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Optimization of Green Solid-Liquid Extraction of Andrographolide from *Andrographis paniculata*

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Abstract

This research paper focused on multi-objective optimization of green solid-liquid extraction (SLE) of andrographolide from Andrographis paniculata (AP), one of the most vital stages for the upstream phytochemical processing. The process was examined under isothermal conditions at different temperatures in a pressurized liquid extractor (PLE). The strategy combines rotatable central composite design (RCCD)—one of the techniques for response surface methodology (RSM), coupled with desirability and penalty functions for the simultaneous numerical optimization based on the concepts of extraction yield and extraction selectivity—a new developed technique for process analysis. Four independent process variables, solid-liquid ratio (1/50–5/50 mg/L), average particle size (0.175–1.200 mm), extraction time (5–25 minutes), and temperature (80–120 °C) were investigated. The temperature process variable was found to have the most significant effect, followed by the particle size, and extraction time on the performance indicators. The optimum overall yield of andrographolide (OYA), overall selectivity of andrographolide (OSA), and overall selectivity of andrographolide with respect to other phytochemical compounds (OSA/OPCC) were found at the solid-liquid ratio of 2/50 g/mL, average particle size of 0.375 mm, extraction time of 20 min, and temperature of 110 ℃ with desirability value of 0.824 to be 8.17%, 34.84% and 53.35%, respectively.

1. Introduction

In recent years, there has been a growing demand for phytochemical compounds for herbal medicinal products, cosmeceuticals, nutraceuticals, and functional food products. This shift is somewhat due to the consumer preference for products containing phytochemical ingredients, which are generally perceived as milder, safer and healthier than their synthetic equivalents [1-3]. More often than not, the demand for andrographolide water extracts, in particular, has been dramatically rising due to the fast-growing awareness, concern and concentrated focus on the quality as well as safety of the extracts, and increased preference for more greener products. Andrographolide is a specialty phytochemical compound, which is considered as the major medicinally active constituent of *Andrographis paniculata* (AP) [4,5]. It is usually produced through solid-liquid extraction (SLE).

SLE is one of the most vital processes in phytochemical industries. It is regarded as the heart of the industry. A poor understanding, design and operation of the SLE can endanger the overall profitability of the phytochemical industry. Green SLE is an approach to processing techniques for extraction through utilization of non-toxic solvents that eliminate or reduce generation of products and by-products that are hazardous to human health and environment [1,6]. It is generally characterized with lower extraction rates and poor performances due to some constraints of the process, its complex nature, and impacts of process variables. The search for the optimum values of the process variables for green SLE of andrographolide is complicated by poorly understood competing side extractions, side reactions, and interactions among process variables, and can be a difficult task. We have depicted in our companion article [7], the possible sequences of multiple competing green SLEs of andrographolide with other phytochemical compounds from *Andrographis paniculata* (AP) in series with multiple reactions of andrographolide and other phytochemical compound (s) in parallel or and series (see compound **3**, Figure 1) using one of the schemes proposed by Isah [1] and Isah et al. [8]. However, finding the optimum extraction temperature, time, average particle size, and solid-liquid ratio that simultaneously maximize the selectivity and yield of the desired product and minimize unwanted products will improve the performance of the process, provide a way for reliable kinetics investigations, scale-up to pilot scales or commercial sizes, and consequently the profitable operation of the entire industry.

In our companion article [7], the results of exploratory investigation of the influences of average particle size, solid-liquid ratio, time, and their interactions on green SLE of andrographolide from AP have been reported based on complete factorial experimental design (CFED). This paper is an extension of our companion article [7] to reveal the optimum values of the earlier investigated process variables with the inclusion of temperature for the green SLE of andrographolide from AP (see Figure 1) through the application of design of experiment (DoE), desirability and penalty functions. This investigative work employs a rotatable central composite design (RCCD), one of the techniques for statistical analysis using response surface methodology (RSM) to examine and simultaneously optimize the influences of the multi-variables on the multi-responses of the process. Simultaneous multi-objective optimization was carried out using modified desirability function proposed by Derringer and Suich [9] and penalty function with the aid of Design Expert Software V9 (Stat-Ease, Inc., Minneapolis, USA). The overall yield of andrographolide (OYA), overall selectivity of andrographolide (OSA), and overall selectivity of andrographolide with respect to other phytochemical compounds (OSA/OPCC) were the multi-responses used as performance indicators of the process.

2. Material and Methods

2.1 Materials

All materials listed in our companion article [7] were used. However, five different classes of average particle sizes of 1.200 mm, 0.805 mm 0.605 mm, 0.375 mm and 0.175 mm of AP plant material were used in the investigation.

2.2 Optimization of green SLE for andrographolide

The optimization of green SLE for andrographolide from AP was conducted in an automated pressurized liquid extractor (PLE), utilizing Dionex ASE 100$^{®}$ in similar manner with exploratory experimentation as described in our companion article [7]. However, the study of temperature effects was incorporated and observed. The values of independent process variables for each run were set according to the RCCD as shown in Table 1.

2.3 Analytical methods

The general tests for phytochemical analysis of water extracts of AP and HPLC analysis of andrographolide were performed in the same way described in the companion article [7]. The determination for andrographolide concentration in the water extracts was analysed using calibration curve with an accuracy of 99.9%. The overall yield of andrographolide ($\hat{Y}$), overall selectivity of the desired extract (i.e. andrographolide), ($\hat{S}_D$), the overall selectivity of undesired extract(s) ($\hat{S}_U$), and overall selectivity of andrographolide with respect to other phytochemical compounds ($\hat{S}_{D/U}$) were calculated according to the Equations 1 to 4, reported by Isah [1] and Isah et al. [8].

Table 1: Ranges and levels of independent process variables for the RCCD

Process variable	Range and levels					
	Symbol	(+2α)	+1	0	-1	(-2α)
Solid-liquid ratio (g/m)	A	5/50	4/50	3/50	2/50	1/50
Particle size (mm)	B	1.200	0.855	0.605	0.375	0.175
Time (min)	C	25	20	15	10	5
Temperature (°C)	D	120	110	100	90	80

2.4 Design of experiments

A rotatable central composite design (RCCD) is one of the efficient DoE techniques of response surface methodology (RSM) useful for empirical modelling, analysis, and optimization of multi-variables and multi-responses. It is widely used in the product and process design, development, as well as in the improvement of the existing ones [10–19]. In 1951, Box and Wilson first reported the development of central composite design (CCD) [16]. The basic CCD for *k* variables consists of a 2^k factorial design with each factor at two levels, -1 and +1, augmented by at least one centre point, and *2 X k* additional axial or star points placed at the coordinates of the experimental design [10, 11,17]. Later in 1957, Box and Hunter improved the CCD by adding the features of rotatability [18]. One of the good features of RCCD estimates the response surfaces of the design points with uniform precisions over the design space. A rotatable design is said to be rotatable only if the variance of the estimated response is a function of the distance of the points from the centre of the design. However, the property of rotatability does not depend on the orientation of the design with respect to the true response surface [10]. The following are the procedures for RSM:

i. Conducting a set of experiments based on any suitable type of RSM design (e.g., RCCD) for reliable measurement of the responses.
ii. Developing an empirical model based on the second-order response surface equation.
iii. Representing the effects of process variables on the response parameter through contour and three dimensional (3D) plots.
iv. Determining the optimum values of process variables that maximize or minimize the response parameter.

Suppose that each of the multi-responses, n is related to the independent process variables, k by the equation as shown in Equation 1.

$$y_i = f(x_1 + x_2 + x_3 \ldots\ldots\ldots\ldots\ldots x_k) + \varepsilon \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where f represents the functional relationship between the response, y_i and the input process variables $(x_1 + x_2 + x_3 \ldots\ldots x_k)$, and ε is a statistical error. In the case of our process, $k = 4$, $x_1 = A$, $x_2 = B$, $x_3 = C$, and $x_4 = D$. Hence, the empirical data based on RCCD were fitted into the second order polynomial equation as shown in Equation 2.

$$y_i = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} {x_i}^2 + \sum_{i<j} \sum_{2}^{k} \beta_{ij} x_i x_j + \varepsilon \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where y_i is the estimated response, β_0 constant, β_i, β_{ii}, and β_{ij} are the coefficients for linear, quadratic, and second order interaction effects, respectively. x_i, and x_j represent the coded independent process variables, and ε is the standard error or residual between the observed and calculated values.

2.5 Multi-objective optimization

The desirability function technique modified by Derringer and Suich [9] were employed for the simultaneous optimization of multiple responses of green solid-liquid extraction of andrographolide from AP. The optimization problem was solved numerically using penalty function approach [19]. In this technique, each empirical model representing response y_i (developed through modeling of RCCD experimental data using second-order response surface equation as shown in Equation 1) was transformed into a desirability function, d_i. If the response, y_i is at its target or goal, then $d_i = 1$, and if the response y_i is outside an acceptable region, then $d_i = 0$. The response y_i has a linear relationship with d_i. Thus, $0 \leq d_i \leq 1$. A weight, w_i is used to add emphasis to the upper/lower bounds, or emphasize the target value. If $w_i = 1$, the d_i will vary linearly. If $w_i > 1$ (maximum 10), it means that more emphasis should be placed on the target. However, if $0 < w_i < 1$ (minimum 0.1), this implies that less importance should be on the target. The change in the value of w_i will have an influence on the ith desirability function.

Let y_i be the ith response (obtained from modeling of RCCD experimental data using Equation 2, d_i be the ith desirability function, T_i be the target or goal of the optimization, L_i be the lower specification limit and U_i be the upper specification limit of the response. If the objective or target, T_i for the response, y_i is a maximum value (the larger the better), the desirability function is given by Equation (3).

$$d_i = \begin{cases} 0, & yi < L_i \\ \left(\dfrac{y_i - L_i}{T_i - L_i}\right)^{w_i}, & L_i \leq y_i \leq T_i \; for \; i = 1,2,3 \ldots\ldots n \ldots\ldots\ldots\ldots\ldots\ldots (3) \\ 1, & yi > T_i \end{cases}$$

On the other hand, if the target T_i for the response, y_i is a minimum value (the smaller the better), the desirability function is given by Equation (4).

$$d_i = \begin{cases} 0, & yi < T_i \\ \left(\dfrac{U_i - y_i}{U_i - T_i}\right)^{w_i}, & T_i \leq y_i \leq U_i \; for \; i = 1,2,3 \ldots\ldots n \ldots\ldots\ldots\ldots\ldots\ldots\ldots (4) \\ 1, & yi > U_i \end{cases}$$

However, if the two-sided desirability function assumes that the target is located between the lower specification limit, L_i and upper specification limit, U_i and the objective for y_i is to be as close as possible to the target T_i (the nominal is the best). The desirability function is defined as shown in Equation (5).

$$d_i = \begin{cases} 0, & yi < L_i \\ \left(\frac{y_i - L_i}{T_i - L_i}\right)^{w_{i1}}, & L_i \le y_i \le T_i \\ \left(\frac{U_i - y_i}{U_i - T_i}\right)^{w_{i2}}, & T_i \le y_i \le U_i \\ 0, & yi > U_i \end{cases} \quad for\ i = 1,2,3 \ldots..n \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

The individual desirability of all transformed responses is combined using geometric mean to give overall desirability objective function, $D_{overall}$ as shown in Equation 6.

$$D_{overall} = \left[\prod_{i=1}^{n} d_i\right]^{\frac{1}{n}} = (d_1 \times d_2 \times d_3 \ldots . d_n)^{\frac{1}{n}} \ldots\ldots\ldots\ldots\ldots\ldots (6)$$

3. Results and Discussion

3.1 Response surface methodology (RSM)

RCCD allowed us to examine the influence of four independent process variables on green solid-liquid extraction of andrographolide from AP with 30 experimental runs. These comprised of 16 factorial points, 8 axial points, and 6 replicates of the central point. The experiments were blocked, and the order was randomized. The levels of the factors used in this experiment were chosen based on the findings and suggestions presented in our companion article [7]. All experiments were performed according to the matrix of the experimental design as shown in Table 2. The responses characterizing the process are also presented in Table 2. The results disclosed that different levels of multi-factor namely solid-liquid ratio, time, particle size, and temperature influenced the measured multi-response parameters. RCCD experimental data for each response was fitted into a full second-order response surface model with its interactions. The empirical models developed through regression analysis are shown in Equations S1 to S6 (see Supporting information).

The models were significant at 95% confidence level. They were demonstrated to cover the following ranges for the AOA extracted from 0.00024 moles to 0.00158 moles, OYE from 16.67 % (w/w) to 23.71 % (w/w), and OYA from 2.17 % (w/w) to 9.21 % (w/w). In addition, the ranges for the OSA were from 13.01 % (w/w) to 39.15 % (w/w), OSOPCC was from 86.99 % (w/w) to 60.85 % (w/w) and OSA/OPCC was from 14.96 % (w/w) to 64.33 % (w/w). The summary of the analysis of variance (ANOVA) results for OYA, OSA and OSA/OPCC are shown in Table 2, and that for AOA, OYE, and OSOPCC are presented in Tables 1S (see supporting information).

From the regression analysis, and ANOVA for OYA, OSA, OSA/OPCC using second-order response surface model as shown in Table 3. The coefficient of determination, R^2 values for OYA, OSA, and OSA/OPCC are 0.965, 0.971, and 0.963 respectively. These results indicate that the models could account between 96.30% and 97.10% of the variability in the data. Meanwhile, the R^2 values for the AOA, OYE, and OSOPCC are also satisfact2zqorily (see supporting information). ANOVA (Table 3) shows the effects of the models' terms B, C, D, CD, and D^2 were significant (p-values < 0.05) whereas A, A^2, AB, AC, AD, B^2, C^2, BD, and BC were insignificant (p-values > 0.05). However, the linear effects of B and D, and the second-order effects of D^2 are the most consequential (p-values < 0.0001). Only the interaction of CD out of the six interaction effects was significant based on the suggested regions for finding the optimum values from our earlier investigation (see companion article) [7]. These results reveal that the temperature was the most influential process variable, followed by the particle size, and then the extraction time on the green SLE of andrographolide from AP.

Table 2. RCCD experimental design matrix and responses for the green SLE of andrographolide

Independent process variable											Responses					
			Codded levels				Actual levels									
Std ord	**Run ord**	**Block**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	y_1 (moles)	y_2 (%)	y_3 (%)	y_4 (%)	y_5 (%)	y_6 (%)
2	5	Day 1	+1	-1	-1	-1	4/50	0.375	10	90	0.00075	19.16	3.29	17.17	82.83	20.73
3	2	Day 1	-1	+1	-1	-1	2/50	0.855	10	90	0.00025	16.67	2.17	13.01	86.99	14.96
5	9	Day 1	-1	-1	+1	-1	2/50	0.375	20	90	0.00043	20.44	3.77	18.45	81.55	22.63
8	6	Day 1	+1	+1	+1	-1	4/50	0.855	20	90	0.00068	18.24	2.99	16.37	83.63	19.57
9	10	Day 1	-1	-1	-1	+1	2/50	0.375	10	110	0.00063	21.33	5.48	25.7	74.3	34.59
12	8	Day 1	+1	+1	-1	+1	4/50	0.855	10	110	0.00101	18.15	4.44	24.45	75.55	32.36
14	7	Day 1	+1	-1	+1	+1	4/50	0.375	20	110	0.00149	21.88	6.55	29.92	70.08	42.69
15	4	Day 1	-1	+1	+1	+1	2/50	0.855	20	110	0.00076	21.03	6.62	31.51	68.49	46
17	1	Day 1	0	0	0	0	3/50	0.605	15	100	0.00067	19.09	3.91	20.49	79.51	25.77
18	3	Day 1	0	0	0	0	3/50	0.605	15	100	0.00065	19.13	3.8	19.88	80.12	24.82
1	18	Day 2	-1	-1	-1	-1	2/50	0.375	10	90	0.00048	19.55	4.17	21.31	78.69	27.09
4	14	Day 2	+1	+1	-1	-1	4/50	0.855	10	90	0.00065	16.47	2.83	17.15	82.85	20.71
6	17	Day 2	+1	-1	+1	-1	4/50	0.375	20	90	0.00092	20.02	4.04	20.16	79.84	25.25
7	15	Day 2	-1	+1	+1	-1	2/50	0.855	20	90	0.00034	18.17	3.01	16.59	83.41	19.89

10	19	Day 2	+1	-1	-1	+1	4/50	0.375	10	110	0.00129	20.69	5.64	27.24	72.76	37.45
11	12	Day 2	+1	+1	-1	+1	4/50	0.855	10	110	0.00046	18.14	4.07	22.44	77.56	28.93
13	11	Day 2	-1	-1	+1	+1	2/50	0.375	20	110	0.00092	22.92	8.09	35.32	64.68	54.61
16	13	Day 2	+1	+1	+1	+1	4/50	0.855	20	110	0.00130	19.88	5.7	28.7	71.3	40.25
19	16	Day 2	0	0	0	0	3/50	0.605	15	100	0.00074	19.66	4.33	22.02	77.98	28.24
20	20	Day 2	0	0	0	0	3/50	0.605	15	100	0.00074	19.11	4.35	22.75	77.25	29.45
21	30	Day 3	-2	0	0	0	1/50	0.605	15	100	0.00024	20.23	4.14	20.47	79.53	25.74
22	23	Day 3	+2	0	0	0	5/50	0.605	15	100	0.00125	19.66	4.41	22.41	77.59	28.88
23	24	Day 3	0	-2	0	0	3/50	0.175	15	100	0.00112	23.71	6.54	27.59	72.41	38.11
24	27	Day 3	0	+2	0	0	3/50	1.200	15	100	0.00049	16.72	2.87	17.18	82.82	20.74
25	22	Day 3	0	0	-2	0	3/50	0.605	5	100	0.00051	18.38	2.98	16.21	83.79	19.34
26	26	Day 3	0	0	+2	0	3/50	0.605	25	100	0.00077	21.36	4.49	21	79	26.58
27	29	Day 3	0	0	0	-2	3/50	0.605	15	80	0.00045	18.41	2.65	14.39	85.61	16.81
28	21	Day 3	0	0	0	+2	3/50	0.605	15	120	0.00158	23.53	9.21	39.15	60.85	64.33
29	28	Day 3	0	0	0	0	3/50	0.605	15	100	0.00069	19.98	4.02	20.14	79.86	25.22
30	25	Day 3	0	0	0	0	3/50	0.605	15	100	0.00069	19.99	4.09	20.44	79.56	25.7

y_1, amount of andrographolide (AOA), y_4, overall selectivity of andrographolide (OSA)
y_2, overall yield of extract (OYE), y_5, overall selectivity of other phytochemical compounds (OSOPCC)
y_3, overall yield of andrographolide (OYA), y_6, overall selectivity of andrographolide with respect to other phytochemical compounds (OSA/OPCC).

Table 3. Analysis of variance (ANOVA) for the selected factorial models of OYA, OSA and OSA/OSPCC

Source	Sum of squares			DF			Mean squares			F-value			Prob > F		
	y_3 (%)	y_4 (%)	y_6 (%)	y_3 (%)	y_4 (%)	y_6 (%)	y_3 (%)	y_4 (%)	y_6 (%)	y_3 (%)	y_4 (%)	y_6 (%)	y_3 (%)	y_4 (%)	y_6 (%)
Model	73.47	1033.86	3462.63	14	14	14	5.25	73.85	23.85	25.78	30.87	23.85	$<0.0001^{(+)}$	$<0.0001^{(+)}$	$<0.0001^{(+)}$
A	0.077	0.021	0.48	1	1	1	0.077	0.021	0.047	0.38	0.009	0.047	$0.5489^{(-)}$	$0.9268^{(-)}$	$0.8322^{(-)}$
B	11.40	87.67	247.75	1	1	1	11.40	87.67	23.89	56.01	36.65	23.89	0.0001 >	$<0.0001^{(+)}$	$0.0003^{(-)}$
C	5.70	60.58	195.80	1	1	1	5.70	60.58	18.88	28.03	25.32	18.88	$<0.0001^{(+)}$	$0.0002^{(+)}$	$0.0008^{(+)}$
D	46.59	754.77	2421.85	1	1	1	46.59	754.77	233.54	228.94	315.52	233.54	$<0.0001^{(+)}$	$<0.0001^{(+)}$	$<0.0001^{(+)}$
AB	0.27	5.53	15.82	1	1	1	0.27	5.53	1.53	1.33	2.31	1.53	$0.2698^{(-)}$	$0.1522^{(-)}$	$0.2386^{(-)}$
AC	0.40	6.59	27.69	1	1	1	0.40	6.59	2.67	1.95	2.76	2.67	$0.1859^{(-)}$	$0.1208^{(-)}$	$0.1262^{(-)}$
AD	0.24	2.36	10.68	1	1	1	0.24	2.36	1.03	1.18	0.99	1.03	$0.2971^{(-)}$	$0.3383^{(-)}$	$0.3288^{(-)}$
BC	0.055	0.85	0.74	1	1	1	0.055	0.85	0.071	0.27	0.36	0.071	$0.6112^{(-)}$	$0.5611^{(-)}$	$0.7942^{(-)}$
BD	0.027	0.52	0.095	1	1	1	0.027	0.52	0.009	0.13	0.22	0.009	$0.7204^{(-)}$	$0.6481^{(-)}$	$0.9254^{(-)}$
CD	2.24	32.18	134.39	1	1	1	2.24	32.18	12.96	10.98	13.45	12.96	$0.0056^{(+)}$	$0.0028^{(+)}$	$0.0032^{(+)}$
A^2	0.025	1.23	1.90	1	1	1	0.025	1.23	0.18	0.12	0.52	0.18	$0.7332^{(-)}$	$0.4852^{(-)}$	$0.6753^{(-)}$
B^2	0.52	5.52	17.21	1	1	1	0.52	5.52	1.66	2.55	2.31	1.66	$0.1344^{(-)}$	$0.1528^{(-)}$	$0.2201^{(-)}$
C^2	0.30	6.76	18.63	1	1	1	0.30	6.76	1.80	1.49	2.83	1.80	$0.2445^{(-)}$	$0.1165^{(-)}$	$0.2031^{(-)}$
D^2	5.40	65.45	351.23	1	1	1	5.40	65.45	33.87	26.54	27.36	33.87			
Pure error	0.009	0.50	1.30	3	3	3	0.003	0.17							
Cor Total	76.67	1081.62	3638.80	29	29	29									
R^2	0.965	0.971	0.963												
Adj. R^2	0.928	0.939	0.922												
Adequate Precision	20.20	22.02	19.66												
Std. Dev	0.45	1.55	3.22												

y_3, overall yield of andrographolide (OYA)

y_4, overall selectivity of andrographolide (OSOPCC)

y_6, overall selectivity of andrographolide with respect to other phytochemical compounds (OSA/OPCC).

(+) Significant, (-) Not significant.

3.2 Influence of process variables and their interactions

The effects of process variables namely, the solid-liquid ratio, time, particle size and temperature and their interactions on the multiple responses of green solid-liquid extraction for andrographolide from AP were further examined within the recommended search space for the optimum values in our previous study (see companion article) [7]. Perturbation plots as depicted in Figures 1S, 2S, and 3S (see supporting information) were used prior to the navigation visualization and examination of the effects using the 3D response surfaces and contour plots. The results presented and discussed here are for the key performance indicators, OYA, OSA (see supporting information), and OSA/OPCC.

3.2.1 The influence of process variables on overall yield of andrographolide (OYA)

The effects of solid-liquid ratio, particle size, and extraction time on OYA at a temperature of 100°C are depicted in Figure 1 (a), (b) and (c) using the 3D response surface and contour plots. OYA can be seen increased by both decrease in the average particle size and solid-liquid ratio, and increase in extraction time. The impact of the solid-liquid ratio was very negligible in comparison with that of the average particle size. A decrease in the average particle size from 1.200 mm to 0.175 mm at 15-minute extraction time, resulted into an increase of 3.7 % in OYA. The effects of temperature and its interaction with average particle size, solid-liquid ratio, and time on OYA are exhibited in Figure 2 (a), (b) and (c). Temperature appears to be the most influential factor for the green SLE of andrographolide. The highest value of 9.21 % for OYA was found at the highest temperature of 120°C with medium average particle size of 0.605 mm and at 15-minute extraction time.

Table 4. Optimum values for green SLE Andrographolide compounds (OSA/OPCC)

Process parameter/variable	Experimental value	Predicted value	(%) Error
Process parameter:			
OYA (%)	8.09	8.17	1.0
OSA (%)	35.32	34.84	1.4
OSA/OPCC (%)	54.61	53.35	2.3
Process variables:			
A = 2/50 g/mL B = 0.375 mm C = 20 min D = 110 ℃			

This led to an overall increase of 6.6 % in the OYA in relation with the findings of the previous studies [7]. However, this high temperature operation may not be suitable for smaller average particle sizes of <0.605 mm, and at the same or longer extraction time. An increase in temperature from 90°C to 110°C for an average particle size of 0.375 mm, and with a decrease in solid-liquid ratio from 4/50 to 2/50 at 20-minute extraction time caused the OYA to increase by 4.3 % and 4.1 % respectively. Likewise, the decrease in the average particle size from 0.855 mm to 0.375 mm at a temperature of 110°C and at 20-minute extraction time resulted in an increase of 1.5 % in the OYA. The contour plots suggest that optimum value of OYA can be found possibly with a lower solid-liquid ratio (2/50 g/mL≤A≤4/50 g/mL), smaller average particle size (0.175 mm≤B≤0.605 mm), shorter extraction time (10 minutes≤C≤20 minutes), and higher temperature (100 °C≤D≤110°C).

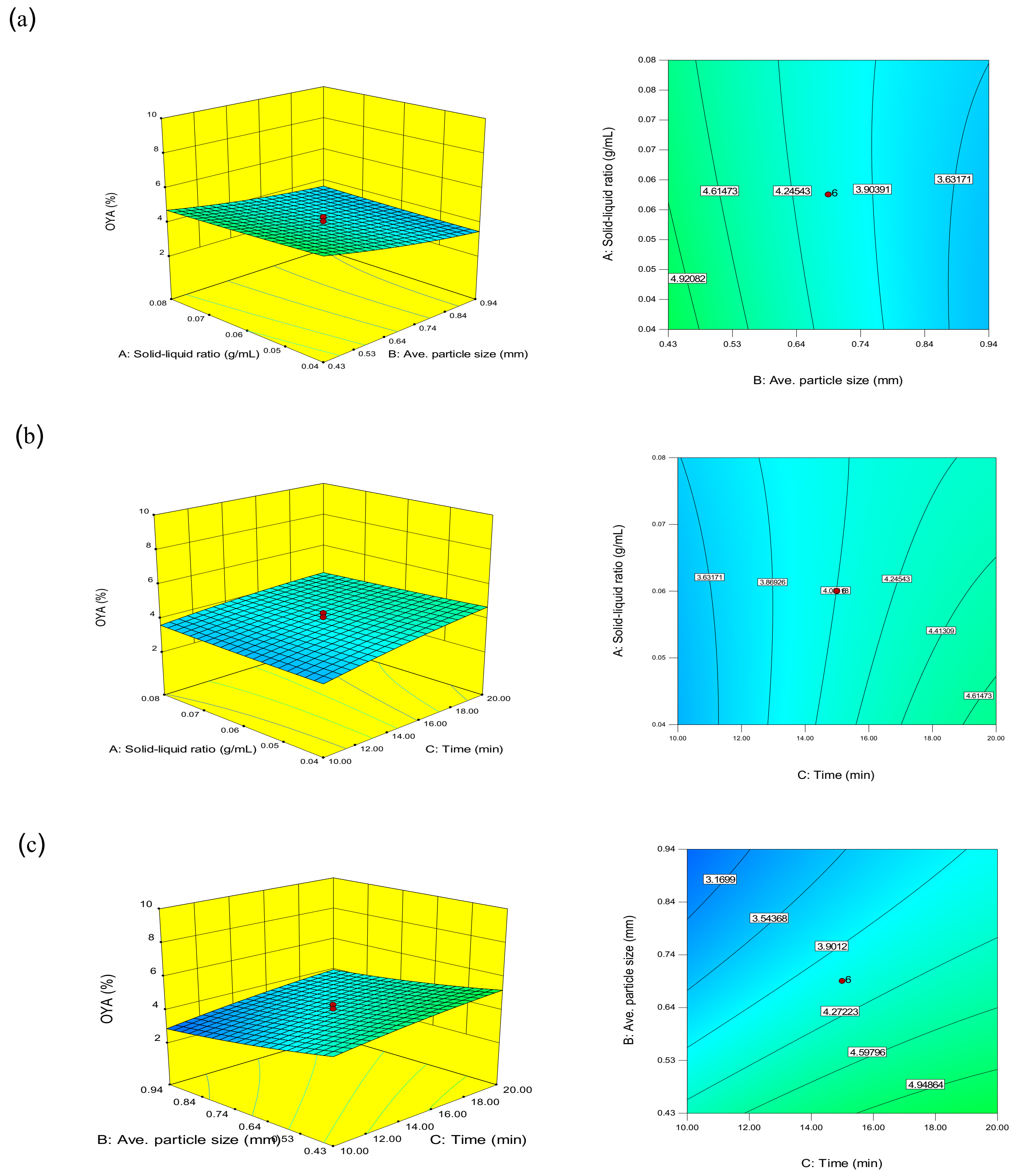

Figure 1. 3D response surface and contour plots for OYA (%) as a function of (a) solid-liquid ratio and ave. particle size at 15 min extraction time and 100 °C temperature, (b) solid-liquid ratio and time at 0.605 mm ave. particle size, and 100 °C temperature, (c) ave. particle size and time at S-L ratio of 3/50 (g/mL), and 100 °C temperature.

3.2.2 Influence of process variables on overall selectivity of andrographolide (OSA)

The effects of solid-liquid ratio, particle size, extraction time, temperature, and their interactions on OSA are shown in Figures 2S (a), (b), (c), and 3S (a), (b), and (c) using the 3D response surface and contour plots (see supporting information). It can be noted that OSA increased by both decrease in the average particle size and solid-liquid ratio, and increase in extraction time. The influence of the solid-liquid ratio was very insignificant in comparison to that of the average particle size. OSA improved 10.4 % because of the decrease in the average particle size from 1.200 to 0.175 mm at 15-minute extraction time.

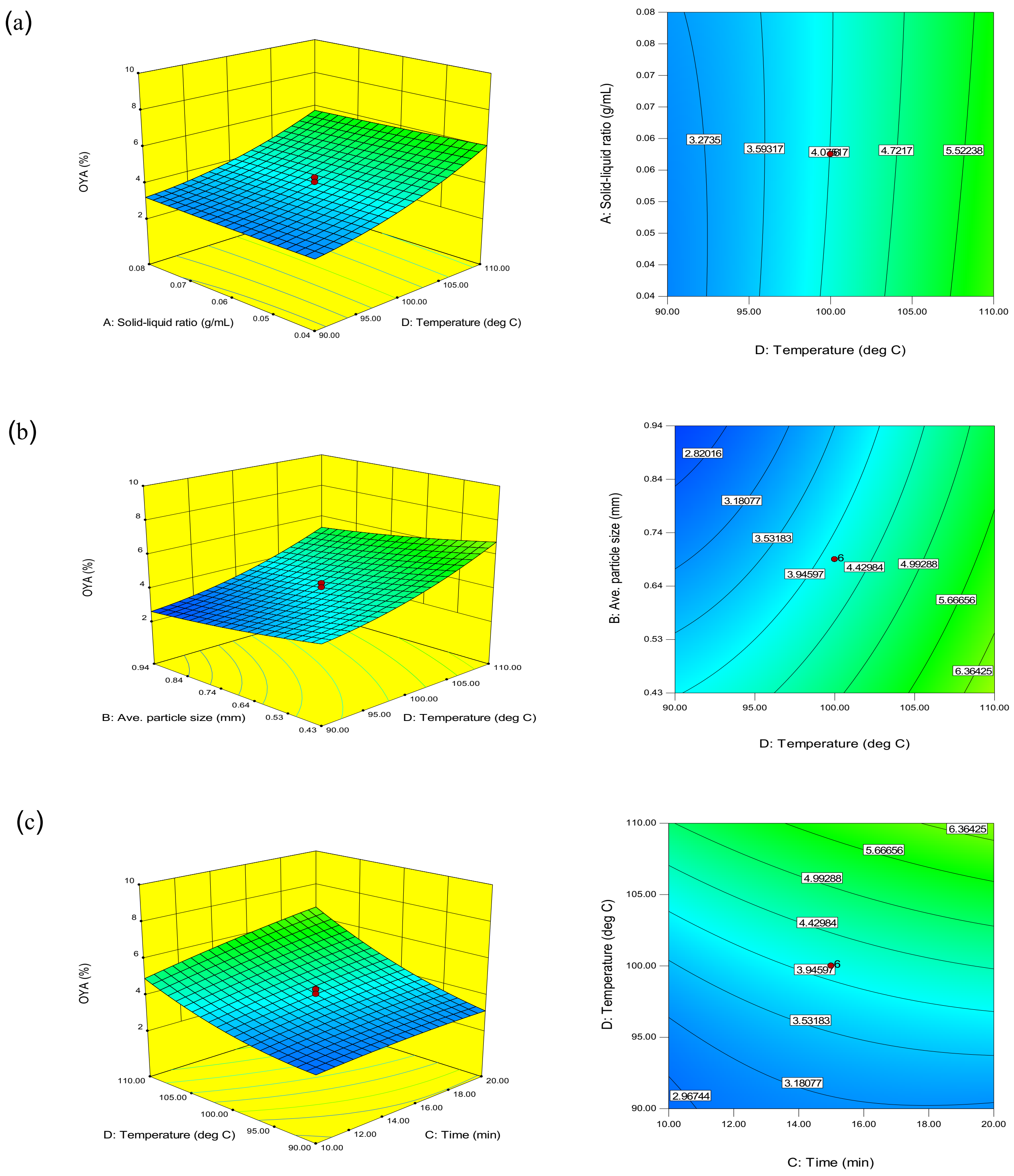

Figure 2. 3D response surface and contour plots for OSA/OPCC (%) as a function of (a) solid-liquid ratio and ave. particle size at 15 min extraction time and 100 °C temperature, (b) solid-liquid ratio and time at 0.605 mm ave. particle size, and 100 °C temperature, (c) ave. particle size and time at S-L ratio of 3/50 (g/mL), and 100 °C temperature.

The highest value of 39.15 % for OSA was achieved at the extreme temperature of 120 °C with medium average particle size of 0.605 mm and at 15-minute extraction time. This resulted to an overall increase of 25.1 % in the OSA in connection with the findings of our previous studies [7]. Nonetheless, for smaller particle sizes of <0.605 mm, and at the same or longer extraction time, this high temperature may not be favourable. An increase in temperature from 90°C to 110°C for an average particle size of 0.375 mm, and with a decrease in solid-liquid ratio from 4/50 to 2/50 at 20-minute extraction time caused the OSA to increase by 16.9 % and 15.2 % respectively. Also, the decrease in the average particle size from 0.855 mm to 0.375 mm at a temperature of 110°C and at 20-minute extraction time resulted in an increase of 3.8 % in the OSA. The contour plots point out that optimum value of OSA can be possibly established with a lower solid-liquid ratio (2/50 g/mL≤A≤4/50 g/mL), smaller average particle size (0.175 mm≤B≤0.605 mm), shorter extraction time (10 minutes≤C≤20 minutes), and higher temperature (100 °C≤D≤110°C).

3.2.3 The Influence of process variables on overall selectivity of andrographolide with respect to other phytochemical compounds (OSA/OPCC)

Figure 3 (a), (b) and (c) displays the effects of solid-liquid ratio, particle size, and extraction time on OSA/OPCC at a constant temperature of 100°C using the 3D response surface and contour plots. It can be observed from the plots that both decrease in the average particle size and solid-liquid ratio, and with an increase in the extraction time causes OSA/OPCC to substantial increase. The impact of the average particle size was more severe than that of the solid-liquid ratio. The OSA/OPC improved 17.4% due to a decrease in the average particle size from 1.200 mm to 0.175 mm at 15-minute extraction time. The influence of temperature and its interaction with particle size, solid-liquid ratio, and time on OSA/OPCC are depicted in Figure 3 (a), (b) and (c). The impact of temperature was the strongest influence for the OSA/OPCC in the green solid-liquid extraction. The highest value of 64.33 % for OSA/OPCC was obtained at the utmost temperature of 120 °C with medium average particle size of 0.605 mm and at 15-minute extraction time. This resulted to an overall increase of 48 % in the OSA/OPCC in comparison with the findings of our previous investigations [7]. Nevertheless, for smaller average particle sizes of <0.605 mm at the same or longer extraction time, this high-temperature operation may not be suitable. The OSA/OPCC increased by 32 % and 29.4 % as results of the increase in temperature from 90 °C to 110 °C, and decrease in solid-liquid ratio from 4/50 to 2/50 for an average particle size of 0.375 mm at 20-minute extraction time, respectively. Similarly, the decrease in the average particle size from 0.855 mm to 0.375 mm at a temperature of 110°C and at 20-minute extraction time resulted in an increase of 8.6 % in the OSA/OPCC. The contour plots indicate that optimum value of OSA/OPCC can be likely obtained with a lower solid-liquid ratio (2/50 g/mL≤A≤4/50 g/mL), smaller average particle size (0.175 mm≤B≤0.605 mm), shorter extraction time (10 minutes≤C≤20 minutes), and higher temperature (100 °C<D≤110°C).

The optimum values for the process variables that maximize the key performance indicators OYA, OSA, and OSA/OPCC are presented in Table 4. The predicted values were determined by transforming the multiple responses into desirability and penalty functions and solved numerically within the ranges of process variables with the aid of Design Expert Software V9. The predicted optimum OYA, OSA, and OSA/OPCC were found at the solid-liquid ratio of 2/50 g/mL, average particle size of 0.375 mm, extraction time of 20 min, and temperature of 110°C with desirability of 0.824 to be 8.17%, 34.84% and 53.35% respectively. The comparison of the predicted and observed experimental values of the process performance indicators shows that the calculated values are slightly lower. The relative errors for OYA, OSA, and OSA/OPCC are 1%, 1.4% and 2.3% respectively. These were considered to be relatively low since the values are within the 5% significant level.

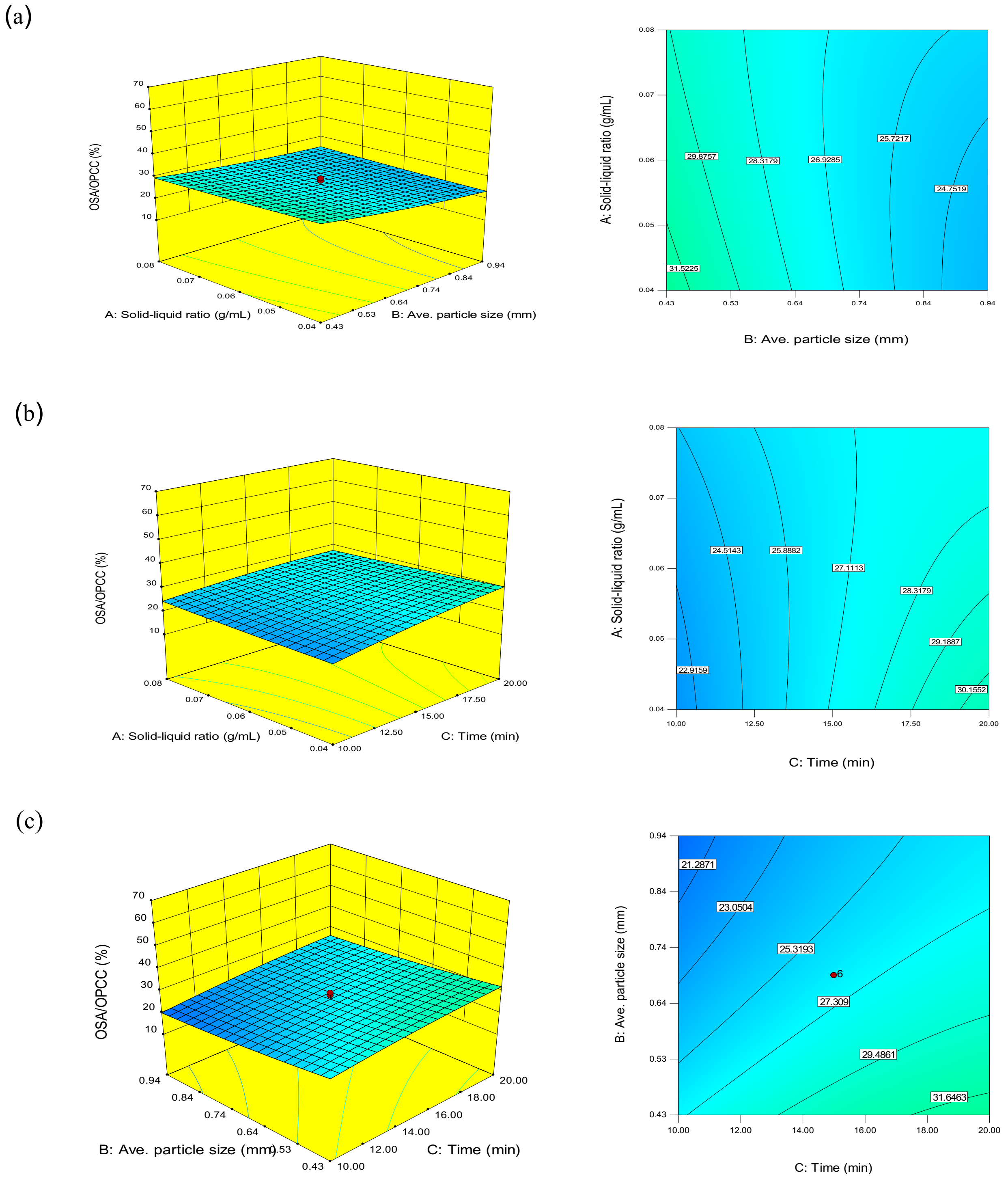

Figure 3. 3D response surface and contour plots for OSA/OPCC (%) as a function of (a) solid-liquid ratio and temperature at 15 min extraction time and 0.605 mm ave. particle size, (b) ave. particle size and temperature at 15 min extraction time and S-L ratio of 3/50 (g/mL), and temperature and time at 0.605 mm ave. particle size and S-L ratio of 3/50 (g/mL).

Conclusions

In this study, a holistic approach for the simultaneous multi-objective optimization of the complex green solid-liquid extraction of andrographolide from AP through the application of RSM based on RCCD coupled with desirability and penalty functions were demonstrated. The linear effects of temperature appeared to the most influential factor among all the process variables. In addition, the quadratic and interactive effects of temperature, temperature and time were found among the most significant effects. The optimum OYA, OSA, and OSA/OPCC were found at the solid-liquid ratio of 2/50 g/mL, average particle size of 0.375 mm, extraction time of 20 min, and temperature of 110 °C with desirability of 0.824 to be 8.17%, 34.84% and 53.35% respectively.

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Disclosure statements

Conflict of Interest: The authors declare that there are no conflicts of interest in this research paper.

Compliance with Ethical Standards: This research does not contain any studies involving human or animal subjects.

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