Optimization of Microwave-Assisted Extraction Saponins from Sapindus mukorossi Pericarps and an Evaluation of Their Inhibitory Activity on Xanthine Oxidase

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A microwave-assisted extraction (MAE) method was applied to separate saponins from Sapindus mukorossi pericarps. The most important factors of the six extraction parameters were selected using Plackett–Burman designs; therefore, the further extraction procedure was optimized using the Box–Behnken designs; meanwhile, the optimum processing parameters and well-pleasing saponin extraction rate were inferred. The final operation conditions were the ethanol concentration of 40%, soaking time of 3 h, particle size of 80–100 meshes, extraction time of 13 min, solvent-solid ratio of 19 mL/g, and microwave power of 425 W. Based on the optimal extraction parameters, the extraction rate of the saponins by means of MAE technique reached 280.55 ± 6.81 mg/g, which exceeds yields acquired using conventional manners. Saponins from S. mukorossi have obvious xanthine oxidase inhibitory properties in vitro compared with allopurinol. The saponins displayed a type of competitive inhibition of xanthine oxidase. In conclusion, a MAE technique in association with a response surface design provides an efficient extraction tactics, which could sufficiently isolate saponins from S. mukorossi pericarps; further, this technique could be applied to the dissociation of other bioactive substances from plant sources. In addition, the saponins may be a promising alternative to conventional medicine to treat gout and other inflammation-associated disorders to mitigate the side effects of traditional drugs.

1. Introduction

Sapindus mukorossi of Sapindaceae family, commonly famous for soapnut, wasnut, reetha, or ritha, is a well-known, handsome, deciduous, and valuable tree species. It is indigenous to the hilly areas of China and Japan below an altitude of 1000 m and is widely cultivated in North India [1, 2]. As it is rich in valuable saponins (possessing the property of outstanding surface activity), S. mukorossi is an ecofriendly and promising alternative material of biosurfactant for producing shampoos, cosmetic cleansers, and detergents in sanitary and cosmetic products [3]. S. mukorossi saponins can be applied to remove heavy metals or hydrocarbons from polluted soil and waste water and for enhancing oil recovery, dye solubilization, and nanoparticle synthesis [4–7]. Moreover, S. mukorossi saponins are widely applied to pharmaceutical industry, due to its manifold pharmacological effects, including antimicrobial activity [8–11], antitumor functions [12–14], anti-inflammatory effects [15, 16], and insecticidal activity [17, 18]. S. mukorossi pericarps are a main source of saponins, which make up 7% to 27% of the whole fruit [19]. Therefore, it is of our great interest to optimize the extraction process of saponins from S. mukorossi pericarps.

Inflammation is the susceptive condition of blood vessel hyperemia. The vascular reaction is the major part of the inflammation process, which results from corporal or biochemical injury factors in living tissue accompanied by redness, swelling, pyrexia, pain, and dysfunction [19, 20]. Xanthine oxidase (XO) is the key enzyme of uricogenesis resulting in a painful inflammation: gout. Moreover, chronic inflammation can cause hyperuricemia or other chronic diseases. Clinically, inflammatory disorders are generally controlled by steroidal or nonsteroidal drugs. Nevertheless,
long-term use of these drugs e.g., betamethasone and acetysalicylic acid can lead to gastrointestinal, renal, and cardiovascular disorders or other side effects [21]. It is important to find a low or even nontoxic anti-inflammatory substitute; therefore, plant products are an ideal potential anti-inflammatory agent.

Nonetheless, in the current investigation, their inhibition activity on XO was still at an exploratory stage. Therefore, it is necessary to evaluate the inhibition model of XO in vitro to provide scientific evidence for further research on S. mukorossi saponins.

Traditional methods, including maceration extraction (ME) [22] and Soxhlet reflux extraction (SE and RE) [23], and nontraditional techniques, supercritical fluid extraction (SCFE), and ultrasonic-assisted extraction (UAE) [24], are generally used for saponins extraction. Nonetheless, some disadvantages are associated with these methods (e.g., long-time consumption, tedious extraction processes, massive organic solvent usage, high energy input, low yield, high apparatus requirements, complex operations, and environmental issues) [25]. Consequently, it is vitally important to select a novel, efficient, and green technique for saponins extraction, which could overcome the drawbacks of traditional techniques. Microwave-assisted extraction (MAE) has attracted much attention for its shorter extraction duration [26], decreased solvent usage, higher extraction efficiency [27], lower operating costs, and environmental friendliness in comparison with traditional and other advanced extraction methods [28, 29]. Specially, microwave energy enables heat to instantaneously and simultaneously transfer to the solvent and the entire material in the extraction process by the special heating mechanism with electromagnetic radiation. In recent years, MAE has been used to separate various bioactive components including saponins, essential oil, flavonoids, and terpenoids from plant materials [17, 26, 28, 29].

Plackett–Burman designs (PBDs) and Box–Behnken designs (BBDs) are generally applied to RSM based on the requirements and experimental conditions. PBD is a first order polynomial design, which is a practical RSM for preliminary studies to screen principal element from packs of associated parameters for the requested response variables [29, 30]. In this study, these variables are either fixed or eliminated in the subsequent investigation. Moreover, as a favorable type of design for supporting a response surface, BBD is further employed to assess second-order multivariate technique in terms of three-level synsemantic factorial design [31]. BBD has been generally performed to optimize the extraction process of bioactive components from natural raw materials since it offers sufficient information about the main and interaction effects, which cannot be easily evaluated by univariate techniques [32, 33]. Additionally, the model outcomes can be clearly exhibited on the response surface plot. As far as we know, there is no study involved in using statistical optimization methodology for the maximum extraction of S. mukorossi saponins. Herein, this paper is mainly concentrated on the optimization of the microwave-assisted extraction procedure by both BBD and PBD. SE, RE, and ME were carried out to compare performance characteristics with MAE. Additionally, the inhibitory activity of saponins on XO in vitro was preliminary studied.

2. Experimental

2.1. Reagents and Materials. Xanthine oxidase (XO) was of biochemical reagent (BR) grade, allopurinol (purity ≥ 98%) and oleandric acid (purity ≥ 98%) were of analytical standard, and xanthine was of pure grade. All of them were purchased from Yuanze Bio-Technology Co., Ltd. (Shanghai, China). Ethanol and dimethyl sulfoxide (DMSO) were of analytical reagent (AR) grade and were purchased from Xilong Scientific Co., Ltd. (Guangdong, China).

S. mukorossi fruits were collected in October 2017 from Jiangxi Normal University (Nanchang, China) and authenticated by Prof. Yisheng Tu (College of Life Sciences, Jiangxi Normal University). A voucher specimen (00049836) had been deposited at Lushan Botanical Garden Herbarium (The Chinese Academy of Sciences, Jiangxi, China). The pericarps were separated from fresh fruits, shade-dried for 15 days at room temperature, and then ground using a 2500Y-grinder (swing-type high-speed traditional Chinese medicine pulverizer, Yongkang Boou Hardware Products Co., Ltd., China) and sieved with different sizes. These homogeneous powder samples were preserved in a sealed desiccator at 4°C before future experiments.

2.2. MAE Apparatus. The apparatus for the MAE process was equipped with a WP800SL23 microwave oven (Guangdong Galanz Enterprise Co., Ltd., China) operating at a frequency of 2.45 GHz, a multimode reactor, and a thermometer IR sensor, by which both time and power can be adjusted through its control panel as described in a previous study of our research group [26]. Microwave energy could be constantly transmitted to the reactor, and during operation, microwave power could be dynamically regulated by the control panel in accordance with the actual demands. A Clevenger condenser was added on the top of the domestic microwave oven to make it suitable for laboratory use, and the condenser was connected to a round-bottom flask (500 mL) through a hole that was wrapped with polytetrafluoroethylene around the external upper part of the round-bottom flask to avoid microwave leakage [34]. The inside dimension of microwave oven chamber was 215 mm × 350 mm × 330 mm. The whole system worked at atmospheric pressure.

2.3. Extraction and Determination Procedures of S. mukorossi Saponins. The initial single-factor experiments were carried out to ascertain the appropriate ranges of the six parameters for subsequent experimental operation. 1 g of pericarps sample was mixed with a definite volume of solvent and then subjected to MAE. After extraction, the mixtures were cooled and the supernatant was collected by centrifugation at 1000 × g for 10 min at 25°C, followed by filtration using a 0.45 µm nylon membrane. The results of HPLC-MS have
shown that oleanolic type is the main compounds of total saponins from pericarps of \textit{S. mukorossi} [35], and the oleanolic acid was always regarded as the standard to determine the content of \textit{S. mukorossi} saponins in previous studies [19, 36]. Herein, we also employed oleanolic-type as the main compounds for the determination the content of saponins from pericarps of \textit{S. mukorossi}. The result of determination showed that the oleanolic-type triterpenoid saponins took a proportion of 76.05% ± 1.52 in the obtained saponins in our study. The content of \textit{S. mukorossi} saponins was determined by HPLC analysis as described by Upadhyay et al. [37].

2.4. Optimizing Processes for S. mukorossi Saponins Extraction. The processes to optimize \textit{S. mukorossi} saponins extraction conditions were performed as follows: (1) screen the most significant factors from the six parameters by PBD and (2) optimize the chosen factors to obtain the optimum operational parameters and forecast an acceptable saponins extraction yield by BBD.

2.4.1. Screening the Most Significant Factors by PBD. PBD determined the most significant variables influencing the extraction of saponins from \textit{S. mukorossi} pericarps. The design standards assumed that no interplay effects existed between each parameter, and they were built on the following polynomial equation:

\[ Y = \beta_0 + \sum_{i=1}^{6} \beta_i X_i + \sum_{i=1}^{6} \beta_{ii} X_i^2 + \sum_{i=1}^{6} \sum_{j=i+1}^{6} \beta_{ij} X_i X_j, \]  

(1)

where \( Y \) denotes the predicted value, \( \beta_0 \) and \( \beta_i \) refer to the invariable describing the equation intercept and the regression coefficient, respectively, and \( X_i \) represents the coded factors (A \ldots F). These six parameters included microwave power (A), irradiation time (B), solvent-solid ratio (C), ethanol concentration (D), soaking time (E), and particle size (F) and were screened at two different levels (-1 and +1) with 12-run experiments by PBD, as presented in Table 1. The levels of independent factors were ascertained in terms of the single-factor experimental results.

2.4.2. Optimization of the Saponins Extraction Conditions by BBD. After determining the most influential independent variables using PBD, the three most significant parameters regarding the extraction yield of saponins were evaluated by BBD at three levels. The ranges and levels of influencing variables are displayed in Table 2 based on the results from pre-experimental runs. The independent parameters were combined with the extraction yields of saponins. Experimental runs were implemented stochastically to decline the effects of uncontrolled factors in the actual experiments introduced by exterior factors (operative and instrumental errors). The following quadratic polynomial equation was used to fit the correlations between the saponins extraction yield and the three selected extraction factors. The complete quadratic equation for the developed model is

\[ Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum_{i=1}^{4} \sum_{j=i+1}^{4} \beta_{ij} X_i X_j, \]  

(2)

where \( Y \) is the predicted response; \( \beta_0, \beta_i, \beta_{ii}, \text{and} \beta_{ij} \) represent the regression coefficients for the intercept, linearity, square, and interaction, separately; and \( X_i \) refers to the variable.

2.5. Comparison of MAE with Traditional Techniques. The conventional techniques including ME, RE, and SE approaches were employed for saponins extraction to compare with the MAE technique. The ME was implemented at 25°C for 48 h while the RE and SE procedures were implemented in a heating mantle with a maximum operating power of 1 kW for the extraction of 4 h. The other operational parameters were identical to the optimized conditions.

2.6. Evaluation of the Inhibitory Activity of Saponins on XO In Vitro. A XO inhibition assay using xanthine as a substrate was performed as reported by Filha et al. [38] with minor modulations. To be brief, 0.48 mL of saponins extracts with a series of concentrations (5, 25, 50, 75, and 100 \( \mu \)g/mL) were blended with 1.5 mL of XO solution (0.28 units/mL in phosphate buffer) and 2.4 mL of phosphate buffer (0.07 mM, pH 7.5), and the mixed solution was preincubated at 25°C for 15 min. After incubation, the reaction was initiated by adding 1.6 mL of xanthine (0.15 mM), and the absorbance was recorded at 295 nm every 2 seconds for 2 min using a UV spectrophotometer. The extractions were replaced with dimethyl sulfoxide solution/phosphate buffer without extract solution as a negative control, and allopurinol (5–100 \( \mu \)g/mL in phosphate buffer) served as a positive control. The trials were repeated three times, and the XO inhibitory rate was calculated as the percent inhibition of XO expressed by the following equation:

\[ \text{Inhibition rate (\%) = } \left( 1 - \frac{K_1}{K_0} \right) \times 100, \]  

(3)

where \( K_0 \) is the slope of linear change in absorbance per second without saponins extract and \( K_1 \) is the slope of the linear change with saponins. Lineweaver–Burk plot was employed to illustrate the enzymatic inhibitory mode of saponins on XO.

2.7. Statistical Analysis. The data from PBD and BBD during the experimental processes were implemented using Design Expert 8.0 software. All the experimental runs were performed in triplicate, and all the values are expressed as the average value ± standard deviation.

3. Results and Discussion

3.1. Screening the Most Significant Factors by PBD. Six parameters were analyzed by PBD for their influence on the extraction rate of saponins. The design matrix was employed to select the most pronounced variables for saponins extraction process, and the corresponding responses are shown
Table 1: Yields of saponins, ANOVA results, and regression analysis of the Plackett–Buran design data for the prediction of significant extraction variables.

<table>
<thead>
<tr>
<th>Run</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>Yield (mg/g)</th>
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<tr>
<td></td>
<td>Actual</td>
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<td></td>
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<td></td>
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<td>Predicted</td>
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<tr>
<td>1</td>
<td>540 (+1)</td>
<td>15 (+1)</td>
<td>10 (−1)</td>
<td>80 (+1)</td>
<td>3 (+1)</td>
<td>80–100 (+1)</td>
<td>241.17</td>
</tr>
<tr>
<td>2</td>
<td>230 (−1)</td>
<td>5 (−1)</td>
<td>10 (−1)</td>
<td>40 (−1)</td>
<td>1 (−1)</td>
<td>40–60 (−1)</td>
<td>160.00</td>
</tr>
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<td>5 (−1)</td>
<td>10 (−1)</td>
<td>40 (−1)</td>
<td>3 (+1)</td>
<td>40–60 (−1)</td>
<td>190.50</td>
</tr>
<tr>
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<td>80 (+1)</td>
<td>3 (+1)</td>
<td>40–60 (−1)</td>
<td>218.50</td>
</tr>
<tr>
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<td>5 (−1)</td>
<td>10 (−1)</td>
<td>80 (+1)</td>
<td>1 (−1)</td>
<td>80–100 (+1)</td>
<td>143.17</td>
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<td>15 (+1)</td>
<td>20 (−1)</td>
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<td>1 (−1)</td>
<td>40–60 (−1)</td>
<td>261.17</td>
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<td>80 (+1)</td>
<td>3 (+1)</td>
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<td>175.83</td>
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<td>80–100 (+1)</td>
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<td>216.83</td>
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<td>12</td>
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<td>40 (−1)</td>
<td>3 (+1)</td>
<td>80–100 (+1)</td>
<td>221.83</td>
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ANOVA

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<th>Mean square</th>
<th>F value</th>
<th>P Value</th>
<th>Inference</th>
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Regression data

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<th>Term</th>
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<th>Coefficient</th>
<th>Standard error</th>
<th>F Value</th>
<th>T Value</th>
<th>P Value</th>
<th>Inference</th>
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<tbody>
<tr>
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<td>38.36</td>
<td>19.18</td>
<td>3.12</td>
<td>37.69</td>
<td>6.15</td>
<td>0.0017</td>
<td>**</td>
</tr>
<tr>
<td>B</td>
<td>37.03</td>
<td>18.51</td>
<td>3.12</td>
<td>35.12</td>
<td>5.93</td>
<td>0.0020</td>
<td>**</td>
</tr>
<tr>
<td>C</td>
<td>31.69</td>
<td>15.85</td>
<td>3.12</td>
<td>25.73</td>
<td>5.08</td>
<td>0.0039</td>
<td>**</td>
</tr>
<tr>
<td>D</td>
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<td>−4.15</td>
<td>3.12</td>
<td>1.77</td>
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</tr>
<tr>
<td>E</td>
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<td>0.80</td>
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<tr>
<td>F</td>
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<td>1.18</td>
<td>3.12</td>
<td>0.14</td>
<td>0.38</td>
<td>0.7210</td>
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T-value limit 2.57 Value of Bonferroni limit 4.88

*aThe results were obtained with Design Expert 8.0 software. A is the microwave power, B is the microwave time, C is the solvent–solid ratio, D is the ethanol concentration, E is the soaking time and F is the particle size. ‘bTotals of all information corrected for the mean; ‘p < 0.05, significant; ‘‘p < 0.01, highly significant; ‘‘‘p < 0.001, extremely significant.

in Table 1. The experimental results are formulated using the following second-order polynomial equation:

\[
\text{yield} = 204.99 + 19.18 \times A + 18.51 \times B + 15.85 \times C - 4.15 \times D + 2.51 \times E + 1.18 \times F
\]  

(4)

Depending on the results from PBD, the absolute T values of each variable are presented in Table 1. The parameters were deemed to be statistically significant, as the T values of parameters are higher than both the T-value limit (2.57) and the Bonferroni limit (4.88). A supporting plot for variable analysis is shown in the Pareto chart of effects (Figure 1), which is composed of several bars reflecting a length proportional to the absolute value. The parameters’ main effects were rank-ordered by significance. The variables, arranged in sequence, were \(A > B > C > D > E > F\). Consequently, three parameters (A, B, and C) of six independent variables had highly pronounced influences on the saponins extraction efficiency as demonstrated in the Pareto chart by their T values, which showed much higher value than the T value of the Bonferroni limit. Similar results were also found in reports by Wei et al. and Gao [39, 40]. Besides, these favorable influences on the saponins extraction rate were exhibited by the three screened crucial variables, meanwhile raising their values contributed to saponins extraction yield. The statistical analysis results were a good fit to the P values acquired from the regression analysis (Table 1) as well. Meanwhile, the influences of other parameters such as the ethanol concentration (negative effect), the soaking time (positive effect), and the particle size (positive effect) were determined to have a relatively insignificant influence on this response value over the range of factors investigated; these abovementioned extraction parameters had a relatively little influence on the saponins extraction efficiency. Consequently, according to the results from Table 1, the ethanol concentration, soaking time, and particle size were fixed at 40%, 3h, and 80–100 meshes, respectively. The other three variables (A, B, and C) were screened for further optimization studies.

3.2. Optimization of Extraction Conditions by BBD

3.2.1. Experimental Design and Statistical Analysis. The effects of three individual target factors (A, B, and C) were evaluated using a BBD, followed by an optimization of the operating process. The levels of different variables and the relevant responses are displayed in Table 2. Seventeen experimental runs were conducted on the basis of the design. Using multiple-regression analysis on the test results, the estimated responses on the yield of saponins, the
The experimental results could be evaluated by the following quadratic polynomial equation:

\[
yield = -370.01 + 2.21 \times A + 17.52 \times B + 7.58 \times C
- 0.01 \times AB - 5.61 \times 10^{-4} \times AC + 0.02 \times BC
- 2.45 \times 10^{-3} \times A^2 - 0.5 \times B^2 - 0.19 \times C^2. \tag{5}
\]

The model adequacy was verified by the F test, determination coefficient \(R^2\), and analysis of variance (ANOVA) for regression as presented in Table 2. The F value, ratio of the regression mean square and residual, indicated the effect of the individually tested variables on the model. The shown P value reflected the significance of every coefficient, which may conversely reveal the model of the interaction effects between the factors [41]. The model was extremely statistically pronounced, as proven by the F test (146.4729). This combined with the low P value (<0.0001) confirmed that the regression equation could be appropriately and adequately applied to elucidate a large proportion of the data variances in the response. The variance of the data round the fitted pattern was defined by lack of fit [42]. The lack of fit of the F value (6.3286) and P value (0.0534) indicated that each of the variables was an insignificant disparity compared with the pure error and the developed models could be well fitted to predict the responses. The value of the \(R^2\) (0.9947) showed that at least 99.47% of the variability in the response suited the model [42]. The significant correlation between the experimental and predicted response values was denoted by the value of the adjusted determination coefficient \((R^2_{Adj} = 0.9879)\). Thus, the high value of \(R^2\) (0.9947), adjusted \(R^2\) (0.9879), and pre-\(R^2\) (0.9288) ensured that the developed model illuminated the practical interactive influences between the responses and the operational factors were satisfactorily associated [43]. A low value of coefficient of variance (C.V., 1.95) expressly displayed a high accuracy and great reliability in the experiments performed. The adequacy accuracy measured the signal-to-noise ratio, whose ratio higher than 4 was satisfying. Therefore, the value of 31.81 indicated a competent signal and indubitably supported the fitness of the developed model [44].

3.2.2. Effect of Process Variables on Saponins Extraction.

The plotted response surfaces were used to investigate both the effects of the screened variables and the interrelations of the tested variables on the saponins yield by 3D response
surface curves with underlying 2D contour plots [45], which showed the optimum extraction conditions as illustrated in Figures 2(a)–2(c). Namely, the response surface plot demonstrated the correlation between responses and test levels of each factor and the pattern of interaction effect between each pair of experimental factors. Every figure displayed how the two parameters affected the saponins yield, while the third parameter was unchanged. These plots (circular or elliptical) statistically showed the significance of the interrelations between parameters. The insignificance of the interaction effects between the relevant parameters was reflected in the circular contour plot, whereas the significance was displayed in the elliptical contour plot [41].

Figure 2(a) shows the interaction effect between A and B on the saponins yield at an invariable value of the parameter C with 15 mL/g. The response curve along with the elliptical contour plot demonstrated that the mutual effects between these two factors were significant. When microwave time was held at a lower level (under 13 min), the extraction yields markedly improved with rising irradiation time for a range of microwave power. The yield reached the maximum value in about 13 min and then declined from 420 to 540 W. There was a quadratic effect which exhibited for the influence of microwave power on saponins yield. An acceptable extraction rate was acquired at approximately 420 W, but the yield was reduced at a higher output power. Saponins yield improved with the enhancement of extraction time at an invariable microwave power. This might be explained by the fact that microwave power played a vital role in saponins extraction before a threshold level. After passing the threshold, no increase was observed on the rate of extraction due to saturation [41]. The microwave irradiation sped up cell rupture by instantaneous heating and internal pressure enhancement inside the cells of target samples, which facilitated sample surface breakdown and in turn promoted the diffusion of saponins from the plant cells into the extracting solution, thus increasing the extraction yield. However, after microwave power reached the threshold level (approximately 420 W), increasing microwave time did not significantly affect the extraction yield. In contrast, over-exposure of plant sample to the microwave field and/or mass saturation impeded the mass transfer rate of saponins into the extracting solution. This may have caused the degradation of saponins, resulting in a decreased yield of saponins [46]. A similar phenomenon was also observed in microwave-assisted extraction of anthraquinones from R. emodi [47].

Figure 2(b) shows the relationship between the extraction parameters A and C on saponins yield at a defined value of the independent variable B of 10 min. A linear response on the saponins yield was presented for the variable A; additionally, a marked quadratic effect on the saponins yield was presented for variable C. Saponins extraction yield improved with the extraction parameter C and a set extraction power from 230 to 427 W. When extraction power was fixed at 427 W, the yield decreased gradually and demonstrated an unfavorable influence on saponins yield. This phenomenon was similar to the previous study [48].

Figure 2(c) displayed the interaction effects between the independent variables B and C on saponins yield with a constant value of the extraction variable A at 385 W. The saponins extraction yield improved with an increased value of the independent variables B and C. The peak of the extraction yield was achieved when the values of the variables B and C slightly exceeded 14 min and 20 mL/g, separately. Nevertheless, saponins extraction yield plateaued when extraction time reached 14 min. This result may be due to the fact that a suitable microwave radiation time could cause sufficient microwave energy accumulation to enhance the saponins dissolution process, resulting in an increased yield. Excessive exposure time under microwave radiation could induce degradation of saponins, resulting in a reduction in saponins extraction yield. A similar phenomenon was found in the process of polysaccharides extraction from Moringa oleifera Lam. leaves [49].

3.2.3. Optimization of MAE by RSM and Validation of the Optimized Conditions. Based on the results of the RSM, the optimized extraction parameters for S. mukorossi saponins are the extraction factors A, B, and C are 425 W, 13 min, and 13 mL/g, separately. Under the abovementioned processing parameters, the maximum saponins extraction rate was forecasted to be 286.31 mg/g by BBD. A validation of the MAE procedure was applied to verify the precision and suitability of the extraction process. The tests were conducted thrice under the optimized processing parameters, and the practical extraction rate of saponins was 280.55 ± 6.81 mg/g. The actual result was very approximate to the predicted values, showing that the obtained extraction conditions are credible.

3.3. Comparison with Reference Methods. In the present study, classical extraction techniques including ME, RE, and
SE were conducted to make a comparison with the MAE extraction process. The saponins extraction rate for ME, RE, and SE were 123.41 ± 3.34 mg/g, 204.16 ± 5.89 mg/g, and 212.58 ± 5.59 mg/g, respectively. Hence, MAE was superior to traditional extraction methods in saponins extraction rate.

With respect to environmental effect, the MAE was carried out at 425 W with 13 min, and the traditional techniques (RE and SE) were performed at 1 KW with 4 h, respectively. The power consumption of MAE was only 0.092 kWh but 4 kWh for RE and SE. The quantity of CO₂ rejected into the atmosphere can be calculated according to the literature: to generate 1 kWh by combustion of fossil fuel (coal or fuel) will release 800 g of CO₂ into the atmosphere [50]. According to the result from calculation, it showed that the quantity of CO₂ rejected into the atmosphere is much lesser in the process of MAE (73.67 g CO₂) than that of RE or SE (3200.00 g CO₂). In summary, it drew a conclusion that MAE can be suggested as a green method as which can provide a shorter extraction time, higher extraction efficiency, less energy consumption, and a smaller quantity of CO₂ release. Therefore, MAE could be applied as a green technique for the isolation of saponins from *S. mukorossi*.

![Graphs](image-url)
At the same time, the RSM in this research confirmed the advantages of identifying the optimal operation conditions and predicting acceptable responses. Therefore, the combination of MAE with RSM has attractive development foreground for industrialized separation and purification of natural compounds from different plant resources in the future.

We deduced that the special heating mechanism produced by microwave radiation power ensured a high saponins extraction rate. In the MAE procedure, the microwaves led to a rapid temperature rise resulting in cytoarchitecture interior changes and internal pressure enhancement, while the saponins diffused in the same direction. This may have ruptured the cell structures to facilitate the quick release of saponins. Moreover, the effect of the molecules’ ionic conduction and dipole rotation caused by microwaves can promote both heat and mass transfer rates [51–53]. In the MAE method, the direct mutual influence of the microwave on the ethanol aqueous solution and the water inside the materials exist in the biomaterial cells to rupture the cells and effuse endocellular components into the solvent, quickly increasing the saponins extraction yield. A similar phenomenon was also observed in the literature [54, 55]. Hence, MAE was more effective than the traditional techniques for saponins extraction.

3.4. Evaluation of XO Inhibitory Activity In Vitro. XO directly regulates the level of uric acid. The effect of *S. mukorossi* saponins on XO activity was demonstrated using spectrophotometry as shown in Figure 3. *S. mukorossi* saponins showed an inhibitory effect on XO. There was a concentration-dependent effect on XO. The lowest inhibitory rate (28.21 ± 0.80%) was at a saponins concentration of 25 μg/mL, and the highest inhibitory rate (89.87 ± 2.25%) was at a saponins concentration of 100 μg/mL. The inhibitory rate of *S. mukorossi* saponins at 100 μg/mL was very close to the rate observed in the positive control, allopurinol, which displayed a rate of 92.25 ± 3.07% at the same concentration. An inhibitory mechanism assay was performed by kinetic analysis using Lineweaver–Burk plots (Figure 4), which indicated that *S. mukorossi* saponins exhibited high XO inhibition. The mode of inhibition for saponins was competitive as determined by the *V*<sub>max</sub> and *K*<sub>m</sub> from Lineweaver–Burk plots. The value of *K*<sub>m</sub> increased as the saponins concentration increased, while the *V*<sub>max</sub> values were constant relative to xanthine as the substrate. Therefore, saponins may compete with the substrate for the active site of XO, preventing the substrate from binding. Similar phenomena have been reported in early studies [56]. *S. mukorossi* saponins may be regarded as triterpene glycosides consisting of the oligosaccharide chain and sapogenins, which shows strong surface activity properties as amphiphilic products. Their inhibition on XO might be attributable to their surface activity. *S. mukorossi* saponins might be specific surfactants that bind to an enzyme with special affinity [57].

The excess of uric acid produced by XO results in hyperuricemia in human, gout, or other chronic inflammatory disorders [58, 59]. The effective inhibition of XO by *S. mukorossi* saponins may inhibit the production of uric acid to reduce the symptoms caused by uric acid. Thus, *S. mukorossi* saponins have great potential as a natural alternative agent for the prevention of, and therapy for, gout and other inflammatory disorders.

4. Conclusion

In conclusion, we investigated the utility of the microwave-assisted method to extract saponins from *S. mukorossi* pericarps. The MAE method was applied to extract the saponins using both PBD and BBD to optimize the operating
pericarps but also offer the basis for using them to achieve the maximum extraction yield of saponins from not only provide a green, efficient, and reliable technique for inflammatory disorders. In summary, the results of this study inferred that saponins may be a potential and natural therapeutic for hyperuricemia and other inflammatory disorders. The optimized operation procedure obtained a yield of 280.55 ± 6.81 mg/g with the following extraction parameters: the ethanol concentration of 40%, soaking time of 3 h, particle size of 80–100 meshes, extraction time of 13 min, solvent-solid ratio of 19 mL/g, and microwave power of 425 W. In comparison with conventional methods, MAE demonstrated a higher extraction efficiency in a shorter extraction time along with decreased energy consumption for saponin extraction. The inhibition activity of XO performed in vitro revealed that saponins from Sapindus mukorossi pericarps demonstrate a type of competitive inhibition on XO compared with the standard drug, allopurinol. It can be inferred that Sapindus mukorossi saponins may be a potential and natural therapeutic for hyperuricemia and other inflammatory disorders. In summary, the results of this study not only provide a green, efficient, and reliable technique for the maximum extraction yield of saponins from Sapindus mukorossi pericarps but also offer the basis for using Sapindus mukorossi saponins to treat gout and other inflammatory disorders.

Data Availability
The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest
The authors declare that there are no conflicts of interest regarding the publication of this paper.

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