Research article

Study on extraction and antibacterial activity of aucubin from *Eucommia ulmoides* seed-draff waste biomass

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A B S T R A C T

Aucubin (AU) is an active ingredient exerting strong antioxidant and anti-inflammatory effects in treating several diseases. This study evaluated the extraction of AU from *Eucommia ulmoides* seed-draff (EUSD) waste biomass using a series of solvents (methanol, ethanol, *i*-propanol, *n*-propanol, *n*-butanol, *n*-pentanol and cyclohexane) assisted with microwave and ultrasound, and proposed the optimized method for extraction. Five factors were investigated by Box-Behnken design (BBD) and response surface methodology (RSM). The optimized extraction conditions were as follows: liquid-solid ratio of 46.37 mL/g, methanol percentage of 89.56%, ultrasonic (extraction) time of 59.95 min, microwave power of 306.73 W, and microwave (extraction) time of 18.93 s. To this end, the AU extraction reached the maximum value (149.1 mg/g), which was consistent with the theoretical value (149.3 mg/g). Furthermore, the kinetics of extraction process were investigated by mathematic modeling. The extraction process analysis was also explored by ^{1}H nuclear magnetic resonance (^{1}H-NMR) spectroscopy, Fourier transform infrared (FTIR) spectroscopy and COSMOtherm program. This study found out that methanol provided better extraction efficiency than the conventional solvents (water, ethanol, *i*-propanol, *n*-propanol, *n*-butanol, *n*-pentanol, cyclohexane) due to possible interactions by the formation of hydrogen bond between AU and methanol, and ultrasound and microwave could significantly enhance mass transfer, which exhibited higher extraction efficiency and lower energy consumptions (149.1 mg/g and 0.102 kW·h *vs.* 73.4 mg/g and 0.700 kW·h for Soxhlet extraction). In the antibacterial activity study, the AU extract exerted strong antibacterial ability against 4 tested pathogens, and the antibacterial effect followed the order of: *Staphylococcus aureus* (35.9 ± 1.32 mm) > *Escherichia coli* (30.7 ± 1.38 mm) > *Bacillus subtilis* (20.5 ± 1.36 mm) > *Salmonella* (15.9 ± 1.39 mm) with the AU concentration of 40 mg/mL. Therefore, the development of this study will help to deepen the further understanding of natural product extraction by methanol-based ultrasonic and microwave, and has certain application value for the development and utilization of natural iridoid glycosides product.

1. Introduction

Eucommia ulmoides (EU) is widely cultivated in China, with an annual output of 300,000 tons. It can be used as a tonic food and herb in traditional Chinese medicine to strengthen the human immune and internal systems [1, 2, 3], such as *E. ulmoides* tea and wine, *E. ulmoides* capsules, *E. ulmoides* slices, *E. ulmoides* oil, feed additives, *etc*. *E. ulmoides* seed-draff (EUSD) waste biomass is the oily residue of the *E. ulmoides* seeds (EUS) after oil expression, which can be extracted as aucubin (AU) as a high-value natural product instead of abandoning it. AU is a C$_4$ desmethyl-cycloalkenyl terpene compound (Figure 1) [4,5], which is

distributed in various parts of EU and has many pharmacological activities, such as liver protection and detoxification, anti-inflammatory, anti-oxidation, anti-aging, anti-osteoporosis and anti-tumor [3, 6]. Since AU is unstable in nature and easy to be oxidatively decomposed, high temperature and long heating time are not conducive to its extraction [7]. Hence, solid-liquid extraction of AU is usually conducted by immersion in a water-based extractant [8]. Nonetheless, this traditional technique requires a long extraction time, a large amount of extractant, and cannot obtain the optimal extraction yield [9]. The solvent is the key factor for an efficient extraction of natural product, because it promotes solvent contact with the target substance [10, 11]. Previous studies on
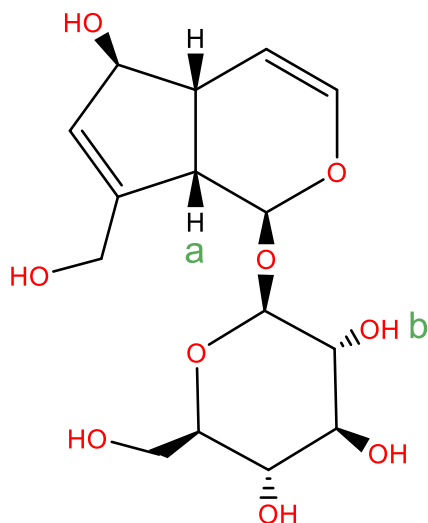

Figure 1. The molecular structure of AU.

AU extraction from EUS or EUSD have proved that a high concentration of organic solvents was effective because of the low aqueous solubility of AU [12, 13, 14]. Ultrasound technology can destroy the cell walls of plant cells while allowing the release of bioactive compounds to the solvent [15, 16]. Therefore, ultrasound can be used as pretreatment strategy to permeate the cell walls or as an extraction technique. The use of ultrasound has been shown to be effective against target substances such as polyphenols in plant extraction and is considered an alternative to conventional extraction methods. It has been reported that ultrasound can extract a large number of bioactive substances from plants such as phenolic compounds [17, 18, 19, 20, 21], polysaccharide [22, 23], carotenoid [15, 24], and essential oils [25, 26]. Besides, microwave can promote the dissolution of effective components in plant cells as it is more uniform and efficient than traditional heating methods, easy to operate, and could save time and energy [27, 28, 29, 30]. Moreover, it was found that the efficiencies of microwave are higher compared to ultrasound which can be explained by a higher rate of cellular structure rupture due higher frequency level of microwave as compared to ultrasound [21, 31, 32]. To date, to the best of our knowledge, there is no detailed research work on the extraction of AU from EUSD waste biomass with regards to potential reuse value.

This paper presented an effective and eco-friendly extraction process of AU. A series of solvents (methanol, ethanol, *i*-propanol, *n*-propanol, *n*-butanol, *n*-pentanol, cyclohexane) for the AU extraction from EUSD were proposed. The effects of extraction conditions (liquid-solid ratio of 10–60 mL/g, solvent percentage of 50–100%, ultrasonic time of 0–100 min, microwave power of 100–600 W and microwave time of 0–50 s) were studied, and these variables were optimized by response surface methodology (RSM) to maximize extraction efficiency. The physical and structural effects of ultrasound and microwave on cell and mass transfer phenomenon were analyzed by scanning electron microscopy (SEM), ^{1}H nuclear magnetic resonance (^{1}H-NMR) spectroscopy, Fourier transform infrared (FTIR) spectroscopy and mathematic modeling. The proposed method was compared with conventional extraction approaches, which has broad prospects in extracting natural products for further scale-up.

2. Material and methods

2.1. Raw material and chemicals

EUSD were supplied by Jishou University (Jishou, China). Dried EUSD were grinded and sieved with the range of 200–300 μm, then kept in a vacuum dryer before use. AU primary reference standard was purchased from Sigma-Aldrich (Shanghai, China). The extraction solvents

(methanol, ethanol, *i*-propanol, *n*-propanol, *n*-butanol, *n*-pentanol, cyclohexane) were purchased from Fuchen reagent (Tianjin, China) and used without further purification. Deionized water was freshly used during all experiment.

2.2. Preparation of standard solution

A 25 mg of AU standard was sonicated with methanol and fixed in a 5 mL volumetric flask to obtain the standard solutions with a concentration of 5 mg/mL. This solution was diluted to 4.2, 3.4, 2.6, 1.8, 1.0 and 0.2 mg/mL respectively, then was filtered through a 0.22 μm microporous membrane (Jinteng Co. Ltd., Tianjin, China) respectively, and finally was taken as a standard solution.

2.3. Selection of optimal solvent for the recovery of aucubin

A 10 g of EUSD was accurately weighed and transferred into a flask, then the extractants (methanol, ethanol, *i*-propanol, *n*-propanol, *n*-butanol, *n*-pentanol, cyclohexane) were added under the same conditions (liquid-solid ratio of 30 mL/g, ultrasonic power of 100 W, ultrasonic time of 20 min, microwave power of 200 W and microwave time of 20 s). Afterwards, the flask was place in the apparatus chamber, connected to condensing tubes, and the controls are set according to the experimental design. Microwave-Assisted, ultrasound-Assisted and microwave-ultrasound assisted extraction treatments were both performed on a microwave ultrasonic combined synthetic extractor (XH-300A, Xianghu Technology, China) equipped with the Titanium alloy probe and three control systems of microwave, ultrasonic and microwave-ultrasonic co-ordination. The extracted solution was filtered through a 0.22 μm microporous membrane (Jinteng Co. Ltd., Tianjin, China) respectively and finally taken as a test solution.

2.4. HPLC analysis and quantification

The test and standard solution were analyzed by Shimadzu LC-20A chromatographic system equipped with a Shimadzu LC-20AB binary pump, a DGU-20A 3 degasser, a SIL-20A auto sampler, a SPD-20A UV detector and an Amethyst C18-H (4.6 mm × 250 mm, 5 μm) series HPLC column (Sepax Technologies). An isocratic elution of 8% methanol-water solution as mobile phase was used and run time was 20 min. The conditions were: flow rate of 1.0 mL/min; column temperature of 298.15 K; detection wavelength of 206 nm, injection volume of 10 μL. Each sample was done with three replicates to ensure accuracy.

2.5. Optimization of methanol-based ultrasonic and microwave assisted extraction by response surface methodology (RSM)

Methanol was chosen as the suitable solvent in the above selection of optimal solvent for the recovery of aucubin. To get a better understanding of the interaction among the factors (liquid-solid ratio, solvent percentage, ultrasonic time, microwave power and microwave time), the optimization of operating conditions using response surface methodology (RSM) was carried out. The bounds of the factors were 30–50 mL/g for liquid-solid ratio, 80–100% for solvent percentage (methanol-water ratio), 40–80 min for ultrasonic time, 200–400 W of microwave power and 10–30 s for microwave time. Design-Expert 12.0.3 software was used to design experiments according to the Box-Behnken center experiment combination. The specific factor level design is shown in Supplementary Table S1.

The interactions between the factors were analyzed by using Design-Expert 12.0.3 software, the final equation in terms of coded factors were:

AU amount $= 147.67 - 0.67A + 5.09B + 0.48C + 0.50D + 0.44E - 0.40AB + 2.50AC + 0.80AD + 0.22AE - 0.65BC - 0.18BD - 2.25BE + 0.20CD + 0.12CE - 0.08DE - 10.42A^2 - 4.19B^2 - 7.53C^2 - 2.68D^2 - 4.69E^2$

Table 1. Extraction of AU with different solvents and the textural parameters of the sample before and after extraction*.

Solvent	Boiling point (K)	Solvent polarity	AU amount (mg/g)**	S_{BET} (m²/g)		V_{pore} (cm³/g)	
				Before extraction	After extraction	Before extraction	After extraction
Water	373.15	10.2	93.76 ± 1.45	1.32	6.53	0.08	0.09
Methanol	337.66	6.6	130.65 ± 1.23	1.32	25.65	0.08	0.26
Ethanol	351.47	4.3	112.84 ± 1.31	1.32	19.40	0.08	0.20
i-propanol	355.55	4.3	106.15 ± 0.89	1.32	16.83	0.08	0.18
n-propanol	370.35	4.0	103.19 ± 0.92	1.32	15.46	0.08	0.15
n-butanol	390.85	3.7	98.41 ± 1.25	1.32	13.01	0.08	0.13
n-pentanol	411.15	0.0	96.26 ± 1.23	1.32	10.88	0.08	0.11
Cyclohexane	353.87	0.1	105.89 ± 1.52	1.32	16.13	0.08	0.16

* Extraction conditions: liquid-solid ratio of 30 mL/g, solvent percentage of 90%, ultrasonic time of 20 min, microwave power of 200 W and microwave time of 20 s.
** The AU amount (mg/g) is expressed based on mg of AU equivalent per gram of raw material (mg AU/g raw material) and the results are expressed as mean ± standard deviation (n = 3).

where A represents the methanol percentage; B represents the liquid-solid ratio, C represents ultrasonic time, D represents the microwave power, E represents the microwave time, respectively.

2.6. Extraction kinetic models

The extraction efficiency of natural product components depends on the control step rate of the extraction process. Therefore, exploring its characteristic kinetic and determining the kinetic model is beneficial to study the factors of mass transfer process and transfer rate, and to find reasonable methods to control and adjust these factors, thereby improving the extraction rate of active ingredients of natural products.

Since the extraction process is a mass transfer process in the research on the extraction kinetic models of AU, we employed four main kinetic models (first-order kinetic model, Fick's second law kinetic model, second-order kinetic model, and So-Macdonald model) to fit the experimental values (Eqs. (1), (2), (3), and (4)). The degree of correlation between the predicted and experimental values was also investigated.

First-order kinetic model equation:

$$\ln[C_e/(C_e - C_t)] = k_{obs} \cdot t \tag{1}$$

where k_{obs} (min⁻¹ or s⁻¹) represents the apparent rate constant; C_t (mg/mL) and C_e (mg/mL) represents the concentration of AU in solution at time t and equilibrium, respectively; and t (min or s) represents the extraction time.

Fick's second law kinetic model equation:

$$\ln[C_e/(C_e - C_t)] = k_{obs} \cdot t + \ln(\pi^2/8) \tag{2}$$
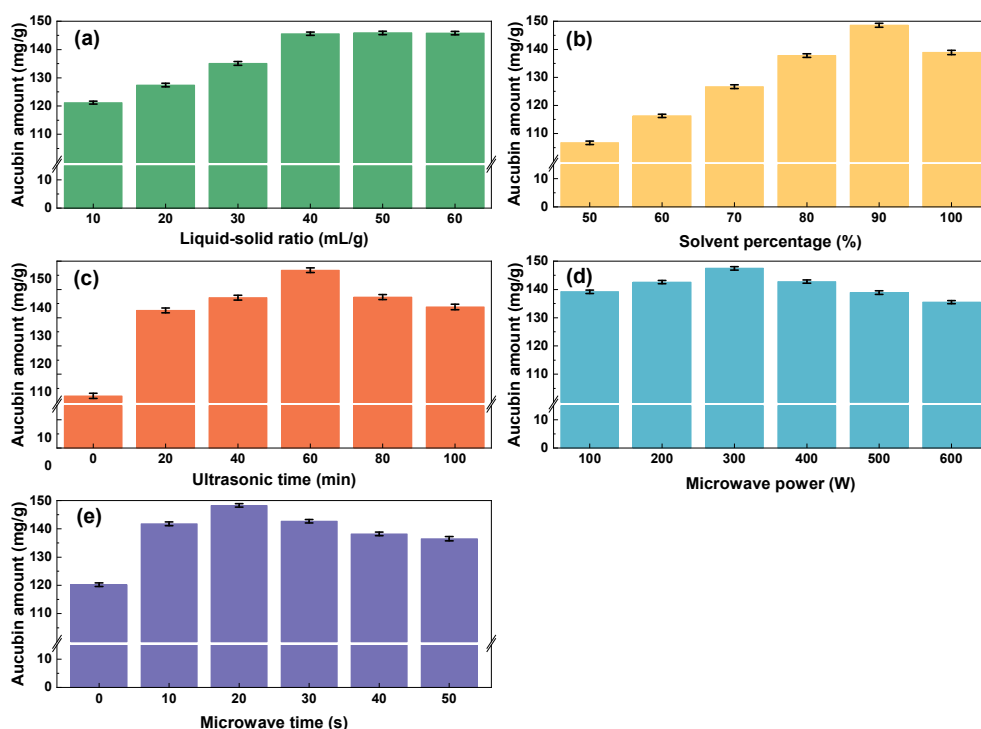
Second-order kinetic model:

$$C_t = C \cdot k_2 \cdot t/(1 + C_e \cdot k_2 \cdot t) \tag{3}$$

where k_2 (min⁻¹ or s⁻¹) represents the second-order kinetic rate constant.

So-Macdonald model:

$$C_t = C_w\ e\cdot[1 - \exp(-k_w \cdot t)] + C_{d1}\ e\cdot[1 - \exp(-k_{d1} \cdot t)] + C_{d2}\ e\cdot[1 - \exp(-k_{d2} \cdot t)] \tag{4}$$

where k_w (min⁻¹ or s⁻¹), k_{d1} (min⁻¹ or s⁻¹), and k_{d2} (min⁻¹ or s⁻¹) represent the extraction rate constants in washing, fast diffusion and slow

Figure 2. Single-factor investigation of the extraction process (a: methanol percentage of 90%, ultrasonic power of 100 W, ultrasonic time of 60 min, microwave power of 300 W and microwave time of 20 s; b: liquid (methanol)-solid ratio of 40 mL/g, ultrasonic power of 100 W, ultrasonic time of 60 min, microwave power of 300 W and microwave time of 20 s; c: liquid (methanol)-solid ratio of 40 mL/g, methanol percentage of 90%, ultrasonic power of 100 W, microwave power of 300 W and microwave time of 20 s; d: liquid (methanol)-solid ratio of 40 mL/g, methanol percentage of 90%, ultrasonic power of 100 W, ultrasonic time of 60 min, and microwave time of 20 s. e: liquid (methanol)-solid ratio of 40 mL/g, methanol percentage of 90%, ultrasonic power of 100 W, ultrasonic time of 60 min and microwave power of 300 W).

Table 2. Significance analysis of regression coefficient.

Source	Sum of squares	df	Mean square	F-Value	P-value	Prob > F
Model	1665.25	20	83.26	130.04	<0.0001	Significant
A-Methanol percentage	7.16	1	7.16	11.18	0.0026	
B-Liquid-solid ratio	415.14	1	415.14	648.35	<0.0001	
C-Ultrasonic time	3.71	1	3.71	5.79	0.0239	
D-Microwave power	4	1	4	6.25	0.0194	
E-Microwave time	3.15	1	3.15	4.92	0.0359	
AB	0.64	1	0.64	0.99	0.3270	
AC	25	1	25	39.04	<0.0001	
AD	2.56	1	2.56	3.99	0.0565	
AE	0.20	1	0.20	0.32	0.5789	
BC	1.69	1	1.69	2.64	0.1169	
BD	0.12	1	0.12	0.19	0.6656	
BE	20.25	1	20.25	31.63	<0.0001	
CD	0.16	1	0.16	0.25	0.6215	
CE	0.06	1	0.06	0.10	0.7573	
DE	0.02	1	0.02	0.04	0.8528	
A^2	948.49	1	948.49	1481.31	<0.0001	
B^2	153.34	1	153.34	239.48	<0.0001	
C^2	494.19	1	494.19	771.81	<0.0001	
D^2	62.84	1	62.84	98.14	<0.0001	
E^2	192.11	1	192.11	300.02	<0.0001	
Residual	16.01	25	0.64			
Lack of Fit	15.07	20	0.75	4.04	0.0639	Not significant
Pure Error	0.93	5	0.18			
Cor Total	1681.26	45				

diffusion stages, respectively; and Cw e (mg/mL), Cd1 e (mg/mL), and Cd2 e (mg/mL) represent the hypothetical concentration of AU at equilibrium in washing, fast diffusion and slow diffusion stages, respectively.

2.7. Data analysis and model evaluation

The reliability of the model was assessed using the correlation coefficient squared (R^2), residual sum of squares (RSS), and chi-square (χ^2). OriginPro 2021 software was used for calculated and analysis of R^2, RSS and χ^2.

2.8. Characterization of EUSD before and after extraction

The Brunauer-Emmett-Teller (BET) surface areas and pore volumes of samples were obtained by measuring the nitrogen adsorption/desorption isotherms at 77.3 K with a Micromeritics TriStar II 3020 analyzer. The samples were degassed at 373.15 K for 12 h prior to measurement.

The SEM images were collected on a Zeiss EVOMA10 scanning electron microscope at 10 kV.

2.9. ^{1}H-NMR and FTIR analysis of AU/methanol solution

^{1}H-NMR measurements were performed by a Bruker Avance NEO 600 spectrometer. The methanol or AU/methanol solutions were loaded in a flame-sealed capillary, and the spectra were collected at 298 K using deuterium oxide (D_2O) as solvent.

FTIR spectroscopy were collected on a Nicolet Nexus 670 FTIR spectrometer operated at a spectral resolution of 2 cm^{-1}. Neat methanol or the AU/methanol solution was spread on the surface of a potassium

bromide (KBr) tablet, and the standard AU was ground with KBr powder to make a tablet for testing.

2.10. Antibacterial activity test

The antibacterial activity of AU extract against 4 pathogens (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Salmonella*) was evaluated according to the diameter of the inhibition zone measured by the experiment. The detailed process was as follows: 100 μL of bacterial suspension was poured into Luria-Bertani (LB) solid medium (Haibo, Qingdao, China) and spread evenly with a spreader under aseptic conditions. Then, the solid medium was punched by a hole puncher. 0.2 mL AU extract with the different concentration (10, 20, 30 and 40 mg/mL) was drawing into the hole, and sterile water was used as a blank control. The solid medium was cultured at 310.15 K for 24 h, and the diameter of the inhibition zone was measured 3 times and averaged.

3. Results and discussion

3.1. Optimal solvents for AU extraction

Different solvents were tested under similar conditions for AU extraction experiment to find the most suitable one, and the results were summarized in Table 1. It was found that increment of AU amount coincided with growing S_{BET} and V_{pore} after extraction, and the methanol was the most effective solvent for AU extraction with an extraction amount of 130.65 mg/g. Therefore, methanol was chosen as the solvent for further study.

3.2. Single-factor investigation of the extraction process

The liquid (methanol)-solid ratio is related to the contact area between the liquid and solid, thus affecting the extraction efficiency. Therefore, the effect of liquid-solid ratio (methanol/EUSD) on the extraction efficiency of AU was studied, and the result was summarized in Figure 2a. It can be found that the concentration of AU increased in the range of 10–40 mL/g and remained constant around 145.8 mg/g. As the content of solvent increases, the dispersion of the EUSD in solvent increases, which promotes the mass transfer between the internal structure and surfaces. Therefore, considering the extraction results and the amount of solvent, the optimized liquid to material ratio is 40 mL/g.

Figure 2b shows the effect of methanol percentage on AU extraction. With the increase of methanol volume fraction, the extraction concentration of AU was significantly increased. When the volume fraction of methanol was 90%, the mass concentration of AU was the highest. Considering the extraction efficiency, the extracted methanol percentage is selected as 90%.

As shown in Figure 2c, the ultrasonic time extended from 10 min to 60 min, and the extraction rate of AU was significantly improved. The extraction amount reached the maximum (146.8 mg/g) at 60 min. With the prolongation of ultrasonic time, the extraction component gradually dissolved through the continuous action of ultrasonic cavitation and mechanical vibration, and the extraction rate gradually increased. The

Table 3. Credibility analysis of regression coefficient.

Index mark	Value
Std. Dev.	0.80
Mean	137.4
C.V. %	0.5824
PRESS	61.641
R^2	0.9905
Adjusted R^2	0.9829
Predicted R^2	0.9633
Adeq Precision	39.951
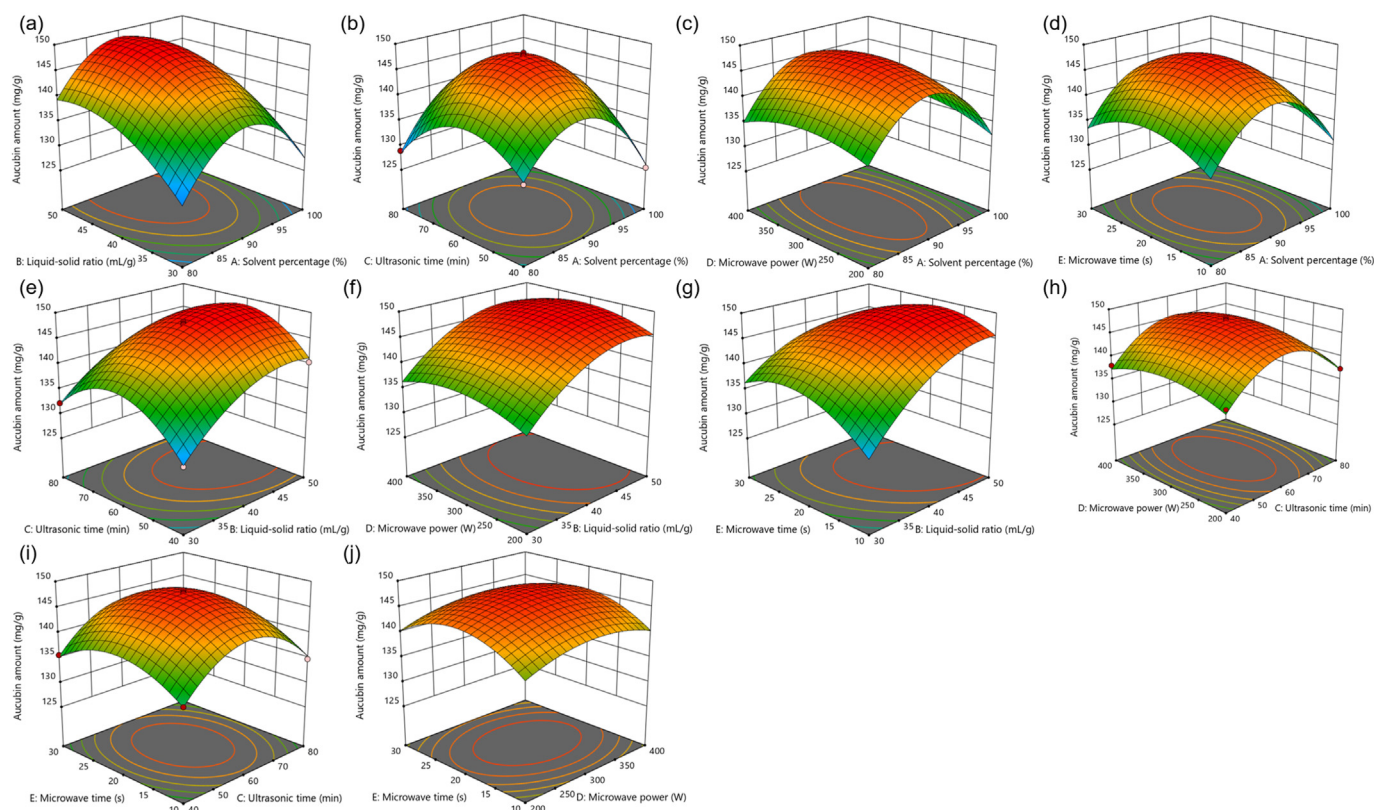
Figure 3. Response surface for the interactions of independent variables on AU amount (a: methanol percentage and liquid-solid ratio; b: methanol percentage and ultrasonic time; c: methanol percentage and microwave power; d: methanol percentage and microwave time; e: liquid-solid ratio and ultrasonic time; f: liquid-solid ratio and microwave power; g: liquid-solid ratio and microwave time; h: ultrasonic time and microwave power; i: ultrasonic time and microwave time; j: microwave power and microwave time).

excessive ultrasonic time led to the rupture of many cells in the EUSD, and the dissolution of other impurities increased. Therefore, the ultrasonic time is selected for 60 min. After prolonging the extraction time, the AU extraction rate gradually decreased. This may be due to AU possessed an enol-type acetal structure [14], and the extraction time is too long to cause oxidation [33].

Microwave power is an important factor for effective mass transfer. Six steps of regulation in power control were analyzed, and the result was presented in Figure 2d. The AU amount increased until 300 W and then decreased from 400 W onwards. The possible reason is that the thermal effect increases with the increase of microwave power, which accelerates the dissolution of AU in the cells and increases the extraction amount. When the microwave power was greater than 300 W, the strong thermal effect generated by the microwave would lead to the oxidation and decomposition of AU [34], and the extraction amount would also be decreased.

As shown in Figure 2e, the AU amount increased with the microwave time increased, reached the maximum at 20 s. Then, a further increase in microwave time led to a decrease in AU amount, since the continuous microwave increased the heating time of the AU, which eventually led to decomposition of AU [35].

### 3.3. Optimization of extraction conditions by response surface methodology (RSM)

3.3.1. Analysis of quadratic multiple regression model

It can be seen from Table 2 that the model P < 0.0001, indicating that the regression model is extremely significant. The missing term (P-0.0639 > 0.05) was not significant, suggested that the experimental design was reliable, and the coefficient R^2-0.9905 (Table 3) was determined, indicating that the model fits well with the test and can be applied to analysis and predict the extraction process of AU.

The determination coefficient $R^2_{adj} = 0.9829$ indicates that the model can better reflect the relationship between the methanol percentage, liquid-solid ratio, ultrasonic time, microwave power and microwave time. The response surface map (Figures 3a–j) visually show the relationship between the impact factors. The steeper the response surface graph is, the more obvious the interaction is. Hence, the smooth graph indicates that the interaction was weak. From the analysis of the F value (Table 2), the order of the effect on the AU amount in the selected range of factors are: liquid-solid ratio (F-648.35), methanol percentage (F-11.18), microwave power

Table 4. Extraction of AU with different method.

Source	Solvent	Technical parameters	AU amount	References
Bark of EU	Ethanol	Enzymatic hydrolysis, 326.15 K, 8 h	22.4 mg/g	[36]
EUS	Methanol	Reflux, 40 min	29.0 mg/g	[37]
EUS	Ethanol	Ultrasonic power of 300 W, 20.5 min	59.1 mg/g	[38]
EUS	Ethanol	Supercritical CO_2, 328.15 K; 26 MPa, 2 h	11.9 mg/g	[39]
EUSD	Ethanol	Ultrasonic power of 160 W, 80 min	52.6 mg/g	[40]
EUSD	Methanol	Soxhlet extraction, 333.15 K, 3 h	73.4 mg/g	This work
EUSD	Methanol	Ultrasonic power of 100 W, 1h	120.2 mg/g	This work
EUSD	Methanol	Microwave power of 300 W, 20 s	102.3 mg/g	This work
EUSD	Methanol	Ultrasonic power of 100 W, 1 h microwave power of 300 W, 20 s	149.1 mg/g	This work
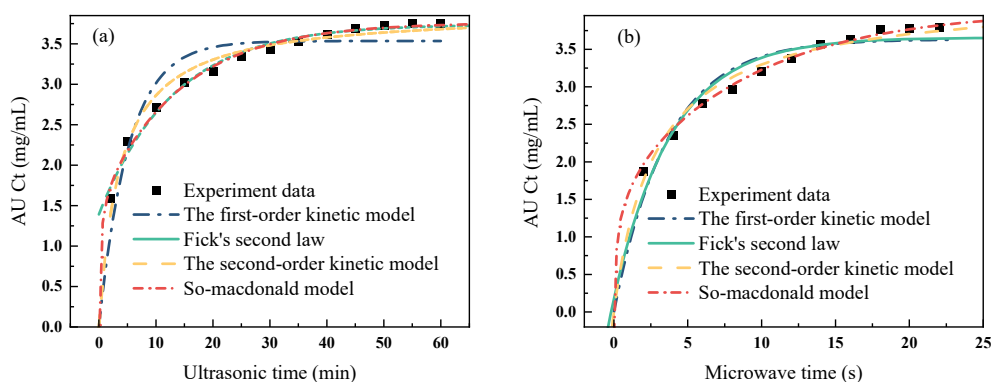
Figure 4. Comparison of four kinetic models (a: methanol percentage of 90%, liquid (methanol) -solid ratio of 40 mL/g, ultrasonic power of 100 W; b: methanol percentage of 90%, liquid (methanol) -solid ratio of 40 mL/g, microwave power of 300 W).

Table 5. Modeling results of experimental data.

| Model	Ultrasound			Microwave		
	R^2	RSS	Chi-square	R^2	RSS	Chi-square
First-order kinetic model	0.9715	0.3927	0.0836	0.8909	0.7090	0.1556
Fick's second law	0.9722	0.3613	0.2462	0.9860	0.0723	0.0157
Second-order kinetic model	0.9913	0.1144	0.0232	0.9755	0.1368	0.0286
So-Macdonald model	0.9972	0.0360	0.0061	0.9897	0.0531	0.0119

(F-6.25), ultrasonic time (F-5.79) and microwave time (F-4.92). According to the P value in Table 2, the influence of the primary term B, the interaction term AC, BE, and the quadratic secondary term A^2, B^2, C^2, D^2, E^2 on extraction amount is extremely significant.

3.3.2. Determination and verification of optimal condition

The maximum response value (149.30 mg/g) was obtained by the response surface methodology under the optimal conditions (liquid-solid ratio of 46.37 mL/g, methanol percentage of 89.56%, ultrasonic time of 59.95 min, microwave power of 306.73 W and microwave time of 18.93 s). In order to test the reliability of the results obtained by the response surface methodology, the verification test was carried out, and the highest AU amount was 149.16 mg/g with the relative standard deviation (RSD%) of 0.05 (Supplementary Table S2).

3.4. Comparison with conventional extraction methods

Table 4 we compared our results with other results in the literature, it can be seen that our approach has the advantage of higher extraction amount in a short extraction time. In addition, we calculated the energy consumptions (Supplementary Section S3) of this method and conventional
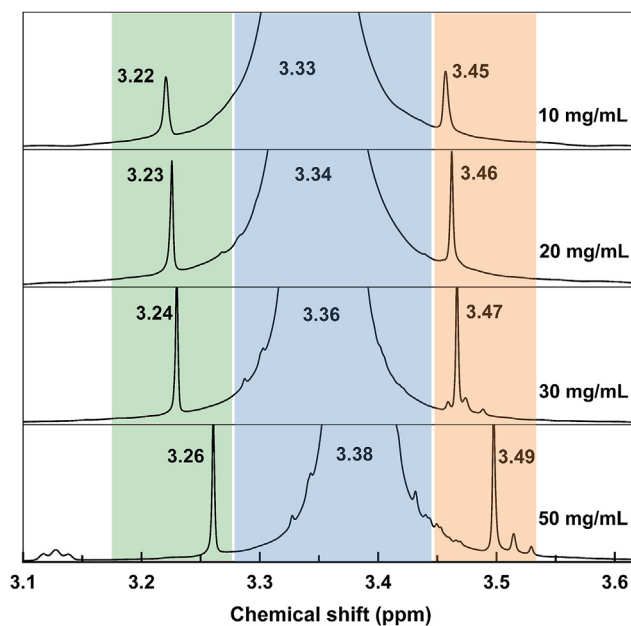

Figure 6. ^{1}H-NMR spectra of AU/methanol solution with different concentrations.

Soxhlet extraction for comparison. The proposed method exhibited higher extraction efficiency and save 85.4% energy consumptions (149.1 mg/g and 0.102 kW·h *vs.* 73.4 mg/g and 0.700 kW·h for Soxhlet extraction).

3.5. Extraction kinetics

According to the results of optimization parameters by RSM, ultrasonic and microwave assisted methanol extraction techniques were the
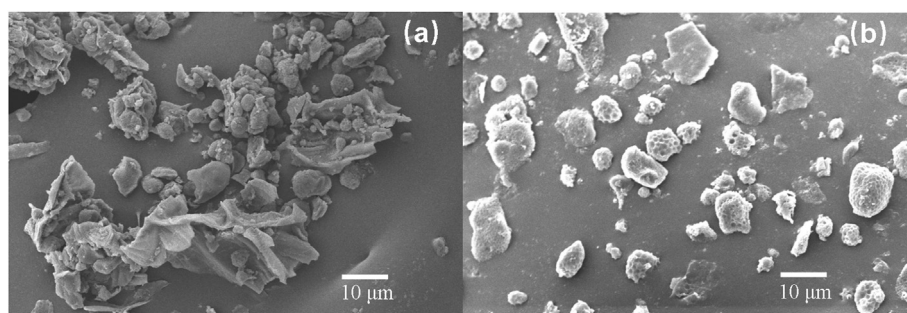
Figure 5. SEM micrographs of EUSD surface: (a) before extraction; (b) after extraction*. *liquid (methanol) -solid ratio of 40 mL/g, methanol percentage of 90%, ultrasonic power of 100 W, ultrasonic time of 30 min, microwave power of 300 W and microwave time of 20 s.
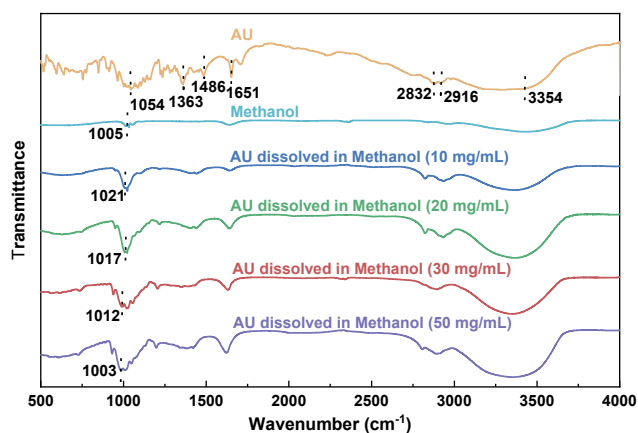
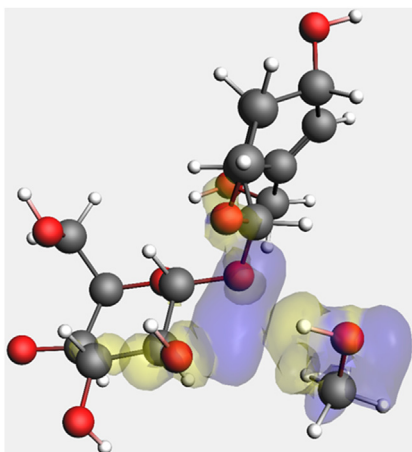**Figure 7.** IR spectra of AU/methanol solution with different concentrations.

Figure 8. Possible interactions between AU and methanol (ball color: white, hydrogen; gray, carbon; red, oxygen).

best extraction process for AU. In order to realize the industrial application of the current method, it is necessary to carry out the extract kinetic experiment, establish appropriate kinetic equations, and explore its mass transfer process.

Four main kinetic models (first-order kinetic model, Fick's second law kinetic model, second-order kinetic model, and So-Macdonald model)

were used to describe the extraction kinetics of natural products. It can be seen from Figure 4a and b that the So-Macdonald model can better fit with the experimental data, since other three kinetic models and experimental data only have good consistency in the initial and equilibrium stages, while a certain deviation from the experimental data can be seen in the intermediate transition stage. In particular, the first-order and Fick's second law kinetic model have large deviations from the experimental data in the intermediate transition stage. It can be seen from Table 5 that the So-Macdonald model has a higher correlation coefficient ($R^2 > 0.9897$) and a lower residual sum of squares (RSS <0.0531).

It can also be seen from Figure 4 that the initial extraction rate of AU is very high, which indicates that the AU and other soluble components on the surface of EUSD particles can quickly dissolve into the extraction solvent, and the AU amount increases rapidly with the increase of extraction time. After the initial stage (ultrasonic time of 0–5 min or microwave time of 0–6 s), as the diffusion turn into the dominant process, the extraction growth rate becomes slower towards the end of leaching, which is exactly fitted to the So-Macdonald model. Similar kinetic behaviors have been reported in previous studies, such as the extraction of oils [41, 42], soluble components [43, 44] and flavonoids [45].

3.6. Extraction process analysis

The extraction process analysis of AU was explored in detail through characterization techniques including SEM, ^{1}H NMR, and IR.

Scanning electron microscopy (SEM) was conducted to observe surface changes of the samples before (Figure 5a) and after extraction (Figure 5b). Figure 5b shows some small pits and grooves appeared after extraction, this led to the specific surface areas and pore volume increased (Table 1). The cell wall structure was damaged, which was conducive to the penetration of the methanol solvent into the cells and reduces mass transfer resistance, thereby accelerating the leaching rate of AU into methanol solution.

To get further information on the interaction between AU and methanol, different AU/methanol concentrations (10, 20, 30 and 50 mg/mL) were collected to characterized by ^{1}H-NMR and IR spectra. Figure 6 shows that a slight increase of the chemical shifts of AU belonging to H-a and H-b (Figure 1) indicates the formation of hydrogen bonds between AU and methanol. The oxygen atoms in the ether bond in AU would benefit the hydrogen bonds formation, thus facilitating the deshielding effect and downfield shifts of hydrogen atoms of AU [46, 47].

Figure 7 exhibits the FTIR spectra of AU/methanol solution with different concentrations. The broad and strong absorption peak at 3354 cm^{-1} was assigned to the stretching vibration of multiple O-H in AU, and
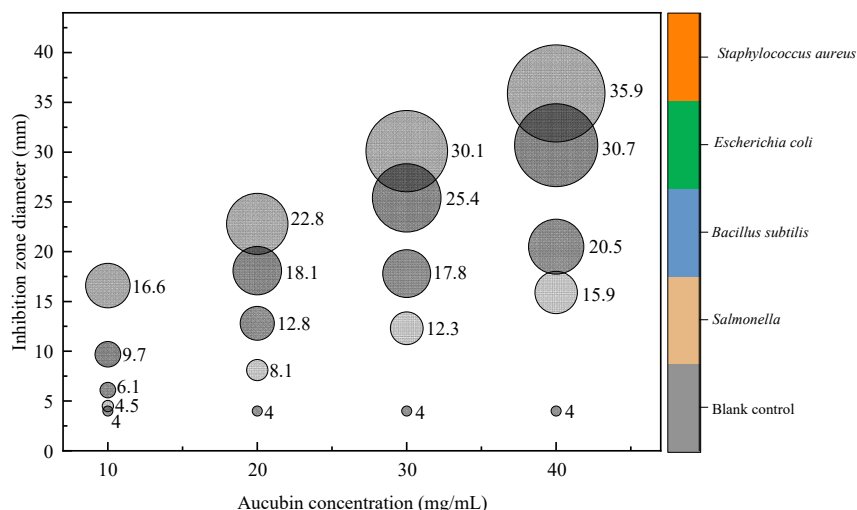

Figure 9. Antibacterial activity of AU extract.

the stretching absorption of –CH$_2$ was at 2916 cm^{-1}. One can observe a special peak at 1651 cm^{-1} which could belong to C=C stretching vibration [48]. The absorption peak at 1486 cm^{-1} and 1363 cm^{-1} were corresponding to the bending vibration of –CH$_2$. Particularly, the band at 1054 cm^{-1} could be attributed to –C–O bond, which had redshifted from 1012 cm^{-1} to 1021 cm^{-1} gradually as the AU concentration increased [48]. This indicates the formation of hydrogen bonds between AU, and supports our observation via the ^{1}H NMR spectroscopy.

Figure 8 display the probable configuration of AU and methanol using COSMOtherm based on the contact probability of molecular surface segments [20, 49, 50]. The hydroxyl group of methanol could act as the hydrogen bond donor and form hydrogen bond with oxygen through the ether bond of AU, resulting in the redshift of -C-O infrared wavenumber in AU [20, 49, 50]. This result is complementary to the infrared characterization.

3.7. Antibacterial activity

The antibacterial activity of AU extract was evaluated against 4 pathogens, and the results were shown in Figure 9. The study showed that the AU extract exerted a strong antibacterial activity against all tested pathogens, the antibacterial effect of AU extract followed the order of: *S. aureus* (35.9 ± 1.32 mm)> *E. coli* (30.7 ± 1.38 mm)> *B. subtilis* (20.5 ± 1.36 mm)> *Salmonella* (15.9 ± 1.39 mm) with the AU concentration of 40 mg/mL.

4. Conclusions

This paper provides a new method for the reutilization of EUSD waste biomass, which can be used as generalized and sustainable extract technology for the pharmaceutical and biochemical applications. The proposed ultrasonic and microwave assisted extraction approach using methanol as a solvent exhibited higher extraction efficiency (2 times higher than conventional Soxhlet extraction) and can save 85.4% energy consumption. The microwave and ultrasound facilitated the penetration of the methanol solvent into the cells and reduces mass transfer resistance, thereby accelerating the leaching rate of AU into methanol solution. Moreover, this study identified the So-Macdonald model to accurately describe and understand the kinetic process of ultrasound and microwave-assisted extraction of AU. Besides, the current study demonstrated possible interactions between AU and methanol, which provide a certain theoretical model and reference data for extraction process and industrial extraction application of AU from EUSD waste biomass. In the antibacterial activity experiment, the AU extract exhibited a strong antibacterial activity against all tested pathogens. We believe that this work could expand possibilities to ultimately develop a novel, green and high-efficiency technique for the certain extraction and application of natural product.

Declarations

Author contribution statement

Yunhui Liao: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Feng Chen: Performed the experiments; Analyzed and interpreted the data.

Lujie Xu: Performed the experiments.

Wubliker Dessie: Analyzed and interpreted the data; Wrote the paper.

Jiaxing Li, Zuodong Qin: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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