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First preliminary evidence of a relationship between feeding selectivity and gastrointestinal parasitism in mandrills (*Mandrillus sphinx*)

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Abstract
Studies have focused the past thirty years on animal self-medication curative or prophylactic self-medication.
This study aims to evaluate whether *Mandrillus sphinx* use certain plants for therapeutic purposes such as species would consume certain plant species for therapeutic purposes as described in other primate species. We have behavioral studied approximately 130 individuals living in the Lékédi Park from Gabon using the focusing method and carried out coprological analyses from fecal material collected on identified animals. Plant parts consumed were subjected to nutritional and pharmacological analyses. The results showed that specifically that the consumption of these plant species such as nuts of *Elaeis guineensis*, fruits of *Palisota ambigua* and above all stems of *Manniophyton fulvum* by parasitized individuals was strongly correlated with parasite prevalence in some individuals. This study suggests that *Mandrillus sphinx*, as described in other primate species. Further phytochemical and pharmacological studies of these plant species are possible.

Keywords: Zoopharmacognosy, *Mandrillus sphinx*, Lékédi Park, Gabon

Introduction
The environment has always played an important role in human health (Morand, 2021) [1]. Research conducted around the world reveals the impact of global changes such as biodiversity loss, habitat degradation, and urbanization on human health (Morand, 2021) [1]. For example, the observed increase in infectious disease epidemics in recent decades appears to be associated with the increase in livestock farming and biodiversity loss (Morand, 2021) [1], as well as deforestation and the increase in commercial plantations (Morand and Lajaunie, 2021) [2].
In addition to these problems related to environmental change, there is the growing number of emerging infectious diseases in wildlife, which, in some circumstances, lead to a change in the ecology of the host, the pathogen, or both (Brugère-Picoux *et al*., 2014) [3]. Thus, wild species can be reservoirs of pathogenic agents capable of contaminating humans (Brugère-Picoux *et al*., 2014) [3].
The use of drugs for to combat these various affections caused by multiple pathogens has long been considered as the preserve of humans. However, traditional ecological data suggest that animals have also long inspired the discovery of certain medicinal plants used to treat humans and their livestock (Huffman, 2022) [4].
Thus, over the past 30 years, several studies have reported that animals would use their environment for to combat or to control diseases (Wrangham, 1995; Huffman and Caton, 2001; Krief *et al*., 2005a; Hart, 2011; Lefèvre *et al*., 2012; Villalba *et al*., 2014; Koto-te-Nyiwa *et al*., 2015) [5-11]. Based on this hypothesis (Huffman, 2001) [6], self-medication studies often referred to as zoopharmacognosy (Rodriguez and Wrangham, 1993; Huffman and Caton, 2001) [6,12] would be a valuable alternative for identifying medicinal plants and drug discovery as revealed by our research bio-prospection program (Krief *et al*., 2005b; Kambale *et al*., 2013; Ngbolua *et al*., 2015) [13-15].
Indeed, the amount of detailed information on self-medication from insects to animals show that use various behavioural strategies for maintain their health

(Wrangham, 1977; Messner and Wrangham, 1996; Lozano, 1998; Krief *et al*., 2005b; Hart, 2011; Huffman *et al*., 2011; Fruth, 2025) [8, 13, 16, 17-20]. Among these strategies, the use of plant parts by animals apparently for their non-nutritive and bioactive properties in their diet for their health needs has been reported by researchers across the world (Lozano, 1998; Krief *et al*., 2005a; Huffman *et al*., 2011) [7,18,19]. The infected female butterflies for example, laid preferentially their eggs on food plants that reduce parasite growth in their offspring (Lefèvre *et al*., 2012) [9].

Also, studies of macronutrient self-medication and the effect of disease-induced anorexia on caterpillars (*Spodoptera exempta*) has shown that larvae infected by a baculovirus restricted to diets high in protein and low in carbohydrate (Povey *et al*., 2013) [21].

Regarding the primates especially great apes, studies provide evident facts of ingestion of plants for medicinal purposes, such as infrequent intake of species which are not a regular part of the diet and ingestion of some plant which is associated with periods of a high risk of parasite infection (Huffman, 1997; Huffman and Caton, 2001; Krief *et al*., 2004) [6,22,23]. Also, consumption of plant parts which have no apparent nutritive value and which are rich in secondary compounds has been also reported (Wrangham and Waterman, 1983; Jisaka *et al*., 1992; Koshimizu *et al*., 1993; Wrangham *et al*., 1998) [24-27].

Other works realized by researchers worldwide describes the diversity of host-parasite relationships in the animal kingdom (Clayton and Moore, 1997) [28].

Some parasitic infections appear to go unnoticed by hosts. In other cases, when homeostasis is disrupted or threatened, it is in the host's interest to actively respond to alleviate discomfort (Huffman, 2016) [29]. The parasites, for example, can dramatically reduce the fitness of their hosts by diverting hosts' nutritional resources for their own growth reproduction and survival causing other fatal or debilitating effects (Schmid-Hempel, 2011) [30].

However, some studies have reported that this primate species and human are infected by the same gastrointestinal parasites responsible of esophagostomies (Remfry, 1978; Ziem *et al*., 2005; Krief *et al*., 2008) [31-33], the diarrhea with dysentery and colic (Euzéby, 1986; Bussiéras and Chermette, 1992; Allela, 2005; Setchell *et al*., 2007) [34-37] in human.

Concerning the mandrill (*Mandrillus sphinx*), diet behaviour is small studied and we know not if this species would be some vegetal species for their health needs. Some diet studies show that is omnivorous species and that consumed various vegetal species from several botanical families (Hoshino, 1985; Lahm, 1986; Norris, 1988, Nsi Akoué *et al*., 2017) [38-41]. Other recent study of parasitism infections realized on wild mandrill population living in Lékédi Park (South-East, Gabon) has revealed a significant gastrointestinal parasite load (Poirotte *et al*., 2015) [42].

Through the mechanisms of natural selection, mandrills, like other animal species, have probably developed protection strategies against these parasites, as suggested by the work of Combes, (2001) [43]. But we don't know what their behavioral strategies are to prevent or combat parasitic infections. In other words, would they consume certain plant species to fight parasitic attacks? Identified these vegetal species that would use for to combat or to control these affections may also to help human for to combat these same affections. The aim of this study is to investigate the vegetal species which would be used by mandrill population living in Lékédi Park from Bakoumba (Gabon) against parasitic affections.

Methods

Study population

The studied population was comprised ~130 free-ranging individuals living in the Lékédi Park and its vicinity (Brockmeyer *et al*., 2015) [44]. Initially, this population from 65 captive individuals initially housed at the CIRMF (Centre International de Recherche Médical de Franceville, Gabon) which has been released into the park (Peignot *et al*., 2008) [45]. At the present time, this population is composed about 85% of wild-born animals (Brockmeyer *et al*., 2015) [44] and forage freely in the park and its vicinity but in the first years after release. During the early years, their diet was supplemented about three-five times a week with bananas and monkey chow. This food supplementation has been progressively decreased throughout the years.

During this study, this diet supplement was already completely stopped at the beginning of a long-term study ("Mandrillus Project": www.projetmandrillus.com), in April 2012, 14 months before the beginning of this study (Brockmeyer *et al*., 2015) [44]. Studied mandrill population occupies a home range of 866 ha including areas both inside and outside the park boundaries (Brockmeyer *et al*., 2015) [44].

Behavioural observations

Between May 2013 and October 2014, data on feeding behavior were collected on 57 individually-recognized mandrills, using 5-min focal samplings (Altmann, 1974) [46]. During focal periods, we recorded all the plant species consumed by the focal individual as well as the plant parts seen eaten (i.e., leave, stem, fruit, seed, nut, root, bark, resin).

During the entire study period, we collected a total of 6350 focal observations representing approximately 529 hours of focal data (Nsi Akoué *et al*., 2017) [41]. We restricted our data set, however, to those animals observed for more than an hour per season per year to improve the quality and reliability of the behavioural data (Nsi Akoué *et al*. 2017) [41]. The analyses performed below were thus based on a total of 22 individuals of both sexes (14 females aged 3.17-15.06 and 8 males aged 3.42-14.75).

Parasitology

We carried out coprological analyses from fecal material routinely collected on unambiguously identified animals. We performed direct microscopic observations after concentration and sedimentation of the fecal samples (Poirotte *et al*. 2015) [42]. We classified nematode eggs and protozoa trophozoites and cystic stages by taxon according to morphological characteristics based on shape, content and size of the eggs and cystic stages (see Table 2 for the criteria used to identify protozoa in: Poirotte *et al*. 2015) [42]. We performed qualitative analyses of the samples (presence/absence of a given species or a set of species, see below) because quantifying some parasite species, such as small protozoa, is challenging and error-prone (Poirotte *et al*. 2015) [42]. We analyzed nematode and protozoa richness's separately because they show contrasting life histories. Moreover, both parasitism (Poirotte *et al*. 2015) [42] and feeding diet (Nsi Akoué *et al*., 2017) [41] are highly seasonal. We calculated therefore, and for each year, parasite richness per animal per season by calculating the average number of parasite species

found in all the samples collected on a same individual over a given season. Gabon experienced two marked seasons (long rainy: Feb-May and long dry: Jun-Sep) and two other short seasons (short rainy: Oct-Nov and short dry: Dec-Jan). We chose to restrict our analyses to the seasons where at least three fecal samples were available per focal animal. Indeed, considering less than three samples limits the ability to detect accurate infectious status because of a non-negligible probability of false negatives (due to e.g., intermittent excretion of parasites).

For nematode species, we failed to identify any plant of interest following our criteria (see below). Consequently, all our analyses were based on protozoa richness retrieved from 279 samples collected on 22 animals during three seasons (no data was available during the short dry season) over two years representing a total of 40 individuals (mean number of samples collected per animal per season per year: 6.98 samples; range: 3-26).

On average, these 22 animals were infected by 3.02 protozoa species including the following seven species (or sets of species): *Balantidium coli*, *Entamoeba coli*, *Endolimax nana*, *Entamoeba hartmanni*, *Entamoeba histolytica*/*dispar* "complex" (see: Poirotte *et al*. 2015) [43], *Pseudolimax butschlii*, and *Coccidia sp*. To select appropriate candidate plant parts, possibly consumed for self-medication purposes (see below), we defined two sets of individuals: the 10 most parasitized individuals were the animals that showed the highest diversity in parasite taxa for a given season and a given year (mean number of TAXA±SEM: 3.76±0.13).

By contrast, the 10 least parasitized animals were those that showed the lowest diversity in parasite taxa (2.26±0.09). Finally, we further studied the impact of feeding diet on the prevalence of each protozoan taxon among the 40 individuals, calculated as the proportion of positive (infected by a given parasite taxon) samples divided by the total number of collected fecal samples per animal per season per year.

Plant selection

Mandrill's feeding diet is extremely diversified with about 150 species consumed all year-round representing more than 340 different plant parts (Nsi Akoué *et al*., 2017) [41]. If mandrills practice self-medication (here to fight-off protozoa), we expected that they would have selected a few specific plant species or plant parts. Consequently, a global analysis based on all consumed plants (Nsi Akoué *et al*., 2017) [41] would certainly obscure any relationship between parasite status and the consumption of a few of them. We therefore applied different filters to first identify appropriate candidate plant parts (analyses were performed on the 219 plant parts seen consumed rather than on each plant species).

First, among the two sets of individuals defined above (most infected *vs*. least infected), we considered as good candidates the plant parts that were seen consumed by more than 50% of the most infected animals (at least five animals) and less than 10% of the least infected animals (none or one individual). Fifteen plant parts were selected following this first criterion (Table 1).

Second, we further considered those plant parts that were on average highly frequently consumed by the most infected individuals compared to the least infected ones. Rates of consumption were calculated by dividing the number of occurrences of consumption of a given item per individual (per season, per year) divided by the total focal time performed on this individual at that time. When the average difference of the rates of consumption between the most and the least infected animals were higher than 0.10, we considered these plant parts as a possible good candidate. With this second criterion, seven additional plant parts were selected (Table 1).

Finally, we performed statistical analyses on the full data set (N=44) using frequencies of consumption of each of these 22 selected plant parts. The botanical samples of plants selected were collected, pressed and identified at the National Herbarium of the Institute of Tropical Medicine and Pharmacopoeia (IPHAMETRA, Libreville, Gabon) after a first identification using relevant standard literature including various regional and local field guides (Walker and Sillans, 1961; Letouzey, 1982a; Letouzey 1982b; White and Abernethy, 1996; Wilks and Issembé, 2000) [47-51]. Specimens were deposited in the herbarium of the University of Sciences and Techniques of Masuku in Franceville, Gabon.

Nutritional analyses

The determination of protein, carbohydrate, and lipid contents of the different plant part samples was determined using standard chemical methods as described by the Association of Official Analytical Chemists (AOAC, 1990) [52]. Soxhlet extraction technique using petroleum ether (40-50°C) was used to evaluate the fat content of the sample (Pearson *et al*., 1981) [53]. Bradford method was used to determine the crude protein content of the sample (Bradford, 1976) [54]. The carbohydrate content of the sample was determined by Dubois method (Dubois *et al*., 1956) [55].

Determination of total energy

The total energy of food mandrill was calculated by according the method of using the formula following (Satter *et al*., 2014) [56]:

Total energy (kcal)=(% Fat×9.3)+(% Carbohydrate×4.1)+(% Proteine×4.1)

Preparation of plant extracts

All plant parts have been collected during the study period (May 2013-Oct 2014). The sample collected were air-dried at room temperature for a total period six weeks and pulverized to powder using a clean electric blender (Model Phillips 190). A 25 g sample of the pulverized of each plant parts was lyophilized and the extract obtained is stored in vials protected from light until the completion of various tests. The yields of the extracts (%) were calculated (Boulenouar *et al*., 2009; Salem, 2009) [57, 58].

Phytochemical analyses

In order to evaluate the variation in phenolic compound contents in mandrills' diets, phytochemical analyses were carried out on 40 most representative plants grouped into two categories (20 candidate plants and 20 non-candidate plants) among all the plants consumed. The 40 candidate plants were those selected based on the criteria mentioned above in the plant selection section. To these criteria we added the criteria of bioavailability combined with the frequency of consumption. Thus, the 40 plants selected were those, in

addition to meeting the other criteria, were abundant in the environment and widely consumed or rare and less consumed. Phytochemical analyses consisted of determining the contents of phenolic compounds (phenols, tannins and flavonoids) in the different total extracts of candidate and non-candidate plant parts.

Determination of total phenols in extracts

The Folin-Ciocalteu method was used to measure total amount of total phenols content as described by Singleton *et al*. (1999) [59]. For that, aliquots of 200 µl of extracts (1 mg/mL) were mixed with 1000 µl Folin-Ciocalteu reagent (0.2 N diluted in Methanol). A reagent blank using methanol instead of sample was prepared. After 5 min incubation at room temperature, 800 µl sodium carbonate solutions (7.5%) were added. Samples were incubated at room temperature for 2 h and the absorbance was measured at 765 nm versus the prepared blank. Gallic acid (3, 4, 5-trihydroxybenzoic acid) was used as standard. All tests were carried out in triplicate and total phenols content was expressed as mg of Gallic acid equivalents (GAE) per 100 g of drug.

Determination of total flavonoids in extracts

The Dowd method was used to measure total amount of flavonoids as described by Arvouet-Grand *et al*. (1994) [60] with slightly modification (Nsi Akoué, 2017) [61]. Globally, 1 mL of 2% $AlCl_3$ in methanol was added to 1 mL of plant extract (1 mg/mL). After 10 min of incubation at room temperature (20 °C), the absorbances were read at 415 nm. All tests were carried out in triplicate and results were expressed as quercetin equivalent (QE). Quercetin was used as standard.

Determination of total tannins in extracts

The reference method of European Community was used to measure total amount of tannins (1994) [62] with small modifications. Globally, aliquots of 250 µl of extract (1 mg/mL) were added at 250 of µl ammonium citrate ferric reagent (3.5 g/L) more 250 µl of ammonia (20%, 8 g/ L) and 1200 µl of H_2O. After 10 min of incubation at room temperature (20 °C), the absorbances were read at 525 nm. All tests were carried out in triplicate and results were expressed as tannic acid equivalent (TAE).

Statistics analysis

First, we performed General Linear Models (LM) with a Gaussian error-structure to study the relationship between the rate of consumption of the 22 candidate plant parts (independent variables) and protozoa richness or protozoa prevalence (a total of 8 dependent variables). We further considered the following possible confounding variables: the sex (class variable with two modalities) and the age (continuous variable in yrs; as per: Poirotte *et al*. 2015) [42] of the studied animals, and the season when the focals were performed and the fecal samples collected (class variable with three modalities). To circumvent multi-colinearity issues (frequencies of consumption of the 22 plant parts may be multi-correlated), we removed all plant parts that did not significantly impact parasite richness and prevalence, using a descending procedure, starting with all predictors and removing the highest p-value until all p-values were inferior to the significance threshold (0.05). Because we performed eight different models on the same data set on feeding diet, we applied sequential Bonferroni corrections to control for multiple testing. In preliminary analyses, we verified that the individual's identity did not impact the results (not shown).

Second, we performed two sets of Generalized Linear Models (GLM) with a binary distribution to study whether the candidate plant parts (Y/N, the dependent variable) showed either different nutritive values or different phytochemical contents compared to non-candidate plant parts for which such data was available. Among the 22 candidate plant parts, we obtained nutritional information for 14 of them (compared to 102 non-candidate plant parts) and phytochemical composition data for 12 of them (compared to 83 non-candidate plant parts). However, for the latter analyses, because of a limited sample size, we first considered the three different classes of presence defined (absent, present, abundant) as ordered continuous variables (0 to 3).

We checked that the significant predictors (see the results) were still significant when considered as class variables in a final model containing only these predictors. Otherwise, full models are presented. Because of a limited sample size of candidate plant parts, we were unable to run any models based on the plant parts found to impact parasitism.

Results

Candidate plant parts

The 22 candidate plant parts were selected according to a combination of the observed rate of consumption and percentage of individuals seen consuming these parts among the 10 most parasitized animals compared to the 10 least parasitized. These plant parts belonged to 12 botanical families, among them, the Rubiaceae (4 species), the Zingiberaceae (4 species) and to a lesser extent the Euphorbiaceae (3 species) were over-represented. However, these three families also appeared to be highly consumed overall by the studied mandrills (Nsi Akoué *et al*., 2017) [42].

Rates of consumption and parasitism

While controlling for possible confounding effects (individual's sex and age, season of data collection) and after correcting for multiple testing, we found that the rate of consumption of three plant parts significantly influenced parasite richness (Table 1): more parasitized individuals were observed consuming more nuts of *Elaeis guineensis*, more fruits of *Palisota ambigua* and above all more stems of *Manniophyton fulvum* (Figure 1).

These relationships rather stemmed from a global influence of parasite richness than linked to the influence of particular taxon, with the notable exception of *M. fulvum* for which several parasites seemed to impact mandrill's rate of consumption. In particular, individuals parasitized with *E. hartmanni* and to a lesser extent by coccidias consumed more frequently the stem of this plant species than less parasitized animals (we observed related effects for *B. coli* and *E. coli* but Bonferroni corrections did not allow to keep the effects). Additionally, we found different positive relationships between the prevalence of four parasites and the rate of consumption of several plant parts (Table 1). Individuals

parasitized with *E. coli* and *E. histolytica/dispar* frequently consumed the leaves of *Nymphaea maculata*, while those infected with *E. hartmanni* highly consumed the fruits of two close species: *Aframomum alboviolaceum* and *Aframomum polyanthum*. Finally, the seeds of *Pentaclethra macrophylla* were also more consumed by individuals highly parasitized with *E. histolytica/dispar* (Table 1).

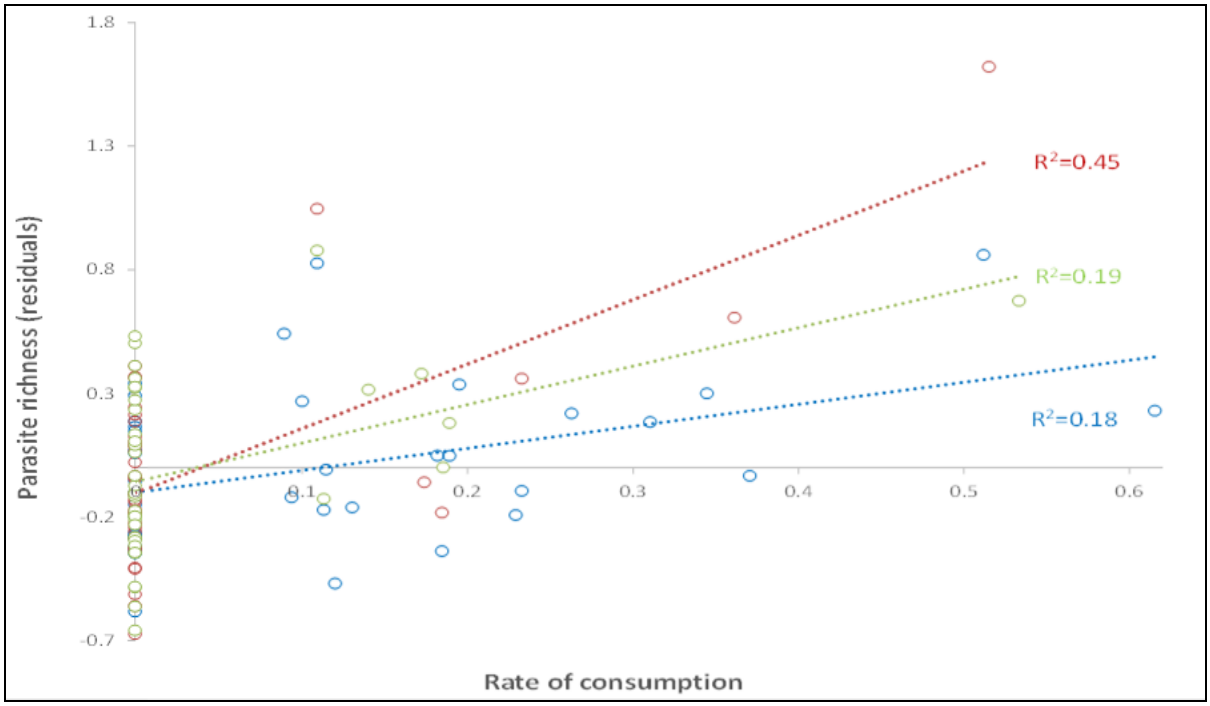

Fig 1: Parasite richness and rate of consumption of three candidate plant parts. *Residuals of parasite richness were obtained by excluding the predictor of interest from the final models. In red: Stems of Manniophyton fulvum, in green: Fruits of Palisota ambigua, in blue: Nuts of Elaeis guineensis.*

Table 1: Twenty-two selected plant parts and their influence on protozoa richness and prevalence. LM with descending procedures were used to select the best predictors ($p < 0.05$). F and P-values are provided; significant predictors are shown in bold and trends in italics after sequential Bonferroni corrections applied to each candidate plant part (significant threshold for the best p-value: 0.05/8; for the second-best p-value: 0.05/7 and so on). Results from confounding factors (sex, age and season) are not shown. The stars indicate the plant parts that were selected according to the second criterion (all others were selected according to the first criterion).

Botanical family	Selected candidate plant part	Protozoa richness	*Bc*	*En*	*Pb*	*Ec*	*Eh*	*Ehd*	*Coccidia sp.*
Zingiberaceae	*Aframomum alboviolaceum* (Ridl.) K. Schum. (fruit)	F=6 P=0.02	F=0.18 P=0.67	F=1.26 P=0.27	F=0.25 P=0.62	F=1.51 P=0.23	F=11.05 P=0.002	F=0.15 P=0.70	F=1.61 P=0.22
Zingiberaceae	*Aframomum cf. polyanthum* (K.Schum.) K.Schum (fruit)	F=0.40 P=0.53	F=0.17 P=0.68	F=0.01 P=0.94	F=0 P=0.97	F=0.03 P=0.85	F=21.71 *P* < 0.0001	F=0.25 P=0.62	F=1.01 P=0.32
Zingiberaceae	*Aframomum daniellii* (Hook.f.) K.Schum (stem)	F=0.06 P=0.80	F=0.18 P=0.67	F=0.26 P=0.62	F=2.92 P=0.10	F=2.17 P=0.15	F=5.10 P=0.03	F=0.53 P=0.47	F=0.89 P=0.35
Anacardiaceae	*Antrocaryon klaineanum* Pierre (fruit)*	F=0.51 P=0.48	F=0.39 P=0.54	F=1.76 P=0.20	F=0.04 P=0.85	F=0.15 P=0.70	F=0.18 P=0.68	F=0.45 P=0.51	F=0.28 P=0.60
Euphorbiaceae	*Croton sylvaticus* Hoschst. ex Krauss (fruit)*	F=0.02 P=0.88	F=0.01 P=0.92	F=0.09 P=0.77	F=0.74 P=0.40	F=0.07 P=0.80	F=0.41 P=0.53	F=0.02 P=0.88	F=0 P=0.96
Aracaceae	*Elaeis guineensis* Jacq. (nut)	F=9.50 P=0.004	F=0.49 P=0.49	F=2.90 P=0.10	F=0.55 P=0.46	F=0.51 P=0.48	F=2.98 P=0.10	F=4.37 P=0.05	F=0.08 P=0.78
Rubiaceae	*Geophila afzelii* Hiern (fruit)	F=0.21 P=0.65	F=0.07 P=0.80	F=2.32 P=0.14	F=0.17 P=0.68	F=0.34 P=0.57	F=1.16 P=0.29	F=0.02 P=0.88	F=0.01 P=0.91
Marantaceae	*Haumania liebrechtsiana* (De Wild. & T.Durand) J.Léonard (seed)*	F=0.13 P=0.72	F=1.12 P=0.30	F=2.57 P=0.12	F=2.90 P=0.10	F=0.07 P=0.79	F=1.85 *P*=0.18	F=5.16 P=0.03	F=0 P=0.96
Aracaceae	*Laccosperma secundiflorum* (P.Beauv.) Kuntze (fruit)	F=5.68 P=0.02	F=0.08 P=0.79	F=0.03 P=0.87	F=0.06 P=0.81	F=0.66 P=0.42	F=0.07 P=0.80	F=0.03 P=0.86	F=3.18 P=0.08
Apocynaceae	*Landolphia hirsuta* (Hua) M. Pichon (fruit)	F=1.13 P=0.30	F=0.47 P=0.50	F=0.05 P=0.83	F=1.41 P=0.24	F=0.24 P=0.63	F=0.06 P=0.80	F=0.33 P=0.57	F=0.27 P=0.61
Rubiaceae	*Lasianthus batangensis* K.Schum (fruit)	F=1 P=0.33	F=1.48 P=0.23	F=2.45 P=0.13	F=0.94 P=0.34	F=0.30 P=0.59	F=0.09 P=0.76	F=0.07 P=0.79	F=0.24 P=0.63
Euphorbiaceae	*Manniophyton fulvum* Müll.Arg. (stem)	F=38.18 *p*<0.0001	F=4.64 P=0.04	F=0.33 P=0.57	F=1.14 P=0.29	F=2.81 P=0.10	F=12.96 P=0.001	F=0 P=0.96	*F=6.79* *P=0.01*
Moraceae	*Musanga cecropioides* R.Br. (fruit)*	F=0.15 P=0.70	F=0.12 P=0.73	F=0.14 P=0.72	F=0.01 P=0.92	F=0 P=0.98	F=5.08 P=0.03	F=0.65 P=0.43	F=0.83 P=0.37
Nymphaeaceae	*Nymphaea maculata* Schum. &	F=0.66	F=0.37	F=3.06	F=0	F=16.62	F=0.05	F=11	F=1.94

	Thonn. (leave)	P=0.42	P=0.55	P=0.09	P=0.95	*p*<0.0001	P=0.84	P=0.002	P=0.17
Commelinaceae	*Palisota ambigua* (P.Beauv.) C.B.Clarke. (fruit)*	F=17.03 *p*<0.0001	F=0.44 P=0.51	F=0.98 P=0.33	F=0.46 P=0.50	F=3.95 P=0.06	F=1.32 P=0.26	F=2.78 P=0.11	F=0.26 P=0.61
Mimosaceae	*Pentaclethra macrophylla* Benth. (seed)*	F=0.35 P=0.56	F=1.11 P=0.30	F=1.21 P=0.28	F=0.01 P=0.91	F=0.53 P=0.47	F=0.02 P=0.88	F=11.40 P=0.002	F=0.31 P=0.59
Rubiaceae	*Psychotria stenostegia* O.Lachenaud (fruit)	F=0.07 P=0.80	F=0.63 P=0.43	F=0.17 P=0.69	F=0 P=1	F=0.43 P=0.52	F=0 P=0.95	F=3.16 P=0.09	F=0.34 P=0.57
Zingiberaceae	*Renealmia macrocolea* K. Schum. (fruit)	F=3.87 P=0.06	F=0.64 P=0.43	F=1.25 P=0.27	F=0.87 P=0.36	F=1.01 P=0.32	F=0.91 P=0.35	F=3.86 P=0.06	F=1.02 P=0.32
Euphorbiaceae	*Ricinodendron heudelotii* (Baill.) Pierre ex Heckel. (seed)	F=0.02 P=0.89	F=0.86 P=0.36	F=0.86 P=0.36	F=0.01 P=0.92	F=1.14 P=0.29	F=2.74 P=0.11	F=0.15 P=0.71	F=0.06 P=0.81
Marantaceae	*Sarcophrynium brachystachyum* (Benth.) K.Schum (stem)	F=0 P=0.97	F=0.02 P=0.88	F=1.09 P=0.31	F=0 P=0.98	F=0.26 P=0.62	F=2.01 P=0.17	F=2.18 P=0.15	F=0.01 P=0.93
Smilaceae	*Smilax anceps* Willd. (fruit)	F=0.02 P=0.88	F=5.32 P=0.03	F=1.11 P=0.30	F=2.36 P=0.13	F=0.05 P=0.83	F=0.44 P=0.51	F=4.18 P=0.05	F=1.37 P=0.25
Rubiaceae	*Tricalysia cf. breteleri* Robbr. (fruit)*	F=0.81 P=0.38	F=0 P=0.95	F=2.28 P=0.14	F=0.02 P=0.89	F=0.36 P=0.55	F=0.04 P=0.84	F=0.03 P=0.87	F=4.24 P=0.05

Legends: BC: *Balantidium coli*, EN: *Endolimax nana*, PB: *Pseudolimax butschlii*, EC: *Entamoeba coli*, EH: *Entamoeba hartmanni*, EHD: *Entamoeba histolytica*/*dispar*.

Nutritional and phytochemical analyses

We did not find any significant differences between the nutritive values of candidate *vs*. non candidate plant parts (Table 2). Regarding the sugar contents that represent directly available energy, the results reveal that sugar contents are higher in plant parts that are considered to have been consumed for nutritional reasons compared to plant parts considered to be consumed for self-medication.

Table 2: Averaged nutritional values (±sem) of candidate vs. non-candidate plant parts.

Nutritional compounds	Candidate plant parts (N=22)	Non-candidate plant parts (N=22)
Sugars (%)	10.7±1.97	15.92±1.59
Lipids (%)	13.33±4.24	7.49±1.13
Proteins (%)	13.92±2.48	11.77±0.81
Energy (Kcal/100g)	210.79±5.23	174.95±3.66

By contrast, these two categories of plant parts differed in their phytochemical composition (Table 4). Quantitative analysis shows that the means total phenols content (TPC) in these two categories of plant parts (Table 4) were different especially for total tannins content ($p< 0.0018$). However, we have not observed of significant different between the two categories of plant parts in TPC (P=0.1229) and total flavonoids content (P=0.0632).

Table 3: Phenolic compound composition of plant parts categories (candidate and non-candidate).

Vegetal species	Plant parts	Total phenols (g EAG/ 100 g)	Total tanins (g EAT/ 100 g)	Total flavonoids (g EQ/ 100 g)
Non-candidates plant parts				
Aframomum sp	Fruit	18.64±2.04	0.96±0.9	6.61±1.2
Alchornea floribunda	Fruit	10.86±1.30	1.85±0.12	2.09±0.5
Barteria bracteosa	Fruit	6.15±1.10	0.08±0.12	0.14±0.2
Colopogonium mucunoides	Fruit	4.15±0.70	0.11±0.01	0.53±0.04
Eriosema glomerata	Fruit	3.69±0.01	2.27±0.08	0.05±0.02
Hylodendron gabunense	Seed	3.18±1.02	1.97±0.12	0.76±0.01
Lannea welwitschii	Fruit	0.69±0.09	0.13±0.03	0.04±1.2
Macaranga schweinfurthii	Petiole	17.34±2.01	4.68±0.12	1.11±0.01
Maesobotrya klaineana	Fruit	0.28±0.01	0.05±0.81	0.09±0.01
Medelina mirabilis	Fruit	3.03±0.05	1.98±0.01	0.38±0.02
Megaphrynium macrostachum	Fruit	4.01±0.01	1.96±0.11	1.62±0.03
Microdesmis haumaniana	Seed	0.98±0.01	0.19±0.01	0.07±0.02
Paspalum scrobiculatum	Seed	1.49±0.41	0.12±0.03	0.11±0.07
Pentaclethra eetveldeana	Flower	9.97±0.07	1.52±0.29	6.08±0.04
Plagiostyles africana	Seed	2.04±0.02	0.26±0.04	0.67±1.01
Rhynchospora corymbosa	Fruit	3.66±0.02	2.26±0.03	0.62±0.07
Trachyphrynium braunianum	Stem	0.66±0.24	0.02±0.01	0.04±0.02
Tristemma mauritianum	Leaf	28.45±1.02	3.21±1.04	3.07±0.04
Uvaria scrabida	Fruit	0.65±0.17	0.07±0.24	0.12±0.03
Uapaca guineensis	Fruit	0.06±0.18	0.005±2.1	0.04±0.01
Means		5.999±1.69	1.185±0.29	1.212±0.43
Candidates plant parts				
Aframomum alboviolaceum	Fruit	35.7±0.03	16.67±0.91	2.75±0.03
Aframomum daniellii	Fruit	8.99±0.03	12.78±1.23	1.78±0.23
Aframomum cf polyanthum	Fruit	74.01±0.01	1.62±0.03	2.33±0.08

Antrocaryon klaineanum	Fruit	4.46±0.10	3.99±0.01	5.94±0.15
Crotons sylvaticus	Fruit	12.7±0.22	6.56±0.46	9.38±0.08
Elaeis guineensis	Nuts	1.01±1.25	0.01±0.65	0.94±0.03
Geophila afzelii	Fruit	1.40±0.11	0.91±0.02	0.49±0.05
Haumania liebrechtsiana	Seed	38.8±0.08	28.15±0.22	0.44±0.01
Landolphia hirsuta	Fruit	7.40±0.11	0.43±0.30	0.02±0.02
Lasianthus batangensis	Fruit	5.76±0.25	2.91±1.05	1.25±0.01
Manniophyton fulvum	Stem	22.7±0.32	11.21±0.57	6.69±0.01
Musanga cecropioides	Fruit	18.53±0.09	2.41±1.05	3.36±0.02
Nymphaea maculata	Flower	14.93±1.01	8.19±0.77	11.4±0.03
Palisota ambigua	Fruit	16.35±0.09	9.03±0.03	2.44±0.03
Pentaclethra macrophylla	Seed	6.37±0.29	2.11±0.21	0.03±0.02
Renealmia macrocolea	Fruit	44.46±0.10	10.69±0.79	0.44±0.01
Ricinodendron heudelotii	Seed	3.01±0.20	0.01±0,49	0.02±0,15
Sarcophrynium brachystachyum	Stem	1.31±0.03	0.11±0.08	1.96±0.10
Smilax anceps	Fruit	6.93±0.05	5.65±0.82	3.31±0.91
Tricalysia cf. breteleri	Fruit	4.45±0.13	3.09±0.78	0.02±0.29
Means		12.971±2.92	6.328±1.58	2.907±0.72

All data were expressed as mean±SD of triplicate experiment (N=3).

Discussion

Our results show that three botanical families (Rubiaceae, Zingiberaceae and Euphorbiaceae) were the most representative in parasitized mandrill diet (Table 1).

The strong representativeness of these botanical families would explain by pharmacological potential of several vegetal species from families who's some are consumed by parasitized individuals.

Indeed, several species of the genus Aframomum belonging to the family Zingiberaceae have been reported to exhibit a wide variety of considerable biological activities (Cousins and Huffman, 2002) [61]. These authors reported antimicrobial activities against a variety of bacterial species such as *Escherichia coli* and *Pseudomonas aeruginosa*, *Yersinia entercolitica*, *Bacillus subtilis*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *Serratia marcescens* (Cousins and Huffman, 2002) [61].

Some studies have revealed that a present also fungicidal activities of certain species of Aframomum against fungal species such as *Candida albicans*, *Trichophyton mentagrophytes*, *Aspergillus Niger*, *Botryodiplodis theobromae* and *Cladosporium cladosporiodes* (Oloke *et al*., 1988) [62]. Studies aimed at evaluating the pharmacological potential of Aframomum daniellii fruits, which are regularly consumed by this population of mandrills, particularly parasitized individuals, have shown that the latter have an inhibitory activity on the growth of pathogens such as *Salmonella enteriditis*, *Pseudomonas fragi*, *P. fluorescens*, *Proteus vulgaris*, *Streptococcus pyogens*, *Staphylococcus aureus*, *Aspergillus flavus*, *A. parasiticus*, *A. ochraceus* and *A. niger* (Adegoke and Skura, 1994) [63].

In addition to *Alchornea floribunda* (Euphorbiaceae) has been reported to have antibacterial activities against the negative and positive Gram as *Bacillus cereus*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Staphylococcus saprophyticus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Moraxella catarrhalis* and *Proteus mirabilis* (Noundou *et al*., 2014) [64], antiprotozoal activity on *Trypanosoma brucei brucei*, *Trypanosoma cruzi*, on *Leishmania infantum* and on the *Plasmodium falciparum* strain resistant to the chloroquine and to the pyrimenthamine (Musuyu Muganza *et al*., 2012) [65].

Also, 90 plant species from Euphorbiaceae family are known to have the potential pharmacological properties and constitute effective remedy for many diseases like antidiarrhea, antioxidants, antibacterial antiamoebic, anticancer, antiplasmodial, HIV/AIDS, Jaundice, infertility, neurosis, syphilis (Bijekar and Gayatri, 2014) [66].

The abundance of species belonging to these three botanical families (*Euphorbiaceae*, *Rubiaceae* and *Zingiberaceae*) in the diet of mandrills with a high parasite load could suggest that permanent access to these plant species belonging to these families would allow them to modulate their parasite load or to fight against diseases caused by these pathogens.

Regarding the rate of consumption and the parasitism, our study shows that the consumption of three vegetal species like *Palisota ambigua*, *Manniophyton fulvum* and *Elaeis guineensis* were correlated with parasitic loads. This result suggests a relationship between parasitism of individuals and the consumption of these three plant species attesting a self-medication behavior in mandrills. Indeed, evidence used of vegetal species by great apes for to combat or control parasitic affections have been reported in several studies (Krief *et al*., 2005b; Fruth *et al*., 2014) [13, 68]. Referring to other studies, leaf and Stem of *Manniophyton fulvum* are known to be consumed by Bonobos for to combat their parasitic infections in Lui-Kotale, Salonga National Park, DR Congo (Fruth *et al*., 2014) [68]. These authors have shown that in spite of year-round availability and abundance of *Manniophyton fulvum* in area, it was ingested only at specific times, in very small amounts and by a small proportion by individuals (Fruth *et al*., 2014) [68]. Also, various parts of *Manniophyton fulvum* are used by human populations in ethnomedicine for to combat various affections across Africa. In DRC, it is used for many applications include a wide range of physical ailments from open wounds and visible bacterial infections to respiratory, gastro-intestinal and sexually transmitted diseases (Bouquet, 1969; Fruth *et al*., 2010, Fruth *et al*., 2011) [69, 70, 71]. The leaves or stem are used for to treat the dysentery, stomach troubles and parasites in Sierra Leone, Ghana and Côte d'Ivoire (MacFoy and Sama, 1983; Abbiw, 1990; Bellomaria and Kacou, 1995) [72-74]. All these ethnopharmacological data show the potential antiprotozoal of *Manniophyton fulvum* reinforcing the assumption that would be consumed by the mandrills for to modulate their parasite load. Phytochemical investigations have revealed that content phytochemical compounds such as tannins, glycosides, terpenes, saponins, and flavonoids. Their pharmacological properties include anti-inflammatory (Nia *et al*., 2005) [75], antioxidant, anti-diarrheal (Ezeigbo *et al*., 2010) [76], antibacterial (Uduak and Kola, 2010) [77], and antiprotozoal agents. Those data would confirm

that these plants would be selected in order to maintain a low level of pathogens and to ameliorate their health status. Thus, it appears evident that use of this three plant species by parasitized mandrills would be related with their parasite burdens as in Bonobos (Fruth *et al*., 2014) [68].

Palisota ambigua species belongs to the Commelinaceae family. The phytochemical screenings revealed that this family produces diverse classes of secondary metabolites like alkaloids, steroids, saponins, fatty acids, and tannins (Crouzet *et al*., 2009) [78]. The tannins and alkaloids are known as herbivore deterrent phytochemicals.

Also, recent studies realized by Tonga *et al*. (2024) [79] reveal that this species presents twelve known compounds namely, N-benzoyl-L-phenylalanyl-L-phenylalaninol acetate (aurantiamide acetate) (1), 20-hydroxyecdysone (2), rubrosterone (3), βsitosterol 3-O-β-D-glucopyranoside (4), 3β-hydroxystigmast-5-en-7-one (5), lupeol (6), betulinic acid (7), bis(2-ethylhexyl) terephthalate (8), 2,3-di-O-dodecanoyl-sn-glycerol 1-O-(6-O-α-D-galactopyranosyl)-β-D-galactopyranoside (9), docosanoic acid (10), pallidol (11), and apigenin (12). All compounds displayed antibacterial activity varying from weak to moderate against the tested pathogenic bacteria (Tonga *et al*., 2024) [79].

The high frequency of consumption by mandrills with a high parasite load suggests that some of these secondary metabolites have antiparasitic effects allowing modulation of the parasite load in this primate species.

A study whose aim was to explore the pharmacological activities of *Elaeis guineensis*, another plant species whose consumption is correlated with the parasite load in the mandrills reveals that Elaeis guineensis has a composition of bioactive constituents including vitamin E, fatty acids, phenolics, flavonoids, phenolics and carotenoids. With samples of leaf, fruit, seed, and root parts has the ability of pharmacological activity. The plant parts have shown significant potential in various biological activities, including high antioxidant activity, significant antimicrobial effects against bacteria (Widyowati *et al*., 2025) [80].

The observations of phytochemical and pharmacological data of these three plant species leads us to conclude that the relationship between the consumption of the latter by mandrills with a significant load of pathogens is for the purpose of self-medication aimed at combating by preventative or curative measure, diseases linked to these different pathogens.

The results of nutritional content have shown that the candidate plant parts present high means protein and lipids values compared non-candidate plant parts. Also, those energy calories were higher in candidate plant parts compared another category. These results would explain by the anorexia stress induced by parasitic infections. Similar observations have been obtained with caterpillar species of the African armyworm (*Spodoptera exempta*) by a baculovirus (Povey *et al*., 2013) [21]. This study has revealed that larvae of this species would increase their consumption of macronutrient when they were infected by baculovirus (Povey *et al*., 2013) [21]. The authors concluded that the observed behaviour was induced anorexia stress caused by infection of baculovirus (Povey *et al*., 2013) [21].

Phytochemical analysis has shown a difference in phytochemical composition of the two categories and that the means total polyphenol content were higher in plant parts consumed by parasitized mandrill especially for total phenols and tannins content there the value was very higher compared total tannins content provide by non-candidate plant parts consumed by small/or non-parasitized mandrills.

Indeed, ingestion secondary compounds such as tannins by animals for to combat parasitic affections have been shown in several observations (Villaba *et al*., 1999; Lefèvre *et al*., 2010; Vallaba *et al*., 2014) [10, 81, 82]. Thus, in a controlled experiment has revealed that lambs experiencing natural gastrointestinal helminthic burdens ate more of a tannin-rich supplement than non-parasitized animals, even when the supplement was of very low nutritional value (Lefèvre *et al*., 2010) [82]. Other study realized on two categories of lambs (with natural gastrointestinal parasitic load and non-parasitized lambs) has also shown that parasitized lambs a greater preference tannin-containing food than non-parasitized and this difference between these two categories disappeared when parasitic burdens were eliminated by chemotherapy (Villaba *et al*., 1999) [81].

Whole of those data would reinforce hypothesis that selection of those three vegetal species such as fruits of *Palisota ambigua*, Stems of *Manniophyton fulvum* and nuts of *Elaeis guineensis* by studied parasitized mandrills would be determined by parasitic burdens. Also, they reveal influence of gastrointestinal parasites in mandrill diet behaviour attesting possible selfmedication behaviour as reported by other great apes studies (Rodriguez and Wrangham, 1993; Huffman and Caton, 2001; Krief *et al*., 2005b; Fruth *et al*., 2014) [6, 12, 13, 68].

Conclusion

At the end of this study, it appears clearly that gastrointestinal parasites influence mandrill diet behaviour. Our study shows that parasitic burden would determine the consumption of fruits of *Palisota ambigua*, stem of *Manniophyton fulvum* and nuts of *Elaeis guineensis*.

The observations of phytochemical and pharmacological data of these three plant species leads us to conclude that the relationship between the consumption of the latter by mandrills with a significant load of pathogens is for the purpose of self-medication aimed at combating by preventative or curative measure, diseases linked to these different pathogens.

Also, other items presenting abundant anti-nutritional compounds like total phenols, tannins and flavonoids were many consumed by parasitized mandrills. Thus, as other primate species, mandrills would use some vegetal species for to prevent parasitism or to control parasitic loads. This preliminary study sets the first steps for further studies of investigation of mandrill self-medication behaviour.

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