

Original Research Paper

Essential Oils from The Dropped Flowers of *Camellia japonica*: Extraction Optimization, Chemical Profile and Antibacterial Property

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Abstract: *Camellia japonica* is one of the most economically valuable species that can be taxonomically placed in the Theaceae family. Many horticultural varieties of *C. japonica* have been cultivated as ornamental trees worldwide, owing to their charming flowers. However, young *C. japonica* flowers usually drop in a “decapitated” form during the peak flowering stage, causing a resource waste. In this study, Essential Oils from the Dropped *C. japonica* Flowers (EODCJF) were extracted with hydrodistillation and process was optimized by response surface methodology. The chemical profile and antibacterial activity were tested by gas chromatography-mass spectrometry and by disc diffusion, respectively. The optimal extraction parameters were: Immersion time of 9 h, extraction time of 8 h and liquid-to-solid ratio of 39: 1 (mL/g), under these conditions, the yield was 3.20%. The actual yield was in compliance with the predicted result with an error margin of 3.61%. Thirty-three volatile compounds accounting for 94.46% were authenticated in EODCJF with alkanes of 53.96% and alcohols of 28.59%, respectively. EODCJF exerted certain antibacterial capacities against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Bacillus pumilus*. Their antibacterial activities could be contributed by the long-chain alcohols in EODCJF and alkanes may potentiate the dispersion of these liposoluble alcohols in EODCJF.

Keywords: Essential Oils, Dropped *Camellia japonica* Flowers, Extraction Optimization, Chemical Profile, Antibacterial Activity

Introduction

Essential Oils (EOs) are a kind of secondary metabolites in plants. They usually comprise diverse components that bear smaller molecular weights and aromatic odors (Zhang *et al.*, 2018a). According to the chemical profile difference, EOs can be classified into aromatics, aliphatics and terpenoids, most of which are featured by a higher volatility and a lower boiling point (Tian *et al.*, 2019). EOs exert a series of bioactivities, such as antioxidant (Imane *et al.*, 2020), antitumor (Andrade *et al.*, 2018), antimicrobial (Zhang *et al.*, 2018b), anti-inflammatory (Sen *et al.*, 2019) and immunomodulatory (Valdivieso-Ugarte *et al.*, 2019) properties. Thus the search for novel bioactive EOs remains ongoing concern.

The genus *Camellia* is native to Southeast Asia and China. Up to now, approximately 280 species of

Camellia have been found to be placed in the Theaceae family taxonomically, such as *Camellia sinensis* var. *sinensis*, *Camellia sinensis* var. *assamica*, *Camellia japonica*, *Camellia oleifera*, *Camellia chrysantha* and *Camellia reticulate* (Li *et al.*, 2019). Among them, *C. japonica* is one of the most economically valuable species. Many horticultural varieties of *C. japonica* have been developed as ornamental trees worldwide, due to the beautiful and charming flowers (Kim *et al.*, 2019; Páscoa *et al.*, 2019). In addition to horticultural application, *C. japonica* and its principles elicit various beneficially physiological and pharmacological effects. The ethanol extract from flowers of *C. japonica* exerts antioxidant capacity via scavenging excessive free radicals and reactive oxygen species and via elevating activities of antioxidases (Piao *et al.*, 2011). The quercetin glycosides, gallic acid and its glucoside conjugates isolated from the hot water extract of *C.*

japonica flowers show higher 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) radical-scavenging capacity than those of other compounds, indicating that the antioxidant activity of *C. japonica* flowers could be chiefly contributed by these bioactive phenolic acids and flavonoid glycosides (Lee *et al.*, 2011). The Oleanane triterpenes isolated from *C. japonica* flowers exhibit inhibitory effects against the replication of porcine epidemic diarrhea virus (Yang *et al.*, 2015) and the methanol extracts from the leaves and flowers of *C. japonica* display certain inhibitory effects against some food-borne pathogen and food spoilage bacteria (Lee *et al.*, 2005). However, to the best of our knowledge, there is little information on the research about the essential oils from *C. japonica* flowers.

Unlike other ornamental flowers, *C. japonica* flowers usually drop in a young “decapitated” form during the peak flowering stage, which causes a tremendous waste of this resource. In present work, Essential Oils from the Dropped *C. japonica* Flowers (EODCJF) were extracted for the first time and the extraction process was optimized. After characterization, the antibacterial potential was appraised. It is hoped that this study will highlight the comprehensive development and utilization of dropped *C. japonica* flowers.

Materials and Methods

Materials

The dropped *C. japonica* flowers were collected from April to June 2020 in the campus of Changshu Institute of Technology (Changshu, Jiangsu, China) and authenticated by Dr. Zhumei Cui, School of Biology and Food Engineering, Changshu Institute of Technology. After being dried at 50-60°C, the dried *C. japonica* flowers were deposited at the School of Biology and Food Engineering, Changshu Institute of Technology with a voucher number of SW-2020-068. Before the experiment began, dried *C. japonica* flowers were sheared and sieved to 40-mesh.

All the reagents and solvents involved were purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd (Shanghai, China) and were of analytical grade.

All the bacterial strains, including *Staphylococcus aureus* (ATCC 49775), *Escherichia coli* (ATCC 33456), *Bacillus subtilis* (ATCC 6633) and *Bacillus pumilus* (ATCC 7065) were from ATCC Global Bioresource Center (Manassas, VA, USA) and stored at -80°C in 30% (v/v) glycerol media.

Essential Oils Extraction

Essential Oils from the Dropped *C. japonica* Flowers (EODCJF) were extracted with hydrodistillation method in a Clevenger-type apparatus (Memarzadeh *et al.*, 2015). Twenty grams of dried powders of *C. japonica* flowers were immersed in a certain volume of distilled

water for several hours. After a certain time of extraction starting from water boiling, EODCJF were obtained and then stored at 0°C in a sealed vial. The extraction yield (w/w, %) was calculated according to the following formula:

$$Y(w/w, \%) = W_1(g) / W_2(g) \times 100$$

Where:

$Y(w/w, \%)$ = Extraction yield of EODCJF (w/w, %)

W_1 = The weight of obtained essential oils (g)

W_2 = The weight of dried powders of *C. japonica* flowers (g)

Experimental Design for Extraction Optimization

First, single-factor experiment was performed to assess the effects of three independent variables, including immersion time (2-12 h), liquid-to-solid ratio (10-60: 1 mL/g) and extraction time (2-12 h) on EODCJF yield. Then, the extraction process was optimized with Response Surface Methodology (RSM) (Cui *et al.*, 2018). A three-factor-three-level Box-Behnken Design (BBD) encompassing seventeen runs was applied at the center point (Table 1).

Regression analysis was conducted to analyze data and fitted to the second-order polynomial model as below:

$$Y = \beta_0 + \sum_{i=1}^4 \beta_i X_i + \sum_{i=1}^4 \beta_{ii} X_i^2 + \sum_{i=1}^3 \sum_{j=i+1}^4 \beta_{ij} X_i X_j$$

Where:

Y = The predicted extraction yield of EODCJF

β_0 = An intercept

β_i = The linear coefficient

β_{ii} = The quadratic coefficient

β_{ij} = The interactive term

X_i and X_j = The coded independent variables

The Chemical Profile of Essential Oils

The chemical composition of EODCJF was determined by a gas chromatography-mass spectrometry (GCMS-QP2010, Shimadzu, Kyoto, Japan) equipped with a Rxi-5Sil MS column (30 m × 0.25 mm ID, 0.25 μm of film thickness) and interfaced with an ion trap detector. Analysis was operated under the following conditions: (1) Helium was adopted as the carrier gas at a flow rate of 1.0 mL/min. (2) Temperatures of transfer line and ion trap were set as 250 and 230°C, respectively. (3) Column temperature was initially raised from 40 to 60°C at 5°C/min and kept for 2 min, then elevated to 110°C at 15°C/min and held for 4 min, from 110 to 180°C at 5°C/min and maintained for 2 min, finally increased up to 280°C at 25°C/min and kept for 5 min. One microliter of EODCJF diethyl ether solution at concentration of 10

mg/mL was injected into the analyzer at a split ratio of 1: 30 under a scan range of 35-500 amu and an electron ionization energy of 70 eV. Compound identification was assigned by referring the retention index of *n*-alkanes (C₈-C₄₀) standards and by comparing the mass spectrum with NIST 05 database or with reported literatures (Tian *et al.*, 2019).

The Antibacterial Activity of Essential Oils

The paper disks with diameter of 6 mm were soaked by EODCJF in Dimethyl Sulfoxide (DMSO) and placed on the surface of solid medium that had been inoculated with the test bacteria (*S. aureus*, *E. coli*, *B. subtilis* and *B. pumilus*) at 6.0 log CFU/mL. Normal saline in Dimethyl Sulfoxide (DMSO) and chloramphenicol in DMSO were served as negative and positive controls, respectively. All the solid medium plates were cultured at 37°C for 24 h. Diameter of inhibition zones was recorded (Zhang *et al.*, 2018b).

Statistical Analysis

Extraction optimization was designed and analyzed with a Design-Expert software 8.0.6.1 (Stat-Ease, Minneapolis, MN, USA). Experimental data was represented as means or means ± SD (standard deviation). SPSS19.0 Software (SPSS Inc., Chicago, USA) was used to appraise the statistical difference. The significance of distances between means was evaluated with *t*-test and one-way Analysis Of Variance (ANOVA).

Results

The Single-Factor Experiments for EODCJF Extraction

Effects of Immersion Time on Yield

As shown in Fig. 1, when liquid-to-solid ratio and extraction time were fixed as 40: 1 (mL/g) and 8 h, respectively, EODCJF yield increased with the prolongation of immersion time ranging from 2 to 8 h, but when immersion time exceeded 8 h, yield decreased. The optimal immersion time was 8 h.

Effects of Extraction Time on Yield

Fix liquid-to-solid ratio of 40: 1 (mL/g) and immersion time of 8 h to explore the effects of extraction time on yield. As shown in Fig. 2, in the range of 2-8 h, EODCJF yield was raised with the increase of extraction time, maximized to 2.83±0.12% at 8 h and then declined with lengthening extraction time.

Effect of Liquid-to-Solid Ratio on Yield

As shown in Fig. 3, when immersion time and extraction time were all set as 8 h, EODCJF yield was heightened within the liquid-to-solid ratio of 10:1 (mL/g)-40: 1 (mL/g), maximized to 3.3±0.1% at the ratio

of 40: 1 (mL/g) and then dropped with the elevation of liquid-to-solid ratio.

The Extraction Optimization of EODCJF with RSM

Based on principles of BBD, according to the results of single-factor experiments, three independent variables, including immersion time (h, X_1), extraction time (h, X_2) and liquid-to-solid ratio (mL/g, X_3) were selected and applied to RSM optimization with EODCJF extraction yield as response variable. Seventeen possible orderings and response values were achieved and represented in Table 2. It can be observed that the EODCJF yields were within a range of 1.88-3.30%, which can be described by the following second-order polynomial equation:

$$Y = 3.26 + 0.29X_1 + 0.13X_2 - 0.032X_3 + 0.11X_1X_2 - 0.04X_1X_3 - 0.02X_2X_3 - 0.44X_1^2 - 0.68X_2^2 - 0.33X_3^2$$

Where:

Y = EODCJF yield (w/w, %)

X_1 = The immersion time (h)

X_2 = The extraction time (h)

X_3 = Liquid-to-solid ratio (mL/g)

The one-way Analysis Of Variance (ANOVA) for fitted equation was shown in Table 3. For the source of model, the high *F*-value (148.11) and low *P*-value (<0.0001) demonstrated that the obtained model is highly significant and the regression equation is desirable (Cheng *et al.*, 2017). The determination coefficient of model (R^2) and adjusted coefficient (Adj R^2) were 0.9948 and 0.9881, respectively. These values implied that the accuracy of model is sufficient to interpret 98.81% of the total variability of model response. For lack of fit, the *F*-value and *P*-value were all greater than 0.05, indicating a non-significance, which further validated the model. The linear terms X_1 and X_2 were highly significant ($P < 0.01$) and X_3 was not significant ($P > 0.05$). The quadratic parameters X_1^2 , X_2^2 and X_3^2 were all highly significant ($P < 0.01$). The interaction parameters X_1X_2 were highly significant ($P < 0.01$), but X_1X_3 and X_2X_3 were not significant ($P > 0.05$).

To better understand the interaction effects between two independent variables, the contour plots (Figs. 4 to 6) were achieved by RSM. From Fig. 4, it can be clearly seen that the increase in immersion time or extraction time potentiated the improvement of EODCJF yield. In Fig. 5, EODCJF yield increased with the increase of immersion time or liquid-to-solid ratio in the initial stage, but experienced a slow elevation with the further increase of any parameters. From Fig. 6, we can observe that EODCJF yield was raised with increasing extraction time or liquid-to-solid ratio at first, but obviously descended afterwards. These results and trends were consistent with those of single-factor experiments.

The optimized extraction parameters obtained from Design-Expert 8.0.6.1 were as follows: Immersion time of 8.69 h, extraction time of 8.24 h, liquid-to-solid ratio of 39.26: 1 (mL/g) and the predicted yield was 3.32%. In order to facilitate practical operation, the predicted optimal conditions were further modified as: Immersion time of 9 h, extraction time of 8 h and liquid-to-solid ratio of 39: 1 (mL/g), under which the actual yield was 3.20%. The actual results were in line with the predicted data with an error margin of 3.61%.

The Chemical Profile of EODCJF

As shown in Table 4, thirty-three volatile compounds accounting for 94.46% were authenticated in EODCJF with GC-MS. It can be noted that EODCJF are abundant in alkanes and alcohols,

corresponding to 53.96 and 28.59% of the total amount, respectively. *N*-tetracosane was the volatile compound with highest content identified in EODCJF (27.7%), followed by 1-tetracosanol (14.3%), *N*-nonacosane (9.03%) and phytol (7.92%).

The In Vitro Antibacterial Activity of EODCJF

As shown in Table 5 and Fig. 7, EODCJF elicited certain antibacterial capacities against *E. coli*, *B. pumilus*, *B. subtilis* and *S. aureus*. The inhibition zone diameters of EODCJF against these test bacteria were larger than those of normal control, but smaller than those of positive control, indicating that the antibacterial activities of EODCJF against these bacteria are inferior to those of chloramphenicol, a well-known chemically obtained antibacterial agent.

Table 2: Box-Behnken design and observed responses

Run	Independent variables			Y
	X ₁	X ₂	X ₃	
1	1	1	0	2.60
2	-1	0	-1	2.17
3	0	-1	-1	2.10
4	0	0	0	3.27
5	0	-1	1	2.10
6	0	0	0	3.20
7	-1	0	1	2.16
8	1	-1	0	2.17
9	-1	-1	0	1.90
10	0	0	0	3.30
11	0	0	0	3.27
12	1	0	1	2.73
13	1	0	-1	2.90
14	0	1	-1	2.44
15	0	1	1	2.36
16	0	0	0	3.26
17	-1	1	0	1.88

Table 3: Test of significance for regression coefficients^a

Source ^b	Sum of Squares	Mean Square	F-Value	P-Value	Significance ^c
Model	4.41	0.49	148.11	<0.0001	**
X ₁	0.66	0.66	198.00	<0.0001	**
X ₂	0.13	0.13	38.52	0.0004	**
X ₃	8.45×10 ⁻³	8.45×10 ⁻³	2.55	0.1542	n.s.
X ₁ X ₂	0.051	0.051	15.29	0.0058	**
X ₁ X ₃	6.40×10 ⁻³	6.40×10 ⁻³	1.93	0.2070	n.s.
X ₂ X ₃	1.60×10 ⁻³	1.60×10 ⁻³	0.48	0.5094	n.s.
X ₁ ²	0.82	0.82	247.62	<0.0001	**
X ₂ ²	1.95	1.95	590.24	<0.0001	**
X ₃ ²	0.46	0.46	137.45	<0.0001	**
Residual	0.023	3.31×10 ⁻³			
Lack of Fit	0.018	5.93×10 ⁻³	4.39	0.0935	n.s.
Pure Error	5.40×10 ⁻³	1.35×10 ⁻³			
Cor Total	4.44				
R-Squared	0.9948				
Adj R-Squared	0.9881				

^aResults were analyzed by design expert 8.0.6.1 software

^bX₁: Immersion time (h); X₂: Extraction time (h); X₃: Liquid-to-solid ratio (mL/g)

^c**P<0.01 highly significant; n.s.: Not significant

Table 4: The chemical compositions of EODCJF

No.	Compound	RI	RI*	Molecular formula	Content (%)
1	1-(2-Hydroxy-5-methylphenyl) ethanone	1363	1419	C ₉ H ₁₀ O ₂	0.21
2	<i>N</i> -Octadecane	1810	1812	C ₁₈ H ₃₈	0.12
3	<i>N</i> -Nonadecane	1910	1906	C ₁₉ H ₄₀	0.09
4	Stearaldehyde	1999	1911	C ₁₈ H ₃₆ O	0.57
5	2-Nonadecanone	2045	2052	C ₁₉ H ₃₈ O	0.12
6	Dibutyl phthalate	2037	2077	C ₁₆ H ₂₂ O ₄	0.12
7	Diocetyl ether	2146	2170	C ₁₆ H ₃₄ O	0.16
8	Ethyl stearate	2177	2196	C ₂₀ H ₄₀ O ₂	0.09
9	Tetraprenol	2192	2203	C ₂₀ H ₃₄ O	1.18
10	Phytol	2208	2219	C ₂₀ H ₄₀ O	7.92
11	Cycloheptadecanol	2226	2228	C ₁₇ H ₃₄ O	0.20
12	Ambrettolid	2246	2272	C ₁₆ H ₂₈ O ₂	0.16
13	Muscalure	2315	2379	C ₂₃ H ₄₆	0.86
14	Diocetyl fumarate	2395	2387	C ₂₀ H ₃₆ O ₄	0.20
15	<i>N</i> -Octadecane	2413	2453	C ₁₈ H ₃₈	0.29
16	Henicosyl formate	2472	2477	C ₂₂ H ₄₄ O ₂	5.43
17	<i>N</i> -Pentacosane	2506	2501	C ₂₅ H ₅₂	6.11
18	Erucic acid	2572	2532	C ₂₂ H ₄₂ O ₂	0.36
19	<i>N</i> -Eicosane	2605	2568	C ₂₀ H ₄₂	0.64
20	9-Hexacosene	2614	2580	C ₂₆ H ₅₂	2.39
21	<i>N</i> -Tetracosane	2637	2603	C ₂₄ H ₅₀	27.7
22	1-Tetracosanol	2681	2650	C ₂₄ H ₅₀ O	14.3
23	Squalane	2702	2714	C ₃₀ H ₆₂	0.40
24	1-Pentacosanol	2772	2802	C ₂₅ H ₅₂ O	3.48
25	Ditetradecyl ether	2880	2850	C ₂₈ H ₅₈ O	0.14
26	<i>N</i> -Nonacosane	2924	2902	C ₂₉ H ₆₀	9.03
27	Squalene	2951	2912	C ₃₀ H ₅₀	1.10
28	<i>N</i> -Triacotane	3003	2918	C ₃₀ H ₆₂	2.97
29	1-Octacosanol	3047	3036	C ₂₈ H ₅₈ O	0.74
30	<i>N</i> -Dotriacotane	3102	3079	C ₃₂ H ₆₆	4.58
31	1-Triacotanol	3246	3107	C ₃₀ H ₆₂ O	0.77
32	<i>N</i> -Tritriacotane	3283	3274	C ₃₃ H ₆₈	1.87
33	<i>N</i> -Tetratriacontane	3312	3298	C ₃₄ H ₇₀	0.16
Total	94.46				
Alkanes	53.96				
Alcohols	28.59				
Carboxyl acids and esters	6.36				
Alkenes	4.35				
Others	1.20				

RI: Retention Index from NIST 05 database; RI*: Retention Index reckoned by referring to that of C₈-C₄₀ *n*-alkanes standard. EODCJF: Essential Oils from the Dropped *C. japonica* Flowers

Table 5: The *in vitro* antibacterial activity of EODCJF

Bacteria	Diameters of inhibition zone (mm)		
	EODCJF	NC	PC
<i>E. coli</i>	7.8	n.d.	10.9
<i>B. pumilus</i>	7.5	n.d.	9.2
<i>B. subtilis</i>	7.4	n.d.	8.9
<i>S. aureus</i>	7.2	n.d.	8.0

Data were expressed as the means (n = 3). EODCJF: Essential Oils from the Dropped *C. japonica* Flowers; NC: Normal Control (normal saline); PC: Positive Control (chloramphenicol); n.d.: Not detected

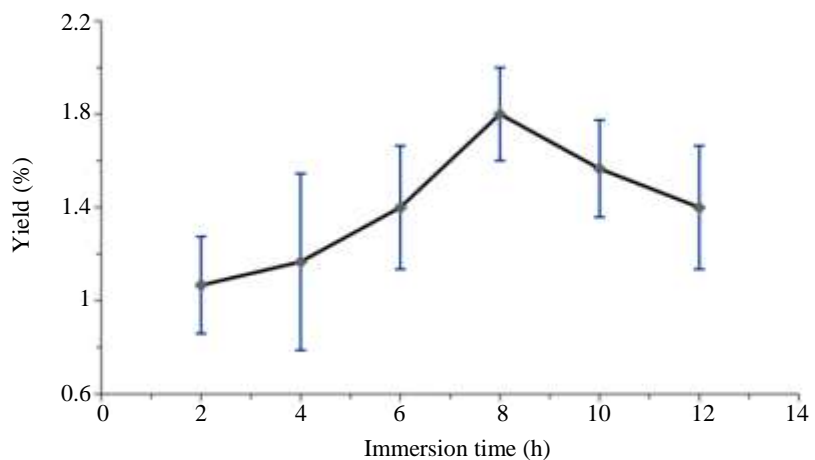


Fig. 1: Effects of immersion time on yield

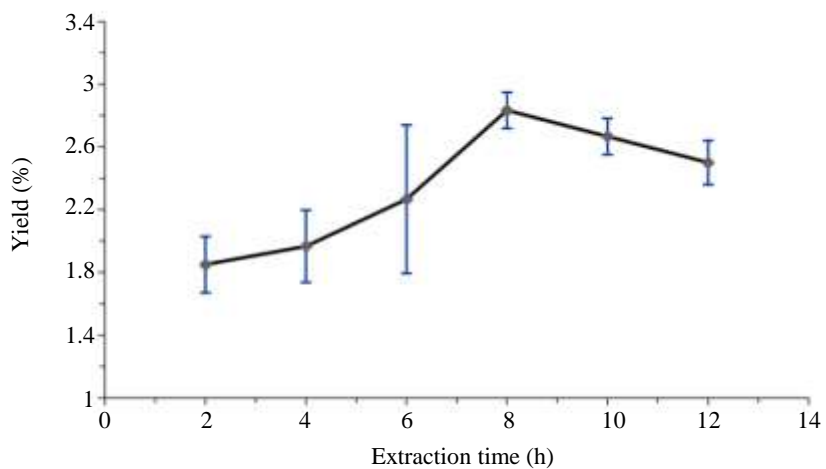


Fig. 2: Effects of extraction time on yield

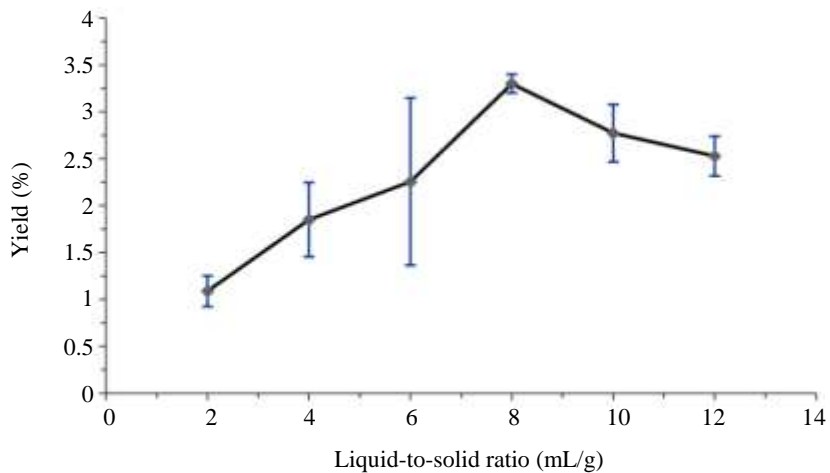


Fig. 3: Effects of liquid-to-solid ratio on yield

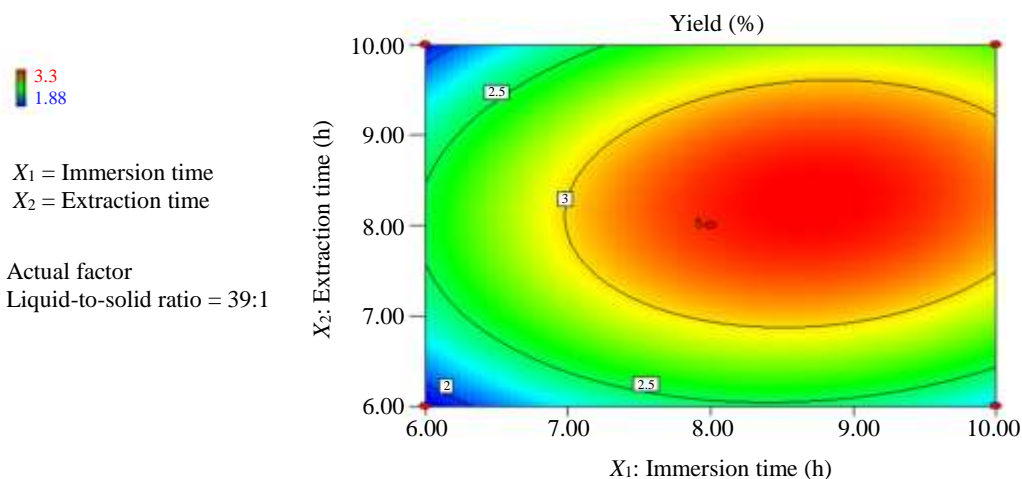


Fig. 4: Contour plot displaying the interaction effect of immersion time (h) and extraction time (h) on the yield of EODCJF

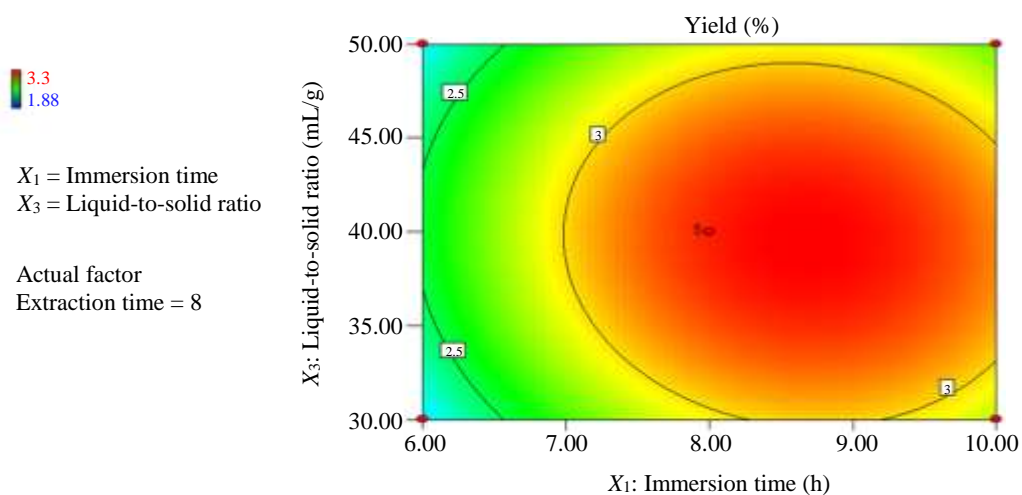


Fig. 5: Contour plot displaying the interaction effect of immersion time (h) and liquid-to-solid ratio (mL/g) on the yield of EODCJF

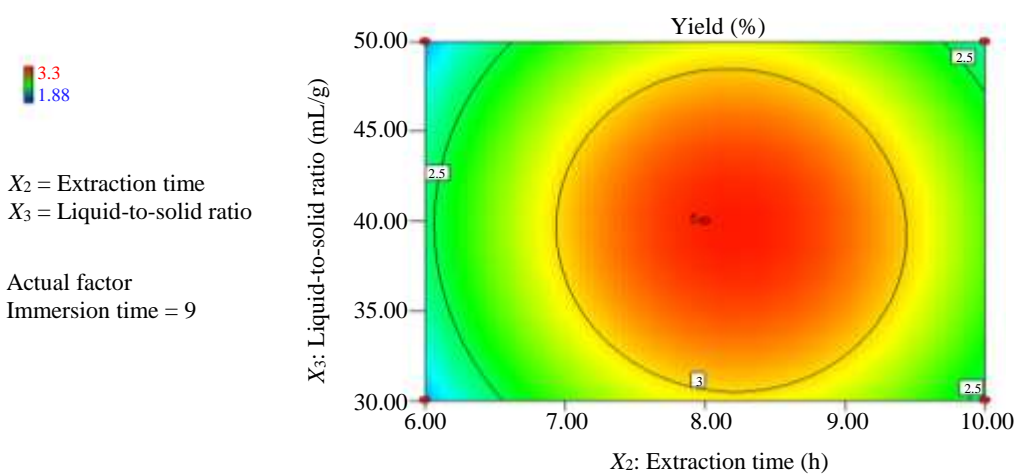


Fig. 6: Contour plot displaying the interaction effect of extraction time (h) and liquid-to-solid ratio (mL/g) on the yield of EODCJF

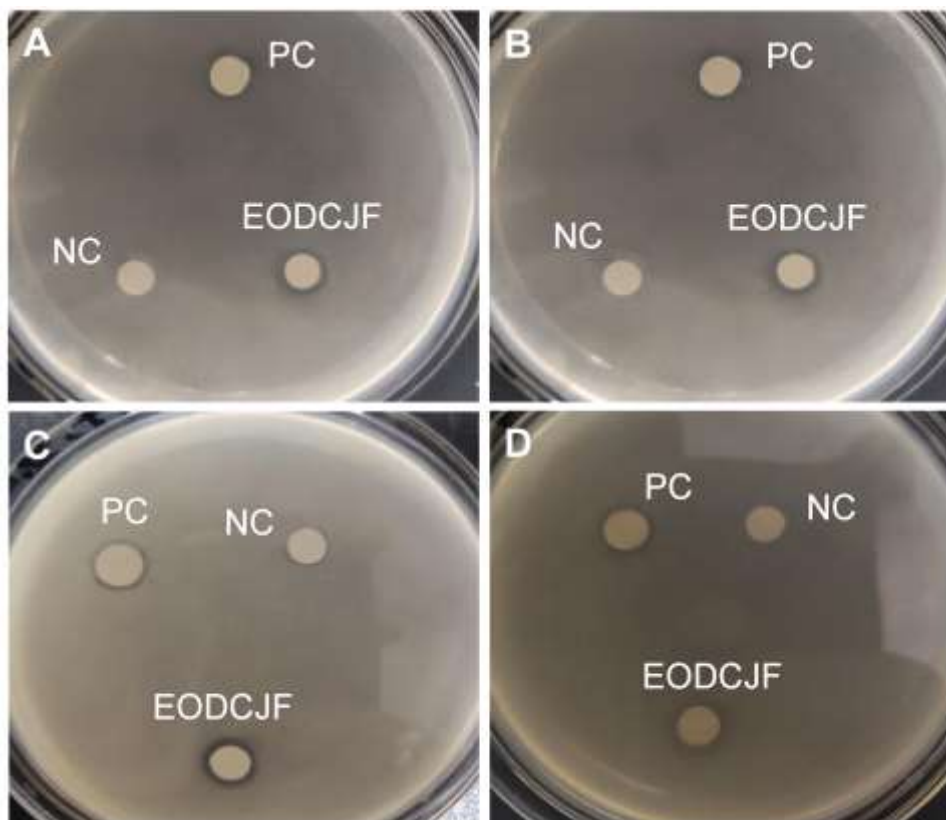


Fig. 7: Selected pictures of discs diffusion. A: *E. coli*; B: *B. pumilus*; C: *B. subtilis*; D: *S. aureus*.

Discussion

At the beginning, single-factor experiments for EODCJF extraction were conducted for obtaining the independent variables of RSM optimization. From Figs. 1 to 3, we can observe that prolonged pre-immersion and extraction time as well as overlarge liquid-to-solid ratio lead to a sharp decline in EODCJF yield. These unexpected phenomena could be explained by the following reasons: (1) pre-immersion of plant materials is beneficial for the dispersion of essential oils in water, thereby increasing extraction yield, however, overtime immersion before extraction would cause the loss of volatile compounds, leading to a decrease in extraction yield (Hui *et al.*, 2015); (2) long-term hydrodistillation at water boiling temperature would alter the chemical compositions of essential oils and make certain substances that are vulnerable to heating degrade, thus causing a decline in extraction yield (Zheljzakov *et al.*, 2014); (3) larger amount of water could result in an excessive swelling of raw materials to absorb some substances in EODCJF, leading to a decrease in extraction yield (Xiao *et al.*, 2008).

Then, a three-factor-three-level RSM was achieved using BBD, followed by giving the predicted optimal

parameters for EODCJF extraction. Under the predicted optimal extraction conditions, the actual yield of EODCJF was almostly equivalent to the predicted yield, and the error margin was smaller than $\pm 5\%$, indicating that RSM coupled with BBD could be a feasible way to optimize the extraction parameters of EODCJF.

GC-MS was performed to disclose the chemical profile of EODCJF (Table 4). The major volatile ingredients in the Essential Oils from *C. japonica* Seeds (EOCJS) are quite different from those of EODCJF. α -Terpineol, α -terpenyl acetate, α -terpinolene, α -pinene and β -pinene were observed to be present more in EOCJS (Ramachandran *et al.*, 2020). The significant differences on the chemical profiles of the essential oils derived from different parts of *C. japonica* would be due to the fact that biological components in plant are apt to assemble in a manner of tissue-specificity and different metabolic enzymes that are responsible for the modulation of secondary metabolites biosynthesis are expressed in different plant organs (Ji *et al.*, 2016). Except *C. japonica*, variations on the essential oils from different parts for a same species were also found in *Blumea balsamifera*, *Ruta chalepensis*, *Artemisia persica* and so on (Tian *et al.*, 2019).

The antibacterial capacities of EODCJF against *E. coli*, *B. pumilus*, *B. subtilis*, and *S. aureus* could be partially contributed by their long-chain alcohols (Kubo *et al.*, 1995). As summarized in Table 4, the total amount of alcohols in EODCJF was found to be 28.59% and overwhelming majority of them can be classified as long-chain alcohols. Meanwhile, according to similarity and intermiscibility principle, the abundant alkanes (Table 4) may ameliorate the dispersity of these liposoluble alcohols in EODCJF as benign solvents, thereby enhancing the interactions with test bacteria (Zheng *et al.*, 2019). Whether or not EODCJF exerts inhibitory effects on other bacteria, such as fungus deserves further investigation. Besides, the determination of minimal inhibitory concentration and the underlying antibacterial mechanisms are equally needed to be explored.

Conclusion

In order to utilize the young dropped flowers of *C. japonica*, a commonly cultivated ornamental tree. In present investigation, the extraction optimization, chemical profile and antibacterial potential of EODCJF were explored for the first time. After being optimized by RSM and BBD, the yield of EODCJF increased up to 3.20%. EODCJF elicited certain inhibitory effects on *E. coli*, *B. subtilis*, *B. pumilus* and *S. aureus*. The antibacterial capacity could be contributed by the long-chain alcohols present in EODCJF. Further studies are still pending: (1) Antimicrobial screenings with wider bacteria spectrum; (2) The underlying antibacterial mechanisms; (3) Fractionate EODCJF to disclose the structure-activity relationship; (4) Comparison on the chemical constituents with the essential oils from fresh *C. japonica* flowers, etc. It is hoped that this work would highlight the comprehensive utilization and development of wasted *C. japonica* flowers.

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Author's Contributions

Yunqi Kong, Gaochao Wang and Xinyu Wang: Carried out the extraction and process optimization as well as wrote the paper.

Tong Wang, Jieyang Shen and Aibe Zhang: Performed the chemical profile and antibacterial assessment.

Yang Zhang and Lixue Zheng: Conceived the project and designed the experiments.

Yang Zhang: Revised and polished the manuscript.

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