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Propithecus Verreauxi Feeding for Anthelmintic Purpose

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Abstract

Lots of scientific findings have been convinced and advanced the hypothetical ability of some animals, such Primate, to preserve daily well-being, using elements from its environment, especially plants. The aim of this study was to determine the possible relationship between the behavior of one Madagascan Lemur specie, living in Berenty Private Reserve, and their observed zero gastrointestinal parasite prevalence. Behavioral monitoring was conducted on eight adult *Propithecus verreauxi*: consumed plants were listed/recorded/harvested, its different plant parts were extracted and subsequent chemical families were identified. Lemur stools were also drawn for coproscopy and anthelminthic biological tests of plant extracts were performed. During the tracking, thirty-two plants composed the Berenty adult *Propithecus verreauxi* diet. Less than half (44.73%) of its extracts exhibited an anthelminthic effect according to the biological tests on *Lemurostongylus* sp. and 34.21% according to scientific literatures. A proportion of 47.06% of these plant extracts contained saponins. After coprological analysis, no Berenty adult *Propithecus verreauxi* stool contained gastrointestinal worm, all studied parasitic parameters were zero. Extracts of Berenty *Propithecus verreauxi* consumed plants demonstrated anthelminthic properties on gastrointestinal parasite: *in vivo* zero prevalence, *in vitro* inhibition of hatching egg and larvae paralysis. In addition to the confirmation of zoopharmacognosy theory, these outcomes may allow a possible establishment of natural anthelmintic ingredients for captive Lemurs.

Keywords: *Propithecus verreauxi* • Madagascar • Plant feeding • Parasitosis • Anthelminthic • Zoopharmacognosy

Introduction

Gastrointestinal helminthiases are one of the major plagues of animals in their natural environment and Madagascan Lemurs are not exempt [1]. Indeed, by their mechanical and traumatic, spoliation, toxic, metabolic disrupting or reproductive inhibiting actions, these helminthiases can harm the animal well-being up to its death by various pathogenic actions.

However, various studies have focused on the intestinal parasitism of an adult Madagascan Lemur (*Propithecus verreauxi*, named also in Malagasy "Sifaka"): it has shown a really low parasite load [2]. Scientists explain this absence of gastrointestinal parasites by putting forward several theories, among them, the zoopharmacognosy. Animals would take advantage of various non-nutritional plant elements or other elements available in nature [3].

Ingestion of plants, rich in secondary compounds, could be interpreted as a preventive as well as curative use and would be very frequently proved as animal behavior, including primates [4]. This phenomenon had motivated researchers to accomplish various works: firstly, there is the advanced notion of preventive zoopharmacognosy for prophylactic purposes, animals would be able to show "food" preference for therapeutic and homeostatic purposes, this behavior was interpreted as "good health prevention"; it would be the result of associative memory use: animal feels the well-being of some items ingestion and perpetuates the act to "preserve" it. For example, an occasional consumption of small amounts of *Trichilia rubescens* leaf by healthy Kibale chimpanzees was intrigued researcher Sabrina Krief; after analyses, isolating the antimalarial plant molecules, chimpanzees may ingest plants for preventive purposes [4]. Secondly, there is confirmed hypothesis of curative zoopharmacognosy: animals could "relieve some of their illnesses" by ingesting items with biologically active compounds. This has been leading to the theory, during the course of evolution: animals have acquired the ability to avoid some plants because of their toxicity, but also to prefer others providing relief and/or heath/well-being improvement.

Instinctive plant use by wild animal was first suggested by the coincidence observation of chimpanzee recovery after consumption of *Vernonia amygdalina* bitter stems [5]. And related to that, the present study aimed to provide information about the relationship between Berenty adult *Propithecus verreauxi* feeding behavior and its corollary to health purpose, leading to support the eventual zoopharmacognosy evidence theory.

Method and Materials

Animal studies

Study protocol was approved by Scientific Committee of Malagasy Institute of Applied Researches (Albert and Suzanne Rakoto-Ratsimamanga Foundation), and all operations were carried out according to university's (Animal Ethic and Well-being Committee, Veterinary School, Faculty of Medicine, University of Antananarivo) and OIE's (Organisation Internationale d'Épizootie) guidelines for animal research.

Study was carried out on adult and sub adult *Propithecus verreauxi* population and conducted in two periods: July 2019 (animal tracking in Gallery and Spiny forests, in Berenty Private Reserve, South of Madagascar: 22°10'00" S and 45°04'00" E) and from August 2019 to April 2020 (plant analyses and biological testing, in Antananarivo, Madagascar Capital: Laboratory of Medical Sciences, Laboratory of Clinical Pharmacology Assessment, Laboratory of Food Cosmetic Remedy and Parc Botanique et Zoologique de Tsimbazaza [PBZT]).

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Each Berenty Lemur (*Propithecus verreauxi*, called "Sifaka" in Malagasy) group was identified beforehand and then monitored for one day without taking parameters: focal individuals were drawn from different localities; two individuals per group with particular traits were followed. Ethological, botanical and coprological data were essentially collected, for all individuals, during a total period of forty days. For each Lemur group and/or animal tacking: first day was observer habituation phase, second and third days were individual follow-up/monitoring/tracking, fourth day was fresh feces collecting and an additional day was focal individual consumed plant harvesting.

Direct observation grid (instantaneous and scan sampling observation for budget activity: every 10-minute sequences recording) and collection sheet (for consumed plants and stools information) were established. Breaking, defecating, feeding, food searching, grooming, moving and watering were mainly considered.

Plant studies

All consumed plants were harvested: information gathered from reserve guides and geographical data were recorded, herbarium and raw extract were prepared later on. Each plant part was collected and dried in the field, in shade at room temperature.

Each collected part of studied *Propithecus verreauxi* consumed plants was macerated in hydro alcoholic solution, filtrate was concentrated and dried. Resulting extracts were preserved in refrigerator for further biological test and chemical analysis.

Information about medicinal properties and chemical compounds of investigated plants, were collected from electronic open access literatures (PubMed, university theses), combining plant and anthelmintic terms.

Biological testing

Focal individual fecal samples were immediately packaged, labeled, and stored at 4°C for further laboratory processing. Hatching and parasite larvae paralyzing tests were executed for the biological assays. The evaluation of the plant extract anthelmintic activity was based on coprological examination and egg counting.

All studied *Propithecus verreauxi*, consuming the harvested plant parts, during its diurnal activities, were monitored, their stools were collected: qualitative methods by sedimentation and flotation were carried out under microscope and followed by the quantitative method from McMaster-technic. Following parameters were resulted: Egg-Per-Gram (EPG), Parasitism-Prevalence (PP, %), Parasite-Specific-Richness (PSR) and Parasitic-Intensity (PI).

Hatching-Egg-Inhibition (HEI, %) was also calculated: parasite eggs were suspended and maintained in culture until hatching, motile and immobile helminth larvae were counted, Larvae-Paralysis-Rate (LPR) value in the presence of extract was compared to that control.

Tests were carried out in triplicate. Plant extracts with LPR and/or HEI equal to or greater than 50% were considered as exhibiting an anthelminthic activity.

Phytochemical screening

Extract qualitative phytochemical screening was performed identifying the chemical constituent main groups regarding the color and physical changing reactions. Screening was applied to plant extracts exhibiting an anthelminthic effect.

Data analyses

Data were recorded using the Microsoft Excel program (Office Version 2016) and some observed parameter values were expressed as average (Mean ± SEM: Standard Error of Mean).

Absence or presence of a significant relationship between studied variables was determined by correlation test based on Principal-Component-Analysis (PCA) using XLSTAT®2019.2.2. program. Results were expressed as factorial map form in biplot diagram.

Results

During the study period, with its 1,000 ha area, Berenty Private Reserve harbored four *Propithecus verreauxi* groups. From three local forests, in total of 19 individuals, 08 were particularly identified (5 males and 3 females), and then, two focal individuals per group were monitored: population characteristics were summarized in Table 1, those of study sites and each studies focal individual specific identification were shown in Figure 1.

Mean proportion of each diurnal focal individual activity was presented in Table 2: almost half of their time (46.16 ± 13.37%) was feeding and as much time (40.92 ± 11.94%) was resting, *Propithecus verreauxi* never drunk.

As observed, *Propithecus verreauxi* only consumed 40.51% (n=79) of all plants present in their territory. Leaf composed the almost third of four of their alimentation (Table 3). Euphorbiaceae – Fabaceae – Meliaceae constituted the more than 40% (in almost same proportion) of consumed family plants by the Berenty *Propithecus verreauxi* and *Azadirachta indica* (Mimy) – *Tamarindus indica* (Kily) – *Commiphora humbertii* (Darosike) composed the 20 to 80% of their alimentation. The prevalence of Berenty adult *Propithecus verreauxi* infested by gastrointestinal helminths was 00.00% (n=19) (Table 4). One milliliter of PBZT Lemurs fecal suspension contained 90 *Lemurostrongylus* sp. eggs (Table 5). Seven (07) plant extracts totally inhibited egg hatching and paralyzed all *Lemurostrongylus* sp. larvae (Table 6).

From coprological examination, *Propithecus verreauxi* fecal contained indigestible alimentary bolus, their aspects were generally green colored with normal consistence. All stools contained no susceptible or visible gastrointestinal parasite, parameter profile values (EPG, PP, PSR, PI) were zero.

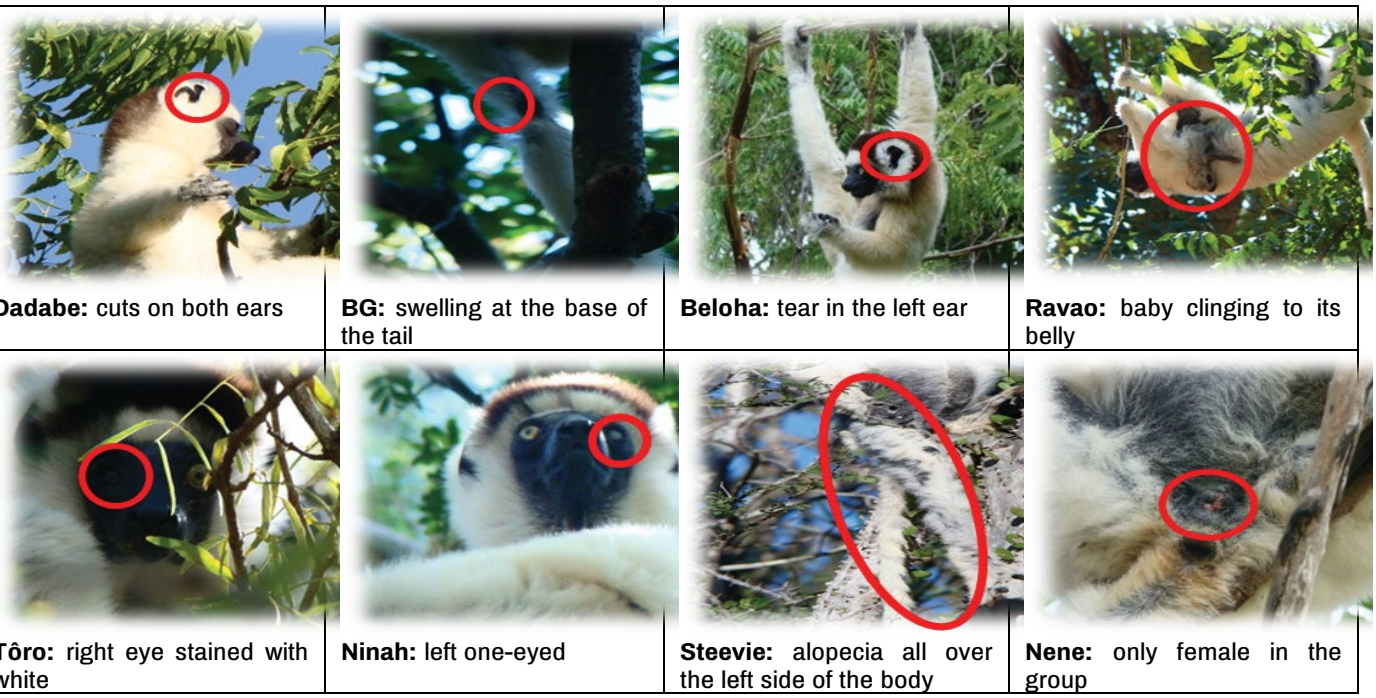
Figure 2A was illustrated the consumed plants by all monitored adult *Propithecus verreauxi*. It summarized the different proportion according to the taken quantities, whether high or low. There was a significant difference between the proportions of each consumed plant. Lemurs in Gallery forest consumed Neem (*Azadirachta indica*) and Kilim-bazaha (*Pithecellobium dulce*), while those from Spiny forest most consumed Darosike (*Commiphora humbertii*) and Daromena (*Commiphora orbicularis*). There was a strong statistically positive correlation between the choices of consumed plant parts of each adult *Propithecus verreauxi*: all individuals consumed leaf in greater quantity (Figure 2B). Illustrated in Figure 3, there was no statistically positive correlation between the population parasitic prevalence and their characteristics (forest of origin, gender, group, physiological stage) of focal individual; contrariwise, it was strongly positive with their consumption of plants with anthelminthic properties: focal individuals, consuming anthelminthic plants according to biological tests and/or the scientific literature information and those from local practitioner guide using human anthelmintic medicinal plants, were completely free from gastrointestinal parasites.

Propithecus verreauxi consumed plant confronted information was summarized in Table 7: seven samples completely inhibited hatching egg

Table 1. Characteristics of Lemur (*Propithecus verreauxi*) population and focal individuals.

Local forest name	Lemur group name	Lemur effective per group	Attributed Lemur name	Gender	Lemur biological development stage	Effective of Lemur focal individuals
Malaza	Voafelaka 2	7	Dadabe	M	A	4
			BG	M	SA	
	Mpantsaka 1	6	Beloha	M	A	
			Ravao	F	A	
Ankoba	Blind	3	Ninah	F	A	2
			Tôro	M	A	
Anefotany	Transition	3	Steevie	M	A	2
			Nene	F	A	
Total=19						**Total**=08

A: Adult; SA: Sub Adult; F: Female; M: Male.



Dadabe: cuts on both ears

BG: swelling at the base of the tail

Beloha: tear in the left ear

Ravao: baby clinging to its belly

Tôro: right eye stained with white

Ninah: left one-eyed

Steevie: alopecia all over the left side of the body

Nene: only female in the group

Figure 1. Study site images and focal individual identifications.

Table 2. Mean proportion repartition of studied *Propithecus verreauxi* diurnal activity.

Activities	Alimentation	Rest	Shifting	Grooming	Food research	Defecation	Others	Watering	Total
Mean ± SEM (%)	46.17 ± 13.37	40.92 ± 11.94	5.87 ± 1.28	3.72 ± 2.20	2.42 ± 1.16	0.40 ± 0.28	0.50 ± 0.68	0.00 ± 0.00	100. 00

Table 3. Mean proportion of plant parts consumed by studied *Propithecus verreauxi*.

Plant part	Young leaf	Mature leaf	Petiole	Ripe fruit	Stem bark	Bud	Green fruit	Total
Mean ± SEM (%)	45.86 ± 5.61	29.07 ± 14.48	5.13 ± 3.27	5.13 ± 3.27	5.13 ± 3.27	5.13 ± 3.27	4.55 ± 7.87	100. 00

Table 4. Parasitic profile of Berenty adult *Propithecus verreauxi*.

Studied Lemur group	Parasitic profile		
	Prevalence (%)	Richness (n)	Intensity (EPG)
Voafelaka 2 (n=7)	00.00	0	0
Mpantsaka 1 (n=6)	00.00	0	0
Blind (n=3)	00.00	0	0
Transition (n=3)	00.00	0	0

n = effective

Table 5. Gastrointestinal egg per millimeter effective of fecal suspension of captured *Eulemur hybrid* in PBZT.

Lemur	EPM
Eulemur hybrid	90

Table 6. Sensitive biological development stage, hatching egg percentage inhibition and *Lemurostrongylus* sp. Larvae paralysis rate exhibited by extracts of Berenty adult *Propithecus verreauxi* consumed plant parts.

Plant species	Consumed plant parts	Sensitive stage (+)		HEI (%)	LPR (%)
		Egg	Larvae		
Alluaudia procera	Young leaf		+	00. 00	100. 00
Azadirachta indica	Young leaf	+		100. 00	00. 00
	Bark	+	+	100. 00	100. 00
	Petiole		+	50. 00	100. 00
Commiphora humbertii	Mature leaf	+		100. 00	00. 00
Cordia sinensis	Young leaf	+	+	100. 00	100. 00
Cordia subcordata	Young and mature leaf	+	+	100. 00	100. 00
Diospyros humbertiana	Young and mature leaf	+		100. 00	81.25
Ficus cocculifolia	Young and mature leaf		+	00. 00	100. 00
Phyllanthus casticum	Mature leaf		+	50. 00	100. 00
Pithecellobium dulce	Young and mature leaf	+	+	100. 00	100. 00
	Bud	+	+	100. 00	100. 00
Salvadora angustifolia	Young and mature leaf	+	+	100. 00	100. 00
Tabernaemontana coffeoides	Young and mature leaf	+		100. 00	56.25
Tamarindus indica	Young leaf		+	50. 00	100. 00
	Ripe and green fruit		+	00. 00	100. 00
Xerosicyos perrieri	Young leaf	+	+	100. 00	100. 00

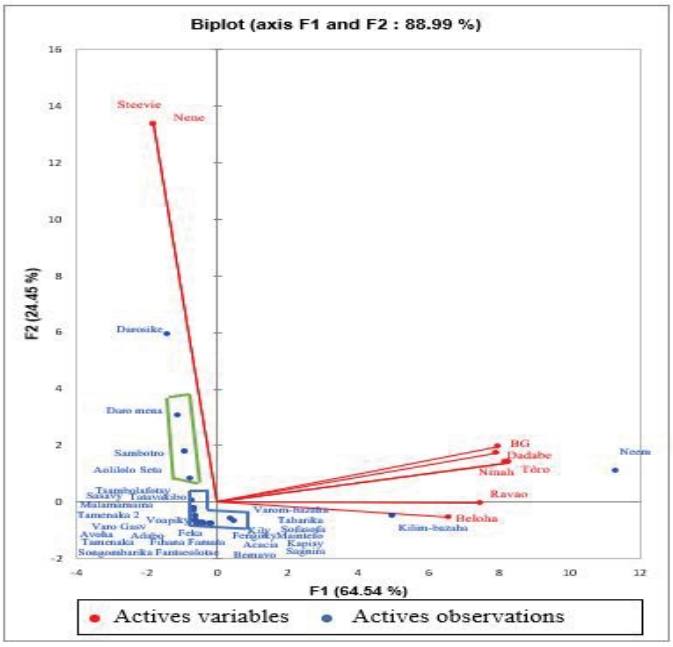
HEI: Hatching-Egg-Inhibition (%); LPR: Larvae-Paralysis-Rate (%)

Table 7. Confronted information about studied *Propithecus verreauxi* consumed plants.

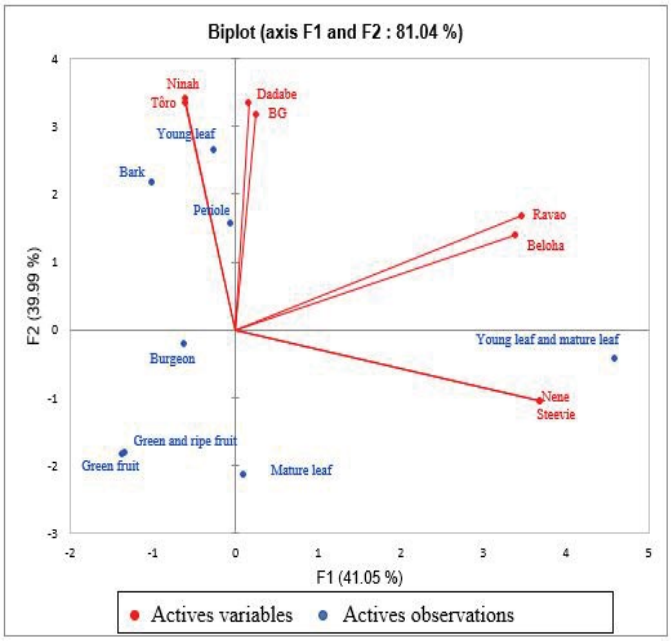
Plant				Literature information		Gastrointestinal anthelminthic property		Phytochemical screening		Focal individuals feeding correspondent plant
Family	Specie	Vernacular name	Part	Biological properties for human	Ref	Scientific literature	Lab	Scientific literature	Lab	
APOCYNACEAE	*Tabernaemontana coffeoides*	Feka	L	Convulsion / Muscle tiredness / Muscular and nervous asthenia / Respiratory depression / Arteriosclerosis / Brain trauma / Circular irregularities / Nasopharyngeal bacterial infections / Cardiac arrhythmias / High blood pressure / Vomiting	1	-	+	A	S/C	Ravao
BORAGINACEAE	*Cordia sinensis*	Varom-bazaha	L	Fever	_	-	++	A	TP/S/C	Ninah, Tôro
			Fr	Oxidation / Malaria / Inflammation / Bacterial infection	1	-	-			
	Cordia subcordata	Varo gasy	L	Bronchitis / Asthma / Liver infections / Liver cirrhosis / Urinary tract infections / Abdominal swelling	3	-	++	A/TP/F/S	TP	Dadabe, BG
BURSERACEAE	*Commiphora humbertii*	Darosike	L	Oral mild inflammatory disease / Coronary heart disease / Gynecological disease / Obesity	2	-	+	S/P	-	Dadabe, Steevie, Nene
	Commiphora orbicularis	Daro mena	L	Liver diseases / ***Helminthiasis*** / Gastrointestinal, respiratory, muscular, urinary tract disorders / Rheumatism / Scurvy / Jaundice / Cancer	2	+	-	-	-	Steevie, Nene
CANNABACEAE	*Celtis bifida*	Bemavo	L	_	_	-	-	-	-	Beloha, Ravao
COMBRETACEAE	*Combretum albiflorum*	Tamenaka	L	Syphilis / Abdominal pain / Diarrhea / Bacterial infections / ***Helminthiasis***	4	+	-	-	-	Beloha
	Combretum phaneropetala	Tamenaka	L	Fever / Liver disorders / Diuretic	2	-	-	-	-	Steevie, Nene
CUCURBITACEAE	*Xerosicyos perrieri*	Tapisaka / Kapisy	L	_	_	-	++	S	A/TP/S/P	Beloha, Ravao
DIDIEREACEAE	*Alluaudia humbertii*	Songombarika	L	_	_	-	-	-	-	Steevie, Nene
	Alluaudia procera	Fantseolotse	L	Toothache / Oral infections	2	-	+	-	TP/P	BG
EBENACEAE	*Diospyros humbertiana*	Maintefo	L	Urinary tract infections / Skin and blood diseases / Syphilis / Malaria	1	-	+	TP/S	F/S/P	Steevie, Nene

EUPHORBIACEAE	*Croton androiensis*	Aolilolo	L	Fever / Malaria / Convulsions / Hypertension / Microbial infections / Dysentery	1	-	-	-	-	Steevie, Nene
	Croton sp.	Tsambolafotsy	L	Fever / Malaria / Convulsions / Hypertension / Microbial infections / Dysentery	1	-	-	-	-	Steevie, Nene
	Euphorbia fiha	Fihana	L	Asthma / ***Helminthiasis***	1	+	-	-	-	Dadabe
	Euphorbia sp.	Famata	L	Osteoarthritis / Convulsion / Diabetes / Eczema / Inflammation / Microbial infection / Cell oxidation / Muscle spasm / Cancer / Cough	3	-	-	-	-	Dadabe, BG
	Margaritaria decaryana	Malamamaina	L	Impotence / Senility	2	-	-	-	-	Steevie, Nene
	Phyllanthus casticum	Sagnira	L	***Helminthiases*** / Dysentery / Dengue fever / Viral hepatitis / Cancer / Wounds / Abscesses / Eczema / Syphilitic ulcers / Edema	6	+	+	A/TP/F	-	BG
FABACEAE	*Acacia rovumae*	Acacia	L	Stomach and mouth inflammation / Stomach and mouth irritation / Wounds / Ulcers / Cough and sore throat	1	-	-	-	-	Beloha, Ravao
	Delonix adansonioides	Fengoky	L	Cough / Fever / Rheumatism	1	-	-	-	-	Dadabe
	Mundulea sp.	Sofasofa	L	Poisoning	1	-	-			
	Pithecellobium dulce	Kilim-bazaha	L	***Helminthiasis*** / Cellular aging / Tuberculosis / Diabetes / Gastric ulcer / Liver disease / Muscle pain / Inflammation / Diarrhea	3	+	++	A/TP/P	-	Dadabe, BG, Beloha, Ravao, Ninah, Tôro
	Tamarindus indica	Kily	B	Inflammation / Bacterial infection	2	-	++	TP/F/S	-	Dadabe, BG, Beloha, Ravao, Ninah, Tôro
			L	***Helminthiasis*** / Stomach ache / Scar	2	+	+	A/TP/S	S/C/P	
			Fr	***Helminthiasis*** / Constipation / Bacterial infection / Fungal infection	2	+	+	F/S/C	S/C	
MELIACEAE	*Azadirachta indica*	Neem / Mimy	L	***Helminthiasis*** / Fungi / Bacterial infection / Pox pox / Chickenpox / Psoriasis / Malaria / Muscle pain / Fever /	1	+	+	TP/S	-	Dadabe, BG, Beloha, Ravao, Ninah, Tôro
			BA	Dental Inflammation	1	-	++	P	-	
			Fr	***Helminthiases*** / Skin diseases / Psoriasis / Urinary diseases / Hemorrhoids	1	+	-	-	-	
			Pt	_	_	-	+	-		
MOLLUGINACEAE	*Mollugo decandra*	Tatavakibo	L	Loss of appetite	1	-	-	-	-	Steevie, Nene
MORACEAE	*Allantsiliodendron alluaudianum*	Avoha	L	_	_	-	-	-	-	Steevie, Nene
	Ficus cocculifolia	Adabo	L	Jaundice / Snake poisoning / Milk retention / ***Helminthiasis***	5	+	+	TP/F/P	-	Beloha, Ravao
PORTULACACEAE	*Talinella grevei*	Sambotro	L	Genitourinary disorder / Gastrointestinal disorder / ***Helminthiasis*** / General fatigue / Skin inflammation / Skin lesion	1	+	-	-	-	Steevie, Nene
SALVADORACEAE	*Salvadora angustifolia*	Sasavy	L	Dental Inflammation	1	-	++	A	TP/F/S/C	Steevie, Nene
SAPINDACEAE	*Allophylus decaryi*	Voapiky	L	Fracture / Rashes / Stomach ache / ***Helminthiasis*** / Oral conditions / Intestinal disorders / Hepatitis / Diabetes / Wounds	5	+	-	-	-	Steevie, Nene
TILIACEAE	*Grewia* sp.	Tabarika	L	Stomach pain / ***Helminthiasis***	1	+	-	-	-	Steevie, Nene

- No anthelmintic effect; **+** Anthelmintic effect in gastrointestinal parasite larvae or egg; **++** Anthelmintic effect in gastrointestinal parasite larvae and egg;
Lab: Laboratory; Ref: Effective of literature reference**;**
BA: Bark; B: Burgeon; Fr: Fruit**;** L: Leaf; Pt: Petiole;
A: Alkaloids**; C**: Coumarins; F: Flavonoids**;** P: Polysaccharides; **S**: Saponins; TP: Tannins/Polyphenols.



(2A)



(2B)

Figure 2. Factorial maps of the relation between the eight studied adults *Propithecus verreauxi* and the consumed plant **(A)** species and **(B)** parts.

and paralyzed all *Lemurostrongylus* sp. larvae (100%, n=17) at 2,400 µg/mL. *Pithecellobium dulce* leaf and bud extracts showed anthelminthic activity in *Lemurostrongylus* sp. egg and larval stages; 11 plant species, out of 32, exhibited anthelminthic activity according to the scientific literatures, with 28 bibliographic citations; 06 plant species among the 32 possessed anthelminthic activity according to both biological tests and scientific literatures, with 19 bibliographic citations. Phytochemical screening, of *Propithecus verreauxi* consumed plant extracts, revealed the abundance/presence of alkaloids, coumarins, flavonoids, polyphenols and tannins, polysaccharides, saponins.

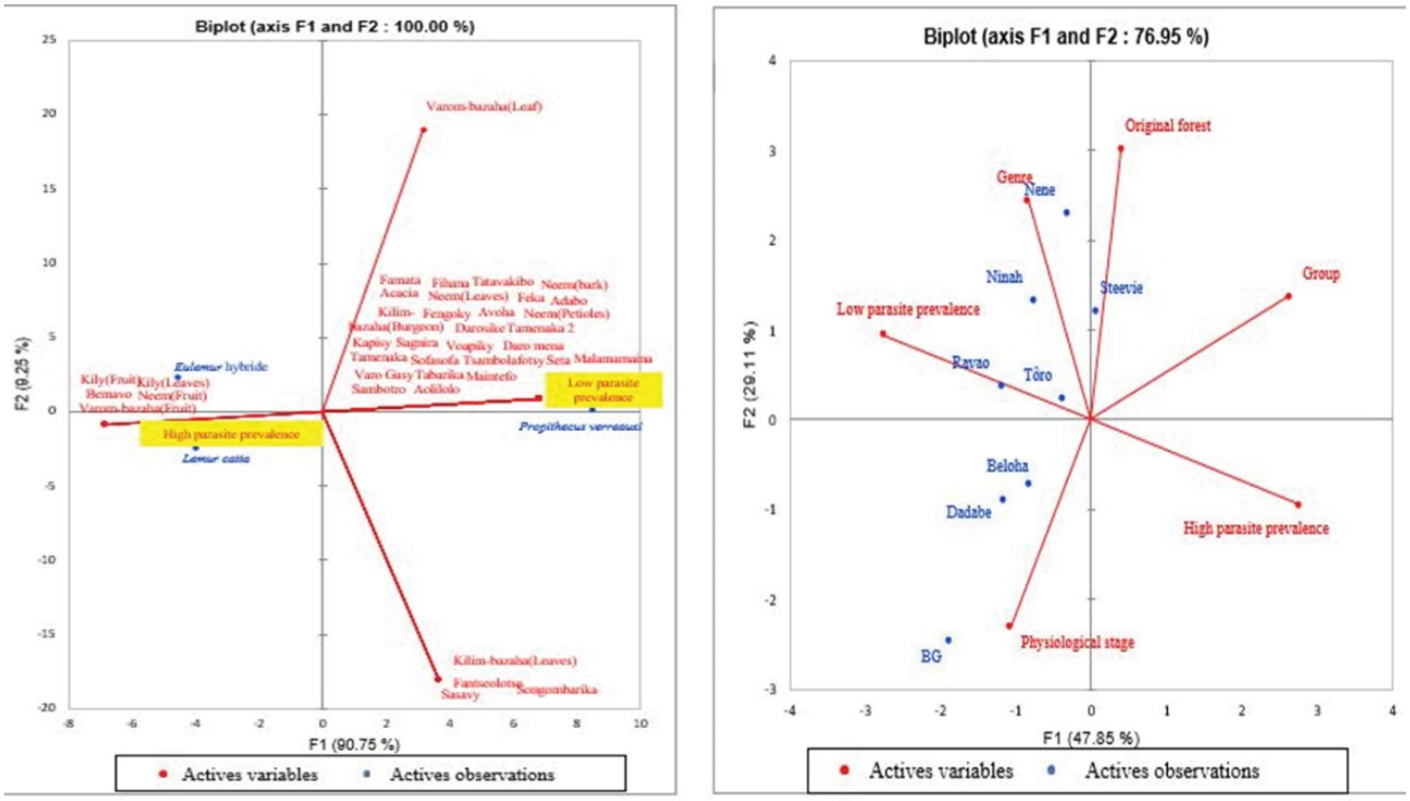
Discussion and Analysis

Berenty Private Reserve was already the study sites of numerous researches [6,7] the present studies were focused on feeding behavior of *Propithecus verreauxi* from Gallery and Spiny forests. Although, most of Lemurs living conditions have been approximately the same over the last decade, some have undergone significant changes: group formation and interchanging, climate; but above all, constituting vegetation that would considerably influence the *Propithecus verreauxi* diets, justifying the monitoring choice of Lemur adult stage [2].

During the study period, in the all area, researchers counted four *Propithecus verreauxi* groups with three to seven individuals each. Animals with apparent/visual body characteristic were chosen, to facilitate tracking. Their main activities were eating, resting and moving with a statistically significant difference between each group activity budget: Spiny forest individuals fed more than others and neglected other activities, they spent very little energy on other activities and consumed maximum effective plants. "Rest-and-Move" activities might be linked: Ninah and Tôro from the ANKOBA forest rested more than the others but also moved with a greater proportion than other individuals. Thirty-two plants have been consumed by all focal individuals during the forty-day tracking. Some plants were consumed by three of four groups: Neem (*Azidirachta indica*), Kilim-bazaha (*Pithecellobium dulce*) and Kily (*Tamarindus indica*); Darosike (*Commiphora humbertii*) was eaten by both groups (Dadabe and BG) in Gallery forest and groups of Steevie and Nene in Spiny forest; Feka (*Tabernaemontana coffeoides*) were only consumed by Ravao. On one hand, climate change, influencing the plant existence, could explain this difference; on the other hand, Lemurs could compensate the absence of some plants by consuming new ones that would providing same well-being. Study period, coincided with the calving season and breastfeeding phase, would be favorable for adult *Propithecus verreauxi*, might probably be synchronized with periods of food maximum resource availability, necessary for their offspring survival. Young leaf was very appreciated by focal individuals (45.85%), followed by mature leaf (29.07%). Other plant parts were eaten in more moderation. Adult *Propithecus verreauxi* would prefer to feed young leaf, fruit, flower when available, because they must contain a high level of carbohydrate and protein, but would not be available throughout the year, unlike mature leaf that have been presented in successive seasons. This study revealed that Sifaka consumed only 40.51% (n=79) of inventoried plant in their territory, demonstrating a wide plant choice but also observing some ones "preference" consumption.

The present work provided pharmacological and chemical data on the seventeen extracts of harvested plants from Berenty. Biological tests were carried out demonstrating anthelminthic properties of the adult *Propithecus verreauxi* consumed plants against *Lemurostrongylus* sp. However, laboratory tests did not predict the complex interactions that can occur in vivo, i.e. in Lemurs and this helminth should not necessarily reflect all the parasites found in adult *Propithecus verreauxi*; but, it was respecting the artificial fulfilling criteria of high enough parasitic intensity. Extraction method, intended for bioassays, could also have altered or failed to pass some bioactive compounds, which could have changed some settings. Most (16 to 35%) consumed plant part extracts were shown anthelminthic properties against *Lemurostrongylus* sp. (Figure 2): *Azadirachta indica* - *Commiphora humbertii* - *Pithecellobium dulce*, bark - leaf - petiole. Study conducted by Ravakiniaina et al. [2] also showed that the consumption of plants with deworming properties by *Propithecus verreauxi* such as *Tamarindus indica*, *Poivrea coccinea* and *Cedrelopsis greveii* would reduce or eliminate the parasites. This could explain the relatively low parasite specific richness in these animals compared to other Lemurs.

According to PCA biplot analysis (Figure 2A), plants gathered and located on the opposite direction of Sifaka individual axes were the consumed plants in small quantities. Correlating to Negre's team hypothesis, fractional but frequent consumption of active plants would be compatible by the maintenance of a long-term ecological benefit and numerous diseases prevention [8]. Anthelminthic plant ingestion would stimulate a synergy action which could be linked to several active plant consumptions hypothetically ensuring an organism health benefit. Adult *Propithecus verreauxi* plant consumption, in minimal quantities, might provide neither energy nor protein in a significant



(3A) **(3B)**

Figure 3. Principal component analysis of the variables; mapping of the Berenty studied *Propithecus verreauxi* parameters (A. consumption of plants with anthelminthic properties, presence of correlation) and (B. population characteristics, absence of correlation) compared to their gastrointestinal parasite prevalence.

way, compared to other consumed plants in vast majority could be interpreted by a search for benefits, other than nutritional ones. In terms of diet (Figure 2a), BG, Dadabe, Ninah, Tôro, Ravao and Beloha were very positively correlated with an acute angle separating each of these variables. It showed a great dissimilarity between the consumed plants by the 06 Gallery forest individuals and the 02 Spiny forest ones: various plants were eaten by both ANKOBA and ANEFOTANY Gallery forest individuals, but only one plant was eaten by both individuals from Spiny and Gallery forests. This result coincided to previous conclusion [9] reflecting that floristic compositions of Gallery were similar but it was different from those of Spiny. In relation to plant part consumption choice, some illustrated in Figure 2B was similar to those indicated in Figure 2A; it only varied from group to group. Individuals in same group ate same plant parts, leaf consumption in significant quantities was common for all individuals. Spiny forest group individuals, unlike individuals in Gallery forest, minimized activities that require them to expend a lot of energy, such as searching for reproductive parts but spend a lot of time-consuming food. These preferences seemed to be driven by availability of these plant parts during the tracking, but also plant ease accessibility and tenderness. It should be noted that plant bark was easy to access as leaf but was more difficult to chew, while petiole was tender but its removal was laborious and required time. As illustrated in Figure 3A, many of adult *Propithecus verreauxi* consumed plants, with or without anthelmintic properties, also consumed by other diurnal species sharing its habitat, such as *Lemur catta* and *Eulemur hybrid* [7]. However, these two Lemur species were exposed to high parasite prevalence, richness and intensity; whereas, adult *Propithecus verreauxi* was almost free from gastrointestinal parasites. Many factors might explain these differences: *Lemur catta* and *Eulemur hybrid* quadrupedal locomotion, their daily dedication to walks on the ground, their physical contact due to their non-territorial way of life, their thirst quenching and food searching, their insectivory, their coprophagy [10]. Figure 3B highlighted too some factors that did not influence Berenty diurnal Lemurs parasitism: neither genus, nor forest origin, nor group and nor physiological stages. Indeed, for the physiological stage, only adults and subadults have been studied, so there were no juveniles whose immune system would not be yet functional, nor elderly subjects whose immune function may be in decline. For the gender, the hormonal factor might not play a significant role with regard to parasitic infestation. Belonging to a group and forest origin were the only variables with a positive correlation, which reinforced the fact that adult *Propithecus verreauxi* were living in isolated groups and were territorial. Conferring to scientific literatures, in addition to anthelminthic activities of adult *Propithecus verreauxi* consumed plants, many of them were exhibited complementary medicinal properties; whether, verified or not by biological tests, some of them were traditionally used by human population, others were used as therapeutic treatments in animals; most of the listed plants demonstrated medicinal properties, except Neem (*Azadirachta indica*) petiole, or leaves of Kapisy (*Xerosicyos perrieri*) - Bemavo (*Celtis bifida*) - Songombarika (*Alluaudia humbertii*) - Avoha (*Allantsiliodendron alluaudianum*). It is possible that phytochemical and biomedical virtues in-depth studies were not yet carried out but it was verified that 84.21% (n=32) of adult Berenty *Propithecus verreauxi* ingested plants that had therefore recognized medicinal virtues. Our study, had proven that Neem (*Azadirachta indica*) petiole and Kapisy (*Xerosicyos perrieri*) leaf exhibited anthelminthic properties against *Lemurostrongylus* sp., might bring a plus in this research findings area. Indeed, these plant parts were not deemed to have any medicinal properties. “Nutraceutical” term, whose use would be more appropriate and fitted perfectly with our hypotheses [4]. Agreeing to results of all extract phytochemical screenings: most of them contained secondary compounds. Visual examination from phytochemical screening, i.e. color and physical changing can therefore differ from one manipulator to another, could explain this difference. Another reason was based on the soil quality where plants were growing: soil composition can taint biological composition of plants collected from different places. Maturity, of plant part (bud, young shoot or old leaf), may play a decisive role on the composition of secondary compounds, because they have only produced during a specific period of its biologic developmental stage.

About the various correlations studied, there was a difference notified between the species of plants ingested by the eight (08) adult *Propithecus verreauxi* individuals. In fact, the zoopharmacognosy hypothesis was born from the fact of a minimal or even zero parasitic infestation, according to some studies in the other sites where the adult *Propithecus verreauxi* have been distributed. However, no study has yet observed the parasitism of individuals from the Berenty Private Reserve. At the end of the coprological examination, on the one hand, no blood, no mucus nor pus, even nature parasitic elements did not note; on the other hand, undigested vegetable elements were noticed. Such information was also observed in chimpanzees which consumed large amount of fiber; this could be interpreted by an increasing intestinal peristalsis that possibly facilitating the expulsion of gastrointestinal parasites, in case of infestation. It can also be interpreted that this consumption may not therefore have a nutritional purpose, but rather or hypothetically therapeutic. Coprological examination was shown no detected parasite; parasite prevalence, parasite richness and parasitic intensity were completely equal to zero, for all studied individuals from the four (04) Lemur groups lived in Berenty Reserve. Various theories could explain this non-existence of gastrointestinal parasites in adult *Propithecus verreauxi*: firstly, their diet which would contain molecules with anthelminthic properties (extracts, from eleven ingested plants and twelve from adult *Propithecus verreauxi* diet, were active against *Lemurostrongylus* sp. eggs); secondly, their arboreality may decrease the likelihood of contracting parasites transmitted through the soil or from stagnant water, cisterns or ponds [10].

With reference to zoopharmacognosy perception, several elements brought to light by this study could reinforce the idea that adult *Propithecus verreauxi* practice it. First of all, according to Negre [8], it seemed delicate to speak of behavior of zoopharmacognosy if the notion of voluntary food choices was not demonstrated. Yet, during the present study, a census of the plants presents on these Sifaka territory, and not consumed, had been carried out, which possibly demonstrated as a "voluntary food choice". The plant petiole and bark consumptions would also reflect a notion of choice because these plant parts were required laborious work of removal, their ingestion would demonstrate a need that must be satisfied despite the loss of time and generated energy. Then, during the tracking observations, some plants with anthelminthic effects against *Lemurostrongylus* sp., were consumed in large quantities, such as *Azadirachta indica*: three of the four consumed parts exhibited an anthelminthic activity against *Lemurostrongylus* sp., as well as *Commiphora humbertii* which constituted the important part of Spiny forest adult *Propithecus verreauxi* feeding. The abundant ingestion of these plants would favor an action by cumulative doses, this could concern the consumption of basic functional food. This Lemur consumed also other anthelminthic plants, but more rarely: it was the case of *Tamarindus indica* or *Tabernaemontana coffeoides*. In general, the duration of consumption and their ingested quantity were key elements of zoopharmacognosy: the shorter this duration, the lower the quantity, the greater the likelihood, the zoopharmacognosy hypothesis could be confirmed. Coprological studies showed non-existent parasitism in adult *Propithecus verreauxi*. Following our bioassays, adult *Propithecus verreauxi* consumed at least 17 plants that its extracts exhibited anthelminthic activity against *Lemurostrongylus* sp. These anthelminthic properties were demonstrated on an experimental model; its choice would be seemed relevant. Indeed, even if *Propithecus verreauxi* was proven no gastrointestinal parasites, previous author studies of coprological analyzes had shown that adult *Propithecus verreauxi* was infested by *Lemurostrongylus* sp. [10]. Nevertheless, bioassays have many limitations: chemical extraction might firstly differ from natural digestion, plant extracts resulting from this mode of food mechanical and chemical transformation into nutrients may not be biologically similar to that achievement from chemical extractions; it would be also necessary to take into account the probable synergies and antagonisms which operate between active compounds of these plants in adult *Propithecus verreauxi* organism. Then, the chosen helminths would reflect only a tiny fraction of the parasites likely to be found in adult *Propithecus verreauxi*. It is agreed that biological tests do not make to know the active fraction of the plant extracts, nor even the precise quantities of fresh matter ingested by these Sifakas for each part of the plant involved in the biological tests; but, 13 plant part extracts of Berenty adult *Propithecus verreauxi* diet were confirmed and used in human/animal ethnomedicine as anthelminthic; since primates are the closest evolutionary animals to humans, these plants could also have the same effects on Lemurs [2].

At the same time, this Lemur gender, their physiological stage, group or even forests of origin have no impact on their parasitism. But their predominantly arboreal life way and absence of watering in contaminating rivers or ponds could also improve adult *Propithecus verreauxi* against gastrointestinal parasite contaminations. Lastly, adult *Propithecus verreauxi* could be able to show a preference for a "plant" for therapeutic and homeostatic purposes allow it to "preserve" their good state of health. This practice could be the result of the use of associative memory: consequently, *Propithecus verreauxi* adult would feel the well-being provided by ingestion of plants with anthelminthic properties and perpetuate the act to “preserve” this well-being [11]. *Propithecus verreauxi* would therefore instinctively opt for the consumption of species or plant parts whose extracts have exhibited anthelminthic properties, according to carried biological tests and confirmed by scientific literatures.

Conclusion

The observed findings were sustained, one more time, the zoopharmacognosy hypothesis practiced by *Propithecus verreauxi*, one Primate lived in Berenty Private Forest Reserve in the South of Madagascar: behavioral feeding, based on plant with anthelmintic (no presented fecal/gastrointestinal adult worm, not-hatching egg and larvae paralysis) properties, contributed to zero parasitic intensity/prevalence/richness of gastrointestinal worm of this Lemur kind.

A largest comparison studies to other Madagascar Lemurs or Primates may extend the confirmation of the actual observations.

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Conflicts of Interest

The authors declare no competing interests.

References

1. Chabaud Alain-G., Edouard-R. Brygoo and Annie-J. Petter. “Les nématodes parasites de Lémuriens malgaches : VI. Description de six espèces nouvelles et conclusions générales.” *Ann Parasitol Hum Comp* 40 (1965): 181-214.

2. Rambeloson Ravakiniaina V., Jeanne Rasamy Razanabolana and Peter M. Kappeler. “Étude des parasites intestinaux chez *Propithecus verreauxi* (Grandidier, 1867) avant et après la période de mise-bas.” *The Newsletter of the Madagascar Section of the IUCN SSC Primate Specialist Group, Göttingen* (2014): 61-67.

3. Fortin, Gaëlle. “Étude bibliographique des phénomènes d’automédication par les plantes et les produits minéraux chez l’animal : Impact de la recherche en zoopharmacognosie.” *DMV Diss* (2012): 17-24.

4. Krief, Sabrina. “Métabolites secondaires des plantes et comportement animal: surveillance sanitaire et observations de l’alimentation des chimpanzés (*Pantroglodytes schweinfurthii*) en Ouganda : Activités biologiques et étude chimique de plantes consommées, Sciences du Vivant [q-bio].” *PhD Diss., Muséum National d’Histoire Naturelle, (2003)*.

5. Huffman, Michael A. and Mohamedi Seifu. “Observations of illness and consumption of a possibly medicinal plant *Vernonia amygdalina* (Del.), by a wild chimpanzee in the Mahale Mountains National Park, Tanzania.” *Primates* 30 (1989): 51-63.

6. Heckman Kellie L, Emilienne Rasoazanabary, Erica Machlin and Laurie R Godfrey et al. “Incongruence between genetic and morphological diversity in *Microcebus griseorufus* of Beza Mahafaly.” *BMC Evol Biol* 6 (2006): 1-11.

7. Simmen Bruno, Annette Hladik and Pierrette Ramasiarisoa. "Food intake and dietary overlap in native Lemur catta and Propithecus verreauxi and introduced *Eulemur fulvus* at Berenty, southern Madagascar." *Int J Primatol* 5 (2003): 949-968.

8. Nègre, Alice, Laurent Tarnaud, J. F. Roblot and Jean-Charles Gantier, et al. "Plants consumed by *Eulemur fulvus* in Comoros Islands (Mayotte) and potential effects on intestinal parasites." *Int J Primatol* 27 (2006): 1495-1517.

9. Ravelonjatovo, Sylvia Aimée. "Contribution à l'étude du comportement et de l'écologie de *Propithecus verreauxi* dans la deuxième parcelle de la Réserve Spéciale de Bezà Mahafaly." *Master Diss., University of Antananarivo* (1998): 74-78.

10. Loudon, James E. and Michelle L. Sauther. "Verreaux's sifaka (*Propithecus verreauxi*) and ring-tailed lemur (*Lemur catta*) endoparasitism at the Bezà Mahafaly Special Reserve." *Madag Conserv Dev* 8 (2013): 21-28.

11. Lozano, George A. "Parasitic Stress and Self-Medication in Wild Animals." *Adv Study Behav* 27 (1998): 291-317.

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