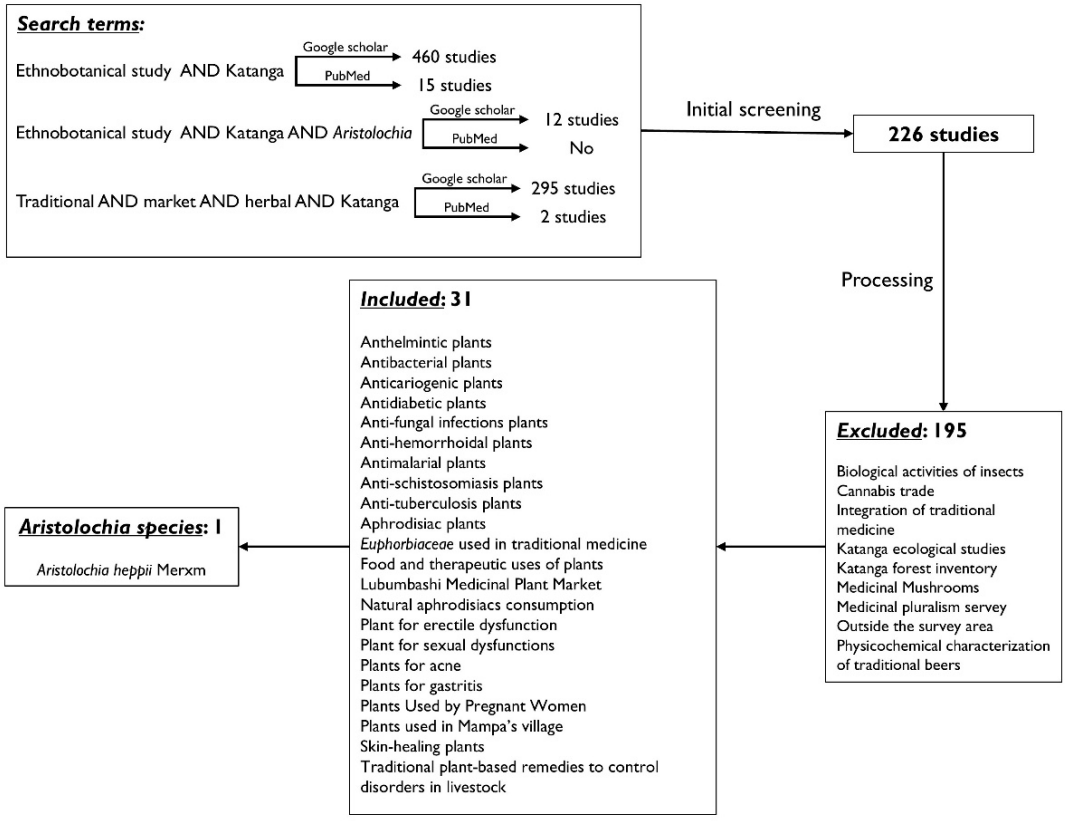

Figure 1. Flow diagram illustrating the literature search strategy and selection process for ethnopharmacological studies reporting *Aristolochia* use in Katanga (DRC).

2.4. Sample Acquisition

Samples of the roots used in traditional medicine were collected using two complementary approaches. On the one hand, field collections were conducted in collaboration with two traditional practitioners to identify the species based on its reported vernacular name ("Kapangapanga" in the Luba language) [13,14], root samples were collected and dried in the shade. Voucher specimens were prepared for botanical identification; the species was authenticated as *Aristolochia heppii* Merxm. by Professor Pierre Meerts at Meise Botanic Garden. On the other hand, plant materials traded as whole roots under the same vernacular name were purchased from local vendors in the medicinal plant markets of the city of Lubumbashi (Figure 2), following the methodology described by Van Andel [21]. Their identification was first performed through macroscopic comparison of botanical characteristics. In addition, microscopic examination of the powdered material confirmed the presence of diagnostic features consistent with the species.

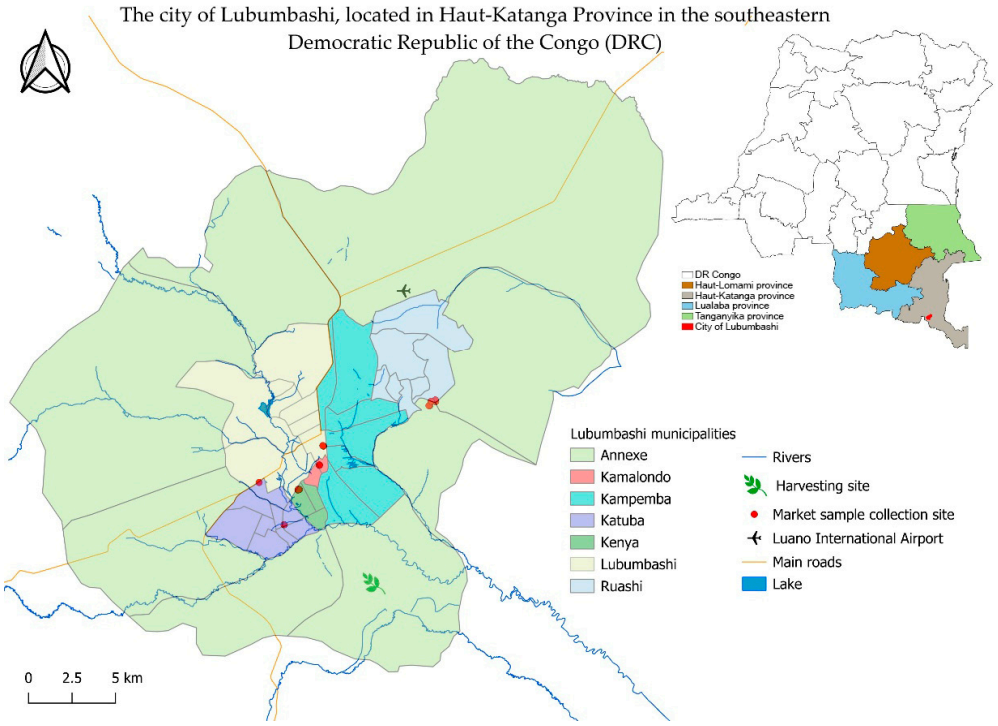

Figure 2. Geographical location of field collection site and medicinal plant markets in Lubumbashi, Haut-Katanga Province, southeastern Democratic Republic of Congo.

Both market-purchased and field-collected samples (Table 1) were stored in plastic bags and preserved from moisture and direct sunlight in the Pharmacognosy Laboratory of the University of Lubumbashi [22].

Table 1. Information on samples used.

Sample Type	Code	Part of the Plant Used	Pre-Analysis Processing (See Section 2.6)
Filed-collected authentic sample	RKPP	Root	hydroalcoholic extraction
Commercial sample	KPP1	Root	hydroalcoholic extraction
Commercial sample	KPP2	Root	hydroalcoholic extraction
Commercial sample	KPP3	Root	hydroalcoholic extraction
Commercial sample	KPP4	Root	hydroalcoholic extraction
Commercial sample	KPP5	Root	hydroalcoholic extraction
Commercial sample	KPP6	Root	hydroalcoholic extraction
Commercial sample	KPP7	Root	hydroalcoholic extraction
Commercial sample	KPP8	Root	hydroalcoholic extraction
Commercial sample	KPP9	Root	hydroalcoholic extraction
Commercial sample	KPP10	Root	hydroalcoholic extraction
Commercial sample	KPP11	Root	hydroalcoholic extraction
Commercial sample	KPP12	Root	hydroalcoholic extraction
Commercial sample	DKPP5	Root	Decoction of KPP5

2.5. Microscopy Analysis

Plant powders were examined according to the European Pharmacopoeia (EDQM, 2025, 2.8.23) using an optical microscope (Olympus CX21, Evident Corporation, Tokyo, Japan) coupled to a camera (Toupcam UCMOS05100KPA) controlled by ToupView software (version 4.11; ToupTek Photonics, Hangzhou, China), under magnifications of 40× and 10× [15]. The preparations were stained using the lactic acid–R reagent procedure (EDQM, 2025, 2.8.23) [23].

2.6. HPLC-DAD/MS Analysis

HPLC analysis was performed using an Agilent 1100 system (Agilent Technologies, Santa Clara, CA, USA), 100.0 mg of powdered plant roots from twelve commercial samples and one authentic sample were extracted four times in an ultrasonic bath for 10 min with 2.5 mL of a formic acid–water–methanol mixture (1:9:40, *v*/*v*/*v*) [23]. To ensure exhaustive extraction of the plant material, a fifth extract was analyzed; however, AAs levels were below the limit of detection (1.1 µg/mL). An aqueous decoction of sample 5 (one of the most concentrated samples) was also prepared by boiling 15 g of powdered plant roots in 300 mL of water to mimic traditional preparation methods [13] (Table 1). The extracts were centrifuged (3900× *g*, 10 min, 25 °C), and the supernatants collected and pooled to obtain final volumes of 10.0 mL for the hydroalcoholic extract and 300.00 mL for the aqueous extract.

HPLC-DAD analysis was carried out using a Kinetex C18 reversed-phase column (150 × 4.6 mm, 2.6 µm particle size, 100 Å pore size) maintained at 40 °C. Gradient elution consisting of acetonitrile (solvent A) and water (solvent B) both acidified with 0.1% trichloroacetic acid, was applied under the following conditions: 0–2 min, 15% A; 2–27 min, 15 to 65% A; 27–32 min, 65 to 100% A; 32–38 min, 100% A; 38–40 min, 100 to 15% A. The flow rate was 1.0 mL/min and the detection wavelength was set at 390 nm. A mixture of AA-I and AA-II (Y0001175, EDQM, Strasbourg, France) was used for calibration and system suitability testing, in accordance with the European Pharmacopoeia (EDQM, 2025, 2.8.21) [23].

HPLC-MS analysis was performed with a linear gradient of acetonitrile (solvent A) and water (solvent B), from 5% to 95% A over 40 min. Mass spectrometric analysis was performed using a single-quadrupole Advion Expression® CMS system (Advion, Ithaca, NY, USA) over an m/z range of 200–400, following electrospray ionization (ESI) in positive ion mode. The interface voltage was set at 3.5 kV. The capillary and ion source temperatures were maintained at 250 °C and 200 °C, respectively.

Different gradients were used to optimize performance for each detector. For HPLC-DAD analysis, an acidified mobile phase was applied in accordance with the European Pharmacopoeia to ensure optimal chromatographic separation and reliable quantification.

In contrast, for HPLC-MS analysis, milder conditions were used to improve ionization efficiency, as aristolochic acids ionize poorly in strongly acidic media. Therefore, different gradient conditions were selected to achieve robust quantification by DAD and efficient ionization for MS confirmation.

Linearity, LOD and LOQ

A stock solution containing a mixture of AA-I and AA-II at a concentration of 0.2 mg/mL was prepared by dissolving the entire content of one 1 mg standard vial in 5 mL of a solvent mixture. Five calibration solutions of AA-I were subsequently prepared by serial dilution to obtain final concentrations of 0.04, 0.035, 0.030, 0.025, and 0.020 mg/mL. Each calibration level was prepared in triplicate.

For each level, chromatographic injections (10 µL) were performed, and calibration curves were constructed using the integrated peak areas. A fit test was performed using Fityk software (version 3.0) [24] to select the best regression model. Method sensitivity was evaluated by calculating the limits of detection (LOD) and quantification (LOQ) based on the standard deviation of the y intercept (σ) and the slope (S) of the calibration curves, according to Equation (1) [25,26].

$$\text{LOD} = \frac{3.3\ \sigma}{S};\ \text{LOQ} = \frac{10\ \sigma}{S} \quad (1)$$

3. Results

3.1. Inventory of Aristolochia Use in Traditional Medicine

According to botanical databases, including Plants of the World Online (POWO) and the Meise Botanic Garden Herbarium, as well as available publications on *Aristolochia* in Africa, Madagascar, and adjacent islands, ten species of the genus *Aristolochia* L. are reported to occur in the DR of Congo: *Aristolochia albida* Duch., *Aristolochia heppii* Merxm., *Aristolochia hockii* De Wild., *Aristolochia inflata* Kunth, *Aristolochia littoralis* Parodi, *Aristolochia labiata* Willd., *Aristolochia macrocarpa* Duch., *Aristolochia promissa* Mast., *Aristolochia triactina* Hook.f. et *Aristolochia zenkeri* Engl. Data gathered in RDC on the traditional uses and phytochemistry of these species is summarized in Table 2.

Table 2. Data on the traditional uses of *Aristolochia* species in the DRC.

Species	Ethnobotanical Uses in DR Congo	Traditionally Used in Other Regions	AAs Identification
A. albida	No	Morocco [27], Nigeria [28]	Yes (in Nigeria [17])
A. heppii	Diabetes, measles, syphilis, dysmenorrhea [13] and Abdominal pain, hemorrhoids, prostatitis [14]	Eastern Africa [29], Zimbabwe, South Africa [7]	No
A. hockii	No [(a)]	Malawi [30]	No
A. inflata	No	Central America [6]	No
A. littoralis	No	Central and South America [6]	No
A. labiata	No	Central and South America [6,31]	Yes (in Brazil [32])
A. macrocarpa	No	No	No
A. promissa	No	No	No
A. triactina	No	No	No
A. zenkeri	No	No	No

[(a)] Although *A. hockii* was previously reported in DR Congo (Katanga) [13], our re-examination of herbarium samples indicates a misidentification; the previously reported plants are actually *A. heppii*.

Although species of this genus are known to be toxic, data on their traditional use in the DRC remain unavailable for local species, except for *A. heppii*. Aristolochic acids have been reported so far only in *A. albida* and *A. labiate* [17,27,32].

To evaluate which species are used in traditional medicine in the Katanga region, all available local ethnobotanical surveys (n = 31) were reviewed. These studies investigated medicinal plants used for various therapeutic purposes, including bacterial infection [33], fungal infections [34], malaria [35,36], diabetes [13,37], sexual dysfunction [38,39], salmonellosis [40], aphrodisiacs [41,42], anti-hemorrhoidal uses [43], tuberculosis [44], skin diseases and wound healing [45], acne [46], schistosomiasis [47], dental caries [48] and gastritis [49]. Additional surveys addressed medicinal plants used in the village of Mampa [50], food and therapeutic uses of plant [51], plants used by pregnant women [52], and members of the *Euphorbiaceae* family used in traditional medicine [53].

An ethnobotanical survey on antidiabetic plants reported the use of a species belonging to the genus *Aristolochia* L. (*Aristolochia heppii* Merxm.) [13]. Furthermore, a study investigating the most frequently species traded in Lubumbashi markets reported the extensive use of *A. heppii* under the vernacular name Kapangapanga in the Luba language, which means "nauseating plant" [14].

3.2. Microscopic Examination

Microscopic examination of the reference sample of *A. heppii* root (Figure 3) indicated the following features: (A) a single-celled trichome with a horizontal internal cavity; (B and D), numerous masses of starch grains and oily material; (C) a sclereid with an irregularly polygonal/arched shape, thick and clearly visible walls, a reduced internal lumen (very limited central cavity), and an isolated appearance, not organized in a bundle. A qualitative microscopic examination was conducted on the powdered material from all market samples and compared with the reference sample. Qualitative examination of powdered market samples showed consistent presence of general root features (starch grains and sclereids), as well as diagnostic traits such as trichome morphology and lipid content. Overall, these characteristic microscopic features were identifiable in all samples.

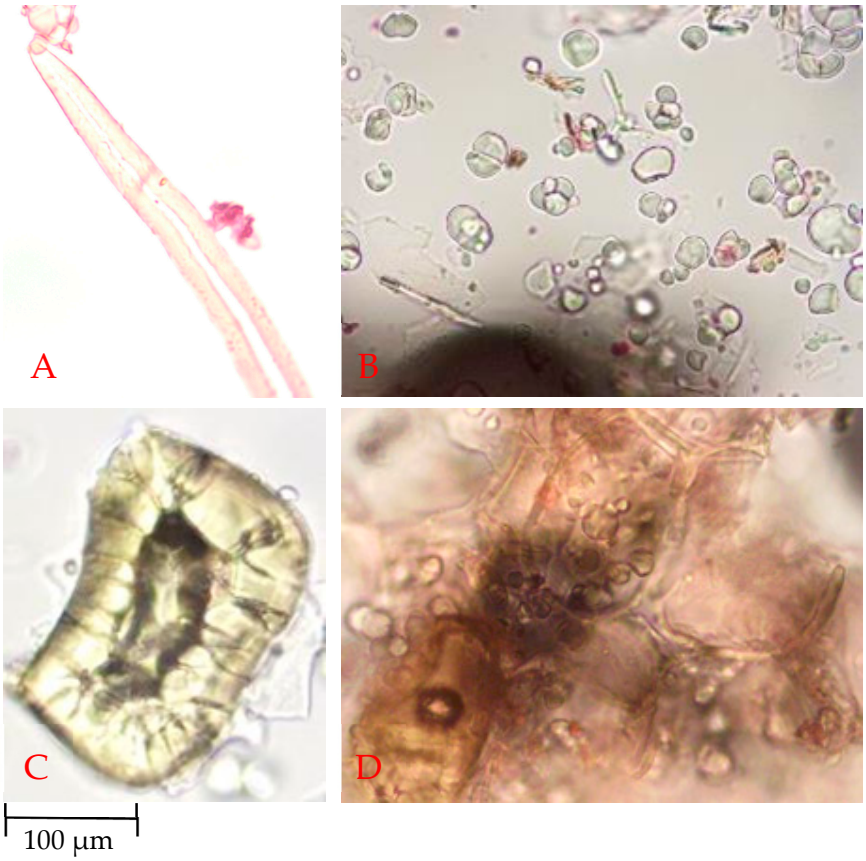

Figure 3. Microscopic features of *A. heppii* (**A**) a single-celled trichome with a horizontal internal cavity; (**B**,**D**), numerous masses of starch grains and essential oils; (**C**) an isolated sclereid with an irregularly polygonal/arched shape, thick and clearly visible walls, a reduced internal lumen.

3.3. HPLC-DAD/MS Analysis of AAs

AA-I was identified by HPLC-DAD (Figure 4) and mass analysis (Figure 5) in *A. heppii* hydroalcoholic extracts and in an aqueous decoction.

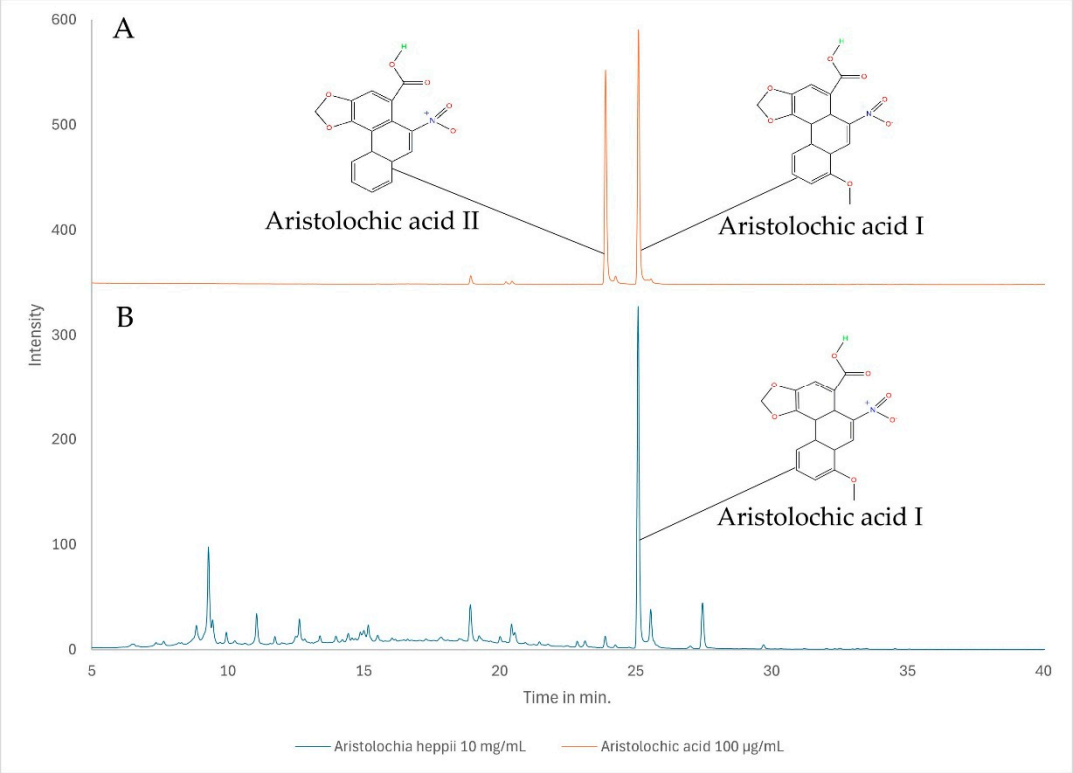

Figure 4. HPLC-DAD chromatograms showing the identification of AAs: (**A**) AA-I and AA-II in the reference mixture (100 µg/mL); (**B**) AA-I in *A. heppii* root hydroalcoholic extract (KPP5 10 mg/mL). Detection wavelength: 390 nm.

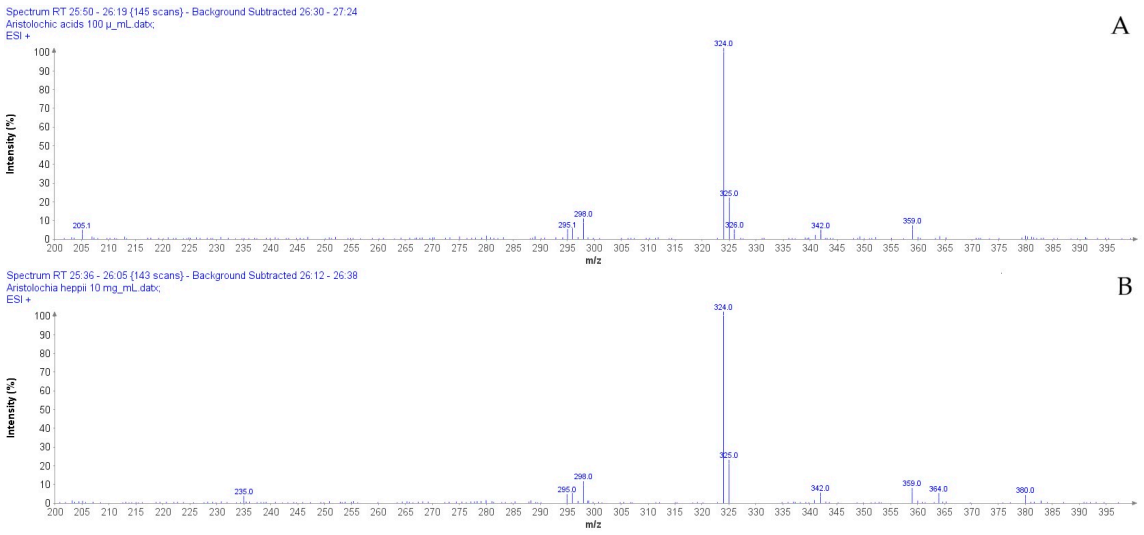

Figure 5. HPLC-MS spectra illustrating the identification of AA-I: (**A**) AA-I in the reference mixture (100 µg/mL), and (**B**) AA-I in *A. heppii* root extract (KPP5 10 mg/mL), recorded in positive ESI mode.

Following the evaluation of the method performance characteristics (Table 3), the major aristolochic acid, AA-I, was quantified (Figure 6).

Table 3. Analytical performances of AA-I assay.

Parameters	Aristolochic Acid I (AA-I; n = 15)	
Linearity	Slope	11,732 ± 173
	Intercept	−6.1 ± 5.1
	R^2	0.9972
	p for the slope significance F-test	$p < 0.0001$
Lower limits of detection and quantitation	LOD (µg/mL)	1.10
	LOQ (µg/mL)	3.32

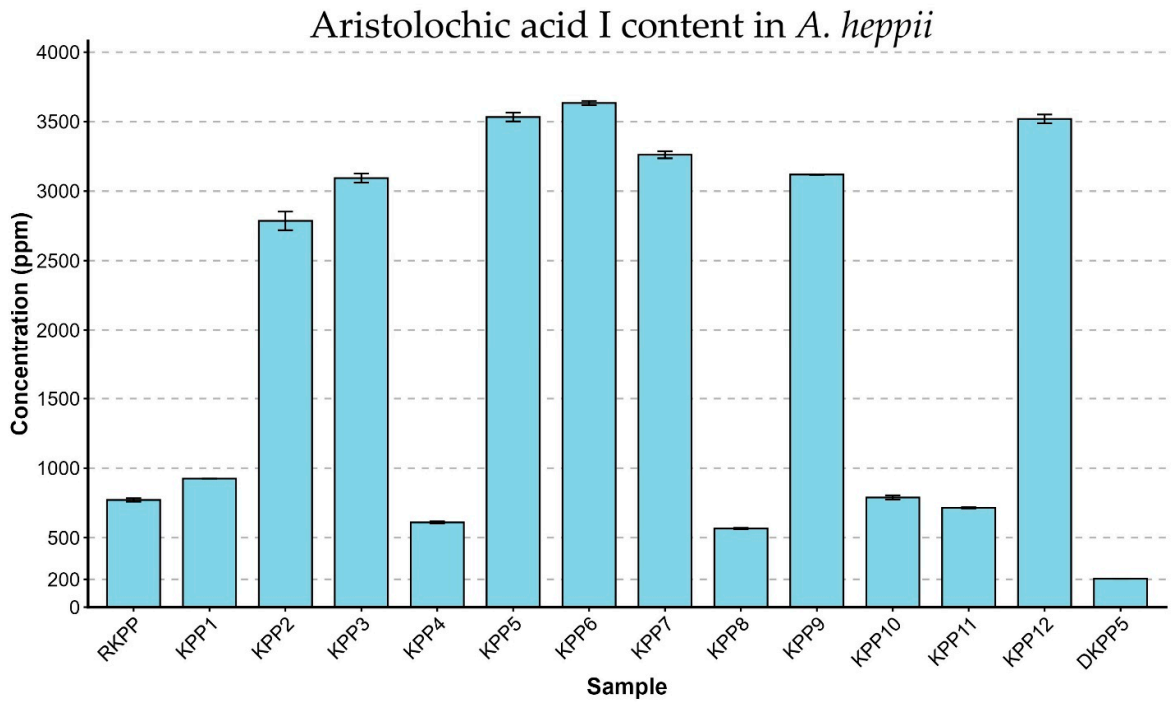

Figure 6. HPLC-DAD quantification of AA-I (mean ± SD, n = 3) in one authenticated reference sample (RKPP), twelve commercial samples purchased in Lubumbashi markets (KPP1–KPP12), and an aqueous decoction prepared from KPP5 (DKPP5).

An AA-I transfer rate was calculated for sample KPP5, according to Equation (2), in which D_{DKPP5} = amount of AA-I in the aqueous decoction (DKPP5) and D_{KPP5} = amount of AA-I in the extracted root powder (KPP5).

$$TR_{AAI} = \frac{D_{DKPP5} \times 100}{D_{KPP5}} \quad (2)$$

The resulting transfer rate was 5.8%, indicating a significant presence of this genotoxic agent in remedies administered to patients. For a traditional decoction, the plant material is typically prepared using approximately 1 g of dried material per liter of water. Based on HPLC-DAD quantification, the concentration of AA-I in the dried plant material was estimated at 200 ppm, corresponding to 200 µg/g of dried plant material. Accordingly, a decoction prepared with 1 g of dried plant material per liter contains approximately 200 µg (M_{AA-I}) of AA-I in 1 L of decoction (V_d). In traditional practice, the daily administered volume is commonly described as one glass (GV), corresponding to approximately 200–250 mL. Therefore, the daily intake of AA-I (40 µg to 50 µg/day) can be estimated as follows (Equation (3)):

$$DI_{AAI} = \frac{GV \times M_{AA-I}}{V_d} \quad (3)$$

4. Discussion

This study aimed to compile an inventory of *Aristolochiaceae* family found in the DRC and list those used in traditional medicine, a useful starting point for toxicity assessment and regulatory actions. Indeed, the genus is well-known for the frequent occurrence of highly toxic aristolochic acids and lactams.

The only species medicinally used in Katanga, *A. heppii* [13,14], was further characterized for microscopical features and aristolochic acid content. *A. heppii* is widely sold on the plant product market in Lubumbashi [13,14]. It is a species endemic to the Miombo forest (DRC, Zambia, Zimbabwe), phylogenetically identified as belonging to the family *Aristolochiaceae*, and is therefore found only in the southeastern part of the DR Congo [19]. Its oblong, woody rootstock, profusely branched at the apex, is the part used in traditional medicine [13,19]. Among its many traditional uses, *A. heppii* is reported for its use to treat diabetes, a property shared with other species of the genus, such as *Aristolochia ringens* Vahl [54]. Moreover, this species is also used in other countries and regions, including Zimbabwe, where it is employed against diarrhea, and South Africa, where it is used as an abortifacient [7].

A. heppii root microscopic characteristics correspond to the general characteristics of underground plant parts, but with a particular type of trichome presenting an horizontal internal cavity; this typical feature allows to apply microscopy to detect the plant presence and search for eventual adulterations of other species.

The chromatographic approach employed in this study combined HPLC-DAD for separation and quantification, together with mass spectrometry for compound confirmation, in accordance with literature recommendations for the analysis of aristolochic acids in herbal materials. The method demonstrated satisfactory system suitability and clear resolution of AA-I and AA-II, consistent with previously reported HPLC-based protocols applied to *Aristolochia* species from other geographic regions [15,16]. The use of HPLC coupled to mass spectrometry allowed identification of AAs based on retention time and m/z information.

The combined HPLC-MS strategy proved suitable for the qualitative confirmation and quantitative assessment of AA-I in *A. heppii*, ensuring the reliability of the reported concentrations and supporting its applicability for the evaluation of potentially toxic constituents in traditional herbal preparations.

Some limitations should nevertheless be acknowledged. Quantification was primarily based on HPLC-DAD, which, although robust and well suited for routine analysis, remains less sensitive than fully MS-based quantitative approaches. The use of mass spectrometry for quantification could allow markedly lower limits of detection and quantification, potentially in the low nanogram range, which would be particularly relevant for trace level exposure assessment and regulatory monitoring. In addition, geographical chemovariability of *A. heppii* may not be fully represented, as only one botanically authenticated field sample was available.

The roots of *A. heppii* were found to contain AA-I at concentrations ranging from 566 $\pm$ 5 ppm (0.057 $\pm$ 0.001%, w/w) to 3533 $\pm$ 32 ppm (0.353 $\pm$ 0.003%, w/w), and in the aqueous decoction at 204.2 $\pm$ 0.4 ppm (0.0204 $\pm$ 0.0001%, w/w). These are over the reported toxicity threshold for AA-I and the regulatory limit set by the Eur. Ph. for herbal products (less than 2 ppm) [5]. As observed in several *Aristolochia* species, the roots contain aristolochic acids and their derivatives [15].

The high amount present in the aqueous extract also exceeds the upper limit established by the Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency (EMA) [5] and as shown in previous studies [41], is therefore likely to induce toxic effects. The manifestation of AAs toxicity may be related to the degree of exposure. High

exposure or ingestion of large doses may lead to acute toxicity (nausea, kidney disease, abdominal pain), whereas repeated ingestion of smaller amounts may result in chronic toxicity [55]. Considering the concentration of AA-I in *A. heppii* aqueous extract (~ 200 ppm) and its resulting oral daily dosage (200 to 250 mL of decoction/day, corresponding to 40 to 50 µg of AA-I/day) over some 7 days (typical use duration), it is plausible that consumers are more likely to be exposed to chronic toxic effects that develop over the long term, including chronic nephropathies, cancers, and other related disorders [55].

Because these effects do not appear acutely and are hardly detectable by tradipraticians, they may obscure the hazardous nature of the species. This situation may be further compounded by the absence of clear scientific identification linking the vernacular name to the correct binomial scientific name, as well as by the lack of formal identification and quantification of AAs in the samples of this species used in DR Congo.

5. Conclusions

AAs are well-established nephrotoxic, carcinogenic, and abortifacient compounds whose adverse effects may manifest as either acute or chronic toxicity, depending on exposure level and duration. Their recognized harmfulness has led to their prohibition or strict regulation in several regions, notably within the European regulatory framework implemented by the European Medicines Agency. Nevertheless, *Aristolochia* species continue to be used in traditional medicine in other parts of the world, particularly in Africa, where regulatory oversight is limited and scientific data remain scarce. In this context, the present study provides new analytical evidence on *A. heppii*, a species commonly used in traditional medicine in the southeastern region of the DRC. The results demonstrate the presence of AA-I at concentrations ranging from 566 $\pm$ 5 ppm (0.057 $\pm$ 0.001%, *w*/*w*) to 3533 $\pm$ 32 ppm (0.353 $\pm$ 0.003%, *w*/*w*). These levels largely exceed established toxicological and regulatory thresholds, indicating a significant potential health risk associated with repeated consumption. Overall, this study contributes to a better understanding of the safety concerns surrounding *A. heppii* and highlights the need for regulatory investigations to support informed public health decision-making in Central Africa.

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