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Study of the differential active components and anti-proliferative effects of *Gardenia jasminoides* J.Ellis at different harvesting periods

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Objective: To investigate the quality standards and anti-proliferative effects of *Gardenia jasminoides* J.Ellis (GJE) at different harvesting periods, and to determine the optimal harvesting time for anti-cancer applications.

Methods: A fast, simple, and accurate analytical method was established using UFLC/Q-TOF-MS technology in positive and negative ion modes to identify differential components in GJE samples collected at seven harvesting periods. The anti-proliferative effects of GJE extracts were evaluated against MCF7 breast cancer and HepG2 hepatocellular carcinoma cell lines using MTT assay.

Results: A total of 52 components were successfully identified from GJE samples, including iridoid glycosides, flavonoids, and phenolic acids. GJE demonstrated stronger anti-proliferative effects against HepG2 cells compared to MCF7 cells. The anti-proliferative activity increased progressively with fruit maturation, reaching peak efficacy at stages 5–6 (orange-red to red fruits) and declining at stage 7 (overripe dark red fruits). Most iridoid glycosides, flavonoids and phenolic acids showed increasing trends during the growth process, correlating with enhanced anti-proliferative effects.

Conclusion: GJE may serve as a potential source for developing anti-cancer therapeutic agents due to its demonstrated anti-proliferative activity. The mature fruits at stages 5–6 are most suitable for anti-cancer applications, providing scientific guidance for optimal harvesting time and quality standardization of GJE as a medicinal material.

KEYWORDS

Gardenia jasminoides J.Ellis, harvesting periods, ultra-fast liquid chromatography-TripleTOF MS, differential active components, anti-proliferative effects

1 Introduction

Gardenia jasminoides J.Ellis, one of the commonly used bulk Chinese medicinal materials, is derived from the dried and mature fruit of *Gardenia jasminoides* J.Ellis, a Rubiaceae plant. It reduces internal heat and inflammation, promotes diuresis, and exerts cooling and detoxifying effects. This traditional Chinese medicine is widely used in clinical practice for various therapeutic applications including cancer treatment (Tang et al., 1992; Shahrajabian and Sun, 2023; Chen et al., 2020). This traditional Chinese medicine, with

over 2000 years of documented therapeutic history, has attracted increasing attention in modern pharmaceutical research due to its diverse pharmacological activities.

Its main active components are iridoid glycosides, flavonoids, lignans, coumarins, volatile oils, phenolic acids, polysaccharides and various trace elements (He et al., 2010; Gan et al., 2023; Liu et al., 2023; Tang et al., 2022; Fang et al., 2022; Tian et al., 2022; Wang et al., 2020; Zhang et al., 2025). However, there has been no research on changes in the chemical composition during the GJE growth process so far, and it has not been reported whether the anticancer effects of GJE are related to its active components and whether they are affected by the processing method.

Liquid chromatography quadrupole time of flight mass spectrometry (LC-Q-TOF/MS) has been regarded as an indispensable tool for qualitative analysis, which could provide sensitive, accurate, and reproducible analysis of multiple components to reveal material basis of traditional Chinese medicine or food (Zhu et al., 2024; Cui et al., 2023; Qiu et al., 2024; Gumus, 2023; Shi et al., 2023; Pellegrino et al., 2022). The content and composition of bioactive compounds in medicinal plants can vary significantly depending on harvesting time and maturity stage (Singh et al., 2023; Song et al., 2023; Hazrati et al., 2024), which highlights the importance of studying harvest timing effects. Understanding the relationship between harvesting periods and phytochemical composition is crucial for optimizing the quality and therapeutic efficacy of traditional Chinese medicines (Wang et al., 2015; Li et al., 2022; Li et al., 2015; Fu et al., 2014; Xia et al., 2020). In order to investigate the changes in the chemical composition during the GJE growth process, and evaluate the quality standards and anti-proliferative effects of GJE, this study established a method for differential identification and activity detection based on UFLC/Q-TOF-MS technology combined with anti-proliferative analysis.

2 Materials and methods

2.1 Materials, reagents and instruments

Gardenia jasminoides J.Ellis (GJE) was harvested in September–November 2022 in the Pharmaceutical Garden of Nanjing University of Chinese Medicine. Seven harvesting stages were defined based on progressive fruit color development from cyan (Stage 1) to dark red (Stage 7). For each harvesting stage, fruits were randomly collected from six different plants ($n = 6$ per stage, total $n = 42$) to ensure biological diversity. Each plant collection constituted one independent biological replicate, and during LC-MS analysis, each sample was analyzed in duplicate as technical replicates. It was authenticated as *Gardenia jasminoides* J.Ellis herb (dried mature fruit of *Gardenia jasminoides* J.Ellis) by Prof. Qinan Wu from Nanjing University of Chinese Medicine. LC/MS-grade acetonitrile, formic acid, methanol, and water were purchased from Merck Co. (Darmstadt, Germany).

An ultra-fast liquid chromatography system (Shimadzu Corporation UFLC XR, Kyoto, Japan) was connected to a triple time-of-flight mass spectrometer (TripleTOF 5600 system, Applied Biosystems/MDS Sciex, Foster City, Canada) with an electrospray ionization source.

2.2 Sample extraction

Gardenia jasminoides J.Ellis (GJE) was processed by removing stalks and impurities, then steamed for 10–15 min until vapor appeared, taken out and dried at 60 °C. The dried fruits were ground into fine powder using a mechanical grinder.

1.5 g of the powdered GJE was accurately weighed and ultrasonically extracted with 20 mL of 70% methanol at 30 °C for 30 min. The extraction conditions were optimized through preliminary experiments comparing different methanol concentrations (70, 80, 95%) and extraction times (20, 30, 40 min). The extraction efficiency was evaluated by measuring the total peak area at 238 nm using HPLC-UV analysis, with geniposide (the main iridoid glycoside in GJE) as the reference compound. The 70% methanol for 30 min yielded the highest total peak area and optimal chromatographic peak number, indicating the highest extraction efficiency and compound diversity. This was followed by centrifugation for 10 min at 12,000 rpm. The extraction supernatants were then diluted 10 times and filtered through a 0.22 μ m filter unit.

2.3 Liquid chromatographic conditions

All samples were separated by a Waters XBridge C₁₈ column (4.6 mm $\times$ 100 mm, 3.5 μ m). A binary solvent gradient consisting of solvent A (water with 0.1% formic acid) and solvent B (acetonitrile with 0.1% formic acid) was used. The flow rate was 800 μ L/min. The total run time was 35 min with the gradient as follows: 0–18 min, 5–35% B, 18–19 min, 35–75% B, 19–28 min, 75–95% B, 28–29 min, 95% B, 29–31 min, 95–5% B, and 31–35 min, 5% B for column equilibration before next run. An injection volume of 2 μ L was used. The column temperature was 40 °C.

2.4 Mass spectrometric conditions

The samples were analyzed on the hybrid system in both positive and negative ion modes. A calibrated delivery system was used to ensure the accuracy of mass less than 1 ppm. The settings were nitrogen gas for nebulization at 55 psi, heater gas pressure at 55 psi, curtain gas at 35 psi, temperature at 500 °C, and ion spray voltage at 5,500 V in positive ion mode, and –4,500 V in negative ion mode. The acquisition of a survey TripleTOF MS spectrum was operated under high resolution settings. The optimized declustering potential (DP) and collision energy (CE) were set at 80 eV and 15 eV in positive ion mode, and at –80 eV and –15 eV in negative ion mode. A sweeping collision energy setting at 35/–35 eV $\pm$ 15 eV was applied for collision-induced dissociation (CID).

2.5 Data processing

TOF-MS data were collected using Analyst® version 1.6 software (Applied Biosystems/MDS Sciex) and processed using PeakView® version 1.2 software (Applied Biosystems/MDS Sciex) with the XIC Manager add-in and MultiQuant™ version 2.1 software (Applied Biosystems/MDS Sciex).

The PeakView® software contained a simple fragment ion predictor to help link the MS/MS spectrum to structures (saved as .mol files) and to provide insights into fragmentation mechanisms.

XIC Manager was used for targeted and non-targeted data processing, which consisted of a table for defining a list of masses or formulae to generate extracted ion chromatograms (XIC), and to review the identification of detected compounds. High confidence in results was based on retention times, accurate mass, isotopic pattern and MS/MS library searching.

The Principal component analysis (PCA) were performed by MarkerView® software version 1.2 (Applied Biosystems/MDS Sciex), where six repeated spectra for each sample were imported and carried out with a Pareto scaling. The *T*-value and corresponding *p*-value were also calculated between each group and all of the other six groups. The program was linked back to the raw data so that differences can be directly visualized in spectra or chromatograms and the converted components can be putatively identified by PeakView® software.

2.6 Cell culture

MCF7 and HepG2 cell lines were maintained in RPMI1640 medium supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin/streptomycin. Cells were plated on a 175 cm² surface area in a 5% CO₂ incubator at 37 °C. The culture medium was changed every 2–3 days for subsequent experiments.

2.7 Anti-proliferative activity

Anti-proliferative ability was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-di-phenyltetrazolium bromide (MTT) colorimetric assay (Boyraz et al., 2021; McCauley et al., 2013; Harvey and Cree, 2010). In brief, MCF7 and HepG2 cancer cell lines were harvested with 0.25% trypsin and plated in 96-well microplates with RPMI1640 medium at a density of 8×10^3 cells/well overnight. After 24-h incubation to allow for cell attachment, cells were divided into the following groups: control group (treated with culture medium only), positive drug control group (treated with doxorubicin hydrochloride), and GJE sample groups representing seven different harvest stages. The GJE sample groups were further subdivided into three concentration subgroups: low concentration (2.5 mg/mL), medium concentration (5 mg/mL), and high concentration (10 mg/mL). All groups were treated with the corresponding samples in medium (200 µL) and incubated for 48 h in a 5% CO₂ incubator at 37 °C. The treated cells were washed with PBS and 200 µL of MTT solution (5 mg/mL MTT stock in PBS diluted to 0.5 mg/mL with RPMI1640 medium) was added to each well. The plates were incubated for 4 h in a 5% CO₂ incubator at 37 °C. Insoluble formazan crystals in the viable cells were dissolved in 100 µL DMSO after removing the medium containing MTT and the absorbance was measured at 570 nm by an ELISA plate reader. Each treatment was performed in triplicate wells and repeated in three independent experiments (*n* = 3 biological replicates). Cell viability was calculated as percentage relative to the control group. The same MTT assay protocol was applied to determine the IC₅₀ values of doxorubicin against MCF7 and HepG2 cell lines under identical experimental

conditions. IC₅₀ values were determined using non-linear regression analysis with four-parameter logistic curve fitting (GraphPad Prism software).

2.8 Statistical correlation analysis

Pearson correlation analysis was performed to assess relationships between individual compound relative contents and anti-proliferative activities (represented as 1/IC₅₀ values). Correlation coefficients (*r*) and significance levels (*p*-values) were calculated using SPSS 26.0 software. Statistical significance was set at *p* < 0.05. Correlation coefficients (*r*) were interpreted according to Evans (1996): 0.00–0.19 (very weak), 0.20–0.39 (weak), 0.40–0.59 (moderate), 0.60–0.79 (strong), and 0.80–1.0 (very strong).

3 Results and discussion

3.1 Appearance changes

The appearance changes of GJE at different harvesting stages are shown in Figure 1, with fresh fruits (unprocessed GJE samples) displayed in the top row and processed, dried herbs (steamed and dried GJE) in the bottom row. The surface colors of fresh GJE fruits transitioned from cyan (stage 1), cyan yellow (stage 2), cyan orange (stage 3), orange yellow (stage 4), orange red (stage 5), red (stage 6), to dark red (stage 7). The cyan fruit at stage 1 was small due to immaturity, while the dark red fruit at stage 7 showed signs of over-ripening.

3.2 Principle component analysis (PCA)

Using UFLC–TripleTOF MS analysis, information on intact precursors and fragment ions were obtained from a single injection.

The base peak chromatograms (BPCs) of GJE samples using both positive and negative ion modes are shown in Figure 2. The separation of peaks is good, and the peak shape is symmetrical. In the positive ion mode, most of the peaks at stage 4 were higher than those at stage 1 and stage 7 GJE, while individual peaks at stage 1 GJE (such as *t*_R = 7.12 and 21.38 peaks) and stage 7 GJE (such as *t*_R = 4.28, 7.15, 13.58, 15.72, 16.78 and 21.54 peaks), reaching the highest value. In the negative ion mode, the peaks obtained at each stages are less than those in the positive ion mode. Most of the peaks at stage 4 GJE are higher than those at stage 1 and stage 7 GJE. With the extension of growth time, some peaks decrease (such as *t*_R = 9.34 and 12.58 peaks). Which shows that the components converted during the GJE growth process.

Observing the aggregation and dispersion of the test samples from the score plots (Figures 3A,C), it can be seen that the PCA model established in this experiment has good clustering. The analysis incorporated all biological replicates (*n* = 6 per stage, total *n* = 42), with individual replicates within each harvesting stage clustering tightly together, demonstrating that within-stage variability was much smaller than between-stage differences. In positive ion mode, GJE at different harvesting periods can be well classified into four significant clusters (*p* < 0.05) for the first and the second principal components



FIGURE 1

Appearance changes of *Gardenia jasminoides* J.Ellis (GJE) during seven harvesting periods. Top row: fresh unprocessed fruits showing progressive color changes from cyan (Stage 1, immature) through cyan-yellow (Stage 2), cyan-orange (Stage 3), orange-yellow (Stage 4), orange-red (Stage 5), red (Stage 6, fully mature), to dark red (Stage 7, overripe). Bottom row: processed dried medicinal herbs obtained after steaming fresh fruits for 10–15 min followed by drying at 60 °C. Samples were collected from the Pharmaceutical Garden of Nanjing University of Chinese Medicine (September–November 2022), with fruits randomly collected from six independent plants per stage ($n = 6$ biological replicates) to ensure biological diversity. Representative images are shown.

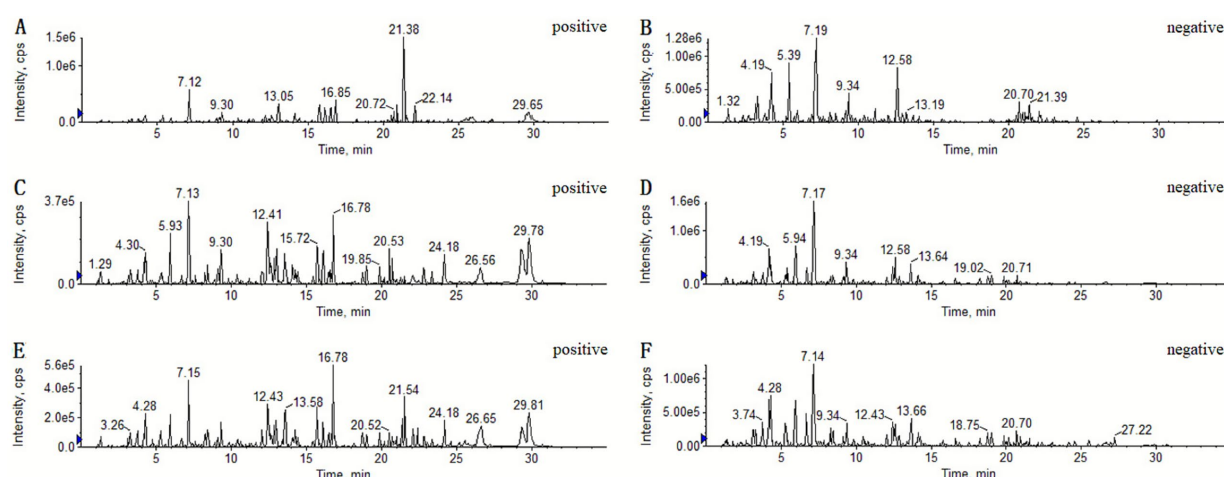


FIGURE 2

Base peak chromatograms (BPCs) of *Gardenia jasminoides* J.Ellis (GJE) extracts analyzed by ultra-fast liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UFLC-Q-TOF-MS). Panels (A,C,E) show positive ion mode; Panels (B,D,F) show negative ion mode. Three representative harvesting stages are presented: Stage 1 (immature cyan fruit; A,B), Stage 4 (mid-maturity orange-yellow fruit; C,D), and Stage 7 (overripe dark red fruit; E,F). Chromatographic conditions: Waters XBridge C18 column (4.6 × 100 mm, 3.5 μm), gradient elution with 0.1% formic acid in water (solvent A) and acetonitrile (solvent B), flow rate 800 μL/min, column temperature 40 °C, injection volume 2 μL, total run time 35 min. The x-axis represents retention time (min) and y-axis represents relative intensity (cps, counts per second). Major peaks with retention times labeled show notable differences across harvesting stages, indicating compositional changes during fruit maturation. Each chromatogram is representative of duplicate injections from one biological replicate.

(PCs), namely stage 1 (red cluster), stage 2 (blue cluster), stage 3–6 (green cluster), and stage 7 (yellow cluster). Here, the blue cluster (stage 2 GJE) and red cluster (stage 1 GJE) are separated by the first PC, whereas the blue cluster, yellow cluster and the green cluster are separated by the second PC. The first and second PCs' values are both 18.8%. In negative ion mode, it can be well classified into three clusters ($p < 0.05$) for the first and the second PCs, namely stage 1 (red cluster), stage 2–6 (green cluster), and stage 7 (yellow cluster). The yellow cluster and the red cluster are separated by the first PC, whereas the green cluster and the yellow cluster are separated by the second PC. The first and second PCs' values are both 18.5%. Loading

plot analysis (Figures 3B,D) revealed the variables contributing to group separation, with the discriminant metabolites showing consistency with the representative compounds selected for correlation analysis.

The fruit at stage 1 is not yet mature, and the fruit maturity at stage 7 is too high, which were far from the middle harvesting period with significant differences. Stage 2–6 are closest, indicating that the differences are relatively small. From the load plots obtained from cluster analysis (Figures 3B,D), it can be seen that most compounds cluster together, and the “outlier” differences are precisely the reason for the significant differences in GJE at different harvesting periods.

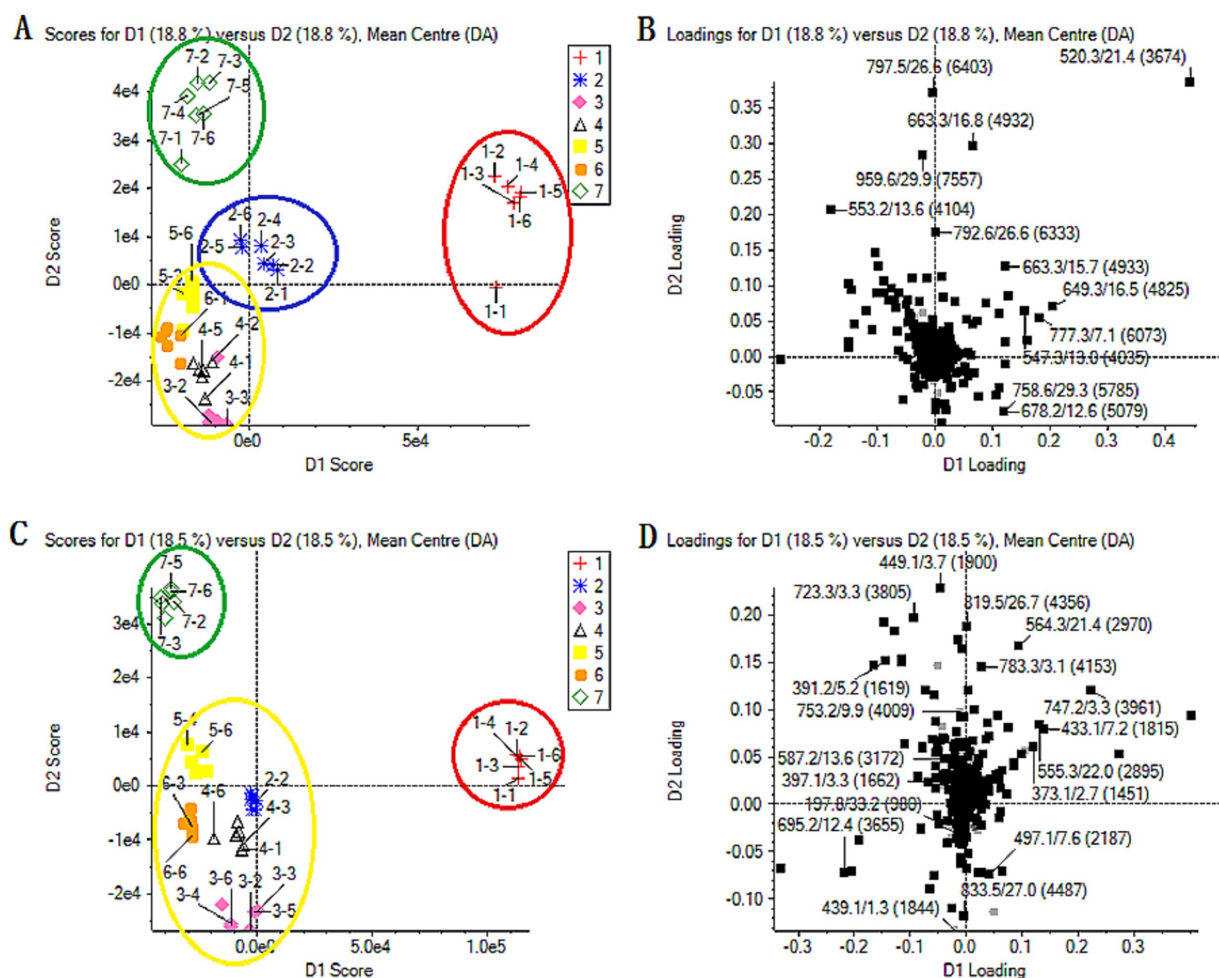


FIGURE 3

Principal component analysis (PCA) of *Gardenia jasminoides* J.Ellis (GJE) metabolic profiles at different harvesting stages. (A) PCA score plot in positive ion mode; (B) Corresponding loading plot in positive ion mode; (C) PCA score plot in negative ion mode; (D) Corresponding loading plot in negative ion mode. Each data point in the score plots represents one biological replicate ($n = 6$ per stage, total $n = 42$ samples). In positive ion mode (A), samples from the seven harvesting stages clustered into four distinct groups: Stage 1 (immature cyan fruit), Stage 2 (cyan-yellow fruit), Stages 3–6 (developing to mature red fruit), and Stage 7 (overripe dark red fruit), as indicated by different colored ellipses. In negative ion mode (C), samples clustered into three groups: Stage 1, Stages 2–6, and Stage 7. Ellipses represent 95% confidence intervals for each cluster. The first two principal components (D1 and D2, equivalent to PC1 and PC2) explained 18.8 and 18.8% of the total variance in positive mode (A), and 18.5% in negative mode (C), respectively. Tight clustering of biological replicates within each stage demonstrates good analytical reproducibility (within-stage variability < between-stage differences), while clear separation between stages indicates significant compositional differences ($p < 0.05$, permutation test with 1,000 iterations). Loading plots (B,D) display the contribution of individual metabolites (m/z features) to the separation between groups, with variables positioned at the periphery having stronger influence on group discrimination. Specific m/z values for key discriminant metabolites are labeled. The combined score and loading plots reveal the metabolic trajectory of GJE during fruit maturation, with early-stage (Stage 1) and late-stage (Stage 7) fruits being distinctly separated from mid-maturation stages (Stages 2–6), reflecting progressive accumulation or depletion of specific bioactive compounds.

3.3 Qualitative identification and relative content analysis of differential components

Based on the chemical composition of GJE from relevant literature, the XIC Manager function of Peakview® software was used to perform components with certain change trend of the seven test samples on the positive and negative ion modes. The screened data was combined with secondary mass spectrometry images. As shown in Tables 1, 2, most of the components could be detected in both positive and negative ion modes. A total of 52 components were identified from GJE samples, including deacetyl asperulosidic acid, shanzhiside methyl ester, geniposidic acid, sesamose, methyl syringate,

scandoside methyl ester, loganic acid, jasminoside B/D/F, gardenoside, citral, 4-hydroxycoumarin/7-hydroxycoumarin, genipin gentiobioside, chlorogenic acid, 10-O-p-hydroxycinnamoyl-scandoside, desacetyl asperulosidic methyl ester, 10-dehydrogenniposide, limonide, 3,6-disinaoyl sucrose, kaempferol-3-O-rutinoside, geniposid-pentaacetat, isochlorogenic acid A, jasminoside R, plumerin, 6''-O-trans-p-coumaroyl genipin gentiobioside, naringenin-4'-glucoside-7-neohesperidin, crocetin mono-(β -d-gentiobiosyl)-ester, 6''-O-trans-sinapyl genipine gentiobioside, 3,5-dicaffeoylquinic acid, crocin-I, jasminoside H/I/S, benzyl o-heptaacetyl- β -d-lactoside, 3,5-di-O-caffeoyl-4-O-(3-hydroxy-3methyl) glutarylquinic acid, 3-O-caffeoyl 4-O-sinapylquinic acid, naringin, gymnospermae

TABLE 1 The differential components of GJE at different harvesting periods in positive ion mode.

Nub.	T _R (min)	Positive ion mode				Molecular formula	Assigned identity
		Mean measured mass (Da)	Mean theoretical mass (Da)	ppm	Ion species		
1	3.23	375.1286	375.1285	−1.5	[M + H] ⁺	C ₁₆ H ₂₂ O ₁₀	Geniposidic acid ^c
		392.1551	392.1550	−1.1	[M + NH ₄] ⁺		
2	3.29	213.0758	213.0759	−1.6	[M + H] ⁺	C ₁₀ H ₁₂ O ₅	Methyl syringate ^d
3	4.27	364.1966	364.1969	−2.2	[M + NH ₄] ⁺	C ₁₆ H ₂₆ O ₈	Jasminoside B/D/F ^a
	5.24	347.1700	347.1699	−0.4	[M + H] ⁺		
4	5.27	185.1172	185.1170	−1.2	[M + H] ⁺	C ₁₀ H ₁₆ O ₃	Citral ^b
5	5.39	163.039	163.0391	−0.4	[M + H] ⁺	C ₉ H ₆ O ₃	4-Hydroxycoumarin/7-Hydroxycoumarin ^d
6	5.96	551.197	551.1958	−2.6	[M + H] ⁺	C ₂₃ H ₃₄ O ₁₅	Genipin gentiobioside ^b
7	6.9	355.1024	355.1021	−1.3	[M + H] ⁺	C ₁₆ H ₁₈ O ₉	Chlorogenic acid ^d
8	12.04	508.2389	508.2381	−3.3	[M + NH ₄] ⁺	C ₂₂ H ₃₄ O ₁₂	Jasminoside R ^a
9	12.41	471.1497	471.1497	−1.9	[M + H] ⁺	C ₂₁ H ₂₆ O ₁₂	Plumerin ^b
10	12.43	697.2338	697.2325	−3.3	[M + H] ⁺	C ₃₂ H ₄₀ O ₁₇	6''-O-trans-p-coumaroyl genipin gentiobioside ^b
11	12.61	670.3069	670.3040	−1.7	[M + NH ₄] ⁺	C ₃₂ H ₄₄ O ₁₄	Crocetin-mono-(β-D-gentiobiosyl)-ester ^b
12	12.64	757.2550	757.2547	−1.3	[M + H] ⁺	C ₃₄ H ₄₄ O ₁₉	6''-O-trans-sinapyl genipine gentiobioside ^g
		774.2815	774.2775	−2.6	[M + NH ₄] ⁺		
13	12.66	994.4126	994.413	−2.5	[M + NH ₄] ⁺	C ₄₄ H ₆₄ O ₂₄	Crocin-I ^a
14	12.84	510.2545	510.2532	−2.6	[M + NH ₄] ⁺	C ₂₂ H ₃₆ O ₁₂	Jasminoside H/I/S ^a
15	12.86	744.2709	744.2699	−3.5	[M + NH ₄] ⁺	C ₃₃ H ₄₂ O ₁₈	Benzyl o-heptaacetyl-β-d-lactoside ^g
16	13.64	561.1603	561.1593	−1.9	[M + H] ⁺	C ₂₇ H ₂₈ O ₁₃	3-O-caffeoyl 4-O-sinapylquinic acid ^b
17	14.11	832.3598	832.3604	−2.3	[M + NH ₄] ⁺	C ₃₈ H ₅₄ O ₁₉	Crocin II ^a
18	14.23	480.2439	480.2420	−2.4	[M + NH ₄] ⁺	C ₂₁ H ₃₄ O ₁₁	Jasminoside T ^a
19	16.87	369.1180	369.1177	−1.1	[M + H] ⁺	C ₁₇ H ₂₀ O ₉	Chlorogenic acid methyl ester ^a
20	20.73	469.3312	469.3294	−4.8	[M + H] ⁺	C ₃₀ H ₄₄ O ₄	Changnanic acid ^b
21	20.98	505.3524	505.3518	−2.9	[M + H] ⁺	C ₃₀ H ₄₈ O ₆	Salic acid ^b
22	23.35	425.2146	425.2129	−4	[M + Na] ⁺	C ₂₀ H ₃₄ O ₈	Acetyl tributyl citrate ^b

^a Increase, ^b Increase and then level off, ^c Level off and then increase, ^d Decrease, ^e Decrease and then level off, ^f Level off and then decrease, ^g Increase and then decrease, ^h Decrease and then increase.

xyloside, crocin II, jasminoside T, jasminoside, rhamnoside A, populin, gnidilatin, chlorogenic acid methyl ester, 6''-O-sinapoyl jasminoside A, 6''-O-sinapoyl geniposide, arjunolic acid, changnanic acid, salic acid, 11-keto mastic acid, α-tocopherol, acetyl tributyl citrate and 3-O-cis coumarinic acid. Among them, geniposidic acid, jasminoside B/D/F, genipin gentiobioside, chlorogenic acid, jasminoside R, crocetin-mono-(β-d-geniobiosyl)-ester, 6''-O-trans-sinapyl genipin gentiobioside, crocin-I, jasminoside H/I/S, 3-O-caffeoyl 4-O-sinapylquinic acid, crocin II and jasminoside T can be detected in both positive and negative ion modes.

Observing the content trend of each component, it was found that the contents of deacetyl asperuloside acid, jasminoside B/D/F desacetyl

asperulosidic methyl ester, 10-dehydrogenniposide, jasminoside R, crocin-I, jasminoside H/I/S, crocin II, jasminoside T, chrysin, chlorogenic acid methyl ester, 6''-O-sinapoylj asminoside A and 6''-O-sinapoylgeniposide showed a continuous upward trend during the growth of GJE. The contents of naringenin-4'-glucoside-7-neohesperidin, citral, genipin gentiobioside, plumerin, 6''-O-trans-p-coumaroyl genipin gentiobioside, crocetin-mono-(β-d-geniobiosyl)-ester, 3-O-caffeoyl 4-O-sinapylquinic acid and acetyl tributyl citrate increased at stage 2 GJE, and there was no significant change in the follow-up. The contents of geniposid pentaacetate, 6''-o-trans-p-coumaroyl genipin gentiobioside and benzyl o-heptaacetyl-β-d-lactoside increased firstly and then decreased during the growth

TABLE 2 The differential components of GJE at different harvesting periods in negative ion mode.

Nub.	T _R (min)	Negative ion mode				Molecular formula	Assigned identity
		Mean measured mass (Da)	Mean theoretical mass (Da)	ppm	Ion species		
1	2.29	389.1089	389.1081	0.2	[M – H] [–]	C ₁₆ H ₂₂ O ₁₁	Deacetyl asperulosidic acid ^a
		371.0984	371.0977	–0.7	[M – H – H ₂ O] [–]		
2	2.69	373.1140	373.1134	–0.3	[M – H·H ₂ O] [–]	C ₁₆ H ₂₄ O ₁₁	Shanzhiside methyl ester ^c
3	3.23	373.1141	373.1134	0.2	[M – H] [–]	C ₁₆ H ₂₂ O ₁₀	Geniposidic acid ^c
4	3.27	419.1195	419.1184	–0.5	[M – H] [–]	C ₁₇ H ₂₄ O ₁₂	Sesamose ^c
5	3.81	403.1246	403.1236	–0.7	[M – H] [–]	C ₁₇ H ₂₄ O ₁₁	Scandoside methyl ester ^c
6	4.06	375.1297	375.1289	–0.7	[M – H] [–]	C ₁₆ H ₂₄ O ₁₀	Loganic acid ^c
7	4.27	345.1555	345.1550	0.3	[M – H] [–]	C ₁₆ H ₂₆ O ₈	Jasminoside B/D/F ^a
	5.24	391.1610	391.1605	–1.2	[M + HCOO] [–]		
8	5.21	449.1301	449.1294	–0.8	[M + HCOO] [–]	C ₁₇ H ₂₄ O ₁₁	Gardenoside ^c
9	5.96	595.1869	595.1871	–0.1	[M + HCOO] [–]	C ₂₃ H ₃₄ O ₁₅	Genipin gentiobioside ^b
10	6.9	353.0878	353.0878	0.5	[M – H] [–]	C ₁₆ H ₁₈ O ₉	Chlorogenic acid ^d
11	6.96	535.1457	535.1455	–0.8	[M – H] [–]	C ₂₅ H ₂₈ O ₁₃	10-O-p-hydroxycinnamoyl-scandoside ^c
12	7.29	385.1140	385.1125	–2.9	[M – H – H ₂ O] [–]	C ₁₇ H ₂₄ O ₁₁	Desacetyl asperulosidic methyl ester ^a
13	7.83	385.1140	385.1131	–1.4	[M – H] [–]	C ₁₇ H ₂₂ O ₁₀	10-Dehydrogenniposide ^a
14	9.54	477.2341	477.2334	–1.3	[M + HCOO] [–]	C ₂₁ H ₃₆ O ₉	Limonide ^c
15	9.87	753.2248	753.224	1.8	[M – H] [–]	C ₃₄ H ₄₂ O ₁₉	3,6-Disinaoyl sucrose ^c
16	10.07	593.1512	593.1499	–0.7	[M – H] [–]	C ₂₇ H ₃₀ O ₁₅	Kaempferol-3-O-rutinosidet ^c
17	10.45	597.1825	597.1815	0.7	[M – H] [–]	C ₂₇ H ₃₄ O ₁₅	Geniposid-pentaacetat ^g
18	11.12	515.1195	515.1193	–1.2	[M – H] [–]	C ₂₅ H ₂₄ O ₁₂	Isochlorogenic acid A ^c
19	12.04	535.2032	535.2005	1.6	[M + HCOO] [–]	C ₂₂ H ₃₄ O ₁₂	Jasminoside R ^a
20	12.43	741.2248	741.2255	–0.9	[M – H] [–]	C ₃₃ H ₄₂ O ₁₉	Naringenin-4'-glucoside-7-neohesperidin ^b
21	12.61	697.2713	697.2713	–0.7	[M + HCOO] [–]	C ₃₂ H ₄₄ O ₁₄	Croctetin-mono-(β-D-gentiobiosyl)-ester ^b
22	12.64	755.2404	755.2421	–1.2	[M – H] [–]	C ₃₄ H ₄₄ O ₁₉	6"-O-trans-sinapyl genipine gentiobioside ^g
23	12.66	601.1199	601.1179	–2.3	[M – H] [–]	C ₂₈ H ₂₆ O ₁₅	3,5-Dicaffeoylquinic acid ^c
24	12.66	975.3715	975.3682	0.4	[M – H] [–]	C ₄₄ H ₆₄ O ₂₄	Crocini-I ^a
		1,021.377	1,021.3748	0.6	[M + HCOO] [–]		
25	12.84	537.2189	537.2171	–2.8	[M + HCOO] [–]	C ₂₂ H ₃₆ O ₁₂	Jasminoside H/I/S ^a
26	12.96	659.1618	659.1625	–1.2	[M – H] [–]	C ₃₁ H ₃₂ O ₁₆	3,5-di-O-caffeoyl-4-O-(3-hydroxy-3methyl) glutarylquinic acid ^c
27	13.64	559.1457	559.1435	–2.9	[M – H] [–]	C ₂₇ H ₂₈ O ₁₃	3-O-caffeoyl 4-O-sinapylquinic acid ^b
28	13.93	579.1719	579.1743	4.4	[M – H] [–]	C ₂₇ H ₃₂ O ₁₄	Naringin ^c
29	14.04	551.2134	551.2111	–2.4	[M – H] [–]	C ₂₇ H ₃₆ O ₁₂	Gymnospermae xyloside ^h
30	14.11	859.3241	859.3218	0.8	[M + HCOO] [–]	C ₃₈ H ₅₄ O ₁₉	Crocini II ^a
31	14.23	507.2083	507.2077	–2.2	[M + HCOO] [–]	C ₂₁ H ₃₄ O ₁₁	Jasminoside T ^a

(Continued)

TABLE 2 (Continued)

Nub.	T _R (min)	Negative ion mode				Molecular formula	Assigned identity
		Mean measured mass (Da)	Mean theoretical mass (Da)	ppm	Ion species		
32	15.57	549.1614	549.1624	−1.6	[M − H] [−]	C ₂₆ H ₃₀ O ₁₃	Jasminoside ^c
33	15.68	563.177	563.1795	4.1	[M − H] [−]	C ₂₇ H ₃₂ O ₁₃	Rhamnoside A ^c
34	15.77	573.1614	573.1596	0.6	[M + HCOO] [−]	C ₂₇ H ₂₈ O ₁₁	Populin ^a
35	16.44	647.2862	647.2872	1.3	[M − H] [−]	C ₃₇ H ₄₄ O ₁₀	Gnidilatin ^c
36	16.87	535.2185	535.2169	−2.2	[M − H] [−]	C ₂₇ H ₃₆ O ₁₁	6''-O-sinapoyljasmnoside A ^a
37	17.5	593.1876	593.1877	−0.5	[M − H] [−]	C ₂₈ H ₃₄ O ₁₄	6''-O-sinapoyl geniposide ^a
38	20.69	487.3429	487.3403	−2.3	[M − H] [−]	C ₃₀ H ₄₈ O ₅	Arjunolic acid ^h
39	22.53	469.3323	469.3303	−2.6	[M − H] [−]	C ₃₀ H ₄₆ O ₄	11-Keto mastic acid ^c
40	22.93	469.3323	469.3318	−1.4	[M + HCOO] [−]	C ₂₉ H ₄₄ O ₂	α-Tocopherol ^h
41	23.55	633.3797	633.3782	−1.1	[M − H] [−]	C ₃₉ H ₅₄ O ₇	3-O-cis coumarinic acid ^h

^a Increase, ^b Increase and then level off, ^c Level off and then increase, ^d Decrease, ^e Decrease and then level off, ^f Level off and then decrease, ^g Increase and then decrease, ^h Decrease and then increase.

process. The contents of 3,6-disinaoyl sucrose and kaempferol-3-O-rutinoside increased only in the late growth stage of GJE.

The contents of other components showed a downward trend. Among them shanzhiside methyl ester, geniposidic acid, sesamose, scandoside methyl ester, loganic acid, gardenoside, 10-O-p-hydroxycinnamoyl-scandoside, limonide, isochlorogenic acid A, 3,5-dicafeoylquinic acid, 3,5-di-O-caffeoyl-4-O-(3-hydroxy-3methyl) glutarylquinic acid, naringin, jasminoside, rhamnoside A, gnidilatin and 11-keto mastic acid did not further decline significantly, while the contents of aijanolic acid, changnanic acid, salicylic acid, α-triene tocopherol and 3-O-cis-p-coumaroyl decreased firstly and then increased. Gymnosperms xyloside, aijanolic acid, changnanic acid, sorbic acid, α-triene tocopherol and 3-O-cis coumarinic acid decreased first and then increased during the growth process. It can be speculated that the active components of GJE changed significantly during the growth process. In view of the increasing trend of most iridoid glycosides, flavonoids and phenolic acids in the growth process, it is presumed that the mature GJE might be easier to play a role in clearing heat and detoxifying effect. This study also plays a guiding role for the subsequent quantitative analysis of GJE.

The biphasic pattern (initial decrease followed by increase) observed for certain compounds warrants further discussion. Based on general patterns of fruit development in medicinal plants, several factors may contribute to this phenomenon. During early to mid-maturation, resource allocation toward the biosynthesis of predominant secondary metabolites (iridoid glycosides and flavonoids, as observed in stages 1–4) could potentially reduce the synthesis of other metabolite classes such as triterpenes (arjunolic acid, changnanic acid) (Figueroa et al., 2021). As fruits approach over-ripening, physiological stress responses typically associated with senescence may induce the accumulation of defensive compounds including antioxidants like α-tocopherol (Yang et al., 2018). Additionally, interconversion between different metabolite forms through enzymatic transformation cannot be excluded. Further metabolic pathway analysis and gene expression studies would be valuable to elucidate the specific mechanisms underlying these dynamics in *Gardenia jasminoides*.

3.4 Anti-proliferative effects of GJE

The anti-proliferative effect of gardenia on MCF7 breast cancer cells and HepG2 hepatocellular carcinoma cells is mainly due to the synergistic effect of its abundant bioactive components through multiple molecular mechanisms. Its possible anti-cancer mechanisms are as follows: (I) the main active components of gardenia, iridoid terpene glycosides, can specifically inhibit the activity of mitochondrial uncoupling protein 2 (UCP2) after intracellular metabolism to genipine (Cho, 2022). The inhibition of UCP2 leads to a decrease in the efficiency of ATP synthesis in mitochondria, a disorder of cellular energy metabolism (Dando et al., 2017), and at the same time promotes the production of reactive oxygen species (ROS) and the release of cytochrome C (Lee et al., 2020), activates the caspase-9 and caspase-3 cascades in the intrinsic apoptotic pathway (Ahani et al., 2019), and finally induces programmed death of cancer cells (Kim et al., 2020); (II) flavonoids work by regulating cell cycle regulatory proteins. These compounds can downregulate the expression of cyclin D1 (Cyclin D1) and cyclin-dependent kinase 4/6 (CDK4/6) (Wang et al., 2017), upregulate the levels of cyclin-dependent kinase inhibitors p21 and p27, arrest cancer cells in the G0/G1 phase, block DNA replication initiation, and thus inhibit cell proliferation (Shin et al., 2013); (III) Phenolic acids exert anti-cancer effects mainly by inhibiting NF-κB and PI3K/AKT signaling pathways (Ahmed et al., 2025). The inhibition of these pathways leads to the downregulation of anti-apoptotic proteins Bcl-2 and survival proteins, and up-regulates the expression of pro-apoptotic proteins Bax and Bad (Singh Tuli et al., 2022), breaking the balance of intracellular apoptosis and anti-apoptotic proteins and promoting cancer cell death.

The anti-proliferative effects of GJE against MCF7 and HepG2 cancer cell lines are illustrated in Figure 4. All GJE samples displayed strong anti-proliferative effects, which increased in a dose-dependent manner. GJE demonstrated stronger anti-proliferative effect against HepG2 cancer cells (IC₅₀ = 20.67 mg/mL, 15.83 mg/mL, 15.93 mg/mL, 12.68 mg/mL, 6.06 mg/mL, 6.43 mg/mL and 9.89 mg/mL), compared with MCF7 cancer cells (IC₅₀ = 18.37 mg/mL, 16.25 mg/mL, 17.09 mg/mL, 15.09 mg/mL, 8.93 mg/mL, 9.06 mg/mL and

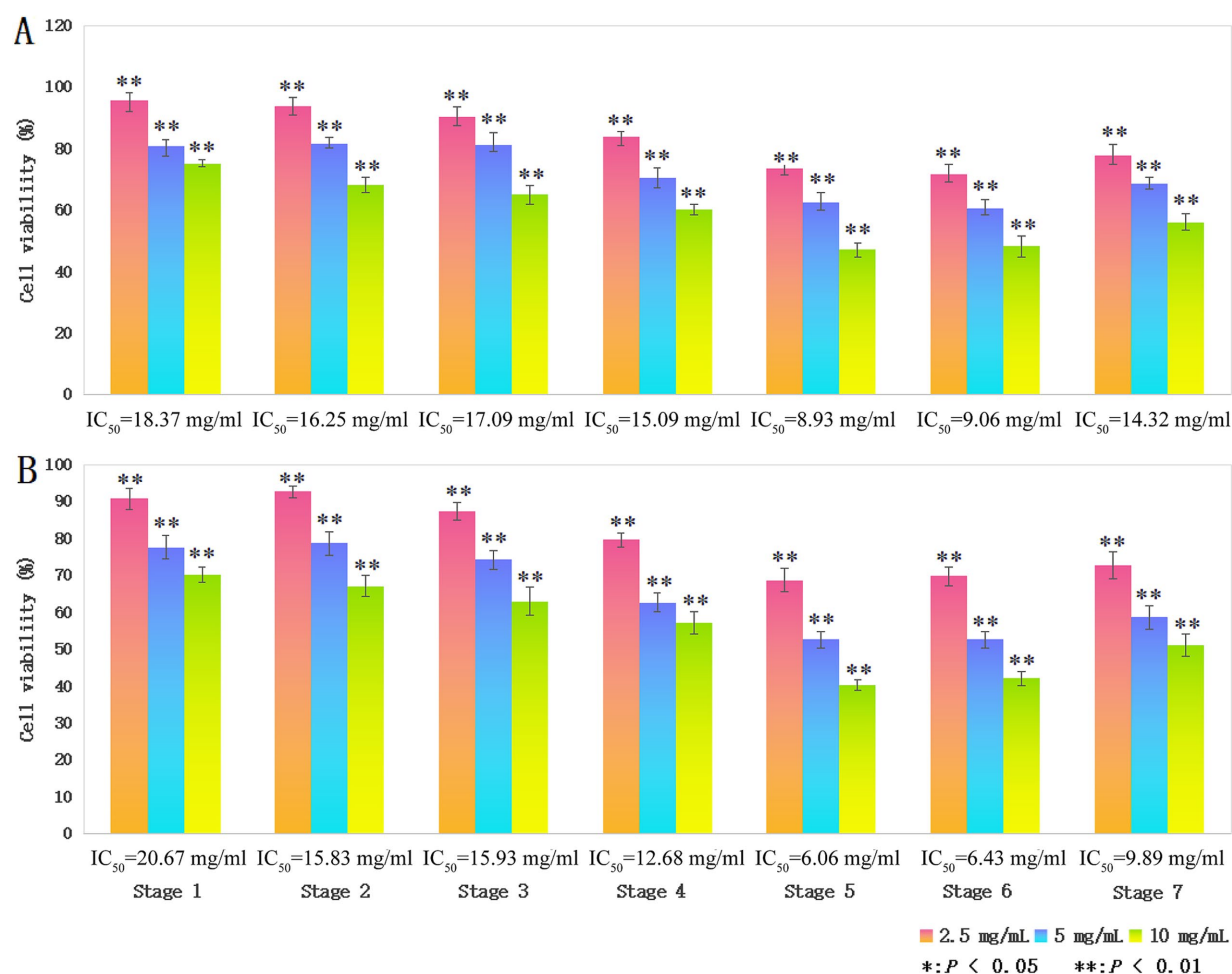


FIGURE 4

Dose-dependent anti-proliferative effects of *Gardenia jasminoides* J.Ellis (GJE) extracts from different harvesting stages against human cancer cell lines. (A) MCF7 breast adenocarcinoma cells; (B) HepG2 hepatocellular carcinoma cells. Cells were seeded at a density of 8×10^3 cells per well in 96-well plates and allowed to attach for 24 h. Cells were then treated with GJE extracts at three representative concentrations (2.5 mg/mL, pink bars; 5 mg/mL, blue bars; 10 mg/mL, yellow-green bars) for 48 h. Cell viability was determined by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay measuring absorbance at 570 nm. Data are presented as mean $\pm$ standard deviation (SD) from three independent experiments, each performed in triplicate wells ($n = 3$ biological replicates). Error bars represent SD. Half-maximal inhibitory concentration (IC₅₀) values were calculated using non-linear regression analysis (four-parameter logistic curve fitting, GraphPad Prism software) and are displayed below each stage on the x-axis. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) *post hoc* test to compare differences among the seven harvesting stages at each concentration. Asterisks indicate significant differences compared to Stage 1: * $p < 0.05$, ** $p < 0.01$. GJE from mature fruits (Stages 5 and 6) exhibited significantly stronger anti-proliferative effects (lowest IC₅₀ values) compared to immature (Stages 1–2) or overripe (Stage 7) fruits in both cell lines. HepG2 cells were significantly more sensitive to GJE treatment than MCF7 cells across all stages ($p < 0.05$, independent samples *t*-test). Doxorubicin hydrochloride was used as positive control (IC₅₀ = 3.78 μ g/mL for MCF7, 3.04 μ g/mL for HepG2).

14.32 mg/mL) ($p < 0.05$, independent samples *t*-test). Doxorubicin (positive control) demonstrated significantly higher potency with IC₅₀ values of 3.78 μ g/mL for MCF7 cells and 3.04 μ g/mL for HepG2 cells. As the growth time increased, the anti-proliferative effect of GJE increased, reaching the peak at stage 5–6 and declining at stage 7. The observed anti-proliferative effects can be specifically attributed to several key bioactive compounds identified in this study. Among iridoid glycosides, deacetyl asperulosidic acid and jasminoside R showed the strongest positive correlations with anti-proliferative activity ($r = 0.78$ – 0.85 , $p < 0.01$), while jasminoside H/I/S and jasminoside T also demonstrated significant positive correlations ($r = 0.65$ – 0.72 , $p < 0.05$), consistent with previous reports that iridoid glycosides induce apoptosis through UCP2 inhibition (Evans, 1996;

Figueroa et al., 2021). For flavonoids, both kaempferol-3-O-rutinoside and naringenin-4'-glucoside-7-neohesperidin showed moderate to strong positive correlations with bioactivity ($r = 0.64$ – 0.74 , $p < 0.05$), consistent with their known cell cycle regulatory effects (Chen et al., 2020; Tian et al., 2022). Phenolic acids also exhibited positive correlations, with chlorogenic acid methyl ester and 3-O-caffeoyl 4-O-sinapylquinic acid showing significant relationships with bioactivity ($r = 0.68$ – 0.76 , $p < 0.05$), likely contributing through anti-inflammatory mechanisms via NF- κ B pathway inhibition (Chen et al., 2020; Tian et al., 2022). However, the underlying mechanisms for the activity decline observed at stage 7 require further investigation. Based on the demonstrated anti-proliferative activity against MCF7 and HepG2 cancer cells, GJE may

serve as a potential source for developing anti-cancer therapeutic agents or functional foods with health benefits.

3.5 Correlation between chemical composition and anti-proliferative activity

Pearson correlation analysis was performed to validate the relationship between major bioactive compound contents and anti-proliferative activities across the seven harvesting stages. Eight representative compounds from three major chemical classes were selected based on their increasing trends during fruit maturation.

All selected compounds demonstrated positive correlations with anti-proliferative activity, supporting the hypothesis that high contents of these compound classes contribute to enhanced bioactivity. Among iridoid glycosides, deacetyl asperulosidic acid and jasminoside R showed strong positive correlations ($r = 0.78\text{--}0.85$, $p < 0.01$), while jasminoside H/I/S and jasminoside T also demonstrated significant positive correlations ($r = 0.65\text{--}0.72$, $p < 0.05$). For flavonoids, both kaempferol-3-O-rutinoside and naringenin-4'-glucoside-7-neohesperidin showed moderate to strong positive correlations with anti-proliferative activity ($r = 0.64\text{--}0.74$, $p < 0.05$). Phenolic acids also exhibited positive correlations, with chlorogenic acid methyl ester and 3-O-caffeoyl 4-O-sinapylquinic acid showing significant relationships with bioactivity ($r = 0.68\text{--}0.76$, $p < 0.05$).

These results suggest that the high anti-proliferative effects observed in mature fruits (stages 5–6) may be related to the increased phenolic, flavonoid and iridoid glycoside contents, as these compounds showed positive correlations with enhanced bioactivity.

4 Conclusion

In this study, based on UFLC/Q-TOF-MS technology, a rapid identification method for the differential components of gardenia at seven harvest stages was successfully established, from which 52 chemical components were identified, and the dynamic changes of chemical components of gardenia at different maturity stages were systematically clarified. The results showed that with the increase of fruit maturity, the main bioactive components in gardenia exhibited time-dependent patterns of change: iridoid terpene glycosides (such as deacetylcephalic acid, jasmonosides, crocinins), flavonoids and phenolic acids generally showed an upward trend during the growth process, reaching a peak in the 5th–6th stage (orange–red to red fruits), and decreasing in the overripe stage (dark red fruits in the 7th stage). The study of anti-proliferative activity showed that the inhibitory effect of gardenia extract on HepG2 hepatocellular carcinoma cells (IC_{50} : 6.06–20.67 mg/mL) was stronger than that of MCF7 breast cancer cells (IC_{50} : 8.93–18.37 mg/mL), and the activity intensity was closely related to the harvest period, and the strongest anti-cancer effect was also achieved at stages 5–6. Pearson correlation analysis further confirmed a significant positive correlation between the content of major bioactive components and antiproliferative activity ($r = 0.64\text{--}0.85$, $p < 0.05$), which verified the intrinsic relationship between “component-effect.” Therefore, this study not only provides a scientific basis for the quality standardization and determination of the optimal harvest date of gardenia, establishes the best harvest period of the 5th and 6th periods for both the content of active ingredients and anti-cancer activity, but also lays a solid

theoretical foundation for the development of anti-cancer drugs or functional health products based on gardenia, which embodies the modern scientific connotation of the concept of “timely harvesting” in traditional Chinese medicine.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

CC: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft. BJ: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft. YC: Data curation, Investigation, Validation, Writing – original draft. XC: Investigation, Methodology, Software, Writing – review & editing. CS: Data curation, Formal analysis, Investigation, Writing – review & editing. SY: Investigation, Resources, Validation, Writing – review & editing. HW: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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Conflict of interest

BJ was employed by China Mobile Group Jiangsu Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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