

FURTHER OBSERVATIONS ON THE USE OF THE  
MEDICINAL PLANT, *Vernonia amygdalina* (Del),  
BY A WILD CHIMPANZEE, ITS POSSIBLE EFFECT ON  
PARASITE LOAD, AND ITS PHYTOCHEMISTRY

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**ABSTRACT** This is the second detailed case study of the use of *V. amygdalina* (Del) by a wild chimpanzee suffering from gastrointestinal upset (flatulence and diarrhea). The female, who was followed for approximately 5 hours over a two-day period, recovered from her symptoms by the afternoon of the second day. Laboratory examination of two fecal samples, one collected approximately 1 hour and another 20.5 hours after ingestion of the plant's bitter pith, revealed a notable drop in the degree of parasitic infection by a *Ternidens* sp. Bioassay of the plant consumed by the female confirmed that the two most abundant and bioactive constituents, vernodalinal and vernonioside B<sub>1</sub>, were present. Vernonioside B<sub>1</sub> was found to occur at significant levels in both the leaves and pith, but the cytotoxic vernodalinal was found only in the leaves. This suggests that vernonioside B<sub>1</sub> and its naturally occurring aglycones are likely to be the bioactive constituents ingested by chimpanzees. The estimated amount of vernonioside B<sub>1</sub> ingested by this female was found to be approximately equal to the amount contained in a traditional Tongwe medicinal preparation from a cold water extract of the leaves to treat similar gastrointestinal disorders in adult human patients. This report provides new evidence for the effectiveness of medicinal plant use in primates and strongly supports the current hypothesis regarding the use of *V. amygdalina* for the control of symptoms from parasitic and gastrointestinal illness by wild chimpanzees.

**Key Words:** *Pan troglodytes schweinfurthii*; Medicinal plants; *Vernonia amygdalina* Del.; Compositae; Parasite load; *Ternidens* sp.; Phytochemistry.

## INTRODUCTION

In primates, and the chimpanzee in particular, various sources of evidence suggest that certain toxic plants are selected for their medicinal value (Huffman & Wrangham, in press).

The use of *Vernonia amygdalina* Del., by chimpanzees for its medicinal value

was first determined from the detailed observations of an ailing female's ingestion of this widely recognized African ethnomedical plant in the Mahale Mountains National Park, Tanzania (Huffman & Seifu, 1989). The symptoms displayed by this female were apparent lack of appetite, malaise, constipation and unusually dark colored urine. She meticulously removed the leaves and outer bark from several young shoots of *V. amygdalina* and chewed on the exposed piths, ingesting only the extremely bitter juice. Within 24 hours, she had regained her appetite and fully recovered from malaise and constipation (Huffman & Seifu, 1989).

At Mahale, there is a trend for greater chimpanzee use of *V. amygdalina* during the rainy season despite year round availability (Huffman et al., 1990). During the rainy season, the number of chimpanzees with detectable parasitic infections also tends to increase, as does the number of different parasite species per individual (Huffman et al., 1990, in prep.).

Based on the female's symptoms and the ongoing investigation of parasitic infection in Mahale M group chimpanzees, it was hypothesized that the use of this plant by chimpanzees aid in the relieve gastrointestinal illness or parasite-related disease (Huffman et al., 1990, 1992).

Subsequent quantitative analysis and assays of the biological activity of *V. amygdalina* specimens (pith, bark, leaf, root) collected from Mahale have revealed the presence of two major classes of bioactive compounds in the plant: the sesquiterpene lactones and the steroid glucosides (Ohigashi et al., 1991a; Jisaka et al., 1992a, in press). The most abundant of these constituents, the sesquiterpene lactone vernodalin, and the steroid glucoside vernonioside B<sub>1</sub>, have been shown to possess antibiotic, anti-parasitic, anti-amoebic, and anti-tumor properties (Asaka et al., 1977; Gasquet et al., 1985; Jisaka et al., 1992 a, b; Jisaka et al., 1993; Kupchan et al., 1969). Recently, *in vitro* anti-schistosomal (*Schistosoma japonicum*) activity tests have shown vernodalin, vernonioside B<sub>1</sub> and its naturally occurring aglycones to be the most bioactive constituents isolated from this species to date (Jisaka et al., 1992b). *In vivo* tests of vernodalin on schistosome-infected mice showed it to be lethal to the parasite at more than 5 mg. (120 mg./kg), but a lower dose (2.5 mg.) had no great effect on the parasite (Jisaka et al., 1992b). While similar *in vivo* tests on vernonioside B<sub>1</sub> are still being conducted, previous *in vitro* tests have failed to find any significant cytotoxicity in this compound, which suggests that vernonioside B<sub>1</sub>'s biological activity is of a different nature than that of vernodalin (Takaoka, pers. comm.).

Preliminary quantitative analyses of a specimen collected at Mahale showed that both vernodalin and vernonioside B<sub>1</sub> occurred in the leaves at significantly high levels (2.18 mg and 0.61 mg/g fresh leaves, respectively). However, while vernonioside B<sub>1</sub> occurred at comparably high levels in the pith (0.75 mg/g fresh pith) vernodalin occurred at levels low enough to be considered insignificant (0.02 mg/g fresh pith) (Jisaka et al., 1992b). It was then suggested that a key to understanding why chimpanzees select the pith rather than the leaves of *V. amygdalina* may lie in the *avoidance* of the highly toxic vernodalin in favor of the equally bioactive, less host-toxic vernonioside B<sub>1</sub> (Jisaka et al., 1992b).

To date, no direct evidence from fecal samples or plant material collected concurrently with observations of the use by chimpanzees of *V. amygdalina*, or any other

medicinal plant, have been available to test these hypotheses. In this paper, behavioral, parasitological, and phytochemical data collected during and subsequent to a recent observation at Mahale of another apparently ill adult female chimpanzee's use of *V. amygdalina* are presented. These data are discussed in relation to the above hypotheses concerning the possible use of the plant by chimpanzees for the control of parasite and/or other gastrointestinal illness.

## MATERIAL AND METHODS

Observations were conducted on the M group of chimpanzees in the Mahale Mountains National Park, Tanzania, between October 1991 and January 1992.

Situated on the eastern shore of Lake Tanganyika, the study area's climate is influenced by weather from the lake and the mountainous terrain, which ranges from 772 m to 2,000 m above sea level. Chimpanzees are supported mainly by the semi-deciduous gallery forests between 780–1,300 m above sea level. The year is divided into two distinct seasons. The rainy season lasts from around mid-October to mid-May, during which rain falls an annual average of 1,800 mm (Nishida, 1990; Takasaki et al., 1990). For further description of the study group and site, see Nishida (1990).

In order to begin the systematic study of medicinal plant use in chimpanzees and to test hypotheses developing from this research, a multi-disciplinary research group called The C.H.I.M.P.P. Group (The Chemo-ethology of Hominoid Interactions with Medicinal Plants and Parasites) has been established and includes specialists in ethology, natural plant products, chemistry, parasitology, pharmacognosy, pharmacology, and traditional medicine (Huffman et al., 1992).

Focal-animal observations were conducted by M.A.H. with the assistance of M.S.K., following focal individuals for as long as possible, recording all social interactions, basic activity patterns, and feeding behavior. Visible cues to an individual's state of health were noted, specifically, stool type, urine color, sinus and respiratory congestion, etc.

During these observations, infrequently used chimpanzee "plant food items" observed to be ingested in a peculiar manner (e.g. for no apparent nutritional gain) or under unusual circumstances (e.g. associated with sickness) were collected. Most plant species utilized were identifiable by the local Kitongwe/Kiswahili vernacular and Latin names (Nishida & Uehara, 1983). Identification of some unknown species were made by G. Mwachala of the East African Herbarium, National Museums of Kenya.

The presence of possible phytochemical activity or ethnomedical use were investigated by cross-referencing species with the medicinal and poisonous plant literature of Africa (e.g. Watt & Breyer-Brandwijk, 1962; Kokwaro, 1976; Burkhill, 1985; Abbiw, 1990). When available, details of local Tongwe ethnomedical use was provided by M.S.K. and two other knowledgeable local informants, R. Nyundo and R. Kasakampe. Promising species were collected in bulk, dried thoroughly, and brought back to Kyoto University for a preliminary screening test of possible biological activity (Ohigashi et al., 1991b, 1993).

During focal-animal and *ad libitum* observations, fecal samples, when available, were collected immediately after discharge and stored individually in 5.0 ml Corning sterile cryogenic vials. At camp, the vials and contents were weighed and 1 gram fecal samples were fixed with a 10% formalin solution. The contents were thoroughly mixed in the vials before being sealed and stored in a cool dark room until transport to the laboratory, where they were examined by S.G. within 6 months after collection.

Each sample was microscopically checked for the presence of parasite eggs, and when present, species or genus level identification was made. Parasite loads were also measured using the McMaster's technique (expressed as eggs/g feces). Egg counts for each fecal sample were carried out three times and the parasite load for each sample was calculated as the mean value derived from those trials.

During the study, as part of an investigation on the chemical ecology and pharmacology of *V. amygdalina*, fresh samples from young shoots of this species (the part utilized most frequently by chimpanzees) were collected by M.A.H. every two weeks from three specific trees. On each sample day, one young shoot from each tree was collected and divided into three parts: young distal leaf, young branch (pith included), and older branch sections (pith included). Two, 2 gram samples of each part were placed separately into small glass specimen bottles, one containing methanol (MeOH) and the other acetone (Me<sub>2</sub>CO), for preservation and later extraction and quantitative analysis of vernodalin and vernonioside B<sub>1</sub> in the laboratory. The samples were then stored in a cool dark place.

In this paper, plant materials collected during the first and last sample periods (October 27 and December 22) were used to assess possible seasonal differences in the relative abundance of the major bioactive compounds. These samples were used to represent the end of the dry season and mid-rainy season states of this species in the study area.

In the laboratory, quantitative analysis of the two most prevalent bioactive constituents, vernodalin and vernonioside B<sub>1</sub>, were conducted by D.I. After Jisaka et al. (1992b), the Me<sub>2</sub>CO extract was used for the analysis of vernodalin and the MeOH extract for that of vernonioside B<sub>1</sub>. Each extract was concentrated to 10 ml, and 4 ml of this solution was poured onto Cosmosil 14OC<sub>18</sub>-OPN gel (Nacalai Tesque, approximately 1g). After the excess solvent was removed *in vacuo*, the gel was transferred into a syringe (7 cm × 0.8 cm i.d.) equipped with a Sep-Pak C<sub>18</sub> cartridge (Waters) at the outlet side. The components absorbed on the Cosmosil gel were successively eluted with 10 ml of water, 10 ml of 90% acetonitril (CH<sub>3</sub>CN), and 100% CH<sub>3</sub>CN under syringe pressure. The 90% CH<sub>3</sub>CN eluate was concentrated, then redissolved with 1 ml of CH<sub>3</sub>CN, and filtered with an H-13-5 filter (TOSOH) and again filled up to 2 ml with CH<sub>3</sub>CN. Five μl of this solution was analyzed for vernodalin using HPLC [AQ-301, Column; ODS, 4.6 × 100 mm (YMC), CH<sub>3</sub>CN-H<sub>2</sub>O (25 : 75), 1 ml/min.] detected by the absorption intensity at UV<sub>220</sub>. By the same procedure, a 90% aq. MeOH eluate was obtained from a sample of the MeOH extract and analyzed by HPLC [CH<sub>3</sub>CN-H<sub>2</sub>O (33 : 67)] detected for vernonioside B<sub>1</sub> by the absorption intensity at UV<sub>254</sub> (Jisaka et al., 1992b). This procedure was repeated for a portion of shoot from the same plant ingested by the adult female reported here.

*V. amygdalina* seeds collected at Mahale were germinated and raised in the experimental green house of the Department of Agriculture, Kyoto University and in the home of K.K. Samples were obtained from these plants to determine average fresh weights of plant parts and for analytical comparison of the occurrence of vernodalin and vernonioside B<sub>1</sub> in fresh (mg/g sample f.W.) versus methanol and acetone extract states ( $\mu\text{g}/\text{mg}$  extract). There was no strong tendency for amounts of these compounds to vary according to state, and, therefore, for the following analyses, all measurements taken from extraction or fresh samples are expressed in the common unit mg/g fresh weight.

## RESULTS

### I. Behavioral Observations

On December 23–24, 1991, an adult female, FT (Fatuma: born circa 1963), was observed for a total of 5 hr. 4 min. The observations were split into two periods as follows: day one (14:37–18:15; 208 min.) and day two (09:32–11:47; 96 min.).

On day one at approximately 14:17, FT was observed by field assistant H. Bunengwa and researcher H. Yoshida to ingest the juice and some fibrous material from the piths of two shoots of *V. amygdalina* (Bunengwa & Yoshida pers. comm.). At approximately 14:33, Bunengwa made verbal contact with M.A.H. and M.S.K., and directed us to the plant used by FT. Two shoots were discarded, and there were distal young strips of bark and leaves left intact and the unchewed proximal portions of the shoot. These two shoots were each approximately 1 cm in diameter, 30 cm in length. A shoot of similar size and maturity from the same tree was collected for further study.

At 14:37, FT was located and focal observations begin. FT and her male infant PM (Pim: born 1988. 2) were traveling with a mixed sub-group of at least 10 members.

During these observations, gastrointestinal upset was evidenced by profuse flatulence and uncontrolled, yellow, liquid stools. FT's urine was clear in color. At 15:22, 1 hr. and 5 min. after ingesting *V. amygdalina*, a fecal sample was collected.

During this day's observations, FT spent 17% of her time resting, once in an elaborately made day-bed in a tree and the rest of the time on the ground. FT's infant PM frequently wandered out of view along with older playmates who solicited him to follow. At such times, FT, apparently waiting until the last possible moment, moved to maintain minimum visible contact. With frequent pauses to rest (36%) she slowly followed PM. When the pair reunited, FT groomed or was groomed by either the infant or his play-partners, which resulted in a few minutes more rest for FT. All of FT's grooming occurred in this context and accounted for 12% of her activity. The remaining 33% of her time was spent intermittently foraging on common food items: *Aframomum* sp. stalks, *Ficus exasperata* Vahl leaves, *Saba florida* (Benth.) Bullock fruits, and a small amount of clay from a termite mound. At 18:15 observations were discontinued.

On day two, members of the previous day's sub-group were located at 08:55 and at 09:32 FT and PM were located in a dense forest thicket with the sub-group, approximately 700 m south of where they were last sighted on the previous evening.

Of the total observation time (96 min.), FT spent 65% of it resting, and 10% grooming. Between rests she spent 23% of her time foraging on *Aframomum* sp. stalks, *Ficus* sp. leaves, and a small amount of clay from a termite mound.

FT's general condition appeared to have improved from the previous day and her stools, although small, were now solid. Fecal samples were collected at 10:55. Focal observations were discontinued at 11:47 when it became impossible to follow her through rough terrain.

At 13:03, 22 hrs. 46 min. after she was first observed to ingest *V. amygdalina*, she was relocated and identified as the capturer of an adult red colobus monkey. She maintained possession and consumed much of the carcass, despite attempts by two adult males, BA and AJ, to take it from her.

## II. Parasitology

According to the analyses of four fecal samples collected from FT between October 29 and December 24, she was found to be harboring two intestinal nematode species, *Trichuris trichiura*, a *Ternidens* sp., and a protozoan commensurate *Troglodytella abrassarti*. Inspection of the two samples collected on December 23 and 24 (20 hr. 38 min. apart), after FT's ingestion of *V. amygdalina* pith, revealed a drop in the *Ternidens* sp. egg count from 130 to 15 eggs/g feces.

Table 1 compares this data for FT with changes in the level of *Ternidens* sp. infection over time with those of 7 other individuals sampled during this study period in which feces containing *Ternidens* sp. eggs occurred on more than one day sampled.

The decrease in egg count observed for FT following ingestion of the pith of *V. amygdalina* was not seen in other cases where multiple samples of the same individual (not observed to ingest this plant) were taken one to several days apart. A decrease was detected in only two (adult males NS, AJ) of the other 7 individuals, for which *Ternidens* sp. data was available (Table 1). For NS, a drop from 20 to 5 eggs/g feces was detected over a 10 day period. Such changes were not considered significant, as they were more likely due to the low level of infection and thus low density of eggs per sample. This is also considered to be the case in those samples in which no eggs were detected in individuals who had tested positive for *Ternidens* sp. at earlier or later trials.

On the other hand, for AJ, the apparently most ill and most seriously infected individual monitored during this study period, a total drop of 510 *Ternidens* sp. eggs/gram feces was detected over an 18 day period (Table 1). During focal observations AJ was not seen to use *V. amygdalina*; his steady recovery, however, may have been associated with extended feeding bouts on the young leaves of *Ficus exasperata* on several days during the period in which he appeared to be the sickest, and showed the highest levels of parasitic infection. The young leaves of this plant contain 5-methoxypsoralen, a well-known furanocoumarin. The young leaves have been estimated to possess effective nematocidal activity when consumed in

**Table 1.** Trends in level\* of *Ternidens* sp. infection in wild chimpanzees of Mahale M Group at the onset of the 1991–92 rainy season.

name	age/sex class	date	infection level (eggs/gram feces)
AJ	adult male	Nov. 1	10
		Nov. 13	15
		Nov. 28	530
		Dec. 16	170
		Dec. 25	20
BA	adult male	Nov. 4	35
		Nov. 16	—
		Nov. 22	270
		Dec. 13	—
BO	old adult female	Oct. 29	<5
		Nov. 11	20
		Nov. 15	—
FT	adult female	Oct. 29	—
		Nov. 19	—
		Dec. 23	130
		Dec. 24	15
NS	adult male	Nov. 2	—
		Nov. 4	20
		Nov. 13	5
SL	adult female	Nov. 16	<5
		Nov. 18	—
		Jan. 6	45
TB	adult male	Nov. 4	—
		Nov. 7	5
		Nov. 16	—
		Jan. 6	33
WL	adult female	Oct. 28	10
		Nov. 27	—
		Dec. 2	140

\* infection rate: eggs/g feces «McMasters technique»; eggs/g feces; —; not detected.

large quantities of 50–100 leaves (Rodriguez & Wrangham, 1993).

For other individuals sampled, it was more often the case for *Ternidens* sp. egg counts to increase over time. The average increase was 69.9 eggs/g feces (S.D. = 84, range 5–236, n = 7). This increase was highly variable between individuals, but the number of days between samples did not appear to affect the degree of measured increase (Table 1). These increases appear, rather, to reflect the frequently observed trend for an increase in parasite infection levels and the number of parasite species infections per individual during the rainy season (Huffman et al., 1990; Kawabata & Nishida, 1991; Huffman et al., in prep.).

### III. Phytochemistry

Quantitative analyses revealed a difference in the relative distribution of ver-

**Table 2.** Comparison of the relative distribution and abundance of two bioactive compounds, vernonioside B<sub>1</sub> and vernodalinal, extracted by plant part from *Vernonia amygdalina* specimens collected in the Mahale Mountains National Park.

sample	compound	young leaf	young stem mg/g fresh weigh	old stem	
FT	vernonioside B <sub>1</sub>	0.51	0.38	0.08	
	vernodalinal	0.47	0.00	0.00	
A, B, C	vernonioside B <sub>1</sub>	1.19	0.69	0.61	mean value
		(0.82)	(0.32)	(0.21)	
	vernodalinal	1.19	0.20	0.11	
		(0.47)	(0.15)	(0.10)	

A sample of the shoot used by FT was collected on December 26, and shoot samples from trees A, B, and C were collected on October 27 and December 22, 1991.

nonioside B<sub>1</sub> and vernodalinal among the test samples A, B, C, and the shoot from the plant ingested by FT (Table 2, 3). The reason for this is not known, but it can be speculated that this is due to either individual differences in plants sampled from different habitats or the longer lapse in time between collection and extraction of the FT sample as compared to that of A, B, C. However, in all samples, vernodalinal occurred at relatively high levels in the young leaf but at very low levels in the young and old stem portions. Vernonioside B<sub>1</sub>, on the other hand, occurred at equally high levels in the young leaf portion and at lower, yet still significant levels in the young and old stem portions.

Comparing the October 27 and December 22 sample sets (Table 3), there was a noticeable, general increase in the relative abundance of vernonioside B<sub>1</sub>, particularly in the leaves. On the other hand, a relative decline in the abundance of vernodalinal, especially in the young and old stems, was noted. Interestingly, vernodalinal was completely absent from the stem portion of FT's sample, strongly sug-

**Table 3.** Comparison of the relative distribution and abundance, by plant part, of two bioactive compounds vernonioside B<sub>1</sub> and vernodalinal, extracted from *Vernonia amygdalina* specimens collected on October 27 (I) and December 22 (II), 1991 in the Mahale Mountains National Park, Tanzania.

sample	young leaf	young stem mg/g fresh weight	old stem
vernonioside B <sub>1</sub>			
I. A	0.47	0.37	0.36
	0.59	0.60	0.39
	0.58	0.53	0.57
II. A	1.76	0.66	0.64
	2.54	1.32	0.93
	1.18	0.66	0.75
vernodalinal			
I. A	1.82	0.51	0.06
	2.77	0.16	0.31
	1.91	0.17	0.06
II. A	1.47	0.15	0.06
	1.54	0.07	0.03
	1.97	0.16	0.14



gesting vernonioside B<sub>1</sub> to be the most likely of the two compounds responsible for the observed effect on parasite load.

From a pharmacological standpoint, it would be valuable to compare the amount of active ingredients contained in a 'standard dose' taken by humans and that contained in the pith ingested by FT. Because neither the chimpanzees nor the Tongwe 'prepare' their medicine from methanol or acetone plant extracts, the rough estimation which follows is based on the amounts yielded by extraction with water.

One traditional Tongwe use of *V. amygdalina* in the treatment of parasitosis or gastrointestinal upset is the preparation of a cold water extract using 2–3 crushed leaves (approximately 10–15 g f.w.) in 300–400 ml of water. An analysis replicating this traditional method using fresh *V. amygdalina* leaves (3 trials) yielded 3.3–5.0 mg of vernonioside B<sub>1</sub>. Based on the data in Table 2, the pith used by FT (60 cm, approximately 50–100 g f.w.; see methods) was estimated to have yielded roughly 3.8–7.6 mg vernonioside B<sub>1</sub> (cold water extraction yielded approximately 20% that of methanol extraction, Izutsu, 1993), or roughly an amount equal to that of the normal dosage prescribed to an adult Tongwe patient. In the future, a more detailed analysis will be necessary to make closer comparison.

## DISCUSSION

Previous studies have discussed a variety of behavioral adaptations for combating parasitosis and other unspecified diseases in primates. These behaviors include the alternation of sleeping sites and feeding sites (Freeland, 1980; Hausfater & Meade, 1982; McGrew et al., 1989) and the ingestion of certain plants, possibly for their pharmacological effect (Hamilton et al., 1978; Wrangham & Nishida, 1983; Phillips-Conroy, 1986; Takasaki & Hunt 1987; Huffman & Seifu, 1989; Huffman & Wrangham, in press).

The latter studies cite a variety of supporting evidence, such as ethnomedical and pharmacological information on bioactive compounds in a plant, its use restricted to certain habitats or seasons where risk of parasitic infection is higher than in other habitats or seasons, and observations of its use by ill individuals.

For example, Phillips-Conroy (1986) reported that *Papio* spp. in Ethiopia living in areas of high susceptibility to the contraction of schistosomiasis ingest the berries of *Balanites aegyptiaca* (L.) Delile, whereas the *Papio* spp. in non-risk areas do not. Based on these observations, she hypothesized that the baboons selected these berries for their medicinal purposes, and identified a steroidal saponin, diosgenin, as the likely agent responsible. Phillips-Conroy & Knopf (1986) tested this hypothesis by feeding diosgenin to schistosome-infected mice. However, instead of decreasing the infection, they found that the diosgenin-altered hormonal environment of the mice actually augmented their vulnerability to the disease, resulting in significantly increased levels of infection.

Another well-investigated example is that of 'leaf swallowing behavior' in chimpanzees which was first reported in detail by Wrangham (1975, 1977) and then by Wrangham and Nishida (1983) from the perspective of its possible non-nutritive

value. The rough surfaced leaves of *Aspilia mossambicensis* (Oliv.), *A. plurisetata* (O. Hoffm.) Wild, and *A. rudis* Oliv. & Hiern are usually selected one at a time and placed into the mouth, whereupon they are not chewed but swallowed whole. In addition to this peculiar method of ingestion, the presence of a powerful bioactive compound, the dithiane polyine thiarubrine A, in the leaves of *A. mossambicensis* and *A. plurisetata* was suggested to be of possibly anti-helminthic value to chimpanzees (Rodriguez et al., 1985; Wrangham & Goodall, 1989). However, Page et al. (1992) was able to confirm the presence of thiarubrine A only in the roots, not in the leaves, of these plants collected from both Mahale and Gombe. Page et al. (1992) succeeded, instead, in isolating two bioactive compounds, kaurenoic and grandifloreonic acid from the leaves of *A. mossambicensis* at Mahale. The anti-parasitic activity of these compounds are unknown.

Where previous studies have been unsuccessful, the present study provides three new types of key evidence, which are as follows: verification of the presence of a bioactive constituent(s) from a sample of the individual plant actually ingested, a clinically measured biological effect (decrease in parasite load) after ingestion, and a quantitative chemical comparison of the amount of active constituent(s) ingested by the study subject with a prescribed dose known to be effective in relieving symptoms in humans.

The observed decrease in the number of *Ternidens* eggs/g of FT's feces over the 20 hour period differed markedly from the other samples collected during this study. These results provide further support for the hypothesis that the consumption of *V. amygdalina* by chimpanzees aids in the relief of gastrointestinal or parasite-related disease (Huffman et al., 1990, 1992).

We do not rule out the possibility that factors other than the detected *Ternidens* sp. infection could have also affected FT's observed condition. However, infection by *Ternidens* can result in ulceration and cystic nodules on the walls of the large bowel where this parasite is found in the host (Beaver et al., 1984), resulting in overall gastrointestinal discomfort. FT's symptoms were consistent with this type of disease. These results do suggest that the ingestion of the bitter juices within the pith of *V. amygdalina*, which are known to contain active anti-parasitic properties *in vitro*, may also have some similar effect in chimpanzees.

Due to the unlikely event of complete eradication of parasites from the host, it is proposed that as a result of the ingestion of such plants, chimpanzees are in effect relieving the symptoms of illness by controlling the number of parasites carried, keeping parasitic infection and its effects on the health of the host to a minimum. Continued monitoring of the behavior and parasite levels of individuals with histories of *V. amygdalina* use over one season is one possible way of testing this hypothesis.

Although preliminary, these observations are in agreement with a previous report of another apparently ill adult female chimpanzee's use of *V. amygdalina* at Mahala (Huffman & Seifu, 1989). In both cases, a visible improvement in the condition of health (e.g. appetite, physical strength, urine and/or stool quality) was apparent within 20–24 hours after observed ingestion of the plant. This recovery time is similar to that recognized for local human inhabitants, the Tongwe, who use a cold water extract prepared from leaves of the same species for 'parasitosis'

or gastrointestinal upset (Huffman & Seifu, 1989).

While it was estimated that FT ingested an amount almost equal to that of the normal dosage prescribe to a Tongwe patient, the estimated figure may be slightly inflated because the above calculation is based on yield by extraction in water, not by chewing. Nonetheless, by this preliminary comparison, it becomes apparent that the amount of vernonioside B<sub>1</sub> ingested by FT is comparable to the effective dose traditionally prescribed for humans.

Investigations to determine the overall biological activity and possible synergistic effect of the primary aglycones of vernonioside B<sub>1</sub>, found to occur in the pith, are now underway. Using a number of parasite species known to infect chimpanzees and humans as models, this information will provide a better understanding of the pith's pharmacological effect for chimpanzees, and will hopefully contribute to the ongoing search for readily available, natural plant-based anti-parasitic agents useful to humans living in tropical regions of the world.

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