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Total tannin content of foods of François' Langur in Fusui, Guangxi, China: Preliminary study

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ABSTRACT

The most valuable plant organs are often particularly rich in essential elements, but also very well defended. The mechanisms through which herbivores obtain essential elements from these organs and avoid being hurt by defensive compounds have long been debated. In order to study the total tannin of the food plants and its potential influence on food selection of François' Langur (*Trachypithecus francoisi*), we studied the feeding behavior of a group of François' Langurs (n = 4) in Fusui Reserve between January and December 2006 via Focal Animal Sampling. We collected food samples confirmed to be used by langurs each month. We measured the total tannins (TT) of these samples using the Folin–Denis method. We compared the TT of the foods across plant parts and among plant species. The food mainly consisted of immature leaves, accounting for 58.5% of the annual diet. The remaining parts were mature leaves (12.1%), fruits (12.1%), seeds (10.6%), flowers (4.3%) and barks (2.4%). The food included trees, accounting for 44.0% of their annual diet, followed by shrubs 22.8%, lianas 19.6% and herbs 13.6%. The average TT found in food was 60.96 mg/g TAE ranging from 25.57 mg/g TAE (September) to 97.89 mg/g TAE (March). The monthly average TT did not vary significantly among months (p = 0.36). Despite the lack of information concerning the relationship between food selection and TT for this langur, the data, however indicate that they can tolerate food with high TT.

1. Introduction

Plant defensive compounds are widespread in plant organs, being particularly higher in those organs with the highest amounts of essential elements [1]. These defensive compounds can render plant parts from unpalatable to toxic in order to prevent consumption by herbivores [2]. Concentrations of defensive compounds vary considerably between [3] and within tissues [4]. One of the best known plant defensive compounds is tannin, the high molecular polymeric phenolics produced by secondary plant metabolism [5]. Tannins are thought to affect the physiology of herbivores in various ways. Tannins can bind with plant proteins in the herbivore's gut inhibiting the digestion of proteins [5], or be toxic to gastrointestinal microorganisms [6]. Furthermore, tannins can have an inhibitory effect due to the reduction of enzyme activity [7]. Thirdly, tannins result in a dysfunction of cellular membranes and deprivation of substrate metal ions and minerals [8] and an inhibition on the absorption of nutrients from the colon [9]. Further, in overdose amounts tannins are known to cause severe gastroenteritis and abdominal pain [9].

The influence of the tannins on primate food selection has been considered for decades [10–13]. Some researchers argue that tannins may have negative effects on primates, and thus may be avoided as food sources [5,7,10,14,15]. While other studies show no obvious preference or avoidance to tannins by other primates [16–18]. Many primates, especially folivore monkeys, consume high amounts of tannin-rich leaves without being hurt [8]. Furthermore, increasing evidence suggests that some tannins may be required components of the mammalian herbivore diet [19,20]. It is found that tannins sometimes have beneficial effects for herbivores by decreasing bloat (a foaming of digesta in the forestomach) and binding to, precipitating, and detoxifying alkaloids [21]. Thus the role tannins play on primate food selection is far from generalization.

François' Langur (*Trachypithecus francoisi*) is an endangered primate species [22] endemic to limestone forests of southwest China and north Vietnam [23,24]). Much attention has been paid on the feeding ecology of the langur, and considerable information has been cumulated since the year 2000 [25–39]. The studies can be roughly classified into two categories. The first category is the food habit of the langur. For example, a study conducted in 2003 in a fragmented habitat in Fusui showed that François' Langurs were selective

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feeders, they use 37 food species, ten food species account for 90% of the total feeding time [25]; another study conducted in 2006 in the same site showed that the langurs used 61 species [26]. This may be related to sampling method and duration. In relative larger habitats, such as Nonggang Nature Reserve [27,28] and Mayanghe Nature Reserve [29,30], langurs also are selective feeders, but they seem to feed on more species and relied less on leaves than on smaller fragment. Spatial variation in feeding ecology is evident. The second category is the food selection and the chemical composition. Due to the difficulties, Li et al. collected 40 food species sampling out of 61 food species, and found that crude protein of the 40 species was not the factor influencing food selection in a year round in Fusui, Guangxi, China [31] and in winter in Mayanghe Nature Reserve, Guizhou, China [32]. The other chemical composition crude fiber is proofed to influence food choice of the langur in captivity [33]. The influence of water on the food selection of langurs seems to be site-dependent. In Fusui, Guangxi, water is not the factor influencing food selection [34]; while in Nonggang Nature Reserve, Guangxi, water is one of the factors influencing food selection of the langur [35].

As stated above, the influence of secondary compound on primate selection is complicated. Langurs are endemic to limestone landscape, which is a special type of habitat characterized by thin, highly alkaline, sandy and dry soils low in mineral nutrients [36]. The

Table 1
Average total tannin (TT) content of plant species and plant parts consumed by François' langur group in Fusui County, China, during January–December 2006 (TT was expressed at mg/g dry weight tannic acid equivalents).

Family	Species	Growth form	Food type	Mean food part TT	Sample size	Food part TT range
Ulmaceae	*Celtis tetrandra*	Tree	Deciduous	M = 26.8	N = 7	17.8–35.9
				IM = 22.3	N = 7	9.3–65.4
Moraceae	*Ficus auriculata*	Tree	Evergreen	IM = 29.6	N = 4	7.8–54.5
				F = 27.0	N = 4	7.2–36.8
Lauraceae	*Litsea glutinosa*	Shrub	Deciduous	IM = 25.6	N = 8	1.6–57.9
				M = 26.8	N = 6	11.0–41.9
				FL = 32.2	N = 2	29.1–35.4
Asclepiadaceae	*Secamone sinica*	Liana	Evergreen	IM = 101.1	N = 1	
				M = 46.5	N = 6	11.5–117.3
				S = 6.6	N = 6	9.5–5.2
Menispermaceae	*Diploclisia glaucescens*	Liana	Evergreen	IM = 84.9	N = 3	55.7–107.7
Moraceae	*Cudrania cochnchinensis*	Shub	Deciduous	M = 44.5	N = 3	37.3–52.3
				IM = 82.4	N = 8	55.0–95.0
Euphorbiaceae	*Mallotus repandus*	Liana	Deciduous	IM = 86.5	N = 4	29.2–130.1
Moraceae	*Ficus virens*	Tree	Evergreen	M = 56.9	N = 1	
Moraceae	*Ficus tinctoria*	Tree	Evergree	F = 38.9	N = 6	17.6–97.1
Poaceae	*Moncladus saxatilis*	Herb	Evergreen	S = 22.4	N = 2	20.5–24.3
				Fl =19.8	N = 6	5.8–50.3
Papilionaceae	*Lespedeza bicolor*	Shrub	Evergreen	IM = 50. 6	N = 4	30.8–71.6
Bignoniaceae	*Stereospermum colais*	Shrub	Deciduous	IM = 102.5	N = 4	16.2–114.6
Sabiaceae	*Sabia japonica*	Liana	Evergreen	IM = 43.0	N = 7	8.7–98.5
				M = 15.9	N = 6	11.3–14.4
Gesneriaceae	*Chirita ophipogoides*	Herb	Evergreen	IM = 66.7	N = 7	15.7–119.5
				Bark = 78.8	N = 3	65.0–108.2
Euphorbiaceae	*Breynia vitisidaea*	Shrub	Evergreen	IM = 126.7	N = 1	
				F = 95.6	N = 4	94.6–96.6
Asclepiadaceae	*Gymnema sylvestre*	Liana	Evergreen	IM = 46.0	N = 6	4.1–102.0
Moraceae	*Ficus coninna*	Tree	Evergreen	F = 28.5	N = 3	26.0–32.4
Menispermaceae	*Pericampylus glaucus*	Liana	Evergreen	IM = 26.8	N = 3	21.0–38.0
Pittosporaceae	*Pittosporum glabratum*	Shrub	Evergreen	M = 28.8	N = 4	18.9–45.1
				IM = 36.9	N = 3	19.1–45.9
				Fl = 33.2	N = 2	24.5–41.8
				F = 54.2	N = 1	
Annonaceae	*Desmos chinensis*	Liana	Evergreen	IM = 68.0	N = 2	61.7–74.3
				F = 22.4	N = 3	20.1–24.5
Urticaceae	*Oreocnide frutescens*	Shrub	Deciduous	IM = 14.5	N = 3	11.3–16.4
Papilionaceae	*Derris fordii*	Liana	Evergreen	IM = 97.3	N = 3	54.2–113.6
Convolvulaceae	*Cuscuta chinensis*	Liana	Evergreen	IM = 73.5	N = 5	30.9–149.8
Euphorbiaceae	*Phyllanthus embalia*	Shrub	Deciduous	IM = 102.2	N = 2	98.2–106.2
Ebenaceae	*Diospyros dumetorum*	Shrub	Deciduous	IM = 77.8	N = 2	29.2–126.5
Moraceae	*Ficus harmandii*	Tree	Evergreen	IM = 17.5	N = 4	5.8–37.4
				F = 33.9	N = 8	9.6–66.5
Rodaceae	*Pyrus calleryana*	Shrub	Deciduous	IM = 68.2	N = 2	59.2–77.1
Sapindaceae	*Boniodendron minus*	Shrub	Deciduous	IM = 122.1	N = 2	104.1–140.1
Myrtaceae	*Syzygium cumini*	Tree	Evergreen	IM = 144.5	N = 1	
Palmaceae	*Guihaia argyrata*	Herb	Evergreen	IM = 4.2	N = 1	
Dioscoreaceae	*Aristolochia kwangsiensis*	Liana	Deciduous	IM = 24.8	N = 2	21.2–28.5
Moraceae	*Morus alba*	Shrub	Deciduous	IM = 22.1	N = 1	
Celastraceae	*Euonymus dielsiana*	Liana	Evergreen	IM = 26.3	N = 3	16.6–40.9
Ranunculaceae	*Clematis loureiriana*	Liana	Evergreen	M = 20.8	N = 1	
Anacardiaceae	*Pistacia weinmannifolia*	Tree	Evergreen	IM = 120.0	N = 5	49.5–128.4
				F = 140.8	N = 2	125.7–185.9
Smilacaceae	*Smilax china*	Liana	Evergreen	IM = 14.5	N = 1	
Moraceae	*Broussonetia papyrifera*	Tree	Deciduous	IM = 30.0	N = 1	
Bignoniaceae	*Oroxylum indicum*	Shrub	Deciduous	IM = 65.4	N = 1	
Icacinaceae	*Apodytes cambodiana*	Shrub	Deciduous	Fl = 34.5	N = 4	31.4–37.6
Total					201	

Key, food part: IM: immature leaves; M: mature leaves; F: fruits; Fl: flowers; B: barks.

vegetation type of such habitats is growth-limited and has therefore been found to be rich in defensive compounds [40]. François' Langur exclusively feeds on limestone plant parts in natural habitats [27,37]. The study of the influence of tannins on the food selection of the langur, therefore, is interesting. Hitherto there is a lack of an integrated study on the content of defensive compounds of François' Langurs' food plants. In this study, we (1) quantify the total tannins (TT) of François' Langurs' food plants and (2) utilize this information to analyze whether they avoid the plant species and plant parts with higher TT or not.

2. Materials and methods

2.1. Study site and study objective

We conducted the study in a fragmented habitat of Francois' Langur of ca 25.7 ha (107°50′E, 22°45′N) in Fusui County, China. Climatically the study site belongs to subtropical monsoon climate, which can be easily divided into rainy season (May–September) and dry season (October–April) [37]. The fragment is a typical karst, which has six steep cliffs and five caves, the langur group can use these caves as sleeping sites at night [37,41]. The highest peak is 268 m a.s.l. The relative altitudes of the peaks are less than 110 m.

During the study period, the average temperature measured by portable thermometer (RC-T601A) was 24.8 °C, the monthly average temperature ranged from 15.2 °C to 25.5 °C and the daily temperature from 3.8 °C to 40.3 °C. Vegetation is classified as secondary broadleaf forest with dominant trees belonging to Euphorbiaceae, Lauraceae, Annonaceae and Meliaceae families [37]. Due to frequent selective deforestation, the vegetation is sparse and few trees have a diameter at breast height (DBH) of over 30 cm [41].

The focal François' Langur group consisted of 4 adults (3 females and 1 male). Due to frequent disturbance by farmers and researchers, the langur group can only tolerate a distance of about 30 m [26]. The animals were well identified by researchers based on age and sex.

2.2. Behavioral observations

We collected behavioral data between January and December 2006 via Focal Animal Sampling [42,43]. Each month we observed the langur group from dawn to dusk, when langurs entered into a night-staying cave. During the observation, we randomly selected a langur as focal animal and observed for 5 minutes followed by 10 minutes of inactive time. We tried to avoid sampling the same individual in the next sample interval. Feeding on a particular food source was considered to start when the animal put the food into its mouth and considered to stop when 20 s had passed without moving within the feeding site, or when the focal animal left the feeding tree, or when the focal animal started eating another item. At this point we designated this to be a completed feeding record. Once the langur fed, we recorded the plant species and the plant part consumed, the feeding duration, as well as the life form of food. Occasionally, we used a telescope (Nikon Fieldscope ED82, 25-75X Zoom, Japan) to improve field observations. Once the langurs fed, we recorded the species and the food parts, as well as food growth forms. Occasionally, due to lack of visibility when langurs were present in clusters around shrubs or lianas, we kept the feeding records even when species identification of food items was not possible. Following Huang [44] we described food parts as mature leaves, immature leaves, fruits, flowers, barks, seeds. Growth forms included trees, shrubs, lianas and herbs. Lianas and herbs were easily identified. Trees and shrubs were distinguished by their height and a readily recognizable stem. Plants with a height ≥5 m and an obvious stem were considered to be trees; otherwise they were recorded as shrubs. If the plant species being fed upon could not be identified simply by observation, a specimen was taken for latter identification in the laboratory. The plant taxonomy follows Flora of Guangxi [45].

We spent 93 days and 1097 h hours observing animals and collected 1682 feeding records. Monthly observations ranged from 5 to 12 days in duration.

2.3. Plant sample collection

The most accurate assessment of nutrient intake was obtained by collecting plant material from the specific trees consumed by animals [46]. Each month once a new food part of a species was confirmed to be fed by an animal, we collected the parts when the langurs were away from the feeding site. We collected the same food part of a specific species only once even if the part was repeatedly consumed in the following observation days. Tree climbing was mostly unnecessary due to the relatively short nature of the considered plant. When plants used by langurs were located in steep cliffs and hence collection proved difficult, we collected the samples from several trees of the species available in the habitat to provide an approximate estimate of chemical content [46]. In these cases, plants of similar habitat were favored. Additionally, many species were not readily accessible for collection, for example, some species, such as *Dracontomelon duperreanum* (Anacardiaceae) and *Cymbidium ensifolium* (Orchidaceae) were only found in steep cliffs in the habitat. Likewise some rare and very small shrubs were only consumed in specific months of the year when it sprout. In occasions, albeit the accessibility of feeding plants insufficient quantities were available for analysis. Therefore 201 samples from 39 species were analyzed in this study, which included 111 samples of immature leaves, 34 of mature leaves, 31 of fruits, 14 of flowers, 8 of seeds and 3 of barks. Samples included 19 samples of herbs, 67 of shrubs, 53 of trees and 62 of lianas in terms of growth forms. After collection, following Waterman et al. [7], we dried off the samples in the sun and subsequently stored in labeled and sealed plastic bags. Upon completion of monthly field observations, we took the samples to laboratory, in which we dried the samples to a constant weight in an oven (101-3-BS-II) at 50 °C. Then we ground the dried samples to powder in an electric power mill (FW-100) and sifted the powder through a 1 mm wire screen, and subsequently we stored the sifted samples in a refrigerator (4 °C) for posterior analysis.

2.4. Measure of TT content

We analyzed the samples in the period from January to March 2007. We used tannic acid as a standard for TT content. The tannins were extracted using 50% (aqueous) methanol [47,48], and chemical procedures followed Dudt and Shure [49]. We used the Folin–Denis method to evaluate the content of TT. All assays were run in triplicate, and results were expressed in mg/g dry weight tannic acid equivalents (mg/g TAE).

2.5. Data analysis

Foraging times for each food species were calculated by summing up all feeding duration across individuals and expressing these as percentages of total foraging time each day, then we averaged daily proportion across days for a month to denote monthly foraging times. Monthly means were further averaged to calculate annual foraging times. Similarly we calculated the proportion of different plant parts and life forms on monthly and annual bases.

We calculated the TT of langur diets via the average TT for all samples of a single species and plant part.

We analyzed TT at three levels, monthly and annual food part TT, species TT and growth form TT. We calculated monthly food part TT by averaging the TT of the same food part across different species for each month, and subsequently averaging across the months to

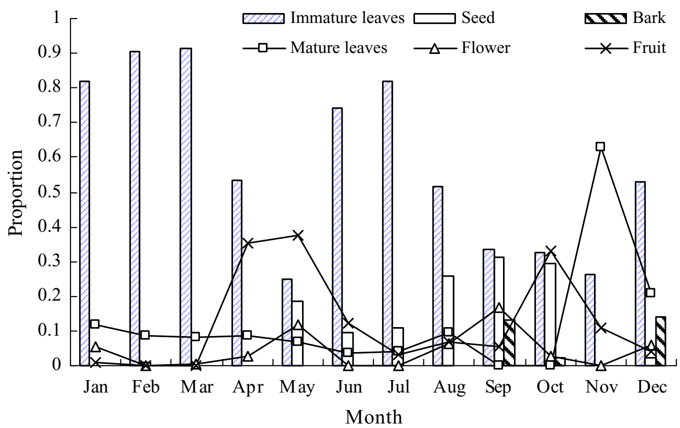

Fig. 1. Monthly variation of time langurs spent on different plant parts in Fusui, China during 2006.

calculate annual food part TT. Analogously, we calculated monthly and annual TT of growth forms. We calculated the monthly TT of a species by averaging the TT of all food parts within a species for each month, and then averaged across all the species. We averaged monthly species TT to denote the annual TT of species.

We used the Chi-square Test to examine the difference of monthly TT of species, food parts and growth forms. Additionally, we ran a Mann–Whitney test to examine the difference between food TT of the first half year and the second half year, TT for the fifteen most preferred food parts and those for the fifteen least preferred food parts. All tests were performed by software SPSS 15.0 (SPSS Inc.). Two-tailed tests of significance were used throughout and significance level was set at 0.05.

3. Results

3.1. Francois' langur diet and its seasonal variation

Food parts consumed by Francois' Langur included immature leaves, mature leaves, fruits, seeds, bark and flowers. Immature leaves were the largest dietary component, accounting for 58.5% of the total feeding time, followed by mature leaves (12.1%), fruits (12.1%), seeds (10.6%), flowers (4.3%) and barks (2.4%). Fruits mainly originated from two species, *Ficus tinctoria* (Moraceae) and *Pistacia weinmannifolia* (Anacardiaceae). Seeds consumed were from only one species, *Moncladus saxatilis* (Poaceae), the seed of which is very small. Only the bark of *Chirita ophipogoides* (Gesneriaceae) was consumed during very dry months (October and December).

There were evident monthly variations in food feeding time on various plant parts (Fig. 1). Immature leaves were dominant food parts in most of the months, ranging from 91.3% in March to 25.1% in May. Mature leaves were the second dominant food in most of the months, it had a maximal value of 63.0% in November, and langurs were not found to use mature leaves in September and October. Seeds and bark were observed to be used in only some months.

Growth forms of food species encompassed trees, shrubs, lianas and herbs. Trees provided the main food resource for individuals, accounting for 44.0% of langurs' feeding record, followed by shrubs (22.8%) and lianas (19.6%). Herbs provided the smallest contribution toward the langurs' diet, accounting for 13.6% of the feeding record.

There were evident seasonal variation in food feeding time on different life forms (Fig. 2), for example, feeding time spent on trees ranged from 75.9% in July to 19.8% in September. Herbs in some months were not found to be used by langurs.

3.2. Monthly average TT of the food species

The average TT of all food species was 60.96 mg/g TAE, ranging from 97.89 mg/g TAE in March to 25.57 mg/g TAE in September (Fig. 3). Monthly average TT did not vary significantly across months (^{2} = 53.19, df = 11, p = 0.36) or between the first half year and the second half year (z = –1.22, p = 0.22). The TT for the fifteen most preferred food species was not significantly different from that of the fifteen least preferred food parts (z = –1.29, p = 0.22).

3.3. TT content of food parts

The fruit of *P. weinmannifolia* had the highest value of TT, 185.9 mg/g TAE; whereas the immature leaves of *Litsea glutinosa* had the lowest value of 1.6 mg/g TAE (Table 1). TT varied among the different life stages and across the different plant parts within a species. For example, in *L. glutinosa* TT of immature leaves was 25.45 mg/g TAE in February and 49.16 mg/g TAE in March; the TT of mature leaves of the same species in April was 11.0 mg/g TAE and that of the species' flowers in the same month was 35.4 mg/g TAE. Though not quantified, samples that appeared to have higher

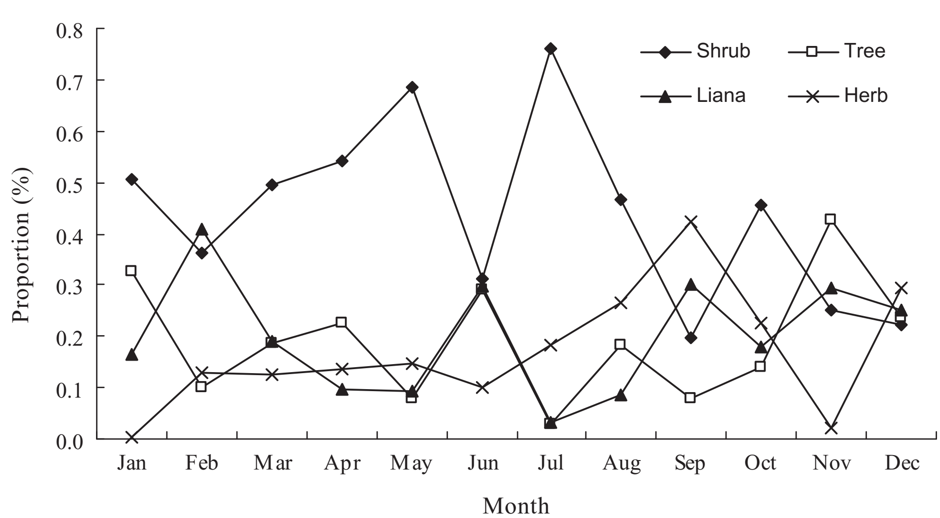

Fig. 2. Monthly variation of time langurs spent on different life forms in Fusui, China during 2006.

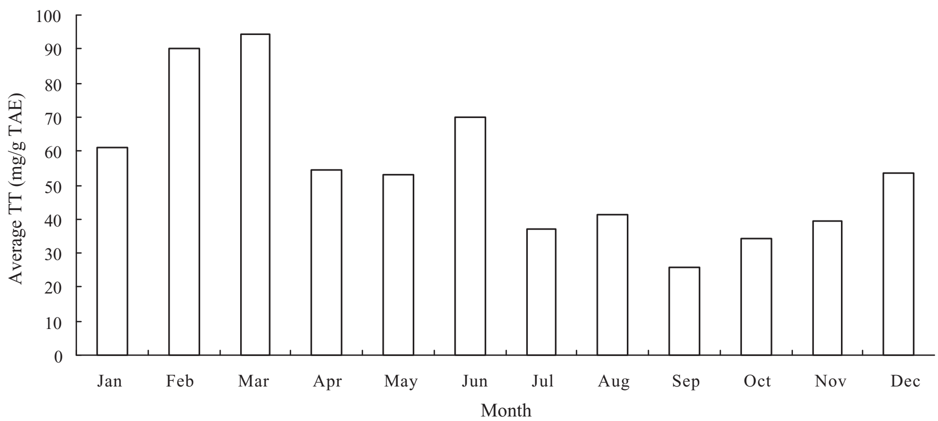

Fig. 3. Monthly averaged total tannins (TT) of plant species used by langurs.

moisture contents and stronger aroma also had a higher TT. For example, *P. weinmannifolia* emanated a strong smell and had a high amount of TT; similarly, *C. ophipogoides* (perennial herb) and *Strophanthus divaricatus* (Family: Apocynaceae) had a high sap concentration and TT.

3.4. Average TT of different food parts and growth forms

TT varied across different plant parts. Fruits had the largest average TT, accounting for 79.8 mg/g TAE, followed by mature leaves (54.4 mg/g TAE) and immature leaves (53.9 mg/g TAE). Flowers contained the least amount of TT, accounting for 30.3 mg/g TAE. TT of food parts varied across the months (Fig. 4). Of the species consumed, only *L. glutinosa* was consumed throughout the year. TT contained in leaves was analyzed throughout the study period (Table 2). The variation of TT for a particular food part for a species is evident.

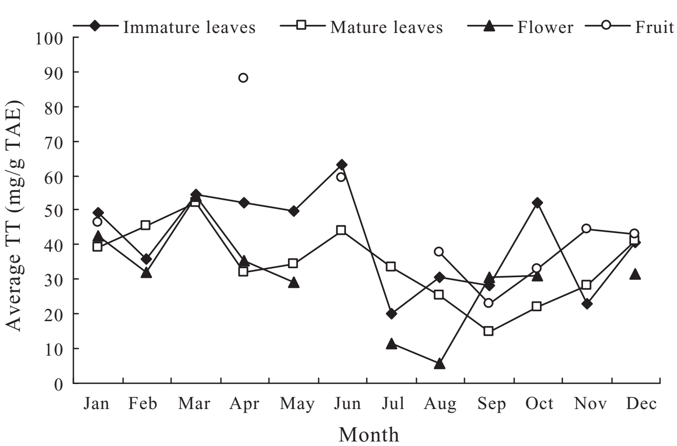

Fig. 4. Monthly total tannins (TT) for different food parts. Discontinuous lines represent food item unavailability.

TT varied among growth forms, shrubs had the largest average TT, which accounted to 86.48 mg/g TAE, followed by herbs (57.2 mg/g TAE) and lianas (48.7 mg/g TAE); trees had the least TT content (42.5 mg/g TAE). Monthly variations across growth forms are shown in Fig. 5.

4. Discussion

Francois' Langurs are folivorous feeders, they spent various time on different food plant and life forms (Figs. 1 and 2). The feeding habit showed in the current result is consistent with that of the previous studies both in the same site [25] and different sites [27,28,30]. But langurs consume more leaves than their counterpart in other sites [27,28] or in the early year in the same site [26]. Inconsistent methodology and sampling duration between these studies may be associated with the variation; the variation, however, imply their spatiotemporal variation in food choice behavior. Francois' Langurs show strong food preferences, their food selection is not based on food availability as a total [26], they selectively use plant species and plant parts in their habitat [27,28,37,39, this study]. Increasingly studies show that primates select food that is "rare" in its habitat as prefer food [12,50–52], obviously this behavior is related to degree of spatial variability and phytochemical quality [52]. François's Langurs have been documented to extensively use some species and compensate by other "rare" food species, so they forage several species within a day [27,28,37]; it seems safe to assume that food selection of the langur also might be influenced by phytochemical factors.

This study shows that TT content varies among species; some species have higher TT contents than others do (Table 1). Compared with other folivorous primates, many food species of Francois' Langur seem to have higher TT contents. Mturi [53] analyzed the two more important food species and found that TT of the plant parts varied between 63.0 and 92.4 mg/g of dry weight. Workman [15] found that Trachypithecus *delacouri* consumed food with the highest TT content of 94.9 mg/g of dry weight. Additionally monthly average TT does not vary across months ($p > 0.05$). In Van Long

Table 2
Total tannins (mg/g TAE dry weight) of *Litsea glutinosa* throughout the study period.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Average
M	10.81	37.80	9.27	11.04	43.99	57.90	16.37	12.48	18.32	15.10	17.52	14.21	22.07
IM	–	13.51	49.17	35.42	–	–	30.80	4.16	1.59	12.07	22.98	17.07	20.75

M: mature leaves; IM: immature leaves; –: this part was unavailable for this month.

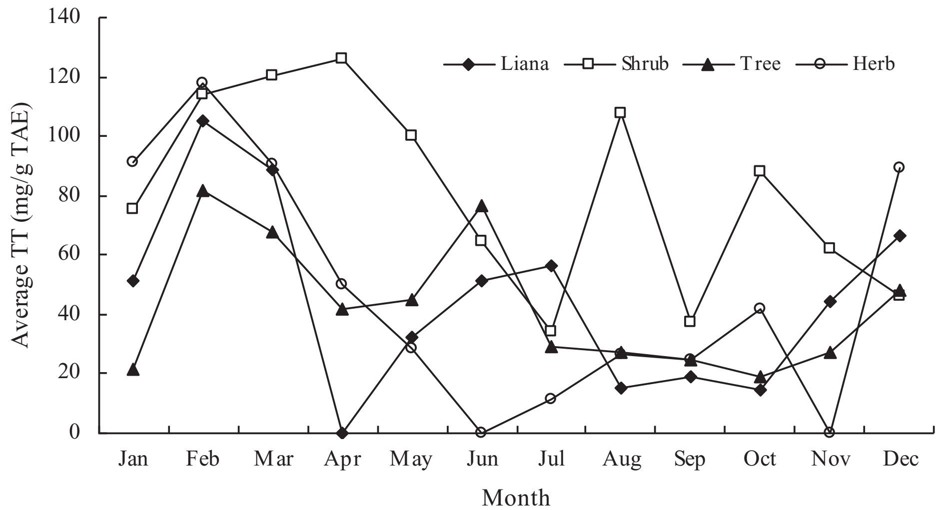

Fig. 5. Monthly variation of total tannins of different plant growth forms. Since in some months, Herbs were not consumed by langurs in some months, the data on herb were not available.

Nature Reserve, the TT of *T. delacouri* food species also has the same trend [15]. They seem to be relatively stable across months as a total.

TT content varied among plant parts and life stage within the same species in the same site (Tables 1 and 2), this result is identical to other studies on primates [48,53,54] and other herbivorous animals [3]. It has been pointed out that the less mature plant parts have higher levels of tannins than mature ones [12], since they provide a good source of protein, water and some structural carbohydrates, and thus are considered to be rich in essential elements [55]. Plant parts rich in essential elements are also very well defended [1]. While other studies indicate that tannin content has little variation in leaf age [56] or the tannin content is higher in mature leaves [57,58]. The current study seems to be the third case if leaves' TT content is considered. But if fruits are considered, it seems to be consistent with the first case. Unripe fruit of *P. weinmannifolia* had the largest TT among all the plant parts.

Food TTs vary among life forms (Fig. 3), shrubs had the largest TT, followed by herbs and lianas, trees had the least TT. The data support the existing conclusions that fast-growing plants have lower defenses [54,59]. The study site has been selectively logged [41], resulting in more edge. It has been confirmed that selective logging opens up the canopy, creating the conditions for the regeneration of colonizing species of plants [60]. Indeed the habitat of the langur is dominant by fast-growing species, such as species from lianas, and seedlings of trees [41].

Though the primary study is far from analyzing the relationship between TT content and food selection, results have two implications. Firstly, TT might have no evident influence on the food selection of langurs. There was no monthly average TT between the first half year and the second half year ($p > 0.05$). The TT for the fifteen most preferred food species was not significantly different from that of the fifteen least preferred food parts ($p = 0.05$), which is similar to *T. delacouri* in Van Long Nature Reserve [15] and other nonhuman primates [16–18]. Secondly, Francois' Langur can tolerate food with high TT level. Langurs in this habitat frequently used the fruit of *P. weinmannifolia*, which has the largest TT among all the analyzed samples; food species and items high in TT were not being avoided. This result coincides with the findings for other langurs, such as Trachypithecus *auratus sondaicus*, which tended to use fruit with higher tannins [61]. The tolerance of high TT in the diet may be an adaptive strategy enabling langurs to survive in such unique habitats.

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References

[1] C.A.M. Robert, N. Veyrat, G. Glauser, G. Marti, G.R. Doyen, N. Vilard, et al., A specialist root herbivore exploits defensive metabolites to locate nutritious tissues, Ecol. Lett. 15 (2012) 55–64.
[2] J. Gershenzon, N. Dudareva, The function of terpene natural products in the natural world, Nat. Chem. Biol. 3 (7) (2007) 408–414.
[3] I. Kaplan, R. Halitschke, A. Kessler, S. Sardanelli, R.F. Denno, Constructive and induced defenses to herbivory in above- and belowground plant tissues, Ecology 89 (2) (2008) 392–406.
[4] R. Shroff, F. Vergara, A. Muck, A. Svatos, J. Gershenzon, Non-uniform distribution of glucosinolates in Arabidopsis thaliana leaves has important consequences for plant defense, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 6196–6201.
[5] K.B. Strier, Primate Behavior Ecology, Allyn and Bacon, Boston, 2003.
[6] T.K. Bhat, B. Singh, O.P. Sharma, Microbial degradation of tannins – a current perspective, Biodegradation 9 (5) (1998) 343–347.
[7] P.G. Waterman, C.N. Mbi, D.B. McKey, African rainforest vegetation and rumen microbes: phenolic compounds and nutrients as correlates of digestibility?, Oecologia 47 (1980) 22–33.
[8] G. Goel, A.K. Puniya, C.N. Aguliar, K. Singh, Interaction of gut microflora with tannins in feeds, Naturwissenschaften 92 (11) (2005) 497–503.
[9] J.M. Arena, C.C. Thomas, Poisoning: Toxicology, Symptoms, Treatments, fourth ed., University of Illinois Springfield, Springfield, 1979.
[10] K.E. Glander, The impact of plant secondary compounds on primate feeding behavior, Yearb. Phys. Anthropol. 25 (1982) 1–18.
[11] K. Milton, Factors influencing leaf choice by howler monkeys: a test of some hypotheses of food selection by generalist herbivores, Am. Nat. 114 (3) (1979) 362–378.
[12] J.F. Oates, P.G. Waterman, G.M. Choo, Food selection by the South Indian leaf-monkey, *Presbytis johnii*, in relation to leaf chemistry, Oecologia 45 (1980) 45–46.
[13] R.W. Wrangham, P.G. Waterman, Feeding behavior of vervet monkeys on *Acacia tortilis* and *A. xanthophloea* with special reference to reproductive strategies and tannin production, J. Anim. Ecol. 50 (3) (1981) 715–731.

[14] P.J. Fashing, E.S. Dierenfeld, C.B. Mowry, Influence of plant and soil chemistry on food selection, ranging patterns, and biomass of *Colobus guereza* in Kakamega Forest, Kenya, Int. J. Primatol. 28 (3) (2007) 673–703.
[15] C. Workman, The Foraging Ecology of the Delacour's Langur (*Trachypithecus delacouri*) in Van Long Nature Reserve, Vietnam, Duke University, Durham, 2010.
[16] C.H. Janson, E.W. Stiles, D.W. White, Selection on plant fruiting traits by brown capuchin monkeys: a multivariate approach, in: A. Estrada, T.H. Fleming (Eds.), Frugivorous and Seed Dispersal, Dr. W. Junk Publishers, Dordrecht, 1986, pp. 83–92.
[17] M. Leighton, Modeling dietary selectivity by Bornean orangutans: evidence for integration of multiple criteria in fruit selection, Int. J. Primatol. 14 (2) (1993) 257–313.
[18] S. Heiduck, Food choice of masked titi monkey (*Callicebus personatus melanochir*): selectivity or opportunism?, Int. J. Primatol. 18 (4) (1997) 487–502.
[19] S. Mole, P.G. Waterman, A critical analysis of techniques for measuring tannins in ecological studies i. technique for chemically defining tannins, Oecologia 72 (1987) 137–147.
[20] L.H. Spelman, K.G. Osborn, K.G. Anderson, Pathogenesis of hemosiderosis in lemurs: role of dietary iron, tannin, and ascorbic acid, Zoo Biol. 8 (3) (1989) 239–251.
[21] S.J. Cork, W.J. Foley, Digestive and metabolic strategies of arboreal mammalian folivores in relation to chemical defenses in temperature and tropical forests, in: D.J. Chivers, P. Langer (Eds.), Plant Defenses against Mammalian Herbivory, CRC Press, Boca Raton, FL, 1991, pp. 133–166.
[22] IUCN, 2008 IUCN Red List of Threatened Species. <http://www.iucnredlist.org>, 2008.
[23] T. Nadler, F. Momberg, N.X. Dang, N. Lormee, Vietnam Primate Conservation Status Review 2002: Part 2: Leaf Monkeys, Fauna & Flora International, Vietnam Program and Frankfurt Zoological Society, Hanoi, 2003, pp. 33–43.
[24] Y.B. Li, C.M. Huang, P. Ding, Z. Tang, C. Wood, Dramatic decline of François' langur (*Trachypithecus francoisi*) in Guangxi Province, China, Oryx 41 (1) (2007) 38–43.
[25] C.M. Huang, H. Hu, Q.H. Zhou, Y.B. Li, X.W. Cai, Feeding strategy of Francois' langur and white-headed langur at Fusui, China, Am. J. Primatol. 70 (2008) 320–326.
[26] Y.B. Li, X.H. Huang, L. Wang, L.L. Yang, Food choice of a group of François' langur in a fragmented habitat in Fusui, China, Acta Theriol. Sin. 32 (3) (2012) 209–215.
[27] Q.H. Zhou, F.W. Wei, M. Li, C.M. Huang, B. Luo, Diet and food choice of the François' langur (*Trachypithecus francoisi*) in the Nonggang Nature Reserve, China, Int. J. Primatol. 27 (5) (2006) 1441–1460.
[28] Z.H. Huang, C.M. Huang, Q.H. Zhou, H. Wei, Y.J. Meng, Diet and the seasonal changes of the François' langur (*Trachypithecus francoisi*), Acta Ecol. Sin. 30 (20) (2010) 5501–5508.
[29] G. Hu, Dietary breadth and resource use of Francois' langur in a seasonal and disturbed habitat, Am. J. Primatol. 73 (2011) 1176–1187.
[30] Y. Luo, Z.R. Chen, S.X. Wang, Observations on the food habit of *Presbytis francoisi*, in Mayanghe Region, Guizhou Province, Chin. J. Zool. 35 (3) (2000) 44–49.
[31] Y.B. Li, P. Ding, C.M. Huang, P.P. Jiang, S.Y. Lu, The crude protein content of main food plants of François' langur (*Trachypithecus francoisi*) in Fusui, Guangxi, China, Acta Ecol. Sin. 33 (23) (2013) 7390–7398.
[32] W. Li, A.W. Guo, B. Wang, N. Liu, G. Hu, Nutritional contents of winter food and its influences on food choice of François' langur at Mayanghe Nature Reserve, Acta Theriol. Sin. 30 (2) (2010) 151–156.
[33] Y.M. Tao, Y.L. Hu, C.M. Huang, Y.B. Li, Effects of crude fiber on the food selectivity of *Trachypithecus francoisi*, Guangxi Sci. 12 (4) (2005) 334–338.
[34] X.H. Huang, Y.B. Li, C.M. Huang, Z.H. Huang, Q.H. Zhou, Food water content of François' langur (*Trachypithecus francoisi*) in Fusui Reserve, J. Sichuan Norm. Univ. 33 (2010) 243–246.
[35] Q. Wu, Z.H. Huang, P.S. Yuan, F.Y. Deng, Q.H. Zhou, Water content of the food and its influence on food choice of Francois' Langur at Nonggang, Guangxi, China, J. Guangxi Norm. Univ. 29 (4) (2011) 117–121.
[36] Z.R. Xu, A study of the limestone forest flora of southern and southwestern China, Guihaia 4 (Suppl.) (1993) 5–54.
[37] Y.B. Li, P. Ding, C.M. Huang, P.P. Jiang, C. Wood, Dietary response of a François' Langur group in a fragmented habitat in Fusui County, China: implications for conservation, Wildlife Biol. 15 (2009) 137–146.
[38] Q.H. Zhou, X.W. Cai, C.M. Huang, Y.B. Li, Y.P. Luo, Feeding activity of François langurs in Karst habitat, Acta Theriol. Sin. 27 (3) (2007) 243–248.
[39] Y.B. Li, C.M. Huang, Z.H. Huang, Q.H. Zhou, Dietary diversity of a group of François' langur (*Trachypithecus francoisi*) in Fusui Reserve: preliminary study, J. Guangxi Norm. Univ. 26 (1) (2008) 96–99.
[40] E.J. Sterling, M.M. Hurley, D.M. Le, Vietnam: A Natural History, Yale University Press, New Haven and London, 2006.
[41] C.M. Huang, X.S. Wei, Q.H. Zhou, Y.B. Li, Z.H. Huang, Effects of habitat quality on activity budgets of the François' langur (*Trachypithecus francoisi*), Acta Theriol. Sin. 27 (4) (2007) 338–343.
[42] J. Altmann, Observational study of behavior: sampling methods, Behaviour 49 (3) (1974) 227–267.
[43] P. Martin, P. Bateson, Measuring Behavior: An Introductory Guide, second ed., Cambridge University Press, Cambridge, 1986.
[44] C.M. Huang, The White-headed Leaf Monkey of China, Guangxi Normal University Press, Guilin, 2002.
[45] Guangxi Institute of Botany Academia Guangxiana, Flora of Guangxi, Guangxi Science and Technology Publishing House, Nanning, 1986.
[46] C.A. Chapman, L.J. Chapman, K.D. Rode, Variation in the nutritional value of primate foods: among trees, time periods, and areas, Int. J. Primatol. 24 (2) (2003) 317–333.
[47] M.F. Wilson, Comparison of tannin levels in developing fruit buds of two orchard pear varieties using tow techniques, Folin-Denis and protein precipitation assays, J. Chem. Ecol. 10 (3) (1984) 493–498.
[48] C.B. Mowry, B.S. Decker, D.J. Shure, The role of phytochemistry in dietary choices of tana river red colobus monkeys (*Procolobus badius rufomitratus*), Int. J. Primatol. 17 (1) (1996) 63–84.
[49] J.F. Dudt, D.J. Shure, The influence of light and nutrients on foliar phenolics and insect herbivory, Ecology 75 (1994) 86–98.
[50] G.L. DaSilva, The western black-and-white colobus as a low energy strategist: activity budgets, energy expenditure and energy intake, J. Anim. Ecol. 61 (1992) 79–91.
[51] A. Koenig, J. Beis, M.K. Chalise, J.U. Ganzhorn, When females should contest for food – testing a hypotheses about resource density, disribution, size, and quality with Hanuman langurs (Presbytis entellus), Behav. Ecol. Sociobiol. 42 (1998) 225–237.
[52] G.A. Preece, Factors influencing variation in the population densities of Colobus guereza within selectively logged forest at the Budongo Forest Reserve: the importance of lianas during a subsistence diet, in: N.E. Newton-Fisher, H. Notman, J.D. Paterson, V. Reynolds (Eds.), Primates of Western Uganda, Springer, New York, 2006, pp. 23–42.
[53] F.A. Mturi, The feeding ecology and behavior of the red Colobus monkey (Ph.D. dissertation), University of Dar Es Salaam, Tanzanian, 1991.
[54] P.G. Waterman, K.M. Kool, Colobine food selection and plant chemistry, in: A.G. Davies, J.F. Oates (Eds.), Colobine Monkeys: Their Ecology, Behavior and Evolution, Cambridge University, Cambridge, 1994, pp. 251–284.
[55] A.F. Richard, Primate in Nature, W. H. Freeman and Company, New York, 1985.
[56] E.L. Bennett, The banded langur: ecology of a colobine in a West Malasian rain-forest (Ph.D. dissertation), University of Cambridge, England, 1983.
[57] P. Feeny, Plant apparency and chemical defense, in: J.N. Wallace, R.L. Mansell (Eds.), Biochemical Interaction between Plants and Insects, Plenum Press, New York, 1976, pp. 168–213.
[58] D.F. Rhoades, R.G. Cates, Towards a general theory of plant anti-herbivore chemistry, in: J.N. Wallace, R.L. Mansell (Eds.), Biochemical Interaction between Plants and Insects, Plenum Press, New York, 1976, pp. 168–213.
[59] P.B. Reich, M.B. Walters, D.S. Ellsworth, Leaf life-span in relation to leaf, plant, and stand characteristics among diverse ecosystems, Ecol. Monogr. 62 (1992) 365–392.
[60] J.U. Ganzhorn, Low-level forest disturbances effects on primary production, leaf chemistry, and lemur populations, Ecology 76 (1995) 2084–2096.
[61] K.M. Kool, Food selection by the silver leaf monkeys, *Trachypithecus auratus sondaicus*, in relation to plant chemistry, Oecologia 90 (1992) 527–533.