



## COMPARATIVE ACTIVITY OF TOTAL POLYPHENOLS AND ANTIOXIDANT COMPOUNDS FROM UNCARIA TOMENTOSA ENHANCED WITH CITRIC ACID

ATIVIDADE COMPARATIVA DE POLIFENÓIS TOTAIS E COMPOSTOS ANTIOXIDANTES DA UNCARIA TOMENTOSA POTENCIADA COM ÁCIDO CÍTRICO 

ACTIVIDAD COMPARATIVA DE POLIFENOLES TOTALES Y COMPUESTOS ANTIOXIDANTES DE UNCARIA TOMENTOSA POTENCIALIZADA CON ÁCIDO CÍTRICO 

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### ABSTRACT

*Uncaria tomentosa*, a climbing vine notable for containing high concentrations of oxindole alkaloids and phenolic compounds, is commonly used in traditional medicine as an anti-inflammatory and antioxidant agent. Also, the citric acid is a food additive widely used for conservation, due to its low cost. In this way, this study aims to evaluate the content of phenolic compounds from *Uncaria tomentosa* and investigate its antioxidant activity when citric acid, at different concentrations, is added to the extract. For this purpose, a gradient of citric acid concentrations was established, and the antioxidant profile from a aqueous extracts of the plant leaves and bark was analyzed by Folin-Ciocalteu essay; inhibition of the free radical of 2,2-diphenyl-1-picrylhydrazyl (DPPH); ferric reducing antioxidant power (FRAP), and scavenging capacity of cationic free radicals of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). The results showed a synergistic effect between citric acid and antioxidant compounds from *Uncaria tomentosa*, presenting highly statistical significance, the synergistic effect was more efficient in the bark than in the leaves.

**Keywords:** ABTS. DPPH. Folin-Ciocalteu. FRAP. Synergistic Effect.



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## 1 INTRODUCTION

The oxidation of food products is one of the major causes of chemical degradation, due to the presence of free radicals that can cause rancidity, in which properties such as taste, smell, texture and color are modified (Suja *et al.*, 2004; Antolovich *et al.*, 2002). Studies based on natural antioxidants have been increased due to the low safety provided by common synthetic antioxidants. Alternatively, the effectiveness of phytochemicals and the fact that these compounds can positively affect the pathology of chronic diseases and the aging process have been emerging as promising natural antioxidants. In addition, consumers believe that natural products are safer than synthetical ones, and more acceptable as natural antioxidants (Dorman & Hiltunen, 2004).

The antioxidant activity *in vitro* can be carried out by means of free radical trapping methods. Among these methods, it can be described the ferric reducing antioxidant power (FRAP), which reduces the capacity of the phenolic reagent of Folin-Ciocalteu, purifying effects in relation to 1,1-diphenyl-2-picrylhydrazyl (DPPH), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (Prior & Schaich, 2005; Brewer, 2011). They are based on the principle of transfer of a single electron and are low-cost and easily accessible, commonly applied in food technology (Pérez-Burillo *et al.*, 2018; Santos *et al.*, 2018).

*U. tomentosa* is a climbing vine native from the Amazon Forest and its active principles are divided into two groups: oxindole alkaloids and phenolic acids (Bors *et al.*, 2011). The polyphenols have the ability to inhibit free radicals, specifically flavonoids, phenolic acids and tannins (Sand-oval *et al.*, 2000). Also, they are used in food conservation, due to some bioactive properties that are beneficial to human health, making it possible for these molecules to replace some synthetic food additives (Caleja *et al.*, 2016; Oak *et al.*, 2005).

Citric acid, or 2-hydroxy-1,2,3-propanetricarboxylic acid, is a weak organic acid, commonly used as a natural preservative to increase the shelf life of products in the food industry (Abdel-Salam *et al.*, 2018; Muñoz-Bernal *et al.*, 2017). Studies have suggested that the use of citric acid can inhibit color change and oxidation effects on vegetable tissue, reducing losses in the appearance of products (Rocculi *et al.*, 2007). Also, the combination of citric acid with other antioxidant components could delay the deterioration of some fruits like mango, inhibiting metabolic reactions by the presence of oxygen (Chiumarelli *et al.*,



2010). It has been previously demonstrated that the interaction of several antioxidants together demonstrated a more efficient synergistic behavior and better stability, regarding the oxidation activity than when antioxidants are applied individually (de Guzman *et al.*, 2009, Tang *et al.*, 2010; Tang *et al.*, 2008; Marinova *et al.*, 2008; Becker *et al.*, 2007; Sharma *et al.*, 2007; Erhan *et al.*, 2006; Rawat *et al.*, 2015).

In this scenario, this study aims to examine the content of phenolic compounds and antioxidant activity of bark and leaves extracts from *U. tomentosa* compounds with an addition of different concentrations of citric acid, in order to synergistically enhance the antioxidant activity. The study was performed by using fast, cheap and classical antioxidant methods, such as Folin-Ciocalteu reducing capacity, inhibition of the free radical of 2,2-diphenyl-1-picrylhydrazil (DPPH), ferric reducing antioxidant power (FRAP), and scavenging capacity of cationic free radicals of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS).

## 2 MATERIALS AND METHODS

### 2.1 VEGETAL MATERIAL

The leaves (L) and barks (B) used were collected from Ucayali region, Peru. This material was washed with 5% sodium hypochlorite and ultrapure water, then stored, protected from light, and refrigerated at 3 °C. For the extraction, samples were vortexed (Scientific Industries Inc., USA), centrifuged (BOECO, Germany) at 3,000 rpm for 30 min, stored in an orbital shaker (BOECO, Germany) in constant agitation of 100 rpm, at room temperature, for 24 h subsequently. The extraction was carried out with a Soxhlet equipment, for 5 h, in a proportion of 95:5 of water:ethanol (v/v) (Adaramola & Onigbinde, 2017). All procedures were performed in the dark. To improve the antioxidant capacity, increasing concentrations of citric acid solutions (0, 5, 10, 15, 20, 30 and 50 mg mL<sup>-1</sup>) were used to promote the synergistic effect, and incubated for 24 h.

### 2.2 CHEMICAL REAGENTS

Citric acid; phospho-molybdenum-tungstic acid (Folin-Ciocalteu reagent); sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>); gallic acid; 2,2-diphenyl-1-picrylhydrazyl (DPPH); 6-hydroxy-2,5,7,8-



tetramethylchroman-2-carboxylic acid (Trolox); 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ); ferric chloride (FeCl<sub>3</sub>); hydrochloric acid (HCl); sodium acetate; 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS), and potassium persulfate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>) were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO). Ethanol (96%) was purchased from Diproquim Productos Químicos (Arequipa, Peru).

## 2.3 TOTAL POLYPHENOLS ASSAY

According to Farahani *et al.* (2019), modified and adapted for this study, this method generates a change in coloration from yellow to a bluish shade, in which its intensity depends on phenol concentration in the sample (Fig. 1A). Therefore, by measuring the absorbance of the resulting product, the concentration can be determined based on a previous calibration curve of gallic acid. This preparation was performed adding 2 mL of a 2% solution of Na<sub>2</sub>CO<sub>3</sub> (w/v), 200 µL of the extract studied and 200 µL of Folin-Ciocalteu reagent to test tubes, in darkness for 30 min. The absorbance was read at 750 nm in a spectrophotometer (BOECO, Germany). The results were expressed in milligrams of gallic acid per gram of *Uncaria tomentosa* extract (EUT) (mg GAE/g EUT).

## 2.4 ANTIOXIDANT ACTIVITY METHODS

### 2.4.1 DPPH radical scavenging capability

For this essay (Fig. 1B), according to the method developed by Grimalt *et al.* (2018), adapted for this study, 3.9 mL of an ethanolic solution of DPPH (25 mg L<sup>-1</sup>) was taken with 100 µL of sample, during 2 h in darkness, and analyzed in a spectrophotometer at a wavelength of 517 nm. The percentage of inhibition of free radicals was calculated following the equation below:

$$I\% = \left[ \frac{Abs_{DPPH} - Abs_{sample}}{Abs_{DPPH}} \right] \quad (\text{Eq. 1})$$

where "Abs<sub>DPPH</sub>" is the absorbance of the DPPH stock solution, and "Abs<sub>sample</sub>" is the absorbance value obtained from samples at the time of the reaction decay. Therefore, the results were expressed as percentage of radical inhibition.

### 2.4.2 Ferric Reducing Antioxidant Power (FRAP)

The present protocol was adapted from Zaouali *et al.* (2010). Briefly, a 10 mmol L<sup>-1</sup> solution of the reagent TPTZ was prepared, using 0.0312 g of TPTZ reagent into a 10 mL flask, which was filled with a solution of 40 mmol L<sup>-1</sup> of hydrochloric acid. To obtain the complete FRAP solution, the resulting solutions were mixed in the following proportions: 2.5 mL of the ferric chloride solution (20 mmol L<sup>-1</sup>), 2.5 mL of the TPTZ solution, and 25 mL of the sodium acetate buffer. The reagent was kept in a thermostatic bath at 37 °C. For the reaction, 90 µL of samples, 270 µL of distilled water and 270 µL of the complete FRAP solution were added. The absorbance of this reaction was measured at 595 nm, with subsequent readings each 30 min (Fig. 1C).

### 2.4.3 ABTS - Cationic Free Radical Scavenging Capacity

This method is faster and more versatile to measuring the antioxidant activity of an extract, due to its high reproducibility rates. Also, the essay is effective to quantify both polar and non-polar compounds (Kuskoski *et al.*, 2005). To generate the radical, a solution of ABTS at a concentration of 7 mmol L<sup>-1</sup> was mixed with a solution of potassium persulfate at 2.45 mmol L<sup>-1</sup>. The mixture was incubated at room temperature in the dark for 16 h. After, an equal volume of phosphate buffer was added, and the preparation was placed in a thermostatic bath at 30°C. A volume of 2.97 µL of this solution was collected and placed in test tubes. Subsequently, 30 µL of the extracts were added. These samples were also diluted in ethanol to measure the absorbance at 734 nm. The white reagent consisted of the solution of the stable radical ABTS●<sup>+</sup> dissolved in ethanol (Fig. 1D). The results were expressed in TEAC (Trolox equivalent antioxidant capacity) (mmol L<sup>-1</sup> TEAC/g of EUt).

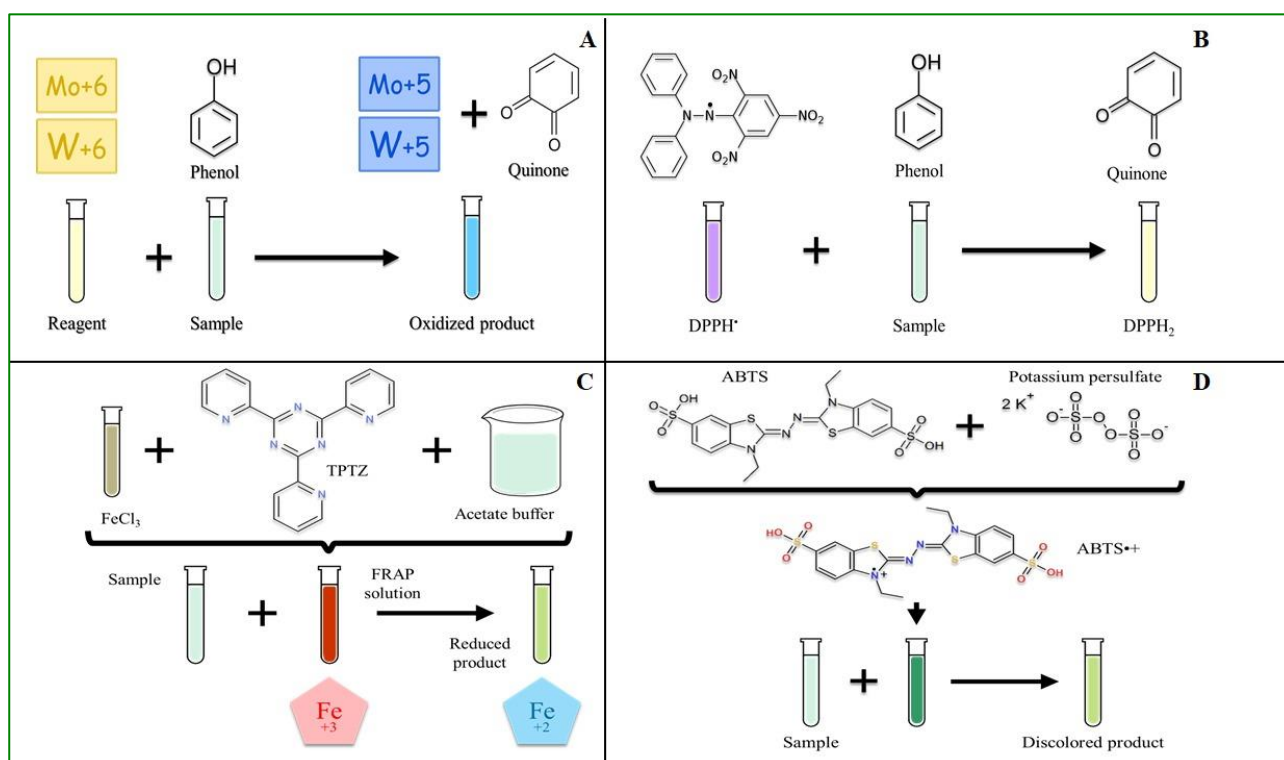
## 2.5 STATISTICAL ANALYSIS

The means were analyzed using ANAVA method in the InfoStat-Statistical Software 2018. In order to investigate the relationships between the study variables, the Pearson Correlation Analysis was adopted, which indicates a positive or negative existence between two variables, and  $\alpha = 5\%$  (correlation coefficient) was adopted to verify the significance. The analysis were performed by Statistica and Sigmaplot software. The principal component multivariate analysis (PCA) was carried out to verify the group of different responses into



three distinct of citric acid concentrations in *U. tomentosa*. The variables adopted were the biochemical parameters: polyphenols and antioxidant activity; biometric parameters: leave and bark of the sample (aerial part) and score plot. In this way, it was possible to simulate the relationship between these variables (Vítolo *et al.*, 2012). For the analysis, the Statistica software was executed using the minimum absorption criterion of 80% in the first two main components and, thus, simulating a possible relationship between the variables (Cruz *et al.*, 2004; Souza *et al.*, 2018).

**Figure 1** – Theoretical basis of the methods used in this work to evaluate the antioxidant profile: (a) Folin-Ciocalteu; (b) DPPH free radical scavenging; (c) Ferric Reducing Antioxidant Power; (d) ABTS cationic free radical scavenging.



Source: the authors.

### 3 RESULTS AND DISCUSSION

#### 3.1 FOLIN-CIOCALTEU REDUCING CAPACITY

The concentration of total polyphenols in EUt from leaves and barks varied significantly (Fig. 2), presenting, as initial values from the pure extracts, 449.1 mg GAE g<sup>-1</sup> EUt and 379.12 mg GAE g<sup>-1</sup> EUt, respectively. The total value of polyphenols observed in



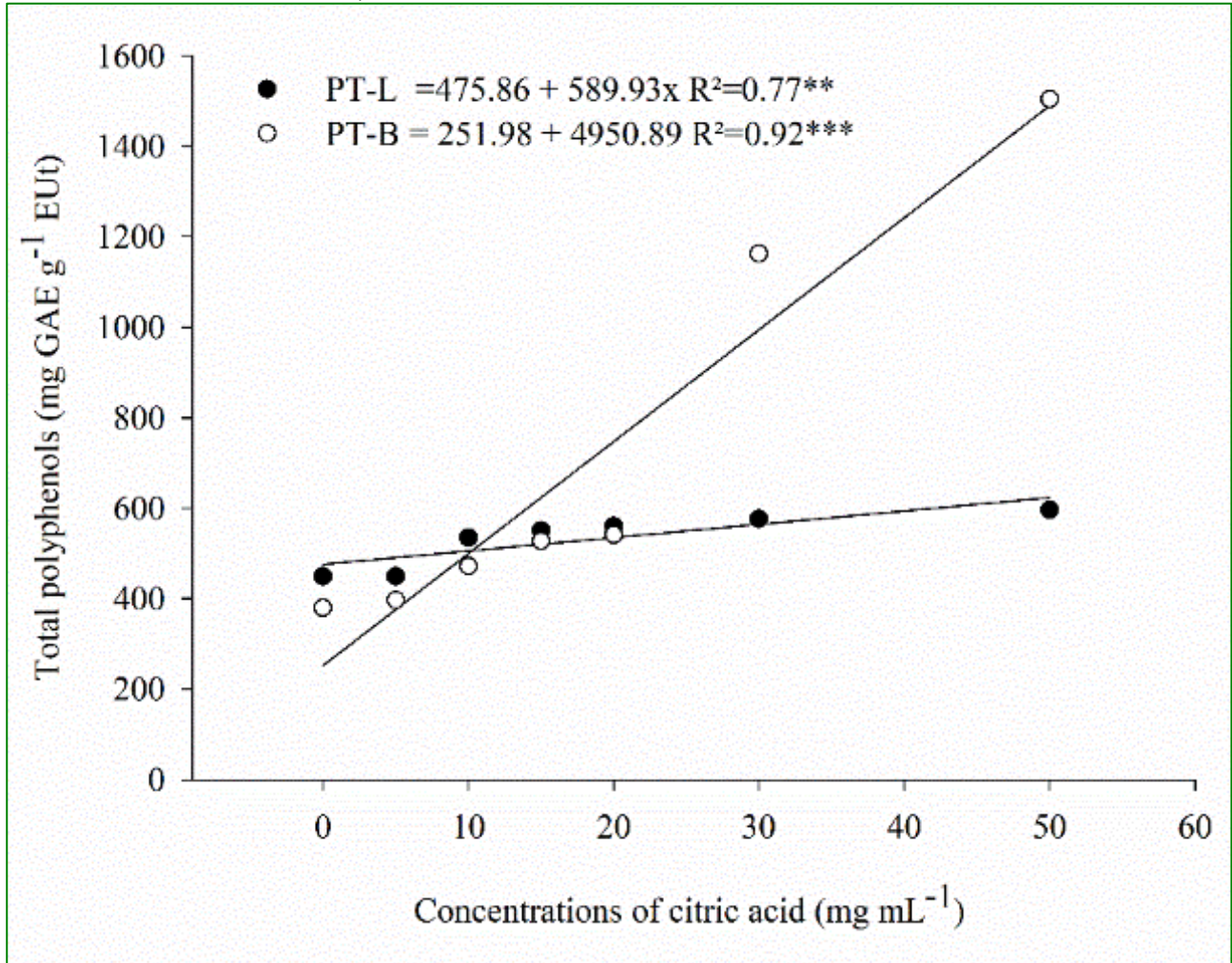
EUt did not show a significant difference when compared to the extracts incubated with citric acid of 5 mg mL<sup>-1</sup>. However, from 10 mg mL<sup>-1</sup> of citric acid, the results for total polyphenol reduction capacity increased proportionally. In addition, it was observed an increasing linear relationship for total polyphenols concentration for both plant structures studies (P<0.05). This effect was more pronounced on the leaves than the barks for higher concentrations (15 and 25 mg mL<sup>-1</sup>), in which there were significative differences between the values found in bark and leaf.

The Folin-Ciocalteu test is not specific only to phenolic compounds, since it reacts to sodium bisulfite, reducing sugars, tricarboxylic acids, some transition metals and aromatic amino acids (i.e. tryptophan and tyrosine), deviating the real value of the total polyphenol content in the sample (Granato *et al.*, 2016; Chen *et al.*, 2015). Besides that, it was found that a large variety of biomolecules present in the extracts can react to the mixture of tungsten and molybdenum (Molyneux, 2004). This overestimation is a product of the interaction between citric acid with the Folin-Ciocalteu reagent, since the REDOX reaction is similar to the one that occurs between acid and polyphenols. These defects in sensitivity can be corrected by means of three different ways: first, by subtracting the concentrations given by the pure extract with those obtained by each one of the components that are not phenolic; second, more selective polyphenol purification methods can be employed, and third, by treating the plant extract with an oxidizing agent such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (Sánchez-Rangel *et al.*, 2013).

Significant and positive correlations were exhibited between phenolic compounds and antioxidant activity, resulting from DPPH and FRAP methods. The antioxidant activity of plant-derived products is known to be influenced not only by their total polyphenol content, but also by the phenolic composition. In general, positive correlations are usually observed between evaluations of antioxidant activity and phenolic compounds (Tagliazucchi *et al.*, 2010; Burin *et al.*, 2014).



**Figure 2** – Total polyphenols of *Uncaria tomentosa* extracted from leaves (black circles) and barks (white circles), in function of different concentrations of citric acid.



Source: the authors.

### 3.2 MEASUREMENT OF ANTIOXIDANT CAPACITY

#### 3.2.1 Free radical scavenging capacity

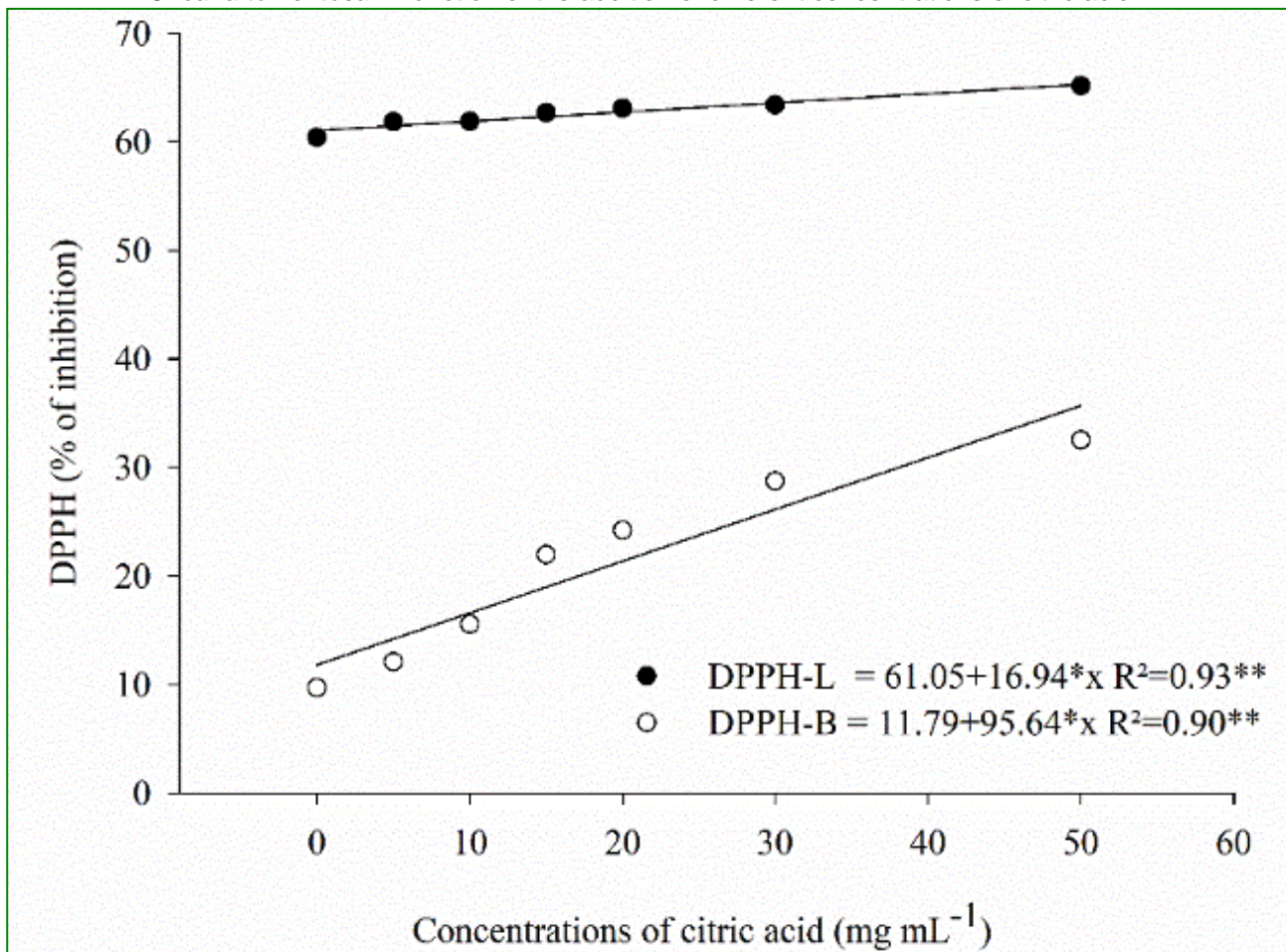
In Fig. 3, it is observed a linear increasing of the free radical inhibition in function of the increment concentration of citric acid for both extractions by the DPPH method. For the leaf's extraction, a similar effect was observed when compared the Folin-Ciocalteu experiment (Fig. 2), with a more pronounced increasing of the free radical inhibition occurred in the bark structure. The antioxidant capacity demonstrated from leaves extracts of the *U. tomentosa* resulted in higher percentage of free radical inhibition, when comparing with the barks. It was also observed that the leaves do not have a significant synergistic effect with



citric acid was added to this extract; on the other hand, the antioxidant activity of barks enriched with citric acid presented a notable raising at higher concentrations.

In ABTS experiments, the leaves and barks extracts without treatment presented an antioxidant activity of 1.34 and 1.14 mmol L<sup>-1</sup> TEAC/g of EUt , respectively, showing that the polyphenols present in the bark extract of *U. tomentosa* are less effective on preventing oxidative damage than their analogues in the plant leaves (Fig. 4). However, when it interacts with citric acid, the antioxidant capacity increases synergistically and significantly in this extract. This activity exceeds three times the antioxidant capacity of the DPPH method and almost twice as much in the ABTS method.

**Figure 3** – DPPH experiments performed in leaves (black circles) and barks (white circles) extracts from *Uncaria tomentosa* in function of the addition of different concentrations of citric acid.

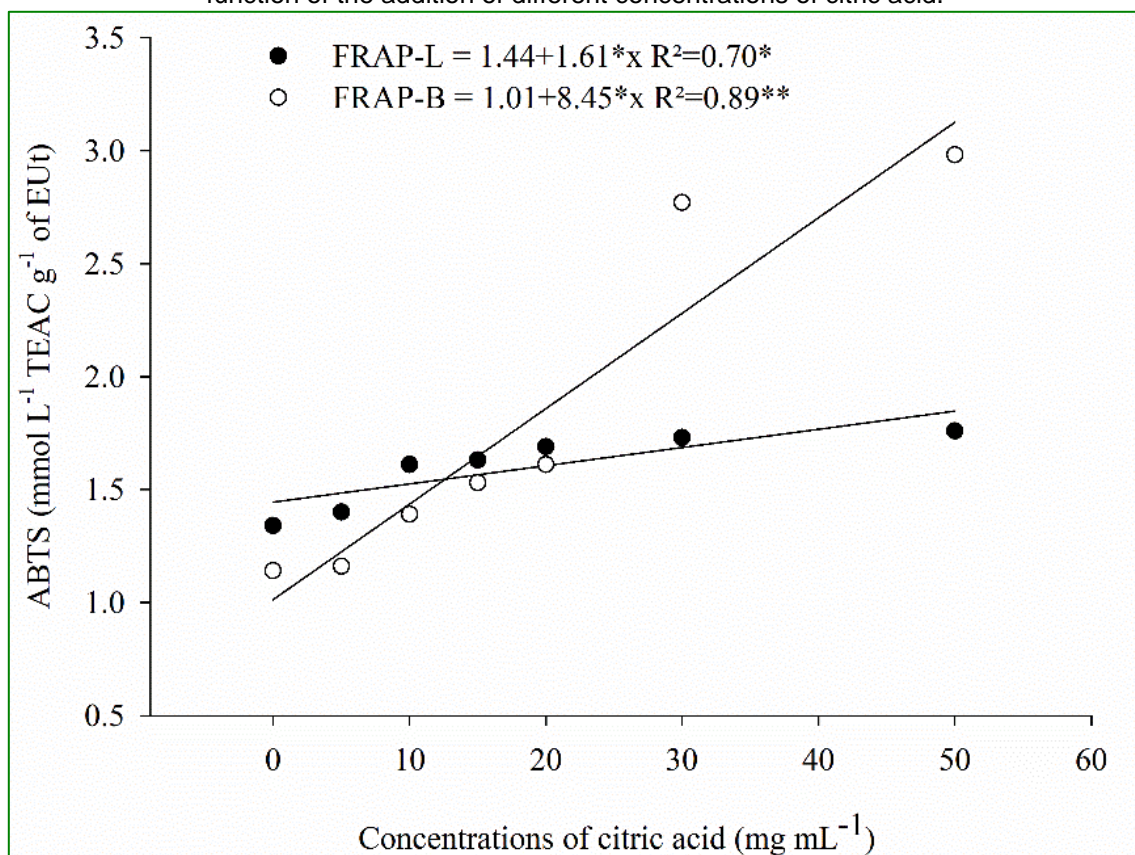


Source: the authors.

Previous studies have stated differences between antioxidant profiles of leaves, barks, and roots (Bors *et al.*, 2011). However, according to Sandoval *et al.* (2002), the EUt have a high capacity to inhibit DPPH radicals, regardless of which part of the plant is used.

The study evaluated lyophilized EUt of bark at different concentrations, and the results ranged from 3.5% for 1  $\mu\text{g mL}^{-1}$  of extract, the minimal concentration used, to 85.5%, when the mass extract was 100  $\mu\text{g}$  of freeze-dried bark for each mL of water. In comparison, the extracts prepared in the present study had a proportion of 5  $\mu\text{g mL}^{-1}$  and inhibited 9.7% of the radicals. Results based on studies of commercial extracts of *U. tomentosa* indicate that this high antioxidant effect is due to the presence of proanthocyanidins, which can be found in the cortex (Navarro *et al.*, 2019). Therefore, this pronounced increase in antioxidant activity could be linked to the interaction between proanthocyanidins and citric acid. It can be inferred that lyophilization and extraction by agitation are techniques of similar performance and give the extracts a very similar antioxidant profile. In overall, these results indicate that it is possible to use the antioxidants of *U. tomentosa* for different processes, where the use of citric acid is involved as, for example, in the use of natural preservatives, which can be optimized by lyophilization.

**Figure 4** - ABTS essays from leaves (black circles) and barks (white circles) extracts from *U. tomentosa* in function of the addition of different concentrations of citric acid.



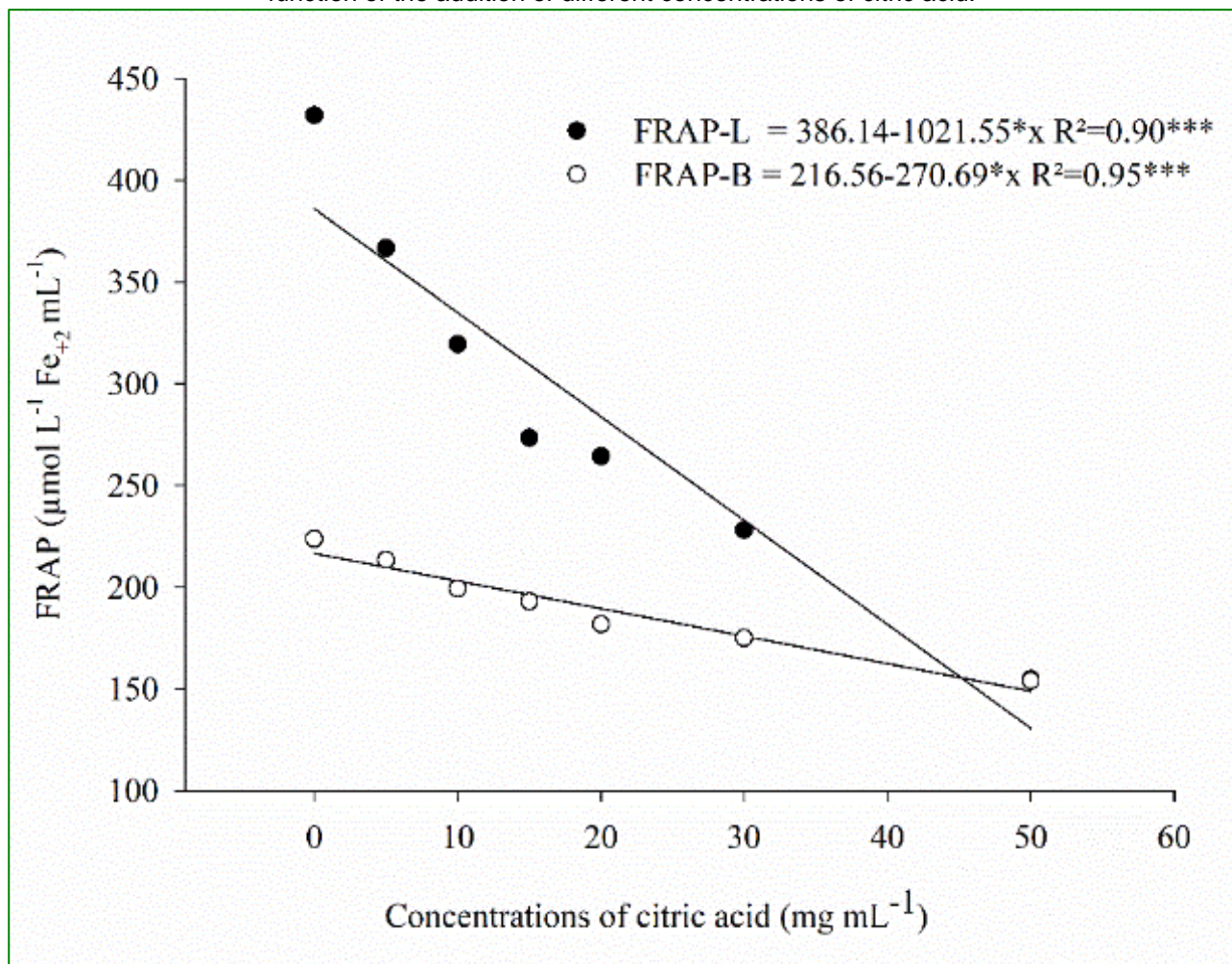
Source: the authors.



### 3.2.2 Ferric Reducing Antioxidant Power

The results obtained show an inversely proportional curves for both samples analyzed, when the citric acid was added, as shown in the Fig. 5.

**Figure 5** - FRAP studies from leaves (black circles) and barks (white circles) extracts from *U. tomentosa* in function of the addition of different concentrations of citric acid.

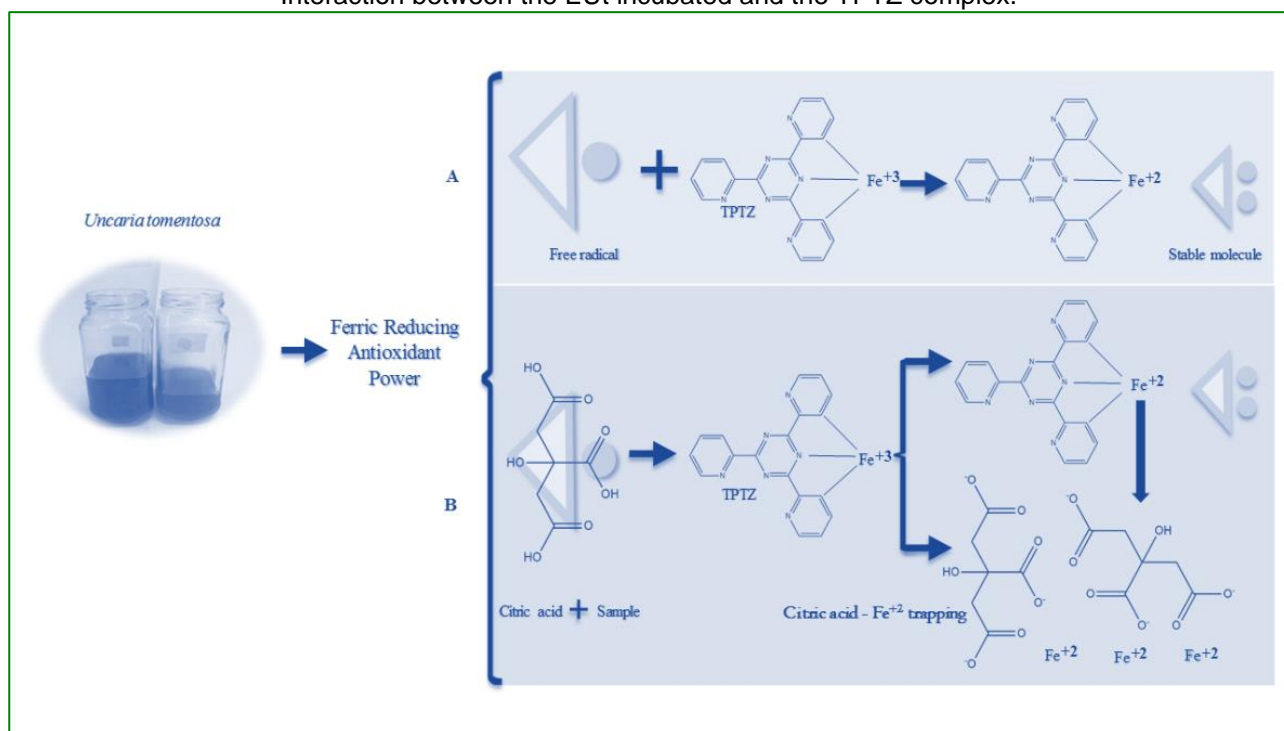


Source: the authors.

The decreasing of the antioxidant effect was notably on the leaves extracts and occurred in less intensity in the bark structure, as the citric acid concentration was arising. Citric acid has a high affinity to metal ions, especially for Fe<sup>+2</sup> (Abdel-Salam *et al.*, 2018). When applying the FRAP method, a citrus-treated plant extract was mixed with the Fe-TPTZ complex reducing the iron to Fe<sup>+2</sup>, which generated the blue color. However, this reaction is almost simultaneous, because when the reaction produces Fe<sup>+2</sup> ions, the citric acid will be converted to ferrous citrate, which does not display absorbance at 595 nm. The greater the amount of citric acid added to reaction, the more salt will be formed. Also, the ferrous citrate

can be formed reacting with some metals present in the plant extract, a fact that explains the inversely proportional results, contrary to those expected. Even so, these can be an evidence that there is a greater increase in synergistic antioxidant activity in leaves than in barks extracts ( $P < 0.001$ ). The reaction occurs normally in the absence of citric acid, fact that does not happen when it is added in different concentrations. Thus, citric acid traps the  $\text{Fe}^{+3}$  ions from the  $\text{Fe(III)-TPTZ}$  complex to form citrate salts and proportionally decreases the amount of  $\text{Fe(II)-TPTZ}$  complex formation (Fig. 6).

**Figure 6** - Possible explanation mechanisms for the results obtained in the FRAP study. A. Explanation of the chemical reaction between the EUt sample (in the form of a free radical) and the TPTZ complex. B. Interaction between the EUt incubated and the TPTZ complex.



Source: the authors.

The multivariate analysis was performed to verify the grouping of the different responses and to obtain additional information about the influence of citric acid concentrations on the biochemical parameters involved with quality, in order to reduce *U. tomentosa* losses. Components F1 and F2 (citric acid concentrations and antioxidant activities, respectively) were found to account for 97.70% of the experiment variance (Fig. 7). However, it can be seen in quadrant (B) that there is a close approximation to the data obtained in the analysis of antioxidant activity by the FRAP method in both leaves and barks, which can be evidenced in the Table 1, which shows a reduction in antioxidant activities as

the citric acid doses increased, contrary to the other observations. It can be verified that all other results showed similar behavior of increases previously evidenced in their contents and activities, as demonstrated in Fig. 7, in quadrants (A) and (C).

**Table 1** - Correlation analysis between the citric acid concentrations used and the biochemical responses in *U. tomentosa* bark and leaves extracts (Pearson method).

Variable	TP-L	TP-B	DPPH-L	DPPH-B	FRAP-L	FRAP-B	ABTS-L	ABTS-B
TP-L	1							
TP-B	0.741	1						
DPPH-L	0.868	0.870	1					
DPPH-B	0.938	0.875	0.952	1				
FRAP-L	-0.942	-0.856	-0.982	-0.977	1			
FRAP-B	-0.936	-0.886	-0.978	-0.981	0.989	1		
ABTS-L	0.992	0.725	0.878	0.935	-0.947	-0.938	1	
ABTS-B	0.798	0.985	0.869	0.914	-0.879	-0.904	0.789	1

\*Significant correlation values for  $\alpha = 5\%$ . Values in bold are different from 0 with a significance level  $\alpha=5\%$ . Table caption: B (bark); L (leaves); - TP (Total Polyphenols); DPPH (antioxidant activity for DPPH method); ABTS (antioxidant activity for ABTS method) e FRAP (antioxidant activity for FRAP method).

Source: the authors.

A principal component analysis (PCA) was performed on the treatment dataset with increasing citric acid concentrations and biochemical parameters, to provide a better visualization of the effects of treatments in a reduced size. In PCA, it was sought to explain the total data variance and the correlations (or covariance) between variables. The main components are linear combinations of the original variables. In factor analysis (FA), the original variables are linear combinations of the factors. The attributes evaluated (eight original variables, TP - total polyphenols, DPPH, ABTS and FRAP in bark and leaves) were grouped into six factors, and the readjusted model was able to explain 99.99% of the variances with eigenvalues greater than 1 (Table 2). The first component explained 91.72% of the parameters studied in the increasing concentrations of citric acid.

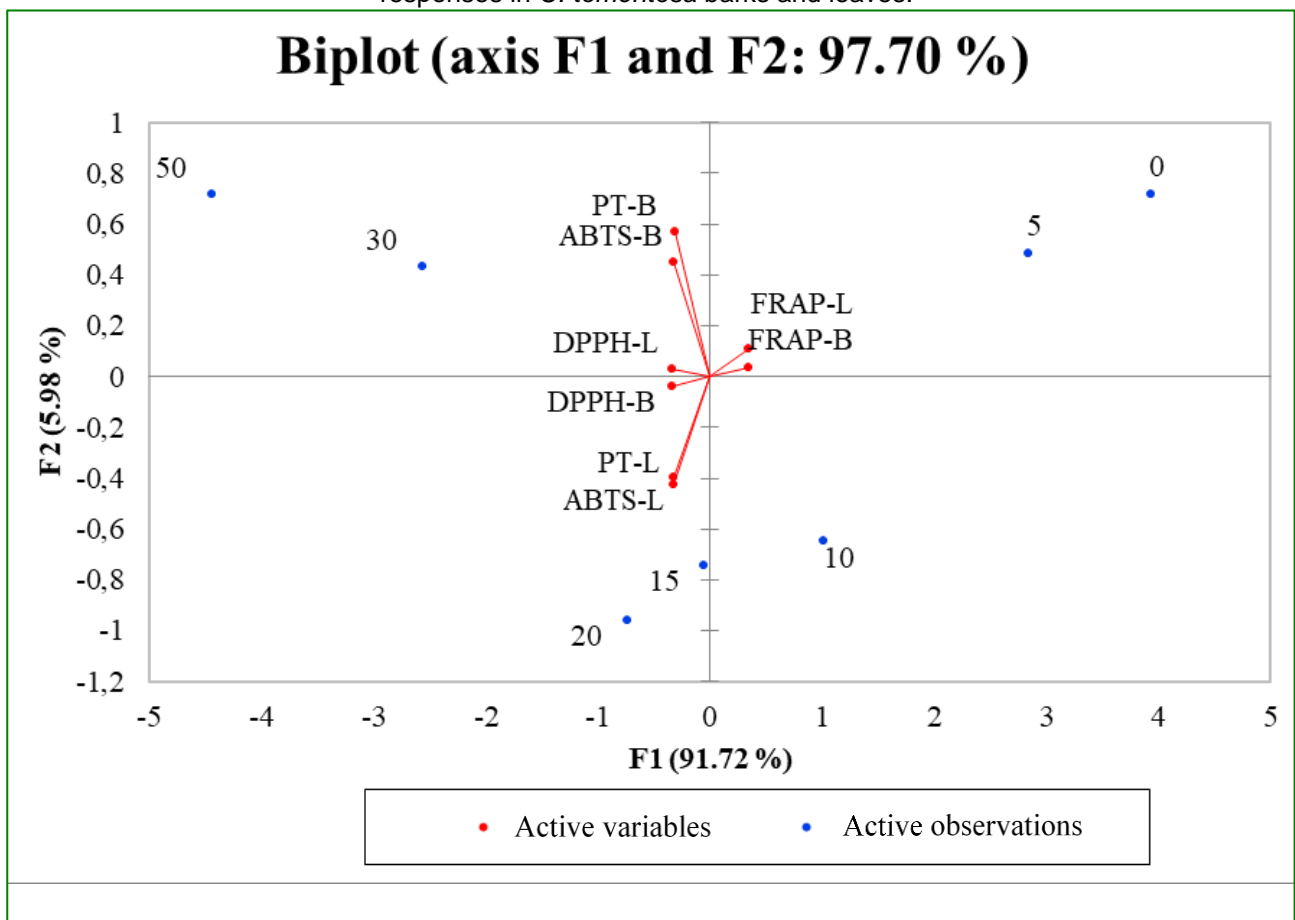
The factor F1, with explains 91,72% of the total variation, was effectively separated by the application of 0, 10, and 0.25 mg mL<sup>-1</sup> of citric acid, whose variables were assessed in function of evaluations in leaves and barks (Fig. 7). Analyzing the F1 loadings, it suggests that there is a separation between DPPH-L, DPPH-B, TP-B, TP-L, ABST-B, ABTS-L, which presented negative loadings. For the factor F2, which explains only 5,98% of the total variation and effectively distanced the citric acid doses applied of 0.1, 0.075 and 0.05 mg.L<sup>-1</sup> from the others. The variables that received the greatest score were TP-B e ABTS-B and,





according to the results, demonstrated high correlated values and thus affecting the respective doses. In addition, it is observed that FRAP-L e FRAP-B were not effective regarding the separation of the applied doses, because they presented similar behaviors. Finally, in Table 2, the correlated variables which explain the PCA factor F3, with positive values, showed only 1,78% of the total variance. Therefore, the analysis of main components was essential for the experiments interpretation and efficient to confirm and corroborate the correlation of the results obtained experimentally in the present study.

**Figure 7** – Principal component analysis plot of citric acid concentrations used and their biochemical responses in *U. tomentosa* barks and leaves.



Source: the authors.

**Table 2** – Summary of auto-values, variability, and percent variability of six PCA principal components (PC) of eight original variables.

Variable	F1	F2	F3	F4	F5	F6
TP-L	-0.348	-0.423	-0.392	-0.198	-0.438	0.396
TP-B	-0.334	0.606	-0.136	-0.349	-0.245	0.162
DPPH-L	-0.357	0.028	0.692	-0.074	0.106	-0.002
DPPH-B	-0.365	-0.040	-0.014	0.867	-0.186	0.061
FRAP-L	0.365	0.118	-0.282	0.107	-0.337	-0.517
FRAP-B	0.367	0.040	-0.189	0.141	0.445	0.627
ABTS-L	-0.348	-0.453	-0.279	-0.177	0.480	-0.371
ABTS-B	-0.344	0.479	-0.395	0.138	0.401	-0.125
	<b>F1</b>	<b>F2</b>	<b>F3</b>	<b>F4</b>	<b>F5</b>	<b>F6</b>
<b>Auto-value</b>	7.338	0.478	0.137	0.027	0.011	0.008
<b>Variability (%)</b>	91.724	5.978	1.708	0.343	0.143	0.104
<b>% accumulated</b>	91.724	97.702	99.410	99.753	99.896	100.000

Table caption: B (bark); L (leaves); - TP (Total Polyphenols); DPPH (antioxidant activity for DPPH method); ABTS (antioxidant activity for ABTS method) and FRAP (antioxidant activity for FRAP method).

Source: the authors.

## 4 CONCLUSIONS

*Uncaria tomentosa* showed a great antioxidant capacity through the results of free radical sweeping tests, DPPH and ABTS, confirming the hypothesis that it has a higher antioxidant capacity in the leaves, when compared to the barks. However, the synergistic effect with the addition of citric acid is more efficient in the bark than in the leaves, indicating remarkably high values of antioxidant activity enhancements. In the FRAP method, the assays showed that there was an increase in antioxidant capacity, which can be correlated to formation of citrate salts, which leads to an inversely proportional results. Statistical analysis confirmed the results of the addition of citric acid in the extracts of *U. tomentosa* improved their antioxidant capacity, showed approximately similar behavior of increases. In conclusion and to the best of our knowledge, the present study shows a very fast, low cost and straightforward evaluation for the use of *U. tomentosa* extracts combined with citric acid for enhancement of antioxidant activity for employment in the food industry. Therefore, this paper can contribute effectively to the obtaining of new combined natural additives and preservatives that help reduce monetary losses, incrementing the development of the agro-food industry.



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## RESUMO

*Uncaria tomentosa*, uma trepadeira notável por conter altas concentrações de alcaloides oxindois e compostos fenólicos, é comumente usada na medicina tradicional como agente anti-inflamatório e antioxidante. Além disso, o ácido cítrico é um aditivo alimentar amplamente utilizado como conservante, devido ao seu baixo custo. Dessa forma, este estudo tem como objetivo avaliar o conteúdo de compostos fenólicos de *Uncaria tomentosa* e avaliar sua atividade antioxidante quando o ácido cítrico, em diferentes concentrações, é adicionado ao extrato. Para isso, foi estabelecido um gradiente de concentrações de ácido cítrico, e o perfil antioxidante de extratos aquosos das folhas e cascas das plantas foram analisados pelo ensaio de Folin-Ciocalteu; inibição do radical livre de 2,2-difenil-1-picril-hidrazil (DPPH); poder antioxidante redutor férrico (FRAP) e capacidade de eliminação de radicais livres catiônicos do ácido 2,2'-azino-bis (ácido 3-etilbenzotiazolina-6-sulfônico) (ABTS). Os resultados mostraram um efeito sinérgico entre o ácido cítrico e os compostos antioxidantes de *Uncaria tomentosa*, apresentando significância estatística, no qual a o efeito sinérgico foi mais eficiente na casca do que nas folhas.

**Palavras-chave:** ABTS. DPPH. Folin-Ciocalteu. FRAP. Efeito sinérgico.

## RESUMEN

*Uncaria tomentosa*, una trepadera notable por contener altas concentraciones de alcaloides oxindoles y compuestos fenólicos, es comúnmente utilizada en la medicina tradicional como agente anti-inflamatorio y antioxidante. Adicionalmente, el ácido cítrico es un aditivo alimentar ampliamente utilizado como conservante, debido a su bajo costo. De esta forma, este estudio tiene como objetivo evaluar el contenido de compuestos fenólicos de *Uncaria tomentosa* y evaluar su actividad antioxidante cuando el ácido cítrico, en diferentes concentraciones, es adicionado al extracto. Para esto, se estableció un gradiente de concentraciones de ácido cítrico, y el perfil antioxidante de extractos acuosos de las hojas y cáscaras de las plantas fueron analizados por el ensayo de Folin-Ciocalteu; inhibición del radical libre de 2,2-difenil-1-picril-hidrazil (DPPH); poder antioxidante reductor férrico (FRAP) y capacidad de eliminación de radicales libres catiónicos del ácido 2,2'-azino-bis (ácido 3-etilbenzotiazolina-6-sulfónico) (ABTS). Los resultados mostraron un efecto sinérgico entre el ácido cítrico y los compuestos antioxidantes de *Uncaria tomentosa*, presentando significancia estadística, siendo que el efecto sinérgico fue más eficiente en la cáscara que en las hojas.

**Palabras clave:** ABTS. DPPH. Folin-Ciocalteu. FRAP. Efecto sinérgico.



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## CONFLITO DE INTERESSES

Os autores declaram que não há conflito de interesses neste trabalho.

## CONTRIBUIÇÕES AUTORAIS

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