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Ex situ cultivation effects on growth performance and secondary metabolite profiles of Securidaca longipedunculata (Polygalaceae) in DRC

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Abstract

S. longipedunculata Fresen (Polygalaceae) is a medicinal tree widely exploited across sub-Saharan Africa, particularly in the miombo woodlands of southeastern Democratic Republic of Congo (DRC), where increasing harvesting pressure threatens its persistence. This study evaluated the effects of ex situ cultivation under Lubumbashi (Upper Katanga, DRC) conditions on both growth performance and secondary metabolite profiles, with the aim of assessing domestication potential for conservation and sustainable use. A field experiment was conducted at the University of Lubumbashi using a completely randomized design with four substrate types (sawdust, sand, forest litter, and agricultural soil), each replicated fifteen times. Growth parameters (survival rate, plant height, leaf number, and collar diameter) were monitored over 18 months. In parallel, thin-layer chromatography (TLC) was employed to qualitatively compare major phytochemical groups (alkaloids, terpenoids, flavonoids, and phenolic acids) in roots, stems, and leaves from both wild and cultivated plants collected in the Lubumbashi region. Substrate type significantly influenced seedling establishment. Sawdust resulted in the highest survival rate (70%), while forest litter promoted superior growth, likely due to enhanced nutrient availability and improved physicochemical properties. Phytochemical screening revealed a consistent presence of flavonoids and phenolic acids in leaves and young stems across both wild and cultivated samples, highlighting their potential as alternative medicinal organs. Terpenoids were mainly detected in roots and stems, in agreement with traditional uses in the region. Alkaloids were not detected, possibly due to methodological limitations or environmental variability affecting their expression. Importantly, chromatographic profiles showed strong similarity between wild and cultivated plants, indicating that ex situ cultivation under Lubumbashi conditions does not significantly alter the qualitative composition of secondary metabolites. These findings support the integration of *S. longipedunculata* into agroforestry and restoration programs in southeastern DRC, providing a viable pathway for biodiversity conservation and sustainable medicinal plant use.

Introduction

Medicinal plants constitute a fundamental component of global healthcare systems and a major source of bioactive compounds underpinning both traditional and modern pharmacopoeias [1, 20, 85]. It is estimated that between 35,000 and 70,000 plant species are used worldwide for therapeutic purposes [3, 22, 84], with a particularly pronounced reliance in sub-Saharan Africa, where up to 80% of the population depends on plant-based remedies for primary healthcare [65, 56]. Beyond their medical importance, medicinal plants are embedded within complex socio-ecological systems [9], contributing simultaneously to biodiversity conservation, local economies, and cultural heritage [21].

However, this dependence is increasingly at odds with the ecological dynamics of resource availability. The intensification of harvesting, coupled with rapid land-use changes driven by urban expansion, mining activities, and agricultural encroachment, is accelerating the degradation of natural ecosystems and threatening the persistence of key medicinal species [63, 94]. In tropical dry forests such as the miombo woodlands of southeastern Democratic Republic of Congo (DRC), these pressures are particularly acute,

resulting in measurable declines in species abundance and regeneration capacity. Despite this, domestication and conservation initiatives remain limited, and exploitation continues to rely predominantly on destructive harvesting of wild populations [5, 27]. This structural imbalance—high demand, low regeneration, and weak domestication—constitutes a critical sustainability challenge for medicinal plant systems [3].

S. longipedunculata Fresen (Polygalaceae) exemplifies this paradox. Widely distributed across African savannah ecosystems and extensively used in traditional medicine for treating a wide spectrum of ailments [62], this species is primarily exploited for its roots and bark, leading to high mortality rates and local population decline [14]. As a consequence, it is increasingly recognized as a species at risk under current exploitation regimes [12]. Ex situ cultivation has been proposed as a strategic pathway to reconcile conservation objectives with sustained utilization [6, 24, 95], particularly within agroforestry and restoration frameworks [18].

Yet, the transition from wild harvesting to cultivation is not merely a technical issue of plant propagation; it raises a fundamental scientific question regarding the biochemical fidelity of cultivated plants [7, 11]. The pharmacological efficacy of medicinal species is intrinsically linked to their secondary metabolite composition, which is known to be highly plastic and responsive to environmental conditions, including soil properties, climatic variability, ontogenetic stage, and biotic interactions [83, 75, 13]. Variations in these factors can induce significant shifts in both the presence and concentration of key compounds, potentially altering therapeutic properties [3]. While previous studies have documented phytochemical variability across ecological gradients and developmental stages, there remains a critical lack of integrative approaches assessing whether ex situ cultivation preserves the qualitative integrity of these metabolites [15] under field conditions, particularly in African tropical ecosystems where environmental constraints differ markedly from controlled experimental systems [8, 46].

This gap is not trivial. Without empirical evidence demonstrating phytochemical stability under cultivation, domestication efforts risk producing biologically viable but pharmacologically altered plants [16, 17], thereby undermining both conservation strategies and medicinal efficacy [13, 28]. In this context, the central scientific challenge is to determine whether cultivated individuals can serve as functional substitutes for wild populations in terms of their chemical profiles [31].

To address this hypothesis, the present study adopts a dual and integrative approach. First, it evaluates the effects of different substrate types on the survival and growth performance of *S. longipedunculata* seedlings under field conditions in Lubumbashi (Upper Katanga, DRC), a region representative of miombo ecosystems under anthropogenic pressure [26]. Second, it compares the secondary metabolite profiles of wild and cultivated plants using thin-layer chromatography (TLC), focusing on key phytochemical groups associated with therapeutic activity [23]. We hypothesize that, despite environmental differences between natural and cultivated habitats, the qualitative composition of major secondary metabolites in *S. longipedunculata* remains stable under ex situ cultivation, provided that appropriate edaphic conditions are maintained. By combining agronomic experimentation with

phytochemical screening, this study aims to generate robust evidence supporting the feasibility of domestication as a sustainable strategy for the conservation and valorization of *S. longipedunculata* in tropical Africa.

Materials and methods

Study sites

Plant material was collected from two contrasting sites located in the Lubumbashi region (Upper Katanga, southeastern DRC, a region characterized by miombo woodland ecosystems under increasing anthropogenic pressure. The climate is tropical sub-humid (Köppen Aw), with a marked seasonality alternating between a rainy season (November–March) and a dry season (May–September) [54], and a mean annual rainfall ranging between 1100 and 1300 mm [19, 57]. Mean annual temperatures fluctuate between 18°C and 25°C. The region is dominated by ferralsols and acrisols, generally acidic, nutrient-poor, and highly weathered, which constrain plant growth and influence vegetation dynamics. The first site consisted of an experimental field located at the Faculty of Agronomic Sciences, University of Lubumbashi (11°36'32.43" S, 27°28'33.8" E; 1270 m a.s.l.). This site is situated within a peri-urban environment subjected to agricultural activities and soil disturbance [68]. The experimental field is characterized by degraded soils with variable structure and fertility, representative of managed agroecosystems in the region. Here, *S. longipedunculata* seedlings were established and monitored under ex situ cultivation conditions, allowing for controlled assessment of growth performance in response to substrate variation. The second site was located in the Jacaranda Salesian concession (11°45'17" S, 27°30'45" E; 1237 m a.s.l.), representing a relatively preserved fragment of miombo woodland. This ecosystem is dominated by tree species such as *Brachystegia, Julbernardia*, and *Isoberlinia*, and is characterized by seasonal leaf phenology, open canopy structure, and nutrient cycling driven by litter decomposition [57]. However, even these natural habitats are increasingly affected by human activities, including charcoal production, selective logging, and land conversion for agriculture and mining [94 Before any fieldwork involving the collection of plant parts (leaves, stem bark, roots) in natural ecosystems and fields, the authorities of the provincial environmental division granted us authorization in response to a request submitted to the APA DRC focal point. Thanks to this authorization and the field mission order obtained from the university's academic authorities, we were able to collect plant parts from specimens. The collection was therefore carried out in accordance with the legislation in force in the Democratic Republic of Congo concerning biodiversity conservation and access to biological resources. During the collection process, only parts suitable for chromatographic analysis and herbarium specimens were collected to ensure that the plant providing the sample was not severely damaged. The collection was carried out using standard plant collecting techniques. For each species encountered, only the organs strictly necessary for botanical identification were collected. Furthermore, the collection was accompanied by the following information: sample numbering, date, collector's name, geographical coordinates, and a description of the vegetation. The collected organs were then placed in a botanical press bearing a unique code and sent directly to the herbarium for

identification and storage for future research. During collection, the aerial parts of the plant (leaves, flowers, fruits, secondary branches) were collected and placed in an herbarium by Chuimika, coded HS/001/024, and deposited at the herbarium. Furthermore, Mr. Ilunga, head of the Lubumbashi herbarium at the Faculty of Agronomic Sciences, University of Lubumbashi, Campus 1825, Lubumbashi, Democratic Republic of Congo, was able to identify the specimen. Thus, this identification was carried out by comparison with three reference herbaria whose specimen characteristics matched those of the collected specimen; these were the Kisimba 543, Malaisse 2356 and Meerts 112 herbaria. After verification of conformity, the species was identified as *Securidaca longipedunculata* and the herbarium was assigned the number Ilunga 107. Confirmation of the specimen's identification was also made by digitizing the herbarium and comparing it with data from virtual digital herbaria such as Herbarium E (https://data.rbge.org.uk/search/herbarium/), Herbarium P (https://science.mnhn.fr/institution/mnhn/search) and via JSTOR Global Plants (https://plants.jstor.org/). The descriptions of morphological characteristics are based on our observations and measurements (life cycle, plant height, root, stem, leaves, flowering head, fruiting head, achenes) and compared with relevant previous studies [62]. From a socio-economic perspective, the Lubumbashi region is marked by rapid urban expansion and strong dependence of local populations on natural resources for energy, income, and healthcare [68]. Medicinal plants such as *S. longipedunculata* are widely harvested and traded through informal markets, often without sustainable management practices, contributing to the decline of wild populations. The selection of these two sites, representing contrasting ecological and management conditions within the same climatic zone, provides a robust framework for assessing the effects of ex situ cultivation on both growth performance and phytochemical stability of *S. longipedunculata*.

Methods

Plant material and experimental setup

Seedlings of *S. longipedunculata* were obtained from seeds collected from mature individuals within the miombo woodland surrounding Lubumbashi, ensuring genetic representativeness of local populations. Seeds were germinated under controlled nursery conditions to standardize initial growth stages and reduce variability associated with germination success [38, 73]. After three months, uniform seedlings (≈ 10 cm in height) were selected based on morphological homogeneity (height, vigor, absence of visible stress symptoms) to minimize initial bias in growth performance [40, 70].

The field experiment followed a completely randomized design (CRD) comprising four substrate treatments (Table1): sawdust, sand, forest litter, and agricultural soil, each replicated fifteen times (n = 60). The choice of substrates was guided by their contrasting physicochemical properties, particularly in terms of porosity, nutrient content, and water retention capacity, which are known to influence root development and early plant establishment [53, 39].

Table 1
Chemical analysis data for the substrate samples used in this study

Substrate/parameter	pH	N	P_2O_5	Mg	K_2O
Agricultural soil	5,2	2,3	1,7	9,1	16,8
Sand	4,4	0,4	0,3	1,8	3,2
Forestry waste	5,9	3,6	2,5	15,8	30,7
Sawdust	4,8	1,2	0,6	7,9	6,3

This design allows for the isolation of substrate effects while minimizing confounding environmental variability.

Planting pits (60 cm in diameter and depth) were manually prepared to ensure sufficient rooting volume and facilitate early root expansion [34]. A spacing of 3 m × 3 m was adopted to reduce competition for light, water, and nutrients, in line with recommendations for woody species establishment [35]. Prior to transplantation, each pit was filled with a single substrate type, previously homogenized to ensure consistency across replicates [8, 74].

To enhance ecological relevance, an intercropping system was implemented, combining *S. longipedunculata* with staple and horticultural crops commonly cultivated in the region. Maize (*Zea mays*) was grown during the rainy season, while tomato (*Lycopersicum esculentum*) and cabbage (*Brassica oleracea var. capitata*) were cultivated during the dry season. This approach was adopted to simulate real agroforestry conditions and evaluate plant performance within multifunctional production systems [49].

Field management and data collection

The experiment was conducted over an 18-month period (March 2022 to August 2023), covering both rainy and dry seasons to account for seasonal variability in growth conditions. During the dry season, irrigation was applied based on locally adapted schedules [49], ensuring adequate soil moisture while avoiding waterlogging [89]. To mitigate the effects of high solar radiation and reduce soil evaporation [95], a mulch layer composed of *Imperata cylindrica* was applied around seedlings, a practice commonly used in tropical agroecosystems [86].

No direct fertilization [58] was applied to *S. longipedunculata* seedlings. Instead, plants relied on residual nutrients from fertilizers applied to associated crops, thereby reproducing low-input agricultural conditions typical [59] of smallholder systems in the region. This methodological choice enhances the external validity of the study by aligning experimental conditions with real-world practices [60].

Growth performance was assessed through repeated measurements at 6, 12, and 18 months after transplantation. The following parameters were recorded: (i) survival rate (%), reflecting establishment

success; (ii) plant height (cm), as an indicator of vertical growth; (iii) number of leaves per plant, representing photosynthetic potential; and (iv) collar diameter (mm), a proxy for structural development and biomass accumulation. These parameters were selected based on their relevance for evaluating early-stage performance of woody species and their sensitivity to environmental and edaphic conditions.

Sampling strategy for phytochemical analysis

To assess the impact of cultivation on secondary metabolite production [61, 63, 64], plant material was sampled from both cultivated and wild populations [45]. At the experimental site, 18-month-old cultivated seedlings were harvested, corresponding to a stage of active vegetative growth. At the natural site, both naturally regenerated seedlings of comparable age and mature individuals were collected to capture ontogenetic variation in phytochemical expression, which is known to influence metabolite composition [83]. For everyone, leaves, stems (or bark in mature plants), and roots were collected separately to account for organ-specific distribution of secondary metabolites [66, 67, 69]. Sampling procedures followed standardized pharmacognostic protocols to ensure reproducibility and minimize contamination [61]. Samples were placed in labeled sterile bags and transported at approximately 20°C to the laboratory. Upon arrival, samples were washed with tap and distilled water to remove debris, then air-dried under controlled conditions, protected from direct sunlight and UV radiation to prevent degradation of sensitive compounds [29]. Dried samples were stored in paper envelopes until further processing.

Extraction procedures

Dried plant materials were processed according to tissue type to optimize extraction efficiency. Leaves and young stems were manually ground using a porcelain mortar to preserve thermolabile compounds, while roots and mature bark were mechanically milled using an electric grinder to ensure uniform particle size [66]. For each sample, 1 g of powdered material was extracted with 10 mL of methanol using ultrasonic-assisted extraction for one hour [50]. Ultrasonication enhances solvent penetration and disrupts plant cell walls, thereby improving the recovery of intracellular compounds. Methanol was selected as the extraction solvent due to its polarity and ability to solubilize a broad spectrum of secondary metabolites, including phenolics, flavonoids, and certain alkaloids. After extraction, samples were filtered and stored under appropriate conditions prior to chromatographic analysis.

Thin-layer chromatography (TLC) analysis

Thin-layer chromatography (TLC) was employed as a qualitative screening technique to compare secondary metabolite profiles across samples. This method was selected for its operational simplicity, low cost, and suitability for comparative phytochemical studies in contexts where access to advanced analytical platforms (e.g., HPLC, LC-MS) is limited [96–97]. Silica gel 60 F254 plates (Merck, Germany) were used as the stationary phase. For each sample, 10 µL of extract was applied, alongside appropriate reference standards to ensure reliability of compound identification.

Different mobile phases and visualization reagents were employed to target specific metabolite classes:

- Flavonoids and phenolic acids: separation using ethyl acetate–formic acid–acetic acid–water system; visualization with Neu reagent; quercetin used as standard [69, 98]
- Alkaloids: separation using chloroform–methanol system; visualization with Dragendorff's reagent; quinine as standard [36, 55]
- Terpenoids: separation using toluene–ethyl acetate system; visualization with vanillin–sulfuric acid reagent [71–72]

Chromatograms were analyzed under UV light and after reagent application, allowing identification of compounds based on color reactions and migration patterns. Although TLC does not provide quantitative data, it enables robust comparative assessment of phytochemical presence and distribution across samples.

Statistical analysis

All statistical analyses were performed using R software (version 4.3.2). Prior to analysis, data were checked for normality using the Shapiro–Wilk test [41] and for homogeneity of variances using appropriate diagnostic methods. Since assumptions of normality were met, one-way analysis of variance (ANOVA) was conducted to evaluate the effect of substrate type on each growth parameter. When significant differences were detected ($p < 0.05$), Tukey's honestly significant difference (HSD) test was applied for post hoc pairwise comparisons. This method was selected for its robustness in controlling Type I error across multiple comparisons and its suitability for balanced experimental designs. All results are presented as mean ± standard deviation. Statistical significance was set at $\alpha = 0.05$. This analytical framework ensures reliable inference of treatment effects while maintaining consistency with standard practices in plant science and agronomy research.

Results

1. S. longipedunculata seedlings after transplanting onto different substrates

Analysis of variance revealed a statistically significant effect of substrate type on the survival rate of *S. longipedunculata* seedlings ($p = 0.04$; Fig. 2). Survival rates varied substantially across treatments, ranging from 50.00 ± 4.32% to 70.00 ± 3.19%. The highest survival was observed in seedlings established on sawdust (70.00 ± 3.19%), indicating favorable conditions for early establishment. In contrast, significantly lower survival rates were recorded in agricultural soil (50.00 ± 3.19%) and sand (53.33 ± 3.94%), suggesting suboptimal physicochemical properties for seedling recovery. Seedlings grown in forest litter exhibited intermediate survival (65.00 ± 4.09%), not significantly different from those grown on sawdust but consistently higher than those observed in mineral substrates. This gradient in survival performance highlights the critical role of substrate characteristics in influencing seedling establishment, likely through differences in aeration, moisture retention, and nutrient availability.

2. Height Growth of S. longipedunculata Seedlings After Transplantation to Different Substrate Types

Analysis of variance indicated a significant effect of substrate type on seedling height growth of *S. longipedunculata* ($p = 0.033$). During the initial six months, seedlings established in forest litter exhibited the greatest height increment (24.45 ± 1.72 cm), whereas those grown in sand showed the lowest growth (22.40 ± 1.44 cm). Seedlings cultivated in agricultural soil (22.75 ± 1.13 cm) and sawdust (23.33 ± 2.07 cm) displayed intermediate growth, with no significant differences between these treatments according to Tukey's test. Over time, growth differences among substrates tended to diminish, with seedling height after 18 months ranging from 35.72 ± 1.70 cm (sand) to 37.72 ± 0.81 cm (forest litter) (Table 2). Despite this convergence, forest litter consistently supported superior growth performance throughout the experiment, suggesting a sustained advantage likely associated with improved nutrient availability and substrate structure.

Table 2
Temporal variation in height growth of *S. longipedunculata* seedlings under contrasting substrate conditions (6, 12, and 18 months).

Substrate	Length at 6 months	Length at 12 months	Length at 18 months
Agricultural land	22,75 ± 1,13ab	31,05 ± 2,45ab	37,16 ± 1,80ab
Forestry waste	24,45 ± 1,72a	32,40 ± 1,61a	37,72 ± 0,81a
Sand	22,40 ± 1,44b	29,00 ± 1,93b	35,72 ± 1,78b
Sawdust	23,33 ± 2,07ab	29,50 ± 1,66b	36,08 ± 1,70ab
p	*0,033*	*0,001*	*0,017*

Legend: Each treatment consists of 15 replicates. The difference between the letters in each column indicates statistical discrimination of the mean values according to Tukey's test at $p < 0.05$.

3. Evolution of the number of leaves in S. longipedunculata transplants under different substrates

Analysis of variance revealed a significant effect of substrate type on the number of leaves per seedling of *S. longipedunculata* across all observation periods, as confirmed by Tukey's post hoc test. Seedlings grown in forest litter consistently produced the highest leaf numbers (29.36 ± 6.59; 28.30 ± 4.99; 25.00 ± 4.05), indicating enhanced vegetative development under this substrate. In contrast, seedlings established in sand and sawdust exhibited the lowest leaf production, reflecting less favorable growth conditions. Seedlings grown in agricultural soil showed intermediate values, without reaching the performance observed in forest litter. This pattern suggests that substrate quality strongly influences leaf development, likely through its effects on nutrient availability and water retention, which are critical factors for sustaining photosynthetic capacity and overall plant vigor.

Table 3
Temporal patterns of leaf production in *S. longipedunculata* seedlings under contrasting substrate conditions (6, 12, and 18 months).

Substrate	Number of leaves after 6 months	Number of leaves after 12 months	Number of leaves after 18 months
Agricultural land	26,77 ± 3,63ab	21,88 ± 3,62ab	21,00 ± 5,56ab
Forestry waste	29,36 ± 6,59a	28,30 ± 4,99a	25,00 ± 4,05a
Sand	20,45 ± 4,71b	18,33 ± 8,07b	14,54 ± 8,29b
Sawdust	21,91 ± 7,35b	18,40 ± 7,30b	14,50 ± 6,57b
p	*0,003*	*0,003*	*0,001*

Legend: Each treatment consists of 15 replicates. The difference between the letters in each column indicates statistical discrimination of the mean values according to Tukey's test at $p < 0.05$.

4. Evolution of the crown diameter of S. longipedunculata seedlings under different substrates

Substrate type had a significant effect on collar diameter of *S. longipedunculata* seedlings at 12 months after transplantation ($p = 0.032$). At this stage, seedlings established in forest litter exhibited the largest collar diameter (4.27 ± 0.90 mm), whereas those grown in sawdust showed the lowest values (3.29 ± 0.62 mm), indicating reduced structural development under this substrate. In contrast, no significant differences among substrates were observed at 6 and 18 months (Table 4). Collar diameter ranged from 2.81 ± 0.60 mm to 3.36 ± 0.67 mm at 6 months, and from 3.41 ± 0.79 mm to 4.27 ± 0.90 mm at 18 months. These results suggest a transient effect of substrate on stem thickening, with differences emerging during intermediate growth stages but tending to diminish over time. This convergence may reflect progressive root system establishment and increased plant capacity to buffer initial substrate constraints.

Table 4
Effect of substrate type on collar diameter dynamics of *S. longipedunculata* seedlings over time (6, 12, and 18 months).

Substrate	Diameter (mm) at 6 months	Diameter (mm) at 12 months	Diameter (mm) at 18 months
Agricultural land	3,00 ± 0,70	3,66 ± 1,00ab	3,88 ± 0,92
Forestry waste	3,36 ± 0,67	4,27 ± 0,90a	4,27 ± 0,90
Sand	2,81 ± 0,60	3,40 ± 0,73ab	3,45 ± 0,82
Sawdust	2,91 ± 0,79	3,29 ± 0,62b	3,41 ± 0,79
p	*0,295*	*0,032*	*0,076*

Legend: Each treatment consists of 15 replicates. The difference between the letters in each column indicates statistical discrimination of the mean values according to Tukey's test at $p < 0.05$.

2. Thin-layer chromatographic analysis of the organs of S. longipedunculata

Thin-layer chromatographic (TLC) analysis did not reveal detectable alkaloid signals in root extracts from either wild adult plants or cultivated and wild seedlings, as no characteristic coloration was observed following Dragendorff's reagent application. In contrast, the quinine reference standard produced a clear positive reaction, confirming the reliability of the analytical procedure (Fig. 3a). These results suggest that alkaloids, if present, may occur at concentrations below the detection threshold of TLC or exhibit variability linked to environmental conditions, developmental stage, or organ specificity. Previous studies have reported the presence of alkaloids in *S. longipedunculata*, indicating that their expression may be modulated by ecological or physiological factors not fully captured under the conditions of this study. Consequently, their occurrence cannot be definitively excluded. TLC screening of terpenoids revealed a more restricted and organ-specific distribution. Terpenoid compounds were detected in a subset of samples, namely stems and roots of wild young seedlings and roots of cultivated seedlings, while no detectable signals were observed in leaf extracts regardless of plant origin or developmental stage (Fig. 3b). This pattern suggests preferential allocation or accumulation of terpenoids in belowground and structural tissues, consistent with their potential ecological and defensive roles. In contrast, flavonoids displayed a broader and more consistent distribution across samples. Chromatographic profiles indicated their presence in leaves of all individuals, irrespective of origin (wild vs. cultivated) or ontogenetic stage (young vs. mature), as well as in stems of young seedlings. Additionally, roots of both cultivated and wild young seedlings exhibited detectable flavonoid signals (Fig. 3c). This widespread occurrence highlights the relative stability of flavonoid expression across environmental conditions and supports their potential role as core metabolites in *S. longipedunculata*.

Discussion

1. Substrate-driven seedling establishment and growth performance

The establishment success of *S. longipedunculata* seedlings under field conditions appears to be strongly mediated by substrate properties, confirming that early plant performance is primarily governed by edaphic constraints. Substrates act simultaneously as mechanical support and as regulators of water and nutrient availability, thereby influencing both root development and aboveground growth [42, 2]. In this study, the superior survival observed in sawdust highlights the importance of physical attributes such as low bulk density, high porosity, and improved aeration, which collectively facilitate root penetration and reduce transplant shock [8, 18]. Root system expansion is a key determinant of seedling establishment, as it directly controls access to limiting resources and enhances resilience to environmental stress [81, 90].

In contrast, the reduced survival observed in sand and agricultural soil likely reflects a combination of physical constraints (e.g., compaction, poor structure) and chemical limitations (low nutrient content,

reduced water-holding capacity). These findings are consistent with previous studies demonstrating that substrate inadequacy can significantly impair seedling establishment [32]. While biotic stressors such as damping-off are commonly implicated in transplant failure [10, 87, 92], their limited occurrence in this study suggests that abiotic stress—particularly high irradiance and soil moisture fluctuations—played a more dominant role in shaping survival outcomes. This highlights the importance of microclimatic buffering (e.g., mulching) in tropical field conditions.

Growth performance, however, followed a different pattern, with forest litter consistently promoting superior development across all measured parameters. This divergence between survival and growth responses underscores a functional trade-off between substrates that optimize establishment (physical properties) and those that maximize biomass accumulation (nutrient availability). Forest litter, enriched in organic matter and mineral nutrients derived from decomposition processes [70, 78], provides both improved nutrient supply and enhanced soil structure, thereby supporting sustained growth. Similar responses have been reported in other woody species, including *Pinus banksiana* [74], suggesting that these mechanisms are broadly applicable across taxa. From a management perspective, this indicates that substrate selection should be strategically aligned with production objectives, distinguishing between early establishment phases and subsequent growth optimization.

2. Alternative substrates and implications for sustainable plant production

The relevance of alternative substrates must be considered within the broader context of soil degradation, which is increasingly recognized as a major constraint to plant production in tropical and subtropical regions [80, 89]. The decline in soil quality, driven by unsustainable agricultural practices and land-use change, undermines ecosystem functions such as nutrient cycling and water regulation. In this context, the use of organic or engineered substrates emerges as a viable strategy to restore plant productivity and improve system resilience [79, 73].

The present results provide empirical support for this approach, demonstrating that locally available materials such as forest litter and sawdust can significantly enhance seedling performance. Beyond their agronomic benefits, these substrates also represent low-cost and accessible resources, which is particularly relevant for smallholder systems. Their integration into agroforestry or restoration frameworks could therefore contribute simultaneously to improved plant production and ecological rehabilitation [24–25].

Importantly, the use of such substrates aligns with circular resource management strategies, whereby organic residues are valorized within production systems. This perspective reinforces the role of ex situ cultivation not only as a conservation tool but also as a component of sustainable land-use systems adapted to local socio-ecological conditions.

3. Phytochemical stability under ex situ cultivation

A critical contribution of this study lies in addressing the question of phytochemical fidelity under ex situ cultivation. The therapeutic value of medicinal plants is intrinsically linked to their secondary metabolite composition [47], which is known to be highly responsive to environmental, physiological, and ontogenetic factors [23, 100, 102]. This raise concerns that cultivation may alter the biochemical integrity of plant material, thereby affecting its pharmacological properties [48].

The absence of detectable alkaloids in all samples should be interpreted cautiously. While TLC analysis did not reveal their presence, previous studies have confirmed that *S. longipedunculata* contains alkaloids [62]. Their non-detection may therefore reflect concentrations below the detection threshold, seasonal variability, or methodological limitations [37]. This highlights an important methodological consideration and underscores the need for complementary analytical approaches in future research.

In contrast, the strong similarity in chromatographic profiles between wild and cultivated plants suggests that ex situ cultivation does not substantially alter the qualitative composition of key secondary metabolites. This finding is consistent with observations in other medicinal species [51, 63] and provides empirical support for the biochemical stability hypothesis underlying domestication strategies. However, the observed differences between developmental stages indicate that ontogeny remains a critical factor influencing metabolite expression [4, 30, 88].

The organ-specific distribution of metabolites further reflects functional specialization within the plant. Terpenoids, predominantly detected in roots and stems, are likely associated with defense mechanisms and structural protection, consistent with their ecological roles and traditional medicinal use [91]. In contrast, flavonoids and phenolic acids exhibited a more ubiquitous distribution, particularly in leaves and young stems, suggesting a more conserved metabolic function related to antioxidant activity and physiological regulation [77, 12]. Environmental factors such as light intensity, humidity, and cultivation practices may further modulate these patterns, highlighting the plasticity of secondary metabolism [43, 58, 76].

4. Implications for conservation and domestication strategies

The convergence of phytochemical profiles between wild and cultivated individuals of *S. longipedunculata* provides critical evidence supporting the feasibility of domestication without compromising biochemical integrity. This finding is particularly significant in the context of increasing harvesting pressure on natural populations, where root extraction remains the dominant practice. Such destructive harvesting methods directly impair regeneration capacity and threaten long-term population viability [99, 14]. These pressures are further intensified by ongoing landscape transformations driven by mining expansion, urbanization, and agricultural intensification in the miombo region [52, 93–94], resulting in a compounded risk of both habitat loss and overexploitation.

In this context, the demonstration that ex situ cultivated plants maintain comparable qualitative phytochemical profiles represents a key step toward reconciling conservation objectives with sustained resource use [82]. By validating the functional equivalence between wild and cultivated plant material,

this study supports the transition from extractive to production-based systems. Such a shift is essential for reducing harvesting pressure on natural populations while ensuring continued access to medicinal resources.

Importantly, the consistent detection of bioactive compounds in leaves and stems opens new avenues for redefining harvesting strategies [101]. Moving from root-based extraction to the use of renewable aerial organs could substantially reduce plant mortality and enhance the sustainability of exploitation practices. This approach aligns with broader conservation frameworks that promote non-destructive harvesting as a means of maintaining both ecological integrity and resource availability [103].

Beyond species-level implications, these findings contribute to a more systemic perspective on medicinal plant management. The integration of *S. longipedunculata* into agroforestry systems offers a multifunctional solution, combining biodiversity conservation, soil restoration, and income diversification. In degraded landscapes, such systems can enhance ecological resilience while providing locally adapted production models. Moreover, the use of locally available substrates (e.g., forest litter, sawdust) reinforces the feasibility of low-cost domestication strategies tailored to smallholder contexts.

From a methodological standpoint, the application of thin-layer chromatography (TLC) demonstrates its relevance as a rapid and accessible tool for comparative phytochemical screening in resource-limited environments [97]. However, to fully validate the biochemical equivalence of cultivated plants, future research should incorporate quantitative and high-resolution analytical techniques (e.g., HPLC, LC-MS), enabling the assessment of metabolite concentrations and potential shifts in bioactive compound profiles.

More broadly, this study highlights the need for an integrated approach to medicinal plant domestication that simultaneously considers ecological, biochemical, and socio-economic dimensions. Such an approach is essential for developing sustainable value chains that balance conservation priorities with local livelihood needs. In this regard, *S. longipedunculata* may serve as a model species for the domestication of other high-value medicinal plants in tropical Africa, particularly within miombo ecosystems where pressures on plant resources are intensifying.

Conclusion

This study provides new insights into the agronomic performance and phytochemical stability of *S. longipedunculata* under ex situ cultivation conditions in southeastern DRC. The results demonstrate that substrate type plays a critical role in seedling establishment and growth, with sawdust enhancing survival through improved physical properties, while forest litter promotes superior vegetative development due to its higher nutrient availability. These findings highlight the importance of optimizing substrate selection according to specific production objectives, particularly during early stages of domestication.

From a phytochemical perspective, the overall similarity in chromatographic profiles between wild and cultivated plants indicates that ex situ cultivation does not fundamentally alter the qualitative composition of major secondary metabolites. The consistent presence of flavonoids and phenolic acids across plant organs, especially in leaves and young stems, suggests that these tissues may serve as viable alternatives to roots for medicinal use. This represents a key opportunity to reduce destructive harvesting practices and enhance the sustainability of resource use. Although alkaloids were not detected under the present analytical conditions, their occurrence cannot be excluded and warrants further investigation using more sensitive techniques.

Taken together, these findings provide a strong scientific basis for promoting the domestication of *S. longipedunculata* as a strategy to reconcile biodiversity conservation with the sustained use of medicinal plant resources. The demonstrated functional equivalence between wild and cultivated individuals supports the integration of this species into agroforestry systems and restoration programs, particularly in miombo ecosystems under increasing anthropogenic pressure.

Future research should focus on quantitative phytochemical analyses and long-term field evaluations to better understand metabolite dynamics and optimize cultivation protocols. More broadly, this study contributes to the development of integrated domestication frameworks that combine ecological, agronomic, and biochemical approaches, thereby supporting the emergence of sustainable medicinal plant value chains in tropical Africa.

Declarations

1. **Plant Guidelines: Plant guidelines have been added.**

2. Herbarium Information: Herbarium information has been completed.

3. Ethical Certifications: Legal authorizations regarding ethical harvesting practices have been obtained and incorporated into the text, and a copy of the application letter has been submitted.

Author Contribution

CM: Monitoring of experiments, laboratory analysis, manuscript writingBA: Chromatographic analysis, manuscript revisionJL: Proposal of experimental setup, manuscript revision, statistical analysisMM: Chromatographic analysis, sample collectionSW: Manuscript revision, provision of laboratory reagents, manuscript translationUS: Manuscript revision, monitoring of experiments, coordination of writingMK: Manuscript revision, monitoring of experiments

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The data used in this study are available from the principal author, who may make them available upon request.

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108. Discrepancy identified between the submission system and the manuscript for Corresponding author Email Addresses ({0}). Please ensure the Corresponding author Email Addresses provided in the manuscript and in the submission, system is identical: magnifiquemika1@gmail.com.

Figures



Figure 1

S. longipedunculataseedling transplanted in ex situ condition (a), S. longipedunculataseedling in full growth in situ (b), collecting of S. longipedunculata bark from an adult plant (c), and collecting of S.

longipedunculataseedlings after 18 months of field rearing (d).

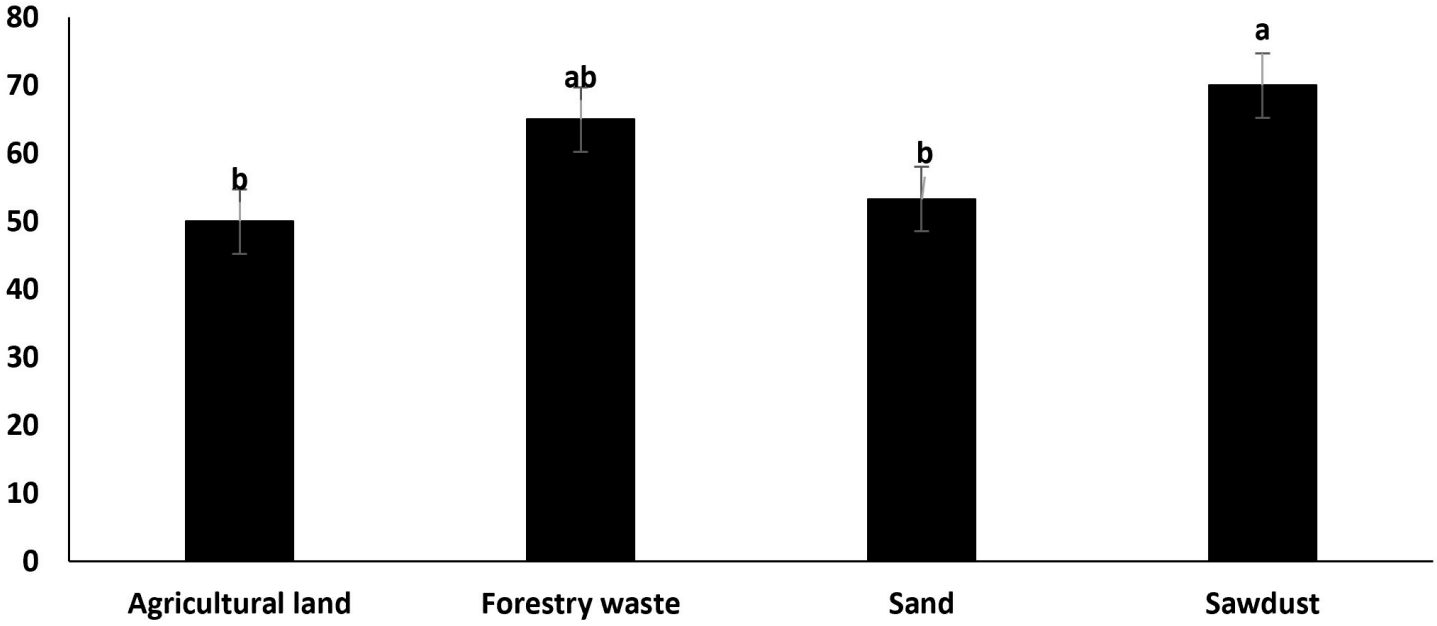


Figure 2

Substrate-driven variation in survival rate of *S. longipedunculata* seedlings. Values are mean ± SD (n = 15); treatments sharing the same letter are not significantly different (Tukey's HSD, $p < 0.05$).

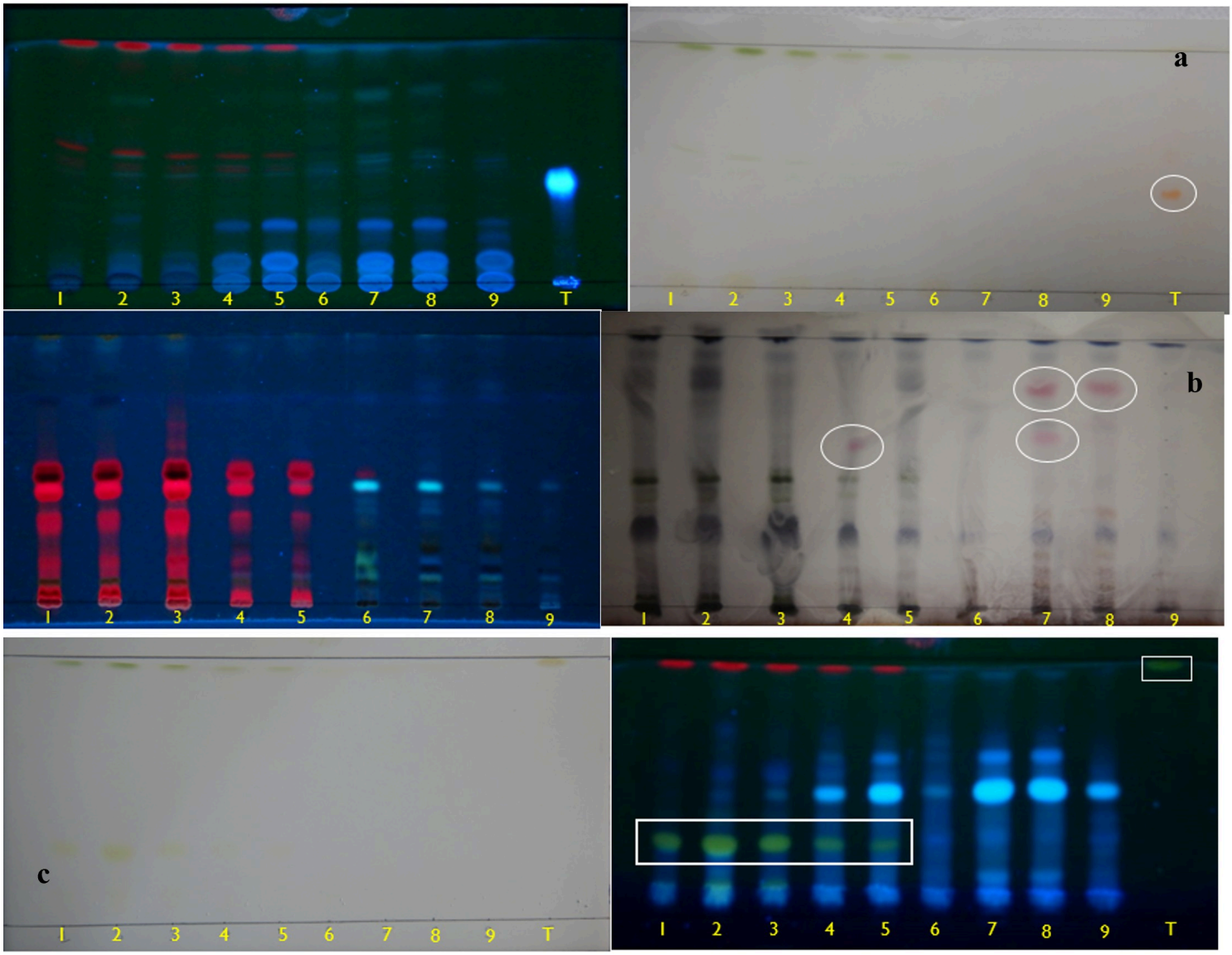


Figure 3

a. TLC analysis of *S. longipedunculata* organ extracts. Mobile phase: chloroform-methanol (90:10); Stationary phase: silica gel 60 F254-Merck; Visualization: UV lamp, Dragendorff's reagent. Samples (10 µL): 1. Young wild leaves; 2. Cultivated leaves; 3. Mature wild leaves; 4. Young wild stems; 5. Cultivated stems; 6. Mature wild stems; 7. Young wild roots; 8. Mature wild roots; T. Quinine (control); **b.** Mobile phase: toluene-ethyl acetate (93:7); Stationary phase: silica gel 60 F254-Merck; Visualization: UV lamp, 5% vanillin-sulfuric acid. Samples (10 µL): 1. Young wild leaves; 2. Cultivated leaves; 3. Mature wild leaves; 4. Young wild stems; 5. Cultivated stems; 6. Mature wild stems; 7. Young wild roots; 8. Cultivated roots; 9. Mature wild roots. **c.** Mobile phase: ethyl acetate-formic acid-glacial acetic acid-water (100:11:11:27); Stationary phase: silica gel 60 F254-Merck; Visualization: UV lamp, Neu reagent. Samples (10 µL): 1. Young wild leaves; 2. Cultivated leaves; 3. Mature wild leaves; 4. Young wild stems; 5. Cultivated stems; 6. Mature wild stems; 7. Young wild roots; 8. Cultivated roots; 9. Mature wild roots; T. Quercetin (reference).