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Development and validation of a prediction model for the extraction of total phenolic and its antioxidant activity from *Dichondra argentea*

Desarrollo y validación de un modelo predictivo para la extracción de fenoles totales y su actividad antioxidante de *Dichondra argentea*

S. Fernández-Avalos[1], L. González-Cruz[1], N.L. Flores-Martínez[2], A. Bernardino-Nicanor[1]*
[1]*Tecnológico Nacional de México/IT de Celaya, Antonio García Cubas Pte. # 600, Celaya 38010, Guanajuato, México.*
[2]*Universidad Politécnica de Guanajuato, Avenida Universidad Sur 1001. Comunidad Juan Alonso, Cortazar 38483, Guanajuato, México.*
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Abstract
Dichondra argentea is used in traditional medicine to treat diseases such as cholecystitis, tonsillitis, gastritis, general body aches and cardiovascular problems. In addition, it is consumed for vomiting, fever, diarrhea and loss of appetite, but the biological activity attributed to it has not yet been demonstrated. Therefore, the aim of the present work was to optimize the extraction of total phenolics and their antioxidant activity from *Dichondra argentea* using water as an extractant. For this purpose, a response surface model was used that included the boiling time, the plant:solvent ratio and the type of sample drying as extraction parameters. The results showed that the optimal extraction conditions are 15 minutes of boiling, a plant-solvent ratio of 1 g of dried sample /55 mL water and convection drying. These conditions made it possible to obtain the highest antioxidant activities for the DPPH and FRAP methods (356.815 μg Trolox eq /g of dried extract and 274.77 μg Fe^{+2} eq /g of dried extract, respectively) as well as the highest content of phenolic compounds (3630.88 μg EAG/g of dried extract). It was also found that time had the greatest influence on the 3 responses. In addition, the optimized extract also had a high content of flavonoids, condensed tannins and saponins.
Keywords: phytochemicals, antioxidant, *Dichondra argentea*, aqueous extract, extraction conditions.

Resumen
Dichondra argentea se utiliza en la medicina tradicional para tratar enfermedades como la colecistitis, amigdalitis, gastritis, dolor general de cuerpo y problemas cardiovasculares. Además, se consume para el vómito, fiebre, diarrea y pérdida de apetito, pero aún no se ha demostrado la actividad biológica que se le atribuye. Por lo tanto, el objetivo del presente trabajo fue optimizar la extracción de fenólicos totales y determinar su actividad antioxidante a partir de *Dichondra argentea* utilizando agua como disolvente de extracción. Para ello, se utilizó un modelo de superficie de respuesta que incluía como parámetros de extracción el tiempo de ebullición, la relación planta:disolvente y el tipo de secado de la muestra. Los resultados mostraron que las condiciones óptimas de extracción son 15 minutos de ebullición, una relación planta-disolvente de 1 g de muestra seca/55 mL de agua y secado por convección. Estas condiciones permitieron obtener las mayores actividades antioxidantes para los métodos DPPH y FRAP (356,815 μg Trolox eq /g de extracto seco y 274.77 μg Fe^{+2} eq /g de extracto seco, respectivamente), así como el mayor contenido de compuestos fenólicos (3630.88 μg EAG/g de extracto seco). También se observó que el tiempo tenía la mayor influencia sobre las 3 respuestas. Además, el extracto optimizado también tenía un alto contenido de flavonoides, taninos condensados y saponinas.
Palabras clave: fitoquímicos, antioxidante, *Dichondra argentea*, extracto acuoso, condiciones de extracción.

*Corresponding author. *E-mail*: aurea.bernardino@itcelaya.edu.mx;
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1 Introduction

The medicinal use of plants in Mexico is currently a living practice, as it is estimated that there are about 23,314 plants in Mexico today, but only about 4,500 are used in traditional Mexican medicine and of these, only 5% have chemical, pharmacological and biomedical validation (Aguirre-Dugua *et al*., 2022). For this reason, much current research is focused on the separation and evaluation of bioactive compounds from medicinal plants against various chronic diseases, as these have few side effects when used by the inhabitants of rural communities. Several authors have reported that the biological properties of plants are due to their secondary metabolites such as coumarins, quinones, phenolic acids, terpenoids, flavonoids, saponins and others (Schinella *et al*., 2002; Mocan *et al*., 2015). One of the biological activities in the treatment of various chronic diseases such as diabetes, cancer, Alzheimer's and Parkinson's is antioxidant activity (Rajendiran *et al*., 2018; Ladas *et al*., 2014; Sindhi *et al*., 2013). But antioxidant activity also contributes to fighting inflammation and the development of microorganisms (Schinella *et al*., 2002; Mocan *et al*., 2015). However, the large number of possible medicinal plants makes their research difficult. For this reason, most of them have not been studied to determine their biological activity and consequently their nutraceutical properties. One of them is *Dichondra argentea* Humb, which belongs to the Convolvulaceae family and is native to the southern United States, Mexico and some South American countries. In Mexico in particular, this plant is consumed in the states of Durango and Aguascalientes (UNAM, 2019) and is known as the "bile´ plant", as it is mainly used to treat biliary and kidney diseases, but also for stomach pain, high blood pressure, colitis and body inflammation, fever, diarrhea, anorexia and back pain, as well as an antiseptic (García, 2015).

One of the most important considerations in the characterization of secondary metabolites is the extraction method, which has a direct impact on biological activity (Ji *et al*., 2020). Therefore, various factors must be taken into account, such as the type and concentration of solvent, temperature, time and pH of the extraction (García-Márquez *et al*., 2012; Gil-Martín *et al*., 2022). In view of the above, the use of predictive methods and experimental designs that allow the possible effects of these factors on biological activity to be assessed, such as the response surface method, a suitable tool for the optimization of the extraction process of bioactive compounds from plants, which combines statistical tools and mathematical models with the aim of creating experimental models and evaluating the effects of different parameters to select the optimal variables of the process and obtain surface responses with benefits by adapting the empirical process to the experimental data obtained in relation to the experimental design created (Bezerra *et al*., 2008; Luo y Chen, 2010; Bruns *et al*., 2006).

For this reason, a response surface method was evaluated in this study, which made it possible to obtain the optimal parameters for extracting the highest concentration of phenolic compounds and the highest antioxidant activity, taking into account the validation of the predicted parameters. The results obtained will form the basis for future pharmacological studies on the importance of *Dichondra argentea* as a source of nutraceutical compounds.

2 Materials and methods

2.1 Materials and methods

Whole plants of *Dichondra argentea* grown in pots were obtained from the town of San Luis Tlaxialtemalco in Xochimilco, Mexico. The plants were transported to the laboratory in boxes.

2.2 Conditioning of the plant material

The plants were taken out of the pot and the soil was removed. Samples of 300 g of the whole plants without soil were placed in polyethylene mesh bags with an opening of approximately 168 μm. The mesh bags with the plants were dried under three different conditions. In the first, the mesh bag was hung in a room at ambient temperature (~29ºC), this treatment was referred to as traditional drying. In the second method, the mesh bag was hung in the refrigerator (Samsung, RS28T5B00B1, México) at a temperature of 3 ºC, this treatment was referred to as cold drying. In the third drying method, the mesh bag was placed in a convection oven (Binder, FD115-UL, Germany) at a temperature of 40 ºC, this treatment was referred to as convection drying. The samples were removed from the drying process when a minimum moisture content of 5 % was reached, which was determined using a thermobalance (Velab, vf-50-5, USA). Finally, the samples were ground and sieved through a sieve with a mesh size of 425 μm. The sample sieved was placed in a glass vial until use.

2.3 Proposed parameters for the response surface method

For the design of the response surface method, the numerical factors and levels were determined according to the report by Goldsmith *et al*, 2014;

Dongmo *et al*, 2017; Muala *et al*, 2021. The first factor considered was the plant-solvent ratio, which was set to a minimum value of 55 mL water and a maximum value of 100 mL water (Tay *et al*., 2014; Benarfa *et al*., 2020). The second factor was the boiling time, for which a low value of 5 min and a high value of 15 min was set according to Papoutsis *et al*. (2016) and Muala *et al*. (2021). A non-numerical factor was considered to evaluate the effects of the drying method, taking into account traditional drying, refrigeration drying and convection drying.

2.4 *Development of the response surface method*

An I-optimal design was used to evaluate the boiling time (A: 5-15 min), plant solvent ratio (B: 55-100 mL), and drying method (traditional drying, cold drying, and convection drying) on antioxidant activity (FRAP and DPPH assays) and total phenolic content (TPC), in addition the optimal conditions of extraction factors were obtained, the design was carried out using the program Design expert v.12.0.3.0 (Stat Ease Inc., Minneapolis, MN, USA), as shown in Table 1.

The effect of the independent variables on the dependent variables was evaluated using the model equations and the graphical representation of the effect can be seen as contour plot and 3D diagram. The fitting of the experimental data was performed using a polynomial equation to determine the relationships between the independent variables and the obtained responses of the dependent variables (Equation 1). A test for lack of fit, the coefficient of determination (R) and the adjusted R^2 were used to assess the validity and appropriateness of the fitted model.

$$Y = \beta_0 + \sum_{i=1}^{k=1} \beta_i X_i + \sum_{i=1}^{k=1} \sum_{j}^{k} \beta_{ij} X_i X_j + \sum_{i=1}^{k} \beta_{ii} X^2{}_i \quad (1)$$

Where:

Y is the dependent variable;

X_i y X_j are the independent variables;

β_0 is the crossover coefficient;

β_i, β_{ii} and β_{ij} are the coefficients of the linear, quadratic and interaction or crossover product terms (Flores-Martínez *et al*., 2016).

2.5 *Extracts preparation*

Considering that a decoction process of *Dichondra argentea* is used for consumption in traditional medicine, water was used as the extraction solvent in the decoction process as reported by Fotakis et al. (2019) with some modifications. 1 g of the sieved sample was mixed with the predicted amount of water and heated to boiling temperature for the predicted time, after treatment the mixture was filtered in a vacuum, the filtrate was freeze-dried and then the dry samples were stored at -20 ºC until analysis.

Table 1. Experimental design plan.

RUN	Factor 1 A: time (min)	Factor 2 B: Plant:solvent ratio (1 g/mL)	Factor 3 C: Drying method	Response 1 TPC (μg EAG/ g of dried extract)	Response 2 DPPH (μg Trolox eq / g of dried extract)	Response 3 FRAP (μg Fe^{+2} eq / g of dried extract)
1	5	1:90.325	Cold	2049.9	216.41	104.11
2	5	1:55	Convection	2157.7	259.83	168.77
3	10	1:77.5	Cold	2425.2	329.07	222.72
4	12.8	1:100	Cold	2296.1	327.22	255.85
5	15	1:64.675	Cold	2944.4	384.19	257.58
6	7.15	1:55	Cold	2775.3	326.06	201.78
7	10	1:77.5	Traditional	2179.6	323.28	213.85
8	5	1:64.675	Traditional	2007.2	197.77	134.95
9	10	1:77.5	Convection	2200.3	320.97	217.57
10	7.15	1:100	Traditional	2147.7	228.22	178.68
11	10	1:77.5	Convection	2216.2	242.11	141.76
12	12.8	1:55	Traditional	2918.4	314.02	269.78
13	10	1:55	Convection	3157.4	383.84	236.52
14	5	1:100	Convection	2100.4	162.34	141.64
15	15	1:100	Convection	2200.3	306.03	215.22
16	15	1:90.325	Traditional	3006.3	237.83	249.16
17	15	1:55	Convection	3629.3	361.96	271.7

μg EAG, micrograms of gallic acid equivalents; μg Trolox eq, equivalent micrograms of Trolox; μg Fe^{+2} eq, equivalent micrograms of Fe^{+2}.

2.6 Dependent variables

2.6.1 Total phenolic content

The total phenolic content was determined as described by Cardador *et al*. (2011). 0.02 mL of the reconstituted extract (0.8 mg/mL) was mixed with 0.1 mL of Folin-Ciocalteu reagent (Folin & Ciocalteu's phenol reagent, Sigma Aldrich, St. Louis, MO, USA). The mixture was allowed to stand at room temperature for 8 min. Then the reaction was stopped by adding 0.3 mL of Na_2CO_3 (20%). The absorbance was measured at 760 nm after 90 min of rest at room temperature in a spectrophotometer (Optima Plus, SP 3000 nano, EE.UU.). The results were expressed in μg EAG/g of dried extract.

2.6.2 Antioxidant activity

*2.6.*2.1 DPPH radical scavenging method

The antioxidant activity was performed as described by Brand-Williams *et al.* (1995). This method is based on the color reduction and consequently on the reduction of absorbance produced by the reaction between the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and the antioxidant present. Briefly, 0.5 mL of the reconstituted extract (0.8 mg/mL) was added to 1.95 mL of a 0.1 M DPPH solution (D9132, Sigma-Aldrich, Mexico). The absorbance of the mixture was measured at 10-minute intervals until the reaction was completed after 1 hour. The results were compared with the negative control prepared under the same conditions but with distilled water added instead of the extract. The results were expressed in μg Trolox eq /g of dried extract.

*2.6.*2.2 Ferric reducing antioxidant power assay (FRAP)

The Fe^{3+} reducing sample assay was performed as described by Benzie and Szeto (1999). The FRAP reagent was added to sodium acetate buffer (300 mM, pH 3.6), TPTZ solution (2,4,6-tris(2-pyridyl)-s-triazine; 10 mM in 40 mM HCl) (T1253, Sigma-Aldrich, Mexico) and hexahydrated ferric chloride ($FeCl_3$-$6H_20$; 20 mM) in a ratio of 10:1:1 (v:v:v). For the assay, 100 μL of the reconstituted extract was mixed with 3000 μL of the FRAP reagent and the mixtures were incubated for 4 min at room temperature (~29ºC). The absorbance was then measured at 593 nm and the extract was replaced with water as a blank. A calibration curve of $FeSO_4$ (2.5-300 μg/mL) (Sigma-Aldrich, USA) was used. The results were expressed as μg Fe^{+2} eq /g of dried extract.

2.7 Qualitative and quantitative phytochemical analysis

With the optimal conditions generated by the response surface method to obtain the highest antioxidant activity and total phenolic content, the extraction process of *Dichondra argentea* was carried out and only with this optimal extract the qualitative and quantitative phytochemical analysis was performed.

2.7.1 Qualitative phytochemical analysis

The saponins, tannins, quinones, flavonoids, terpenoids coumarins and cardiac glycoside were qualitative tested according as described by Soni and Sosa (2013).

2.7.2 Quantitative phytochemical analysis

*2.7.*2.1 Quantification of the saponins

For this assay, the extract was reconstituted to a concentration of 3 mg/mL and quantification was performed according to the report of Hiai *et al*. (1976). To 1 mL of extract was added 1 mL vanillin (8 %) and also 1 mL sulfuric acid (72 %). The mixture was incubated at 60 ºC for 10 min. After the incubation time, the mixture was placed in ice for 15 min to stop the reaction and then the absorbance was recorded at 538 nm. The saponins concentration was determined using Diosgenin (Sigma-Aldrich, St. Louis, MO, EE.U.) as an external standard, and the results were expressed as mg diosgenin equivalents / g of dried extract.

*2.7.*2.2 Quantification of the condensed tannins

To 1 mL of the reconstituted extract (3 mg/mL) was added 1 mL of vanillin hydrochloride (hydrochloric acid and vanillin in methanol). The mixture was incubated at room temperature for 20 min then the absorbance at 500 nm was recorded. The concentration of condensed tannins was determined using (-)-epicatechin (Sigma-Aldrich, St. Louis, MO, EE.UU.) as an external standard and expressed as mg (-)-epicatechin eq / g of dried extract (Kunatsa *et al*., 2020).

*2.7.*2.3 Quantification of the total flavonoids

In a flask, to 0.5 mL of the reconstituted extract (3 mg / mL) were added 1.5 mL methanol (80 %), 0.1 mL potassium acetate (1 M) and 2.8 mL distilled water. The mixture was incubated at room temperature (~29ºC) for 30 min. After the incubation time, the absorbance was recorded at 410 nm. Quercetin was used as an external standard and the concentration of total flavonoids was expressed as mg quercetin eq / g of dried extract (Sagar *et al*., 2020).

Table 2. ANOVA of the dependent variable TPC.

Source	Sum of squares	Mean square	F-value	*p*-value	
Model	36436.25	2802.79	34.48	0.0070	significant
A-Time	14168.87	14168.87	174.31	0.0009	
B-Plant:solvent ratio	11122.73	11122.73	136.83	0.0013	
C-drying method	90.21	45.10	0.5549	0.6237	
AB	2808.60	2808.60	34.55	0.0098	
AC	2.56	1.28	0.0158	0.9844	
BC	3868.28	1934.14	23.79	0.0144	
A^2	956.77	956.77	11.77	0.0415	
ABC	2799.44	1399.72	17.22	0.0227	
A^2B	2072.88	2072.88	25.50	0.0150	
Residual	243.86	81.29			
Lack of Fit	242.60	121.30	95.96	0.0720	not significant
Pure Error	1.26	1.26			
Cor Total	36680.11				

3 Results and discussion

3.1 *Influence of the extraction factors on the total phenolic content (TPC)*

A reduced cubic model with a polynomial test was used to evaluate the dependent variable TPC. It was taken into account that in the analysis of variance (ANOVA) the values $p < 0.05$ indicate that the terms of the model are significant while that with values $p > 0.1$ the terms of the model are not significant. In this study, the values for the factors A, B, AB, BC, A^2, ABC, A^2B are statistically significant. However, as can be seen in Table 2, factor C (drying method) had a smaller effect on the dependent variable TPC, while factor A (boiling time) had a larger effect. Furthermore, considering that the values of the coefficient of determination (R^2 = 0.9934) and the adjusted coefficient of determination (R^2_{adj} = 0.9645) are close to 1, it can be concluded that a perfect relationship between the experimental results and the theoretical values predicted by the polynomial model was achieved.

The predicted data indicate that the highest concentration of TPC was obtained during convection drying (Figure 1c), which according to Juániz *et al*. (2016) could be due to the denaturation of the components of the cell wall by the drying temperature (40 ºC). It has also been reported that an increase in temperature causes a higher solubilization and diffusion coefficients of the polyphenols, resulting in a higher extraction ratio (Silva *et al*., 2007).

On the other hand, the lowest TPC values were obtained with cold and traditional drying. This result could be due to the enzymatic reactions and degradation of compounds caused by the long time at the drying temperature used, as reported by Sultana (2012). This is because the traditionally dried samples required three weeks to reach a moisture content of 5%, while the cold-dried samples required three and a half weeks to reach the same moisture content. In the case of the cold-dried samples, in addition to the exposure time, the temperature used (3 ºC) influence on loss of TPC since according to Galani *et al*. (2017) a storage to low temperature (4 ºC) generate the loss of TPC caused by the polyphenol's degradation in addition of a change in the pattern activity in the involved enzymes.

As can be seen in Figures 1a and 1b, a larger amount of solvent and a shorter boiling time (less than 13 min) resulted in a lower concentration of TPC, as indicated by the blue coloration. With a boiling time between 11 and 15 min and a solvent amount between 55 and 64 mL, the TPC concentration is higher, as indicated by the red coloration in Figure 1c. These results are in agreement with those of Dongmo *et al*. (2017) for *Boscia senegalensis* and Yapo *et al*. (2015) for *Combretum micranthum*, which indicate that the highest TPC concentration is achieved at a boiling time between 10 and 20 min.

3.2 *Influence of the extraction factors on the antioxidant capacity*

3.2.1 *DPPH*

Table 3 shows the influence of the three factors evaluated. As with the TPC, a reduced cubic model with a polynomial test was used to assess the dependent variable antioxidant capacity by performing analysis of variance (ANOVA). An F-value of 11.3 was obtained for this model, indicating that the model is significant. For the F-value of the lack of fit, a value of 0.27 was obtained, which means that the lack of fit is not significant in terms of pure error. The values obtained for the factors A, B and A^2 with a p value < 0.05 indicate that these terms are significant, while the

values p> 0.1 are not significant. This shows that time is the factor that has a greater influence on antioxidant activity, while the effect of the drying method (C) had no influence on antioxidant activity. Considering also the values of the coefficient of determination (R^2 = 0.8371) and the adjusted coefficient of determination (R^2adj = 0.7631), and according to (Makkiyah *et al*., 2023), which indicate that values > 0.70 are considered good, it can be concluded that a good relationship between the experimental results and the theoretical values predicted by the polynomial model was obtained.

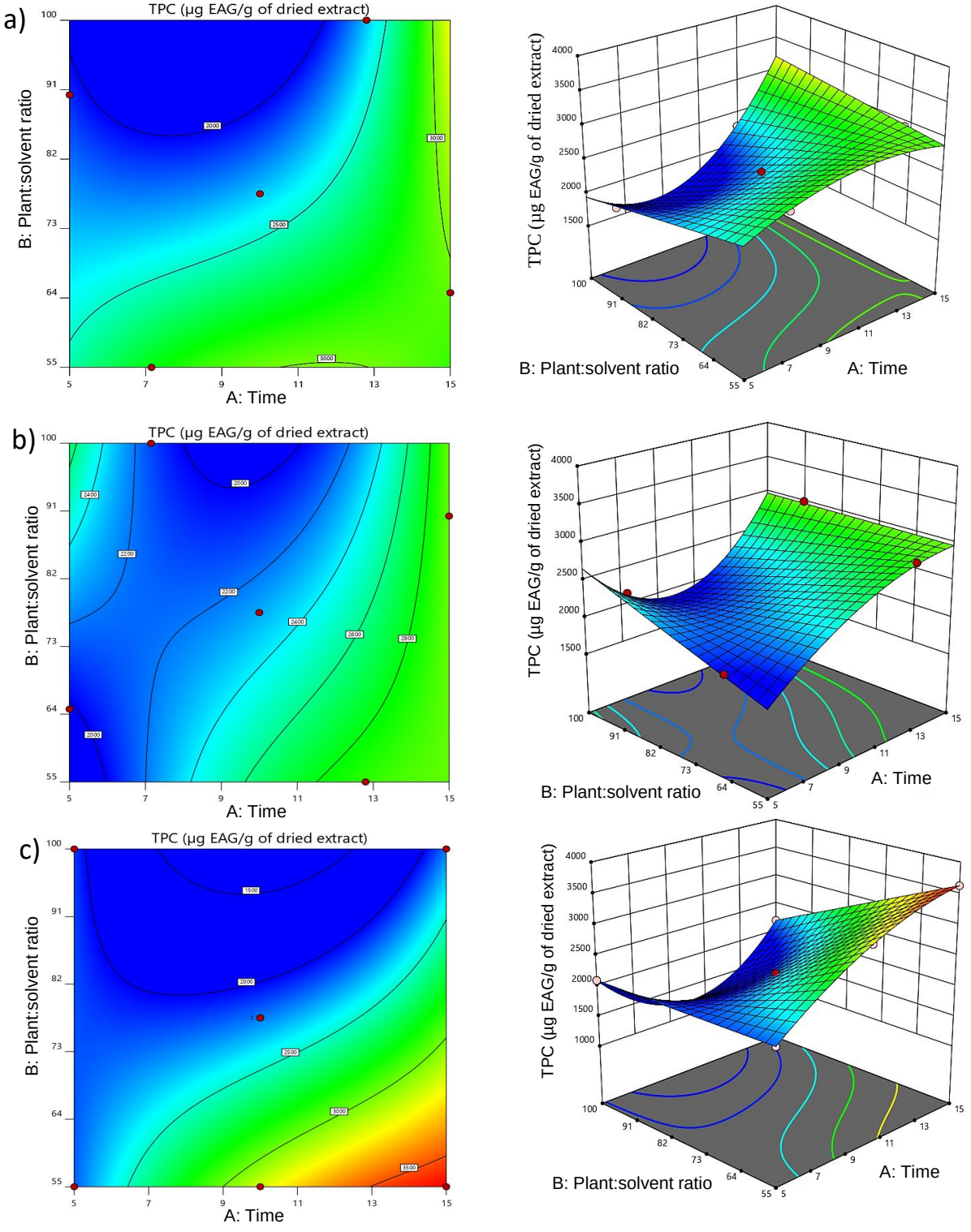


Figure 1. Contour graphic and 3D surface of the TPC response, a) cold drying, b) traditional drying, c) convection drying.

Table 3. ANOVA of the dependent variable DPPH.

Source	Sum of squares	Mean square	F-value	*p*-value	
Model	58535.83	11707.17	11.31	0.0005	significant
A-Time	27227.63	27227.63	26.30	0.0003	
B- Plant:solvent ratio	13847.60	13847.60	13.37	0.0038	
C-drying method	7945.11	3972.55	3.84	0.0544	
A^2	7292.47	7292.47	7.04	0.0224	
Residual	11389.34	1035.39			
Lack of Fit	8279.89	827.99	0.2663	0.9186	not significant
Pure Error	3109.45	3109.45			
Cor Total	69925.17				

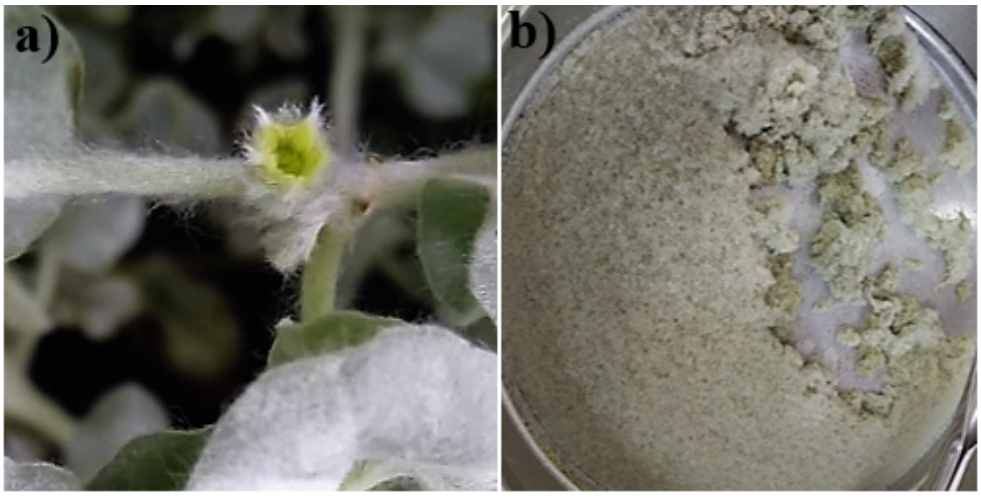


Figure 2. *Dichondra argentea*, a) Complete plant, b) Dried, ground and sieved plant.

The lowest antioxidant activity, determined by the DPPH method, was obtained when a larger volume of solvent was used and the boiling time was between five and seven min, which corresponds to the blue zone in Figures 3b and 3c. In contrast, the highest antioxidant activity values, determined by the same method, were obtained with the lowest volume of solvent and a boiling time between 11 and 15 min., located in the yellow zone of Figures 3b and 3c.

For the drying method, all three methods showed similar behavior between the factor and the dependent variable (antioxidant activity, DPPH). Considering that the drying method is the determining factor, the cold drying method (Figure 3a) showed the broadest yellow-red color spectrum, indicating a greater response with boiling times between 8 and 15 min.

Previous reports have shown that optimization of the decoction process in several plants with boiling times between 10 and 11 minutes achieved the highest antioxidant activity as determined by the DPPH method. For example, *Boscia Senegalensi* (Dongmo *et al*. 2017) and *Pelargonium graveolens* (Ennaifer *et al*., 2018), whose result for DPPH radical inhibition was around 60% at a concentration of 136.10 ± 0.62 mg ET/g. Gogoi *et al*. (2019) also reported that the use of a small amount of solvent during decoction process produces higher antioxidant activity, as is the case with *Pleurotus citrinopileatus*, and that the appropriate ratio between plant and solvent depends on the type of tissue and the phytochemicals that constituent the plant (Bitwell *et al.,* 2023). For these reasons, the lower volume was set in 55 mL was considered in this study, taking into account that, as shown in Figure 2a, the villi of the plant form a lint-like structure during grinding (Figure 2b), which increases its water absorption capacity, but the homogeneous water absorption allows a high extraction of compounds with antioxidant activity.

Considering that a boiling time between 11 and 15 min gave the highest antioxidant activity determined in this study using the DPPH method, a simulation was performed with a time longer than 15 min up to 18.94 min to determine whether increasing the boiling time causes an increase in the extraction of compounds with antioxidant activity. The results predicted from the model showed a decrease in antioxidant activity when the boiling time was more than 15 min, as shown in Figures 4a, 4b and 4c. The decrease in antioxidant activity when the boiling time exceeds 15 min apparently leads to a degradation of the compounds and consequently to a decrease in

antioxidant activity determined by the DPPH method, as Muala et al. (2021) indicate that these compounds have thermolabile properties.

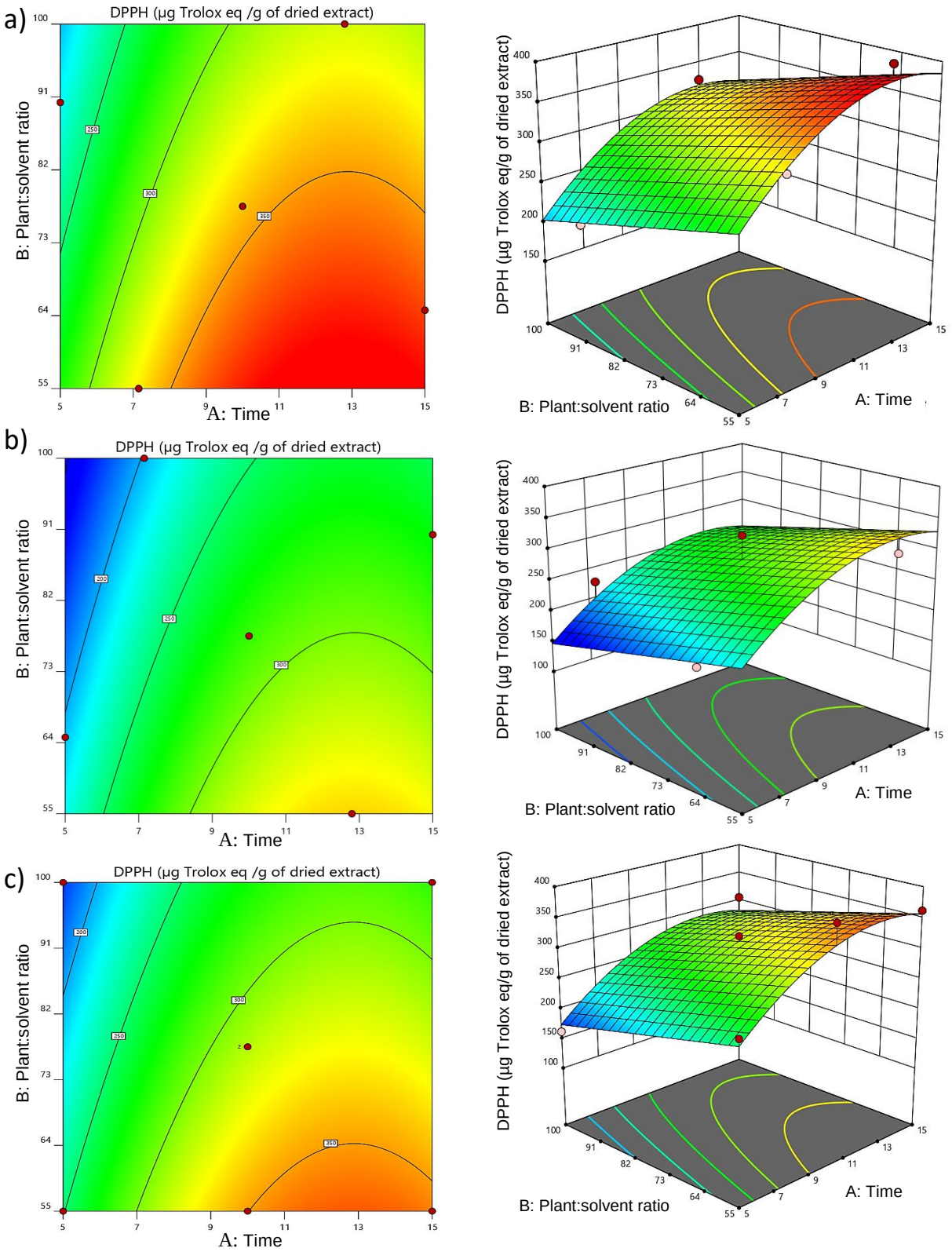


Figure 3. Contour graphic and 3D surface of the DPPH response, a) cold drying, b) traditional drying, c) convection drying.

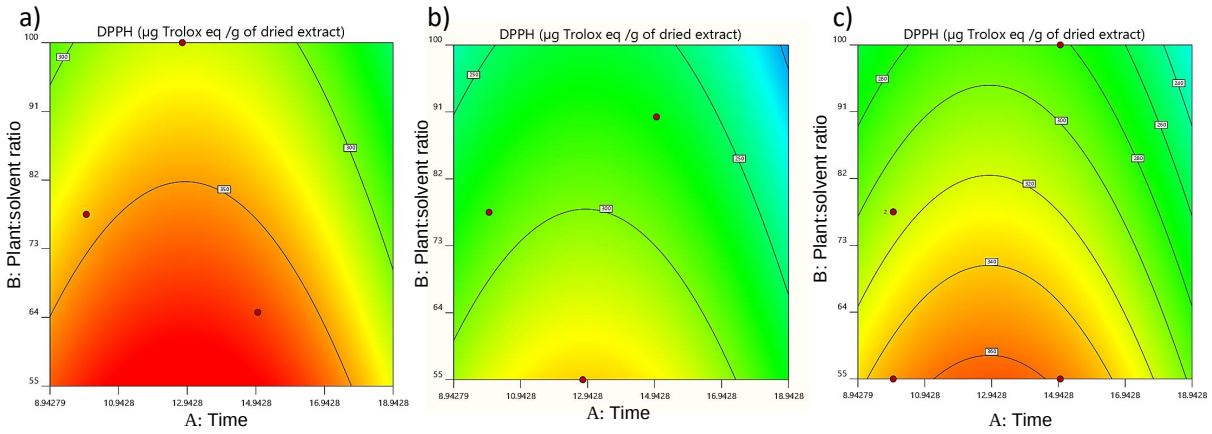


Figure 4. Simulation of times longer than 15 minutes, a) cold drying, b) traditional drying, c) convection drying.

Table 4. ANOVA of the dependent variable FRAP.

Source	Sum of squares	Mean square	F-value	*p*-value	
Model	36255.66	9063.92	16.86	< 0.0001	significant
A-Time	29798.09	29798.09	55.41	< 0.0001	
B- Plant:solvent ratio	2123.15	2123.15	3.95	0.0702	
C-drying method	1143.56	571.78	1.08	0.3771	
A^2	2077.59	2077.59	3.86	0.0729	
B^2	2442.42	2442.42	4.54	0.0544	
Residual	6453.09	537.76			
Lack of Fit	3579.51	325.41	0.1132	0.9873	not significant
Pure Error	2873.58	2873.58			
Cor Total	42708.75				

3.2.2 FRAP

Similar to TPC and antioxidant activity determined by the DPPH method, a reduced quadratic model with a polynomial test was used to evaluate the dependent variable of antioxidant capacity determined by the FRAP method by performing an analysis of variance (ANOVA), as shown in Table 4. With this model, an F value of 16.86 was obtained, indicating that the model was significant. On the other hand, an F-value of 0.11 was obtained for the lack of fit, which means that the lack of fit is not significant in terms of pure error. For the factor time (A), a $p< 0.05$ was obtained, which means that this factor is a significant term of the model, while that for the B and C factors the value of p obtained was > 0.05 for this reason, these factors is not significant. The experimental results obtained showed a good relationship with the predicted values based on the values of the coefficient of determination (R^2= 0.8489) and the adjusted coefficient of determination (R^2adj = 0.7985).

The categorical factor C-drying method and B-solvent-to-plant ratio, showed no significant difference compared to the dependent variable antioxidant activity (Figures 5a, 5b and 5c) determined by the FRAP method. The antioxidant activity determined by the FRAP method increases when a lower volume in the solvent-plant ratio is used, which is consistent with the data of Tan *et al*. (2011) and Dailey y Voung (2015).

The highest antioxidant activity was obtained with a boiling time between 11 and 15 min. This time range is consistent with findings reported for water extracts of olive leaf, *Zingiber officinale*, and lemon pomace (Goldsmith *et al*., 2014; Ramírez-Godínez *et al*., 2017; Papoutsis *et al*., 2016), which indicate that the best antioxidant activity, as determined by the FRAP method, is achieved with a 15-min boiling time. These results are likely due to the fact that, during the boiling process, insoluble high-molecular-weight compounds in the plant material are hydrolyzed, releasing low-molecular-weight compounds (Lee *et al*., 2017). An interval of 11 to 15 min appears to be sufficient for the adequate breakdown of insoluble compounds, resulting in higher antioxidant activity as determined by the FRAP method. Also have been considered that the contact of the plant material with the water in boiling increase the solubilization of the compounds with antioxidant activity with a greater probability of extract the target analytes (Muala *et al*., 2021). On the other hand, Kumar *et al.,* (2023) indicate that the

water heating improves the extraction efficiency since the dielectric constant of the water decrease which increase the dissolution of the compounds from the vegetal matrix.

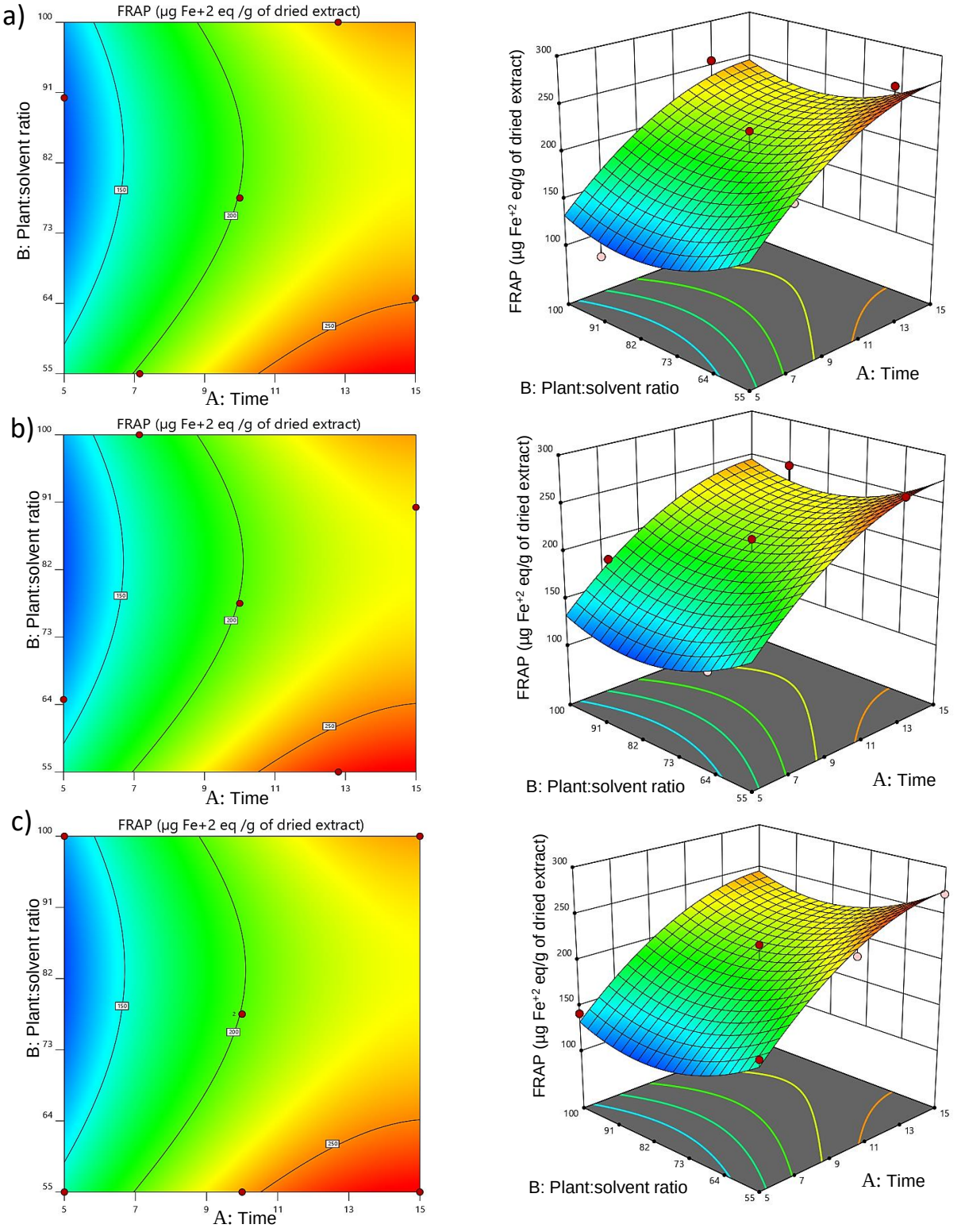


Figure 5. Contour graphic and 3D surface of the FRAP response, a) cold drying, b) traditional drying, c) convection drying.

Table 5. Correlation values of Total Phenolics Content (TPC) versus antioxidant activity using the DPPH and FRAP methods.

Antioxidant method	Correlation values (R) TPC (μg EAG/g of dried extract)
FRAP (μg Fe^{+2} eq /g of dried extract)	0.736
DPPH (μg Trolox eq /g of dried extract)	0.629

Table 6. Predicted and experimental results of the prediction model.

Assay	Predicted value	Experimental value (n=3)	% Deviation
TPC	2332.26	2329.73 ± 7.54	0.11
(μg EAG/g of dried extract)	3630.88	3643.77 ± 13.02	0.35
	2960.81	2953.96 ± 12.43	0.23
DPPH	341.485	341.11 ± 4.17	0.01
(μg Trolox eq /g of dried extract)	356.815	356.86 ± 2.44	0.01
	328.122	329.07 ± 1.75	0.29
FRAP	208.52	208.03 ± 1.50	0.24
(μg Fe^{+2} eq /g of dried extract)	274.779	274.30 ± 1.19	0.17
	278.703	279.38 ± 2.18	0.24

μg EAG, micrograms of gallic acid equivalents; μg Trolox eq, equivalent micrograms of Trolox; μg Fe^{+2} eq, equivalent micrograms of Fe^{+2}.

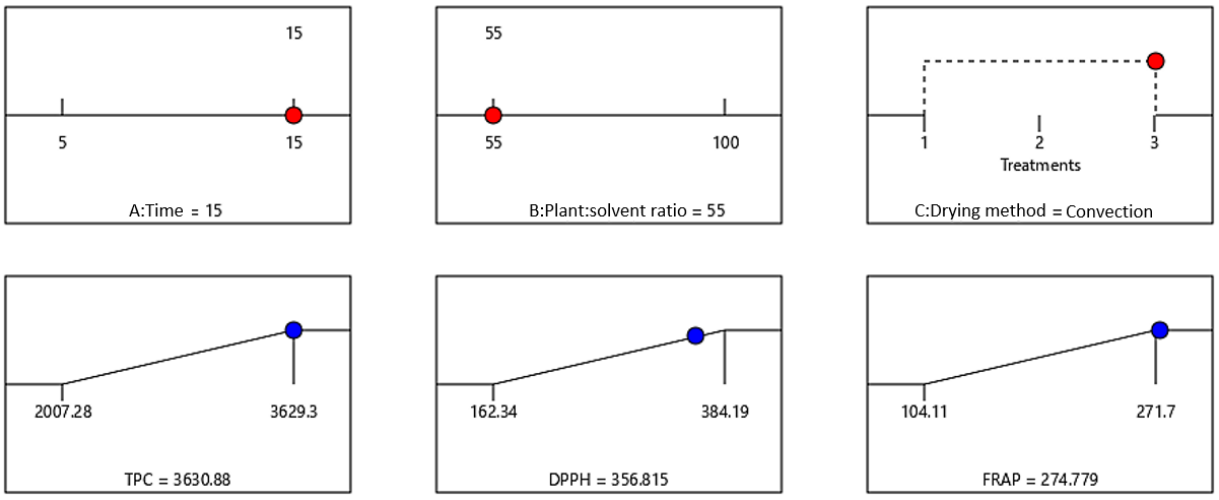


Figure 6. Optimal extraction conditions.

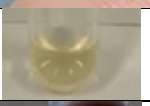
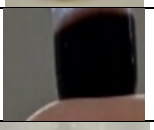
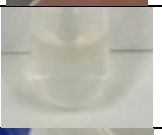
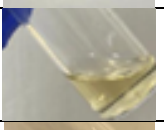
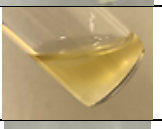
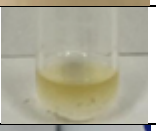
3.3 Pearson correlation analysis between antioxidant activity and total phenolic content (TPC)

A partial correlation between TPC and antioxidant activity determined by both methods, DPPH and FRAP, was obtained with R values of 0.629 and 0.736, respectively. As can be seen in Table 5, the antioxidant activity is not only caused by the phenolic compounds, but also by the presence of other compounds such as proteins, vitamins, minerals, alkaloids and saponins (Dangles, 2012). Considering the above, under the conditions determined in this study, there is no adequate correlation that allows the prediction of antioxidant activity as a function of TPC, since, as stated by Kähkönen *et al*. (1999), different chemical structures of phenolic compounds lead to different antioxidant activity. However, other authors such as Cardenas-Sandoval *et al*. (2012) and Estrada-Zúñiga *et al*. (2012) point out that in plants the most important compounds that contribute to antioxidant activity are the phenolic compounds.

3.4 Validation of the prediction model

To validate the conditions generated by the prediction model, the three highest values of each dependent variable were used for the experimental tests. The results are shown in Table 6. The values obtained in the experimental tests confirm that the created and proposed prediction model is reliable and reproducible, with percentage deviations between 0.01

Table 7. Secondary metabolites identified in water extract of *Dichondra argentea*.

Secondary metabolite	Reference for a positive result	Result	Visualizations
Saponins	Generation of stable foam	++	
Flavonoids	Yellow color	+++	
Quinones	Blue, green or intense red color	++	
Glucosides	Pink color	-	
Cardiac glucosides	Brown ring at interphase	-	
Terpenoids	Yellow-brown color	++	
Coumarins	Yellow color	+	
Tannins	Blue-black color	+++	

and 0.35 between the experimental results and the predicted ones.

Numerical optimization and several plots were performed to determine the optimal values of the independent variables. Only one set of optimal conditions was considered, namely the boiling time (15 min), the plant-solvent ratio (1g / 55 mL) and the drying method (convection drying). The predicted values for the dependent variable with these conditions were 356.815 μg Trolox eq/g for DPPH, 274.779 μg Fe^{+2} eq/g for FRAP and 3630.88 μg EAG/g for TPC. With these values for the three factors, the response for the three dependent variables was favorable. However, for the antioxidant activity determined by the DPPH method, the response of the predicted value is not at its maximum point, despite this the predicted value is inclined towards the vertical line on the right side, which represents the highest values of the response (Figure 6).

3.5 Phytochemical analysis of the optimized extract

3.5.1 Qualitative phytochemical analysis

Considering that the prediction model did not show a complete correlation between TPC and antioxidant activity, a phytochemical analysis of the optimized extract was performed for the secondary metabolites reported by several authors to possess antioxidant activity. Several secondary metabolites were detected in the optimized extract, mainly tannins and flavonoids, while coumarins, saponins and quinones were found in lower concentrations. Glucosides and cardiac glucosides were not identified (Table 7).

In the identification of secondary metabolites, it has been reported that the chemical nature of phytochemicals can vary depending on the plant material used and affects their solubility in different

Table 8. Quantitative concentration of phytochemical compounds.

Sample	Total flavonoids mg quercetin eq / g of dried extract	Condensed tannins mg (-)-epicatechin eq / g of dried extract	Saponins mg diosgenin eq / g of dried extract
Water extract of *Dichondra argentea*	12.22 ± 0.34	49.69 ± 0.96	142.00 ± 0.86

mg quercetin eq, milligram equivalents of quercetin; mg (-)-epicatechin eq, milligram equivalents of (-)-epicatechin; mg diosgenin eq, milligram equivalents of diosgenin.

solvents (Abubakar and Misau, 2017). Considering that the water has a polarity index of 10.2 is capable of extract a broad number of phytochemicals in comparison with other solvent wit lower polarity such as chloroform which due its lower polarity is not possible extract both tannins and saponins (Dhawan y Gupta, 2017). However, Khalil and Mustafa (2020) reported that the extraction of coumarins usually requires the use of more than one solvent in addition to methanol; for this reason, a lower concentration of coumarins was found in this study. For the glucosides, similar conditions are required, since, as mentioned by Martins *et al*. (2016), for their extraction it is necessary to use a solvent with medium polarity such as ethanol (70%), which allows a greater extraction of these compounds. It has also been reported that the decoction generates Maillard compounds that lead to a reduction in the extraction of glucosides (Ibrahim *et al*., 2020). In other studies, using the same species or other species of the same genus, the presence of triterpenoids, coumarins, flavones and isoflavones was also reported (Chou *et al*., 1993; Yuming *et al*., 2002; Sheu *et al*., 2012).

3.5.2 Quantitative phytochemical analysis

The quantification of phytochemicals compounds (Table 8) showed that in the with the conditions obtained of the prediction model, the optimized extract have a high concentration of saponins, condensed tannins and total flavonoids. However, is necessary consider that the composition can vary depending on the plant although remain to same genus (Rubanza *et al.,* 2005).

The obtained results are according with those reported by Hoyos-Martínez *et al*., (2019) who indicated that concentrations of tannins between 29 y 887 mg/g of dry extracts are recovery when water is used as solvent of extraction.

Conclusions

The obtained results are the first in indicating the best conditions for the extraction of phenolic compounds and antioxidant activity from *Dichondra argentea*, since with the experimental assays was observed a deviation lower than 1 % in comparison with the predicted results, indicating that the prediction model is reliable and reproducible. Under this consideration, the extraction of the highest TPC and antioxidant activity is obtained with a treatment of *Dichondra argentea* at 15 min of boiling time, a ratio plant-solvent of 1 g of sample/ 55 mL of water and convection drying as ideal conditions. The prediction model optimized for extraction of phenolic compounds showed also a high concentration of tannins and flavonoids, medium concentration of saponins, in addition, quinones, terpenoids and coumarins were identified. This study is relevant considering that can be the basis for further research in the pharmacological area with *Dichondra argentea.*

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