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RESEARCH ARTICLE

Bioactive Properties of Plant Species Ingested by Chimpanzees (*Pan troglodytes schweinfurthii*) in the Kibale National Park, Uganda

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We measured the biological activities of a selected sample (84 crude extracts) of 24 species eaten by wild chimpanzees (*Pan troglodytes schweinfurthii*) in the Kibale National Park, western Uganda, to assess their potential chemotherapeutic values. Antibacterial, antimalarial, and/or antileishmania activities were observed in some crude extracts, and five of these extracts showed a significant cytotoxicity against human tumor cells. Active compounds isolated from three plant parts occasionally ingested by chimpanzees (*Diospyros abyssinica* (Ebenaceae) bark, *Uvariopsis congensis* (Annonaceae) leaves, and *Trichilia rubescens* (Meliaceae) leaves) showed highly significant medicinal properties. Two novel antiparasitic limonoids were isolated from *Trichilia rubescens* and their molecular structures were determined. In addition to elucidating the natural equilibrium maintained between hosts and pathogens, our investigation of the diet of wild chimpanzees may serve as a guideline to discovering plants with bioactive properties that should be preserved from destruction because of their health maintenance value for great ape populations. Am. J. Primatol. 68:51–71, 2006.

Key words: zoopharmacognosy; chimpanzee; chemical ecology; secondary compounds; bioassays

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INTRODUCTION

In an effort to fully appreciate and understand primate foraging behavior and plant selection criteria, recent studies have looked at the macronutrient and mineral contents of items in the diet [e.g., Chapman et al., 2003; Conklin-Brittain & Wrangham, 1994; Conklin-Brittain et al., 1998; Rode et al., 2003]. Behavioral, ecological, and pharmacological studies have shown that the great ape diet contains a variety of plant parts of no apparent nutritional significance, which may be consumed because of their secondary compounds [e.g., Huffman et al., 1998a; Jisaka et al., 1992; Koshimizu et al., 1993; Wrangham & Waterman, 1983; Wrangham et al., 1998]. It has been suggested that some of these dietary plants may also have medicinal properties [Huffman, 2003; Huffman et al., 1998a,b; Ohigashi et al., 1994]. Chemical investigations of the medicinal hypothesis have focused mainly on the consequences of two types of non-nutritional ingestion (i.e., ingestion of items that appear to be of little or no nutritive significance) in chimpanzees: 1) swallowing whole leaves of various species, and 2) ingesting the bitter pith of *Vernonia amygdalina* [Huffman & Seifu, 1989; Wrangham & Goodall, 1989; Wrangham & Nishida, 1983]. The antiparasitic properties of the plants ingested by chimpanzees in these cases were also investigated [Huffman et al., 1993, 1996b; Koshimizu et al., 1994; Jisaka et al., 1992, 1993; Ohigashi et al., 1994]. While leaf-swallowing has been convincingly implicated as an agent of parasite expulsion [Huffman & Caton, 2001; Huffman et al., 1996b], and may be involved in alleviating digestive pain caused by parasites [Wrangham, 1995], its known effects are due to physical rather than chemical causes [Huffman & Caton, 2001; Huffman et al., 1996a; 1997; Page et al., 1997]. Messner and Wrangham [1996] examined the in vitro anthelmintic activity of *Rubia cordifolia* rough leaves, but no significant activity was observed. On the other hand, bitter pith has been shown to have potential pharmacological consequences because it contains chemical compounds that apparently are responsible for the control of nematode infections [Huffman et al., 1993, 1996a; Koshimizu et al., 1993; Ohigashi et al., 1994]. Furthermore, it has been suggested that compounds found in the ordinary diet of animals may have important positive effects on health and may prevent risks of infection and illness [Huffman, 1997; Huffman et al., 1998a; Janzen, 1978]. This hypothesis is supported by the results of a previous study [Vitazkova et al., 2001] in which mice repeatedly sampled bitter substances as a likely feeding strategy to achieve chemoprophylaxis against parasite-related diseases. Mice that were infected by malaria parasites, and mice that were not infected spontaneously ingested the amount of chloroquine sufficient to contain the infection, even if the solution was unpalatable. Although some naturally occurring plants with a bitter taste are poisonous, by sampling a variety of bitter substances in small amounts, animals may reduce the likelihood of ingesting lethal doses and increase the chance of ingesting a plant with medicinal prophylactic value. We studied the chemistry of plants in the chimpanzee diet to assess their possible pharmacological value, as a contribution to zoopharmacognosy [Rodriguez & Wrangham, 1993].

Our goal was to screen the biological properties of plant species from Kibale National Park, Uganda, that may be of benefit to the health of chimpanzees. In addition, this approach offered us the opportunity to describe important secondary compounds in the chimpanzee diet, and to isolate previously undescribed natural compounds. As part of our survey of secondary compounds in the diet of chimpanzees, species ingested by the chimpanzees were investigated and different tissues (bark, leaves, fruits, and flowers) were tested even if they

were not consumed. This was done for three reasons: 1) to fully understand the criteria for plant selection used by Kanyawara chimpanzees, 2) to take into account the potential differences in consumption of plants according to study site [Goodall, 1986] and use by other animal species, and 3) to gain a better knowledge of plant species, which constitute an important natural resource that should be preserved for the health of both local animals and humans. Today the use of plant products is common to both traditional and western medicine. New approaches are needed in the search for drugs to combat widespread infectious diseases such as malaria, leishmania, and cancer. Such studies also provide a database for researchers investigating different primate species that eat the same plant species, and emphasize the necessity of large-scale screening. On the basis of previous findings [Huffman et al., 1998a], and in an attempt to highlight the most potentially biologically active plant parts in the diet of chimpanzees, we collected items that were ingested rarely or only in small amounts. Another rationale for focusing on rarely ingested items in the diet is the ''dose-dependent'' distinction made between poison and medicine. Our selection criteria were used as a simple guideline, and were not intended to represent an absolute measure of a plant's medicinal value to chimpanzees. The collected plant materials were tested for their pharmacological properties against parasites, bacteria, and yeasts. To test the hypothesis regarding the potential effect of plant ingestion on health, we noninvasively monitored the health of the chimpanzees by using feces and urine analysis, veterinary diagnosis, and behavioral observations [Krief et al., in press]. The levels of activity and the context of consumption may help to highlight different aspects of the potential medicinal effects of the plants ingested.

MATERIALS AND METHODS

Study Site

This study was conducted in the Kibale National Park between December 2000 and March 2001 (dry season), and in October 2001 (rainy season). The park, which is located between 0°13′ to 0°41′N and 30°19′ to 30°22′E, occupies 795 km^2 of mid-altitude moist forest, secondary forest, grassland, swamps, and plantations of *Eucalyptus* and pines, and includes elements of lowland tropical rainforest, montane rainforest, and mixed deciduous rainforest. The elevation is between 800 and 1500 m, and the rainfall averages 1,700 mm per year.

Behavioral Observations

S.K. observed the habituated Kanyawara community of chimpanzees (*Pan troglodytes schweinfurthii*), which contains about 50 individuals, including 10 adult males and 16 adult females. We recorded feeding observations in 10-min focal-animal sessions and made ad libitum observations during particular sequences related to possible medicative behavior (especially in sick chimpanzees) or to ingestion of items that were rarely eaten and/or were most likely non-nutritional. In total, 3,864 focal sessions were conducted. The health state of the animals was monitored daily by noninvasive methods [Krief et al., 2005].

Veterinary Survey

The veterinary work consisted of daily clinical observations (respiratory, digestive, reproductive, and urinary functions, and locomotion). Intestinal parasite evaluation was carried out by examination of feces. While following the

focal animal throughout the day, we paid particular attention to any abnormal behavioral activity, especially clear signs of potential illness, such as decreased appetite, long and frequent resting, sneezing, coughing, or intestinal disorder, as described by Huffman et al. [1997]. Whenever possible, feces were collected from all individuals that were clearly identified.

Fecal samples were inspected immediately after discharge to check for the presence of macroscopic parasites, and then collected and stored individually in vials. Part of each sample was stored in formalin. Fresh samples were analyzed microscopically using the MacMaster flotation method, and a direct method to ascertain the load and species present was applied to the formalin samples, as described by Krief et al. [2003, 2005].

A total of 252 dung samples from identified chimpanzees were collected, including 187 samples collected during the dry season and 65 obtained during the rainy season. These samples came from 38 individuals (mainly adults: 127 samples from 18 females, and 125 samples from 20 males).

Plant Collection

Our plant collection procedure was based on the observation that some types of food appeared more likely than others to have medicinal effects. Therefore, the collected plants included 1) bark, which appears to provide minimal nutritional gain and seems energetically costly to remove from the tree; 2) rough-surfaced leaves (e.g., *Ficus exasperata* and *Ficus asperifolia*), which may have physical properties that are important for parasite expulsion; 3) plants with spines (e.g., *Chaetacme aristata* or *Acanthus pubescens*), which are difficult to eat; and 4) urticant plants (e.g., *Urera* sp.). Of the plant parts we collected that are eaten by chimpanzees, all but one (*Celtis africana* leaves) are consumed only infrequently and thus can not be considered as a major component of their nutritional intake, according to long-term data (Table I). In addition to the plant parts specifically eaten by chimpanzees, we collected other parts from the same species. This was done for comparison purposes and to increase our general knowledge of the entire plant's chemistry. The plant specimens were identified with the help of field assistants, and then compared and identified in the herbarium of the Laboratoire de Phanérogamie at the Muséum National d'Histoire Naturelle (Paris, France). The plant material was sheltered from the sun and air-dried in the field.

Chemical Analysis

The air-dried, finely powdered plant material was extracted three times consecutively with ethyl acetate and methanol heated at 40°C for 3 hr. Eighty-four crude extracts were made from the different parts of 24 plant species from 13 botanical families. The extracted solutions were concentrated in vacuo to obtain crude extracts. Thin layer chromatography (TLC), column chromatography, and high-performance liquid chromatography (HPLC) were used to fractionate the bioactive extracts and to isolate the active compounds. Spectroscopic analyses (^{1}H-NMR, ^{13}C-NMR, LC-MS, EI-MS, IR, and UV) were employed to determine the chemical structures.

In Vitro Bioassays

Dried extracts dissolved in dimethyl sulfoxide (DMSO) were assessed for in vitro activities against three parasites (*Leishmania donovani*, *Trypanosoma brucei brucei*, *Plasmodium falciparum*) and a free-living worm (*Rhabditis*

TABLE I. Plant Parts Collected and Frequence (1: Rare, 2: Common, 3: Frequent) of Ingestion by Kanywara Chimpanzees

Plant part collected and					
Ingested by chimpanzees (frequency)			Not Ingested by chimpanzees		
Species	Family, form	Part and frequence	Species	Family, form	Part
Albizia grandibracteata Taub.	Fabaceae, tree	Bark (1)	*Chionanthus africanus* (Welw. ex Knobl.) Steam	Oleaceae, tree	Bark
Dombeya kirkii Mast.	Sterculiaceae, tree	Bark (1)	*Strombosia scheffleri* Engl.	Olacaceaea, tree	Bark
Pterygota mildbraedii Engl.	Sterculiaceae, tree	Bark (1)	*Trilepisium madagascariense* Thouars ex DC.	Moraceae, tree	Bark
Olea capensis L. subsp. *welwitschii* (Knobl.) Friis & Green	Oleaceae, tree	Bark (1)	*Pancovia pedicellaris* Radlk. & Gilg	Sapindaceae, tree	Bark
Ficus saussureana DC.	Moraceae, tree	Bark (1)	*Monodora myristica* (Gaertn. Dunal)	Annonaceae, tree	Bark
Diospyros abyssinica (Hiern) F. White	Ebenaceae, tree	Bark (1)	*Mimusops bagshawei* S. Moore	Sapotaceae, tree	Bark
Chaetacme aristata Planch.	Ulmaceae, tree	Bark (1)	*Uvariopsis congensis* Robyns & Ghesq.	Annonaceae, tree	Bark
Ficus exasperata Vahl	Moraceae, tree	Bark (1)	*Chrysophyllum albidum* G. Don	Sapotaceae, tree	Bark
Ficus natalensis Hochst.	Moraceae, tree	Bark (1)	*Celtis africana* Burm.f	Ulmaceae, tree	Bark
Urera sp.	Urticaceae, vine	Leaves (1)	*Celtis gomophophylla* Bak.	Ulmaceae, tree	Bark
Ficus asperifolia Miq.	Moraceae, shrub	Leaves (1)	*Chrysophyllum albidum* G. Don	Sapotaceae, tree	Leaves
Ficus exasperata Vahl	Moraceae, tree	Leaves (1)	*Acanthus pubescens* (Thomson ex Oliv.) Engl.	Acanthaceae, shrub	Leaves
Monodora myristica (Gaertn.) Dunal	Annonaceae, tree	Leaves (1)	*Potomorphe umbellata* (L.) Miq. (ex *Piper umbellatum*)	Piperaceae, herb	Leaves
Mimusops bagshawei S. Moore	Sapotaceae, tree	Leaves (1)	*Ficus saussureana* DC.	Moraceae, tree	Leaves
Celtis gomphophylla Bak.	Ulmaceae, tree	Leaves (1)	*Pancovia pedicellaris* Radlk. & Gilg	Sapindaceae, tree	Leaves
Uvariopsis congensis Robyns & Ghesq.	Annonaceae, tree	Leaves (1)	*Dombeya kirkii* Mast.	Sterculiaceae, tree	Leaves
Celtis africana Burm.f.	Ulmaceae, tree	Leaves (2)	*Chionanthus africanus* (Welw. ex Knobl.) Steam	Oleaceae, tree	Leaves
Albizia grandibracteata Taub.	Fabaceae, tree	Leaves (1)	*Potomorphe umbellata* (L.) Miq. (ex *Piper umbellatum*)	Piperaceae, herb	Flowers
Trilepisium madagascariense Thouars ex DC.	Moraceae, tree	Leaves (1)	*Acanthus pubescens* (Thomson ex Oliv.) Engl.	Acanthaceae, shrub	Flowers
Strombosia scheffleri Engl.	Olacaceae, tree	Leaves (1)			
Trichilia rubescens Oliv.	Meliaceae, tree	Leaves (1)			
Pterygota mildbraedii Engl.	Sterculiaceae, tree	Leaves (1)			
Urera sp.	Urticaceae, vine	Fruits (1)			

pseudoelongata) that has almost the same sensibility to Ivermectin as a parasite worm. Antitumor activities were assayed by measuring the cytotoxicity against KB cells from human tumor tissue. Extracts were also tested against bacteria (*Staphylococcus aureus* and *Escherichia coli*), yeast, and fungi (*Candida tropicalis* and *Penicillium crustosum*). All of the tests were conducted in duplicate or triplicate, and the means of the assays are presented below. The results are expressed as the concentrations that inhibited parasite or cell growth by 50% (IC_{50}) or killed 50% of the population of worms (LD_{50}; lethal dose=50%).

Antitrypanosomal screening was carried out according to the method described by Loiseau et al. [2000]. *Trypanosoma brucei brucei* GVR 35/Cl.2 bloodstream forms were used for in vitro screening. *Leishmania donovani* (MHOM/IN/80/DD8) promastigote forms were used for in vitro screening. Antileishmania screening was performed according to the method described by Mbongo et al. [1997].

Antimalarial activity was tested against intraerythrocytic asexual forms of the human malaria parasite *Plasmodium falciparum*, following the method of Desjardin et al. [1979]. We prepared an asynchrone culture of parasites [Trager & Jensen, 1976] with human serum containing parasitemia of 1% and a hematocrit of 2%. For the anthelmintic assay, *Rhabditis pseudoelongata* were isolated from wild rabbit feces and maintained on sterilized rabbit feces. They were recovered from a 10-day-old culture by the Baerman method. The isolated worms were stage I–IV larvae and male and female adults. Approximately two hundred worms were deposited in each of the 24 wells of the plate. Extracts in DMSO were added and the wells were filled up to 500 μl with sterile water. Motile and nonmotile worms were counted and compared with those in the control wells. Cytotoxicity assays were conducted on a KB cell line. The KB cell line was derived from an epidermoid carcinoma in the mouth of an adult human [Eagle, 1955; Shoemaker et al., 1983]. A suspension of 75,000 cells in 2 ml of 199/bicarbonate medium was deposited in every well. Two control tests with solvent and adriblastine 10^{-7} M were prepared. The trays were incubated for 3 days. Then 200 μl of neutral red were added and the trays were incubated one more night. The following day the suspension was removed and the cells were washed rapidly with PBS, and 1 ml of SDS was deposited in each well. Living cells incorporate neutral red, whereas dead cells do not [Borenfreund & Puerner, 1985]. Absorbance of neutral red was measured with a 540-nm filter. Antimicrobial assays against bacteria, yeast, and fungi were conducted in two steps: antimicrobial screening, and evaluation of the minimum inhibition concentration (MIC). The disks were soaked with 500 μg of extract in 20 μl of methanol, and then dried and laid down in petri dishes inundated with colonies in culture medium. They were incubated at 37°C for bacteria, and at 27°C for fungi for 18 hr. The diameter of the inhibition zone was read. The bacteria used were *Staphylococcus aureus* ATCC 6538 (culture medium: Mueller-Hinton) and *Escherichia coli* ATCC 8739 (culture medium: Mueller-Hinton), and the fungi were *Candida tropicalis* ATCC 66029 (culture medium: Sabouraud) and *Penicillium crustosum* LCP 75.3045 (culture medium: malt agar extract). The extracts selected by this method for their activities were tested in liquid medium for antimicrobial activity against *Staphylococcus aureus* to determine the MIC. Other microbial targets did not exhibit significant activities. Extracts in DMSO and a suspension of bacteria in exponential growth in Luria Bertoni medium (0.2 nm < DO < 0.4 nm) were deposited in 96-well trays. Control tests were performed using DMSO and bacterial suspension. The trays were incubated at 30°C and shaken at 250 rpm for 18 hr. Absorbancy at 620 nm was measured. The lowest concentration of test extracts in which no growth

occurred was defined as the MIC. The contents of the well were removed and cultured in petri dishes with Mueller-Hinton medium to check the bactericidal or bacteriostatic activity. The growth of bacteria was assessed after 48 hr by visual inspection.

Fractionation of bioactive extracts and isolation of pure products

According to the TLC results, we selected appropriate solvents to fractionate the crude extracts that appeared to be the most active, using the following specific methods:

1. The crude methanol extract of *Diospyros abyssinica* bark was chromatographed on silica gel with heptane-ethyl acetate. Fractionation was bioguided by activities on *Leishmania donovani*. The active fractions were purified by HPLC (Thermo-Hypersil 250 × 21.2 mm × 5 μ Kromasyl HS C18).
2. Dried bark powder of *Uvariopsis congensis* was first defatted with heptane and then extracted with ethyl acetate. Crude ethyl acetate extract was fractionated on silica gel with heptane-acetone. Column chromatography with isopropanol-dichloromethane and HPLC (Thermo-Hypersyl 250 × 21.2 mm × 5 μ Kromasyl HS C18) on the more active fractions on KB cells were used to isolate compounds.
3. Purification of the extract of *Uvariopsis congensis* leaves was performed by HPLC (Thermo-Hypersyl 250 × 21.2 mm × 5 μ Kromasyl HS C18).
4. The methanolic extract of the bark of *Albizia grandibracteata* was partitioned with butanol-water, and the butanol fraction was washed with ether. Four fractions were separated with Sephadex LH 20.
5. Crude ethyl acetate extract of the *Trichilia rubescens* leaves was fractionated, bioguided by its antimalarial activity. The extract was chromatographed on silica gel with dichloromethane-methanol, and the active fractions against *P. falciparum* were rechromatographed with heptane-ethyl acetate.

RESULTS

Pharmacological Properties of Plant Parts Ingested by Chimpanzees

According to data extracted from the Dictionary of Natural Products on CD-Rom, edited by Chapman and Hall [2003], natural pure products have been isolated from 30 (25%) of the species eaten by chimpanzees in the Kibale Forest, which shows that we still know very little about the chemistry of the plants in their diet. Moreover, these compounds are not always extracted from the part ingested by the chimpanzees.

Table II shows the most active extracts screened from a total sample of 84 plant parts collected from 24 species ingested by Kanyawara chimpanzees. During the 3,864 focal-animal sessions conducted in the present study, the chimpanzees were feeding or foraging for 47% of the time. Of the 46 plant items consumed in the present study, two were previously known to have pharmacological properties (i.e., *Antiaris toxicaria* pith and leaves, the latex of which is used as dart poison, and *Phytolacca dodecandra* berries, which contain bioactive saponins). The current pharmacological assays revealed three bioactive items (*Albizia grandibracteata* bark, *Trichilia rubescens* leaves, and *Ficus exasperata* stems bark) that were consumed during the present study. Their activities are reported below and in Table II. All of the five items were consumed in less than 0.5% of the feeding time during the study period.

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TABLE II. Biological Activities of Plants Species Ingested by Chimpanzees in Kibale National Park, Uganda*

				Biological activities in µg/ml							
									Staphylococcus aureus		
Species	Part extracted	Part eaten	Extraction solvent	IC_{50} *Plasmodium falciparum*	IC_{50} *Leishmania donovani*	LD_{50} *Rhabditis pseudoelongata*	KB cytotoxicity at 10 µg/ml (%)	KB cytotoxicity at 1 µg/ml (%)	Inhibition diameter (mm)	Bacteriostatic (BS) or bactericidal (BC)	MIC
Monodora myristica	Bark	No	Ethyl acetate	21.6	>10	**50C<<100**	25		<6		
Monodora myristica	Bark	No	Methanol	25.6	>10	**50<<100**	**92**	37	<6		
Potomorphe umbellata	Flower	No	Ethyl acetate	36.6	>10	**50<<100**	19		8	BS	142
Chrysophyllum albidum	Leaves	No	Methanol	44.0	>10	>100	20		10	BC	**11**
Potomorphe umbellata	Leaves	No	Ethyl acetate	**9.0**	>10	>100	32	24	**14**	**BS**	**46**
Acanthus pubescens	Flower	No	Ethyl acetate	45.8	>10	>100	18	12	**16**	**BC**	**25**
Acanthus pubescens	Flower	No	Methanol	67.2	>10	**25<<50**	0	0	<6		
Uvariopsis congensis	Bark	No	Ethyl acetate	**4.1**	>10	>100	**90**	39	8	BS	>100
Uvariopsis congensis	Bark	No	Methanol	**2.8**	>10	>100	**65**	**50**	8	BS	187
Chionanthus africanus	Leaves	No	Ethyle acetate	29.9	>100	**50<<100**	0		10	BS	50
Chionanthus africanus	Leaves	No	Methanol	71.9	>100	**50<<100**	0		<6		
Olea capensis	Bark	Yes	Ethyl acetate	42.9	>10	>100	21		8	BS	210
Olea capensis	Bark	Yes	Methanol	66.6	>10	>100	21		<6		
Ficus saussureana	Bark	Yes	Ethyl acetate	35.2	>10	>100	28		11	BS	369
Ficus saussureana	Bark	Yes	Methanol	67.6	>10	>100	8		11	BC	202.3
Diospyros abyssinica	Bark	Yes	Ethyl acetate	**5.6**	**1.5**	**25<<50**	**93**	**89**	**12**	**BC**	**12**
Diospyros abyssinica	Bark	Yes	Methanol	68.1	>10	>100	**87**	23	11	BC	1085
Chaetacme aristata	Bark	Yes	Ethyl acetate	29.7	>10	>100	11		10	BS	225
Chaetacme aristata	Bark	Yes	Methanol	59.9	>10	>100	0		<6		
Ficus exasperata	Bark	Yes	Ethyl acetate	24.3	>10	>100	0		**12**	**BS**	197.6
Ficus exasperata	Bark	Yes	Methanol	**17.6**	>10	>100	0		**12**	**BS**	487.5
Ficus natalensis	Bark	Yes	Ethyl acetate		>10	>100	9		<6		
Ficus natalensis	Bark	Yes	Methanol	41.7	>10	>100	0		11	BC	99
Albizia grandibracteata	Bark	Yes	Ethyl acetate	52.9	>100	>100	28		10	BC	50

Albizia grandibracteata	Bark	Yes	Methanol	90.2	>100	**25<<50**	**98**	**90**	<8		
Dombeya kirkii	Bark	Yes	Ethyl acetate	**16.6**	>100	>100	0		10	BC	>100
Dombeya kirkii	Bark	Yes	Methanol	76.9	>100	>100	0		<8		
Pterygota milbraedii	Bark	Yes	Ethyl acetate	25.6	>100	>100	19		**15**	**BC**	**25**
Pterygota milbraedii	Bark	Yes	Methanol	77.1	>100	>100	12		10	BS	>100
Urera sp.	Fruits	Yes	Ethyl acetate	89.6	>10	>100	0		<6		
Urera sp.	Fruits	Yes	Methanol	86.1	>10	>100	0		8	BS	180
Urera sp.	Leaves	Yes	Ethyl acetate	84.1	>10	>100	0		<6		
Urera sp.	Leaves	Yes	Methanol	51.9	>10	>100	0		<6		
Ficus asperifolia	Leaves	Yes	Ethyl acetate	64.1	>10	**100**	38		<6		
Ficus asperifolia	Leaves	Yes	Methanol	41.6	>10	>100	38		<6		
Ficus exasperata	Leaves	Yes	Ethyl acetate	43.1	>10	>100	18	4	<6		
Ficus exasperata	Leaves	Yes	Methanol	37.4	>10	>100	18	10	<6		
Monodora myristica	Leaves	Yes	Ethyl acetate	72.2	>10	>100	23	18	<6		
Monodora myristica	Leaves	Yes	Methanol	37.4	>10	>100	4	0	<6		
Mimusops bagshawei	Leaves	Yes	Ethyl acetate	**41.4**	>10	>100	33	18	<6		
Mimusops bagshawei	Leaves	Yes	Methanol	55.7	>10	>100	6	3	<6		
Celtis gomphophylla	Leaves	Yes	Ethyl acetate	40.7	>10	>100	25	23	<6		
Celtis gomphophylla	Leaves	Yes	Methanol	54.7	>10	>100	34	17		BS	219
Uvariopsis congensis	Leaves	Yes	Ethyl acetate	**14.7**	>10	>100	46	**45**	<6		
Uvariopsis congensis	Leaves	Yes	Methanol	**15.1**	>10	>100	**53**	**49**	<6		
Celtis africana	Leaves	Yes	Ethyl acetate	68.7	>10	>100	23	21	<6		
Celtis africana	Leaves	Yes	Methanol	60.1	>10	>100	12	0	<6		
Albizia grandibracteata	Leaves	Yes	Ethyl acetate	>100	>100	>100	0		10	BS	>100
Albizia grandibracteata	Leaves	Yes	methanol	>100	>100	**25**	**95**	**59**	9	BS	>100
Trillepisium madagascariense	Leaves	Yes	Ethyl acetate	64.7	>100	>100	0		**12**	**BS**	>100
Trilepisium madagascariense	Leaves	Yes	Methanol	62.4	>100	>100	15		<8		
Strombosia scheffleri	Leaves	Yes	Ethyl acetate	69.4	12≪25	>100	0		**14**	**BC**	>100
Strombosia scheffleri	Leaves	Yes	Methanol	63.5	25≪50	>100	0		<8		
Trichilia rubescens	Leaves	Yes	Ethyl acetate	**<3.1**	25≪50	>100	0		**12**	BC	>100
Trichilia rubescens	Leaves	Yes	Methanol	**<3.1**	25≪50	>100	33		11	BC	>100
Pterygota milbraedii	Leaves	Yes	Ethyl acetate	76.7	>100	>100	0		**12**	**BC**	>100
Pterygota milbraedii	Leaves	Yes	Methanol	>100	>100	>100	0		<8		

*IC_{50}, inhibiting concentration 50%; LD_{50}, lethal dose 50%; bold, most significant activities.

Of the 84 items tested, 45% (n=38) were bark extracts. Bark extracts represent 63% of the items that exhibited a significant activity in the assays conducted. The difference between the two proportions is significant (Fisher test, $P=0.04$), consistent with our hypothesis that bark may often be a high source of pharmacological compounds. However, in addition to the rarely eaten plant parts that exhibited significant IC_{50} values, food plants that had weaker IC_{50} values but were consumed in larger quantities may also have an important long-term pharmacological effect.

Various kinds of bioactivity were detected in the crude extracts investigated. Antiparasite activities were found for several plant extracts. Six crude extracts (7%) displayed noticeable antimalarial activity (i.e., their IC_{50} values were below 10 mg/ml). Three of the six samples were extracted from plant parts eaten by chimpanzees. Ethyl acetate and methanol crude extracts of *Trichilia rubescens* leaves and the ethyl acetate crude extract of *Diospyros abyssinica* bark had a significant IC_{50} value against *Plasmodium falciparum*. The ethyl acetate crude extract of *Diospyros abyssinica* bark exhibited high antileishmania activity after 24-hr and 72-hr incubation times ($IC_{50}=1$ mu/μ). Ten crude extracts had an LD_{50} below 100 mu/μ against *Rhabditis pseudoelongata*.

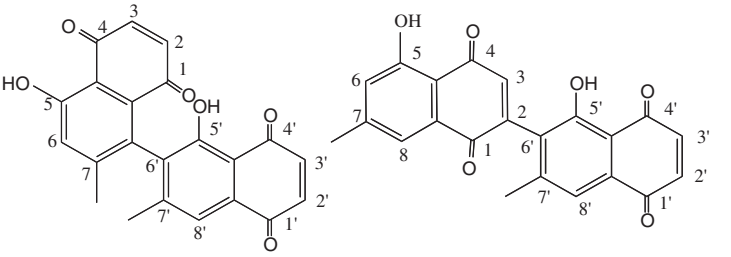
Antimicrobial activities against bacteria, yeast, and fungi were investigated. An inhibition zone with a diameter of 12 mm or more was observed for 17 crude extracts, 11 of which were bactericidal against *Staphylococcus aureus*. The MIC was below 25 mu/μ for four crude extracts. Three crude extracts (bark of *Mimusops bagshawei, Chrysophyllum albidum*, and *Pancovia pedicellaris*) had an inhibition diameter of ≥ 9 mm, but less than 12 mm against *Escherichia coli*. No extracts exhibited any significant activity against *Trypanosoma brucei brucei, Candida tropicalis*, or *Penicillium crustosum*. We noted that the methanol crude extract of *Acanthus pubescens* flowers, which are not eaten by Kanyawara chimpanzees but are consumed by Sonso chimpanzees [Pebsworth et al., in press] showed important anthelmintic activity at a range of 25–50 mu/μ. Its ethyl acetate extract was bactericidal (antimicrobial screening with disks) and its MIC against *S. aureus* was 25 mu/μ.

In vitro tests of the biological activity of six crude extracts showed a >85% cytotoxic activity on human KB cells at 10 mu/μ. Five crude extracts (methanol crude extracts of *Uvariopsis congensis* bark and leaves, *Albizia grandibracteata* bark and leaves, and ethyl acetate crude extract of *Diospyros abyssinica* bark) had an $IC_{50} \leq 1$ mu/μ.

According to the biological screening results, extracts from plant parts eaten by the chimpanzees (*Diospyros abyssinica*, *Albizia grandibracteata, Trichilia rubescens*, and *Uvariopsis congensis*) exhibited such high bioactivity that it leads us to strongly consider the chimpanzees selecting them were using a criterion other than nutritive intake alone. Such items are rarely ingested by Kanyawara chimpanzees, as was the case for those plant foods with medicinal value for parasite-related maladies in Mahale chimpanzees [Huffman et al., 1998a]. For these reasons, we decided to further investigate the chemical content of the extracts.

Chemical Investigations

To isolate the bioactive constituents of the plants from the chimpanzee diet, we focused our chemical analyses on the four plant species that exhibited the most significant biological properties: *Diospyros abyssinica*, *Uvariopsis congensis*, *Albizia grandibracteata*, and *Trichilia rubescens*.



Isodiospyrin Diospyrin

Fig. 1. Two bioactive bisnaphtoquinones isolated from the bark of *Diospyros abyssinica* (Ebenaceae), eaten by the chimpanzees of Kanyawara, Kibale National Park, Uganda.

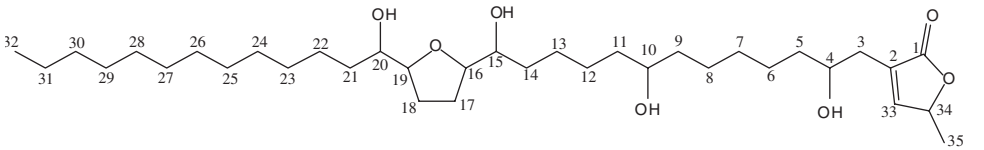


Fig. 2. A bioactive acetogenin, the annonacin, isolated from the leaves of *Uvariopsis congensis* (Annonaceae) eaten by the chimpanzees of Kanyawara, Kibale National Park, Uganda.

Fractionation of *Diospyros abyssinica* bark (which is rarely consumed by chimpanzees) yielded isodiospyrin and diospyrin (Fig. 1), two bisnaphtoquinones identified by NMR, LCMS, UV, and IR spectroscopy. Their IC_{50} value against *Leishmania donovani* was 0.5 mu, μ (IC_{50} *L. donovani* for the reference product: pentamidine=7 mu, μ). Their activity against *Plasmodium falciparum* was weaker (IC_{50}=1.5 mu, μ; IC_{50} chloroquine=0.1 mu, μ).

These quinones were previously isolated from other *Diospyros* species, and are known to exhibit activities against protozoan parasites such as *Leishmania donovani*, *Trypanosoma cruzi*, and *Trypanosoma brucei brucei* [Yardley et al., 1996]. They have also shown inhibitory activities against murine tumors in vivo [Norhamon & Hazra, 1997; Pal et al., 1996] and inhibition of blood platelet aggregation [Kuhe et al., 1998].

The activities against *Plasmodium falciparum* and KB cells of the crude extract of *Uvariopsis congensis* were investigated. The crude extract of dried bark powder was purified. One of the compounds isolated was identified by NMR, LC-MS, and EI-MS methods as cis-annonacin, an acetogenin (Fig. 2). This compound was the most common product of the leaves that were sporadically eaten by chimpanzees. Its IC_{50} on KB cells is 0.24 mu, μ (IC_{50} on KB cells for the reference product: adriblastine=0.1 mu, μ). Gigantetrocine, another acetogenin that is potentially cytotoxic to tumoral cells (2.2 mu), was isolated.

The methanol extract of *Albizia grandibracteata* bark (which was eaten by OK on 20 October 2001) had anthelmintic activity against *Rhabditis pseudo-elongata* and was cytotoxic (90% at 1 mg) to KB cells. A mixture of saponins was contained in the active fraction. Three products were isolated and found to be structurally novel saponins (Krief et al., in press).

After the crude ethyl acetate extract of *Trichilia rubescens* leaves was fractionated, its antimalarial activity increased up to 4 ng/ml. During this fractionation, novel limonoids (trichirubines A and B) (Fig. 3) with high antimalarial activities were extracted and identified [Krief et al., 2004].

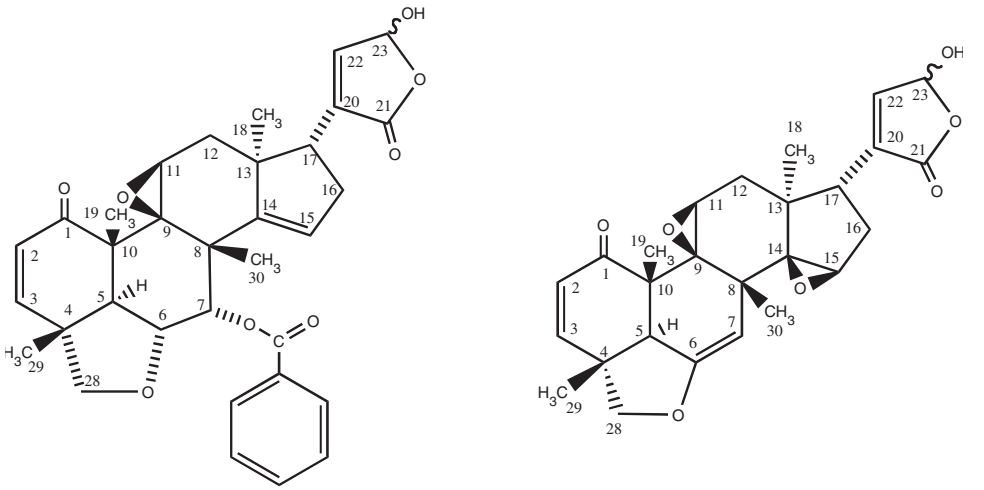


Fig. 3. Antimalarial compounds (trichirubines A and B) from the leaves of *Trichilia rubescens* (Meliaceae) ingested by the chimpanzees of Kanyawara, Kibale National Park, Uganda.

Chimpanzee ingestion of plants with bioactive compounds, and the chimpanzees' state of health

Most of the chimpanzees monitored during the 4 months of this survey appeared to be in good health, or at least improved quickly after they incurred small injuries or exhibited flu-like symptoms [Krief, 2003]. However, during the study period at least 10 cases of patently ill chimpanzees were observed. Five cases involved respiratory infections with nasal discharge, sneezing, and coughing, including two severe cases of lethargy, dyspnea, and anorexia. Two chimpanzees were detected suffering from gastrointestinal disorders. Bacterial infections were diagnosed in one wounded chimpanzee (LB) and in another individual (TU) that suffered from tooth abscess. An old female (LP) with severe abdominal distension also had a left-hand injury. The swollen hand was not used for tree climbing.

These wounded or sick chimpanzees were closely monitored in the field. Since the symptoms were totally absent after 1 week, we consider that all of these animals recovered from their respective diseases. Their utilization of plants with possible medicinal functions was examined in each instance (see case studies below).

Fecal samples showed the presence of parasites, with 93% and 95% prevalence for feces collected in the dry and rainy seasons, respectively. However, 96% of the samples had less than 1,000 parasites per gram [Krief et al., 2003]. This corresponds to a low parasitic load for humans [Hercberg et al., 1986].

From the 10 case studies of illness presented in Table III, we selected two because of their particularly clear diagnosis and because it was possible to make sufficiently continuous observations to test the hypothesis regarding the effect of plants on health. These two case studies are described below:

Case 1

On 15 February 2001, a fight was observed between two adult males (YB and LB). YB bit LB's foot. LB's fifth toe was severely cut and hung from his foot by

TABLE III. Diagnosis of Ill Health Status as Evaluated With Non-invasive Methods in Kanyawara's Chimpanzees, Kibale National Park, Uganda

Symptoms	Individual	Age	Veterinary observations	Urinalysis	Coprological analysis	Behavioral observations [as partly reported in Krief et al., 2005]
Influenza-like syndrom	KK	17 yrs	15/02/01: frequent cough, 16/02/01: weakness, dyspnea, sneezing	15/02/01: low pH	16/02/01: *Probstmayria* sp., eggs and larvae of strongyles, eggs of *Trichuris* sp.	16/02/01: resting=77% for KK (33% for the 13 individuals from the party)
	AR	>50 yrs	9/02/01: deep cough, sneezing, very thin, slow digestive transit	7/02/01: Nitrites, blood, leukocytes 9/02/01: Nitrites, blood	7/02/01 et 9/02/01: (*Strongyloides fulleborni*, ancylostoma, *Probstmayria* sp)., soft consistency F#108: *Bertiella studeri*	7/02/01: frequent resting 9/02/01: left her nest 1:20 later than her offspring
	OU	~25 yrs	15/02/01: frequent and deep cough, sneezing, dyspnea	No sample	15/02/01:2 samples with multispecific infection (*Strongyloides fulleborni* ancylostoma. *Probstmayria* sp.); parasite load=700/ml	
	ES	7 yrs	19/02/2001: sneezing	No sample	No sample	Normal behavior
	PG	13 yrs	19/02/2001: sneezing	No sample	No sample	Normal behavior
Intestinal disorder	LP	~50 yrs	Intestinal disorder	3/02/2001: leukocytes, proteins	2/02/01: coccidiosis and multispecific infection	5/02/01: seed-eating in elephant dung, 10/02/01: consumption of fine fiber material from a hollow trunk
	OK	6 yrs	16–20/10/01: alternately dry, soft and runny stools	No sample	16–20/10/01: high parasite numeration by direct examination and Mac Master flotation, multispecific infection including *Probstmayria* sp.	20/10/01: consumption of the bark of *Albizia grandibracteata*, never recorded as food before
Infectious lesions	LB	~35 yrs	Toe wounded after a fight 16/02/01: wound sweeping, 20/02/01: locomotion painful, toe black on dry, 24/02/01: the tow fell off	16/02/01: low pH		Did not join the male group
	TU	~40 yrs	14/02/01: gingival abcess	No sample	16/02/01: ancylostoma and *Strongyloides fulleborni* in 2 samples	
Multiple infections	LP	~50 yrs	22/12/00: abdominal distension and painful left arm	23/12/00: blood (++) et leukocytes (+)	No sample	22/12/00: geophagy, spent much time in nest:5:20 PM to 7:50 AM

TABLE IV. Ethnomedicinal Information on Plants Ingested by a Wounded Chimpanzee (LB) in the Kibale National Park, Uganda (UPU: unknown part used, DRC: Democratic Republic of Congo, CAR: Central Africa Republic)

Species and part ingested	Date of ingestion	Use in traditional medicine for humans and domestic animals
Acanthus pubescens (stems)	21/02/2001	Burundi: stems against dermatosis, skin infections and sterility of cattle (prelude website) DRC: anti-inflammatory for limping (UPU)
Cordia africana (fruits)	19, 20, 21, 24/02/2001	Burundi: anti-hemorrhage for humans (UPU) (prelude website)
Ficus sur (fruits)	19/02/2001	West Africa: immature fruits for reproductive stimulation (prelude website) Congo: piece of fruits on abscess, edema and leprous ulcer [Bouquet, 1969]
Ficus natalensis (fruits)	16/02/2001	South and East Africa: entire plant against infectious veneral disease [Watt & Breyer-Brandwijk, 1962]
Ficus exasperata (leaves and stems)	20/02/2001	Congo leaves against edema, leprous ulcer [Bouquet, 1969] and in application on abscess (prelude website) Sierra-Leone: leaves against dermatosis (prelude website) Congo: edema, abscess (UPU) (prelude website) CAR: dermatosis (UPU) (prelude website)

only a strip of skin. On 19 February, LB's locomotion appeared to be painful, and the following day his toe was black and dry. The wound on the edge of his foot grew to be 1 cm deep and 4 cm wide. Nine days after the aggression, LB's toe fell off and the wound looked clean. LB's diet during this period contained five plants that are typically used in traditional medicine for wounds and other ailments in humans and domestic animals (Table IV). None of these plants were unusual dietary items, except for the young leaves and stems of *Ficus exasperata*. Four chimpanzees from the same party fed on other items while LB consumed the *F. exasperata*. We investigated the chemistry of three of these species to ascertain whether they could have aided the recovery of LB's wound (results in Table III). The disks soaked with an ethyl acetate extract of *F. exasperata* bark were bacteriostatic, and the leaves and stems of this species were eaten by the wounded chimpanzee.

Case 2

Starting about 16 October 2001, OK, a 6-year-old female, suffered from an intestinal disorder, as evidenced by the production of alternately dry, soft, and diarrheic stools. Fecal analysis revealed a high load of parasitic infection (strongyle species and *Probstmayria gombensis*) [Krief et al., 2003]. We observed OK eating *Albizia grandibracteata* bark at 0942 hr on 20 October 2001. This was the first recorded instance since the observations began in Kanyawara in 1987 that a chimpanzee was seen consuming the bark of this species, although the animals were occasionally observed eating its leaves. OK was the only one of the group that ate the bark. She ate it for 3 min while her mother and siblings waited for her. Feces collected 2 days after OK's ingestion of *A. grandibracteata*

bark (October 22) had a normal consistency, and the parasitic load was nil. *A. grandibracteata* bark is traditionally ingested as a medicine in Uganda and Democratic Republic of Congo (DRC) against intestinal parasites and bloat [Defour, 1994; Heine & König, 1988]. This observation raises the possibility that OK's bark-eating was responsible for the reduction in the high parasite load and digestive symptoms observed after October 16.

Regular presence of bioactive compounds in the diet

Along with some other unusual behaviors, we observed that Kanyawara chimpanzees feed occasionally on *Trichilia rubescens* leaves. Such consumption bouts were short (1–21 min, 21 observations, mean=5 min) and infrequent (chimpanzees were observed to ingest these plant species 0–4 times per month across the 8 months of observation). Only a few leaves were eaten each time (about seven per minute). This plant generally grows in a cluster, so the leaves would be available for several chimpanzees. For each observation made, usually only one chimpanzee (rarely more) ate this item while it was in a group of two to 28 individuals. On two occasions, chimpanzees were observed ingesting 100 leaves or more at a time. In one case (11 June 2003), a wounded chimpanzee (AJ) consumed more than 100 leaves from 0737 to 0758 hr. Twelve minutes later, ST was observed feeding on *Trichilia rubescens* leaves (about 20). ST was observed 4 weeks later ingesting around 100 leaves, and showed no observable or measurable symptoms. He was observed by LK, who was tasting a few leaves of the item. In one case, one individual (MS, 22 December 2003) ingested many leaves (60) and was observed by three individuals (BB, ST, and TU) that did not consume the item. On 25 March 2004, UM changed her travel route to consume a few leaves of this plant. Two minutes later, MS consumed 17 leaves. During the 8-month study period, three individuals were observed ingesting the plants on multiple occasions (MS: three times, AJ: three times, ST: twice). In each case the chimpanzee left the *Trichilia* shrub before it had eaten all the leaves of the tree, except for AJ, who consumed leaves from two trees on 6 November 2003. These observations are a strong indication that *T. rubescens* leaves may provide benefits other than nutritional ones, since usually only a small amount is consumed. Significant antimalarial activities on the human protozoan parasite *Plasmodium falciparum* have been demonstrated for the crude extracts, and two novel limonoids with a CI_{50} roughly equivalent to chloroquine have been isolated [Krief et al., 2004]. The human parasite is very similar to *P. reichenowi*, which occurs in west, central, and east tropical Africa, and affects gorillas and chimpanzees [Töft, 1986]. The several species of *Plasmodium* that affect great apes are generally considered to be mildly pathogenic; however, few details have been reported for wild individuals [Toft, 1986]. No symptom that could be related to malaria was detected in the chimpanzees in the present study; however, a more accurate diagnosis is needed to detect discrete symptoms. In one case, a larger amount of leaves was consumed by a wounded chimpanzee. In addition, *T. rubescens* is easily confused with a closely related species, *T. dregeana*. In fact, *T. rubescens* was previously identified as *T. dregeana* in Kibale National Park. Accordingly, it is noteworthy that *T. dregeana* bark is used in traditional Zulu medicine to treat headaches and inflammatory diseases [Jäger et al., 1996]. Among the 39 ethanolic plant extracts tested by Jäger et al. [1996], *Trichilia dregeana* showed high inhibitory activity for prostaglandin synthesis. Prostaglandins are involved in the process of inflammation and are responsible for the feeling of pain. Such properties may have benefited the wounded chimpanzee, since the pain felt by the

chimpanzee would have been mitigated by the increased consumption of a plant with an analgesic effect.

DISCUSSION

The results of the biological screening of plant parts eaten by chimpanzees demonstrate that some items that are ingested only rarely possess very significant pharmacological properties. This suggests that in addition to nutritional value, there may be other motivational factors for choosing certain food items. Other plant parts exhibited weaker effects, and thus may act by cumulative effect or repeated ingestion. As suggested by Lozano [1998], there may be two aspects of self-medicative behavior: preventive and therapeutic. Without passing judgment here on whether the ingestion of some plants with a preventive function is a conscious act, it seems clear to us that the low-level consumption of some plants with minimal nutritional benefits in the regular diet may serve to prevent the risk of parasitism or infection, and to maintain a low level of pathogens. This study provides new evidence that chimpanzees forage on some plants that are also used in traditional human and veterinary medicine [Huffman et al., 1996a, 1998a]. Of the 117 plant species known from long-term records to be ingested by chimpanzees in the Kibale Forest, at least 27 (23%) are used in traditional African medicine, according to published references and data available on websites [reviewed in Krief, 2003] (Krief et al., in press). Although the names of the plant parts used by healers are often not included in the description of the preparation, we found uses in traditional medicine for 35 of the 163 parts ingested by chimpanzees in Kanyawara. While a larger sample set including regularly-eaten plants is necessary to demonstrate a correlation between the rate of consumption and bioactivities of the plant species, we do not believe that every species ingested by chimpanzees has some kind of biologically active property. Cases of *Albizia grandibracteata* consumption related to intestinal disorders, and increased *Trichilia rubescens* consumption by a wounded chimpanzee provide evidence that ingestion of plants with medicinal properties is not just a random event and may very well be driven by a desire for certain secondary plant compounds. It would be interesting to screen for other properties, such as antiinflammatory activity, in future studies.

The noninvasive health monitoring used during this study is a viable way to test the hypothesis of a regulation process induced by plant antiparasitic activities or the immunostimulating properties of natural compounds. Thus, four points are consistent with the hypothesis that elements of the chimpanzee diet can contribute to reducing the effect of parasites and microbes on their health: 1) some items ingested by chimpanzees are also common to traditional African medicine; 2) the chimpanzees had a relatively low parasite load; 3) most of the Kanyawara chimpanzees were in relatively good health, as evaluated by urinalysis, fecal analysis, and veterinary observations [Krief, 2003]; and 4) the results of the biological property screening were confirmed by the isolation of pure bioactive compounds. It would be interesting to conduct similar studies on other primate species in the region.

The related observations of ill or wounded chimpanzees were consistent with the hypothesis that they benefited from the use of some plants with bioactive properties. Such plants are also used by the local population to treat similar symptoms [Huffman & Wrangham, 1994; Huffman et al., 1996a, 1998b]. For example, the diet of LB when he was wounded contained *Ficus exasperata* stems and leaves. The stem bark of this species has antibacterial properties, and its

leaves are used in Africa to cure cutaneous infections and to relieve pain in humans and livestock [Bouquet, 1969]. Leaves of *F. exasperata* have also been prescribed as an anti-ulcer remedy, and a study on rats revealed that the extract of these leaves has significant anti-ulcerogenic properties [Akah et al., 1998]. In one study [Koshimizu et al., 1993], *F. exasperata* showed antitumor activities and remarkable inhibition of trypsin activity; however, the authors did not describe the parts tested. These points suggest to us that LB's health may have been improved by the inclusion in his diet of some of these plants that contain bioactive compounds.

The unusual behavior of feeding on *Trichilia rubescens* leaves led us to discover its interesting antimalarial activity. No symptom related to an infectious disease, such as malaria, was detected in the chimpanzees that ingested this item; however, malaria in apes is known to be subclinical or mildly pathogenic. Nevertheless, when coupled with other diseases, malaria may become deleterious [Skinner & Hopwood, 2004]. Malaria was examined as the cause of linear enamel hypoplasia in apes by Skinner et al. [1995] and Guatelli-Steinberg & Skinner [2000]. In a recent survey on the prevalence of malaria in wild-born, formerly captive Bornean orangutans, M.J. Reid and colleagues (personal communication) found that more than 25% of the individuals tested were infected by a *Plasmodium* parasite. Such clues suggest that pathogen pressure may be reduced by the ingestion of certain plants. This hypothesis was most clearly supported by the case in which OK ate bark of *Albizia grandibracteata* while she suffered from intestinal upset and had a heavy parasitic load. This unusual dietary item apparently increased OK's intake of compounds that were bioactive against helminths, because we found that *A. grandibracteata* bark contained bioactive saponins. Subsequently, OK's condition improved, and fecal examination confirmed reduced parasitism in vivo. These observations support the apparent importance of *Albizia grandibracteata* bark as a traditional medicine.

While OK's case suggests that her diet was effectively medicinal, further work will be required to prove it. First, chemical extractions with methanol or ethyl acetate solvents exclude the digestion process from pharmacological testing. Second, detoxification mechanisms could interfere with potential medicinal properties. Third, we need to quantify the amount of each plant that is consumed to estimate what bioactivity level may be expected in the chimpanzee's body. As proposed by Huffman [2003], the next step will be to conduct in vivo tests to determine the efficiency of different plant parts in suppressing different pathogens relevant to chimpanzees.

According to the results of our in vitro tests, several plant species ingested by chimpanzees are active against human pathogens. Some of these agents, such as *Staphylococcus aureus* and *Escherichia coli*, can affect both humans and chimpanzees. In such instances the observation that chimpanzees chose bioactive plant products supports the self-medication hypothesis. However, despite the phylogenetic proximity of humans and chimpanzees, additional tests are needed regarding pathogens specific to chimpanzees. Finally, even if such medicinal activity is supported by further data, we acknowledge that in the case of plant parts that provide nutrients and minerals, it is difficult to assess the relative importance of nutritional signals vs. cues of bioactivity or painkilling as influences on chimpanzee food choice.

In conclusion, this study shows that the chimpanzee diet includes compounds that likely offer medicinal benefits, such as reducing the level of pathogens and keeping chimpanzee health problems to a subclinical level. The implications from this survey of chimpanzee diet, feeding behavior, and health may lead to the

discovery of new natural products with potential medicinal properties for human beings. The chimpanzees' diet may allow them to maintain a good state of health and prevent illness, while avoiding potential toxicity and resistance induced by repeated consumption. Some plants with stronger biological properties are consumed in small quantities and only on rare occasions, and thus are likely of curative value. Between these two mechanisms, we should consider that health is a continuum, and the positive feedback of a medicinal substance may act during the sampling of the regular diet by an individual that feels weak discomfort. Therapeutic medication may also be learned individually, and food aversion may be attenuated by observation of conspecifics feeding on an item.

At a conference at the Max Planck Institute in Leipzig, 2004, during which the Great Apes Health Monitoring Unit (GAHMU) was created, disease was considered to be the third major threat for great ape populations today. Habitat destruction caused by the opening of roads into a forest has a major impact because it not only facilitates poaching and the bushmeat trade, it also increases the potential for pathogen introduction and transmission. Environmental disruption may change the balance between the host and the pathogen by depriving animals of important plants that provide both essential nutrients that affect immunologic status, and secondary compounds that may help the animals fight against disease. The effect on animals' health from the deprivation of secondary compounds is probably underestimated today because the actual medicinal value of plants in primate habitats is still unknown. Thus, a pharmacological approach to the study of their diet may be a valuable new tool for primate conservation.

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