Cost-effective Isolation of Bioactive Compounds from a Discarded Bioresource – Defatted Seeds of *Camellia oleifera*

Yong Ye,* Yue Li, and Fei Fang

The *Camellia oleifera* oil industry is the economic mainstay in many high-poverty mountain regions of China, but the defatted seeds are currently discarded, leading to a waste of bioresources. In this work, a cost-effective technique was designed to isolate the flavonoid, saponins, and polysaccharides by ultrasonic-assisted acid-base alternative extraction. The activities of these compounds were evaluated by their DPPH and ABTS radical scavenging abilities *in vitro* and inflammatory inhibition in mice, and the economic efficiency was assessed. The optimal extraction conditions by response surface design were 1.6% HCl, water/seed ratio 16, extraction time 89 min, and ultrasonic power 310 W. The yields of the flavonoid, saponin, and polysaccharide were, respectively, 1.4 ± 0.2%, 6.7 ± 0.8%, and 22.5 ± 1.7%. The extracts could eliminate DPPH and ABTS radicals and alleviate inflammation with concentration dependence, and showed excellent capacity in the order of flavonoid ≥ saponin > polysaccharides. The residue after extraction was cellulose, with a yield of 63.0 ± 2.4%. The defatted seeds could produce a value of $11.35 per kilogram after production of the four valuable extracts, which would greatly increase the income in these high-poverty areas. This integrated extraction technique provides valuable recycling utilization of a typically discarded bioresource.

Keywords: Camellia oleifera; Defatted seeds; Isolation; Bioactive compounds; Recycling

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INTRODUCTION

Camellia oleifera is an evergreen plant that mostly grows in the middle region of China. Its seeds are used for the extraction of edible oil (Lee and Yen 2006). The oil industry has become an economic mainstay in the impoverished mountain region, where there are 128 million farmers and the average income per person is less than $400 per year (He and Wang 2013). The yield of these seeds has dramatically increased in recent years due to the edible oil requirement and cultivation; the production of defatted seeds is up to 800,000 tons per year in China (Li *et al.* 2011). Although the defatted seeds are rich in active compounds (Luo *et al.* 2003), they have been discarded without isolation and further exploitation, leading to great waste of this bioresource.

Flavonoids, saponins, and polysaccharides, active compounds found in many plants, have pharmacological effects such as anti-inflammation, anti-bacteria, anti-tumor, and immune enhancement and can reduce the risks of cardiovascular, cerebrovascular, and respiratory diseases (Wei *et al.* 2011; Jin 2012; Jin *et al.* 2013).

For use in health food and medicines, these seed extracts are priced at $100 per kilogram on the urban market, which is much higher than the cost of the raw material. The flavonoid, saponin, and polysaccharide were detected in the seeds of *C. oleifera* with the contents of 1 to 3%, 10 to 14%, and 15 to 20%, respectively (Chen *et al.* 2010; Du *et al.* 2011). The isolation of these compounds will provide large amounts of bioactive compounds, and it is therefore valuable to extract them by low cost methods for industrialization.

Flavonoids and saponins are soluble in methanol, ethanol, acetone, and other solvents, which are widely used for their extraction and purification (Jones and Kinghorn 2012; Khoddami *et al.* 2013). Polysaccharides are insoluble in organic solvents; however, they can be purified by organic solvents to remove other soluble impurities (Zhang *et al.* 2013). A large quantity of organic solvent not only increases cost, but also leads to air or water pollution in incomplete recycling. New methods have been studied for natural compounds extraction, including supercritical fluid extraction and microwave extraction (Delazar *et al.* 2012; Santos-Buelga *et al.* 2012), but they have limited application and require expensive equipment.

In the present research, to fully utilize the natural resource and achieve industrial production in the future, a cost-effective way of ultrasonic assisted acid-base alternative extraction without organic solvent was designed to extract the flavonoids, saponins, and polysaccharides from the defatted seeds of *C. oleifera*. Ultrasonic power can expedite dissolution at a lower temperature, which protects the thermosensitive compounds during extraction (Ghafoor *et al.* 2009). Based on the difference in solubility of the compounds, an acid-base alternative was applied to separate them. The extraction conditions were optimized, the activities of the extracts were evaluated by free radical scavenging ability, and the economic efficiency was assessed. Results showed that this method is a good prospect for the recycling of the discarded resource and increasing incomes in the high-poverty mountain regions.

EXPERIMENTAL

Materials

The defatted seeds of *Camellia oleifera* Abel were collected from an oil manufacturing company (Meizhou, China). The 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Wako Pure Chemical Industry, Ltd. (Osaka, Japan) and 2,2´-azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) from Sigma-Aldrich Chemical Company (St. Louis, USA). Analyzed reagents kaempferol (purity 98%) (Wuhan Chemical Co.; Wuhan, China), camelliagenin, vitamin C and aspirin (Shanghai Chemical Co.; Shanghai, China), monosaccharides (*i.e.*, arabinose, galactose, mannose, glucose, rhamnose, and xylose) (Shanghai Biochemical Co.; Shanghai, China), and dextrans (*i.e.*, T-10, T-40, T-70, T-100, and T-500) (Shanghai Pharmaceutical Group; Shanghai, China) were purchased for the experiments. All other reagents were of analytical grade and purchased from Guangzhou Reagent Co. (Guangzhou, China).

Bioactivity experiment was carried out on Kunming mice weighing 20 ± 2 g (half male and half female). The animals were housed under conditions of 25 ± 2 ºC, 50 ± 10 % humidity with a 12 h light/dark cycle. All animals used in this work were treated according to the guideline of animal handling in South China University of Technology.

Methods

Integrated procedure of the extraction

One kilogram of the defatted seeds of *C. oleifera* was crushed to pass through a 20-mesh sieve. The crushed seeds were then submerged in a 0.5 to 2.5% HCl aqueous solution (water/seeds = 5/1 to 25/1 in mL/g) with ultrasonic power at 200 to 400 W for 30 to 150 min. To protect the compounds from heat, the temperature was kept below 60 °C with cooling water. The extraction conditions were optimized by the response surface method described in the next section. The extract was then allowed to sit for 5 h. The supernatant was neutralized by 5% NaOH, concentrated by ultrafiltration (10,000 Da cut-off), and lyophilized to obtain the polysaccharide extract. The precipitate was extracted with 1000 mL of 10% NaOH, and the filtrate was adjusted to a neutral pH with 10% HCl and kept for 5 h. The residue and the precipitate were separately washed with 500 mL of water and dried in a vacuum to obtain the saponin and flavonoid extracts. After extraction, the resulting residue was cellulose. The procedure is illustrated in Fig. 1.

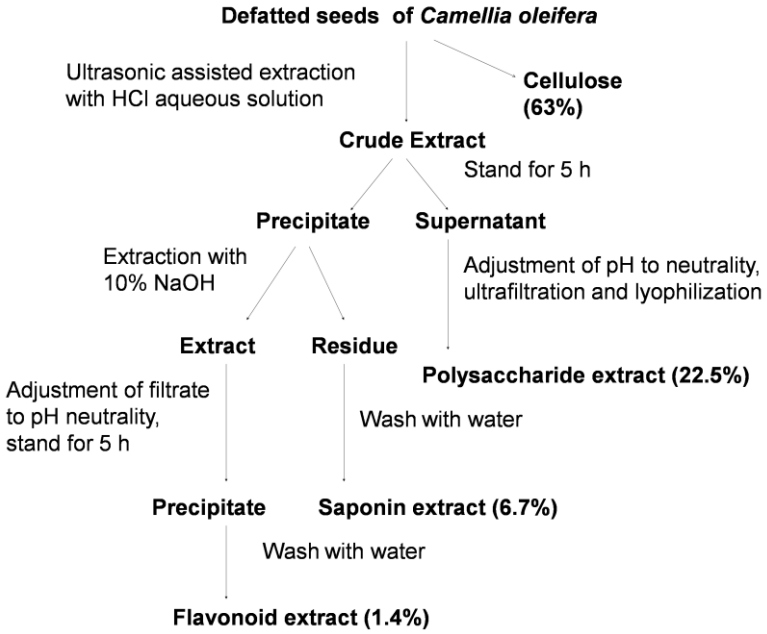


Fig. 1. Extraction procedure on the defatted seeds of *Camellia oleifera*

Optimization of extraction conditions

The HCl content, water/seed ratio (in mL/g), extraction time, and ultrasonic power during extraction were optimized by a response surface method, and the total yield of the extract served as the index. According to a central composite experimental design (Wu and Cui 2000), the HCl content, water/seed ratio, extraction time, and ultrasonic power were selected as the factors. Five levels of each factor were set up on the basis of single-factor experiments.

Each experimental level to each independent variable was encoded as -2, -1, 0, 1, and 2. The factors and levels are shown in Table 1. Design-Expert 8.0 software (Stat-Ease Inc., MN, USA) was applied to arrange the experiments at the corresponding levels of each factor (Mart and Gamse 2000).

Table 1. Factors and Levels in the Extraction

Factors	Levels				
	-2	-1	0	1	2
A: HCl content (%)	0.5	1	1.5	2	2.5
B: Water/seed ratio (mL/g)	5	10	15	20	25
C: Extraction time (min)	30	60	90	120	150
D: Ultrasonic power (W)	200	250	300	350	400

Purity determination of the extracts

The purities of the flavonoid and saponin were measured on an HP 1100 HPLC (Agilent Technologies Co., Ltd.; CA, USA). The operating conditions were as follows: column, Hypersil ODS (250 × 4.6 mm, 5 μm); mobile phase, methanol/water (80/20); injection volume, 10 μL; flow rate, 1 mL/min; temperature, 30 °C; and wavelength, 366 nm (flavonoids) and 280 nm (saponins).

The operating conditions for the polysaccharide extract were as follows: column, TSKgel G4000 PWXL (300 × 7.8 mm, 10 μm); mobile phase, 0.2 M phosphate buffer (pH 6.9); injection volume, 100 μL; flow rate, 1.0 mL/min; temperature, 40 °C; and detection, refractive index.

Purification of the compounds

One gram each of the flavonoid extract and the saponin extract were dissolved in 5 mL of 80% methanol and purified by silica gel chromatography, with elution by chloroform/methanol (8/2). The peak fractions were collected and dried in a vacuum; 0.3 g of the purified flavonoid aglycone and 0.5 g of the purified sapogenin were obtained for structural analysis.

The polysaccharide extract (1 g) was dissolved in 10 mL of a 0.2 M phosphate buffer (pH 6.9) and purified by Sephadex G-50 gel chromatography, with elution by the same buffer. The peak fractions were collected and lyophilized, and 0.6 g of the purified polysaccharide was obtained for compositional analysis.

Structural analysis of the purified compounds

Ultraviolet spectra analysis was carried out on a UV-3010 ultraviolet spectrometer (Hitachi, Japan) scanning from 200 to 600 nm. Fourier transform infrared spectra were measured on a Nicolet 380 FI-IR spectrograph (Nicolet Instrument Corporation; WI, USA) using KBr tablets from 4000 to 400 cm^{-1}, with a resolution of 2 cm^{-1}. Mass spectra were recorded on a Bruker Esquire HCT Plus Mass spectrometer with ESI (Bruker, Germany) in m/z of cation model scanning from 150 to 1200 for 60 min. Nuclear magnetic resonance spectra were determined on a 400 MHz AM NMR (Bruker, Switzerland) in DMSO-d_6, operating at 101 MHz for ^{13}C NMR and 400 MHz for ^{1}H NMR.

The molecular weight of the polysaccharide was determined by gel permeation chromatography (Ultrahydrogel column, 7.8 mm × 300 mm; flow rate, 0.1 mL/min) according to previous work (Rong and Takashi 2003). The polysaccharide was hydrolyzed by 2 mol/L trifluoroacetic acid at 100 °C for 8 h under nitrogen protection, and collected by methanol extraction. The monosaccharides were further acetylated with acetic anhydride and extracted with chloroform for composition analysis by an Agilent 6890 gas chromatograph (Agilent Technologies Co., Ltd.; CA, USA) under the following

conditions: DB225 capillary column, 30 m × 0.32 mm; gas speed, 1 mL/min; detector, FID; and column temperature, 220 °C (Wang *et al.* 2001).

Measurement of DPPH radical scavenging activity

The DPPH free radical scavenging activity was analyzed by a previously reported method (Gao *et al.* 2008). The extract (0.1 g) was dissolved in 10 mL of 50% ethanol and diluted to 1, 2, 3, 4, and 10 mg/mL. Vitamin C as the control was treated as the same as the extract.

Each sample (2 mL) was mixed with DPPH (2×10^{-4} M, 2 mL) in the ethanol solution, incubated in the dark at 25 °C for 30 min, and detected at 517 nm (absorbance Ai; 2 mL of ethanol as blank absorbance A0); 2 mL of the sample solution was added to 2 mL of ethanol and used as the background absorbance Aj. Each concentration was repeated three times, and the average value was recorded. The DPPH radical scavenging activity was calculated using Eq. 1.

$$\text{DPPH radical scavenging activity (\%)} = [A0-(Ai-Aj)] / A0 \times 100 \quad (1)$$

ABTS radical scavenging activity

The ABTS radical scavenging activity was measured by the improved method of Cai *et al.* (2004). A 7 mM ABTS working solution was prepared from 2.45 mM potassium persulfate, stored in the dark at room temperature for 12 to 16 h, then diluted with phosphate buffer (10 mM, pH 7.4) so the absorbance at 734 nm was 0.700 ± 0.020. The diluted ABTS (3 mL) was mixed with 30 μL of the extract solution, shaken for 30 s, and kept for 6 min; then, the absorbance (As) at 734 nm was measured. The ABTS radical scavenging activity of the samples was then calculated using Eq. 2.

$$\text{ABTS radical scavenging activity (\%)} = (1 - As/0.700) \times 100 \quad (2)$$

Anti-inflammatory test

Mice were divided into 8 groups of 10 mice each. The control group, positive group, and other groups respectively received normal saline, aspirin (100 mg/kg), and the extracts (the flavonoid, the saponin and the polysaccharide) at high dose (100 mg/kg) and low dose (25 mg/kg) by intragastric administration. Croton oil irritant solution (0.2 mL) was applied to the inner surface of the right ear of mice according to the reference (Brooks *et al*. 1985).

Aspirin and the extracts were administered 30 min before croton oil application. Four hours later, the mice were sacrificed by cervical dislocation and 7 mm punches were made in the ear by a cork borer. Each ear disc was weighed; the difference between right and left punched ear was calculated as swelling value. Anti-inflammatory activity was recorded as percent inhibition of swelling value in control.

Statistical analysis

All the data are presented as the mean ± standard deviation ($\bar{x} \pm s$). The statistical analysis was carried out with the statistical software SPSS 11.0 (SPSS Inc.; IL, USA). Analysis of variance (ANOVA) and Dunnett's test were used for significance testing between groups.

RESULTS AND DISCUSSION

Optimized Extraction Conditions

Flavonoids, saponins, and polysaccharides are heat-sensitive compounds, and high temperatures should be avoided during extraction. Ultrasonic extraction can help the compounds diffuse into water at a low temperature. At a temperature below 60 °C, the other extraction conditions were optimized by a response surface method, which is a method of mathematical experimental design using a polynomial function to obtain the optimum factors by a series of specified level experiments (Shao *et al.* 2011).

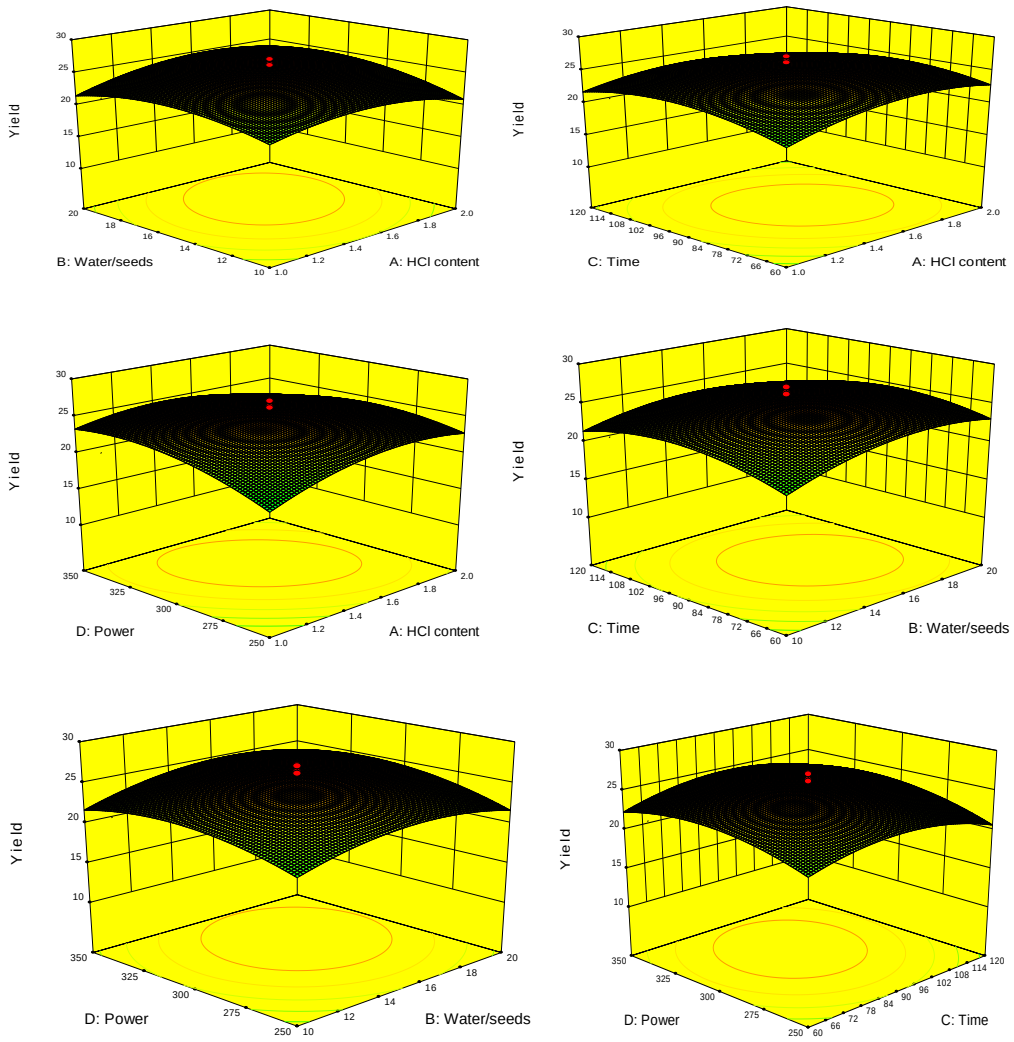


Fig. 2. Effects of interaction between the factors on yield of total extract

Each of the thirty treatments was performed in the arrangement of corresponding encoded level of factors listed in Table 2, and the yield of the extract in each treatment was obtained. The ANOVA testing showed that a response surface quadratic model reached a significant level ($F=4.69$, $p < 0.01$). The regression equation of the yield (y) and the factors (x) was $y = -118.11 + 37.82x_A + 2.04x_B + 0.47x_C + 0.49x_D + 0.26x_{AB} - 0.04x_{AC} - 0.04x_{AD} - 7.77x_A^2 - 0.08x_B^2$. The optimal conditions were 1.6% HCl content, water/seed ratio 16, extraction time 89 min, and ultrasonic power 310 W; the target yield

was 26.0%. Three repetitions of extraction were carried out on the optimum conditions, and the yields were 24.8%, 25.3%, and 23.7%, proving that the optimized results were accurate. The interaction of factors is shown in Fig. 2, and the apexes of surfaces were the optimal values, indicating that interactions among the four factors were obvious. The yields of the flavonoid extract, the saponin extract, and the polysaccharide extract were, respectively, 1.4 ± 0.2%, 6.7 ± 0.8%, and 22.5 ± 1.7%. The residue was cellulose, with a yield of 63.0 ± 2.4%. The extract is mainly composed of flavonoid, saponin, polysaccharide, and cellulose in accord with the references (Chen *et al.* 2010; Du *et al.* 2011).

Table 2. Arrangement of Treatments by Response Surface Design

Run	Factor A HCl content (%)	Factor B Water/seeds (mL/g)	Factor C Extraction time (min)	Factor D Ultrasonic power (W)	Yield (%)
1	0	0	0	0	21.8
2	1	1	-1	-1	19.6
3	0	0	0	0	25.6
4	-1	-1	-1	1	19.3
5	-1	1	-1	1	20.9
6	0	0	0	0	27.2
7	1	1	-1	1	19.1
8	0	2	0	0	21.6
9	0	0	0	0	26.2
10	1	1	1	-1	17.5
11	1	1	1	1	18.7
12	0	0	0	-2	16.4
13	0	0	0	0	27.1
14	0	0	0	0	26.4
15	2	0	0	0	22.6
16	-1	1	-1	-1	16.2
17	0	0	0	2	19.9
18	0	0	2	0	17.5
19	1	-1	1	1	16.7
20	1	-1	1	-1	15.5
21	-1	-1	-1	-1	14.8
22	1	-1	-1	1	15.3
23	-1	1	1	1	19.5
24	0	0	-2	0	16.9
25	-1	1	1	-1	15.9
26	-2	0	0	0	12.9
27	0	-2	0	0	14.3
28	-1	-1	1	1	21.8
29	-1	-1	1	-1	18.8
30	1	-1	-1	-1	19.4

Natural flavonoids and saponins generally exist in the form of glycosides, and different kinds of glycosides of flavonoid and saponin have been found in seeds of *C. oleifera* (Murakami *et al.* 2000; Gao *et al.* 2011). Flavonoid glycosides and saponin glycosides are soluble in water and other aqueous solvents. The extracts are a mixture of different glycosides, although they may have same basic skeleton of flavone or sapogenin.

An aqueous HCL solution (1.6%) with ultrasonic extraction, as applied in this research, can hydrolyze flavonoid glycosides and saponin glycosides into flavone and sapogenin, which are insoluble in water and easily isolated by precipitation to get the purified products. Polysaccharide is another compound in the seeds of *C. oleifera*, but has not been utilized effectively. The polysaccharide is currently found as an acidic polysaccharide (Tian *et al.* 2004), but no integrated extraction process has been reported, and its structure and properties are not fully understood. An acid solution was designed in this research to help the polysaccharide dissolve in water, and the polysaccharide was purified by ultrafiltration to remove salts and other small compounds. The yield and purity of the extracts show that the procedure is practical. This research provides an integrated separation process of flavonoid, saponin, and polysaccharide, and does not require organic solvents and expensive equipment; therefore, it is cost effective and suitable for industry use.

Purity of the Extracts and Structure of the Purified Compounds

These compounds were identified and structurally elucidated after further purification of the extracts. The purity of the extracts was calculated by peak areas from HPLC. The contents of flavonoids, saponins, and polysaccharides in the extract were, respectively, 86.7 ± 2.1%, 81.3 ± 4.2%, and 74.3 ± 3.5%. It suggests that they are the main constituent of the extract.

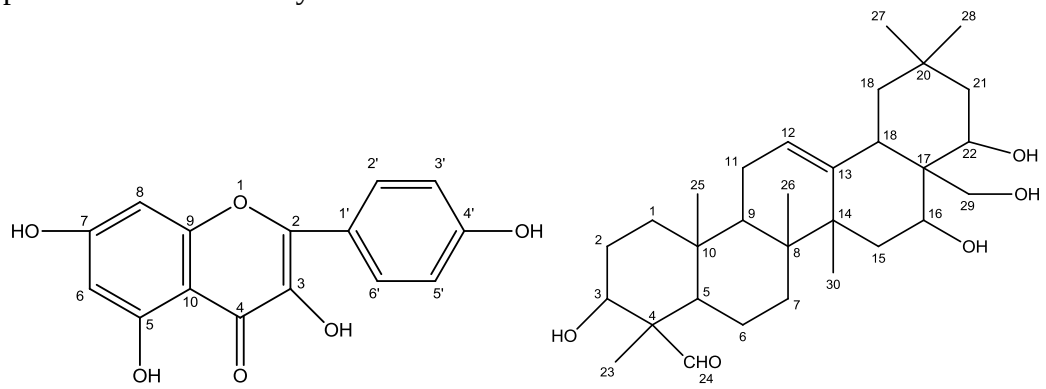
The melting point of the purified flavonoid was 261.6 to 262.0 °C. There were three absorption peaks in the UV spectra, at 207, 265, and 366 nm. The IR spectra displayed a hydroxyl group at 3423 cm^{-1}, a C-O-C group at 1261 cm^{-1}, aromatic rings at 1514 and 1459 cm^{-1}, and an aromatic ketone at 1648 cm^{-1}. Mass spectroscopy showed its mass-to-charge ratio (m/z) was 286. In ^{1}H NMR (400 MHz, DMSO-d_6) spectra, δ12.48 (s, 1H), 10.44 (d, J = 270.7 Hz, ^{2}H), and 9.37 (s, ^{1}H) were four signals of hydroxyl protons on the aromatic ring, 8.12 (d, J = 8.8 Hz, ^{2}H), 6.87 (d, J = 8.8 Hz, ^{2}H), 6.53 (d, J = 1.9 Hz, ^{1}H), and 6.31 (t, J = 7.8 Hz, ^{1}H) were six protons on the benzene ring. The ^{13}C NMR (101 MHz, DMSO-d6) spectra showed a carbonyl carbon and seven double bonds, attributable to the following: δ177.2 (C-4), 164.9 (C-7), 161.4 (C- 5), 157.2 (C-4′), 153.5 (C-9), 147.4 (C-2), 136.2 (C-3), 126.7 (C-2′, 6′), 121.3 (C-1′), 118.5 (C-3′,5′), 106.4 (C-10), 96.2 (C-6), and 94.6 (C-8). Its molecular formula was $C_{15}H_{10}O_6$. The data above are consistent with previous work (Chen *et al.* 2009), which showed that the compound was kaempferol. Its structure is illustrated in Fig. 3a.

The purified saponin was an amorphous powder, with a melting point 251.4 to 251.8°C and a m/z 488. There was a UV absorption peak at 207 nm. The IR spectra showed hydroxyl (3430 cm^{-1}) and aldehyde (2860 cm^{-1} and 1710 cm^{-1}) peaks. There was one carbonyl (d 9.51, J = 12.51) and two olefinic protons (d 7.63, 7.51) in the ^{1}H NMR spectra. The signals of ^{13}C NMR spectra were attributable to the following: δ203.5 (C-24), 144.3 (C-13), 122.7 (C-12), 73.5 (C-3), 72.2 (C-22), 67.4 (C-16), 65.3 (C-29), 56.7 (C-4), 46.8 (C-5), 43.4 (C-9, 18), 42.1 (C-14, 21), 40.6 (C-17, 18), 35.7 (C-8), 33.4 (C-1, 15), 32.1 (C-10), 30.3 (C-7, 20), 27.6 (C-27, 28), 25.4 (C-2), 23.5 (C-11, 30), and 17.6 (C-6, 23, 25, 26). It had a sapogenin structure named camelliagenin ($C_{30}H_{48}O_5$), which was consistent with the literature (Toshiyuki *et al.* 1999). Its structure is shown in Fig. 3b.

The average molecular weight of the polysaccharide was 12,570, based on three repetitions. It was primarily composed of six kinds of monosaccharides: arabinose (31.80 ± 0.36%), galactose (20.67 ± 0.40%), mannose (16.47 ± 0.55%), glucose (13.80 ± 0.36%), rhamnose (11.43 ± 0.41%), and xylose (5.83 ± 0.35%). These results had some

differences from the literature, probably because of the use of different plant varieties (Li *et al.* 2012).

Flavonoid glycosides and saponins are different in the varieties of *C. oleifera*, but they may have same or similar structure of flavonoid aglycone and sapogenin (Chen *et al.* 2010; Du *et al.* 2011). The extract mainly consists of one kind of flavonoid aglycone and sapogenin, suggesting that single compound could be easily obtained by this process. The major compounds isolated from the seeds of *C. oleifera* deserve to be harnessed in food and pharmaceutical industry.

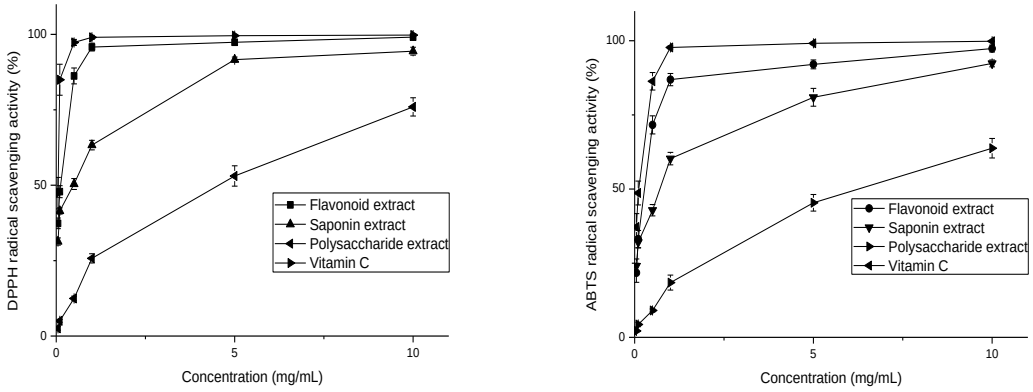


a **b**

Fig. 3. Structures of (a) the flavonoid aglycone and (b) the sapogenin

Free Radical Scavenging Activities of the Extracts

The inhibition concentrations on 50% DPPH radical (IC_{50}) of the extracts of the flavonoid, saponin, and polysaccharides were 0.12, 0.48, and 4.6 mg/mL, respectively, and their IC_{50} on ABTS were 0.27, 0.70, and 6.4 mg/mL, suggesting that the extracts all have stronger radical scavenging activities even though their IC_{50} is higher than vitamin C (0.07 mg/mL). The sequence of activity was flavonoid > saponin > polysaccharides. The results are shown in Fig. 4, which indicates that the extracts are better antioxidants with free radical scavenging activities.



a **b**

Fig. 4. (a) DPPH and (b) ABTS radical scavenging activity of the extracts ($\bar{x} \pm s$, n = 3)

The DPPH and ABTS methods have been widely used in determination of the ability of anti-free radicals in biological samples (Zheng *et al.* 2010). 1,1-diphenyl-2-picrylhydrazyl can form a stable free radical in organic solvent with typical characteristic absorption peaks, and antioxidants in the reactive system provide oxygen atoms and electrons to the DPPH radical to reduce the absorption. 2,2´-azinobis (3-ethylbenzothiazoline-6-sulfonic acid can make stable ABTS water soluble radicals by oxidation, bind to antioxidants, and cause no absorption at 734 nm. The flavonoid extract had the strongest ability of scavenging DPPH and ABTS radicals among the three extracts, probably because of the phenolic hydroxyl groups (Shaidi *et al.* 1992). Kaempferol contains four phenolic hydroxyl groups, but the saponin and the polysaccharide do not contain phenolic hydroxyl groups and have a weaker ability to eliminate free radicals.

Anti-inflammatory Activity of the Extracts

The extracts showed anti-inflammatory activities in animal test. The results are shown in Table 3. The flavonoid and the saponin had similar anti-inflammatory effects to aspirin at dose of 100 mg/kg, and the polysaccharide had weaker inhibition on inflammation, but had significant difference (p<0.01) from the normal control.

Table 3. Effect of the Extracts on Croton Oil Induced Ear Inflammation in Mice

Group	Dose (mg/kg)	Difference in weight between left and right punched ear in mg Mean ± SD (n=10)	Inhibition (%)
Normal control	/	34.2 ± 5.2	/
Aspirin	100	11.9 ± 4.3**	65.1
Flavonoid	25	29.4 ± 4.4	14.2
	100	18.1 ± 4.5**	47.2
Saponin	25	28.6 ± 5.3	16.5
	100	18.4 ± 3.9**	46.2
Polysaccharide	25	31.6 ± 5.8	7.6
	100	26.9 ± 4.2**	21.4

**, p<0.01, compared to Normal control.

Economic Efficiency Analysis

Based on the above optimized extraction technique, the yields of the flavonoid, saponin, polysaccharide, and cellulose were respectively 1.4%, 6.7%, 22.5%, and 63.0%. The former three products can be used in health foods or medicines with higher prices of $80, $50, and $30 per kilogram crude product (purity 70-80%) individually (Hang *et al.* 2007). Cellulose can be used as biofuel materials with a lower price of $0.2 per kilogram. Therefore, one kilogram of defatted seeds can yield $11.35.

Direct cost of products is made up of salary, water, and electricity expenditures, and depreciation of fixed assets. Salary is about $0.4 per kg, since the present salary is $20 per day (Xi 2009), and each worker can treat 50 kg of defatted seeds every day. Water and electricity expenditure is about $1.2 per kg, and depreciation of fixed assets is about $0.6 per kg. This leads to a total cost of $2.2 per kg.

The calculated profit becomes $9.15 per kg by subtracting total cost from sales. An annual defatted seeds production of 800,000 tons can create a value of $9.08 billion and profit of $7.32 billion. This can greatly promote the income and improve the living quality of farmers in the high-poverty mountain areas of China.

CONCLUSIONS

1. An integrated extraction process has been designed by ultrasonic assisted acid-base alternative extraction to isolate flavonoid, saponin, and polysaccharides from defatted seeds of *C. oleifera*. The technique is cost-effective because no organic solvent and expensive equipment are used in the process.
2. The extraction conditions were optimized by a response surface method. The optimal conditions were 1.6% HCl content, water/seed ratio 16, extraction time 89 min, and ultrasonic power 310 W. The yields of the flavonoid, saponin, and polysaccharides were, respectively, 1.4%, 6.7%, and 22.5%. The residue was cellulose, with a yield of 63.0%.
3. The structures of flavonoid, saponin, and polysaccharide were elucidated. The extracts could eliminate DPPH and ABTS radicals and alleviate inflammation.
4. This integrated extraction technique provided four valuable products: the flavonoid, the saponin, the polysaccharide, and the cellulose. This technique could create large profit and will not only achieve full utilization of a typically discarded bioresource, but also increase the income of farmers in the high-poverty mountain regions of China.

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