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Inventory of the best-selling medicinal plants on the Lubumbashi markets (DR Congo) and authentication of samples from the 3 most popular species

Cedrick S. Mutombo [a,b,*], Papy M. Moke [a,b], François N. Ntumba [b,c], Salvius A. Bakari [a], Gaël N. Mavungu [b,d], Desiré M. Numbi [e], Alex M. Kolela [a], Cynthia M. Kibwe [a], Vianney N. Ntabaza [a], Victor E. Okombe [d], Amandine Nachtergael [b], Jean-Baptiste S. Lumbu [f], Pierre Duez [b], Joh B. Kahumba [a]

[a] *Laboratory of Pharmacognosy, Department of Pharmacology, Faculty of Pharmaceutical Sciences, Université de Lubumbashi (UNILU), 1825, Lubumbashi, the Democratic Republic of the Congo*
[b] *Unit of Therapeutic Chemistry and Pharmacognosy, Faculty of Medicine and Pharmacy, University of Mons (UMONS), 7000, Mons, Belgium*
[c] *Département d'économie agricole, Faculté des Sciences Agronomiques, Université de Lubumbashi (UNILU), 1825, Lubumbashi, the Democratic Republic of the Congo*
[d] *Unit of Pharmacology and Therapeutic, Faculty of Veterinary Medicine, Université de Lubumbashi (UNILU), 1825, Lubumbashi, the Democratic Republic of the Congo*
[e] *Laboratoire d'écologie et restauration écologique du paysage, Faculté des Sciences Agronomiques, Université de Lubumbashi (UNILU), 1825, Lubumbashi, the Democratic Republic of the Congo*
[f] *Service de Chimie Organique, Département de Chimie, Faculté des Sciences, Université de Lubumbashi (UNILU), 1825, Lubumbashi, the Democratic Republic of the Congo*

A R T I C L E I N F O

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A B S T R A C T

Ethnopharmacological relevance: Knowledge of the high-sale medicinal plants and their authentication are essential parameters to ensure the safety of people using herbal medicine and to plan the safeguarding of medicinal species threatened with extinction.
Aims: The present study, carried out in Lubumbashi, Democratic Republic of Congo, aimed to geolocate medicinal plant sales points, list the best-selling species, and authenticate samples of the most popular species on the market.
Methods: A survey was conducted among the medicinal plant sellers in Lubumbashi's markets and other public spaces, to identify the best-selling species. Samples of the species reported as the most sold were purchased for sales unit weight measurement, and authentication. For the 3 most popular plant species, the identity of 92 samples, purchased from some 25 herbalists, was assessed by combining the microscopic characteristics of powdered drugs with the HPTLC fingerprints of methanolic extracts; for each species, these samples were compared with 1 or 2 botanically authenticated reference samples. As abundant starch granules were detected by microscopy, some samples were suspected of heavy flour contamination, which was confirmed by an enzymatic determination of their starch content.
Results: A total of 108 herbalists (48.1 % women) with a median age of 37 years (range, 20–67 years), and a median seniority of 5 years (0.7–30) were interviewed. From a total of 514 purchased samples, 396 (77.0 %) corresponded to 56 species that could be identified, including 92 samples representing the 3 most frequently sold plant species. The identities of 118 samples (having 82 different vernacular names), could not be determined, due to the lack of voucher specimen. Roots were the most sold organ (56.0 %; n = 514), mainly in powder form (78.7 %), and the median price was 21 USD/kg, at the time of the survey (January to May 2021). The identified specimens were, predominantly, the roots of *Terminalia mollis* M.A.Lawson (33.3 %), *Securidaca longepedunculata* Fresen (28.7 %), and stem barks of *Nauclea pobeguinii* Hua ex Pobég. (23.1 %); from the recorded sales figures, the amounts of material annually sold for these 3 species are estimated at about 5.7, 6.0, and 3.1 tons, respectively. Some sellers reported problems in identifying and/or preserving *S. longepedonculata* and *T. mollis*. Among the 92 samples analyzed for the three species, 18.5 % raised problems, including species substitutions (14/17), dilution with flour (1/17), dilution with flour and species substitution (1/17), and detection of a

* Corresponding author. Laboratory of Pharmacognosy, Department of Pharmacology, Faculty of Pharmaceutical Sciences, Université de Lubumbashi, B.P. 1825 Lubumbashi, the Democratic Republic of the Congo.
E-mail address: ShakalengaM@Unilu.ac.cd (C.S. Mutombo).

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phytochemical variant (1/17), that could arise either from contamination by another species, a particular plant growth environment, or poor storage conditions.

Conclusion: The confusion/adulteration rate measured here for highly popular species is quite alarming (18.5 %); but, indeed, roots, especially as powders, are often difficult to differentiate based on the coarse organoleptic examination practiced by herbal traders. Microscopic and phytochemical characteristics reported in this study, quite easy to obtain with basic laboratory equipment, should be systematically applied by the health products regulatory authorities to control the quality of herbals and ensure that patients get the drug desired for their treatment.

1. Introduction

The commercial exploitation of medicinal plants involves questions about both forest management and quality control of traded materials (Hilonga et al., 2018; Tanga et al., 2018). Indeed, uncontrolled harvesting and/or overexploitation of medicinal plants can constitute a threat of local extinction and, in some cases, to the looting of other sites, which may lead to the disappearance of certain species (Okigbo et al., 2008; Van Andel et al., 2012; Lawin et al., 2016). The combined effects of the gradual increase in demand, the revenues generated by medicinal plant sales, and the fact that most medicinal plants are harvested in the wild by the unsustainable harvesting practices mean that commercialized species are considered overexploited and significantly threatened (Towns et al., 2014). Also, the expensive and best-selling species may be subject to adulteration or substitution, especially because of their economic importance and/or the difficulty in discriminating them from related species (Raman et al., 2015; Srirama et al., 2017).

Studies conducted on the medicinal plant sector in different African countries reported relevant information related to *(i)* the economic importance of medicinal plants (Van Andel et al., 2012); *(ii)* the risk of species misidentification from similar vernacular names (Veldman et al., 2020); *(iii)* the risk of species substitution or adulteration (Ouarghidi et al., 2012); *(iv)* the threat of extinction of certain species (Williams et al., 2013; Towns et al., 2014); and *(v)* the lack of sufficient data related to their trade, especially in DR Congo, recognized as one of the world's important forest reserves (Kobongoso et al., 2019). To date, two studies documented approximately 60 medicinal plant species sold in more than 10 markets in Kinshasa (DR Congo) and their uses, as well as the characteristics of sellers (Ngbolua et al., 2016, 2019). In Lubumbashi (Haut-Katanga), medicinal plants flow, actors and socio-economic issues, as well as the trade-related disappearance of *Securidaca longepedunculata*, has recently been investigated (Chuimika et al., 2023; Ntumba et al., 2024).

Recently, Stévart et al. (2019) stated that (*i*) 1/3 of the flora of tropical Africa is potentially threatened with extinction; (*ii*) DR Congo is among the countries affected by this threat; and (*iii*) it is in the region of the ex-Katanga province (area concerned by this study) where this threat is very high. Studies carried out in the Lubumbashi region (Haut-Katanga province) also reported high rates of gradual decrease in forest area (Useni et al., 2017, 2020), which is inversely proportional to the degree of urbanization (Useni et al., 2018). In several African countries, there is evidence that commercial harvesting of medicinal plants to cater to a growing urban population has become an environmentally destructive activity (Towns et al., 2014). In Lubumbashi, no study has yet been conducted in relation to the best-selling medicinal plants, or the vulnerability level of plant species with medicinal interest.

In African countries, including DR Congo, the quality and safety of traded medicinal plants is notably compromised by the lack of compendial standards, as very few pharmacopoeia-type analytical monographs are available. It is also essential to understand the trade of plant species and to develop simple and effective methods to control the identity and quality of their drugs offered for sale (Van Andel et al., 2012). A wealth of analytical techniques involving microscopy, chromatography, spectroscopy and genomics are proposed to control the quality of traded medicinal plants (Vermaak et al., 2010; Nicoletti, 2011; Nithaniyal et al., 2017; Masondo and Makunga, 2019; Veldman et al., 2020; Muyumba et al., 2021), but it's important for developing countries to rely on locally available methods; both microscopy and (high-performance) thin-layer chromatography appear to be methods of choice, given their reasonable feasibility with limited equipment and laboratory facilities. And, indeed, reliable data on the exploitation and quality control of medicinal plants are essential for sustainable and efficient ecosystem management (Volenzo and Odiyo, 2020) and for the integration of traditional medicine into the official health system (Mutombo, 2022).

The present study aims to develop knowledge of the commercial exploitation circuit of medicinal plants in Lubumbashi, authenticate samples from the most popular species, and appraise the sustainability of forest resources in medicinal plants. These data intend to orientate future strategies to (*i*) preserve and/or multiply species of medicinal interest; (*ii*) control the quality of traded herbal products; and (*iii*) to develop adequate and sustainable integration of traditional medicine into the official health system.

2. Methods

2.1. Ethnobotanical survey

2.1.1. Study area

This study was conducted in Lubumbashi, Haut-Katanga province, in the southeast of the DR Congo, near the Republic of Zambia border (Fig. 1). Lubumbashi is the economic capital of DR Congo and the second largest city (11°27′ to 11°47′ S; 27°20′ to 27°40′ E) (Kasongo et al., 2018). With an area of 747 km^2, Lubumbashi is subdivided into 7 municipalities and 44 neighborhoods (Mutombo et al., 2021). This city is home to a diverse population, coming from all provinces of DR Congo. The local health profile is typical of tropical regions, characterized by the predominance of infectious and parasitic diseases; so-called *"local diseases,"* i.e., uncharacterized in conventional medicine but specifically treated by traditional practitioners, have also been reported (Crump, 2012; Becker et al., 2013; Kalunga et al., 2014; Mutombo et al., 2018, 2022). In Lubumbashi, the health care offer is based on a rather independent/unconnected mix of conventional, traditional (i.e., recourse to a traditional practitioner) and folk (i.e., self-medication with natural substances, predominantly medicinal plants) medicines (Chenge et al., 2014; Bashige et al., 2020; Manya et al., 2021; Mutombo et al., 2022). Medicinal plants are either collected in the nearby environment or purchased from herbalists located in different municipalities (Toirambe, 2007; Kahumba et al., 2015).

2.1.2. Surveyed population

Due to the lack of reliable data on the population size of herbalists in Lubumbashi, we sought to interview all the medicinal plant sellers who were accessible through markets and other public spaces. And so, all herbalists encountered from January to May 2021 were asked to participate in this survey. Out of a total of 124 herbalists met, 108 agreed to participate.

2.1.3. Ethical considerations

This study was approved by the Ethics Committee of the Université de Lubumbashi, under the number UNILU/CEM/087/2022. The ethical requirements of international organizations, including the Helsinki Declaration (World Medical Association, 2013), the European general regulations on personal data (EU, 2018), and the Nagoya Protocol (United Nation, 2011), were referred to for guidance on informed consents, on protection and anonymization of personal data, and on access to genetic resources, including the fair and equitable sharing of benefits. Before beginning the interview, each herbalist was briefed on the objectives of the study, the importance of their responses, the privacy and data use policy, and their freedom to participate or not in the survey.

2.1.4. Data collection

The coordinates of each surveyed site were collected in decimal degree (DD) format with a Garmine Etrex H GPS. A 22-item questionnaire (Supplementary data S1) was administered in semi-structured interviews, for approximately 50–60 min per herbalist, in Swahili. The

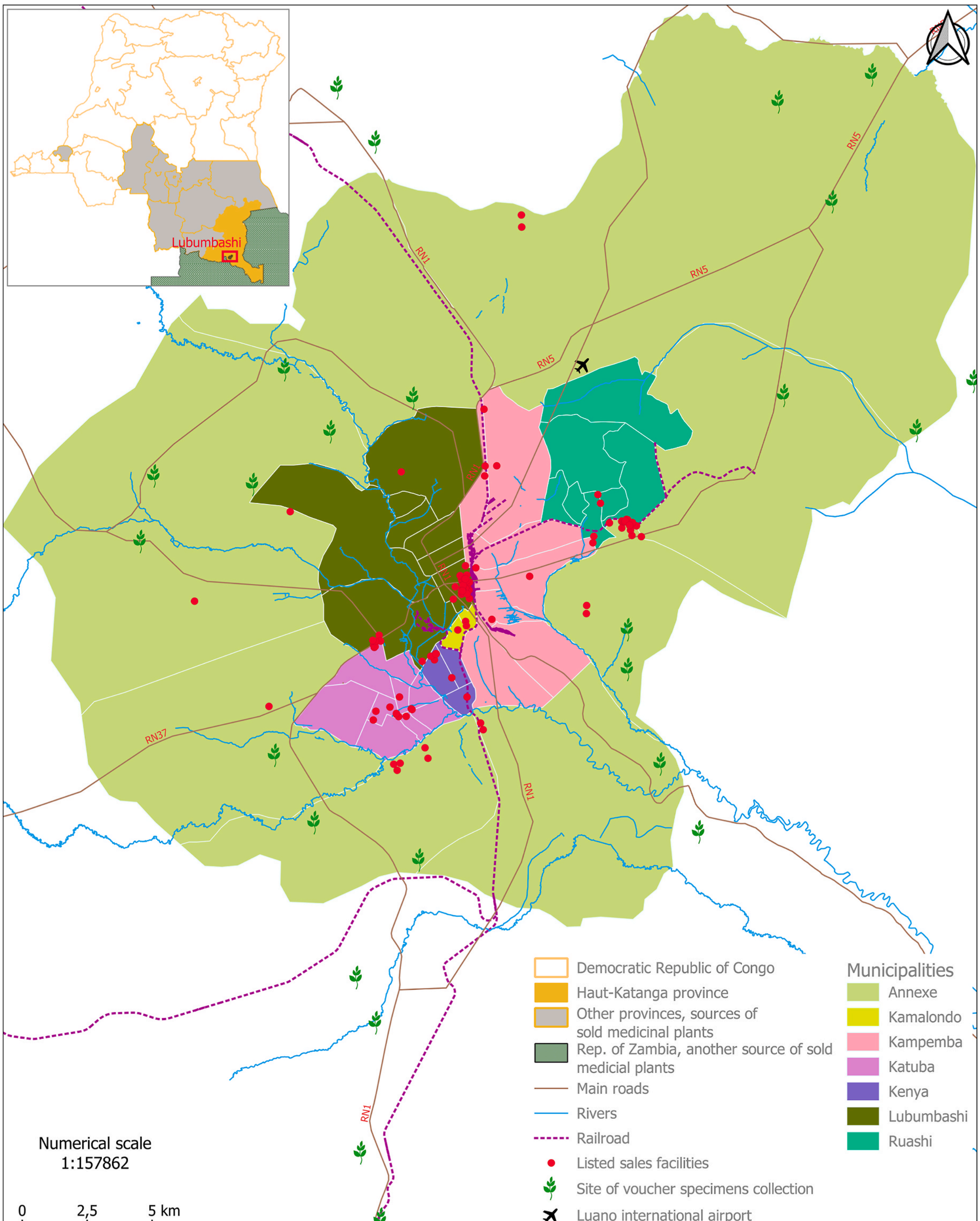

Fig. 1. Map of Lubumbashi municipalities indicating the geolocation of medicinal plant sales points and locations for collecting voucher specimens. The upper map presents the other regions of DR Congo (gray) and Zambia (green) from which medicinal plants were stated to come from (Map created using QGis 3.28.3 Firenze). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

information sought mainly concerned (*i*) the geolocation of sales points; (*ii*) the medicinal plant supply and sale chain; and (*iii*) the informant sociodemographic profile. For each medicinal plant cited among the best-selling, a sample was purchased and packaged in a plastic bag. To trace the samples to their respective sellers, a coding system was applied by combining an abbreviation for the municipality where the sample was purchased (AN = Annexe, KM = Kamalondo, KN = Kenya, KT = Katuba, LB = Lubumbashi, RS = Ruashi), a stall number arbitrary assigned for this municipality (Et1, Et2, … Etn), and a sample number for this stall (P1, P2, P3, … Pn). All dried or powdered samples were stored in the Laboratory of Pharmacognosy (Université de Lubumbashi), while the fresh materials were dried at room temperature, protected from humidity and sunlight.

2.1.5. Identification of species

Although some species (best-known medicinal plants, very popular in our region, and very easily identifiable by macroscopic observation, such as cloves or ginger rhizomes) could be identified by simply examining the purchased specimens, others were to be collected in their natural environment (Fig. 1), with the help of experienced herbalists – harvesters. Voucher specimens of the purchased and *in natura*-collected samples were deposited at the herbarium of INERA – Kipopo (Lubumbashi) for their botanical identification by D.M. Mbangu, who used taxonomic keys. For the 3 most abundant species on the market, a second voucher specimen was also deposited at the herbarium of the botanical garden of Meise (Brussels) for a second botanical identification by P. Meerts (Université Libre de Bruxelles). Correspondence between the scientific names attributed by the botanists and the vernacular names used on the market was also verified from previous ethnobotanical works in the study area (Malaisse, 1997; Lumbu et al., 2004; Meerts and Hasson, 2016). The accepted scientific names and updated botanical families were checked with The World Flora Online (http://www.worldfloraonline.org/) and the Kew Herbarium Catalogue (http://www.plantsoftheworldonline.org/). Certain species not native to the Lubumbashi region could not be formally identified due to the lack of herbaria and samples presenting conclusive macroscopic characteristics.

2.2. Analysis of samples of the 3 highest-sales species

Given the risk of adulteration, substitution, or confusion of the high-sales species (Srirama et al., 2017), these were prioritized for quality control. Microscopic and phytochemical profiling (Muyumba et al., 2021) were performed to authenticate the species found in more than 20 % of plant sales stalls. A total of 92 samples (36 for *Terminalia mollis* M.A. Lawson, 31 for *Securidaca longepedunculata* Fresen, and 25 for *Nauclea pobeguinii* Hua ex Pobég.) were analyzed. For each of these species, a reference sample, corresponding to the organ found on the market, was collected from a plant identified *in natura*.

2.2.1. Light microscopy

Micromorphological analyses were performed at the Laboratory of Pharmacognosy (Faculty of Pharmaceutical Sciences, Université de Lubumbashi, 1825 Lubumbashi, DR Congo) with a Nikon Eclipse E200 microscope, equipped with a camera (Xcam Family 1080p), and a monitor. Briefly, a few mg of powder from each sample were finely spread on a slide, and then a drop of 50 % chloral hydrate was added (Council of Europe, 2020). The preparations were then covered with coverslips gently heated for a few minutes and observed under the 10x objective after cooling, correcting magnification to 100×. The microscopic elements were identified from reference documents (Zhao et al., 2005; Chanda et al., 2010; Upton et al., 2011; Chothani and Patel, 2012; Salih et al., 2013).

2.2.2. Phytochemical profiling

These analyses were performed at the Unit of Therapeutic Chemistry and Pharmacognosy (Faculty of Medicine and Pharmacy, University of Mons, 7000 Mons, Belgium). The collection and transport of medicinal plant specimens was authorized by the Ministry of Environment of the DR Congo through its letter n° 745/CAB/MIN/EDD/AAN/TNT/SAA/2018 concerning the PhytoKat project.

2.2.2.1. High performance thin-layer chromatography. High performance thin layer chromatography (HPTLC) of methanolic extracts was performed according to the European Pharmacopoeia requirements (Council of Europe, 2021) with equipment provided by *CAMAG* and consisting of (*i*) an automatic TLC sampler (ATS 4), (*ii*) an automatic development chamber (ADC 2), (*iii*) a semi-automatic derivatizer, (*iv*) a heating plate, and (*v*) a visualizer. This equipment was controlled by VisionCats 2.5 software. Extracts were prepared by mixing 1 g of the powder of each sample with 10 mL of methanol, sonicating for 30 min at 40 °C, macerating for 24 h, and centrifuging (4000 g, 25 °C, 10 min). The clear supernatants were recovered and stored at 4 °C for a maximum of 2 months. Aliquots of 2.5–4.0 μL were applied in 7.5–8.0 mm wide bands onto 10 × 20 cm HPTLC Silica gel 60 F_{254} plates (Merck, Germany). Prior to development, the plates were exposed for 20 min to ~33 % relative humidity through an air stream obtained with a saturated $MgCl_2$ solution (Council of Europe, 2021). The migration distance was 70 mm. Applied mobile phases, reference substances, and derivatization reagents are listed in Table 1.

2.2.2.2. HPLC – DAD analyses. Samples that seemed different from references, according to microscopy and HPTLC profiles, were analyzed in high-performance liquid chromatography (HPLC). A 5 μL aliquot of the HPTLC extracts (with 5x dilution for *T. mollis*) was injected into an Agilent Analytical HPLC Systems (1260 Infinity II LC System; Luna® 5 μm, C18, 100 Å, 250 × 4.6 mm column, with a precolumn, C18, 5 μm, 7.5 mm × 4.6 mm). The mobile phase was acetonitrile (A) and 1.0% formic acid in Milli-Q water (B), with 3 gradients corresponding to the 3 analyzed species (Table 2). The flow rate was 1.0 mL/min, the column temperature was maintained at 25.00 ± 0.03 °C, and the diode array detector (DAD) was set at 254 and 280 nm for chromatogram acquisition.

2.2.2.3. Total starch determination. Microscopic analysis detected in 2 commercial samples (Figs. 4, 2h and 2i) of *S. longepedunculata*, very large amounts of starch grains, morphologically different from those observed in the reference samples and suggestive of contamination. To assess the level of contamination in these samples, total starch was quantified in these 2 suspect samples, 2 reference samples (harvested), and 5 samples randomly selected from those purchased on the market and authenticated by microscopy and HPTLC.

The moisture content of each sample was determined according to the (Official Association of Analytical Chemists, 1990) and the starch was enzymatically quantified in 100 mg of sample power using the commercial kit K-TSTA-100A (Megazyme International, Ltd., Wicklow, Ireland) (McCleary et al., 2019), following the instructions of the manufacturer. Analyses were performed in triplicate, and the values were calculated in % of total starch vs. dried drug.

2.3. Data processing

2.3.1. Cartography

The Global Positioning System (GPS) coordinates collected during the survey were used to geolocate the medicinal plant sales points in Lubumbashi, and the site where voucher specimens were collected. Shapefiles showing the administrative subdivision of Lubumbashi, its road, hydrographic, and railway networks were obtained from the Geography Department of the Faculty of Sciences (Université de Lubumbashi); the maps were generated with QGis 3.28.3 Firenze (QGIS).

Table 1
HPTLC conditions.

Phytochemical group	Species	References	Mobile phases	Detection
Phenolic compounds	*Terminalia mollis*	Rosmarinic acid, ferulic acid, vitexin	Toluene - acetone - formic acid - water (60:30:10:10; v/v)	The plate is dried at 100 °C for 3 min and then derivatized while still warm with 2 mL of Neu 1% reagent (Green Nozzle, Level 3) and 2 mL of PEG400 5% (Blue Nozzle, Level 3) and photographed under UV at 366 nm after each reagent
	Securidaca longepedunculata	Sakuranetin, magnolol, sinapic acid, caffeic acid	Dichloromethane - methanol - acetone - water (70:20:5:3; v/v)	
	Nauclea pobeguinii	p-Coumaric acid, sinapic acid, resveratrol	Ethyl acetate - glacial acetic acid - formic acid - water (100:11:11:26; v/v)	
Terpenoids	*Terminalia mollis* *Securidaca longepedunculata*	Friedelin, Maslinic acid	Dichloromethane - methanol - water (75:20:3; v/v)	The plate is derivatized with 2 mL of anisaldehyde - sulfuric acid (Nozzle blue, level 4), heated at 110 °C for 3 min, then photographed under visible light and at 366 nm.
	Nauclea pobeguinii	Ursolic acid, maslinic acid		
Alkaloids	*Nauclea pobeguinii*	Strictosamide	Toluene - acetone - methanol (65:20:10; v/v)	The plate is derivatized with 2 mL of Dragendorff' reagent (Red Nozzle, level 3) and photographed under visible light.

Table 2
Gradients applied for HPLC analyses.

Species	Gradient		
	Time (min)	% Solvent A	% Solvent B
T. mollis	0	5	95
	2	5	95
	9	10	90
	15	20	80
	20	35	65
	25	95	5
	27	100	0
	37	100	0
S. longepedunculata	0	15	85
	2	15	85
	10	40	60
	20	40	60
	30	100	0
	40	100	0
	42	15	85
N. pobeguinii	0	5	95
	2	5	95
	9	10	90
	15	20	80
	30	65	35
	35	100	0
	45	100	0

2.3.2. Survey data

The abundance of each species on the markets studied was calculated as the percentage of herbalists who sell it among all interviewed. The vulnerability (*Vu*) of a species listed on the Lubumbashi market was estimated (Equation (1)) from *(i)* the number of sellers (Ab); *(ii)* an arbitrary sales score (Fs; set to 2 for sale ≥1 kg/day; to 1 for sale ≥500 g; to 0.5 for sale <500 g); *(iii)* the estimated amount harvested per month (Rq) in kg; *(iv)* the number of sold organs (Up) and their vital importance for the plant (Pi; set to 3 for the whole plant; to 2 for roots or rhizomes; to 1.5 for stem barks; to 1 for leaves, flowers, and fruits) (Lawin et al., 2016); *(v)* the number of stated harvesting sites (Hs); and *(vi)* the plant availability in the harvesting site (D_m, as declared by the harvester). The harvester declarations have been assigned numerical values, including 4 = very abundant, 3 = abundant, 2 = rare, and 1 = very rare.

$$Vu = \frac{(A_b + F_s) \times \left(R_q + U_p + \sum P_i\right)}{H_s + D_m} \quad \text{(Equation 1)}$$

Species were considered vulnerable or threatened with extinction for $Vu > 50$. Each species was also checked for its eventual presence on the *Red List of the International Union for Conservation of Nature and Natural Resources* (https://www.iucnredlist.org/).

2.3.3. Volumes sold and market values

Purchased samples were weighed in the laboratory, and purchase prices in Congo Democratic Francs (CDF) were converted to USD at the rate of 2000 CDF to 1 USD. The weight of the sales unit was multiplied by the number of units sold per day to estimate (*i*) the average amount of material sold per day per herbalist; and (*ii*) the sum in kg of material sold per day, per month, and per year in the whole city. The monthly harvest quantities in kg reported by interviewed herbalists allowed to estimate an average per herbalist per month and for all herbalists per year.

2.3.4. Statistical analysis

Statistical tests (Pearson, Sperman, Chi-square, Fischer's exact test, Student's t-test, Mann-Whitney test, one-way ANOVA, and Kruskal-Wallis) were performed with GraphPad Prism 10 software (GraphPad Software, La Jolla, USA), with the confidence level and margin of error set at 0.95 and 0.05, respectively.

3. Results and discussion

3.1. Survey data

3.1.1. Interviewed herbalists

Our extensive survey of the medicinal plant markets identified 124 medicinal plants sales points in 7 Lubumbashi municipalities (Fig. 3) and therefore 124 herbalists, from which 108 accepted to answer our survey. From these herbalists, 48.1 % were women, a proportion statistically comparable to that of men ($p = 0.5862$). In TM practice, women are generally numerous in the sale of medicinal plants, which is often reported in African countries, e.g., in Ghana (Van Andel et al., 2012).

Most (75.9 %; n = 108) of herbalists self-identify as "healers" ($p < 0.0001$), most of whom started as traditional practitioners before beginning herbalism (Table 3). The predominance of *"healers"* among herbalists can be considered an asset as consumers may benefit from adequate advice and precautions for use (Table 3). All stated to be motivated mainly by the need for money and, at 79.6 %, reported being satisfied with the income generated. This confirms previous reports from Pakistani communities that the sale of medicinal plants is an important source of income (Sher et al., 2014).

The median age of interviewed herbalists was 37 years (range 20–67 years) with a median seniority of 5 years (range: 9 months–30 years); age and seniority were statistically comparable ($p = 0.3181$ and 0.7204, respectively) between men and women. In Lubumbashi, most herbalists have accumulated experience in this activity, which probably give them

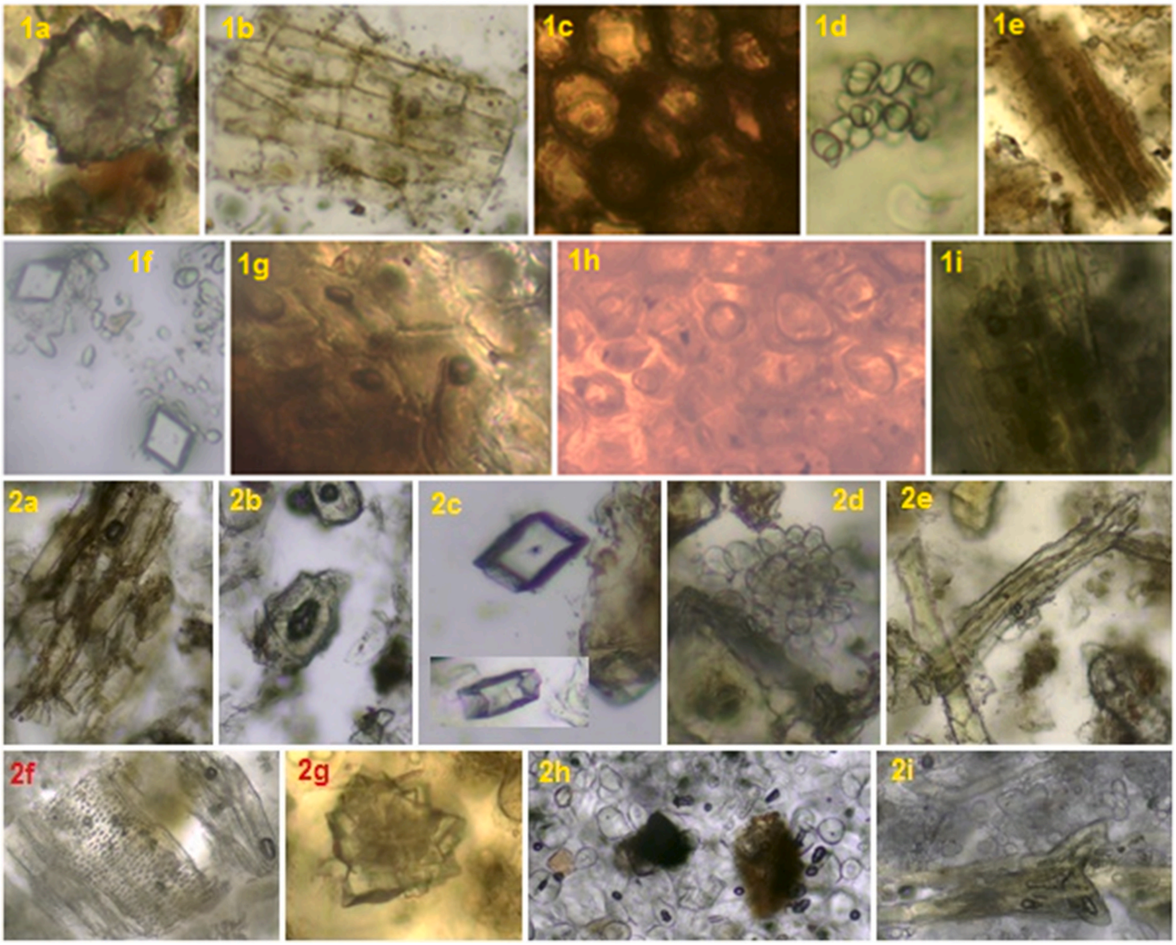

Fig. 2. Microscopic characteristics of reference samples of *T. mollis* (1) and *S. longepedunculata* (2), as well as atypical samples purchased as *"Kibobo"*, *"Mbubu"*, or *"Tshibangu mutshi"* (*T. mollis*) and *"Mweyeye"* or *"Lupapi"* (*S. longepedunculata*). Observation in 50 % chloral hydrate, under a 10× objective. For *T. mollis*: references (1a = oxalate prism crystal; 1b = parenchyma tissue; 1c = sclerenchyma tissue; 1d = starch grains, and 1e = Xylem); atypical samples (1f = oxalate crystals; 1g and 1h = sclerenchyma tissue; 1i = parenchyma tissue). For *S. longepedunculata*: references (2a = parenchyma tissue; 2b = sclerenchyma cells; 2c = oxalate crystals; 2d = starch grains and 2e = fibers); atypical samples (2f = punctate vessels; 2g = oxalate prism crystal; 2h = starch grains in sample 10 and 2i = starch grains and sclerenchyma tissue in sample 11).

a certain mastery in the identification and harvesting of species, processing of drugs, selection of the most sought-after species, setting of prices, *etc*. The age and seniority of the interviewed herbalists were not correlated (Pearson r = 0.3317; R^2 = 0.1100), as most of them started their activity quite late in their lives.

The education level is dominated by high school (58.3 %), most frequently for men compared to women ($p = 0.013$) (Table 3); this is in line with the general population (INS, 2014). Overall, 45.4 % were engaged in the sale of medicinal plants as their sole activity, with the remainder also engaged in other activities, notably farming (45.8 %; n = 59) and the sale of vegetables and spices (23.7 %; n = 59); the latter activity was primarily cited by women ($p = 0.031$).

3.1.2. Sold species and therapeutic uses

A total of 514 medicinal plant samples were purchased from the *"most sold"* (as stated by each herbalist), from which 396 (77.0 %) represented 56 identified species and 118 (corresponding to 82 vernacular names) we could not yet formally identify, as they were sold as powder, root, or stem bark pieces. The number of different species we could identify is similar to Ethiopia (50 species) (Kebede et al., 2016), higher compared to Kinshasa markets (Ngbolua et al., 2016, 2019), but lower compared to Gabon (Towns et al., 2014), Tanzania (Hilonga et al., 2018; Veldman et al., 2020), Ghana (Van Andel et al., 2012), Madagascar (Randriamiharisoa et al., 2015), and Ecuador (Tinitana et al., 2016). These data are certainly difficult to compare as, according to Randriamiharisoa et al. (2015), the number of species that can be identified in each market is limited both by the length of the survey and the fact that herbalists only list species available at the time of the survey.

Regarding the 118 unidentified samples, 73.7 % (74 vernacular names) were each sold by only one or two herbalists, who reported bringing them from distant provinces (Kongo Central, Maniema) or from foreign countries, notably Zambia. These distant origins may explain the absence of these species in the Lubumbashi area where we collected voucher specimens. Such unidentified samples were also reported in Gabon (Towns et al., 2014), and Tanzania (Hilonga et al., 2018).

The identified species belong to 55 genera grouped in 36 families, with a predominance of Fabaceae (10.7 %) and Euphorbiaceae (5.4 %), which is in line with previous works (Hilonga et al., 2018; Kebede et al., 2016). These 2 families are generally classified among those containing the largest number of medicinal plant species (Allkin, 2017) and Fabaceae have also been reported to be the most abundant family in Haut-Katanga and Lubumbashi areas (Useni et al., 2019; Mavungu et al., 2023).

Overall, for the 514 *"most sold"* herbs, the organs are mainly roots (56.0 %) and stem barks (23.3 %), generally in powder form (78.7 %), to be prepared by maceration, decoction, or infusion, for oral administration (91.7 %); this confirms previous ethnobotanical studies conducted

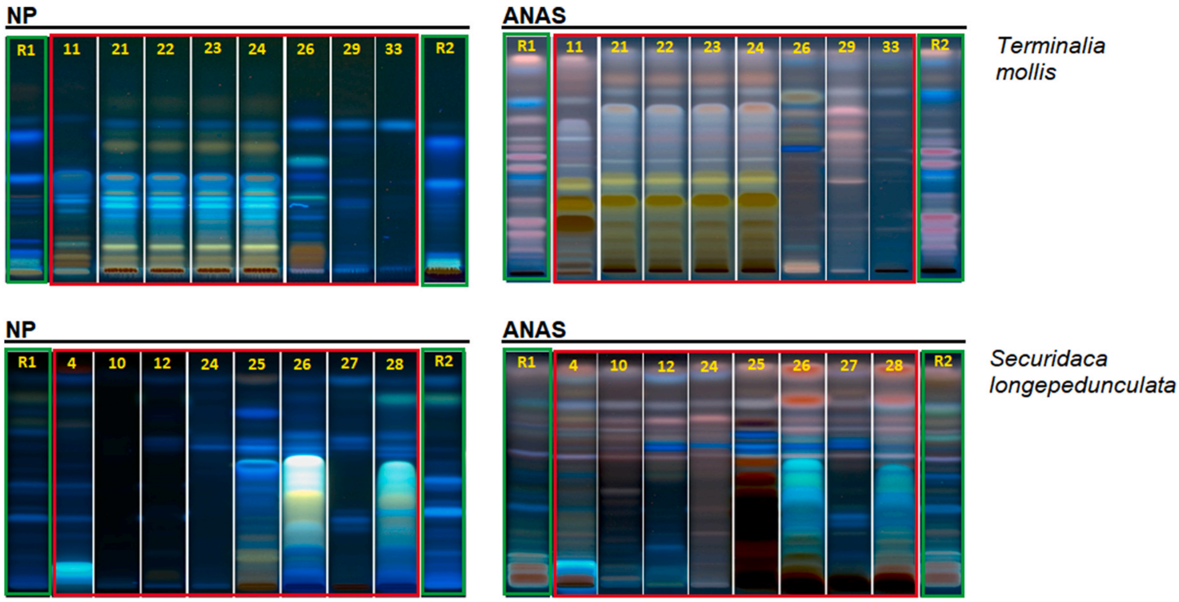

Fig. 3. HPTLC fingerprints of the methanol extracts of reference samples (R1 & R2) of *T. mollis* and *S. longepedunculata*, as well as the atypical samples purchased as *"Kibobo"*, *"Mbubu"*, or *"Tshibangu mutshi"* (*T. mollis*) and *"Mweyeye"* or *"Lupapi"* (*S. longepedunculata*). HPTLC profiles of reference samples are boxed in green and those of samples that showed atypical are boxed in red. The profiles of the other samples are presented in Supplementary data S2. These profiles were developed by applying 2.5 and 4.0 μL for *T. mollis* and *S. longepedunculata* respectively onto 10 x 20 Silica gel F_{254} HPTLC plates; (NP) derivatized with NEU reagent [2-aminoethyl diphenyl borinate (1 % in methanol) and polyethylene glycol 400 (5 % in methanol)] and eluted with a mixture of toluene/acetone/formic acid/water (60:30:10:10; v/v) for *T. mollis* and Dichloromethane/methanol/acetone/water (70:20:5:3; v/v) for *S. longepedunculata*; (ANAS) derivatized with anisaldehyde - sulfuric acid (2 mL) and eluted with a mixture of dichloromethane/methanol/water (75:20:3; v/v), observed under UV (366 nm). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

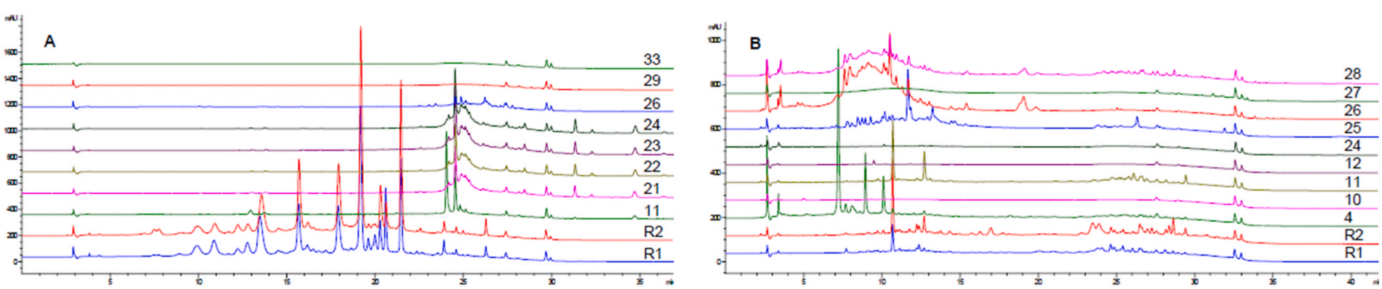

Fig. 4. HPLC-DAD fingerprints (254 nm) of the methanolic extracts of the reference samples (R1 & R2) of *T. mollis* and *S. longepedunculata*, as well as the atypical samples purchased as *"Kibobo"*, *"Mbubu"*, or *"Tshibangu mutshi"* (*T. mollis*) and *"Mweyeye"* or *"Lupapi"* (*S. longepedunculata*). HPLC chromatograms obtained with an Agilent Analytical HPLC Systems (1260 Infinity II LC System; Luna® 5 μm, C18, 100 Å, 250 × 4.6 mm column, pre-column, C18, 5 μm, 7.5 mm × 4.6 mm), mobile phase: acetonitrile (A) and 1 % formic acid in Milli-Q water, Gradient for *T. mollis*: 5 % (A) 0–5 min, 10–95 % (A) 9–25 min and 100 % (A) 27–37 min), injection 5 μL (with 1/5 dilution). Gradient for *S. longepedunculata*: 15 % (B) 0–2 min, 40 % (B) 10–30 min, 100 % (B) 30–40 min and 15 % (B) 42 min), injection 5 μL.

in Lubumbashi (Okombe et al., 2014; Bashige et al., 2017; Bakari et al., 2018; Maloba et al., 2020; Mavungu et al., 2023). Of note, roots are vital organs for plants (Lawin et al., 2016) and their large exploitation could contribute to highly traded species vulnerability (Lawin et al., 2016; Tanga et al., 2018). Powders appear to be the preferred form of herbalists, stated to facilitate dispensing in uniform measures but also use by the consumer. Nevertheless, this drug form singularly complicates the identification of samples (Muyumba et al., 2021); indeed, the adulteration and falsification of powders is reported to be much more common compared to samples sold in whole form (Amritha et al., 2020). This predominance of powders on Lubumbashi markets indicates a definite need for rapid and efficient methods to assess their identity (Van Andel et al., 2012), a problem even more important for the numerous samples still unidentified.

In Lubumbashi, these 514 *"most sold"* herbs are destined for the treatment of various disorders, predominantly hemorrhoids (20.0 %), erectile dysfunction (18.6 %), and infectious and parasitic diseases (18.2 %) (Table 5).

Considering the number of species per stall, the presence of different organs/forms from the same species, and the similarities in aspects and organoleptic characteristics (Zhao et al., 2011; Muyumba et al., 2021), a correct labeling would be essential to ensure the quality of dispensing (Vredenburgh and Zackowitz, 2009). However, in the stalls visited, these 514 *"most sold"* samples were either unlabeled (49.6 %), or labeled by their vernacular names (29.1 %), by the disease they treat (18.9 %), or by both (2.4 %). As a vernacular name meaning and accuracy can vary depending on the ethnic origin of the seller and customer (Bashige et al., 2015; Veldman et al., 2020; Mutombo, 2022), the risk of species substitution is not negligible. For herbalists, a correct labelling of medicinal plants would require the language of origin to be indicated alongside the vernacular name used and/or combined with the botanical name, especially for drugs to be sold on distant markets. The predominance of unlabeled products on the market has been explained by the predominance of orality in traditional medicine (Maluleka and Ngulube, 2017) but, in the absence of quality control, this most likely adds confusion, especially when labeled by disease name; indeed, the same

Table 3
Socio-demographic characteristics of the interviewed herbalists.

Variables	Modalities	Gender			Total	
		M n = 56	F n = 52	Correlation between variable and gender [a]	n	%
Reasons for starting to sell medicinal plants	Treatment of diseases with plants	42	35	0.780 ($p = 0.377$)	77	71.3
	Lack of income-generating activities	13	15	0.445 ($p = 0.505$)	28	25.9
	Sale of vegetables, fruits, and spices	1	2	0.402 ($p = 0.526$)	3	2.8
Categories	"Healer"	45	37	1.249 ($p = 0.264$)	82	75.9
	"Not a healer"	11	15	1.249 ($p = 0.264$)	26	24.1
Age range (years)	25–30	10	18	3.943 ([b])	28	25.9
	31–40	21	12	2.643 ($p = 0.104$)	33	30.6
	41–50	17	9	2.512 ($p = 0.113$)	26	24.1
	51 and more	8	13	1.976 ($p = 0.160$)	21	19.4
Seniority in the sale of medicinal plants	1–5 years	30	25	0.326 ($p = 0.568$)	55	50.9
	6–10 years	11	18	3.077 ($p = 0.079$)	29	26.8
	11–15 years	10	4	2.469 ($p = 0.116$)	14	13.0
	16 years and more	5	5	0.015 ($p = 0.902$)	10	9.3
Frequency of sales per week	Every day	33	31	0.005 ($p = 0.942$)	64	59.3
	Monday to Saturday	18	20	0.472 ($p = 0.492$)	38	35.2
	Other special schedules	5	1	2.522 ($p = 0.112$)	6	5.5
Study levels	No studies	8	11	0.877 ($p = 0.349$)	19	17.6
	Primary	4	10	3.492 ($p = 0.062$)	14	13.0
	Secondary	39	24	6.121 ([b])	63	58.3
	University	4	1	1.664 ($p = 0.197$)	5	4.6
	No answer	1	6	4.231 ([b])	7	6.5
Other major professions or occupations	None	25	24	0.025 ($p = 0.875$)	49	45.4
	Agriculture	18	9	3.165 ($p = 0.075$)	27	25.0
	Sales of vegetables and other items	3	11	5.963 ([b])	14	13.0
	Other activities	3	4	0.243 ($p = 0.622$)	7	6.5
	No answer	7	4	0.681 ($p = 0.409$)	11	10.1

[a] Chi-square or Fischer's exact test.
[b] Statistically significant correlation or dependence.

Table 4
Species reported by at least 5 sellers as being the most sold. In this table, species are listed in descending order of abundance on the Lubumbashi markets.

Scientific name (Family)/ Voucher number	Names used in the market (language [a])	Abundance n (%)	Used organ[b]	Forms	Most frequent indications	Price (USD [c]/kg)	Daily sold quantity (kg) per seller	Monthly supply [d] (kg)/ seller	Hs[e]	St [f]	D [g] [Vu][h]	UICN [i] threatens status
Terminalia mollis M.A. Lawson. (Combretaceae)/ KIP154964751	Kibobo (La, Bb) Mbubu (Lb) Tshibangu mutshi (Tshiluba)	36 (33.3)	R	Pieces [Pr], Powder	Hemorrhoids, abdominal pain, low back pain	16.5 ± 14.4	0.51 ± 0,32	12.9 ± 8.2	17	Wld	3 [24.0]	LC
Securidaca longepedunculata Fresen. (Polygalaceae)/ KIP143459255	Mweyeye (Lb) Lupapi (Bb)	31 (28.7)	R	Powder [Pr], Pieces	Sexual weakness, typhoid fever, sinusitis, constipation	24.4 ± 19.0	0.62 ± 0.52	20.9 ± 10.1	22	Wld	3 [27.7]	LC
Nauclea pobeguinii Hua ex Pobég. (Rubiaceae)	Mujilanga (Tsb) Kibe (Lb)	25 (23.1)	SB	Pieces [Pr], Powder	Hemorrhoids, malaria, digestive disorders	14.2 ± 9.9	0.40 ± 0.16	20.8 ± 9.3	7	Wld	2 [61.2]	LC
Senna petersiana (Bolle) Lock (Leguminosae)/ KIP452336122	Kafungunansha (Bb) Kavungu mbele (Lb)	22 (20.4)	R	Powder, Pieces	Hemorrhoids, hernia, malaria, sexual weakness	22.7 ± 19.2	0.29 ± 0.14	17.4 ± 8.2	15	Wld	4 [20.8]	LC
Albizia adianthifolia (Schumach.) W. Wight. (Fabaceae)/ KIP365059075	Kapeta nzovu (Bb)	18 (16.7)	R	Pieces [Pr], Powder	Dentary decay, sexual weakness, genital infection	27.4 ± 24.2	0.28 ± 0.12	12.1 ± 7.9	11	Wld	4 [16.7]	LC
Piper nigrum L. (Piperaceae)/ KIP343445554	Nketu (Sw)	16 (14.8)	SD	Seed	Hemorrhoids, oxyurosis, hip pain	43.9 ± 29.3	0.13 ± 0.8	14.3 ± 9.8	7	Wld	3 [13.3]	Not listed
Phyllanthus muellerianus (Kuntze) Exell. (Phylantaceae)/ KIP596015377	Lulembalemba (Lb), Mulembalemba (Hb)	13 (12.0)	R	Pieces [Pr], Powder	Menstrual disorders, sores, hernia, genital infection	32.1 ± 19.7	0.45 ± 0.32	7.5 ± 5.6	10	Wld	3 [8.0]	Not listed
Senegalia brevispica (Harms) Seigler & Ebinger (Fabaceae)/ KIP423441865	Munga (Lb) Mutonge	13 (12.0)	R	Pieces [Pr], Powder	Diabetes, hernia, menstrual disorders	32.6 ± 29.0	0.49 ± 0.34	12.0 ± 8.4	11	Wld	3 [11.8]	LC

(continued on next page)

Table 4 (*continued*)

Scientific name (Family)/ Voucher number	Names used in the market (language [a])	Abundance n (%)	Used organ[b]	Forms	Most frequent indications	Price (USD [c]/kg)	Daily sold quantity (kg) per seller	Monthly supply [d] (kg)/ seller	Hs[e]	St [f]	D [g] [Vu][h]	UICN [i] threatens status
Crossopteryx febrifuga (Afzel. ex G. Don) Benth. (Rubiaceae)/ KIP386319397	Mutotshi	13 (12.0)	ST, R	Powder [Pr], Pieces	Abdominal pain, hemorrhoids, pinworms	20.7 ± 11.2	0.13 ± 0.12	14.4 ± 9.5	4	Wld	3 [28.7]	LC
Cussonia cobisieri De Wild (Araliaceae)/ KIP338686183	Ntambo mutshi (Lb) Ntambwe mutshi (Tsb) Pudriko (Sw)	13 (12.0)	R, ST	Powder [Pr], Pieces	Sexual weakness, rheumatism, sexual impotence	67.6 ± 26.3	0.30 ± 0.28	16.6 ± 9.2	10	Wld	3 [17.6]	Not listed
Fagara mortehanii De Wild. (Rutaceae)/ KIP157475759	Pupwe kyulu (Lb)	11 (10.2)	R	Powder [Pr], Pieces	Stomachache, ovarian cyst, genital infection	17.0 ± 15.7	0.18 ± 0.12	8.6 ± 3.6	11	Wld	3 [8.5]	Not listed
Ekebergia benguelensis Welw. ex C.DC. (Meliaceae)/ KIP536010668	Kalayi (Bb) Kalaya (Lb)	11 (10.2)	R	Powder [Pr], Pieces	Headache, sinusitis, hip pain	42.5 ± 37.9	0.19 ± 0.9	12.7 ± 3.4	7	Wld	3 [14.9]	LC
Cymbopogon densiflorus (Steud.) Stapf (Poaceae)/ KIP346657443	Kikotshi (Lb) Tshikota (Tsb)	11 (10.2)	AP	Arial part	Musanvu, abdominal pain, typhoid fever	30.3 ± 8.8	0.45 ± 0.18	11.9 ± 8.6	4	Wld	3 [20.0]	Not listed
Osodendron leptophyllum (Harms) E.J.M.Koenen (Fabaceae)/ KIP256290468	Munyenze (Lb)	11 (10.2)	R	Pieces [Pr], Powder	Hemorrhoid, digestive disorders, genital infection	8.3 ± 5.6	0.69 ± 0.67	21.3 ± 12.9	10	Wld	2 [21.6]	LC
Oldifielda dactyllophylla (Well. ex. Oliv.) J. Léonard. (Picrodendraceae)/ KIP586478488	Kikoto mutshi (Lb), 6h à 6h (Fr)	10 (9.3)	R	Powder	Sexual weakness, hernia	32.4 ± 23.8	0.21 ± 0.17	14.4 ± 4.6	6	Wld	3 [15.5]	Not listed
Parinari curatellifolia Planch. ex Benth. (Chrysobalanaceae)/ KIP226300166	Kifulu mutshi (Lb)	9 (8.3)	R	Pieces [Pr], Powder	Hemorrhoids, menstrual disorders, female infertility	32.7 ± 28.5	0.54 ± 0.50	20.8 ± 6.7	9	Wld	3 [17.6]	LC
Calea urticifolia (Mill.) DC. (Asteraceae)/ KIP453326432	Kabutshungu (Sw)	8 (7.4)	R, L	Leaves [Pr], Powder	Malaria, diabetes, typhoid fever	32.9 ± 9.2	0.14 ± 0.10	12.7 ± 9.5	5	Wld, Cult	3 [14.1]	Not listed
Antidesma venosum Meyer (Euphorbiaceae)/ KIP294562545	Kifubia (Lb)	8 (7.4)	R	Pieces [Pr], Powder	Hemorrhoids, female infertility	25.6 ± 24.9	0.14 ± 0.13	12.6 ± 7.2	9	Wld	3 [12.0]	LC
Erythrina abyssinica Lam. ex DC (Fabaceae)/ KIP361645844	Kisungwa (Bb)	8 (7.4)	R, ST	Powder [Pr], Pieces	Stomachache, sexual weakness	13.8 ± 8.5	0.17 ± 0.13	7.5 ± 3.8	7	Wld	3 [6.9]	LC
Zingiber officinale Roscoe. (Zingiberaceae)/ KIP142459684	Gingembre (Fr) Tangawisi (Sw)	7 (6.5)	RH	Powder [Pr], Rhizom	Sexual weakness, cough	14.6 ± 9.3	0.49 ± 0.47	17.9 ± 4.9	4	Wld	4 [17.2]	DD
Gladiolus klattianus Hutch (Iridaceae)/ KIP486882628	Kabolelubamba (Lb)	7 (6.5)	BL	Powder [Pr], Bulbe	Sexual weakness, hemorrhoids, hernia	20.2 ± 14.9	0.28 ± 0.26	6.1 ± 4.8	5	Wld	3 [6.1]	Not listed
Bobgounia madagascariensis (Desv) J.H.Kirkbr. & Wiersema (Fabaceae)/ KIP336657434	Mpampi (Lb)	7 (6.5)	R	Pieces [Pr], Powder	Sexual weakness, malaria, epilepsy	27.4 ± 24.3	0.18 ± 0.15	15.1 ± 10.4	7	Wld	3 [11.6]	Not listed
Annona senegalensis Pers. (Annonaceae)/ KIP154964757	Mulolo (Lb)	7 (6.5)	R	Pieces [Pr], Powder	Sexual weakness, cough, high blood pressure	27.7 ± 13.8	0.50 ± 0.43	13.3 ± 11.7	6	Wld	3 [12.2]	LC
Becium anyanum G. Tayl. (Lamiaceae)/ KIP533550698	Kafupa (Sw)	6 (5.6)	R	Pieces [Pr], Powder	Sexual weakness	25.6 ± 12.0	0.73 ± 0.68	3.7 ± 1.2	5	Wld	2 [4.5]	Not listed
Aristolochia hockii De Wild. (Aristolochiaceae)/ KIP475859291	Kapangapanga (Lb)	6 (5.6)	R	Pieces [Pr], Powder	Abdominal pain, hemorrhoids, prostatitis	33.9 ± 12.4	0.52 ± 0.20	19.9 ± 4.8	3	Wld	3 [22.1]	Not listed
Justicia secunda Vahl. (Acanthaceae)/ KIP555753135	Majani y aba postolo (Sw)	6 (5.6)	L	Dry leaves	Anemia	24.9 ± 8.4	0.13 ± 0.8	20.2 ± 5.7	3	Cult	3 [22.1]	Not listed
Moringa oleifera Lam. (Moringaceae)/ KIP277884610	Moringa (Fr)	5 (4.6)	SD, L	Seed, Powder [Pr]	Typhoid fever, diabetes, poisoning	24.2 ± 10.5	0.14 ± 0.12	7.9 ± 4.7	11	Cult	4 [3.9]	LC

[a] Languages (La = Lamba, Lb = Luba, Bb = Bemba, Hb = Hemba, Tsb = Tshiluba, Fr = Français, Sw = Swahili).
[b] Sold organs (R = Roots, SB = Stem barks, SD = Seeds, BL = Bulb, RH = Rhizome, L = Leaves, Fr = Fruits; AP = Aerial parts); Pr: Market predominant drug form.
[c] Prices in U.S. dollars calculated from prices in Congo Democratic Francs (CDF) at the exchange rate in effect during the survey period, i.e., 2000 CDF for $1 USD.
[d] Monthly supply (kg) per seller, refers to both harvested and wholesale quantities per month; these values were estimated by the sellers, based on their experiences.
[e] Number of harvesting sites reported by harvesting herbalists for each species.
[f] Status, (Wld = wild plant, Cult = cultivated plant).
[g] Empirical estimate value of species availability in supply area (4 = very abundant, 3 = abundant, 2 = rare, 1 = very rare).
[h] Calculated species vulnerability index.
[i] IUCN = International Union for Conservation of Nature, that determines the vulnerability and the levels of threats of extinctions on different species (LC: minor priority or non-vulnerable species, DD: insufficient data).

herbal product can be used against various diseases, and some diseases can be managed by different species (Table 4).

Our 108 herbalists report delivering herbal products based on their clients' description of *"illnesses/symptoms"* (71.3 %) or required vernacular name(s) (28.7 %). Logically, sales based on *"illnesses/symptoms"* are much more practiced when herbalists declare themselves *"healers"* (90.2 %; n = 82) than simple sellers (11.5 %; n = 26); this self-proclaimed knowledge, if truly based on experience or teaching, may contribute to minimizing the risk of misuse and/or toxic effects.

From the 514 *"most sold"* herbs, only a few are harvested by herbalists themselves (17.1 %), the rest being obtained from members of the herbalists' families (48.8 %) or wholesalers (34.1 %). Herbalists identify samples through macroscopic and organoleptic characters, either by themselves (90.7 %) or by referring to colleagues (9.3 %); macroscopic and organoleptic characteristics have in fact always been used by traditional healers to assess medicinal plants (Zhao et al., 2011). But, considering that the drugs are mostly delivered already cut or ground, these characteristics can be insufficient to correctly identify a species, with a high risk of confusion or adulteration (Zhao et al., 2011; Muyumba et al., 2021).

As stated from the 108 visited stalls, the most frequent samples were roots from *Terminalia mollis* M.A.Lawson, (33.3 %) and *Securidaca longepedunculata* Fresen (27.7 %) as well as stem barks from *Nauclea pobeguinii* Hua ex Pobég (23.1 %); indeed, these drugs are offered to treat pathologies (hemorrhoids, and sexual weakness) for which Lubumbashi traditional practitioners are most frequently consulted (Table 4; Table 5). Macro- and microscopic aspects indicate that the samples sold on the Lubumbashi market were only harvested from mature plants. Given the abundance of these species on the market, they may require special attention regarding their availability/vulnerability, as well as the identity and quality of the drugs offered for sale.

Table 5
Therapeutic indications for 514 drugs purchased on the Lubumbashi market.

Indications	n	%
Hemorrhoids	103	20.0
Sexual weakness	81	15.8
Genital infection	43	8.4
Inguinal hernia	32	6.2
Digestive disorders	25	4.9
Malaria	18	3.5
Typhoid fever	17	3.3
Tooth decay	15	2.9
Abdominal pain	14	2.7
Stomach aches	14	2.7
Menstrual disorders	14	2.7
Diabetes	13	2.5
Hip pain	13	2.5
Cough	11	2.1
Anemia	9	1.8
Oxyurosis	9	1.8
Frigidity	7	1.4
Sexual impotence	7	1.4
High blood pressure	6	1.2
Sinusitis	6	1.2
Ovarian cyst	5	1.0
Rheumatism	4	0.8
Osteoporosis	3	0.6
Prostatitis	3	0.6
Female infertility	3	0.6
Gonorrhea	3	0.6
Tuberculosis	3	0.6
Other (indications for less than 2 drugs)	67	13.0

Table 6
Anthropogenic activities observed in medicinal plant harvesting sites.

Observed activities	n	%
Farming activities	69	63.8
Farming activities and charcoal making	14	13.0
No idea	9	8.3
None	8	7.4
Charcoal making	4	3.7
Farming activities and mining	2	1.9
Mining	2	1.9

3.1.3. Market values

Prices observed on the market show great variability (see standard deviations in Table 4) both between species and between herbalists; such a variability, also observed e.g., in Ghana (Van Andel et al., 2012), can be partly due to the diversity of sales units proposed by herbalists. The median price at the time of the survey was 21 US$/kg (range, 1 to 390 US$/kg), somewhat lower than reported in other countries, such as Gabon (56 US$/kg) (Towns et al., 2014).

No significant correlation was found between the abundance of species on the market and their average prices ($p = 0.5246$); the minus sign of correlation (Spearman $r = -0.1280$) indicates that, logically, some of rarer species are more pricey than high-sales species. There were significant differences (test; $p < 0.0001$) in the median prices/kg according to the purchasing municipality [Annex (18 US$), Kamalondo (10 US$), Katuba (26 US$), Kampemba (35 US$), Kenya (12 US$), Lubumbashi (34 US$) and Ruashi (13 US$)], with significantly higher prices in Kampemba and Lubumbashi.

3.1.4. Volumes sold in relation to species vulnerability

Based on the *"most sold"* herbs average sales weight, the number of herbalists selling a given species and the number of units sold per day, the Lubumbashi total volume of sales could be estimated at 145.7 kg/day, i.e. 3.8 tons/month (26 days) and 45.5 tons/year (312 days); based on herbalist own estimations, the total supply of medicinal plants in Lubumbashi could reach 5.3 tons/month, or about 63.5 tons/year. The medicinal plants are sold in Lubumbashi in considerable amounts, especially compared to those observed in other African countries (Towns et al., 2014; Hilonga et al., 2018); this can be explained by the considerable population of Lubumbashi (~3,000,000 inhabitants in 2023) (World Population Review, 2023) which heavily recourses to TM (~80 %; Mutombo et al., 2022). These drugs come from the Haut-Katanga province as well as from other DR Congo provinces (Haut-Lomami, Tanganyika, Kasaï, Central Kongo, Maniema, and Kinshasa), and Republic of Zambia; in some cases, the same species can be harvested from several sites (Fig. 1, Table 4). Of note, herbalists who buy in bulk can even be unaware of the real harvesting sites. Most of the 108 herbalists (91.7 %) reported observing anthropogenic activities at medicinal plant

Table 7
Additional information on the samples that showed different microscopic and phytochemical profiles.

Sample data								Data on the herbalist				Probable problem on the sample
Species declared by the herbalist	Code [a]	No.[b]	Declared organ	Found form	SL [e]	Sample source	Harvesting environment	Category	Gender	Age (year)	SNR (year)	
Terminalia mollis	LBEt8P1	11	Root	Powder	DN	Personal harvesting	LB (HK)	Healer	F	49	8	Substitution
	KTEt4P1	21	Root	Powder	UL	Harvested by a family member	ML(HL)	Healer	F	27	4	Substitution
	ANEt22P2	22	Root	Powder	UL	Wholesale purchase	TP (KS)	Healer	F	29	15	Substitution
	KTEt21P5	23	Root	Powder	UL	Wholesale purchase	ML (HL)	Healer	F	34	7	Substitution
	ANEt30P7	24	Root	Powder	DN	Harvested by a family member	TL (KO)	Healer	M	38	10	Substitution
	KPEt1P1	26	Root	Powder	PD	Personal harvesting	KM (HL)	Healer	M	32	18	Substitution
	ANEt2P4	29	Root	Powder	DN	Personal harvesting	LU (HK)	Healer	M	38	6	Substitution
	KTEt1P4	33	Root	Powder	UL	Harvested by a family member	ZB (PL)	Healer	F	32	2	Substitution
Securidaca longepedunculata	KNEt4P1	4[c]	Root	Powder	UL	Wholesale purchase	ML (HL)	Non-healer	F	41	3	Variation due to harvesting environment or storage
	LBEt12P3	10	Root	Powder	DN	Harvested by a family member	LP (KO)	Healer	M	36	3	Substitution
	LBEt17P7	11[d]	Root	Powder	DN	Wholesale purchase	BK (HL)	Healer	M	34	11	Dilution with flour
	LBEt8P4	12	Root	Powder	DN	Wholesale purchase	KL (HK)	Healer	M	33	5	Substitution and dilution with flour
	RSEt4P4	24	Root	Powder	UL	Harvested by a family member	KS (MN)	Healer	F	37	6	Substitution
	RSEt18P1	25	Root	Powder	UL	Harvested by a family member	MU (HL)	Healer	F	47	4	Substitution
	ANEt1P4	26	Root	Powder	DN	Wholesale purchase	KB (HK)	Healer	F	35	2	Substitution
	ANEt3P1	27	Root	Powder	PN	Personal harvesting	LU (HK)	Healer	M	43	17	Substitution
	KPEt4P3	28	Root	Powder	UL	Harvested by a family member	TB (HK)	Healer	M	55	5	Substitution

[a] The meaning of sample code is explained in methodology section 2.1.4 (data collection).
[b] Sample number in the HPTLC and HPLC chromatograms.
[c] Different only at the phytochemical level.
[d] Different only at the microscopic level.
[e] Sample's labeling (DN: disease name; UL: unlabeled; PD: plant and disease names; PN: plant name); Harvesting sites (LB: Lubumbashi; ML: Malemba Nkulu; TP: Tshikapa; TL: Tshilenge; KM: Kamina; LU: Luambo; ZB: Zambia; LP: Lupatapata; BK: Bukama; KL: Kilwa; KS: Kasongo; MU: Mulongo; KB: Kasumbalesa; TB: Tumbwe; HK: Haut-Katanga; HL: Haut-Lomami; KS: Kasai; KO: Kasai Oriental; PL: bordering country; MN: Maniema); SNR: seniority.

harvest sites, with farming and charcoal making being the most cited (Table 6). There is therefore a risk of extinction of some species, as the above activities often result in the cutting of ligneous species and the destruction of herbaceous habitat (Chen et al., 2016). Notably, the local *Miombo* woodland is considered an ecosystem at high risk of disappearance (Useni et al., 2020); sustainable management should be encouraged for its preservation, notably by showing that harvesting medicinal plants is a great part of cultural economies that can prevent overexploitation of soils for other purposes.

Among the 56 species we could identify, 72.9 % were reported as *"abundant at harvest sites"* and their estimated vulnerability values appear reassuring (Table 4). This is consistent with the fact that most species are available in several areas (Lawin et al., 2016). Of note, 19 (33.9 %) of identified species are listed in the International Union for Conservation of Nature and Natural Resources (IUCN) red list; from these, 94.7 % are still under minor priority (LC) status and qualified as *"not vulnerable"* (Table 4). Accordingly, in Lubumbashi, at least for identified species, a strong exploitation by herbalists may seem sustainable; however, given the level of traditional medicine use (about 80% of about 3,000,000 inhabitants in 2023) and the current population growth, combined with progressive local destruction of wildlife habitats (Useni et al., 2017, 2018, 2020), conservation measures must be considered before these species reach critical levels of vulnerability.

Especially for the 3 highest sales species, the estimated sales volumes of *S. longepedunculata* (19.3 kg/day, i.e., 6 tons/year), *T. mollis* (18.2 kg/day, i.e., 5.7 tons/year) and *N. pobeguinii* (9.9 kg/day, i.e., 3.1 tons/year) indicate a possible sustainability concern (Lawin et al., 2016), especially for *N. pobeguinii* that was qualified by the interviewed herbalists as *"rare in harvesting sites,"* with the highest estimated vulnerability value (Table 4). Although this species is listed in the IUCN red list with "*minor priority*" (LC) status. This indicates that special attention must be given to this species, given its probable pharmacological value (Dhooghe et al., 2008; Mesia et al., 2010; Kuete et al., 2015; Seukep et al., 2016; Mfotie Njoya et al., 2017; Marius et al., 2018; Yüce et al., 2019; Tsafack et al.,

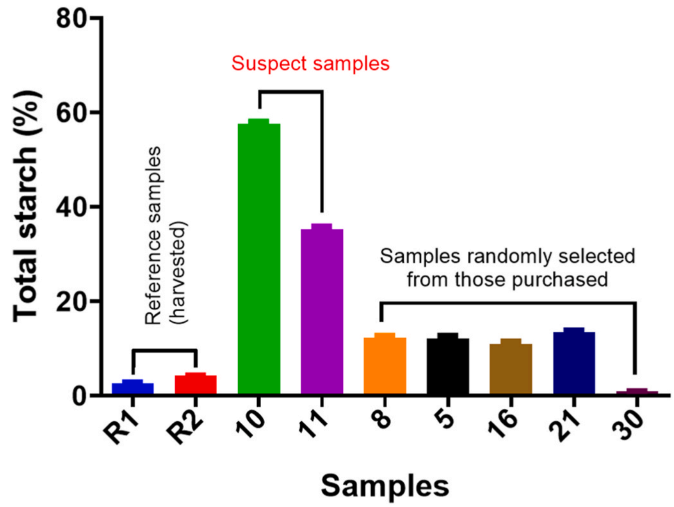

Fig. 5. Proportions of total starch in *S. longepedunculata* samples sold in Lubumbashi (n = 3). The abundance of starch grains in samples 10 and 11 is also illustrated by the microscopy photos (Fig. 2 and h and 2i respectively). The sample numbers are the same as in the HPTLC and HPLC profiles for this species (Figs. 3 and 4; Supplementary data S2).

2021; Peeters et al., 2022). Species with LC status in the IUCN red list but estimated by herbalists as *"vulnerable"* or *"highly vulnerable"* have been reported in other countries, notably in Benin (Lawin et al., 2016).

3.2. Analysis of samples from the 3 highest-sales species

3.2.1. Terminalia mollis (root barks)

This species sold in Lubumbashi mainly as powders, under the name of *"Kibobo"* (Bemba language), *"Mbubu"* (Luba), or *"Tshibangu mutshi"* (Tshiluba), was collected twice *in natura* with help of herbalists – harvesters from the Bemba, Luba-Kat, and Luba-Kasaï ethnic groups. Reference 1 (R1) and 2 (R2) voucher specimens were identified as *Terminalia mollis* M.A.Lawson (Combretaceae) and were deposited at the Kipopo (KIP154964751) and Meise herbarium (Brussels), respectively. Light microscopy of the root barks powder shows oxalate prism crystals (1a), parenchyma tissue (1b), sclerenchyma tissue (1c), starch grains (1d) and xylem bundles (1e) (Fig. 2).

From the 36 root barks samples purchased in Lubumbashi as Kibobo (Bemba language), Mbubu (Luba), or Tshibangu mutshi (Tshiluba), 28 (77.8 %) showed the presence of microscopic elements like those observed in the 2 reference samples (Fig. 2). The other 8 samples show oxalate crystals (1f), sclerenchyma tissue (1g and 1h), and parenchyma tissue (1i), but in different shapes than those observed in reference samples (Fig. 2); these microscopically different samples also showed differences in their HPTLC profiles, both after derivatization with NP/PEG400 reagent, and anisaldehyde-sulfuric acid (Fig. 3). Among these 8 atypic samples, 5 (tracks 11, 21 to 24) showed similar HPTLC profiles, implying the same species, but different from *T. mollis*; the other 3 samples (tracks 26, 29, and 33) are different from each other but show some bands observed in the *T. mollis* references (R1 and R2). HPLC profiling of these 8 atypical samples confirms *(i)* their difference from *T. mollis* (Fig. 4); and *(ii)* the similarities observed in HPTLC for 5 samples, corroborating they come from the same species, but different from *T. mollis* (Figs. 3 and 4; tracks 11, 21 to 24). These results indicate, on one hand, that the combination of microscopy and HPTLC profiling is an easy way to control the identity of traded plant drugs and, on another hand, that some herbalists clearly have difficulties in correctly identifying *T. mollis*. To identify the main adulterant species (tracks 11, 21 to 24), they were compared, by microscopy and HPTLC, with 2 local species, morphologically close to *T. mollis, Uapaca kirkiana* var. gossweileri (Hutch.) Meerts. and *Uapaca sensibarica* Pax. (Supplementary data S3), but to no avail. Given the possibility of a same vernacular name for different species (Veldman et al., 2020), it would be important to compare data with those of collected and identified species named *"Kibobo"*, *"Mbubu"*, *"Tshibangu mutshi"*, and other phonetically related names in different ethnic groups. This would allow to evaluate eventual risks of this confusion and/or the pharmacological similarities of those species confused with *T. mollis*.

Interestingly, the 8 atypical samples have been purchased from mature and experienced herbalists, self-identifying as healers, with median age and seniority of 33 (range 27–49) and 7.5 (2–18) years, respectively (Table 7). The samples were collected by the interviewed herbalist (3/8), by others (3/8), or purchased in bulk (2/8) and sold as powders, either unlabeled or labeled with disease names. It is fundamental to ensure feedback of such errors to herbalists but, in the absence of a botanical identification of adulterants, it is not possible so far to assess eventual health risks.

3.2.2. Securidaca longepedunculata (root barks)

This species, sold in Lubumbashi mainly as root bark powders, under the vernacular name of *"Mweyeye"* (Luba language) or *"Lupapi"* (Bemba), was collected twice *in natura* with help of herbalists – harvesters. Reference 1 (R1) and 2 (R2) voucher specimens were identified as *Securidaca longepedunculata* Fresen. (Polygalaceae) and were deposited at the Kipopo (KIP143459255) and Meise herbarium (Brussels), respectively.

Fig. 2 shows the microscopic characteristics of reference samples, with the presence of sclerenchyma tissue (2a), sclerenchyma cells (2b), oxalate crystals (2c), starch grains spherical in shape, with the hilum not visible, small, and clustered (2d) and fibers (2c). From the 31 samples collected from the market, 23 (74.2 %) presented comparable microscopic profiles, but 1 was heavily contaminated with starch and 7 marked a clear difference with the presence of punctate vessels (2f), oxalate prism crystals (2g) and other elements (2g). The 7 samples different according to microscopy also showed major differences in their HPTLC profiles, for both after derivatization with NP/PEG400 reagent, and anisaldehyde-sulfuric acid. Among these samples, 6 showed differing HPTLC and HPLC profiles (Figs. 3 and 4), implying that they would be from 6 different adulterant species, 1 presented chromatographic bands common with the reference samples but with additional spots, indicating either a contamination or a phytochemical variant (Sample 4). Such variation would be due to the drugs processing and preserving methods, which would have resulted in contamination or phytochemical variation. Drying *S. longepedunculata* root barks in sun or shade has been associated with significant chemical variations in phenolics and terpenoids (Mutombo et al., 2024). As 2 samples (10 and 11) showed large amounts of starch grains reminding of cassava flour (Supplementary data S2), bulky, non-clustered, irregular and variable in shape, with visible hilum (Fig. 2; 2h & 2i), total starch was enzymatically determined as *(i)* 2.7 % and 4.2 % in reference samples R1 and R2, respectively; *(ii)* 9.9 % in 5 samples randomly selected among those presenting microscopic and HPTLC profiles typical of *S. longepedunculata* root barks; and *(iii)* 57.6 % and 35.3 % in samples 10 and 11, respectively (Fig. 5). These values show some quantitative variability of starch in *S. longepedunculata* samples but may also indicate possible contamination of drugs during grinding, as the same mortars being used for drugs, cassava, or maize, pointing to a need of proper mortar cleaning. The high starch content (>50 %) found mainly in sample 10 seems to have been intentionally added for economic reasons (Srirama et al., 2017), *S. longepedunculata* root barks being in high demand in Lubumbashi (Chuimika et al., 2023). This adulteration would be facilitated by the macroscopic resemblance between cassava flour and the root bark powder of this medicinal plant; such substitution has also been reported in Iran between the leaves of *Ziziphora* sp and those of *Thymus* sp (Sheidai et al., 2019). As sample 11 presented microscopic and HPTLC features of authentic samples, it was identified as *S. longepedunculata* heavily diluted with starch.

Table 7 shows that the 9 atypical samples (7 in microscopy and phytochemistry, 1 only in microscopy and 1 only in phytochemistry) were purchased from 9 herbalists, 8 of whom qualified as "healers" and whose median age and seniority were 37 (33–55) and 5 (range 2–17) years, respectively. These herbalists reported that these 9 atypic samples were collected by themselves (1), family members (4) and wholesalers (4); drugs were sold as powders and 4 were unlabeled, while 4 were labeled by disease names and 1 by a vernacular name.

3.2.3. Nauclea pobeguinii (stem barks)

This species, sold in Lubumbashi mainly as stem bark powders, under the vernacular name of *" Mujilanga"* (Tshiluba language), was collected *in natura* with the help of an herbalist – harvester from the Luba Kasaï ethnic group. A reference voucher specimen was identified as *Nauclea pobeguinii* (Hua ex Pobég.) Merr. (Rubiaceae) and was deposited at the Meise herbarium (Brussels).

Light microscopy of the authentic sample showed oxalate crystals (3a), fibers (3b), starch grains (3c) and sclerenchyma tissue (3d) (Supplementary data S2). The HPTLC profiles, after derivatization with NP-PEG400, are characterized by blue and greenish bands, and, after derivatization with anisaldehyde - sulfuric acid, by blue, yellowish, and light purple bands (Supplementary data S2). The alkaloid profiles showed several bands, including 3 near to deposition line (Rf 0–0.05), two at Rf 3.4 and one at Rf 6.8 (Supplementary data S2). Microscopy, HPTLC, and HPLC analyses showed that all the 25 purchased samples were correctly identified, implying 100 % identification fidelity for this species (Supplementary data S2). Such high authenticity of this species would be due to its popularity in the interviewed population and/or the fact that all samples were reported to originate from the same region, Kasaï. Furthermore, authenticity of this species is an encouraging and reassuring element for its users.

3.2.4. General conclusion on samples quality

From 92 samples analyzed for the 3 species, 18.5 % showed problems, including species substitution (14/17), dilution with flour (1/17), dilution with flour and species substitution (1/17), as well as one phytochemical variant (1/17) that may be due either to the harvesting environment, or the conditions under which the drug was stored before our survey or to contamination (Table 7). Herbal products samples of poor-quality are continually detected in various countries around the world, with percentages of adulterated or low-quality products varying from 0 to 100% depending on the country, the products analyzed, and the methods used (Ichim, 2019; Ichim et al., 2020; Ichim and Booker, 2021; Orhan et al., 2024). At the African level, proportion of samples with problems observed in the present study is much lower than those previously reported in other countries i.e., Tanzania (>60 %) (Veldman et al., 2020), Morocco (>50 %) (Ouarghidi et al., 2012), South Africa (>40 %) (Mankga et al., 2013; Raman et al., 2015; Pretorius et al., 2017) or Egypt (40 %) (Abdel Kawy et al., 2012; Kamal et al., 2017). The level we record here can probably be explained by the targeted species, the present study concentrating only on top-selling species for which the seniority and experience of herbalists on the Lubumbashi markets is likely an important factor. To get a global idea of herbal drug quality, it will be necessary to yield this type of information for the low-selling and/or unidentified species.

4. Conclusion

In Lubumbashi, medicinal plants are generally sold at very affordable prices and the market is held by mostly mature people, most of whom self-identify as *"healers"*. The present survey allowed to detail demographic data on local herbalists, approaching trade habits, and to list a series of 514 herbal drugs as the most selling. From these, however, only 56 botanical species could be formally identified; most of these 56 identified species appear as non-vulnerable but this may be different for unidentified/low-selling species. Given an estimated local trade level of 45.5 tons per year for herbal drugs and the anthropogenic pressures on ecosystems, targeted safeguarding measures or multiplication are certainly worth developing for the rarest species. *Terminalia mollis* root barks, *Securidaca longepedonculata* root barks, and *Nauclea pobeguinii* stem barks appear as the most abundant drugs on the market. Among the 92 samples analyzed from these 3 species, 18.5 % showed problems, including species substitution, dilution with flour, and phytochemical variation. A complete assessment of atypical samples should consider whether the detected differences are within the natural range of variation of the species or not. This would need a huge series of trade samples coming from authenticated botanical sources; however, in the local context, such samples are not available. And so, collecting samples and comparing them with each other and with harvested voucher samples is the only palliative we could develop. Macro- and microscopic characters allowed to confirm that all our root samples came from aged plants (general appearance and presence of lignified tissues) and to detect "atypical samples" for which typicity differences (natural variant, difference in age or in botanical species) remain to be assessed.

It seems that most herbalists correctly identified these popular species, but it is intriguing that some presumably experienced healers confuse them; it seems important to encourage herbalists to obtain materials in their whole form rather than in powders and to develop appropriate morphological and sensory assessment of ingredients. To get a global idea of herbal drug quality, it will however be necessary to also yield this type of information for the low-selling and/or unidentified species. This study demonstrates the utility of microscopic and chemical profiling analyses in the authentication of herbal samples derived from markets and nature and shows possibilities for health products regulatory authorities to implement systematic control of the traded medicinal plants with microscopy and HPTLC, methods that require relatively simple technical means.

CRediT authorship contribution statement

Cedrick S. Mutombo: Writing – original draft, Visualization, Validation, Supervision, Software, Methodology, Funding acquisition, Data curation, Conceptualization. **Papy M. Moke:** Writing – review & editing, Methodology, Investigation, Funding acquisition. **François N. Ntumba:** Writing – review & editing, Supervision, Resources, Investigation, Funding acquisition. **Salvius A. Bakari:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization. **Gaël N. Mavungu:** Writing – review & editing, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Desiré M. Numbi:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Data curation. **Alex M. Kolela:** Writing – review & editing, Visualization, Software, Resources, Methodology, Investigation, Formal analysis. **Cynthia M. Kibwe:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis. **Vianney N. Ntabaza:** Writing – review & editing, Supervision, Methodology, Data curation. **Victor E. Okombe:** Writing – review & editing, Resources, Methodology, Data curation. **Amandine Nachtergael:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Formal analysis. **Jean-Baptiste S. Lumbu:** Writing – review & editing, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation. **Pierre Duez:** Writing – review & editing, Visualization, Validation, Supervision, Software, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Joh B. Kahumba:** Writing – original draft, Validation, Supervision, Resources, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

All authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2024.119029.

Data availability

All data are available in the manuscript and supplementary data.

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