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ORIGINAL ARTICLE

GC×GC–MS analysis of volatile components extracted by spinning cone column from *Forsythia suspensa* and its antimicrobial assessment

Zheng-Guang Sun[1], Li He[1], Shuai Zhang[2] and Hai-Jian Su[1,2*]

[1]Technology Center, China Tobacco Jiangxi Industrial Co., Ltd., Nanchang 330000, People's Republic of China
[2]Technology Center, China Tobacco Shandong Industrial Co., Ltd., Qingdao 266000, People's Republic of China

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Abstract: *Forsythia suspensa* is a traditional medicinal plant rich in volatile bioactive compounds with important pharmacological and application value. In this study, volatile compounds from *F. suspensa* were extracted using spinning cone column (SCC) technology for the first time. The compounds were qualitatively characterized using gas chromatography–mass spectrometry (GC–MS) and comprehensive two-dimensional gas chromatography–mass spectrometry (GC×GC–MS), as well as quantified using the area normalization method. The antibacterial activity of the resulting essential oil against six pathogenic microorganisms was evaluated using the broth microdilution method. The essential oil yield obtained through SCC extraction reached 2.16%. A total of 38 compounds were identified from the essential oil, with β-pinene, 4-terpinenol, sabinene, and α-pinene as the major constituents. The GC×GC–MS analysis enabled the detection of 90 compounds, including those identified by GC–MS, and revealed additional bioactive compounds, such as guaiacol, myrtenal, citronellol, carvacrol, and α-bisabolol, that were not detected by GC–MS. The antimicrobial assays demonstrated that the essential oil exhibited antimicrobial activity against susceptible test microorganisms, with minimum inhibitory concentration values ranging from 31.3 µg/mL to 62.5 µg/mL. These findings highlighted the potential of SCC technology for extracting *F. suspensa* essential oil and demonstrated the value of GC×GC–MS for comprehensive characterization of its volatile constituents, providing a basis for further investigation of its antibacterial potential.

Keywords: *Forsythia suspensa*, volatile compounds, spinning cone column, comprehensive two-dimensional gas chromatography–mass spectrometry, antimicrobial activity

1 Introduction

Forsythia suspensa (Thunb.) Vahl, a member of the Oleaceae family, produces dried fruits widely utilized in traditional Chinese medicine and as a food additive (Jiao et al., 2012). Previous studies have reported that *F. suspensa* essential oil exhibits various biological activities, including antibacterial, antiviral, antioxidant, and anti-inflammatory effects (Dong et al., 2017; Wang et al., 2018; Kang et al., 2008), indicating its potential applications in the healthcare, pharmaceutical, and food industries. Recent studies have further demonstrated the antimicrobial and insecticidal potential of *F. suspensa* essential oil, highlighting its potential as a natural bioactive ingredient (Kačániová et al., 2026). Conventional methods for extracting *F. suspensa* essential oil include steam distillation, microwave-assisted hydrodistillation, and supercritical carbon dioxide (CO_2) extraction (Ni et al., 2009). However, these methods may have limitations associated with processing time, thermal exposure, or operational requirements (EI Horsy et al., 2026). To improve extraction efficiency and bioactive compound recovery, alternative approaches, including ultrasound-assisted extraction, ultrasonic nebulization extraction, and ionic liquid-mediated microwave-assisted extraction, have been investigated (Ming et al., 2019; Yang et al., 2014; Xu et al., 2026). Despite these studies, which have primarily focused on extraction optimization and the recovery of bioactive constituents, the application of low-temperature vacuum separation techniques to *F. suspensa* volatiles remains relatively unexplored. Furthermore, spinning cone column (SCC) technology has attracted increasing attention due to its unique low-temperature separation characteristics. Upon combining centrifugal forces generated by rotating cones with vacuum operation, SCC enables the rapid separation and recovery of volatile compounds under reduced pressure, potentially

*Corresponding Author: Hai-Jian Su. Email: qdsuhj@163.com
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minimizing thermal degradation and volatile losses. These characteristics offer potential advantages in processing efficiency as well as aroma preservation, and SCC technology has been applied in the food, beverage, flavor, and fragrance industries (Yang et al., 2025; Sun et al., 2024). To our knowledge, SCC technology has not previously been applied to recover volatile constituents from *F. suspensa*.

The chemical characterization of *F.* suspensa essential oil has primarily relied on gas chromatography–mass spectrometry (GC–MS). Studies have shown that its volatile composition varies considerably with fruit maturity and extraction conditions, particularly in the relative abundances of monoterpenes and oxygenated compounds (Xie et al., 2025; Ming et al., 2019). Recent investigations have also characterized the chemical composition and biological activities of *F. suspensa* essential oils obtained using different extraction methods (Xu et al., 2026; Kačániová et al., 2026). For example, Tian et al. identified 39 volatile compounds in *F. suspensa* essential oil using conventional GC–MS (Tian et al., 2018). However, the complex composition of essential oils, particularly the presence of structurally similar and co-eluting peaks, can limit the separation and identification capabilities of conventional one-dimensional GC–MS. Conversely, comprehensive two-dimensional gas chromatography–mass spectrometry (GC×GC–MS) combines two chromatographic columns with different stationary-phase selectivities and a modulator to enhance chromatographic resolution and peak capacity. This technique enables more comprehensive characterization of complex volatile mixtures by improving the separation of co-eluting compounds and, in some cases, structural isomers. Consequently, GC×GC–MS has emerged as a powerful tool for characterizing complex volatile mixtures, including essential volatile oils (Jin et al., 2026). However, systematic characterization of *F. suspensa* essential oil using GC×GC–MS has not yet been reported.

In this study, volatile components from *F. suspensa* were extracted using SCC technology and characterized using GC–MS and GC×GC–MS. The antibacterial activity of the resulting essential oil was also evaluated. Compound identification was based on mass spectral matching, retention indices (RI), and comparison with authentic standards, while relative compound abundances were determined using the area normalization method. A total of 90 volatile compounds were identified. The results demonstrated the potential of SCC technology for recovering *F.* suspensa volatiles and provided a more detailed characterization of their chemical composition using complementary chromatographic techniques. These findings elucidated the volatile profile of *F. suspensa* and provided a basis for further investigation of its potential applications.

2 Materials and Methods

2.1 Plant Material

F. suspensa was collected from Lushi County, Henan Province, China, in September 2025. The plant specimen has been submitted to Institute of Botany, Chinese Academy of Sciences, where it was identified and authenticated as *Forsythia suspensa* (Thunb.). The voucher specimen has been deposited in this herbarium (Index Herbariorum acronym: PE) under accession number PE02726057.

2.2 Isolation of Essential Oil (EO)

A 20 kg sample of *F. suspensa* was ground using a pulverizer, mixed with 200 kg of purified water, and soaked for 15 h. The resulting mixture was transferred to the feed tank of the SCC system. The feedstock was preheated to 50°C and introduced at the top of the cone assembly. The bottom and overhead temperatures were maintained at 60°C and 45°C, respectively. The column efficiency was set to 8%, and the feed flow rate was maintained at 500 kg/h. The extraction process lasted 0.5 h. The volatile components were condensed using the overhead condensation system and collected, while the residual material was discharged from the bottom of the main column. The process yielded 38 kg of extract liquid. After static phase separation, the upper oil layer was collected, dried over anhydrous sodium sulfate, and weighed. The obtained essential oil was 0.432 g, with an extraction yield of 2.16% (w/w). Aliquots (0.1 mL) of the essential oil were collected separately from the upper, middle, and lower fractions of the collected oil and diluted to 2 mL with dichloromethane for subsequent compositional analysis.

2.3 GC–MS Analysis

The volatile compounds in the essential oil were qualitatively analyzed using a GC–MS system (8890A-5977C, Agilent Technologies, Santa Clara, CA, USA) equipped with a DB-5ms capillary column (60 m × 0.25 mm × 0.25 μM). High-purity helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature was initially maintained at 50°C for 5 min, increased to 280°C at a rate of 5°C/min, and held at 280°C for 5 min. The injector temperature was set to 280°C, with a split ratio of 10:1. Mass spectra were acquired at an electron ionization energy of 70 eV over an m/z range of 35–450.

2.4 GC×GC–MS Analysis

The volatile constituents of the essential oil were qualitatively analyzed using a GCxGC–MS system (8890–5977C, Agilent Technologies, Santa Clara, CA, USA) equipped with an SSM1810 solid-state modulator (J&X Technologies, Shanghai, China), following a previously reported method with modifications (Jin et al., 2026). The two-dimensional separation system comprised a nonpolar DB-5ms column in the first dimension (30 m × 0.25 mm × 0.25 μM) and a mid-polarity DB-17 column in the second dimension (2 m × 0.18 mm × 0.18 μM), both supplied by Agilent Technologies. The SSM1810 modulator was positioned between the two columns to enable periodic thermal modulation, with a modulation period of 6 s. Ultrahigh-purity helium (99.999% purity) was used as the carrier gas at a constant flow rate of 1.0 mL/min. Samples were introduced using splitless injection. The oven temperature was initially maintained at 50°C for 5.0 min, increased to 280°C at a rate of 5°C/min, and held at 280°C for 5 min. The detector and transfer line

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temperatures were set to 280°C, and the quadrupole temperature was maintained at 150°C. Electron ionization was conducted at 70 eV, with the ion source temperature set to 230°C. Mass spectra were acquired in full-scan mode over an m/z range of 35–450. Notably, the GC×GC–MS system was used specifically to improve the separation and qualitative verification of compounds that were not adequately resolved by conventional one-dimensional GC–MS.

2.5 Quantitative Analysis

The volatile constituents of the essential oil were quantified using an Agilent 6890N gas chromatograph equipped with a flame ionization detector (GC–FID). The chromatographic conditions and analytical column were consistent with those used for GC–MS analysis. The relative content of each volatile constituent was calculated using the peak-area normalization method. All measurements were conducted in triplicate, and the results were expressed as mean values.

2.6 Antimicrobial Activity Assay

The antibacterial activity of the essential oil was evaluated using the broth microdilution method, with modifications to a previously reported procedure (Gürer et al., 2026). Detailed experimental procedures are provided in the Supplementary Materials.

3 Results and Discussion

3.1 Extraction Yield of Essential Oil

In this study, *F. suspensa* was extracted using SCC technology, yielding 432 g of essential oil, corresponding to an extraction yield of 2.16%. A literature search of the China National Knowledge Infrastructure, Web of Science, and PubMed databases using the keywords "*Forsythia suspensa*", "essential oil", and "volatile components" identified reported essential oil yields of 0.6%–1.5% for conventional steam distillation and supercritical CO_2 extraction. Conversely, microwave-assisted steam distillation yielded ~ 2.0% (Jiao et al., 2012). However, the practical application of microwave-assisted extraction at the industrial scale remains limited. The SCC technology combines low-temperature, low-pressure mass transfer with dynamic thin-film separation and multistage countercurrent processing. These features facilitated the recovery of a broad range of volatile constituents, particularly low-boiling and thermosensitive compounds that are susceptible to losses or degradation during conventional extractions. To examine differences in the recovery of such constituents, representative oxygenated monoterpenes in the SCC-extracted oil were compared with the composition of *F. suspensa* essential oil obtained via hydrodistillation, as reported by Jiao et al. (2012). The reported relative contents in the hydrodistilled oil were 1.62% for 4-terpinenol, 4.92% for linalool, 4.35% for α-terpineol, and 3.95% for camphor. In comparison, the SCC-extracted oil contained a substantially higher relative content of 4-terpinenol (20.60%), whereas the relative contents of linalool (0.11%) and α-terpineol (1.74%) were lower. The related compound 4-thujanol accounted for 3.01% (Table 1). Additionally, guaiacol, citronellol, and carvacrol were detected in SCC-extracted oil but were not reported in the reference hydrodistilled oil. The enrichment of 4-terpinenol suggests that SCC extraction facilitated the recovery of certain volatile constituents under the conditions employed. However, the differences in chemical composition between the two oils cannot be attributed solely to the extraction technique because botanical origin, harvest stage, raw material particle size, extraction duration, and liquid–solid ratio also influenced the results. Accordingly, the 2.16% yield obtained using SCC is presented as a reference value rather than definitive evidence of superior performance relative to steam distillation, supercritical CO_2 extraction, or microwave-assisted distillation. Furthermore, SCC equipment is available in three specifications, with maximum processing capacities of 100 kg/h, 1000 kg/h, and 10000 kg/h. This range of capacities suggests potential applicability at different production scales, although practical suitability depends on the specific processing requirements and operating conditions.

3.2 GC–MS Analysis of Chemical Components of F. suspensa EO Extracted by SCC

The chemical constituents of the essential oil extracted from *F. suspensa* using SCC technology were analyzed through GC–MS. Compounds were identified through mass spectral library matching, retention index comparison, and verification against authentic standards, where available. A total of 38 compounds were successfully identified, and their relative contents are summarized in Table 1. Terpenes and alcohols were the predominant chemical classes, accounting for 58.54% and 35.57%, respectively, whereas alkanes, aldehydes, ketones, and esters occurred at comparatively low relative abundances. The major individual constituents were β-pinene (28.13%), 4-terpinenol (20.6%), sabinene (12.25%), α-pinene (6.51%), limonene (5.63%), and 1,8-cineole (eucalyptol, 3.12%). In comparison with previously reported profiles of *F. suspensa* essential oil studies (Jiao et al., 2012; Dong et al., 2017; Wang et al., 2018), the SCC-extracted oil differed in both the identified constituents and their relative abundances. Although β-pinene, α-pinene, limonene, and α-terpineol have been reported previously, the oil obtained in this study exhibited remarkably high contents of 4-terpinenol and sabinene. Additionally, eucalyptol was identified in the oil analyzed in this study; to our knowledge, this compound has not been reported previously in *F. suspensa* essential oil. These compositional variations are closely associated with the unique mass-transfer characteristics of SCC technology. Furthermore, during operation, centrifugal forces distribute the feed liquid over rotating cones, forming thin liquid films, increasing the interfacial area available for mass transfer, and shortening diffusion distances. Cone rotation also enhanced liquid mixing and reduced mass-transfer limitations at the liquid–liquid or liquid–solid interface, depending on the extraction system. These effects facilitated the transfer of volatile constituents into the recovered extracts. Studies have described the mass-transfer characteristics and processing advantages of SCC systems (Luo et al., 2022; Makarytchev et al., 2005).

Table 1. GC–MS analysis of chemical components of the essential oil extracted by SCC from *F. suspensa*

Compound	RRI		%[d]	IM[c]	Ref[d]		
	Exp[a]	Lib[b]			MD	HD	EAMHD
Terpenes							
α-Thujene	912	910–935	0.73	t_R, MS			
α-Pinene	935	921–944	6.51	t_R, MS			
Camphene	950	936–959	0.46	t_R, MS			
Sabinene	974	961–981	12.25	t_R, MS			
β-Pinene	977	964–988	28.13	t_R, MS			
β-Myrcene	994	980–995	1.24	t_R, MS			
α-Phellandrene	1006	995–1013	0.18	t_R, MS			
Limonene	1031	1019–1039	5.63	t_R, MS			
β-Ocimene	1025	1025–1047	0.07	t_R, MS			
γ-Terpinene	1064	1049–1069	2.17	t_R, MS	0.24	0.34	0.22
Allo-ocimene	1132	1128–1132	0.02	MS			
α-Copaene	1395	1363–1391	0.21	t_R, MS			
Cedrene	1434	1397–1435	0.14	t_R, MS			
Caryophyllene, (*Z*)-	1428	1392–1426	0.10	t_R, MS			
α-Bergamotene, cis-	1431	1403–1433	0.04	MS			
Thujopsene, cis-	1452	1424–1450	0.01	t_R, MS			
β-Bisabolene, (*Z*)-	1513	1495–1509	0.09	MS			
δ-Cadinene	1530	1498–1531	0.24	t_R, MS			
Caryophyllene oxide	1582	1563–1595	0.01	t_R, MS			
Alcohols							
1-Hexanol	871	859–885	0.15	t_R, MS	-	-	-
1,8-Cineole	1037	1021–1044	3.12	t_R, MS	-	-	-
4-Thujanol	1035	1033–1077	3.01	t_R, MS	-	-	-
Linalool	1110	1097–1102	0.11	t_R, MS	4.92	7.36	8.99
trans-Pinocarveol	1141	1124–1163	1.56	MS	-	-	-
Borneol	1171	1152–1177	2.52	t_R, MS	-	-	-
4-Terpinenol	1181	1165–1189	20.6	t_R, MS	1.62	2.96	3.68
p-Cymen-8-ol	1186	1178–1191	0.27	t_R, MS	1.68	1.33	1.49
α-Terpineol	1194	1178–1203	1.74	t_R, MS	4.35	5.57	6.18
Myrtenol	1201	1177–1206	1.39	t_R, MS	-	-	-
Nerol	1231	1216–1250	1.10	t_R, MS	-	-	-
Aldehydes, Ketones, Esters							
Isothujone	1120	1106–1124	0.01	t_R, MS	-	-	-
Cryptone	1191	1172–1199	0.22	MS	-	-	-
Neral	1245	1237–1244	0.23	t_R, MS	-	-	-
Bornyl acetate	1290	1264–1297	0.31	t_R, MS	-	-	-
Citronellyl acetate	1354	1337–1358	0.07	t_R, MS	-	-	-
Neryl acetate	1366	1345–1378	0.08	t_R, MS	-	-	-
Alkanes							
p-Cymene	1027	1011–1033	0.81	t_R, MS	-	-	-
Dodecane	1200	1200	0.06	t_R, MS	-	-	-

Note: a: Relative retention indices experimentally determined against n-alkanes; b: Relative retention indices reported in the literature: https://webbook.nist.gov/chemistry/ (12.10.2026) (Linstrom & Mallard, 2001; Babushok et al., 2011; Xu et al., 2025); c: IM: Identification method; t_R: identification based on the retention times of standard compounds; MS: identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data; d: Data in the literature: (Jiao et al., 2012).

3.3 GC×GC–MS Analysis of Chemical Components of F. suspensa EO Extracted by SCC

To further characterize the chemical composition of *F. suspensa* essential oil, GC×GC–MS was used to analyze the SCC-extracted oil, and its chromatographic separation performance was systematically compared with that of conventional GC–MS. The results are shown in Figure S1. Conventional GC–MS using a 60 m capillary column identified 38 compounds (Table 1), whereas GC×GC–MS using a 30 m primary column identified 90 compounds (Table S1), encompassing all components detected by conventional GC–MS and nearly doubling the number

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of identified constituents. Severe co-elution was observed during conventional GC–MS analysis. As illustrated in Figure S1, only two chromatographic peaks appeared within the retention-time interval of 18.54 min–19.54 min. These peaks were identified as myrtenol and nerol through mass spectral library matching, retention index verification, and comparison with authentic standards. Conversely, GC×GC–MS resolved six distinct peaks within the same retention-time interval, corresponding to myrtenol, myrtenal, (*S*)-verbanone, citronellol, nerol, and carveol. Furthermore, GC×GC–MS also provided improved separation of isomeric components. Conventional GC–MS detected only *δ*-cadinene, whereas GC×GC–MS additionally resolved its isomer, *σ*-cadinene. Similarly, conventional GC–MS identified only *γ*-terpinene, whereas GC×GC–MS separated and identified both *α*-terpinene and *γ*-terpinene. These comparisons demonstrated that the orthogonal separation achieved using nonpolar and medium-polarity columns, coupled with modulation, substantially improved chromatographic resolution. This configuration enhanced the separation of co-eluting compounds and structural isomers, facilitated the detection of additional constituents, and broadened the chemical profile of *F. suspensa* essential oil.

The relative contents of the 90 identified components were estimated using the peak-area normalization method (Table S1) and compared with those obtained using conventional GC–MS. The distributions of compounds across chemical categories are shown in Figure 1. The relative abundances of the major compound categories decreased in the following order: terpenes > alcohols > alkanes. The GC×GC–MS also revealed higher total peak-area percentages for the other compound categories than conventional GC–MS, potentially reflecting the co-elution limitations of one-dimensional separation. Both methods identified *β*-pinene as the most abundant constituent, corroborating previous reports identifying it as a predominant compound in *F. suspensa* (Wei et al., 2016). Additionally, GC×GC–MS enabled the detection of several compounds reported to exhibit biological activity, including *α*-terpinene, camphene, *β*-myrcene, and *α*-phellandrene (Rudbäck et al., 2012; Freitas et al., 2020; Radice et al., 2022). These findings provided a more comprehensive chemical profile of *F. suspense* essential oil and a useful basis for further investigating the chemical constituents potentially responsible for its biological activities.

3.4 Antimicrobial Activity Assay

The antimicrobial activity of *F. suspensa* essential oil extracted using SCC technology was evaluated by measuring the MIC and MBC against six microbial strains: two Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*), two Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), and two fungi (*Candida albicans*, *Aspergillus fumigatus*). The essential oil exhibited MIC values of 31.3 μg/mL–125 μg/mL and MBC values of 62.5 μg/mL–250 μg/mL. The results demonstrated broad-spectrum antimicrobial activity against all tested microorganisms, with distinct variations in microbial susceptibility. Among all strains, *P. aeruginosa* and *C. albicans* exhibited the most susceptible organisms, with the lowest MIC (31.3 μg/mL) and MBC (62.5 μg/mL) values. Conversely, the two Gram-positive bacterial strains were less susceptible. *Staphylococcus aureus* showed an MIC of 125 μg/mL and an MBC of 125 μg/mL, whereas *B. cereus* exhibited an MIC of 125 μg/mL and the highest MBC of 250 μg/mL. In addition, *E. coli* and *A. fumigatus* exhibited intermediate susceptibility, with MIC and MBC values of 62.5 μg/mL and 125 μg/mL, respectively. The positive controls, including ampicillin, gentamicin, and amphotericin B, exhibited MIC values of 0.25 μg/mL–2 μg/mL and MBC values of 1 μg/mL–4 μg/mL, indicating greater antimicrobial potency compared with the essential oil under the tested conditions. Overall, the essential oil exerted more

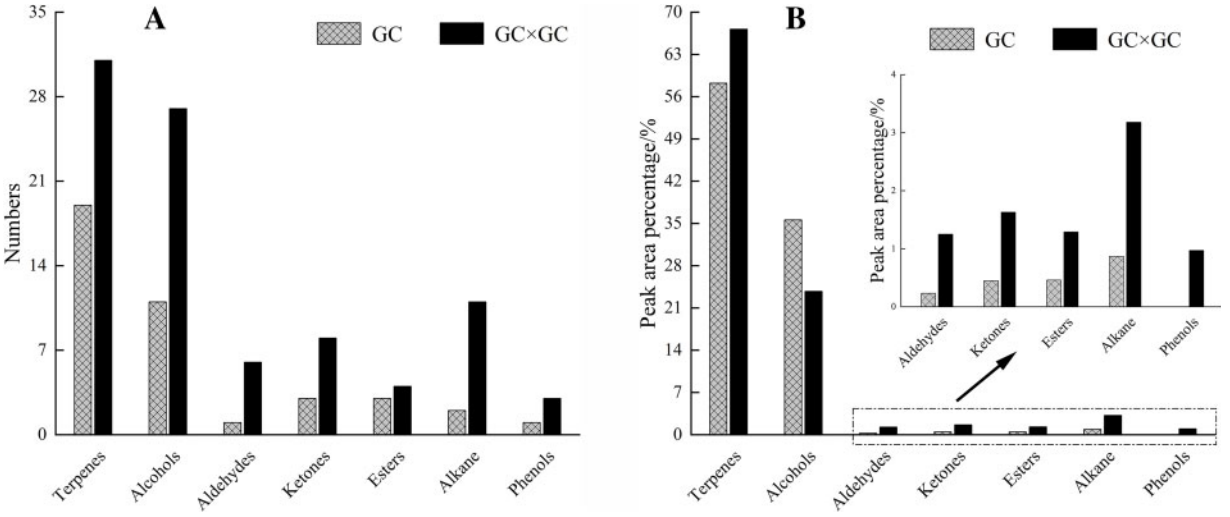


Figure 1. Comparison of the number and peak-area percentage of volatile compounds classified into different chemical categories as analyzed using GC–MS and GC×GC–MS. A: Number of identified compounds in each category; B: Total peak-area percentage of each category

potent inhibitory effects on Gram-negative bacteria and fungi than against Gram-positive bacteria. In comparison with *F. suspensa* essential oils obtained using conventional extraction techniques, including steam distillation and ultrasonic extraction, the SCC-extracted oil reportedly exhibited stronger antimicrobial activity. The difference correlated with variations in chemical composition arising from the extraction method. Notably, 4-terpinenol, sabinene, and eucalyptol were identified as potentially important contributors to antimicrobial capacity. Furthermore, 4-Terpinenol disrupted bacterial cell walls and membranes as well as interfered with intracellular deoxyribonucleic acid and protein synthesis, thereby disrupting bacterial metabolism (Zhang et al., 2018). Sabinene has been reported to suppress cariogenic virulence factors, including those associated with bacterial growth, adhesion, and biofilm formation (Park et al., 2019). Eucalyptol disturbed membrane integrity and interfered with bacterial energy metabolism and the synthesis of essential metabolites, thereby strengthening its bactericidal effect (Yao et al., 2025). Furthermore, GC×GC–MS also identified several oxygenated monoterpenes, including myrtenol, (S)-verbenone, citronellol, nerol, and carveol, that contributed to the overall antimicrobial activity of the essential oil. Myrtenol exhibited antibacterial and antibiofilm activities against *S. aureus*, potentially through interference with cell wall synthesis and modulation of biofilm-associated virulence factors (Cordeiro et al., 2020; Lopez-Romero et al., 2015). Citronellol demonstrated antibacterial activity against *E. coli* and *S. aureus* by altering bacterial surface properties and disrupting membrane integrity, resulting in intracellular potassium ion leakage (Kotan et al., 2007). Similar membrane-disrupting effects have been reported for carveol, which affected bacterial membrane permeability and cellular surface characteristics (Kotan et al., 2007). Nerol has also exhibited antibacterial activity against various bacterial strains, suggesting a potential contribution to the antimicrobial properties of essential oils (Petrovic et al., 2022). Additionally, verbenone enantiomers exhibited antibacterial and antifungal activities against clinically relevant microorganisms, although their precise mechanisms of action remain unclear (Abhirami et al., 2024). The low-temperature vacuum conditions employed in SCC facilitated the retention of thermosensitive volatile compounds, potentially contributing to the antimicrobial activity of the resulting essential oil. However, the antimicrobial effects of essential oils generally reflect interactions among multiple volatile constituents rather than the independent bioactivity of individual compounds. Accordingly, the contribution of each compound, including those newly identified by GC×GC–MS, cannot be quantitatively established from chemical profiling alone.

4 Conclusion

This study employed SCC technology to extract essential oil from *F. suspensa*, achieving an extraction yield of 2.16%. The combination of GC–MS and GC×GC–MS enhanced the separation and identification of volatile constituents. A total of 90 compounds were identified, including numerous trace bioactive components and isomers that were not detected using GC–MS. These findings provided a more comprehensive chemical profile of *F. suspensa* essential oil. Antimicrobial assays demonstrated that the SCC-extracted essential oil inhibited all six tested microorganisms, including Gram-positive bacteria, Gram-negative bacteria, and fungi. The strongest inhibitory effects were observed against *Pseudomonas aeruginosa* and *Candida albicans*, both of which exhibited an MIC of 31.3 μg/mL. The relatively high abundance of 4-terpinenol and the presence of sabinene and eucalyptol contributed to the observed antimicrobial activity. However, several limitations remain. The contributions of individual compounds to antimicrobial activity cannot be quantified through chemical profiling alone, and direct comparisons with other extraction methods using identical raw materials were not conducted. Furthermore, the bioactivity assessment was limited to *in vitro* assays, with no *in vivo* validation. The scalability of SCC and the long-term storage stability of the essential oil under industrial conditions also remain unassessed. Future research should address these gaps through comparative extraction experiments, identification of key bioactive compounds and elucidation of their structure-activity relationships, *in-vivo* validation, mechanistic investigations using multi-omics approaches, and pilot-scale optimization of SCC for practical applications.

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Author Contributions

Zheng-Guang Sun: Conceptualization, investigation, writing and editing. Li He: Investigation, data analysis. Shuai Zhang: Investigation, data analysis. Hai-Jian Su: Conceptualization, methodology, supervision, manuscript revision. All authors have read and agreed to the published version of the manuscript.

Availability of Data and Materials

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Artificial Intelligence (AI) Statement

The authors affirm that no Artificial Intelligence (AI) tools were employed in the preparation of this manuscript, which

includes language editing, graphical abstract preparation, figure generation, or image editing.

Conflicts of Interest

The authors declare that they do not have any conflict of interest.

Supporting Information

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ORCID

Zheng-Guang Sun: 0009-0005-1258-0924
Li He: 0009-0002-5436-7046
Shuai Zhang: 0009-0008-1889-8978
Hai-Jian Su: 0009-0007-8199-6979

References

Abhirami, N., Chandran, M., Ramadasan, A., Bhasura, D. & Ayyappan, J. P. (2024). Myrtenal exhibits cardioprotective effects by attenuating the pathological progression associated with myocardial infarction. *Fundamental & Clinical Pharmacology*, *38*(2), 276–289. DOI: 10.1111/fcp.12965.

Babushok, V. I., Linstrom, P. J. & Zenkevich, I. G. (2011). Retention indices for frequently reported compounds of plant essential oils. *Journal of Physical and Chemical Reference Data*, *40*(4), 1–47. DOI: 10.1063/1.3653552.

Cordeiro, L., Figuriredo, P., Souza, H., Souza, A., Andrade-Júnior, F., Barbosa-Filho, J. & Lima, E. (2020). Antibacterial and antibiofilm activity of myrtenol against *Staphylococcus aureus*. *Pharmaceuticals*, *13*(6), 133. DOI: 10.3390/ph13060133.

Dong, Z. L., Lu, X. Y., Tong, X. L., Dong, Y. Q., Tang, L. & Li, J. (2017). *Forsythiae Fructus*: A review on its phytochemistry, quality control, pharmacology and pharmacokinetics. *Molecules*, *22*(9), 1466.

EI Horsy, L., Lteif, S., Nehme, A., Cayot, N. & Bou-Maroun, E. (2026). Advances in extraction and analytical techniques for food aroma compounds: A critical review of methods, challenges, and future directions. *Food Research International*, *243*(1), 120366. DOI: 10.1016/j.foodres.2026.120366.

Freitas, B. C., Queiroz, P. A., Baldin, V. P., Amaral, P. H. R., Rodrigues, L. L. F., Vandresen, F., Caleffi-Ferracioli, K. R., Scodro, R. B. D., Cardoso, R. F. & Siqueira, V. L. D. (2020). (-)-Camphene-based derivatives as potential antibacterial agents against *Staphylococcus aureus* and *Enterococcus spp*. *Future Microbiology*, *15*(16), 1527–1534.

Gürer, Ç. Ü., Tütünis, S. Y., Toplan, G. G., Iscan, G., Demirci, B. & Tan, N. R. (2026). GC–MS characterization of the essential oil from *Teucrium kotschyanum* Poech (Lamiaceae) and its antimicrobial assessment. *Records of Natural Products*, *20*(3), e25123750. DOI: 10.25135/rnp.2512.3750.

Jiao, J., Fu, Y. J., Zu, Y. G., Luo, M., Wang, W., Zhang, L. & Li, J. (2012). Enzyme-assisted microwave hydro-distillation essential oil from *Fructus forsythia*, chemical constituents, and its antimicrobial and antioxidant activities. *Food Chemistry*, *134*(1), 235–243. DOI: 10.1016/j.foodchem.2012.02.114.

Jin, R. B., Kuang, F. Y., Meng, Q., Song, H. L., Feng, X. P. & Zou, M. S. (2026). Volatile compound fingerprinting for traditional sesame oil authentication using GC–MS with GC×GC–MS-assisted identification. *Journal of Food Composition and Analysis*, *155*, 109277.

Kačániová, M., Qiao, M., Bianchi, A., Elizondo-Luevano, J. H., Ben Hsouna, A., Ben Saad, R., Ban, Z., Li, L., Gao, Y., Mnif, W. & Garzoli, S. (2026). GC–MS and MALDI-TOF-MS integrated techniques for a comprehensive evaluation of the effect of Forsythia fruit essential oil application on vacuum-packaged broccoli. *Journal of Food Biochemistry*, *2026*, 4063394.

Kang, H. S., Lee, J. Y. & Kim, C. J. (2008). Anti-inflammatory activity of arctigenin from *Forsythiae Fructus*. *Journal of Ethnopharmacology*, *116*(2), 305–312. DOI: 10.1016/j.jep.2007.11.030.

Kotan, R., Kordali, S. & Cakir, A. (2007). Screening of antibacterial activities of twenty-one oxygenated monoterpenes. *Zeitschrift für Naturforschung C*, *62*(7–8), 507–513. DOI: 10.1515/znc-2007-7-808.

Linstrom, P. J. & Mallard, W. G. (2001). The NIST chemistry WebBook: A chemical data resource on the internet. *Journal of Chemical & Engineering Data*, *46*(5), 1059–1063. DOI: 10.1021/je000236i.

Lopez-Romero, J. C., González-Ríos, H., Borges, A. & Simoes, M. (2015). Antibacterial effects and mode of action of selected essential oils components against *Escherichia coli* and *Staphylococcus aureus*. *Evidence-based Complementary and Alternative Medicine*, *2015*, 795435. DOI: 10.1155/2015/795435.

Luo, Y., Wang, K., Zhuang, H. N., Li, D. J., Meng, X. L., Shi, M. L., Yao, L. Y., Song, S. Q., Sun, M., Wang, H. T. & Feng, T. (2022). Elucidation of aroma compounds in passion fruit (*Passiflora alata* Ait) using a molecular sensory approach. *Journal of Biochemistry*, *46*, e14224.

Makarytchev, S. V., Langrish, T. A. G. & Fletcher, D. F. (2005). Exploration of spinning cone column capacity and mass transfer performance using CFD. *Chemical Engineering Research and Design*, *83*(12), 1372–1380. DOI: 10.1205/cherd.03413.

Ming, L. S., Huang, H., Jiang, Y. M., Cheng, G. J. S., Zhang, D. Y. & Li, Z. (2019). Quickly identifying high-risk variables of ultrasonic extraction oil from multi-dimensional risk variable patterns and a comparative evaluation of different extraction methods on the quality of *Forsythia suspensa* seed oil. *Molecules*, *24*(19), 3445. DOI: 10.3390/molecules24193445.

Ni, L. J., Zhang, L. G., Hou, J., Shi, W. Z. & Guo, M. L. (2009). A strategy for evaluating antipyretic efficacy of Chinese herbal medicines based on UV spectra fingerprints. *Journal of Ethnopharmacology*, *124*(1), 79–86. DOI: 10.1016/j.jep.2009.04.006.

Park, B. I., Kim, B. S., Kim, K. J. & You, Y. O. (2019). Sabinene suppresses growth, biofilm formation, and adhesion of *Streptococcus mutans* by inhibiting cariogenic virulence factors. *Journal of Oral Microbiology*, *11*(1), 1632101. DOI: 10.1080/20002297.2019.1632101.

Petrovic, J., Kovalenko, V., Svirid, A., Stojkovic, D., Ivanov, M. & Kostic, M. (2022). Individual stereoisomers of verbenol and verbenone express bioactive features. *Journal of Molecular Structure*, *1251*, 131999. DOI: 10.1016/j.molstruc.2021.131999.

Radice, M., Durofil, A., Buzzi, R., Baldini, E., Martínez, A. P., Scalvenzi, L. & Manfredini, S. (2022). Alpha-phellandrene and alpha-phellandrene-rich essential oils: A systematic review of biological activities, pharmaceutical and food applications. *Life*, *12*(10), 1602. DOI: 10.3390/life12101602.

Rudbäck, J., Bergström, M. A., Börje, A., Nilsson, U. & Karlberg, A. T. (2012). α-terpinene, an antioxidant in tea tree oil, autoxidizes rapidly to skin allergens on air exposure. *Chemical Research in Toxicology*, *25*(3), 713–721.

Sun, Z. G., Yang, X. D., Wu, W., Yang, P. F., Yang, J., Bai, B., Huang, K., Liu, Y. Z., Liu, S. & Cui, F. X. (2024). Characteristic aroma components of cucumber based on odor activity value analysis before and after rotary spinning cone column treatment. *Food and Fermentation Industries*, *50*(18), 309–316.

Tian, D., Shi, M. Q. & Wan, Y. (2018). Volatile oil from *Forsythia suspense*: Chemical constituents and pharmacological effects. *Natural Product Research and Development*, *30*(10), 1834–1842.

Wang, Z. Y., Xia, Q., Liu, X., Liu, W. X., Huang, W. Z., Mei, X., Luo, J., Shan, M. X., Lin, R. C., Zou, D. X. & Ma, Z. Q. (2018). Phytochemistry, pharmacology, quality control and future research of *Forsythia suspensa* (Thunb.) Vahl: A review. *Journal of Ethnopharmacology*, *201*, 318–339.

Wei, S., Wu, T., Li, M. & Zhang, S. R. (2016). Analysis of major components and antibacterial activity of volatile oil from *Forsythiae Fructus* in different origins. *Chinese Journal of Experimental Traditional Medical Formulae*, *22*(4), 69–74.

Xie, Q., Yuan, H., Liu, S., Liang, L., Luo, J., Wang, M., Li, B. & Wang, W. (2025). Mid-level data fusion techniques of LC-MS and HS-GC–MS for distinguishing green and ripe Forsythiae Fructus. *Molecules*, *30*(7), 1404. DOI: 10.3390/molecules30071404.

Xu, F., Chen, X. R., Zhang, J. Y., Qu, C. L., Gu, L. & Xing, X. (2025). Phytochemical constituents, antibacterial, synergistic antibacterial, cytotoxic, and antioxidant activities of the essential oil of Rhizomes of *Davallia bullata* Wall. ex Hook. *Records of Natural Products*, *19*(1), 84–94. DOI: 10.25135/rnp.498.2501-3404.

Xu, W., Tang, Y., Wu, M., Zhang, N., Wang, B., Ben, A., Zhao, R. & Liu, T. (2026). An integrated amino acid ionic liquid mediated microwave-assisted extraction for simultaneously obtaining essential oils, forsythoside A, forsythin and polysaccharides from *Forsythia suspensa* fruits. *Industrial Crops and Products*, *250*, 124005. DOI: 10.1016/j.indcrop.2026.124005.

Yang, P. F., Liu, Y. Z., Sun, Z. G., Wu, W., Shang, Z. B., Kong, L. Q., Sun, H. F. & Mao, D. B. (2025). Differential analysis of volatile components of pineapple peel extracted by spinning cone column with different temperatures. *Food and Fermentation Industries*, *51*(10), 312–320.

Yang, J. J., Wei, H. M., Teng, X. E., Zhang, H. Q. & Shi, Y. H. (2014). Dynamic ultrasonic nebulisation extraction coupled with headspace ionic liquid-based single-drop microextraction for the analysis of the essential oil in *Forsythia suspensa*. *Phytochemical Analysis*, *25*(2), 178–184. DOI: 10.1002/pca.2490.

Yao, B., He, B., Peng, J. H., Song, X., Zhao, R., Sun, Y. & Zhang, Y. F. (2025). The comprehensive review of eucalyptol: Synthesis, metabolism, and therapeutic applications in disease treatment. *Molecular Biology Reports*, *52*(1), 346. DOI: 10.1007/s11033-025-10461-y.

Zhang, Y. T., Feng, R. Z., Li, L. X., Zhou, X., Li, Z. W., Jia, R., Song, X., Zou, Y. F., Yin, L. Z., He, C. L., Liang, X. X., Zhou, W. H., Wei, Q., Du, Y. H., Yan, K., Wu, Z. L. & Yin, Z. Q. (2018). The antibacterial mechanism of terpinen-4-ol against *Streptococcus agalactiae*. *Current Microbiology*, *75*(9), 1214–1220. DOI: 10.1007/s00284-018-1512-2.