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ORIGINAL RESEARCH PAPER

EXTRACTION OPTIMIZATION OF *TRADESCANTIA SPATHACEA* SW. LEAF CRUDE EXTRACT AND ANTHOCYANIN CONTENT

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Abstract: Considering both the health and energy areas where anthocyanins have been revealing their real and potential applications, research on their extraction optimization methods is a priority. Conditions to achieve the highest mass of crude extract from *Tradescantia spathacea* leaves with the highest absorbance (A) in the region of 500 - 540 nm linked with anthocyanins, were investigated through a Box-Behnken design and response surface methodology. Independent variables involved in the Box-Behnken design were solvent (ethanol) concentration (30, 50, 70 % v/v), solvent/fresh leaf ratio (1.67:1, 2.34:1; 3:1 v/w) and extraction temperature (30, 50, 70 °C). Dependent (response) variables were crude extract amount and the extract with the maximum A in the region of 500-540 nm. Experimental values of A 500 - 540 nm were 0.3562 and 0.7558; crude extract mass ranged between 0.285 g and 0.7157 g, corresponding to a yield between 144 to 347 mg dried extract per g of dried leaf. Using a solvent/fresh leaf ratio of 3:1 v/w, 70 °C, 65 % ethanol, the highest values of both crude extract mass and A 500 - 540 nm were obtained.

Keywords: *extraction, health and energy applications, natural red pigments*

INTRODUCTION

The plant included in this study has a currently accepted scientific name of *Tradescantia spathacea* Sw., also known as *Rhoeo spathacea* (Sw.) Stearn, *Rhoeo discolor* (L'Her.) Hance ex Walp and *Tradescantia discolor* L'Her of the Commelinaceae family [1]. It is native to southern Mexico, Guatemala and Belize and in traditional medicine has been specifically described with the name of T'ni ay by the Zoque-Popoluca Indian group in the south of the state of Veracruz, Mexico (Magallanes, Veracruz) and used to remedy or control long menstrual cycles [2, 3]. In relation to its chemical composition, the presence of the acylated anthocyanin rhoenin has been reported [4, 5]. The following activities have also been reported: anti-inflammatory [6]; antioxidant [7 - 9]; antigenotoxic and antimutagenic [7]; antigonorrhea and abortifacient [10]; antibacterial or antimicrobial [9, 11]; treatment for cancer at the preclinical level or cytotoxic activity against cancer cells at the preclinical level [12, 13].

Besides research in the health area, the presence of anthocyanin (a secondary metabolism product of the flavonoid pathway) in *Tradescantia spathacea* Sw. has attracted the attention for its potential use as a natural food colorant [14] and also in the energy area where anthocyanins have been used as photosensitizers in dye-sensitized solar cell (DSSC) prototypes [15 – 17] and recently, its aqueous extract has been reported as an alternative less polluting ingredient during SnO_2 and Ni-SnO_2 nanoparticle synthesis [18, 19].

References to *Tradescantia spathacea* Sw. leaves have frequently appeared in the above-cited research studies, however, studies of anthocyanin extraction optimization as a crude extract have not been published as far as can be ascertained. This lack of information delays an efficient use of *Tradescantia spathacea* Sw. leaves for the production of crude or purified extracts in their potential application as a food colorant or as a photosensitizer.

One of the most potent tools for optimizing processes is Response Surface Methodology (RSM) using different experimental designs including a central composite or Box-Behnken design [20]. The Box Behnken design has been used for modelling and optimizing different industrial processes to permit the evaluation of the effect of multiple independent factors on dependent responses. Some examples include the extraction of molecules of biotechnological interest such as anthocyanins from *Hibiscus sabdariffa* L. [21 – 24] and *Ixora siamensis* [25], betalains from *Opuntia ficus-indica* [26] and *Beta vulgaris* [27], or the evaluation of the capacity of sugarcane bagasse fly ash during removal of acrylonitrile from wastewater [28]. The present study shows the statistical relationship between independent variables: ethanol concentration, solvent: leaf ratio, and temperature for obtaining the best conditions to generate the highest amount of crude extract from leaves of *Tradescantia spathacea* Sw. using RSM.

MATERIALS AND METHODS

Materials

Purple maguey leaves were collected in the municipality of Veracruz, Ver. (Latitude:

19 ° 10'51 "N, Longitude: 96 ° 08'34" W) and botanical identification of one plant specimen was made by Dr. Sergio Avendaño Reyes of the Herbarium of the Institute of Ecology (INECOL).
Absolute ethanol was purchased from JT Baker (min. 99.9 % (v/v)). Deionized water was used in all the extraction solutions.

Extraction Method

The leaves were washed with running water to remove impurities and the lower ends of the leaves were cut; leaf size was subsequently reduced to approx. 1 cm x 1 cm, weighed according to what was required for the extraction and placed in a round bottom flask with three inlets, adding solvent (30 %, 50 %, 70 % ethanol), as suggested, in solvent: mass ratios of 1.67:1, 2.34:1 and 3:1. Extraction was carried out at three different temperatures (30 °C, 50 °C, 70 °C) for one hour. After the operation, the liquid extract was dried in a rotary evaporator (Büchi, E120, Sweden), recovering the solvent used and the crude extract. The latter was brought to constant weight in a vacuum oven (Gallenkamp, AF874/86, United Kingdom). Subsequently, the dried extract was resuspended with distilled water, and anthocyanin content was analyzed in a UV-VIS spectrophotometer (Hewlett-Packard, 8452 A, Germany) between 500 and 540 nm. Each experiment was performed in triplicate, and the average value was taken for data analysis.

Experimental design

In this study, Response Surface Methodology (RSM) was used to evaluate three independent variables (percentage of ethanol, solvent: leaf ratio and temperature) to obtain optimum raw extract and purple maguey leaf anthocyanin contents. The experimental sequence was established based on a Box-Behnken design with three levels for each of three independent variables, labelled as -1, 0 and +1, indicating lower, intermediate, and upper levels (Table 1).

Table 1. *Process independent variables, factors, and levels of the Box-Behnken design*

Independent variables, units	**Factors**	**Levels**		
	X	-1	0	1
Ethanol concentration, [%]	X_1	30	50	70
Solvent: solid ratio, [mL·g^{-1}]	X_2	1.67	2.34	3
Temperature, [° C]	X_3	30	50	70

The predicted response function (Y) was established according to generalized response surface model (Eq. 1):

$$Y = \beta_0 + \sum_i^k \beta_i X_i + \sum_{i=i}^k \beta_{ii} X_i^2 + \sum_{i>j}^k \beta_{ij} X_i X_j \qquad (1)$$

where β_0 is the model constant; β_i and β_{ii} are the linear and quadratic coefficients of X_i and X_i^2 factors, respectively; β_{ij} is the second order coefficient generated by the interaction between X_i and X_j factors and k refers to the number of evaluated factors (k=3).

Statistical analysis

Statistical analysis was performed using a Design Expert Statistical version 11 Software package (Stat-Ease, Inc, Minneapolis, USA, trial version). Data were analyzed through an analysis of variance (ANOVA) and the quality of the models was evaluated by a correlation coefficient (R^2) value. A Fisher's F test for each response was carried out to verify the statistical significance of the models. Differences were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Box-Behnken design analysis

In this study, a Box-Behnken design was used to propose a model of the optimal conditions of the extraction process based on three independent variables (solvent concentration, solvent: solid ratio, and temperature) at three levels for two response variables (extract weight and maximum absorbance between 500 and 540 nm). Experiments were performed in the order established by the design (Table 2).

Table 2. *Box-Behnken experimental design with three independent variables*

Run	Ethanol [%]	Solvent: Leaves ratio [mL·g^{-1}]	Temperature [°C]
1	50	3	30
2*	50	2.335	50
3	50	1.67	30
4	70	3	50
5	70	2.335	70
6	30	2.335	70
7	50	1.67	70
8	30	1.67	50
9*	50	2.335	50
10*	50	2.335	50
11	70	1.67	50
12	30	3	50
13	30	2.335	30
14	70	2.335	30
15	50	3	70

* Central points used to determine experimental error

Predicted values were obtained from the modelled design by Design Expert 11 software and experimental data were correlated with a percentage of error for each run. The predicted results for the two response variables are shown in Table 3.

Table 3. *Experimental and predicted responses of extraction process*

Run	Y1 = Dried extract weight [g]			Y2 = Maximum A between 500 and 540nm		
	Y1 exp	Y1 pre	% Error	Y2 exp	Y2 pre	% Error
1	0.433	0.367	15.218	0.486	0.447	7.980
2	0.513	0.490	4.353	0.528	0.537	-1.766
3	0.462	0.308	33.365	0.401	0.464	-15.644
4	0.393	0.499	-26.968	0.658	0.721	-9.559
5	0.606	0.622	-2.700	0.505	0.503	0.355
6	0.706	0.665	5.864	0.504	0.527	-4.522
7	0.716	0.614	14.266	0.392	0.429	-9.487
8	0.285	0.482	-69.114	0.752	0.688	8.524
9	0.388	0.490	-26.412	0.582	0.537	7.676
10	0.488	0.490	-0.508	0.504	0.537	-6.612
11	0.434	0.440	-1.321	0.486	0.447	7.939
12	0.592	0.541	8.561	0.623	0.660	-6.000
13	0.322	0.359	-11.366	0.488	0.488	-0.049
14	0.303	0.316	-4.409	0.356	0.332	6.781
15	0.716	0.673	5.991	0.756	0.692	8.511

Model analysis shows significant differences between models generated by the software (linear, 2FI or interactive, quadratic, and cubic) for both response functions, dried extract weight (Y1) and maximum A between 500 and 540 nm (Y2). For Y1, the linear model is suggested for having a lower p-value than 0.05, despite its low R2 (0.6479) value; on the other hand, Y2 is suggested to be evaluated using a quadratic model according to its higher R2 (0.8699) value (Table 4). For both equations, cubic models were aliased.

Table 4. *Model adequacy tested for dependent variables*

Sequential model sum of squares for dried extract weight (Y1)						
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Mean vs Total	3.61	1	3.61			
Linear vs Mean	**0.1979**	**3**	**0.066**	**6.75**	**0.0076**	**Suggested**
2FI vs Linear	0.0321	3	0.0107	1.14	0.3912	
Quadratic vs 2FI	0.0524	3	0.0175	3.79	0.0928	
Cubic vs Quadratic	0.0143	3	0.0048	1.09	0.5116	Aliased
Residual	0.0087	2	0.0044			
Total	3.91	15	0.2609			

Sequential model sum of squares for Maximum A between 500 and 540nm (Y2)						
Mean vs Total	4.29	1	4.29			
Linear vs Mean	0.0693	3	0.0231	1.93	0.184	
2FI vs Linear	0.0465	3	0.0155	1.45	0.2987	
Quadratic vs 2FI	**0.0593**	**3**	**0.0198**	**3.77**	**0.0935**	**Suggested**
Cubic vs Quadratic	0.023	3	0.0077	4.81	0.1771	Aliased
Residual	0.0032	2	0.0016			
Total	4.49	15	0.2994			
Model Summary Statistics for dried extract weight						
Source	**Std. Dev.**	**R^2**	**Adjusted R^2**	**Predicted R^2**	**PRESS**	
Linear	**0.0989**	**0.6479**	**0.5519**	**0.2818**	**0.2194**	**Suggested**
2FI	0.0971	0.7531	0.5679	-0.1643	0.3556	
Quadratic	0.0679	0.9246	0.7888	0.1871	0.2483	
Cubic	0.0661	0.9713	0.7994		*	Aliased
Model Summary Statistics for Maximum A between 500 and 540 nm						
Linear	0.1096	0.3443	0.1655	-0.3883	0.2795	
2FI	0.1034	0.5754	0.257	-1.1941	0.4418	
Quadratic	**0.0724**	**0.8699**	**0.6357**	**-0.864**	**0.3753**	**Suggested**
Cubic	0.0399	0.9841	0.889		*	Aliased

Representative equations for linear and quadratic models

Based on the Box-Behnken design, the relationships between the experimental values and the variables proposed in the design were expressed in two empirical models, one linear and one quadratic, as presented below (Eq. 2 and 3):

$$Y1=0.056758 - 0.001056A + 0.044549B + 0.00765C \quad (2)$$

$$Y2= 2.16160 – 0.025411A - 1.17733B + 0.010729C + 0.005658AB + 0.000083AC + 0.005244BC + 0.000058A^2 + 0.155181B^2 -0.000245C^2 \quad (3)$$

where: A = percentage of ethanol, B = solvent: mass ratio, C = temperature. The positive and negative signs in the equations relate to whether the value of the independent variable referred to (A, B or C) should be added or subtracted from the base value and their values indicate the relative impact of each one.

Applying an ANOVA test on these two models, followed by a Fisher's test (Table 5) the equation for dried extract weight shows a low p-value (p = 0.0076) for the model correlating to a high F value, indicating the model is significant. Contrary to Y_1 (dried extract weight), ANOVA analysis for Y_2 (maximum anthocyanin A between 500 and 540

nm) indicated a p-value for the model equal to 0.0812. This value could be due to noise; however, this model can be re-fitted. Additionally, the temperature (C) is a significant term in both models because of its low p-values C=0.0011 and C^2 = 0.0485, for Y_1 and Y_2, respectively.

Table 5. *ANOVA and Fisher´s test analysis*

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Dried extract weight						
Model	0.1979	3	0.066	6.75	0.0076	significant
A	0.0036	1	0.0036	0.3652	0.5579	
B	0.007	1	0.007	0.7182	0.4148	
C	0.1873	1	0.1873	19.16	0.0011	
Residual	0.1075	11	0.0098			
Lack of Fit	0.0988	9	0.011	2.51	0.3175	not significant
Pure Error	0.0087	2	0.0044			
Cor Total	0.3054	14				
Maximum A between 500 and 540 nm						
Model	0.1751	9	0.0195	3.71	0.0812	not significant
A	0.0164	1	0.0164	3.13	0.1373	
B	0.0303	1	0.0303	5.77	0.0614	
C	0.0227	1	0.0227	4.33	0.092	
AB	0.0227	1	0.0227	4.32	0.0922	
AC	0.0044	1	0.0044	0.844	0.4004	
BC	0.0195	1	0.0195	3.71	0.1119	
A^2	0.002	1	0.002	0.3768	0.5662	
B^2	0.0174	1	0.0174	3.32	0.1281	
C^2	0.0354	1	0.0354	6.75	0.0484	
Residual	0.0262	5	0.0052			
Lack of Fit	0.023	3	0.0077	4.81	0.1771	not significant
Pure Error	0.0032	2	0.0016			
Cor Total	0.2013	14				

Model fit

Diagnostic plots were generated to evaluate predicted data vs experimental data. Figure 1 shows how predicted and experimental data are related; only some points are placed near the straight line, others not, indicating a lack of fit. Points close to the straight line signify normal distribution (Figure 2). Some points are observed outside the centerline of the linear regression plot of studentized residuals. An outlier, that is a value considered to be outside the expected distribution, was detected by this scatterplot (Figure 2). This could influence the statistical analysis of the extraction process by exerting a strong bias shown by the results of a DFFIT test (Figure 3), not adequate for extraction optimization, and were thus excluded.

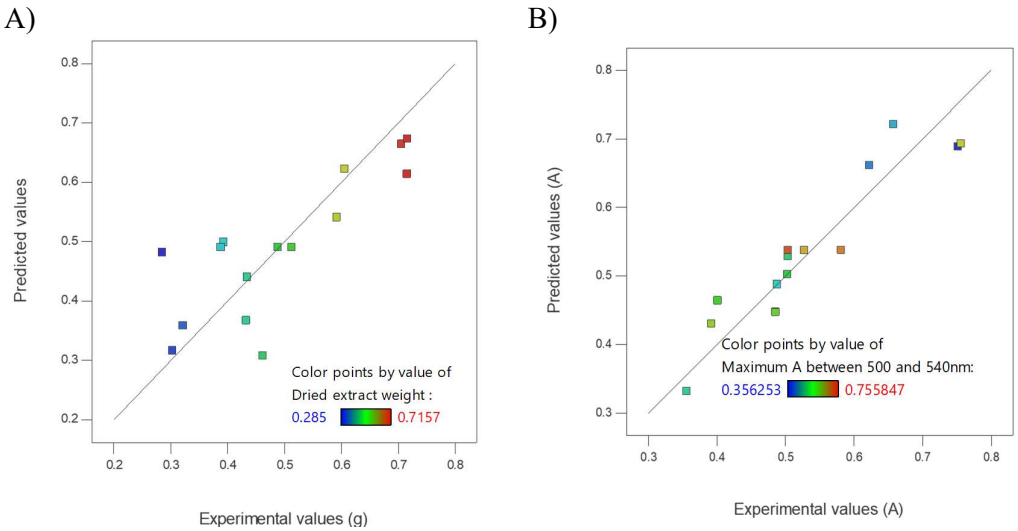

Figure 1. *Comparison between predicted and experimental values. For extract weight (A) and for maximum absorbance between 500 and 540 nm (B), it is observed only some points fit to plot while other points nearly fit the predicted value*

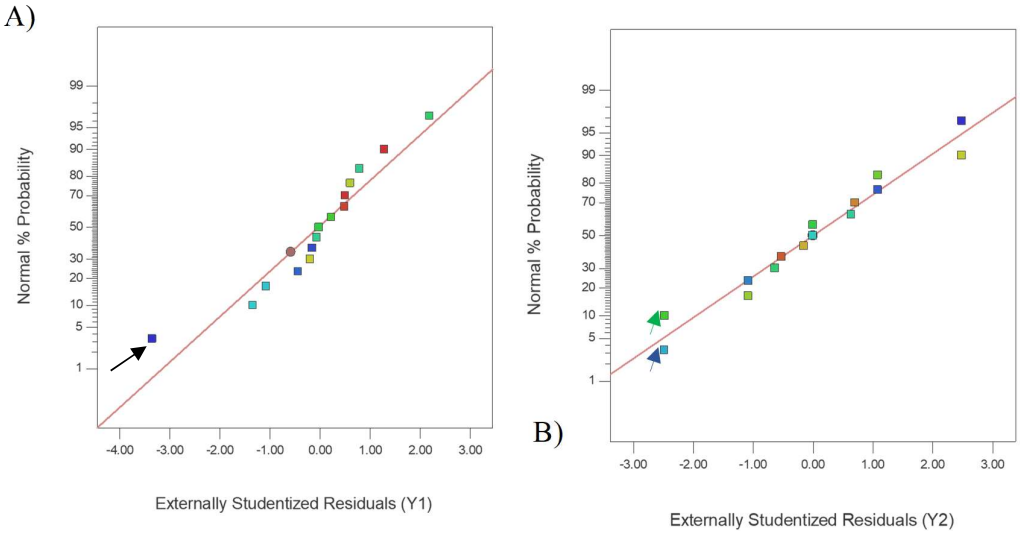

Figure 2. *Normal probability graphs of studentized residuals for Y_1 and Y_2 variables. Black arrow indicates at the left (A) run 14, as it shows, corresponds to an outlier for both variables. For Y_2, additional outliers have been observed, indicate by green arrow (run 10) and blue arrow (run 6) (B)*

Differences between fitted values known as DIFFTs indicate if a particular point influences the regression. For Y1, there's a point (run 8) outside the horizontal reference lines – 1.549, 0, +1.549 however, the linear model for the dried extract weight response was significant, and the model fits. In the case of Y2 there are 4 points exceeding the range of DIFFTs (Runs 3, 4, 8 and 15). Those points could influence the quadratic model generated for Y2, which was not significant ($p = 0.0812$). A refit for the model was carried out (an ANOVA test excluding the non-fitting runs 4 and 8), and the results are observed

in Table 6. Without those 2 points, a lineal model was suggested, in a similar fashion to dependent variable Y1. R^2 value diminished for this model 28 % from the quadratic model previously generated for the same response (Y2).

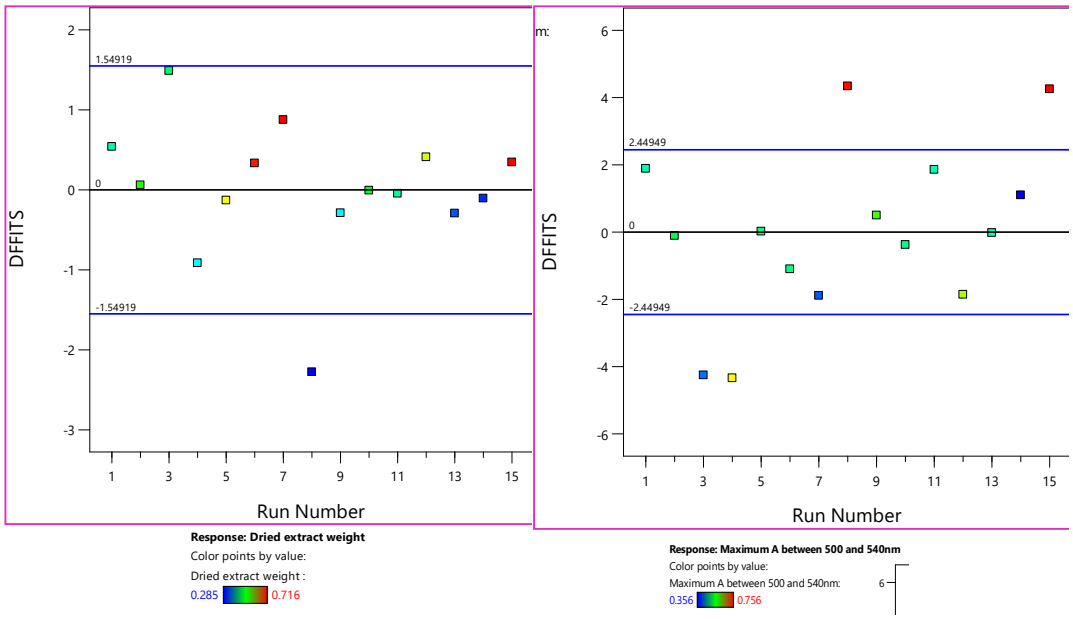

Figure 3. *Differences of fit (DFFITs) for each run. Each square corresponds to each run of analysis; the color of each square represents the gradient of maximum and minimum levels indicated down the graph. A) DFFITs graph associated with response Y1. B) DFFITS graph of response Y2*

Table 6. *Refit model Summary Statistics for Y2 response*

Model adequacy tested for dependent variables						
Sequential model sum of squares for Maximum A between 500 and 540nm (Y2)						
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Mean vs Total	3.36	1	3.36			
Linear vs Mean	**0.0809**	**3**	**0.027**	**4.94**	**0.0269**	**Suggested**
2FI vs Linear	0.0289	3	0.0096	2.85	0.127	
Quadratic vs 2FI	0.01	2	0.005	1.97	0.2539	Aliased
Residual	0.0102	4	0.0025			
Total	3.49	13	0.2686			
Model Summary Statistics for Maximum A between 500 and 540 nm						
Source	**Std. Dev.**	**R^2**	**Adjusted R^2**	**Predicted R^2**	**PRESS**	
Linear	**0.0739**	**0.6223**	**0.4964**	**0.0802**	**0.1196**	**Suggested**
2FI	0.0581	0.8444	0.6887	-0.0589	0.1377	
Quadratic	0.0505	0.9216	0.7647	-0.3075	0.17	Aliased

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An equation 4 for the new model was obtained and used for calculating the predicted values for Y2 (Table 7) and for use in the ANOVA analysis (Table 8).

$$Y2= 0.082488 – 000681A + 0.140038B + 0.002663 \quad (4)$$

where: A = ethanol concentration, B = solvent: mass ratio, C = temperature.

Table 7. *Experimental and refitted-predicted responses of the extraction process*

Re-fitting analysis			
Run	**Y2 exp**	**Y2pre**	**% Error**
1	0.486	0.085	82.479
2	0.528	0.088	83.369
3	0.401	0.090	77.437
5	0.505	0.096	81.029
6	0.504	0.098	80.463
7	0.392	0.101	74.202
9	0.582	0.106	81.709
10	0.504	0.109	78.350
11	0.486	0.112	77.000
12	0.623	0.114	81.630
13	0.488	0.117	76.003
14	0.356	0.120	66.357
15	0.756	0.122	83.805

Table 8. *ANOVA and Fisher´s test analysis for refitted model*

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	0.0809	3	0.027	4.94	0.0269	significant
A	0.001	1	0.001	0.1814	0.6801	
B	0.0463	1	0.0463	8.48	0.0173	
C	0.0227	1	0.0227	4.16	0.0719	
Residual	0.0491	9	0.0055			
Lack of Fit	0.0459	7	0.0066	4.11	0.2096	not significant
Pure Error	0.0032	2	0.0016			
Cor Total	0.13	12				

According to the ANOVA-Fisher analysis for the re-fitting analysis, the lineal model shows a low p-value = 0.0269, contrary to the ANOVA Test for the quadratic model, where p-value = 0.0812. P-values less than 0.0500 indicate model terms are significant. In this case, B is a significant model term (p = 0.0173).

Effect of dependent variables

The relationship between temperature, ethanol concentration, and solvent: solid ratio can be observed on the 3D response surface graph. Response surface graphs generate interactions between 3 dependent factors, maintaining one of them constant [27]. As mentioned before, the temperature has an influence on both independent variables shown by its p-values. In figure 4, the effect of temperature related to ethanol is shown; an increment of temperature and a low ethanol value (Figure 4a) yield a high value of dry extract weight. In a similar way, the best relationship between temperature and solvent: leaf ratio (Figure 4b) was achieved when both factors were used at their highest values. Temperature has been reported to improve extraction processes considering the nature of plant matrix [27, 29]. There is no observable interaction between solvent: leaf ratio and ethanol (Figure 4c) for improving dry extract weight yield.

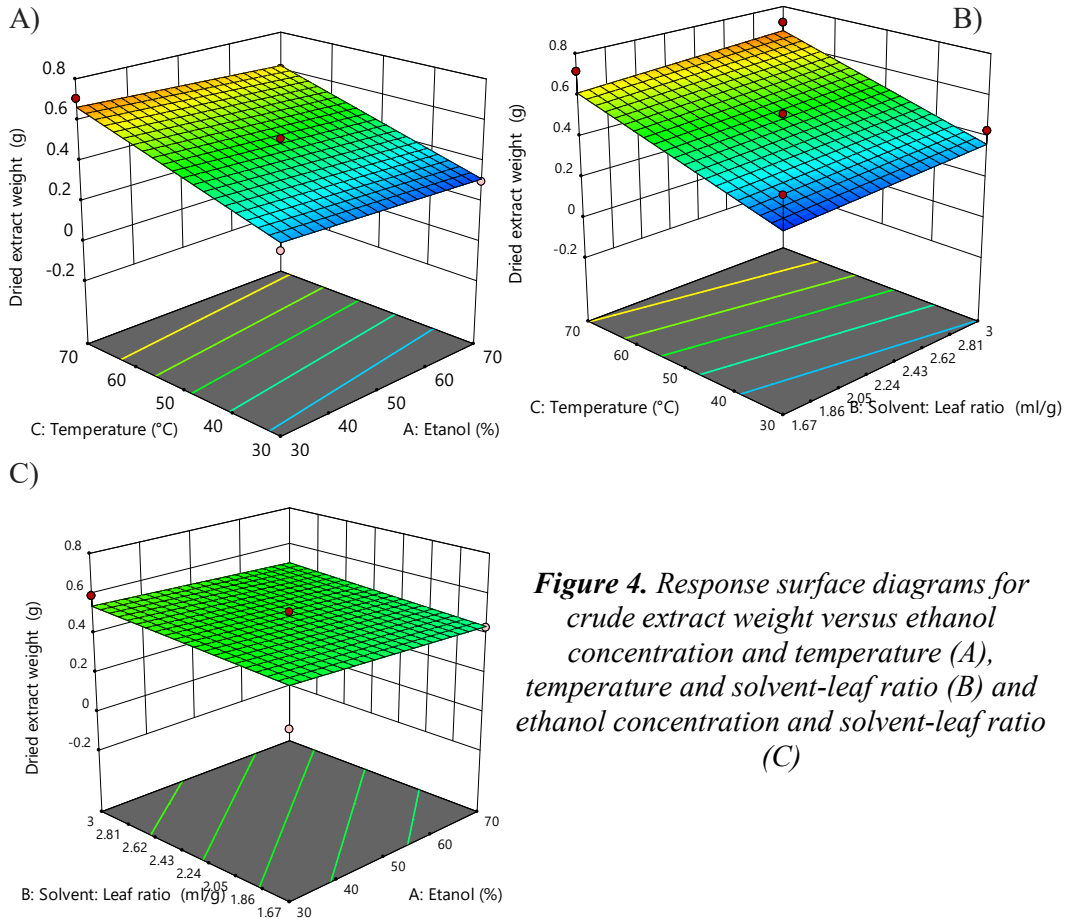

Figure 4. *Response surface diagrams for crude extract weight versus ethanol concentration and temperature (A), temperature and solvent-leaf ratio (B) and ethanol concentration and solvent-leaf ratio (C)*

Anthocyanin absorbance value ranges from 500 to 540 nm [30] and, considering the characteristic purple color of the plant leaf, that is directly related to this metabolite group. The generated response surface graph for this independent variable (Y_2) evaluating the interaction between solvent:leaf ratio-ethanol concentration (Figure 5a) generates high values for absorbance when evaluated at extreme, mean or low ethanol concentrations. When temperature is compared with solvent:leaf ratio (Figure 5b), both factors at their

highest condition, absorbance increases. The temperature and ethanol concentration interaction (Figure 5c) shows, in a similar fashion to Y_1, that temperature influences maximum absorbance data generating an intermediate value of absorbance independent of ethanol concentration.

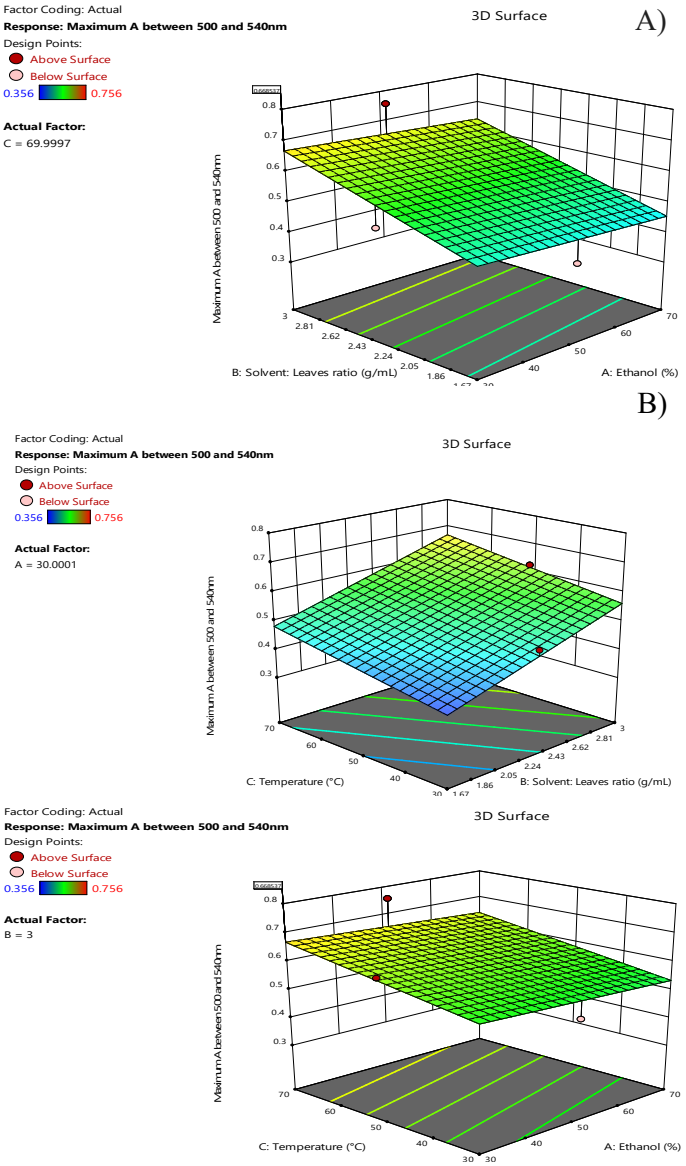

Figure 5. *Response surface diagrams for maximum absorbance between 500 and 540 nm versus ethanol concentration and solvent-leaf ratio (A), temperature and solvent-leaf ratio (B) and ethanol concentration and temperature (C)*

Optimization

Optimal extraction process conditions are shown in optimization ramps [31] for the greatest extract weight and the maximum absorbance between 500 and 540 nm (Figure 6). Optimum conditions of the extraction process of purple maguey leaves are 30 % ethanol, a 3:1 solvent:leaf ratio and a temperature of 70 °C, yielding 0.6567 g extract and a maximum A value between 500 and 540 nm of 0.7558 (Table 9).

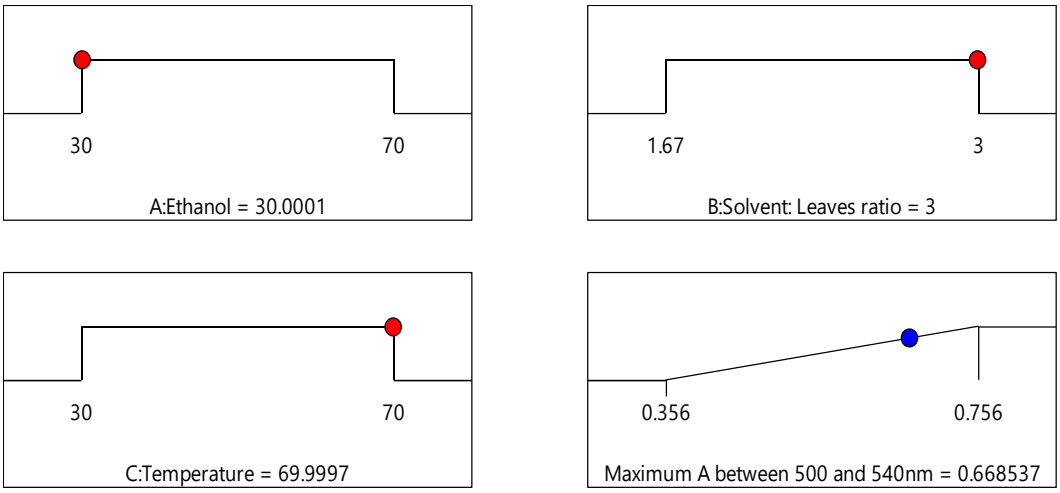

Figure 6. *Desirability ramp of optimized extraction process. Schematic representation of conditions to obtain the highest yields of dried extract weight and maximum A between 500 and 540 nm during Tradescantia spathacea leaf extraction*

Table 9. *Experimental and predicted values of responses under optimum conditions*

Optimum levels for process parameters	**Optimized conditions**		**Experimental values**	
	Extract weight [g]	Maximum A 500 -540 nm	Extract weight [g]	Maximum A 500-540 nm
Solvent concentration [% ethanol] = 30 %	0.6567	0.7558	0.7157 ± 0.10	0.7558 ± 0.09
Solvent: Fresh leaf ratio [$mL \cdot g^{-1}$] = 3:1				
Temperature [°C] = 70				

CONCLUSIONS

Every condition has an impact on the extraction process yield; however, temperature represents the most influential variable to optimize conditions of the extraction experiment. Regardless that some of the experimental data generated noise for statistical and optimization analysis, according to data generated by Design Expert 11, predictable conditions to reach the greatest level for both dependent variables were obtained. It is necessary to evaluate some other parameters like *p*H, antioxidant activity (DPPH assay) or total phenolic composition that could improve or generate information to reach optimal conditions for high-quality extraction. *Tradescantia spathacea* has the potential to obtain some interesting compounds for different fields, from the food industry to solar cells.

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REFERENCES

1. Integrated Taxonomic Information System ITIS: *Tradescantia spathacea* Sw. 2021,Taxonomic Serial No.: 505554;
2. http://www.medicinatradicionalmexicana.unam.mx/fmim/termino.php?l=4&p=zoque-popoluca&cr=25&t=maguey-morado&id=1016 Biblioteca Digital de la Medicina Tradicional Mexicana (Mexican Traditional Medicine Digital Library) Flora Medicinal Indígena de México (Indigenous Medicinal Flora of Mexico),accessed February 5, 2022;
3. Martínez, M.: *Las plantas medicinales de México*, 5th edition, Botas, México, **1969**, 368-372;
4. Idaka, E., Ogawa, T., Kondo, T., Goto, T.: Isolation of highly acylated anthocyanins from Commelinaceae plants, *Zebrina pendula*, *Rhoeo spathacea* and *Setcreasea purpurea*, *Agricultural and Biological Chemistry*, **1987**, **51**, 2215-2220;
5. Idaka, E., Suntory Limited: *U.S. Patent 4,999*, *423*, **1991**;
6. Longuefosse, J.L., Nossin, E.: Medical ethnobotany survey in Martinique, *Journal of Ethnopharmacology*, **1996**, **53**, 117-142;
7. González-Avila, M., Arriaga-Alba, M., de la Garza, M., Hernández-Pretelín, M.C., Domínguez-Ortiz, M.A., Fattel-Fazenda, S., Villa-Treviño, S.: Antigenotoxic, antimutagenic and ROS scavenging activities of a *Rhoeo discolor* ethanolic crude extract, *Toxicology in Vitro*, **2003**, **17**, 77-83;
8. Reyes-Munguía, A., Azuara-Nieto, E., Beristain, C.I., Cruz-Sosa, F., Vernon-Carter, E.J.: Purple maguey (*Rhoeo discolor*) antioxidant properties, *CyTA Journal of Food*, **2009**, **7**, 209-216;
9. Tan, J.B.L., Lim, Y.Y., Lee, S.M.: Antioxidant and antibacterial activity of *Rhoeo spathacea* (Swartz) Stearn leaves, *Journal of Food Science and Technology*, **2015**, **52**, 2394-2400;
10. Halberstein, R.A.: Medicinal plants: historical and cross-cultural usage patterns, *Annals of Epidemiology*, **2005**, **15**, 686-699;
11. García-Varela, R., García-García, R.M., Barba-Dávila, B.A., Fajardo-Ramírez, O.R., Serna-Saldívar, S.O., Cardineau, G.A.: Antimicrobial activity of *Rhoeo discolor* phenolic rich extracts determined by flow cytometry, *Molecules*, **2015**, **20**, 18685-18703;
12. Rosales-Reyes, T., de la Garza, M., Arias-Castro, C., Rodríguez-Mendiola, M., Fattel-Fazenda, S., Arce-Popoca, E., Hernández-García, S., Villa-Treviño, S.: Aqueous crude extract of *Rhoeo discolor*, a Mexican medicinal plant, decreases the formation of liver preneoplastic foci in rats, *Journal of Ethnopharmacology*, **2008**, **115**, 381-386;
13. García-Varela, R., Fajardo-Ramírez, O.R., Serna-Saldívar, S.O., Altamirano, J., Cardineau, G.A.: Cancer cell specific cytotoxic effect of *Rhoeo discolor* extracts and solvent fractions, *Journal of Ethnopharmacoly*, **2016**, **190**, 46-58;
14. Tan, J.B.L., Lim, Y.Y., Lee, S.M.: *Rhoeo spathacea* (Swartz) Stearn leaves, a potential natural food colorant, *Journal of Functional Foods*, **2014**, **7**, 443-451;
15. Lai, W.H., Su, Y.H., Teoh, L.G., Hon, M.H.: Commercial and natural dyes as photosensitizers for a water-based dye-sensitized solar cell loaded with gold nanoparticles, *Journal of Photochemistry and Photobiology A: Chemistry*, **2008**, **195**, 307-313;
16. Hug, H., Bader, M., Mair, P., Glatzel, T.: Biophotovoltaics: Natural pigments in dye-sensitized solar cells, *Applied Energy*, **2014**, **115**, 216–225;
17. Zolkepli, Z., Lim, A., Ekanayake, P., Tennakoon, K.: Efficiency enhancement of cocktail dye of *Ixora coccinea* and *Tradescantia spathacea* in DSSC, *Journal of Biophysics*, **2015**, **5**, 1-8.
18. Matussin, S.N., Tan, A.L., Harunsani, M.H., Mohammad, A., Cho, M.H., Khan, M.M.: Effect of Ni-doping on properties of the SnO_2 synthesized using *Tradescantia spathacea* for photoantioxidant studies, *Materials Chemistry and Physics*, **2020**, **252**, 123-293;
19. Khan, M.M., Matussin, S.N., Rahman, A.: Recent progress of phytogenic synthesis of ZnO, SnO_2 and CeO_2 nanomaterials, *Bioprocess Biosystem Engineering*, **2022**, **45** (4), 619-645, https://doi.org/10.1007/s00449-022.02713.z;
20. Gilmour, S.: Response Surface Designs for Experiments in Bioprocessing, *Biometrics*, **2006**, **62**, 323-331;
21. Chumsri, P., Sirichote, A., Itharat, A.: Studies on the optimum conditions for the extraction and concentration of roselle (*Hibiscus sabdariffa* Linn.) extract, *Songklanakarin Journal of Science and Technology*, **2008**, **30** (Suppl. 1), 133-139;
22. Cisse, M., Bohuon, P., Sambe, F., Kane, C., Sakho, M., Dornier, M.: Aqueous extraction of

anthocyanins from *Hibiscus sabdariffa*: Experimental kinetics and modelling, *Journal of Food Engineering*, **2012**, **109**, 16-21;
23. Diessana, A., Parkouda, C., Cissé, M., Diawara, B., Dicko, M.H.: Optimization of aqueous extraction of anthocyanins from *Hibiscus sabdariffa* L. calyces for Food Application, *Food Science and Quality Management*, **2015**, **45**, 23-31;
24. Miranda-Medina, A., Hayward-Jones, P.M., Carvajal-Zarrabal, O., Ladrón de Guevara-Vela, L.A., Ramírez-Villagómez, Y.D., Barradas-Dermitz, D.M., Luna-Carrillo, G., Aguilar-Uscanga, M.G.: Optimization of *Hibiscus sabdariffa* L., anthocyanin aqueous-ethanol extraction parameters using response surface methodology, *Scientific Study & Research Chemistry & Chemical Engineering, Biotechnology, Food Industry*, **2018**, **19**, 053-062;
25. Mat Nor, N.A, Arof, A.K.: Optimization of extraction parameters for anthocyanin from *Ixora siamensis* by response surface methodology, *Optical and Quantum Electronics*, **2016**, **48**, 1-9;
26. Maran, J.P., Manikandan, S., Mekala, V.: Modeling and optimization of betalain extraction from *Opuntia ficus-indica* using Box–Behnken design with desirability function, *Industrial Crops and Products*, **2013**, **49**, 304-311;
27. Swamy, G.J., Sangamithra, A., Chandrasekar, V.: Response surface modeling and process optimization of aqueous extraction of natural pigments from *Beta vulgaris* using Box–Behnken design of experiments, *Dyes and Pigments*, **2014**, **111**, 64-74;
28. Kumar, A., Prasad, B., Mishra, I.M.: Optimization of process parameters for acrylonitrile removal by a low-cost adsorbent using Box–Behnken design, *Journal of Hazardous Materials*, **2008**, **150**, 174-182;
29. Sulaiman, I.S.C., Basri, M., Masoumi, H.R.F., Chee, W.J., Ashari, S.E., Ismail, M.: Effects of temperature, time, and solvent ratio on the extraction of phenolic compounds and the anti-radical activity of *Clinacanthus nutans* Lindau leaves by response surface methodology, *Chemistry Central Journal*, **2017**, **11** (1), 54;
30. Woodall, G.S., Stewart, S.R.: Do anthocyanins play a role in UV protection of the red juvenile leaves of *Syzygium*?, *Journal of Experimental Botany*,**1998**, **49**, 1447–1450;
31. Anuar, N., Adnan, A.F.M., Aziz, N.S.N., Taha, R.M.: Optimization of extraction parameters by using response surface methodology, purification, and identification of anthocyanin pigments in *Melastoma malabathricum* fruit, *The Scientific World Journal,* **2013,** 810547, https://doi.org/10.1155/2013/810547;
32. Tan, J.B.L., Lim, Y.Y., Lee, S.M.: *Rhoeo spathacea* (Swartz) Stearn leaves, a potential natural food colorant, *Journal of Functional Foods*, **2014**, **7**, 443-451;
33. Lai, W.H., Su, Y.H., Teoh, L.G., Hon, M.H.: Commercial and natural dyes as photosensitizers for a water-based dye-sensitized solar cell loaded with gold nanoparticles, *Journal of Photochemistry and Photobiology A: Chemistry*, **2008**, **195**, 307-313;
34. Hug, H., Bader, M., Mair, P., Glatzel, T.: Biophotovoltaics: Natural pigments in dye-sensitized solar cells, *Applied Energy*, **2014**, **115**, 216–225;
35. Zolkepli, Z., Lim, A., Ekanayake, P., Tennakoon, K.: Efficiency enhancement of cocktail dye *of Ixora coccinea* and *Tradescantia spathacea* in DSSC, *Journal of Biophysics*, **2015**, **5**, 1-8.