Identification and Quantification of Chemical Constituents in DIGESTIVE CARE

1. Objective

1.1 To investigate the main chemical components in DIGESTIVE CARE using high-performance liquid chromatography-mass spectrometry (HPLC-MS) technology.

1.2 To determine the contents of total proteins, total polysaccharides, total polyphenols, and total flavonoids in DIGESTIVE CARE using ultraviolet spectrophotometry.

2. Method

2.1 Identification of Chemical Constituents in DIGESTIVE CARE

Ten batches of the DIGESTIVE CARE sample (1.0 g per batch) were accurately weighed for parallel experiments. For each batch, 10 mL of chromatographic-grade methanol was precisely added, followed by ultrasonic extraction for 2 h. After allowing the extracts to settle and clarify, 1 mL of the supernatant was collected for mass spectrometric analysis.

Reference standards were dissolved and diluted with methanol to six different concentrations for mass spectrometric analysis.

High-performance liquid chromatography (HPLC) was performed on an UltiMate 3000 HPLC system (Thermo Fisher Scientific, CA, USA) equipped with an AQ C18 column (1.9 μm, 2.1 mm × 100 mm). The flow rate was 0.3 mL/min, and the injection volume was 3 μL. The mobile phase consisted of 0.1% acetonitrile (A) and 0.1% formic acid in water (B). The gradient elution program is shown in Table 1. UV detection wavelengths were set at 210, 254, 280, and 365 nm.

Mass spectrometric analysis was performed on a Q-Exactive mass spectrometer (Thermo Fisher Scientific, CA, USA) equipped with a heated electrospray ionization (HESI) source. The ion source temperature was set to 310 °C and the capillary temperature to 320 °C. The sheath gas flow was 30 arb. units and the auxiliary gas flow

10 arb. units. The spray voltage was set to 3 kV in positive ion mode and 2.8 kV in negative ion mode. Data were acquired in data-dependent acquisition (DDA) mode with a loop count of 10. Fragmentation was performed using higher-energy collisional dissociation (HCD) with stepped normalized collision energies of 10, 28, and 35 eV. For qualitative analysis: Full MS scan range: *m/z* 100–1500, resolution: 70,000, AGC target: 3e6, max injection time: 200 ms. MS/MS resolution: 17,500, AGC target: 1e5, max injection time: 50 ms. For quantitative analysis: For quantitative analysis: The mass spectrometer SIM (Selected Ion Monitoring) mode is used to monitor the quasi-molecular ion peaks of the target compounds.

Table 1 Gradient elution program in HPLC

Retention (min)	Mobile phase A	Mobile phase B	Flow rate (mL/min)	Colum temperature (℃)
0.00	10	90	0.3	40
15.00	100	0	0.3	40
17.00	100	0	0.3	40
17.10	90	10	0.3	40
20.00	90	10	0.3	40

2.2 Determination of Total Polysaccharides Content in DIGESTIVE CARE

A total of 1 g DIGESTIVE CARE was accurately weighed, mixed with 50 mL of distilled water, and refluxed in a boiling water bath for 2 hours. Subsequently, 5 mL of 10% trichloroacetic acid (TCA) was added to precipitate proteins, followed by centrifugation (4000 rpm, 10 min) to collect the supernatant. The supernatant was precipitated with 80% ethanol (overnight at 4°C), and after centrifugation, the precipitate was redissolved in distilled water.

A total of 50 mg of anhydrous glucose was accurately weighed and dissolved in purified water to prepare a series of standard solutions at concentrations of 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, and 2 mg/mL. To each solution, 6% phenol reagent was added and

mixed thoroughly, followed by the rapid addition of concentrated sulfuric acid. After mixing, the solutions were allowed to stand for 5 min, then placed in boiling water for 15 min. After removal and cooling to room temperature, the absorbance was measured at 490 nm using a microplate reader. A blank control was prepared by subjecting 0.5 mL of purified water to the same procedure. The standard curve was constructed based on the measured absorbance values.

The prepared DIGESTIVE CARE sample was diluted to six different concentrations and subjected to the same procedure as described for the glucose standard solutions. The absorbance of the final solutions was measured at 490 nm using a microplate reader to ensure that the calculated concentrations fell within the linear range of the standard curve.

2.3 Determination of Total Proteins Content in DIGESTIVE CARE

A total of 5 g DIGESTIVE CARE sample was accurately weighed. Extraction was performed with a sodium hydroxide solution (pH 11) at a solid-to-solvent ratio of 1:10 (g/mL). The extraction process was conducted at 35°C for 1.5 hours.

Reagent A and Reagent B from the Thermo Scientific Pierce BCA Protein Assay Kit were combined to prepare the working reagent. A standard curve was generated using the provided bovine serum albumin (BSA) standard, prepared at a series of concentrations: 0.3125, 0.625, 1.25, 2.5, 5, and 10 μg/mL. Following incubation at 37°C for 30 minutes in the dark, the absorbance of the standards was measured at 562 nm using a microplate reader.

The DIGESTIVE CARE extract was diluted to six different concentration gradients. The working reagent (mixture of Reagent A and B) was added to each diluted sample. After incubation at 37°C for 30 minutes in the dark, the absorbance was measured at 562 nm using a microplate reader. Dilutions were optimized to ensure the final measured protein concentrations fell within the linear range of the standard curve.

2.4 Determination of Total Polyphenols Content in DIGESTIVE CARE

A total of 1 g of the DIGESTIVE CARE sample was accurately weighed, mixed with 20 mL of 70% methanol, and ultrasonically extracted for 30 minutes. The mixture was filtered, and the subsequent filtrate was collected as the test solution.

A total of 50 mg of gallic acid standard was accurately weighed and dissolved in 30% methanol to prepare standard solutions with concentrations of 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, and 2 mg/mL. The Folin-Ciocalteu reagent and 7.5% Na_2CO_3 solution were sequentially added to each solution. After thorough mixing, the absorbance was measured at 760 nm using a microplate reader. The standard curve was constructed based on the measured absorbance values.

The prepared DIGESTIVE CARE sample was diluted to six different concentrations and subjected to the same procedure as described for the gallic acid standard solutions. The absorbance of the final solutions was measured at 760 nm using a microplate reader, ensuring that the calculated concentrations fell within the linear range of the standard curve.

2.5 Determination of Total Flavones Content in DIGESTIVE CARE

A total of 1 g of the DIGESTIVE CARE sample was accurately weighed, mixed with 20 mL of 70% methanol, and ultrasonically extracted for 30 minutes. The mixture was filtered, and the subsequent filtrate was collected as the test solution.

A total of 50 mg of rutin standard was accurately weighed and dissolved in 70% ethanol to prepare standard solutions with concentrations of 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, and 2 mg/mL. The 5% sodium nitrite solution, 10% aluminum nitrate solution, and 4% sodium hydroxide solution were sequentially added to each solution. After thorough mixing, the absorbance was measured at 510 nm using a microplate reader. The standard curve was constructed based on the measured absorbance values.

The prepared DIGESTIVE CARE sample was diluted to six different concentrations and subjected to the same procedure as described for the rutin standard

solutions. The absorbance of the final solutions was measured at 510 nm using a microplate reader, ensuring that the calculated concentrations fell within the linear range of the standard curve.

3. Results

3.1 Identification and Quantification of Chemical Constituents in DIGESTIVE CARE

The LC-MS data was processed using MS-DIAL software. MS^2 fragment ions were extracted and searched against natural product databases, including MassBank and GNPS. Through comparative analysis, a total of 11 major chemical constituents in DIGESTIVE CARE were identified through comparative analysis. Their HPLC fingerprint is shown in Figure 1. The mass spectrometric identification results are listed in Table 2.

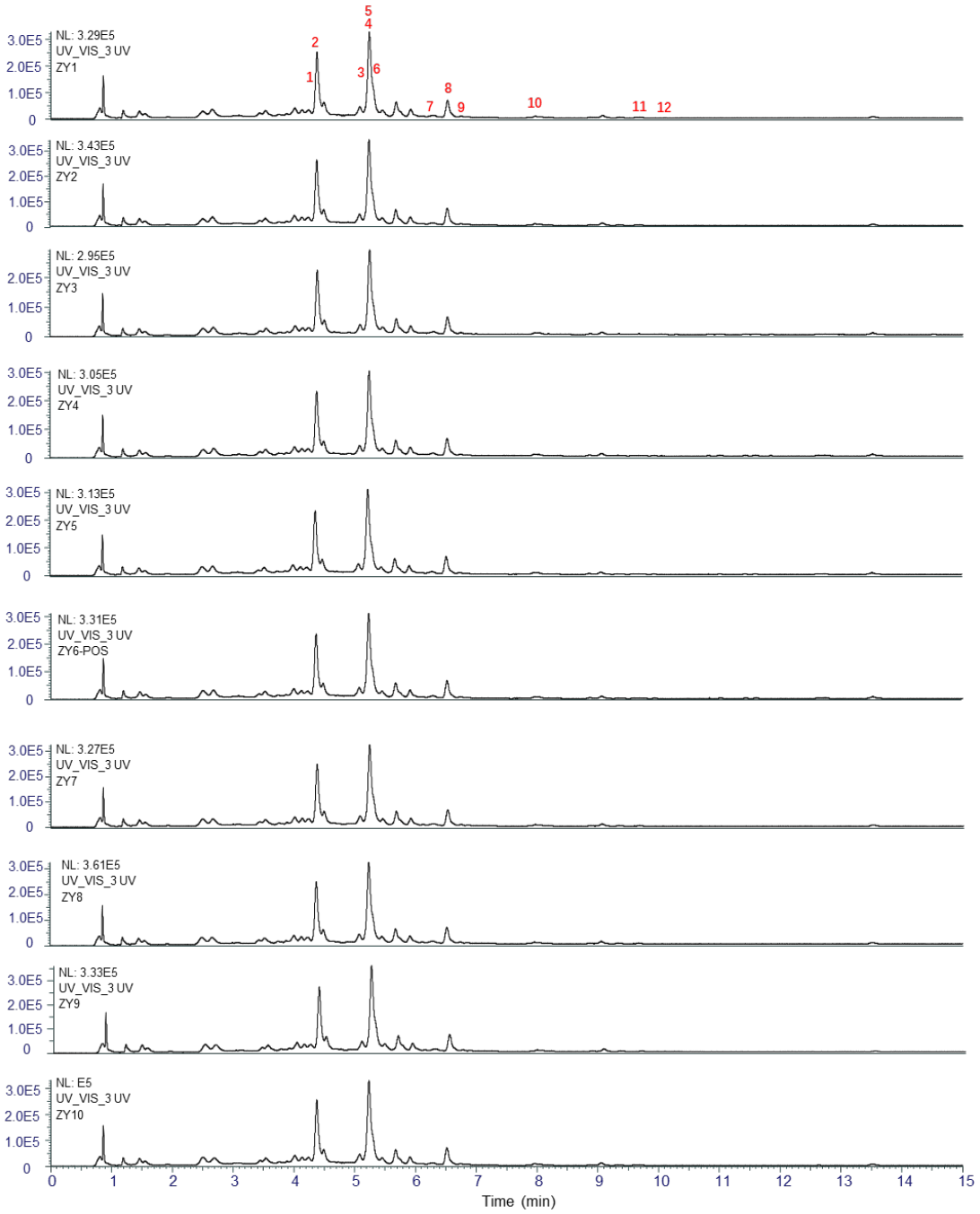

Figure 1. HPLC fingerprint chromatograms of the main chemical constituents identified in ten batches of DIGESTIVE CARE

Among the 11 identified chemical constituents, reference standards for 7 compounds are commercially available. Therefore, these 7 standards are diluted to twelve different concentrations: 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 1000, 2000, 5000 ppb. Standard curves are plotted (Figures 2-8), and the corresponding regression equations are obtained. Based on the regression equations, the final content of these 7 chemical constituents in the DIGESTIVE CARE samples is calculated (Table 2).

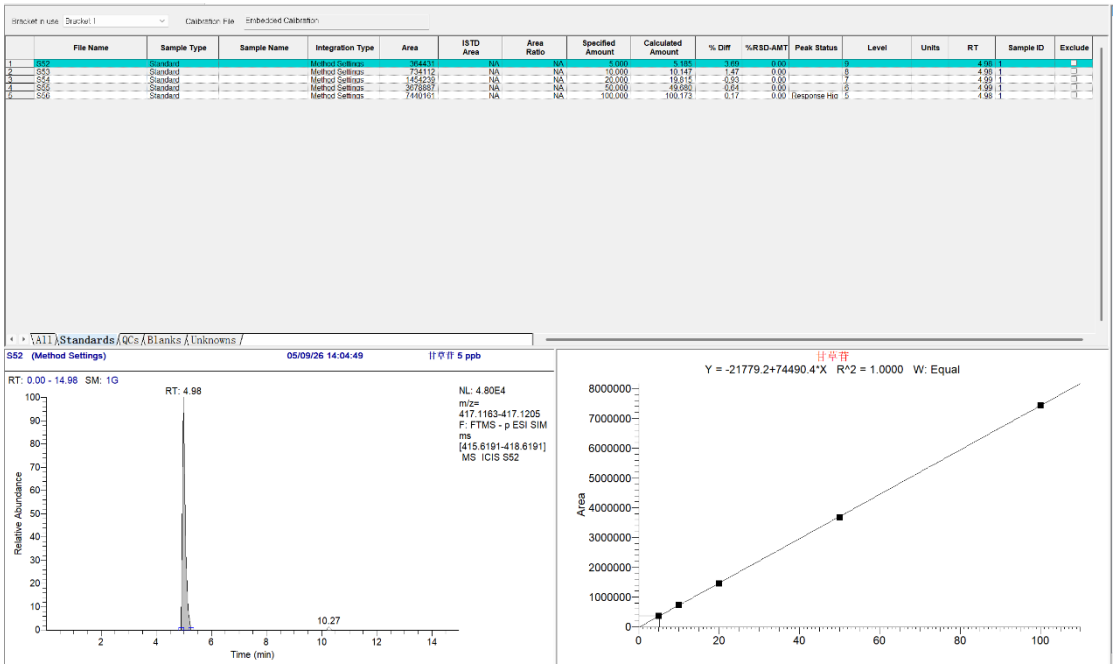

Figure 2. Standard Curve of Liquiritin

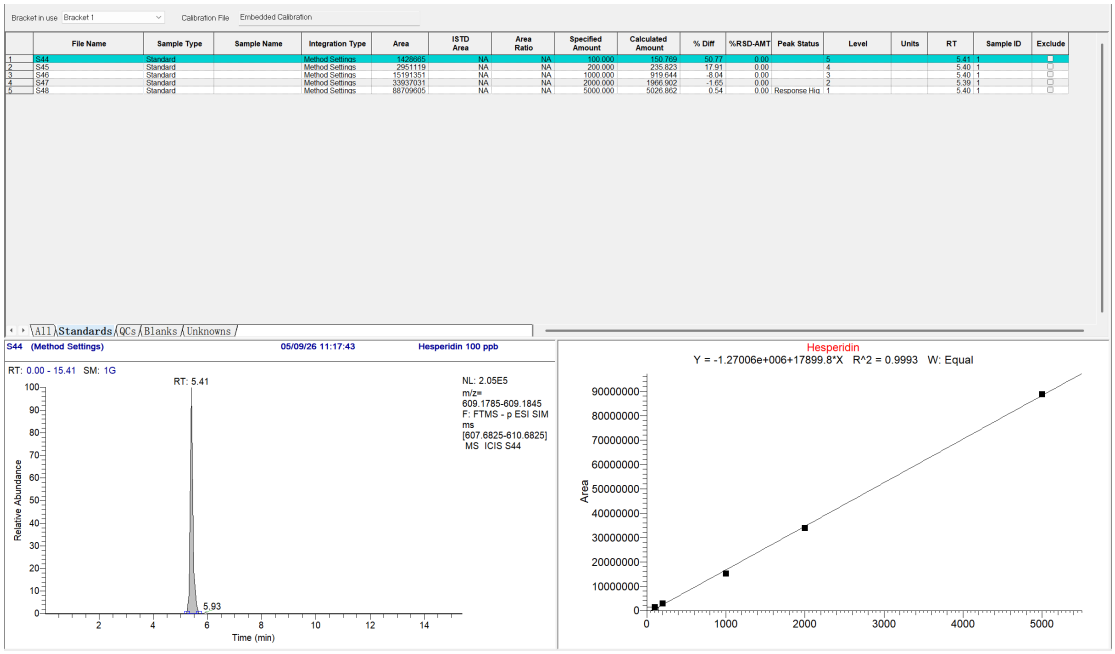

Figure 3. Standard Curve of Hesperidin

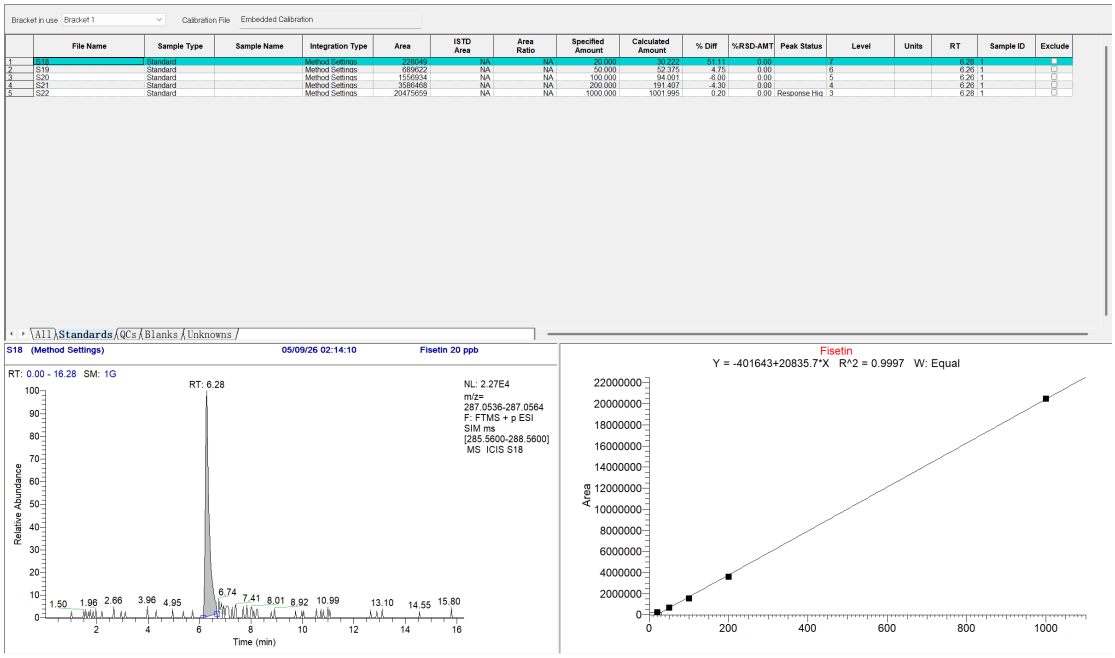

Figure 4. Standard Curve of Fisetin

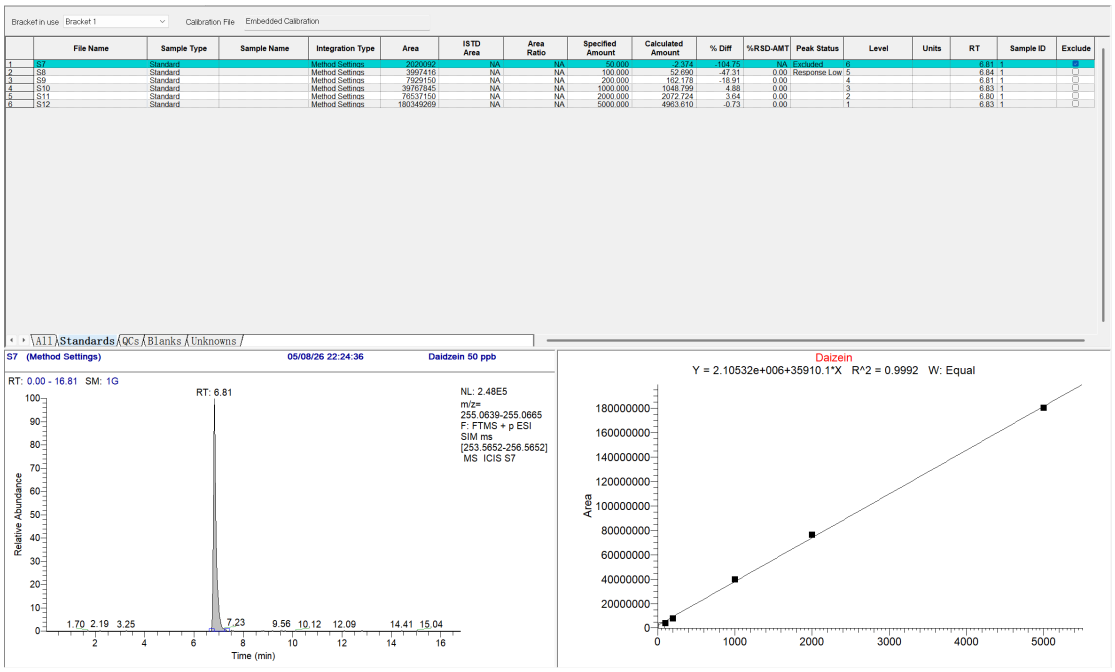

Figure 5. Standard Curve of Daidzein

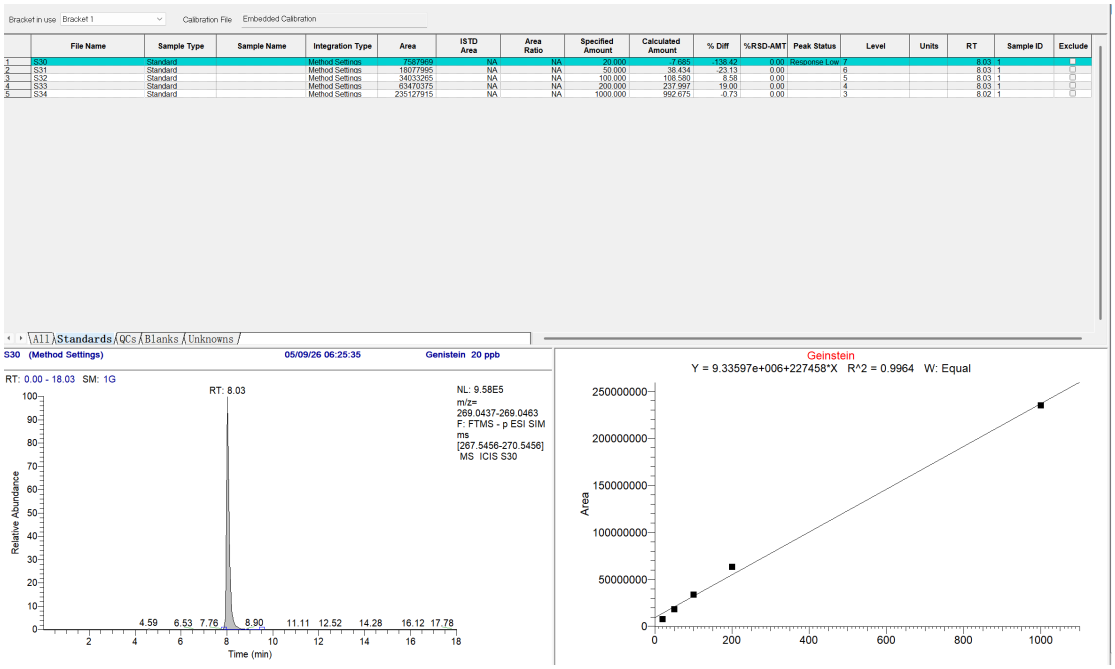

Figure 6. Standard Curve of Genistein

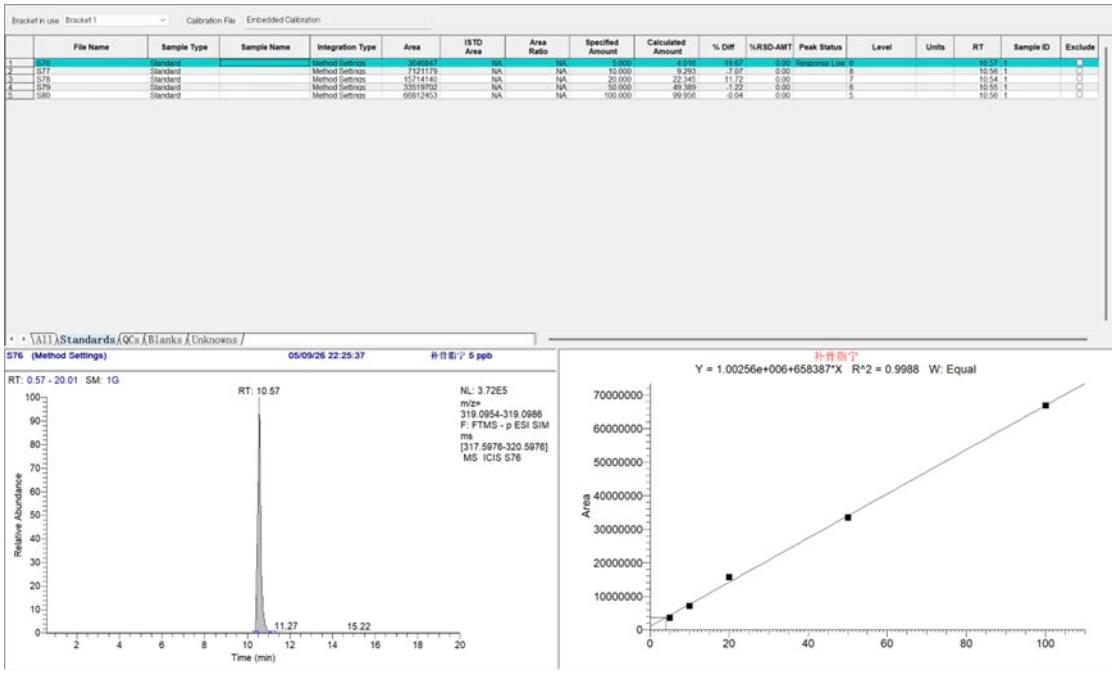

Figure 7. Standard Curve of Corylin

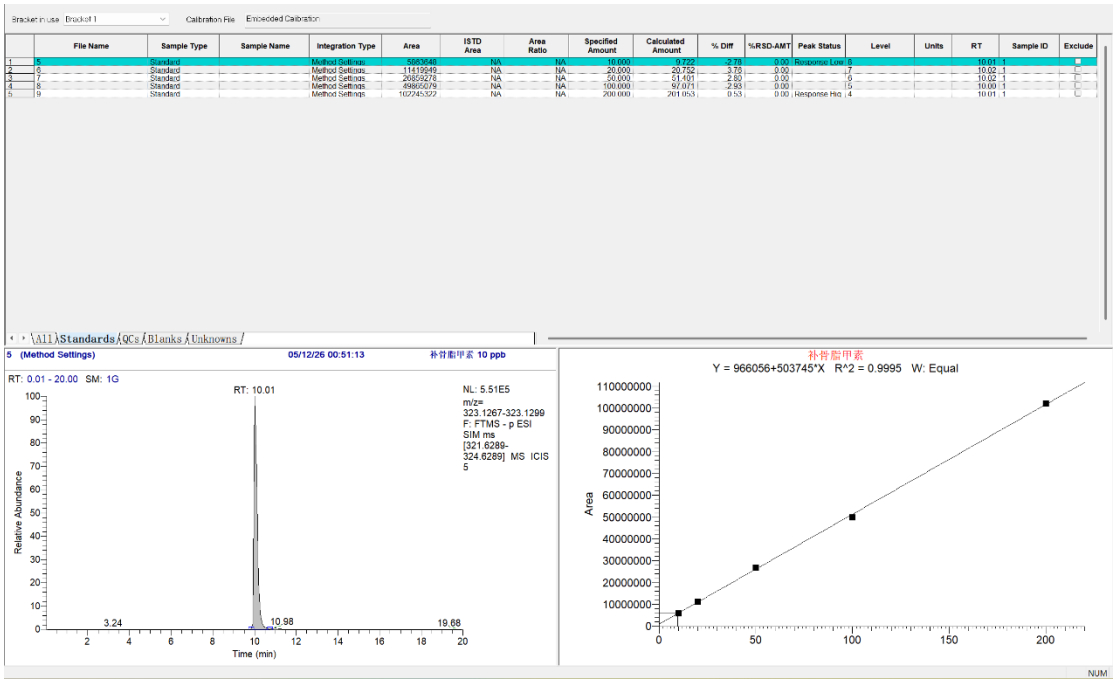

Figure 8. Standard Curve of Bavachin

Table 2. Summary of qualification and quantification results for the main constituents in ten batches of DIGESTIVE CARE

No.	Retention time (min)	Content (μg/g)	Ion type	Chemical name	Chemical attribute	Chemical formula
1	4.24	/	$[M-H]^-$	Leonoside A/ [(2R,3R,4R,5R,6R)-4-[(2S,3R,4R,5R,6S)-4,5-dihydroxy-6-methyl-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyoxan-2-yl]oxy-6-[2-(3,4-dihydroxyphenyl)ethoxy]-5-hydroxy-2-(hydroxymethyl)oxan-3-yl] (E)-3-(3,4-dihydroxyphenyl)prop-2-enoate	Phenylpropanoid glycosides	$C_{35}H_{46}O_{20}$
2	4.38	/	$[M+H]^+/[M-H]^-$	Isoflavone base + 2*O*, *O*-Hex	Isoflavonoid *O*-glycosides	$C_{24}H_{22}O_{12}$
3	5.15	6.56	$[M+H]^+$	Liquiritin	Flavonoid *O*-glycosides	$C_{21}H_{22}O_9$
4	5.24	/	$[M+H]^+$	Isoflavone base + 2*O*, *O*-MalonylHex	Isoflavonoid *O*-glycosides	$C_{24}H_{22}O_{12}$
5	5.24	/	$[M+H]^+/[M+HCOO]^-$	Apigetrin/cosmosiin /apigenin 7-*O*-glucoside	Flavonoid-7-*O*-glycosides	$C_{21}H_{20}O_{10}$
6	5.38	1173.74	$[M-H]^-$	Hesperedin	Flavonoid-7-*O*-glycosides	$C_{28}H_{34}O_{15}$
7	6.25	2.53	$[M+H]^+$	Fisetin	Flavonols	$C_{15}H_{10}O_6$

8	6.5	/	$[M+H]^+$	2,3-dihydro-1H-carbazol-4(9H)-one	Carbazoles	$C_{12}H_{11}NO$
9	6.81	29.60	$[M+H]^+/[M-H]^-$	Daidzein	Isoflavones	$C_{15}H_{10}O_4$
10	8.02	48.97	$[M+H]^+$	Genistein	Isoflavones	$C_{15}H_{10}O_5$
11	10.03	17.94	$[M+H]^+$	Bavachin	6-Prenylated flavanones	$C_{20}H_{20}O_4$
12	10.50	2.03	$[M+H]^+$	Corylin	Pyranoisoflavonoids	$C_{20}H_{16}O_4$

3.2 Total Polysaccharides Content in DIGESTIVE CARE

The glucose standard curve demonstrated excellent linearity within the concentration range of 0.001 to 0.015 mg/mL, with a coefficient of determination (R^2) of 0.9966, confirming the validity of the analytical method (Figure 9).

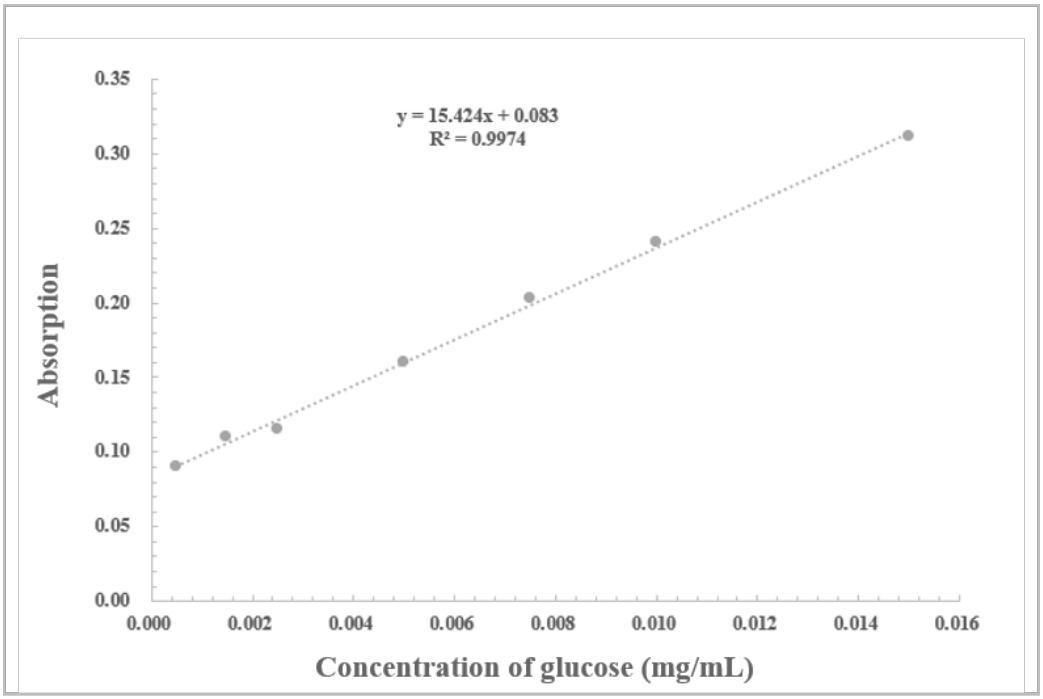

Figure 9. Standard curve for total polysaccharide determination

Based on the regression equation derived from the standard curve, the total polysaccharide content in the Digestive Care sample was calculated to be 64.53 mg/g of sample.

3.3 Total Proteins Content in DIGESTIVE CARE

The BSA standard curve demonstrated excellent linearity within the concentration range of 0.3125 to 10 µg/mL, with a coefficient of determination (R^2) of 0.9992, meeting the required methodological criteria (Figure 10).

Based on the regression equation derived from the standard curve, the total protein content in the Digestive Care sample was calculated to be 194.23 mg/g of sample.

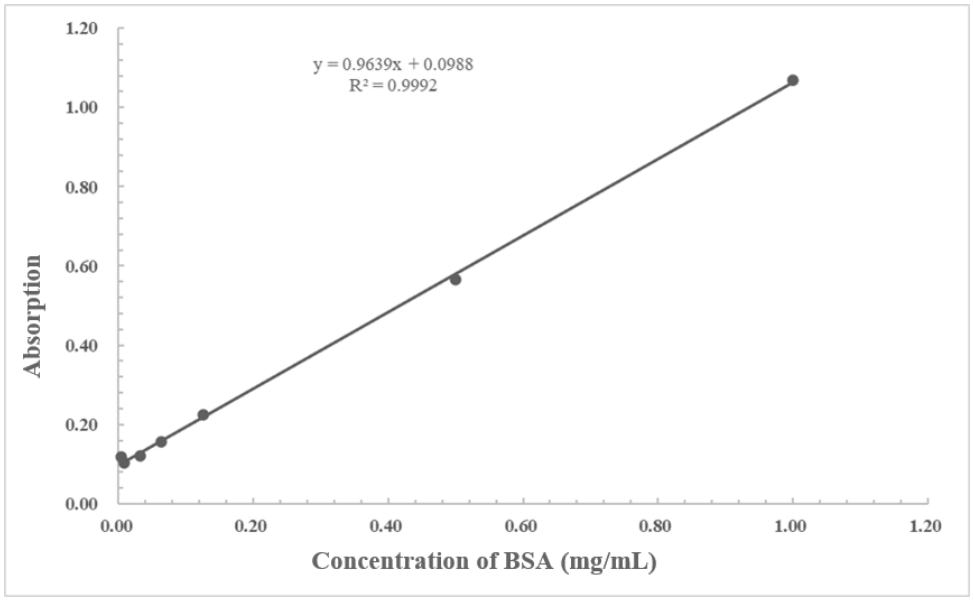

Figure 10. Standard curve for total protein determination

3.4 Total Polyphenols Content in DIGESTIVE CARE

The gallic acid standard curve exhibited excellent linearity in the range of 0.01–0.5 mg/mL (R^2=0.9941), meeting the methodological requirements, as shown in Figure 11.

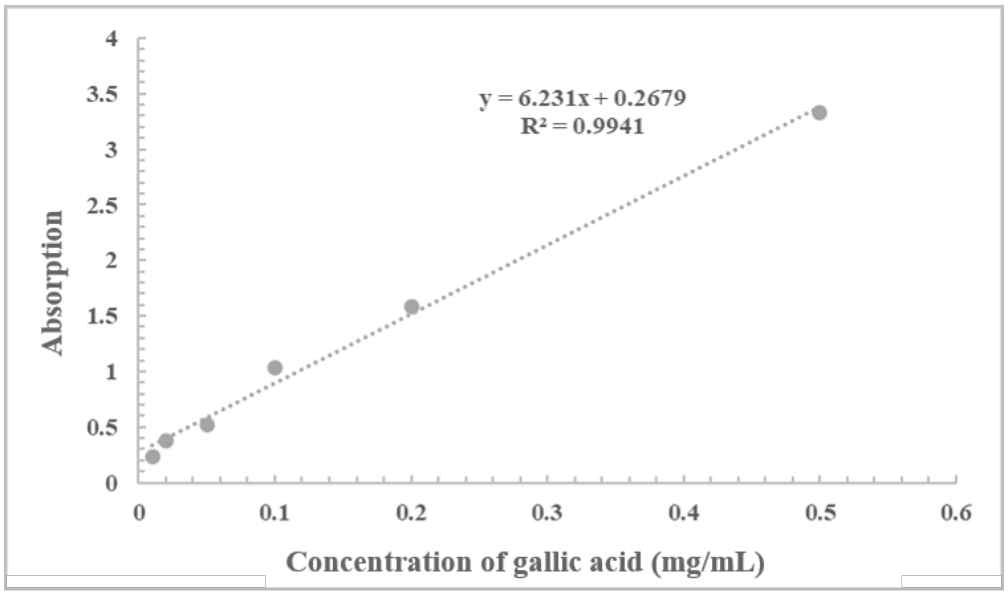

Figure 11. Standard curve for total polyphenols determination

Based on the regression equation of the above standard curve, the total polyphenol content in the DIGESTIVE CARE sample was calculated to be 6.29 mg/g.

3.5 Total Flavones Content in DIGESTIVE CARE

The standard curve exhibited excellent linearity in the range of 0.01–2 mg/mL (R^2=0.9966), meeting the methodological requirements, as shown in Figure 12.

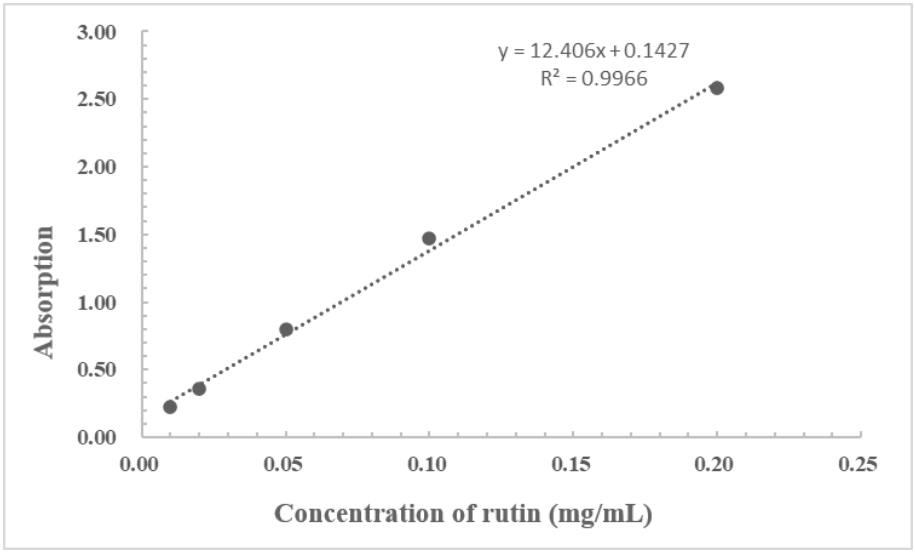

Figure 12 Standard curve for total flavones determination

Based on the regression equation of the above standard curve, the total flavonoid content in the DIGESTIVE CARE sample was calculated to be 24.73 mg/g.

4. Summary of Nutritional Profile in DIGESTIVE CARE

Nutritional Component	Content
Total protein	194.23 mg/g
Total Polysaccharide	65.53 mg/g
Total polyphenol	6.29 mg/g
Total flavones	24.73 mg/g
Liquiritin	6.56 μg/g
Hesperedin	1173.74 μg/g
Fisetin	2.53 μg/g
Daidzein	29.60 μg/g
Genistein	48.97 μg/g
Bavachin	17.94 μg/g
Corylin	2.03 μg/g

5. Summary of Bioactivities of the Main Chemical Constituents in DIGESTIVE CARE

No.	Chemical name	Bioactivity
1	Leonoside A	**Bioactivity Profile:** Its primary reported biological activity is antioxidant action. **Detailed Summary:** Currently, the reported biological activity of Leonoside A is primarily its antioxidant effect[1]. Furthermore, the Leonoside compound Leonoside F has been confirmed to possess activity protecting hepatocytes from chemically induced injury[2], hinting that Leonoside A may have similar hepatoprotective effects.
2	Isoflavone base + 2*O*, *O*-Hex	**Bioactivity Profile:** Specific biological activities have not been reported. However, as an isoflavone O-glycoside compound, it holds potential for applications in anti-inflammation, anti-tumor, anti-aging, and anti-diabetes.
3	Liquiritin	**Bioactivity Profile:** Its biological activities include anti-tumor, anti-inflammatory, and antioxidant effects, among others.

Detailed Summary:

Firstly, the role of liquiritin in anti-tumor applications has been confirmed by several studies. Research has found that liquiritin can effectively inhibit the proliferation of breast cancer cells and induce apoptosis by suppressing the epidermal growth factor receptor (EGFR) and mitogen-activated protein kinase 8 (MAPK8) signaling pathways[3]. Additionally, liquiritin inhibits angiogenesis in breast cancer by suppressing the lysosomal degradation of CXCL1 in tumor-associated macrophages (TAMs)[4].

In terms of anti-inflammatory effects, liquiritin acts by regulating multiple signaling pathways. Studies show that liquiritin can alleviate inflammation damage induced by lipopolysaccharide (LPS) in human keratinocytes (HaCaT cells) by inhibiting the NF-κB and JNK signaling pathways[5]. Furthermore, liquiritin can reduce chondrocyte apoptosis by modulating the P53/PUMA signaling pathway, thereby mitigating the progression of osteoarthritis[6].

Regarding its antioxidant activity, liquiritin exerts its protective effects through various mechanisms. Research has found that liquiritin can enhance the survival ability of neuroblastoma cells by increasing the expression of glucose-6-phosphate dehydrogenase[7]. Additionally, liquiritin protects osteoblasts from oxidative stress-induced cytotoxic damage by modulating oxidative stress and

		mitochondrial dysfunction[8]. In summary, as a natural compound with multiple biological activities, liquiritin shows broad application prospects in anti-tumor, anti-inflammatory, and antioxidant therapies. Future research can further explore the potential therapeutic effects of liquiritin in other diseases and develop its clinical applications.
4	Isoflavone base + 2*O*, *O*-MalonylHex	Bioactivity Profile: Specific biological activities have not been reported. However, as an isoflavone *O*-glycoside compound, it holds potential for applications in anti-inflammation, anti-tumor, anti-aging, and anti-diabetes.
5	Apigenin 7-*O*-glucoside	**Bioactivity Profile:** Its biological activities include antibacterial, anti-tumor, anti-inflammatory, and antioxidant effects, among others. **Detailed Summary:** Firstly, apigenin 7-*O*-glucoside (A7G) exhibits significant activity in antibacterial and anti-biofilm aspects. Research has found that A7G shows potent inhibitory effects on biofilms of *Staphylococcus aureus* and *Escherichia coli*, with mechanisms including inhibition of

exopolysaccharide (EPS) production, quorum sensing (QS), and cell surface hydrophobicity (CSH)[9]. Moreover, A7G has been shown to improve hyperuricemia by inhibiting xanthine oxidase activity and modulating renal urate transporters, indicating potential application in the treatment of gout and related metabolic syndromes[10].

In anti-tumor aspects, A7G functions through multiple pathways. For instance, in cervical cancer HeLa cells, A7G induces apoptosis and inhibits cell migration by suppressing the PTEN/PI3K/AKT signaling pathway[11]. Additionally, in oral cancer cells, A7G inhibits cell migration and invasion by regulating matrix metalloproteinase-2 (MMP-2) expression and the extracellular signal-regulated kinase (ERK) pathway. These studies suggest the potential application value of A7G in treating various cancers.

A7G also demonstrates significant anti-inflammatory and antioxidant activities. In inflammatory responses, A7G reduces the production of inflammatory factors by inhibiting the NF-κB/AP-1/PI3K-Akt signaling pathway[12]. Furthermore, under oxidative stress conditions, A7G enhances cellular antioxidant capacity by inducing heme oxygenase-1 (HO-1) expression[13]. These properties endow A7G with potential application prospects in treating various inflammatory diseases.

In summary, apigenin 7-*O*-glucoside, as a multifunctional natural compound, exhibits broad

		biological activities in antibacterial, anti-tumor, anti-inflammatory, and antioxidant aspects. These studies provide a scientific basis for the application of A7G in fields such as food, pharmaceuticals, and cosmetics, and offer new research directions for its use in treating related diseases. Future research can further explore the mechanisms and clinical applications of A7G to fully realize its potential health benefits.
6	Hesperedin	**Bioactivity Profile:** Its biological activities include antioxidant, anti-inflammatory, and anti-tumor effects, among others. **Detailed Summary:** Firstly, hesperidin exhibits strong antioxidant capacity by inhibiting reactive oxygen species (ROS) generation and enhancing intracellular antioxidant defense mechanisms. This antioxidant effect is primarily achieved by activating the ERK/Nrf2 signaling pathway, thereby enhancing cellular resistance to oxidative stress[14]. Furthermore, hesperidin also shows significant effects in anti-inflammation. Studies have found that hesperidin can exert anti-inflammatory effects by inhibiting the expression of nuclear factor-κB (NF-κB), inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2), thereby reducing the production of inflammatory mediators[14]. This anti-inflammatory mechanism has been validated

in various inflammation models, further supporting the potential of hesperidin as an anti-inflammatory agent.

In terms of anti-tumor activity, hesperidin inhibits cancer cell proliferation and induces apoptosis through various mechanisms. Research indicates that hesperidin can induce cell cycle arrest and apoptosis in cancer cells by regulating the expression of cyclins and apoptosis-related proteins[15]. Additionally, hesperidin further promotes cancer cell apoptosis by activating the c-Jun N-terminal kinase (JNK) pathway, enhancing the transmission of apoptotic signals[15].

The bioavailability of hesperidin is an important limiting factor for its clinical application. To improve its bioavailability, researchers have developed various novel formulations, such as nanoparticles, liposomes, and cyclodextrin inclusion complexes. These novel formulations significantly enhance the solubility and stability of hesperidin, thereby increasing its biological activity[16]. Moreover, metabolites of hesperidin, such as glucuronide and sulfate forms, have also been found to possess significant biological activity, further supporting its potential application in cardiovascular diseases and cancer[17,18].

In summary, hesperidin, as a natural compound with multiple biological activities, demonstrates significant effects in antioxidant, anti-inflammatory, and anti-tumor aspects. Through the development

		of novel formulations and the study of its metabolites, the clinical application prospects of hesperidin are broader. However, future clinical trials are still needed to verify its safety and efficacy to better guide its application in disease prevention and treatment.
7	Fisetin	**Bioactivity profile:** Exhibits diverse activities including effects on bone health, anti-inflammatory, and anti-tumor actions. **Detailed summary:** Primarily, fisetin shows remarkable effects on bone health. Studies indicate that fisetin promotes osteoblast differentiation and bone formation by facilitating the phosphorylation of glycogen synthase kinase-3β (GSK-3β) at Ser9, thereby activating β-catenin and inhibiting the onset of osteoporosis[19]. Additionally, fisetin promotes osteoblast differentiation and mineralization by upregulating Runx2 transcriptional activity[20]. These studies suggest that fisetin holds significant potential for application in maintaining bone health. Regarding its anti-inflammatory effects, fisetin acts through multiple signaling pathways. Research shows that fisetin can significantly reduce the production of inflammatory mediators by inhibiting the NF-κB and ERK1/2 signaling pathways[21]. Furthermore, fisetin reduces the expression and secretion of inflammatory cytokines and promotes autophagosome-

		lysosome fusion and degradation by inhibiting the PI3K/AKT/mTOR signaling pathway[22]. These mechanisms indicate the potential application value of fisetin in treating inflammation-related diseases. In terms of anti-tumor activity, fisetin exerts its effects through various mechanisms. Studies demonstrate that fisetin can inhibit tumor growth and metastasis by suppressing key signaling pathways such as PI3K/Akt/mTOR, NF-κB, and MAPK[22]. Moreover, fisetin enhances the efficacy of chemotherapeutic agents by inducing apoptosis and autophagy[23,24]. These studies highlight the important adjuvant role of fisetin in cancer treatment. In summary, fisetin, as a multifunctional natural compound, demonstrates significant biological activities in various fields including bone health, anti-inflammation, and anti-tumor effects. Future research should further explore its potential in clinical applications and optimize its bioavailability to better realize its therapeutic effects.
8	2,3-dihydro-1H-carbazol-4(9H)-one	**Bioactivity Profile:** Its biological activities include antibacterial and anti-tumor effects, among others. **Detailed Summary:**

Firstly, the antibacterial activity of 2,3-dihydro-1H-carbazol-4(9H)-one has garnered widespread attention. Studies show that certain carbazole compounds exhibit significant inhibitory effects against both Gram-positive and Gram-negative bacteria. For example, Murrayaquinone A and 6-methoxy-3,7-dimethyl-2,3-dihydro-1H-carbazol-1,4(9H)-dione show good antibacterial activity against *Staphylococcus aureus*and *Escherichia coli*, with a minimum inhibitory concentration (MIC) of 50 μg/mL. This antibacterial property gives carbazole compounds like 2,3-dihydro-1H-carbazol-4(9H)-one important research value in developing new antibacterial drugs.

Furthermore, 2,3-dihydro-1H-carbazol-4(9H)-one also demonstrates potential anti-tumor activity. Through synthesis and cytotoxicity evaluation, researchers have found that certain carbazole derivatives can effectively inhibit the growth of cancer cells. For instance, 1-[3-(9H-carbazol-4-yloxy)-2-hydroxypropyl]-3-aryl-1H-pyrazole-5-carboxylic acid derivatives show significant inhibitory effects *in vitro*on human neuroblastoma (SK-N-SH), human lung cancer (A549), and human breast cancer (MCF-7) cell lines, with compound 3d being the most effective in inhibiting SK-N-SH cell growth[25]. These findings indicate the potential application of carbazole derivatives like 2,3-dihydro-1H-carbazol-4(9H)-one in anti-tumor drug development.

Finally, the synthetic methods for 2,3-dihydro-1H-carbazol-4(9H)-one also facilitate the study of its

		biological activities. Through ruthenium(III)-catalyzed C-H activation/cyclization reactions, various N-acyl-2,3-dihydro-1H-carbazol-4(9H)-ones can be efficiently synthesized under mild conditions. This method is not only simple and scalable but also exhibits broad functional group tolerance, producing only water and nitrogen gas as by-products[26]. This synthetic strategy provides an important foundation for further research into the biological activities of 2,3-dihydro-1H-carbazol-4(9H)-one. In summary, carbazole compounds like 2,3-dihydro-1H-carbazol-4(9H)-one show broad application prospects in biological activities such as antibacterial and anti-tumor effects. Future research can further explore their potential in other biological functions and enhance their application value by optimizing synthetic methods.
9	Daidzein	**Bioactivity Profile:** Possesses significant effects in anti-tumor, anti-inflammatory, antioxidant, and immune system regulation. **Detailed Summary:** Firstly, the potential of daidzein in anti-tumor applications has been confirmed by multiple studies. Research indicates that daidzein can inhibit the growth of ovarian cancer cells by inducing apoptosis and cell cycle arrest, and reduce tumor formation by suppressing the Raf/MEK/ERK

signaling pathway[27]. Additionally, daidzein's metabolite, Equol, exhibits significant anti-inflammatory and neuroprotective effects, capable of inhibiting microglial activation, thus potentially playing a role in treating neurodegenerative diseases[28].

In terms of anti-inflammatory and antioxidant activities, daidzein, by modulating the gut microbiota-brain axis, can alleviate chronic stress-induced depression-like behaviors and improve neuroinflammation and synaptic plasticity [29]。Moreover, daidzein enhances glucose uptake by promoting the translocation of glucose transporter 4 (GLUT4), thereby playing a role in diabetes management[30]. Studies also found that daidzein exerts its antioxidant effects by inhibiting 5-lipoxygenase and myeloperoxidase activities[31].

Regarding immune regulation, daidzein exhibits immunosuppressive activity by inhibiting the maturation and function of dendritic cells[32]. Furthermore, daidzein can mitigate insecticide-induced liver injury by regulating the TGF-β1, PI3K/PIP3/Akt, Nrf-2/Keap-1, and NF-κB signaling pathways[33].

Despite its various biological activities, the bioavailability and metabolism of daidzein remain critical factors affecting its efficacy. Advances in nanotechnology and formulation strategies can improve the bioavailability and therapeutic application of daidzein [34]。For example, the solubility,

		permeability, and bioavailability of daidzein have been significantly enhanced by forming a cocrystal with piperazine[35]. In conclusion, as a natural product with broad therapeutic potential, daidzein shows promising prospects in managing various chronic diseases such as cancer, diabetes, and neurodegenerative disorders. However, further clinical trials are needed to verify its efficacy and safety in different populations and to explore synergistic effects with other bioactive compounds to maximize its clinical application value.
10	Genistein	**Bioactivity Profile:** Its biological activities include anti-tumor, antioxidant, and anti-inflammatory effects, among others. **Detailed Summary:** Firstly, genistein exerts its anti-tumor effects by inducing apoptosis and inhibiting cell proliferation. In human leukemia HL-60 cells, genistein reduces cell numbers by inducing G2/M phase cell cycle arrest and DNA damage, and induces apoptosis through endoplasmic reticulum stress and mitochondrial-dependent pathways[36]. Additionally, genistein inhibits the proliferation of MCF-7 human breast cancer cells by downregulating the IGF-1R/PI3K/Akt signaling pathway[37].

The antioxidant effects of genistein have also been widely studied. It protects cells from oxidative stress damage by enhancing the activity of antioxidant enzymes. For example, in ovarian granulosa cells, genistein attenuates oxidative stress-induced cell damage and improves cell survival via the cAMP-PKA signaling pathway[38]. Furthermore, genistein protects intervertebral disc cells from degeneration through the Nrf2-mediated antioxidant defense system[39].

In terms of anti-inflammatory effects, genistein alleviates inflammatory responses by inhibiting the NF-κB and MAPK signaling pathways. In rat models, genistein significantly inhibits the expression of inflammatory factors in d-galactosamine-induced acute liver failure[40]. Moreover, genistein alleviates endotoxin-induced colonic toxicity by inhibiting the TLR4/MyD88, JAK1/STAT3, and NF-κB pathways[41].

The versatility of genistein is also reflected in its impact on metabolic diseases. Studies show that genistein reduces lipid accumulation in chicken hepatocytes by activating the SIRT1-AMPK signaling pathway, suggesting its potential as a nutritional supplement to prevent lipid metabolism-related diseases[42]. Additionally, genistein shows potential therapeutic effects on non-alcoholic fatty liver disease (NAFLD) by regulating lipid and glucose metabolism[43].

In summary, genistein, as a natural isoflavone, possesses broad biological activities, including

		anti-tumor, antioxidant, anti-inflammatory, and metabolic regulatory effects. Its multiple mechanisms of action make it an important candidate for the research and development of novel therapeutic agents. However, further research is needed to explore its specific mechanisms of action in different disease models and its clinical application potential.
11	Bavachin	**Bioactivity Profile:** It demonstrates potential medicinal value in treating various diseases, especially in metabolic diseases, cardiovascular diseases, immune regulation, and anti-tumor fields. **Detailed Summary:** Firstly, the role of bavachin in metabolic diseases is particularly significant. Studies show that bavachin significantly enhances insulin-dependent glucose uptake in 3T3-L1 adipocytes by activating insulin signaling and AMPK pathways, indicating its potential application in treating type 2 diabetes[44]. Additionally, bavachin alleviates high-fat diet-induced obesity and hepatic steatosis in mice by inhibiting the expression of lipogenic genes and promoting thermogenesis in adipose tissue[45]. In cardiovascular diseases, bavachin protects human aortic smooth muscle cells from β-glycerophosphate-mediated vascular calcification and apoptosis by activating mTOR-dependent autophagy and inhibiting the β-catenin signaling pathway[45]. Furthermore, bavachin alleviates LPS-

induced inflammatory responses by inhibiting NLRP3 inflammasome activation, demonstrating its potential in treating inflammatory and autoimmune diseases[46].

In immune regulation, bavachin enhances T cell activation and antigen-specific immune responses by activating the NFAT signaling pathway, showing its potential as an immune adjuvant [47]. Additionally, bavachin exerts anti-neuroinflammatory effects by modulating the A20 ubiquitin-editing complex and inhibiting the NF-κB signaling pathway, providing new insights for its application in neurological diseases[48].

In the field of anti-tumor therapy, bavachin induces apoptosis in multiple myeloma cell lines by inhibiting the activation of NF-κB and STAT3, demonstrating its potential as an anti-tumor agent. Moreover, bavachin induces apoptosis in colorectal cancer cells by activating the MAPK signaling pathway, further validating its anti-tumor activity [49].

In summary, bavachin, as a multifunctional natural compound, exhibits broad biological activities and potential clinical application value. However, its hepatotoxicity at different doses also requires further study to ensure its safety and efficacy in clinical applications[50]. Future research should continue to explore the molecular mechanisms and clinical applications of bavachin to provide new treatment strategies for various chronic diseases.

12	Corylin	**Bioactivity Profile:** Its biological activities include anti-tumor, anti-inflammatory, and antioxidant effects, among others. **Detailed Summary:** In terms of anti-tumor activity, in ovarian cancer SKOV3 cells, corylin significantly inhibits cancer cell proliferation and clonogenicity by inducing apoptosis and G0/G1 phase cell cycle arrest. This process involves the downregulation of signal transducer and activator of transcription 3 (STAT3) phosphorylation and the inhibition of its nuclear localization and target gene expression by corylin [51]. Furthermore, the role of corylin in metabolic diseases has also been validated. Research has found that corylin promotes the browning of white adipose tissue by activating SIRT1 and *β*3-adrenergic receptor (*β*3-AR), thereby reducing obesity and insulin resistance. In terms of anti-aging, corylin extends lifespan and improves various aging-related phenotypes in *Caenorhabditis elegans* by activating the DAF-16 and SKN-1 signaling pathways[52]. The anti-inflammatory effects of corylin have also been extensively studied. In a lipopolysaccharide (LPS)-induced acute lung injury model, corylin significantly alleviates inflammatory responses by inhibiting the MAPK and IL-6/STAT3 signaling pathways[52]。In studies

of chronic ulcerative colitis, corylin improves colitis symptoms by modulating the gut-brain axis and promoting serotonin production in the colon[53]. Additionally, corylin improves sepsis-associated cardiac dysfunction by downregulating microRNA-214-5p[54].

Regarding bone health, corylin promotes osteoblast differentiation by activating estrogen and Wnt/β-catenin signaling pathways and exhibits significant osteogenic activity *in vitro* and *in vivo*. Moreover, corylin inhibits the proliferation and migration of non-small cell lung cancer cells by suppressing the NF-κB signaling pathway[55]。

In summary, corylin, as a multifunctional natural compound, demonstrates broad biological activities and potential clinical application value. Its mechanisms of action in anti-tumor, anti-inflammatory, antioxidant, anti-aging, and metabolic regulation provide an important scientific basis for future drug development and disease treatment.

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