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Ethnobotanical and phytochemical studies of *Crotalaria* species (Fabaceae) from the Vakinankaratra region of Madagascar

Herizo Lalaina Andriamampianina [1, 2], Maholy Pricille Ratsimiebo [1, 2], Mihajasoa Stella Razanatseheno [1, 2], Hanitra Ranjàna Randrianarivo [1, 2], Lovarintsoa Judicaël Randriamampianina [1, 2], Danielle Aurore Doll Rakoto [1, 2] and Victor Louis Jeannoda [1, 2, *]

[1] *Laboratory of Applied Biochemistry to Medical Sciences (LABASM), Fundamental and Applied Biochemistry Department, Faculty of Sciences, University of Antananarivo, P.O. Box 906, Antananarivo 101, Madagascar.*
[2] *Life and Environmental Sciences Doctoral School (SVE), University of Antananarivo, P.O. Box 906, Antananarivo 101, Madagascar.*

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Abstract

This study was conducted to assess the potential of species of the genus *Crotalaria* (Fabaceae) found in the Vakinankaratra region of Madagascar, with a view to exploiting them in various areas of interest. The work focused on inventorying locally known species, analysing their biogeographical origin, describing their traditional uses and characterising their phytochemical composition. Data from literature reviews and ethnobotanical field surveys identified 19 species of *Crotalaria* in this region, including 6 endemic (31.6%), 5 native (26.3%) and 8 introduced (42.1%) species. The identified uses fell into 3 categories: medicinal (53%), agronomic (47%), and toxic (32%). Whole plants (45%) and leaves (39%) were the parts used most frequently, while seeds and stem bark were used less frequently (less than 10%). Some species fell into more than one category of use. The most common forms of preparation were based on the use of fresh plants (50%), followed by decoctions, infusions, macerations and steam baths (27%), and powders prepared from plant parts for external use (17%). Endemic species were distinguished by their medicinal and toxic properties, whereas introduced species were mainly used for agricultural and fodder purposes. A phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins and polyphenols, which were bioactive compounds known for their pharmacological and toxic properties. These results highlighted the specific richness of the *Crotalaria* genus in the Vakinankaratra region, as well as its potential interest for pharmaceutical and agronomic research in Madagascar.

Keywords: *Crotalaria*; Fabaceae; Vakinankaratra; Ethnobotanical survey; Phytochemistry

1. Introduction

The genus *Crotalaria*, one of the largest genera in the Fabaceae family, comprises around 700 species growing in tropical and subtropical areas, most of which are found in Africa [1, 2]. They are annual or perennial plants, herbaceous, shrubs or small trees. They vary greatly in size from species to species and can reach up to 4 m in height. Their flowers are usually yellow, sometimes purple or whitish [1, 3]. Many species have significant medicinal, ecological and agricultural potential. Economically, *Crotalaria* species are valuable as green manure, cover crop and in intercropping farming systems, helping to restore soil fertility and rehabilitate degraded farmlands [4, 5]. Several species have been shown to have antimicrobial properties, and many have long been used in folk medicine to treat a variety of diseases [6, 7, 8, 9]. Their wide range of biological activities includes anti-inflammatory, antioxidant, anticancer, cytotoxic and anti-HIV

* Corresponding author: Victor Louis Jeannoda

effects [10]**.** These diverse properties make them highly promising for further ethnobotanical studies and exploration of their potential uses in traditional medicine.

In Madagascar, 53 species of *Crotalaria* are present, of which 34 are endemic, 12 are introduced, and 7 are native [2]. These plants are distributed in almost all regions of the island, with the south and the highlands being the richest areas.

The *Crotalaria* species covered by this study grow in the Vakinankaratra region, one of Madagascar's 23 regions. This region has a rich floral resource base with 242 species distributed in 161 genera and 64 families, of which 26 species are endemic [11]. *Crotalaria* species have traditionally been used for a variety of purposes, including in herbal medicine to treat ailments, to enrich soil and in cultural practices. Despite their traditional use, scientific data on the ethnobotanical importance and phytochemical composition of *Crotalaria* species in the Vakinankaratra region remain insufficiently studied. Most of the traditional knowledge associated with these plants has yet to be documented.

The objective of this study was to identify the traditional uses of these plants in the Vakinankaratra region and to analyse their phytochemical properties in order to gain a better understanding of these species and enhance local ethnobotanical knowledge.

2. Experimental

2.1. Relevant data of the area studied

This study was carried out in the Vakinankaratra region. It is located in the central part of Madagascar between latitudes 18°59' and 20°03' South and longitudes 46°17' and 47°19' East, approximately 170 km south of the capital Antananarivo. The region comprises 7 districts, including 2 urban (Antsirabe I and Ambatolampy) and 5 rural (Antanifotsy, Faratsiho, Antsirabe II, Betafo and Mandoto) (Figure 1) [12].

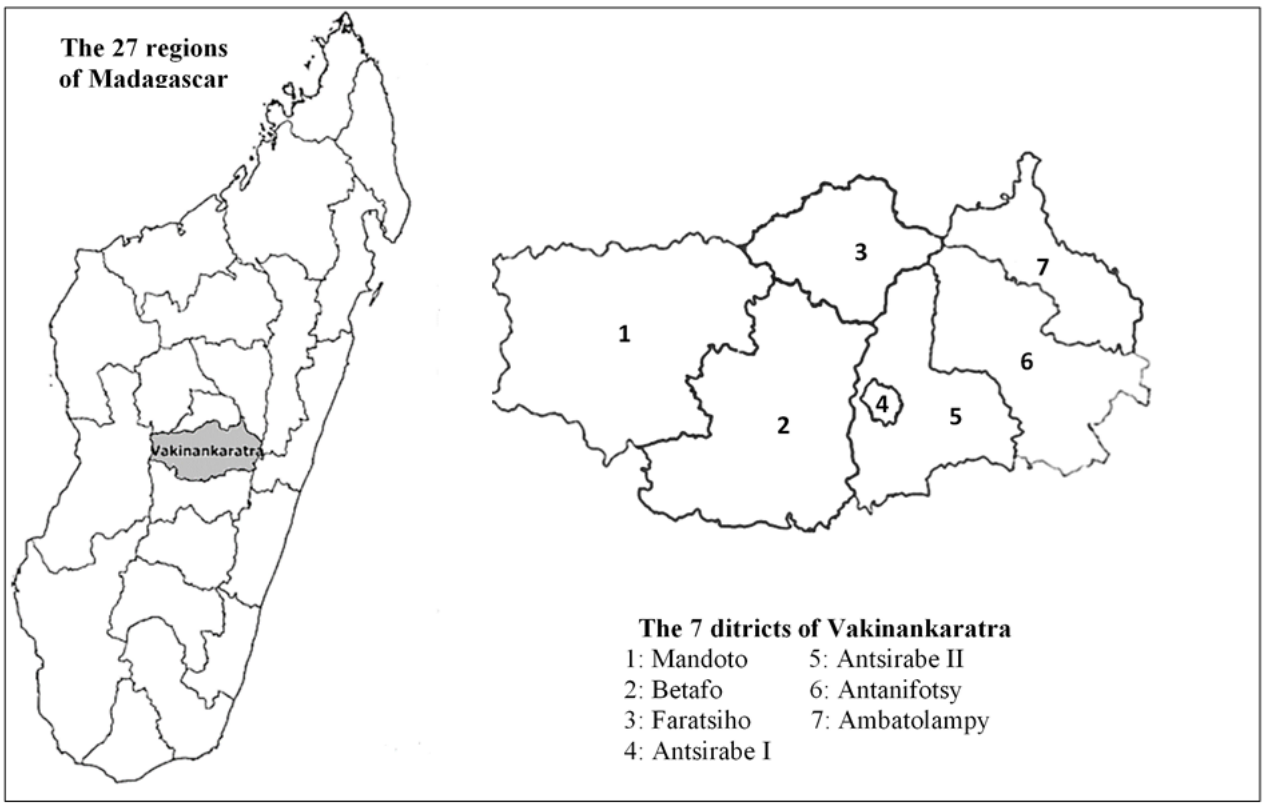

Figure 1 Location of the Vakinakaratra region and its 7 districts

Covering an area of 19,250 km^2, it is one of the most densely populated and economically productive regions of Madagascar, with a strong agricultural and agro-industrial orientation and an economy based mainly on agriculture. The region is characterised by volcanic highlands with altitudes ranging from 600 to 2,600 m. The climate is of the tropical highland type, with an average annual temperature of 20°C or less. Minimum temperatures can reach 5°C and

can fall below 0°C in winter, especially in June and July [13]. In terms of flora, Vakinankaratra has remarkable plant biodiversity with endemic, medicinal and useful species. The region has a forest cover of about 3% of the total area [12].

2.2. Ethnobotanical surveys

Ethnobotanical surveys were conducted in May and June in several areas of the Vakinankaratra region, including rural and peri-urban areas. In each locality visited, they were conducted with local agents (NGOs or projects), village communities, farmers and other resource persons such as guides, forest rangers and traditional healers. The methodology used followed approaches commonly employed in ethnobotany [14]. Data were collected using semi-structured interviews, direct observations at the sites, and guided visits by informants to the various harvesting locations. A questionnaire was completed for each recorded species in order to collect essential information such as vernacular names, therapeutic uses, potential toxicity, other uses, parts of the plant used, methods of preparation, and any other data deemed relevant. Herbarium specimen were created for the systematic identification of each species collected.

The inventory of *Crotalaria* species present in the study areas was compiled from rigorously selected bibliographic data. These sources, in particular the Tropicos website (Catalogue of the Vascular Plants of Madagascar), provided information on the botanical description, geographical distribution and location of the species. Furthermore, field observations enabled the collection of species not found in the references consulted.

2.3. Botanical identification

An initial identification was carried out by comparison with reference specimens kept at the Tsimbazaza Botanical and Zoological Park Herbarium (PBZT). The final botanical identification of each sample was then carried out with the support of Roger Marcus PolhilL, botanist at the Royal Botanic Gardens, Kew, and specialist in Fabaceae systematics. The voucher specimens were deposited at the herbarium of the Department of Plant Biology and Ecology at the Faculty of Sciences of the University of Antananarivo.

2.4. Study materials

For each plant identified, the various parts available at the time of the surveys were collected. For all plants, these were leaves and stem bark. Seeds and fruit pods were only available for certain plants.

2.5. Preparation of extracts

The harvested organs were dried separately in the shade, in a dry, clean and well-ventilated place. Once dry, they were ground using a blender-type grinder (Robot Coupe GT 550), sieved, and the fine powders obtained were stored at room temperature in airtight jars.

2.6. Phytochemical screening

The reactions of chemical group detection were those developed by Fong *et al.* [15] and Marini-Bettolo *et al.* [16].

3. Results

3.1. Identified species of *Crotalaria*

In the region, 19 species of *Crotalaria* were found at the study sites and identified (Table 1; Figure 2).

Table 1 List of species found at survey sites

Species cited in the bibliography and found at survey sites	**Species not cited in the bibliography but found at survey sites**		
C. diosmifolia *C. tanety* *C. ibityensis* *C. spinosa* *C. uncinella* *C. pallida*	*C. cytisoides* *C. emirnensis* *C. xanthoclada* *C. bernieri* *C. cleomifolia*	*C. incana* *C. incana* var. *purpurascens* *C. retusa* *C. grahamiana* *C. fulva*	*C. micans* *C. trichotoma* *C. zanzibarica*
Total: 6 species (31.6%)	Total:13 species (68.4%)		

Figure 2 The *Crotalaria* species collected and identified

Common names, geographical origin, collection sites, main traditional uses, parts of plants used, and associated methods of preparation for each species are presented in Table 2.

Of the 19 species recorded, six were endemic, accounting for 31.6% of the total; five were native, accounting for 26.3%; and eight were introduced, accounting for 42.1%. The richest collection sites were in the Ibity district, homed to almost 40% of the species, primarily endemic ones. This high concentration emphasised the area's ecological importance. The districts of Manandona and Betafo were home to a large proportion of native species. Introduced species were mainly collected in the districts of Behenjy and Manandona.

Table 2 Data on the different species of *Crotalaria* collected during ethnobotanical surveys

Species and origin	Vernacular names (local name)	Harvest location	Uses	Plant part used	Method of preparation
C. cytisoides (E)	Lakamisikely	Ibity	Diarrhoea, dysentery	L	Cooked with rice broth and leafy vegetables (« vary amin'ny anana »), infusion
			Scabies	WP	Topically applied powder
			Fishing poisons	L	Crushed fresh leaves thrown into the water to stun the fish
C. diosmifolia (E)	Ambarivatendolo, Korintsampotsy	Ibity	Treatment of poisoning by plants	L	Decoction of fresh plant: a soup spoonful per day
C. emirnensis (E)	Famamo	Betafo, Andraikiba	Fishing poisons	L	Crushed fresh leaves thrown into the water to stun the fish
C. ibityensis (E)	Angefotsy, Famamo	Ibity	Insecticides	WP	Fresh plant: aqueous maceration
C. tanety (E)	Akahomilahy, Amberivatrindolo	Ibity	Dysentery	L	Infusion of fresh leaves
C. xanthoclada (E)	Famakiasa, Hazombevavy	Manandona	Fishing poisons	L	Crushed fresh leaves thrown into the water to stun the fish
C. bernieri (N)	Ranomanja	Ibity	Stomach ache, diarrhoea	L	Fresh leaves decoction
			Food for oxen	WP	Fresh plant
C. cleomifolia (N)	Amberivatrindolo Kitsakitsan'akoho	Manandona	Green manure (compost)	WP	Mixed with *Tephrosia* and *Psiadia altissima* leaves
			Coffee substitute	S	Roasted seeds
C. incana (N)	Aikamanga, Kirintsana	Betafo	Green manure (compost)	WP	Fresh plant
			Feed for livestock		Fresh plant
			Fishing poisons		Crushed fresh leaves thrown into the water to stun the fish
C. incana var. *purpurascens (N)*	Kirintsana	Betafo (Tatamarina lake)	Feed for livestock	WP	Fresh plant
C. spinosa (N)	Tsilomboasary, Voasarimbiby, Voasarinalika	Ibity, Betafo	Fever	WP	Steam bath using the whole fresh plant
C. retusa (I)	Akondrondolo, Akondronjaza, Famonakoho	Behenjy	Fever	L	Fresh leaves: decoction
			Scabies		Topically applied powder
			Ground cover plant		Fresh plant
C. uncinella (I)	Ambarivatrandololahy, Kirintsana, Lakamisilahikely	Ibity	Dysentery	L	Decoction of fresh leaves
C. grahamiana (I)	Alokalok'akoho	Behenjy	Insecticides	WP	Fresh plant: aqueous maceration
			Green manure		Fresh plant
C. fulva (I)	Ambarivatrindolo	Behenjy	Scabies and skin tumours	S	Topically applied powder
			Green manure	WP	Fresh plant
C. micans (I)	Antsotry, Manarak'andro, Odiandro	Manandona	Green manure	WP	Fresh plant
			Scabies, boils	L, S	Powder: topical application
C. pallida (I)	Aika, Aikaberavina	Behenjy	Green manure,	WP	Fresh plant
			Urinary problems		Decoction
C. trichotoma (I)	Ahimatavy, Marofolehana	Andraikiba	Food for cattle	L, SB	Fresh plant
C. zanzibarica (I)	Ahimatavy	Manandona	Livestock feed, green manure, cover crop	WP	Fresh plant

N: native, E: endemic, I: introduced, L: leaves, SB: stem barks, S: seeds, WP: whole plant

3.2. Plant utilizations and modes of recipes preparation

The uses of the 19 *Crotalaria* species identified in the study area fell into three main categories (Table 3). Ten species were used in traditional medicine, particularly for the treatment of diarrhoea, dysentery, fever, scabies, skin tumours, boils and urinary tract infections. Nine species were used in agriculture, particularly as green manure, fodder plants or cover crops, and more occasionally as food resources or coffee substitutes (roasted seeds). Finally, six species were exploited for their toxic properties, mainly as fishing poisons or insecticides. It should be noted that some species fall into more than one category of use.

Table 3 The *Crotalaria* species concerned in the three main use categories

Main categories of use	Species concerned	Number of Species
Medicinal	*C. cytisoides*, *C. diosmifolia*, *C. tanety*, *C. bernieri*, *C. spinosa*, *C. retusa*, *C. uncinella*, *C. fulva*, *C. micans*, *C. pallida*	10
Agronomic	*C. bernieri*, *C. cleomifolia*, *C. incana*, *C. incana var. purpurascens*, *C. retusa*, *C. grahamiana*, *C. fulva*, *C. micans*, *C. zanzibarica*	9
Toxic	*C. cytisoides*, *C. emirnensis*, *C. ibityensis*, *C. xanthoclada*, *C. incana*, *C. grahamiana*	6

The same species of *Crotalaria* could be associated with several uses within local communities. The most frequently mentioned uses were green manure (18.9%), followed by the treatment of scabies and use as fishing poisons (10.8%), the treatment of dysentery and use as livestock feed (8.1%). Less common uses, including treating diarrhoea, use as an insecticide, managing fever, and as a cover crop, accounted for approximately 5.4%. Finally, the occasional use as a coffee substitute or for treating skin tumours, boils or urinary tract conditions was mentioned only once (2.7%).

The majority of preparations used the plant in its fresh state (50%), mainly for fodder, fertiliser or fishing. Aqueous preparations, such as decoctions, infusions, macerations, or steam baths, were also common (27%) and were mainly used to treat human or animal diseases. Powders of the plant were applied locally (17%) to treat skin conditions. Roasted organs or organs mixed with other plants (6%) were used in food recipes or in the preparation of mixtures (Figure 3).

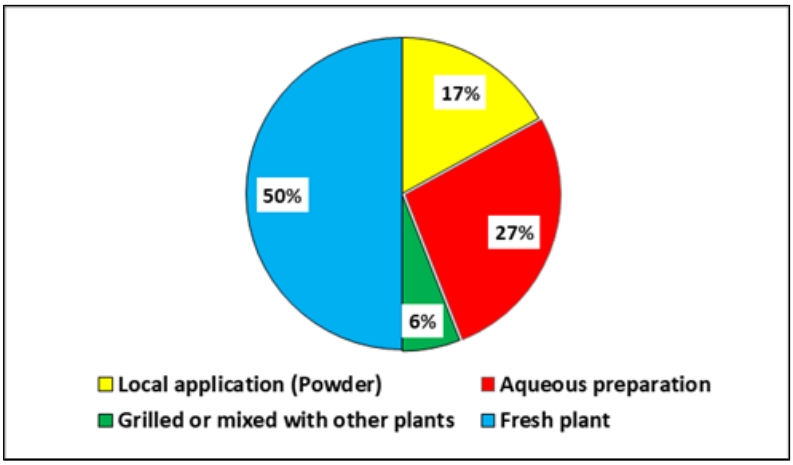

Figure 3 Modes of plant preparation and their uses

3.3. Used parts of plants

Analysis of the data (Figure 4) revealed that the whole plant and leaves were by far the most widely used parts, accounting for 45% and 39% of uses respectively. Seeds and stem bark were rarely exploited, accounting for less than 10%. The leaves were mainly associated with medicinal uses. The whole plants were more commonly used in agro-ecological and food applications.

Table 4 Phytochemical screening of different organs of various *Crotalaria* species from the Vakinankaratra region

Species	Alkaloids				Saponins				Flavonoids				Tannins and Polyphenols				Steroids				Triterpenes				Unsaturated sterols				Iridoids				Leucoantho-cyanins				Quinones			
	L	B	S	P	L	B	S	P	L	B	S	P	L	B	S	P	L	B	S	P	L	B	S	P	L	B	S	P	L	B	S	P	L	B	S	P	L	B	S	P
C. cytisoides	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
C. diosmifolia	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	+	+	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
C. emirnensis	+	-	*	*	-	-	*	*	-	-	*	*	+	+	*	*	-	-	*	*	-	-	*	*	-	-	*	*	-	-	*	*	-	-	*	*	-	-	*	*
C. ibityensis	-	-	*	*	-	-	*	*	+	-	*	*	+	+	*	*	-	-	*	*	-	+	*	*	-	-	*	*	-	-	*	*	-	-	*	*	-	-	*	*
C. tanety	-	-	*	*	+	-	*	*	+	+	*	*	+	+	*	*	-	-	*	*	+	-	*	*	+	+	*	*	-	-	*	*	-	-	*	*	+	+	*	*
C. xanthoclada	+	-	+	+	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
C. bernieri	+	-	-	+	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+	+	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
C. cleomifolia	+	-	+	-	-	-	+	-	-	-	-	-	+	+	+	+	-	-	-	-	-	+	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
C. incana	-	-	-	-	-	-	-	-	+	-	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
C. incana var. *pur*	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. retusa	-	-	+	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. spinosa	+	-	+	+	-	-	+	-	+	-	-	-	+	+	+	+	-	+	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
C. uncinella	+	-	-	-	-	-	-	-	-	+	-	-	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
C. fulva	-	-	*	*	-	-	*	*	-	+	*	*	+	+	*	*	-	-	*	*	-	-	*	*	-	-	*	*	-	-	*	*	-	-	*	*	-	-	*	*
C. micans	+	-	+	-	-	+	+	-	-	-	-	-	+	+	+	-	-	+	-	+	-	+	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
C. pallida	+	+	+	+	-	-	+	-	-	-	-	-	+	+	+	+	-	-	-	-	-	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
C. grahamiana	+	-	-	-	-	-	+	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
C. trichotoma	+	-	+	+	+	+	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
C. zanzibarica	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	-	+	-	-	-	+	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-

L: leaves; B: stem barks; S: seeds; P: pods; +: positive test; -: negative test
*: undetermined due to the absence or insufficiency of plant material

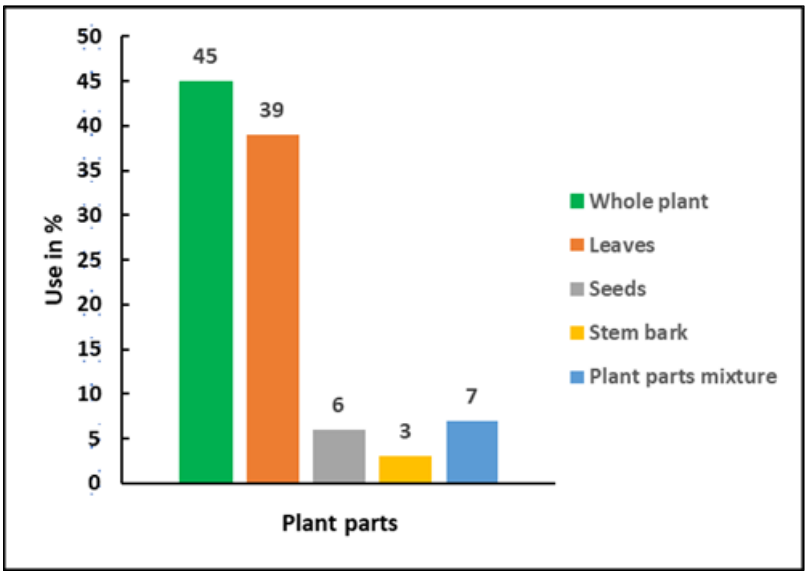

Figure 4 Percentage of use of the different parts of the plant

3.4. Qualitative phytochemical analysis

A phytochemical analysis was carried out on powder from various organs (leaves, stem bark, seeds and pods). The results are presented in Table 4. Eight of the 10 chemical families studied were detected in at least 1 species, while 2, leucoanthocyanins and iridoids, were not found in any of the 19 species. Tannins and polyphenols were present in all species. The number of chemical groups detected ranged from 2 (*C. emirnensis*) to 6 (*C. uncinella, C. tanety* and *C. micans*). With the exception of tannins and polyphenols, the other secondary metabolites were not present in all organs. For example, alkaloids were present in all organs for *C. pallida* and *C. zanzibarica*, in leaves and seeds only for *C. cleomifolia*, in leaves only for *C. uncinella*, and absent in all organs for *C. citisoides, C. diosmifolia*, etc.

4. Discussion

Ethnobotanical surveys conducted in the Vakinankaratra region allowed to identify 19 species of *Crotalaria*, including 6 endemic, 5 native and 8 introduced species. These species represented approximately 36% (19/53) of the taxa currently reported in the country [2]. The floristic composition showed a predominance of widely distributed species compared to endemic species, the latter being mainly located in southern Madagascar [1]. The 19 species recorded, corresponding to 35.8% of the *Crotalaria* species currently known in Madagascar, included 13 taxa (68.4%) that were not listed in the existing literature on the Vakinankaratra region.

The surveys conducted have documented the various uses associated with the species identified, including their medicinal properties, toxicity, and uses for food and agriculture.

Several uses of the 6 species mentioned in the literature were confirmed during the surveys, such as the use of *C. cytisoides* and *C. uncinella* against dysentery [17, 18] or *C. ibityensis* as an insecticide [18, 19]. Conversely, certain local practices, such as cooking *C. cytisoides* leaves with rice and other leafy vegetables (vary amin'ny anana) or using *C. cleomifolia* seeds as a coffee substitute, were not documented in the literature.

Depending on the locations visited, several species had multiple uses. For example, *C. bernieri* was not only used as feed for cattle, but was also as part of the traditional pharmacopoeia of the Ibity district, where the leaves were prepared as a decoction to treat abdominal pain and diarrhoea. Similarly, *C. zanzibarica* was used as fodder for livestock, ground cover and green manure, thereby contributing to improve soil fertility, which confirmed the ecological role of Fabaceae in biological nitrogen fixation [20].

Endemic species were mainly used for medicinal purposes or for toxic applications (*C. cytisoides, C. diosmifolia, C. ibityensis*), while introduced species were mainly used in agricultural applications or as fodder (*C. zanzibarica, C. micans, C. grahamiana*). Native species, on the other hand, have mixed medicinal and agricultural uses. This trend of using plants according to their origin (native, introduced, etc.) has been observed in other plants in other countries [21, 22].

Most preparations (50%) were made from fresh plants, particularly for uses related to fodder, green manure and fishing. Water-based preparations, such as decoctions, infusions, macerations, or steam baths, accounted for 27% of preparation methods and were mainly intended for medicinal purposes. The preferred use of fresh plants could be explained by the desire to preserve the integrity of bioactive metabolites, while the use of aqueous extractions reflected empirical mastery of techniques that promoted optimal extraction of active ingredients [23].

Regarding the parts used, the whole plant (45%) and leaves (39%) were the most popular, while seeds and stems were rarely used (<10%). This preference for leaves was also reported in other ethnobotanical studies in Africa [24, 25]. This predominance could be explained, on the one hand, by the permanent availability of the leaves, which were easy to harvest in large quantities, and on the other hand, by their high content of secondary metabolites compared to other parts of the plant [26]. Furthermore, harvesting this part did not compromise the survival of the plant, unlike other organs, and should be encouraged within local communities as a sustainable practice that promotes the conservation of valuable medicinal plants. As for the seeds, their use remained relatively rare, probably due to their limited availability, bitter taste, or low content of bioactive compounds compared to the leaves.

Phytochemical analysis of the various organs of the 19 species of *Crotalaria* surveyed revealed the presence of various secondary metabolites. It should be noted that the production of secondary metabolites depended on the species, location and harvest period (phenological stage) of the plant [27]. Alkaloids, flavonoids, tannins and polyphenols were the predominant compounds. These compounds are relatively common in the *Crotalaria* genus [28, 29, 30], and are widely known for their antimicrobial, anti-inflammatory and antioxidant activities [10], thus validating the traditional uses of the plant. However, it should be remembered that the toxicity of certain alkaloids, particularly pyrrolizidine alkaloids commonly reported in the genus *Crotalaria*, is well known [31, 32, 28]. This highlighted the importance of controlling the doses used in traditional preparations and the need for toxicological testing for species used for food or medicinal purposes.

5. Conclusion

This study supplemented the existing scientific data on the diversity, uses and phytochemical composition of *Crotalaria* species in the Vakinankaratra region. It provided valuable baseline information for future in-depth research aimed at improving knowledge and promoting the potential of these plants. Ultimately, this study will enhance our understanding of Madagascan plant biodiversity, contributing to the conservation and promotion of the *Crotalaria* genus.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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