

Phytochemical Analysis and Antioxidant Activities of Various Extracts from the Aerial Part of *Anemone baicalensis* Turcz.: In Vitro and In Vivo Studies

Shuang Sun ^{1,†}, Guangqing Xia ^{1,2,3,†}, Hao Pang ^{2,4}, Junyi Zhu ^{2,3}, Li Li ^{2,3,*} and Hao Zang ^{1,2,3,4,*}

¹ College of Pharmacy, Yanbian University, Yanji 133000, China; SS11721@163.com (S.S.); qingguangx@thnu.edu.cn (G.X.);

² School of Pharmacy and Medicine, Tonghua Normal University, Tonghua 134002, China; panghao0447@163.com (H.P.); swx0527@163.com (J.Z.)

³ Key Laboratory of Evaluation and Application of Changbai Mountain Biological Gerplasm Resources of Jilin Province, Tonghua 134002, China;

⁴ School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Benxi 117004, China;

* Correspondence: zanghao@thnu.edu.cn (H.Z.); lili1984@thnu.edu.cn (L.L.);

Tel.: +86-435-320-2678 (H.Z. & L.L.)

† These authors contributed equally to this work.

Reagents and Chemicals

p-Nitroblue tetrazolium chloride (NBT) and horseradish peroxidase (EC 1.11.1.7) were purchased from Sigma-Aldrich. Curcumin, salicylic acid, *L*-ascorbic acid, 2,4,6-*tri*(2-pyridyl)-*s*-triazine (TPTZ), ammonium acetate (NH₄Ac), ferrous sulfate heptahydrate (FeSO₄·7H₂O), copper sulfate (CuSO₄), sodium sulfate (Na₂SO₄), taurine, 4-aminoantipyrine, lipoic acid, ferulic acid, sulfanilamide, cupric chloride dihydrate (CuCl₂·2H₂O), phosphoric acid (H₃PO₄), ninhydrin hydrate, quercetin, naphthylethylenediamine dihydrochloride, *D*-(+)-glucose, butylated hydroxytoluene (BHT), butyl hydroxyanisole (BHA), α -naphthol, iodine, 2,9-dimethyl-1,10-phenanthroline (Neocuproine, Nc), gelatin, ABTS, tertiary butylhydroquinone (TBHQ), 3,5-dinitrosalicylic acid (DNS), potassium iodide (KI), ferric chloride (FeCl₃), 4-nitroaniline, sodium nitrite, antimony trichloride, calcium hydroxide (Ca(OH)₂), vanillin, copper sulfate pentahydrate (CuSO₄·5H₂O), phosphomolybdic acid hydrate, hydroxylamine hydrochloride, potassium hydroxide, 3,5-dinitrobenzoic acid, phenol, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, ginsenoside Re, sodium dihydrogen phosphate, linalool, berberine hydrochloride, dibasic sodium phosphate, sodium nitroprusside dehydrate, sodium hypochlorite (NaClO) (10% active chloride), tannic acid, potassium persulfate, potassium chloride (KCl), sodium acetate, gallic acid, sodium molybdate, arbutin, *L*-tyrosine, urea, phloroglucinol, and potassium iodate were purchased from Energy Chemical. Benedict's reagent was purchased from Adamas. DPPH was purchased from Alfa Aesar. Bromocresol green, Trolox, pyrocatechol violet, Sudan III, and Sudan IV were purchased from TCI. Folin and Ciocalteu phenol reagent (FC reagent), aluminum chloride hexahydrate (AlCl₃·6H₂O), linoleic acid, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4''-disulfonic acid sodium salt (Ferrozine), ferrous chloride tetrahydrate (FeCl₂·4H₂O), sodium potassium tartrate tetrahydrate (Rochelle salt), ethylenediaminetetraacetic acid disodium salt dihydrate (EDTANa₂·2H₂O), potassium ferricyanide (K₃[Fe(CN)₆]), lead(II) acetate trihydrate, tungstosilicic acid hydrate, bismuth subnitrate, mercury(II) chloride (HgCl₂), pepsin (32 U/mg), pancreatin, bovine bile extract, magnesium acetate, sodium thiosulfate standard solution (0.1 M), potassium hydroxide standard solution (0.1 M), phenolphthalein, tween 40, and 1,3-dinitrobenzene were purchased from Xiya Reagent. Concentrated sulfuric acid (H₂SO₄), phenol, sodium carbonate (Na₂CO₃), methanol, ethanol, pyridine, dimethyl sulfoxide (DMSO), petroleum ether (60–90°C), sodium hydroxide (NaOH), concentrated hydrochloric acid (HCl), sodium chloride (NaCl), chloroform, magnesium powder, acetic acid, citric acid, ammonium hydroxide (NH₃·H₂O), acetic anhydride, 30% hydrogen peroxide (H₂O₂), formaldehyde, ethyl ether, and 3% bromine water were purchased from Sinopharm. Trypsin (2500 U/mg) was purchased from Aladdin. All reagents and solvents used were of analytical grade. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde (MDA), catalase (CAT), glutathione (GSH), and nitric oxide (NO) kits were purchased from Nanjing Jiancheng. Blue litmus paper was purchased from Jinda.

Preparation of Preliminary Experimental Solutions for Qualitative Phytochemical Analysis

Aqueous Extraction Solutions

Anemone baicalensis aerial part (ABAP) powder (5 g) was weighed and passed through a sieve (20 mesh). Distilled water (50 mL) was added, and the mixture was allowed to stand overnight at room temperature. Next, 5 mL of filtrate was obtained by filtration, and this filtrate was analyzed to check for amino acids and proteins. The remaining residue and leaching solution were heated at 60°C for 10 min. After heating, the mixture was filtered immediately. This filtrate was used to check for carbohydrates, organic acids, saponins, glycosides, phenolics, tannins, and cyanogenic glycosides.

Methanol Extraction Solutions

ABAP powder (5 g) was weighed and passed through a sieve (20 mesh). Ethyl ether (50 mL) was added, and the mixture was heated under reflux for 10 min. The filter residue was transferred back into the bottle after filtration. Next, 35 mL of methanol was added, and the mixture was heated under reflux for 10 min. After heating, the mixture was filtered immediately. This filtrate was used to check for flavonoids, anthraquinones, cardiac glycosides, coumarins and lactones, volatile oils, terpenoids, steroids, lipids, and alkaloids.

Petroleum Ether Extraction Solutions

ABAP (3 g) was weighed and passed through a sieve (20 mesh). Petroleum ether (15 mL) was added, and the mixture was allowed to stand at room temperature for 4 h. Next, 5 mL of filtrate was obtained by filtration, and this filtrate was analyzed to check for volatile oils, lipids, steroids, and triterpenoids.

Qualitative Phytochemical Analysis

Tests for Proteins

Ninhydrin Tests

Here, 1 mL of aqueous extraction solution was mixed with 1 mL of 0.2% ninhydrin solution. The mixture was boiled for 5 min. The development of a purple color indicated the presence of amino acids or proteins.

Biuret Tests

Here, 1 mL of aqueous extraction solution was mixed with 1 mL of solution A (0.1 g/mL NaOH), and then two drops of solution B (0.01 g/mL CuSO₄) were added. This mixture was shaken, and a purple, red, or purplish-red color indicated the presence of amino acids or proteins.

Tests for Carbohydrates

Fehling's Tests

Equal volumes of solution A (34.66 g of CuSO₄·5H₂O dissolved in 500 mL of distilled water) and solution B (173 g of sodium potassium tartrate tetrahydrate and 50 g of NaOH dissolved in 500 mL of distilled water) were mixed together. A sample (1 mL) of this mixture was then mixed with 1 mL of aqueous extraction solution. The resulting mixture was boiled gently. The formation of a brick-red precipitate indicated the presence of reducing sugars.

Benedict's Tests

Here, 1 mL of aqueous extraction solution was mixed with 1 mL of Benedict's reagent, and the resulting mixture was boiled gently. The formation of a reddish-brown precipitate indicated the presence of carbohydrates.

Molisch's Tests

For this, 1 mL of aqueous extraction solution was mixed with 1 mL of Molisch's solution (2 g of α -naphthol dissolved in 100 mL of 95% ethanol). The mixture was then poured carefully into another test tube containing 1 mL of H₂SO₄. A purple ring at the aqueous phase/organic phase interface indicated the

presence of carbohydrates.

Iodine Tests

For this, 1 mL of aqueous extraction solution was mixed with 1 mL of iodine solution (127 mg of iodine and 200 mg of KI dissolved in 10 mL of distilled water). The development of a dark blue or purple color indicated the presence of carbohydrates.

Tests for Phenolics

FeCl₃ Tests

Here, 1 mL of aqueous extraction solution was mixed with 1 mL of 2% FeCl₃ solution. The development of a blue-green or black color indicated the presence of phenolics.

FeCl₃-K₃[Fe(CN)₆] Tests

A few drops of aqueous extraction solution were added onto a thin-layer chromatography plate, and the chromogenic reagent (1% K₃[Fe(CN)₆] solution was mixed with 2% FeCl₃ solution in equal volumes) was sprayed onto the plate, thus generating a blue color. Then, 2 M HCl was sprayed onto the plate, and a darker color indicated the presence of phenolics.

Diazotization Tests

For this, 1 mL of aqueous extraction solution was mixed with 1 mL of 3% Na₂CO₃ solution. The resulting mixture was boiled for 3 min and then cooled in ice water. Two drops of newly prepared diazotization reagent were added. The development of a red color indicated the presence of phenolics.

Tests for Organic Acids

pH Tests

The pH of the aqueous extraction solution was measured with a pH meter. A pH value below 7.0 indicated the presence of organic acids.

Blue Litmus Paper Tests

A few drops of aqueous extraction solution were placed on blue litmus paper. The development of a red color indicated the presence of organic acids.

Bromocresol Green Tests

A few drops of aqueous extraction solution were added onto a thin-layer chromatography plate. Chromogenic reagent (0.1 g of bromocresol green dissolved in 500 mL of ethanol and mixed with 5 mL of 0.1 N NaOH) was sprayed onto the plate. The development of a yellow color on a blue background indicated the presence of organic acids.

Tests for Tannins

FeCl₃ Tests

The experimental procedure was the same as that described in FeCl₃ tests. The development of a blue-green or black color indicated the presence of tannins.

Bromine Water Tests

Bromine water (3%) was added to 1 mL of aqueous extraction solution. The formation of a precipitate indicated the presence of tannins.

Lead Acetate Tests

For this, 1 mL of lead acetate solution was added to 1 mL of aqueous extraction solution; a precipitate was considered evidence for the presence of tannins.

Lime Water Tests

Clear lime water (1 mL) was added to 1 mL of aqueous extraction solution. The formation of a precipitate indicated the presence of tannins.

Gelatin Tests

In this, 1 mL of aqueous extraction solution was mixed with 1 mL of a 0.5% gelatin dissolved in a 10% NaCl solution. The turbidity indicated the presence of tannins.

Tests for Flavonoids

Shinoda Tests

An appropriate amount of magnesium powder was added to 1 mL of methanol extraction solution, followed by two drops of HCl. The development of a red to red-purple color indicated the presence of flavonoids.

Alkaline Reagent Tests

For this, 1 mL of methanol extraction solution was mixed with 1 mL of 2% NaOH solution. The development of an intense yellow color followed by a change to colorless on addition of a few drops of diluted HCl indicated the presence of flavonoids.

AlCl₃ Tests

A few drops of methanol extraction solution were added onto a thin-layer chromatography plate. A 1% AlCl₃ methanol solution was sprayed onto the plate. Observation of yellow-green fluorescence under an ultraviolet lamp indicated the presence of flavonoids.

Lead Acetate Tests

A few drops of lead acetate solution were added to 1 mL of methanol extraction solution. The formation of a yellow precipitate indicated the presence of flavonoids.

Tests for Saponins

Foam Tests

Here, 1 mL of aqueous extraction solution was mixed with 5 mL of distilled water. This mixture was shaken and then left to stand for 10 min. The formation of a stable foam indicated the presence of saponins.

Tests for Steroids and Triterpenoids

Liebermann-Burchard Tests

For this, 5 mL of the aqueous extraction solution was placed in an evaporating dish and then evaporated. The residue was dissolved in 1 mL of acetic anhydride. One drop of H₂SO₄ was added, and the development of a red or purple color indicated the presence of triterpenoids. The development of a blue-green color indicated the presence of steroids.

Salkowski Tests

In this test, 1 mL of methanol extraction solution was mixed with 1 mL of CHCl₃. Then, 1 mL of H₂SO₄ was added carefully, and the mixture was shaken gently. A reddish-brown color in the CHCl₃ layer and green fluorescence in the H₂SO₄ layer indicated the presence of steroids or triterpenoids.

Tests for Terpenoids

CHCl₃-H₂SO₄ Tests

In this test, 1 mL of methanol extraction solution was mixed with 2 mL of CHCl₃ and then evaporated. H₂SO₄ (2 mL) was added carefully, and the mixture was heated at 60°C for 2 min. The development of a gray color indicated the presence of terpenoids.

Vanillin-H₂SO₄ Tests

A few drops of petroleum ether extraction solution were added onto a thin-layer chromatography plate. Chromogenic reagent was prepared by dissolving 5 g of vanillin in 100 mL of 10% H₂SO₄ ethanol solution, and then sprayed onto the plate. The development of a red, blue, or purple color indicated the presence of volatile oils, terpenoids, and steroids.

CH₃COOH-CuSO₄ Tests

In this test, 4 mg of methanol extract was weighed into a test tube, and subsequently, 2 mL of acetic acid and 2 mg of anhydrous copper sulfate were sequentially added. Following 3 min of sonication, the mixture was heated in a 70°C water bath for an additional 3 min. The development of a green color indicated the presence of iridoid glycosides.

Tests for Alkaloids

Bertrad's Reagent Tests

For this, 1 mL of methanol extraction solution was mixed with 1 mL of tungstosilicic acid reagent. The reagent was prepared by dissolving 5 g of tungstosilicic acid hydrate in 100 mL of distilled water and adding a small amount of HCl to adjust the pH to 2.0. The formation of a pale yellow or off-white precipitate indicated the presence of alkaloids.

Dragendorff's Reagent Tests

Here, 1 mL of methanol extraction solution was mixed with 1 mL of Dragendorff's reagent. For the reagent, solution A (850 mg of bismuth subnitrate dissolved in 40 mL of distilled water and 10 mL of acetic acid) and solution B (8 g of KI dissolved in 20 mL of distilled water) were mixed in equal volumes to prepare a stock solution. A sample of this stock solution (10 mL) was then mixed with 20 mL of acetic acid and diluted to 100 mL with distilled water. The formation of a light yellow or reddish brown precipitate indicated the presence of alkaloids.

Mayer's Reagent Tests

Here, 1 mL of methanol extraction solution was mixed with 1 mL of Mayer's reagent. For the reagent, solution A (1358 mg of HgCl_2 dissolved in 60 mL of distilled water) and solution B (5 g of KI dissolved in 10 mL of distilled water) were mixed and then diluted to 100 mL with distilled water. The formation of a white or light yellow precipitate indicated the presence of alkaloids.

Tests for Anthraquinones

Borntrager's Tests

For this test, 1 mL of methanol extraction solution was mixed with 1 mL of 10% NaOH solution. A red color was developed. Next, a small volume of 30% H_2O_2 solution was added, and the mixture was heated at 60°C. HCl solution was then added, and the red color disappeared. Finally, NaOH solution was added, and the development of a red color indicated the presence of anthraquinones.

Magnesium Acetate Tests

Three drops of 1% magnesium acetate methanol solution were added to 1 mL of methanol extraction solution. The development of a red color indicated the presence of anthraquinones.

Tests for Coumarins and Lactones

Hydroxamic Acid Iron Tests

Three drops of 7% hydroxylamine hydrochloride methanol solution and 10% KOH methanol solution were added to 1 mL of methanol extraction solution. After heating at 60°C, 5% HCl was added to adjust the pH to 3.0-4.0. Next, two drops of a 1% FeCl_3 ethanol solution were added. The development of an orange or purple color indicated the presence of coumarins and lactones.

Diazotization Tests

Methanol extraction solution was used. The experimental procedure was the same as that described in diazotization tests. The development of a red color indicated the presence of coumarins and lactones.

Fluorescence Tests

A few drops of methanol extraction solution were added onto a thin-layer chromatography plate, and blue-green fluorescence was observed under an ultraviolet lamp. Then, a 1% KOH solution was sprayed onto the plate. Generation of intense fluorescence indicated the presence of coumarins.

Tests for Volatile Oils and Fats

Phosphomolybdic Acid Tests

A few drops of petroleum ether extraction solution were added onto a thin-layer chromatography plate, and 25% phosphomolybdic acid solution (2.5 g of phosphomolybdic acid hydrate dissolved in 10 mL of absolute ethanol) was sprayed onto the plate. The development of a blue color indicated the presence of lipids, triterpenoids, and steroids.

Vanillin-H₂SO₄ Tests

Methanol extraction solution was used. The experimental procedure was the same as that described in vanillin-H₂SO₄ tests. The development of a red, blue, or purple color indicated the presence of volatile oils, terpenoids, and steroids.

Sudan Tests

One drop of Sudan III solution (0.1 g of Sudan III dissolved in 10 mL of 95% ethanol) was added to 1 mL of methanol extraction solution. The development of an orange color indicated the presence of oils and fats. One drop of Sudan IV solution (0.01 g of Sudan IV dissolved in 5 mL of acetone, followed by addition of 5 mL of 70% ethanol) was added to 1 mL of methanol extraction solution. The development of a red color indicated the presence of oils and fats.

Tests for Cardiac Glycosides

Kedde Tests

A few drops of methanol extraction solution were added onto a thin-layer chromatography plate. Chromogenic reagent was prepared by mixing solution A (2% methanol solution of 3,5-dinitrobenzoic acid) and solution B (2 M KOH solution) in equal volumes. The reagent was sprayed onto the plate. The development of a purple-red color followed by a change to colorless indicated the presence of cardiac glycosides.

Raymond Tests

Methanol extract (1 mg) was dissolved in 50% ethanol. Both 2% *m*-dinitrobenzene ethanol solution (0.1 mL) and 20% NaOH solution (0.2 mL) were added. The development of a blue-purple color indicated the presence of cardiac glycosides.

Legal Tests

Methanol extract (1 mg) was dissolved in two drops of pyridine. One drop of 3% sodium nitroprusside solution and one drop of 2 M NaOH solution were added. The development of a dark red color followed by a change to colorless indicated the presence of cardiac glycosides.

Tests for Cyanogenic Glycosides

Prussian Blue Tests

For this, 1 g of ABAP powder was placed in a test tube, 2 mL of distilled water was added, and the test tube was immediately wrapped with filter paper. Then, one drop of 10% KOH solution was added onto the filter paper, and the system was heated at 60°C for 30 min. Next, one drop each of 10% ferrous sulfate, 10% HCl, and 5% FeCl₃ were sequentially added onto the filter paper. A blue color on the filter paper indicated the presence of cyanogenic glycosides.

Preparation of Different Extracts of ABAP for Quantitative Phytochemical Analysis, Antioxidant Activity Assays, and UHPLC-MS Analysis

The collected samples of ABAP were dried in a cool, ventilated place and then pulverized to powder. The powder (20 g) of ABAP was added to a single-neck round-bottomed flask (glass, 500 mL), followed by the addition of 200 mL of various solvents (double-distilled water, absolute methanol, absolute ethanol, or 80% ethanol; in the following text, the first three are referred to as water, methanol, and ethanol, respectively) and refluxing using a hotplate magnetic stirrer employing methyl silicone oil as the heating medium for 6 h at the respective boiling points of the solvents. The same solvent is employed once for each individual extraction process. To minimize errors, three separate but parallel experiments were conducted, and the data collected from these experiments were subsequently utilized to describe the observed statistical differences. Extracts were filtered through a Whatman No. 1 filter paper and evaporated under a reduced pressure at < 50°C until dry using a rotary evaporator. All solvent extracts utilized in this experiment underwent rigorous drying to achieve a constant weight prior to their application, thereby ensuring minimal residual solvent content. All dried extracts were weighed and stored at -20°C until use. Yield was calculated as % yield = (weight of dry extract/initial weight of dry sample) × 100.

Determination of Total Carbohydrate Content (TCC)

Briefly, 250 μL of ABAP extract in distilled water, 125 μL of phenol solution (5%), and 625 μL of H_2SO_4 were mixed in an Eppendorf tube and incubated for 30 min. Subsequently, 200 μL of the sample was pipetted from each Eppendorf tube onto a microplate. A calibration curve was produced based on glucose (0–200 mg/L) as a standard. The absorbance of the sample was recorded at 490 nm against a blank sample consisting of ABAP extract with distilled water. The mean of three readings was used, and TCC was expressed in milligrams of glucose equivalents (GE)/g of ABAP extract.

Determination of Total Protein Content (TP_{roC})

Briefly, 200 μL of bicinchoninic acid (BCA) working solution and 20 μL of ABAP extract in distilled water were mixed in a microplate and incubated at 37°C for 30 min. A calibration curve was produced based on bovine serum albumin (BSA) (0–500 mg/L) as a standard. The absorbance of the sample was recorded at 562 nm against a blank sample consisting of ABAP extract with distilled water. The mean of three readings was used, and TP_{roC} was expressed in milligrams of BSA equivalents (BSAE)/g of ABAP extract.

Determination of Total Monoterpenoid Content (TMC)

Briefly, 160 μL of ABAP extract in methanol and 40 μL of H_2SO_4 were mixed in a microplate and allowed to stand at room temperature for 30 min. The absorbance was obtained at 538 nm against a blank sample consisting of ABAP extract with methanol. A calibration curve was produced based on linalool (0–20 mg/mL) as a standard. The mean of three readings was used, and TMC was expressed in milligrams of linalool equivalents (LE)/g of ABAP extract.

Determination of Total Alkaloid Content (TAC)

Briefly, 1 mL of ABAP extract dissolved in 2N HCl at 1 mg/mL concentration was mixed with 5 mL of citrate buffer solution (pH 4.7, comprising 0.5M K_2HPO_4 and 0.2 M citric acid) and 5 mL of bromocresol green solution. After thorough mixing, 5 mL of chloroform was added, vigorously shaken, and left to settle at room temperature for 5 min. A separatory funnel was employed to isolate the chloroform layer, which was then dried by adding a suitable quantity of anhydrous sodium sulfate and allowed to stand at room temperature for 30 min. The absorbance of this layer was measured at 420 nm, utilizing a chloroform blank as the reference. A calibration curve was produced based on berberine hydrochloride (1.24–12.36 mg/L) as a standard. The mean of three readings was used, and TAC was expressed in milligrams of berberine hydrochloride equivalents (BHE)/g of ABAP extract.

Determination of Total Phenolic Content (TP_{heC})

Briefly, 100 μL of Folin and Ciocalteu phenol reagent (FC reagent) (1 M) and 200 μL of ABAP extract in distilled water were mixed in an Eppendorf tube and incubated for 5 min. Subsequently, 500 μL of Na_2CO_3 solution (20%) was added and allowed to stand at room temperature for 40 min in the dark (with mixing every 10 min). Subsequently, 200 μL of the sample was pipetted from each Eppendorf tube onto a microplate. A calibration curve was produced based on gallic acid (0–100 mg/L) as a standard. The absorbance of the sample was recorded at 750 nm against a blank sample consisting of ABAP extract with distilled water and Na_2CO_3 . The mean of three readings was used, and TP_{heC} was expressed in milligrams of gallic acid equivalents (GAE)/g of ABAP extract.

Determination of Total Phenolic Acid Content (TPAC)

Briefly, 20 μL of ABAP extract in distilled water, 20 μL of Arnow reagent, 20 μL of HCl solution (0.1 M), 120 μL of distilled water, and 20 μL of NaOH solution (1 M) were mixed in a microplate and recorded immediately at 490 nm against a blank sample (Arnow reagent was replaced with distilled water). A

calibration curve was produced based on caffeic acid (0–100 mg/L) as a standard. The mean of three readings was used, and TPAC was expressed in milligrams of caffeic acid equivalents (CAE)/g of ABAP extract.

Determination of Total Flavonoid Content (TFC)

Briefly, 100 μ L of AlCl_3 (2%) in methanol and 100 μ L of ABAP extract in methanol were mixed in a microplate and incubated at room temperature for 10 min. A calibration curve was produced based on quercetin (0–100 mg/L) as a standard. The absorbance of the sample was recorded at 415 nm against a blank sample consisting of ABAP extract with methanol. The mean of three readings was used, and TFC was expressed in milligrams of quercetin equivalents (QE)/g of ABAP extract.

Determination of Total Tannin Content (TT_{anC})

Briefly, 200 μ L of FC reagent (1 M) and 200 μ L of ABAP extract in distilled water were mixed in an Eppendorf tube and incubated for 5 min. Subsequently, 100 μ L of Na_2CO_3 solution (20%) and 1500 μ L of distilled water were added and allowed to stand at room temperature for 30 min in the dark (with mixing every 10 min). Subsequently, 200 μ L of the sample was pipetted from each Eppendorf tube onto a microplate. A calibration curve was produced based on tannic acid (0–200 mg/L) as a standard. The absorbance of the sample was recorded at 725 nm against a blank sample consisting of ABAP extract with distilled water and Na_2CO_3 . The mean of three readings was used, and TT_{anC} was expressed in milligrams of tannic acid equivalents (TAE)/g of ABAP extract.

Determination of Gallotannin Content (GC)

Briefly, 875 μ L of ABAP extract in methanol and 375 μ L of saturated KIO_3 solution were mixed in an Eppendorf tube and incubated at 15°C for 120 min. A calibration curve was produced based on gallic acid (0–400 mg/L) as a standard. The absorbance of the sample was recorded at 550 nm against a blank sample (KIO_3 was replaced with distilled water). The mean of three readings was used, and GC was expressed in milligrams of GAE/g of ABAP extract.

Determination of condensed tannin content (CTC)

Briefly, 4 mg of phloroglucinol was added to 2 mL of ABAP extract in distilled water. Subsequently, 1 mL of HCl solution and 1 mL of formaldehyde solution were added and mixed in an Eppendorf tube and incubated at room temperature overnight. The precipitate was separated by filtration, and the unprecipitated phenolics were measured in the filtrate according to the method of TP_{heC} .

Determination of Total Triterpenoid Content ($\text{TT}_{\text{T}}\text{C}$)

Briefly, 180 μ L of ABAP extract in acetic anhydride and 20 μ L of H_2SO_4 were mixed in a microplate and incubated at room temperature for 10 min. A calibration curve was produced based on ginsenoside Re (0–400 mg/L) as a standard. The absorbance of the sample was recorded at 350 nm against a blank sample consisting of ABAP extract with acetic anhydride. The mean of three readings was used, and $\text{TT}_{\text{T}}\text{C}$ was expressed in milligrams of ginsenoside Re equivalents (GRE)/g of ABAP extract.

Antioxidant Activity Assay

DPPH Assay

Briefly, 100 μ L of ABAP extract in methanol and 100 μ L of DPPH in methanol (50 μ M) were mixed in a microplate and allowed to stand at room temperature for 20 min in the dark. The absorbance of the sample was recorded at 515 nm. The half-maximal inhibitory concentration (IC_{50}) values were calculated and expressed as the mean \pm standard deviation (SD) in μ g/mL.

ABTS Assay

Briefly, 190 μL of diluted ABTS solution and 10 μL of ABAP extract in DMSO were mixed in a microplate and incubated for 20 min in the dark. The absorbance of the sample was recorded at 734 nm. The IC_{50} values were calculated and expressed as the mean \pm SD in $\mu\text{g/mL}$.

Hydroxyl Radical Assay

Briefly, 50 μL of ABAP extract in DMSO, 50 μL of FeSO_4 solution (3 mM), and 50 μL of H_2O_2 solution (3 mM) were mixed in a microplate and incubated for 10 min. After that, 50 μL of salicylic acid solution (6 mM) was added and incubated at room temperature for 30 min in the dark. The absorbance of the sample was recorded at 492 nm. The scavenging activity was expressed as % scavenging rate and was calculated as follows:

$$\% \text{scavenging} = \left(1 - \frac{\Delta A_{\text{sample}} - \Delta A_{\text{control}}}{\Delta A_{\text{control}}} \right) \times 100\%$$

Superoxide Radical Assay

Briefly, 45 μL of ABAP extract in DMSO (10 mg/mL), 15 μL of *p*-nitroblue tetrazolium chloride (NBT) in DMSO (1 mg/mL), and 150 μL of NaOH in DMSO (50 μM) were mixed in a microplate, and the absorbance of the sample was recorded immediately at 560 nm against a blank sample (NBT was replaced with DMSO). Curcumin was used as a positive reference. The scavenging activity was expressed as % scavenging rate and was calculated as follows:

$$\% \text{scavenging} = \left(1 - \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{control}}} \right) \times 100\%$$

FRAP Assay

Briefly, 20 μL of ABAP extract in DMSO and 180 μL of FRAP reagent were mixed in a microplate and incubated at 37°C for 30 min in the dark. A calibration curve was produced based on FeSO_4 (0–600 mg/L) as a standard. The absorbance of the sample was recorded at 595 nm. The standard curve of the ferrous ion is $y = 4.416x + 0.087$. Trolox was used as positive reference. The FRAP was expressed as the Trolox Equivalent Antioxidant Capacity ($\text{TEAC}_{\text{FRAP}}$).

CUPRAC Assay

Briefly, 20 μL of CuCl_2 solution (100 mM), 50 μL of neocuproine in 96% ethanol (7.5 mM), 50 μL of NH_4Ac solution, 20 μL of ABAP extract in DMSO, and 30 μL of distilled water were mixed in a microplate and incubated at 50°C for 20 min. This mixture was allowed to stand at room temperature for 10 min. The absorbance of the sample was recorded at 450 nm. Trolox was used as positive reference. The CUPRAC was expressed as the Trolox Equivalent Antioxidant Capacity ($\text{TEAC}_{\text{CUPRAC}}$).

Iron Chelating Assay

Briefly, 50 μL of ABAP extract in methanol, 110 μL of ultrapure water, and 20 μL of FeCl_2 solution (0.5 mM) were mixed in a microplate and incubated for 5 min. Subsequently, 20 μL of ferrozine solution (2.5 mM) was added and incubated for 10 min. The absorbance was recorded at 562 nm against a blank sample (ferrozine solution was replaced with water). EDTANa_2 was used as a positive reference. The IC_{50} values were calculated and expressed as the mean \pm SD in $\mu\text{g/mL}$.

Copper Chelating Assay

Briefly, 40 μL of ABAP extract in ultrapure water, 140 μL of acetic acid-sodium acetate buffer solution (pH 6.0, 50 mM), and 10 μL of CuSO_4 solution (5 mM) were mixed in a microplate and incubated for 30 min. Subsequently, 10 μL of pyrocatechol violet solution (4 mM) was added and incubated for 30 min. The

absorbance was recorded at 632 nm against a blank sample (pyrocatechol violet was replaced with water). EDTANa₂ was used as a positive reference. The IC₅₀ values were calculated and expressed as the mean ± SD in µg/mL.

H₂O₂ Assay

Briefly, 70 µL of phenol solution (pH 7.0, 12 mM, in 84 mM phosphate buffer (PBS)), 20 µL of 4-aminoantipyrine solution (pH 7.0, 0.5 mM, in 84 mM PBS), 32 µL of H₂O₂ solution (pH 7.0, 0.7 mM, in 84 mM PBS), 8 µL of horseradish peroxidase (EC 1.11.1.7) solution (pH 7.0, 1 U/mL, in 84 mM PBS), and 70 µL of ABAP extract (pH 7.0, in 84 mM PBS) were mixed in a microplate, and the absorbance of the sample was recorded immediately at 504 nm against a blank sample (phenol solution was replaced with PBS). Gallic acid was used as a positive reference. The IC₅₀ values were calculated and expressed as the mean ± SD in µg/mL.

Singlet Oxygen Assay

Briefly, 40 µL of ABAP extract (pH 7.4, in 45 mM PBS), 50 µL of *N,N*-dimethyl-4-nitrosoaniline (pH 7.4, 0.2 mM, in 45 mM PBS), 20 µL of histidine solution (pH 7.4, 0.1 mM, in 45 mM PBS), 20 µL of NaClO solution (pH 7.4, 0.1 mM, in 45 mM PBS), 20 µL of H₂O₂ (pH 7.4, 0.1 mM, in 45 mM PBS), and 50 µL of PBS (pH 7.4, 45 mM) were mixed in a microplate and allowed to stand at room temperature for 40 min. The absorbance of the sample was recorded at 440 nm against a blank sample (ABAP extract was replaced with PBS). Ferulic acid was used as a positive reference. The IC₅₀ values were calculated and expressed as the mean ± SD in µg/mL.

β-Carotene Bleaching Assay

Briefly, β-carotene solution was prepared by dissolving β-carotene (2 mg) in CHCl₃ (10 mL). Then, 2 mL of the solution was pipetted into a flask and vortex-mixed with linoleic acid (40 mg) and Tween 40 (400 mg). After the removal of CHCl₃, 100 mL of oxygenated ultrapure water was added, and the emulsion was shaken vigorously. Aliquots (2.4 mL) of the emulsion were pipetted into different test tubes containing 0.1 mL of ABAP extract in methanol (5 mg/mL). Butylated hydroxytoluene and tertiary butylhydroquinone were used as positive controls. In the control group, ABAP extract was replaced with methanol. When the sample was added to the emulsion, it was recorded as *t* = 0 min. The tubes were capped and placed in a water bath at 60°C. The absorbance was recorded at 470 nm every 15 min until 120 min. The antioxidant activity coefficient (AAC) was calculated according to the following equation:

$$AAC = \frac{A_{A(120)} - A_{C(120)}}{A_{C(0)} - A_{C(120)}} \times 1000$$

where *A*_{A(120)} is the absorbance of the antioxidant at 120 min, *A*_{C(120)} is the absorbance of the control at 120 min, and *A*_{C(0)} is the absorbance of the control at 0 min.

HClO Assay

HClO was freshly prepared by adjusting the pH of a 1% (*v/v*) of NaClO to 6.2 with 1% H₂SO₄. The concentration of HClO was determined by reading the absorbance at 235 nm and using the molar extinction coefficient of 100 M⁻¹ cm⁻¹. Briefly, 20 µL of ABAP extract aqueous solution, 20 µL of 150 mM taurine aqueous solution, 20 µL of 0.5 mM HClO solution, and 140 µL of PBS (pH 7.4, 50 mM) were mixed in a microplate and incubated for 10 min. Subsequently, 2 µL of 2 M KI aqueous solution was added and mixed. The absorbance was recorded at 350 nm against a blank sample (taurine and HClO were replaced with water). Trolox was used as positive reference. IC₅₀ values were calculated and expressed as the mean ± SD in µg/mL.

NO Assay

Briefly, 3 mL of ABAP extract in methanol (1 mg/mL) and 3 mL of sodium nitroprusside solution (pH 7.4, 5 mM, in 0.1 M PBS) were mixed in an Eppendorf tube and incubated at 25°C for 150 min. At intervals,

100 μ L of the sample was pipetted from each Eppendorf tube onto a microplate containing 100 μ L of Griess reagent. In the control group, ABAP extract was replaced with methanol. The absorbance was recorded at 546 nm against a blank sample (Griess reagent was replaced with distilled water). Curcumin (0.1 mg/mL) was used as a positive reference.

UHPLC-MS

Methanol extract of ABAP was analyzed using UHPLC (Agilent 1290 system) with Q-TOF-MS (Agilent 6545 system). A ZORBAX SB-C₁₈ column (150 \times 3.0 mm, 1.8 μ m; Agilent) was used. The column temperature was set to 40°C. The mobile phase was a mixture of 0.1% formic acid in water (solvent A) and a mixture of 0.1% formic acid in acetonitrile (solvent B) at a flow rate of 0.4 mL/min. Linear gradient elution was applied (0–1 min, 95% A; 1–30 min, 95–70% A; 30–50 min, 70–30% A; 50–56 min, 30–1% A; 56–60 min, 1% A). The extract was diluted to 1 mg/mL with methanol and filtered using a 0.22 μ m membrane before use. The sample injection volume was 5 μ L. The Q-TOF-MS (Agilent) was operated in positive-ion mode with scan range m/z 100–1700. Data were recorded and analyzed with qualitative analysis software (version B. 07.00, Agilent).

Stability Studies of Methanol Extract of ABAP

pH Stability

The stability in acidic and basic environments was investigated using a methanol extract of ABAP dissolved in deionized water with the pH adjusted to 1, 3, 5, 7, 9, or 11 using 1 M HCl or 1 M NaOH. The final concentration of methanol extract was 3 mg/mL. After incubation at room temperature for 1 h, the pH of the mixture was adjusted to 7, and the TP_{he}C and the ABTS scavenging abilities were examined.

Thermal Stability

To evaluate the thermal stability, a methanol extract of ABAP dissolved in deionized water (3 mg/mL, pH 7) was placed in test tubes with screw caps. The test tubes were placed in a boiling water bath (100°C). Samples were removed after 0, 15, 30, 60, 120, 180, and 240 min and cooled in an ice water bath. and the TP_{he}C and the ABTS scavenging abilities were examined.

Modeling of the Stability in the Gastrointestinal Tract

For this, 100 mL of methanol extract of ABAP in distilled water (3 mg/mL) were mixed with 10 mL of PBS (pH 6.8, 10 mM) and incubated at 37°C for 2 min (oral condition). Then, 0.5 mL of 1 M HCl-KCl buffer (pH 1.5) and 5 mL of pepsin solution (pH 1.5, 32 U/mL in 1 M HCl-KCl buffer) were added to samples. The mixtures were incubated at 37°C for 60 min (stomach condition). Thereafter, 1 mL of 1 M NaHCO₃ together with 1 mL of a mixture of bile and pancreatic juice (pH 8.2, 10 mg/mL of pancreatin, 14,600 U/mL of trypsin, and 13.5 mg/mL of bile extract in 10 mM PBS) was added to the mixture, and the pH was adjusted to 6.8. The mixtures were incubated at 37°C for 3 h (duodenal condition). The results were used for the determination of TP_{he}C and ABTS scavenging abilities of methanol extract during simulated gastrointestinal digestion and were taken at 0, 0.5, 1–4 h.

Oxidative Stability of the Oils

Extra virgin olive oil (EVOO) and cold-pressed sunflower oil (CPSO) were placed in separate flasks. Methanol extract of ABAP was added to the EVOO and CPSO flasks at concentrations of 100 and 25 μ g/g, respectively. To compare with the stabilizing effect of methanol extract, EVOO and CPSO were supplemented with synthetic antioxidants TBHQ and BHA at 20 μ g/g. A control group was prepared without antioxidants. The flasks were left open and placed in an oil bath at 160°C to simulate frying. Two samples from each category were removed from the flasks every 4 h for duplicate analysis. The oxidative stability of the oils was evaluated by measurement of the free acidity (percentage of oleic acid), peroxide values (milliequivalents of O₂/kg oil), and ultraviolet absorption at 232 and 270 nm (K₂₃₂ and K₂₇₀).

Oral Acute Toxicity Study

Twenty adult Kunming mice (19–22 g) were acquired by Liaoning Changsheng Biotechnology Co., Ltd. (animal license number SCXK (Liao) 2020–0001; Liaoning, China). Housed mice had free access to food and

water under a 12 h light–dark cycle. All mice were reared adaptively for 3 d before starting the experiment. We followed the relevant policies in the Guidelines for the Use of Laboratory Animals developed by Tonghua Normal University. The Institutional Animal Care and Use Committee of Tonghua Normal University approved the experimental protocol (Ethic approval code: 20230068), and the experimental protocol follows the rules of the Declaration of Helsinki. The mice were divided into two groups ($n = 20$), with ten males and ten females in each group. The mice in the healthy control group received vehicle treatment. The methanol extract of ABAP was dissolved in water to a final volume of 10 mL/kg mouse body weight (BW) in a single dose of 2000 mg/kg methanol extract. The mice were then continuously observed for 1 h for behavioral changes and toxicity. Intermittent observations were made for the next 6 h, and a final observation was conducted at 24 h. At this stage, the survival rate was calculated, and we found that no mice died. All mice were euthanized using isoflurane. On the basis of the study results, two doses (200 and 400 mg/kg) were selected for further study.

Gastric Protective Experiment

Animals

Adult male Wistar rats (170–200 g) were acquired by Liaoning Changsheng Biotechnology Co., Ltd. (animal license number SCXK (Liao) 2020–0001; Liaoning, China). Housed rats had free access to food and water under a 12 h light–dark cycle. All rats were reared adaptively for 1 week before starting the experiment. We followed the relevant policies in the Guidelines for the Use of Laboratory Animals developed by Tonghua Normal University. The Institutional Animal Care and Use Committee of Tonghua Normal University approved the experimental protocol (Ethic approval code: 20230068), and the experimental protocol follows the rules of the Declaration of Helsinki.

Experimental Protocol

A total of forty rats were randomly assigned to five groups, each containing eight rats. Each group received a distinct oral treatment. The control group (group I) was administered 0.5% carboxymethylcellulose sodium. The positive control group (group II) received omeprazole at a dosage of 30 mg/kg BW. The model group (group III) was given anhydrous ethanol along with 0.5% carboxymethylcellulose sodium. The low-dose group (group IV) was treated with methanol extract at a dosage of 200 mg/kg BW, while the high-dose group (group V) received methanol extract at 400 mg/kg BW. All the treatments were administered daily for 7 d. The rats were then fasted for 24 h with access to water. Then, 2 h after the conclusion of the final treatment, rats in group I were administered 4 mL/kg BW of normal saline via gavage, whereas rats in groups II–V were orally given 4 mL/kg BW of anhydrous ethanol. After all the above processes were completed, we observed that no rats succumbed to death after being orally administered anhydrous ethanol. Next, all rats were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg BW). Abdominal aortic blood was collected, and the gastric tissue was rapidly excised. After leaving the blood samples at room temperature for 1 h, they were centrifuged for 10 min at 3500 rpm and 4°C. The serum was stored at –80°C. The tissue was washed with iced normal saline, dried using filter paper, and weighed. A 10% homogenate of the gastric tissue was prepared using normal saline, centrifuged for 10 min at 8000 rpm and 4°C, and the supernatant was stored at –80°C.

Assessment of Gastric Mucosal Damage

The stomach was dissected along the curved side, inverted to reveal the mucosal surface, and thoroughly rinsed with iced normal saline. The gastric mucosal damage was evaluated macroscopically. The length and width of the lesion are measured to calculate the score. The ulcer index of the obtained rat gastric tissue is evaluated, and the ulcer inhibition rate is determined. The scoring criteria are as follows: For lesions with a length greater than 1 mm, award 1 point for every 1 mm. If the width exceeds 1 mm, double the score by the number of mm in width. If both length and width are between 0.5 mm and 1 mm, award 0.5 points. For lesions with both dimensions less than 0.5 mm, award 0.25 points. The sum of these scores represents the

ulcer index for the animal. The average ulcer index is obtained by calculating the mean of all scores. Calculate the ulcer inhibition rate using the following formula:

$$GR = \frac{U_I - U_S}{U_I} \times 100\%$$

where GR is the gastric ulcer inhibition rate, U_I is the average gastric ulcer index of rats in group III, and U_S is the average gastric ulcer index of rats in the sample administration group.

For microscopic histologic examinations, gastric tissue specimens from three rats in each group underwent a series of processing steps. Initially, the specimens were fixed in 4% paraformaldehyde, followed by dehydration and washing. Subsequently, they were embedded in paraffin through infiltration and sliced into 4 μ m thick sections. These sections were then dewaxed and stained with hematoxylin and eosin for microscopic observation. Under a microscope, pathological changes in the tissue were carefully observed and recorded.

Biochemical Analyses

To assess oxidative stress parameters related to stomach function, both serum and gastric tissue samples were evaluated for various biomarkers, including nitric oxide, glutathione, alanine aminotransferase, aspartate aminotransferase, catalase, and malondialdehyde. All samples underwent analysis using commercial kits, adhering strictly to the manufacturer's guidelines for accurate and reliable results.

Table S1. Phytochemical analysis of *Anemone baicalensis* aerial part.

Phytochemicals	Types of Tests	Sample Solution		
		Water	Methanol	Petroleum Ether
Proteins/amino acids	1. Ninhydrin tests	+	○	○
	2. Biuret tests	+	○	○
Carbohydrates	1. Fehling's tests	+	○	○
	2. Benedict's tests	+	○	○
	3. Molisch's tests	+	○	○
	4. Iodine tests	+	○	○
Phenolics	1. FeCl ₃ tests	+	○	○
	2. FeCl ₃ -K ₃ [Fe(CN) ₆] tests	+	○	○
	3. Diazotization tests	+	○	○
Organic acids	1. pH tests	+	○	○
	2. Blue litmus paper tests	+	○	○
	3. Bromocresol green tests	+	○	○
Tannins	1. FeCl ₃ tests	+	○	○
	2. Bromine water tests	–	○	○
	3. Lead acetate tests	+	○	○
	4. Lime water tests	–	○	○
	5. Gelatin tests	–	○	○
Flavonoids	1. Shinoda tests	○	+	○
	2. Alkaline reagent tests	○	–	○
	3. AlCl ₃ tests	○	–	○
	4. Lead acetate tests	○	+	○
Saponins	1. Foam tests	+	○	○
Steroids and triterpenoids	1. Liebermann–Burchard tests	○	+	○
	2. Salkowski tests	○	+	○
Terpenoids	1. CHCl ₃ -H ₂ SO ₄ tests	○	+	○
	2. Vanillin-H ₂ SO ₄ tests	○	○	+
	3. CH ₃ COOH-CuSO ₄ tests	○	+	○
Alkaloids	1. Bertrad's reagent	○	–	○
	2. Dragendorff's reagent	○	+	○
	3. Mayer's reagent	○	–	○
Anthraquinones	1. Borntrager's tests	○	–	○
	2. Magnesium acetate tests	○	–	○
Coumarins and lactones	1. Hydroxamic acid iron tests	+	○	○
	2. Diazotization tests	+	○	○
	3. Fluorescence tests	○	–	○
Volatile oils and fats	1. Phosphomolybdic acid tests	○	+	○

Phytochemicals	Types of Tests	Sample Solution		
		Water	Methanol	Petroleum Ether
Cardiac glycosides	2. Vanillin-H ₂ SO ₄ tests	○	+	○
	3. Sudan tests	○	+	○
	1. Kedde tests	○	—	○
	2. Raymond tests	○	—	○
	3. Legal tests	○	—	○
	1. Prussian blue tests	—	○	○

(+) indicates presence; (—) indicates absence; (○) indicates no test.

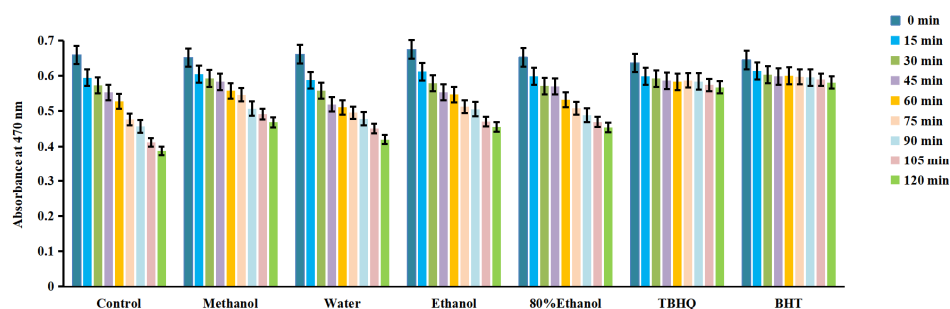


Figure S1. Alterations in the absorbance of β -carotene in the presence of different extracts of *Anemone baicalensis* aerial part.

TBHQ: tertiary butylhydroquinone; BHT: butylated hydroxytoluene.

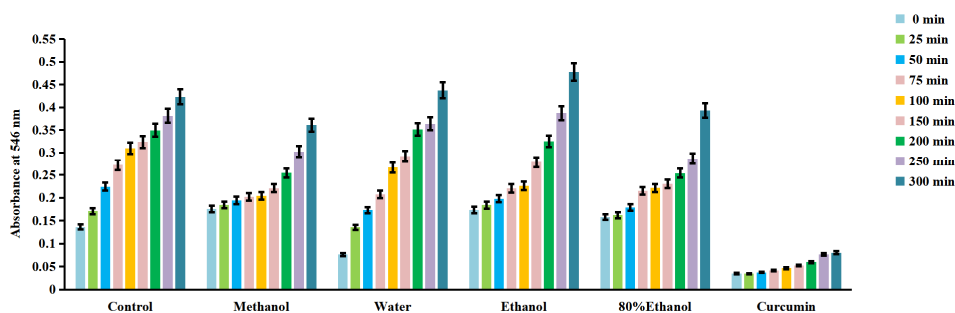
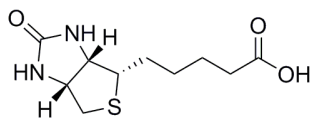
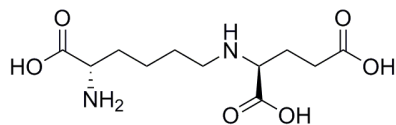


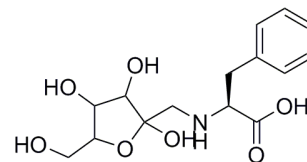
Figure S2. Evolution of absorbance of different extracts of *Anemone baicalensis* aerial part over time assessed by the nitric oxide scavenging method.



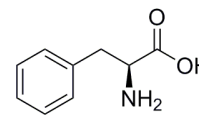
Biotin



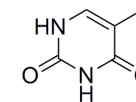
L-Saccharopine



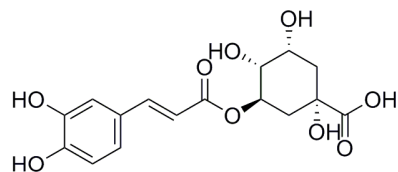
N-Fructosyl phenylalanine



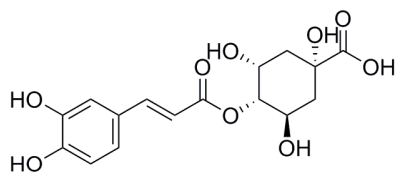
L-Phenylalanine



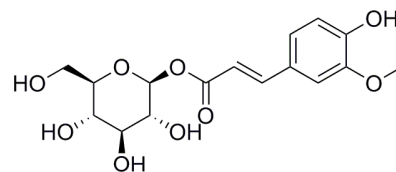
Thymine



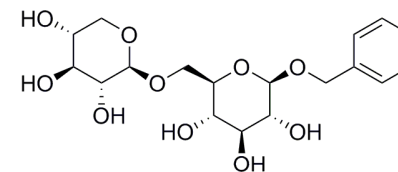
Chlorogenic acid



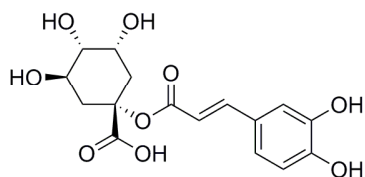
4-O-Caffeoylquinic acid



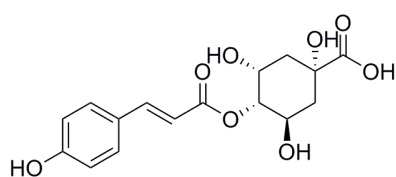
1-O-Feruloyl- β -D-glucose



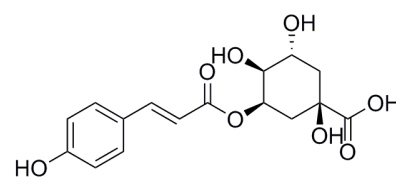
Benzyl 6-O- β -D-xylopyranosyl- β -D-glucopyranoside



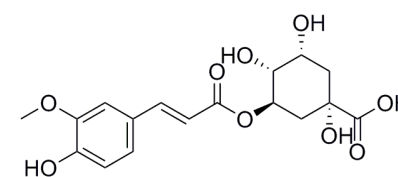
1-O-Caffeoylquinic acid



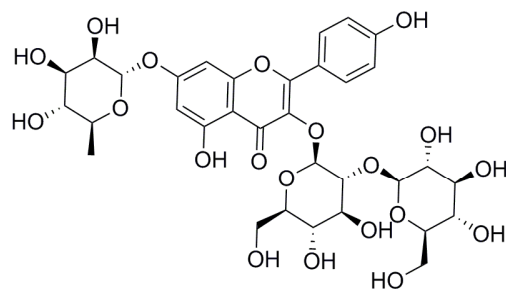
4-p-Coumaroylquinic acid



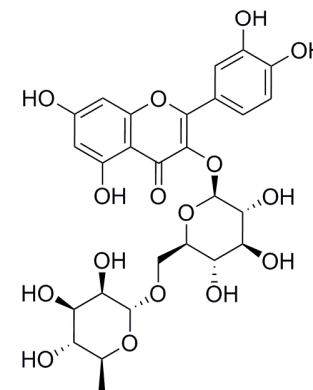
3-p-Coumaroylquinic acid



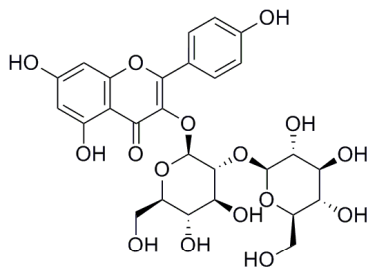
3-O-Feruloylquinic acid



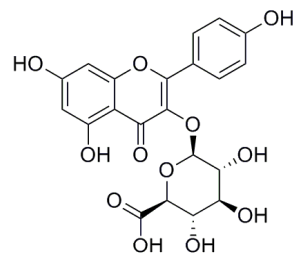
Kaempferol 3-O-sophoroside 7-O-rhamnoside



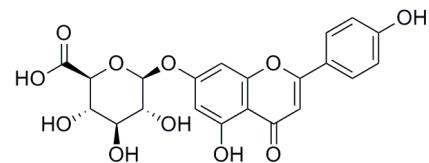
Rutin



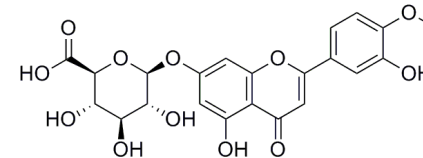
Kaempferol 3-O-sophoroside



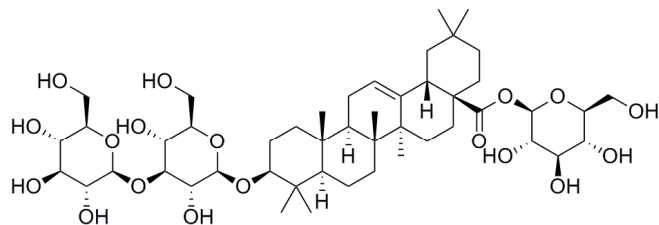
Kaempferol 3-glucuronide



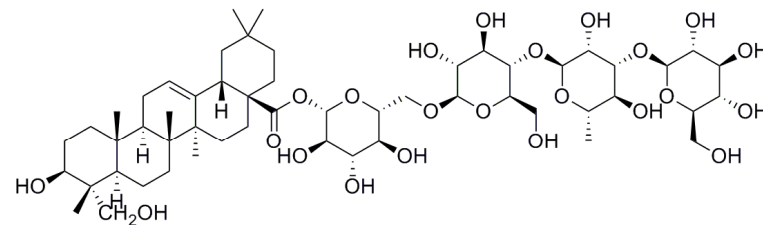
Apigenin 7-glucuronide



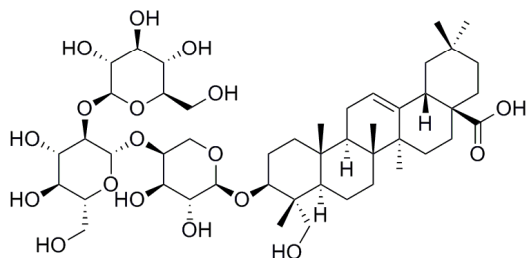
Diosmetin 7-glucuronide



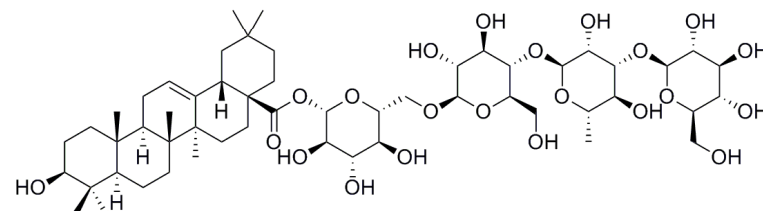
1-O-{3-[(3-O-Hexopyranosyl)hexopyranosyl]oxy}-28-oxoolean-12-en-28-yl}hexopyranose



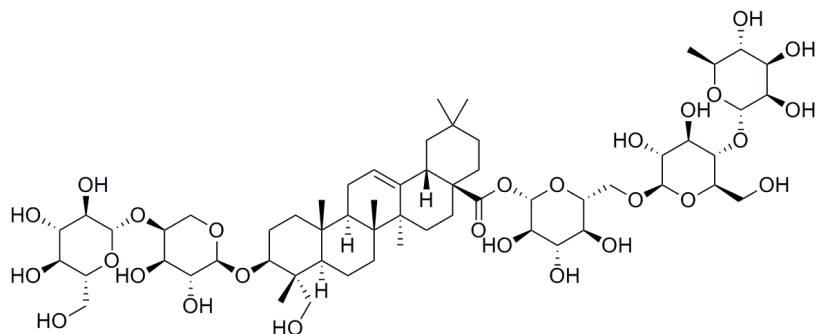
Hederagenin 28-O- β -D-glucopyranosyl-(1-3)- α -L-rhamnopyranosyl-(1-4)- β -D-glucopyranosyl-(1-6)- β -D-glucopyranosyl ester



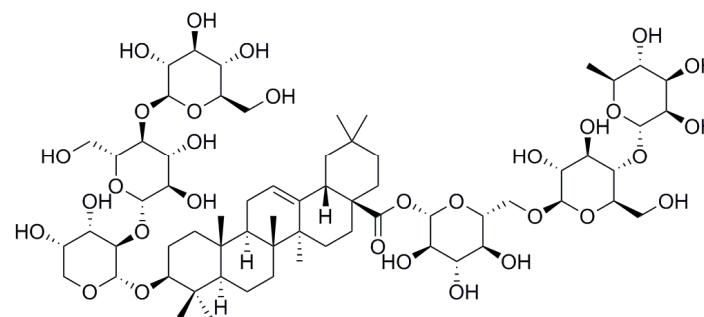
Pulsatilloside A



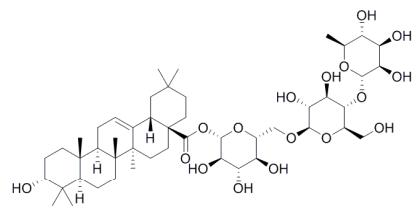
Oleanolic acid 28-O- β -D-glucopyranosyl-(1-3)- α -L-rhamnopyranosyl-(1-4)- β -D-glucopyranosyl-(1-6)- β -D-glucopyranosyl ester



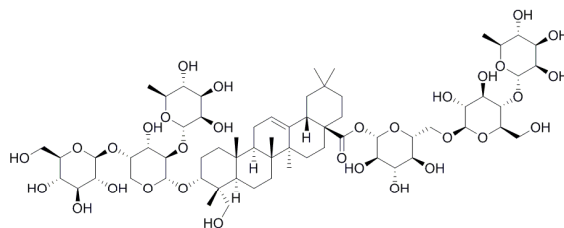
Leonloside D



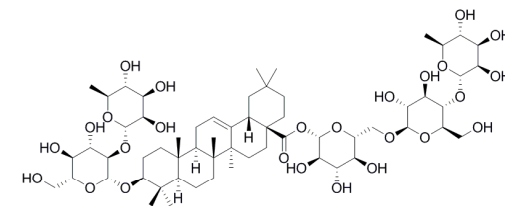
Raddeanoside R18



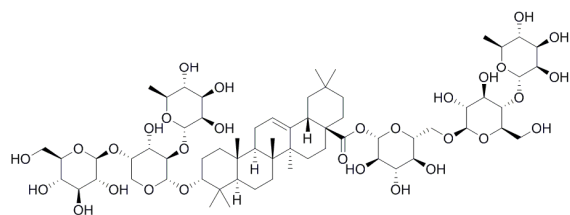
Cussonoside B



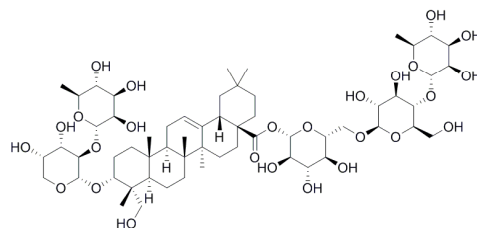
Hederacolchiside F



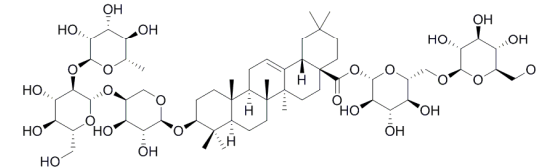
Anhuenoside E



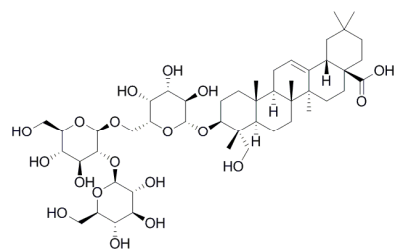
Hederacolchiside E



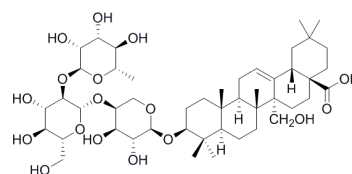
Hederacoside C



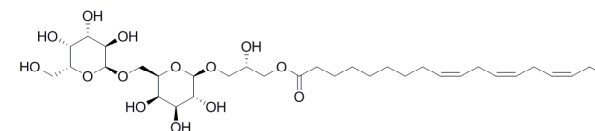
Raddeanoside R14



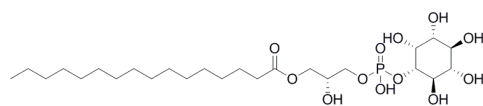
3-O-β-D-Glucopyranosyl-(1-2)-β-D-glucopyranosyl-(1-6)-β-D-galactopyranosyl-hederagenin



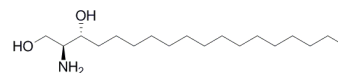
Raddeanoside R13



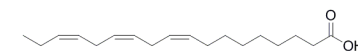
Gingerglycolipid A



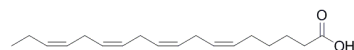
1-Palmitoylglycerophosphoinositol



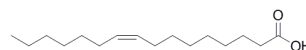
Sphinganine



Linolenic acid



Stearidonic acid



Palmitoleic acid

Figure S3. Chemical structures of the compounds identified in the methanol extract of *Anemone baicalensis* aerial part.

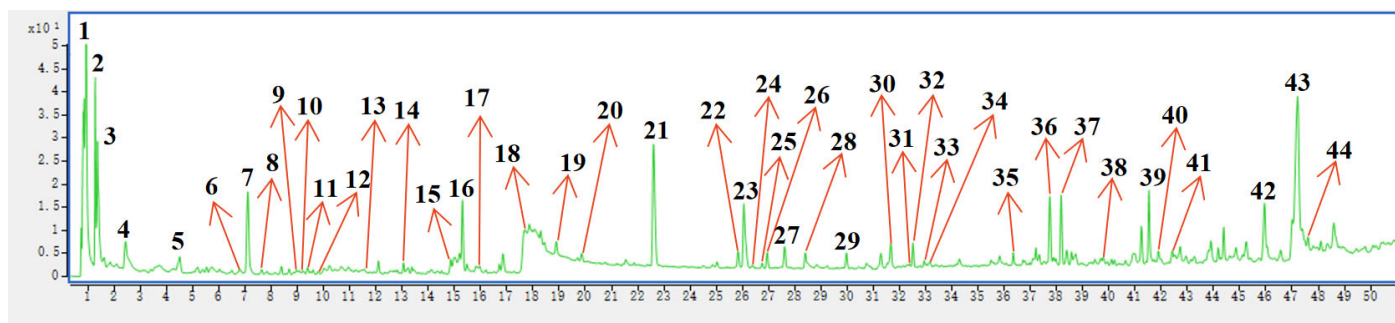


Figure S4. Positive-ion mode UHPLC-MS findings of methanol extract of *Anemone baicalensis* aerial part.

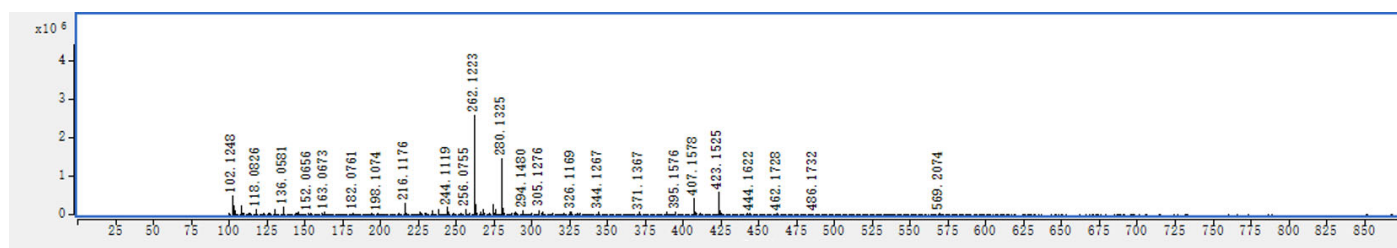


Figure S5. MS spectrum of peak 1.

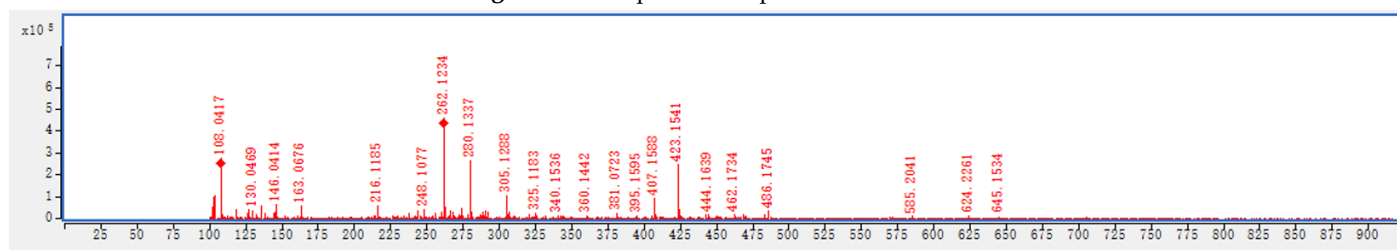


Figure S6. MS/MS spectrum of peak 1.

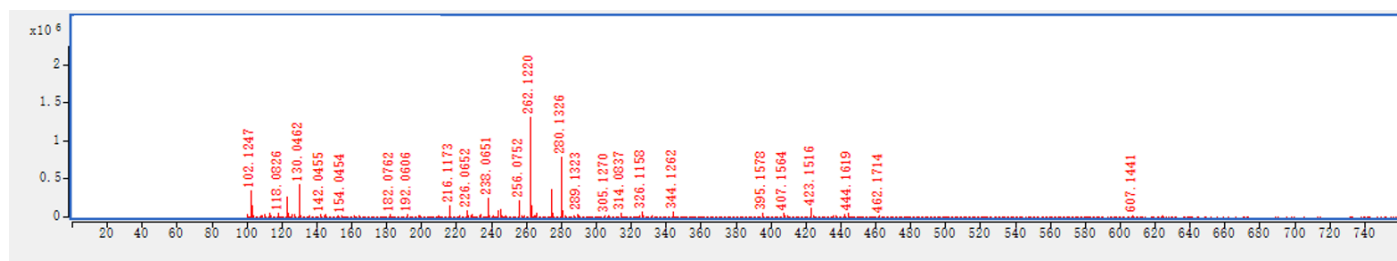


Figure S7. MS spectrum of peak 2.

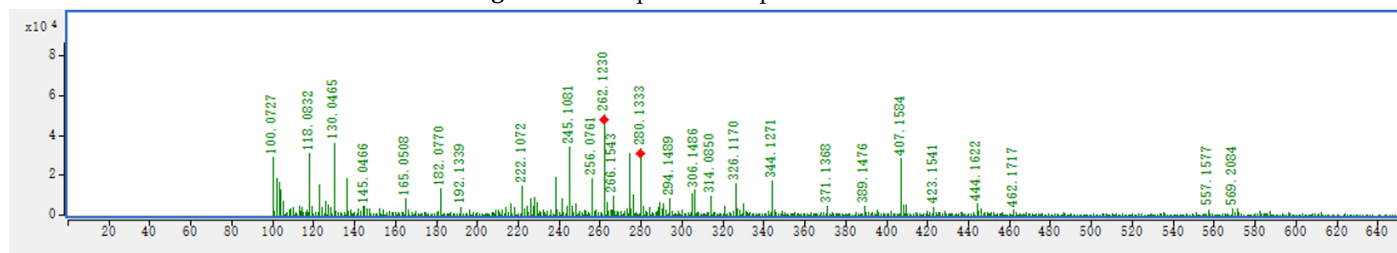


Figure S8. MS/MS spectrum of peak 2.

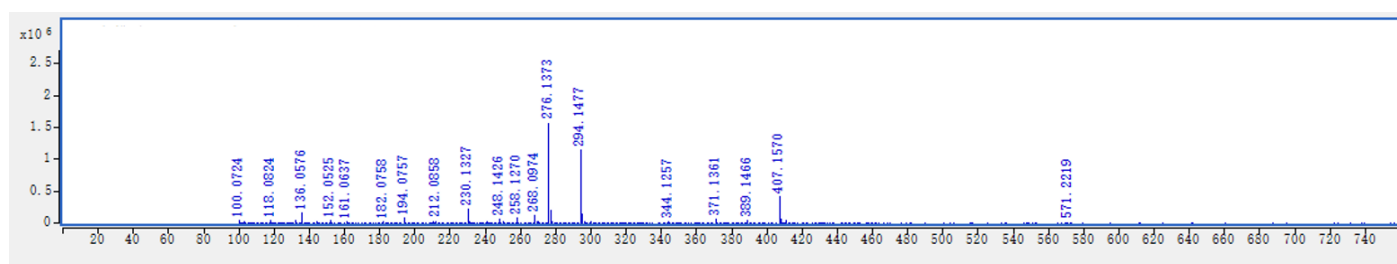


Figure S9. MS spectrum of peak 3.

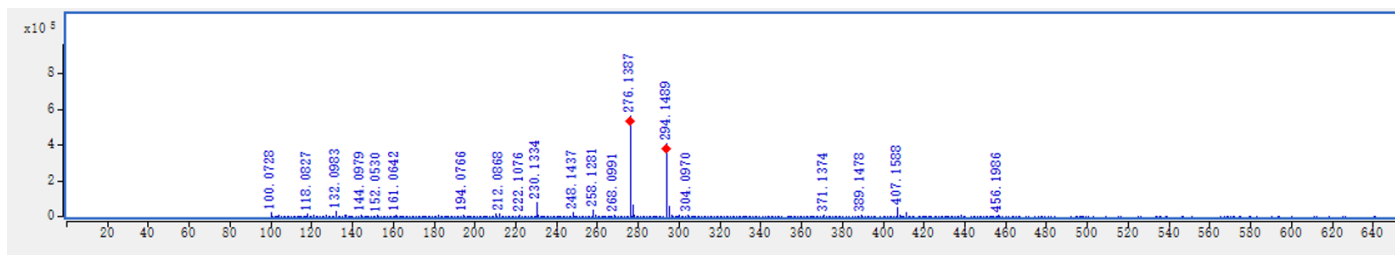


Figure S10. MS/MS spectrum of peak 3.

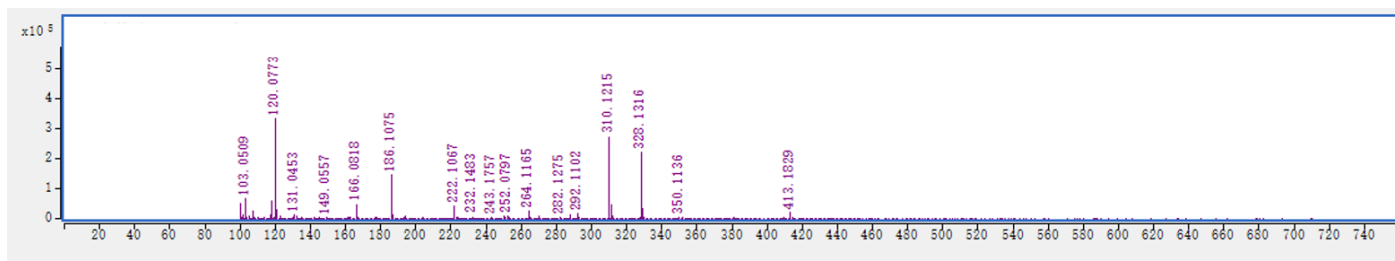


Figure S11. MS spectrum of peak 4.

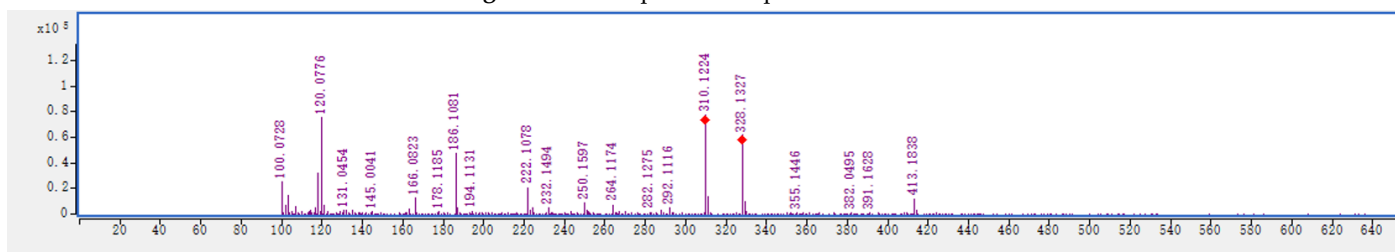


Figure S12. MS/MS spectrum of peak 4.

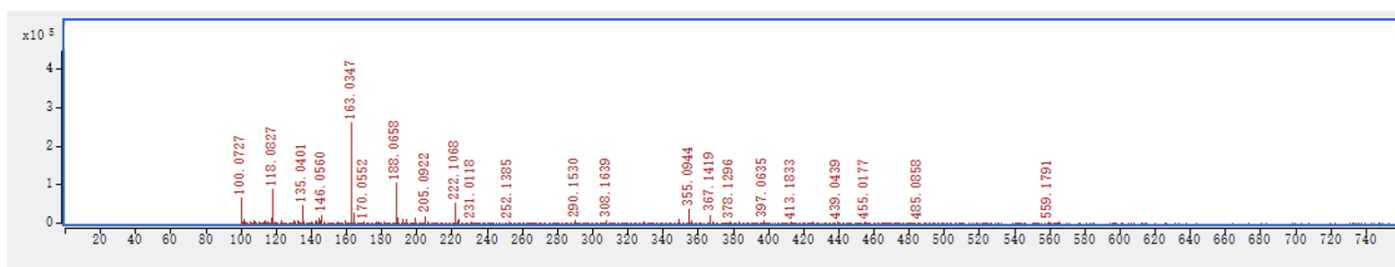


Figure S13. MS spectrum of peak 5.

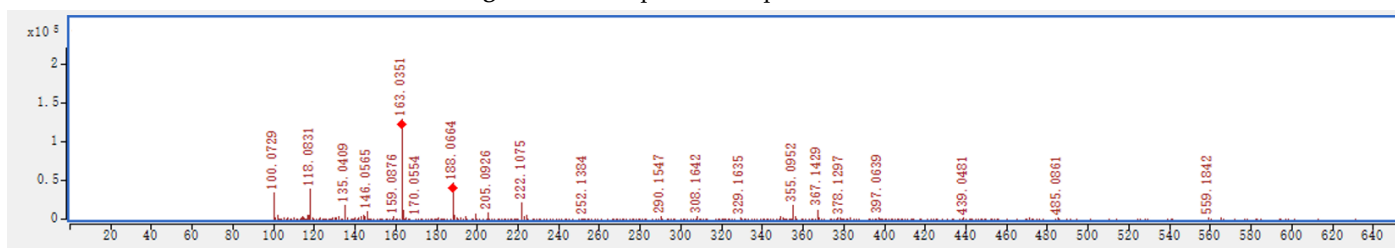


Figure S14. MS/MS spectrum of peak 5.

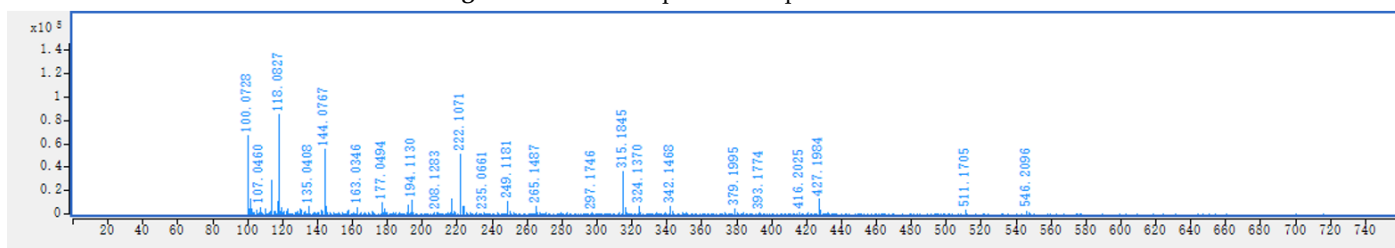


Figure S15. MS spectrum of peak 6.

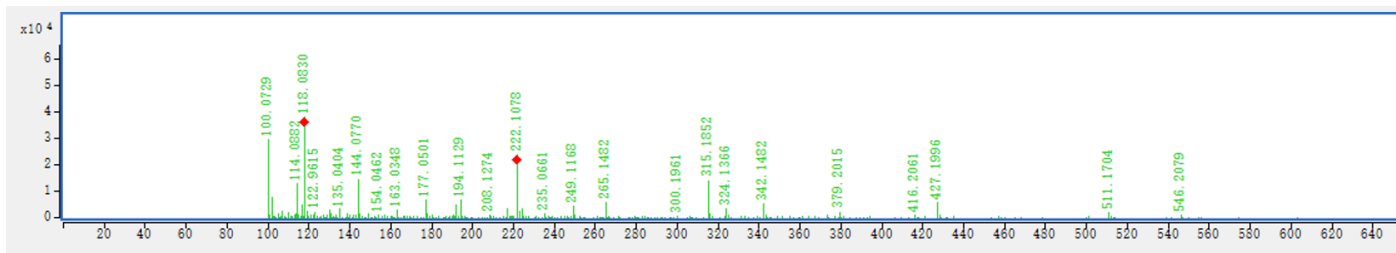


Figure S16. MS/MS spectrum of peak 6.

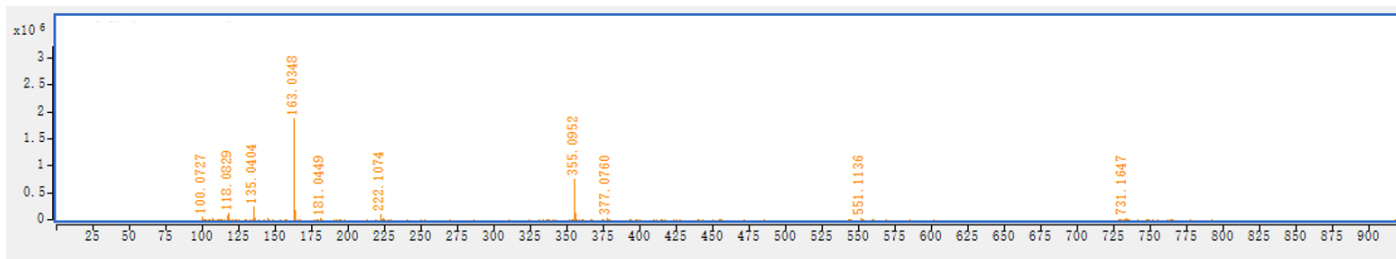


Figure S17. MS spectrum of peak 7.

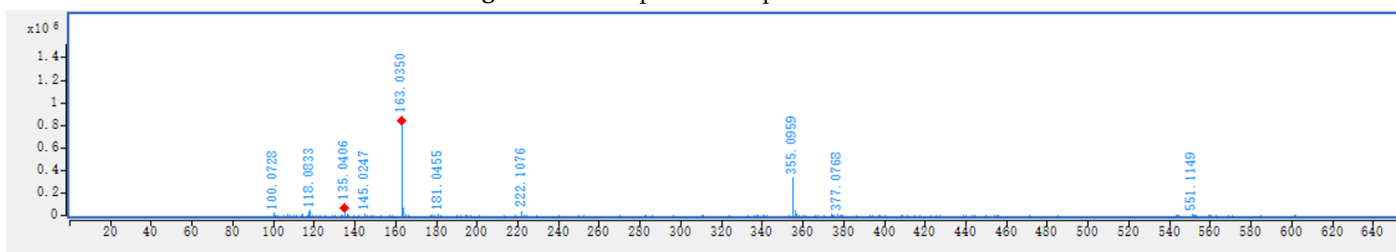


Figure S18. MS/MS spectrum of peak 7.

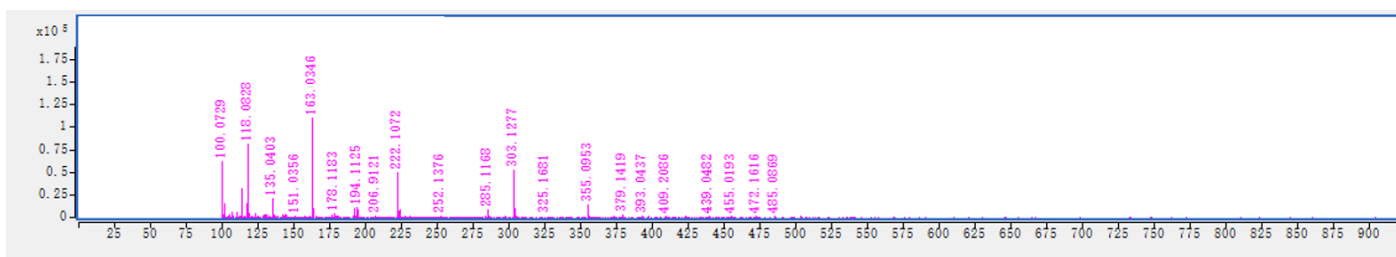


Figure S19. MS spectrum of peak 8.

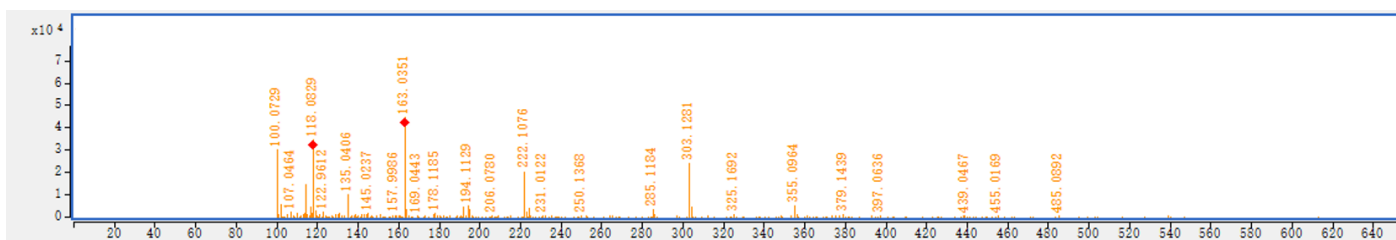


Figure S20. MS/MS spectrum of peak 8.

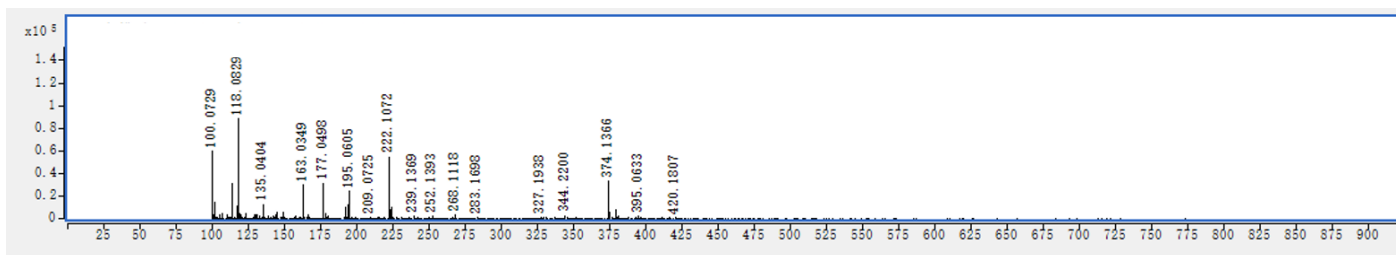


Figure S21. MS spectrum of peak 9.

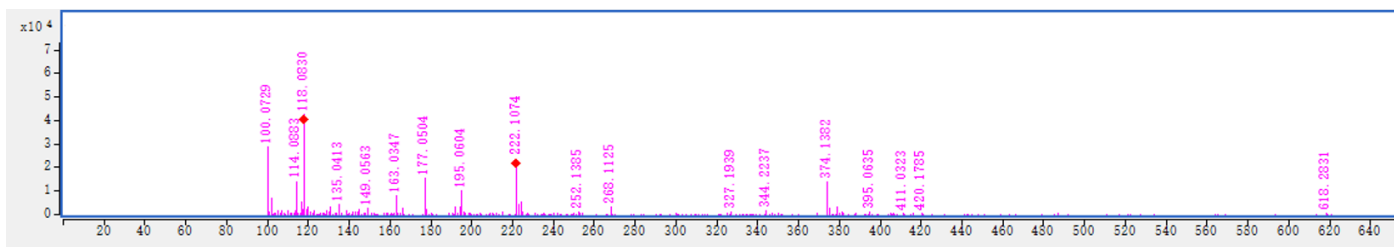


Figure S22. MS/MS spectrum of peak 9.

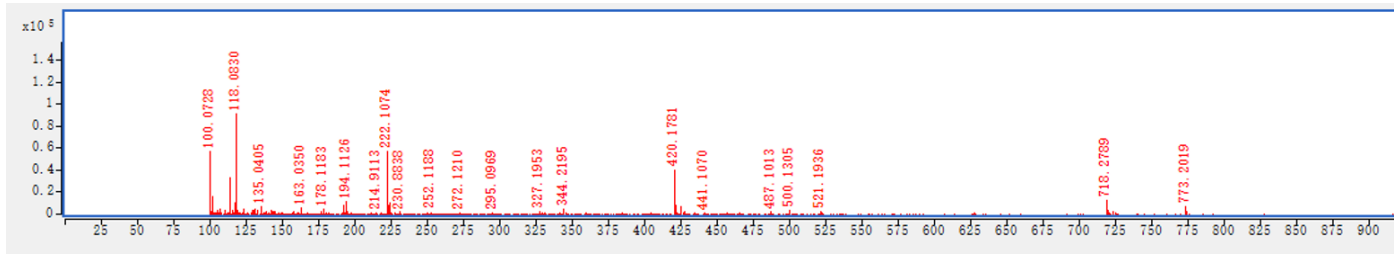


Figure S23. MS spectrum of peak 10.

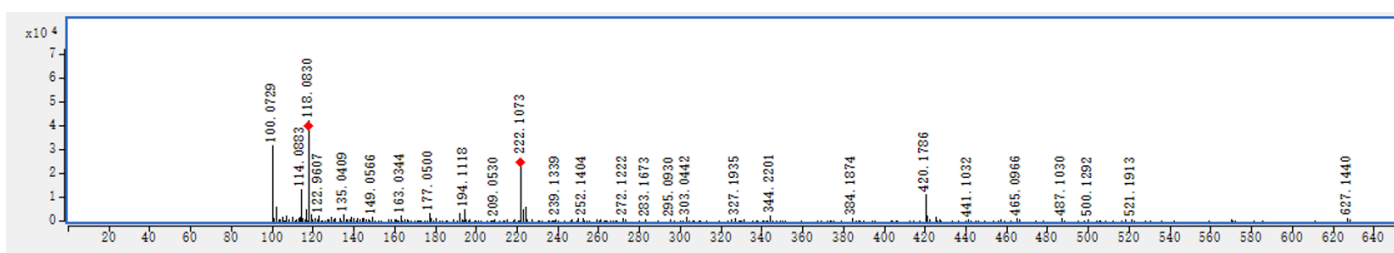


Figure S24. MS/MS spectrum of peak 10.

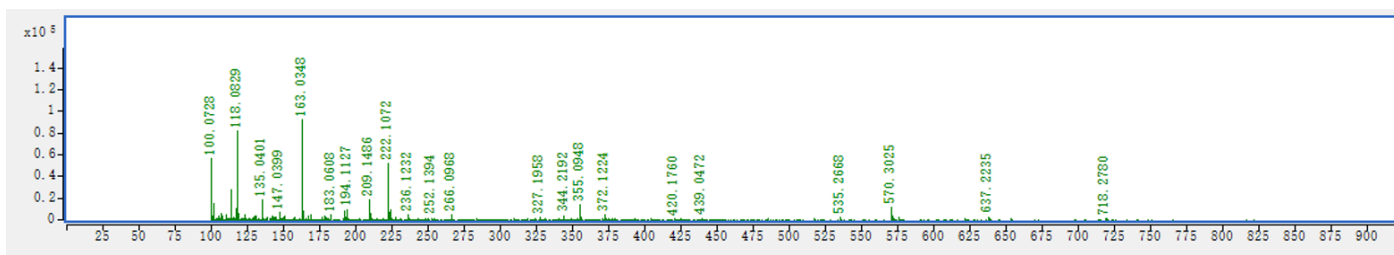


Figure S25. MS spectrum of peak 11.

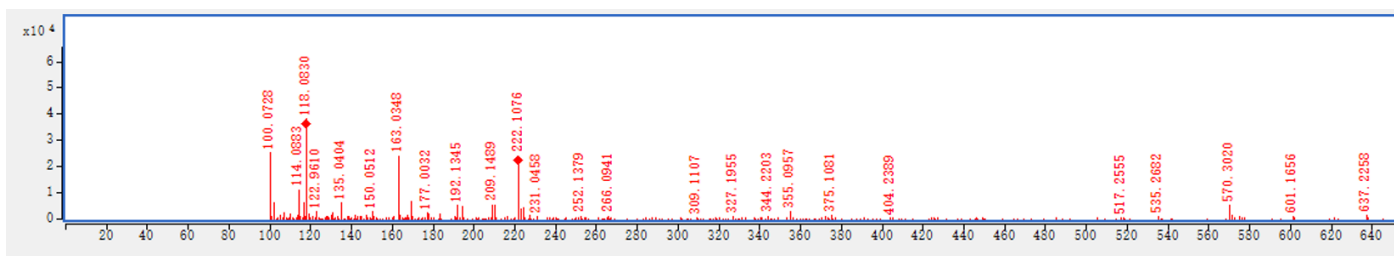


Figure S26. MS/MS spectrum of peak 11.

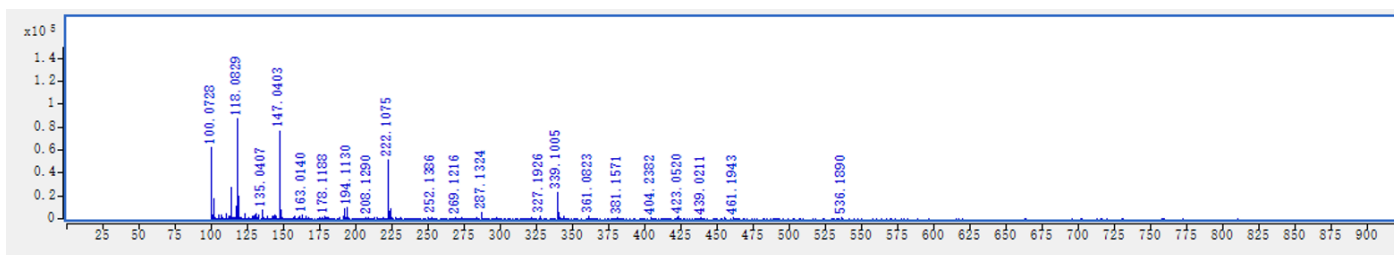


Figure S27. MS spectrum of peak 12.

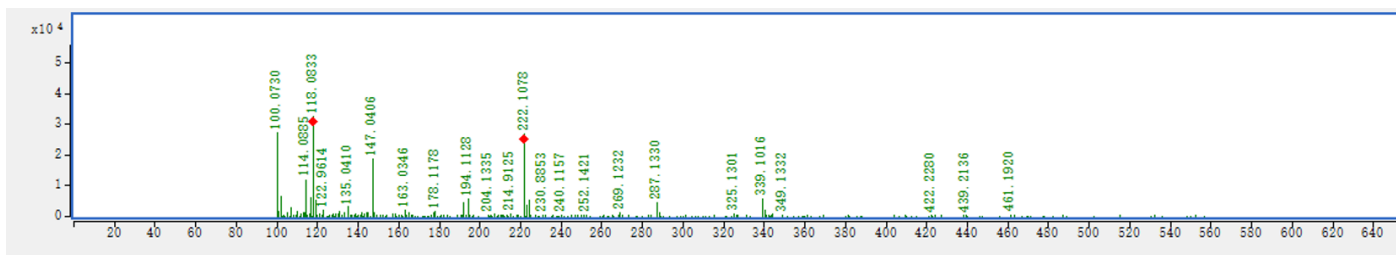


Figure S28. MS/MS spectrum of peak 12.

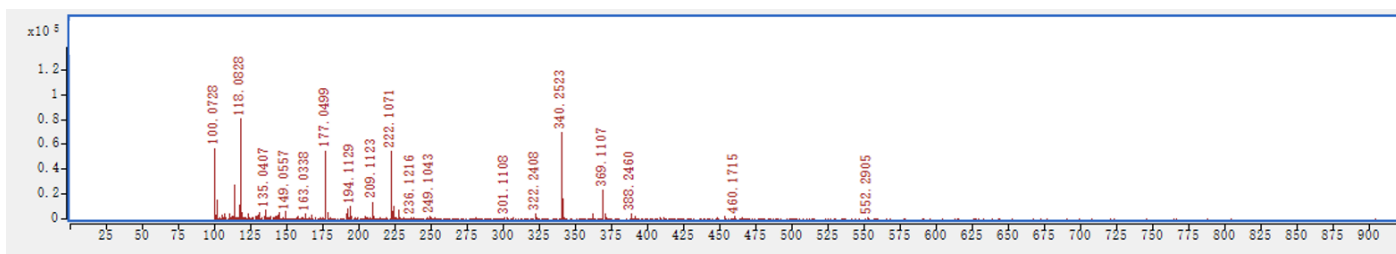


Figure S29. MS spectrum of peak 13.

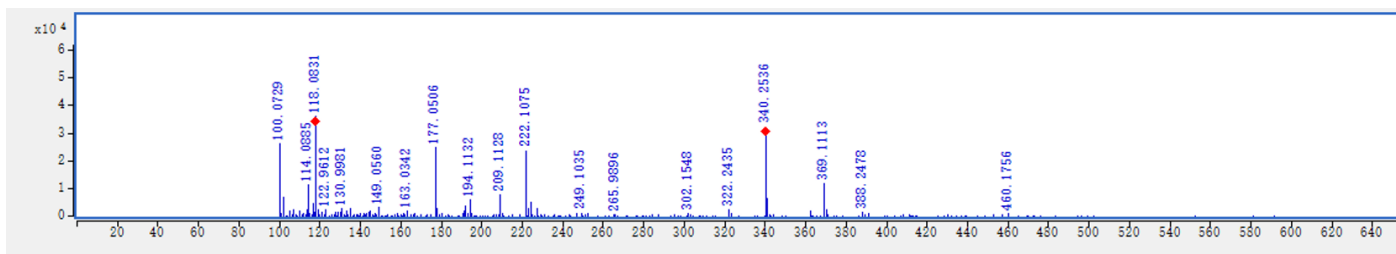


Figure S30. MS/MS spectrum of peak 13.

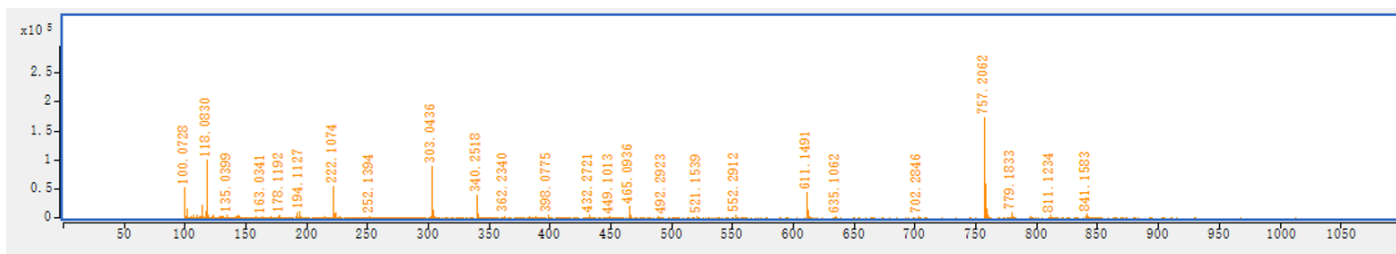


Figure S31. MS spectrum of peak 14.

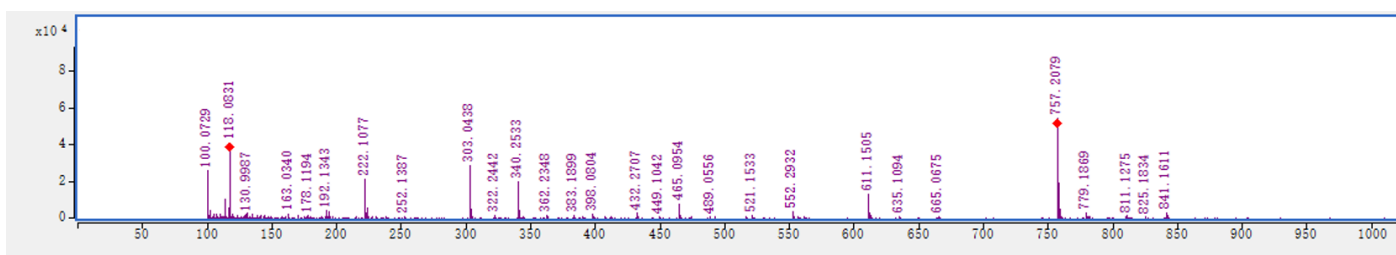


Figure S32. MS/MS spectrum of peak 14.

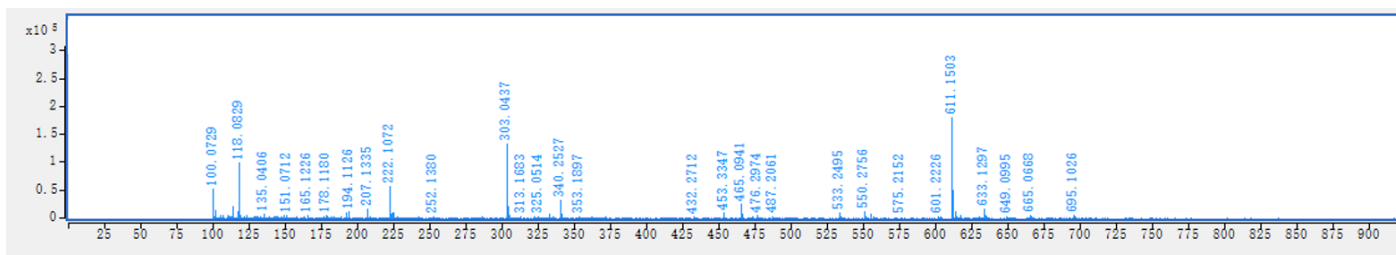


Figure S33. MS spectrum of peak 15.

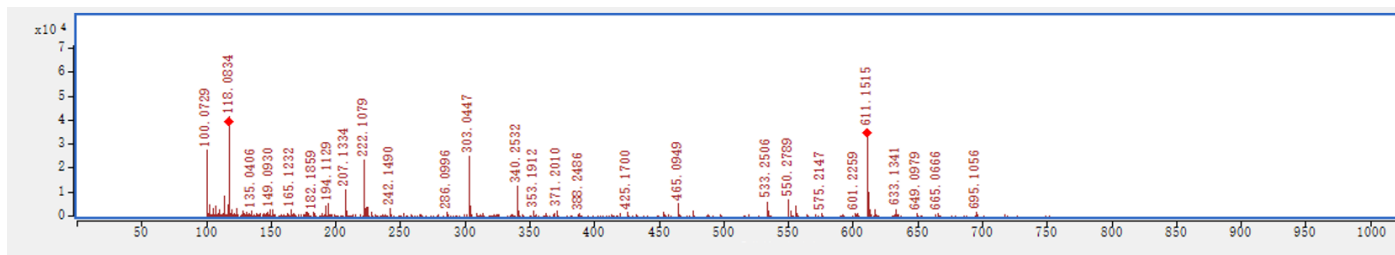


Figure S34. MS/MS spectrum of peak 15.

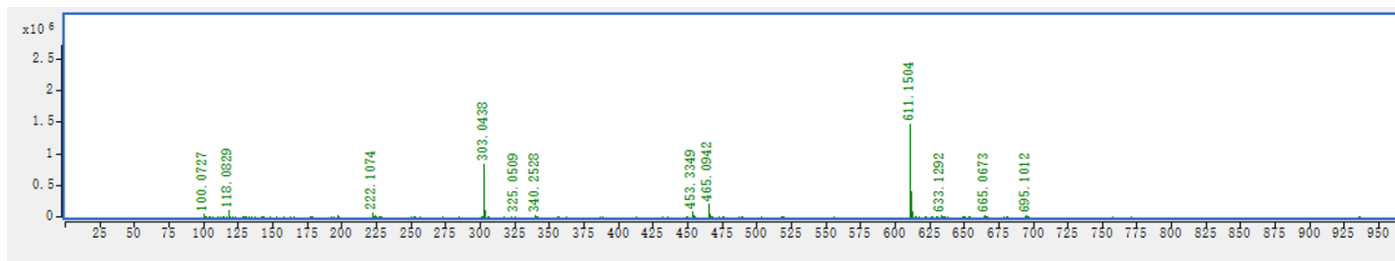


Figure S35. MS spectrum of peak 16.

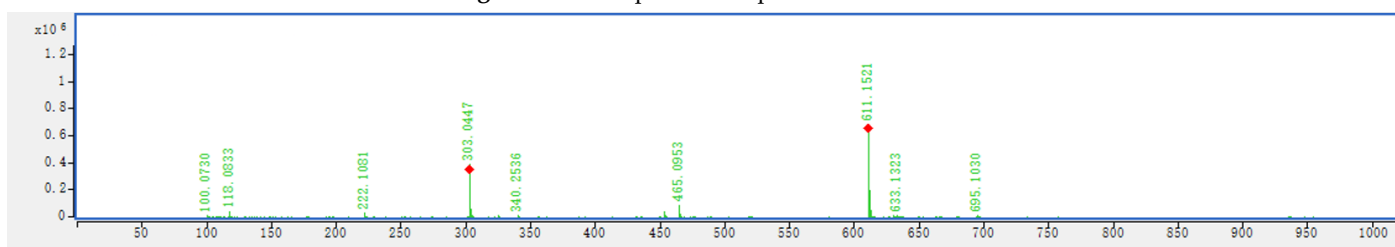


Figure S36. MS/MS spectrum of peak 16.

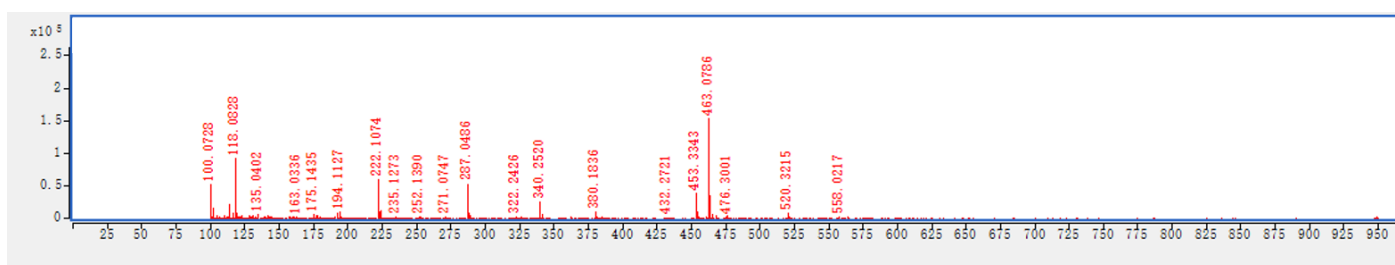


Figure S37. MS spectrum of peak 17.

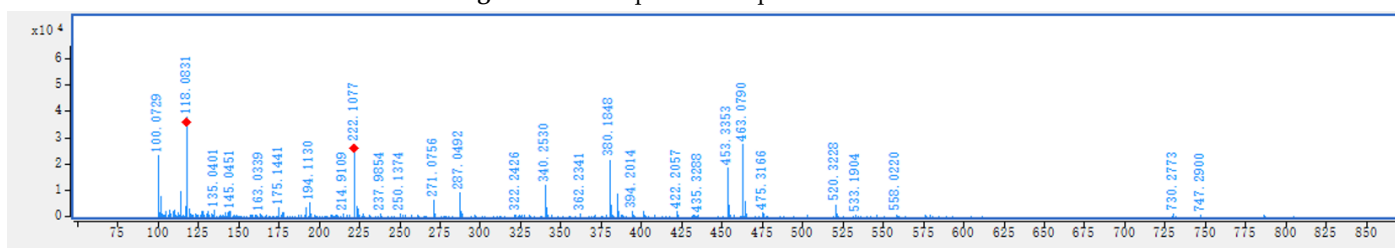


Figure S38. MS/MS spectrum of peak 17.

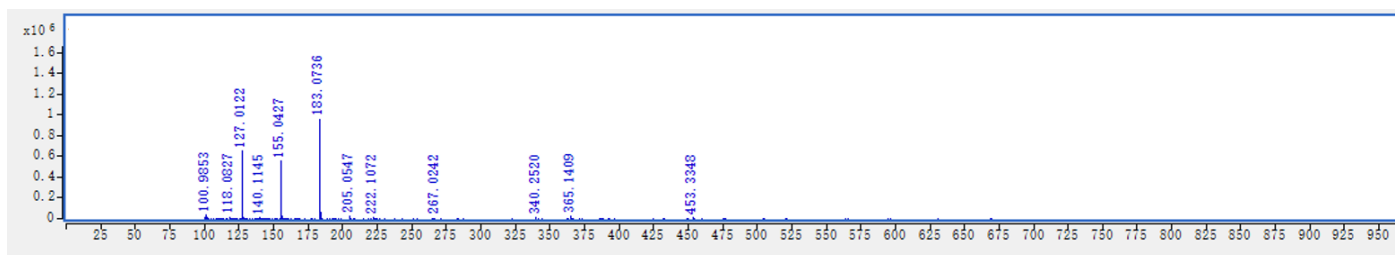


Figure S39. MS spectrum of peak 18.

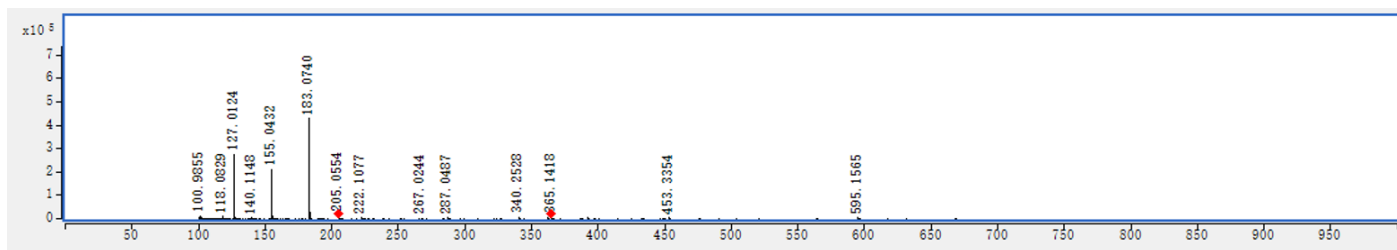


Figure S40. MS/MS spectrum of peak 18.

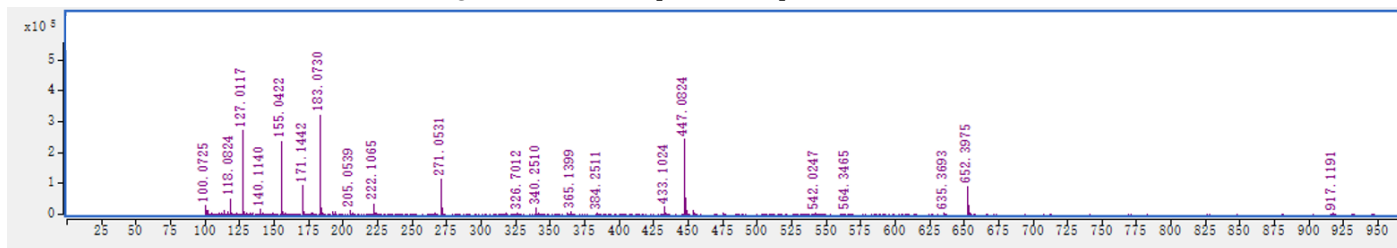


Figure S41. MS spectrum of peak 19.

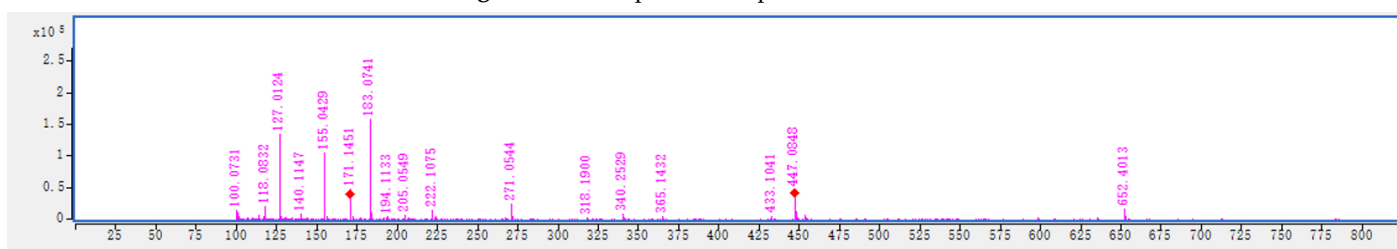


Figure S42. MS/MS spectrum of peak 19.

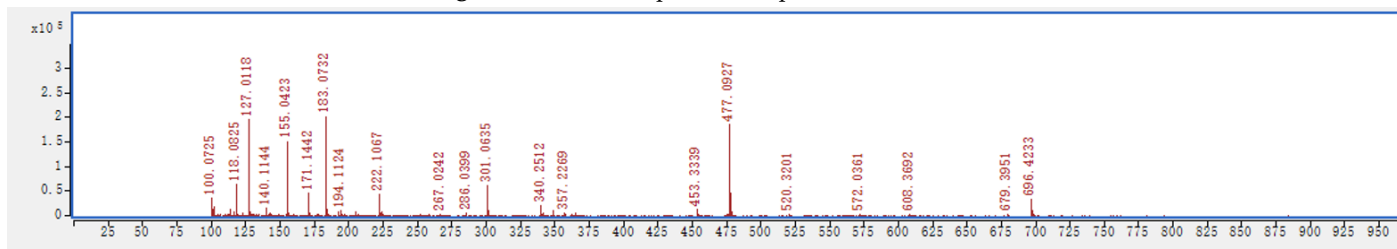


Figure S43. MS spectrum of peak 20.

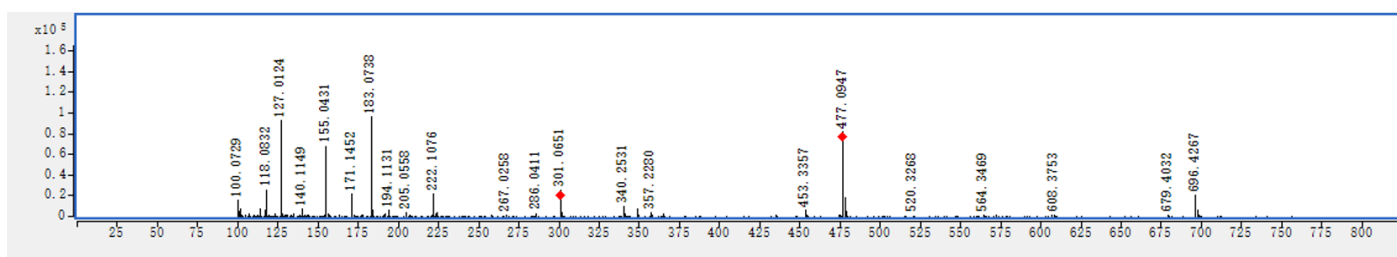


Figure S44. MS/MS spectrum of peak 20.

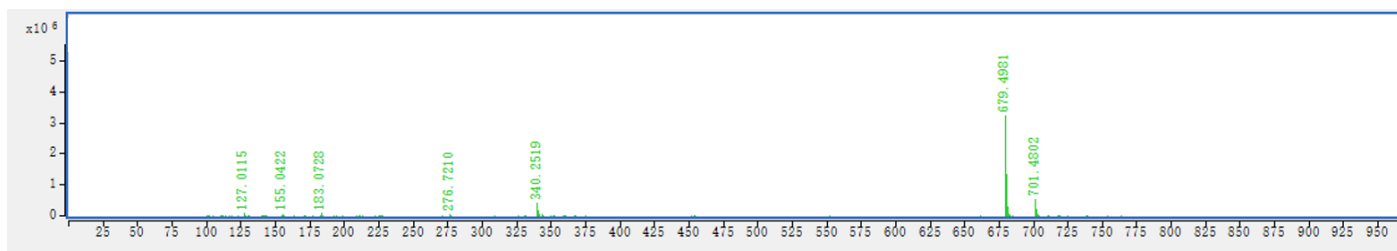


Figure S45. MS spectrum of peak 21.

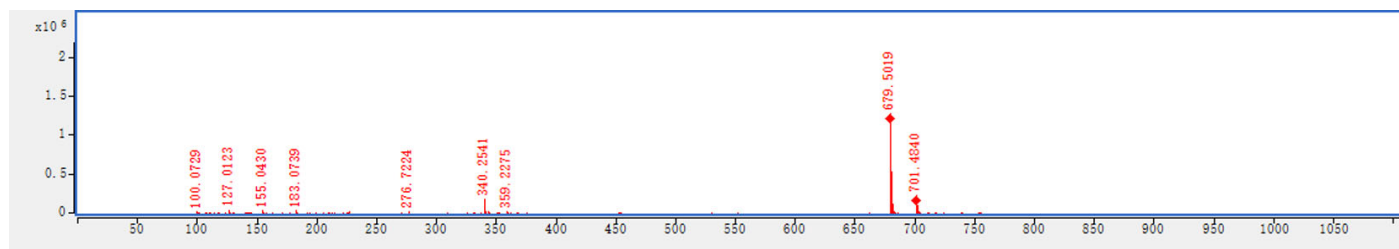


Figure S46. MS/MS spectrum of peak 21.

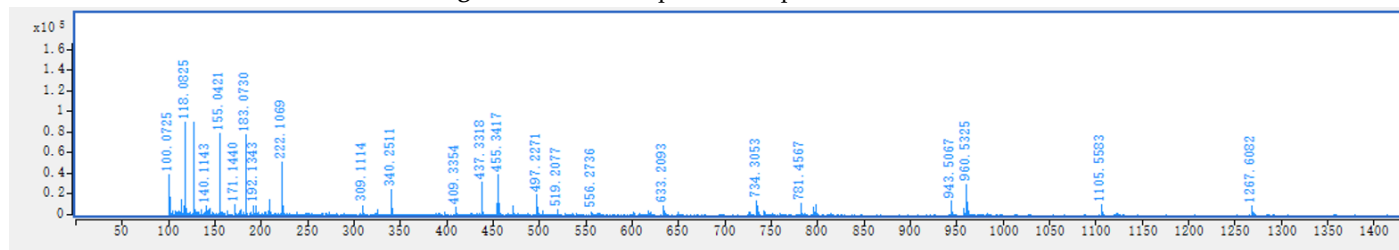


Figure S47. MS spectrum of peak 22.

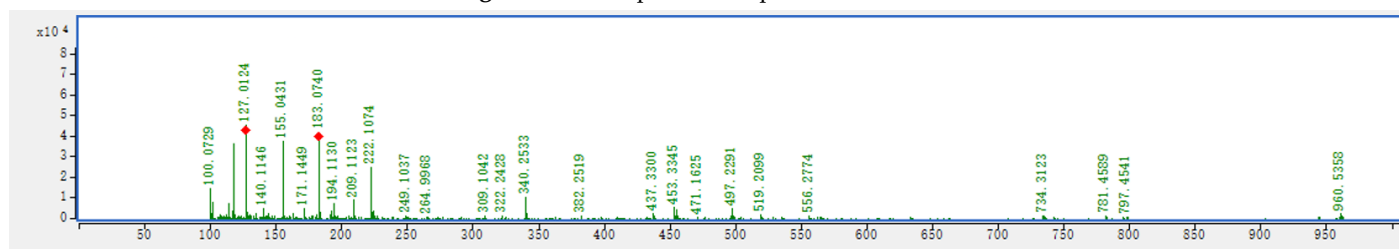


Figure S48. MS/MS spectrum of peak 22.

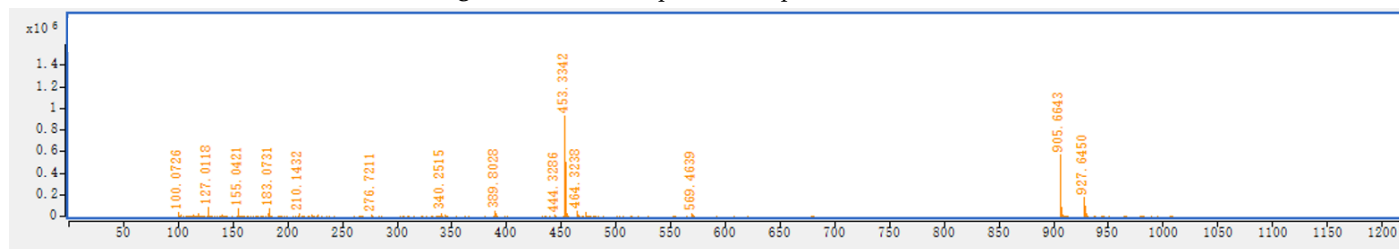


Figure S49. MS spectrum of peak 23.

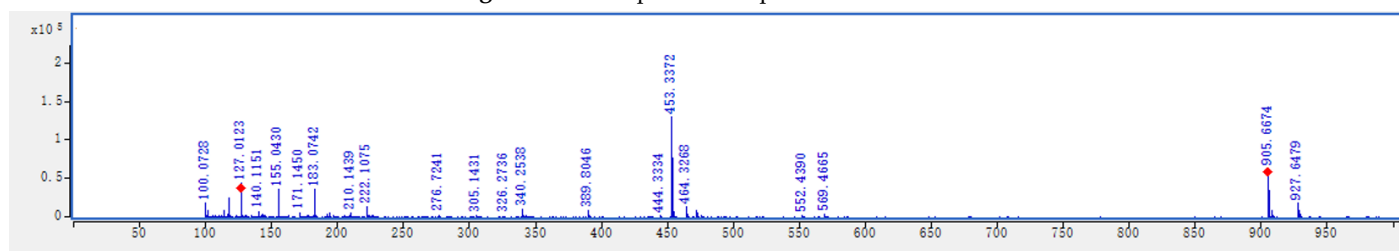


Figure S50. MS/MS spectrum of peak 23.

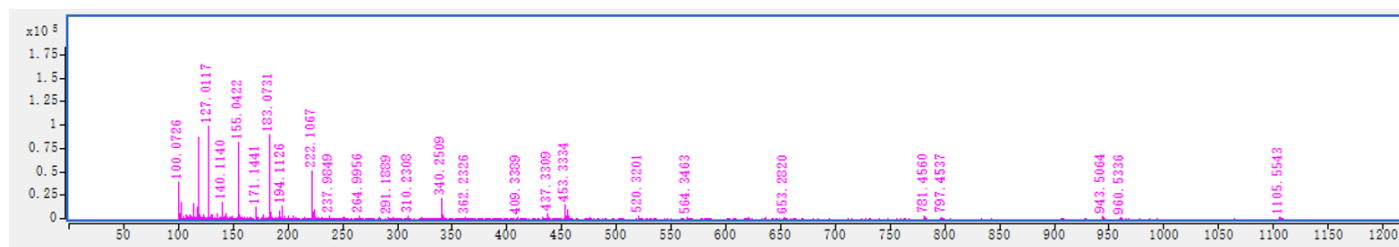


Figure S51. MS spectrum of peak 24.

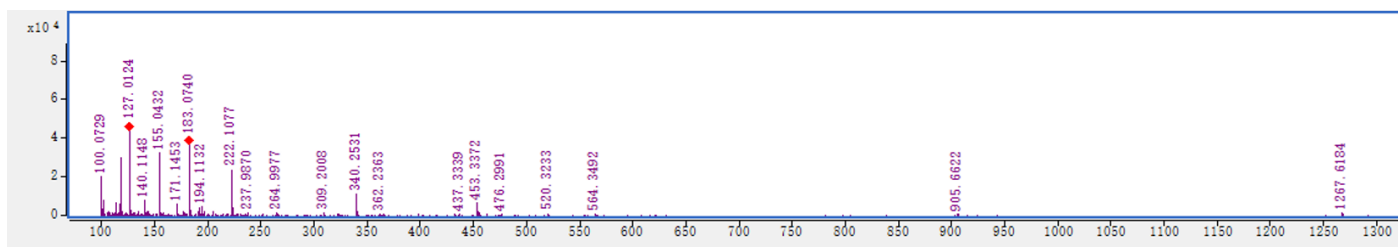


Figure S52. MS/MS spectrum of peak 24.

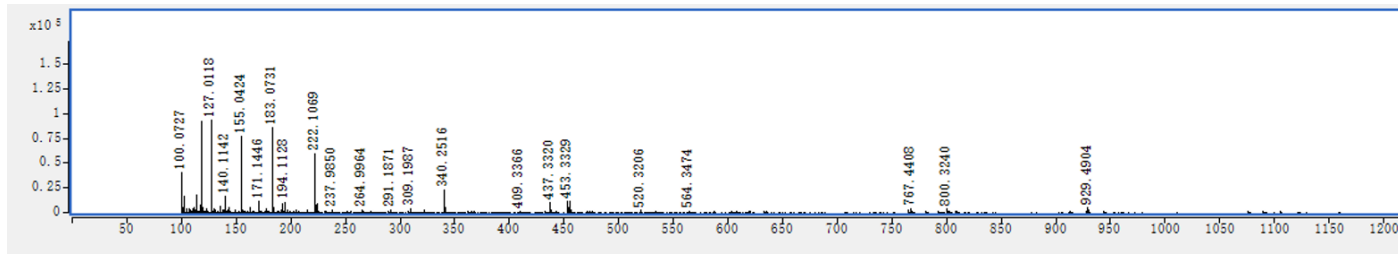


Figure S53. MS spectrum of peak 25.

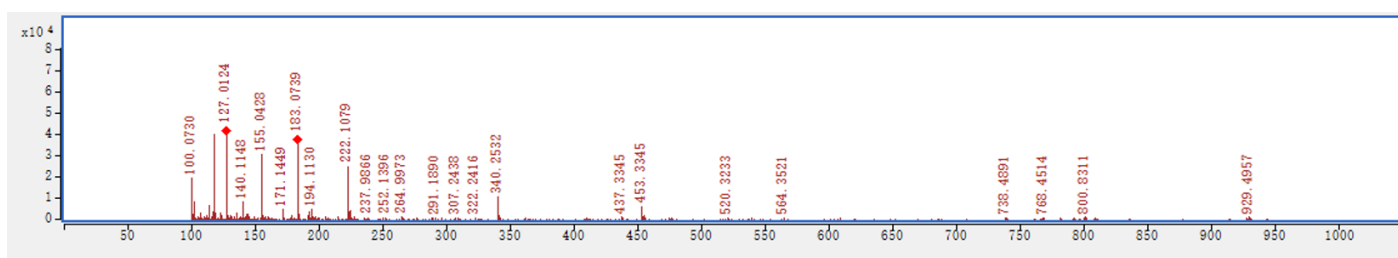


Figure S54. MS/MS spectrum of peak 25.

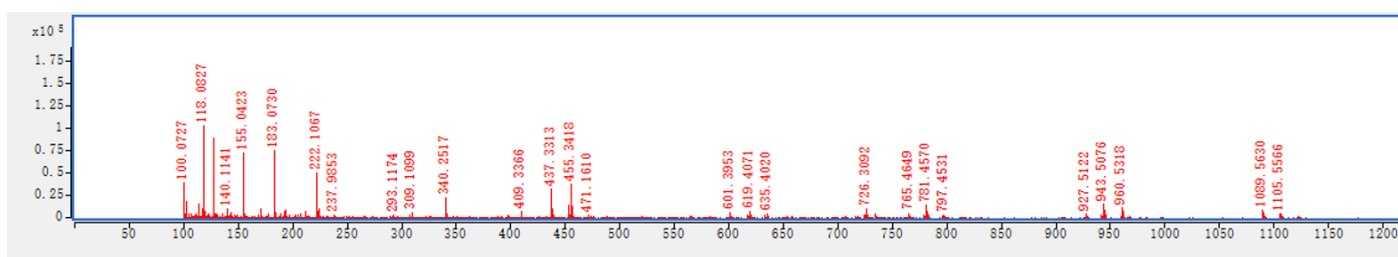


Figure S55. MS spectrum of peak 26.

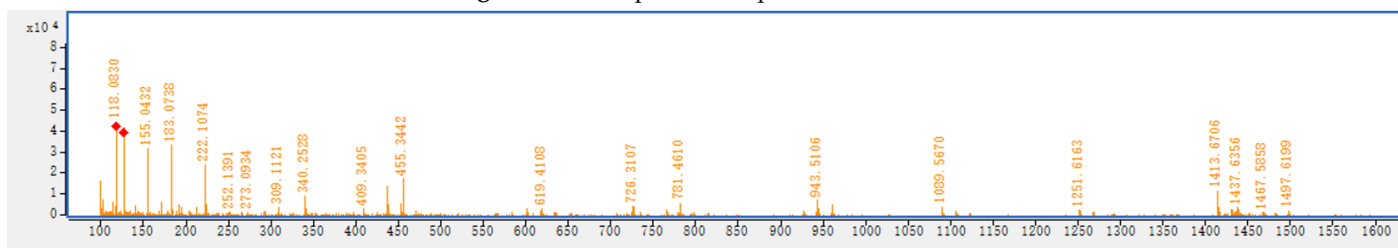


Figure S56. MS/MS spectrum of peak 26.

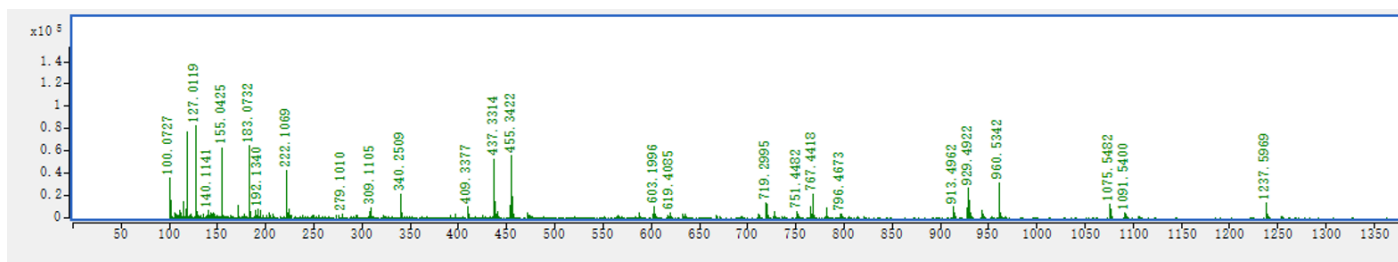


Figure S57. MS spectrum of peak 27.

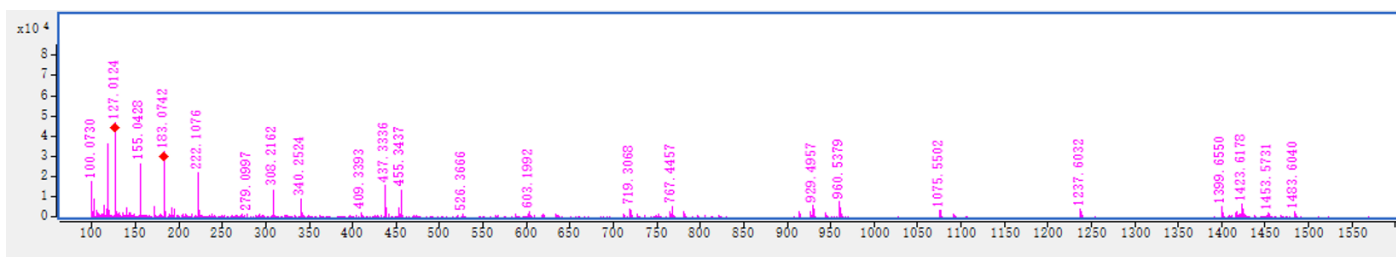


Figure S58. MS/MS spectrum of peak 27.

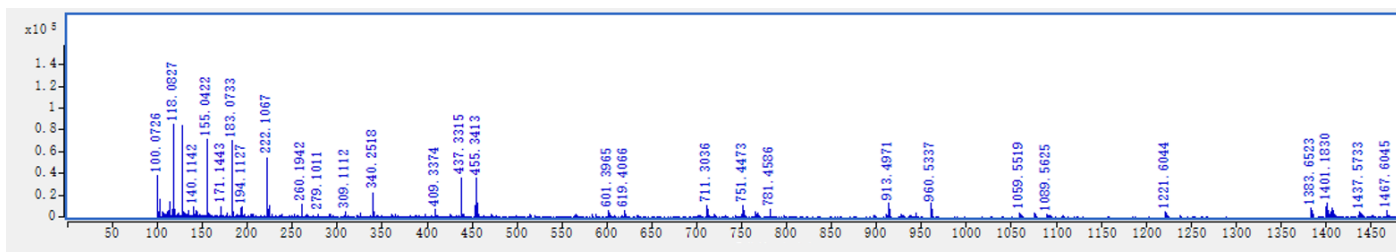


Figure S59. MS spectrum of peak 28.

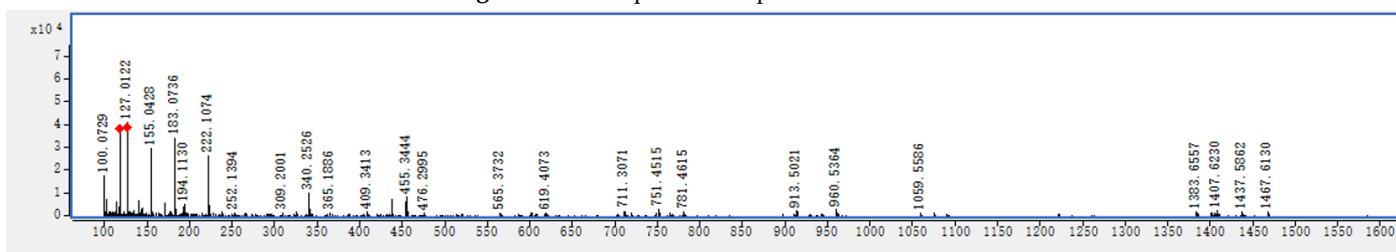


Figure S60. MS/MS spectrum of peak 28.

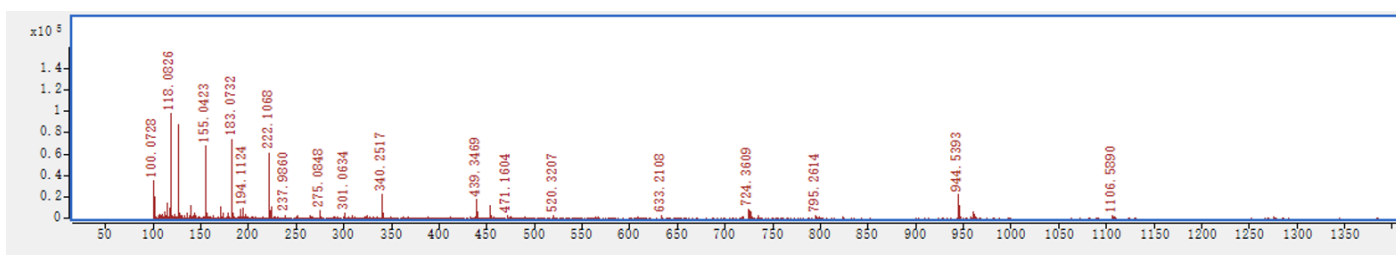


Figure S61. MS spectrum of peak 29.

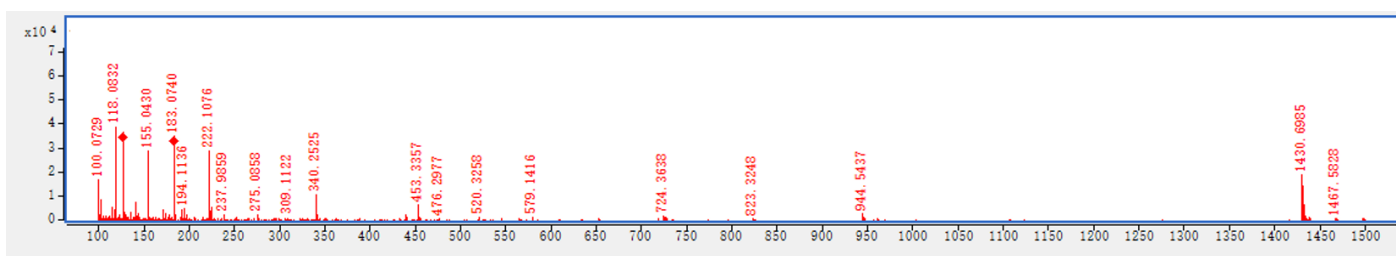


Figure S62. MS/MS spectrum of peak 29.

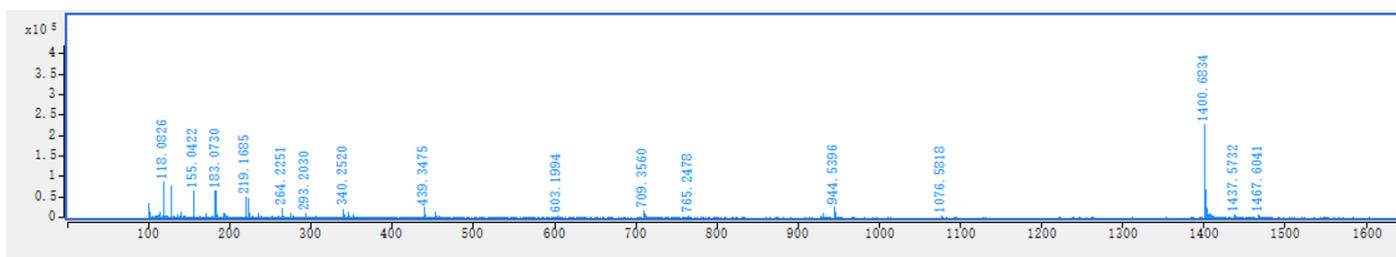


Figure S63. MS spectrum of peak 30.

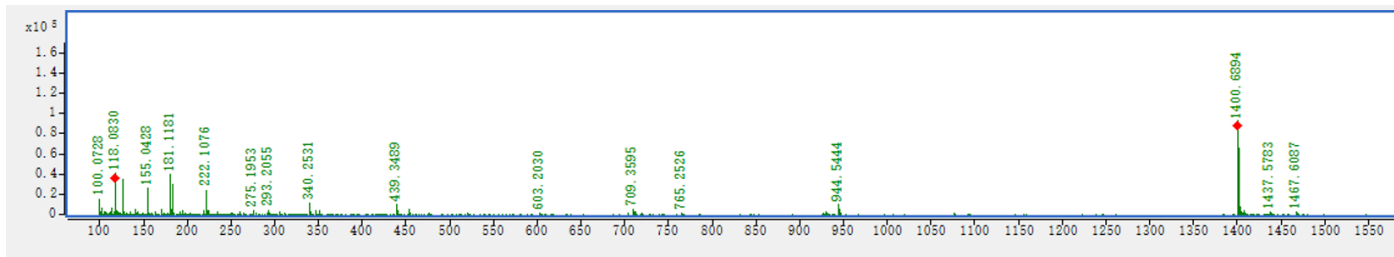


Figure S64. MS/MS spectrum of peak 30.

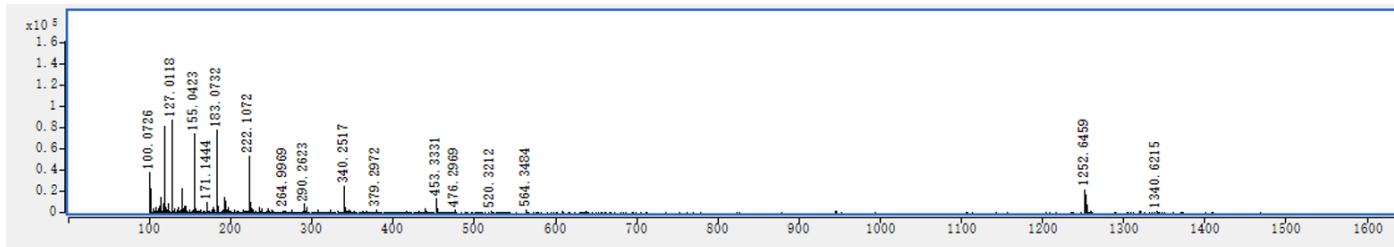


Figure S65. MS spectrum of peak 31.

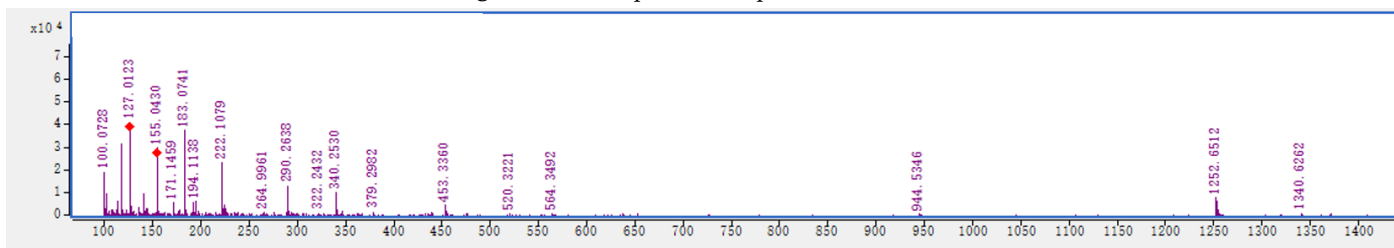


Figure S66. MS/MS spectrum of peak 31.

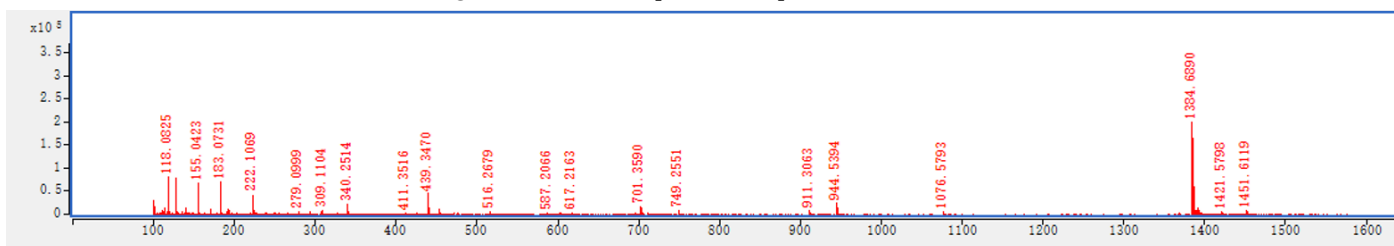


Figure S67. MS spectrum of peak 32.

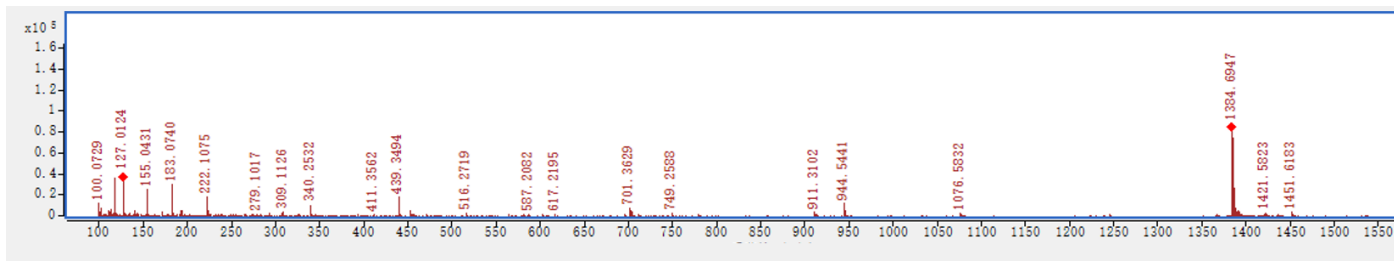


Figure S68. MS/MS spectrum of peak 32.

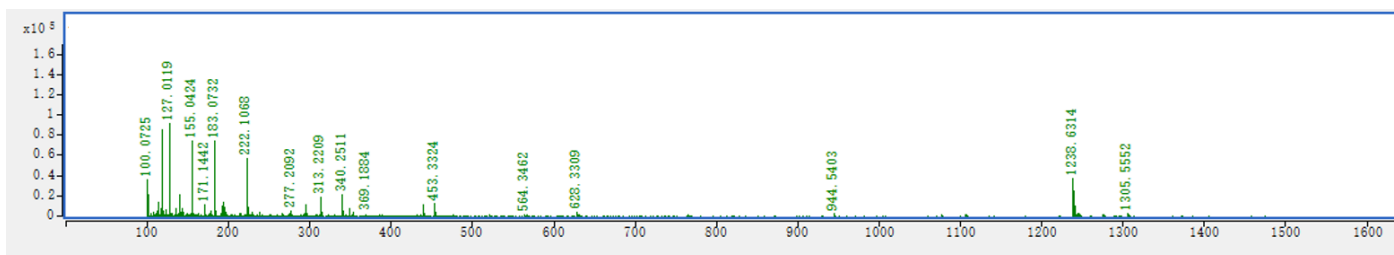


Figure S69. MS spectrum of peak 33.

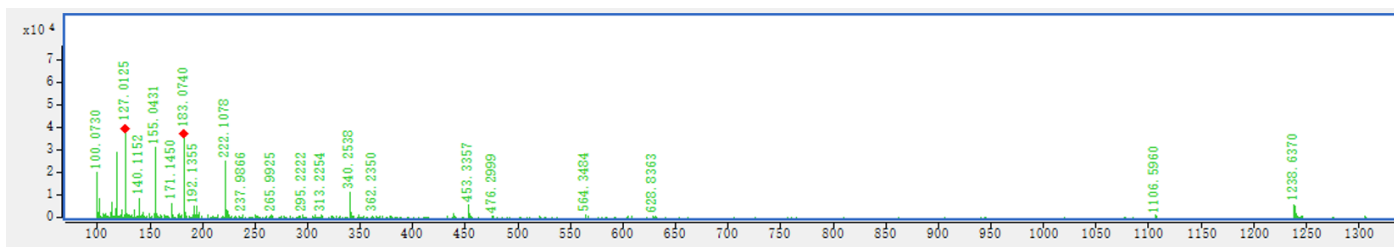


Figure S70. MS/MS spectrum of peak 33.

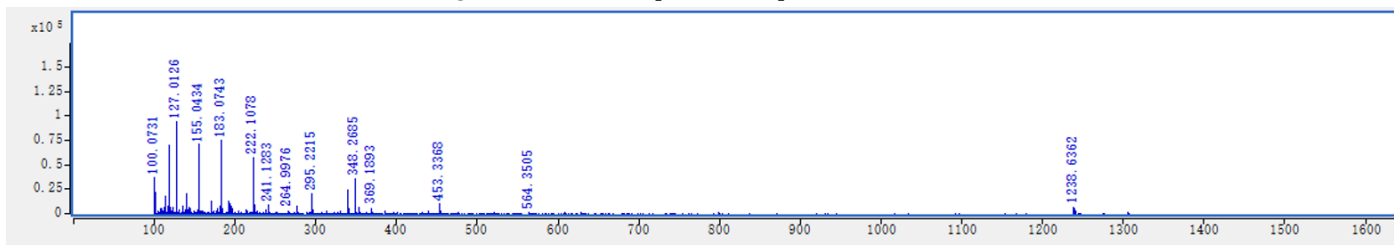


Figure S71. MS spectrum of peak 34.

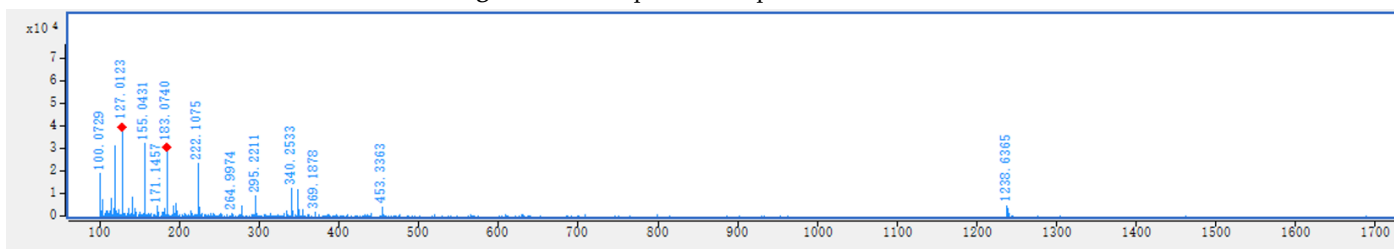


Figure S72. MS/MS spectrum of peak 34.

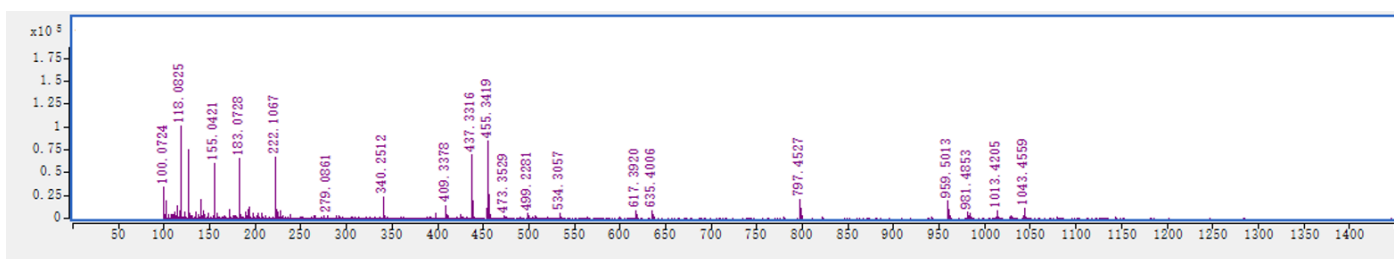


Figure S73. MS spectrum of peak 35.

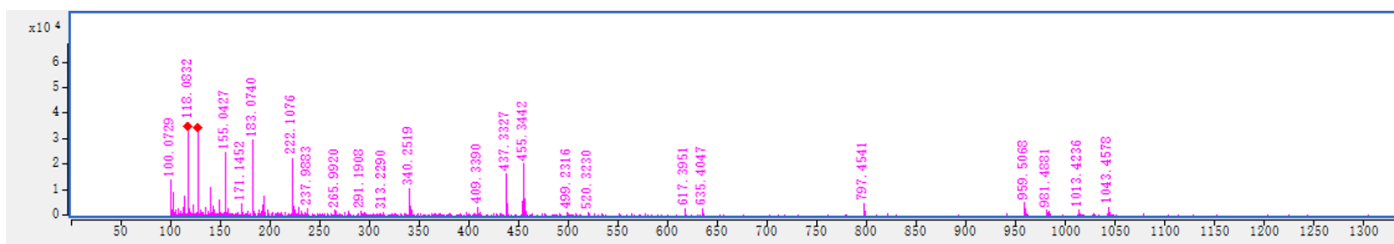


Figure S74. MS/MS spectrum of peak 35.

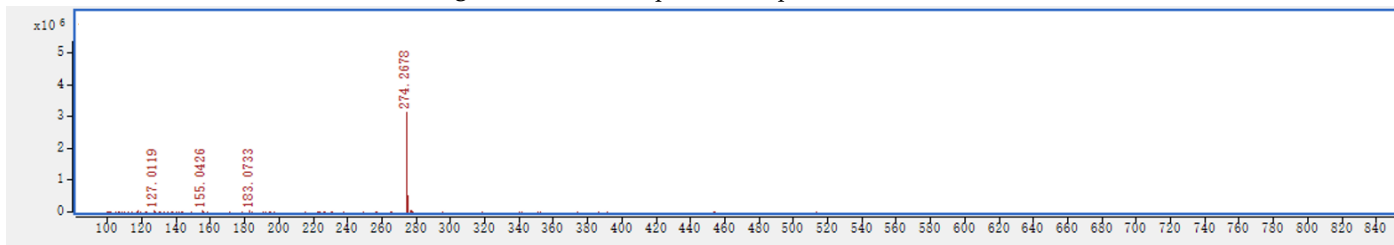


Figure S75. MS spectrum of peak 36.

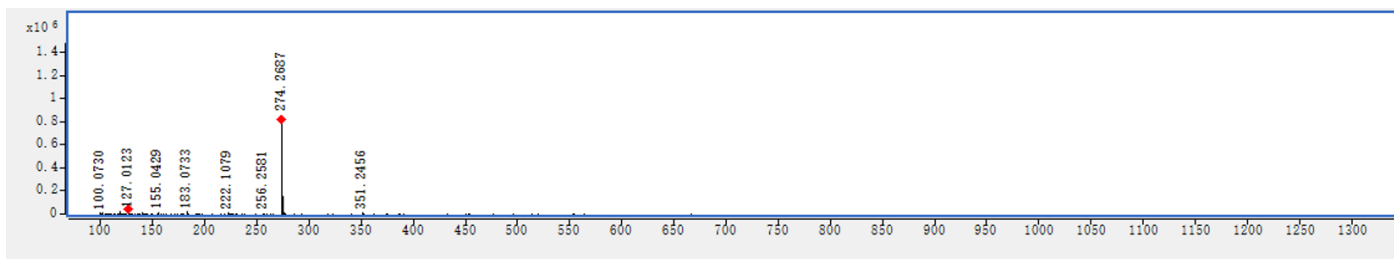


Figure S76. MS/MS spectrum of peak 36.

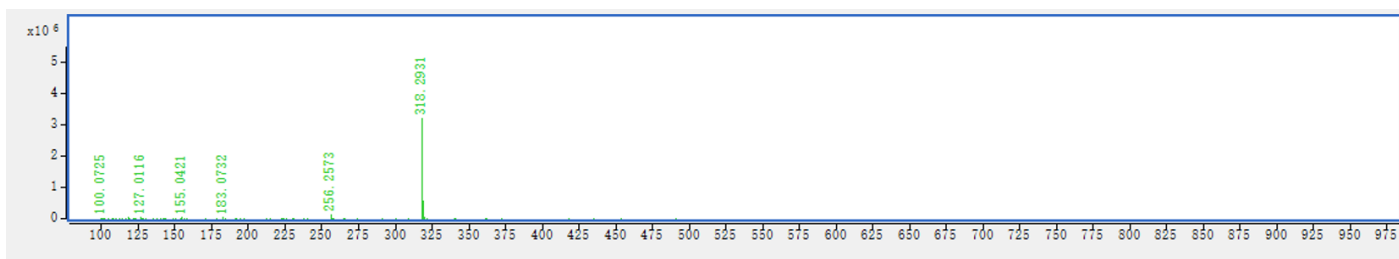


Figure S77. MS spectrum of peak 37.

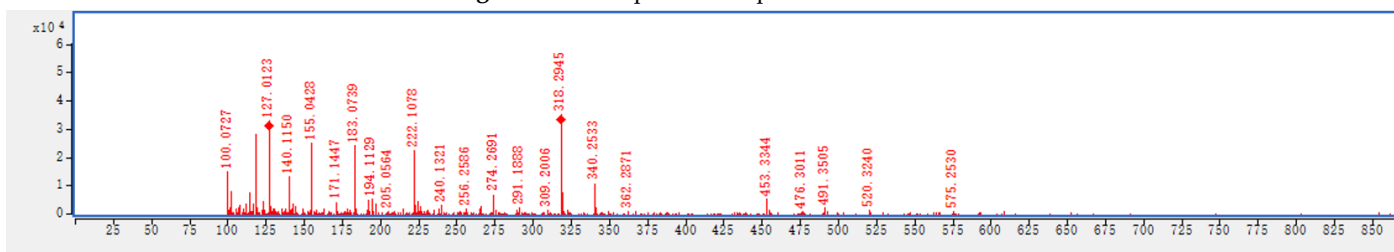


Figure S78. MS/MS spectrum of peak 37.

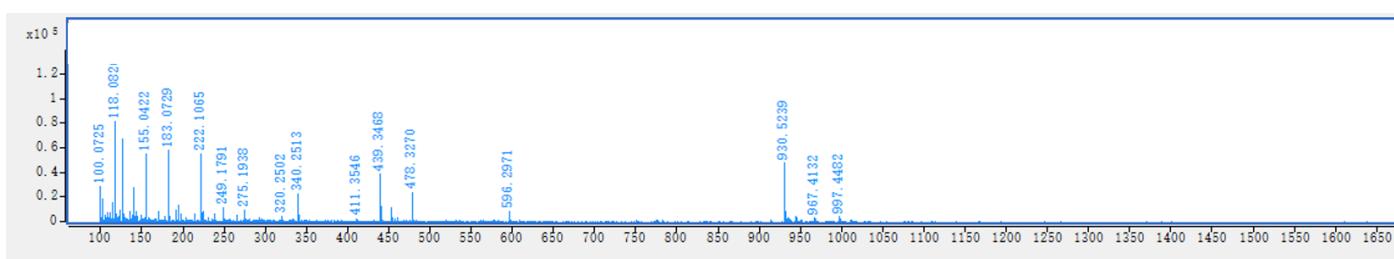


Figure S79. MS spectrum of peak 38.

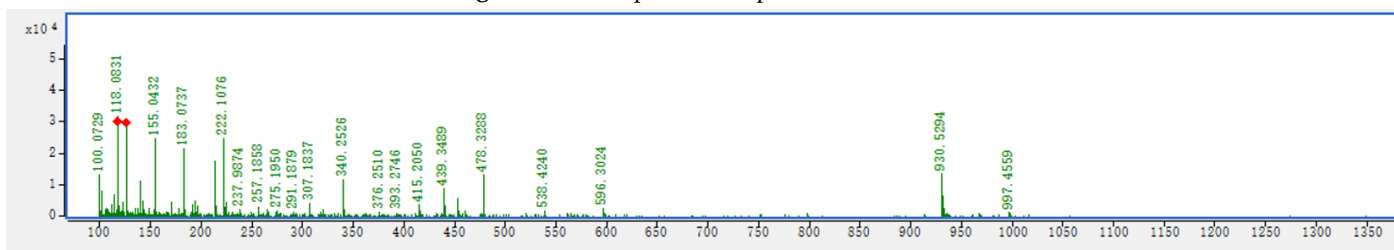


Figure S80. MS/MS spectrum of peak 38.

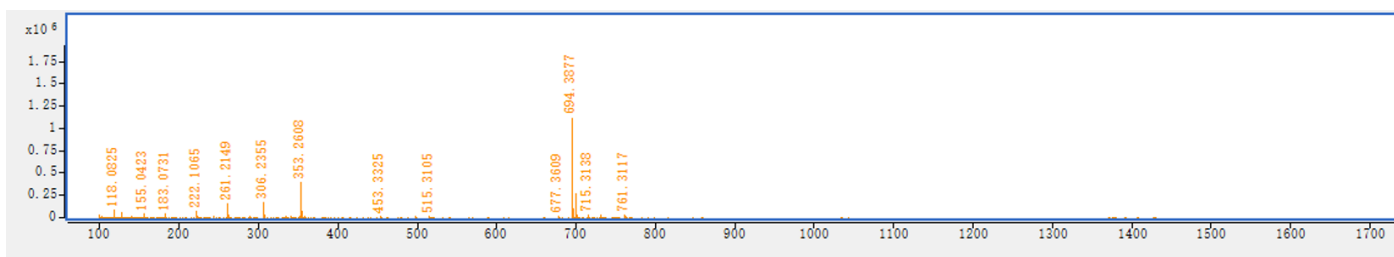


Figure S81. MS spectrum of peak 39.

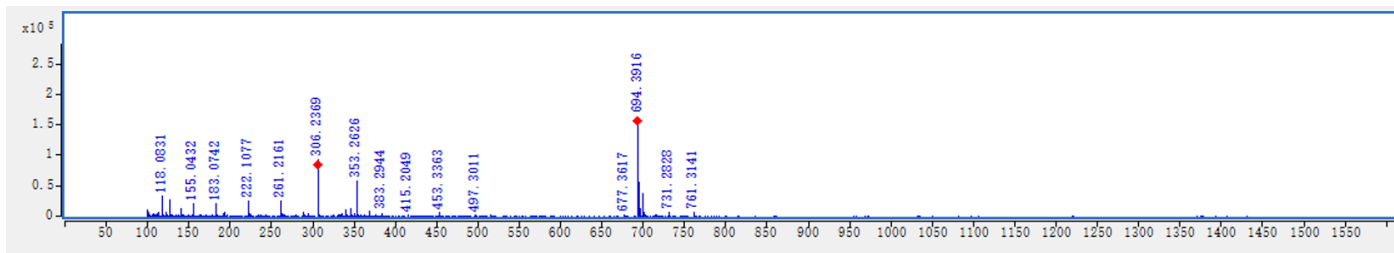


Figure S82. MS/MS spectrum of peak 39.

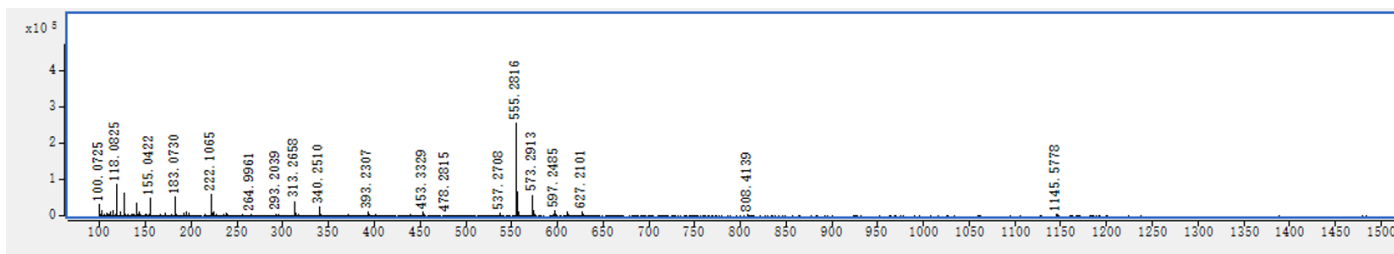


Figure S83. MS spectrum of peak 40.

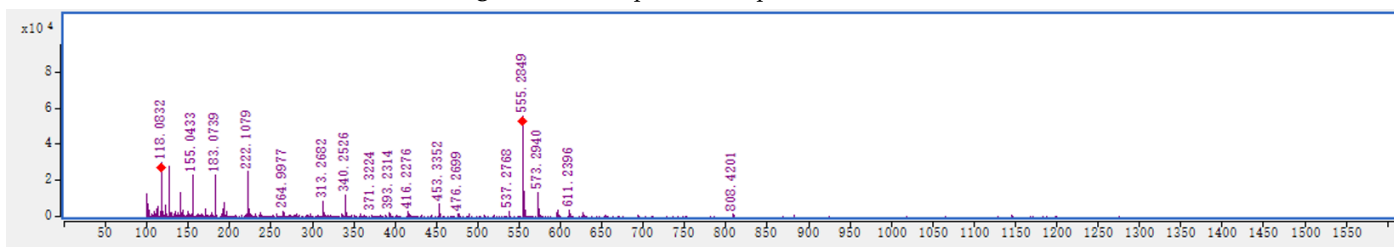


Figure S84. MS/MS spectrum of peak 40.

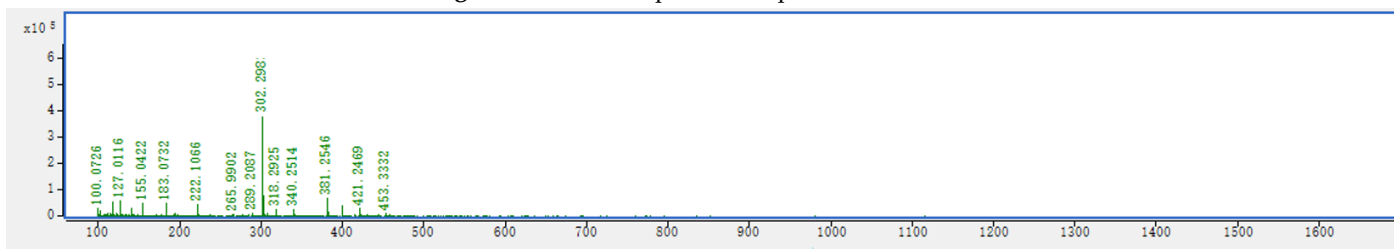


Figure S85. MS spectrum of peak 41.

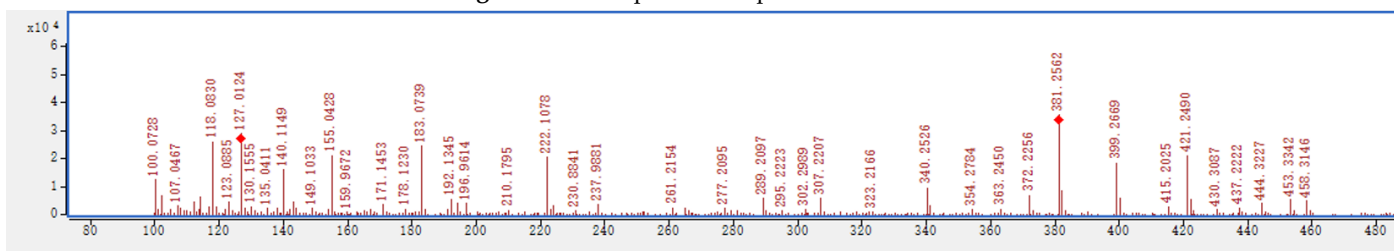


Figure S86. MS/MS spectrum of peak 41.

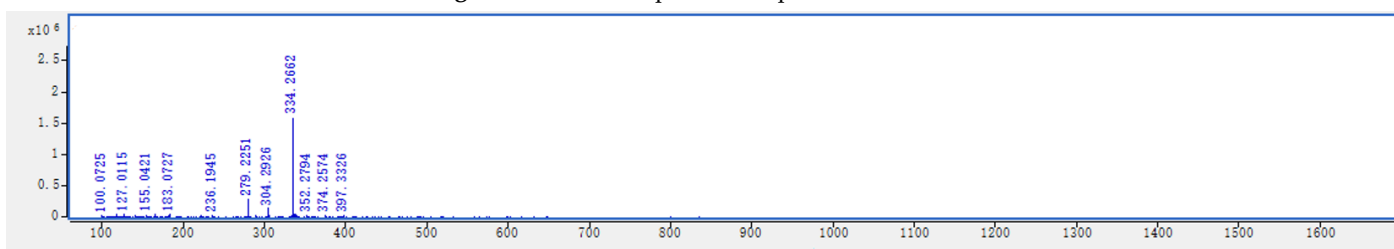


Figure S87. MS spectrum of peak 42.

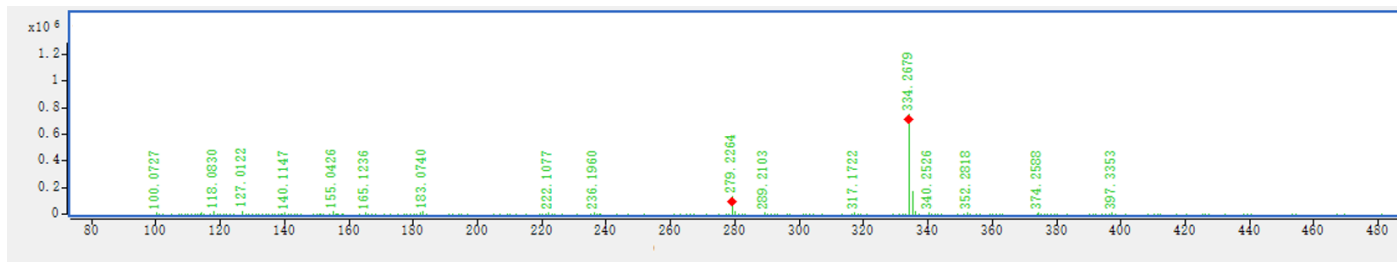


Figure S88. MS/MS spectrum of peak 42.

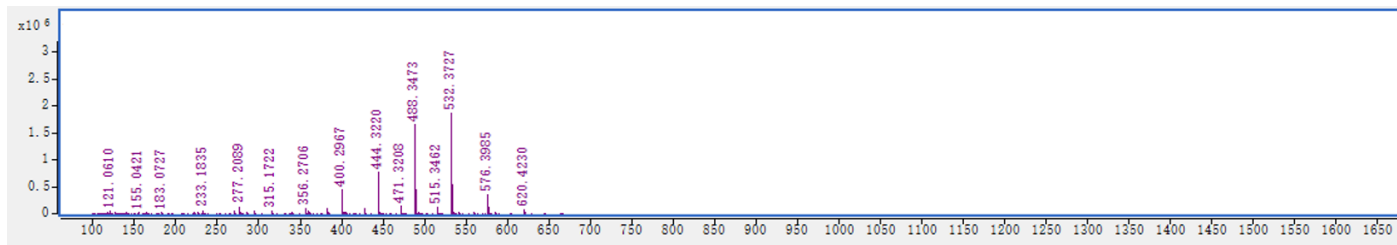


Figure S89. MS spectrum of peak 43.

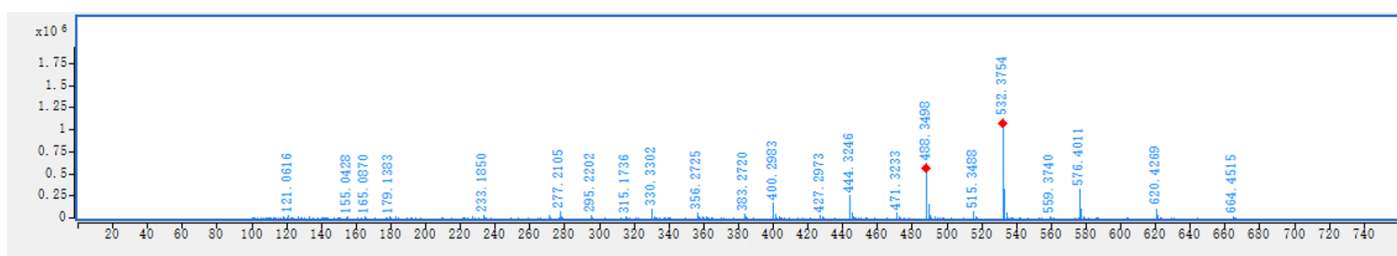


Figure S90. MS/MS spectrum of peak 43.

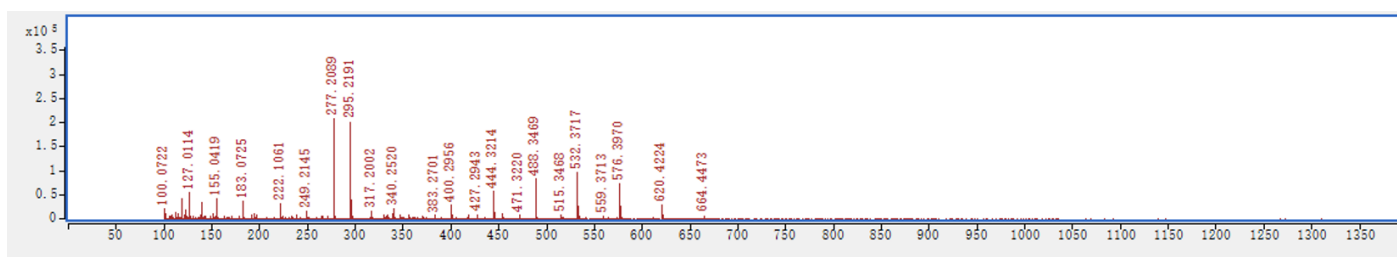


Figure S91. MS spectrum of peak 44.

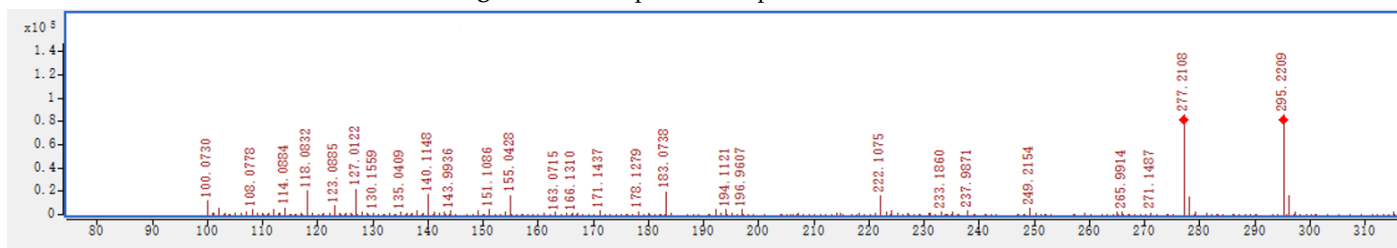


Figure S92. MS/MS spectrum of peak 44.