Original Article

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Optimization of bioactive compound extraction from Brazilian forest bark species: effects of solvent mixtures on metal ion chelation and urease enzyme inhibition

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Abstract: The bark of forest species is a resource with a high concentration of bioactive compounds that have antioxidant, chelating and antimicrobial properties. Bioprospecting bioactive compounds from bark can generate income for local communities and contribute to the social and economic development of rural areas, especially in regions where traditional logging activity is in decline. Optimizing extraction is crucial to ensure that extracts with a high content of bioactive compounds are obtained. In this study, we optimized the extraction of bioactive compounds from the bark of *Stryphnodendron adstringens* and *Myrcia eximia*, evaluating the effects of mixtures of water, ethanol, and acetone on the chelation of Fe(II), Zn(II), and Cu(II) ions and on the inhibition of the urease enzyme. The results showed that both extracts have chelation and urease inhibition capacity. The simplex–centroid experimental design identified the ideal formulations for each response. Notably, maximum urease inhibition was achieved with a mixture of 7% water, 50% ethanol, and 43% acetone for *S. adstringens* and a mixture of 8% water, 75% ethanol, and 17% acetone for *M. eximia*. These findings highlight the importance of optimizing extraction conditions for each species, as efficiency is sensitive to solvent polarity and material matrix. © 2025 Society of Industrial Chemistry and John Wiley & Sons Ltd.

Supporting information may be found in the online version of this article.

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Key words: solvent mixture design; bioactive compounds; urease inhibition; metal chelation; *Stryphnodendron adstringens*; *Myrcia eximia*

Introduction

Bioprospecting bioactive compounds from the bark of forest species has emerged as a promising alternative in the fight against the rampant deforestation that has been taking place in recent years, especially in Brazil's two largest biomes, the Cerrado and the Amazônia. Extracting these resources offers a new source of income for local communities and promotes the sustainability of the forestry industry. The barks of forest species are rich in bioactive compounds that have antioxidant, chelating and antimicrobial properties, making them attractive for application in various areas, from agriculture to medicine.[1–3] Alkaloids, flavonoids, tannins and other compounds can be extracted and used in the production of medicines, cosmetics, food supplements and other high value-added products. It is important to note that the extraction of bioactives from the bark does not cause damage to the tree, allowing it to be reused in subsequent cycles.[4,5]

Extracting bioactives can generate income for local communities, who can get involved in collecting, processing and marketing the bark. This can contribute to the social and economic development of rural areas, especially in regions where traditional logging activity is in decline.[4,5] In this study, bioprospecting is understood as the exploration of native species biodiversity, with the aim of discovering new bioactive compounds and optimizing their extraction for innovative applications. For the bioprospecting of bark bioactives to become a viable alternative to deforestation, some challenges need to be overcome.[3,6] The first is to find species whose bark is rich in bioactive compounds. In the literature, several studies using the species *Stryphnodendron adstringens* and *Myrcia eximia*, characteristic of the Cerrado and Amazônia biomes, respectively, report that the concentration of bioactive compounds in *S. adstringens* bark can reach 30% of the bark, while in the species *M. eximia* this value can reach up to 32%.[1,7,8]

The second challenge is that optimizing extraction is crucial to ensure that extracts with a high content of bioactive compounds are obtained, which can be improved using statistical tools such as simplex–centroid design.[9,10] The simplex–centroid mixture experimental design was chosen because it is a robust and efficient statistical tool for optimizing multicomponent extraction systems.[9,10] Its use is one of the main highlights of this work, as it allows for a systematic exploration of the complex synergistic interactions between the solvents. By using this methodology, it was possible not only to evaluate the impact of each solvent individually but also to determine the exact proportions needed to precisely optimize the extraction of bioactive compounds.[9–11] The simplex–centroid experimental design is an effective tool for evaluating the impact of different process variables, such as solvent type, temperature, and extraction time.[12,13]

The use of design for solvent mixtures is one of the simplest and most efficient ways to improve the extraction of bioactive compounds, since each solvent in the mixture has specific properties that, when combined, create a synergy to optimize extraction.[9–11] The combination of the three solvents makes it possible to extract a wider range of bioactive compounds with different polarities, from the most polar to the most apolar, in addition, the synergy between the solvents has the ability to promote the disruption of the interactions between the phenolic compounds and the plant matrix, increasing the efficiency of the extraction and the yield of the process.[11–13] The appropriate choice of the proportion of solvents in the mixture also makes it possible to target the extraction to specific phenolic compounds of interest, reducing the extraction of undesirable compounds.

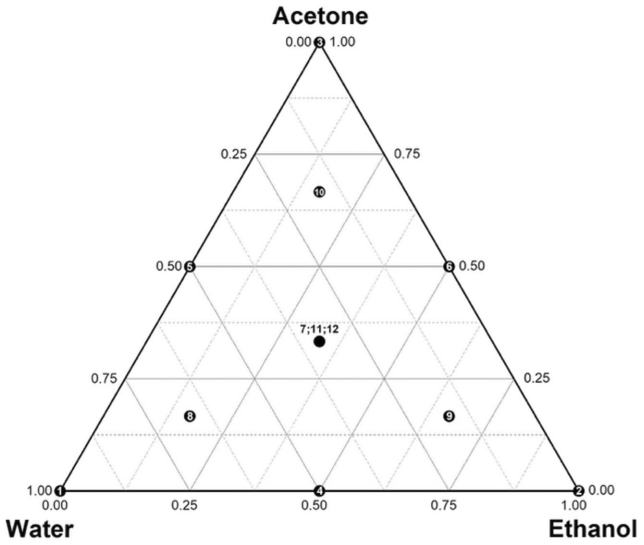
The aim of this study was to evaluate the effects of solvents (water, ethanol and acetone) and their mixtures on the content of total phenols, the chelating activity of Fe(II), Zn(II) and Cu(II) ions and the inhibition of the urease enzyme in the extracts obtained from the barks of *S. adstringens* and *M. eximia* using simplex–centroid solvent mixture design. In addition, the optimum combination of solvents was determined for each of the responses measured.

Materials and methods

Collection and sampling

The barks of *S. adstringens* were collected in the city of Ouro Branco (October, 2020), located in the central region of the state of Minas Gerais, Brazil (20°31′06.0″ S and 43°42′58.3″ W). The barks of *M. eximia*, on the other hand, were collected in the city of São João da Ponta (January, 2018), in the northwestern region of the state of Pará, Brazil (0°51′3″ S and 47°55′17″ W). Both barks were removed manually from the trunks of six trees with an average height and diameter of 3.3 m and 45.2 cm for *S. adstringens* trees and 13 m and 14.1 cm for *M. eximia*, respectively (measured at 1.30 m above ground level).[14,15]

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Experimental point	Independent variables		
	X_1 (Water)	X_2 (Ethanol)	X_3 (Acetone)
1	1	0	0
2	0	1	0
3	0	0	1
4	0.5	0.5	0
5	0.5	0	0.5
6	0	0.5	0.5
7	0.33	0.33	0.33
8	0.66	0.17	0.17
9	0.17	0.66	0.17
10	0.17	0.17	0.66
11	0.33	0.33	0.33
12	0.33	0.33	0.33

Figure 1. Representation of the augmented simplex–centroid design for three components and identification of the solvent system factors.

The bark of the six individuals was dried and ground into a composite sample for each species. The particles were classified in terms of particle size and the fraction of barks with a size of 250 µm (60 mesh) was used in all the analyses in this work.

Experimental design of mixtures and statistical analysis

The design of the simplex–centroid mixture experiment was carried out using Statistica 14.1.0.8 software (Cloud Software Group, Inc.), and utilized to explore the effect of different solvents on the extraction of phenolic compounds from the bark of *S. adstringens* and *M. eximia* species and to determine the ideal mixtures of solvents to maximize the responses studied. To define the simplex–centroid experiment, the different solvents were evaluated from a triangle (Fig. 1), with pure components at the vertices, representing 100% of each of the solvents. Each solvent in the system was studied at six levels, according to the 12 experimental points shown in Table 1. The linear (Eqn 1), quadratic (Eqn 2) and special cubic (Eqn 3) mathematical models were evaluated.

$$\text{Linear: } y_n(x) = \sum_{i=1}^{q} \beta_i x_i \quad (1)$$

$$\text{Quadratic: } y_n(x) = \sum_{i=1}^{q} \beta_i x_i + \sum_{i<j}^{q-1} \sum_{j}^{q} \beta_{ij} x_i x_j \quad (2)$$

$$\text{Special cubic: } y_n(x) = \sum_{i=1}^{q} \beta_i x_i + \sum_{i<j}^{q-1} \sum_{j}^{q} \beta_{ij} x_i x_j + \sum_{i<j}^{q-2} \sum_{j<k}^{q-1} \sum_{k}^{q} \beta_{ijk} x_i x_j x_k \quad (3)$$

In the equations, y_n represents the predicted response function. Five responses ($n = 5$) were studied: the concentration of total phenolics, chelating activity for the metal ions iron (II), zinc (II) and copper (II) and the inhibition of the urease enzyme. The independent variables x_i, x_j and x_k correspond to the components used in the mixture design (water, ethanol and acetone), with $1 > x_{ijk} > 0$. β_i, β_{ij} and β_{ijk} represent, respectively, the linear coefficient related to pure component *i*, the quadratic binary interaction coefficient for components *i* and *j*, and the cubic ternary interaction coefficient for components *i*, *j* and *k*.

Analysis of variance (ANOVA) and regression analysis were used to evaluate the mathematical prediction models (P-value<0.10). Contour and Pareto charts were generated from the fitted models. The desirability function was used to optimize the five response variables separately.

Preparation of bark extracts

The extracts of the *S. adstringens* and *M. eximia* species were obtained in the proportions shown in Fig. 1. In this way, 0.5 g of ground bark was added to 60 mL of mixture and extracted in an ultrasonic bath at 60 °C for 1 h. The suspension was

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Table 1. Experimental conditions of the mixture design, observed and predicted values for each response for the *Stryphnodendron adstringens* species.

Experimental point	Independent variables			Total phenols (mg GAE g of bark^{-1})		CHFe(II) (%)		CHZn(II) (%)		CHCu(II) (%)		UI (%)	
	X_1 (water)	X_2 (ethanol)	X_3 (acetone)	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted
1	1	0	0	167.68	160.71	28.50	27.59	51.86	52.15	39.99	39.15	2.45	–1.53
2	0	1	0	157.84	153.38	11.80	12.46	51.61	47.96	34.46	33.98	1.05	–8.91
3	0	0	1	123.22	137.95	33.36	30.63	51.83	47.46	28.83	29.95	1.26	9.08
4	0.5	0.5	0	238.84	227.41	28.92	28.68	58.14	54.78	45.40	44.08	8.72	–5.23
5	0.5	0	0.5	238.69	246.45	28.09	24.45	55.98	51.90	43.77	44.05	2.90	6.74
6	0	0.5	0.5	180.61	190.88	11.51	9.44	87.63	79.61	44.55	45.19	66.73	64.59
7	0.33	0.33	0.33	217.32	220.62	33.36	30.38	88.80	81.07	47.23	47.03	64.13	58.00
8	0.66	0.17	0.17	205.26	222.86	25.52	31.22	57.72	64.58	42.70	45.41	3.65	21.73
9	0.17	0.66	0.17	191.81	201.89	20.18	21.17	53.74	72.42	42.42	44.06	2.53	38.55
10	0.17	0.17	0.66	250.60	203.10	14.64	25.82	50.47	71.30	45.87	42.71	65.88	48.54
11	0.33	0.33	0.33	217.32	220.62	33.36	30.38	88.80	81.07	47.23	47.03	64.13	58.00
12	0.33	0.33	0.33	217.32	220.62	33.36	30.38	88.80	81.07	47.23	47.03	64.13	58.00

filtered using a glass crucible with a porosity of 1. The extracts were roto-evaporated to remove the solvents and resuspended in 50 mL of deionized water. The extracts were kept refrigerated at 4 °C until analysis.[16]

Determination of total phenol content

The concentration of total phenols (TPh) was determined using the Folin–Ciocalteu method, adapted for microplates.[17] To do this, a 4 µL aliquot of the bark extract was added to 148 µL of a 10% Folin–Ciocalteu solution. After resting for 8 min, another 148 µL of a 7.5% calcium carbonate solution was added to the mixture and the microplate was shaken for 20 s. After 45 min of reaction in an oven at 45 °C, the absorbance of the mixture was measured at 765 nm using a microplate reader (EZ Read 2000, biochrom, UK). The total phenol content was expressed in mg of gallic acid equivalent (GAE) per gram of bark.

Determination of the chelating activity of iron (II) ions

The chelating activity of ferrous ions (CHFe(II)) was determined following a previous methodology[18] adapted for microplates. In short, in each well of the microplate, 0.1 mM $FeSO_4$ (100 µL) was added to the diluted extracts (100 µL; 1:10), followed by 0.25 mM ferrozine (100 µL). The microplate was left to stand for 10 min before absorbance measurements were taken at 562 nm using a microplate reader (EZ Read 2000, biochrom, UK). The results of the extracts were compared with the ethylenediamine tetraacetic acid (EDTA) salt. The ferrous ion chelating capacity of the extracts was calculated using the following formula:

$$\text{Chelating activity Fe (II)}\ (\%) = \left(\left(A_{\text{control}} - A_{\text{sample}}\right)/A_{\text{control}}\right) \times 100 \quad (4)$$

where $_{\text{control}}$ and $_{\text{sample}}$ are the absorbance of the negative control and the extracts, respectively.

Determination of the chelating activity of zinc (II) and copper (II) ions

To determine the chelating capacity of zinc (II) ions (CHZn(II)) and copper (II) ions (CHCu(II)), the methodology described in the literature was followed, adapted for microplates.[19] The bark extracts were dissolved in 0.01 mol L^{-1} hexamine/HCl buffer with the addition of 0.01 mol L^{-1} KCl, pH 5.0 (1:10). $CuSO_4.5H_2O$ or $ZnCl_2$

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Table 2. Experimental conditions of the mixture design, observed and predicted values for each response for the *Myrcia eximia* species.

Experimental point	Independent variables			Total phenols (mg GAE g of bark^{-1})		CHFe(II) (%)		CHZn(II) (%)		CHCu(II) (%)		UI (%)	
	X_1 (water)	X_2 (ethanol)	X_3 (acetone)	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted
1	1	0	0	139.65	133.63	25.52	26.55	38.36	38.65	34.00	31.62	65.66	65.27
2	0	1	0	108.23	107.22	30.73	29.12	41.26	39.32	31.40	32.72	69.73	68.73
3	0	0	1	61.33	66.44	15.12	15.88	36.87	37.74	11.72	11.78	24.86	27.34
4	0.5	0.5	0	147.49	140.46	23.50	22.93	53.21	51.57	37.26	36.20	65.61	64.23
5	0.5	0	0.5	151.82	150.91	26.01	27.79	35.28	36.44	37.65	35.34	70.77	72.87
6	0	0.5	0.5	126.88	130.99	23.16	22.31	48.26	47.19	37.94	39.32	68.43	69.92
7	0.33	0.33	0.33	147.45	145.53	20.60	20.78	53.53	52.75	38.30	37.30	68.21	69.31
8	0.66	0.17	0.17	130.45	150.43	27.33	24.06	47.24	47.14	28.35	36.49	70.08	70.14
9	0.17	0.66	0.17	130.02	134.98	18.45	23.09	44.36	50.95	41.13	38.18	68.43	70.32
10	0.17	0.17	0.66	138.30	124.88	22.75	20.29	47.20	45.38	30.10	30.91	67.95	59.40
11	0.33	0.33	0.33	147.45	145.53	20.60	20.78	53.53	52.75	38.30	37.30	68.21	69.31
12	0.33	0.33	0.33	147.45	145.53	20.60	20.78	53.53	52.75	38.30	37.30	68.21	69.31

were dissolved in the same buffer at concentrations of 3 and 8 mmol L^{-1}, respectively. An aliquot of 143 µL of the extracts was added to the well of the microplate, along with 143 µL of $CuSO_4.5H_2O$ or $ZnCl_2$ and 14 µL of 5 mM murexide. The microplate was incubated in the dark at room temperature for 3 min. The absorbance was measured at wavelengths of 482 nm (Cu(II)) or 462 nm (Zn(II)) and 530 nm (Cu(II) and Zn (II)) using a microplate reader (EZ Read 2000, Biochrom, UK). The results of the extracts were compared with those for EDTA. The chelating capacity of the extracts for Zn(II) and Cu(II) ions was calculated using the following formula:

$$\text{Metal chelating activity (II)}(\%) = \left[\frac{\left(\left(\frac{A_{initial}}{A_{530}}\right)_0 - \left(\frac{A_{initial}}{A_{530}}\right)_s\right)}{\left(\frac{A_{initial}}{A_{530}}\right)_0}\right] \times 100 \quad (5)$$

where $A_{initial}$ is the initial absorbance measured according to the metal, A_0 is the ratio between the measured absorbances of the negative control and A_S is the ratio between the absorbances of the samples.

Determination of the ability to inhibit the urease enzyme

The urease enzyme inhibition test (UI) was carried out in microplates following the methodology described in the literature.[20] To do this, 25 µL of potassium phosphate buffer (100 mM and pH 6.8) containing 4 IU of pure urease was added to each well of the microplate. Next, 25 µL of the bark extract was added to each well already containing the urease. The plate was incubated for 10 min at room temperature to inactivate the enzyme. After this period, another 200 µL of potassium phosphate buffer (100 mM and pH 6.8) containing 150 mM urea and 0.002% phenol red was added to each well. Changes in absorbance at 570 nm were measured on a microplate reader (EZ Read 2000, biochrom, UK) every 3 min up to 30 min and then read at 45 and 60 min. The inhibition of urease activity was calculated in relation to the percentage decrease in absorbance of the positive control. For the simplex centroid experiment, the inhibition capacity at 60 min was used.

Results and discussion

The results obtained on the experimental variable for the simplex–centroid mixture design for both species of *S. adstringens* and *M. eximia* are presented in Tables 1 and 2,

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respectively, which show the proportion of the mixture as the independent variable and the answers as the observed and predicted quantity of TPh, CHFe(II), CHZn(II), CHCu(II) and UI.

The ANOVA of the mathematical models obtained, which can be seen in Table S1, allowed the contour graph to be drawn for the *S. adstringens* (Fig. 2) and *M. eximia* (Fig. 3) species for each of the responses evaluated, as a function of the solvent proportions. The vertices of the contour graph show the value of the response, and the edges of the triangle indicate the concentration, which represents the individual components and their binary and ternary mixtures, respectively. It was also possible to draw Pareto graphs (Figs S1 and S2) for each response. As the Pareto chart displays the absolute value of the effects, it is possible to determine which effects are significant, but it is not possible to determine which effects increase or decrease the response.[12] To determine the magnitude and direction of the effects, the coefficients of the equations available in Tables S2 and S3 (*S. adstringens* and *M. eximia*) were used, from which non-significant interactions were eliminated.[13,21]

According to the ANOVA results (Table S1), the quadratic interaction model was satisfactory for the analyses of total phenolics, copper (II) chelation and enzyme inhibition for the *S. adstringens* species and for the analyses of total phenolics, zinc (II) chelation, copper (II) and urease inhibition for the *M. eximia* species.

In the *S. adstringens* species, for the iron (II) and zinc (II) chelation responses, the interaction models with the lowest *P*-values were linear and quadratic, respectively. However, even outside the confidence interval ($P > 0.1$), they showed predicted results close to the observed values (Table 1). The lack of interaction between the solvents (Fig. S1) for the enzyme inhibition response affected the model, which did not show good predictability of the results with the lowest inhibition values but, managed to predict the results with the highest values well (Table 2).

For the *M. eximia* species, the linear interaction model for the iron (II) chelating activity response showed the lowest *P*-value (Table S1) but, similarly to the *S. adstringens* species, even with $P > 0.1$, the model was able to predict results close to those obtained experimentally.

The reduced special cubic model equations for both species were used to optimize the responses individually (Table 3). All the formulations calculated showed a good desirability value.

Concentration of total phenols

The results obtained for TPh using different solvents ranged from 123.22 to 250.60 mg GAE g of bark^{-1} for *S. adstringens* bark (Table 1) and from 61.33 to 151.82 mg GAE g of bark^{-1} for *M. eximia* bark (Table 2). The mathematical equations for TPh show that the extraction efficiency is affected by

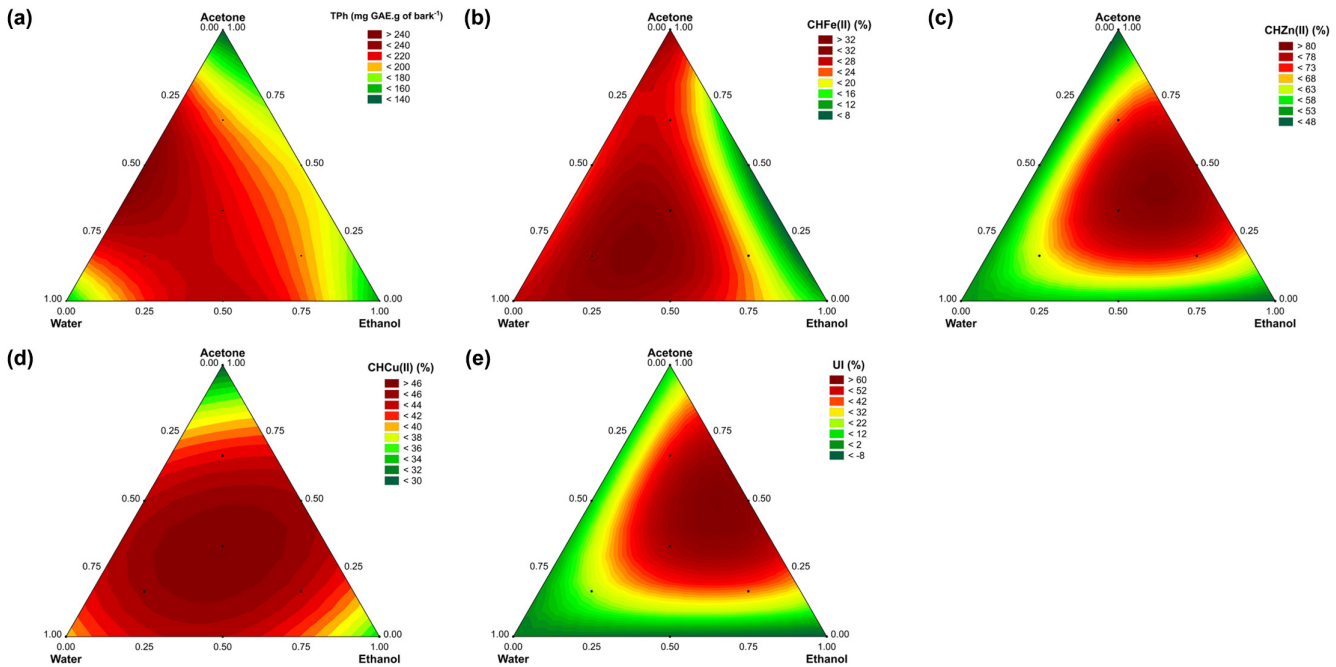


Figure 2. Contour plot for the effects of different solvent proportions on (a) total phenolic concentration, (b) Fe(II), (c) Zn(II), (d) Cu(II) ion chelating activities and (e) urease inhibition capacity of *Stryphnodendron adstringens* bark.

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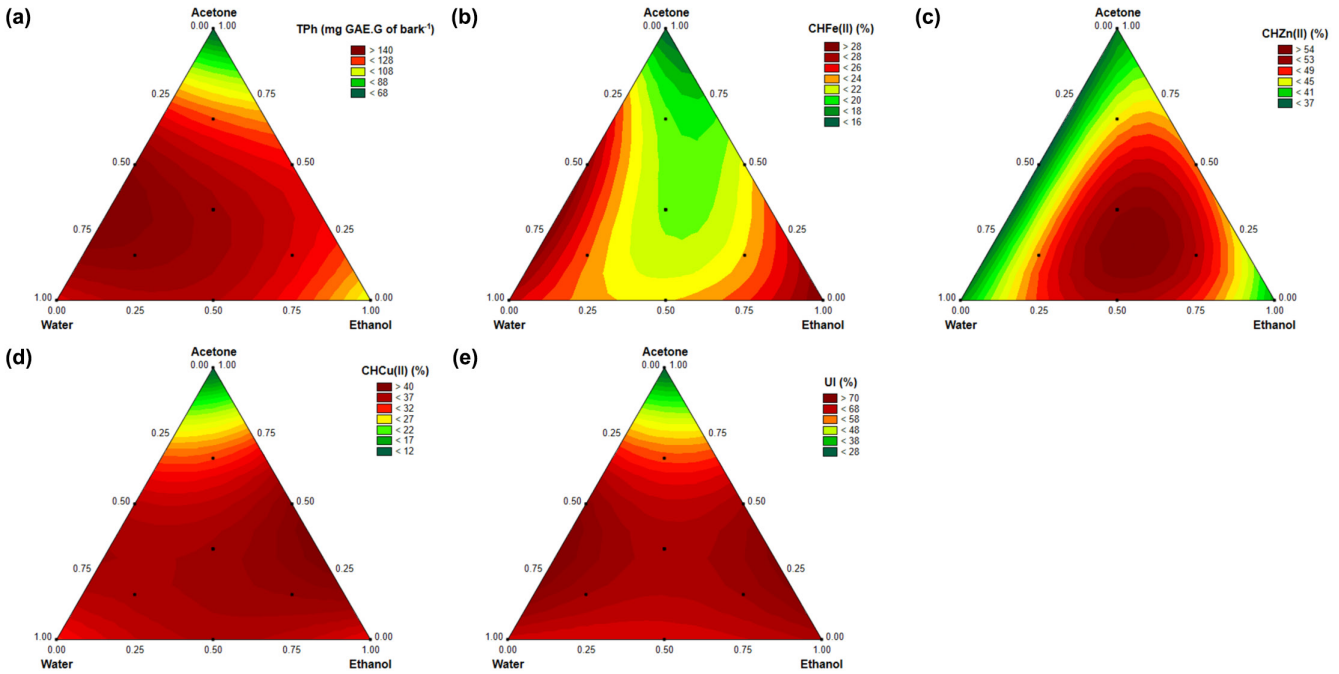


Figure 3. Contour plot for the effects of different solvent proportions on (a) total phenolic concentration, (b) Fe(II), (c) Zn(II), (d) Cu(II) ion chelating activities and (e) urease inhibition capacity of *Myrcia eximia* bark.

Table 3. Optimized formulation for both species based on the desirability function.

Analysis	*Stryphnodendron adstringens*				*Myrcia eximia*			
	Water	Ethanol	Acetone	Desirability	Water	Ethanol	Acetone	Desirability
Total phenols	0.50	0.00	0.50	0.96745	0.75	0.05	0.20	1
CHFe(II)	0.50	0.30	0.20	0.96190	0.00	1.00	0.00	0.89705
CHZn(II)	0.25	0.38	0.38	0.85519	0.25	0.50	0.25	1
CHCu(II)	0.25	0.38	0.38	0.97948	0.00	0.67	0.33	0.99253
UI	0.07	0.50	0.43	1	0.08	0.75	0.17	1

the following decreasing order: water–acetone, water–ethanol, water, ethanol and acetone (*S. adstringens*) and water–acetone, ethanol–acetone, water, ethanol and acetone (*M. eximia*).

Comparing the contour plots for total phenolics of both species, we can see that the phenolics present in *S. astringens* (Fig. 2(a)) are more soluble in solvent mixtures than in isolated solvents. A mixture of 50% water and acetone manages to extract higher concentrations of phenolic compounds than a mixture of ethanol and acetone, which is in line with the phenolic extraction optimization equation for *S. adstringens* (Table 3).

As for the *M. eximia* species, the contour graph (Fig. 3(a)) shows that acetone alone is not a good solvent for extracting phenolic compounds in this species, unlike water alone. However, when small concentrations of ethanol and acetone are mixed, the solubility of the phenolics tends to increase. The optimum extraction point for *M. eximia* is 75% water, 5% ethanol and 20% acetone. According to the literature, water increases the permeability of cell tissue and mass transfer by molecular diffusion, in addition to the recovery of water-soluble bioactive compounds.[13,22] In addition, the use of water in a mixture with organic solvents provides a moderately polar medium that is more favorable for extraction, since the chemical nature of phenolic compounds changes from simple to highly polarized.[13]

Fe(II) ion chelating activity

Analyzing the contour plots for the chelating activity of phenolics for ferrous ion, we can see that the polarity of the solvents interferes significantly with the response. The *S. adstringens* species (Fig. 2(b)) showed good results both with water and acetone alone and in mixtures of the three solvents,

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with the best point for extraction being a ternary mixture (Table 3). On the other hand, the binary mixture of ethanol and acetone and ethanol alone did not give good results. For the *M. eximia* species, the contour plot (Fig. 3(b)) showed that the solvent mixtures did not favor the chelation capacity of the ferrous ion, a fact that was proven by the optimization of this response, which showed only ethanol as the best solvent in this case. We can also see, from the difference between the species, that there is no specificity between the phenolic compounds involved in chelating the ferrous ion since the difference in solubility and polarity between the solvents and the concentration of phenolic compounds had no impact on the chelating activity. From the coefficients of the model equation for CHFe(II), the extraction efficiency is affected by the following decreasing order: acetone and water (*S. adstringens*) and ethanol, water and acetone (*M. eximia*). Both species showed values close to the maximum chelating activity of the ferrous ion (Tables 1 and 2) and below the values shown by EDTA (100%).

Chelating activity of Zn(II) ions

The chelating capacities of zinc (II) ions for both species (Tables 1 and 2) were higher than the value presented by the EDTA salt (34.24%). The contour plots for the zinc (II) ion chelation response show that, for both species, isolated solvents are not suitable for this response. For the *S. adstringens* species (Fig. 2(c)), the best results were in the vicinity of the region between acetone and ethanol, with the optimization equation showing a maximum response in a 25% water, 37.5% ethanol and 37.5% acetone solution. In the *M. eximia* species (Fig. 3(c)), closer to the mixture between water, ethanol and acetone, the chelating activity of this mixture increases, which can also be observed in the optimized formulation (25% water, 50% ethanol and 25% acetone). The chelating capacity of *S. adstringens* bark was most affected by the following decreasing order: water, ethanol and acetone. For *M. eximia* bark, the extraction capacity was most affected by the following decreasing order: water–ethanol, ethanol, water, acetone and ethanol–acetone.

Chelating activity of Cu(II) ions

The results obtained for the chelating capacity of copper (II) ions for both species were lower than the values obtained for EDTA (83.60%). Both barks also showed values close to the maximum chelating activity and the results show that the mixtures are better than the solvents alone. In the case of *S. adstringens* bark, the contour graph (Fig. 1(d)) shows that acetone and ethanol alone were not good solvents. The region of the graph with the highest values was close to the central point of the experiment. In optimizing this response, a mixture of 37.5% ethanol, 37.5% acetone and 25% water is the best formulation for this response. For the *M. eximia* species (Fig. 3(d)), acetone alone was not a good solvent, indicating that the polarity of phenolics is not as low as the polarity of acetone. However, when acetone is mixed with ethanol, the chelating activity of the extract obtained tends to increase. This observation is in line with the optimization of this response, which shows that the optimum point is in the formulation containing 67% ethanol and 33% acetone. According to the coefficients of the fitted model equation, the chelating capacity of cupric ions was most affected by the following decreasing order in *S. adstringens* bark: ethanol–acetone, water, water–acetone, ethanol, water–ethanol and acetone. In the *M. eximia* species, the capacity was most affected by the following decreasing order: ethanol–acetone, water–acetone, ethanol, water and acetone.

As described in the literature, phenolic compounds can only complex metal ions when their functional groups are properly oriented, i.e. they must be completely organized spatially.[19,23] The glycosylation of phenolic groups also hinders the ability of phenolics to bind to metal ions.[23] In general, condensed tannins bind to metal ions mainly *via* catechol groups, while hydrolyzed tannins bind *via* galloyl groups.[19] In a study carried out by Fedenko and collaborators, several phenolic compounds have the ability to bind to metal ions of various different valences.[23] Among the most found in tree bark are gallic, vanillic, ellagic, cinnamic, *p*-coumaric, ferulic and tannic acids, as well as catechin, quercertin, rutin, catechin and condensed tannins.[12,21,23]

Based on previous studies, the phenolic profile of *S. adstringens* and *M. eximia* barks has been comprehensively studied, revealing the presence of compounds that exhibit a high capacity for chelating metal ions.[7,15] This aligns with the literature indicating that the phenolic extracts from the barks of these species are consistent with their role in chelation activity.

Another important field of study into the metal chelating activity of phenolic compounds is in medicine, more precisely in the treatment of Alzheimer's. Dishomeostase of metals, i.e. an imbalance in concentration, specifically of copper (Cu), zinc (Zn) and iron (Fe), plays a key role in the progression of the disease. As these transition metal ions are important for cell function, their imbalance can cause oxidative stress that leads to cell death and eventual cognitive deterioration.[24]

Comparing the results of the chelating activity of Fe(II), Zn(II) and Cu(II) ions is difficult, as each author expresses the chelating activity of a specific amount of chelating agent in different ways. According to Wettasinghe & Shahidi, 62% of ferrous ions were chelated by 100 ppm of sesame

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husk extracts.[25] In comparison, in this experiment the concentration of the extracts was around 1 ppm for both species for the metal chelating activity analyses. In another study, the iron chelating capacity of tannins extracted from buckwheat groats at a concentration of 5 ppm and obtained a 16% binding capacity for Fe(II).[19]

Urease inhibition capacity

The inhibition capacity of the urease enzyme showed similar inhibition values when comparing the maximum obtained for the two species only at the 60 min point. While in the *S. adstringens* species the maximum (66.73%) was obtained at the ethanol–acetone point (0.5–0.5), the maximum inhibition obtained by the *M. eximia* species (70.08%) was at the point with the highest concentration of water along with the other solvents (0.66–0.17-0.17). The contour plots for urease inhibition can be seen in Figs 2(e) and 3(e) for *S. adstringens* and *M. eximia*, respectively. While for the species *S. adstringens*, the extracts obtained from isolated solvents did not show good results, for the bark of *M. eximia* only the extract in isolated acetone did not achieve good urease inhibition results. In terms of optimization, the ideal formulation for *S. adstringens* bark is 7% water, 50% ethanol and 43% acetone. For the *M. eximia* species, the optimized formulation is 8% water, 75% ethanol and 17% acetone. The different maximum points between the species show that the inhibition of the urease enzyme is not related to a specific phenolic compound, since the difference between the solvents means there is a difference in polarity and, consequently, extraction of phenolic compounds of different solubility. In the *S. adstringens* species, the inhibition capacity was only influenced by the ethanol–acetone mixture, while in the *M. eximia* species, inhibition was influenced in the following decreasing order: water–acetone, ethanol–acetone, ethanol, water and acetone.

Simorì and colleagues studied the inhibition of urease from grape pomace extracts. Even though deep eutectic solvents were used, which are mixtures of two or three chemical compounds that have a lower melting point than any of the isolated components, the inhibition values were lower than those presented for the extracts of *S. astringens* and *M. eximia* barks. The maximum value of reduction in urease inhibition for the treatments carried out was 25%.[26]

The evaluation of urease inhibition capacity over time (Fig. 4) revealed an interesting kinetic behavior for the extracts. For both species, maximum inhibition was observed between 9 and 12 min. This phenomenon can be attributed to the time required for the inhibitory compounds present in the extracts to bind to the urease enzyme and reach a state of kinetic equilibrium.[27] Urease is a metalloenzyme that contains two nickel (Ni) ions in its active site, and its inhibition can occur through different interaction mechanisms, such as inhibitors binding to the active site, blocking the access of the substrate (urea), or through interactions with nearby amino acid residues that affect the enzyme's conformation.[26,27]

After the peak inhibition, the enzymatic activity begins to be slowly restored. The rate of inhibition decline varies

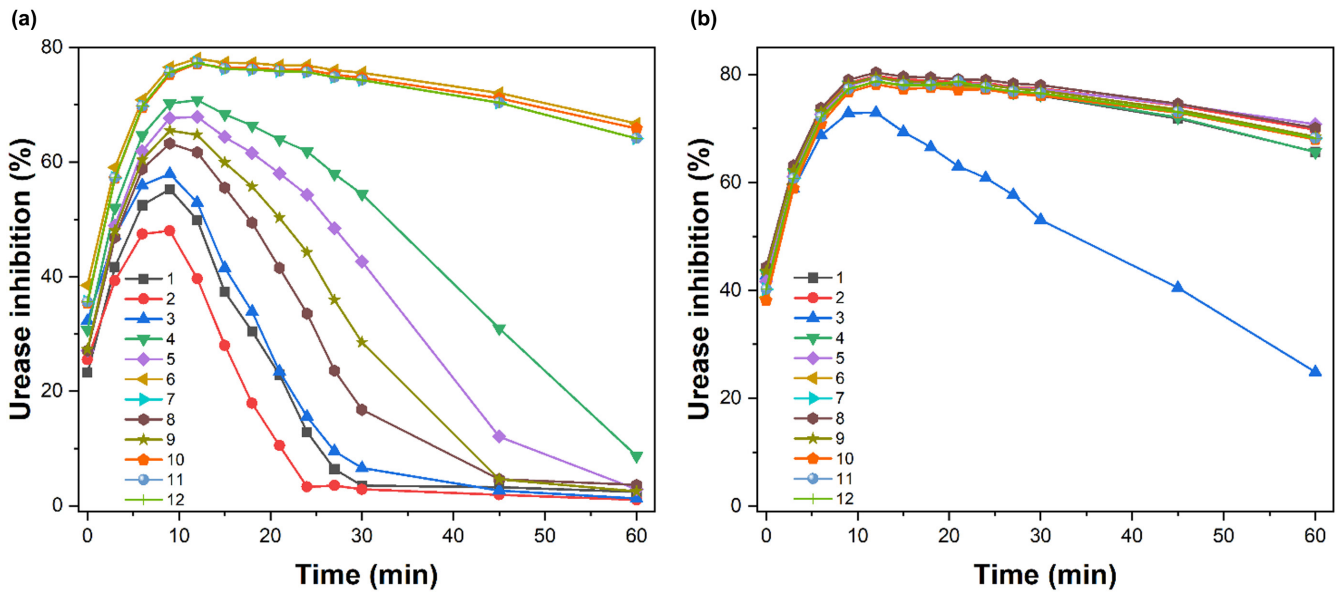


Figure 4. Inhibition capacity of the urease enzyme over time (minutes) for the extracts obtained in the experiments for the barks of (a) *S. adstringens* and (b) *M. eximia*.

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considerably among the *S. adstringens* extracts (Fig. 4(a)), with formulations containing at least 33% acetone (treatments 6, 7 and 10) maintaining inhibition for a more prolonged period compared to the isolated solvent extracts. This suggests that the extracts from solvent mixtures are more effective in the long term, possibly owing to the presence of a broader spectrum of inhibitory compounds that act through different mechanisms or have different degradation kinetics. In the case of the *M. eximia* species (Fig. 4(b)), most of the extracts showed a more stable inhibition profile after the peak, except for the 100% acetone extract. This reinforces the inference that the urease-inhibiting compounds in this species have a high polarity, as they are effectively extracted in water and ethanol. The variation in the temporal inhibition profile is an important indicator of the complexity and diversity of the bioactive compounds present in each species, as well as their interaction with the enzyme.

Conclusion

The simplex–centroid solvent mixture design was effective in determining the appropriate mixture for the responses studied for the barks of the two species. The lack of a universal extraction condition demonstrates the importance of considering the unique characteristics of each plant material. Assuming generic solutions can compromise the quality and efficiency of the process. Differences in extraction efficiency were attributed to solvent polarities, material matrix, and extractable compounds. For example, for urease inhibition, the ideal formulation for the species *S. adstringens* was 7% water, 50% ethanol, and 43% acetone, while for the species *M. eximia* it was 8% water, 75% ethanol, and 17% acetone. For Zn(II) ion chelation, the ideal mixture for *S. adstringens* was 25% water, 38% ethanol, and 38% acetone, and for Cu(II) ion chelation in *M. eximia*, the optimal formulation was 0% water, 67% ethanol, and 33% acetone. These results highlight the need to optimize solvents for each type of compound and desired activity.

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Supporting information

The supplementary material contains the ANOVA results for the simplex–centroid design regression models for both species, the ANOVA results for the reduced special cubic model of general fit for the responses obtained in the *S. adstringens* and *M. eximia* species and the pareto chart showing the significant interactions for concentration of total phenolics, chelating activities of Fe(II), Zn(II), Cu(II) and urease inhibition capacity of *S. adstringens* and *M. eximia* barks.

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