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Research Article

Microwave-Assisted Extraction of Polysaccharides From the *Abelmoschus sagittifolius*: Optimization, Structure, and Bioactivity

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The aim of this study was to extract polysaccharides from *Abelmoschus sagittifolius* using the microwave-assisted extraction (MAE) method. Three independent variables, including extraction temperature, microwave power, and microwave time, were chosen for investigation through single-factor experiments. Response surface methodology (RSM) based on a Box-Behnken design (BBD) was utilized to optimize the extraction conditions. The study found that the optimal conditions for polysaccharide extraction were a temperature of 63°C, a microwave power of 650 W, and a duration of 58 min. The polysaccharide yield under these conditions was 29.8 g/100 g, closely matching the experimental yield of 30.1 g/100 g. This yield was approximately 1.15 times higher than the yield achieved through conventional extraction methods (heat reflux extraction (HE) method). The study also demonstrated that polysaccharides extracted using the MAE method exhibited improved antioxidant capacity and enzyme inhibition and displayed significant inhibitory effects on HepG2 cells compared to the conventional extraction method. Moreover, the polysaccharides obtained from the optimized MAE process had a lower molecular weight and higher polydispersity than the HE method. These findings indicate that MAE is a promising, efficient, and environmentally friendly technology for extracting polysaccharides from *A. sagittifolius*.

Keywords: *Abelmoschus sagittifolius*; antioxidant; polysaccharides; response surface methodology (RSM)

1. Introduction

Abelmoschus sagittifolius (Kurz) Merr., known as "Sam Bo Chinh" in Vietnam, is a plant from the *Abelmoschus* genus in the Malvaceae family. It is found in tropical and subtropical regions worldwide, including China, India, Australia, Africa, and Southeast Asia [1–3]. In Asian countries, the roots of *A. sagittifolius* have long been used for treating fever, backache, headache, stomachache, and cough [3–5]. These roots are rich in bioactive compounds like polysaccharides, flavonoids, and alkaloids [1, 4]. Among these compounds, polysaccharides exhibit multiple beneficial properties like antitumor, anti-inflammatory, antiviral, and antioxidant effects [3–6]. However, the bioactivity of these polysaccharides can be affected by different extraction methods, which influence their molecular weight, structure, monosaccharide composition, and branching [6, 7]. Therefore, an efficient technique for the extraction of polysaccharides from *A. sagittifolius* is imperative.

Currently, polysaccharides are typically extracted using heat reflux, alkaline, and dilute acid solutions to efficiently break down the cell wall [8, 9]. However, these methods have limitations such as low yields, impurities, and high molecular weights [9]. They are also energy-intensive, require significant amounts of organic solvents, and pose environmental risks that can harm bioactive components [8, 9]. To address these challenges, new extraction methods like pulsed electric fields (PEF)-assisted extraction, microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), and ultrasonic-assisted extraction (UAE) have been developed [8, 10, 11]. Among these, MAE is a cost-effective and efficient technique for extracting polysaccharides from herbs and plants [7, 8,

12]. MAE utilizes microwave radiation to break cell walls, decrease particle sizes, and improve solvent interaction with target compounds [5, 7, 8]. Previous studies have shown that polysaccharides extracted from natural plants using MAE exhibit stronger antioxidant properties compared to those obtained through traditional methods [13, 14]. However, there is limited research on the characterization and antioxidant effects of polysaccharides extracted from *A. sagittifolius* using MAE. Therefore, it is crucial to establish an MAE method for *A. sagittifolius* polysaccharides.

Response surface methodology (RSM) based on the Box-Behnken design (BBD) is a valuable approach for effectively interpreting and organizing experiments, providing advantages over other methods [15, 16]. It is a strong statistical technique that helps optimize complex experimental procedures by reducing the number of trials required for process optimization, assessing main variables and their interactions, and determining optimal levels [11, 15, 16]. In this study, a three-level, three-variable BBD was utilized to model and optimize extraction parameters, including extraction temperature, microwave power, and microwave time, for polysaccharides from *A. sagittifolius* using the MAE method. A mathematical model was developed using RSM to assess the influence of the three independent variables and their interactions on the extraction yield of the desired products. A comparison between the MAE and conventional heat reflux extraction (HE) methods was conducted. Furthermore, the extracted polysaccharides were analyzed for composition using high-performance liquid chromatography and gel permeation chromatography (GPC) spectrometry, and their antioxidant and antitumor properties were assessed through in vitro tests.

2. Materials and Methods

2.1. Materials

2.1.1. Plant Material. The research utilized 2-year-old roots of *A. sagittifolius*, cultivated at the Viet Trung Farm Area in Bo Trach District, Quang Binh Province, Vietnam. These roots, provided by Sam Tien Vua International Technology Joint Stock Company, were washed, sliced into 2–3-mm pieces, and dried at 50°C for 8 h until the moisture content fell below 10%. Moisture content was measured using the AND MF-50 moisture analyzer (Mettler Toledo, United States). After drying, the slices were milled and sieved through a 0.25-mm mesh to obtain *A. sagittifolius* root powder, which was then stored in a dry, dark environment until needed [8].

2.1.2. Chemicals. The analysis utilized a range of chemicals from various sources. These included ethanol (99.6% purity, from China), sodium hydroxide (> 99.8% purity, from China), hydrochloric acid (36%–38% purity, from China), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$), all sourced from Sigma-Aldrich. Additionally, 5-dinitrosalicylic acid (98% purity), D-glucose (Sigma-Aldrich), phenol (Sigma-Aldrich), and sulfuric acid (96% purity, from Sigma-Aldrich) were used. Monosaccharide standards, including galactose, rhamnose, arabinose, glucose, xylose, mannose, galacturonic acid, and glucuronic acid, were obtained from Yuanye Bio-Technology Co. (Shanghai, China). Dulbecco's modified eagle medium (DMEM) (Hyclone, Utah, United States), fetal bovine serum (FBS) (Hyclone, Utah, United States), and sulforhodamine B (SRB) (Rhodamine B, Jiangnanjie, China) were also used. All chemicals employed in the analysis met the required technical specifications.

2.2. Extraction Procedure

2.2.1. Extraction of polysaccharides using microwave assistance (MAE). The aim of the study was to investigate the impact of MAE on the content of polysaccharides. The microwave power, temperature, and irradiation time were modified based on the previous studies between 400–800 W, 40°C–90°C, and 40–80 min, respectively [13]. The mixture was then treated with 95% ethanol at 4°C overnight [16]. After that, the mixture was centrifuged at $5000 \times g$ for 20 min to collect the supernatant. The precipitate was subsequently dialyzed continuously for 8 h in deionized water, with regular changes of the water, and then lyophilized to obtain polysaccharide powder for further analysis. The polysaccharide extraction efficiency (%) was determined by comparing the dry weight obtained postethanol precipitation with the dry weight of *A. sagittifolius* powder used in each experiment.

2.2.2. Experimental Design. Based on the results of the single-factor experiments, a three-level BBD with three factors was utilized to optimize the conditions for MAE. The independent variables preferred for the BBD design were extraction temperature (X_1, °C), microwave power (X_2, W), and microwave extraction time (X_3, min). Seventeen experimental runs with one block and five center points were performed in accordance with the BBD design. Table 1 presents the variables and their levels, both in coded and actual values. The obtained data were analyzed using the statistical Design-Expert software 8.0.5 (Stat-Ease Inc., Minneapolis, Minnesota, United States), and the significance of the model was evaluated by analysis of variance (ANOVA). The experimental data from BBD were explained by the second-order polynomial model, as follows [15, 16]:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X^2 + \sum_{i>j}^{k} \beta_{ij} X_{ij} \quad (1)$$

where X_i and X_j denote the different independent variables. Y is the predicted response. β_0, β_j, β_{jj}, and β_{ij} denote the regression coefficients corresponded to intercept, linearity, square, and interaction, respectively. All the statistical analyses were done using Design-Expert 8.0.5 package (State-Ease Inc., Minneapolis, Minnesota, Unite States).

2.2.3. Traditional Extraction Method (HE). The HE method is a widely used technique for extracting polysaccharides. In this study, we used the HE method with some minor adjustments based on a previously documented technique for HE of polysaccharides [16]. The first step involved

Table 1: Box-Behnken design of experimental conditions and observed responses for polysaccharide content of *A. sagittifolius* extracted using microwave-assisted extraction.

Run	Independent variable			Polysaccharide yield (Y, g/100 g)		
	X_1 (°C)	X_2 (W)	X_3 (min)	Actual value	Predicted value	Residual
1	65 (0)	800 (+1)	40 (−1)	27.00 ± 0.81	27.03	−0.0250
2	65 (0)	600 (0)	60 (0)	29.20 ± 0.85	28.58	0.6200
3	90 (+1)	600 (0)	40 (−1)	22.90 ± 1.03	23.34	−0.4375
4	65 (0)	600 (0)	60 (0)	28.70 ± 1.01	28.58	0.1200
5	65 (0)	600 (0)	60 (0)	28.50 ± 0.91	28.58	−0.0800
6	65 (0)	600 (0)	60 (0)	28.7 ± 0.95	28.58	0.1200
7	65 (0)	400 (−1)	80 (+1)	25.00 ± 0.90	24.98	0.0250
8	40 (−1)	400 (−1)	60 (0)	17.40 ± 0.84	17.86	−0.4625
9	65 (0)	600 (0)	60 (0)	27.80 ± 0.89	28.58	−0.7800
10	90 (+1)	400 (−1)	60 (0)	21.60 ± 0.81	21.16	0.4375
11	40 (−1)	600 (0)	80 (+1)	26.70 ± 0.77	26.26	0.4375
12	65 (0)	400 (−1)	40 (−1)	23.50 ± 0.64	23.50	0.0000
13	65 (0)	800 (+1)	80 (+1)	25.50 ± 0.69	25.50	0.0000
14	90 (+1)	800 (+1)	60 (0)	17.40 ± 1.12	16.94	0.4625
15	90 (+1)	600 (0)	80 (+1)	20.50 ± 0.85	20.96	−0.4625
16	40 (−1)	800 (+1)	60 (0)	25.70 ± 1.01	26.14	−0.4375
17	40 (−1)	600 (0)	40 (−1)	24.40 ± 0.92	23.94	0.4625

refluxing 1.0 g of *A. sagittifolius* powder with 10 mL of 80% (*v*/*v*) ethanol at 80°C for 2 h to eliminate small molecules. The polysaccharides were then extracted twice with 30 mL of deionized water at 90°C for 2 h. Finally, the resulting polysaccharides were obtained following the same procedures mentioned earlier.

2.3. Experimental Methods

2.3.1. Solubility Index, Water, and Oil Retention Capacity. Solubility tests were conducted following the procedure outlined by Alizadeh et al. [17], with some adjustments. Solutions containing the polysaccharide (1.0%, *w*/*v*) were prepared using water and solvents (hexane and methanol) at different temperatures (25°C, 40°C, and 55°C). The solutions were agitated at $300 \times g$ for 30 min and then centrifuged in a high-speed centrifuge (MF80, Hanil Science Ind., Seoul, Korea) for 30 min at $3000 \times g$. The resulting supernatants were collected and dried in an oven at 100°C for 12 h. The solubility was determined using Equation (2):

$$\text{Solubility index} = (\text{weight of dried supernatant/weight of the sample}) \times 100. \quad (2)$$

To determine the water-holding capacity (WHC) and oil-holding capacity (OHC), we used the method described by Oh and Kim [18] with some modifications. First, we took 250 mg of each sample and mixed it separately with 100 mL of distilled water or 25mg/10 mL of olive oil in Erlenmeyer flasks. The mixtures were then thoroughly stirred and left at room temperature for 1 h. After centrifugation for 10 min, we weighed the residues. WHC and OHC were expressed as milliliters per gram of sample.

2.3.2. Antioxidant Properties

2.3.2.1. $ABTS^{\bullet+}$ Radical Scavenging Activity. To assess the $ABTS^{\bullet+}$ radical scavenging activity of polysaccharides, the method described by Oh and Kim [18] was adapted with some modifications. Initially, equal volumes of a 7-mM ABTS stock solution and a 2.45-mM potassium persulfate solution were combined to generate $ABTS^{\bullet+}$. This mixture was incubated in the dark at 0°C for 12–16 h. The resulting $ABTS^{\bullet+}$ solution was then diluted with ethanol until its absorbance was 0.700 ± 0.020 at 734 nm. For the assay, 0.2 mL of the polysaccharide solution (ranging from 0.05 to 0.3 mg/mL) was added to 1.8 mL of the $ABTS^{\bullet+}$ solution. The reaction mixture was kept in the dark for 10 min before measuring the absorbance at 734 nm. A control solution was prepared by mixing 0.2 mL of ethanol with 1.8 mL of $ABTS^{\bullet+}$ solution. Additionally, ascorbic acid (0.05–3.0 mg/mL in water) served as a reference compound. The $ABTS^{\bullet+}$ scavenging activity was then calculated using Equation (3):

$$\begin{aligned} &ABTS^{\bullet+}\ \text{scavenging activity}\ (\%) \\ &\quad = \left(\left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right)/\text{Abs}_{\text{control}}\right) \times 100 \end{aligned} \quad (3)$$

where $\text{Abs}_{\text{control}}$ is the absorbance value of blank and $\text{Abs}_{\text{sample}}$ is the absorbance value of extract.

2.3.2.2. Cell Viability Assay. HepG2 cells were obtained from the American Type Culture Collection (ATCC) in Manassas,

Virginia, United States. These cells were cultured in DMEM, which was supplemented with 10% FBS and 1% penicillin-streptomycin, following the method described by Jia et al. [19]. The cells were then incubated at 37°C in a 5% CO_2 atmosphere. To screen the polysaccharide activity, the SRB experiment was employed. HepG2 cells were seeded in 96-well plates and exposed to varying concentrations of polysaccharide (5.5–160 μg/mL) for 48 h. After the treatment, the cells were fixed with a 50% TCA solution and stained with 0.4% SRB staining solution. The OD at 540 nm was measured using a microplate reader (BioTek ELX800, United States). The experiments were repeated at least three times.

2.3.2.3. Collagenase Inhibition Assay. The activity of the collagenase inhibitor was assessed by using azo dye-impregnated collagen as a substrate, following the method explained in Wang et al. [20]. To conduct the test, tubes were prepared by adding 1 mg of azo dye-impregnated collagen, 800 μL of Tris-HCl (0.1 M, pH 7), and 100 μL of the sample solution with varying concentrations (0.05–0.3 mg/mL). The mixture was shaken for 1 min before adding 100 μL of collagenase (200 units/mL). Following this, the mixture was incubated at 43°C for 1 h. The solid was separated by centrifugation at 3000 rpm for 10 min, and the supernatant was placed in a 96-well plate to measure absorbance at 550 nm. In addition, a blank was also prepared using all the reaction reagents except for the sample solution.

2.3.2.4. α-Amylase Inhibition Assay. The method used by Savran et al. [21] with some modifications was employed to determine the inhibitory activity of α-amylase. Lyophilized polysaccharide was dissolved in distilled water to create a polysaccharide extract (25 μL) with varying concentrations (0.05–0.3 mg/mL), which was then mixed with a 0.05% starch solution (50 μL) and an α-amylase solution from porcine pancreas (50 μL) in a phosphate buffer (pH 6.9 with 6-mM sodium chloride). The mixture was incubated at 37°C for 10 min. To stop the reaction, 1-M HCl (25 μL) and iodine-potassium iodide solution (100 μL) were added. The absorbance was measured at 630 nm using a microplate reader (PR2100, Bio-Rad, United States). The α-amylase inhibitory activity was reported as millimoles of acarbose equivalents (mmol ACE)/g extract. A blank was prepared by including the sample solution in all reaction reagents, except the α-amylase solution.

2.3.3. Determination of Polysaccharide Composition. The following method was used to determine the monosaccharide compositions of the polysaccharides, with some modifications to the method reported by Li et al. [22]. Initially, a sample of the polysaccharide (5 mg) was hydrolyzed using trifluoroacetic acid (TFA) (2 M, 4 mL) in a sealed flask, at a temperature of 110°C for 6 h. The excess TFA was then removed by using a nitrogen gas–blowing instrument for three times. After that, the residue was redissolved in 100 mL of deionized water and filtered through a 0.22-μm nylon membrane for analysis. To analyze the filtrate, a Dionex ICS 3000 system (Sunnyvale, California, United States) equipped with a CarboPac PA1 guard column (50 × 2 mm) and a CarboPac PA20 analytic column (250 × 2 mm) coupled with pulsed amperometric detection (PAD) was used. The monosaccharide compositions of the polysaccharides were determined by comparing the retention times with those of standards. Finally, the monosaccharide content was calculated according to the calibration curve (peak area vs. concentration) of each monosaccharide standard.

2.3.4. GPC Spectrometry. Polysaccharide molecular weight was determined using GPC with a Waters 1525 HPLC system and a Waters 2414 differential refractive index detector. The eluent was 0.02-mol/L potassium dihydrogen phosphate, with a flow rate of 0.6 mL/min. Column temperature was kept at 35°C ± 0.1°C. One-microliter samples were injected for each run, and polysaccharide molecular weights were reported as dextran equivalent molecular weight. Dextran standards from 1×10^3 to 1×10^7 Da were used for calibration through linear regression.

2.3.5. Scanning Electron Microscopy. The morphology of *A. sagittifolius* powder particles was examined using a Hitachi Ion Sputter E-1010 scanning electron microscope at a 15-kV voltage. The sample was fixed on aluminum stubs, coated with 40-nm gold.

2.4. Statistical Analysis. The experimental results were expressed using the mean ± SD of three repeats, and data were analyzed using one-way ANOVA followed by Duncan's multiple-range test. All statistical analyses were processed using Design-Expert software (version 11.0; Stat-Ease Inc., Minneapolis, Minnesota).

3. Results and Discussions

3.1. Single-Factor Experimental Analysis. Figure 1(a) illustrates the effect of varying extraction temperatures on the yield of polysaccharides from *A. sagittifolius*, with the extraction time fixed at 60 min, a raw material to solvent ratio of 1:30 g/mL, and microwave power set to 600 W. The figure shows significant variations in polysaccharide yield with changes in extraction temperature. Notably, as the temperature increased from 40°C to 70°C, the polysaccharide yields significantly improved. The highest yield of 28.9 g/100 g was achieved at 70°C, which was double the yield obtained at 40°C. This enhancement in yield is attributed to the increased solubility and dissolution of polysaccharides at higher temperatures. Previous studies have similarly indicated that elevated microwave temperatures facilitate dipole rotations and enhance thermal energy in the reaction mixture, leading to increased polysaccharide yields [14, 16]. However, polysaccharide content decreased when the temperature was raised from 80°C to 90°C, likely due to polysaccharide hydrolysis. Therefore, maintaining the extraction temperature at 70°C is recommended to maximize polysaccharide yield.

Figure 1(b) shows the influence of microwave power on polysaccharide yield at a constant extraction temperature of 70°C, a duration of 25 min, and a solid to solvent ratio of 1:30 g/mL. As microwave power increased from 400 to 500 W, polysaccharide yield slightly improved from 15.6 to

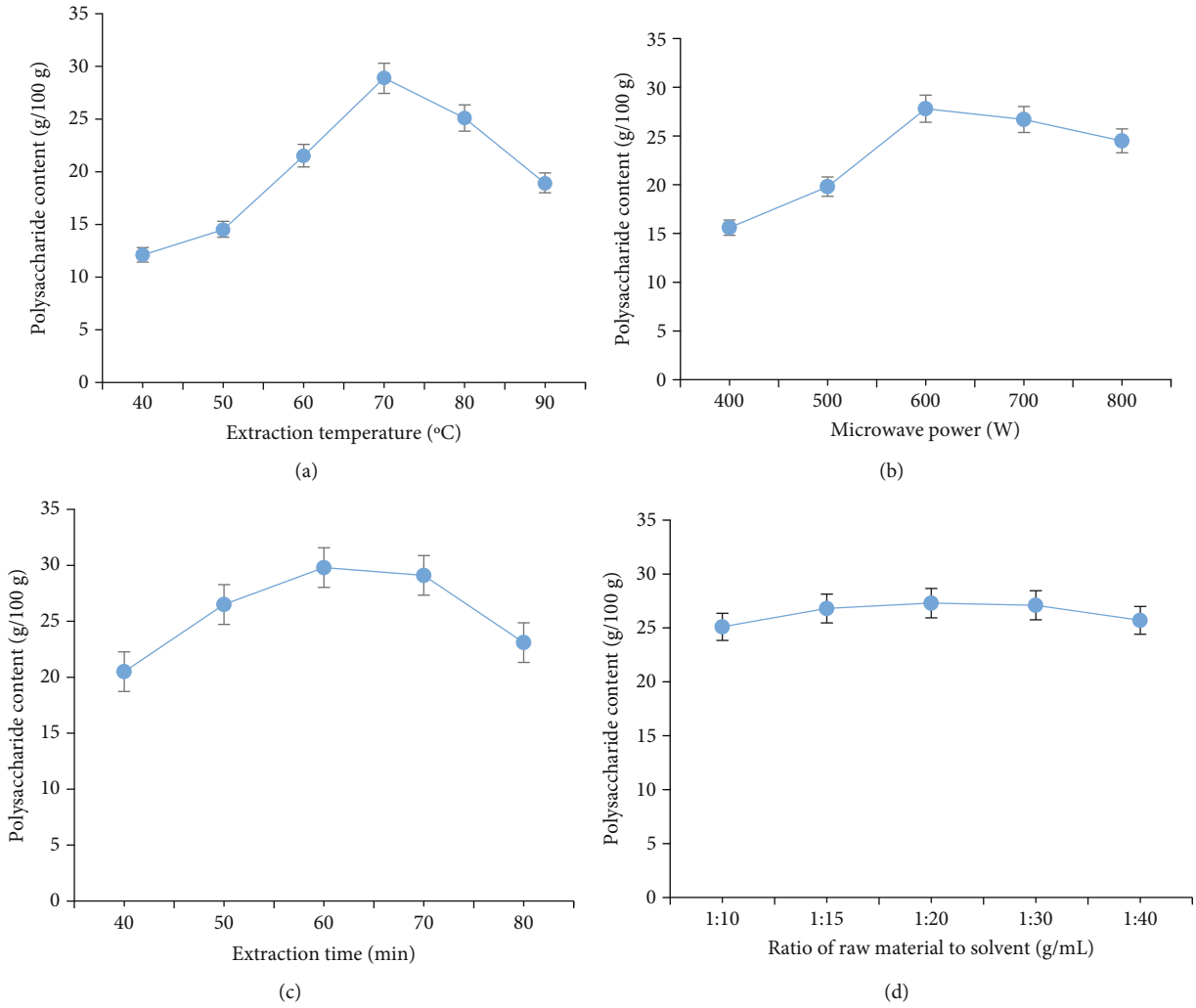


Figure 1: Effect of difference: (a) extraction temperature, (b) microwave power, (c) extraction time, and (d) ratio of raw material to solvent on polysaccharide content.

16.8 g/100 g. The highest yield of 27.8 g/100 g was observed at 600 W, after which the yield declined. This reduction in yield may be due to polysaccharide degradation and the dissolution of undesirable components. Similar patterns have been reported in studies on MAE of natural products [5, 7, 8, 16]. Consequently, a microwave power of 600 W is determined to be optimal for extracting polysaccharides.

Figure 1(c) presents the effect of extraction time on polysaccharide recovery using the MAE method. The results show that polysaccharide yield increased with longer extraction times, peaking at 29.8 g/100 g after 60 min. Beyond this point, further increases in extraction time led to a decline in polysaccharide content, which dropped to 23.1 g/100 g. This decrease is attributed to polysaccharide degradation due to hydrolysis, oxidation, and interactions with macromolecular proteins that result in precipitation. The study also explored the impact of the raw material to solvent ratio on polysaccharide yield. A slight increase in yield was observed when the ratio was adjusted from 1:10 to 1:20 g/mL, but the yield remained constant when the ratio was increased further to 1:30 g/mL. A significant reduction in polysaccharide yield occurred when the solid to solvent ratio exceeded 1:30 g/mL. This reduction is likely due to solute saturation in the solvent, which negatively affected mass transfer efficiency. Similar findings were reported by Zhang et al. [6] and Ren et al. [16] for polysaccharides extracted from *Lentinus edodes* and *Sargassum thunbergii*. However, in this study, the effect of varying solid to solvent ratios on polysaccharide yield was not pronounced, indicating that this factor had a limited impact on polysaccharide extraction.

The study determined that extraction temperature, microwave power, and extraction time had a more significant influence on polysaccharide yield from *A. sagittifolius*. Using a single-factor experimental design, the optimal conditions for extraction were identified as 70°C, 600 W, and 60 min. To further refine these conditions, a BBD with 17 experimental runs was employed. This RSM approach evaluated extraction temperature (X_1) ranging from 40°C to 90°C, microwave power (X_2) ranging from 400 to 800 W, and extraction time (X_3) ranging from 40 to 80 min, as outlined in Table 1.

Table 2: Results of the ANOVA for the response surface quadratic model.

Source	Sum of squares	df	Mean square	*F* value	*p* value
Model	223.25	9	24.81	65.51	< 0.0001
X_1	17.40	1	17.40	45.97	0.001
X_2	8.20	1	8.20	21.66	0.003
X_3	0.001	1	0.001	0.004	0.96*
X_1X_2	39.06	1	39.06	103.16	< 0.0001
X_1X_3	5.52	1	5.52	14.58	0.007
X_2X_3	2.25	1	2.25	5.94	0.045
X_1^2	98.63	1	98.63	260.49	< 0.0001
X_2^2	43.52	1	43.52	114.94	< 0.0001
X_3^2	0.06	1	0.056	0.15	0.73*
Lack of fit	1.62	3	0.55	2.10	0.25*
R^2	0.99		Adeq precision	24.46	
Adjusted R^2	0.97		C.V. %	2.49	
Predicted R^2	0.88				

Note: $p < 0.05$ indicates statistical significance.
Abbreviations: Adj R^2: adjusted R^2; df: degree of freedom; MS: mean square; Pred R^2: predicted R^2; R^2: coefficient of determination; SS: sum of squares.
*Stands for insignificant differences ($p < 0.05$).

3.2. Fitting Model and Respond Surface Analysis. RSM was used to optimize the parameters for polysaccharide recovery. The experiment consisted of three factors at three levels: extraction temperature (X_1), microwave power (X_2), and extraction time (X_3). In total, there were 17 runs, including five central points. Table 1 shows the polysaccharide yield responses (Y), while Table 2 compares the observed and predicted values. The highest yield obtained was 29.20 g/100 g, which was achieved at 65°C for 60 min and with 600 W (run 2).

To evaluate the predicted model's adequacy, the ANOVA results in Table 2 were analyzed. The results showed that the model was highly significant, with a p value of less than 0.001. Additionally, the high R^2 (0.99) and predicted R^2 (0.97) values, along with a low coefficient variation (C.V.) (2.49%), suggested a small difference between the experimental and predicted response values. The lack of fit for the polysaccharide recovery model was 0.25 (not significant), indicating a good fit with the experimental results. Analytical plots (Figure 2) were also used to further evaluate the model's adequacy. Figure 2(a) demonstrates a normal distribution of residuals, with most points being located on the diagonal line. In Figure 2(b), it can be seen that the prediction versus actual values for each experimental run were closely fitted to the regression line. Figure 2(c) displays randomly scattered residuals, indicating independence from the factors. Finally, Figure 2(d) confirms the homogeneous variance assumption with randomly scattered data points of internally standardized residuals plotted against predicted values. Thus, the findings indicated that the developed model was highly adequate.

Table 2 shows how the independent variables (X_1, X_2, and X_3) affect the recovery of polysaccharides from *A. sagittifolius*. The linear coefficients (X_1 and X_2), quadratic terms (X_1^2, X_2^2), and all the interaction coefficients (X_1X_2, X_1X_3, and X_2X_3) had a significant impact on polysaccharide recovery ($p < 0.05$). Meanwhile, the linear coefficient (X_3) and quadratic term (X_3^2) did not have a significant effect and were excluded from the analysis. Consequently, a second-order polynomial equation (Equation 4) was formulated. The relationship between different factors and responses was depicted using three-dimensional (3D) response surface plots in Figure 3, with red indicating the highest values and blue representing the lowest values.

$$Y = 28.58 - 1.47X_1 + 1.01X_2 - 3.13X_1X_2 - 1.18X_1X_3 - 0.75X_2X_3 - 4.84X_1^2 - 3.22X_2^2. \quad (4)$$

Figure 3(a) illustrates the impact of extraction temperature (X_1) and microwave power (X_2) on the recovery of polysaccharides while keeping treatment time (X_3) constant at 60 min. The research indicates that microwave power has a more significant impact on polysaccharide yield compared to extraction temperature. Increasing microwave power (X_2) can enhance polysaccharide content by breaking down the cell membrane and improving polysaccharide permeability into the solvent [7, 8, 23]. Conversely, the influence of extraction temperature (X_1) is less pronounced. The polysaccharide content slightly increased as X_1 increased from 40°C to 50°C at a fixed X_2 of 600 W, reaching its highest value at medium X_1 (55°C–60°C). But, as the extraction temperature increased from 60°C to 80°C, the polysaccharide content decreased due to degradation. These findings are consistent with previous studies on natural polysaccharides from black garlic and sweet tea [24, 25]. Therefore, the study suggests that the optimal conditions for obtaining maximum polysaccharide content are between 60°C–65°C and 550–600 W.

In Figure 3(b), a 3D response surface illustrates how varying extraction temperature (X_1) and MAE time (X_3)

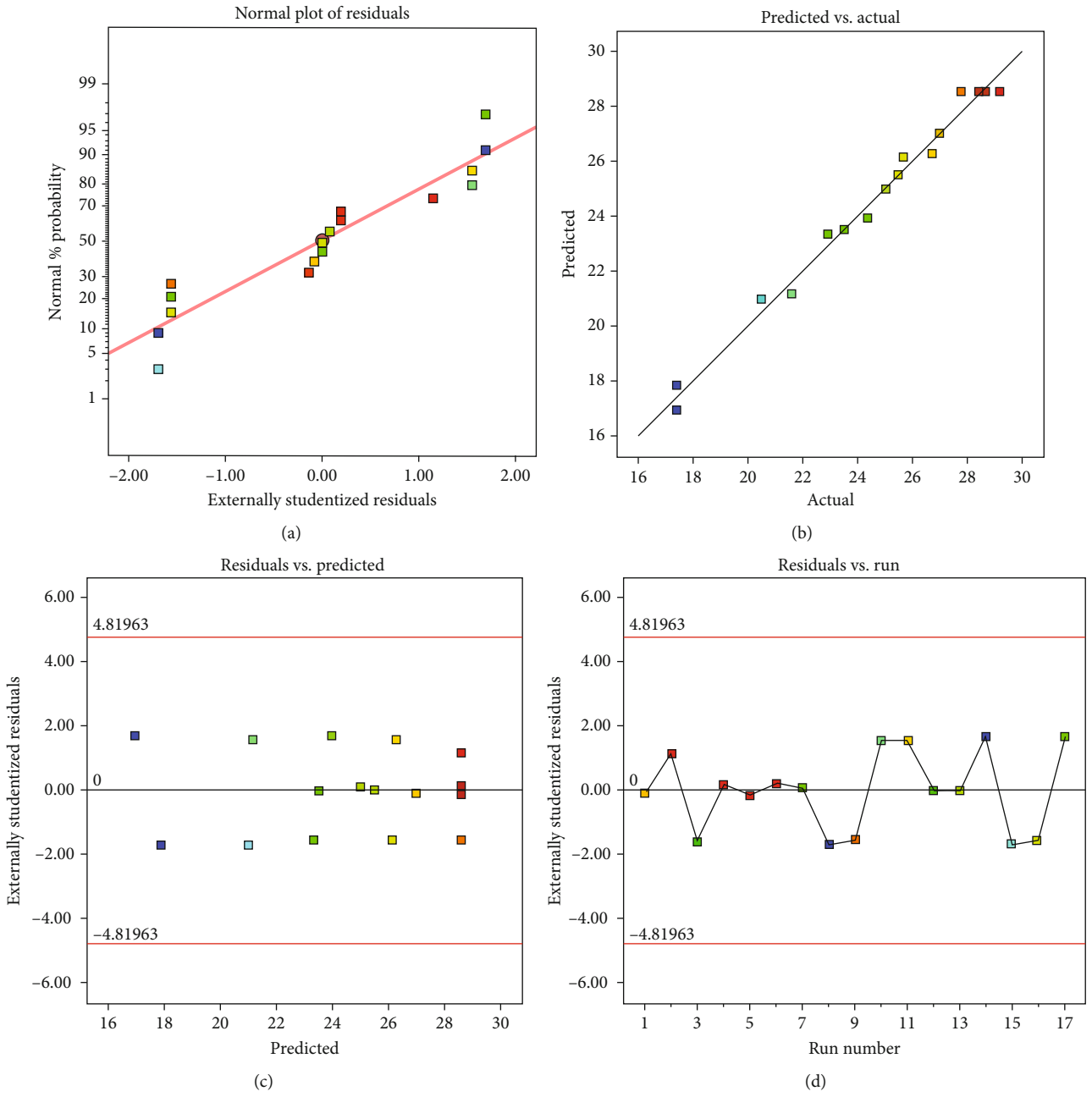
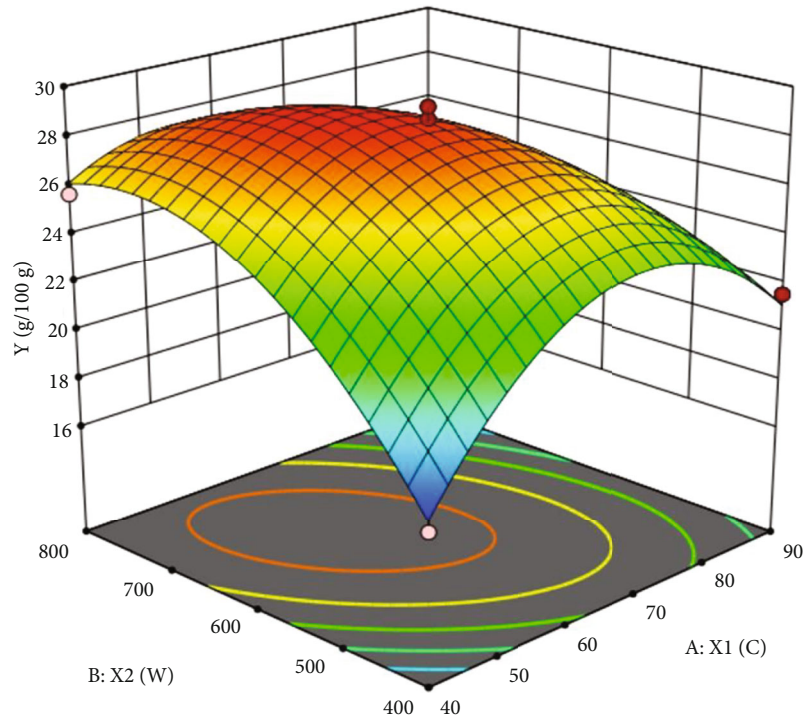


Figure 2: Analytical plots to verify the appropriateness of the model for the polysaccharide yield response: (a) normal plot, (b) predicted versus actual plot, (c) residuals versus predicted plot, and (d) residuals versus run plot.

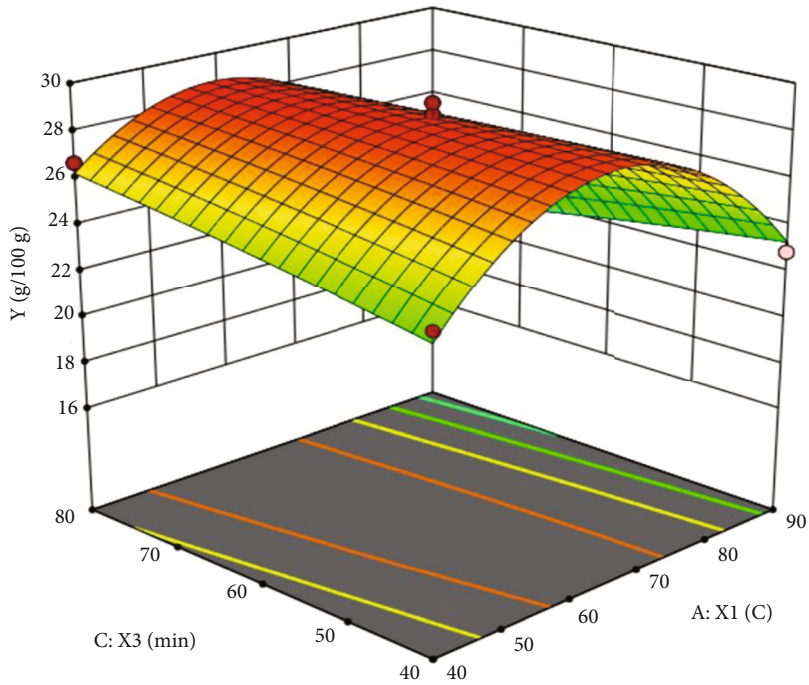
affects polysaccharide yield while maintaining microwave power (X_2) at a constant 600 W. Increasing X_1 and X_3 between 40°C–65°C and 40–60 min, respectively, improved polysaccharide production by breaking down plant cell walls at higher temperatures, aiding polysaccharide diffusion. However, exceeding the optimal range of 60°C–65°C and 55–60 min reduced polysaccharide content, highlighting the ideal conditions for maximum content between 60°C–65°C and 55–60 min. In Figure 3(c), with temperature at 65°C, the impact of X_2 on yield is significant for longer X_3 durations but insignificant for shorter ones. The highest polysaccharide content is achieved after a 60-min treatment, decreasing afterward, consistent with Xu et al.'s [8] findings that extended extraction times lower polysaccharide content in *Eucommia ulmoides* Oliver leaf.

3.3. Optimization of Extraction Condition and Bioactivities

3.3.1. Optimization of Extraction Condition. According to the RSM results, the best conditions for extracting the highest amount of polysaccharide using UAE were found to be at a temperature of 62.5°C, a duration of 57.71 min, and a microwave power of 650 W. However, for practical purposes, the operating parameters were slightly adjusted to a

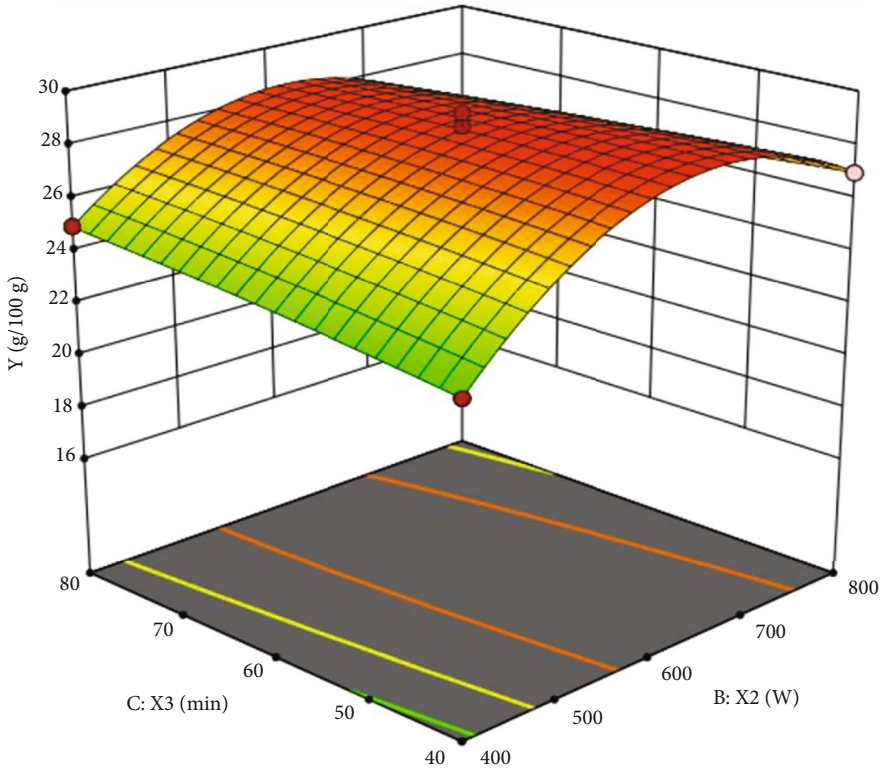


(a)



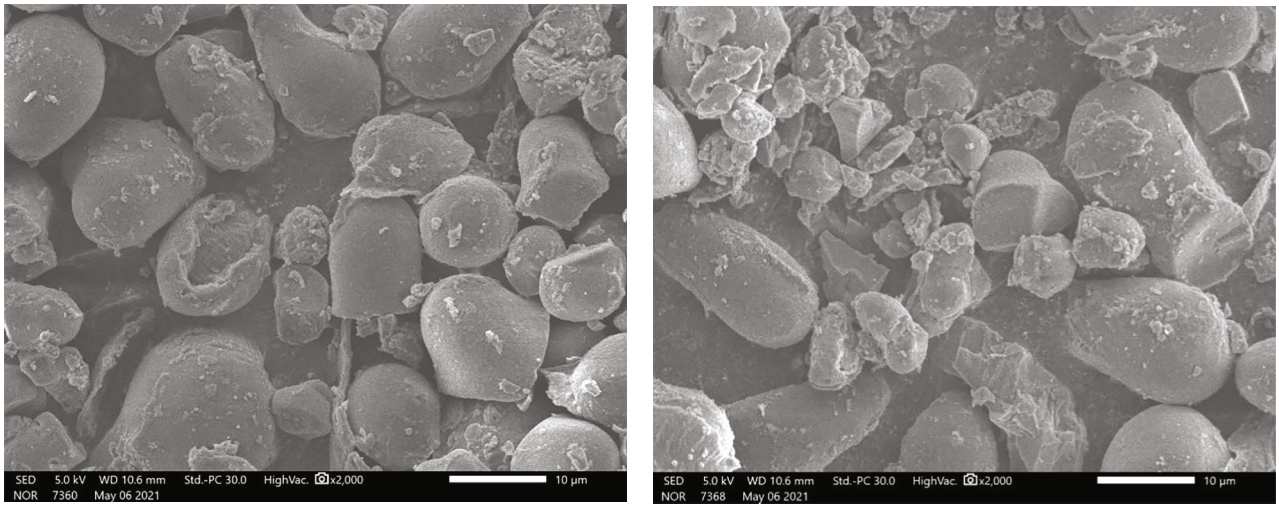
(b)

Figure 3: Continued.



(c)

Figure 3: Response surface plots (3D) for the microwave-assisted extraction of polysaccharide. (a) Extraction temperature versus microwave power. (b) Extraction time versus temperature. (c) Extraction time versus microwave power.



(a) (b)

Figure 4: SEM images of *A. sagittifolius* polysaccharides extracted with different methods: (a) HE method and (b) at optimal MAE (650 W, 63°C, 58 min).

temperature of 63°C, a microwave power of 650 W, and a duration of 58 min. This adjustment led to a polysaccharide content of 29.8 g/100 g, which was very close to the experimental yield of 30.1 g/100 g. These results indicate that the mathematical model accurately predicted the polysaccharide extraction yield.

A comparison between the MAE method and the conventional HE method for extracting polysaccharides from *A. sagittifolius* powder was conducted, as shown in Figure 4. The images revealed that all *A. sagittifolius* polysaccharides had an irregular granular shape. However, polysaccharides extracted by HE exhibited an irregular granular

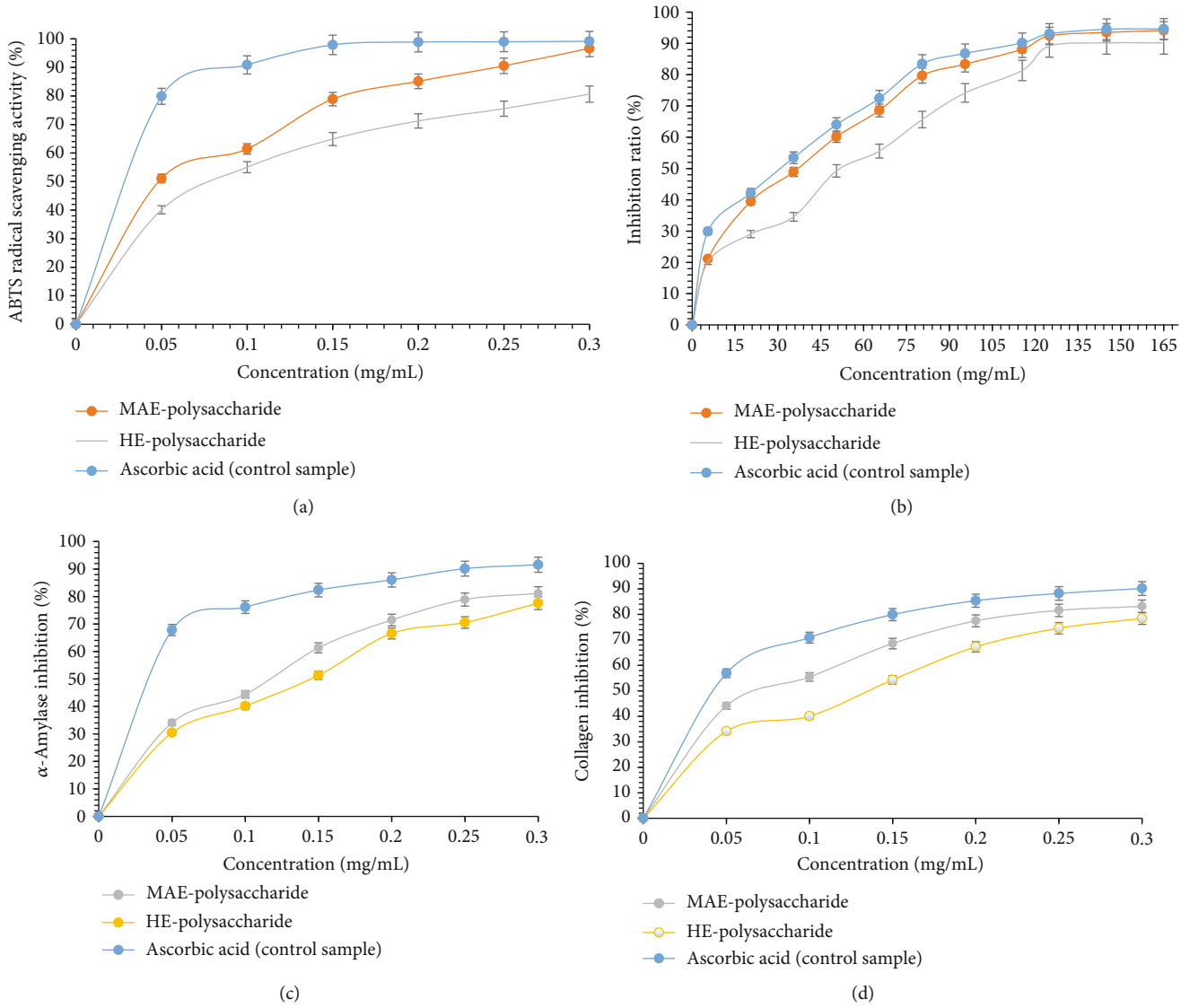


Figure 5: (a) ABTS radical scavenging activity. (b) Inhibition rate in HepG2 cell. (c) α-Amylase inhibition (%). (d) Collagen inhibition (%) of polysaccharide at different concentrations.

shape with smooth cell surfaces and less deformation compared to those extracted by MAE under optimal conditions, which displayed an irregular granular shape with numerous holes and broken cells (Figure 4(b)). This indicates that prolonged microwave irradiation and high power led to changes in the polysaccharide structure, in line with previous studies suggesting that MAE significantly affects the polysaccharide microstructure [19, 26]. Yuan et al. [26] proposed that MAE-induced changes in microstructure could enhance polysaccharide properties such as antioxidant and emulsifying activity, which is consistent with our findings in Figure 5.

3.3.2. Bioactivities. Figure 5(a) shows that MAE polysaccharides had a maximum ABTS scavenging rate of 96.67% at a concentration of 0.3 mg/mL, slightly lower than ascorbic acid. In contrast, HE polysaccharides only reached a peak ABTS scavenging rate of 80.65% at the same concentration. Moreover, *A. sagittifolius* polysaccharides, especially those extracted via MAE, significantly inhibited HepG2 cells. In Figure 5(b), polysaccharides in the concentration range of 5.5–160 μg/mL notably decreased HepG2 cell viability in both time- and concentration-dependent ways. After a 48-h treatment, MAE polysaccharide showed higher cytotoxicity towards HepG2 cells compared to HE polysaccharide, with an IC50 value of 48.36 μg/mL, similar to ascorbic acid (41.32 μg/mL). Conversely, HE polysaccharides exhibited lower cytotoxicity with an IC50 value of 62.96 μg/mL, indicating that variances in MAE and HE polysaccharide structure may impact cytotoxicity, as noted by Jia et al. in their study on *Panax ginseng Meyer* polysaccharides [19].

The study also found that polysaccharides extracted using MAE reduced α-amylase activity more than those extracted with the HE method, leading to a slower breakdown of starch. Results from Figures 5(c) and 5(d) indicated that the inhibitory effects of MAE and HE polysaccharides

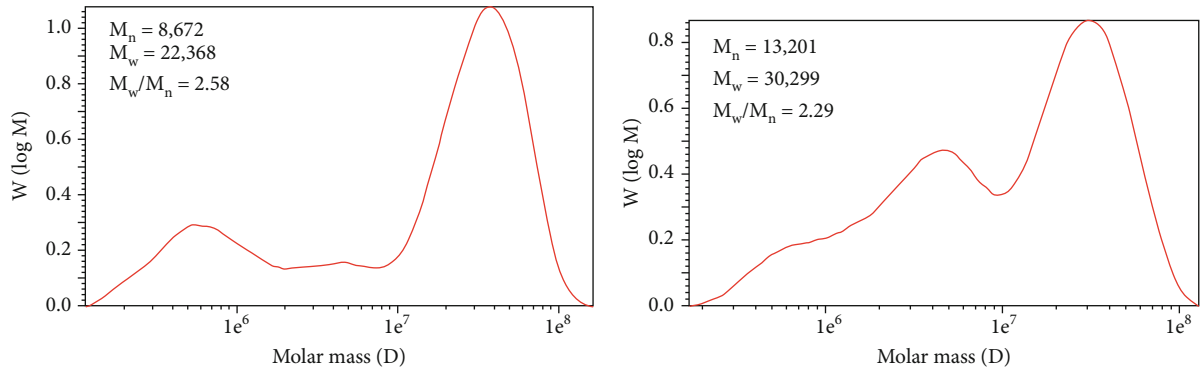


Figure 6: GPC chromatogram of (a) MAE and (b) HE polysaccharides.

on enzymes increased with higher concentrations. Specifically, at 0.3 mg/mL, MAE polysaccharide exhibited significant α-amylase inhibition, reaching 81.12%, surpassing the HE polysaccharide at the same concentration (Figure 5(c)). A similar trend was observed for collagenase inhibition by polysaccharides extracted using MAE and HE methods (Figure 5(d)). These results were statistically significant ($p < 0.05$) and performed better than results from previous studies [27, 28]. However, the α-amylase and collagenase inhibition by MAE polysaccharide were still lower than that of ascorbic acid ($p < 0.05$). Strong α-amylase inhibition can help regulate blood sugar levels by slowing down the conversion of carbohydrates to glucose and glucose absorption in the intestines [29, 30]. Maaiden et al. [29] pointed out that collagenase inhibition can assist in reducing skin damage and wrinkles. These results suggest that polysaccharides extracted using MAE can effectively decrease HepG2 cell viability, combat skin aging, and manage blood glucose levels, indicating that MAE extraction is a promising method for obtaining highly antioxidant polysaccharides from *A. sagittifolius*.

3.4. Structural Characterization of Polysaccharides

3.4.1. Molecular Weight Distribution and Constituent Monosaccharides. The study investigated the impact of MAE on the molecular structures of polysaccharides by analyzing molecular weights (Mw and Mn), polydispersity (Mw/Mn), and monosaccharide composition, in comparison to the HE method. The results from the GPC analysis (Figure 6) revealed that the average molecular weight (Mw) of polysaccharides treated with the MAE method was 22,368 g/mol, significantly lower than that of polysaccharides extracted using the HE method (30,299 g/mol). Furthermore, the polydispersity index (Mw/Mn) for MAE samples was 2.58, approximately 1.2 times higher than that of HE samples (2.29), indicating increased degradation of polysaccharides during the MAE process. These findings are consistent with previous studies on polysaccharide extraction from *Artemisia sphaerocephala* and *E. ulmoides Oliver* leaf [8, 13].

The influence of MAE treatment on the composition of polysaccharides is illustrated in Table 3. The results depicted significant differences in glucose, glucuronic acid, and galacturonic acid levels of MAE polysaccharides compared to HE polysaccharides. Conversely, no significant variations were observed in other monosaccharides such as rhamnose, xylose, galactose, arabinose, ribose, and arabinose. These changes in sugar composition result from the partial breakdown of linkages, like hydrogen bonds, between the polysaccharides and other components in the plant cell walls of *A. sagittifolius*, thereby enhancing the antioxidant and antitumor properties of the extracted polysaccharide, as depicted in Figure 5. The ABTS radical scavenging activity, α-amylase inhibition, collagenase inhibition, and cytotoxicity towards HepG2 cells by polysaccharides extracted using MAE were found to be higher than those by the HE polysaccharide. These results are in line with Zhao et al. [31], who demonstrated that reducing molecular weight and increasing glucuronic acid and galacturonic acid content in short-chain polysaccharides improve the antioxidant and antitumor properties of the extracted polysaccharide. Additionally, Shi et al. [32] observed that short-chain polysaccharides containing arabinose, glucose, fucose, and galacturonic acid in the side chain exhibit high biological activity and are more easily fermented by intestinal bacteria. Consequently, our results suggest that the MAE method utilized in this study significantly influenced the polysaccharide structure and exhibited notable in vitro activity.

The study found that glucose and galactose were the predominant sugar components in both the MAE and HE polysaccharides, accounting for 46.90% to 48.28% and 27.15% to 27.44% of the total sugar content, respectively. Smaller quantities of mannose (1.15% to 1.27%) and rhamnose (1.03% to 1.04%) were also present. The findings suggest that while the extraction methods did not alter the types of sugars extracted from *A. sagittifolius*, they did influence their quantities. However, due to the limited research on the monomeric composition of *A. sagittifolius* polysaccharides, making direct comparisons is challenging. In conclusion, microwave radiation significantly affects the molecular composition of polysaccharides extracted from *A. sagittifolius*, which in turn has a substantial impact on the antioxidant activity of the extracts.

3.4.2. Functional Properties. The examination of the technological properties of polysaccharides is essential for predicting their potential industrial applications. In this research,

Table 3: Constituent monosaccharides of MAE and HE polysaccharide.

Extraction method	MAE polysaccharide	HE polysaccharide
Polysaccharide yield (g/100 g)	29.80 ± 0.51^{a}	25.91 ± 0.62^{b}
Constituent monosaccharides (%)		
Rhamnose	1.03 ± 0.03	1.04 ± 0.01
Galacturonic acid	2.35 ± 0.05^{a}	1.68 ± 0.03^{b}
Xylose	3.74 ± 0.10	3.89 ± 0.09
Ribose	4.0 ± 0.12	4.09 ± 0.15
Glucuronic acid	2.69 ± 0.03^{a}	2.00 ± 0.04^{b}
Arabinose	10.58 ± 0.45	10.72 ± 0.33
Glucose	$46.9^{b} \pm 2.10$	$48.28^{a} \pm 2.02$
Mannose	1.27 ± 0.36	1.15 ± 0.03
Galactose	27.44 ± 1.02	27.15 ± 1.05

Note: Different superscript letters in the same row indicate statistically significant differences between treatments ($p < 0.05$). The values are mean ± SD ($n = 3$).

Table 4: Solubility (%) of polysaccharide from *A. sagittifolius* as a function of temperature.

Solvent	Extraction method	Temperature (°C)		
		25°C	40°C	55°C
Water	MAE	32.1 ± 1.10^{d}	48.02 ± 1.13^{b}	60.33 ± 2.04^{a}
	HE	30.3 ± 1.33^{d}	44.54 ± 1.30^{c}	58.30 ± 2.40^{a}
Methanol	MAE	1.10 ± 0.05^{e}	2.02 ± 0.11^{e}	2.03 ± 0.10^{e}
	HE	1.07 ± 0.02^{ef}	1.79 ± 0.21^{e}	2.11 ± 0.12^{e}
Hexane	MAE	0.51 ± 0.02^{f}	0.92 ± 0.01^{f}	1.33 ± 0.05^{ef}
	HE	0.77 ± 0.11^{f}	0.92 ± 0.04^{f}	1.30 ± 0.05^{e}

Note: Different superscript letters indicate statistically significant differences among temperature treatment ($p < 0.05$). The values are mean ± SD ($n = 3$).

Table 5: Oil-holding capacity (OHC) and water-holding capacity (WHC) from *A. sagittifolius* as a function of temperature.

	Extraction method	Temperature (°C)		
		25°C	40°C	55°C
OHC (g oil/g polysaccharide)	MAE	$3.58^{b} \pm 0.13$	$6.01^{a} \pm 0.26$	$6.89^{a} \pm 0.45$
	HE	$4.14^{b} \pm 0.21$	$6.45^{a} \pm 0.21$	$6.15^{a} \pm 0.45$
WHC (g water/g polysaccharide)	MAE	$24.81^{c} \pm 0.23$	$28.14^{b} \pm 0.27$	$32.79^{a} \pm 0.16$
	HE	$25.42^{b} \pm 0.52$	$27.54^{a} \pm 0.27$	$27.50^{a} \pm 0.23$

Note: Different superscript letters in the same row indicate statistically significant differences between treatments ($p < 0.05$). The values are mean ± SD ($n = 3$).

we assessed the solubility of polysaccharides in various solvents at different extraction temperatures (25°C, 40°C, and 55°C) and present the results in Table 4. The study revealed that with increasing temperature, the solubility of polysaccharides in distilled water also notably increased. Furthermore, the solubility of MAE and HE polysaccharides was lower in hexane and methanol compared to distilled water at the same temperature. There was no significant difference in solubility between hexane and methanol, indicating that polysaccharides extracted from *A. sagittifolius* were highly polar. Our investigation demonstrated that the MAE polysaccharide exhibited higher water solubility compared to the HE polysaccharide ($p < 0.05$), a favorable attribute in food processing for enhancing texture, flavor, and overall quality [6, 7, 10].

The study also indicated a similar trend for WHC and OHC, where an increase in extraction temperature from 25°C to 55°C led to elevated WHC and OHC, as shown in Table 5. This could be attributed to enhanced molecular motion at higher temperatures, facilitating water absorption and increasing the amount of water and oil retained by the sample [18, 33]. The OHC values of MAE and HE polysaccharides in this study ranged from 3.5 to 7 g oil/g polysaccharide, akin to guar and xanthan gum (4–6 g oil/g fiber),

suggesting that polysaccharides from *A. sagittifolius* could be beneficial for promoting emulsification and stabilization in food and pharmaceutical products.

4. Conclusion

This study has demonstrated that MAE is an efficient method to increase the yield of polysaccharides extracted from *A. sagittifolius*. The use of RSM based on the BBD has successfully determined the optimal conditions for polysaccharide extraction. The experimental and predicted data regarding the extraction yield did not significantly differ. Based on contour plots and variance analysis, the optimum conditions for MAE polysaccharide extraction from *A. sagittifolius* were a temperature of 63°C, a time of 58 min, and a microwave power of 650 W. The average yield was 29.8 g/100 g, which closely matched the experimental yield of 30.1 g/100 g. The results showed that MAE offered substantial advantages over traditional extraction methods, particularly in terms of superior extraction efficacy and antioxidant activity of the extract, with the shortest extraction time. The MAE polysaccharide had a lower moisture content of 1.20%, ash content of 5.90%, and a dry mass basis of 3.45%–4.23% than the polysaccharides extracted using traditional extraction methods. The MAE polysaccharides were mainly composed of glucose (46.9%) and galactose (27.16%), followed by arabinose (10.58%), and a small number of other sugar components. The optimized MAE process resulted in polysaccharides with a low molecular weight (Mw 2236 g/mol) and high polydispersity (Mw/Mn 2.58) than traditional extraction methods (HE) (3029 g/mol and 2.19, respectively), leading to high solubility, OHC, and WHC. These results offer great potential for food processing. Therefore, the polysaccharide extracted from *A. sagittifolius* showed physicochemical characteristics, antioxidant activity, and functional properties that allow it to compete with commercial hydrocolloids in various fields.

Data Availability Statement

The authors have confirmed that the data supporting this study can be found in the article and its supporting materials. The raw data that back up the findings of this study can be obtained from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

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