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*Full Length Research Paper*

## ***Uncaria guianensis* (Aubl.) J.F. Gmel. extracts reduce bronchial hyper responsiveness and inflammation in a murine model of asthma**

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***Uncaria guianensis* (Aubl.) J. F. Gmel. (“cat’s claw”, Rubiaceae) is a plant with potential to treat asthma because of its anti-inflammatory and antioxidant activities. The aim of this study was to evaluate the effects of two extracts of *U. guianensis* in an animal model of allergic asthma. Balb/c mice were sensitized twice with ovalbumin intraperitoneally one week apart, then challenged with intranasal ovalbumin for three days. Animals were treated with aqueous or hydroethanolic extracts (100 mg/kg) for three days, simultaneously with ovalbumin challenges. Control mice received saline solution on the same days. *In vivo* bronchial hyper responsiveness, airway and lung inflammation, IgE levels, and total antioxidant capacity were measured. Treatment with the hydroethanolic extract significantly reduced total cell and eosinophil counts in bronchoalveolar lavage, and *in vivo* bronchial hyper responsiveness. Moreover, *U. guianensis* hydroethanolic extract significantly reduced interleukin 13 levels in lung homogenate. Total antioxidant capacity and IgE serum levels were not affected with the extract administration. Of note, treatment with the aqueous extract did not elicit significant effects on asthma-like characteristics. Only the hydroethanolic extract of *U. guianensis* reduced lung inflammation and bronchial hyper responsiveness in asthmatic mice.**

**Key words:** Anti-inflammatory agents, asthma, oxindolics, phenols, respiratory hypersensitivity, *Uncaria guianensis*, Rubiaceae.

### **INTRODUCTION**

Asthma is a highly prevalent chronic inflammatory disease whose main characteristics are bronchial hyper responsiveness, variable limitation of airflow, and airway inflammation. The disease leads to significant morbidity

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worldwide, and its prevalence is increasing in the last 20 years (Global Initiative for Asthma, 2019).

Asthmatic patients may also be in an oxidative state in which oxygen and nitrogen reactive species are linked to inflammation and disease severity (Kirkham and Rahman, 2006; Mishra et al., 2018; Nadeem et al., 2003; Sahiner et al., 2011).

Although oxidative stress can play a role in the pathophysiology of asthma, inflammation is the hallmark of the disease, with involvement of Th2 cytokines such as interleukins (IL) 4, 5, 10 and 13, interferon-gamma (IFN- $\gamma$ ), and tissue growth factor beta (TGF- $\beta$ ) (Hogan, 2007; Oeser et al., 2015). The first-choice drugs for asthma are inhaled corticosteroids and long-acting bronchodilators, but some patients may need short-acting bronchodilators (for immediate symptom relief), leukotriene antagonists, muscarinic antagonists, and monoclonal antibodies (Global Initiative for Asthma, 2019). However, not all asthmatic patients achieve good disease control with current treatments (Olin and Wechsler, 2014). Therefore, new, safer, effective drugs for asthma are still needed. This can be accomplished by screening plants with anti-inflammatory activity.

*Uncaria guianensis* (Aubl.) J.F. Gmel. (Rubiaceae) and *Uncaria tomentosa* (Willd. ex Roem. & Schult.) DC. (Rubiaceae) are Amazonian plants popularly known as cat's claw. *U. guianensis* is found in Bolivia, Brazil, Colombia, Ecuador, Guyana, French Guyana, Peru, Suriname, and Venezuela, whereas *U. tomentosa* is found in Belize, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, Guatemala, Guyana, French Guyana, Honduras, Nicaragua, Panama, Peru and Venezuela. These species grow best in tropical and subtropical humid climates, in soils of alluvial origin and sandy loam or open clay texture, with abundant organic matter in poorly drained or flooded areas. Their barks, roots, and leaves have been traditionally used in the treatment of rheumatism, arthritis, gastrointestinal disorders, infections, wounds, and asthma, among other conditions. The harvest of *Uncaria* barks is an important income source for many Amazonian indigenous communities. For a more comprehensive review on ethnobotanical and ethnopharmacological aspects of *U. tomentosa* and *U. guianensis* (Honório et al., 2016).

Cat's claw is sold over the counter worldwide as an anti-inflammatory drug. The anti-inflammatory effects of *Uncaria* species have been attributed to alkaloids, which are the most important components of the plant (Honório et al., 2016). Nevertheless, triterpenes, flavonoids, and phenylpropanoids are also present (Pereira and Dantas, 2016; Zhang et al., 2015). The anti-inflammatory properties of *Uncaria* species make them suitable for the treatment of inflammatory diseases, such as asthma (Akinbami et al., 2012). In fact, we have previously shown that extracts from barks and leaves of *U. tomentosa* were effective in reducing the production of pro-inflammatory

cytokines and ameliorating lung mechanics in asthmatic mice (de Azevedo et al., 2018). In that study, pentacyclic alkaloids and phenolic compounds were the major constituents of the extracts. Similarly, the anti-inflammatory effect of an ethanolic extract of *U. guianensis* leaves was confirmed when it inhibited zymosan-induced paw edema and pleural exudation (Carvalho et al., 2006); however, *U. guianensis* was never evaluated for the treatment of asthma. Therefore, the hypothesis is that two extracts of leaves of *U. guianensis* (aqueous and hydroethanolic) can reduce bronchial inflammation, assessed by cell count and Th2 cytokine measurements in bronchoalveolar lavage and lung tissue, oxidative stress, assessed by measurement of total antioxidant capacity in serum, and bronchial hyperresponsiveness, assessed *in vivo*, in an animal model of allergic asthma.

## MATERIALS AND METHODS

All animal experiments were carried out at the Laboratory of Experimental Pulmonary Pathophysiology, Ribeirao Preto Medical School, University of Sao Paulo (FMRP-USP). The study was approved by the local institutional review board on animal experimentation (protocol #072/2013) and followed the ARRIVE guidelines (Kilkenny et al., 2014) and the EU Directive 2010/63/EU for animal experiments (European Parliament and Council, 2010).

### Plant material

*U. guianensis* was grown in the rural area of Jardinópolis, Sao Paulo, Brazil (latitude 21°4'33" S, longitude 47°44'48" W) with authorization from the Brazilian government. The material was identified by Dr. Pietro Giuseppe Delprete (Herbier de Guyane, Institut de Recherche pour le Développement, Cayenne, French Guiana) and a voucher specimen was deposited in the Herbarium of Medicinal Plants at the University of Ribeirao Preto (UNAERP, voucher #HPMU-3133).

### Plant extract preparation

The leaves were collected at 9 a.m., washed in water and dried with paper sheets; then, they were further dried in a circulating-air oven at 45°C for 24 h. After being completely dried, the leaves were powdered in a knife-mill and sieved to 40-mesh particle size.

Two extracts were prepared, aqueous (UGA) and hydroethanolic (UGH), at the Laboratory of Plant Biotechnology, UNAERP. For the aqueous extract (UGA), 100 g of powdered plant material were added to 1 L of boiling water; heating was turned off, and the mixture was left in infusion for 1 h; then the mixture was filtered in a paper filter and, finally, lyophilized. For the hydroethanolic extract (UGH), 100 g of powdered plant material were added to 1 L of ethanol 70% (v/v in water); the mixture was left in maceration for 10 days; then the mixture was filtered in paper filter and, finally, rotaevaporated.

### Quality control

The identities of the quinic acid and chlorogenic acid, present in the

extracts of *U. guianensis*, were confirmed by high-performance liquid chromatography-mass spectrometry (HPLC-MS) analysis using a Waters (Milford, MA, USA) Acquity UPLC *H-Class* system equipped with a PDA detector and a Waters Xevo TQ-S tandem quadrupole mass spectrometer with a Z-spray source operating in the positive mode. Both extracts were injected (5  $\mu$ L) in a Sigma-Aldrich Ascentis Express C<sub>18</sub> column (100  $\times$  4.6 mm i.d.; 2.7  $\mu$ m particle size) from SUPELCO Analytical. The mobile phase used for gradient elution consisted of 0.1% formic acid (solvent A) and methanol with 0.1% formic acid (solvent B) at a flow rate of 0.5 mL.min<sup>-1</sup>. The gradient elution program started with 3% B between 0 and 4 min, followed by 3-60% B between 4-15 min and 60-90% B between 15-19 min. Then, it returned to the initial condition (3% B) within the following 5 min. The source and operating parameters were optimized as follows: Capillary voltage = 3.4 kV; Z-spray source temperature = 150°C, desolvation temperature (N<sub>2</sub>) = 300°C, desolvation gas flow = 600 L.h<sup>-1</sup>, and mass range from *m/z* 100 to 700 in the full-scan mode.

Quantifications of quinic acid and chlorogenic acid in UGA and UGH were carried out by liquid chromatography tandem-mass spectrometry (HPLC-MS/MS) in multiple reaction monitoring (MRM) mode. Quinic acid (catalogue no. 46944-U, Supelco) was purchased from Sigma-Aldrich (São Paulo, SP, Brazil), while chlorogenic acid (catalogue no. 00500590, HWI Analytik GMBH) was purchased from Pharma Solutions. The working standard solutions were prepared by diluting the stock solutions (1.0 mg/mL) of the target compounds at the following concentrations: 5, 10, 50, 250, 1250, 2500 and 5000 ng/mL.

The identities of mitraphylline and isomitraphylline, present in the extracts of *U. guianensis*, were confirmed by UPLC-MS analysis using authentic standards of compounds on a Waters (Milford, MA, USA) Acquity UPLC *H-Class* system equipped with a PDA detector and a Waters Xevo TQ-S tandem quadrupole mass spectrometer with an electrospray source operating in the positive mode. The sample injection volume was 5  $\mu$ L in a Zorbax Eclipse XDB-C18 column (150  $\times$  4.6 mm i.d.; 3.5  $\mu$ m particle size) from Agilent. The mobile phase used for gradient elution consisted of 0.2% ammonium acetate (solvent A) and acetonitrile (solvent B) at a flow rate of 0.6 mL.min<sup>-1</sup>. The gradient elution program started with 35% B, and remained at 35% for 18 min, raised B to 50% in the following 14 min, remained at 50% B for 3 min, and returned to the initial condition (35% B) within the following 5 min. The source and operating parameters were optimized as follows: Capillary voltage = 3.2 kV, source temperature = 150°C, desolvation temperature (N<sub>2</sub>) = 350°C, desolvation gas flow = 600 L h<sup>-1</sup>, and mass range from *m/z* 100 to 600 in the full-scan mode.

Quantification of mitraphylline was carried out by UPLC-MS/MS in the multiple reaction monitoring (MRM) mode with the crude extract of *U. guianensis*. The reference standard solution was prepared by appropriate dilutions of the stock solution (1.0 mg/mL) with CH<sub>3</sub>OH, resulting in the following concentrations of mitraphylline: 5, 25, 50, 125, 250 and 500 ng/mL.

The crude extract sample was accurately weighed (1.0 mg) and dissolved in 1.0 mL of CH<sub>3</sub>OH, resulting at a concentration of 1.0 mg/mL. Then this stock solution was filtered through a Millipore filter (0.45  $\mu$ m) and a serial dilution with CH<sub>3</sub>OH was performed to obtain the concentrations of 50, 10, 1, 0.1 and 0.01  $\mu$ g/mL. Ten microliters of the crude extract solution were injected into an Ascentis Express C<sub>18</sub> column (100  $\times$  4.6 mm i.d.; 2.7  $\mu$ m particle size) from SUPELCO Analytical. The mobile phase used for gradient elution consisted of formic acid 0.1% as system A and Acetonitrile containing 0.1% of formic acid as system B. The flow rate was 0.5 mL/min and the gradient elution program started with 30% B, raised B to 90% in the following 3 min, remained at 90% B for 2 min, and returned to the initial condition (30% B) within the following 5 min. Triplicate injections were made for mitraphylline standard solution

and crude extract sample. The optimum condition of MRM was determined (Supplementary Table 1). The calibration curve obtained in MRM mode was used for quantification of mitraphylline and the concentration in the crude extract sample was expressed in  $\mu$ g/mL. For this purpose, each tested concentration was corrected using a dilution factor (20, 100, 1000, 10000 and 100000 times) from the stock solution at a concentration of 1000  $\mu$ g/mL. The data was acquired and processed using TargetLynx™ Application Manager software (Waters, Corporation).

## Mice

Specific pathogen-free, male Balb/C mice (6-8 weeks of age) were obtained from the local breeding facility of Ribeirão Preto Medical School, Ribeirão Preto, SP, Brazil. Water and food were provided *ad libitum*. Mice were housed in separate cages, grouped according to the treatment they were assigned to: SAL-SAL (sensitized with saline, challenged with saline), OVA-SAL (sensitized with ovalbumin, challenged with saline), SAL-UGA (sensitized with saline, challenged with *U. guianensis* aqueous extract), OVA-UGA (sensitized with ovalbumin, challenged with *U. guianensis* aqueous extract), SAL-UGH (sensitized with saline, challenged with *U. guianensis* hydroethanolic extract), and OVA-UGH (sensitized with ovalbumin, challenged with *U. guianensis* hydroethanolic extract).

## Allergen sensitization/challenge protocol

Briefly, mice were sensitized two times, seven days apart, with intraperitoneal (i.p.) injection of 10  $\mu$ g of ovalbumin (OVA; Sigma grade V, Sigma-Aldrich, Dorset, UK) plus 1 mg of aluminum hydroxide (Al(OH)<sub>3</sub>; Sigma-Aldrich, Dorset, UK). After one week, mice were challenged with intranasal instillation of OVA (10  $\mu$ g) under light anesthesia (ketamine and xylazine, i.p.) for three consecutive days. More details are given elsewhere (Borges et al., 2013).

## Treatment with extracts of *U. guianensis*

Aqueous and hydroethanolic extracts were administered for three consecutive days under light anesthesia (ketamine and xylazine, i.p.), beginning with the first challenge, at a dose of 100 mg/kg/day; control groups received saline on the same days.

## Assessment of *in vivo* respiratory mechanics

Measurements of respiratory parameters were performed as previously described (Borges et al., 2013; Hantos et al., 1992; Marchica et al., 2011). Briefly, 24 h after the last challenge, animals were anesthetized (xylazine and pentobarbital, i.p.) and they were then connected to a mechanical ventilator for small animals (FlexiVent, Scireq, Montreal, Canada) under a respiratory rate of 150 per minute and positive end-expiratory pressure (PEEP) of 3 cm H<sub>2</sub>O. They were also paralyzed with pancuronium bromide i.p. for measurement of lung mechanics. Bronchial hyperresponsiveness was measured at baseline and with increasing concentrations of aerosolized methacholine (Mch: 6.25, 12.5, 25, and 50 mg/mL; Hudson RCI ultrasonic nebulizer, Teleflex Medical, Temecula, USA). Total resistance (RRS), total elastance (ERS), central airway resistance (Rn), tissue resistance (G), and tissue elastance (H) were determined from curves with a coefficient of determination (COD)  $\geq$  0.85.

### Collection of bronchoalveolar lavage, blood and lung tissue

Bronchoalveolar lavage, blood, and lung tissue processing was previously described (Erel, 2004; Fonseca et al., 2017). Briefly, after the measurement of lung mechanics, the animals were disconnected from the ventilator and the bronchoalveolar lavage (BAL) was collected. Blood was then obtained from puncture of the right ventricle and serum was stored at  $-80^{\circ}\text{C}$  for determination of levels of IgE anti-OVA. Finally, the chest was opened, and blood was washed from the lungs. The right lung was stored in RNAlater (Qiagen, Austin, USA) at  $-80^{\circ}\text{C}$  for cytokine measurement. The left lung was insufflated with 10% buffered formalin under 25 cm  $\text{H}_2\text{O}$  of pressure for 25 min. It was then included in paraffin blocks for histological analysis.

### Total and differential cell counts in BAL

BAL samples were stained with trypan blue and used for total cell counts on Neubauer chambers. Then, samples were centrifuged (Cytospin IV, Thermo Scientific, Runcorn, Cheshire, EUA) and stained with a rapid panoptic staining kit (Laborclin, Pinhais, Brasil) for differential cell count (300 cells per sample).

### Cytokine levels in lung homogenate

From the right lung, 50 mg were homogenized with a protease inhibitor cocktail (Complete EDTA-free, Roche). The supernatant was collected and stored at  $-80^{\circ}\text{C}$  for cytokine levels measurement. Commercially available ELISA kits (BD Biosciences BD OptEIA™, San Diego, USA, and eBioscience, San Diego, USA) were used for measurement of IL-4, 5, 10 and 13, IFN- $\gamma$  and TGF- $\beta$  levels.

### Anti-ovalbumin IgE serum levels

Serum levels of anti-OVA IgE were measured by ELISA (BD OptEIA, BD Bioscience), as previously described (Fonseca et al., 2017).

### Histological analysis

Histological analysis was performed as previously described (Fonseca et al., 2017). Lung sections (5- $\mu\text{m}$  thick) were obtained and stained with hematoxylin-eosin (H&E). For morphological analysis, 4 to 5 airways from each animal with intact epithelium and maximum-to-minimum diameter ratio  $\geq 0.5$  were studied. The airways were digitally photographed at 200 $\times$  for computer-assisted analyses. Tissue inflammation was semi-quantified at the basal membrane by using a previously described score (Ford et al., 2001): 0, absent; 1, a few; 2, a single layer; 3, two to four layers; and 4, five, or more layers of inflammatory cells. Each airway was scored by three different persons, blinded to the allocation.

### Total antioxidant capacity (TAC)

TAC was quantified as previously described (Erel, 2004), in which oxidation of 2,2V-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS+) is analyzed. In the presence of antioxidants, the solution loses its color. The results are expressed as Trolox equivalents.

### Statistical analysis

All data were summarized with means and standard deviations, medians and interquartile ranges, or counts (proportions), where applicable. Results of the different treatment groups were compared with 1-way or 2-way repeated-measurements ANOVA, with Bonferroni correction for multiple comparisons. Significance level was set at 0.05. All statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA).

## RESULTS

### Identification and quantification of major compounds in the extracts

Both extracts contained mitraphylline and isomitraphylline (Supplementary Figure 1), although the concentration of mitraphylline in UGH extract ( $9.7 \pm 0.6 \mu\text{g/mL}$ ) was greater than in UGA extract ( $4.6 \pm 1.0 \mu\text{g/mL}$ ). Phenolic compounds such as quinic and chlorogenic acids were also detected in *U. guianensis* extracts (Supplementary Figure 2 and Supplementary Figure 3). Quinic acid was detected in higher concentrations in UGA ( $288.4 \pm 20.8 \mu\text{g/mL}$ ) than in UGH extract ( $141.3 \pm 20.1 \mu\text{g/mL}$ ), while chlorogenic acid was detected in much lower concentrations in both extracts, being higher in UGH ( $15.0 \pm 1.0 \mu\text{g/mL}$ ) than in UGA extract ( $9.9 \pm 1.2 \mu\text{g/mL}$ ).

### Total and differential cell counts

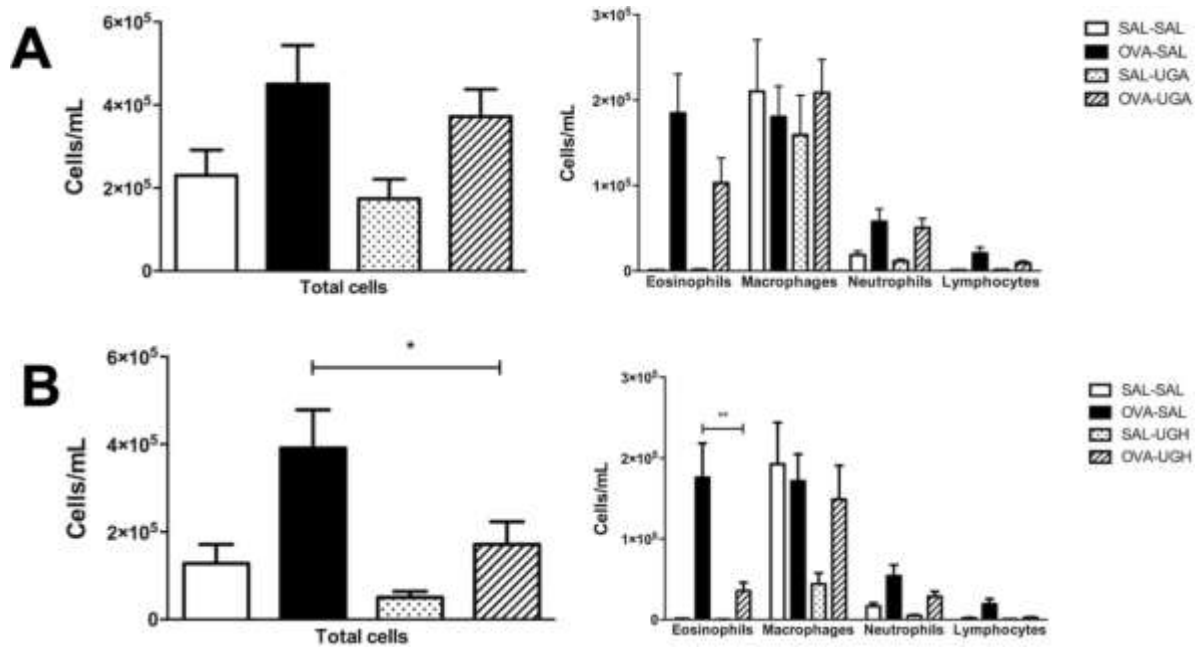
Asthmatic animals had a significantly higher number of total inflammatory cells and eosinophils in BAL, compared to controls. Only the administration of UGH extract significantly decreased the number of inflammatory cells and eosinophils in BAL (Figure 1).

### *In vivo* measurements of lung mechanics

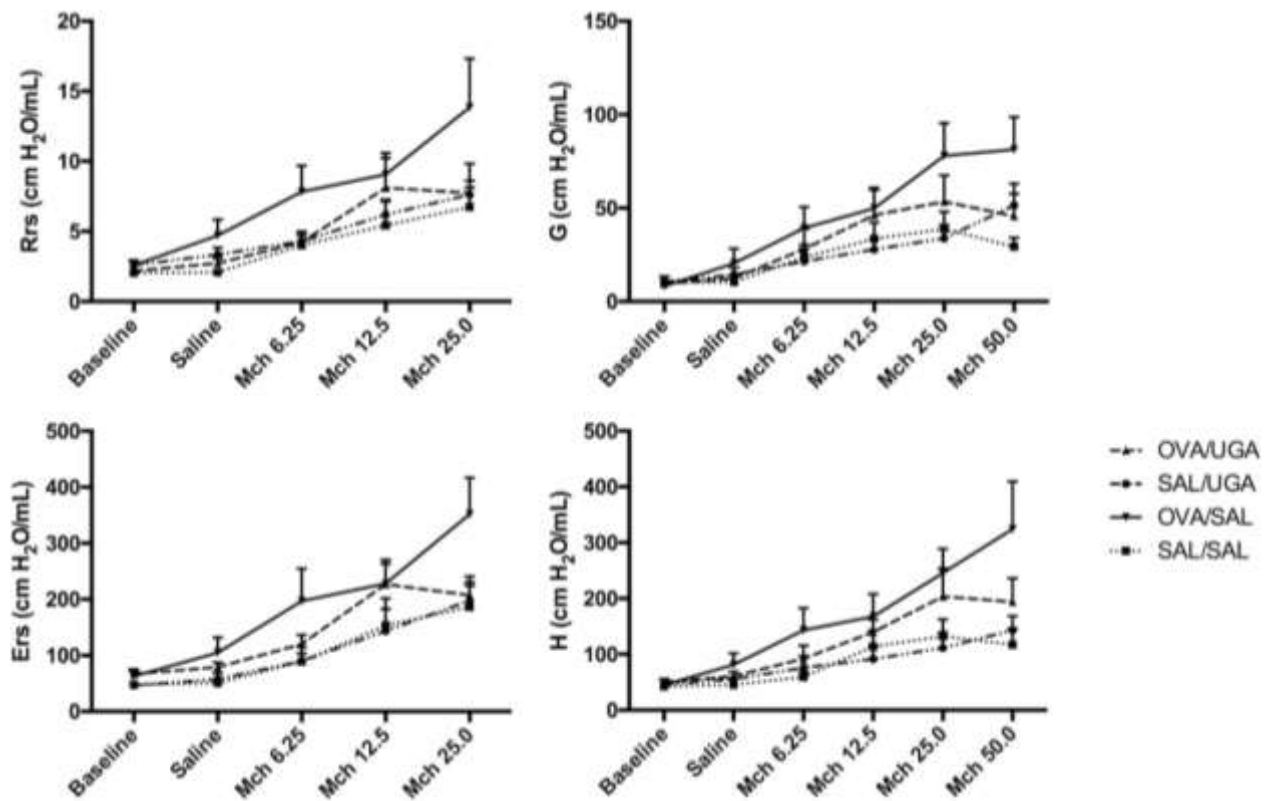
Results of lung mechanics are shown in Figures 2 and 3. OVA-challenged mice had a significant increase in bronchial hyperresponsiveness when compared to saline-challenged mice. Of note, treatment with UGH extract significantly attenuated bronchial hyperresponsiveness, demonstrated by a decrease in RRS, ERS, G, and H. Treatment with UGA extract did not reduce bronchial hyperresponsiveness when compared to OVA-challenged mice.

### Cytokine levels in lung homogenate

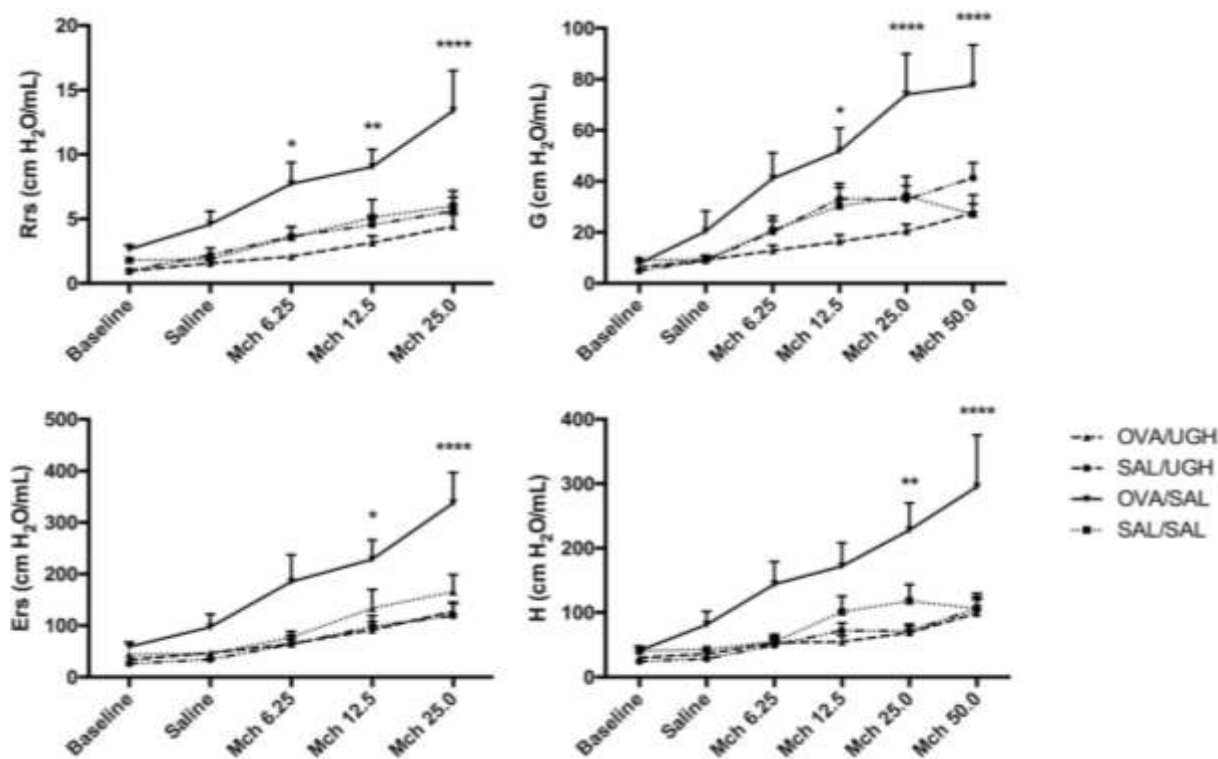
Levels of IL-4, IL-5, IL-10, IL-13, IFN- $\gamma$ , and TGF- $\beta$  in lung homogenate are shown in Figure 4. Levels of IL-13 were significantly increased in OVA-challenged mice when



**Figure 1.** Total and differential cell counts in bronchoalveolar lavage (BAL) in animals treated with aqueous (UGA, panel A) or hydroethanolic (UGH, panel B) extract of *Uncaria guianensis*. Only significant treatment effects are marked. Legend: \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; SAL, normal saline; OVA, ovalbumin.



**Figure 2.** *In vivo* measurements of lung mechanics under increasing concentrations of aerosolized metacholine (Mch) in animals treated with aqueous extract of *Uncaria guianensis* (UGA). SAL, Normal saline; OVA, ovalbumin.



**Figure 3.** *In vivo* measurements of lung mechanics under increasing concentrations of aerosolized metacholine (Mch) in animals treated with hydroethanolic extract of *Uncaria guianensis* (UGH). Only significant treatment effects are marked. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*\*,  $p < 0.0001$ ; SAL, normal saline; OVA, ovalbumin.

compared to saline-challenged mice. Treatment with UGA or UGH extracts decreased IL-13 levels significantly, when compared to OVA-challenged mice. Levels of IL-4, IL-5, IL-10, IFN- $\gamma$ , and TGF- $\beta$  did not reach statistical significance among groups.

#### Anti-OVA IgE, TAC and tissue inflammation

Levels of anti-OVA IgE and TAC, and inflammation airway score did not reach statistical significance among groups (Figures 5 and 6).

#### DISCUSSION

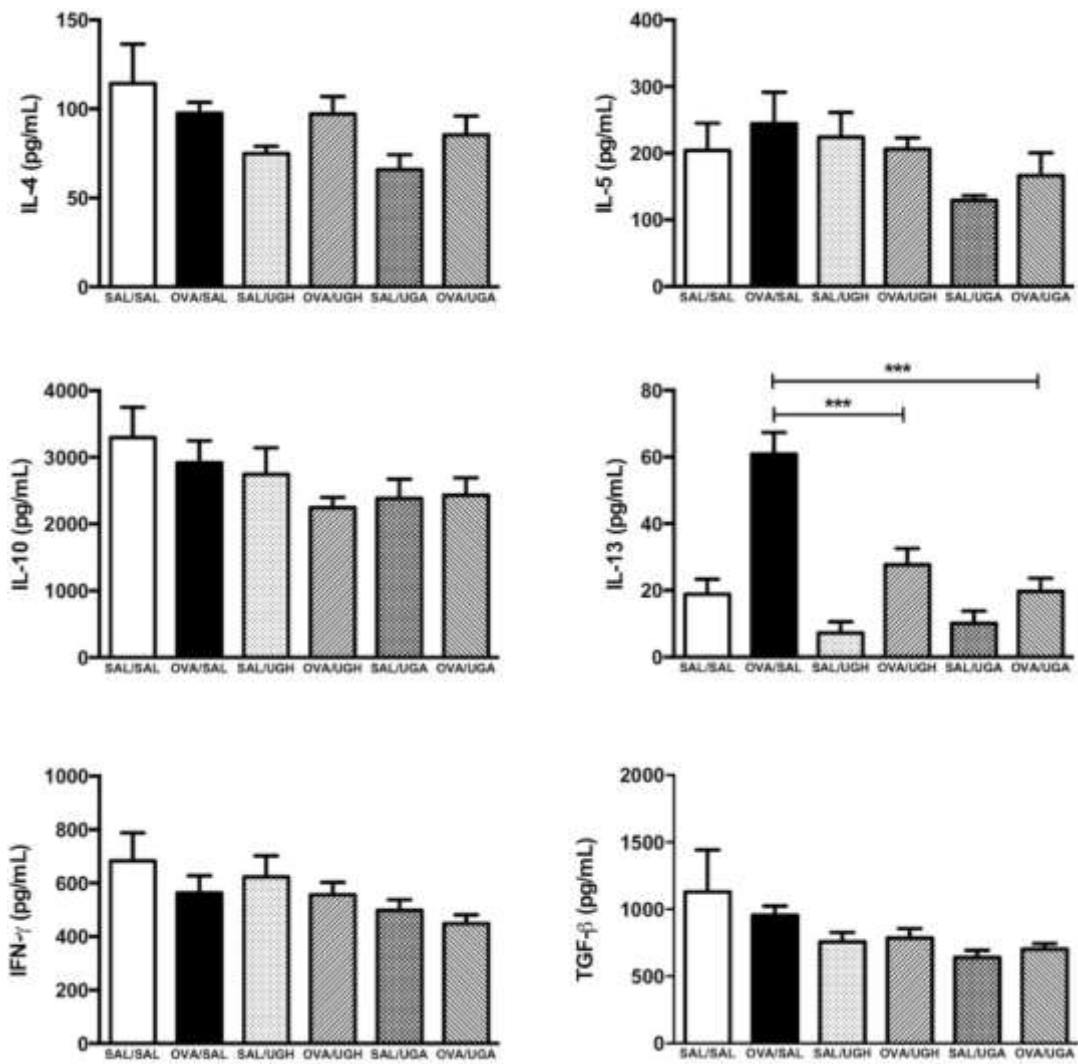
This study showed herein, for the first time, that a hydroethanolic extract from leaves from *U. guianensis* (UGH) significantly decreased bronchial hyperresponsiveness, lowered BAL cellularity and eosinophilia, and diminished levels of IL-13 in lung homogenate of asthmatic mice. On the other hand, the aqueous extract (UGA) was not effective in attenuating asthma-like characteristics.

The anti-inflammatory activity of extracts from leaves of

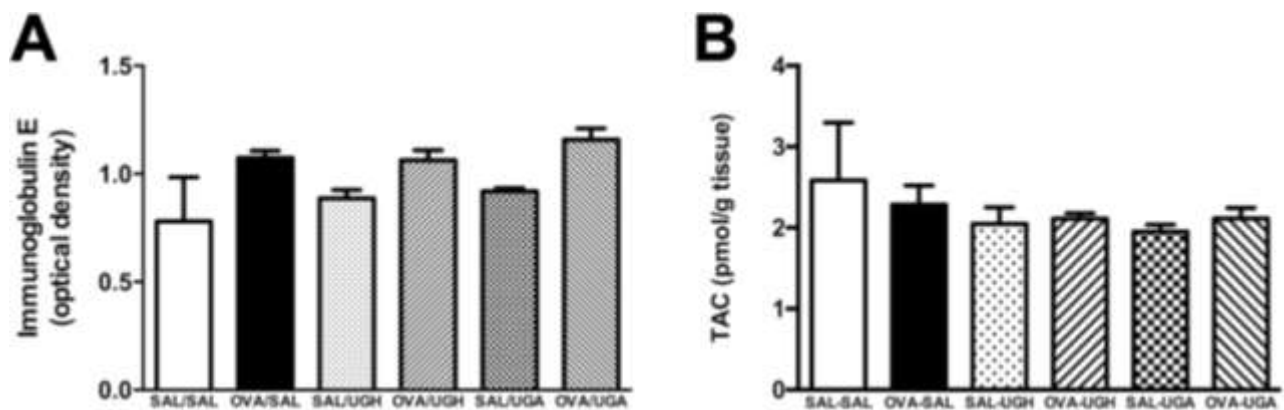
*Uncaria* species has been extensively demonstrated *in vitro* and *in vivo*, and the mechanisms of action include inhibition of nuclear factor  $\kappa$ -B (NF- $\kappa$ B) activation, among others (Aguilar et al., 2002). In fact, anti-inflammatory activity was reported for all the compounds identified in the extracts of *U. guianensis*, mitraphylline (Montserrat-De La Paz et al., 2016; Rojas-Duran et al., 2012) and chlorogenic (Hwang et al., 2014; Yun et al., 2012) and quinic acids (Åkesson et al., 2005).

The group has previously reported a similar chemical profile while analyzing extracts of barks and leaves from *U. tomentosa*, especially mitraphylline (de Azevedo et al., 2018). Although the anti-inflammatory effect of *Uncaria* species can be mostly attributed to its mitraphylline content, other alkaloids are present and may contribute to the effect, such as rhynchophylline, isorhynchophylline, speciophylline and uncarine E (Sandoval et al., 2002). Additionally, a significant amount of quinic acid, which is a compound responsible for the anti-inflammatory activity in commercial extracts of *U. tomentosa* was detected (Åkesson et al., 2005). Therefore, it was speculated that the use of quinic acid as an active marker for standardization of extracts of *U. guianensis* should be considered.

Interestingly, in an animal model of OVA-induced

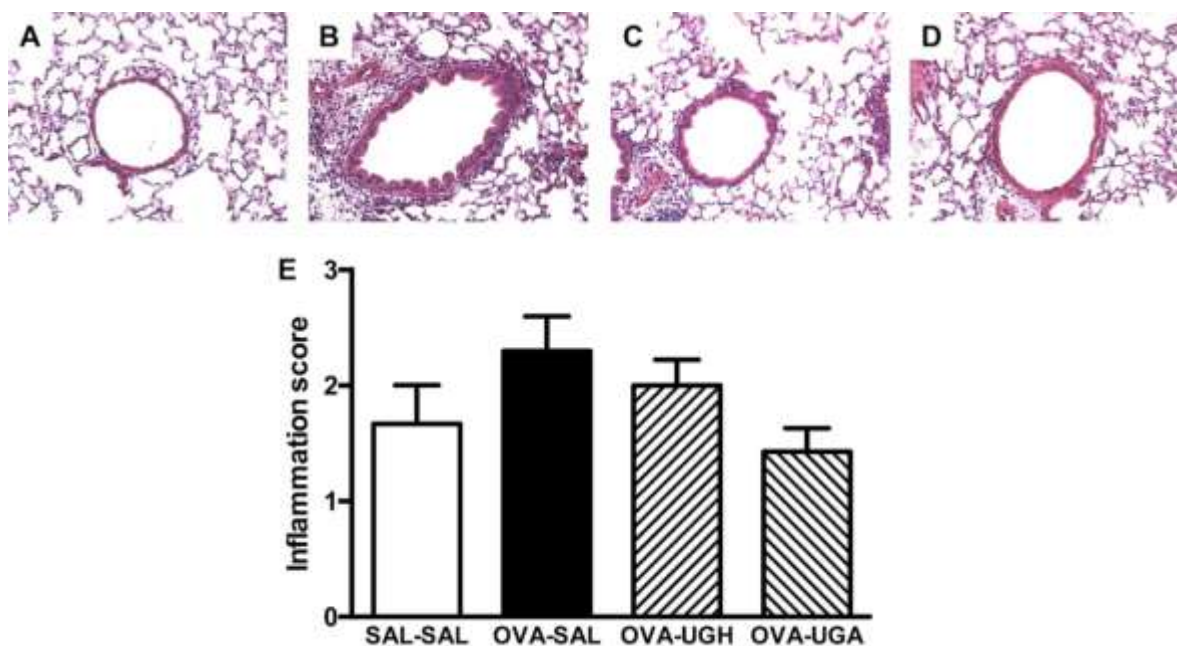


**Figure 4.** Levels of cytokines in lung homogenate. \*\*\*,  $p < 0.001$ ; SAL, normal saline; OVA, ovalbumin; UGH, hydroethanolic extract of *Uncaria guianensis*; UGA, aqueous extract of *U. guianensis*.



**Figure 5.** Panel A: Anti-ovalbumin IgE serum levels. Panel B: Total antioxidant capacity (TAC) in lung tissue. SAL, normal saline; OVA, ovalbumin; UGH, hydroethanolic extract of *Uncaria guianensis*; UGA, aqueous extract of *U. guianensis*.





**Figure 6.** Quantification of inflammatory cells in lung tissue. Panels A, B, C, and D are selected airways representing groups SAL/SAL (A), OVA/SAL (B), OVA/UGH (C), and OVA/UGA (D). SAL, normal saline; OVA, ovalbumin; UGH, hydroethanolic extract of *Uncaria guianensis*; UGA, aqueous extract of *U. guianensis*.

allergic asthma, administration of chlorogenic acid significantly decreased eosinophilia and levels of IL-4, IL-5, and TNF- $\alpha$  in the lungs, and also decreased serum anti-OVA IgE (Kim et al., 2010).

Considering that mitraphylline, quinic acid, and chlorogenic acid all present anti-inflammatory activity, it is therefore speculated that all these compounds, combined, were responsible for the effects observed. This, however, remains to be proven.

The anti-inflammatory activity of *U. guianensis* has also been demonstrated in clinical trials. In a randomized, double-blind, placebo-controlled clinical trial, aqueous extract of *U. guianensis* significantly reduced the pain associated with activity within the first treatment week, as compared to placebo. In addition, the treatment with *U. guianensis* did not cause deleterious effects on blood or liver functions, or side effects (Piscoya et al., 2001).

In this study, *U. guianensis* significantly decreased total cell and eosinophil counts in BAL. Other authors have also shown, in different models, a similar effect of *U. guianensis* on eosinophils (Carvalho et al., 2006). Th2 cytokines such as IL-4 and IL-13 are involved in asthmatic bronchial hyper responsiveness (Oeser et al., 2015) and eosinophil recruitment (Hogan, 2007), which play an important role in the pathogenesis of asthma (Davoine and Lacy, 2014). Interestingly, a reduction in IL-13 levels on lung homogenate in response to UGH and UGA was observed. It was hypothesized that the

reduction in IL-13 was the driver to the decreased eosinophil counts in BAL, since IL-13 is involved in eosinophil recruitment (Hogan, 2007).

In fact, recent clinical trials on biologic therapies targeting IL-4 and IL-13 have found promising results (Parulekar et al., 2018). However, other Th2 cytokines were not affected by this protocol. Further studies with higher doses or longer treatment protocols should be done to better clarify *U. guianensis* effects on Th2 cytokines.

It was also shown that UGH attenuated bronchial hyper responsiveness and improved lung mechanics. The attenuation of bronchial hyper responsiveness observed with *U. guianensis* may be related to the decrease of total cells and eosinophils in BAL and IL-13 levels. Overall, UGH elicited better results than UGA, which can be explained by different extraction methods, which, in turn, may produce extracts with different compositions. Indeed, fractions of extracts of *U. guianensis* with different polarities had different effects (Urdanibia et al., 2013). In addition, results showed that concentrations of chlorogenic acid and mitraphylline were higher in UGH, compared to UGA.

Regarding toxicity of *U. guianensis*, little information is available, but there are some reports on the toxicity of *U. tomentosa* (Mukhtar et al., 1992; Sheng et al., 2000). It is noteworthy that while *Uncaria* species seem to be non-toxic, some of its purified compounds can be toxic (Zhou

et al., 2016). In addition, the alkaloids present in *Uncaria* species can also have effects on the central nervous system (Shi et al., 2003); on that account, it is mandatory that the chemical profile of any *Uncaria* extract be well known before conducting preclinical or clinical trials.

Limitations of this study include the short treatment course. Although UGH attenuated the main characteristic of asthma, a longer treatment protocol could have resulted in effects on other inflammatory markers and cytokines. Additionally, a dose-response curve was not performed. Therefore, smaller doses could have been equally effective, while higher doses could have resulted in effects on other inflammatory markers.

## Conclusion

In conclusion, only the hydroethanolic extract from leaves of *U. guianensis* was effective in treating ovalbumin-induced asthma in mice, decreasing bronchial hyperresponsiveness and inflammation. The anti-inflammatory effect was observed in this study can be attributed to the major compounds present in the extracts (quinic acid, chlorogenic acid, mitraphylline and isomitraphylline), but not exclusively. This study supports further studies on the efficacy of *U. guianensis* for asthma treatment.

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## CONFLICT OF INTEREST

All contributing authors declare no conflicts of interest.

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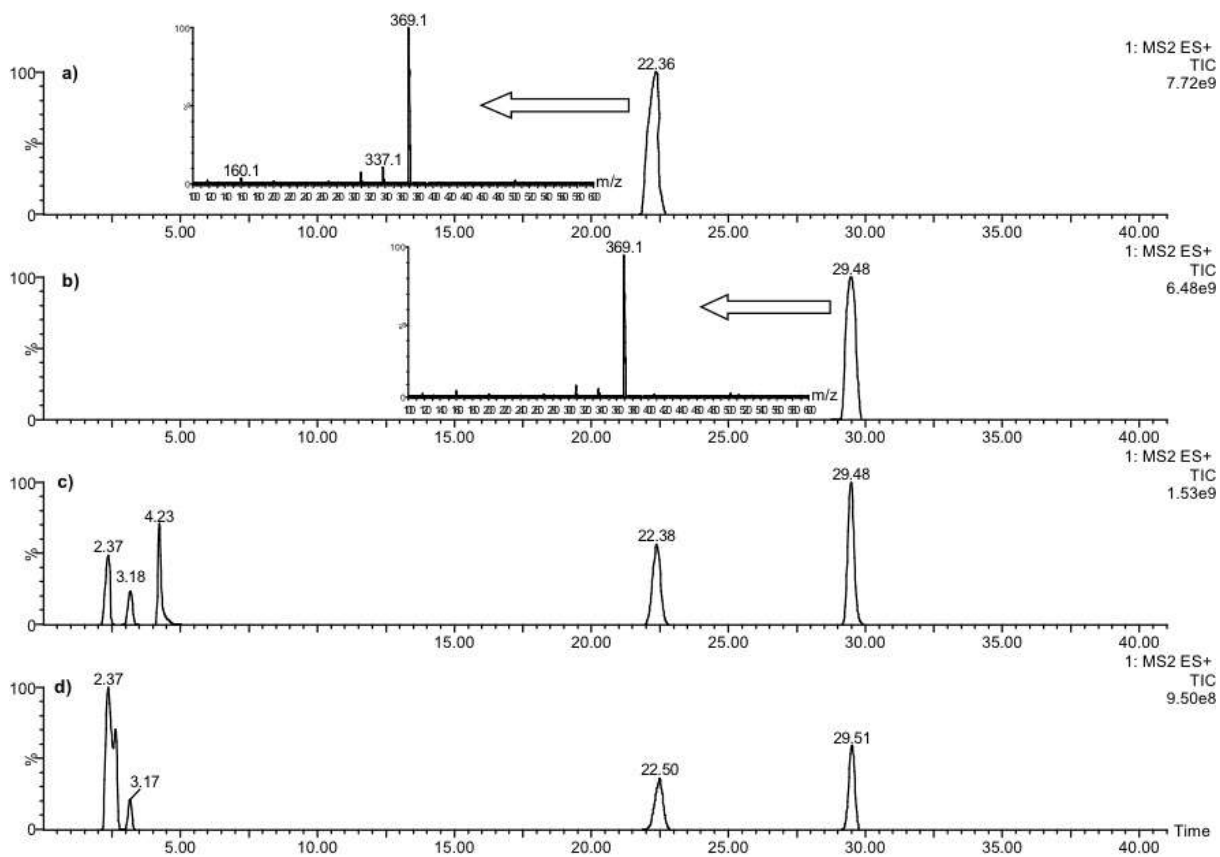
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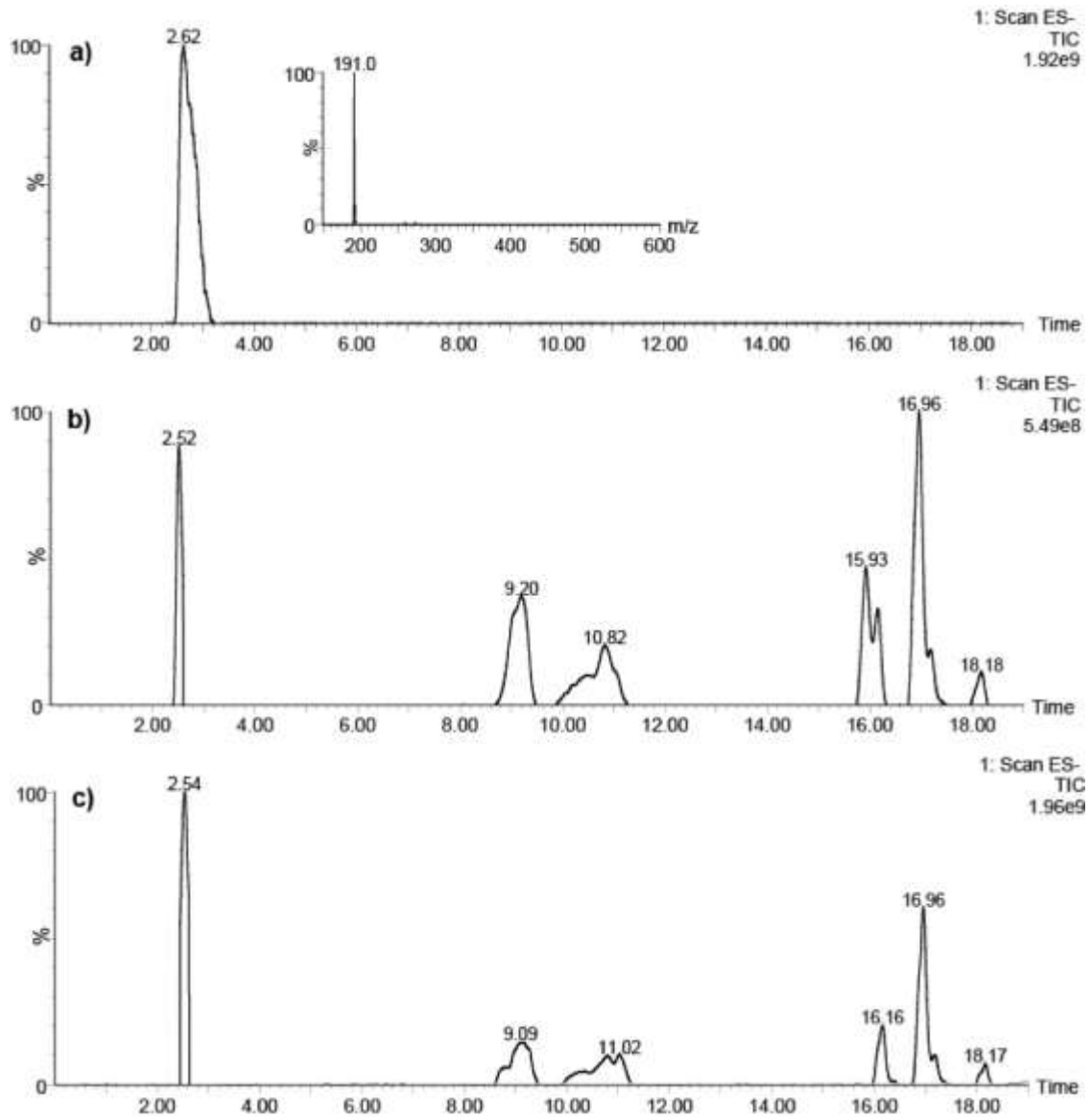
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**Supplementary Table 1.** Ion transitions, instrument settings and retention times for each quantified compound.

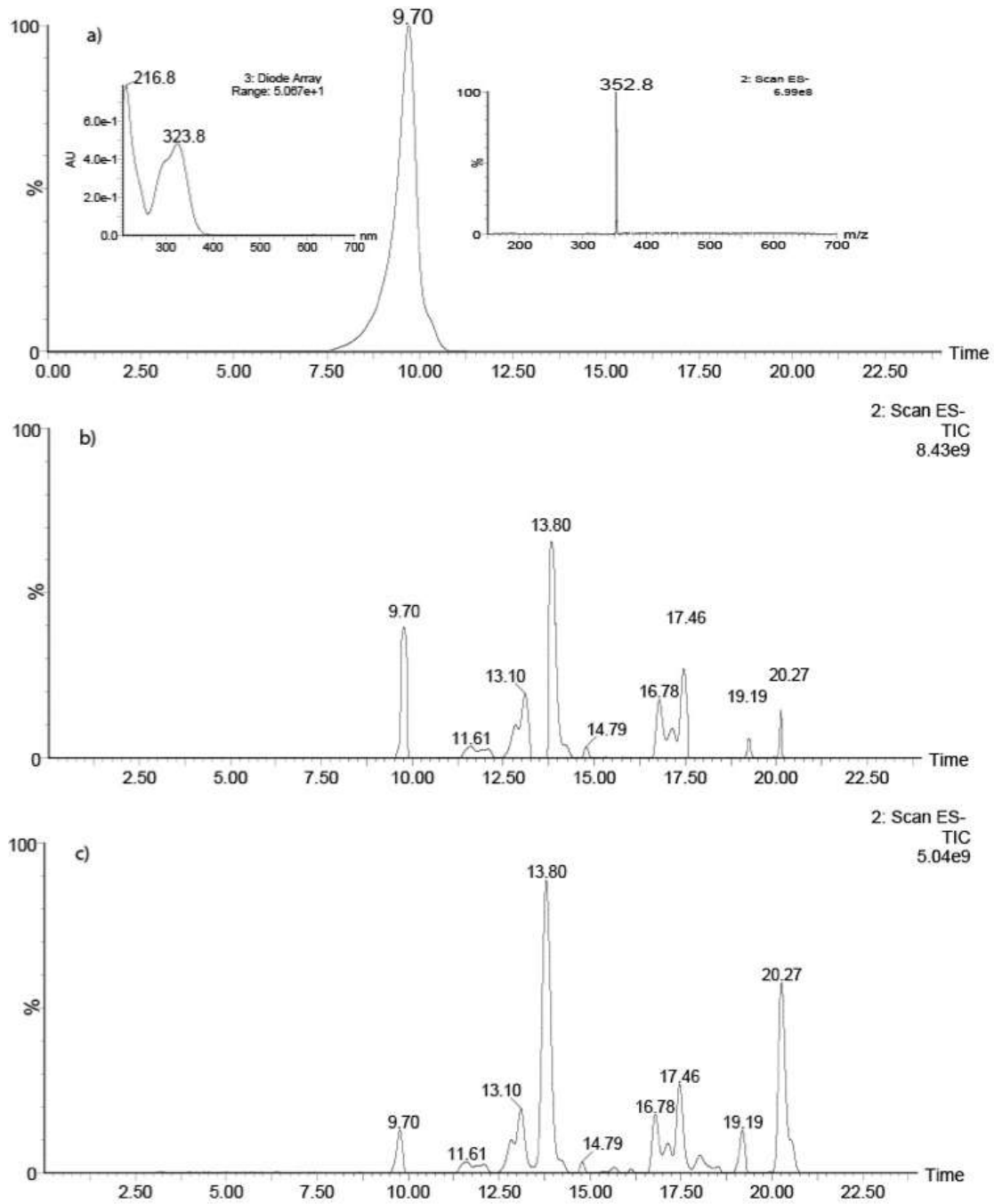
Compound	Precursor ion ( $m/z$ )	Product ion ( $m/z$ )	DP <sup>a</sup> (V)	CE <sup>b</sup> (eV)	RT <sup>c</sup> (min)
Quinic acid	191.1	85.2	40	25	1.92
Chlorogenic acid	353.2	191.3	40	25	2.11
Mitraphylline	421.4	201.1	2	26	4.95

<sup>a</sup>DP, Declustering Potential; <sup>b</sup> CE, Collision Energy (Ar was used as collision gas); <sup>c</sup> RT, Retention time.

**Supplementary Figure 1.** UPLC-MS chromatograms of mitraphylline (a) and isomitraphylline (b) standards, and of hydroethanolic leaf extract (UGH) (c) and aqueous leaf extract (UGA) (d) from *U. guianensis*.



**Supplementary Figure 2.** UPLC-MS chromatograms of quinic acid (a) standard, and of hydroethanolic leaf extract (UGH) (b) and aqueous leaf extract (UGA) (c) from *U. guianensis*.



**Supplementary Figure 3.** UPLC-MS chromatograms of chlorogenic acid (a) standard, and of hydroethanolic leaf extract (UGH) (b) and aqueous leaf extract (UGA) (c) from *U. guianensis*.