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Plants Consumed by *Eulemur fulvus* in Comoros Islands (Mayotte) and Potential Effects on Intestinal Parasites

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The study of self-medication among animals—zoopharmacognosy—is founded on observations that suggest that wild animals use plants with specific biological properties that may be beneficial to them. To verify whether self-vermifugation occurs among Eulemur fulvus *in the wild, we studied their feeding behavior in both the dry and humid forests of Mayotte (Comoros Islands). We used the focal individual sampling method over an annual cycle. We conducted a complementary study during the 2-mo mating season, via the scan sampling method (at 10-min intervals). Among the 29 plant species brown lemurs consumed, we tested 16 in vitro as antiparasitic agents on 3 experimental parasite models* (Rhabditis pseudoelongata, Trichomonas vaginalis, Entamoeba invadens). *We obtained crude extracts to be tested after 2 successive chemical extractions (ethyl acetate and methanol), and 7 of them, belonging to 4 different plant species, showed an antiparasitic property: lemurs consumed* Annona squamosa *and* Mimusops comorensis *in large amounts, but ingested* Ixora cremixora *and* Syzygium jambos *sporadically. The 4 plants were active on the flagellate but only one of them* (Ixora cremixora) *also demonstrated antinematode properties. Humans use 2 of the plants as intestinal antiparasitic agents in traditional medicine and include*

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numerous other plants in the diet. The relative lake of amoebas and flagellates in stools of Eulemur fulvus *may be related to the consumption of plants with antiprotozoal properties. Nevertheless, in the absence of specific behavior that could be linked to a voluntary therapeutic action during our study, self-vermifugation in* Eulemur fulvus *remains elusive.*

KEY WORDS: antiparasitic property; *Eulemur fulvus*; feeding behavior; zoopharmacognosy.

INTRODUCTION

Since the 1960s, observations of feeding ecology in primates have suggested that they consume plants that could be useful in medicine (Cousins and Huffman, 2002; Glander, 1994; Huffman, 1997; Huffman and Wrangham, 1994; Huffman *et al.*, 1998a,b; Rodriguez and Wrangham, 1993). In 1991, Rodriguez and Wrangham introduced the concept of zoopharmacognosy: "the process by which wild animals select and use specific plants with medicinal properties for the treatment and prevention of disease" (Glander, 1994). Huffman and Seifu reported the first accepted example of therapeutic ingestion of plants in 1989. They described the recovery of an adult female chimpanzee after consumption of *Vernonia amygdalina*. They suggested that the low consumption frequency of the plant, known for its ethnobotanical value, indicated that it was ingested for reasons other than nourishment, and could represent medicinal use. Later, Huffman *et al.* (1993) demonstrated a reduction in parasite load within 20 h after consumption of the species, with demonstrated antiparasitic properties, by a second sick female chimpanzee. Wrangham and Nishida (1983) also observed unusual feeding behavior in chimpanzees that consumed leaves of different species of *Aspilia* sp. without chewing them. In 1985, Rodriguez *et al.* proposed that the leaves may function as a vermicide. More detailed field and laboratory investigations (Huffman *et al.*, 1996; Page *et al.*, 1997) failed to replicate the original findings and revealed no other significant nematocidal activity. Complementary studies (Huffman and Caton, 2001; Huffman *et al.*, 1996) showed that adult nematodes are expelled by the physical action of swallowing rough leaves of *Aspilia* sp. and several others species, which suggests that leaf-swallowing behavior is a mechanical way to control nematodes. Recently, in Uganda, Krief (2003a) observed a young female chimpanzee eating the bark of *Albizzia grandibracteata*. The plant, which the chimpanzee community had never consumed before, is used as vermicide in traditional medicine and could have positive effects on the primate's health. Indeed, the female, suffering from both diarrhea and constipation, with a high number of parasites detected in stools, was in good health again 2 d after consuming the plant. Most previous observations of unusual feeding behavior concern great apes. In the literature on lemurs, researchers have reported no observation of sick individuals consuming particular plants and only a few observations could be related to zoopharmacognosy. In 1999, Birkinshaw described a female of *Eulemur macaco* rubbing her fur with a myriapod (*Charactopyrus* sp.) having repellent effect on external parasites. The anointment behavior seems voluntary and closely resembles that which Valderama *et al.* (2000) described in wild capuchins, wherein a strong chemical deterrent produced by the myriapod provides the important repellent effect. Recent work (Carrai *et al.*, 2003) has shown unusual feeding habits by periparturient sifaka females (*Propithecus verreauxi verreauxi*) selecting tannin-rich plants and suggests multiple benefits, such as anthelmintic effects (Athanasiadou *et al.*, 2001).

Within the theoretical framework, we hypothesized that prosimians benefit from the medicinal properties of plants that they consume. We propose to verify whether plants ingested by brown lemurs (*Eulemur fulvus*) have antiparasitic properties, irrespective of whether or not overt signs of illness are associated with the consumption. We conducted a field study in Mayotte on wild lemurs in their natural habitat. We further tested the activity of plants consumed on 3 experimental models of parasites.

METHODS

Study Sites and Subjects

We conducted the study in Mayotte, an island of the Comoro archipelago in the Mozambique channel 350 km from the African coast. The climate comprises 2 distinct seasons: 1) a wet season from November to April and 2) a dry season from May to October. We obtained data during 2 different field studies on wild adult brown lemurs. Between 1999 and 2001, during 10 different mo (excepted May and June), we observed 4 adults from 2 groups in the dry forest of Saziley in the southeast of Mayotte (Tarnaud, 2004b). In May and June 2002, we conducted complementary observations on 3 adults from 1 of the groups (Nègre, 2003). In June 2002, we made additional observations on another group (12 individuals) in the rain forest of Combani at the center of the island.

Observations of Feeding Behavior

We observed the feeding behavior of brown lemurs to identify the composition of their diet and the proportion of time they consumed food items. Feeding behavior refers to the actual ingestion of foods. We used 2 methods per Altmann (1974) related to the previous studies: 1) focal individual sampling (Tarnaud, 2004a) and 2) instantaneous and scan sampling, with scans separated by periods of 10 min. At Saziley, good observation conditions allowed us to identify and to follow several individuals, whereas in the rain forest of Combani we recorded only the main activity of visible individuals. We conducted observations from dawn to dusk.

Physical and Chemical Preparation of Plant Material

Botanists at the Paris National Museum of Natural History authenticated plant parts that brown lemurs ingested via preserved samples at the museum's herbarium. We collected items consumed during observations of May and June at Saziley and Combani for bioassays. Seeds were mostly undigested in lemur feces; hence we removed them from fruits and studied only pulp and peel. We dried plant materials carefully in a botanic dyer (Service de l'Environement et de la Forêt de Mayotte) and then reduced them to powder with 2 different grinders adapted to item size (Laboratoire de Chimie des Substances Naturelles, Université Paris Sud). We obtained crude extracts after 2 successive chemical extractions with EA followed by that in methanol, followed by 3 macerations of plant material (40 g dry mass) for 1 h at 40°C and evaporation of the solvent (Rotovapor®; Krief, 2003). Then, we made solutions of the crude extracts at 5, 10, and 25 g/l in ethanol with 6% dimethyl sulfoxide (DMSO). However, when it became impossible to dissolve the extracts fully in the mixture, we changed the solvent (ethanol + 6% DMSO) to pure DMSO. We kept the solutions at 4°C.

Assay Models

We evaluated the activity of the crude plant extracts *in vitro* on 3 parasites available at the Chatenay-Malabry Laboratory of Parasitology (France): *Rhabditis pseudoelongata* (nematode), *Trichomonas vaginalis* (flagellate protozoa), and *Entamoeba invadens* (amoeba). We used them as experimental models, representative of nematodes, flagellate protozoa, and amoeba parasites in *Eulemur fulvus* (Table I). We introduced parasites to the solutions to be tested and, after incubation, counted the parasites microscopically to determine the presence or absence of plant activity, compared to the control culture composed of the solvent (ethanol + 6% DMSO or pure DMSO) and the same quantity of parasites. In primary assays, carried out in duplicate, we incubated parasites in the presence of 100 mg/l of crude extracts. We tested the plant extracts selected in

Table I. *Eulemur fulvus* intestinal parasites

Type of parasite	Parasite species	Way of life of *Eulemur fulvus* host	References
Nematode			
Oxyure	*Callistoura brygooi*	Wild/captivity	Chabaud and Petter (1958), Coiffier (2000)
Oxyure	*Callistoura blanci*	Wild/captivity	Chabaud *et al.* (1965), Coiffier (2000)
Oxyure	*Lemuricola vauceli*	Wild/captivity wild	Chabaud *et al.* (1965), Nègre (2003)
Oxyure	*Lemuricola baltazardi*	Wild/captivity	Chabaud *et al.* (1965)
Oxyure	*Enterobius lemuris*	Wild/captivity	Baer (1935), Chabaud and Petter (1958)
Oxyure	*Enterobius anthropopitheci*	Captivity (uncertainly determined)	Baylis and Daubney (1922)
Connected to oxyures	*Subulura prosimiae*	Captivity	Baer (1935)
Trichure	*Trichuris lemuris*	Wild	Chabaud *et al.* (1965), Chabaud *et al.* (1964)
Protozoa			
Amoeba	*Entamoeba histolytica*	Captivity	Smith and Merrovitch (1985)
Coccidia	*Cryptosporidium* sp.	Captivity	Gomez *et al.* (1992)
Flagellate	*Trichomonas* sp.	Wild	Nègre (2003)
Ciliate	Undetermined	Wild	Nègre (2003)

complementary assays, carried out in triplicate, on the same 3 parasites, at 100, 200 and 500 mg/l, to confirm any antiparasitic activity.

Activity on Flagellates and Amoebas

We observed anti-*Trichomonas* activity over cultures of the parasite in T.Y.M. modified medium (Diamond, 1957; Taylor and Baker, 1968) in which we replaced sheep serum by calf serum. The strain, from a woman suffering from trichomoniasis, is sensitive to metronidazole (Loiseau *et al.*, 2002). We cultured *Entamoeba invadens* (MNHN strain), from the diarrhea of a python, in Pavlova's medium (Kreier, 1978) supplemented with 10% of heat-inactivated fetal calf serum. We carried out experiments on protozoan parasites using a glass tube containing *ca.* 10,000 amoeba/ml (20,000 *Trichomonas*/ml), in a final volume of 5 ml with 100 μl of the solution to be tested. We counted viable protozoa via a Malassez cell after 1 week of incubation at 22°C for amoeba, and after 48 h at 37°C for *Trichomonas*.

Activity on Nematodes

We isolated *Rhabditis pseudoelongata* (IP strain) from wild rabbit feces and maintained them on a solid medium composed of sterilized rabbit feces. We recovered worms from 7–10-d-old culture via a Baermann apparatus. We carried out bioassays in 24-cell culture plates (Costar®, Corning). Each cell contained *ca.* 1000 worms (Bories, 1993) in a volume of 500 μl of water (Volvic®). Then, we added 10 μl of the crude plant extract solutions. After 24 h of incubation in darkness at 25–27°C, we counted motile worms in 50 μl of each cell.

Data Analysis

We used descriptive statistics to express the diet of brown lemurs and unilateral tests to determine which extracts killed more parasites than in the control culture. In primary assays we adapted the risk α to investigation possibility ($\alpha = .01$ for *Rhabditis pseudoelongata*; $\alpha = .1$ for *Entamoeba invadens* and *Trichomonas vaginalis*). We tested results on the nematodes via reduced space, and on protozoa via Student *t*-test. Interpretation of complementary assays is graphic, based on a linear regression (Microsoft Excel) to show the tendency (direction of slope *a* and gradient *b*) and its interpretation (*t* absolute value of the statistic *t*).

RESULTS

Annual Diet of the Brown Lemur

The main part of feeding behavior data is from the dry forest of Saziley with 1028 h of observation by day and 892 h by night (Tarnaud, 2004b) complemented by 299 scans (Nègre, 2003). From the humid forest of Combani, we have only a few additional observations (152 scans). On both study sites in Mayotte, brown lemurs are mainly frugivorous-folivorous, also consuming flowers (Table II). The majority of the diet comprised very few plant species.

At Saziley, the annual quantities of ripe and unripe fruits ingested represent two-thirds of the diet (Table IIA and B). The remaining third consists of young and mature leaves. The subjects ingested 40 plant parts belonging to 24 identified species. Each month, the consumption of 3 or 4 food items accounted for 75% of the food ingested, thus forming the bulk of the diet. Individuals consumed some food plants in large amounts during

Table II. Annual diet of *Eulemur fulvus* at Saziley and Combani

A. Proportions of solid food ingested by adult females at Saziley over 1 yr excluding May and June (1999–2001)

Plant species	Part eaten	October	November	December	January	February	March	April	July	August	September	Mean
Mangifera indica	Ripe fruit	57.9	54.3	48.7	51.8	18.4	0	0	0	0	49.4	28.05
Mimusops comorensis	Ripe fruit	4.0	1.3	0	0	21.5	0	4.7	8.1	48.3	24.6	18.45
Ancylobotrys petersiana	Ripe fruit	0	0	0	28.7	11.5	0	25.7	0	0	.8	6.67
Cordia myxa	Ripe fruit	0	0	0	0	35.3	17.6	0	0	0	0	5.29
Albizia lebbeck	Flower	16.0	0	1.7	0	0	9.1	0	0	0	1.8	3.76
Annona squamosa	Flower	12.3	1.3	1.5	1.2	0	2.6	0	0	0	0	3.69
A. squamosa	Young leaves	0	7.3	0	0	0	0	0	3.6	15.9	8.7	3.55
M. comorensis	Mature leaves	.5	5.7	.3	8.5	0	3.3	0	0	14.6	1.3	3.43
A. squamosa	Ripe fruit	0	0	0	0	1.4	0	27.7	0	0	0	2.91
Grewia sp.	Ripe fruit	0	0	0	0	6.3	21.5	1.2	0	0	0	2.90
A. petersiana	Young leaves	4.2	13.3	2.7	2.6	.3	0	1.8	.2	0	3.6	2.89
Tamarindus indica	Young leaves	0	1.1	.2	3.7	.7	18.3	2.5	0	2.3	0	2.88
T. indica	Unripe fruit	0	0	0	0	0	12.1	15.1	0	0	0	2.72
Salacia leptoclada	Mature leaves	2.7	.3	0	0	0	6.4	8.7	1.1	2.7	3.5	2.54
Ehretia cymosa	Ripe fruit	0	0	22.8	0	0	0	0	0	0	0	2.28
T. indica	Mature leaves	.5	.6	.1	0	8	.3	.2	8.6	7.5	3.5	2.22
T. indica	Ripe fruit	.1	.3	.5	0	0	8.1	0	5.2	0	0	1.41
M. indica	Unripe fruit	.0	0	0	0	0	0	0	0	7.0	2.7	.98
Adansonia digitata	Flower	.8	1.4	0	0	0	0	5.9	0	0	0	.82
Lantana camara	Flower	.3	1.4	0	0	2.8	0	2.5	0	0	0	.69
L. camara	Ripe fruit	0	0	1.2	.5	0	0	3.3	0	0	0	.50
S. leptoclada	Young leaves	0	.1	1.6	2.1	.3	0	0	0	0	0	.41
Broussonetia greveana	Young leaves	0	2.0	0	0	0	0	0	0	0	0	.20
S. leptoclada	Ripe fruit	0	0	.1	.9	.8	0	0	0	0	0	.17
A. squamosa	Mature leaves	0	0	0	.0	0	0	0	0	1.6	0	.16
M. indica	Young leaves	0	0	.1	0	0	.8	.6	0	0	0	.15
Terminalia catappa	Ripe fruit	0	0	0	0	0	0	0	1.2	0	0	.12
Leptadenia madagascariensis	Mature leaves	.4	.1	.5	0	0	0	0	0	0	0	.11
A. petersiana	Flower	.1	.4	0	0	0	0	0	0	0	0	.05
Erytrhoxylum lanceum	Ripe fruit	0	0	0	.1	0	0	0	0	0	0	.02
Total		100	100	100	100	100	100	100	100	100	100	100

Table II. Continued

B. Proportion of time devoted to the consumption of plant parts at Saziley in May and June (2002)

Plant species	Food items	May	June	Mean
Mimusops comorensis	Ripe fruit	49.24	20.12	34.68
Tamarindus indica	Mature leaves	9.75	22.09	15.92
T. indica	Unripe fruit	9.59	18.08	13.83
Annona squamosa	Ripe fruit	10.03	13.34	11.68
Musa sp.	Ripe fruit	.00	13.30	6.65
AN 7[a]	Mature leaves	7.45	.00	3.73
A. squamosa	Mature leaves	.00	3.75	1.88
T. indica	Flower	.00	2.77	1.39
Senna singueana	Mature leaves	2.57	.00	1.29
Mangifera indica	Mature leaves	.71	1.39	1.05
Terminalia catappa	Ripe fruit	.62	1.39	1.00
Allophyllus bicruris	Mature leaves	1.86	.00	.93
Mystroxylon aethiopicum	Mature leaves	1.65	.00	.83
Tarenna supra-axillaris	Ripe fruit	1.65	.00	.83
Secamone pachystigma	Mature leaves	.00	1.57	.78
M. indica	Young leaves	1.42	.00	.71
M. comorensis	Mature leaves	1.42	.00	.71
T. indica	Young leaves	.00	1.25	.63
Ancylobotrys petersiana	Mature leaves	.00	.96	.48
Ficus sp.	Mature leaves	.71	.00	.35
AN 15[a]	Mature leaves	.71	.00	.35
Albizzia lebbeck	Mature leaves	.62	.00	.31
Total		100	100	100

C. Proportion of time devoted to the consumption of plant parts at Combani in June (2002)

Plant species	Food items	June
Mimusops comorensis	Unripe fruit	50.0
Litsea glutinosa	Ripe fruit	31.3
L. glutinosa	Mature leaves	15.6
Syzygium jambos	Unripe fruit	3.1
Ixora cremixora	Ripe fruit	*b*
Ficus pyrifolia	Unripe fruit	*b*
Ceiba pentandra	Flowers	*b*
C. pentandra	Mature leaves	*b*

Note. Highlighted when solid food consumption represented >10% of the overall monthly diet.
[a]Plant species unidentified.
[b]Consumptions observed between scan sampling.

several successive mo. Indeed, the ripe fruit of *Mangifera indica* accounted for >45% of the diet from September to January. From May to August >30% of the diet consisted of ripe fruit of *Mimusops comorensis*. Lemurs ingested a few other food items, such as the ripe fruit of *Cordia myxa,* in large amounts only over a short time (35.3% in February and 17.6% in

March). Lemurs consumed leaves of *Annona squamosa* in large quantities during August (15.9%). Subjects ingested others items more rarely, such as young leaves of *Salacia leptoclada*, *Broussonetia greveana* and *Mangifera indica,* which represent $<2\%$ of the diet, whatever the period of the year.

At Combani, they consumed 6 additional plant parts from 5 species (Table IIC). *Mimusops comorensis* and *Litsea glutinosa* accounted for $>95\%$ of the diet and unripe fruits of *Mimusops comorensis* alone represent 50% of it. Lemurs consumed 4 others plants occasionally: *Syzygium jambos, Ixora cremixora, Ficus pyrifolia,* and *Ceiba pentandra*. They consumed the latter 3 species during hours of observation but between 2 successive scans. We observed the ingestion of fruits of *Ixora cremixora* and unripe fruits of S*yzygium jambos* only once.

In Vitro Antiparasitic Properties of Several Food Plants

Of the plant species brown lemurs consumed in May and June both at Saziley and Combani, we collected 14 in quantities suitable for bioassays. We also sampled 2 other items available in the forest: Flowers of *Albizia lebbeck* and young leaves of *Annona squamosa* consumed during others months of the dry season (Table IIA). Twenty-seven botanic samples were available from the items, providing 56 crude extracts (Table III). During preparation of solutions to be tested, one-third of the extracts were insoluble in ethanol + DMSO 6%, and therefore required the use of pure DMSO as solvent.

After primary assays, 15 extracts from 7 species displayed significant antiparasitic activity compared with control cultures. We confirmed the activity of 7 of them via complementary assays (Table IV). The crude extracts belonged to 4 plant species: *Mimusops comorensis, Annnona squamosa*, *Syzygium jambos*, and *Ixora cremixora*. They were all effective at least against the flagellate parasite, and 1 of them, ethyl acetate extract from fruits of *Ixora cremixora*, was active on the 3 parasitic models (Fig. 1a–c). There was no surviving nematode or amoeba at 500 mg/l ($a = -1.18E^{-03}$; $|t| = 7.53$ and $a = -.216$; $|t| = 10.81$, respectively). Moreover, the extract of *Ixora cremixora* was lethal for the flagellate culture from 200 mg/l ($a = -.64$; $|t| = 10.6$). Ethyl acetate extracts from ripe fruits ($a = -.68$; $|t| = 11.2$) and mature leaves of *Mimusops comorensis* ($a = -.17$; $|t| = 3.84$) were active on *Trichomonas* (Fig. 2a, b). The ripe fruits of the plant killed all of the parasites at a concentration of just 200 mg/l. However, unripe fruits showed no significant activity on any parasitic species at primary essays ($p > .01$). Methanol extract from young

Table III. Exhaustive list of crude extracts

			Solution and extract number		
Plant family	Plant species	Plant parts	Ethyl acetate	Methanol	Plant samples
Anacardiaceae	*Mangifera indica*	Young leaves	E12	E13	AN3
		Mature leaves	***E14***[a]	E15	AN3
Annonaceae	*Annona squamosa*	Young leaves	E1	E2/***E3***[a]	LT38
		Mature leaves	E8	E9	LT92
		Ripe fruit	E57	***E58***[a]	AN2
Apocynaceae	*Ancylobotrys petersiana*	Mature leaves	E33	E34	AN21
Asclepiadaceae	*Secamone pachystigma*	Mature leaves	***E53***	E54	AN22
Bombaceae	*Ceiba pentandra*	Flowers	E41	***E42***[a]	AN44
		Mature leaves	E43	E44	AN44
Caelastraceae	*Mystroxylon aethiopicum*	Mature leaves	***E45***[a]	E46	AN19
Caesalpiniaceae	*Senna singueana*	Mature leaves	***E39***[a]	E40	AN16
	Tamarindus indica	Mature leaves	E16	E17	AN4
		Unripe fruit	E18	E19	AN4
		Bark	E20	E21	AN4
Combretaceae	*Terminalia catappa*	Mature leaves	E24	E25	AN12
		Ripe fruit	E49	***E50***[a]	AN12
Lauraceae	*Litsea glutinosa*	Mature leaves	E35	E36	AN32
		Ripe fruit	***E47***[a]	***E48***[a]	AN32
Mimosaceae	*Albizzia lebbeck*	Flowers	E6	***E7***	LT77
		Mature leaves	E26	***E27***	AN13
Myrtaceae	*Syzygium jambos*	Unripe fruit	E51	E52	AN35
Rubiaceae	*Ixora cremixora*	Unripe fruit	E37	***E38***[a]	AN39
	Tarenna supra-axillaris	Ripe fruit	E28	E29/***E30***[a]	AN17
Sapindaceae	*Allophyllus bicruris*	Mature leaves	E22	E23	AN10
Sapotaceae	*Mimusops comorensis*	Mature leaves	E4	E5	LT26
		Ripe fruit	E10	***E11***[a]	AN1
		Unripe fruit	E55	E56	AN30

[a]Samples dissolved in DMSO are in bold italic; others were dissolved in alcohol mixed with DMSO (6%).

leaves ($a = -.19$; $|t| = 4.55$) and ethyl acetate extract from mature leaves of *Annona squamosa* ($a = -.54$; $|t| = 6.7$) were active on the flagellates (Fig. 33, b). Ripe fruits of the species (methanol extract) also seemed to have an anthelmintic effect at 500 mg/l ($b = -2.7E^{-06}$; $|t| = 7.24$). But the trend observed was not uniform (as at the low doses tested the solution seemed to increase the nematode survival rate), and the gradient was very small (Fig. 3c). Unripe fruit of *Syzygium jambos* (ethyl acetate extract) produced a lethal effect on the *Trichomonas* ($a = -.6$; $|t| = 9.9$; Fig. 4). The number of surviving flagellates was near zero from a concentration of 200 mg/l. We noticed no activity for the other extracts tested: slope *a* or gradient *b* of graphs were not significantly different from zero.

Table IV. Plant extracts with significant effects on parasites

Plant species	Plant parts	Active extracts	Parasites		
			Entomoeba invadens	*Rhabditis pseudoelongata*	*Trichomonas vaginalis*
Ixora cremixora	Fruit	E37 (EA)	+	+	+
	Ripe fruit	E58 (M)		+	
Annona squamosa	Mature leaves	E8 (EA)			+
	Young leaves	E2 (M)			+
Mimusops comorensis	Ripe fruit	E10 (EA)			+
	Mature leaves	E4 (EA)			+
Syzygium jambos	Unripe fruit	E51 (EA)			+

Note. EA = ethyl acetate; M = methanol.

DISCUSSION

Plants Consumed by Brown Lemurs and Their Antiparasitic Properties *In Vitro*

The frugivorous-folivorous diet of brown lemurs that we observed is similar to that reported for those in Mayotte and Madagascar (Overdorff, 1992, 1993; Simmen *et al.*, 2003; Sussman, 1974; Tarnaud, 2004a; Tattersall, 1977; Vasey, 2000, 2002). In the dry forests of Mayotte, brown lemurs ingest fruits in addition to leaves all year round. They consume flowers in small quantities, except during certain months. As such, the opportunistic species (Pereira and Kappeler, 1997) is able to exploit many different types of habitat and adjust its diet accordingly. Moreover, the ability to adjust quickly to environmental variations seems to be related to the timing of reproduction (Tarnaud, 2004a). Finally, we observed consumption of few botanic species, such as fruits of *Ixora cremixora,* for the first time during May and June.

Among the 16 plants included in bioassays, 4 exhibited antiparasitic properties *in vitro*. Two of them come from the dry forest of Saziley (*Mimusops comorensis* and *Annona squamosa*) and the 2 others from the humid forest of Combani (*Ixora cremixora* and *Syzygium jambos*). All 4 demonstrated antiprotozoan properties against the flagellate *Trichomonas vaginalis* and 1 of them, *Ixora cremixora*, was clearly active on the 3 parasites tested. We demonstrated the *in vitro* antinematode properties of the plant fruit against *Rhabditis pseudelongata* and the antiprotozoal properties against *Trichomonas vaginalis* and *Entamoeba invadens.* In spite of the discovery of antiparasitic properties in fruit of *Ixora cremixora*, a native plant of Comoros, Madagascar and East Africa, the plant does not

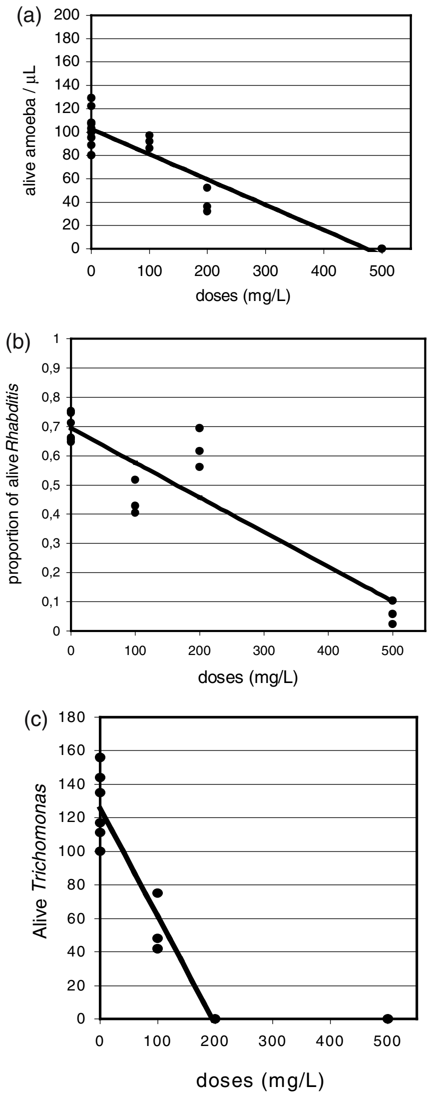

Fig. 1. (a) *In vitro* activity of fruit of *Ixora cremixora* (E37, ethyl acetate) on amoeba. (b) *In vitro* activity of fruit of *Ixora cremixora* (E37, ethyl acetate) on *Rhabditis.* (c) *In vitro* activity of fruit of *Ixora cremixora* (E37, ethyl acetate) on *Trichomonas.*

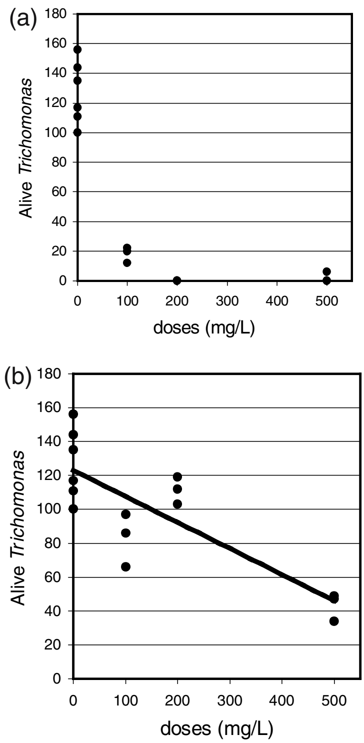

Fig. 2. (a) *In vitro* activity of ripe fruit of *Mimusops comorensis* (E10, ethyl acetate). (b) *In vitro* activity of mature leaves of *Mimusops comorensis* (E4, ethyl acetate).

seem to be used in traditional medicine, and researchers have studied it very little until now. It would be interesting to deepen chemical research on *Ixora cremixora*. *Mimusops comorensis*, endemic to Comoros, is largely represented in dry forest habitats, and is also quite frequent in rain forests (Pascal and Labat, 2002). The ethyl acetate extracts from the leaves and ripe fruits that brown lemurs consumed at Saziley were active *in vitro* on *Trichomonas* but not the unripe fruits (yellow colored) consumed at Combani, suggesting that the active fraction is linked to the maturity of fruits (Goldstein and Swain, 1963). *Annona squamosa* is indigenous to South America (Brazil) and has now spread over the tropics (Adjanohoun *et al.*, 1989). It was introduced later in Mayotte, where it is cultivated for its fruits. The trichomonacidal properties of leaves is likely to be due

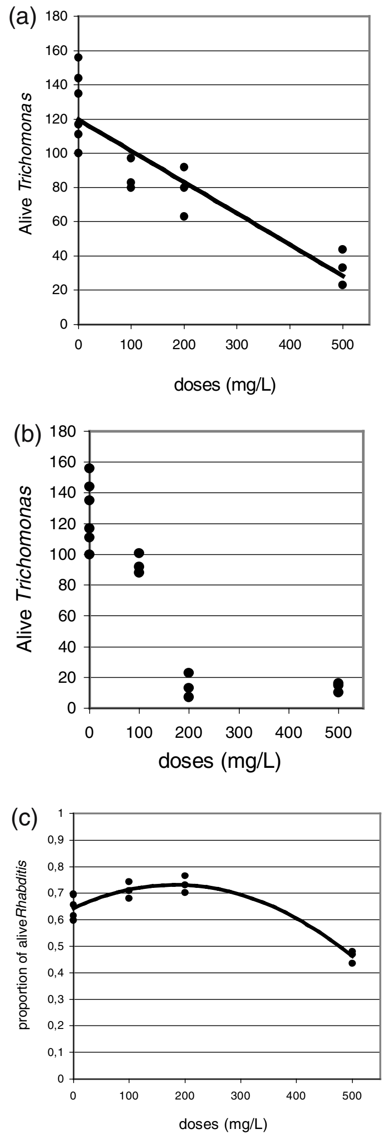

Fig. 3. (a) *In vitro* activity of young leaves of *Annona squamosa* (E2, methanol). (b) *In vitro* activity of mature leaves of *Annona squamosa* (E8, ethyl acetate). (c) *In vitro* activity of ripe fruit of *Annona squamosa* (E58, methanol).

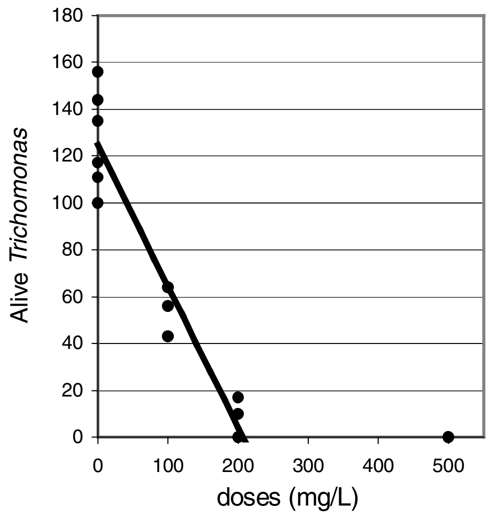

Fig. 4. *In vitro* activity of unripe fruit of *Syzygium jambos* (E51, ethyl acetate).

to 2 different substances present at different stages of maturity. Indeed, it is the methanol extract from young leaves that is active, while from mature leaves, it is the ethyl acetate extract. In the case of fruits, the trend is not so clear, and could be the result of an experimental artefact. But the anthelmintic activity of ripe fruit of *Annona squamosa* would be compatible with the use of unripe fruit, seeds, and leaves as a vermicide (Ambasta and Shri, 1994; El Tahir *et al.*, 1999; Kothar *et al.*, 2001; Sharma and Behari, 1992; Watt and Breyer-Brandwijk, 1962). The roots of the plant are also used for their purgative properties. Moreover, several bioassays have already confirmed the antiparasitic activities in seeds of *Annonaceae* (Bories *et al.*, 1991; Sahpaz *et al.*, 1994). *Syzygium jambos* grows in Reunion, Asia, South America, and Africa (Djipa *et al.*, 2000) and was introduced later in Comoros, where it tends to invade the rain forest. The effect of unripe fruit of *Syzygium jambos* on *Trichomonas* protozoa can be related to traditional uses of the plant as a remedy for intestinal problems (Djipa *et al.*, 2000; Slowing *et al.*, 1994).

Humans use many others plants consumed by *Eulemur fulvus* for intestinal problems, and biological assays on some of them have already confirmed antiparasitic activities against gastrointestinal parasites. For example, the green parts of *Lantana camara* and roots of *Jatropha curcas* have anthelmintic activity (Begum *et al.*, 2000; Fagbenro-Beyioku *et al.*, 1998). Several *Ficus* spp. are traditionally used as vermicides (Valdizan and Maldonado, 1922) and researchers have confirmed their activity *in vitro* and *in vivo* (Amorin *et al.*, 1999; Hansson *et al.*, 1986; Rodriguez and Wrangham, 1993). In 1996, Coe and Anderson found an anti-amebaean activity in bark of *Mangifera indica*, which is traditionally used against amoebiasis, diarrhea, and digestive problems in Equatorial Africa, Indian Ocean, and Central America (Aderibigde *et al.*, 2001; Adjanohoun *et al.*, 1989; Le Grand, 1989; Pernet and Meyer, 1957). Ross *et al.* (1980) confirmed the laxative properties of *Tamarindus indica,* different parts of which are used all over the world as a purgative (Adjanohoun *et al.*, 1982, 1983, 1989; Boiteau and Allorge-Boiteau, 1993; Coe and Anderson, 1996; Pernet and Meyer, 1957; Watt and Breyer-Brandwijk, 1962). Moreover, in the dry forest of Saziley, we saw in lemur tools partly chewed and partly digested leaves of *Tamarindus indica* and *Mimusops comorensis*. Though the leaves are not particularly rough, some plants consumed by brown lemurs could also have mechanical antiparasitic properties, as in the case of leaf-swallowing behavior in chimpanzees (Huffman and Caton, 2001 Huffman *et al.*, 1996; Wrangham and Nishida, 1983).

Antiparasitic Properties of Plants and Potential Control of Parasitic Gastrointestinal Fauna

In bioassays, one-quarter of the plants tested showed activity *in vitro*. Thus, among the 72 different species consumed by brown lemurs, many other plants could have antiparasitic properties. We can hypothesize that the consumption of plants demonstrating antiparasitic properties could have a beneficial effect on lemur health through the combined effect of all of the antiparasitic substances ingested. A plant with clear antiparasitic properties but rarely consumed, such as *Ixora cremixora,* could have an effect on brown lemur health by association with other bioactive plants. Moreover, certain plants are regularly consumed throughout the year, such as *Annona squamosa* and *Mimusops comorensis* (Fig. 5), and could have a chemical effect on the parasitic gastrointestinal fauna of lemurs via the cumulative effect of daily doses ingested.

The primary intestinal parasites in *Eulemur fulvus* are nematodes (Table I). Researchers identified several species of oxyures and 1 trichure in wild (Madagascar) and captive individuals (Baer, 1935; Baylis and Daubney, 1922; Chabaud and Petter, 1958; Chabaud *et al.*, 1964, 1965; Coiffier, 2000). Gomez *et al.* (1992) and Smith and Merrovitch (1985) identified 1 species of amoeba and 1 of coccidian protozoa in the intestinal tracts of captive individuals. On Mayotte island, Nègre (2003) studied the level of parasitic infestation in natural habitat of the same 2 lemur groups

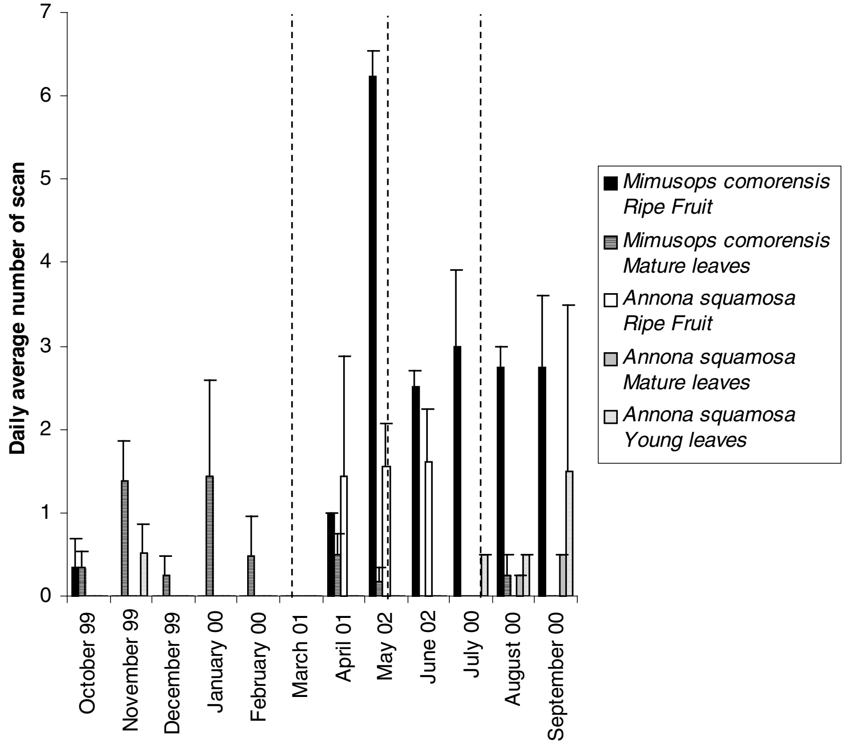

Fig. 5. Scan proportion (in time) of the annual consumption of *Mimusops comorensis* and *Annona squamosa.*

at Saziley (45 stool samples) and Combani (33 stool samples). Nematodes [*Lemuricola (Madoxyuris) vauceli*] were visible at macroscopic exam in 65% of fresh stools and microscopic exam after concentration allowed identification of nematode eggs (*Lemuricola* sp. and *Callistoura* sp.) in 84.8% of stools. In contrast, prevalence of stools infested by protozoan parasites (flagellate such as *Trichomonas* sp. and an undetermined ciliate) was quite low (26%). The relative absence of protozoan parasites may be related to the consumption of the 6 plant extracts with activity against them. Jansen (1978) was the first to suggest a link between the absence of protozoa parasites in the great apes of the Kibale forest (Uganda) and the regular consumption of plants rich in secondary compounds. Moreover, the quantitative approach based on the McMaster technique showed that lemur stools were weakly infested. Indeed, 78.8% of stools had a parasitic load <200 eggs/g (Hecberg *et al.*, 1986). The coprological analysis was extended to a third site: Mbouzi islet, in front of the main town of Mayotte (Mamoudzou). The natural environment is comparable to that of Saziley but the lemur diet was modified by human contributions (plates composed of 50% bread, rice, and sugar and 50% fruits, mostly banana). Parasitic infestation among lemurs is comparable at both sites where feeding is natural (Saziley and Combani); we detected oxyures in two-thirds (*Callistoura* sp.) and one-third (*Lemuricola* sp.) of the stool samples examined. Conversely, when humans assist feeding, the level of infestation is higher, with a prevalence of infested stools 20% higher than that in a natural feeding ground. Thus a diversified, balanced diet, including regular consumption of antiparasitic plants, helps to limit parasitic infestation among Mayotte lemurs.

Potential Effects of Plants Consumed by Eulemur fulvus and Self-vermifugation

Consumption of plants with antiparasitic properties associated with a weak level of infestation suggests an adaptation of dietary behavior. Nevertheless, a careful interpretation of antiparasitic properties of plants consumed by Mayotte lemurs is required. First, our results come from *in vitro* bioassays, so they did not prove a chemical effect directly on lemur physiology after digestion and assimilation. Further, species used in bioassays are not the same as the ones identified in brown lemur intestines but experimental models representative of the main types of parasites in brown lemurs. Finally, our approach is only qualitative and not quantitative. The assays allowed us to identify antiparasitic properties in plants, but not to determine the level of activity of plants or to compare activities of different plants. Within this framework, ethnobotanical uses of plants against digestive problems and coprological results on lemurs represent a link between theoretical *in vitro* activities of plant extracts and a real effect of vermifugation on lemurs in their natural habitat.

Nevertheless, it is still difficult to confirm voluntary self-vermifugation for 2 main reasons: 1) contrary to observations in great apes, we did not observe consumption of a specific plant by a sick lemur followed by improvement of its health and 2) currently we do not know whether antiparasitic properties do or do not affect Mayotte lemur choices of foods. In the south of Madagascar, secondary compounds in plants have an effect on lemur diets. *Propithecus verreauxi* and *Indri indri* select plants rich in tannins and alkaloids (Simmen *et al.*, 1999), while *Lemur catta* and 2 sympatric *Eulemur* spp. consume less astringent plants (Ganzhorn, 1988; Hladik *et al.*, 2000). We analyzed 16 plants among 29 identified in brown lemur diet; 4 of them had *in vitro* antiparasitic properties. Complementary assays on the 13 other species would be interesting to confirm the significant proportion (1/4). Moreover, with a systematic screening of plants in the environment of lemurs we could verify whether the dietary proportion of bioactive plants is more important than the proportion available in the forest, as in Krief (2003), which would argue for a process favoring the consumption of plants with antiparasitic properties.

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