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Extraction optimization of total triterpenoids from *Jatropha curcas* leaves using response surface methodology and evaluations of their antimicrobial and antioxidant capacities

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ABSTRACT

Background: Triterpenoids are multifunctional secondary metabolites in plants. But little information is available concerning the actual yield, optimal extraction method and pharmacologic activity with regard to triterpenoids from *Jatropha curcas* leaves (TJL). Hence, response surface methodology (RSM) was used to optimize the extraction parameters. The effects of three independent variables, namely liquid-to-solid ratio, ethanol concentration and extraction time on TJL yield were investigated. TJL obtained by silica column chromatography was tested against bacterial and fungal species relevant to oral disease and wounds through broth microdilution. Antioxidant activity was assessed using the 2,2-diphenyl-2-picrylhydrazyl and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assays.

Results: A second order polynomial model produced a satisfactory fitting of the experimental data with regard to TJL yield ($R2 = 0.983$, $P < 0.01$). The optimum extraction conditions were 16 mL/g (liquid-to-solid ratio), 70% (ethanol concentration) and 50 min (extraction time). Predicted values agreed well with the experimental values. TJL had extraordinarily strong antibacterial and antifungal activities (24.42 μg/mL < MIC < 195.31 μg/mL) against all the tested human pathogens except *Bacteroides vulgatus* (390.62 μg/mL) and *Bacteroides stercoris* (781.25 μg/mL). The DPPH and ABTS assays revealed a moderate antioxidant activity of TJL compared with ascorbic acid.

Conclusion: These results provided reliable scientific basis for further investigation of triterpenoids from *J. curcas*.

1. Introduction

Triterpenoids are the largest group of secondary metabolites in plants. They have received extensive attention due to their broad spectrum pharmacological activities coupled with a low toxicity profile [1]. They are known to be responsible for antiangiogenic, antipruritic, antiallergic, antitumor, antiviral, antimicrobial, antioxidant and spasmolytic activities [2,3,4,5]. *Jatropha curcas* is a drought-resistant medical crop belonging to the family Euphorbiaceae [6]. Many plants of this family exude tree sap latex containing large amounts of triterpenoids, and some of them could be used for biofuel, rubber and medicinal applications [7]. There are high content triterpenoids in *J. curcas* leaves as reported by Suharti et al. [8]. However, little information is available concerning the actual yield, optimal extraction method and pharmacologic activity with regard to *J. curcas* leaves (TJL).

In the recovery and purification of bioactive compounds from plant materials, extraction is the initial step and plays an important role. Many factors such as solvent-to-solid ratio, extraction time, solvent composition and number of steps may influence the extraction efficiency and compounds yield [9,10,11]. Hence, it is necessary to optimize the extraction parameters to obtain the highest triterpenoid recovery. Several traditional extraction techniques such as heating, boiling, refluxing and soxhlet extraction have been used to extract triterpenoids [12,13,14,15], which consume a large amount of solvent and time. However, ultrasound-assistant extraction has been developed with advantages of shortening extraction time, decreasing solvent consumption and increasing extraction yield. The enhancement in extraction obtained by using ultrasound is mainly attributed to the effects of acoustic cavitations produced in the solvent by the passage of an ultrasonic wave. Ultrasound also exerts a mechanical effect, allowing greater penetration of solvent into the sample matrix, increasing the contact surface area between solid and liquid phase; as a result the solute diffuses quickly from solid phase to the solvent [16,17].

Classical optimization method is laborious, time consuming and expensive, since it uses one-factor-at-a-time approach. Meanwhile,

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the interactions between variables are ignored and dubious conclusions may be drawn. The response surface methodology (RSM) can overcome these limitations, for it enables evaluation of the effects of several variables [18]. RSM has been successfully used in optimizing the extraction of phenolic compounds from plants such as wheat [19] and onion [20].

Almost all parts of *J. curcas* are used in traditional medicine, including the treatment of mouth disease and wounds [6,21]. Meanwhile, triterpenoids also have evident activity against oral pathogens in *Miconia* [22]. Therefore, the objective of the work is to establish the optimal conditions of ultrasound-assistant extraction of TJL and investigate TJL using *in vitro* models of relevance to oral disease and wounds: antibacterial, antifungal and antioxidant assays. The results will provide scientific evidence for further investigation of triterpenoids from *J. curcas*.

2. Materials and methods

2.1. Plant materials

J. curcas roots, stems, leaves, flowers and barks were collected from a *Jatropha* nursery in Yanyuan County, Sichuan Province, China in August 2012. They were authenticated by a plant taxonomist from the Department of Botany, Sichuan University, Chengdu. A voucher specimen of the plant (NO. 10/08) was deposited in the herbarium of Sichuan University. All plant materials were dried, ground into powder in a mill, passed through a 60-mesh sieve and stored in an airer.

2.2. Microorganisms

16 common human pathogenic microbes were selected here and some were closely associated with oral disease and wounds. Bacteria were *Porphyromonas gingivalis* ATCC 33277, *Aggregatibacter actinomycetemcomitans* ATCC 33384, *Fusobacterium nucleatum* ATCC 25586, *Prevotella buccae*, *Peptoniphilus asaccharolyticus*, *Bacteroides fragilis*, *Bacteroides vulgatus*, *Peptostreptococcus anaerobius*, *Bacteroides stercoris*, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Escherichia coli*. ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 700603, *Salmonella dublin* and *Stenotrophomonas maltophilia*. Three fungi were two *Monilia albican* species ATCC 90029 and 10231, and *Candida guilliermondii*. The strains were recovered from clinical samples obtained from the Clinical laboratory of West China Second University Hospital of Sichuan University. All the strains were maintained at 4°C until use.

2.3. Experimental design

A Box–Behnken design (BBD) was used to determine the optimal conditions of ultrasound-assisted extraction for TJL. Liquid-to-solid ratio (*A*), ethanol concentration (*B*) and extraction time (*C*) were chosen as independent variables. The range and center point values of them presented in Table 1 were based on the results of preliminary single factor experiments. The experimental design consisted of 12 factorial experiments and three replicates of the central point (Table 2). The yield of TJL was selected as the response for the combination of independent variables given in Table 2. Three triplicate experiments were carried out at each experimental design point and the mean values were stated as observed responses. Experimental runs were randomized to minimize the effects of unexpected variability in the observed responses. The general form of the second degree polynomial equation is

$$Y = a_0 + b_1A + b_2B + b_3C + c_{12}AB + c_{13}AC + c_{23}BC + d_1A^2 + d_2B^2 + d_3C^2 \quad \text{[Equation 1]}$$

where Y is the predicted response; a_0 is the interception; b_1, b_2 and b_3 are the linear coefficients of liquid-to-solid ratio (*A*), ethanol concentration (*B*) and extraction time (*C*), respectively; c_{12}, c_{13}, and c_{23} are the interaction coefficient of liquid-to-solid ratio, ethanol concentration and extraction time, respectively; d_1, d_2 and d_3 are the squared coefficient of liquid-to-solid ratio, ethanol concentration and extraction time, respectively. Analysis of the experimental design data and calculation of predicted responses were carried out using Design Expert software (Version 7.0.0, Stat-Ease, Inc., Minneapolis, MN). Additional confirmation experiments were subsequently conducted to verify the validity of the statistical experimental design.

Table 1
Coded and actual levels of three variables.

Variables	Coded levels of variables		
	-1	0	1
Liquid-to-solid ratio/(mL/g) (*A*)	8	14	20
Ethanol concentration/% (*B*)	60	72.5	85
Extraction time/min (*C*)	40	50	60

Table 2
Experimental designs using Box–Behnken and results.

Test run no.	Coded levels of variables			TJL mg/g DM
	A	*B*	*C*	
1	+1	+1	0	2.16
2	+1	-1	0	2.57
3	-1	+1	0	2.34
4	-1	-1	0	2.08
5	+1	0	+1	2.15
6	+1	0	-1	2.18
7	-1	0	+1	2.05
8	-1	0	-1	2.06
9	0	-1	+1	2.39
10	0	-1	-1	2.48
11	0	1	+1	2.32
12	0	1	-1	2.12
13	0	0	0	2.65
14	0	0	0	2.68
15	0	0	0	2.69

2.4. Ultrasound-assistant extraction

The single factors for solvent extraction procedures were set as below: Firstly, the effect of liquid-to-solid ratio on the extraction was investigated. Leaf powder (1.0 g) was put into a 50 mL conical flask, 75% ethanol added and extraction performed for 30 min at different liquid-to-solid ratios (4–20 mL). Secondly, we studied the influence of ethanol concentration on TJL yield. 8 mL of different ethanol concentrations (60–100%) was added and the extraction performed for 30 min. Thirdly, the extraction time was studied in a range of 10–60 min. 8 mL of 75% ethanol was added. Finally, the optimal extraction times were investigated in a range from 1 to 5 times. 8 mL of 75% ethanol was added and the extraction performed for 30 min. The ultrasonic power was fixed at 100 W, 25°C for all extraction experiments performed in optimization procedure.

The RSM ultrasound-assistant extraction procedure was set as below: leaf powder was put into a 50 mL conical flask, then different concentrations of ethanol were added (60–85%) with different liquid-to-solid ratios (8–20 mL/g) and time (40–60 min) for 4 times. Similarly, the ultrasonic power was also fixed at 100 W, 25°C. The extracts for 4 times were merged and filtered using filter papers. And the filtrate was used to determine the content of triterpenoids.

2.5. Determination of total triterpenoid content

The content of TJL obtained by the aforementioned method was determined according to Lu et al. [23] with a slight modification and

then expressed as milligram ursolic acid equivalent/gram dry weight (DM). Briefly, after a 200-μL sample solution in a 10 mL volumetric flask was heated to evaporation in a water-bath, 1 mL new mixed 5% (W/V) vanillin-acetic solution and 1.8 mL sulfuric acid were added, mixed and incubated at 70°C for 30 min. Then the mixed solution was cooled and diluted to 10 mL with acetic acid. The absorbance was measured at 573 nm against blank using a spectrophotometer. The blank consisted of all reagents and solvents without sample solution. The content was determined using the standard ursolic acid calibration curve. The calibration equation for ursolic acid was $Y = 0.0605X - 0.0122$ ($R^2 = 0.9991$), in which Y was the absorbance value; X was the concentration of ursolic acid (μg/mL). The linear range of ursolic acid was 1–10 μg/mL.

2.6. Triterpenoid contents in different tissues of J. curcas

The optimized conditions by RSM were employed to investigate the triterpenoid content in different tissues of *J. curcas* including root, annual stem, two year stem, leaf without petiole, petiole, flower and bark.

2.7. TJL preparation for pharmacologic activity tests

The crude ethanol extract of leaves was collected and evaporated to dryness under reduced pressure using a rotary evaporator, then mixed with 500 mL of distilled water. The mixture was treated by means of liquid-liquid extraction in a separatory funnel with petroleum ether and ethyl acetate in sequence. Petroleum ether extraction could remove phytochrome and some lipids in favor of subsequent isolations. The ethyl acetate residue was subjected to column chromatography over silica gel (100–200 mesh). It was eluted ordinally with petroleum ether, the combination of petroleum ether: acetone from 9:1 to 0:10, and acetone:methanol from 9:1 to 0:10. The fractions were collected in 250 mL conical flasks.

Triterpenoid tests were performed on each fraction respectively using two methods. One was Liebermann–Burchard and Salkowski test [24]. Concisely, a chloroform solution of the extract was mixed with acetic anhydride and three drops of sulfuric acid. The development of a red to brown color indicated the presence of triterpenoids. The other was a thin layer chromatograph detection [25,26]. It was achieved by spraying with 10 mL vanillin-sulfuric acid reagent, prepared by mixing 0.5 mg vanillin, 49 mL glacial acetic acid, and 1 mL concentrated sulfuric acid, and then heating at 105°C for 10 min. A red-brown color spot showed the existence of triterpenoids. Fractions those tested containing triterpenoids were merged together, dried in a rotary evaporator set to 40°C, and stored at 4°C for further analysis.

2.8. Antimicrobial capacities

Broth microdilution method using 96-well microplates for determination of the minimal inhibitory concentration (MIC) values was applied according to the Clinical and Laboratory Standard Institute (CLSI) [27].

The sample stored at 4°C was diluted using double dilution method. The concentrations ranged from 3125 μg/mL to 6.10 μg/mL. Metronidazole, cephalothin and nystatin were positive controls against anaerobes, aerobes and fungi, respectively. The strains aforementioned at 4°C were sub-cultured on a fresh appropriate agar plate for 24–48 h, and regulated to $OD_{600} = 0.5$ in their respective broth. Anaerobic strains were cultured in Wilkins Chalgren anaerobe broth, aerobic strains in beef-protein broth and fungi in potato dextrose broth.

An equal volume of 100 μL samples of different concentrations and strain broths were added to each well relatively. Microplates were covered with lids and incubated anaerobically and aerobically as required at 37°C for 24–48 h. The presence of turbidity and a pellet on the bottom indicated microbial growth. The lowest concentration of the sample that inhibited the microbial growth was the MIC of TJL against the relative strain. The lowest sample concentration at which 99.9% of the bacteria and fungi had been killed was taken as minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC), respectively. If the test strains from microplates had no viability completely after 48 h of reincubation on their appropriate agar plates at 37°C, the relative concentration was considered as the MBC or MFC value.

2.9. Antioxidant activity

2.9.1. 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

Spectrophotometric method based on the use of the free radical DPPH was employed to determine the antioxidant activity according to Blois with a slight modification [28]. In brief, a 0.2 mM solution of DPPH radical solution in methanol was prepared, and then 100 μL of this solution was mixed with 100 μL TJL sample solution in methanol containing 0.2–3.2 mg/mL of dried sample. After 30 min at 37°C, the absorbance was measured at 517 nm. A reference commercial synthetic antioxidant (ascorbic acid) was tested with the same method. The results were expressed as percentage reduction absorbance shown by the sample with respect to DPPH solution. The formula was:

$$\text{DPPH scavenging}\ (\%) = [(\text{control absorbance} - \text{sample absorbance})\ /\text{control absorbance}] \times 100.$$

2.9.2. 2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$) radical scavenging activity

A 7 mM $ABTS^+$ radical solution in water was prepared. The method was getting the ABTS stock solution and 2.45 mM potassium persulfate (final concentration) to react and allowing in the dark at room temperature for 12–16 h before use. For the test of TJL, the $ABTS^+$ solution was diluted with phosphate-buffered saline 0.2 mM (pH 7.4) to an absorbance of 0.7 at 734 nm. After the addition of 100 μL diluted $ABTS^+$ solution to 100 μL sample with the concentration from 0.025 to 1.6 mg/mL, the absorbance reading was taken 6 min later [29]. The activity was given as percentage $ABTS^+$ scavenging that was the same as DPPH assay.

3. Results and discussion

3.1. Effect of liquid-to-solid ratio on the extraction yield of TJL

The effect of liquid-to-solid ratio on the extraction yield of TJL was shown in Fig. 1a. The extraction yields of TJL significantly increased from 8.23 to 12.65 mg/g DM as the liquid-to-solid ratio increased within the range of 4 to 8 mL/g, due to the increase of the driving force for the mass transfer of the TJL. However, the extraction yields no longer significantly changed when the liquid-to-solid ratio continued to increase.

3.2. Effect of ethanol concentration on the extraction yield of TJL

The concentration of extraction solvent influences the efficiency of extracting TJL. In general, lower concentration of ethanol is suitable for the extraction of polar triterpenoid compounds and higher concentration of ethanol is suitable for the extraction of low polar or non-polar ones. Extraction was carried out with different ethanol concentrations (50–100%, v/v) while keeping other extraction parameters constant. The yield of TJL significantly increased from 14.39 to 19.51 mg/g DM when the concentration of ethanol increased from 50% to 75%. However, as the concentration continued to increase

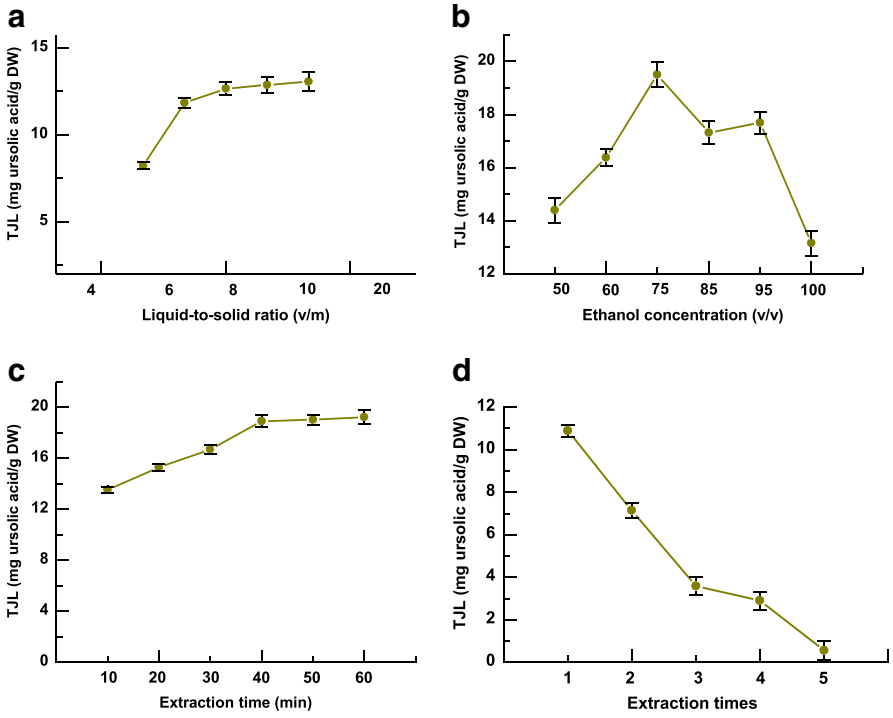

Fig. 1. The effects of extraction variables on TJL yield. (a) Effect of liquid-to-solid ratio on TJL yield, the other extraction conditions were 75% ethanol, 40 min extraction time, 25°C extraction temperature and 40 W ultrasonic frequency; (b) effect of ethanol concentration on TJL yield, the other extraction conditions were 8 mL/g liquid-to-solid ratio, 40 min extraction time, 25°C extraction temperature and 40 W ultrasonic frequency; (c) effect of extraction time on TJL yield, the other extraction conditions were 8 mL/g liquid-to-solid ratio, 75% ethanol, 25°C extraction temperature and 40 W ultrasonic frequency; and (d) effect of extraction times on TJL yield, the other extraction conditions were 8 mL/g liquid-to-solid ratio, 75% ethanol, 40 min extraction time, 25°C extraction temperature and 40 W ultrasonic frequency. Each value represents a mean ± SD (n = 5).

up to 100%, the TJL decreased to 13.15 mg/g DM (Fig. 1b). The reason may be that higher concentration of ethanol makes more liposoluble components dissolve out, which is adverse to extract triterpenoids.

3.3. Effect of time on the extraction yield of TJL

Extraction time would significantly influence the extraction efficiency of triterpenoid compounds from medicine plants or edible vegetables. The effect of different time (10–60 min) on the extraction yield of TJL was shown in Fig. 1c. When extraction time varied from 10 min to 40 min, the yield of TJL was significantly increased from 12.50 to 18.93 mg/g DM. While there was no significant change as time varied from 40 min to 60 min. It indicated that 40 min was sufficient to obtain TJL. Meanwhile, the increase of extraction time may lead to the degradation of triterpenoid compounds. Therefore, 40 min was selected as the favorable extraction time.

3.4. Effect of extraction times on the extraction yield of TJL

Investigation on extraction times makes the maximum amount of triterpenoid compounds dissolve out from *J. curcas* leaves. The effect of different extraction times on the extraction yield of TJL was shown in Fig. 1d. Extraction was carried out with different times (1–5) while other extraction parameters were constant. TJL content decreased from 11.92 to 2.89 mg/g DM as the extraction times increased from 1 to 4. However, the fifth extraction yield was a little (0.55 mg/g DM). Therefore, 4 times was selected to quantify TJL for the sake of saving experimental time and consumables.

3.5. Optimization of extraction conditions of TJL by RSM

System error is characterized as categorical error in which a signal is changed so that it indicates a different category than the one from which it actually comes [30]. The presence of system error limits the application of optimization techniques in digital simulation experiments including RSM. Therefore, as a more widely accepted design strategy to settle this problem, the assignment of common pseudorandom number streams was used in our study. Meanwhile, test equipments were calibrated, experimental procedures were kept precise and all experiments were performed by exactly the same person.

The BB design was employed to study the interactions among *A*, *B* and *C* variables and also determine their optimal levels. The extraction times in this study was 4. The results were represented in Table 2. Multiple regression analysis was used to analyze the data and a quadratic polynomial equation was derived from regression analysis as follows:

$$Y = -10.73516 + 0.46440A + 0.13183B + 0.21600C - 2.79167 \times 10^{-2}AB - 8.33333 \times 10^{-5}AC + 7.25000 \times 10^{-4}BC - 8.56481 \times 10^{-3}A^2 - 9.08333 \times 10^{-4}B^2 - 2.68333 \times 10^{-3}C^2. \quad \text{[Equation 2]}$$

Table 3
Analysis of variance (ANOVA) for the regression equation.

SD	SS	DF	*F* value	Prob > *F*	S
Model	0.79	9	32.20	0.0007	**
A	0.035	1	12.82	0.0159	*
B	0.042	1	15.36	0.0112	*
C	6.125E-004	1	0.22	0.6562	
AB	0.11	1	40.98	0.0014	**
AC	1.000E-004	1	0.037	0.8560	
BC	0.021	1	7.68	0.0393	*
A^2	0.35	1	128.19	<0.0001	**
B^2	0.030	1	11.13	0.0207	*
C^2	0.27	1	97.09	0.0002	**

c.v. % = 2.25; R^2 = 0.983; AdjR^2 = 0.9525; residual = 0.014; SD: source of deviation; DF: degree of freedom; SS: sum of squares; S: significant; *P < 0.05, **P < 0.01.

The adequacy of the model was checked using the Analysis of Variance (ANOVA) which was tested using Fisher's statistical analysis and the results were showed in Table 3. According to the ANOVA table, the experimental data fitted well to the quadratic models. The ANOVA for response surface quadratic regression model showed that the model was highly significant ($P < 0.01$) with a very high F-value (32.20). The R^2 value (multiple correlation coefficient) closer to one denotes better correlation between the observed and predicted values [31]. In this case the value of R^2 (0.983) indicated good correlation between the experimental and predicted values, which showed that the model was significant. The adjusted R^2 (Adj $R^2 = 0.9525$) was also satisfactory to confirm the significance of the model. This indicated

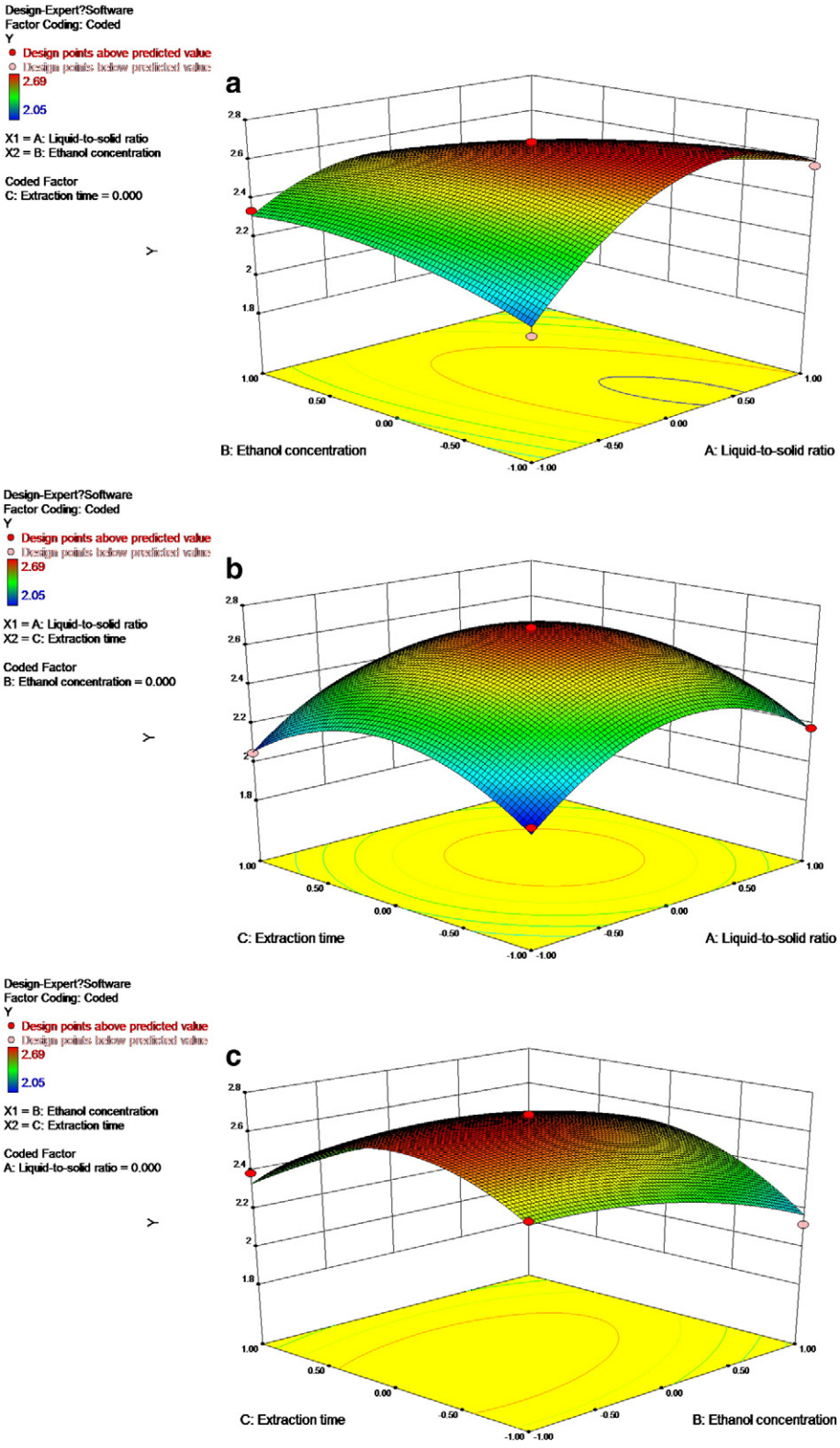

Fig. 2. Three-dimensional plot of TJL. (a) Response plot of liquid-to-solid ratio (*A*) *vs.* 559 ethanol concentration (*B*); (b) response plot of liquid-to-solid ratio (*A*) *vs.* extraction 560 time (*C*); (c) response plot of ethanol concentration (*B*) *vs.* extraction time (*C*).

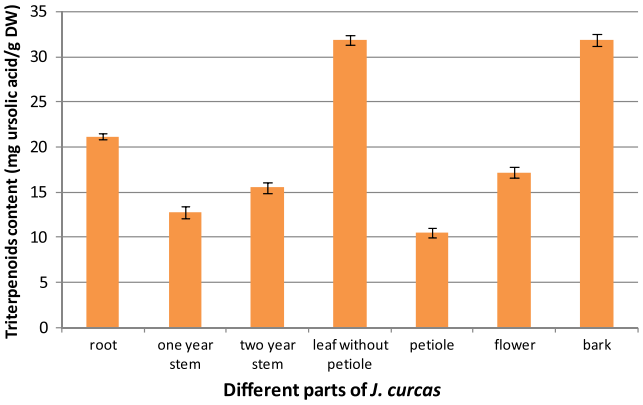

Fig. 3. Triterpenoid contents of different tissues of *J. curcas*. Each value represents a mean ± SD (n = 5).

that the quadratic polynomial equation was suitable to describe the response of the experiment pertaining to triterpenoids. A low value of coefficient of the variance (c.v.) (2.25) clearly indicated a high degree of precision and reliability of the experimental values. No abnormality was obtained from the diagnoses of residuals. Therefore, it can be concluded that the model was statistically sound.

To determine optical levels of the test variables for the yield of TJL, the 3D response surfaces described by the regression model were represented in Fig. 2a–c. The maximum yield of TJL was recorded under the experimental conditions of $A = 16$ mL/g, $B = 70\%$, $C = 50$ min, with the corresponding $Y = 27.2$ mg/g DM. To confirm these results, three triplicate tests were performed under optimized conditions. The TJL yield was 26.7 ± 0.2 mg/g DM, which clearly showed that the model fitted the experimental data and thus suitable for optimization of the TJL extraction procedure.

3.6. Triterpenoid contents in different tissues of J. curcas

As shown in Fig. 3, leaves without petioles and barks had the highest triterpenoid contents compared with other tissues of *J. curcas*. This was consistent with the report by Suharti et al. [8]. Chipping off the barks would damage the plants seriously. Therefore, the extraction of triterpenoids from leaves was valuable, which could have a great potential to put leaves into medicinal use and raise comprehensive utilization of *J. curcas*.

3.7. Antimicrobial activity

The antimicrobial activity of TJL showed low values of MICs from 24.42 to 781.25 μg/mL (Table 4). Broadly, aerobic strains were more sensitive to TJL than anaerobes in this investigation. *P. gingivalis* was most sensitive to TJL among the anaerobes with MIC of 48.83 μg/mL. For aerobic strains, *E. faecalis*, *S. aureus* and *E. coli* showed the most sensitivity with the same MIC of 24.42 μg/mL. Besides, TJL also showed significant capacities against the fungi tested here. The MBC/MFC values were found to be 2 to 4 times higher than MICs. Therefore, TJL had excellent broad spectrum antimicrobial properties.

In antimicrobial susceptibility tests, MICs of rude solvent extracts and isolated phytochemicals should be less than 8 mg/mL and 1 mg/mL, respectively, which can be considered potentially useful therapeutically [32,33]. In our study, the MIC values, ranging from 24.42 to 781.25 μg/mL, were less than 1 mg/mL, which conformed the existence of a significant activity against all microbes tested. Especially for oral pathogens (*P. gingivalis*, *A. actinomycetemcomitans* and *F. nucleatum*), TJL had low MIC values ranging from 24.42 to 195.31 μg/mL, even lower than certain pure triterpenoids [22]. Therefore, TJL has a great potential to be used as therapeutic agents for some oral disease.

Table 4
Antimicrobial activity of TJL expressed as MICs and MBCs or MFCs.

Type	Microbes	Gram stain	MIC[a]	MBC/MFC[a]
Anaerobes	*P. gingivalis*	-	48.83	97.66
	A. actinomycetemcomitans	-	97.66	195.31
	B. fragilis	-	97.66	195.31
	P. anaerobius	+	97.66	195.31
	F. nucleatum	-	97.66	390.62
	P. asaccharolyticus	-	195.31	390.62
	P. buccae	-	195.31	781.25
	B. vulgatus	-	390.62	781.25
	B. stercoris	+	781.25	1562.51
Aerobes	*E. faecalis*	+	24.42	48.83
	E. coli	-	24.42	97.66
	S. aureus	+	24.42	195.31
	P. aeruginosa	-	48.83	97.66
	S. maltophilia	-	48.83	195.31
	K. pneumonia	-	97.66	195.31
	S. dublin	-	97.66	390.62
Fungi	*M. albican*		97.66	390.62
	C. guilliermondii		24.42	48.83
	M. albican		24.42	97.66

"+" was Gram-positive; "-" was Gram-negative. The positive controls were active against all reference microbes (MIC range: 0.1–25 μg/mL).
[a] μg/mL.

Up to now, most antibacterial medicinal plants are active against Gram-positive bacteria but few against Gram-negative strains because of the susceptibility difference between them [34,35]. This susceptibility difference is caused by the difference of their morphological constitutions. The outer phospholipidic membrane of negative strains contains lipopolysaccharide components as an effective permeability barrier, while the positive only have an outer peptidoglycan layer [36]. But in our investigation, TJL were found a remarkable antibacterial activity on negative strains besides the positive ones. *P. aeruginosa* is an important cause of infection in patients with compromised host defense mechanisms. The infections are complicated and can be life threatening [37]. However, this bacterium is resistant to many antibiotics and disinfectants, which makes it a difficult pathogen to treat. Our study showed that the MIC value was 48.83 μg/mL, which indicated that TJL had a strong antibacterial activity compared with seed extracts (MIC: 250–500 μg/mL) [38]. Thus, it could be used to develop new antibacterial drugs, especially against the resistant Gram-negative strains.

The MIC values obtained in the antifungal assays were of 24.42 to 97.66 μg/mL. *M. albican* and *C. guilliermondii* were more susceptible to TJL than seed extracts (MIC: 62.5–250 μg/mL) [38]. Aiyelaagbe et al. [39] found moderate antifungal activity against *C. albicans* by hexane, chloroform and methanol extracts from roots of *Jatropha podagrica* at a concentration of 20 mg/mL. Compared with them, TJL showed a strong antifungal activity. It can be exploited to treat skin diseases in the future.

3.8. Antioxidant activity

The radical-scavenging activities of TJL were estimated by comparing the percentage inhibition of formation of DPPH and ABTS radicals between the sample and ascorbic acid. It was found that the radical-scavenging activities of TJL and the positive control increased with the increasing of concentration (Fig. 4). When the concentration of TJL was 3.2 mg/mL, the percentage inhibition of DPPH radical reached 79.51%. For ABTS radical, the percentage inhibition was 65.25% with TJL concentration of 1.6 mg/mL.

Using specific antioxidant nutrients is an efficient method in the field of oral disease prevention and wound healing [40,41]. According to our research, TJL should belong to relatively mild antioxidant activity groups compared with ascorbic acid, but is stronger than that of crude extracts from some plants such as *Platycodon grandiflorus*

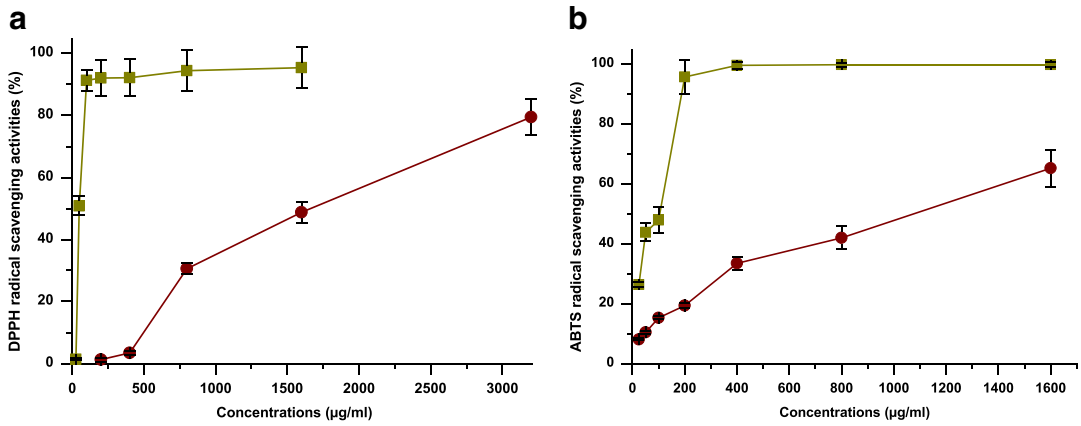

Fig. 4. Antioxidant activities of TJL at different concentrations. (a) DPPH radical-scavenging activities of TJL. Each value represents a mean ± SD (n = 5). (●) TJL; (■) ascorbic acid; (b) ABTS radical-scavenging activities of TJL. Each value represents a mean ± SD (n = 3). (●) TJL; (■) ascorbic acid.

[42]. It is noteworthy that the leaf extract has the highest scavenging activity according to Oskoueian et al. [43], so triterpenoids might not be the main natural products responsible for antioxidant activity in Jatropha leaves.

4. Conclusions

RSM was successfully used to optimize the extraction parameters of triterpenoid compounds from *J. curcas* leaves. The liquid-to-solid ratio, ethanol concentration and extraction time were all important factors influencing the extraction efficiency. The best combination of them was 16 mL/g liquid-to-solid ratio, 70% ethanol, 50 min extraction time and 40 W ultrasonic frequency for 4 times at 25°C. The yield of TJL reached 26.7 mg/g DM under this condition.

TJL showed extraordinarily strong antibacterial and antifungal activities and relatively moderate antioxidant ability, which tallied with the traditional use of *J. curcas* leaves in oral and wound treatments. These results make it reasonable and valuable to carry out further investigations on the triterpenoids from *J. curcas*.

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