



## Influence of Process Variables for Green Solid-Liquid Extraction of Andrographolide from *Andrographis Paniculata*

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- ✓ Multiple extraction.

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### Abstract

In this study, the influence of process variables for green solid-liquid extraction of andrographolide from *Andrographis paniculata* (AP) was investigated using a 2<sup>3</sup> complete factorial experimental design (CFED) augmented with center points. The main and interaction effects of the solid-liquid ratio (1/50 and 5/50 mg/L), particle size (0.175 and 1.200 mm), and time (5 and 25 minutes) were examined. The process was studied at 80 C in a pressurized liquid extractor (PLE). 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 process responses used as performance indicators. The results were determined using high-performance liquid chromatography (HPLC), analyzed using half-normal probability plots, Pareto charts, analysis of variance (ANOVA), and 3D response surface and contour plots. Among the three process variables, the particle size was found to have the greatest significant effect, followed by time, and the smallest was the solid-liquid ratio on OYA, OSA and OSA/OPCC. In addition, the double interactions of particle size and time had the most pronounced effects, followed by solid-liquid ratio and time on both OSA and OSA/OPCC, while double interactions of solid-liquid ratio and particle size had the least influence.

## 1. Introduction

Solid-liquid extraction (SLE) is one of the most crucial processes in phytochemical industries. It is viewed as the heart of phytochemical processing because once it is jeopardized the whole industry will be affected [1,2]. SLE is the process of leaching out soluble components from multi-component solid mixture by contacting with suitable solvent that selectively dissolves some but not all components in the solid [3,4]. Many process variables such as solid-liquid ratio, solvent, degree of agitation, particle size, time and temperature affect the rate of SLE for phytochemical compounds [1,2,3]. The influence of a process variable can be defined as the change in response produced by a shift in the level on that process variable averaged over the levels of the other input process variable [5].

*Andrographis paniculata* (AP) is one of the most popular herbs that has been successfully used against a wide range of diseases in East Asia, South-Central Asia and South-East Asia for many centuries [6,7]. AP has been used for anti-cancer [8], anti-inflammatory [9], anti-human immune deficiency virus

1 (HIV-1) [10], anti-diabetic [11], anti-venom [12], anti-hypertensive [13]. In addition, it has been utilized for anti-microbial and hepatoprotective [13], anti-thrombotic [14], antileishmanial activity [15], anti-malarial [16], anti-oxidant [17], immunomodulatory [18], cardiovascular protective effects [19]. It has been also employed for the treatment of appetite loss and cold [20], skin condition eruption and scabies [21]. Andrographolide was found as the main medicinally active component of AP [22,23,24]. The phytochemical andrographolide and its derivatives such as 14-deoxy 11,12-didehydro andrographolide, neoandrographolide, and 14-deoxyandrographolide obtained from AP are recently discovered to have promising therapeutic potentials against SARS-CoV-2 (Covid-19) [25].

In reality, the green or non-green solid-liquid extraction of most phytochemical compound(s) from plant materials is a complex process involving multiple competing extractions in series with multiple reactions in parallel or series. The sequences of multiple competing green solid-liquid extractions of andrographolide with other phytochemical compounds from AP in series with multiple reactions of andrographolide and other phytochemical compound (s) in parallel or and series can be depicted using one of the schemes proposed by Isah et al. [3] as shown in Figure 1. The numbers in the scheme as shown in Figure 1 (a) and (b) represent the following: 1–AP, 2–green solvent (water), desired product extract (3–andrographolide), undesired product extracts (4–apigenin, 5–glucose, 6–7-O-methyl wogonin, 7–skullcapflavone I, 8–andrographidines A, B, C, D, E, and F; and 9–unknown competing product extracts in relatively larger amounts from free reducing sugars, cardiac glycosidase, tannins, alkaloids, saponins, and anthraquinones derivatives), undesired products of andrographolide transformations (10–isoandrographolide, 11–14-deoxyandrographolide, 12–deoxyandrographolide, 13–andrograpanin, 14–neoandrographolide, 15–andrographiside, 16–14-deoxy–11,12–didehydroandrographolide, 17dehydroandrographolide, 18–14-deoxy–11–hydroxyandrographolide, and 19–14-deoxy–12–hydroxyandrographolide). Due to process complexity (see compound 3, Figure 1), the effects of multiple process variables and their combined interactions on the key performance indicators of the green solid-liquid extraction of andrographolide from AP must be carefully and simultaneously investigated prior to multi-objective optimization.

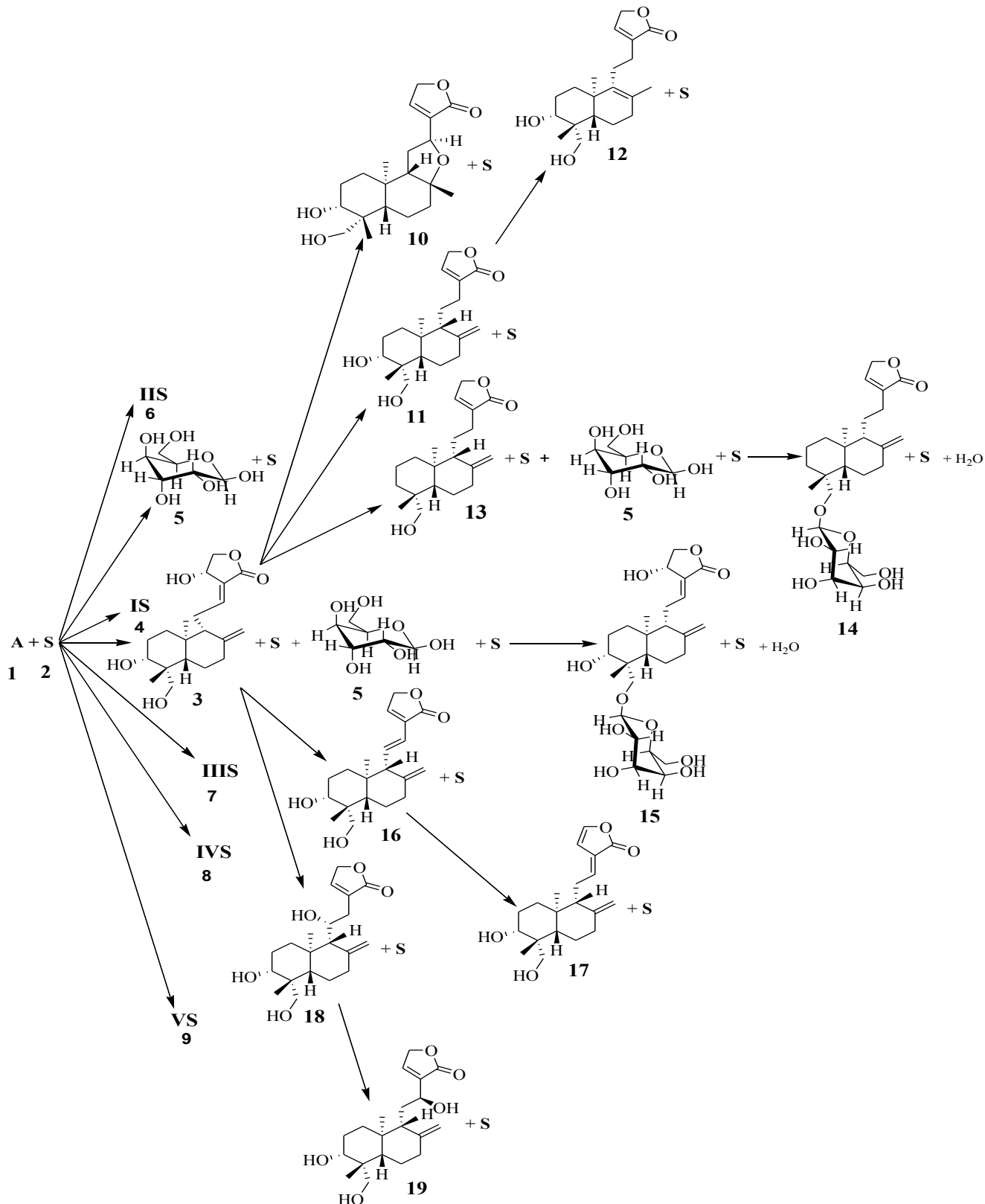
In this work, we have applied the design of experiment (DoE) to study the effects of three process variables, namely solid-liquid ratio, particle size and time on the green solid-liquid extraction of andrographolide from AP in a pressurized liquid extractor (PLE) under isothermal condition at 80 °C. In addition, the study has examined the double and triple-interactions of the input variables on the performance measures of the process. It has also identified and found the practicable domains of the values for each independent variable. Temperature is one of the most influential factors for green solid-liquid extraction of phytochemical compounds involving water. However, in this systematic exploration, the temperature process variable was kept constant to avoid its dominant effect over other important factors, and reduce the number of experiments. This is in order to examine, quantify and clearly visualize other independent variables' influences. The experiments were planned using a 2<sup>3</sup> complete factorial experimental design (CFED) augmented with center points.

## 2. Material and Methods

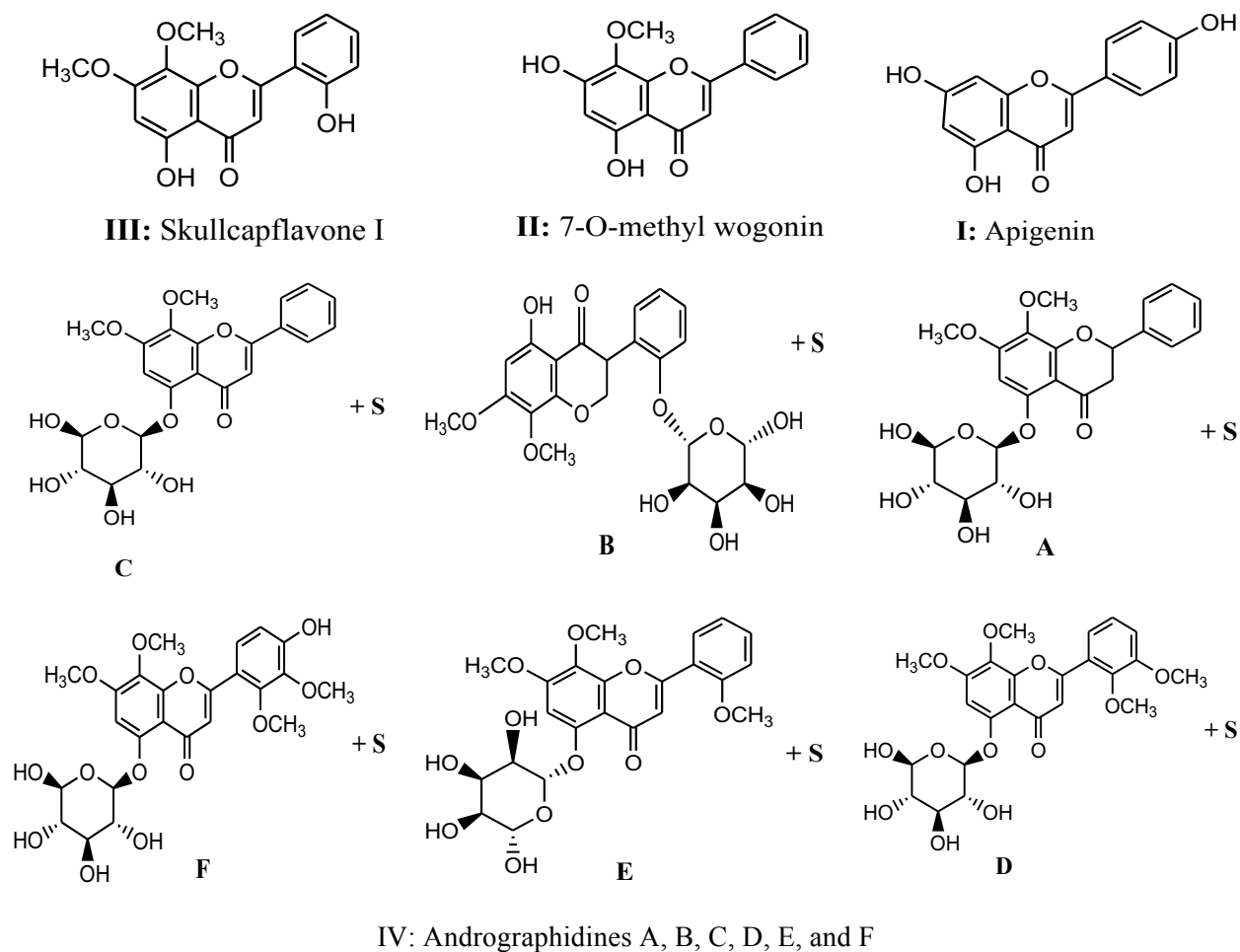
### 2.1 Materials

Dried leaves of AP were procured in ground form from Fidea Resource Sdn Bhd, and authenticated by Forest Research Institute of Malaysia (FRIM) with ID No.: SBID002/13. The ground AP was stored in a cool room at Institute of Bioproduct Development (IBD), Universiti Teknologi Malaysia (UTM). It was sifted into various mesh sizes and classified into groups with standard sieves, and unit tests shaker (UTS) Malaysia. Three different classes of average particle sizes of 1.200 mm, 0.688 mm and 0.175 mm

were used in the investigation. The samples were conditioned in an oven at 50 °C for 24 hours before extraction. Distilled water was collected from IBD pilot plant. Standard chemical of andrographolide (98 %) was procured from Sigma Aldrich<sup>®</sup>, USA. Deionized water was produced from NANOpure Diamond<sup>™</sup> Barnstead. The methanol HPLC grade was obtained from QRëc<sup>®</sup>.



**Figure 1(a).** Multiple competing green solid-liquid extractions of andrographolide (desired product extract) and other phytochemical compounds (undesired product extracts) in series with multiple transformations of andrographolide and its complex reactions with other phytochemical compounds.



**Figure 1(b).** Undesired product extracts in multiple competing green solid-liquid extractions.

## 2.2 Green solid-liquid extraction of andrographolide

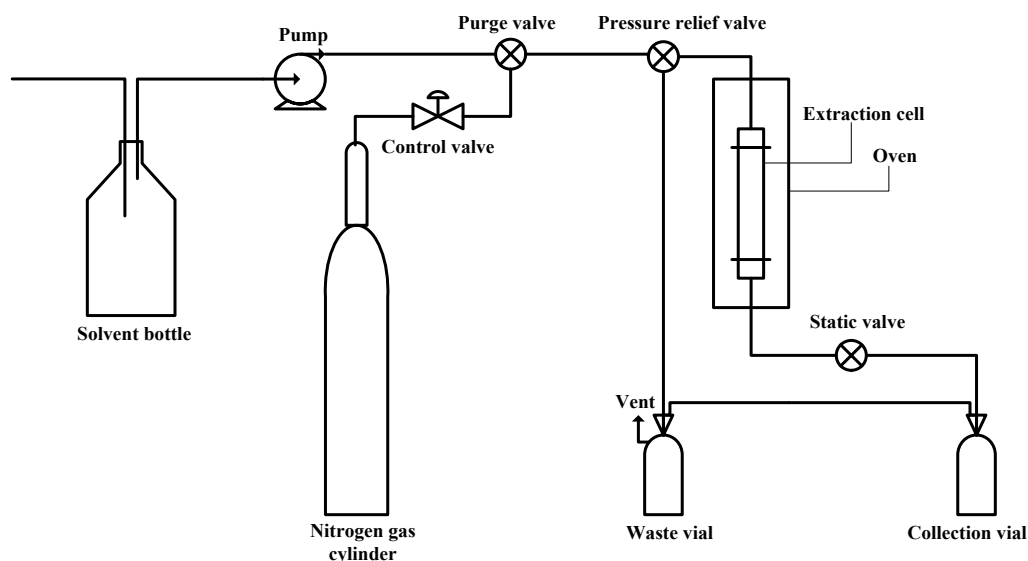
The green solid-liquid extraction of andrographolide from AP using water as the greenest solvent was performed under isothermal condition in an automated pressurized liquid extractor (PLE), employing Dionex ASE 100<sup>®</sup>, USA as shown in Figure 2. The values of independent process variables for each run were set according to the complete factorial experimental design (CFED) with center point requirements as shown in Table 1.

**Table 1.** Ranges and coded levels of process variables for CFED with centre points.

Process variable	Range and levels			
	Symbol	+1	0	-1
Solid-liquid ratio (g/mL)	A	5/50	3/50	1/50
Particle size (mm)	B	1.200	0.605	0.175
Time (min)	C	25	15	5

For instance, for run 1, 2g sample of average particle size 1.200 mm was packed into a 100 mL extraction cell (to have a ratio of solid-liquid: 1g: 50 mL). Two stainless steels frits of 10µm aperture size were

installed at the bottom of both ends of the cell caps. One of the frits was tightened on the bottom of the cell body, and a disposable cellulose filter was added before loading of the sample. The other frit was screwed after packing of the sample and placing of another disposable cellulose filter. That was done to prevent blockage of the frits and entrainment of fine particles into the extracts.



**Figure 2.** Schematic diagram of pressurized liquid extraction (PLE) system.

The ASE 100<sup>®</sup> was programmed to the set point temperature of 80 °C, static extraction time of 5 min, purge time of 60 seconds, flushing volume of 30%. When the system was under steady state and the oven was ready, the extraction cell was placed on the cell holder, and the cell door was closed to allow the cell to be inside the oven. Then, the filling of the extraction cell was commenced by moving the solvent via a fast pump from the solvent bottle into the cell. The extraction started after the pressure, and volume reaches 1,300 psi and above 80 mL, respectively. The pressure and volume of the system kept increasing as the extraction progresses for the static extraction time. After the process was completed, compressed nitrogen gas together with the fixed volume of fresh solvent for flushing moved all the extract into the collection vial. The ASE 100<sup>®</sup> was reprogrammed according to specified values for each of the remaining experimental runs, and the above procedure was repeated.

### 2.3 Phytochemical analysis of green extracts of *Andrographis paniculata*

The green extracts of *Andrographis Paniculata* (AP) were analysed in order to find their constituents through phytochemical analysis prior to HPLC analysis. Different techniques for the identification of carbohydrates of primary metabolites, free reducing sugars, cardiac glycosidase, tannins, flavonoids, terpenes/steroidal rings, alkaloids, saponins, and anthraquinones derivatives of secondary metabolites were employed.

### 2.4 HPLC analysis of andrographolide

The identification and quantification of andrographolide in the water extracts were carried out on a Waters 2690 Alliance High-Performance Liquid Chromatography (HPLC), equipped with Waters 996 Photodiode Array (PDA) detector, connected to a reversed-phase column (Lichrospher 100 RP-18, 5 µm, 250 X 4.6mm), Germany. A stock solution was prepared from pure crystals of andrographolide standard with purity of 98% that was purchased from Sigma-Aldrich. Each sample was clarified with a 0.45 µm Whatman nylon syringe filter, and then transferred into a 1.5 mL vial. The volume of 20 µL

was programmed and injected with the aid of an auto sampler. The detection and measurement of andrographolide concentration were best observed at 223 nm using an isocratic method [26] with methanol/water (60:40) as the mobile phase at constant flow rate of 0.7 mL/min under column temperature of 23 °C. Chromatographic peaks of andrographolide were identified by comparing with the retention time of the standards. One of the typical HPLC chromatograms obtained from AP water extracts is shown in Figure S1 (see supporting information). The quantification for andrographolide in the water extracts was analyzed using calibration curve of the standard with an accuracy of 99.8%. The overall yield of andrographolide and extracts were calculated from Equation 1.

$$\hat{Y}(\%) = \frac{A_D}{A_{SM}} \times 100 \dots \dots \dots (1)$$

Where  $A_D$  and  $A_{SM}$  are the amount of desired extract (andrographolide or whole extracts), and the amount of solid material (*Andrographis paniculata*) used in the solid-liquid extraction. The 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 2 to 4 reported by Isah et al. [3], respectively. Where  $A_{AASM}$  and  $\hat{S}_U$  are the actual amount of solid material disappeared in the solid-liquid extraction and the overall selectivity of undesired extracts (other phytochemical compounds).

$$\hat{S}_D(\%) = \frac{A_D}{A_{AASM}} \times 100 \dots \dots \dots (2)$$

$$\hat{S}_U(\%) = \frac{A_U}{A_{AASM}} \times 100 \dots \dots \dots (3)$$

$$\hat{S}_{D/U}(\%) = \frac{\hat{S}_D}{\hat{S}_U} \times 100 \dots \dots \dots (4)$$

## 2.5 Design of experiments

Design of experiments (DoE) can be described as a systematic approach for planning experiments when studying the effects of multiple variables of a process simultaneously through statistical technique [27]. In other words, DoE is a planned approach for determining a variable and response relationships. The goal of statistically designing an experiment is to collect a maximum amount of information with a minimum number of experiments [28,29,30]. This guarantees not only the reduction of a number of experiments needed in the research but also to maintain the maximum information about the process. Complete factorial experimental design (CFED) is a  $2^k$  plan, which is one of the most commonly used multivariable exploratory experiments [31,32].

CFED was selected over fractional factorial experimental design (FFED) in order to quantify all effects of process variables and their interactions on the performance indicators of the process. A set of design of experiments consisted of 12 runs were randomly conducted based on the CFED with centre points. The objective was to explore and identify the domain of values for each of the process variable and the state of the response variables when an input variable of the process change, and examine the magnitude and direction of effects of process variables. It was also to find the relative importance of each independent variable, and hence, develop an empirical model for the responses of solid-liquid extraction for andrographolide. The range and coded levels of three process variables for a complete factorial experimental design with centre points are presented in Table 1. Each factor was coded to the (-1, 0, +1) interval where the low, centre and high levels were coded as -1, 0 and +1, respectively. The total number of experiments conducted was 8 factorial points and 4 replicates at the center points.

Meanwhile, the four replicated experiments ran at the centre points (run 2, 4, 8 and 11) were used to estimate the pure error associated with the experiment and evaluate the possible effect of curvatures (as measured by difference between the average of the centre points and the average of the factorial points) in the design space.

### 3. Results and Discussion

#### 3.1 Process research and development

The research for the green SLE of andrographolide and the development of solid-liquid extractor in a situation where multiple competing extractions occur should be focused on complete understanding of the SLE of the desired product extract (i.e. andrographolide) and the process characteristics. Table 2 shows the presence of various vital phytochemical compounds discovered in the green extracts of AP.

**Table 2.** Phytochemical analysis of AP green extracts

Constituents	Test	Observation	Inference
Carbohydrates	Molisch's test	A purple to violet coloration at interface	Positive
Free reducing sugars	Fehling's test	A brick red precipitate formed	Positive
Cardiac Glycosides	Salkowsk's test	A layer of reddish-brown color formed	Positive
Tannins	Lead Sub-Acetate test	A color precipitate formed	Positive
	FeCl <sub>3</sub> test	A blue-black precipitate formed	Positive
Flavonoids	Shinoda's test	A green color and heavy precipitate formed	Positive
	NaOH test	Yellow coloration formed	Positive
Terpenes/steroids	Liebermann	A violet blue and finally green formed	Positive
	Burchard's test		Positive
Alkaloids	Dragendoff's test	A blue-blackish precipitate	Positive
	Meyer's test	A precipitate formed	Positive
	Wagner's test	A white precipitate	Positive
Saponins	Frothing test	A honey comb formed	Positive
Anthraquinones derivatives	Borntrager's test	A pink color formed	Positive

These include carbohydrates of primary metabolites; free reducing sugars, cardiac glycosidase, tannins, flavonoids, terpenes/steroidal rings, alkaloids, saponins, and anthraquinones derivatives of secondary metabolites. The matrix of experimental design with their responses are listed in Table 3. The performance indicator(s) that characterize the extraction should be the basis for the selection of optimal extractor, extractor design and scale up to industrial size [32]. In this circumstance where the multiple competing SLE of desired product extract **3** (see Figure 1) with other phytochemical compounds **4**, **5**, **6**, **7**, **8** and **9** (undesired product extracts) from AP undergoes series reactions in parallel with **10**, **15** (through reaction with **5**), and consecutive with **11**, **12**, **13**, **14**, **16**, **17**, **18**, and **19** (undesired products of andrographolide transformations), the most important variable for controlling the process is the real time for a batch extractor. For instance, it will be extremely difficult to obtain the desired product extract **3** as illustrated in Figure 1, if the rate of extraction of **3** is slower than the rate of reaction (transformations of **3** into **11**, **12**, **13**, **14**, **16**, **17**, **18**, and **19**). On the other hand, if the rate of extraction of **3** is faster than the rate of its transformation to unwanted products, a high selectivity and yield of **3** can be achieved. However, if the extraction is allowed to proceed for a long time in a batch extractor, the desired product extract **3** will be transformed into the undesired products **11**, **12**, **13**, **14**, **16**, **17**, **18**, and **19**.

Thus, it is important to know the exact time for getting the maximum selectivity of the desired product extract with the minimum selectivity of undesired product extracts of multiple competing extractions and minimum reaction selectivity of multiple reactions. Therefore, the process research and development should be focused on maximizing selectivity of the desired product extract **3** while minimizing selectivity of undesired product extracts of multiple extraction(s), and reaction selectivity (RS) of multiple reactions.

### 3.2 Empirical modeling and analysis

From Table 3 of the experimental design matrix with their corresponding outcome of the experiments for green SLE of andrographolide, the results revealed that different levels of independent variables (solid-liquid ratio, particle size and extraction time) influenced the measured process responses. Experimental data were fitted into a full first-order model with double and triple factor interactions. The least squares regression was used in generating the empirical models as shown in eqs. S2 to S7 (see Supporting information).

**Table 3.** CFED Experimental design matrix and responses for the green SLE of andrographolide

		Independent process variables						Responses					
		Coded levels			Actual levels								
Run order	Std order	A	B	C	A	B	C	y <sub>1</sub> (millimoles)	y <sub>2</sub> (%)	y <sub>3</sub> (%)	y <sub>4</sub> (%)	y <sub>5</sub> (%)	y <sub>6</sub> (%)
1	6	+1	-1	+1	5/50	0.175	25	6.48	22.69	2.98	13.11	86.89	15.09
2	7	-1	+1	+1	1/50	1.200	25	0.87	15.19	2.11	13.89	86.11	16.13
3	1	-1	-1	-1	1/50	0.175	5	1.11	19.51	4.36	22.32	77.68	28.73
5	9	0	0	0	3/50	0.605	15	3.18	18.55	2.59	13.97	86.03	16.24
4	10	0	0	0	3/50	0.605	15	3.12	18.24	2.5	13.69	86.31	15.87
6	2	+1	-1	-1	5/50	0.175	5	6.41	22.47	3.25	14.45	85.55	16.89
7	5	-1	-1	+1	1/50	0.175	25	0.10	20.83	1.82	8.75	91.25	9.58
8	11	0	0	0	3/50	0.605	15	3.14	18.36	2.55	13.91	86.09	16.16
9	12	0	0	0	3/50	0.605	15	3.20	18.69	2.67	14.29	85.71	16.68
10	4	+1	+1	-1	5/50	1.200	5	4.37	15.33	1.55	10.1	89.9	11.23
11	8	+1	+1	+1	5/50	1.200	25	4.76	16.69	2.47	14.8	85.2	17.36
12	3	-1	+1	-1	1/50	1.200	5	0.69	12.06	1.3	10.82	89.18	12.13

*y*<sub>1</sub>, amount of andrographolide (AOA),

*y*<sub>2</sub>, overall yield of extract (OYE),

*y*<sub>3</sub>, overall yield of andrographolide (OYA),

*y*<sub>4</sub>, overall selectivity of andrographolide (OSA)

*y*<sub>5</sub>, overall selectivity of other phytochemical compounds (OSOPCC),

*y*<sub>6</sub>, overall selectivity of andrographolide with respect to other phytochemical compounds (OSA/OPCC).



The models were significant at 95% confidence level. They were demonstrated to cover the following ranges for the amount of andrographolide extracted from 0.000104 moles to 0.006476 moles, overall yield of extract from 12.06% (w/w) to 22.69% (w/w), and overall yield of andrographolide from 1.3% (w/w) to 4.36% (w/w). In addition, the ranges for the overall selectivity of andrographolide was from 8.75% (w/w) to 22.32% (w/w), overall selectivity of other phytochemical compounds was from 77.68% (w/w) to 91.25% (w/w) and overall selectivity of andrographolide with respect to other phytochemical compounds was from 9.58% (w/w) to 28.73% (w/w). The summary of ANOVA results for all the models are presented in Tables 1S and 2S (see supporting information).

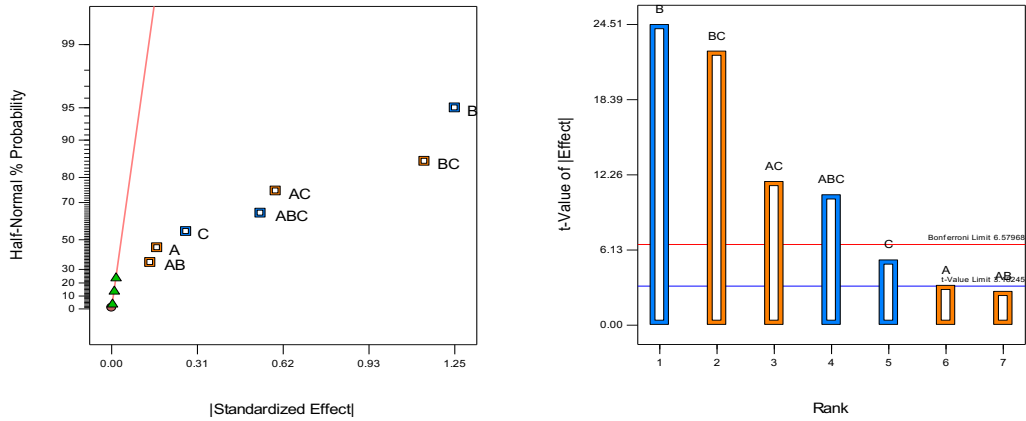
### 3.3 Statistical analysis

The half-normal probability and Pareto charts of the effects were first used to identify and visualize the magnitude of the significant effects of process variables and their interactions prior to the detailed ANOVA for the confirmation of the validity of the results as shown and discussed in Tables S1 and S2 (see supporting information). A plot of fraction of design space (FDS) as depicted in Figure 2S (see supporting information) was used to assess and quantify the prediction variability using the standard error mean at any given fraction of the whole design space. Perturbation plots as exhibited in Figures 3S, 4S, and 5S (see supporting information) were also used prior to navigation of the 3D response surfaces and contour plots. OYA ( $y_3$ ), OSA ( $y_4$ ), and OSA/OPCC ( $y_6$ ) are more important process parameters and appear to be more sensitive than the other process responses. Hence, only their detailed results are shown and considered to use for qualitative discussions.

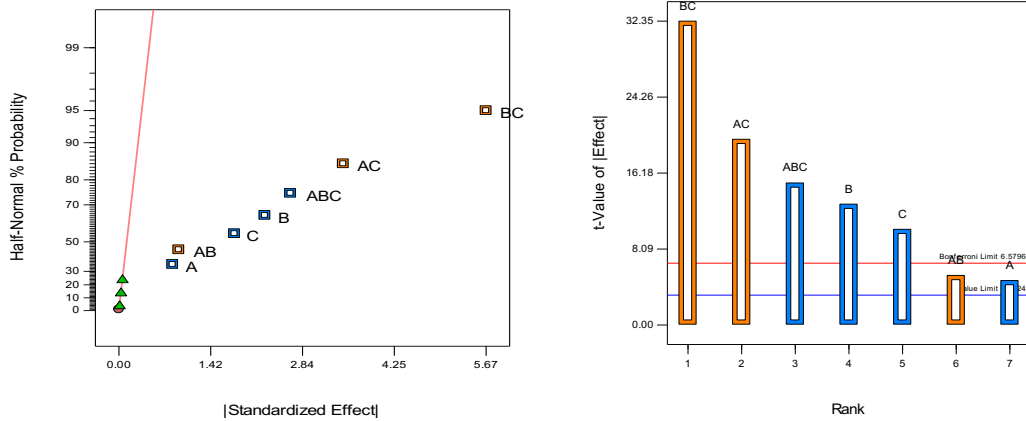
Figure 3 displays the plots of half-normal percent probability against the absolute value of all standardized effects, and ranks t-value distributions of the absolute effects on Pareto charts for OYA, OSA and OSA/OPCC. It can be observed from Figure 3a, b, and c for the plots of OYA, OSA and OSA/OPCC, respectively that the error obtained from four replicate runs falls on the line near zero indicated a very insignificant effect. On the other hand, the significance of effects for main process variables (A, C, B) with their double (AB, AC, BC) and triple (ABC) interaction increases from left to right. Positive effects include A, AB, AC and BC for OYA and excluding A in the case of OSA and OSA/OPCC, while the negative effects are A, C, B and ABC for OSA and OSA/OPCC and leaving out A in the case of OYA response.

The significant effects were categorized as B>BC>AC>ABC>C>A>AB on OYA and BC>AC>ABC>B>C>AB>A on OSA and OSA/OPCC responses. Thus, large absolute values show up as outliers in the upper right-hand section of the graphs. The Pareto chart of t-values of effects with t-limit and Bonferroni adjusted t-limits were used to visualize the relative size of effects as shown in Figure 4 (a), (b) and (c) for OYA, OSA and OSA/OPCC, respectively. It was found B, BC, AC and ABC in the case of OYA and BC, AC, ABC, B and C on OSA, and with addition of AB on OSA/OPCC were above Bonferroni limit indicating effects were most likely important. Similarly, all factors of main-effects (A, B, C) with their double and triple interactions on OSA, OSA/OPCC and except for A and AB in the case of OYA were above t-value limit showing the effects were possibly

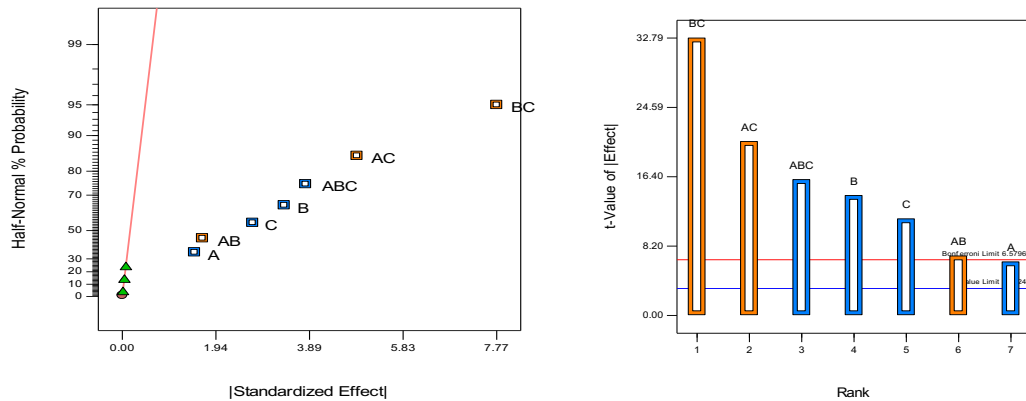
(a)



(b)



(c)



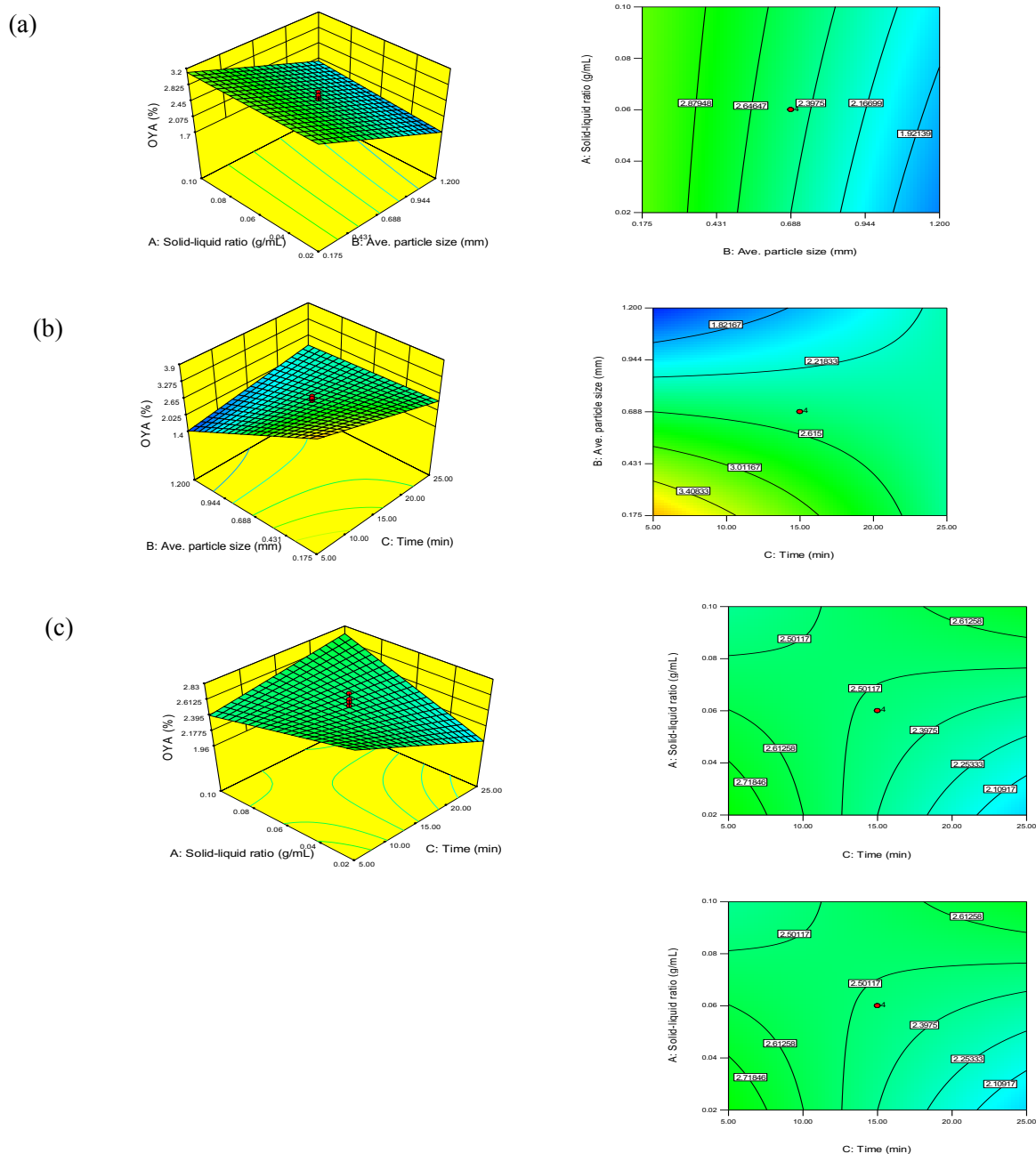
**Figure 3.** Half-normal probability plots and Pareto charts of t-values of effects for (a) OYA (%), (b) OSA (%), (c) OSA/OPCC (%).

### 3.4 Effect of process variables and their interactions

The three-dimensional (3D) response surface and contour plots were employed to navigate the design space and examine the effects of process variables and their interactions within the design domain. It was found from the model and statistical analyses that there were three double and one triple interaction-effects in addition to the main-effects of process variables, which were significant to the green SLE of andrographolide from AP.

### 3.4.1 The effects on OYA

It can be observed from the 3D response surface and contour plots as exhibited in Figure 4 (a), (b) and (c) that OYA increases with the decrease in both solid-liquid ratio and average particle size at shorter extraction time with stronger negative effects for the lower solid-liquid ratio and smaller average particle size at longer extraction time.



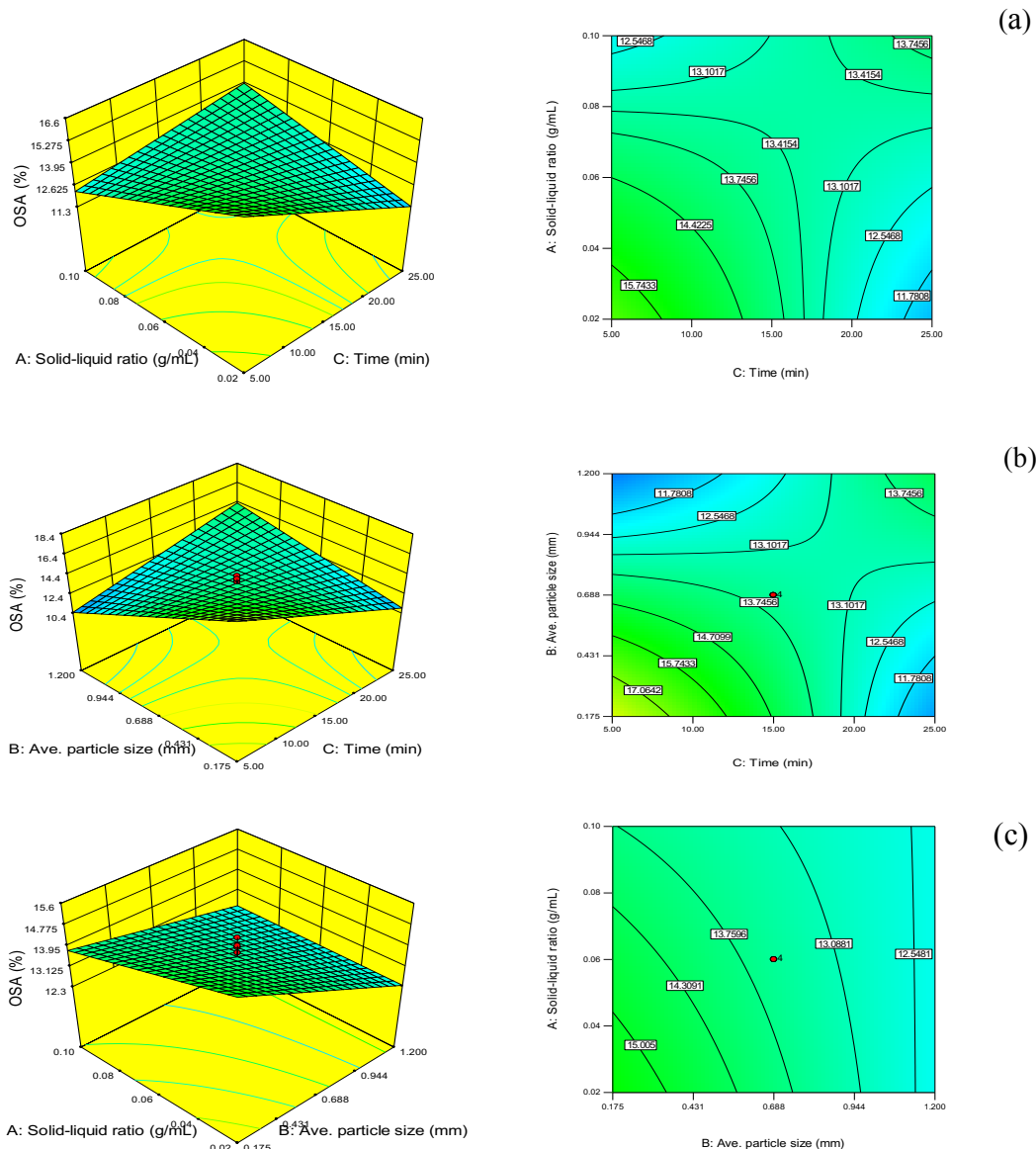
**Figure 4.** 3D response surface and contour plots for OYA (%) as a function of (a) solid-liquid ratio and time at ave. particle size of 0.69 mm, (b) ave. particle size and time at S-L ratio of 3/50 (g/mL) and (c) solid-liquid ratio and ave. particle size at time of 15 min.

The contour plots suggest that higher OYA are only possible with smaller average particle size ( $0.175 \text{ mm} < B < 1.200 \text{ mm}$ ), a lower solid-liquid ratio ( $1/50 \text{ g/mL} < A < 5/50 \text{ g/mL}$ ) and shorter extraction time ( $5 \text{ minutes} < C < 25 \text{ minutes}$ ). It has also been reported in the solid-liquid extraction of andrographolide from

AP using aqueous ethanol that OYA increases with decrease in average particle size [33]. However, OYA increases at an extreme higher solid-liquid ratio ( $\geq 5/50$  g/mL) and larger average particle size ( $\geq 1.200$  mm) with the increase in extraction time ( $\geq 25$  min) as shown in Figure 4(a) & (b). At extraction time of 15 minutes, OYA appears to have a linear, and inverse relationship with particle size and solid-liquid ratio respectively, and with strong negative effect as average particle size increases (Figure 4b).

### 3.4.2 The effects on OSA

The 3D response surface and contour plots in Figure 5 (a), (b) and (c) show that OSA also increase with the decrease in both solid-liquid ratio and particle size at shorter extraction time with stronger negative effects for the lower solid-liquid ratio and smaller average particle size at longer extraction time. The contour plots also suggest that higher OSA are only achievable with smaller particle size ( $0.175$  mm  $< B < 1.200$  mm), a lower solid-liquid ratio ( $1/50$  g/mL  $< A < 5/50$  g/mL) and shorter extraction time.

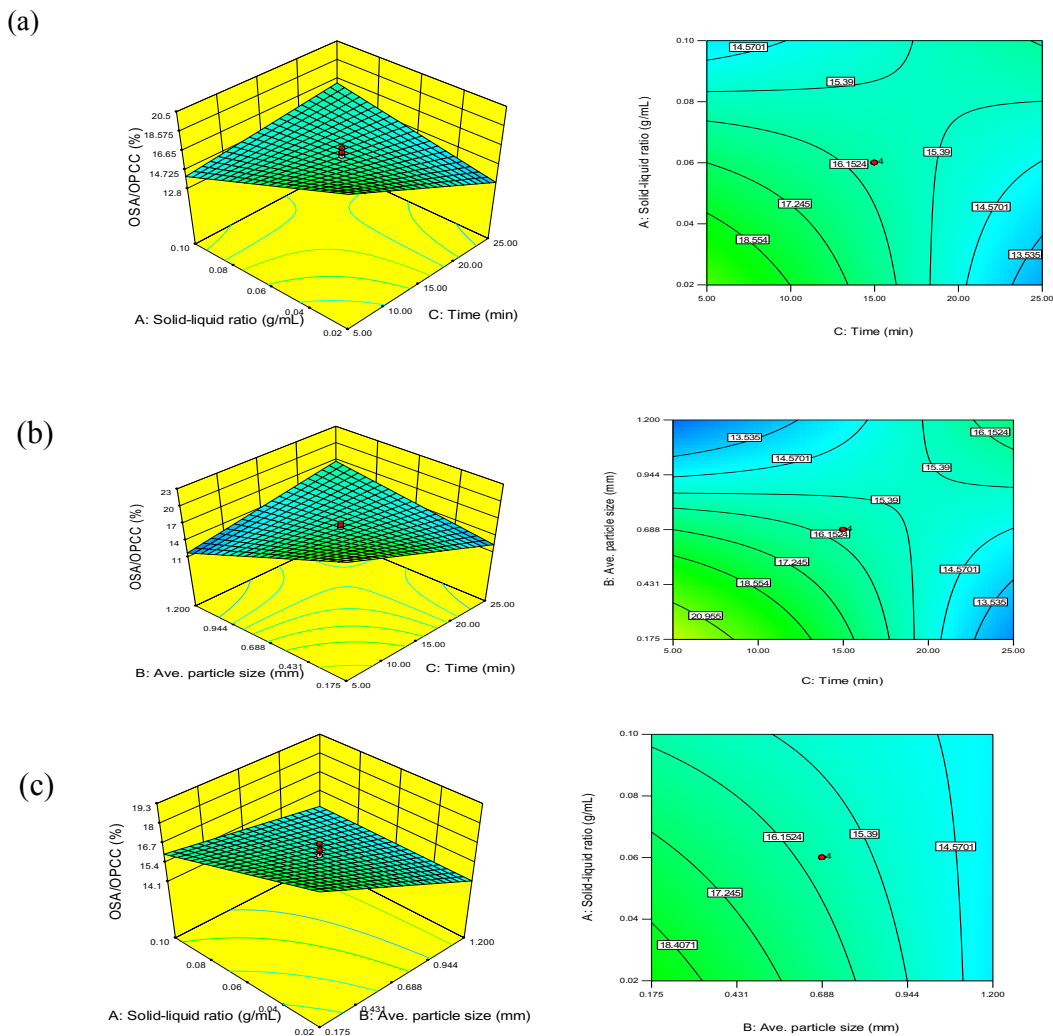


**Figure 5.** 3D response surface and contour plots for OSA (%) as a function of (a) solid-liquid ratio and time at ave. particle size of 0.69 mm, (b) ave. particle size and time at S-L ratio of 3/50 (g/mL) and (c) solid-liquid ratio and ave. particle size at time of 15 min

(5 minutes <math>C < 25</math> minutes). Nonetheless, OSA tends to increase with the increase in higher solid-liquid ratio ( $\geq 5/50</math> g/mL) and larger average particle size ( $\geq 1.200</math> mm) at longer extraction time ( $\geq 25</math> min) as presented in Figure 5(a) and (b). Unlike OYA, OSA increases with both decrease in particle size and solid-liquid ratio at extraction time of 15 minutes with strong negative effect as particle size increases (Figure 5b).$$$

### 3.4.3 The effects on OSA/OPCC

It can be visualized and examined from the plots as displayed in Figure 6 (a), (b) and (c) that OSA/OPCC increases with the decrease in both solid-liquid ratio and average particle size at shorter extraction time with stronger negative effects for the lower solid-liquid ratio and smaller average particle size at longer extraction time. Likewise, the contour plots suggest that higher OSA/OPCC are only feasible with smaller average particle size ( $0.175< B < 1.200</math> mm), a lower solid-liquid ratio ( $1/50< A < 5/50</math> g/mL) and shorter extraction time ( $5< C < 25</math> minutes).$$$



**Figure 6.** 3D response surface and contour plots for OSA/OPCC (%) as a function of (a) solid-liquid ratio and time at ave. particle size of 0.69 mm, (b) ave. particle size and time at S-L ratio of 3/50 (g/mL) and (c) solid-liquid ratio and ave. particle size at time of 15 min.

However, OSA/OPCC tends to increase with the increase in higher solid-liquid ratio ( $\geq 5/50$  g/mL) and larger average particle size ( $\geq 1.200$  mm) at longer extraction time ( $\geq 25$  min) as displayed in Figure 6 (a) and (b). Similarly, OSA/OPCC increases with decrease in both average particle size and solid-liquid ratio at extraction time of 15 minutes with strong negative effect as average particle size increases (Figure 6b).

## Conclusion

In the present work, a  $2^3$  CFED was used to explore the experimental design space and examine the effects of solid-liquid ratio, particle size and extraction time on the green solid-liquid extraction of andrographolide from *Andrographis paniculata*. The findings from the analyses reveal that the statistically significance of the main-effects of process variables, their double and triple interaction-effects are in following order as  $B > BC > AC > ABC > C > A > AB$  on OYA and  $BC > AC > ABC > B > C > AB > A$  on OSA and OSA/OPCC responses. It was found that the green production of a high amount of andrographolide water extracts would require smaller average particle size ( $0.175 \text{ mm} < B < 1.200 \text{ mm}$ ), a lower solid-liquid ratio ( $1/50 \text{ g/mL} < A < 5/50 \text{ g/mL}$ ) with shorter extraction time ( $5 \text{ minutes} < C < 25 \text{ minutes}$ ). Likewise, the process operation would require the green extraction to be at longer extraction time ( $\geq 25$  min) when larger particle size ( $\geq 1.200$  mm) and higher solid-liquid ratio ( $\geq 5/50$  g/mL) are employed. The process optimization should be focused on maximizing the overall selectivity of andrographolide while minimizing the selectivity of undesired products (other phytochemical compounds) by manipulating the process variables with the inclusion of temperature within the feasible region of the design space.

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## Disclosure statements

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

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

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