Salivary response of spider monkeys (*Ateles geoffroyi*) to consumption of plant secondary metabolites (#110850)

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Salivary response of spider monkeys (*Ateles geoffroyi*) to consumption of plant secondary metabolites

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Plants produce a wide variety of secondary metabolites which elicit a bitter taste. Black-handed spider monkeys feed on various plant species and consume different concentrations of secondary metabolites. Monkeys can modulate the acidity-alkalinity (pH) and salivary expression of total proteins (TP) and proline-rich proteins (PRP) dependent on the concentration of tannins, helping to counteract negative post-ingestive effects. However, it is unclear whether there is modulation of pH, TP, and PRP to other secondary metabolites than tannins and whether this effect also occurs towards bitter substances not associated with secondary metabolites. Our objective was to determine if there are adjustments in salivary pH, TP, and PRPs expression towards bitter substances or if spider monkeys display a specific response to secondary metabolites present in their diet and substances not associated with secondary metabolites. We presented six adult spider monkeys (*Ateles geoffroyi*) with different concentrations of tannic acid, caffeine, and rutin (0.1, 0.3, 0.6 and 1 mM) and denatonium benzoate (0.001, 0.003, 0.006 and 0.01 mM) dissolved in 30 mM sucrose. We administrated each concentration and collected saliva using swabs (SalivaBio). We used test paper strips to measure the pH and determined the TP concentration using the Bradford method at 595 nm. We also determined the percentage of PRPs using SDS-PAGE electrophoresis. The results showed an increase in salivary pH in response to consumption of secondary metabolites, no variations in TP concentration, variations in the percentage of PRPs associated with tannic acid concentrations, and no significant changes when the animals consumed denatonium benzoate. Our results showed that spider monkeys specifically modulate acidity-alkalinity towards secondary metabolites and salivary PRPs expression towards tannic acid in their diet, and that they do not have a generalized salivary response to bitter compounds that

are typically considered as toxic substances.

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Abstract

Plants produce a wide variety of secondary metabolites which elicit a bitter taste. Black-handed spider monkeys feed on various plant species and consume different concentrations of secondary metabolites. Monkeys can modulate the acidity-alkalinity (pH) and salivary expression of total proteins (TP) and proline-rich proteins (PRP) dependent on the concentration of tannins, helping to counteract negative post-ingestive effects. However, it is unclear whether there is modulation of pH, TP, and PRP to other secondary metabolites than tannins and whether this effect also occurs towards bitter substances not associated with secondary metabolites. Our objective was to determine if there are adjustments in salivary pH, TP, and PRPs expression towards bitter substances or if spider monkeys display a specific response to secondary metabolites present in their diet and substances not associated with secondary metabolites. We presented six adult spider monkeys (*Ateles geoffroyi*) with different concentrations of tannic acid, caffeine, and rutin (0.1, 0.3, 0.6 and 1 mM) and denatonium benzoate (0.001, 0.003, 0.006 and 0.01 mM) dissolved in 30 mM sucrose. We administrated each concentration and collected saliva using swabs (SalivaBio). We used test paper strips to measure the pH and determined the TP concentration using the Bradford method at 595 nm. We also determined the percentage of PRPs using SDS-PAGE electrophoresis. The results showed an increase in salivary pH in response to consumption of secondary metabolites, no variations in TP concentration, variations in the percentage of PRPs associated with tannic acid concentrations, and no significant changes when the animals consumed denatonium benzoate. Our results showed that spider monkeys specifically modulate acidity-alkalinity towards secondary metabolites and salivary PRPs expression towards tannic

acid in their diet, and that they do not have a generalized salivary response to bitter compounds that are typically considered as toxic substances.

Keywords: Frugivorous primates, bitter compounds, salivary proteins, salivary pH, proline-rich proteins.

Introduction

Primates use plants as an essential food source because they contain nutrients such as carbohydrates, lipids, and proteins (Mattes, 2011; Lambert & Rothman, 2015). However, the risk of consuming plant parts is that some compounds can be nutritionally limiting or even toxic, such as secondary metabolites whose function is to protect plants from herbivory (Akula & Ravishankar, 2011; War et al., 2012; Villalba et al., 2017; Kariñho-Betancourt, 2018; Yang et al., 2018). The type of secondary metabolites varies depending on the plant species (Wink, 2003; 2008), and their concentration depends on the plant part and stage of maturity. For example, they are found in higher concentration in young leaves and unripe fruits compared to ripe fruits, and during the ripening process of fruits, the concentration of organic acids varies (Sun et al., 2010; Huang et al., 2012; Da Silva et al., 2014).

The secondary metabolites classification considers terpenes, alkaloids, and phenolic compounds, the latter in turn divided into two groups: tannins and flavonoids (Bourgaud et al., 2001; Wink, 2008; Kariñho-Betancourt, 2018). Secondary metabolites generally produce a bitter taste and an astringent sensation and can generate negative post-ingestive effects such as nausea, abdominal pain and liver and kidney damage (Frutos et al., 2004; War et al., 2012; Stevenson et al., 2017). However, at low concentrations, some secondary metabolites can also act as antioxidants and immunostimulants (Crozier et al., 2009).

The presence of secondary metabolites has been documented in the diet of all major primate taxa, including great apes (Rogers et al., 1990; Takemoto, 2003; Beaune et al., 2017), strepsirrhines (Carrai et al., 2003; Norscia et al., 2006; 2012), catarrhines (Chapman & Chapman, 2002; Fashing et al., 2007; Ta et al., 2017) and platyrrhines (Felton et al., 2009; Espinosa-Gómez et al., 2018). In addition, most primate studies have focused on identifying the presence and concentration of tannins and alkaloids (Rogers et al., 1990; Hamilton & Galdikas, 1994; Wakibara et al., 2001; Chapman & Chapman, 2002; Norscia et al., 2012; Ta et al., 2017; Thurau et a., 2021), without considering other compounds such as flavonoids and terpenes. Considering the role that secondary metabolites play in the diet of primates (Glander, 1978; Rogers et al., 1990; Leighton, 1993; Kar-Gupta & Kumar, 1994; Fashing et al., 2007; Windley et al., 2022), it is relevant to conduct work that delves into the relationship between specific secondary metabolites present in their diet and their physiological response (Windley et al., 2022).

Some primate species appear not to be physiologically affected by the secondary metabolites in the plants they consume (Oates et al., 1980; Rothman et al., 2009). This suggests that they have defense mechanisms that counteract the negative effect of these compounds, for example, modulation of salivary pH (Ramírez-Torres et al., 2022) and the expression of proline-rich proteins and histatins, which capture and precipitate tannins and alkaloids, preventing them from interacting with proteins necessary for digestion (Oates et al., 1980; Shimada, 2006; Fashing et al., 2007; Morzel et al., 2022; Windley et al., 2022).

PRPs have been identified in the saliva of different primate species such as macaques (*Macaca fascicularis* and *Macaca arctoides*), baboons (*Papio hamadryas*), howler monkeys (*Alouatta palliata mexicana* and *Alouatta pigra*) and spider monkeys (*Ateles geoffroyi*) (Oppenheim et al., 1979; Schlesinger et al., 1989; Mau et al., 2011; Espinosa-Gómez et al., 2015; 2018; 2020; Ramírez-Torres et al., 2022). Similarly, histatins have been identified in the saliva of crab macaques (*Macaca fascicularis*), gorillas (*Gorilla gorilla gorilla*), gibbons (*Nomascus leucogenys*), vervet monkeys (*Cercopithecus aethiops*) and langurs (*Presbytis cristata*) (Xu et al., 1990; Padovan et al., 2010).

Spider monkeys are primates that feed on more than 250 plant species, of which they mainly consume ripe fruits, although they may also feed on unripe fruits as well as on other plant parts such as leaves, flowers, and seeds (González-Zamora et al., 2009; Schaffner et al., 2012; Pablo-Rodríguez et al., 2015; Hartwell et al., 2021). This dietary diversity exposes them to different types and concentrations of secondary metabolites and to variations in the acidity of the nutrients that make up their diet. Black-handed spider monkeys (*Ateles geoffroyi*) can perceive the bitter taste elicited by different secondary metabolites and synthetic substances such as denatonium benzoate (Laska et al., 2009), and they have been shown to modulate their salivary pH, TP concentration, and PRPs percentage when consuming tannic acid (Ramírez-Torres et al., 2022). However, it is unclear if this salivary response is specific to tannins or is a general response towards other secondary metabolites, such as flavonoids, alkaloids or synthetic bitter compounds. Therefore, we addressed the following questions: Which secondary metabolites are present in the plants consumed by black-handed spider monkeys? What are the secondary metabolites concentrations of plant parts according to their stage of maturity? What are the salivary responses of spider monkeys to ingestion of alkaloids and flavonoids? Is there a general salivary response of spider monkeys to bitter stimuli?

Materials and Methods

Ethics statement

The experiments reported here comply with the Guidelines for the care and use of mammals in neuroscience and behavioral research (National Research Council, 2011), the American Society of Primatologists' Principles for the Ethical Treatment of Primates, and Mexican laws (NOM-062-ZOO-1999 and NOM-051-ZOO-1995). The protocol was approved by the Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT; official permit numbers SGPA/DGVS/000041/22 and SPARN/DGVS/01767/22).

Collection of plant parts

We collected unripe fruits, ripe fruit, young leaves, and mature leaves from 64 trees of nine species reported as part of the diet of black-handed spider monkeys in the region of Los Tuxtlas, Veracruz, Mexico. The species collected were: (4) *Ficus americana*, (3) *F. colubrinae*, (3) *F. rzedowskiana*, (5) *F. insipida*, (8) *F. yoponensis*, (11) *Spondias mombin*, (7) *S. radlkoferi*, (12) *Brosimum alicastrum* and (11) *Poulsenia armata*. These species represent more than 40% of the spider monkey´s diet (González-Zamora et al., 2009).

Secondary metabolite analysis

Once the plant parts were collected, they were dehydrated until they reached a constant weight. We used a dehydrator (SANGKEE®) for the fruits at 35 ^{o}C and the leaves were dehydrated in a cardboard box (50 cm x 50 cm) under a 70-watt incandescent bulb. The dehydrated samples were ground to a fine powder. One pool was formed for each plant part (UF, RF, YL, and ML) per plant species. We took 5 g of each pool and added 5 ml of HPLC-grade methanol (1:1). Each mixture was vortexed for one minute and allowed to stand for 5 min. We took the supernatant with a 5 ml syringe and a 0.045 µm pore nylon filter placed at the bottom of the syringe to recover the supernatant after passing through the filter.

Once the sample was filtered, we determined the presence and concentration of tannic acid (tannin), caffeine (alkaloid), and rutin (flavonoid) using high-performance liquid chromatography (HPLC) Agilent Technologies HPLC System (model: 1200 infinity). HPLC grade methanol was used as a solvent at a temperature of 20 ˚C with a mobile phase of acetonitrile, methanol, and water (15:15:70) with a flow rate of 1 ml/min. The concentration of the secondary metabolites was quantified using the HPLC equipment's LC-UV-100 UV/VIS detectors. We performed the secondary metabolites analyses at the Institute of Applied Chemistry, Universidad Veracruzana.

Site and study subjects

We performed the behavioral tests at the Environmental Management Unit "Hilda Ávila de O'Farrill" of the Universidad Veracruzana, located near Catemaco in Los Tuxtlas, Veracruz, Mexico. We worked with three female and three adult male spider monkeys (n=6). During the tests, we kept the animals in individual enclosures (4 x 4 x 4 m). When individuals completed the test, the sliding doors between the enclosures were opened, allowing them to interact with each other. The enclosures were enriched with trunks and vines to stimulate animal welfare. All individuals were exposed to natural conditions of temperature, relative humidity, and light-dark cycle. The monkeys were feed on cultivated fruits and vegetables one time a day, and all tests were conducted two hours before their feeding time to ensure they were motivated to participate.

Study design

Each individual was presented with one bottle with a metal drinking spout, containing 100 ml of sucrose at a concentration of 30 mM (control solution) or an experimental solution containing the secondary metabolites tannic acid, rutin, or caffeine at concentrations 0.1, 0.3, 0.6, and 1 mM, mixed with sucrose (30 mM). We used solutions of denatonium benzoate, a bitter-tasting synthetic compound at concentrations 0.001, 0.003, 0.006 and 0.01 mM mixed with sucrose (30 mM) (Fig. 1). All monkeys had access to one bottle for one minute (control or experimental). The sucrose concentration used is above the taste preference threshold of spider monkeys, which makes it attractive to induce their consumption, however, this concentration is low enough to avoid masking effects to other taste substances (Laska et al., 1996; 2000). The concentrations of the secondary metabolites and of the artificial bitter tastant were above the spider monkeys' taste threshold (Laska et al., 2000; 2009), which ensured that the individuals could reliably perceive them. The different solutions and saliva collection were administered between 09:00 and 11:00 h. Sucrose (CAS# 57-50-1) was obtained from Merck, tannic acid (CAS# 1401-55-4) from Meyer, rutin (CAS# 207671-50-9), caffeine (CAS# 58-08-2) and denatonium benzoate (CAS# 3734-33-6) from Sigma-Aldrich.

Figure 1. Approximately here.

Saliva collection

We collected saliva samples using a swab (SalivaBio Children's Swab, Salimetrics 5001.08, SalivaBio, State College, PA, USA) 13 cm adjusted to a size of 4.3 cm to allow the complete introduction of the swab into the mokey's oral cavity. To encourage the monkeys to chew the swab, they were soaked with 0.5 ml of corn syrup for 60 s following the method reported by Ramírez-Torres et al., (2022).

Determination of salivary acidity/alkalinity (pH)

We determined salival pH using colorimetric paper strips and compared the colour of the test strips (OF®) with the standards (Ramírez-Torres et al., 2022).

Saliva sample processing

A pool was formed from the saliva obtained from each individual on days 6 to 8 by offering them each of the different concentrations of the secondary metabolites and denatonium benzoate. We processed the samples following the method used by Espinosa-Gómez et al., (2018). We performed the saliva analysis at the Veterinary School of the Universidad Popular Autónoma del Estado de Puebla (UPAEP, University).

Determination of the total protein concentration (TP)

The Bradford method was using a spectrophotometer measuring the absorbance of total proteins at 595 nm (Bradford, 1976).

Determination of the presence of proline-rich proteins (PRPs)

We determined the presence of PRP by identifying protein bands in one-dimensional SDS-PAGE gel electrophoresis (Beeley et al., 1996), with modifications by Espinosa-Gómez et al., (2018). Once the proteins were fixed on the gels, they were stained in a Coomassie-R250 bath for four hours and washed out with 10% acetic acid baths. We used 3 µl of the molecular weight marker Flash Protein Ladder (Gel Company), which was loaded into the first lane.

Densitometric analysis of protein bands identified as proline-rich proteins (PRP) on 1D-SDS-PAGE gels.

The electrophoresis gels were scanned at 1,200 dpi quality by HP Digital Sender Flow 8500 fn2 scanner to calculated the percentage of PRPs (% PRPs). Densitometry analysis of the gel images was performed using IMAGEJ software (Tiago & Wayne, 2012) following the method reported by Ramirez-Torres et al., (2022).

Statistical analysis

A factorial analysis was performed to determine if there were significant differences in each of the salivary physicochemical characteristics recorded for the different concentrations of the substances. Post hoc Tukey tests were performed using the R program version 4.1.1 to determine where these differences were found.

Results

Secondary metabolite analysis

The presence of tannic acid, caffeine, and rutin was identified in the plant species reported as part of the diet of spider monkeys, except caffeine in *Ficus americana*. Variations in the concentration of these secondary metabolites were found depending on the plant part and its maturity stage (Table 1). The highest concentration of tannic acid (252.43 µg/g) was recorded in the young leaves of *F. insipida*, ripe fruit of *P. armata* had the highest concentration of caffeine (5.26 µg/g), and mature leaves of *F. american*a presented the highest concentration of rutin (247.24 µg/g).

Table 1. Approximately here.

Salivary pH

Factor analysis indicated that there were statistically significant differences in the salivary pH of spider monkeys by compound ($F = 21.72$, $p < 0.05$), by concentration ($F = 57.38$, $p < 0.05$) and in the interaction of concentration and compounds ($F = 18.05$, $p < 0.05$). Tukey's test identified the differences in salivary pH when the spider monkeys consumed the different concentrations of the secondary metabolites solutions (Table 2). There was a significant increase in salivary pH with increasing concentrations of tannic acid and caffeine. We did not find significant differences in salivary pH with increasing the concentration of rutin and denatonium benzoate. The most alkaline salivary pH was 8.1 ± 0.25, recorded when the monkeys consumed the 1 mM concentration of tannic acid.

Table 2. Approximately here.

Total protein concentration

Factor analysis indicated significant differences in salivary TP of spider monkeys by compound ($F = 6.35$, $p < 0.05$). No significant differences were found by concentration nor by the interaction of the compounds and their concentration. Tukey's test identified significant differences between the TP concentration when monkeys consumed the 0.3 mM caffeine solution, and the control solution offered during the rutin administration period. There were significant differences in TP expression between the 0.3 mM concentration of caffeine and the 0.1 and 0.3 mM rutin concentrations.

Percentage of protein-rich proline

Factor analysis indicated statistically significant differences in the percentage of PRPs by compound ($F = 71.24$, $p < 0.05$), by concentration ($F = 11.65$, $p < 0.05$), and in the interaction of concentration and compounds ($F = 6.43$, $p < 0.05$). Tukey's test identified the differences in the percentage of PRPs when offered the concentrations of the different solutions (Table 3). There was a significant increase in the percentage of PRPs with increasing concentrations of tannic acid. At the same time, there was no significant increase in the percentage of PRPs with increasing concentrations of caffeine and rutin and denatonium benzoate. The highest percentage of PRPs recorded was 27.63 ± 4.64, obtained when the monkeys consumed the 1 mM concentration of tannic acid.

Table 3. Approximately here.

Discussion

Our results demonstrated the presence of tannic acid, caffeine and rutin in the fruits (unripe and ripe) and leaves (young and mature) of the nine plant species which have been reported to represent a large proportion in the diet of black-handed spider monkeys in Los Tuxtlas region (González-Zamora et al., 2009). Thus, our results confirmed that spider monkeys are naturally exposed to these secondary metabolites in their diet. We found tannic acid, caffeine, and rutin in some plant parts (young leaf, mature leaf, unripe fruit, and ripe fruit) in all plant species considered here, except for caffeine in *F. americana*.

The rutin was found in the highest concentration in the plant parts, followed by tannic acid and caffeine. We found that the highest concentration of rutin was in mature leaves of *F. americana*, tannic acid in young leaves of *F. insipida*, and caffeine in ripe fruits of *P. armata*. Although there were variations in the concentrations of the three secondary metabolites between fruits and leaves, unlike what has been reported in the literature (Sun et al., 2010; Huang et al., 2012; Da Silva et al., 2014), ripe fruits did not always present lower secondary metabolites concentration. For example, in *S. radlkoferi*, *F. colubrinae*, *F. insipida*, *F. yoponensis*, and *P. armata* tannic acid had higher concentration in ripe fruits compared to unripe fruits, and in most plant species higher concentration of caffeine was found in fruits compared to leaves, except for *F. americana* where no caffeine was found. Research has shown that plant stress can be caused by herbivory and various environmental factors, such as light incidence (Akula & Ravishankar, 2011; Kariñho-Betancourt, 2018; Yang et al., 2018).

To our knowledge, this is one of the first studies that delve into the identification of particular secondary metabolites in species that are part of the diet of spider monkeys and even in non-human primates since most studies only focus on determining the presence and concentration of groups of substances such as tannins and alkaloids (Rogers et al., 1990; Hamilton & Galdikas, 1994; Wakibara et al., 2001; Chapman & Chapman, 2002; Norscia et al., 2012; Ta et al., 2017; Thurau et a., 2021; Li et al., 2022). However, tannic acid and caffeine have repeatedly been used to determine the behavioral and physiological response to bitter or astringent substances in western gorillas (*Gorilla gorilla gorilla gorill*), pigtailed macaques (*Macaca nemestrina*), squirrel monkeys (*Saimiri sciureus*) and spider monkeys (*A. geoffroyi*) (Critchley & Rolls, 1996; Remis & Kerr, 2002; Laska et al.., 2009; Ramírez-Torres et al., 2022). However, this is the first report of rutin in plants consumed by non-human primates. Rutin, a flavonoid that has been studied in functional foods, provides an astringent/sour sensation (Kim et al., 2023) and, like other flavonoids, has been shown to have effects as a glycemic level controller, anti-inflammatory, antioxidant and neuroprotective agent (Crozier et al., 2009; Hosseinzadeh & Nassiri-Asl, 2014; Ghorbani et al., 2017; El-Beltagi et al., 2019) and its consumption in primates could have a potentially beneficial effect, as has been described in female primates that consume them to solve stressful situations such as the postpartum period (Colombage et al., 2024).

The salivary pH value of spider monkeys, when offered the control solution (30 mM sucrose) at the beginning of each experimental period, shown that saliva maintains a neutral pH. However, an increase in salivary pH (which became more alkaline) was generally recorded as the concentration of the three secondary metabolites consumed by the spider monkeys increased, with the highest value obtained when the animals consumed the highest concentration of tannic acid (1 mM).

Changes in salivary pH in spider monkeys when consuming different secondary metabolites act as a defense mechanism to avoid acid-associated damage from food (Lavy et al., 2012; Ramírez-Torres et al., 2022). The differential response in the salivary pH levels to the tested compounds could be due to the fact that they present different degrees of acidity (tannic acid - pH 3.5, caffeine - pH 4.4 to 5.5, rutin - pH 6.5 to 7 and denatonium benzoate - pH 6.8 to 7. 8), whereby the body releases only the necessary amount of salivary alkalinity-promoting buffers such as bicarbonate ions, thus avoiding damage to the oral cavity associated with the organic acids in the food (Foley et al., 1995; Pedersen & Belstrøm, 2019). Changes in pH levels could be related to their food selection, as spider monkeys accept acidic tastes as may occur in other primate species such as squirrel monkeys (*Saimiri sciureus*) and rhesus macaques (*Macaca mulatta*) (Pritchard et al., 1995; Laska,1996; 1999; Laska et al., 2000). It has been proposed that this ability allows them the detection of acids and essential amino acids in fruits and thus to assess their nutritional value (Larsson et al., 2014). In addition, these changes could contribute to the ability of spider monkeys to consume unripe fruits when ripe fruits are unavailable (Pablo-Rodríguez et al., 2015; Batista-Silva et al., 2018).

On the other hand, we found statistically significant differences in TP concentration between caffeine and rutin. However, although there was an increase in TP with increasing concentrations of tannic acid consumed by the monkeys, this was not as significant as with the caffeine and rutin solutions. We did not find a significant increase in TP when the monkeys consumed different concentrations of denatonium benzoate.

Diet type and taste sensations can influence salivary protein expression (Dawes & Shaw, 1965; Neyraud et al., 2006; Quintana et al., 2009; Morzel et al., 2012; Torregrossa et al., 2014). Furthermore, in some primates, it has been shown that in addition to secondary metabolites, fiber also influences TP expression and concentration (Neyraud et al., 2006; Quintana et al., 2009; Canon et al., 2010; Ramirez-Torres et al., 2022). Although we did not estimate the amount of fiber in the plant parts, our study demonstrates that the content of secondary metabolites present confers a bitter taste, which is not sufficient to cause significant changes in the concentration of salivary TP in spider monkeys, which could be because the type of proteins related to this taste does not represent a high percentage of salivary TP. Spider monkeys feed on a predominantly frugivorous diet (with a high proportion of ripe fruits) and therefore may not need to express significant changes in TP concentration as could be the case in folivorous or granivorous species, when exposed to higher amounts of fiber and secondary metabolites (Klein & Klein, 1977; Milton, 1978; Dias & Rangel-Negrín, 2015; Masi et al., 2015).

Statistically significant differences were found in the percentage of PRPs by compound, concentration, and interaction of concentration with compounds. The percentage of PRPs in the saliva of spider monkeys when offered the control solution ranged from 10.04 ± 2.21 % to 12.32 ± 1.61 %. There was a significant increase in PRPs when the monkeys consumed tannic acid from its lowest concentration (0.1 mM), with the salivary expression of PRPs increasing at higher concentrations of this solution (1 mM), reaching 27.63 ± 4.64 %. However, no statistically significant changes were recorded in the PRPs for caffeine and rutin, and they remained at 14.1 ± 2.57 % and 13.53 ± 4.59 %, respectively, when offering the 1 mM concentrations. Similarly, no significant differences were recorded in the PRPs percentage with the different concentrations of denatonium benzoate, which remained in a range of 11.03 ± 1.62 % when offering the highest concentration (0.01 mM).

Our results agree with the literature that PRPs act as a defense mechanism against the consumption of tannins (Shimada, 2006; Mau et al., 2011; Espinosa-Gómez et al., 2015; 2018; 2020; Morzel et al., 2022; Windley et al., 2022). However, the production of this type of protein does not increase with the consumption of other secondary metabolites, such as alkaloids and flavonoids. The salivary response of the monkeys may be linked to the diverse taste receptors that detect bitter flavors which are sensitive to various molecules responsible for this taste quality (Matsunami et al., 2000; DuBois et al., 2008; Kuhn et al., 2010). The differences in the chemical structures of tannins, alkaloids, and flavonoids lead to their perception by specific receptors, resulting in varied responses in protein production. Additionally, tannins can create astringency, which has been shown to stimulate the production of PRPs (Kauffman & Keller, 1979; Wróblewski et al., 2001). This may explain why an increase in PRPs levels was observed only when the monkeys consumed tannic acid, but not with caffeine or rutin. Accordingly, we propose that the expression of salivary PRPs may be a specific mechanism for tannins. This does not rule out the existence of other defense responses against other secondary metabolites that prevent the damage associated with them, for example, histatin-rich proteins which are effective in capturing secondary metabolites (Naurato et al., 1999; Shimada, 2006) and the intestinal microbiota which produces a wide variety of enzymes with the ability to form a protective biofilm and detoxify potentially toxic compounds such as secondary metabolites (McKey et al., 1981; Zhang et al., 2020; Xia et al., 2023). Furthermore, it is essential to highlight that no significant changes were found in the percentage of PRPs when presenting the spider monkeys with denatonium benzoate which is a synthetic bitter-tasting substance. Accordingly, we conclude that the expression of this type of protein does not occur in response to the perception of bitter taste as such (Frutos et al., 2004; War et al., 2012; Stevenson et al., 2017). Thus, spider monkeys, despite perceiving bitter taste as something negative, possess physiological mechanisms that allow them to react differentially and specifically to the wide variety of compounds that cause an avoidance response (Fig. 2). In this context, it is important to consider that the vegetative parts consumed by spider monkey do not have the same concentration of secondary metabolites. This physiological discrimination associated with the type of secondary metabolites is an essential mechanism for these primates that allows them to be selective in obtaining quick energy, but with the ability to include a wide variety of food species and plant parts, and to respond physiologically to compounds that do not represent the same adverse effects on their health, and whose consumption could even benefit them (Schaffner et al., 2012; War et al., 2012; Pablo-Rodríguez et al., 2015; Kariñho-Betancourt, 2018; Yang et al., 2018; Hartwell et al., 2021).

Figure 2. Approximately here.

Conclusion

This study demonstrates the presence of different secondary metabolites in plants consumed by spider monkeys and provides the first evidence of the flavonoid rutin in the diet of primates. The results support the hypothesis that spider monkeys have specific salivary mechanisms for some secondary metabolites, such as tannins, while for other compounds, such as caffeine or rutin, salivary protein expression is not affected. Data obtained with denatonium benzoate showed no significant trigger for bitter taste in protein expression or changes in salivary acidity.

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Figure 1

Experimental design

Each substance was administered to all individuals for a period of 49 days. In each period, the monkeys were initially offered the control solution containing 30 mM of sucrose and then, in ascending order, the four concentrations of tannic acid, rutin, caffeine (0.1, 0.3, 0.6 and 1 mM) or denatonium benzoate (0.001, 0.003, 0.006 and 0.01 mM) mixed with 30 mM of sucrose. Each concentration was offered for eight days, and on days six, seven and eight of each period salivary pH was recorded, and saliva samples were taken. After finishing the administration of a substance in its different concentrations, a three-day rest period was given, during which the control solution was offered, so that the analysis would reflect the result of the concentration of each solution and not be an accumulated response. At the end of each period of administration of the substances, a two-week break was implemented, after which a new experimental period began.

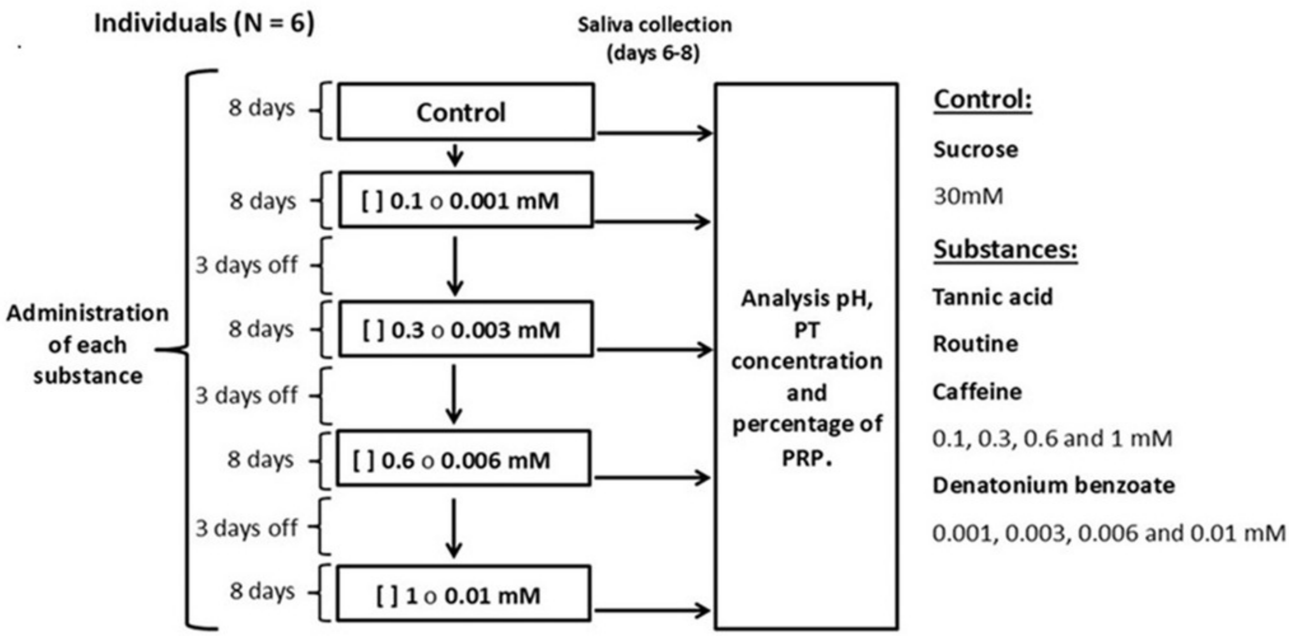
Individuals (N = 6)
Saliva collection (days 6-8)
Administration of each substance
8 days
Control
8 days
[ ] 0.1 o 0.001 mM
3 days off
8 days
[ ] 0.3 o 0.003 mM
3 days off
8 days
[ ] 0.6 o 0.006 mM
3 days off
8 days
[ ] 1 o 0.01 mM
Analysis pH, PT concentration and percentage of PRP.
Control:
Sucrose
30mM
Substances:
Tannic acid
Routine
Caffeine
0.1, 0.3, 0.6 and 1 mM
Denatonium benzoate
0.001, 0.003, 0.006 and 0.01 mM

Figure 2

Response of salivary physicochemical characteristics.

Variations in salivary pH, TP concentration and PRPs percentage of spider monkeys when consuming different (A). secondary metabolites (tannic acid, caffeine and rutin) and (B) denatonium benzoate.

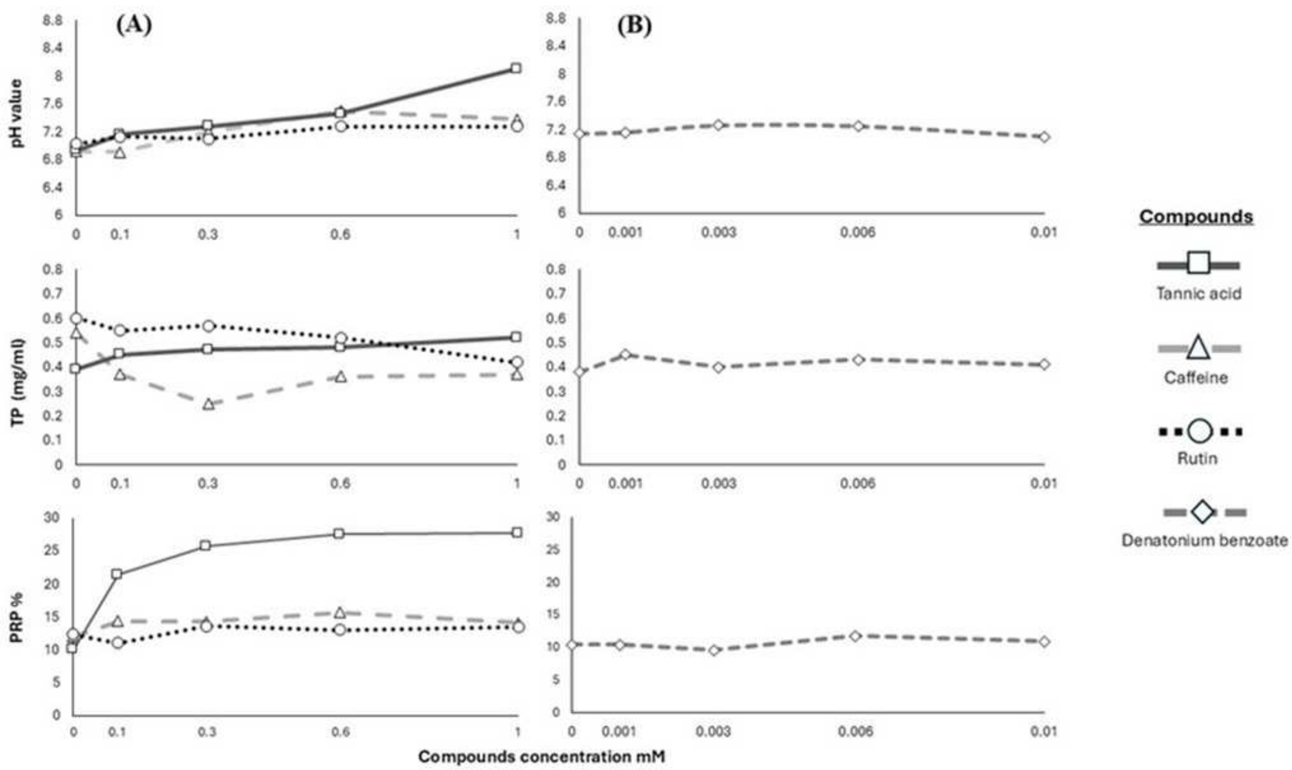

Table 1 (on next page)

Secondary metabolites in plants reported as consumed by spider monkeys (*Ateles geoffroyi*).

Concentration of tannic acid, caffeine and rutin in plant parts of the nine species reported as consumed by spider monkeys in Los Tuxtlas, Veracruz, Mexico.

Table 1. Secondary metabolites in plants reported as consumed by spider monkeys (*Ateles geoffroyi*)

Species	Plant part	Concentration (µg/g)		
		Tannic acid	Caffeine	Rutin
Spondias mombin	young leaves	-	-	-
	mature leaves	-	-	-
	Unripe fruits	-	0.16	-
	Ripe fruits	3.85	1.8	0.38
Spondias radlkoferi	young leaves	-	-	22.5
	mature leaves	2.28	-	1.1
	Unripe fruits	5.86	-	1.51
	Ripe fruits	16.72	0.65	5.15
Ficus americana	young leaves	-	-	0.14
	mature leaves	1.1	-	247.24
	Unripe fruits	-	-	4
	Ripe fruits	-	-	-
Ficus colubrinae	young leaves	2.27	-	21.21
	mature leaves	3.81	-	10.47
	Unripe fruits	0.58	0.05	0.23
	Ripe fruits	2.09	-	3.2
Ficus insípida	young leaves	252.43	-	1.94
	mature leaves	8.81	-	0.84
	Unripe fruits	-	-	-
	Ripe fruits	5.19	5.15	0.17
Ficus rzedowskiana	young leaves	6.71	0.44	27.96
	mature leaves	0.3	0.49	26.97
	Unripe fruits	3.19	0.74	1.92
	Ripe fruits	0.38	1.7	1.31
Ficus yoponensis	young leaves	-	-	18.14
	mature leaves	-	-	19.22
	Unripe fruits	8.27	3.46	10.37
	Ripe fruits	14.47	0.41	0.95
Brosimum alicastrum	young leaves	-	-	-
	mature leaves	16.01	-	-
	Unripe fruits	13.28	0.63	18.22
	Ripe fruits	12.08	1.82	19.29
Poulsenia armata	young leaves	0.8	0.41	0.38
	mature leaves	3.54	0.79	-
	Unripe fruits	-	-	-
	Ripe fruits	4.15	5.26	1.72

Table 2 (on next page)

Comparison of salivary pH of spider monkeys (*Ateles geoffroyi*).

Comparisons of salivary pH values of spider monkeys when offered different concentrations of secondary metabolites and denatonium benzoate. We put < when the pH of the X axis was significantly lower than that of the Y axis, while > when it was higher.

Table 2. Comparison of salivary pH of spider monkeys (*Ateles geoffroyi*)

Substance		**Tanic acid**					**Caffeine**				
	Concentration [mM]	0	0.1	0.3	0.6	1	0	0.1	0.3	0.6	1
Tanic acid	0			<	<	<			<	<	<
	0.1				<	<				<	
	0.3	>				<	>	>			
	0.6	>	>			<	>	>			
	1	>	>	>	>		>	>	>	>	>
Caffeine	0			<	<	<			<	<	<
	0.1			<	<	<			<	<	<
	0.3	>				<	>	>			
	0.6	>	>			<	>	>			
	1	>				<	>	>			
Rutin	0				<	<				<	<
	0.1				<	<				<	
	0.3				<	<				<	<
	0.6	>				<	>	>			
	1	>				<	>	>			
Denatonium benzoate	0				<	<				<	
	0.001				<	<				<	
	0.003	>				<	>	>			
	0.006	>				<	>	>			
	0.01				<	<				<	

Table 3(on next page)

Comparison of % PRPs of spider monkeys (*Ateles geoffroyi*).

Comparison of PRPs percentage values of spider monkeys when offered different concentrations of secondary metabolites and denatonium benzoate. We put < when the PRPs percentage of the X axis was significantly lower than that of the Y axis, while > when it was higher.

Table 3. Comparison of % PRPs of spider monkeys (*Ateles geoffroyi*)

Substance		**Tanic acid**				
	Concentration [mM]	0	0.1	0.3	0.6	1
Tanic acid	0		<	<	<	<
	0.1	>				
	0.3	>				
	0.6	>				
	1	>				
Caffeine	0		<	<	<	<
	0.1		<	<	<	<
	0.3		<	<	<	<
	0.6			<	<	<
	1		<	<	<	<
Rutin	0		<	<	<	<
	0.1		<	<	<	<
	0.3		<	<	<	<
	0.6		<	<	<	<
	1		<	<	<	<
Denatonium benzoate	0		<	<	<	<
	0.001		<	<	<	<
	0.003		<	<	<	<
	0.006		<	<	<	<
	0.01		<	<	<	<