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OPTIMIZATION OF ULTRASOUND-ASSISTED EXTRACTION OF TRITERPENOID SAPONINS CONTENT FROM DRIED *Gomphrena celosioides* USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT. *The objective of this study was to apply ultrasound-assisted extraction (UAE) as a novel technique for optimization of triterpenoid saponins yield from dried Gomphrena celosioides, using a response surface methodology. The results indicated that the optimal UAE parameters consisted of extraction time of 33.6 min, extraction temperature of 78.2°C and aqueous solvent/sample ratio of 26.1/1 mL/g. Under these optimal parameters, the highest triterpenoid saponins content was 2.337%. Using scanning electron microscope to analyze the morphological residues obtained after extraction referred that the ultrasound-assisted extraction destroyed the structural components significantly. Hence, this extraction helped to improve the yield of triterpenoid saponins content (TSC).*

KEY WORDS: *Extraction, Gomphrena celosioides, RSM, triterpenoid saponins, ultrasound.*

INTRODUCTION

Despite the rapid development of allopathic medicines during the 20^{th} century, plants still remain one of the major sources of drugs in modern as well as in traditional system. The traditional medicines, plant-based drugs, in the health care system consist of huge plants with various medicinal and pharmacological benefits and offer effective treatments for many diseases without any risk of side-effects while synthetic drugs do not (Ilyas et al. 2013).

The *Amaranthaceae* family, which comprises of many species, has been used in nutrition and different traditional systems of medicines due to treatments for diseases such as bacterial infections, jaundice, urinary problems, high cholesterol, cough, fever, diarrhea, liver disorders and kidney disorders. *Gomphrena* genus is commonly known as an edible, medical and commercial plant, also named as Globe Amaranth or Bachelor Button, which belongs to the family *Amaranthaceae*. *Gomphrena* genus has been found all over the world. Its stem is 10-20 cm height, rather sparse branches, appressed pilose with white silky hair. Besides, its leaves are spiral, deltoid to elliptic and ovate, 1.5 - 4.5 cm length and 0.5 - 0.3 cm wide. The flowers are in terminal and axillary, cylindrical spikes with 1 - 2 cm length and 1 cm in diameter. Flowers mainly bloom from June to December (Onocha et al. 2005).

An early pharmacochemical study of *Gomphrena celosioides* revealed the phytoconstituent presence of saponins, terpenoid, steroids, phytosterol, amino acids and non-reducing sugars in all the plant parts. The study also found phenolics and flavonoids in leaves, inflorescence and stem of the plant (Ilyas et al. 2013, Adeoti et al. 2016). In recent years, triterpenoid saponins have been increasingly used in pharmaceuticals as they are anti-inflammatory, anti-atherosclerosis, anti-diabetes, anti-allergy, antibacterial, antiviral, antifungal and antioxidant properties (Ke et al. 2014, Singh et al. 2016). Saponins are popular in plants, mainly in seeds, leaves, roots, fruits and stems. Triterpenoid saponins were found in legumes (soybeans, lentils, green beans, quinoa seeds, ginseng roots, etc. Saponins were also found in medicinal plants such as *Gomphrena celosioides* (Ogundipe et al. 2008).

Saponins can be extracted with different methods. Traditional techniques (maceration, soxhlet extraction) are time-consuming, require various materials and high purity solvents, yet show low selectivity and productivity. Many non-conventional technologies such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), electromagnetic, CO_2 supercritical have been applied to maximize saponin content in extraction. Among the most methods to extract bioactive compounds from plants, UAE is novel and effective as it greatly reduces extraction time, less energy consumption and higher extraction efficiency (Cheok et al. 2014).

When it comes to UAE, Hierroa et al. (2018) extracted saponin from edible seeds (quinoa, soybean, lupine, fenugreek, red lentil) in ethanol or aqueous ethanol solution, obtained the highest saponin content in ethanol extract from fenugreek and red lentil with 12% and 10%, respectively. Besides, Luo et al. (2017) extracted saponin from flowers of *Coreopsis tinctoria* in ammonium sulfate solvent concentration of 37.76% (w/w), ethanol 35.62% (w/w) (frequency of 40 kHz, 250 W), optimized the extraction process by response surface methodology (RSM), and the total saponins content obtained was 33.4 g/kg. In addition, Pham et al. (2018) also optimized the extraction process of saponins from *Catharanthus roseus* stems by RSM with Box - Behnken design to evaluate the interaction effects of ultrasonic parameters, including ultrasonic temperature, time, solvent/sample ratio, etc. These mean that saponins content has been extracted from many different raw materials with various solvents using UAE. However, there are scarcely researches on triterpenoid saponins content of the *Gomphrena celosioides* extracted by UAE. Moreover, using RSM to optimize experimental parameters is more benefits than other methods, as it reduces the number of experimental trials as well as a simple method, saving time and resources.

Hence, this study focused on optimizing the extraction of triterpenoid saponins from *Gomphrena celosioides* by UAE using RSM to estimate the effect of the interaction of parameters, including ultrasonic temperature, time, and solvent/sample ratio (v/w) on the yield of total triterpene saponins from dried *Gomphrena celosioides*. Aqueous solvents will ensure the safety of the extraction solution in food and pharmaceutical products.

MATERIAL AND METHODS

Chemicals and reagents

All chemicals and reagents used in this study originated from Merck or Sigma with analytical grade. In there, oleanolic acid was purchased from Sigma (Germany) and used as standard reagent.

Sample preparation

Fresh *Gomphrena celosioides* were collected from Cu Chi district, Ho Chi Minh city (Vietnam). This plants were removed yellow leaves and broken branches. Then, they were washed by tap water, drained and dried at 60ºC to reduce the moisture content to less than 10%. Dried samples were ground into small pieces and sieved with Retsch AS - 200 sieve to uniform size materials (particle diameter of 4 mm). After that, samples were put into polyethylene bags, sealed and stored at 4ºC. These samples were used for further experiments.

Ultrasound-assisted extraction (UAE)

The dried sample (2 g) was extracted in an ultrasonic bath (Elmasonic S60 H, 550 W, 37 kHz, Germany) at different extraction conditions with deionized water as a solvent. The received extracts were immediately cooled to room temperature by using an ice water bath, filtered for removal of the residue and determined triterpenoid saponins yield in the extracts.

Determination of triterpenoid saponins content (TSC)

The content of triterpenoid saponins was measured as the method described by Chen et al. (2007). Briefly, the received extract was made up to 100 mL by distilled water. Using 2 mL of diluted extract was sequentially mixed with 0.2 mL of 8% (w/v) vanillin – acetic acid solution and 1.2 mL of perchloric acid. Then, the mixture was incubated in a water bath at 60ºC for 15 min. After that, ethyl acetate was made up to 5 mL. After being cooled to room temperature, with a blank solution as a reference, the absorbance was measured at 550 nm. Oleanolic acid was used to generate the standard curve. The result as expressed as gam oleanolic acid equivalents per 100 gram dry weight (%).

Scanning electron microscopy (SEM)

The structural variations of materials extracted with UAE were observed by SEM. After extraction and vacuum filtration, the residues were dried at 60°C for about 4 hours to achieve the required moisture content (less than 10%). Then, the dried and initial samples were put on a plate with double-sided adhesive tape and vacuum coated with platinum. After that, a scanning electron microscope system (Nanosem 450, Holland) was used to examine morphological changes of them at various magnifications.

Experimental design

RSM with Box–Behnken design was employed to evaluate the impact of variables (ultrasonic temperature, time and solvent/sample ratio) and to optimize UAE conditions for increased triterpenoid saponins yield of dried *Gomphrena celosioides* in an ultrasonic bath. The levels of independent variables and Box–Behnken design are presented in Table 1 and Table 2, respectively.

To predict the optimal extraction conditions, a second order polynomial equation (Equation 1) was used to assess the relationship between independent variables and triterpenoid saponins yield (Y)

$$Y = a_o + a_1X_1 + a_2X_2 + a_3X_3 + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3 \quad (1)$$

Statistical data analysis

Modde software version 5.0 (Umetrics AB company, Sweden) was used to design the experiment and analyze the data. The optimal extraction conditions were performed by contour plots and three-dimensional (3D) response surfaces.

Table 1. Coded level and actual values of independent factors.

Independent factors	Symbols	Coded levels		
		-1	0	1
Solvent/material ratio (v/w)	X_1	20/1	25/1	30/1
Extraction time (min)	X_2	20	30	40
Extraction temperature (ºC)	X_3	60	70	80

RESULTS AND DISCUSSION

Optimization of extraction process

The effects of some factors of the extraction process were determined by the preliminary studies. There are only three major factors such as solvent/material ratio, extraction temperature and extraction time which were selected to optimize of the saponins extraction conditions. Table 3 showed that the multivariable linear regression was performed to analyze and predict the constants, coefficients of linear, quadratic and interaction effects of extraction factors. The regression equation was obtained below:

$$Y = 2.208 + 0.053X_1 + 0.108X_2 + 0.222X_3 – 0.137X_1^2 – 0.156X_2^2 – 0.137X_3^2$$

Table 2 showed that TSC of *Gomphrena celosioides* powder ranged from 1.398 to 2.254%. The received results pointed out that TSC was strongly affected by the independent factors (solvent/material ratio, extraction temperature, extraction time). Besides, the p_{value} of the regression equation model is 0.000 (<0.05) (Table 3). These results noticed that this model was significant. Moreover, the lack of fit was 0.221 (>0.05) and was not significant for this model. This indicated that the model was the good predictability of the response.

In addition, the coefficient of determination was higher than 0.9 (R^2=0.973), this can explain more than 97.3% of the actual data in the response; while the adjusted coefficient of determination (R^2_{adj}=93.7%) was quite close to the received R^2 value, indicating that the actual and predicted data had a high fitting precision, proving the feasibility of the experimental method. These results were illustrated in Table 2. Furthermore, Q^2 parameter in the model is the goodness of prediction and estimates the predictive power of the model. Q^2 value for TSC in this study was 0.746; it should be regarded as good (Q^2>0.5 and R^2-Q^2<0.3) (Eriksson et al. 2008). For this reason, the model is quite accurate for predicting the response. Based on the received regression equation, TSC depends on all variables of the extraction process, for example, solvent/material ratio, extraction temperature and extraction time.

Table 2. Experimental design matrix and predicted results for TSC.

Run	Independent factors						Responses	
							Y, TSC (%)	
	X_1	X_2	X_3	Solvent/material ratio (v/w)	Extraction time (min)	Extraction temperature (°C)	Experimental value	Predicted value
1	-1	-1	-1	20/1	20	60	1.398	1.39773
2	1	-1	-1	30/1	20	60	1.459	1.51153
3	-1	1	-1	20/1	40	60	1.593	1.63333
4	1	1	-1	30/1	40	60	1.667	1.67413
5	-1	-1	1	20/1	20	80	1.801	1.79373
6	1	-1	1	30/1	20	80	2.009	1.96853
7	-1	1	1	20/1	40	80	2.119	2.06633
8	1	1	1	30/1	40	80	2.168	2.16813
9	-1	0	0	20/1	30	70	1.997	2.0169
10	1	0	0	30/1	30	70	2.144	2.1247
11	0	-1	0	25/1	20	70	1.948	1.9435
12	0	1	0	25/1	40	70	2.156	2.1611
13	0	0	-1	25/1	30	60	1.948	1.8483
14	0	0	1	25/1	30	80	2.193	2.2933
15	0	0	0	25/1	30	70	2.254	2.20861
16	0	0	0	25/1	30	70	2.193	2.20861
17	0	0	0	25/1	30	70	2.180	2.20861

For the linear term, all factors were statistically significance (p_{value}<0.05) and have the positive effects on TSC. In there, the extraction temperature (X_3) was the positive strongest effect (The coefficient of X_3 achieved the maximum value in the linear term) and solvent/material ratio (X_1) was the smallest effect on TSC.

Table 3. Analysis of variance and regression equation coefficients of the model.

Y	Coefficient	Std. Err	p_{value}
Constant	2.208	0.029	1.91×10^{-11}
X_1	0.053	0.021	0.042
X_2	0.108	0.021	0.0015
X_3	0.222	0.021	1.79×10^{-5}
X_1X_1	-0.137	0.041	0.013
X_2X_2	-0.156	0.041	0.007
X_3X_3	-0.137	0.041	0.013
X_1X_2	-0.018	0.024	0.476*
X_1X_3	0.015	0.024	0.549*
X_2X_3	0.009	0.024	0.714*
N=17	R^2=0.973	R^2_{adj}=0.937	Q^2=0.746
Regression equation model: p_{value} = 0.000			
Lack of fit (F=3.67), p_{value}=0.221*			

*: p_{value}>0.05, insignificant effect.

Regarding the quadratic effect, all variables are negative effects on TSC. The effect of extraction temperature and solvent/material ratio was lower than that of extraction time which also has the strongest influence on TSC. This result is in agreement with study of Deng et al. (2019) for both of the linear and quadratic term, they extracted saponins from *Sapindus mukorossi* pericarp by microwave-assisted method. Besides, Kwon et al. (2003) also determined that the most important effect of crude saponins extraction from ginseng for the quadratic term in the regression equation was the extraction time. In another study, Wang et al. (2018) extracted TSC from germinated quinoa by the ultrasound probe; the results proved that TSC depended on all parameters for both of the linear and quadratic term (for example ultrasonic time, ultrasonic temperature, ethanol volume fraction and liquid ratio).

For the interactions between factors, there were not any interactions between all variables. The results were similar to study of Kwon et al. (2003); however, they were contrary to other studies, for example, Deng et al. (2019) noticed that there was the interactions between extraction time and temperature, solvent/solid ratio and extraction time, solvent/solid ratio and extraction temperature. Besides, the concentration of solvent could also interact with the other parameters such as solvent/solid ratio, extraction time and temperature (Wang et al. 2018).

Response surface plots

The three-dimensional response surfaces of TSC were used to determine all the effect of factors and their interactions by 3D response surface curves and 2D contour plots. Typically, the response surface plot illustrated the correlation between response and factors. Each plot showed how the two factors affected TSC, while the third factor was not changed.

As shown in Figure 1A and 1A', increase of solvent/material ratio from 20/1 to 26/1 (v/w) with extraction time increasing from 20 to 32 min enhanced the extraction yield of TSC from 1.7 to 2.323%. This showed that extending extraction time or higher solvent/material ratio can improve TSC. Extraction time of this case was different to that of studies of Zhao et al. (2012) and Wang et al. (2018), they extracted TSC from *Codonopsis lanceolata* for 60 min by reflux extraction method and from germinated quinoa for 15 min by the ultrasound probe, respectively. Besides, Figure 1B and 1B' described the interactive effect of extraction time and temperature on TSC. TSC increased from 1.7 to 2.255% when extraction time increased from 20 to 28 min and extraction temperature also increased from 60 to 72^{o}C. The viscosity of solvent decreases and the dissolving capacity of bioactive compounds increases at higher temperature. Moreover, their diffusion into the solvent was enhanced with the increasing temperature. Extraction time of this study was higher than that of study of Wang et al. (2018), who extracted saponins from germinated quinoa by the ultrasound probe at 54^{o}C.

In addition, solvent/material ratio (v/w) was one of the most important factors. Figure 1C and 1C' showed that with an increase in solvent/material ratio from 20/1 to 26/1 (v/w) along with an increase in temperature from 60 to 77^{o}C, TSC also increased from 1.65 to 2.324%. During the extraction process, the matrix of materials will swell; thus, the solvent volume must be sufficient to ensure that the entire samples are immersed. Essentially, this optimum ratio was lower than that of Li et al. (2010), they used microwave-assisted extraction to extract saponins from the defatted residue of yellow horn with the optimum solvent/material ratio of 32/1 (v/w). This proved that TSC depends on many factors such as extraction methods, initial material and extraction conditions.

However, the yield of extraction process will almost decrease if the material is kept at high temperature for a long period of time or added overmuch solvent. This is due to the rapid degradation of bioactive compounds at high temperature for a long time. Furthermore, these

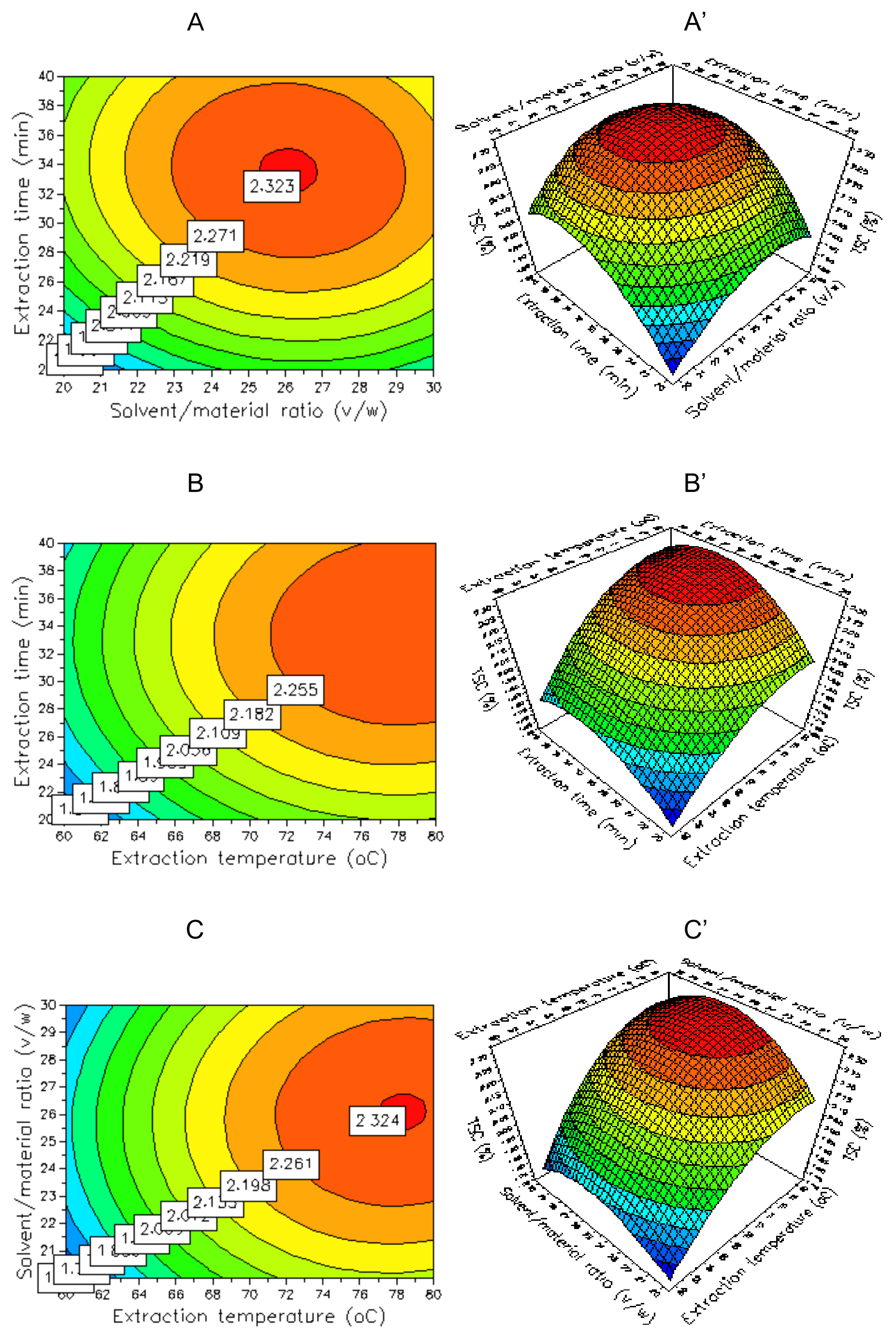

Figure 1. Contour (left) and response surface (right) plots of solvent/material ratio, extraction temperature and extraction time and its mutual interaction to TSC at the optimal conditions.

compounds easily oxidized on longer exposure to the surrounding atmosphere (Naczk & Shahidi 2004, Tao & Sun 2015).

Determination and validation of optimal conditions

Based on the results of the RSM, the optimized extraction parameters for collecting triterpenoid saponin from *Gomphrena celosioides* were the extraction factors such as solvent/material ratio, extraction time and temperature which were 26.1 mL/g, 33.6 min, and 78.2ºC, respectively. Under the above-mentioned processing parameters, the optimum TSC was estimated to be 2.327% (Table 4). The validation of the UAE method was used to determine the suitability and precision of the model. The tested result was performed under optimum conditions and the received TSC was 2.337±0.034%. It was quite close to the predicted TSC, indicating that the obtained extraction conditions are reliable.

Table 4. Results of optimal conditions.

Factors			Predicted result	Experimental result
X_1 (v/w)	X_2 (min)	X_3 (ºC)	Y_p (%)	Y_e (%)
26.2/1	33.6	78.2	2.327	2.337±0.034

Basically, the optimized parameters in this study were quite different to the research of Hu et al. (2012), they indicated that the optimization conditions for saponins extraction from *Eclipta prostrasta L.* with UAE method were extraction temperature of 70ºC, extraction time of 3 hours, ethanol (70%, v/v)/solid ratio of 14/1 (v/w), and TSC obtained 2.096%. In the other hand, the TSC in this study was slight different to result of Wang et al. (2018) with the highest saponins content obtained of 27.40 mg/g (about 2.74%), they extracted saponins from germinated *Quinoa* by the UAE methods in the optimized conditions including extraction temperature of 54°C, extraction time of 15 min, ethanol (75%, v/v)/solid ratio of 50/1 (v/w). These different optimum parameters might be due to the differences in research materials and equipment.

Effect of ultrasound-assisted extraction on the structure of material

Figure 2 showed the scanning electron micrographs of *Gomphrena celosioides* without and with ultrasonic treatment. The shape of the initial material changed clearly, and the surface was seriously decayed. Besides, the number of the pores in the plant cell walls also increased steadily, which would improve the mass transfer and the diffusion of the bioactive compounds into the solvent (Vinatoru 2001). Furthermore, there was an increase in the number of the swelling cell tissues after the UAE treatment, so these swelling ones would be broken and damaged. Then, cell disintegration can take place quickly. The internal pressure and temperature that increase rapidly by the UAE-generated cavitation bubbles might cause those consequences (Aspé & Fernández 2011). In addition, Dadi et al. (2019) also recorded that the degree of disruption of the structure of the plant cell dramatically changed with the prolongation of the extraction time during UAE due to the effects of the ultrasonic wave and vibration. In this study, the extraction time reached 33.6 min, that is enough to detroy structual cell wall, it was longer than that of studies of Quoc & Muoi (2018) (15 min) or Dadi et al. (2019) (10-30 min).

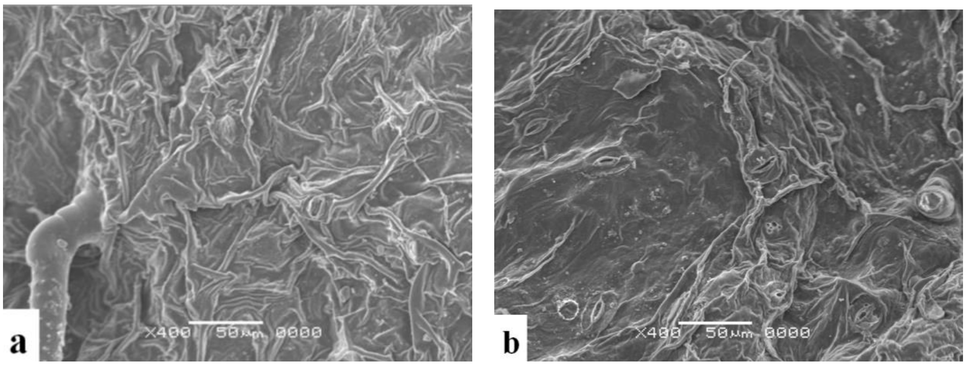

Figure 2. Scanning electron micrographs of initial material (a) and residue (b) after treatment by UAE.

In this study, the surface of the initial material was lumpy and rough. Then, these particularities were broken down and the surface became smoothly after sonication although there were few slight ruptures on the surface. This result contrasted with that of other materials, for example, *Orthosiphon stamineus* powder (Ho et al. 2014), *Polygonum multiflorum* Thunb. roots powder (Quoc & Muoi 2018). They noticed that a large number of wrinkles appeared on the surface of the cell wall of residue. Basically, the above-mentioned results also pointed out that UAE influenced the structure of the plant cells strongly, which improved the yield of TSC.

CONCLUSIONS

In general, the application of RSM pointed out that the optimum conditions for saponins extraction were solvent/material ratio of 26.1/1 (mL/g), extraction time of 33.6 min and extraction temperature of 78.2°C with TSC of 2.337%. There was not the interaction between factors in the quadratic term. In addition, the predicted results were similar to the experimental results, indicating the suitability of the model. This study noticed that the ultrasound-assisted extraction process was an effective method for obtaining triterpenoid saponins from plants.

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