MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN

Faculty of Chemical and Biopharmaceutical Technologies

Department of Pharmaceutical Industry

# *Master's thesis*

onthetopic TECHNOLOGY OF ISOLATION, IDENTIFICATION AND RESEARCH OF ANTIOXIDANT ACTIVITY OF POLYPHENOLS FROM PINGYIN ROSAE RUGOSAE FLOS

Completed:student of the group MPhch-20 of the

specialty 226 Pharmacy, industrial pharmacy

Gao Jie

Supervisor Roman Kachan; Wang Xiao

Reviewer _____

Kyiv 2021

KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN
Institute, faculty Chemical and Biopharmaceutical Technologies
Department Pharmaceutical Industry
Specialty 226 Pharmacy, industrial pharmacy

**APPROVE**
HeadofDepartment Industrial Pharmacy,
Professor, Doctor of Pharmaceutical Science
Vladyslav STRASHNYI
"14" December 2021

# ASSIGNMENTS

# FOR THE MASTER'S THESIS

Gao Jie

**1. Thesis topic** Technology of isolation, identification and research of antioxidant activity of polyphenols from Pingyin Rosae Rugosae Flos

**2. Scientific supervisor** Roman Kachan**,** Associate professor , Doctor; Wang Xiao, Professor, Doctor

approved by the order of the higher educational institution on 4th October 2021, N286

**3. Deadline for student submission of work** 14th December 2021

**4. Initial data to work** scientific and information sources, methodological and technological literature, scientific periodicals, international and domestic regulations and standards for the development and production of medicines.

**5. Content of the thesis (list of questions to be developed)** Research progress on rose, dandelion and plant polyphenols; optimization of polyphenol extraction technology; rose polyphenol isolation technology and identification; dandelion polyphenol isolation technology and identification; research on antioxidant activity of rose and dandelion polyphenols.

## 6.Consultants of the master's thesis sections

| Section | Surname, initials and position of the consultant | Signature | |
|---|---|---|---|
| | | the task was issued | the task accepted |
| Section 1 | Roman Kachan, Associate professor, Doctor | | |
| Section 2 | Wang Xiao, Professor, Doctor | | |
| Section 3 | Roman Kachan, Associate professor, Doctor | | |

## 7. Date of issuance of the assignments EXECUTION SCHEDULE

| No | The name of the stages of the master's thesis | Terms of performance of stages | Note on performance |
|---|---|---|---|
| 1 | Introduction | 20.09 –27.09.2021 | |
| 2 | Section 1<br>Theoretical | 28.09. – 11.10.2021 | |
| 3 | Section 2<br>Materials and Methods | 12.10 – 25.10.2021 | |
| 4 | Section 3<br>Results and Analysis | 26.10 - 08.11.2021 | |
| 5 | Conclusions | 09.11.-15.11.2021 | |
| 6 | Draw up a master's thesis<br>*(final version)* | 16.11.-04.12.2021 | |
| 7 | Submission of master's thesis to the department for review *(14 days before the defense)* | 06.12.-14.12.2021 | |

| | | | |
|---|---|---|---|
| 8 | Checking the master's thesis for signs of plagiarism *(10 days before the defense)* | 14.12-18.12.2021 | |
| 9 | Submission of master's thesis to the master's department to check the implementation of the appendix to the individual curriculum *(10 days before the defense)* | 16.12-18.12.2021 | |
| 10 | Submission of master's thesis for approval by the head of the department (*from 7 days before the defense*) | 18.12-21.12.2021 | |

Student_____

Scientificsupervisor_____

Head of the scientific and methodological center

for the management of specialist training_____

## Abstract

Master's Thesis in the specialty 226 - Pharmacy, Industrial Pharmacy. - Kyiv National University of Technologies and Design, Kyiv, 2021.

*Rosa rugosa* is a kind of Chinese medicine for both medicine and food, which has high ornamental value. Rose contains a variety of active ingredients, except the most famous volatile oil, polyphenols are also one of the main active ingredients. Firstly, the optimal extraction conditions of rose polyphenols were determined through single factor experiment and $L_9$ ($3^4$) orthogonal experiment. The result was that the ethanol concentration was 60%, the material-to-liquid ratio was 1:25 (w/v), the ultrasonic power was 150W, and the ultrasonic time was 60min. Under these conditions, the extraction rate of rose polyphenols extracted by auxiliary reflux method was (3.21±0.02)%. Secondly, rose polyphenols were screened for the most suitable macroporous resin AB-8 for purification. 30% and 70% of the elution fractions were collected as R-30 and R-70, respectively. R-30 and R-70 were tested in vitro anti-oxidant capacity by DPPH, ABTS, FRAP method. The results showed that the antioxidant capacity of R-30 was higher than that of R-70. At the same time, the total rose sample R was identified by UHPLC-Q-TOF-MS. Finally, a total of 10 polyphenolic compounds were identified. They were Quinic acid, Gallic acid, Di-O-galloyl-glucoside, Bis-HHDP-glucoside, Catechin, Di-O-galloyl-HHDPglucoside, Di-O-galloyl- HHDPglucoside, Galloyl-HHDP-glucoside, Ellagic acid, Kaermpferol-3-O-rutinoside.

In order to further study the separation and biological activity of plant polyphenols, *Taraxaci Herba* was selected as a material, which is a kind of Chinese medicine for both medicine and food. The best extraction method was used to extract dandelion, and the dandelion was separated and purified by a combination of polyamide atmospheric pressure column and chromatography. A total of 9 compound monomers were obtained and 8 of them were identified by MS and NMR. Finally, 4 monomer compounds were selected for in vitro antioxidant activity research. The results show that the four compounds have good in vitro antioxidant activity. The order of antioxidant capacity was protocatechuic acid (V)> caffeic acid (I)> luteolin (VIII)> chlorogenic acid (VII).

Key words: *Rosa rugosa;* polyphenols; isolation and purification; in vitro antioxidation; *Taraxaci Herba*

## Анотація

Магістерська робота за спеціальністю 226 – фармація, промислова фармація. – Київський національний університет технологій та дизайну, Київ, 2021.

Rosarugosa — це різновид китайської медицини як для медицини, так і для їжі, яка має високу декоративну цінність. Троянда містить безліч активних інгредієнтів, крім найвідомішої ефірної олії, поліфеноли також є одними з основних активних інгредієнтів. По-перше, оптимальні умови екстракції поліфенолів троянд були визначені за допомогою однофакторного експерименту та $L_9$ ($3^4$) ортогонального експерименту. Результатом стало те, що концентрація етанолу становила 60%, відношення матеріалу до рідини становило 1:25 (вага/об'єм), потужність ультразвуку становила 150 Вт, а час ультразвуку – 60 хвилин. За цих умов швидкість екстракції поліфенолівтроянди, екстрагованих допоміжним методом дефлегмації, становила (3,21±0,02)%. По-друге, поліфенолитроянди були перевірені на найбільш придатну для очищення макропористу смолу AB-8. 30% і 70% фракцій елюції збирали як R-30 і R-70, відповідно. R-30 та R-70 тестували invitro антиоксидантну здатність методом DPPH, ABTS, FRAP. Результати показали, що антиоксидантна здатність R-30 була вищою, ніж у R-70. У той же час загальний зразок троянди R був ідентифікований за допомогою UHPLC-Q-TOF-MS. Нарешті було ідентифіковано 10 поліфенольнихсполук. Це були хінова кислота, галова кислота, Di-O-галлоіл-глюкозид, біс-HHDP-

глюкозид, катехін, Di-O-галлоіл-HHDP-глюкозид, Di-O-галлоіл-HHDP-глюкозид, галлоіл-HDP-глюкозид, еллагова кислота, Kaermpferol -3-O-рутинозид.

З метою подальшого вивчення поділу та біологічної активності рослинних поліфенолів, TaraxaciHerba був обраний як матеріал, який є різновидом китайської медицини як для медицини, так і для їжі. Для вилучення кульбаби використовували найкращий метод екстракції, кульбабу відокремили та очистили комбінацією поліамідної колонки з атмосферним тиском та хроматографією. Всього було отримано 9 з'єднаних мономерів і 8 з них ідентифіковано за допомогою МС та ЯМР. Нарешті, для дослідження антиоксидантної активності invitro було відібрано 4 мономерні сполуки. Результати показують, що ці чотири сполуки мають гарну антиоксидантну активність invitro. Порядок антиоксидантної здатності був протокатехової кислоти (V)> кавова кислота (I)>лютеолін (VIII)>хлорогенова кислота (VII).

Ключові слова: Rosarugosa; поліфеноли; ізоляція та очищення; антиоксидантне окислення invitro; TaraxaciHerba

**CONTENT**

## Introduction

*Rosa rugosa* Thunb. Is a perennial deciduous shrub, belonging to *Rosa* of *Rosaceae* [1]. Rose is native to China, but because of its high practical and medicinal value, it is widely planted all over the world, mainly in France, Russia, Bulgaria and other countries. The main planting areas of roses in China are Shandong, Gansu, Yunnan and other places [2]. Rose not only can be used for gardening and viewing because of its gorgeous flowers, but also has a long history in China as one of the important plants with the same medicine and food. The medicinal value of roses is recorded in Chinese ancient books such as "Materia Medica", "Materia Medica Supplements" and "Food Materia Medica"[3]. Roses contain volatile oil, polyphenols, alkaloids, vitamins, amino acids, soluble sugars, minerals and other active components and nutrients[4]. Current research shows that rose has the functions of reducing and eliminating free radicals, antioxidation, antithrombotic, anticancer, anti-inflammatory, antibacterial, immune regulation, reducing blood lipid and preventing heart disease[5]. Thus, in addition to the outstanding ornamental and edible value, the medicinal value of roses can not be ignored.

*Taraxaci Herba*, a traditional Chinese medicine, is a dry whole plant of Taraxacum mongolicum Hand.-Mazz., Taraxacum sinicum Kitag. or several plants of the same genus[6]. Dandelion is distributed all over the world and mostly in the northern hemisphere. It has strong adaptability and strong tolerance to harsh environments in nature, so it grows on hillsides, grasslands and roadsides[7]. It is

not only an edible mountain vegetable with high nutritional value, but also a Chinese herbal medicine with high medicinal value. The medicinal value of dandelion has been recorded in ancient Chinese books such as "Tang Materia Medica", "Compendium of Materia Medica", and "Ben Sketch Classic" [8]. Up to now, domestic and foreign research scholars have isolated more than 50 compounds from dandelion, including polyphenols, polysaccharides, coumarins, terpenes, sterols, and volatile oils. Many studies have shown that the traditional Chinese medicine dandelion mainly has pharmacological effects such as protecting liver and choleretics, anti-inflammatory, anti-bacterial, anti-fatigue, anti-tumor, anti-cancer, anti-mutation, lowering blood sugar, anti-oxidation, promoting gastrointestinal function and enhancing immunity [9] . It can be seen that dandelion has huge resource reserves, high medicinal value, and great development and utilization value.

Plant polyphenol is one of the main active components which is a secondary metabolite of complex phenols in plants. A large number of experiments had proved that polyphenols have good antioxidant[10], anticancer[11], antiviral[12], bacteriostatic and anti-inflammatory[13], and alleviating cardiovascular and cerebrovascular diseases[14]. Therefore, many Chinese herbal extracts containing phenolic acids were also widely used in medicine and functional foods. For example, chlorogenic acid in honeysuckle was the main active component of ShuangHuangLian injection that played an antibacterial role[15]. However, polyphenols have similar structure, complex components , so it is difficult to

separate and prepare them.

In order to further study the pharmacological activities of plant polyphenols, separation and preparation are necessary. Rose and dandelion are rich in polyphenols, but there are few reports on the separation of their polyphenols. Therefore, this study used rose and dandelion as materials to study the separation of polyphenols and test their antioxidant activity. Provide technical support for the pharmacological activity and quality standard research of polyphenol compounds.

# Section 1

## 1.1 Overview of rose

As a common medicinal and edible homologous plant, rose is widely used in life. Its bioactive components are very complex. At present, there are about 300 kinds of chemical components known. Its main bioactive components are volatile oil, flavonoids, polyphenols, polysaccharides and so on.

### 1.1.1 Volatile oil

Volatile oil generally refers to essential oil components, which is the general name of volatile aromatic substances extracted from spice plants or fragrant secreting animals. Rose essential oil is a kind of aromatic volatile substance extracted and separated from edible roses, which has the characteristics of non-toxic, safe and unique fragrance [16]. Rose essential oil smell fragrant and sweet. It is known as "liquid gold" because of its small extraction amount and extremely expensive price. It is often widely used in food additives, intermediate cosmetics, drug care and so on [17]. Rose essential oil can activate male hormones and sperm, alleviate female dysmenorrhea, and treat gynecological diseases, cardiovascular and cerebrovascular diseases. In daily life, it can also be used to calm emotions, boost mood, relieve nervous tension and pressure, and has powerful beauty and health care functions [18].

The components of rose volatile oil are very complex, mainly composed of volatile alcohols, aldehydes and terpenes. The identification of aroma components is the focus of rose volatile oil research. Li Fei et al. [19] used bitter water rose as

raw material, optimized the process of preparing rose purified oil by subcritical butane extraction through three factor and three-level orthogonal test, analyzed it by GC-MS and matched it with the computer standard spectrum library, and preliminarily identified 47 compounds, accounting for 90.48% of the total peak area, of which the main aroma characteristic components are benzyl alcohol, eugenol, 2-tridecanone β- Phenylethanol, citronellol, geraniol and so on, and they are considered to be the key aroma compounds leading to the aroma difference of different rose purified oils. Yao Chenyang et al. [20] identified and analyzed the volatile components of petals in full bloom of five rose varieties: single petal white rose, double petal red rose, Fenghua rose, Purple Branch rose and Japanese four seasons rose. A total of 49 volatile components were detected and identified by headspace solid phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC-MS), belonging to alcohols, esters, aldehydes, terpenes, phenols, ketones, alkanes, acids and ethers. At the same time, the main volatile substances identified include phenylethanol, citronellol, Neroli alcohol, geraniol, Citronellyl acetate, geranyl acetate, geranal, Neroli aldehyde α– Farnesene, etc. Zhao Guojian et al. [21] used molecular distillation technology to separate the rose essential oil of 'Fenghua No. 1' in Pingyin County, and systematically studied the components after three-stage distillation. After GC-MS analysis, 29 compounds were determined, mainly including citronellol, geraniol, linalool, Neroli alcohol, phenylethanol, methyl eugenol, citronella acetate and farnesene. Zhang Fengmei [22] and others analyzed Bulgarian rose essential oil, Turkish rose essential oil,

Moroccan Rose essential oil, Gansu rose essential oil in China and caravan Moroccan Rose essential oil samples by HS-SPME-GC - MS. A total of 191 aroma components were detected from five rose essential oil samples, including 52 alcohols, 42 olefins, 39 esters 15 aldehydes, 15 alkanes, 8 ketones and 20 other components; The main aroma components are alcohols. Zheng Xiaoga et al. [23] used gc-ims to detect 54 monomers of volatile substances and dimers of some substances in three kinds of roses: double petal rose, Damascus rose and glass rose in Pingyin County, in which monomers mainly include alcohols, esters, ketones, aldehydes, olefins, acids and ethers. After comparing the volatile components of different roses, it was found that the essential oil of each rose had characteristic flavor compounds different from other varieties.

According to the literature, although the main volatile components of rose essential oil are alcohols, esters, terpenes and aldehydes, there are some differences in the composition of volatile substances among different varieties of roses. The type and content of volatile components in roses are affected by the variety, origin and flowering period of roses. There is a certain relationship between their sensory quality and the type and proportion of volatile components; Moreover, different varieties of rose essential oils have their unique characteristic volatile components, which can be used to quickly distinguish and identify different rose varieties.

### 1.1.2 Polyphenols

Rose polyphenols are another kind of active components of roses. They are not

only the basis of pharmacodynamic substances of roses, but also one of the hotspots of rose research. Zhang LIANLI et al. [24] extracted three main edible roses (Kushui, Phnom Penh and Pingyin) with water and methanol respectively. Through comparison, it was concluded that the content of total flavonoids in rose tea soup was higher than that in methanol extract and the antioxidant activity was different. A total of 9 flavonoids were identified from methanol extract by High performance liquid chromatography tandem quadrupole time of flight mass spectrometry (HPLC-Q-TOF-MS). Jinjing [25] used methanol: water: formic acid: trifluoroacetic acid (70:27:2:1) to extract 22 kinds of rose freeze-dried flower powder of 4 kinds of roses (Chinese rose, Mahalanobis rose, louver rose and French Rose), and detected 7 kinds of 35 flavonoids by UPLC-DAD-Triple-TOFMS method. Zhang Ling [26] extracted the dried rose samples of 'White Purple Branch', 'Pink Purple Branch' and 'Purple Branch' with 11% acetic acid methanol, and identified 13, 10 and 18 flavonoids respectively. Among them, paeoniflorin in 'Purple Branch' rose is more than 80%, followed by delphinidin 10%, Sagittarius chamomile 5% and Geranium 0.3%. Xiao et al.[27] Purified two flavonoids with macroporous adsorption resin, quercetin and kaempferol flavonoids. Wu Mengqi et al.[28] Preliminarily separated the rose residue extracted by reflux with 75% ethanol through macroporous adsorption resin HP20, and further purified by high-speed countercurrent chromatography to obtain ellagic acid with a purity of 96.2%. Zhang juanmei [29] extracted and prepared the polyphenols from rose dregs with macroporous resin through ultrasonic

assisted extraction. At the same time, the components of the obtained rose polyphenols were analyzed by HPLC-MS / MS. A total of 23 compounds were identified, including 6 hexosides of gallic acid, 6 glycosides of hexahydroxybiphenyl dicarbonyl (HHDP) and 7 glycosides of quercetin, Two glycosides of kaempferol, quercetin and sage phenol.。Li Sainan [30] extracted roses with 60% ethanol solution and purified them with D101 macroporous resin. 13 compounds were isolated from rose bold by HPCCC, of which 6 were single products with high purity. Gao Jianing et al. [31] estimated the polyphenol content of dry roses from different varieties and producing areas by HPLC and UV Spectrophotometry with catechin, gallic acid, quercetin, total proanthocyanidins and total tannic acid as the standard control, and measured that the polyphenol content of different Roses was significantly different. Zheng [32] et al. Determined the contents of caffeic acid, apigenin, p-coumaric acid and chlorogenic acid in roses by high performance liquid chromatography combined with diode array and electrochemical detector, and determined that the phenols in roses include gallic acid, vanillic acid, caffeic acid, coumaric acid, etc. Chen Dongming [33] measured that the extraction amount of rose polyphenols under the optimal conditions was 2.732mg/g. Baydar et al. [34] took gallic acid as the standard, measured that the total amount of phenolic compounds in the methanol extract of fresh rose was 344.45 ± 10.52 mg / g, and identified that it contained gallic acid and quercetin.

### 1.1.3 Polysaccharides

Polysaccharide is a natural polymer compound with large molecular weight,

complex structure and special function. Polysaccharide compounds are widely distributed in plants and have many biological activities, such as antiviral and immune regulation.

Rose polysaccharide is also one of the important active components of rose, with antioxidant, moisturizing and other biological activities [35]. Ma Menghua [36] et al. Deproteinized by Sevage method, removed the pigment by repeated washing with high concentration ethanol, and determined by phenol sulfuric acid method that the sugar content in Rose residue is as high as 29.5%, including soluble sugar of 8.8%. Chen Dongming [33] took the remaining rose residue after extracting essential oil as raw material and extracted rose polysaccharide by ultrasonic method. It was measured that the extraction amount of rose polysaccharide under the optimal conditions was 4.126mg/g. Liang Qichao et al. [37] optimized the extraction process of rose polysaccharide by response surface methodology. Under the optimal conditions, the yield of rose polysaccharide was 1.46%. Wu et al. [38] detected and isolated an immunomodulatory polysaccharide compound wsrp-1b with a molecular weight of 1.11 from the distillation waste of bitter water rose × 104 Da, composed of glucose (42.6%), mannose (21.4%), arabinose (9.9%), xylose (2.2%) and galactose (23.9%).

Although the content of rose polysaccharide is not high, it can be extracted from rose residue extracted from rose essential oil, which has high research value for the reuse of by-products extracted from rose essential oil.

### 1.1.4 Other components

In addition to the above active ingredients, roses also contain amino acids and minerals. Wei Qin [39] et al. Analyzed the nutritional components of rose residue and the chemical components of wastewater. The results showed that rose residue contained nutrients such as sugar, amino acids and mineral elements (iron, calcium and manganese). The total amount of sugar was 29.5%, the total amount of soluble sugar was 8.8%, the total amount of amino acids was 10.205%, and the content of essential amino acids for human body was 3.853%. Ma Xueyi and Chen Yaozu [40] analyzed the amino acids and trace elements in Rose dregs in Kushui, Gansu Province. The results showed that rose dregs contained 19 trace elements and 17 amino acids. The contents of calcium and phosphorus in trace elements were high, and there were 7 kinds of essential amino acids in amino acids.

## 1.2 Overview of dandelion

Dandelion has a wide range of uses, both edible and medicinal. It is a Chinese medicinal resource with the same medicine and food, and it is also a wild vegetable resource with a promising development and utilization[41]. Dandelion roots, leaves and flowers contain very rich active ingredients, which have high medicinal value. The biologically active ingredients contained in it are extremely complex. There were currently more than 50 active ingredients known. Its main biologically active ingredients include polyphenols, polysaccharides, coumarins, terpenes, sterols, and volatile oils.

### 1.2.1 Polyphenols

Dandelion polyphenol is one of the main active components of dandelion, and

it is one of the hotspots of dandelion research. Lingyun[42] first extracted dandelion with 95% ethanol with petroleum ether, ethyl acetate and n-butanol, and then separated it with silica gel H by low-pressure column chromatography, obtaining a total of 4 polyphenols. Shi Shuyun[43-44] used Inner Mongolia dandelion as raw material, separated the chemical components by chromatography, and identified its structure by spectroscopy. A total of 16 flavonoid derivatives and 15 phenolic acid compounds were separated and identified. In addition, an on-line rapid screening method, high-performance liquid chromatography–diode array detection–radical-scavenging detection–electrospray ionization mass spectrometry (HPLC–DAD–RSD–ESI-MS) system, had been developed for the separation and identification of radical scavengers from the methanolic extract of T. mongolicum. Peng Deqian[45] extracted the ethanol extract of Mongolian dandelion root with n-hexane, ethyl acetate and n-butanol in sequence, and then used silica gel column chromatography and high performance liquid chromatography for separation and purification. The structure was identified based on physical and chemical properties and spectral data analysis. Results A total of 16 monomer compounds were isolated from the Mongolian Dandelion root extract, 9 of which were polyphenols. Yadava [46] isolated a new flavonoid glycoside from the stem of dandelion, it was characterized as 3,5,7,3',4'-pentahydroxy 8-C methyl flavones 7-O-β-D-xylopyranose (14)O-β-D glucopyranosyl 3'- O-α-L-rhamnopyranoside by various chemical degradations and spectral analysis. Kenny O et al.[47] used LC-SPE-NMR method to isolate and identify three phenolic acids,

vanillin, conifer aldehyde, and *p*-methoxyphenylglyoxylic acid from crude extracts of Dandelion root, and reported their antibacterial activity for the first time. Wu Zhe et al.[48] optimized the extraction process of caffeic acid, chlorogenic acid and total phenolic acid in dandelion by using methanol as the extractant, assisted extraction by ultrasonic and enzymatic hydrolysis, using response surface design method, combined with fingerprint comprehensive quality evaluation method . The results showed that the optimal extraction conditions were 0.1% cellulase, pH 4, liquid-to-solid ratio 300:1 (mL:g), methanol volume fraction 40%, and ultrasonic extraction time 120 min. Xu Shulai[49] studied the effects of ultrasonic-assisted extraction and microwave-assisted extraction on the yield of dandelion flavonoids, using spectrophotometry and high performance liquid chromatography (HPLC) to determine the yield of total flavonoids and the composition of flavonoids in dandelion. By orthogonal experiment method, it was concluded that the best process of ultrasonic assisted extraction was ethanol concentration of 60%, material-to-liquid ratio of 1:80 g/mL, extraction time of 35 min, and extraction temperature of 60°C. Under these conditions, the yield of dandelion flavonoids was 3.97%. The optimal process conditions for microwave-assisted extraction were ethanol concentration of 60%, material-liquid ratio of 1:80 g/mL, extraction time of 10 min, extraction temperature of 55°C, and microwave power of 800 W. Under these conditions, the yield of dandelion flavonoids was 4.57%. And think that the method of microwave-assisted extraction was better.

### 1.2.2 Terpenes

Terpenoids are a class of compounds derived from methylglutaric acid, and the basic carbon framework mostly has 2 or more isoprene units ($C_5$) with structural characteristics. In addition to their existence in the form of terpene hydrocarbons, a large number is the formation of various oxygen-containing derivatives, including alcohols, aldehydes, ketones, carboxylic acids, esters and glycosides. Secondly, there are nitrogen-containing derivatives, and a few sulfur-containing derivatives exist. According to the number of isoprene units included in the molecule, terpenes can be divided into monoterpenes, sesquiterpenes, diterpenes, dipterpenes, triterpenes, tetraterpenes, and polyterpenes. All compounds derived from the polymerization of isoprene and whose molecular formula conforms to $(C_5H_8)_n$ are generally called terpenoids. Among them, monoterpenoids and sesquiterpenoids are called "low terpenoids", most of which are volatile and often exist in higher plants in the form of volatile oils. Compounds above diterpenoids are called "high terpenoids", which are generally not volatile, and generally exist in plants in the form of resins[50]. Most plants continuously synthesize various terpenoids during the growth and development stages. Among them, sesquiterpenoids are the largest category of terpenoids, with about 7000 species[51].

As early as 1938, British scholars separated taraxerol, φ-taraxasterol, taraxasterol and β-amyrin from five ring triterpenoids from dandelion. Katrin Schutz also reported such compounds and isolated arnidiol from dandelion

flowers,and faradiol [52]. Ageta H used silica gel chromatography to separate two new triterpenoids from the n-hexane extract of T. Japaoniucm Kodiz dandelion roots, namely neo-lupenol and tarolupenol and the ethanol esters of these alcohols [53]. Ling Yun et al. [54] separated two triterpenoids from the petroleum ether extract of Taraxacum sinicum, namely φ-taraxasteryl palmitate and φ-taraxasteryl acetate, both of which were isolated from this plant for the first time. Wu Yanling et al. [55] isolated the triterpenoid oleanic acid from dandelion, and confirmed that the compound can promote the gastrointestinal motility in mice through the mouse gastrointestinal motility test. Jiang Xing et al. [56] used chromatographic technology to isolate 8 eucalyptane-type sesquiterpenoids from 95% ethanol extract of dandelion for the first time, namely plebeiolide A, plebeiafuran, and 9β-acetoxy-1β-hydroperoxy-3β ,4β-dihydroxygermacra-5,10(14)-diene,4-O-acetyl-3-O-(3'-acetoxy-2'-hydroxy-2'-methylbutyryl)-cuauhtemone,chlorantholide A,chlorantholide C,daucucarotol And 1α,6β-dihydroxy-cis-eudesm-3-ene-6-O-β-D-glucopyranoside. And they verified that some of the compounds have potential anti-inflammatory activity.

### 1.2.3 Phytosterols

Phytosterols are steroid derivatives whose $C_{17}$ side chain of the steroidal nucleus is a chain-like side chain of 8-10 carbon atoms. It is widely distributed in the plant kingdom and exists in almost all plants who is an important component of plant cells. In plants, it mostly exists in a free state, and it often coexists with oils in plant seeds or pollen. It also exists in the form of glycosides or higher fatty

acid esters with sugars. Sterol can promote metabolism and anti-tumor, so it has been studied by people in recent years.

Ling Yun et al. [42] first extracted the dandelion extracted with 95% ethanol with petroleum ether, ethyl acetate and n-butanol, and then separated it with silica gel H by low-pressure column chromatography, and finally obtained β-sitosterol, st igmasterol and daucosterol 3 sterol compounds. Dong Changying et al. [57] used reflux extraction method to extract dandelion sterol, the extraction rate was 8.761%, and recrystallization method was used to purify the sterol, and the content of dandelion sterol was 0.62%. Secondly, in vivo experiments proved that when dandelion sterol was above 0.150 g/ml, it had good anti-inflammatory effect on mouse ear swelling model, and it had anti-inflammatory effect on mouse hind limb toe swelling model. In addition, British scholars studied medicinal dandelion and learned that sitosterol is the most abundant in monomeric sterols, followed by stigmastrol, and campestrol. Except for stigmasterol and campesterol, most other sterols exist in the form of esters, and less forming glycosides[58]. In 1983 a study by American scholars found that dandelion pollen contains relatively large amounts of 5α-stigmast-7-en-3β-ol and campesta-7, 2,4 (28) -dien-3β-ol, and a small amount of sitosterol, iso-fucosterol, 2,4-methylenecholesterol [59].

### 1.2.4 Volatile oils

In dandelion, volatile oil is also one of its active ingredients, which has the effects of anti-inflammatory, anti-allergic, deworming, and regulating the central nervous system.

Hook et al.[60]conducted tissue culture of T. officinale, collected the volatile products of apple flavor in the air above the culture, and used GC-MS for detection, indicated the presence of acetic acid butyl ester,2-methyl-I-propanol, n-butyl alcohol, 4-phenyl-1-butanol, 4-hydroxy-4-methyl-2-pentanone, acetic acid, 4-terpineol,fl-terpineol and z-terpineol. Lingyun [61] used gas chromatography-mass spectrometry technology to analyze the components of dandelion volatile oil, and successfully separated 19 components and identified 15 of them at the same time. And the results show that the main component of dandelion volatile oil is 2-furaldehyde. Lingyun [62] also pointed out in the report that using GC to compare the volatile oil components in the flowers, leaves, and roots of Japanese dandelion T. japonicum and Taraxacum leucanthum T. albidum Dahlst, the results show that the composition of the volatile oil is basically the same, accounting for 95% of the volatile oil. It include 36 kinds of alcohols and phenolic compounds, 14 kinds of aldehydes and ketones, 7 kinds of esters and ethers, 46 kinds of alkane and 14 kinds of organic acids. Also they found the content of 6,10,14-trimethyl-2-pentadecanone in Japanese dandelion is higher than that in white flower dandelion. Yang Chao et al. [63] used supercritical fluid CO2 extraction method to extract dandelion volatile oil, analyzed the composition of the volatile oil by GC-MS combined instrument, supercritical fluid CO2 extraction method extracted dandelion volatile oil, the extraction rate was 5.55%; GC- MS combined analysis identified 26 dandelion volatile oil components, accounting for 83.72% of the total volatile oil components. The main components were linolenic acid (51.70%),

palmitic acid (18.41%), oleic acid (2.75%), and ethyl palmitate (1.94%). , Behenic acid (1.40%), Nonadecanic acid (1.23%), etc. Among them, oleic acid and ethyl palmitate were obtained from dandelion volatile oil for the first time. And they used animal and cell experiments to prove that dandelion volatile oil has obvious anti-inflammatory activity and anti-breast cancer effect.

### 1.2.5 Other ingredients

In addition to the above active ingredients, dandelion also contains pigments, polysaccharides, minerals and other ingredients. Roberfroid[64] proved through experiments that dandelion root contains a large amount of polysaccharide inulin, which has multiple effects, such as eliminating gastrointestinal pathogens, inhibiting obesity, cancer and osteoporosis. Guo Huijing [65] used Xinjiang wild dandelion as the main raw material to extract and separate the polysaccharides from the whole plant. Under the optimal extraction conditions, the polysaccharide yield was 14.27% and purified. Kleinig et al. [66] found that dandelion petals contain cryptoxanthin and lutein and other epoxides (zeaxanthin), antheraxanthin, violaxanthin, and neoxanthin. Most of these compounds form mono- or di-esters with some common saturated fatty acids. Wu Xiaochun [67] reported that there are 66 kinds of trace elements in dandelion, among which 12 kinds of elements are relatively high, including Cu, Zn, Fe, Mn, Mo, these 5 essential trace elements. In addition, dandelion also contains a lot of Na, K, Ca, as well as $V_C$, $VB_1$, $VB_2$ and so on.

## 1.3 Overview of the research on polyphenols

### 1.3.1 Overview of polyphenols

Plant polyphenols, also known as plant tannins, are complex phenolic secondary metabolites in plants. They have a polyphenol structure and are mainly found in the bark, roots, leaves, shells and pulp of plants[68]. Plant polyphenols exist in many plants, and the number of phenolic compounds and their derivatives contained in the entire Plantae is more than 8,000[69], and they are one of the most abundant natural products in nature. As early as 1796, Seguin extracted polyphenol compounds in the water which extracts that can transform raw hides into leather are called "plant tannins". The term "plant polyphenols" was proposed by Haslam in 1981 based on the molecular structure and molecular weight of tannins. The basic skeleton of plant polyphenols is phenol, which is easily oxidized, so its properties are lively and have antioxidant properties. Polyphenols have a complex structure. Structurally, they include simple phenols with low molecular weights to polymeric tannins with molecular weights as large as thousands of Daltons, and they exist in a variety of forms[70].

Polyphenols have a variety of classification methods due to their complex structure. Generally, according to the different chemical bonds between polyphenol monomers, it can be divided into hydrolyzed tannins (phenolic acid polyphenols) and condensed tannins (flavanol polyphenols or procyanidins). And then it can also be divided into flavonoids ($C_6$-$C_1$-$C_6$, $C_6$-$C_3$-$C_6$), phenolic acids ($C_6$-$C_1$, $C_6$-$C_3$), simple phenols ($C_6$), coumarins, wood Lipids, tannins and stilbene ($C_6$-$C_2$-$C_6$) and other compounds. Or they can be classified according to different

raw materials from which polyphenols are extracted, such as honeysuckle polyphenols, apple polyphenols, grape polyphenols, or rose polyphenols etc.

### 1.3.2 Extraction method of polyphenols

(1) Organic solvent extraction method

Organic solvent extraction is currently one of the commonly used methods for extracting polyphenols at home and abroad. This method uses the principle of similar compatibility and the difference in solubility of compounds in different solvents, and by selecting an appropriate solvent, the target compound is separated from the raw material. When the solvent comes into contact with the raw material, the solvent begins to diffuse and permeate, enter the cell from the outside of the cell and dissolve the soluble substance, so that the concentration difference is formed inside and outside the cell. Then the soluble substance in the cell diffuses to the main body of the solution with the concentration difference as the driving force, finally to the inside and outside of the cell. The active ingredient concentration reaches dynamic equilibrium, and the solution is filtered out [71]. The types of compounds contained in Chinese herbal medicines are complex, and the choice of the extracted solvent has a significant impact on the extraction effect. Therefore, how to choose the most effective solvent for extraction is the key to the entire extraction process. Polyphenol compounds have a Polyphenolic hydroxyl structure and have a certain polarity, so hydrophilic solvents such as water, ethanol, methanol, etc. are often selected for extraction. This kind of solvent not only has good solubility for polyphenol compounds, but also has strong penetrating ability

to the cells of Chinese medicinal materials, therefore a higher extraction rate can be obtained. Bao Chen et al.[72] used ethanol to extract Agrocybe cha tree polyphenols. Under the conditions of extraction temperature of 80℃, ethanol concentration of 50%, and material-to-liquid ratio of 1:30 for 3 h, the extraction rate of tea polyphenols was 14.96%. The organic solvent extraction method is simple in equipment and convenient to operate, but it also has the disadvantages of low extraction rate, large solvent consumption, time-consuming, and high cost.

(2) Ultrasonic assisted extraction method

Ultrasound refers to electromagnetic waves with a frequency of about 20~50kHz, which is a kind of mechanical wave. The ultrasonic-assisted extraction method is the use of ultrasonic waves to strengthen the extraction of effective ingredients from plants, which is a physical crushing process. When the ultrasonic intensity reaches a certain level, the structure of the plant cell wall is destroyed, causing the cell to rupture in a very short time, and the effective components in the cell are released and enter the solvent to achieve the extraction effect [73]. Leandro[74] studied ultrasound-assisted extraction (UAE) of antioxidant polyphenols from Aronia melanocarpa berries. The influence of various parameters (time and temperature of extraction, solvent composition, solid–solvent ratio, particle size, and ultrasonic irradiations) on the extraction kinetics and yields was evaluated. Very clear effect of ultrasound was observed (up to 85% increase of the yield of extracted polyphenols). Ultrasonic extraction has low energy consumption, short extraction time, less impurity dissolution, low

equipment accuracy, safe and convenient operation, high extraction rate, wide operating temperature range, and is suitable for the extraction of heat-sensitive substances[75].

(3) Microwave-assisted extraction method

Microwave can penetrate plant cells. Polar substances in the cells absorb this energy and release heat. Then the temperature in the cell rises rapidly. The internal water vaporizes to produce instantaneous pressure to break the cell wall and cell membrane, allowing the extracellular solvent to enter the cell and dissolve it. Finally, the intracellular material is quickly leached out. Therefore, the extraction rate can be increased, the extraction time can be reduced, and the solvent consumption can be reduced by this method. Pan Xuejun[76] determined the extraction rate of tea polyphenols and caffeine in green tea leaves under different experimental conditions. The results showed that under optimal conditions, extraction at room temperature for 20 hours, ultrasonic assisted extraction for 90 minutes, and heat reflux extraction for 45 minutes, the extraction rate of polyphenols was about 28 %. The extraction rate of polyphenols was 30% by using microwave-assisted extraction method to extract for 4 minutes. And they thought microwave-assisted extraction method was more effective than the conventional extraction methods studied. The microwave-assisted extraction process is simple, efficient and fast, and saves solvents. And the required equipment is simple and has high development potential.

(4) Enzyme-assisted extraction method

The enzyme-assisted extraction method uses cellulase, hemicellulase, pectinase, etc. to act on plant cells, destroy the cell wall of plant cells. And then cause local loosening, swelling, collapse and other changes in the cell wall and intercellular structure, which is beneficial to The leaching of active ingredients. Fu Xiaoyan et al. [77] compared the traditional solvent extraction method and enzyme-assisted extraction method for the total phenol extraction of oats before and after germination, and the results showed that the enzyme-assisted extraction method is more conducive to the extraction of oat phenols. Enzymatic extraction can not only increase the extraction rate of polyphenols, but also effectively shorten the extraction time. In addition, the method has mild conditions and can maintain the original three-dimensional structure and biological activity of natural products to the utmost extent.

(5) Supercritical fluid extraction method

Supercritical extraction technology refers to a method of extracting effective ingredients from solid materials or liquid materials at its critical pressure and temperature. The pressure of the supercritical fluid affects the solubility of the active ingredients, and the solubility of the active ingredients increases with the increase of fluid pressure. Supercritical fluid is a new type of high-efficiency extractant, usually non-toxic, chemically stable, high solubility and selective $CO_2$ as the extractant. Gu Renyong et al. [78] used supercritical $CO_2$ extraction technology to extract polyphenols from August melon. The results show that the 95% ethanol solution is used as the extraction solvent, the conventional extraction

method August melon polyphenol yield is 4.37%, and the product purity is 81.46%; while the supercritical $CO_2$ extraction technology uses 95% ethanol solution as the entrainer to effectively strengthen The extraction efficiency, the polyphenol yield increased by 2.45 times, and the product purity increased by about 15.49%. The method can effectively avoid the oxidative decomposition of biologically active substances and the extraction purity is high, but the extraction efficiency is low and the production cost is high.

### 1.3.3 Separation and purification method

(1) Ion precipitation separation method

Ion precipitation separation method is a classic chemical separation method. The method is to select a suitable ion precipitation agent to produce complex precipitation in the crude plant polyphenol extract solution under a certain pH value. After subsequent filtration, washing, extraction and other processes, the plant polyphenols with higher purity are finally obtained[79,80]. Ouyang Yuzhu et al. [81] used calcium ion precipitation method to separate the total polyphenols of August melon, and used response surface methodology to optimize the process conditions. The results showed that: in the 50 mL extract, 90 mL saturated lime water was used as the precipitant, the reaction temperature was set to 70 ℃, and the precipitation was 38 min. The extraction rate of total polyphenols was 5.103 7% and the purity was 84.417%. The method has simple equipment, low energy consumption, and fast separation effect, but the pH in the solution easily affects the biological activity of polyphenols, so the scope of use is limited.

(2) Column chromatography separation method

Column chromatography separation method mainly uses macroporous resin or gel particles as adsorbents to separate and purify the effective components in plants. One of the phases is fixed, which is called stationary phase, and the other flowing through the stationary phase is called mobile phase. It uses the qualitative difference between the physical and chemical properties of the components in the sample to make the components distributed in two phases in different degrees. Each component moves at different speeds to achieve separation. Xu Dandan et al.[82] took the crude extract of Kuiren polyphenols as the research object, and discussed the process conditions for the separation and enrichment of macroporous resins. The recovery rate of polyphenols obtained after resin separation and purification was 82.3%, and the purity was super The filtered 15% increased to 77%. Luo Chaohua et al.[83] used dextran gel as a filler to explore the effect of column chromatography on the separation of epigallocatechin gallate in tea polyphenols. It was found that the tea polyphenols were obtained by this method to obtain a white powdery solid, and the purity of the tea polyphenols was more than 90% detected by high performance liquid chromatography, and they considered the process was scaled up and verified to be suitable for industrial production. Compared with other separation methods, column chromatography has the advantages of large preparation volume and simple operation, but it takes a long time and consumes a large amount of solvent.

(3) Membrane separation method

The separation medium of the membrane separation method is a natural or synthetic polymer membrane with high selectivity. This method uses external energy or chemical potential as the driving force to make the target product penetrate the membrane, so as to achieve the selective separation of molecules of different particle sizes at the molecular level, ultimate to achieve the purpose of separation and purification. Semipermeable membranes can be divided into microfiltration membranes, ultrafiltration membranes, nanofiltration membranes, reverse osmosis membranes, etc. according to their pore size. Wang Yonggang et al. [84] used peanut shell polyphenol extract as raw material and used membrane separation technology to purify it. Studies have shown that the purity of polyphenols in the extract increased from 9% to 18.67%, and the solid content increased from 5.4% to 43.9% after the removal and concentration of impurities by the microfiltration membrane and nanofiltration membrane system. Membrane separation method has the characteristics of continuous operation, small footprint, high efficiency and energy saving, special membrane allocation, no pollution, etc., but the permeation membrane is expensive and the separation period is long [85,86].

(4) Chromatographic separation method

Chromatographic separation methods mainly include high-performance preparative liquid chromatography, gas chromatography, thin-layer chromatography, supercritical fluid chromatography and high-speed countercurrent chromatography. Among them, the most commonly used methods

for the separation of polyphenols are high-speed countercurrent chromatography and high-performance preparative liquid chromatography. They make use of the differences in the forces of each component in the system composed of the stationary phase and the mobile phase. When the two phases move relative to each other, these substances move with the mobile phase and are repeatedly distributed between the two phases. So that the substances are separated. Xia Yajun et al.[87] used reverse-phase medium pressure preparative liquid chromatography to separate and prepare polyphenols in olive leaves, and obtained 5 phenolic compounds, whose mass fractions were all greater than 90% calculated according to the normalization method. Wang Wei[88] et al. used high-speed countercurrent chromatography combined with preparative liquid chromatography to separate 8 polyphenols from grape seed ethanol extracts. Among them, the purity of the five polyphenols proanthocyanidin B1, proanthocyanidin B2, gallic acid, epicatechin gallate and catechin are all higher than 96%. In recent years, chromatographic technology has been widely used in the extraction and separation of natural products and the pharmaceutical industry, with the advantages of high speed, high efficiency and high sensitivity. Among them, high-speed countercurrent chromatography also has the advantages of large preparation volume and no irreversible adsorption. This method has high research and utilization value in the separation and extraction of natural products.

### 1.3.4 Pharmacological activity of polyphenols

The research on the physiological activity of plant polyphenols is one of the

hotspots of polyphenol research in recent years, and it is also one of the fastest growing directions in polyphenol chemistry. A lot of research work had shown that plant polyphenols have good effects in anti-mutagenic, anti-tumor, anti-virus, anti-microbial, anti-aging and many other aspects. [89]

(1) Antioxidant

Plant polyphenols can effectively remove excess free radicals in the body, inhibit lipid peroxidation, protect the damage of biological macromolecules induced by free radicals, and slow down the aging of human tissues and organs [90]. Zhang Juanmei [29] used five evaluation systems to determine the in vitro antioxidant activity of rose polyphenols using iron ion reducing power, reducing potential and DPPH free radical, hydroxyl free radical and ABTS free radical scavenging ability. The results show that under the experimental conditions, the in vitro antioxidant activity of rose polyphenol is significantly better than that of Vc, and it has the potential to be developed as a food antioxidant. Zhang Juanmei also used the commercial pUC18 plasmid DNA as the material to verify the antioxidant capacity of rose polyphenols. The results showed that rose polyphenols can effectively protect against the oxidative damage of Fenton reagent. At the same time, the experiment of rose polyphenols on the life extension of nematodes further shows that rose polyphenols have a certain effect on the life extension of nematodes, and it is consistent with the increase in total antioxidant capacity and total SOD enzyme activity in nematodes. Sui Hongyu et al. [T48] used Kunming healthy mice as a model to study the 75% ethanol extract of dandelion. The

research results showed that the total flavonoids extract of dandelion could increase the activity of superoxide dismutase (SOD) in the brain tissue of aging model mice, and reduce the content of malondialdehyde (MDA), leukocytosis factor (LPF), and monoamine oxidase (MAO). The results show that total flavonoids had antioxidant effects.

(2) Antibacterial and anti-inflammatory

Polyphenols have obvious inhibitory ability to a variety of bacteria, fungi and yeasts, and do not affect the growth of animals at the corresponding inhibitory concentration. Apple polyphenols can effectively prevent food poisoning and other diseases caused by E. coli and Staphylococcus aureus, and the critical growth drug concentration of Staphylococcus aureus and E. coli are 0.2186 and 1.558 0 mg/mL, respectively[90]; Black wattle bark polyphenols have significant inhibitory effects on Escherichia coli, Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, Shigella dysentery, etc.[91]; Catechin can effectively inhibit A Type and B influenza viruses[92]; tea polyphenols have good inactivation effects on influenza viruses[93], adenovirus[94], HIV [95] and other viruses. The inhibitory method of polyphenols on viruses is generally considered to be that polyphenols combine with the protein coat of the virion or the cell membrane of the host, so that the virus cannot attach to the host cell, and the virus loses its invasiveness[96].

(3) Anti-cancer

Polyphenols have a wide range of biological effects in mammalian cells. A large number of epidemiological studies and animal experiments have proved that

polyphenols can induce tumor cell apoptosis and inhibit tumor cell proliferation, and have a good preventive effect on cancer. . H.A. Bawadi et al. [97] found that 0.24-24 μmolL of black soybean polyphenols can significantly inhibit the formation of ATP in cancer cells and accelerate the apoptosis of cancer cells; at this concentration, black soybean polyphenols will not affect the metabolism of normal cells. In addition, cocoa polyphenols can achieve the function of anti-colon cancer by inhibiting the biosynthesis of polyamines in cancer cells.

## Conclusions to section 1

Rose（*Rosa rugosa* Thunb.) Is a perennial deciduous shrub, belonging to Rosa of Rosaceae. It has the functions of reducing and eliminating free radicals, antioxidation, antithrombotic, anticancer, anti-inflammatory, antibacterial, immune regulation, reducing blood lipid and preventing heart disease. Roses contain volatile oil, polyphenols, alkaloids, vitamins, amino acids, soluble sugars, minerals and other active components and nutrients. Therefore, in addition to the outstanding ornamental and edible value, the medicinal value of roses can not be ignored.

Dandelion (*Taraxaci Herba*) is a perennial herbaceous plant in the Compositae, which has the effects of clearing away heat, detoxifying, and eliminating carbuncle. Up to now, domestic and foreign research scholars have isolated more than 50 compounds from dandelion, including polyphenols, polysaccharides, coumarins, terpenes, sterols, and volatile oils. Dandelion, as a kind of medicinal and edible

plant, is widely cultivated and rich in resources, which contains rich biologically active ingredients and has high utilization value.

Plant polyphenols, also known as tannins, are the general term for polyhydroxy compounds, which have many pharmacological properties. They can regulate the activity of most enzymes and cell receptors. And they have antioxidant, anti-cancer, antibacterial and anti-inflammatory effects. Concurrently, they havd a positive therapeutic and preventive effect on nerve tissue degeneration.Because the chemical components were very complicated, the separation of the polyphenols from the complex natural extracts was a difficult task. Currently commonly used extraction methods are organic solvent extraction method, ultrasonic assisted extraction method, microwave-assisted extraction method, enzyme-assisted extraction method, and supercritical fluid extraction method.And the separation methods are ion precipitation separation method, column chromatography separation method, membrane separation method, chromatographic separation method.

## Section 2 Materials and Methods

### 2.1 Experimental materials

Rose was purchased from Jinan Jiumei Rose Products Co., Ltd., Shandong, and was identified as a *R rugosa*. by Professor Liu Wei, School of Pharmacy, Qilu University of Technology. Dandelion was purchased from Traditional Chinese Medicine Decoction Pieces Factory of Shandong Jianlian Shengjia Traditional Chinese Medicine Co., Ltd., and was identified as *Taraxaci Herba* by Professor Liu Wei of the School of Pharmacy of Qilu University of Technology.

### 2.2 Experimental reagents and instruments

The instruments and reagents used in the experiment are shown in Table 2-1 and Table 2-2

Table 2-1 Experimental reagents

| Experimental reagent | Manufacturer |
|---|---|
| 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) | Sigma–Aldrich |
| ABTS assay kit | Biyuntian Biotechnology Co., Ltd. |
| Ammonia (Analytical Pure) | Tianjin Fuyu Fine Chemical Co., Ltd. |
| Ascorbic acid (Vc) | Sinopharm Chemical Reagent Co., Ltd |
| Ethanol (Analytical Pure) | Tianjin Fuyu Fine Chemical Co., Ltd. |
| Ethyl acetate (analytical grade) | Tianjin Fuyu Fine Chemical Co., Ltd. |
| Folin-phenol reagent | Sinopharm Chemical Reagent Co., Ltd. |
| FRAP assay kit | Biyuntian Biotechnology Co., Ltd. |

| | |
|---|---|
| Gallic acid | Tianjin Komiou Chemical Reagent Co., Ltd. |
| Methanol (analytical grade) | Tianjin Fuyu Fine Chemical Co., Ltd. |
| Methanol (chromatographically pure) | American World Corporation |
| N-Butanol (Analytical Pure) | Tianjin Fuyu Fine Chemical Co., Ltd. |
| N-hexane (analytical purity) | Tianjin Fuyu Fine Chemical Co., Ltd. |
| Petroleum ether (analytical pure) | Tianjin Fuyu Fine Chemical Co., Ltd. |
| Polyamide | Beijing solabao Technology Co., Ltd |
| Sodium carbonate | Tianjin kemio Chemical Reagent Co., Ltd |
| Trifluoroacetic acid (analytical grade) | Tianjin Fuyu Fine Chemical Co., Ltd. |

Table 2-2 Experimental equipment

| Main instrument | Manufacturer |
|---|---|
| 3057-11 portable recorder | Chongqing Chuanyi Automation Co., Ltd. |
| 8823B UV detector | Beijing Binda Yingchuang Technology Co., Ltd. |
| Agilent 1120 High Performance Liquid Chromatograph | Agilent Corporation |
| Benchtop centrifuge | Pingfan Instrument Factory |
| Bruker ADVANCE DPX 400 Nuclear Magnetic Resonance Spectrometer | Bruker Impact II™, Germany |

| | |
|---|---|
| Bruker AVIII HD 600 Nuclear Magnetic Resonance Spectrometer | Bruker Impact II™, Germany |
| BUCHI Rotary Evaporator | Swiss BUCHI Company |
| DC-0506 low temperature thermostat | Shanghai Tongtian Biotechnology Co., Ltd. |
| Electronic analytical balance | Mettler Toledo, Switzerland |
| Freeze dryer | Ningbo Xinzhi Biological Technology Co., Ltd. |
| Grinder | Tianjin Test Instrument Co., Ltd. |
| High-power CNC ultrasonic instrument | Shanghai Jiuwu Industrial Co., Ltd. |
| Microplate reader | TECAN, Switzerland |
| Q-TOF mass spectrometer equipped with ESI interface | Bruker Impact II™, Germany |
| TBE-300C high-speed countercurrent chromatograph | Shanghai Tongtian Biotechnology Co., Ltd. |
| TBP-5002 infusion pump | Shanghai Tongtian Biotechnology Co., Ltd. |
| UB-7 pH meter | Denver Instruments Co., Ltd. |
| Ultra-High Performance Liquid Chromatography (UHPLC） | American Waters Technology Co., Ltd. |
| Waters high performance liquid chromatograph (HPLC) | American Waters Technology Co., Ltd. |

## 2.3 Rose experiment method

### 2.3.1 Research on the extraction technology of rose polyphenols

At present, the extraction methods for plant polyphenols include organic solvent extraction, ultrasonic-assisted extraction, microwave-assisted extraction, enzyme-assisted extraction, and supercritical fluid extraction. Among them, the ultrasonic-assisted extraction method requires simple equipment, safe and convenient operation, high extraction rate, and can prevent the structure of polyphenols from being damaged to a large extent, and is suitable for large-scale preparation and production.

Orthogonal experimental design is a design method for studying multiple factors and multiple levels. With the characteristics of "uniform dispersion, neat and comparable", orthogonal experimental design is the main method of fractional factorial design[98], which has the advantages of high efficiency and speed.

In this experiment, ethanol was used as the solvent, and ultrasonic assisted extraction was used to extract polyphenols from dandelion. The experiment took the polyphenol content in the extract and the extraction rate of the rose sample as the inspection index, and used the method of combining single factor influence factor experiment and orthogonal design experiment to study the extraction effect. First, the four single factors of ethanol concentration, material-to-liquid ratio, ultrasonic time, and ultrasonic power were tested to determine the value range of each factor. And then on the basis of single factor experiments, the process parameters of ethanol extraction of dandelion polyphenols were optimized through

orthogonal experiments, and the optimal process conditions for extracting dandelion polyphenols by this method were screened. At the same time, it was compared with ultrasonic-assisted reflux extraction method to lay the foundation for subsequent research.

#### 2.3.1.1 The extraction process of rose polyphenols

Accurately weighed 1.0 g of crushed rose, added ethanol solution, and put it in an ultrasonic cleaner to extract polyphenols from rose under different conditions. After ultrasonic-assisted extraction, it was centrifuged at 4000 r/min, and the supernatant was taken as the rose polyphenol extract.

#### 2.3.1.2 Determination of polyphenol content by Folin method

In this study, the extraction rate of rose polyphenols was used as an inspection indicator, and the polyphenol content in the extract was determined by the Folin-Ciocalteu colorimetric method[99]. The principle of this method is that polyphenol compounds can reduce tungstate to produce blue compounds in alkaline solution, with maximum absorption at 765 nm, and the content of polyphenols has a positive relationship with the color depth. Gallic acid was used as the standard control, a standard curve was drawn from a standard solution prepared by the standard substance to determine the content of polyphenols in the extract.

(1) Drawing of polyphenol standard curve

Used gallic acid as the standard substance to determine the polyphenol content, accurately weighed 0.10 g of the gallic acid standard substance, dissolved it with distilled water, and diluted to 100 mL. And then accurately measure the gallic acid

standard solution 0.0, 1.0, 2.0, 4.0, 6.0, 8.0 mL. Respectively place in a 100 mL volumetric flask to constant volume, prepared into a standard solution of gallic acid with a concentration of 0, 0.01, 0.02, 0.04, 0.06, and 0.08 mg/mL.

Accurately measured 1.0 mL of the standard solution in a graduated test tube, added 5.0 mL of 10% Folin-phenol reagent to each test tube, and shook well. Added 4.0 mL of 7.5% sodium carbonate ($Na_3CO_4$) solution within 3 to 8 minutes of reaction, and shook it. Lay it at room temperature for 60 min. Took 200 μL of each in a 96-well plate, and measured the absorbance with a microplate reader at a wavelength of 765 nm.

(2) Determination of samples

Accurately measure 10 mL of sample, place it in a 100 mL volumetric flask and dilute it to the scale, which was the sample diluted 10 times.Accurately measured 1.0 mL of the diluted sample in a graduated test tube, added 5.0 mL of 10% Folin-phenol reagent to each test tube, and shook well. Added 4.0 mL of 7.5% sodium carbonate ($Na_3CO_4$) solution within 3 to 8 minutes of reaction, and shook it. Lay it at room temperature for 60 min. Took 200 μL of each in a 96-well plate, and measured the absorbance with a microplate reader at a wavelength of 765 nm. Compared the mass concentration of the extracted polyphenols with the standard curve, and calculated the extraction rate according to the following formula:

$$\text{Extraction rate of rose polyphenols}(\%) = \frac{\mathrm{C} \times \mathrm{V} \times \mathrm{N} \times 10^{-3}}{\mathrm{m}} \times 100\%$$

In this formula, C: the concentration of flavonoids in the extract (mg/mL); V: the volume of the test solution (mL); N: the dilution factor; m: the mass of sample

(g).

#### 2.3.1.3 Single factor experiment

(1) Single factor experiment of ethanol concentration

The crushed rose was accurately weighed into 6 parts, each 1.0 g, added 0%, 20%, 40%, 60%, 80%, 100% ethanol as the extraction solvent, and fixed the material-to-liquid ratio (w/v) 1:20, ultrasonic time 60 min, ultrasonic power 100 W for extraction of rose polyphenols. After ultrasonic-assisted extraction, centrifuged at 4000 r/min, and took the supernatant to determine the extraction rate of polyphenols according to the method of 2.3.1.2, and compared the extraction rates of rose polyphenols at various ethanol concentrations.

(2) Single factor experiment of material-to-liquid ratio

The crushed rose was accurately weighed into 6 parts, each 1.0 g, and added them for extraction according to different material-to-liquid ratios (w/v) 1:10, 1:15, 1:20, 1:25, 1:30, 1:35 solvent ethanol, fixed ethanol concentration of 60%, ultrasound time 60min, ultrasound power 100 W for extraction of rose polyphenols. After ultrasonic-assisted extraction, centrifuged at 4000 r/min, the supernatant was taken to determine the extraction rate of polyphenols according to the method of 2.3.1.2, and the extraction rate of rose polyphenols under different material-to-liquid ratios was compared.

(3) Ultrasound time single factor experiment

The crushed rose was accurately weighed into 6 parts, each 1.0 g, and ultrasonicated them for 20, 40, 60, 80, 100, and 120 min. The fixed material-to-

liquid ratio (w/v) was 1:20, the ethanol concentration was 60%, and the ultrasonic power was 100W for the extraction of rose polyphenols. After ultrasonic-assisted extraction, centrifuged at 4000 r/min, the supernatant was taken to determine the extraction rate of polyphenols according to the method of 2.3.1.2, and the extraction rate of rose polyphenols under different ultrasonic time was compared.

(4) Ultrasonic power single factor experiment

The crushed rose was accurately weighed into 6 parts, each 1.0 g, and performed ultrasound under the conditions of ultrasonic power of 50, 100, 150, 200, 250 and 300 W respectively. The fixed liquid-to-material ratio (w/v) was 1:20, the ethanol concentration was 60%, and the ultrasonic time was 60 min for extraction of rose polyphenols. After ultrasonic-assisted extraction, centrifuged at 4000 r/min, the supernatant was taken to determine the extraction rate of polyphenols according to the method in 2.3.1.2, and the extraction rate of rose polyphenols under different ultrasonic powers was compared.

**2.3.1.4 Orthogonal method optimized experimental design**

Based on the single-factor experiment results in 2.3.1.3, several factors that had a greater influence on the extraction rate of rose polyphenols were selected for orthogonal design (Table 2-3), and the $L_9(3^4)$ orthogonal table was selected for orthogonal design. For further optimized the ultrasonic extraction process conditions of rose polyphenols.

Table 2-3 Orthogonal factor level table

| Level | Factor |
|---|---|

| | A<br>Ethanol concentration (%) | B<br>Material-to-liquid ratio (w/v) | C<br>Ultrasound time (min) | D<br>Ultrasonic power (W) |
|---|---|---|---|---|
| 1 | 40 | 1:10 | 40 | 100 |
| 2 | 60 | 1:20 | 60 | 150 |
| 3 | 80 | 1:25 | 80 | 200 |

**2.3.1.5 Comparison of extraction of rose polyphenols by reflux method and ultrasound-assisted method**

The two commonly used polyphenol extraction methods, reflux method and ultrasonic-assisted reflux method, were compared and evaluated. Under the same ethanol concentration, material-to-liquid ratio and extraction time, there are differences in the extraction amount of rose polyphenols.

First, rose polyphenols were extracted by reflux method. Weighed 1.0 g of crushed rose in a 100 mL single-necked flask, added 60% ethanol as the extraction solvent according to the material-to-liquid ratio 1:25 (w/v) , and weighed. The reflux condenser was connected. And the sample was heated by a temperature-controlled electric heating mantle, and was refluxed for 60 min. Finally, made the sample to cool to room temperature, weighed and used 60% ethanol to make up the weight to the weight before reflux. The samples were mixed, and centrifuged at 4000r/min. Drew the supernatant to determine the polyphenol content and calculated according to the method in 2.3.1.2 extraction rate.

Secondly, the ultrasonic-assisted method was used to extract rose polyphenols

for comparison. 1.0 g of crushed rose was weighed and added it to a 100 mL single-neck flask, added 60% ethanol as the extraction solvent according to the liquid-to-material ratio of 1:25 (w/v), and weighed. The reflux condenser was connected. The sample was put into the ultrasonic cleaner, and the heating was turned on. The sample was ultrasonically refluxed for 60 min at a power of 150 W. Finally, the sample was cooled to room temperature, weighed, supplemented to the weight before reflux with 60% ethanol, mixed, and centrifuged at 4000 r/min. Drew the supernatant to determine the polyphenol content and calculated according to the method in 2.3.1.2 extraction rate.

The above experiment was repeated three times. Compared and evaluated the extraction rate of reflux method and ultrasonic-assisted reflux method for extracting rose polyphenols.

### 2.3.2 Purification of rose polyphenols by macroporous resin

#### 2.3.2.1 Extraction of rose polyphenols

1.6 kg of crushed roses were weighed, and ultrasonic reflux extraction was carried out according to the optimal conditions obtained in 2.3.1. The obtained crude extract was concentrated to 900 mL under reduced pressure. After adding 10 times of water to suspend, 0.1 g/mL chitosan solution was added in a ratio of 1:10 (v/v), which purpose is to flocculate and remove the tannin in the rose crude. After centrifugation at 4000 r/min for 10 min, the supernatant was extracted and concentrated under reduced pressure. The resulting sample was labeled R.

#### 2.3.2.2 Pretreatment of macroporous resin

The macroporous resin must be pretreated before use, the purpose is to remove a small amount of polymer, organic matter and harmful ions contained in the resin. First, the resin was soaked in 95% ethanol solution for 8 h, and then washed with 95% ethanol until the effluent was diluted with water and not turbid, and then washed with distilled water until there was no alcohol smell. Secondly, the resin was soaked in 5% NaOH solution for 4 h and then washed with distilled water to neutrality. Then it was soaked in 5% HCL solution for 4 hours, and then washed with distilled water to neutrality. After each loading and elution, the resin column was first washed with 5% NaOH solution until it was colorless, then washed with distilled water until the pH was neutral, then washed with 5% HCL until it was colorless, and finallywashed with distilled water until the pH was neutral.

**2.3.2.3 Screening of macroporous resin**

Generally speaking, organic compounds with low polarity are suitable for separation on non-polar resins, and organic compounds with higher polarity are suitable for separation on medium and polar resins. Polyphenolic compounds with phenolic hydroxyl groups and glycoside chains have a certain degree of polarity, hydrophilicity and strong hydrogen bond generation ability, so they are easily adsorbed by weakly polar and polar resins.

Table2-4 Physical properties and types of macroporous resins

| Resin type | Specific surface area/($m^2$/g) | Particle size/(nm) | Average pore diameter/(nm) | Polarity |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| D101 | 500-550 | 0.3-1.25 | 90-100 | Non-polar |
| X-5 | 500-600 | 0.3-1.0 | 290-300 | Non-polar |
| AB-8 | 480～520 | 0.3-1.25 | 130-140 | Weak polarity |
| BS-75 | 90-150 | 0.3-1.2 | 250-300 | Mid-polarity |

The surface moisture of the pretreated macroporous adsorption resin was absorbed by the filter paper. 2.00 g of pretreated D101 (non-polar), X-5 (non-polar), AB-8 (weak polarity), BS-75 (mid-polarity) resins were accurately weighed into a 150 mL plug cone Shaped bottle. A stoppered Erlenmeyer flask was charged with 50 mL of the crude rose polyphenol extract R, placed in a constant temperature water bath shaker, and shaken at 25°C and 120 r/min for 24 h for sufficient adsorption. Determine the concentration of polyphenols in the solution at this time. After filtering the extract, the polyphenol solution on the resin surface was washed away with distilled water, and then each 50 mL 70% ethanol solution was desorbed under the same conditions, and the polyphenol concentration in the desorbed solution was determined. The data obtained afterwards were calculated according to the following formulas for adsorption capacity, adsorption rate, desorption rate and recovery rate, with the purpose of screening out resins with excellent performance for subsequent experiments.

$$\text{Adsorption capacity (mg)} = C_0V_0 - C_1V_1$$

$$\text{Adsorption rate (\%)} = \frac{C_0V_0 - C_1V_1}{C_0V_0} \times 100\%$$

$$\text{Desorption rate (\%)} = \frac{C_2V_2}{C_0V_0 - C_1V_1} \times 100\%$$

$$\text{Recovery rate}(\%) = \frac{C_2 V_2}{C_0 V_0} \times 100\%$$

$C_0$: initial solution mass concentration, mg/mL; $V_0$: initial solution volume, mL; $C_1$: adsorption equilibrium solution mass concentration, mg/mL; $V_1$: adsorption equilibrium solution volume, mL; $C_2$: desorption solution Mass concentration, mg/mL; $V_2$: volume of desorption solution, mL.

**2.3.2.4 Dynamic adsorption experiment**

A chromatography column with an inner diameter of 2.5 cm (specification 350*30 mm) was selected, and 60 mL of the processed AB-8 macroporous resin was accurately measured, then the column was wet packed (diameter-to-height ratio 1:5). The crude rose polyphenol liquid R was diluted with 1 time water, and the polyphenol concentration was 5.56 mg/mL at this time. The sample R was added to the chromatography column using a peristaltic pump at a volume flow rate of 2 BV/h. Test tubes were used to receive the eluent, and each test tube collected 10 mL. Measure the polyphenol content of each tube according to the method of 2.3.1.2, and draw the leakage curve. The purpose was to determine the optimal sample load.

**2.3.2.5 Separation of macroporous resin**

The best macroporous resin selected was selected, and the sample R was added to the macroporous resin column according to the best sample loading ratio. First, the macroporous resin column was rinsed with water until the eluent was colorless, in order to remove impurities that were not adsorbed. Then it was rinsed with 30%, 70% ethanol and washed until the eluent was colorless. The two components were

collected, concentrated under reduced pressure, and lyophilized in vacuo. They were marked as R-30 and R-70 respectively.

#### 2.3.2.6 HPLC analysis

The total rose sample and the components separated and purified by the macroporous resin column were analyzed by HPLC. The analysis conditions of HPLC were carried out according to Table 2-5. The column was a Waters C18 column (250×4.6 mm, 5μm), the mobile phase was acetonitrile (A)- 0.1% formic acid in water(B), and the flow rate was 1.0 mL/min. The wavelength was 280nm, and the injection volume was 10 μL.

Table 2-5 HPLC gradient elution conditions of Rose

| Time (min) | Flow (mL/min) | A - Acetonitrile (%) | B - 0.1% formic acid in water (%) |
|---|---|---|---|
| 0.00 | 1.0 | 5.0 | 95.0 |
| 7.00 | 1.0 | 9.0 | 91.0 |
| 27.00 | 1.0 | 13.0 | 87.0 |
| 31.00 | 1.0 | 16.0 | 84.0 |
| 52.00 | 1.0 | 28.0 | 72.0 |
| 57.00 | 1.0 | 40.0 | 60.0 |
| 60.00 | 1.0 | 100.0 | 0.0 |

### 2.3.3 Identification of polyphenols in roses by mass spectrometry

#### 2.3.3.1 Mass spectrometry analysis conditions

The chromatographic column was a Waters C18 column (250×4.6 mm, 5 μ

m), the flow rate was 1 m L/min, the column temperature was 45 ℃, and the mobile phase: acetonitrile (A)-0.1% formic acid in water (B). The elution gradient method was consistent with 2.3.2.6. The total time was 60 minutes.

The mass spectrometer used an electrospray ion source. Ion source parameters: capillary voltage, 3500 V in positive ion mode, 3000V in negative ion mode; drying gas flow rate was 8 L/min; atomizing gas pressure was 200 kPa; drying gas temperature was 200 ℃. The quadrupole ion energy was 3 eV.

Collision cell parameters: collision energy was 7 eV, transmission time was 60 μs, collision cell RF voltage amplitude was 750 Vpp, and the waiting time before pulse was 5 μs. The mass scanning range was m/z 50~1500. The collision energy in the secondary mass spectrometry analysis was 10 ~ 50 eV.

**2.3.3.2 Data analysis**

The data deconvolution and peak alignment were carried out by MS-DIAL33 and peak table with m/z, retention time and area was obtained.

**2.3.3.3 Stability investigation**

The sample solution was tested repeatedly at 0, 2, 4, 6, 12, and 24 h after the sample solution was prepared according to the chromatographic conditions of 2.3.2.6 to determine its stability.

**2.3.4 Study on the antioxidant activity of rose polyphenol extract**

Three antioxidant activity indicators, DPPH free radical scavenging, ABTS free radical scavenging, and total antioxidant capacity determination (FRAP), were used to evaluate the antioxidant activity of 2 parts of rose, which were

separated by macroporous resin.

#### 2.3.4.1 Determination of DPPH free radical scavenging ability

As a stable free radical, DPPH can exist stably in organic solvents. It has a single electron, so it can accept an electron or hydrogen ion, and has a maximum absorption at a wavelength of 517nm. When there is a free radical scavenger, the single electron of DPPH is captured to make its color lighter, and the absorbance value at the maximum light absorption wavelength decreases, and the decrease degree is linear. The decrease in absorbance level indicates the increase in oxidation resistance. By this way can be evaluated the oxidation resistance of the test sample. This antioxidant capacity is expressed by the inhibition rate. The greater the inhibition rate, the stronger the oxidation resistance. $V_C$ is a polyhydroxy compound with strong antioxidant capacity. Therefore, in this experiment, $V_C$ was chosen as a reference for the antioxidant capacity of other samples.

(1) Preparation of DPPH working fluid

6.5 mg of 1,1-diphenyl-2-pyridyl hydrazine (DPPH) was accurately weighed and placed in a 250 mL Brown volumetric flask. Then it was dissolved with absolute ethanol and fixed to volume, which was prepared into 26 μg/mL DPPH radical solution and store in the dark at 0 ~ 4 ℃.

(2) Configuration of standard solution and sample solutions

First, 3.0 mg of $V_C$ standard substance and 3.0 mg of samples were accurately weighed in a 100 mL volumetric flask, then dissolved with water and diluted to

the scale. Which were $V_C$ standard solution and sample solutions. Secondly, the prepared solutions were diluted to 1.0, 3.0, 5.0, 10, 20 and 30 μg/mL.

(3) Determination of antioxidant capacity

In the 96 well plate,100 μL water was added in 100 μL DPPH solution, stirred quickly, and then put in the dark at room temperature for 30 min. The absorbance value was measured at 517 nm and recorded it as $A_0$. 100 μL $V_C$ standard solution of different concentrations and sample solutions of different concentrations were added in 100 μL DPPH solution, stirred quickly, and then put in the dark at room temperature for 30 min. The absorbance value was measured at 517 nm and recorded it as $A_1$. 100 μL $V_C$ standard solution of different concentrations and sample solutions of different concentrations were added in 100 μL absolute ethanol, stir quickly, and then put in the dark at room temperature for 30 min. The absorbance value was measured at 517 nm and recorded it as $A_2$. The experimental design was shown in table 2-6.

Table 2-6 The experimental design of the capacity of scavenging DPPH·

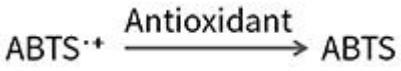
| | DPPH Solution | Solvent for DPPH solution | Sample solutions | Solvent of sample solutions |
|---|---|---|---|---|
| $A_0$ | 2ml | 2ml | - | - |
| $A_1$ | 2ml | - | 2ml | - |
| $A_2$ | - | - | 2ml | 2ml |

Calculate the DPPH free radical scavenging rate by the following formula:：

$$\text{DPPH free radical scavenging rate (\%)} = \frac{A_0 - (A_1 - A_2)}{A_0} \times 100$$

### 2.3.4.2 Determination of ABTS free radical scavenging ability

The principle of the ABTS method to determine the total antioxidant capacity is shown in the formula:




ABTS is oxidized to green ABTS+ under the action of appropriate oxidants. The production of ABTS+ will be inhibited in the presence of antioxidants. The absorbance of ABTS+ can be measured at 734 nm or 405 nm to determine and calculate the total antioxidant capacity of the sample. After the substance is added to the ABTS free radical solution, if the absorbance at 734 nm decreases, it means that the substance has free radical scavenging activity and is an antioxidant. This method is called ABTS free radical scavenging method, which can be used to evaluate the antioxidant capacity of plants (or Chinese herbal medicine extracts) and pure compounds. Trolox is an analog of vitamin E, which has antioxidant capacity similar to vitamin E. In this experiment, Trolox was selected as a reference for the total antioxidant capacity of other antioxidants. For example, the total antioxidant capacity of Trolox is 1. Under the same concentration, the antioxidant capacity of other substances is expressed by the multiple of its antioxidant capacity compared with Trolox.

(1) Preparation of ABTS working fluid

First, equal volumes of ABTS solution and oxidizer solution were used to prepare ABTS working mother liquor, and stored it in the dark at room temperature for 12-16 h before use. Before use, the ABTS working mother liquor was diluted

with 80% ethanol to make the ABTS working solution. When the absorbance of ABTS working solution was 0.7 ± 0.05 in A734 and about 1.4 in A405 after subtracting the corresponding 80% ethanol blank control, it indicated that it can be used.

(2) Preparation of standard curve

The 10 mM Trolox standard solution was diluted with distilled water to 0.15, 0.3, 0.6, 0.9, 1.2 and 1.5 mM.

(3) Determination of antioxidant capacity

Because the color of the sample itself can not interfere with the detection, the sample to be tested can be directly used for the anti-oxidation detection.

First, 200 μL ABTS working solution was added to each test hole of 96 well plate. Next, 10 μL of distilled water was added into the blank control well; 10 μL of Trolox standard solutions of various concentrations were added into the standard curve detection hole; 10 μL of various samples were added into the sample detection hole. Mixed gently. A734 was measured after 2-6 min incubation at room temperature. Finally, the total antioxidant capacity of the sample was calculated according to the standard curve. The experimental design is shown in Table 2-7.

Table 2-7 The experimental design of the capacity of scavenging ABTS+·

| | ABTS Solution | Solvent for ABTS solution | Sample solutions or Trolox solutions |
|---|---|---|---|
| $A_0$ | 200μL | 10μL | - |

| | | | |
|---|---|---|---|
| $A_1$ | 200μL | - | 10μL |

The calculation formula of ABTS free radical scavenging rate is:

$$\text{ABTS free radical scavenging rate (\%)} = (1 - \frac{A_1}{A_0}) \times 100$$

**2.3.4.3 Determination of total antioxidant capacity by FRAP method**

The principle of the Ferric ion reducing antioxidant power method to determine the total antioxidant capacity is that under acidic conditions, antioxidants can reduce Ferric-tripyridyltriazine (TPTZ–Fe（III）) to produce blue TPTZ–Fe（II）. TPTZ–Fe（II）has an absorbance at 593 nm, which can be used as An indicator of the total antioxidant capacity in a sample. Since the reaction is carried out under acidic conditions, some endogenous interference factors can be suppressed. And since the total concentration of iron ions or ferrous ions in the sample is usually less than 10 μM, the iron ions or ferrous ions in the sample will not significantly interfere with the detection reaction of the FRAP method. And because the iron or ferrous ion in the reaction system is chelated with TPTZ, the small amount of metal ion chelating agent contained in the sample itself usually does not significantly affect the detection reaction. Therefore, it is widely used in the analysis of antioxidant capacity of food and health products.

The principle of FRAP method to determine the total antioxidant capacity is shown in the formula:

$$Fe^{3+}\text{-TPTZ} \xrightarrow{\text{Antioxidant}} Fe^{2+}\text{-TPTZ (blue)}$$

(1) Preparation of FRAP working fluid

First, 3000 μL of TPTZ diluent was added into 300 μL of TPTZ solution,

mixed well. Then it was added 300 μL of detection buffer. After mixing, incubate at 37°C and should use it within 2 h.

(2) Preparation of standard curve

278 mg of $FeSO_4$•$7H_2O$ provided by the kit was weighed in volumetric flask, dissolve and dilute to 10 mL, and at this time the concentration was 100 mM. 100 mM $FeSO_4$ solution was diluted to 0.15, 0.3, 0.6, 0.9, 1.2 and 1.5 mM.

(3) Determination of antioxidant capacity

Since the color of the sample itself does not interfere with the detection, the sample to be tested can be directly used for the detection of anti-oxidation.

First, 180 μL of FRAP working solution was added into each detection well of the 96-well plate. Next, 5 μL of distilled water was added into the blank control well; 5 μL of various concentrations of FeSO4 standard solutions were added into the standard curve detection hole; 5 μL of samples were added into the sample detection hole. Mixed gently. After incubating at 37°C for 3-5 min, A593 was measured. Finally, the total antioxidant capacity of the sample was calculated according to the standard curve. The experimental design was shown in Table 2-8.

Table 2-8 The experimental design of the capacity of scavenging FRAP

| | FRAP working fluid | Solvent for FRAP working fluid | FeSO4 solutions | Sample solutions |
|---|---|---|---|---|
| $A_0$ | 180μL | 5μL | - | - |
| $A_1$ | 180μL | - | 5μL | - |
| $A_2$ | 180μL | - | - | 5μL |

## 2.4 Experimental method of dandelion

### 2.4.1 Extraction of dandelion polyphenols

1kg of dandelion was taken, and the optimal extraction technology was selected by referring to the method in 2.3.1. It with 60% ethanol, with a material-to-liquid ratio of 1:25 (w/v), ultrasound assisted reflux extraction at 200W power for 60min, filter, and concentrated the extract under reduced pressure. The concentrated solution was suspended in water, and extracted 3 times with the same amount of petroleum ether for degreasing and removing impurities such as chlorophyll. The petroleum ether phase was discarded, and the remaining part was extracted 5 times with an equal amount of ethyl acetate. The aqueous phase and the ethyl acetate phase were concentrated under reduced pressure to a paste, and were named T-W and T-E respectively. T-E was preliminarily purified using a polyamide resin column. The sample was filled into a 30-80 mesh polyamide atmospheric column by dry loading. After eluted with water, then eluted with 30%, 60%, 95% ethanol. The 3 components obtained were concentrated under reduced pressure to dryness and were named T-30, T-60, T-95 respectively. The obtained samples were stored in the refrigerator.

### 2.4.2 Countercurrent chromatography combined with semi-preparative high performance liquid chromatography to separate and purify polyphenols in dandelion

There are many kinds of active ingredients of natural plants, and different plants or similar plants from different places of production also have differences

in structure and content of active ingredients. The study of medicinal components based on monomer compounds is the basis of the research of natural medicinal substances. Therefore, in order to further study the pharmacological activities of dandelion polyphenol compounds, it is necessary to separate and prepare more monomer compounds. Dandelion polyphenols are complex in composition and difficult to separate and prepare. Currently, the commonly used separation and preparation method is mainly column chromatography [7], and its sample preparation efficiency is low and time-consuming. Chromatography has the characteristics of large preparation volume, good separation effect, convenient operation, etc., and has been widely used in the separation and preparation of natural products. At present, there are relatively few research reports on the separation of dandelion polyphenols by chromatography [100]. Among them, countercurrent chromatography (CCC) and semi-preparative high performance liquid chromatography (SP-HPLC) are commonly used in chromatography. Among them, countercurrent chromatography uses the partitioning effect of the sample between two immiscible solvents. When the sample passes through the two solvent phases, it is separated due to the different partition coefficients. It is a full liquid chromatography without solid support. Countercurrent chromatography can also be divided into high-speed countercurrent chromatography (HSCCC), pH-zone-refining counter-current chromatography (pH-ZRCCC), and dual countercurrent chromatography (DuCCC) according to different separation methods. And the high performance preparative liquid chromatography, like

countercurrent chromatography, which separation system is also composed of two phases, the difference is that the stationary phase of liquid chromatography is a solid. It can be separated according to the adsorption capacity, partition coefficient, ion exchange effect or molecular size difference of each component in the stationary phase and the mobile phase by selecting the chromatographic column of different materials.

In this experiment, dandelion medicinal materials were used as raw materials, and two counter-current chromatography methods of pH-ZRCCC and HSCCC were combined with Pre-HPLC to separate and purify the dandelion polyphenols, and use ESI-MS and NMR to identify its structure. Finaly provide technical support for quality standard research.

#### 2.4.2.1 Determination of Partition Coefficient in the Solvent System of Countercurrent Chromatography

According to the theory of countercurrent chromatography as partition chromatography, the necessary condition for sample separation is the appropriate partition coefficient of each component. Choosing a suitable solvent system is the key to successful countercurrent chromatography. The choice of solvent system can be determined by measuring the partition coefficient (K). The K value can be measured by high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), ultraviolet absorption method and other methods. Considering the complexity of the components contained in the samples in this experiment and the efficiency of the experiment, the HPLC method was selected

to determine the partition coefficient.

(1) Determination of the partition coefficient of pH-ZRCCC

Prepared the solvent system, each of the upper and lower phases were took 5 mL and were added to the test tube. First, ammonia solution was addes until the pH of the solvent system was about 10. The sample of 5 mg was added in it, and shook to completely dissolve. After standing to complete stratification, each of the upper and lower phases were took 10 μL for detection by HPLC. The peak area ($A_U$) of the upper phase was compared the peak area ($A_L$) of the lower phase to calculate the partition coefficient $K_{base}$ of the sample under alkaline conditions. The calculation formula was as follows: $K_{base}=A_U/A_L$.

When separating acidic substances, if $K_{base}\ll 1$, the next measurement could be carried out. Trifluoroacetic acid was added in the above test tube, adjusted the pH to about 2. The test tube was shaked again, and then standed to balance. Each of the upper and lower phases were took 10 μL for detection by HPLC. Then the distribution coefficient $K_{acid}$ was calculated. If $K_{acid}\gg 1$, the solvent system could be used to pH-ZRCCC method for sample separation.

If $K_{base}$ was not small enough, should be used a solvent system with a weaker polarity to recalculate $K_{base}$ and $K_{acid}$. If $K_{acid}$ was not large enough, should be used a solvent system with a strong polarity to repeat the above steps.

(2) Determination of the partition coefficient of HSCCC

The sample was weighed about 5 mg into a 10 mL test tube. The solvent system was configured, and 5 mL each of the upper and lower phases was took in the test

tube which contained the sample. The test tube was shook vigorously, and stood it for layering. Each of the upper and lower phases were took 10 μL for detection by HPLC. The peak area ($A_1$) of the upper phase was compared the peak area ($A_2$) of the lower phase to calculate the distribution coefficient K. The calculation formula was as follows: $K=A_1/A_2$.

**2.4.2.2 Preparation of two-phase solvent system and sample solution**

(1) Preparation of a two-phase solvent system and sample solution for pH-ZRCCC

Ethyl acetate-acetonitrile-water (4:1:5, v/v/v) was selected as the two-phase solvent system, and the pH-ZRCCC was used for separation. Ethyl acetate, acetonitrile and water were placed in a separatory funnel according to the ratio and shook vigorously. Put it stood until the upper and lower phases were separated. trifluoroacetic acid (10 mM) was added to the upper phase, ammonia (10 mM) was added to the lower phase. Then, they were degassed by ultrasound 3min.

The sample was weighed, the same amount of acidified upper phase and the lower phase without alkali were took to dissolve the sample for subsequent countercurrent chromatographic separation.

(2) Preparation of a two-phase solvent system and sample solution for HSCCC

Petroleum ether-ethyl acetate-methanol-water (1:4:1:4, v/v/v/v) was selected as the solvent system, and was placed in a separatory funnel according to the proportions, shook the separatory funnel. Then put it stood until the upper and lower phases were separated, and degassed ultrasonically for use.

The sample was weighed, equal amounts of the upper and lower phases were took to dissolve the sample for later use.

#### 2.4.2.3 Countercurrent chromatographic separation

(1) Separation procedure of pH-ZRCCC

First, the stationary phase (upper phase) was pumped into the column at a flow rate of 30.0 mL/min until the column was full. Then, the sample solution was injected into the separation columns through the sample loop, and the rotational speed was adjusted to make the separation column rotate clockwise at a speed of 800 rpm. While the mobile phase (lower phase) was pumped at a speed of 2 mL/min. Samples were collected at 5-min intervals into test tubes, and monitored it at 280 nm using an ultraviolet detector. After the separation was finished, the pH The solvent in the column was blown out by a compressed gas, and was put into a measuring cylinder. The retention ratio of the fixed phase was calculated as the volume of the residual stationary phase divided by the column volume.

(2) Separation procedure of HSCCC

First, the stationary phase (upper phase) was pumped into the column at a flow rate of 30.0 mL/min until the column was full. Then the rotational speed was adjusted to make the separation column rotate clockwise at a speed of 800 rpm. While the mobile phase (lower phase) was pumped at a speed of 2 mL/min. When the system reached hydrodynamic equilibrium, the sample solution was injected into the separation column through the sample loop. Samples were collected at 5-min intervals into test tubes, and monitored it at 280 nm using an ultraviolet

detector. After the separation was finished, the pH The solvent in the column was blown out by a compressed gas, and was put into a measuring cylinder. The retention ratio of the fixed phase was calculated as the volume of the residual stationary phase divided by the column volume.

**2.4.2.4 Separation of SP-HPLC**

Selecting a suitable mobile phase is the key to successful separation in semi-preparative high performance liquid chromatography. First, the polarity of the target compound was judged by the result of liquid chromatography. Then, according to the polarity of the target compound, appropriate separation conditions were established to separate T-W and T-60 samples. The column was YMC-Pack ODS-A (250×10.0 mml, 5μm), and the organic phase was methanol. When the flow rate was 3 mL/min and the absorbance was 280 nm, the most appropriate mobile phase and injection volume were studied.

(1) Composition of mobile phase

The sample was dissolved in methanol and filtered with a 0.45 μm nylon filter membrane.

According to the results of HPLC, the target compound in sample T-W has high polarity. Therefore, a lower methanol concentration was selected for separation, and the composition of the mobile phase was changed according to the chromatogram. Eventually the optimal separation conditions were selected from the chromatogram. The polarity of the target compound in sample T-60 is small. Therefore, a higher methanol concentration was selected for separation, and the

composition of the mobile phase was changed according to the chromatogram. Eventually the optimal separation conditions were selected from the chromatogram.

(2) Determination of injection volume

The concentration of the fixed sample solution was 3 mg/mL. The injection volume was gradually increased under optimal conditions. Observe the changes in the chromatogram and select the most appropriate injection volume according to the results of the chromatogram.

**2.4.3.5 HPLC analysis and structure identification**

The total dandelion sample and the separated and purified components were analyzed by HPLC. The analysis conditions of HPLC were carried out according to Table 2-9. The chromatographic column was a Waters C18 column (250×4.6 mm, 5 μm), the mobile phase was methanol (A)-0.1% formic acid in water solution (B), the flow rate was 1.0 mL/min, and the detection wavelength was 280 nm, and the injection volume was 10 μL. Finaly the normalization method was used to calculate the purity of the sample. The separated and purified components were dissolved in DMSO and analyzed by ESI-MS and NMR to identify their chemical structures.

Table 2-9 HPLC gradient elution conditions of Dandelion

| Time (min) | Flow (mL/min) | A - Methanol (%) | B - 0.1% formic acid in water (%) |
|---|---|---|---|
| 0.00 | 1.0 | 5.0 | 95.0 |

| | | | |
|---|---|---|---|
| 5.00 | 1.0 | 20.0 | 80.0 |
| 15.00 | 1.0 | 24.0 | 76.0 |
| 25.00 | 1.0 | 24.0 | 76.0 |
| 26.00 | 1.0 | 34.0 | 66.0 |
| 35.00 | 1.0 | 40.0 | 60.0 |
| 50.00 | 1.0 | 55.0 | 45.0 |
| 60.00 | 1.0 | 70.0 | 30.0 |
| 70.00 | 1.0 | 100.0 | 0.0 |

### 2.4.3 Study on antioxidant activity of dandelion total polyphenol extract

#### 2.4.3.1 Determination of DPPH free radical scavenging ability

Refer to the method in 2.3.4.1 for determination

#### 2.4.3.2 Determination of ABTS free radical scavenging ability

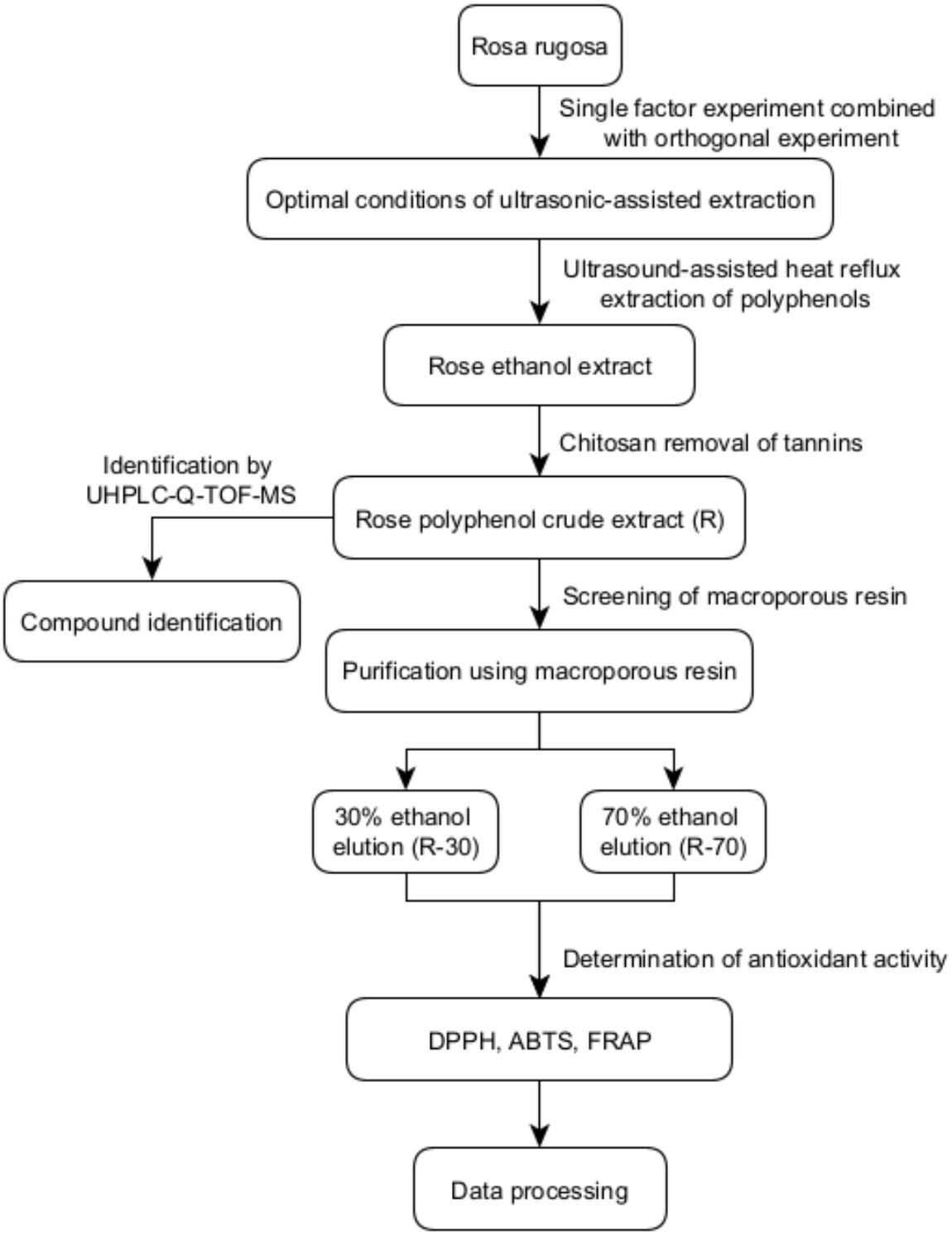
Refer to the method in 2.3.4.2 for determination

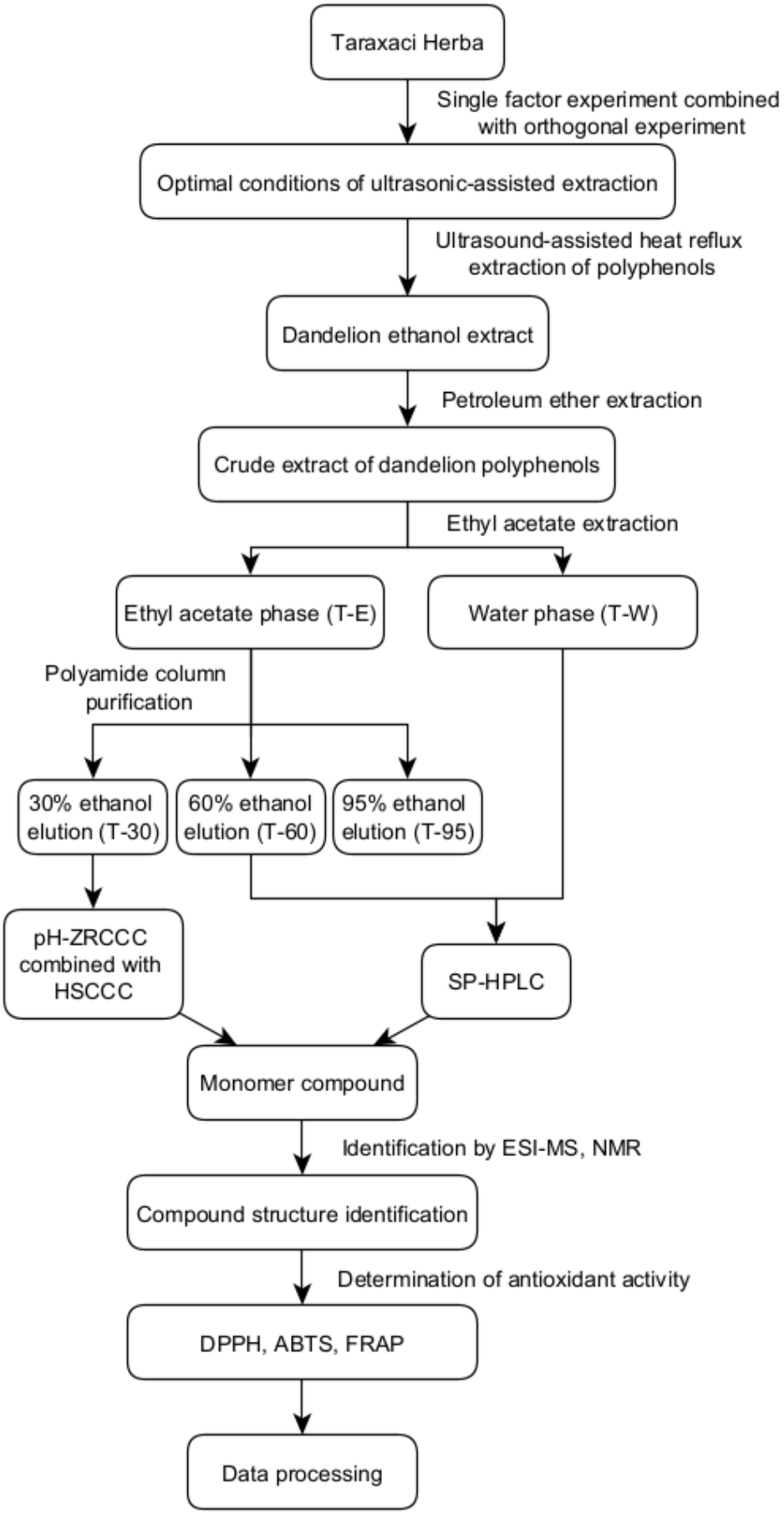
#### 2.4.3.3 Determination of total antioxidant capacity by FRAP method

Refer to the method in 2.3.4.3 for determination

## Conclusions to section 2

Through experimental design, the specific flow chart of the experiment was finally determined:


Rosa rugosa
Single factor experiment combined with orthogonal experiment
Optimal conditions of ultrasonic-assisted extraction
Ultrasound-assisted heat reflux extraction of polyphenols
Rose ethanol extract
Chitosan removal of tannins
Identification by UHPLC-Q-TOF-MS
Rose polyphenol crude extract (R)
Compound identification
Screening of macroporous resin
Purification using macroporous resin
30% ethanol elution (R-30)
70% ethanol elution (R-70)
Determination of antioxidant activity
DPPH, ABTS, FRAP
Data processing


Taraxaci Herba
Single factor experiment combined with orthogonal experiment
Optimal conditions of ultrasonic-assisted extraction
Ultrasound-assisted heat reflux extraction of polyphenols
Dandelion ethanol extract
Petroleum ether extraction
Crude extract of dandelion polyphenols
Ethyl acetate extraction
Ethyl acetate phase (T-E)
Water phase (T-W)
Polyamide column purification
30% ethanol elution (T-30)
60% ethanol elution (T-60)
95% ethanol elution (T-95)
pH-ZRCCC combined with HSCCC
SP-HPLC
Monomer compound
Identification by ESI-MS, NMR
Compound structure identification
Determination of antioxidant activity
DPPH, ABTS, FRAP
Data processing

## Section 3 Results and Analysis

### 3.1 Rose research results

### 3.1.1 Research results of the extraction technology of rose polyphenols

#### 3.1.1.1 Standard curve of gallic acid

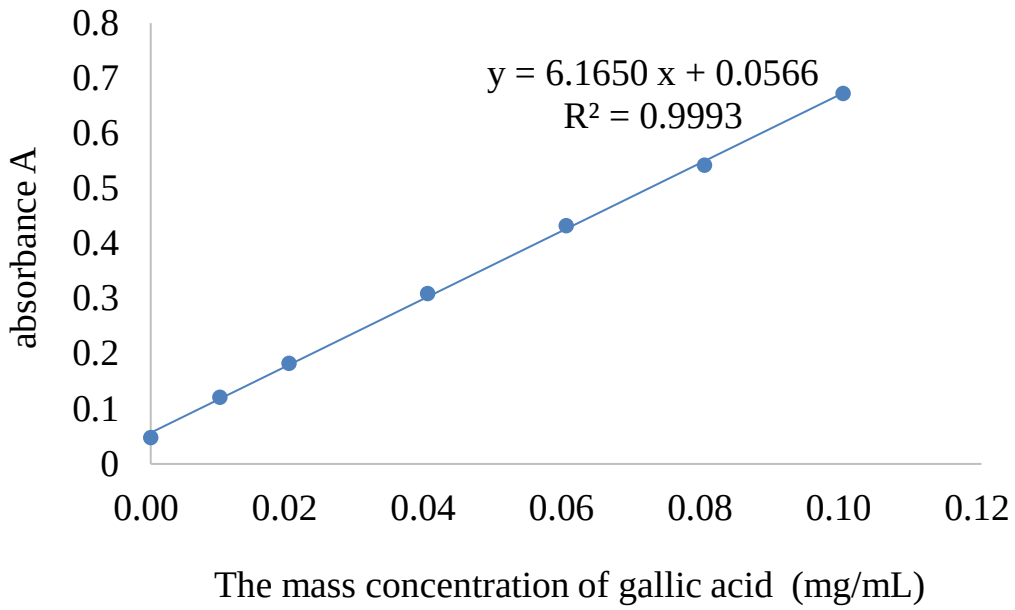



Fig.3-1 Standard curve of polyphenols

According to the method in 2.3.1.2, with the mass concentration of gallic acid (mg/mL) as the abscissa and the absorbance value as the ordinate, the standard curve of gallic acid was drew (Figure 3-1). The content of samples polyphenols were calculated with gallic acid as the reference substance. The linear regression equation of the obtained gallic acid was: Y=6.0903X+0.0619 (Y-absorbance A; X-mass concentration, mg/mL), and the correlation coefficient was $R^2$=0.9993. Experiments shown that within the absorbance range of 0.0-0.67, the gallic acid concentration had a good linear relationship with absorbance. Therefore it could be used to measure and calculate the concentration of polyphenols in rose.

### 3.1.1.2 The influence of ethanol concentration

According to the method of 2.3.1.3, the extraction experiment of rose polyphenol was carried out, and the experimental results were shown in Figure 3-2.

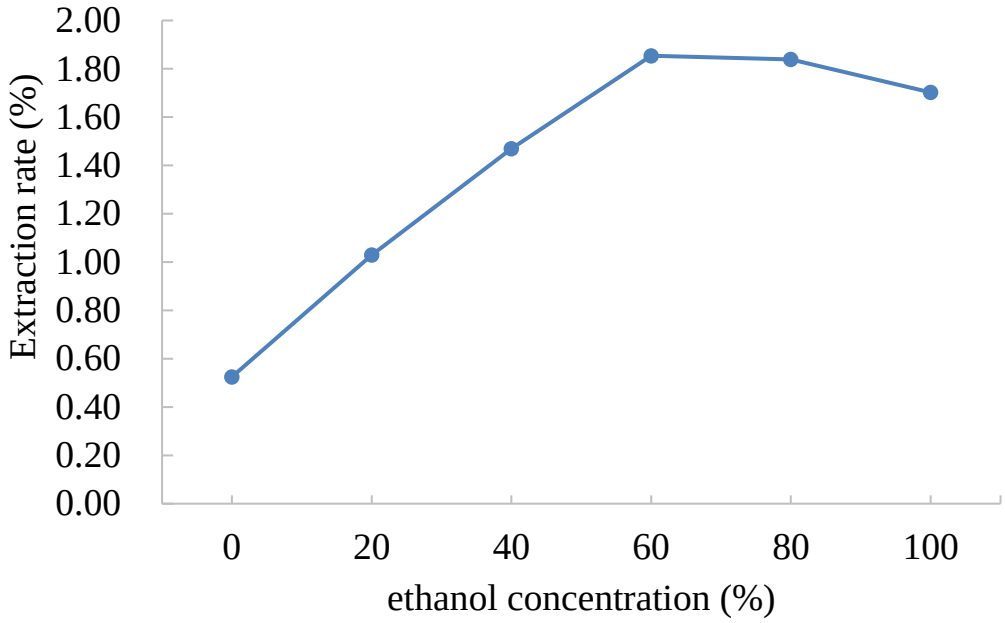



Figure 3-2 The effect of ethanol concentration on the extraction rate of rose polyphenols

Through the Figure 3-2 when the concentration of ethanol increases, the extraction rate of rose polyphenols also increases.But when the concentration was close to 60%, the extraction rate was highest. However, when the ethanol concentration reaches 80%-100%, the extraction rate of polyphenols no longer increased, or even slightly decreased. It was speculated that roses may not only contain polyphenols, but also polysaccharides and proteins. The polysaccharides and proteins on the cell surface might be coagulated by the high concentration of ethanol and form a barrier, thereby limiting the dissolution of rose polyphenols.

Therefore, too high ethanol concentration was not conducive to the extraction of rose polyphenols. The results showed that the extraction of rose polyphenols with 60% ethanol was better.

### 3.1.1.3 The influence of material-to-liquid ratio

According to the method of 2.3.1.3, the extraction experiment of rose polyphenol was carried out, and the experimental results were shown in Figure 3-3.

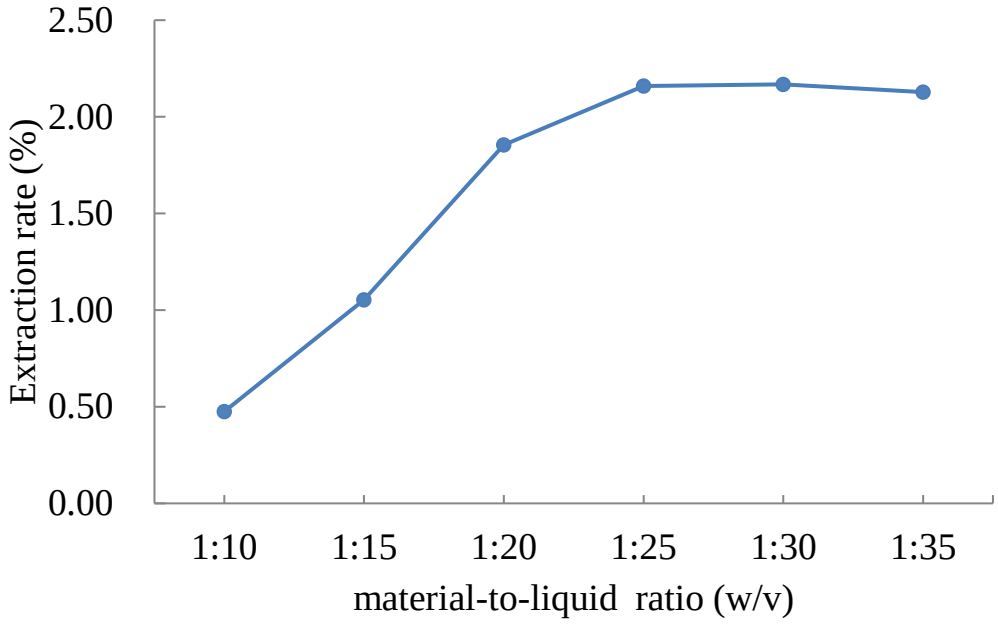



Figure 3-3 The effect of material-to-liquid ratio on the extraction rate of rose polyphenols

As could be seen from Figure 3-3, when the amount of solvent was increased, the extraction rate of polyphenols showed a gradually increasing trend. When the material-to-liquid ratio was 1:25 (w/v), if the solvent ratio was increased, the extraction rate of polyphenols did not increase significantly. First of all,

considering if the material-to-liquid ratio was too large, the cost of extraction would be increased. Second, too much solvent would complicate the later concentration process. Finally, as the amount of solvent increased, the dissolved impurities also increased, which might affect the purification of flavonoids in the later stage. The results showed that it was better to select 1:25 (w/v) material-to-liquid ratio as the extraction solvent for the extraction of rose polyphenols.

**3.1.1.4 The influenceof ultrasound time**

According to the method of 2.3.1.3, the extraction experiment of rose polyphenol was carried out, and the experimental results were shown in Figure 3-4.

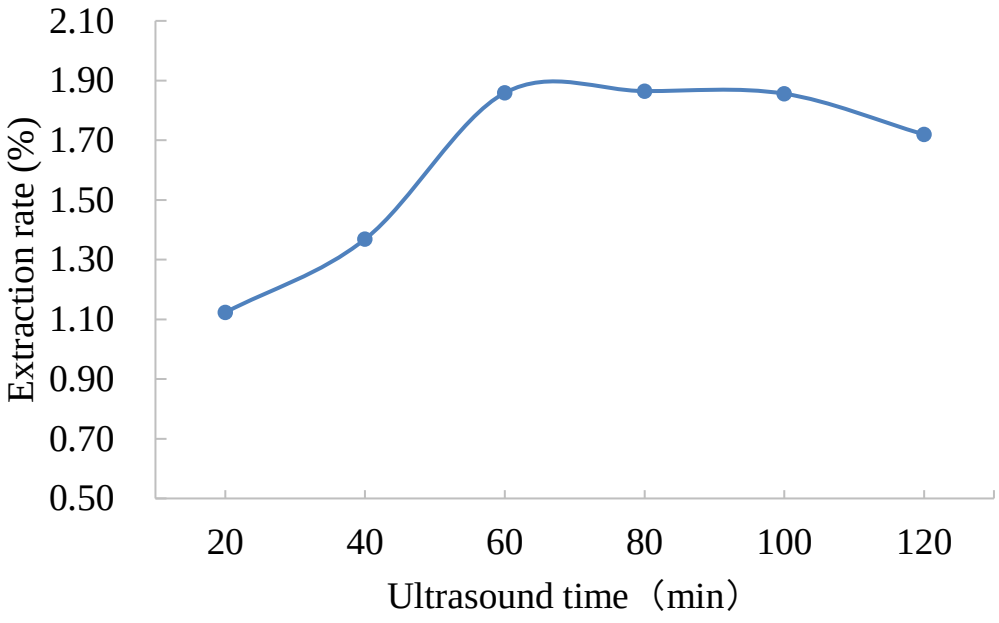



Figure 3-4 The effect of ultrasound time on the extraction rate of rose polyphenols

As could be seen from 3-4, before 60 min, the extraction rate of rose polyphenols showed a significant growth trend with the increase of time. After 60

min, the extraction rate of rose polyphenols did not change much, and even tended to decrease. It was speculated that with the increase of ultrasonic time, the extraction amount of rose polyphenols first increased, but at 60 min, the extraction amount of rose polyphenols reached the maximum value. Moreover if the ultrasound time was extended, due to the influence of light, heat and other factors, some rose polyphenols will be oxidized, which might affect the amount of extraction. Therefore, 60 min was chosen as the best extraction time.

**3.1.1.5 The influenceof ultrasound power**

According to the method of 2.3.1.3, the extraction experiment of rose polyphenol was carried out, and the experimental results were shown in Figure 3-5.

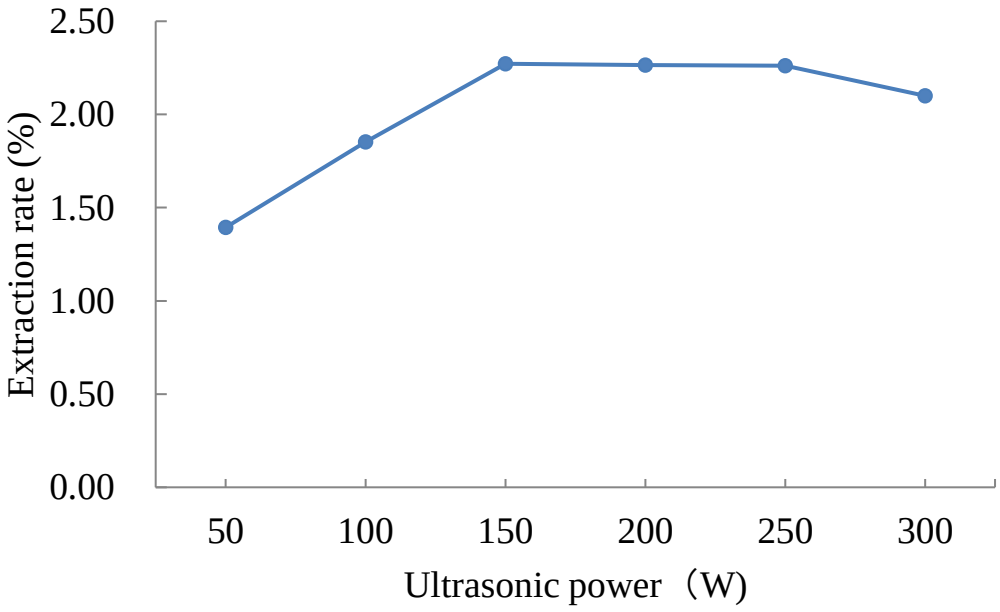



Figure 3-5 The effect of ultrasonic power on the extraction rate of rose polyphenols

As could be seen from Figure 3-5, when the ultrasonic power was increased,

the extraction rate of rose polyphenols was on the rise. When the ultrasonic power reached 150W, the extraction rate reached the maximum. When the ultrasonic power exceeded 150W, the extraction rate began to decrease. As the ultrasonic power increased, the mechanical action and cavitation were significantly enhanced, which made the mediator particles in the rose moved more rapidly. Finally the faster the molecular diffusion on the interface diffusion layer, the greater the amount of polyphenols exuded; but when the ultrasonic power was too high, The attenuation of ultrasonic propagation increased, which would cause the extraction rate to decrease. Therefore, 150W was considered the best extraction power.

**3.1.1.6 Orthogonal test results**

According to the single factor test results, the ethanol volume fraction, extraction temperature, material-to-liquid ratio, and extraction time were selected as the test factors, and the polyphenol content was used as the test index. The $L_9(3^4)$ orthogonal test was used to further optimize the alcohol extraction technology of rose polyphenols. The orthogonal test results were shown in Table 3-1.

Table 3-1 The results of orthogonal test

| | A Ethanol concentration | B Material-to-liquid ratio | C Ultrasound time | D Ultrasonic power | Polyphenol extraction rate（%） |
|---|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 1 | 1.315 |
| 2 | 1 | 2 | 2 | 2 | 2.014 |
| 3 | 1 | 3 | 3 | 3 | 2.226 |

| | | | | | |
|---|---|---|---|---|---|
| 4 | 2 | 1 | 2 | 3 | 1.611 |
| 5 | 2 | 2 | 3 | 1 | 2.059 |
| 6 | 2 | 3 | 1 | 2 | 2.317 |
| 7 | 3 | 1 | 3 | 2 | 1.573 |
| 8 | 3 | 2 | 1 | 3 | 1.993 |
| 9 | 3 | 3 | 2 | 1 | 2.359 |
| $k_1$ | 1.852 | 1.500 | 1.875 | 1.911 | |
| $k_2$ | 1.996 | 2.022 | 1.995 | 1.968 | |
| $k_3$ | 1.975 | 2.301 | 1.953 | 1.944 | |
| R | 0.144 | 0.801 | 0.120 | 0.057 | |

Note: $k_1$, $k_2$, $k_3$- the average value of the sum of the test results of rose polyphenols at corresponding levels, R - the range of rose polyphenols.

As shown in table 3-1, that for factor A, the extraction rate of rose polyphenols was significantly different between $A_1$ and $A_3$, while the difference between $A_2$ and $A_3$ was small, therefore $A_2$ was the best. For factor B, the extraction amount of rose polyphenols differed significantly among various levels, and $B_3$ had the best result. For C factor and D factor, the difference between each level was small, inside $C_2$ and $D_2$ had the best results. Comprehensive comparison showed that the best extraction process for rose polyphenols was: $A_2B_3C_2D_2$, that was, the ethanol concentration at 60%, material-to-liquid ratio was 1:25 (w/v), the microwave power was 150 W, and the extraction time was 60 min.

From the extremely poor results, it could be seen that the primary and

secondary order of the influence of the four factors on the extraction rate of rose polyphenols was B material-to-liquid ratio>A ethanol concentration>C ultrasonic time>D ultrasonic power. In addition, ethanol volume fraction, extraction temperature, material-to-liquid ratio and extraction time all had extremely significant effects on the extraction effect of rose polyphenols.

**3.1.1.7 Verification experiment**

A verification experiment was carried out on the basis of the results of the orthogonal experiment. The rose polyphenols were extracted under the optimal conditions, and 3 sets of parallel experiments were carried out. The extraction rate reached (2.46±0.06)%, which was greater than the maximum value in the orthogonal experiment. The feasibility of this condition was verified.

**3.1.1.8 Comparison of extraction results of rose polyphenols by reflux method and ultrasonic assisted reflux method**

Table 3-2 Comparison of different extraction methods of polyphenols from rose

| No. | Extraction methods | Concentration of extract（mg/ml） | Extraction ratio（%） | Average extraction rate（%） | Standard deviation（%） |
|---|---|---|---|---|---|
| 1 | reflux method | 1.1029 | 2.76 | 2.78 | 0.02 |
| 2 | | 1.1166 | 2.79 | | |
| 3 | | 1.1188 | 2.80 | | |
| 4 | | 1.2730 | 3.18 | 3.21 | 0.02 |

| | | | |
|---|---|---|---|
| 5 | ultrasound-assisted reflux | 1.2798 | 3.20 |
| 6 | | 1.2957 | 3.24 |

In order to compare the effectiveness of the ultrasonic extraction process of rose polyphenols and the conventional heating extraction process, the two processes were compared and verified according to the experimental method of 2.3.1.5. The experimental results were shown in Table 3-2 below.

The experimental results showed that the extraction rate of reflux extraction method was (2.78±0.02) %, and the extraction rate of ultrasonic-assisted reflux method was (3.21±0.02) %. It could be seen from the above data that ultrasound-assisted reflux method had obvious advantages.

### 3.1.2 Isolation and purification by macroporous resin

Rose polyphenols were extracted using the ultrasonic-assisted reflux method according to the optimal extraction conditions. The bold material was processed according to the method of 2.3.2.2, and then sample R was obtained. The content of polyphenols was 11.16mg/ml.

#### 3.1.2.1 Screening of macroporous resin

The method of 2.3.2.3 was used to compare the properties of four kinds of macroporous resins. The result was shown in Figure 3-6. It could be seen from the figure that when the amount of macroporous resin was the same, BS-75 had the strongest adsorption capacity for polyphenols, followed by AB-8. Which two had the smaller difference. The adsorption capacity of X-5 was poor, while the

adsorption capacity of D101 was the worst. Secondly, about the desorption capacity. A-8 and D101 had the best desorption ability, followed by X-5, and finally was BS-75. Through comprehensive comparison of recovery rates, AB-8 was more suitable for the isolation and enrichment of rose polyphenols.

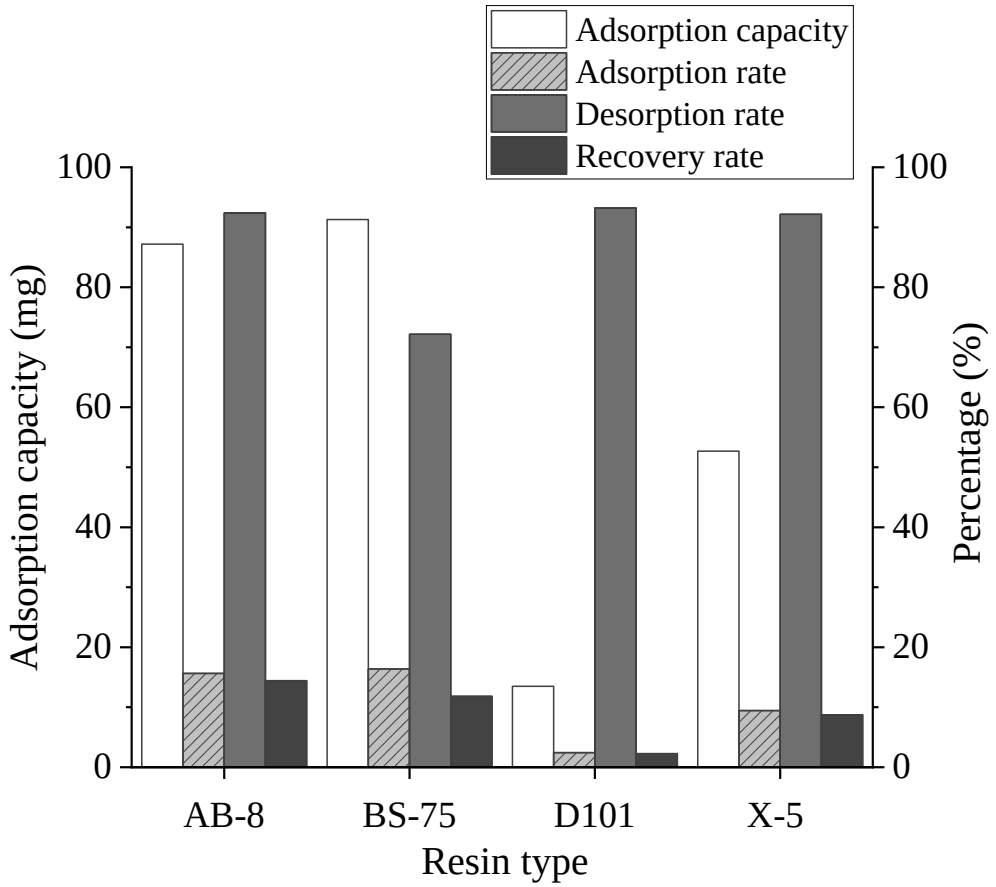



Figure 3-6 Screening results of macroporous resin

### 3.1.2.2 Dynamic adsorption experiment

According to the method of 2.3.2.4, the dynamic adsorption experiment was carried out, and the leakage curve was obtained (Figure 3-7). It could be seen from the figure that the concentration of polyphenols in the eluate of 0-10 tubes was close to 0, and there was a significant leakage of polyphenols in tube 11 (0.210mg/ml). At 13 tubes (120ml), the concentration reached 0.675mg/ml, which

the leakage exceeded 10% of the original mass concentration of rose polyphenol. To ensure that the rose polyphenol could be adsorbed completely, 120mL was finally selected as the best sample amount, namely 2BV .

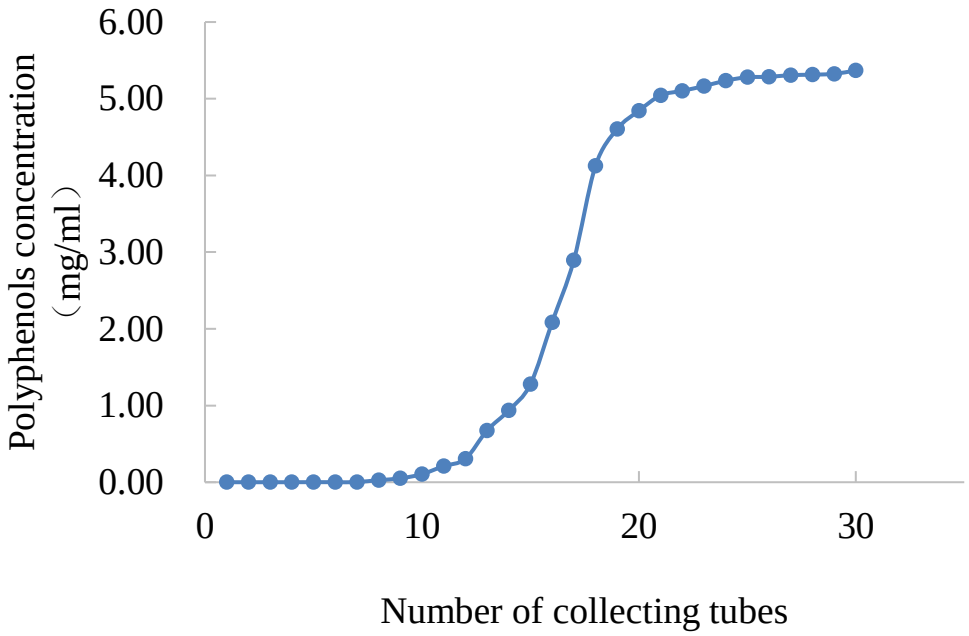



Figure 3-7 The leakage curve of rose polyphenols

### 3.1.2.3 Isolation results

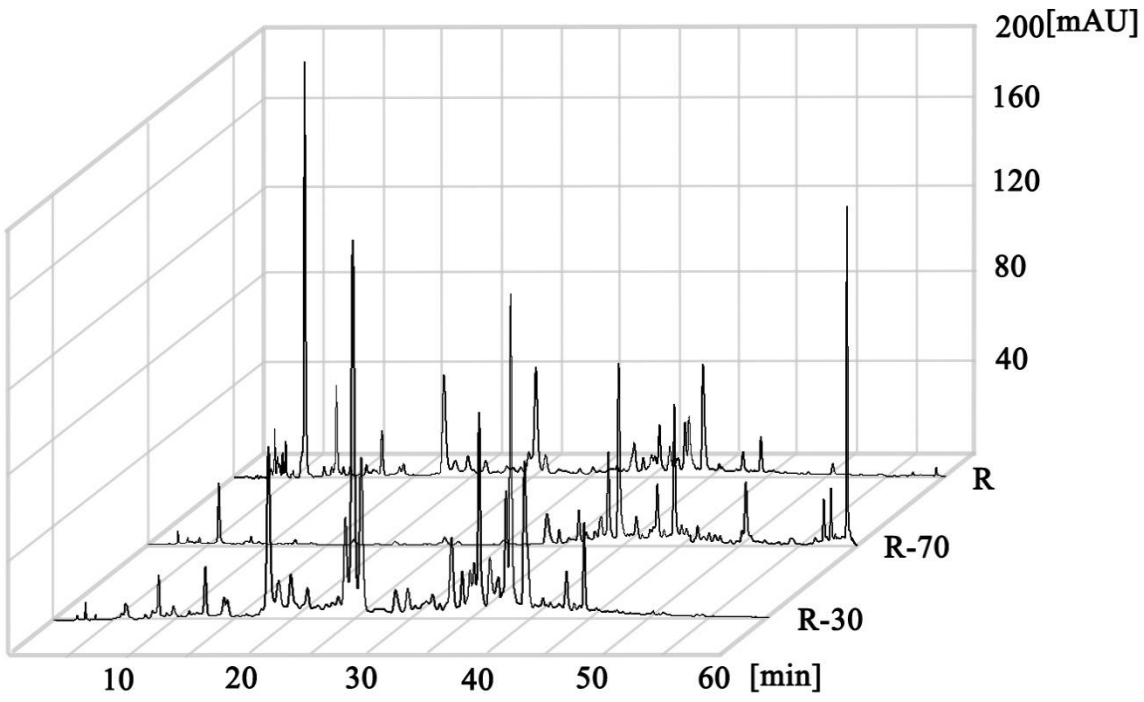
According to the method of 2.3.2.5, select AB-8 for separation. The sample was added to the macroporous resin column at a ratio of 2BV. The final component R-30 eluted with 30% ethanol and the component R-70 eluted with 70% ethanol were isolated. The samples were tested by HPLC using the method 2.3.2.6, and the results were shown in Figure 3-8. It could be seen from the figure that R-30 contains most of the main active ingredients. And the content of each component was significantly improved compared with the total sample R. The low-polarity components were mainly concentrated in R-70, and the enrichment effect was obvious after purification by the macroporous resin. The results showed that the

macroporous resin AB-8 could effectively isolate and purify rose polyphenols.

Figure 3-8 HPLC analysis diagram of each fraction after column separation

### 3.1.3 Mass spectrometry identification results

Figure 3-9 was a liquid chromatogram of the analysis of polyphenols in the total sample R of roses. By analyzing the mass spectrum data and the UV absorption characteristics of each peak, combined with relevant literature data, infer the polyphenols contained therein. Ten polyphenols had been identified,




and the specific identification results were shown in Table 3-3.

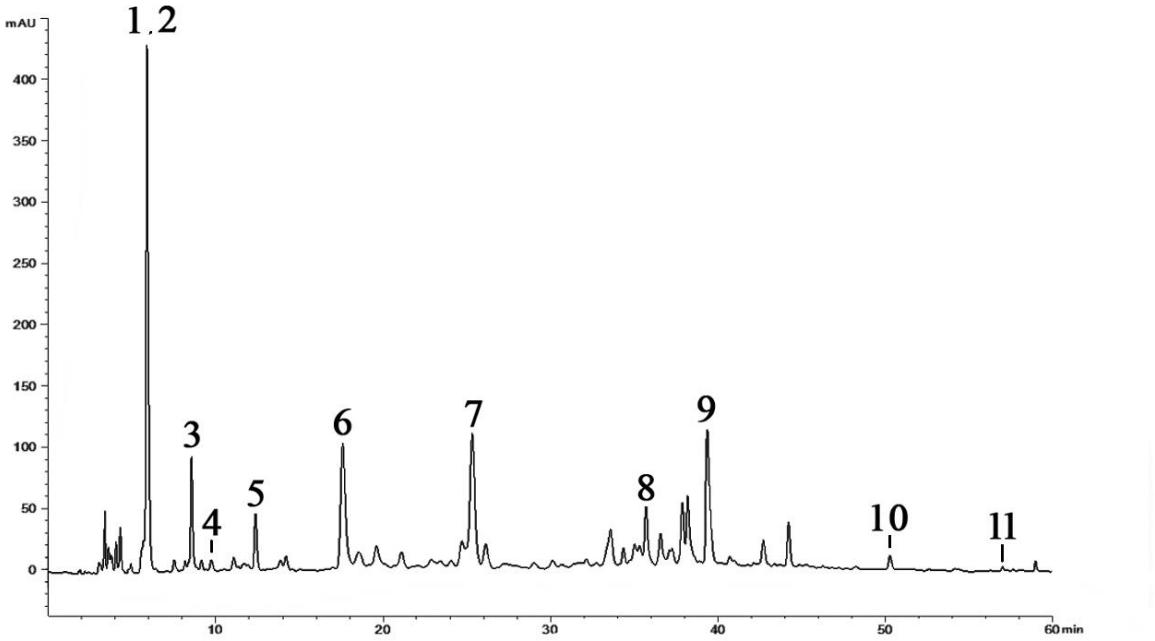



Figure 3-9 HPLC chromatogram of rose ( 280 nm)

Table 3-3 LC-MS analysis result of rose

| Common peaks | $t_R$ (min) | [M-H]- | Formula | Peak identification |
|---|---|---|---|---|
| 1 | 5.7 | 191.0523 | $C_7H_{11}O_6$ | Quinic acid |
| 2 | 6.0 | 169.0123 | $C_7H_5O_5$ | Gallic acid |
| 3 | 8.7 | 483.0747 | $C_{20}H_{19}O_{14}$ | Di-O-galloyl-glucoside |
| 4 | 10.1 | 289.0423 | $C_{34}H_{23}O_{22}$ | Bis-HHDP-glucoside |
| 5 | 12.8 | 633.0677 | $C_{15}H_{13}O_6$ | Catechin |
| 6 | 18.1 | 785.0768 | $C_{34}H_{25}O_{22}$ | Di-O-galloyl-HHDPglucoside |
| 7 | 25.0 | 785.0771 | $C_{34}H_{25}O_{22}$ | Di-O-galloyl-HHDPglucoside |
| 8 | 35.0 | 633.0678 | $C_{27}H_{21}O_{18}$ | Galloyl-HHDP-glucoside |
| 9 | 39.6 | 300.9974 | $C_{14}H_5O_8$ | Ellagic acid |

| | | | | |
|---|---|---|---|---|
| 10 | 50.4 | 497.0315 | - | Unknown |
| 11 | 56.9 | 593.1246 | $C_{27}H_{30}O_{15}$ | Kaermpferol-3-O-rutinoside |

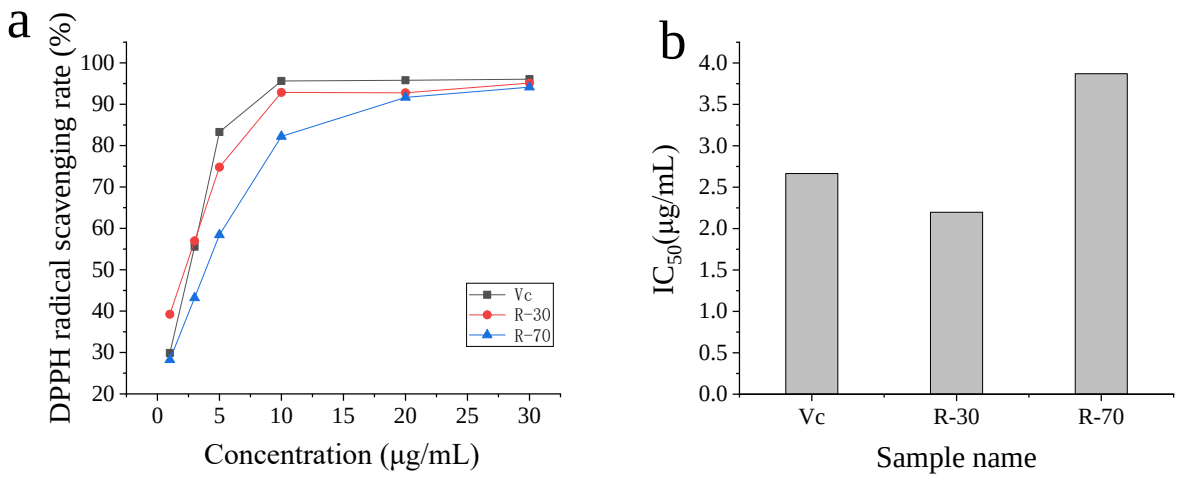
### 3.1.4 Results of determination of rose antioxidant activity

In order to evaluate and compare the antioxidant activity of roses separated and purified by macroporous resin, two isolated components R-30 and R-70 were tested.

#### 3.1.4.1 DPPH free radical scavenging ability

Samples R-30 and R-70 and $V_C$ were measured according to the method of 2.3.4.1, and the results were shown in Figure 3-10. According to Figure 3-10 a, it could be seen that in the concentration range of 0-30 μg/mL, the ability of the two samples and the positive control $V_C$ to scavenge DPPH free radicals increases as the concentration increases. Show that they had a certain dose effect. Among them, the activity of R-30 in scavenging DPPH free radicals was similar to that of the positive control $V_C$, and higher than that of R-70. The $IC_{50}$ of $V_C$, R-30 and R-70 were 2.66 μg/mL, 2.20 μg/mL, 3.87 μg/mL, respectively (Figure 3-10 b).

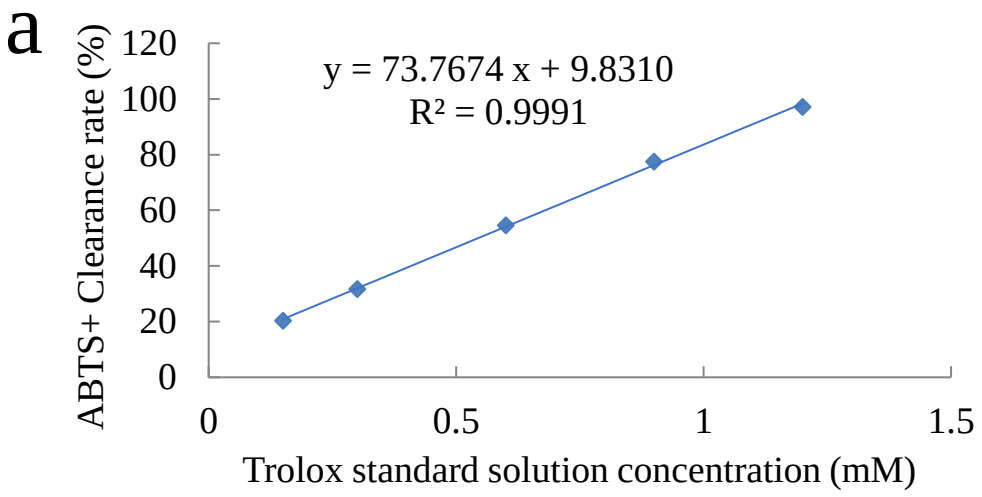
Figure 3-10 The measurement result of DPPH free radical scavenging ability.



(a) Rose polyphenols and $V_c$ on the scavenging rate of DPPH free radicals, (b) Rose polyphenols and $V_c$ in addition to DPPH free radical half scavenging rate $IC_{50}$.

### 3.1.4.2 ABTS free radical scavenging ability

Two samples and Trolox were tested according to the method of 2.3.4.2 for the scavenging ability of ABTS free radical cations. With Trolox as the positive control, the standard curve y=73.7674x+9.8310, $R^2$=0.9991 (Figure 3-11 a) was drawn. The results of the ABTS free radical scavenging activity of the samples were represented by the Trolox equivalent antioxidant capacity (TEAC) (Figure 3-11 b). It could be seen from the figure that both samples had a certain scavenging effect on ABTS free radicals. At the same concentration, the scavenging activity of sample R-30ABTS+ was higher than that of R-70. The TEAC of R-30 was 5.37 mmol/g and R-70 was 1.83 mmol/g.



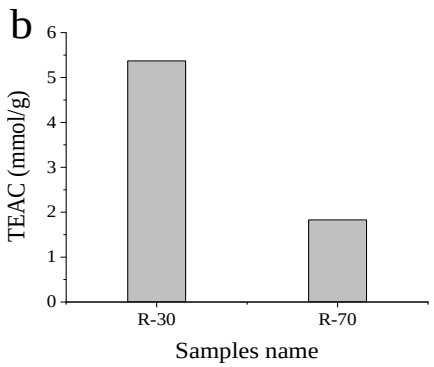



Figure 3-11 Measurement results of ABTS radical scavenging ability. (a) Trolox standard curve, (b) Trolox equivalent antioxidant capacity of rose polyphenols.

### 3.1.4.3 Determination of total antioxidant capacity by FRAP method

The reduction potential of an antioxidant reaction with the TPTZ–Fe (III) complex was measured through 2.3.4.3 method, producing a TPTZ–Fe (II) complex, which was adopted in this study. Using FeSO4 to draw the standard curve,and the linear equation $y = 0.3364 x + 0.0107$, $R^2 = 0.9992$ was obtained (Figure 3-12 a).The $FeSO_4$ values were calculated by the standard curve and linear equation (Figure 3-12 b). The $FeSO_4$ value was higher, the iron reduction ability was better. Therefore, the antioxidant capacity of sample R-30 was stronger than that of R-70, which was the same as the results of DPPH and ABTS. The total antioxidant capacity of R-30 was 0.73 mM Fe(II)/g DW, and that of R-70 was 0.43 mM Fe(II)/g DW.

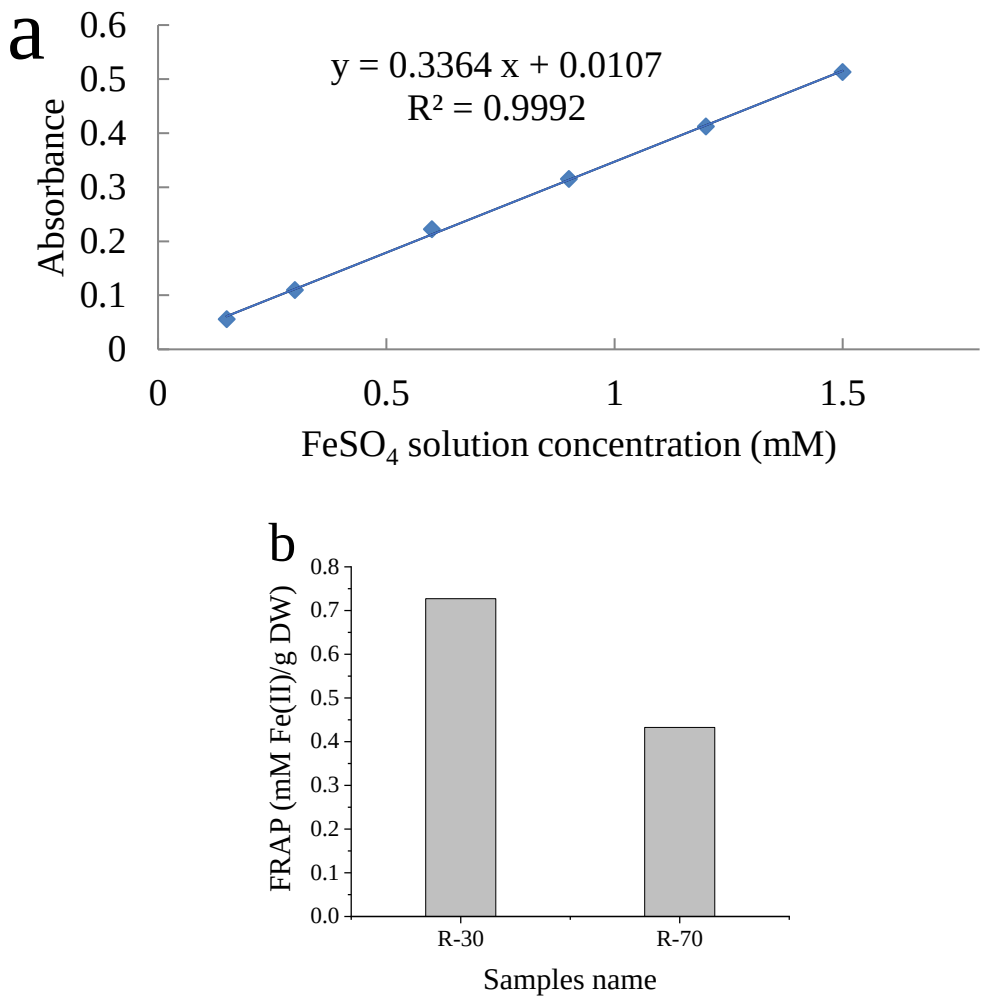



Figure 3-12 FRAP method to determine the results of total antioxidant capacity. (a) FeSO4 standard curve, (b) The antioxidant activity of rose polyphenols were expressed by the concentration of FeSO4 standard solution.

## 3. 2 Dandelion research results

### 3.2.1 Extraction results of dandelion polyphenols

Through screening, the final selection of ethanol concentration was 60%, material-to-liquid ratio was 1:25 (w/v), and under the condition of ultrasonic power of 200W, 1kg of dandelion was ultrasonically assisted for reflux extraction for 60min. The obtained dandelion alcohol extraction was first extracted by petroleum ether according to the method of 2.4.1, and the total sample of the crude

dandelion crude extract was obtained. Then the total sample was extracted with ethyl acetate, and the two components T-W and T-E were obtained. The total sample and T-W, T-E were analyzed by HPLC according to the method of 2.4.2.5, and the results were shown in Figure 3-13. According to the HPLC results, the components in the sample T-W obtained by concentration in the aqueous phase after extraction were relatively simple. Therefore, it could be directly separated and prepared by SP-HPLC. The active ingredients contained in T-E obtained by concentrating the ethyl acetate phase were complicated. In order to make the subsequent separation more effective, a polyamide column was used to further process the T-E.

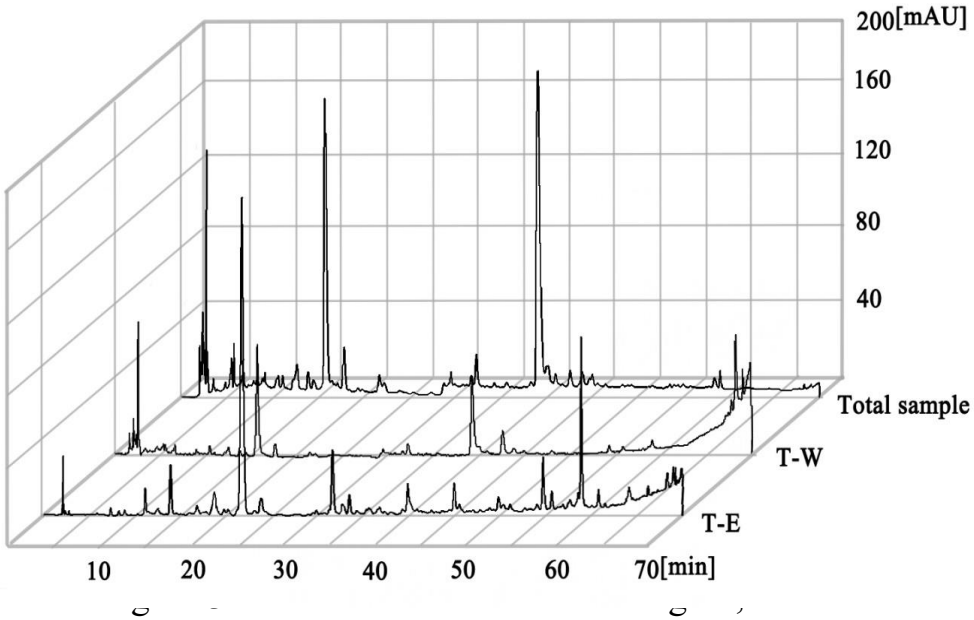



Figure 3-13 H

Sample T-E

column according

obtained. The co

The results were

seen that the content of the effective components in the 95% ethanol elution part was low, and it was of little significance to separate and prepare again. Therefore, the 30% and 60% ethanol elution parts were collected for further separation and purification. Among them, the sample T-30, which was eluted and concentrated

with 30% ethanol, had complex components and contained many kinds of compounds. So it was separated and purified by countercurrent chromatography which had a relatively large amount of preparation. Sample T-60, which was eluted and concentrated with 60% ethanol, had a relatively simple composition and the main components had similar polarities. So it could be directly purified and separated by semi-preparative liquid chromatography.

Figure 3-14 HPLC analysis diagram of each fraction after column separation

### 3.2.2 Separation and purification of polyphenols in dandelion by countercurrent chromatography combined with semi-preparative high performance liquid chromatography

#### 3.2.2.1 Separation of pH zone countercurrent chromatography

pH-ZRCCC has a large amount of preparation and is suitable for components with a concentration greater than 0.1 mmol, preferably greater than 1 mmol [101]. Therefore, the first choice of pH-zone countercurrent chromatography was used to separate the higher content of polyphenol components in T-30, and at the same time to enrich the trace components.

Table 3-4 Distribution coefficients of target compound I-VI in solvent system $K_{acid}$ and $K_{base}$

| K | Target compound | | | | | |
|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI |
| $K_{acid}$ | 29.92 | 6.43 | 9.29 | 4.61 | 0.20 | 1.94 |

| | | | | | | |
|---|---|---|---|---|---|---|
| $K_{base}$ | 0.00 | 0.75 | 0.00 | 0.05 | 0.02 | 0.00 |

Proper partition coefficient (K) is the key to successful separation of pH-zone countercurrent chromatography. When separating acidic substances, $K_{base} \ll 1$ and $K_{acid} \gg 1$ of the solvent system were required, and the sample should have good solubility in the solvent system [102]. The solvent system ethyl acetate-n-butanol-water (4:1:5, v/v/v) was select according to literature reports and the experience of this laboratory. Then the partition coefficients $K_{acid}$ and $K_{base}$ of the target compound in the solvent system were determined according to the method of 2.4.2.1. The measurement results were shown in Table 3-4. The results showed that this solvent system was suitable for the separation of this sample. So ethyl acetate-n-butanol-water (4:1:5, v/v/v) was used as the solvent system. The upper phase was added trifluoroacetic acid (10 mM) as the stationary phase. The lower phase was added ammonia water (10 mM) as the mobile phase. The solvent system was used to separate 1.6 g T-E according to the method of 2.4.2.3. As shown in Figure 3-15, the target compound was eluted in the form of irregular rectangular peaks. The retention rate of the stationary phase was 61.1%. According to HPLC detection, compounds I and II were successfully separated within 10 hours, and the purity was 98.1% and 98.8%. But the resolution of other compounds was bad, so they were combined into M-A. The liquid chromatogram was shown in Figure 3-16. After freeze-drying each component sample, 60.2 mg of compound I, 6.3 mg of compound II and 590 mg of M-A were obtained.

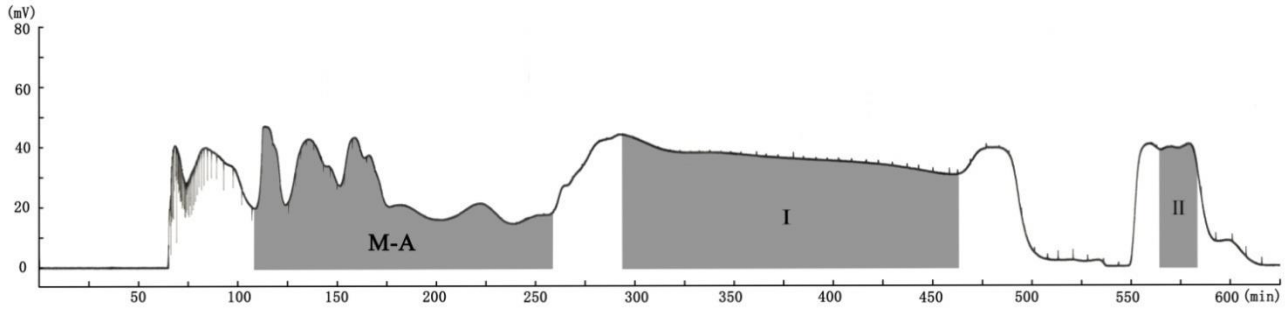



Figure 3-15 pH-ZRCCC separation diagram of crude extract of Dandelion. Solvent system:ethyl acetate-n-butanol-water (4:1:5, v/v/v); TFA (10mM) in upper organic phase, ammonia (10mM) in lower aqueous phase; revolution speed: 800 rpm; flow-rate: 2.0mL/min; sample size: 1.6g; UV detection wavelength: 280nm.

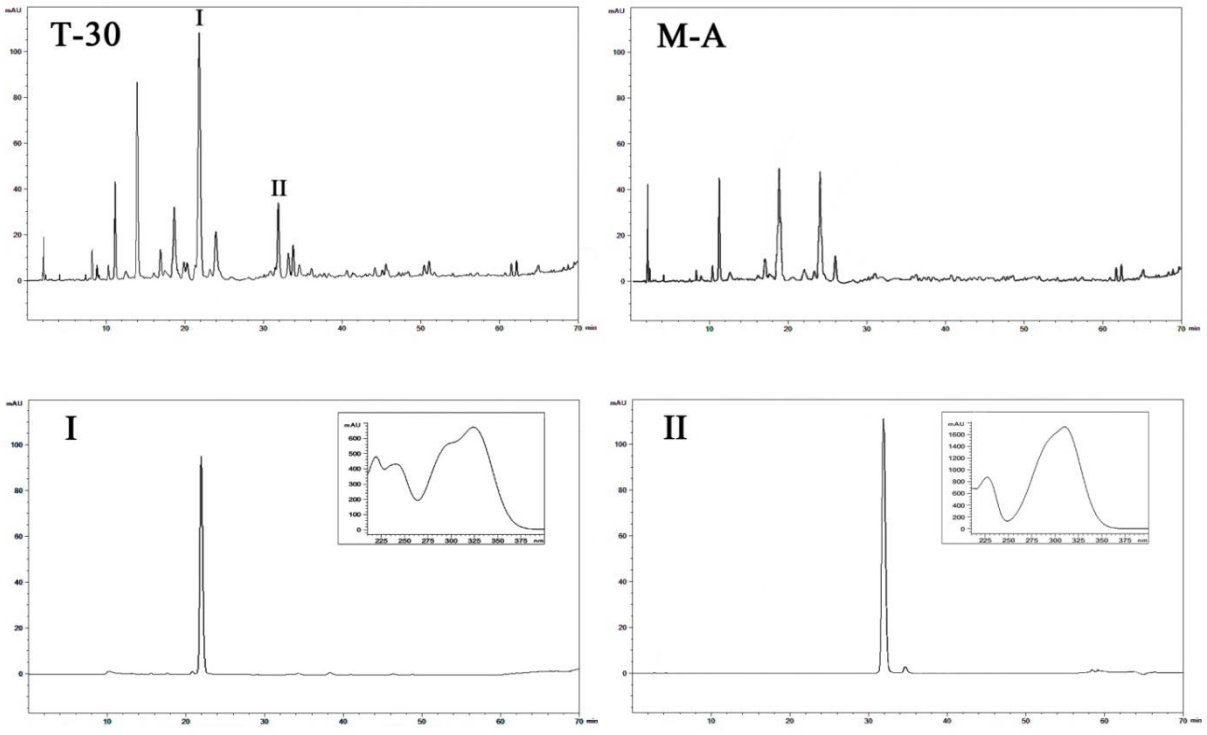



Figure 3-16 HPLC chromatograms of dandelion bold sample T-30 and three components separated by pH-ZRCCC. (T-30) Sample T-30; (M-A) Mixture M-

A; (I) Caffeic acid; (II) *p*-hydroxycinnamic acid.

### 3.2.2.2 Separation of high-speed countercurrent chromatography

After M-A was separated by pH zone countercurrent chromatography, the separation effect could not be achieved. It was speculated that its acidity and alkalinity might be similar or the content of components was small, so it was suitable for the application of high-speed countercurrent chromatography to separate it again.

The key to the separation of high-speed countercurrent chromatography is the choice of the solvent system. The K value of the target compound in the solvent system should be between 0.5 and 2.0 [101]. First, the commonly used solvent system ethyl acetate-acetonitrile-water (4:1:5, v/v/v) was selected, and it was found that the K value of the target compound in this solvent system was far greater than 2.0. It was speculated that the polarity of the target compound might be low, so petroleum ether was added to the solvent system to adjust the polarity of the solvent system. Finally, petroleum ether-ethyl acetate-acetonitrile-water (1:4:1:4, v/v/v/v) was selected as the solvent system, and the K values of compounds III, IV, V, and VI were determined to be 1.03, 0.97, 0.86, 0.78,which were in line with the selection range of the solvent system. Therefore, petroleum ether-ethyl acetate-acetonitrile-water (1:4:1:4, v/v/v/v) was selected as the solvent system for HSCCC separation. And 400mg M-A was separated by the method of 2.4.2.3. The results were shown in Figure 3-17. M-A was separated within 6.5 h, and the retention rate of the stationary phase was 69.4%. Detected by HPLC,

compound III (7.7 mg, purity 82.2%), compound IV (5.4 mg, purity 24.8%), compound V (6.2 mg, purity 95.3%), compound VI (3.4 mg, purity 89.0%). After purification by semi-preparative high performance liquid chromatography, the purity could reach more than 90%. The liquid chromatogram was shown in Figure 3-18, and multiple enrichments were used for subsequent structural identification.

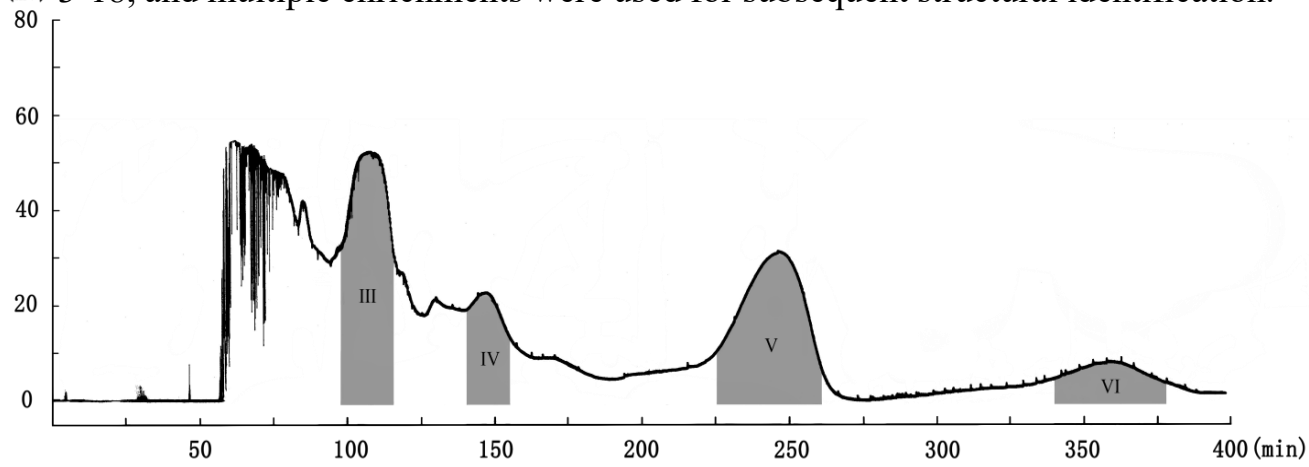


Figure 3-17 HSCCC separation diagram of recovered components of Dandelion. Solvent system:petroleum ether-ethyl acetate-acetonitrile-water (1:4:1:4, v/v/v/v); revolution speed: 800 rpm; flow rate:2.0mL/min; sample size: 400mg; UV detection wavelength: 280nm.

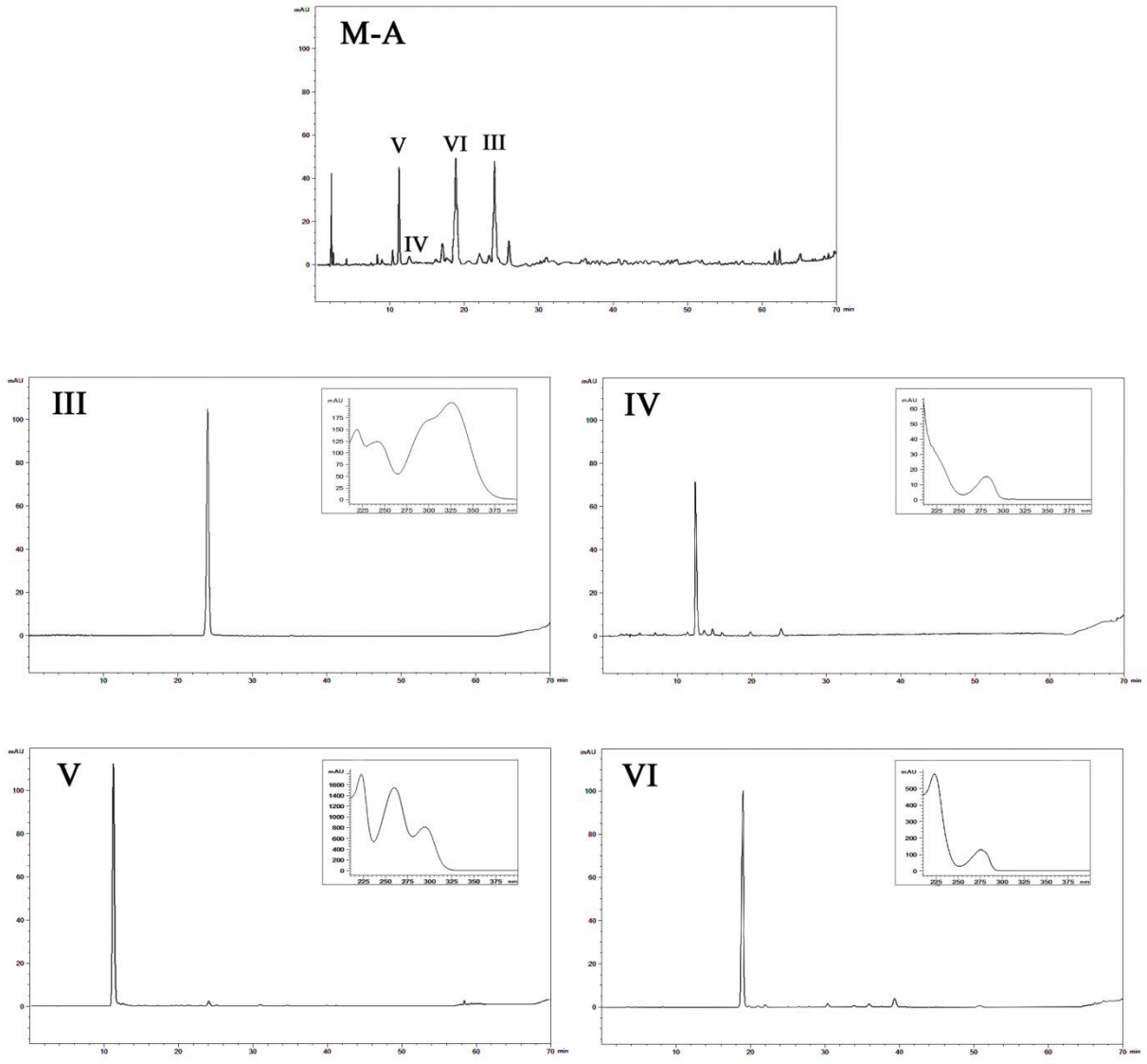



Figure 3-18 HPLC chromatograms of Mixture M-A and components separated by HSCCC. (M-A) Mixture M-A; (III) 1-O-caffeoylglycerol; (IV) 3,4-dihydroxyphenylacetic acid; (V) protocatechin; (VI) *p*-hydroxyphenylacetic acid.

**3.2.2.3 Separation of semi-preparative high performance liquid**

**chromatography**

(1) Composition of mobile phase

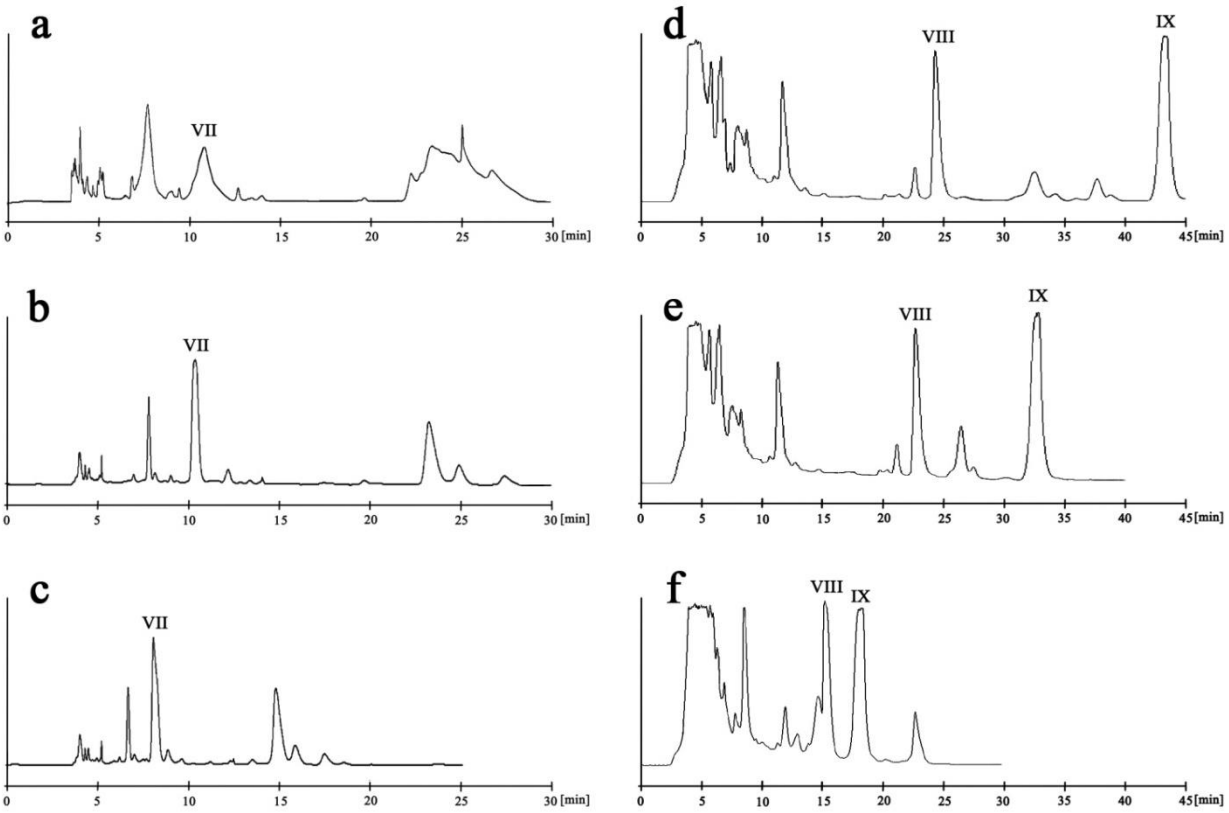



Figure 3-19 Effects of composition of mobile phase on separation. (a) Sample T-W, methanol-water (28:72, v/v); (b) Sample T-W, methanol-0.1% formic acid water (28:72, v/v); (c) Sample T-W, methanol-0.1% formic acid water (32:68, v/v); (d) Sample T-60, methanol-water (55:45, v/v); (e) Sample T-60, methanol-water (58:43, v/v); (f) Sample T-60, methanol-water (60:40, v/v)

For sample T-W, when methanol-water (28:72, V/V) was used as the mobile phase, the target peak tailing in the sample was severe and the resolution between peaks was not good (Figure 3-19 a). Considering that the target compound was a polyphenol compound, it was more stable in an acidic system. Therefore, formic

acid was added to the water to prepare a 0.1% formic acid aqueous solution. When methanol-0.1% formic acid water (28:72, V/V) was used as the mobile phase, the target compound was separated within 20 minutes, and the resolution was good (Figure 3-19 b). With the same injection volume, the methanol ratio was increased to 68%, and the resolution between the target peak and adjacent impurity peaked begins to decrease (Figure 3-19 c). In order to the purity of the separated compounds, methanol-0.1% formic acid water (28:72, V/V) was selected as the mobile phase to separate the T-W.

For sample T-60, with the same injection volume, when the mobile phase was methanol-water (55:45, V/V), better separation results could be achieved, but the separation time was longer (Figure 3-19 d). The proportion of methanol was Increased in the mobile phase could significantly shorten the separation time. When 58% methanol was used as the mobile phase, the main target compounds have a good separation effect, and they can be separated within 35 min (Figure 3-19 e). While the methanol ratio was continued to increase , the resolution between the target peak and adjacent impurity peaked began to decrease (Figure 3-19 f). In order to ensure the purity of the separated compounds, methanol-water (58:42, V/V) was selected as the mobile phase to separate the T-60.

(2) Injection volume

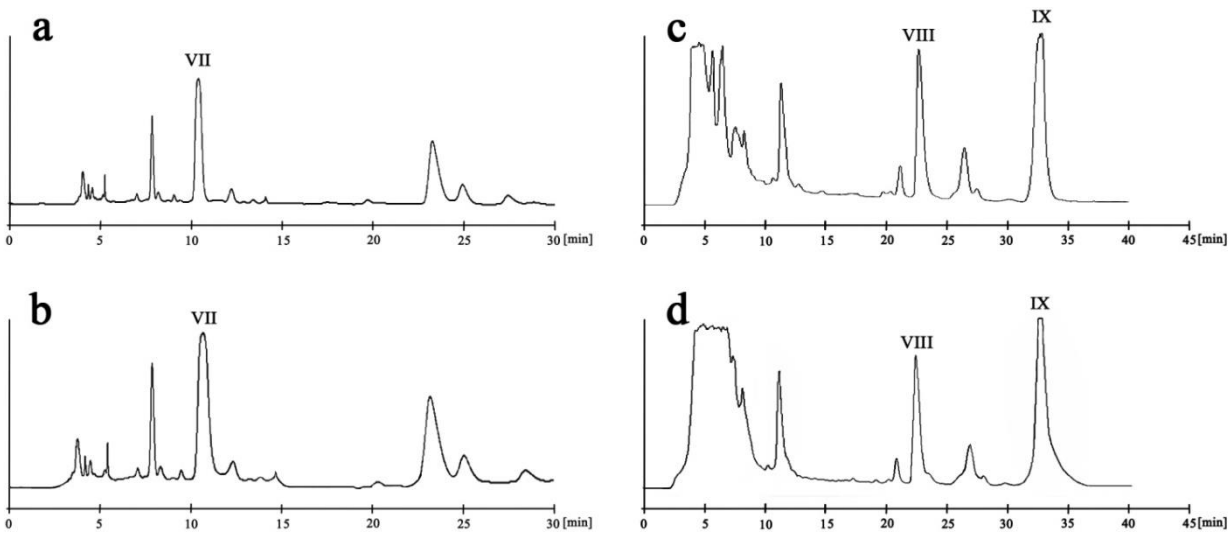



Figure 3-20 Effects of sampling amount on separation. (a) T-W, 50 μL; (b) T-W, 80 μL; (c) T-60, 50 μL; (d) T-60, 80μL.

The sample concentration was fixed to 3 mg/mL. By the injection volume was gradually increased, the effect of the injection volume on the separation effect was investigated. The results were shown in Figure 3-20. It could be seen from the figure that when the sample volume was 50 μL, both samples had a good separation effect. After the injection volume was increased, the separation between two adjacent components decreased, and the adjacent components could not be separated. Therefore, considering the purity and separation efficiency of the obtained product, the injection volume was selected as 50 μL.

(3) Semi-preparative high performance liquid chromatography separation results

According to the selected mobile phase, the samples T-W and T-60 were prepared by semi-preparative high performance liquid chromatography. The collected components were tested by HPLC, and the liquid chromatogram was shown in the Figure 3-21. The components were concentrated under reduced pressure and dried in vacuo to finally obtain compound VII (32.8 mg, purity 98.2%), compound VIII (20.4 mg, purity 96.9%), and compound IX (28.1 mg, purity 91.2%).

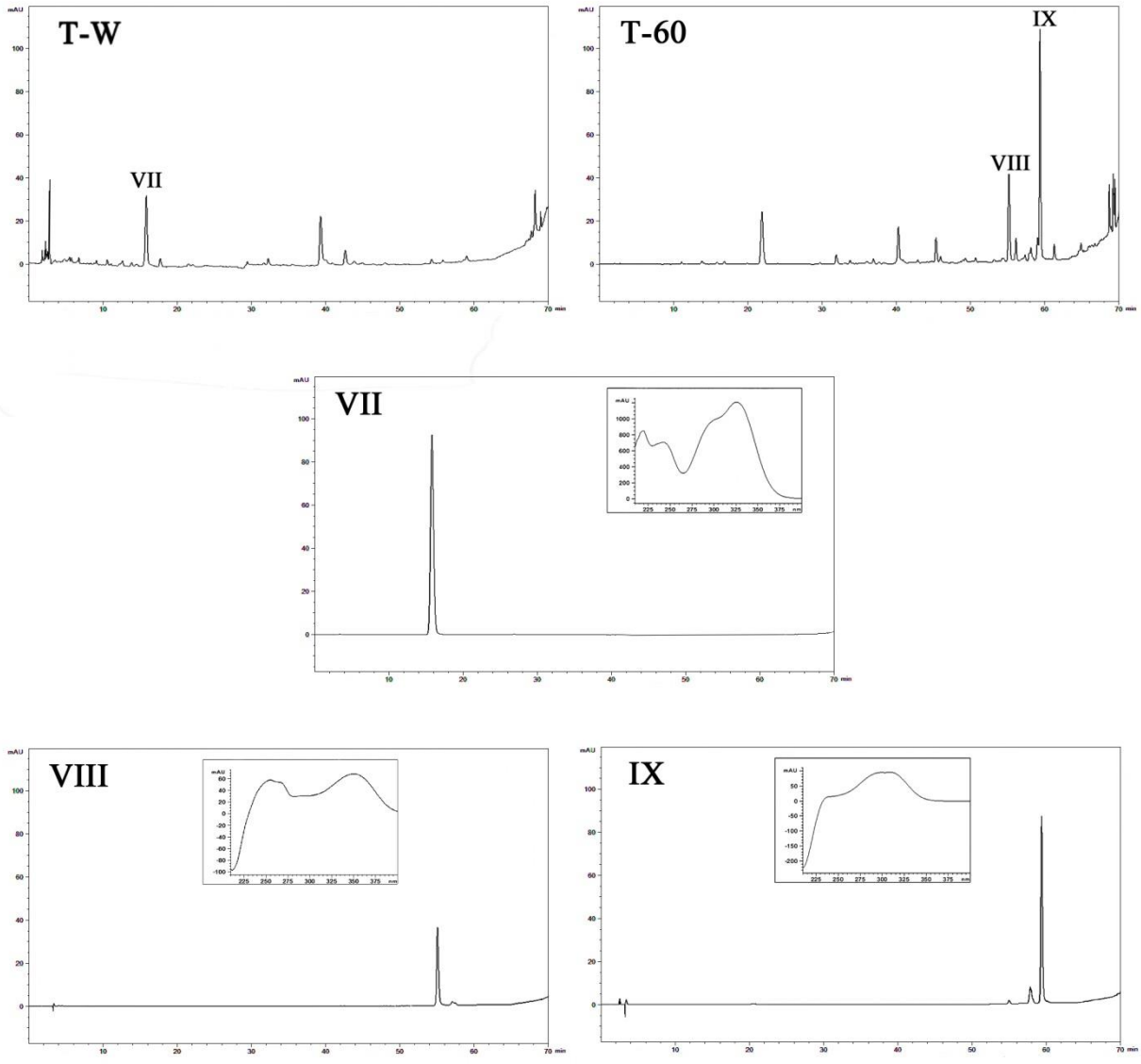


Figure 3-21 HPLC chromatograms of sample T-W, T-60 and components separated by Pre-HPLC. (T-W) Sample T-W; (T-60) Sample T-60; (VII) Chlorogenic acid; (VIII) Luteolin; (IX) Unknown

### 3.2.2.4 Structural identification of compounds

Compound I: ESI-MS, *m/z*:179.0332[M-H]$^-$. $^1$H NMR (400 MHz, DMSO) δ 9.79 (s, 3H), 7.41 (d, J = 15.9 Hz, 1H), 7.03 (s, 1H), 6.76 (d, J = 8.1 Hz, 1H), 6.17 (d, J = 15.9 Hz, 1H). $^{13}$C-NMR (100 MHz, DMSO) δ 169.35 (s), 148.64 (s), 146.30 (s), 143.50 (s), 126.40 (s), 121.16 (s), 117.61 (s), 116.38 (s), 115.06 (s). It is consistent with the caffeic acid data reported in the literature [103], so the compound I was identified as caffeic acid.

Compound II: ESI-MS, *m/z*:163.0288[M-H]$^-$ . $^1$H NMR (400 MHz, DMSO) δ:7.49 (t, *J* = 11.7 Hz, 3H), 6.79 (d, *J* = 8.5 Hz, 2H), 6.29 (d, *J* = 15.9 Hz, 1H), 1.23 (s, 1H).$^{13}$C NMR (100 MHz, DMSO) δ 159.88 (s), 144.27 (s), 130.48 (s), 116.20 (s). It is consistent with the data of *p*-hydroxycinnamic acid reported in the literature [104], so compound II is identified as *p*-hydroxycinnamic acid.

Compound III: ESI-MS, *m/z*:253.0653[M-H]$^-$.$^1$H NMR (400 MHz, DMSO) δ 7.49 (d, *J* = 15.9 Hz, 1H), 7.05 (s, 1H), 7.00 (d, *J* = 8.2 Hz, 1H), 6.77 (d, *J* = 8.1 Hz, 1H), 6.26 (d, *J* = 15.9 Hz, 1H), 4.15 (dd, *J* = 11.2, 4.0 Hz, 1H), 4.00 (dd, *J* = 11.1, 6.5 Hz, 1H), 3.75 – 3.66 (m, 1H), 1.24 (s, 2H).$^{13}$C NMR (100 MHz, DMSO) δ 167.09 (s), 148.92 (s), 146.08 (s), 145.55 (s), 125.96 (s), 121.80 (s), 116.24 (s), 115.23 (s), 114.46 (s), 69.90 (s), 66.08 (s), 63.17 (s). It is consistent with the 1-O-caffeoylglycerol data reported in the literature [105], so compound III is identified

as 1-O-caffeoylglycerol.

Compound IV: ESI-MS, *m/z*:167.0277[M-H]$^-$. $^1$H NMR (600 MHz, DMSO) δ 8.84 (s, 1H), 6.64 (d, *J* = 2.6 Hz, 2H), 6.63 (s, 1H), 6.48 (dd, *J* = 8.0, 2.1 Hz, 1H), 3.32 (s, 3H).$^{13}$C NMR (150 MHz, DMSO) δ 173.77 (s), 145.43 (s), 144.43 (s), 126.32 (s), 120.46 (s), 117.13 (s), 115.79 (s). It is consistent with the data of 3,4-dihydroxyphenylacetic acid reported in the literature [106], so the compound IV is identified as 3,4-dihydroxyphenylacetic acid.

Compound V: ESI-MS, *m/z*:153.0089[M-H]$^-$.$^1$H NMR (400 MHz, DMSO) δ 9.46 (s, 2H), 7.33 (s, 1H), 7.31 – 7.25 (m, 1H), 6.78 (d, *J* = 8.2 Hz, 1H).$^{13}$C NMR (100 MHz, DMSO) δ 167.77 (s), 150.45 (s), 145.34 (s), 122.34 (s), 122.14 (s), 117.01 (s), 115.60 (s). It is consistent with the protocatechin data reported in the literature [107], so compound V is identified as protocatechin.

Compound VI: ESI-MS, *m/z*:151.0288[M-H]$^-$。$^1$H NMR (400 MHz, DMSO) δ 12.15 (s, 1H), 9.27 (s, 1H), 7.03 (d, *J* = 8.3 Hz, 1H), 6.69 (d, *J* = 8.3 Hz, 1H).$^{13}$C NMR (100 MHz, DMSO) δ 173.59 (s), 156.50 (s), 130.71 (s), 125.60 (s), 115.48 (s). It is consistent with the data of *p*-hydroxyphenylacetic acid reported in the literature [108], so compound VI is identified as *p*-hydroxyphenylacetic acid.

Compound VII: ESI-MS, *m/z*:352.98[M-H]$^-$. $^1$H NMR (400 MHz, DMSO) δ 12.42 (s, 1H), 9.60 (s, 1H), 9.17 (s, 1H), 7.42 (d, *J* = 15.9 Hz, 1H), 7.04 (s, 1H), 6.99 (dd, *J* = 8.2, 1.6 Hz, 1H), 6.77 (d, *J* = 8.1 Hz, 1H), 6.15 (d, *J* = 15.9 Hz, 1H), 5.55 (s, 1H), 5.00 (dt, *J* = 101.9, 33.1 Hz, 3H), 3.98 – 3.83 (m, 1H), 2.02 (dd, *J* = 13.0, 6.9 Hz, 2H), 1.94 (dd, *J* = 13.1, 3.1 Hz, 1H), 1.79 (dd, *J* = 12.9, 7.8 Hz, 1H).

$^{13}$C NMR (101 MHz, DMSO) δ 175.38 (s), 166.18 (s), 148.80 (s), 146.02 (s), 145.41 (s), 126.06 (s), 121.81 (s), 116.20 (s), 115.24 (s), 114.76 (s), 73.92 (s), 71.34 (s), 70.81 (s), 68.51 (s), 37.66 (s), 36.68 (s). It is consistent with the chlorogenic acid data reported in the literature [109], so the compound VII was identified as chlorogenic acid.

Compound VIII: ESI-MS, *m/z*:284.88[M-H]$^-$. $^1$H NMR (400 MHz, DMSO) δ 12.99 (s, 1H), 7.44 – 7.37 (m, 2H), 6.88 (d, *J* = 8.3 Hz, 1H), 6.67 (s, 1H), 6.44 (d, *J* = 2.0 Hz, 1H), 6.18 (d, *J* = 1.9 Hz, 1H). $^{13}$C NMR (101 MHz, DMSO) δ 182.09 (s), 164.36 (s), 161.94 (s), 157.77 (s), 150.35 (s), 146.25 (s), 119.47 (s), 116.47 (s), 113.75 (s), 104.99 (s), 103.27 (s), 94.32 (s). It is consistent with the luteolin data reported in the literature [109], so the compound VIII was identified as luteolin.

Compound IX: The purity of the current separation could not identify this compound, and further research was needed.

### 3.2.3 Study on the antioxidant activity of dandelion polyphenol extract

The main components and total samples of dandelion were selected for antioxidant activity detection, and the antioxidant capacity of each component in dandelion was compared.

#### 3.2.3.1 Determination of DPPH free radical scavenging ability

In order to evaluate the antioxidant activity of the dandelion components separated by chromatography, the higher content of the components was determined and compared with their DPPH free radical scavenging activity.

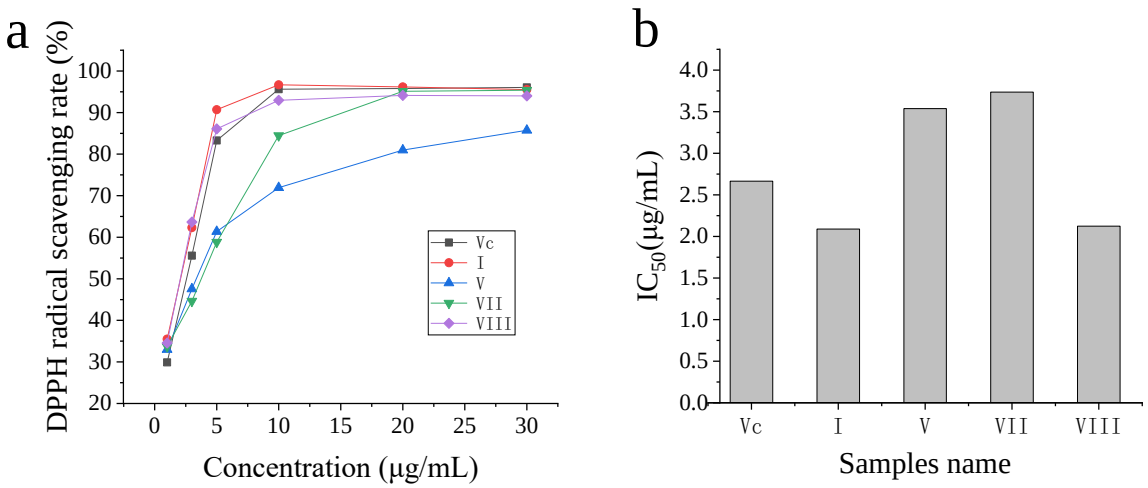



Figure 3-22 The measurement result of DPPH free radical scavenging ability. (a) Dandelion polyphenols and $V_c$ on the scavenging rate of DPPH free radicals, (b) Dandelion polyphenols and $V_c$ in addition to DPPH free radical half scavenging rate $IC_{50}$

Figures 3-22 illustrates the DPPH radical scavenging activities of $V_C$ and the four polyphenol compounds which was isolated from dandelion. Among the results of DPPH scavenging activities (Figure 3-22 a), within the concentration range of 0-30 μg/mL, the selected target compound and the positive control $V_C$'s ability to clear DPPH free radicals all increase as the concentration increases. Indicated that they had a certain dose effect. Among them, compound I had the highest DPPH free radical scavenging activity, followed by VIII. Their activities are higher than the positive control $V_C$. In order to quantify the antioxidant activity further, the $IC_{50}$ was calculated (Figure 3-22 b). The lowest $IC_{50}$ of compound I was 2.09 μg/mL, reflecting the highest DPPH radical scavenging activity among all samples. The $IC_{50}$ of Compound VIII was 2.12μg/mL, which was only slightly higher than the compound I. They were all lower than Vc, which the $IC_{50}$ was

2.66μg/mL. And then the $IC_{50}$ of compound V was 3.54 μg/mL and compound VII was 3.74 μg/mL. Comprehensive comparison of the performance order of its clearance ability is: I>VIII>Vc>V>VII.

### 3.2.3.2 Measurement results of ABTS radical scavenging ability

The ABTS radical cation scavenging capacities of each compounds and Trolox were examined.Using Trolox as a positive control, drawn a standard curve $y=73.7674x+9.8310$, $R^2=0.9991$ (Figure 3-23 a). The results of ABTS radical scavenging activity were expressed by Trolox equivalent antioxidant capacity (TEAC) (Figure 3-23 b). In this assay, the selected pure dandelion compounds had a certain scavenging effect on ABTS radicals. As the results, when the concentration was same, the ABTS+ scavenging activity of compound V was the highest, which was different from the result of DPPH free radical scavenging activity determination. At the same time, compound I and compound VIII were lower, but they were little different with compound I. The TEAC of compound VII was 1.66 mmol/g, which was still the lowest among the 4 compounds. The descending order of the ABTS radical scavenging activity value was: V>I>VIII>VII. The antioxidant capacity of each compound had a small difference, except compound VII.

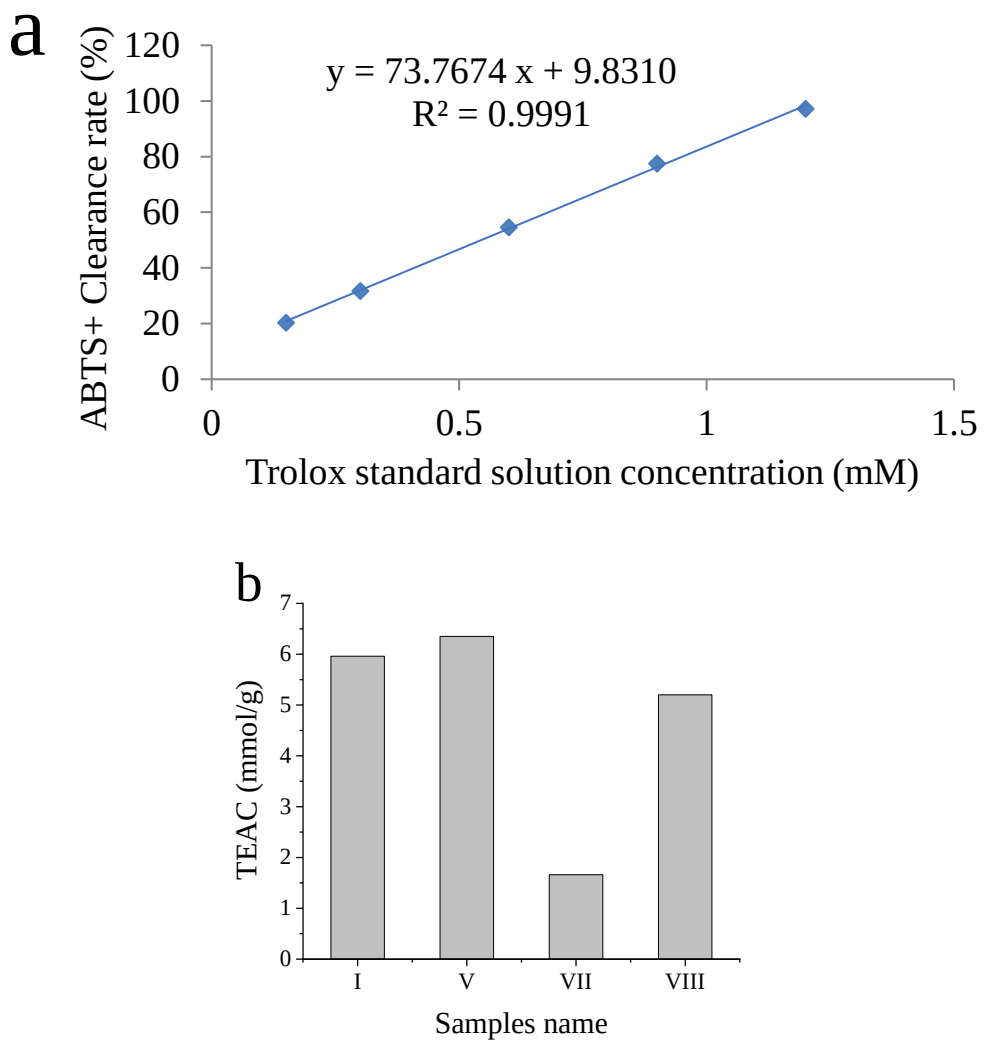



Figure 3-23 Measurement results of ABTS radical scavenging ability. (a) Trolox standard curve, (b) Trolox equivalent antioxidant capacity of four dandelion polyphenols.

**3. 2.3.4 FRAP method to determine the results of total antioxidant capacity**

The reduction potential of an antioxidant reaction with the TPTZ–Fe(III) complex was measured through simple and reliable experiments, producing a TPTZ–Fe(II) complex, which was adopted in this study. Using $FeSO_4$ to draw the standard curve,and the linear equation $y = 0.3364x+0.0107$, $R^2 = 0.9992$ was obtained (Figure 3-24 a).The $FeSO_4$ values were calculated by the standard

curve and linear equation (Figure 3-24 b). The $FeSO_4$ value was higher, the iron reduction ability was better. Compound V showed the highest antioxidant capacity, with a total antioxidant capacity of 1.38 mM Fe(II)/g DW, which was similar to the ABTS radical scavenging test. At the same time, compound VII had the weakest antioxidant capacity, with a total antioxidant capacity of 0.43mM Fe(II)/g DW, which was the same as the results of DPPH and ABTS. In general, the antioxidant capacity of the isolated compounds was: V>I>VIII>VII.

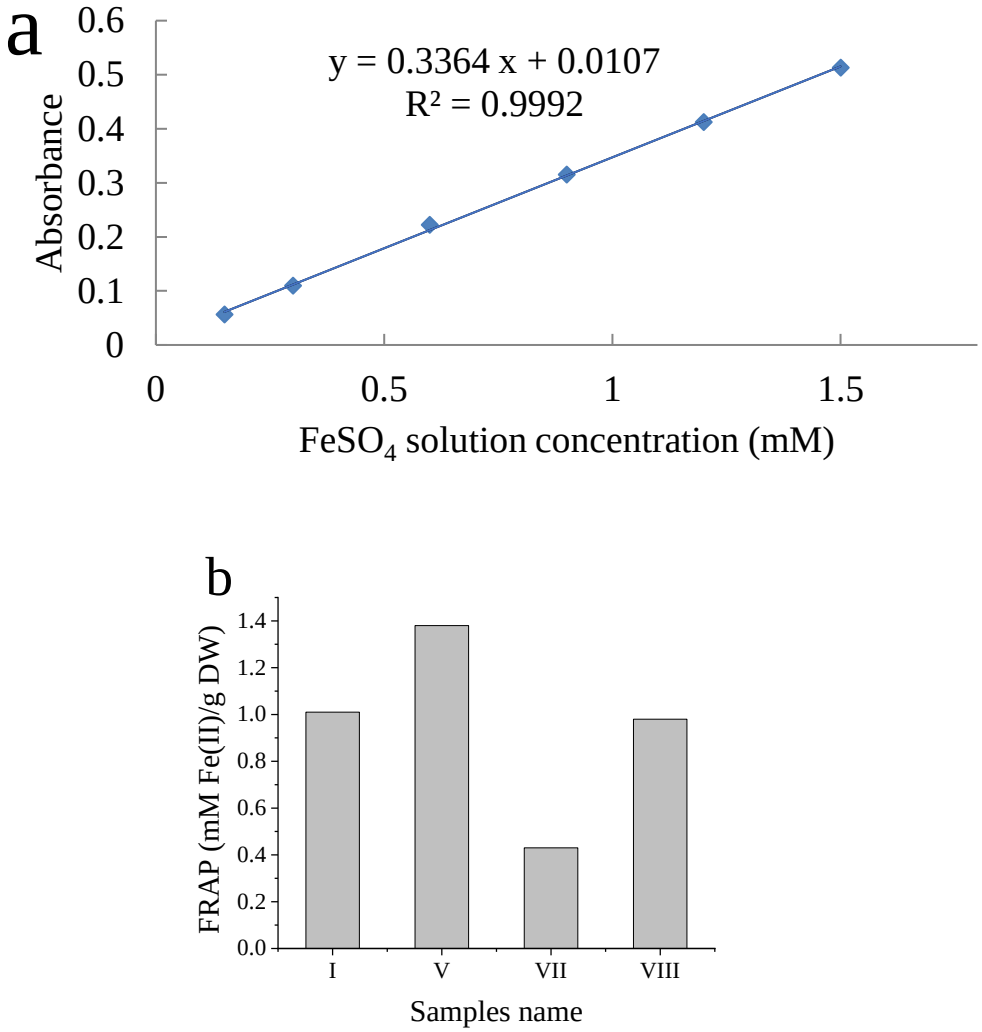



Figure 3-19 FRAP method to determine the results of total antioxidant capacity. (a) $FeSO_4$ standard curve, (b) The antioxidant activity of 4 dandelion polyphenols

were expressed by the concentration of $FeSO_4$ standard solution.

## Conclusions to section 3

1. Single factor test and $L_9$ ($3^4$) orthogonal test were used to optimize the technological parameters of ultrasonic-assisted extraction of polyphenols, and compared with the reflux method. The experimental result indicated that the optimal extraction process parameter of rose polyphenols is: ethanol concentration of 60%, dandelion and solvent ratio of 1:25 (w/v), ultrasonic power of 150 W, and ultrasonic time of 60 min. Under these conditions, the extraction rate of dandelion polyphenols was (2.46 ± 0.06)%. The process was reliable, stable, and has a certain reference value. The primary and secondary order of the influence of the four factors on the extraction rate of dandelion polyphenols is: B material-to-liquid ratio>A ethanol concentration>C ultrasonic time>D ultrasonic power. At the same time, the reflux method and the ultrasonic-assisted reflux method were compared. The experimental results show that ultrasonic assistance has a significant auxiliary effect on the thermal reflux method.

2. The experimental results of macroporous resin purification of rose polyphenols shown that the type AB-8 macroporous resin was more conducive to the separation and enrichment of rose polyphenols. In addition, the optimal load capacity was determined to be 2BV through dynamic adsorption experiments. Two different rose polyphenol components R-30 and R-70 were obtained through separation of macroporous resin resin.

3. UHPLC-Q-TOF-MS was used to identify the polyphenols contained in the total rose sample R. A total of 10 polyphenolic compounds were identified. They

were Quinic acid, Gallic acid, Di-O-galloyl-glucoside, Bis-HHDP-glucoside, Catechin, Di-O-galloyl-HHDPglucoside, Di-O-galloyl-HHDPglucoside, Galloyl-HHDP-glucoside, Ellagic acid, Kaermpferol-3-O-rutinoside.

4. Based on the results of DPPH, ABTS, and FRAP, it could be seen that the in vitro antioxidant capacity of sample R-30 was higher than that of R-70, which might be caused by the fact that R-30 contains more active ingredients than R-70. And it may also be due to the stronger antioxidant activity of the active ingredients in R-30. Further research is needed.

5. Using a combination of pH-zone-refining counter-current chromatography (pH-ZRCCC) and high-speed counter-current chromatography (HSCCC), six polyphenolic compounds were separated from the dandelion crude T-30. In the separation of pH-ZRCCC, ethyl acetate-acetonitrile-water (4:1:5, v/v/v) was selected as the solvent system, trifluoroacetic acid (10 mM) was added to the upper phase as the stationary phase, and ammonia (10 mM) was added to the lower phase as the mobile phase. Caffeic acid (60.2 mg, purity 98.1%), p-hydroxycinnamic acid (6.3 mg, purity 98.8%) and mixture A (590 mg) were separated from 1.6 g of crude ethyl acetate extract of dandelion. After further using HSCCC, petroleum ether-ethyl acetate-methanol-water (1:4:1:4, v/v/v/v) was elected as the solvent system. As a result, 1-O-caffeoylglycerol (4.0mg, purity 99.7%), 3,4-dihydroxyphenylacetic acid (0.4mg, purity 93.3%), protocatechuic acid (6.2mg, purity 95.3 %) and p-hydroxyphenylacetic acid (2.1mg, purity 94.3%) were obtained from 400 mg of mixture A.

6. The column YMC-Pack ODS-A (250×10.0mml, 5μm) was used to investigate the influence of the composition of the mobile phase and the amount of sample on the separation effect, and the dandelion crude TW and T-60 were separated. Two polyphenol compounds and one unknown compound. In the separation of the sample T-W, the mobile phase was methanol-0.1% formic acid water (28:72, V/V), the injection volume was 50μL (containing 3mg crude extract), the flow rate was 3mL/min, and the wavelength was 280nm. Chlorogenic acid (32.8mg, purity 98.2%) was isolated from T-W. For the separation of T-60, the mobile phase was methanol-water (58:42, V/V), the injection volume was 50μL (including 3mg crude extract), the flow rate was 3mL/min, and the detection wavelength was 280nm. Luteolin (20.4mg, purity 96.9%) and unknown compound IX (28.1mg, purity 91.2%) were isolated from T-60.

7. The antioxidant activity of four higher-content polyphenols, which were isolated from dandelion, had been preliminarily studied by DPPH method, ABTS method, and FRAP method. As the result, all the four polyphenols has antioxidant activity in vitro. Among them, the results measured by the DPPH method were slightly different from the two other methods. Comprehensively judge the strength of antioxidant activity as follows: protocatechuic acid > caffeic acid > luteolin > chlorogenic acid.

# CONCLUSIONS

In this subject research, rose and dandelion was used as the research object, and a more in-depth experimental study was carried out on the separation and extraction of polyphenols.

First, rose was used as the material, the ultrasonic-assisted extraction process of rose polyphenols was optimized by single factor test and $L_9$ ($3^4$) orthogonal test, and the optimal process condition for ultrasonic-assisted extraction of rose polyphenols was determined to be 60% ethanol. The ratio of rose concentration to solvent was 1:25 (w/v), the ultrasonic power was 200w, the ultrasonic time was 60min, and the heating reflux method was used to extract rose polyphenols. Then the rose extract was preliminarily separated and purified using macroporous resin. Two components were obtained and tested for antioxidant activity. Finally, the antioxidant activity of the part R-30 eluted with 30% ethanol was higher than that of the part R-70 eluted with 70% ethanol. At the same time, the total rose sample R was identified by UHPLC-Q-TOF-MS. A total of 10 polyphenolic compounds were identified.

Then dandelion was used as the material. The extraction of polyphenols was carried out under the optimal extraction conditions selected. After the petroleum ether and ethyl acetate were extracted, the polyamide atmospheric pressure column was used for preliminary separation. And then using pH-zone-refining counter-current chromatography (pH-ZRCCC), high-speed counter-current

chromatography (HSCCC) combined with semi-preparative high performance liquid chromatography (SP-HPLC) for dandelion polyphenols. Last, 9 compounds were separated and purified from dandelion. Through HPLC, MS, NMR detection, 8 polyphenolic compounds could be determined, namely caffeic acid, p-hydroxycinnamic acid, 1-O-caffeoylglycerol, 3,4-dihydroxyphenylacetic acid, protocatechuic acid, p-hydroxyphenylacetic acid, chlorogenic acid and luteolin, which purity were all over 90%. The method has large preparation volume and good reproducibility, and is suitable for the separation and purification of dandelion polyphenol compounds. Finally, the antioxidant activity of four higher-content polyphenols, which were isolated from dandelion, had been preliminarily studied by DPPH method, ABTS method, and FRAP method. As the result, all the four polyphenols has antioxidant activity in vitro, and the order of their antioxidant activity is: protocatechuic acid> caffeic acid> luteolin> chlorogenic acid.

According to the research results of this article, it is recommended to conduct in-depth research in the following aspects:

(1) This subject had optimized the extraction conditions for the ultrasonic-assisted extraction of polyphenols, but the scale-up extraction experiment had not been carried out. Consider that when the amount of sample increases, it may have a certain interference effect on the efficacy of ultrasound. Consequently, the conditions for large-scale extraction of polyphenols can be optimized in the future, so that they can be better put into industrial production.

(2) This subject had studied the preliminary separation and identification of

rose polyphenols, but failed to separate and prepare pure products. In the future, the separation and purification technology of rose polyphenols can be further studied, the purpose is to more fully study the activity of rose polyphenols and to better develop and utilize them.

(3) This subject used pH-zone-refining counter-current chromatography (pH-ZRCCC), high-speed counter-current chromatography (HSCCC) and semi-preparative high performance liquid chromatography (SP-HPLC) to separate and purify dandelion polyphenols. These three methods have their own advantages and characteristics. In the future, we can continue to explore more efficient and suitable separation techniques to separate and prepare other compounds in dandelion to obtain more kinds of dandelion polyphenol compounds, which purpose is to better study the active ingredients contained in dandelion and its pharmacological activities.

(4) This subject has conducted a preliminary in vitro antioxidant activity study on rose and dandelion polyphenols. In order to further study its antioxidant capacity, in vivo antioxidant experiments may be necessary, such as cell anti-oxidation experiments. The antioxidant mechanism of rose and dandelion polyphenols needs to be further explored.

## References

[1] The Flora Committee of the Chinese Academy of Sciences. Flora of China [M]. Beijing: Science Press, 1985.

[2] Ma Menghua, Cui Bo, Yu Haifeng, Yuan Chuanjian. Research progress of roses[J]. Journal of Shandong Institute of Light Industry (Natural Science Edition), 2008, 22(04): 38-42.

[3] Zhang Feng, Wang Zhiyang, Shi Yiqiang. Extraction of rose essential oil[J]. Chemical Industry Times, 2007(09): 70-71+75.

[4] Sun Di, Fu Benning, Zhang Zhiguo. Research progress on bioactive substances from roses[J]. Food Industry, 2016, 37(07): 243-245.

[5] Liu Jie, Jiang Zhumao, Yang Baoyu, Shan Jing, Ai Chunmei, Zhao Yanjun, Wan Tengteng. Research on the production technology of rose Greek yogurt[J]. China Food Additives, 2018(02):161-165.

[6] National Pharmacopoeia Commission. The Pharmacopoeia of the People's Republic of China (2020 edition) [S]. Beijing, China Medical Science and Technology Press, 2020: 367.

[7] Gong Zhunan, Zhang Weiming. Plant Resources of Taraxacum in China[J]. China Wild Plant Resources, 2001, 20(3): 9-14.

[8] Nie Wenjia, Xu Shuishi, Zhang Yongmei. Research progress on the effective ingredients and pharmacological effects of dandelion[J]. Journal of Liaoning University of Traditional Chinese Medicine, 2020, 22(07): 140-145.

[9] Xie Shimin, Zhou Changzheng. Research progress of dandelion

pharmacological effects and clinical application[J]. Journal of Liaoning University of Traditional Chinese Medicine, 2020, 22(05): 138-142.

[10] ZHENG J, YU X, MANINDER M, et al. Total phenolics andantioxidants profiles of commonly consumed edible flowersin China[J]. International Journal of Food Properties, 2018,21(1): 1524-1540.

[11] A.B. Oyenihi,C. Smith. Are polyphenol antioxidants at the root of medicinal plant anti-cancer success?[J]. Journal of Ethnopharmacology,2018,229:

[12] LI J, SONG D, WANG S, et al. Antiviral effect of epigallocatechin gallate via impairing porcine circovirus type 2 attachment to Host cell receptor [J]. Viruses, 2020, 12(2): 176.

[13] Sun Aidong,Sun Jianxia,Bai Weibin, et al.Study on the antibacterial effect of apple polyphenols[J].Journal of Beijing Forestry University,2008,30 (4):150-152.

[14] Shimada K, Kawarabayashi T, Tanaka AK, et al. Oolong tea increase plasma adiponectin levels and low density lipoprotein particle size in patients with coronary artery disease[J]. Diab Res Clin Pract, 2004, 65(3): 227-234.

[15] Wang Lin, Yang Yang, Zhang Yiying, et al. Current status of literature research on antibacterial effects of traditional Chinese medicine[J].World Journal of Integrated Traditional Chinese and Western Medicine, 2017;12(12): 1663-1665+1763.

[16] Wang Huiying, Wang Jinting. Current status of research and application of rose essential oil[J]. Food and Oils, 2015, 28(10): 5-9.

[17] Liang Xiangping, Xie Huichun. Research progress of rose essential oil[J]. Anhui Agricultural Science Bulletin, 2020, 26(23): 24-25.

[18] Teng Xiangjin, Hao Zaibin, Meng Teng. Development and utilization of rose essential oil[J]. Northern Horticulture, 2011(07): 172-173.

[19] Li Fei, Cheng Xiaohui, Li Quanhong. Study on the subcritical fluid extraction process for the preparation of kushui rose absolute oil and its key aroma compounds[J]. Fragrance, Flavor and Cosmetics, 2019(05):1-5+16.

[20] Yao Chenyang, Ge Hong, Wu Hua, Jia Ruidong, Zhao Xin, Lu Yingmin, Yang Shuhua. Analysis of volatile components in petals of different varieties of roses[J]. Acta Horticulture, 2019, 46(02): 375-384.

[21] Zhao Guojian, Zhao Yuehan, Lu Longxiao, Hou Zhaohua. Molecular distillation technology to extract rose essential oil and its component analysis[J]. Specialty Research, 2020, 42(03): 53-58.

[22] Zhang Fengmei, Liu Zhe, Si Xiaoxi, Liu Zhihua, Shen Qinpeng, Liu Chunbo, Zhu Ruizhi, Zhang Tao, He Pei, Tang Shiyun. Analysis of the aroma components of rose essential oil and its application in cigarette flavoring[J]. Tobacco Science and Technology, 2020, 53 (05):47-56.

[23] Zheng Xiaoga, Wu Aidong, Li Jifeng, Wei Xulun, Liu Jianjun, Chen Qingcai, Wang Yingxin, Yan Changyong. The GC-IMS fingerprint analysis of the volatile components of three different varieties of rose essential oil[J]. Agricultural Products Processing, 2020(20) :60-64+67.

[24] Zhang Lianli, Dai Weifeng, Qin Hao, Zhang Min. Analysis of flavonoids in

three rose teas and their antioxidant activity[J]. Journal of Kunming University of Science and Technology (Natural Science Edition), 2019, 44(05): 84-90.

[25] Jin Jing. Comparative analysis of the antioxidant activity and flavonoids of the four types of rose petals [D]. Guiyang: Guizhou Normal University, 2018.

[26] Zhang Ling. Study on the coloring mechanism of rose (Rosa rugosa Thunb.) petals [D]. Taian: Shandong Agricultural University, 2015.

[27] Xiao Z. P., Wu H. K., Wu T., Shi H., Hang B., Aisa H. A.. Kaempferol and quercetin flavonoids from Rosa rugosa[J]. Kluwer Academic Publishers-Consultants Bureau, 2006, 42(6).

[28] Wu Mengqi, Huang Hai, Cai Dihong, Xu Zhizhen, Xia Wei, Zhang Wenqing. Separation and Purification of Ellagic Acid from Bitter Water Rose Residue by Macroporous Adsorption Resin-High Speed Countercurrent Chromatography[J]. Food Industry Science and Technology, 2021, 42(05) :182-187.

[29] Zhang Juanmei. Ultrasonic-assisted extraction preparation, component analysis and biological activity of polyphenols in rose dregs[D]. Nanjing Agricultural University, 2014.

[30] Li Sainan. Isolation, extraction and activity evaluation of flavonoids in traditional Chinese medicine Apocynum venetum and rose [D]. Changchun Normal University, 2012.

[31] Gao Jianing, Zhang Dan, He Haiyan, Huang Tian Francium. Study on the polyphenol content and antioxidant activity of different varieties of roses in

different producing areas [J]. Guizhou Science, 2021, 39(01): 1-9.

[32] Zheng J.B., Liu X.X., Zhou Y.Z. Simul-Taneous Determination of Five Phenolic Compoundsin Dried Flowers by LC using DAD Combined Electrochemical Detection [J]. Chromatographia, 2007, 12(11): 707~712

[33] Chen Dongming. Ultrasonic extraction of polyphenols and polysaccharides from rose dregs [D]. Zhengzhou University, 2014.

[34] Nilgün Gktürk Baydar a, B H B. Phenolic compounds, antiradical activity and antioxidant capacity of oil-bearing rose (Rosa damascena Mill.) extracts[J]. Industrial Crops and Products, 2013, 41(2):375-380.

[35] Liu Fang, Ren Qifei, Ma Jinghua, Ou Mingzhu, Chen Yunfei, Yang Shuo, Zhou Yan. Research progress in the extraction and purification of the main functional components of roses[J]. Agriculture and Technology, 2021, 41(06): 25 -29.

[36] Ma Menghua, Yu Haifeng, Cui Bo, Yue Zongcui, Yuan Chuanzhen, Bai Weifang. Extraction of polysaccharides from rose dregs[J]. Food Industry Science and Technology, 2009, 30(11): 218-220.

[37] Liang Qichao, Zou Yulong, Zhang Xiuping, et al. Study on the optimization of rose polysaccharide extraction process and its antioxidant activity[J]. Food Research and Development, 2018, 39(22): 41−46.

[38] Wu M Q, Feng H F, Song J X, et al. Structural elucidation and immunomodulatory activity of a neutral polysaccharide from the Kushui Rose (Rosa setate x Rosa rugosa) waste[J]. Carbohydrate Polymers, 2020, 232:

115804.

[39] Wei Qin, Wang Yonghong, Hu Yayun, etc. Study on the chemical and nutritional components of rose dregs[J]. Journal of Northwest Agriculture and Forestry University, 2005, 20(3): 140 ~141

[40] Ma Xueyi, Chen Yaozu. Research on the chemical constituents of Kushui Rose (Fourth Report)——Analysis of Amino Acids and Trace Elements[J]. Journal of Lanzhou University, 1986(03): 96-99.

[41] Xia Yan. Anti-inflammatory effect of dandelion extract on LPS-induced RAW264.7 cells and regulation of NF-κB signaling pathway [D]. Jilin Agricultural University, 2017.

[42] Ling Yun, Zhang Yalin, Cai Shaoqing, Zheng Junhua. Study on the flavonoids and sterols of dandelion[J]. Chinese Journal of Medicinal Chemistry, 1998(01): 49-51.

[43] Shi Shuyun, Zhou Changxin, Xu Yan, et al. Study on the chemical constituents of Mongolian dandelion [J]. Chinese Journal of Chinese Materia Medica, 2008, 33 (10): 1147-1157.

[44] SHI S Y，ZHAO Y，ZHOU H H，et al. Identification ofantioxidants from Taraxacum mongolicum by high-performance liquid chromatography-diode array detection-radical-scavenging detection-electrospray ionization mass spectrometry and nuclear magnetic resonance experiments[ J ] . Journal of ChromatographyA，2008，1209 ( 1 )：145-152.

[45] Peng Deqian, Gao Juan, Guo Xiumei, et al. Study on the chemical constituents

of Mongolian Taraxacum officinale [J]. Chinese Patent Medicine, 2014, 36 (7): 1462-1466.

[46] Yadava R N , Khan S . A New Flavonoidal Constituent from Taraxacum officinale (L.) Weber[J]. Asian Journal of Chemistry, 2013, 25(7):4117-4118.

[47] Kenny O , Brunton N P , Walsh D , et al. Characterisation of Antimicrobial Extracts from Dandelion Root (Taraxacum officinale) Using LC-SPE-NMR[J]. Phytotherapy Research, 2015, 29(4):526-532.

[48] Wu Zhe, Li Zhaojia, Feng Wei, Meng Ran, Wang Xiuping. Optimization and quality evaluation of comprehensive extraction technology of dandelion phenolic acid[J]. Jiangsu Agricultural Sciences, 2020, 48(23): 190-195.

[49] Xu Shulai, Wang Li, Ren Hongbo, Luan Xueyan, Chen Jingquan. Optimization of the extraction process of dandelion flavone and analysis of its main components[J]. Food Industry Science and Technology, 2020, 41(19): 172-178.

[50] Langenheim, J.H .Plantresins[J].Am.Sci., 1990, 78:16～24.

[51]Bohlmann,J .etal.Plantterpenoidsynthases:Molecularbiologyandphylogenetic analysis[J].Proc.NatiAcadSci., 1998, 95 (8) :4126～4133.

[52] K Schütz, Carle R , Schieber A . Taraxacum —A review on its phytochemical and pharmacological profile[J]. Journal of Ethnopharmacology, 2006, 107( 3):313-323.

[53] H, Ageta, and, et al. Composite constituents: four new triterpenoids, neolupenol, tarolupenol and their acetates isolated from roots of a japanese

dandelion, taraxacum japonicum[J]. Tetrahedron Letters, 1981.

[54] Ling Yun, Xu Yang, Zheng Junhua. Study on the chemical constituents of dandelion triterpenoids[J]. Chinese Herbal Medicine, 1998, 29(4): 2.

[55] Yan-Ling W U , Hui-Shan P . Research on the gastrointestinal propulsive motivity and chemical constituents of Dandelion extraction[J]. Journal of Medical Science Yanbian University, 2005.

[56] Jiang Xing, Zhao Min, Gao Xiaobo, Zhu Jingxin, Lu Hongmei, Wang Jiazhi, Yuan Guangjun. Study on the chemical constituents of eucalyptane-type sesquiterpenoids in dandelion[J]. Zhongnan Pharmaceutical, 2016,14(12):1293 -1297.

[57] Dong Changying, Wu Junbo. The extraction of dandelion sterol and its anti-inflammatory activity[J]. Heilongjiang Animal Science and Veterinary Medicine, 2011(05):133-134.

[58] Xie Shenyang, Yang Xiaoyuan, Ding Zhanggui, Li Minggang, Zhao Jiangyuan. The chemical constituents and pharmacological effects of dandelion[J]. Research and Development of Natural Products, 2012, 24(S1):141-151.

[59] Svoboda JA, et al. Camporison of sterols of pollens, honeybee workersand prepupae from field sites. Arch Insect Biochem, 1983, 1: 25-31.

[60] Hook I, Sheridan H, Wilson G. Volatile metabolites from suspension cultures of Taraxacum officinale[J]. Phytochemistry, 1991, 30(12):3977.

[61] Ling Yun, Zhang Weihua, Guo Xiufang, Cai Shaoqing, Zheng Junhua. Gas

Chromatography-Mass Spectrometry Analysis of Volatile Oil Constituents of Dandelion[J]. Northwest Pharmaceutical Journal, 1998(04): 154.

[62] Ling Yun, Zheng Junhua. Research progress of traditional Chinese medicine Dandelion[J]. China Modern Applied Pharmacy, 1998(03): 10-13.

[63] Yang Chao, Yan Qingzi, Tang Jie, Xia Bohou, Lin Limei, He Qingzhi, Liao Duanfang. Analysis of the volatile oil of dandelion and its anti-inflammatory and anti-tumor activity[J]. Chinese Journal of Traditional Chinese Medicine, 2018, 33(07): 3106 -3111.

[64] Roberfroid M . Concepts in functional foods: the case of inulin and oligofructose.[J]. journal of nutrition, 1999, 129(7 Suppl):1398S.

[65] Guo Huijing. Preliminary study on extraction, separation, purification, identification and biological activity of dandelion polysaccharides[D]. Shihezi University, 2019.

[66] Hans Kleinig, Helfried Nietsche. Carotinoidester-muster in gelben Blütenblättern[J]. Phytochemistry, 1968, 7(7):1171-1175.

[67] Wu Xiaochun, Du Shengli, Chen Haisheng. Research and application of dandelion[J]. Journal of Pharmaceutical Practice, 2002(04):246-248.

[68] Peng Rujie, Wang Jiadan, Han Wei. Research progress on the extraction, separation and purification and analysis methods of plant polyphenols[J]. Electromechanical Information, 2016(14): 21-29.

[69] Balasundram N,Sundram K,Samman S. Phenolic compounds in plants and Agri-industrialby-products:antioxidant activity,occurrence,and potential

uses[J],Food Chemistry,2006,99(1)：191 ~ 203

[70] Nagendran Balasundrma,Kalyana Sundarm,Samir Samman.Phenolic compounds in Plnatsand agri-industrial by-products ： Antioxidant activity,occurrence,and potential uses[J].FoodChemistry,2005,5：1 ~ 13

[71] Zhang Ling. Study on the coloring mechanism of rose (Rosa rugosa Thunb.) petals [D]. Taian: Shandong Agricultural University, 2015.

[72] Bao Chen, Zheng Baodong. Study on the extraction technology of Agrocybe cylindrica polyphenols by organic solvent method[J]. Chinese Journal of Tropical Crops, 2012, 33(11): 2070-2074.

[73] Chen Dongming. Ultrasonic extraction of polyphenols and polysaccharides from rose dregs [D]. Zhengzhou University, 2014.

[74] Leandro Galvan d'Alessandro, Karim Kriaa, Iordan Nikov, et al. Ultrasound assisted extraction of polyphenols from black chokeberry [J]. Separation and Purification Technology, 2012(93): 42-47.

[75] Li Qunmei, Yang Lvpeng, Li Jian, etc. Research on the extraction and separation methods of plant polyphenols is intense [J]. Preservation and Processing, 2010, 10(1): 16-19.

[76] Pan X, Niu G, Liu H. Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves[J]. 2003, 42(2):129-133.

[77] Fu Xiaoyan, Sui Yong, Xie Bijun, et al. Comparison of the content and antioxidant activity of phenolic substances extracted from germinated oatmeal by different methods[J]. Food Industry Science and Technology, 2014, 35(15):

54-57 .

[78] Gu Renyong, Yang Wangen, Yu Ji, et al. Response surface optimization of supercritical CO2 extraction of polyphenols from August melon young fruit[J]. Food Science, 2015, 36(10): 76-80.

[79] Huang Huihua, Wang Shaobin, Wang Zhi, Liang Hanhua. Research on the complexation and precipitation recovery between tea polyphenols and proteins[J]. Food Science, 2002(01): 26-30.

[80] Gust J, Wawer I. Relationship Between Radical Scavenging Effects and Anticorrosive Properties of Polyphenols[J]. Corrosion, 1995, 51(1):37-44.

[81] Ouyang Yuzhu, Li Xuefeng, Yao Yihuan. Ion precipitation method for separation of total polyphenols in August melon peel[J]. Food Science, 2014, 35(16): 76-79.

[82] Xu Dandan, Zhang Wenbin, Yang Ruijin, et al. Separation and enrichment of sunflower polyphenols by macroporous adsorption resin[J]. Food and Machinery, 2015, 31(5): 210-216.

[83] Luo Chaohua, Li Jun. The process optimization of gel column separation and purification of epigallocatechin gallate in tea polyphenols[J]. Pharmaceutical Research, 2015 (1): 17-19.

[84] Wang Yonggang, Li Wei. The effect of membrane technology separation and purification on the quality of peanut shell polyphenol extract[J]. Food Research and Development, 2010, 31(9): 72-74.

[85] Xiang Li. Extraction, purification and antioxidant activity of black wheat

polyphenols[D]. Wuhan Institute of Technology, 2012.

[86] Zuo Chunlin. Extraction and purification of polyphenols from Robinia pseudoacacia flowers and their antioxidant properties[D]. Shandong Normal University, 2013.

[87] Xia Yajun, Liu Yongfeng, Pei Dong, et al. Separation and preparation of polyphenols in olive leaves by medium pressure preparative chromatography [J]. Chinese Herbal Medicine, 2014, 45(12): 1689-1692.

[88] Wang Wei, He Tianyu, Lan Tao, Xi Xingjun, Zhao Xinxin. The separation and preparation of polyphenols in grape seeds by high-speed countercurrent chromatography combined with preparative liquid chromatography[J]. Chromatography, 2019, 37(11): 1193-1199.

[89] Song Lijiang, Di Ying, Shi Bi. Significance and development trend of research and utilization of plant polyphenols[J]. Progress in Chemistry, 2000(02):161-170.

[90] Zhao Baolu. The protective effect of tea polyphenols on brain nerves from Parkinson's disease and its molecular mechanism[J]. Advances in Biochemistry and Biophysics, 2008(07):735-743.

[91] He Youjie, Di Ying, Zhao Yu, et al. Antibacterial properties of degradation products of black wattle bark tannins[J]. Chemistry and Industry of Forest Products, 1999, 19 (2): 1-7.

[92] Zheng Qun, Ding Lixin, Shi Weixian, et al. Study on the inhibitory effect of catechins on influenza virus proliferation [J]. Public Health and Preventive

Medicine, 2005, 16 (4): 12-14.

[93] Peng Huiqin, Cai Weimin, Xiang Shao. In vitro anti-influenza virus A3 effect of tea polyphenols[J]. Tea Science, 2003, 23 (1): 79-81.

[94] Joseph M Weber, Angelique Ruzindana-Umunyana, Lise Imbeault,et al. Inhibition of adenovirus infection andadenain by green tea catechins [J]. Antiviral Research,2003, 58:167-173.

[95] Pilar Almajano M. Rosa Carbo, Angel Lopez JimenezJ, et al. Antioxidant and antimicrobial activities of tea infusions [J .Food Chemistry, 2008, 108 (1): 55-63.

[96] Wang Jinlian, Qiu Yexian, Chen Hongwei, et al. The inhibitory effect and mechanism of tea polyphenols on several plant pathogenic fungi[J]. Natural Products Research and Development, 2008, 20 (4): 690-694.

[97] Bawadi H A，Bansode R R,Trappey I A,etal.Inhibition of Caco-2 colon，MCF -7 and Hs578T breast ,and DU 145 prostatic cancer cell proliferation by water -soluble black bean condensed tannins [J]. Cancer Letters，2005，218 (2):153-162.

[98] Wang Xueshen. Orthogonal design method[J]. Shanxi Chemical Industry, 1989(3):6.

[99] Tian Shuge, Wei Yulong, Liu Hongbing. Determination of total polyphenols in different parts of pomegranate by Folin-Ciocalteu colorimetry[J]. Spectroscopy Laboratory, 2009, 26(02): 341-344.

[100] Shi Shuyun, Zhang Yuping, Zhao Yu, Huang Kelong, Liu Suqin. Separation

and Preparation of Flavonoid Glycosides in Dandelion by High Speed Countercurrent Chromatography[A]. Chinese Pharmaceutical Association. The 9th National Symposium on Traditional Chinese Medicine and Natural Medicines and Proceedings[A]. C]. Chinese Pharmaceutical Association: Academic Affairs Department of Chinese Pharmaceutical Association, 2007: 5.

[101] Zhang Tianyou, Wang Xiao. High-speed countercurrent chromatography technology [M]. Beijing: Chemical Industry Press, 2011, first edition: 25-28, 74-75.

[102] Y. Ito, pH-zone-refining counter-current chromatography: origin, mechanism, procedure and applications, J. Chromatogr. A 1271 (2013) 71-85.

[103] Lee K, Whang W . Inhibitory effects of bioassay-guided isolation of anti-glycation components from Taraxacum coreanum and simultaneous quantification [J]. Molecules, 2018; 23(9): 2148-2161.

[104] Talapatra B，Das AK，Talapatra SK. Defuscin，a new phenolic ester from Dendrobium fuscescens：Conformation of shikimic acid [J]. Phytochemistry，1989，28（1）：290-291.

[105] Li Dingxiang, Feng Guixin, Wang Zhengtao. Study on the phenolic acid chemical constituents in Senecio Senecio[J]. Henan Journal of Traditional Chinese Medicine, 2014, 34(09): 1847-1849.

[106] Li Huaxuan, Cui Shujun, Xiao Chaojiang, Dong Xiang, Shen Yi, Jiang Bei. The phenolic components of pachyderm and their analgesic activity[J].

Chinese Patent Medicine, 2019, 41(07): 1582-1586.

[107] Liang Yonghong, Ye Min, Zhang Lingzhi, Li Huifang, Han Jian, Wang Baorong, Guo Dean. Two new phenolic acid compounds in Drynaria fortunei [J]. Acta Pharmaceutica Sinica, 2010, 45(07): 874- 878.

[108] Duan Lixin, Yu Zhengjiang, Feng Baomin, Pei Yuehu, Hua Huiming, Wang Yongqi. Phenolic acids in compound Sanzi Yangqin Decoction[J]. Journal of Shenyang Pharmaceutical University, 2007(11): 679-681.

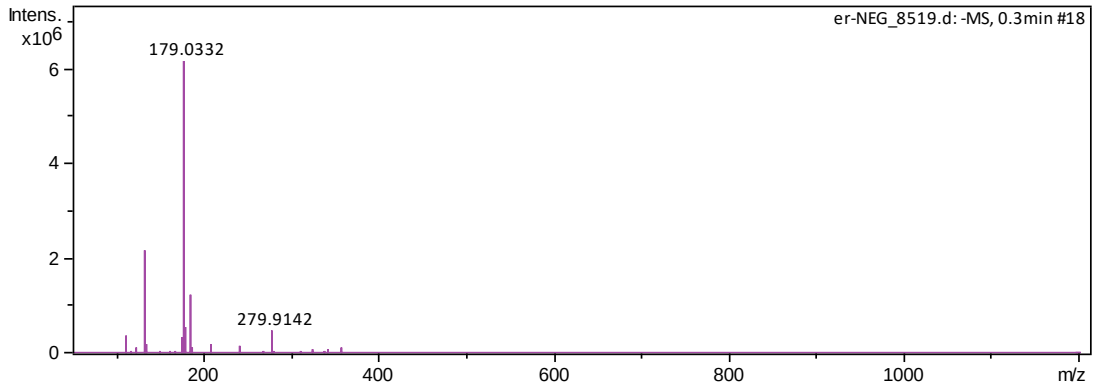
[109] Song Yaling, Wang Hongmei, Ni Fuyong, Wang Xuejing, Zhao Yiwu, Huang Wenzhe, Wang Zhenzhong, Xiao Wei. Study on phenolic acids in honeysuckle and their anti-inflammatory activity[J]. Chinese Traditional and Herbal Medicine, 2015, 46(04): 490-495 .

**ANNEXES**

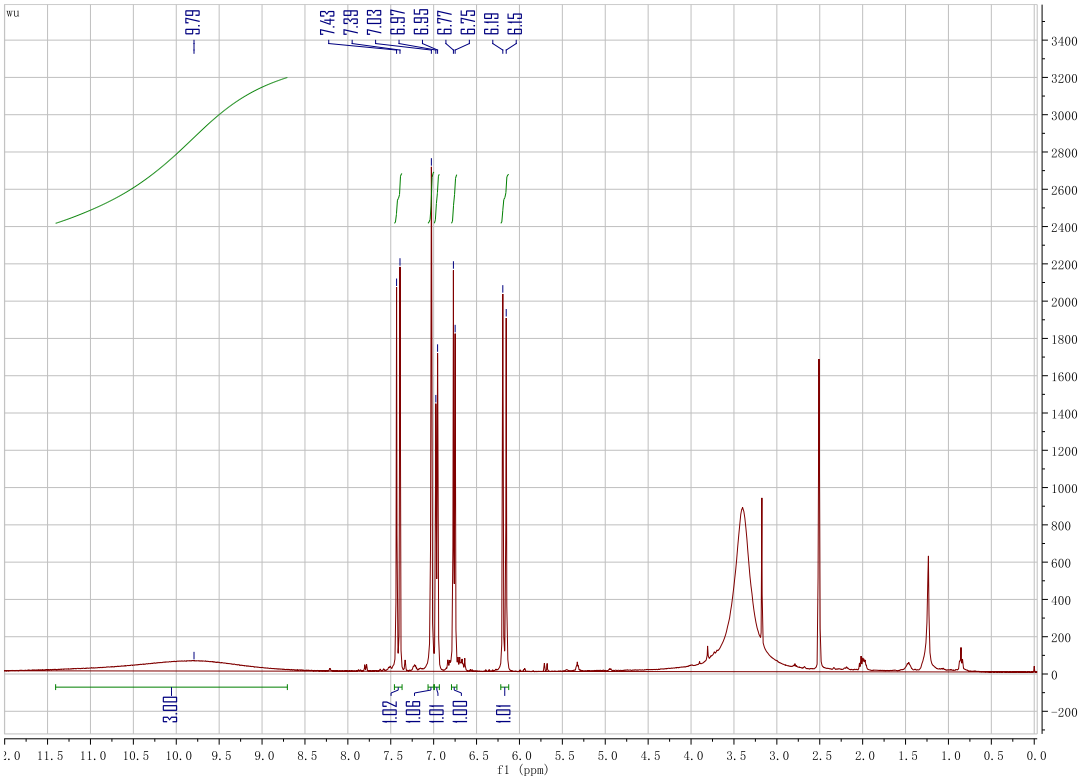
ESI-MS, $^{13}$C-NMR and 1H-NMR diagrams of the compounds isolated from dandelion

1. Compound I: caffeic acid

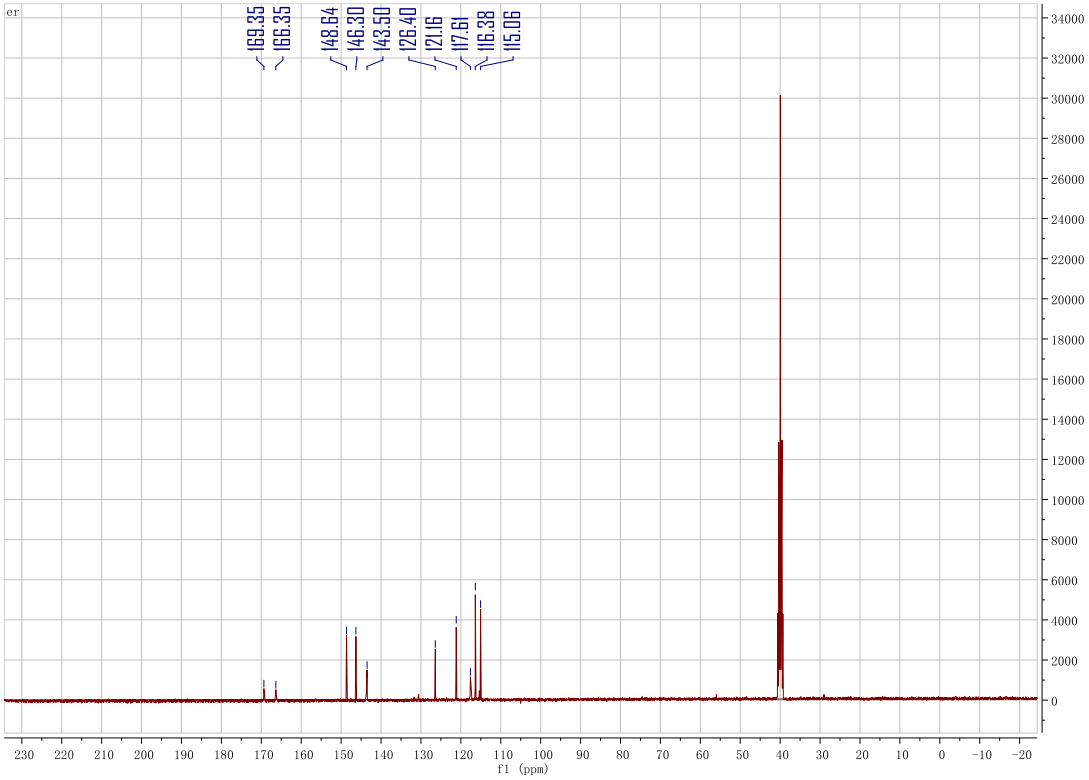
Mass spectrum in negativeion mode




$^{1}$H NMR (400 MHz, DMSO)

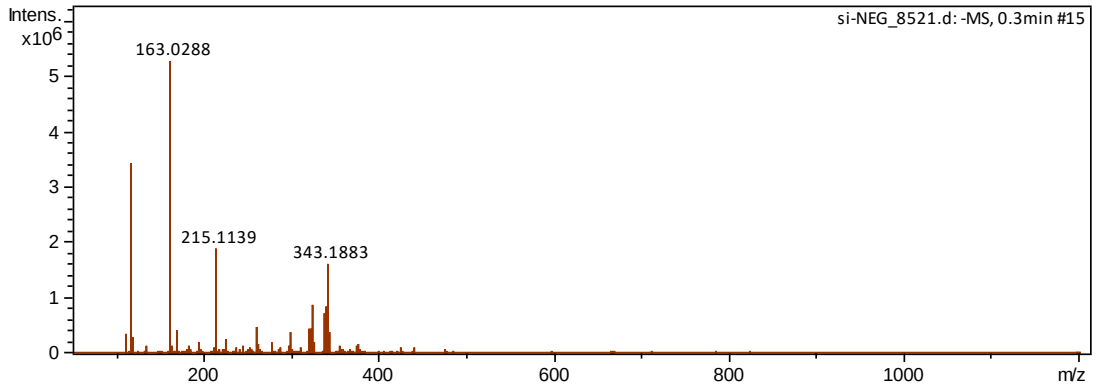



$^{13}$C-NMR (100 MHz, DMSO)

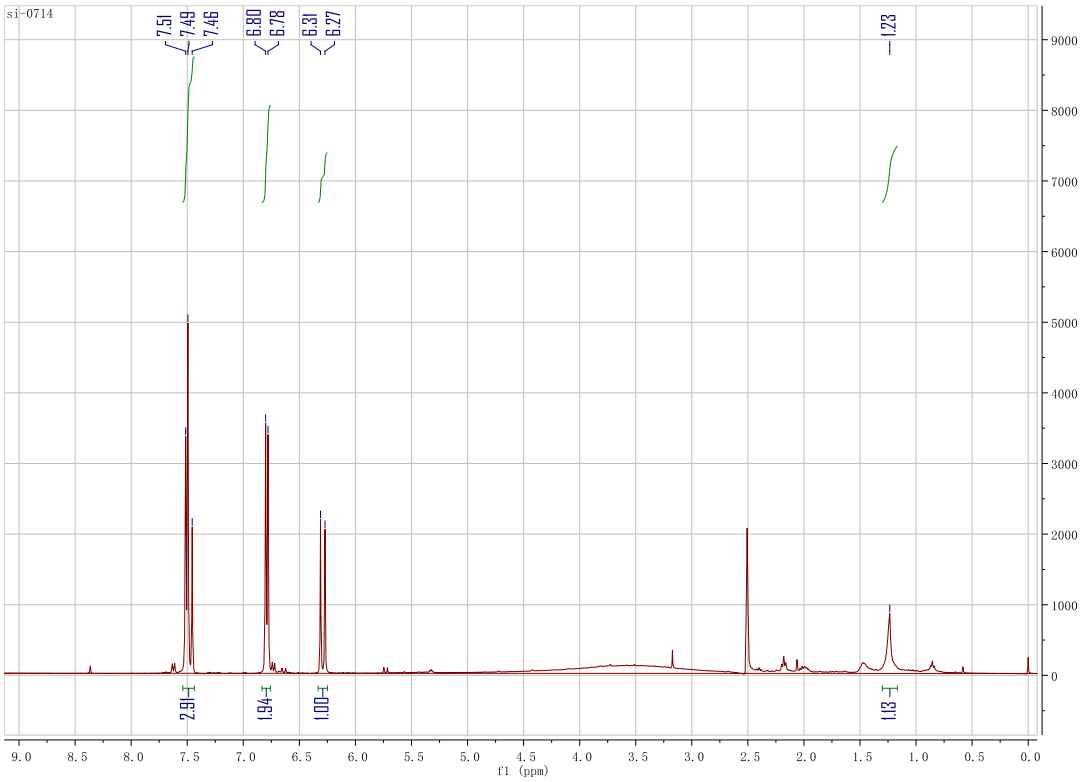



2. Compound II: *p*-hydroxycinnamic acid

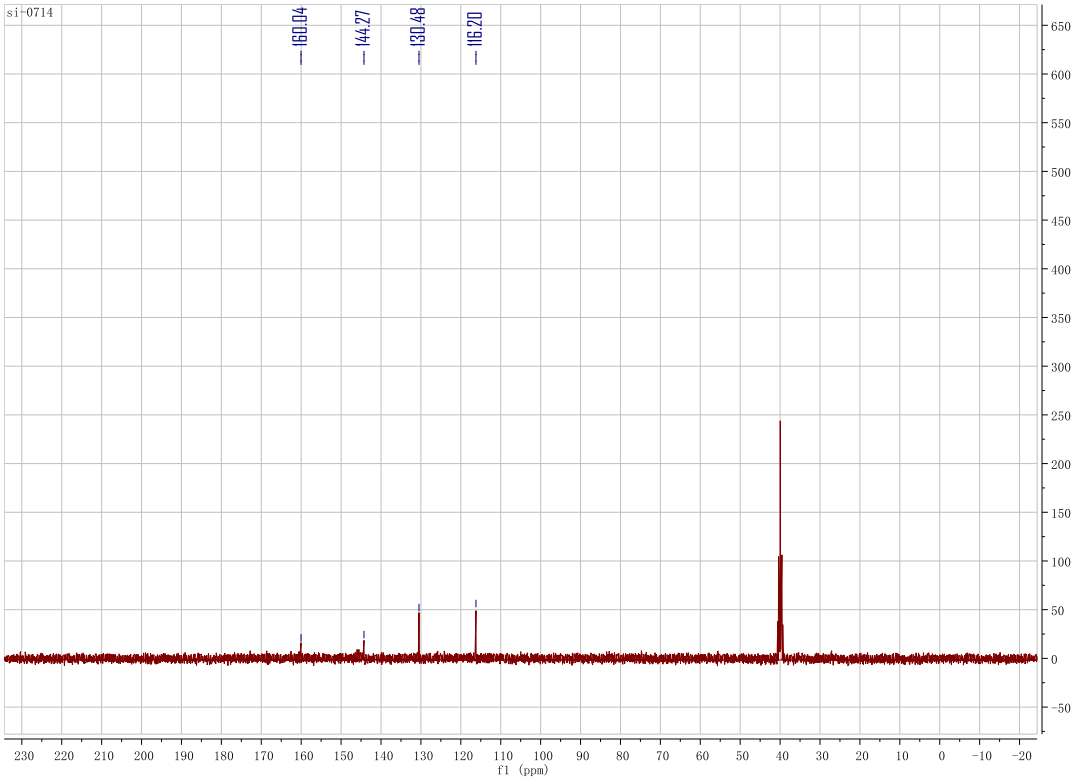
Mass spectrum in negativeion mode




$^{1}$H NMR (400 MHz, DMSO)

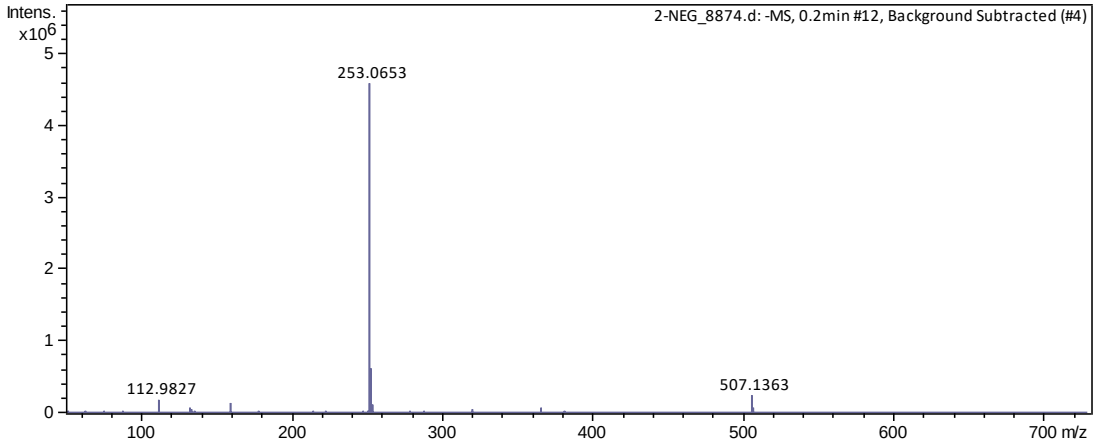



$^{13}$C-NMR (100 MHz, DMSO)

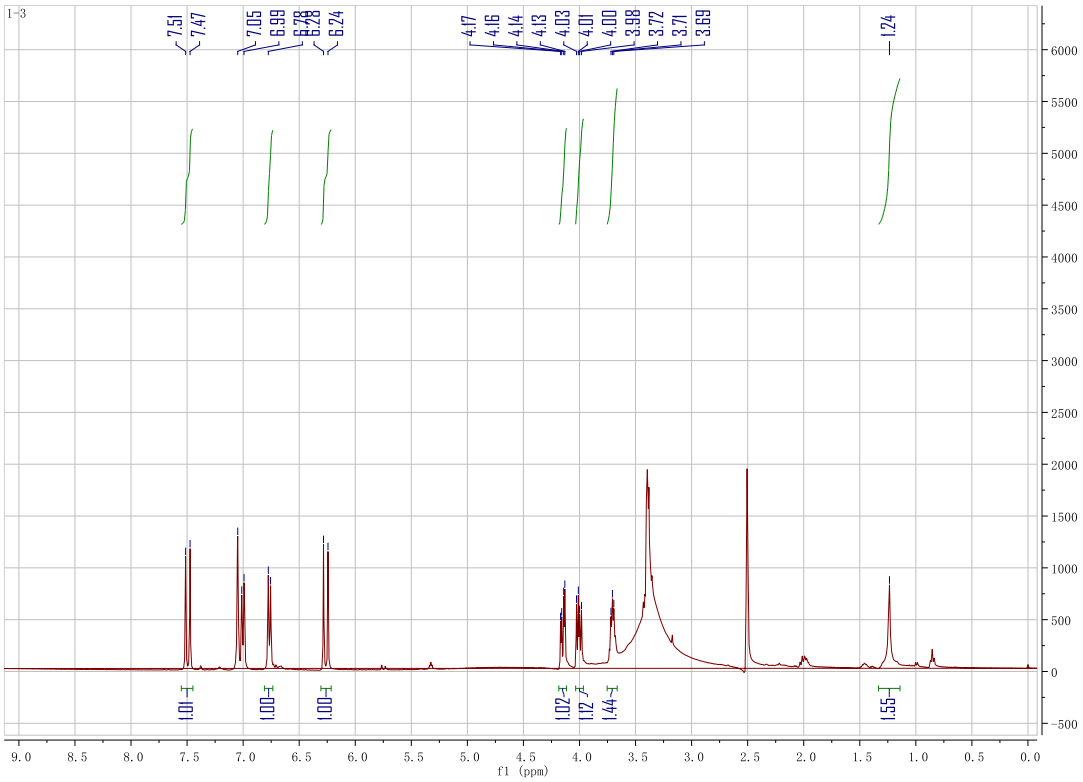



3. Compound III: 1-O-caffeoylglycerol

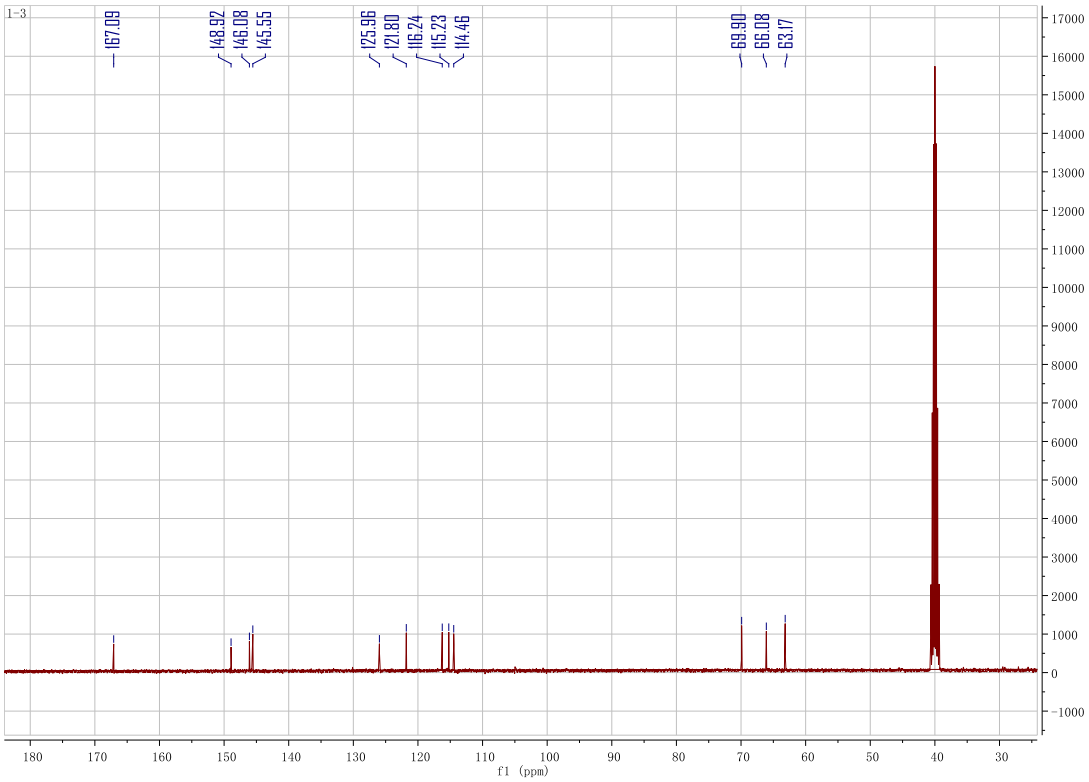
Mass spectrum in negativeion mode




$^{1}$H NMR (400 MHz, DMSO)

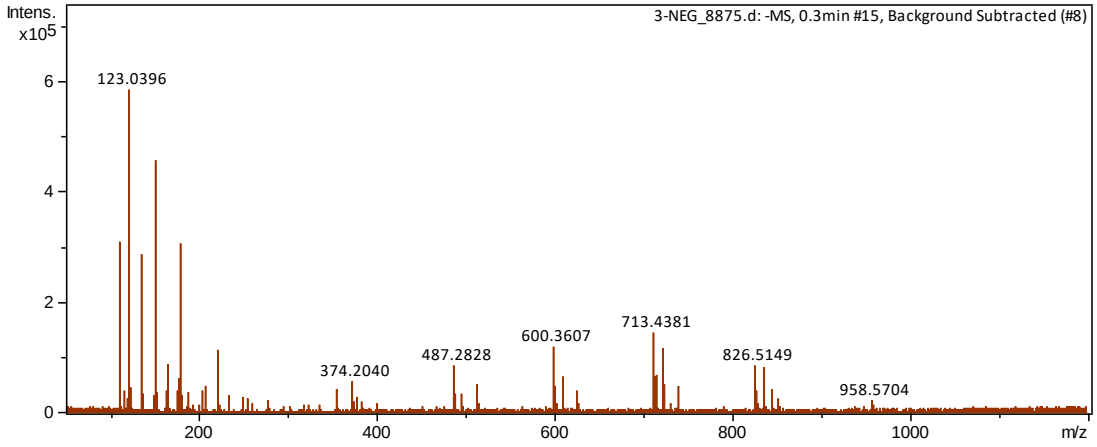



$^{13}$C-NMR (100 MHz, DMSO)

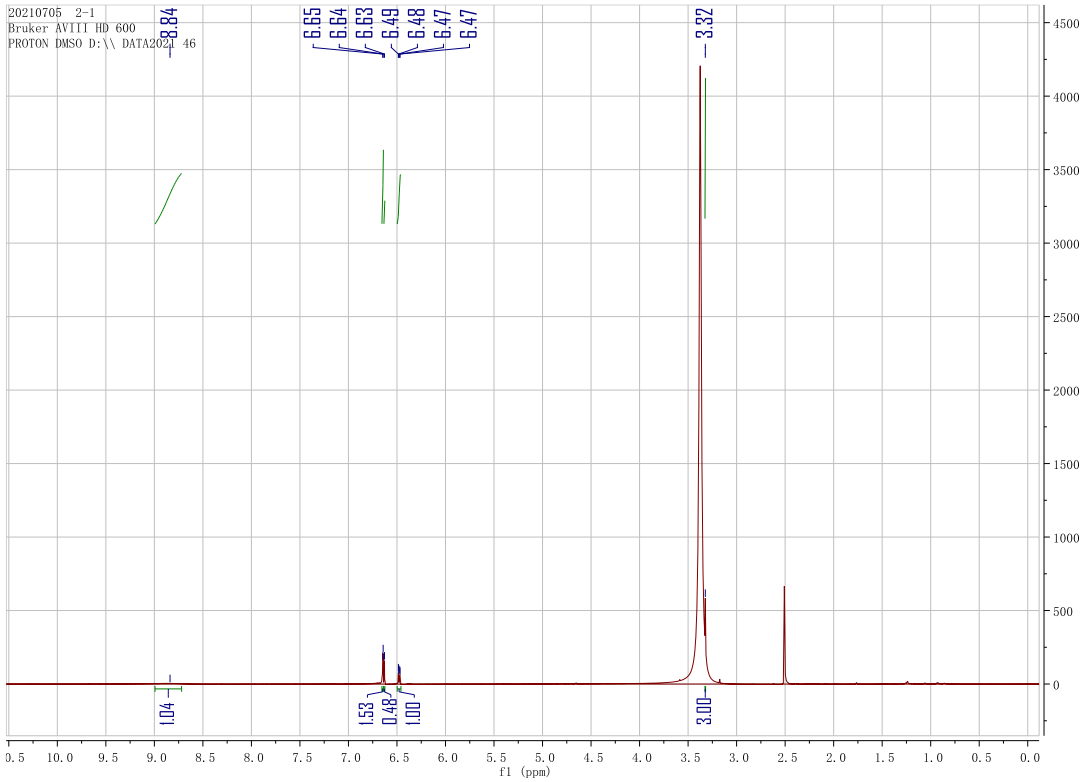



Compound IV: 3,4-dihydroxyphenylacetic acid

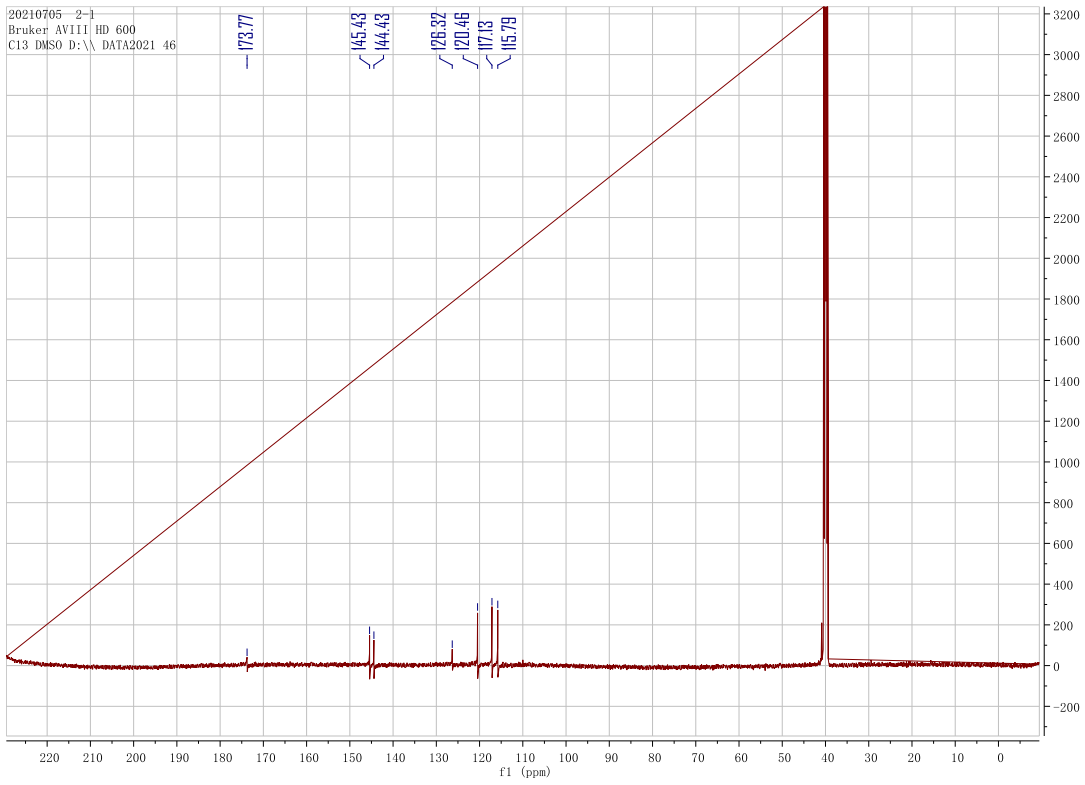
Mass spectrum in negativeion mode




$^{1}$H NMR (600 MHz, DMSO)

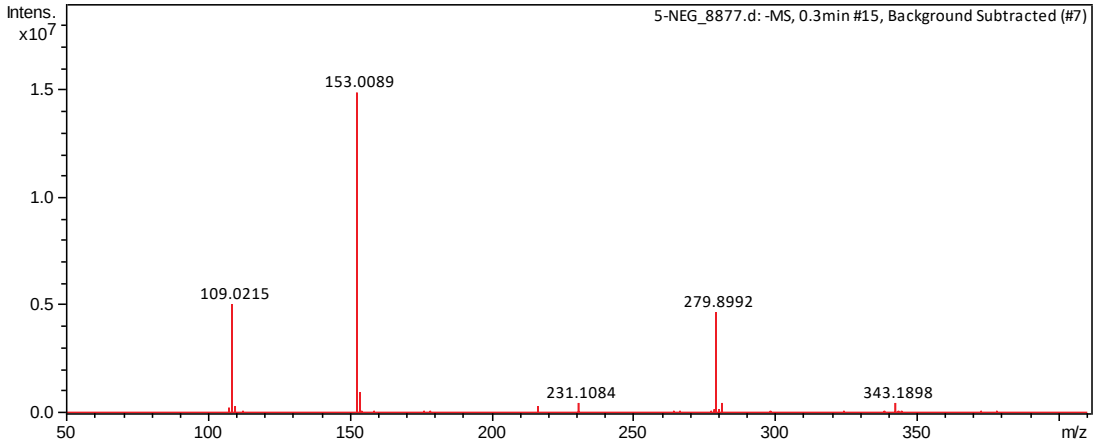



$^{13}$C NMR (150 MHz, DMSO)

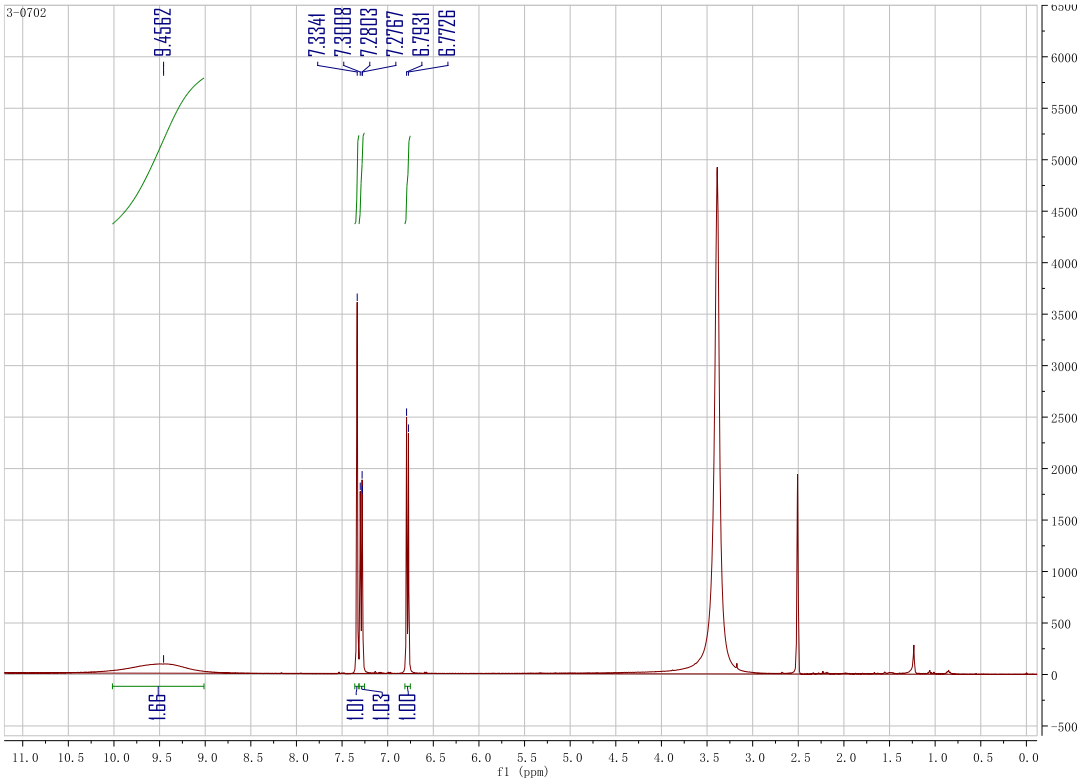



Compound V: protocatechin

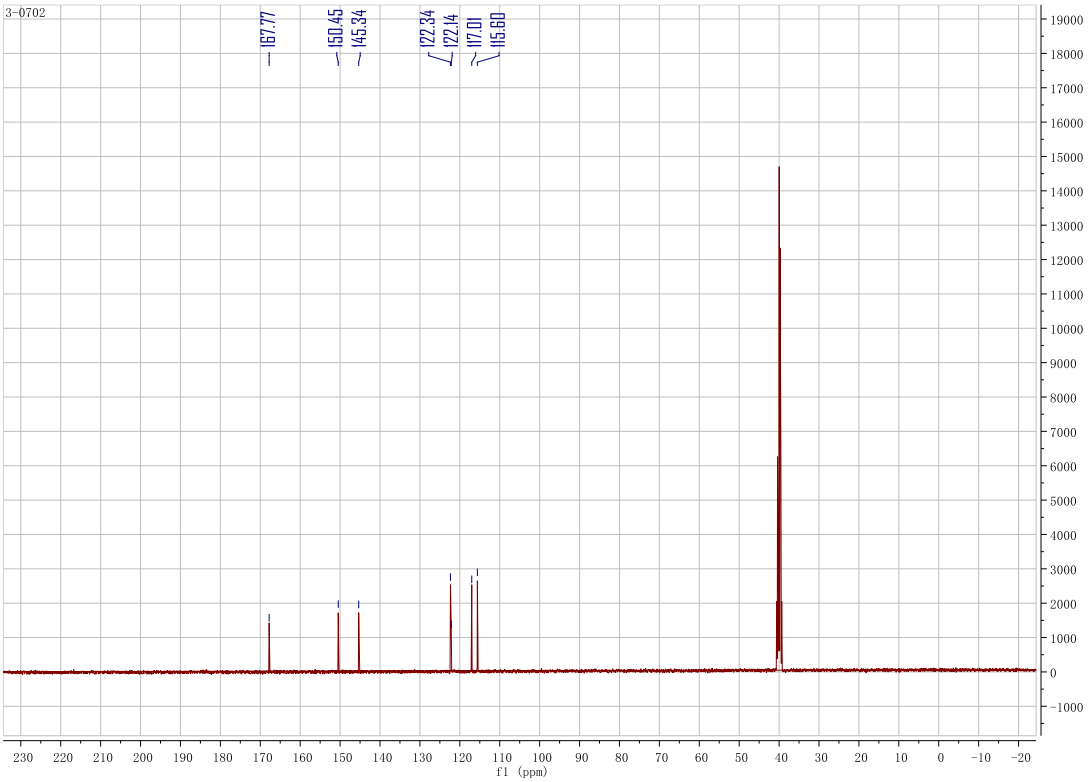
Mass spectrum in negativeion mode




$^{1}$H NMR (400 MHz, DMSO)

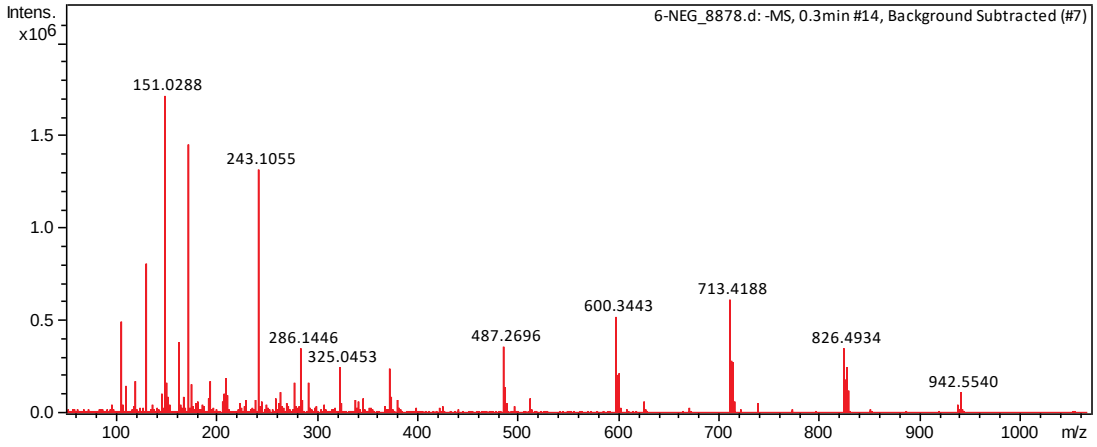



$^{13}$C-NMR (100 MHz, DMSO)

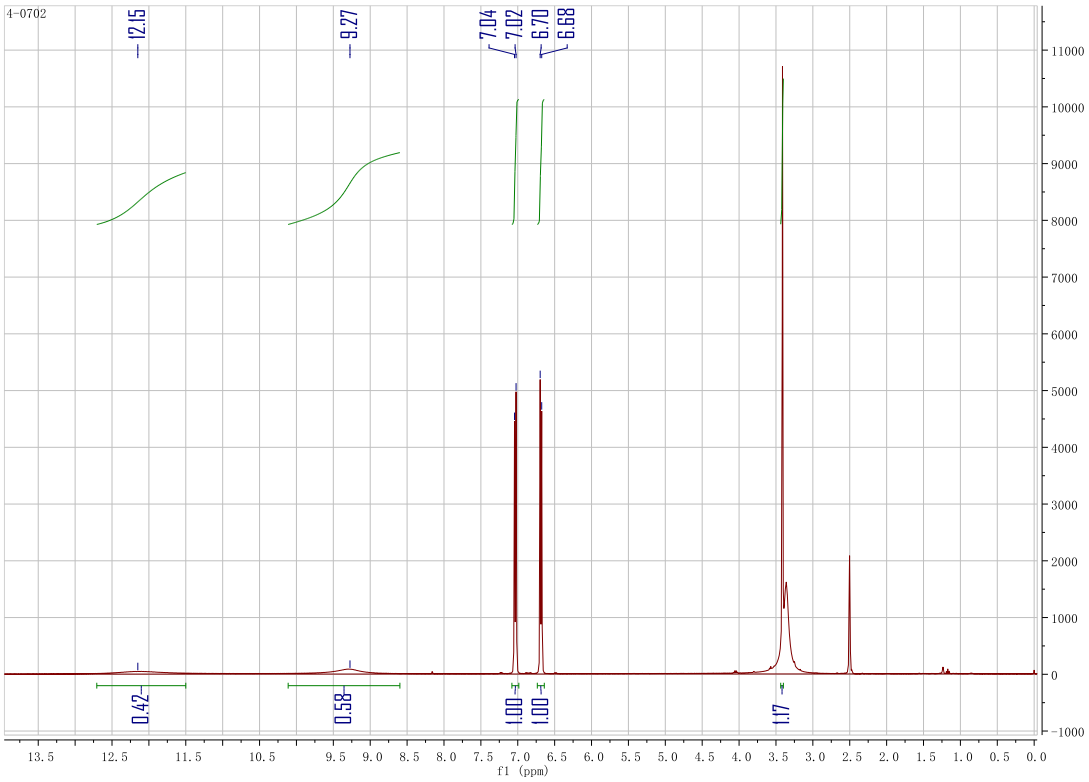



Compound VI: *p*-hydroxyphenylacetic acid

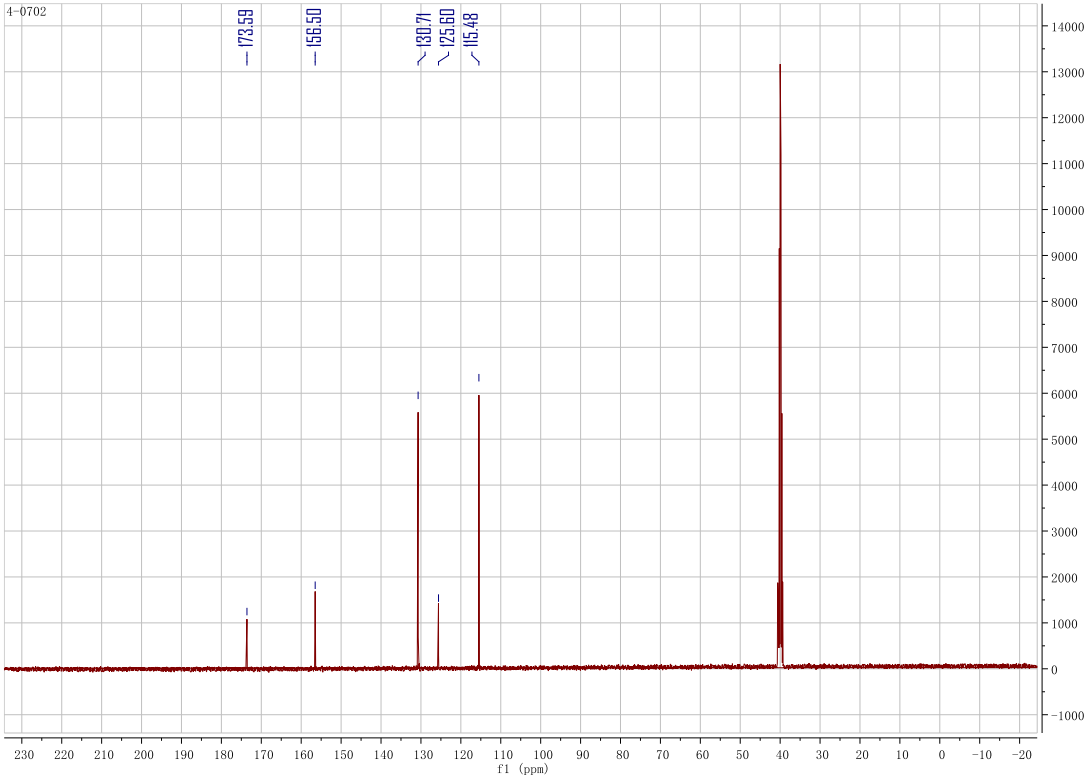
Mass spectrum in negativeion mode




$^{1}$H NMR (400 MHz, DMSO)

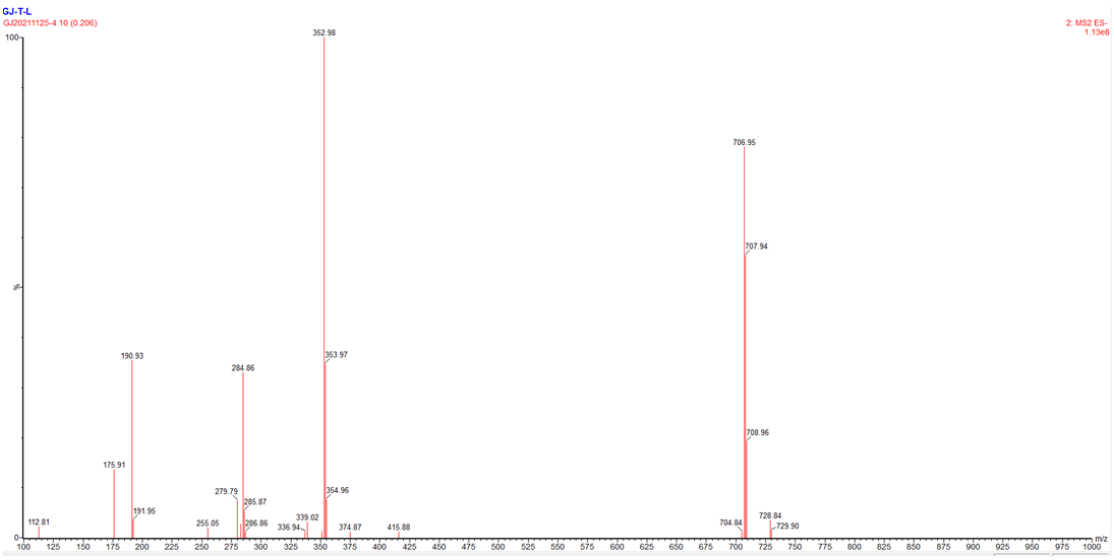



$^{13}$C-NMR (100 MHz, DMSO)

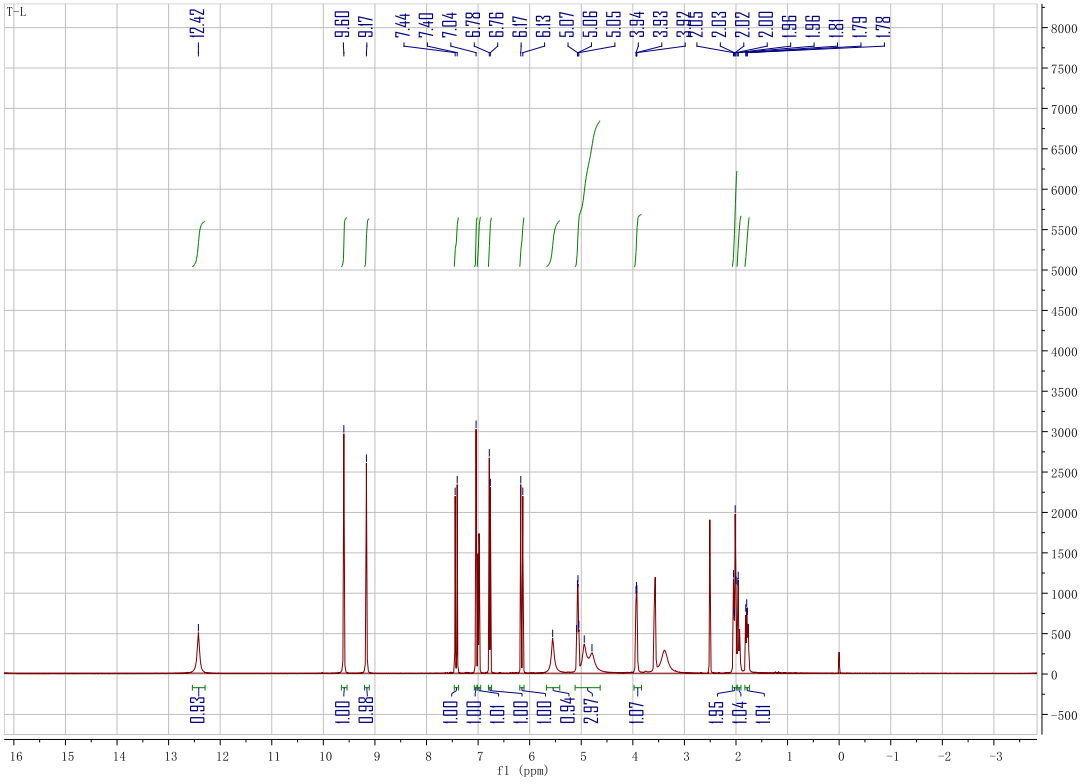



Compound VII: chlorogenic acid

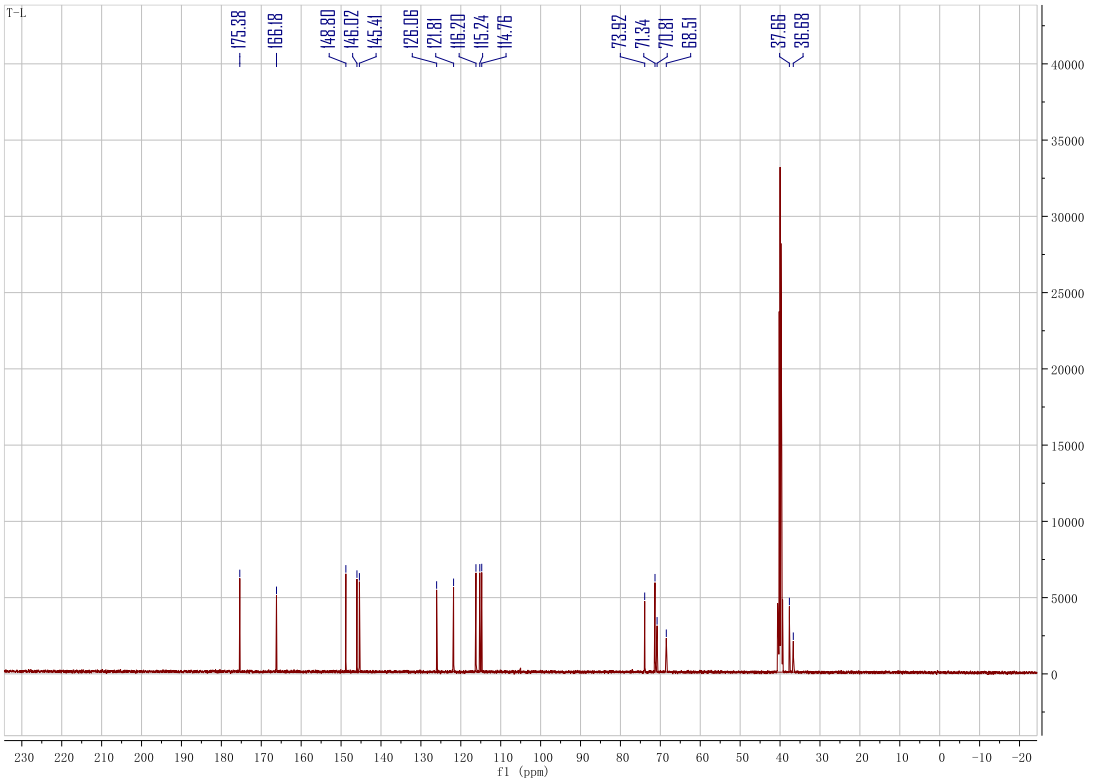
Mass spectrum in negativeion mode




$^{1}$H NMR (400 MHz, DMSO)



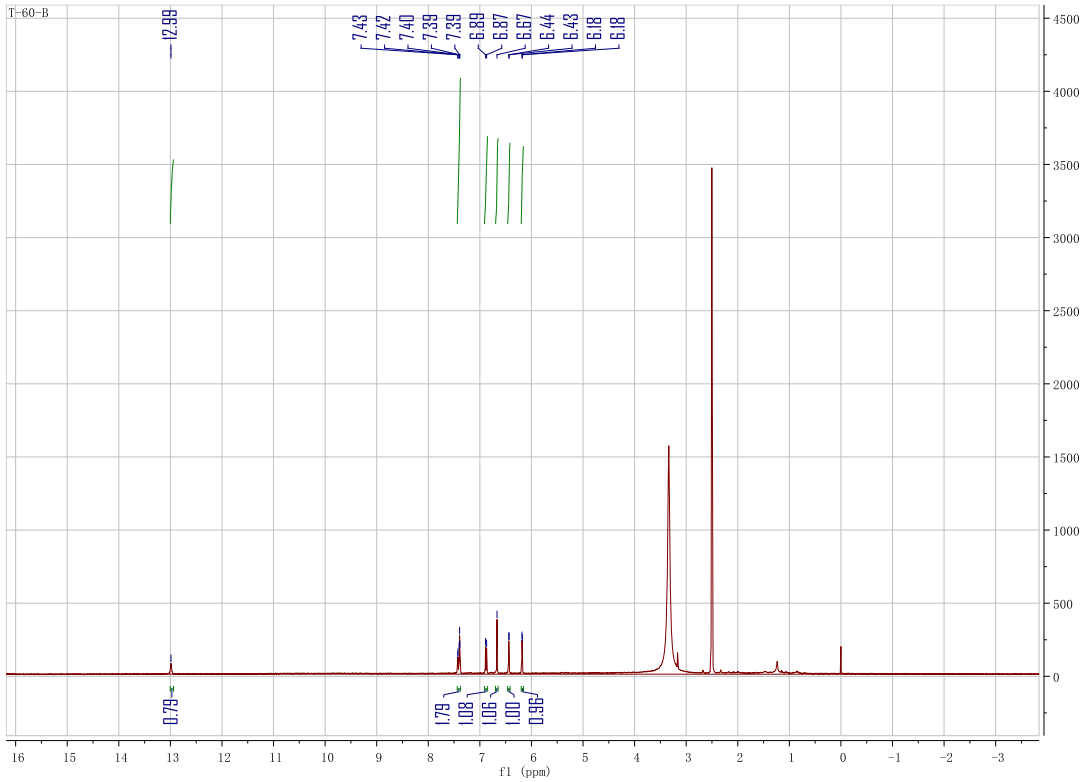

$^{13}$C-NMR (100 MHz, DMSO)



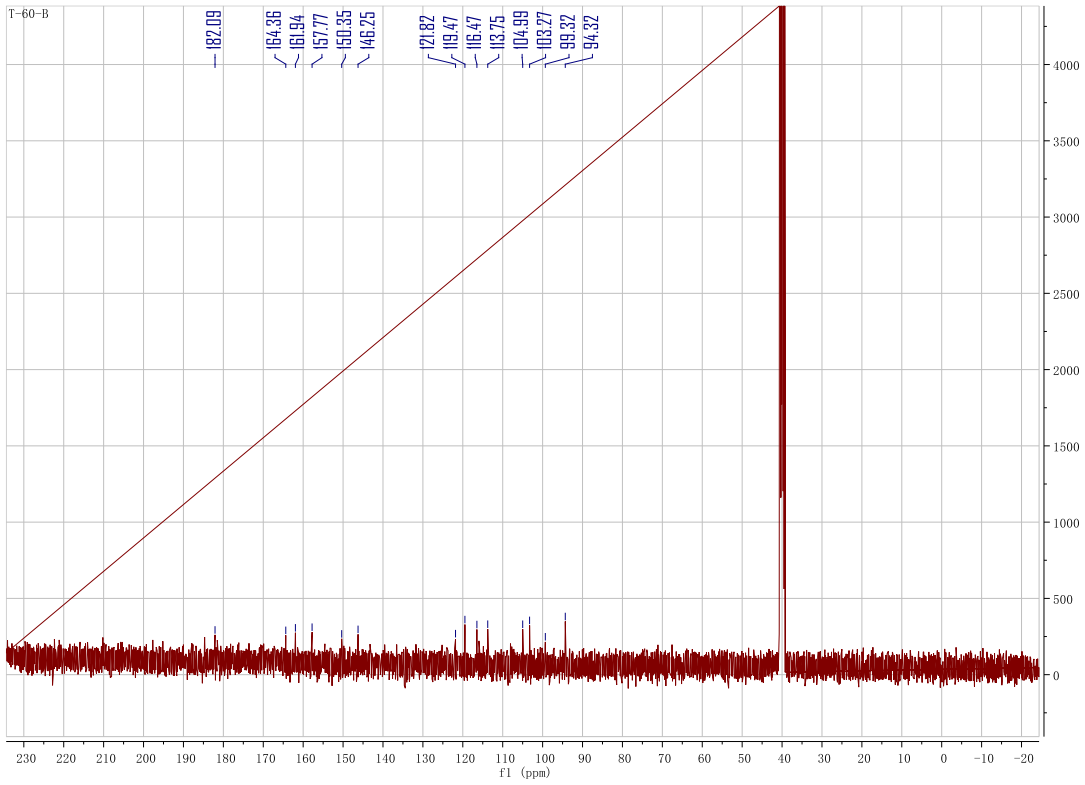

Compound VIII: luteolin

Mass spectrum in negativeion mode


$^{1}$H NMR (400 MHz, DMSO)

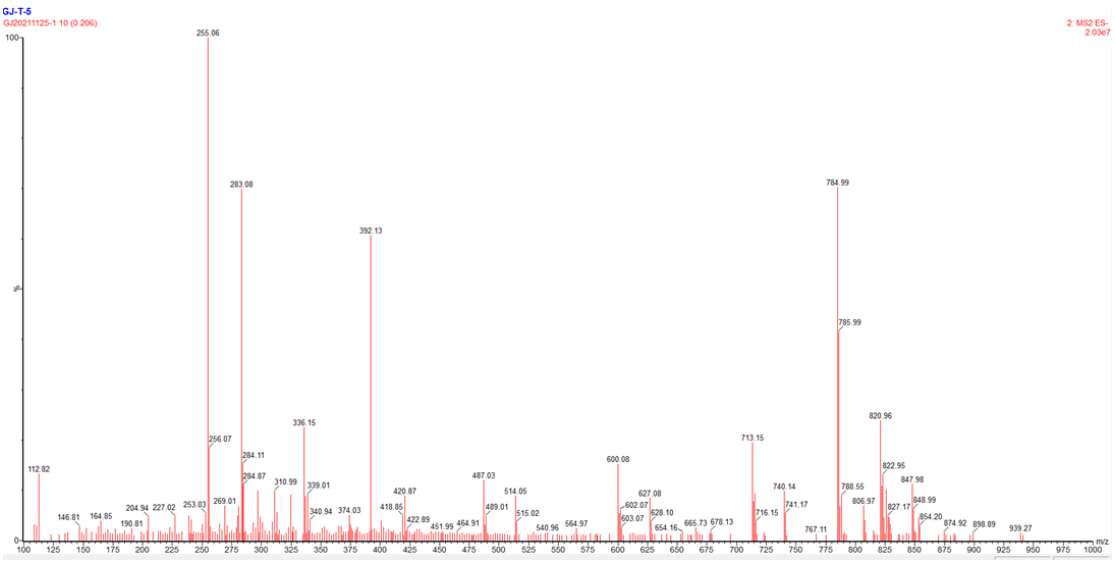



$^{13}$C-NMR (100 MHz, DMSO)

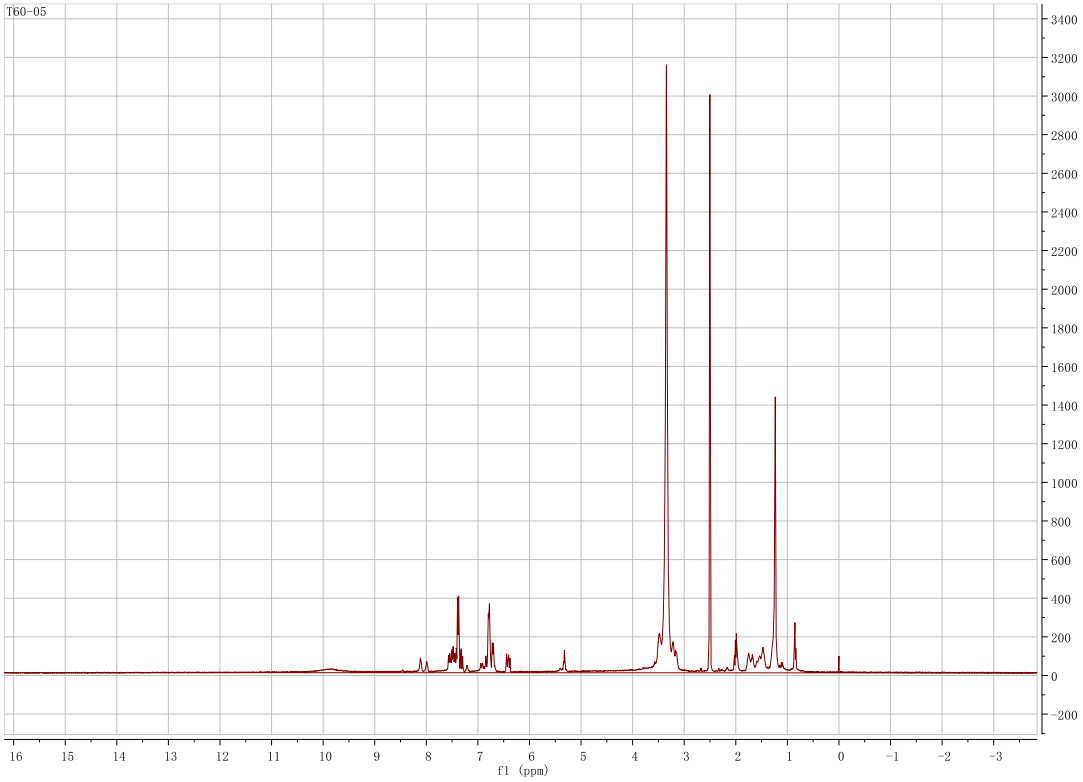



Compound IX

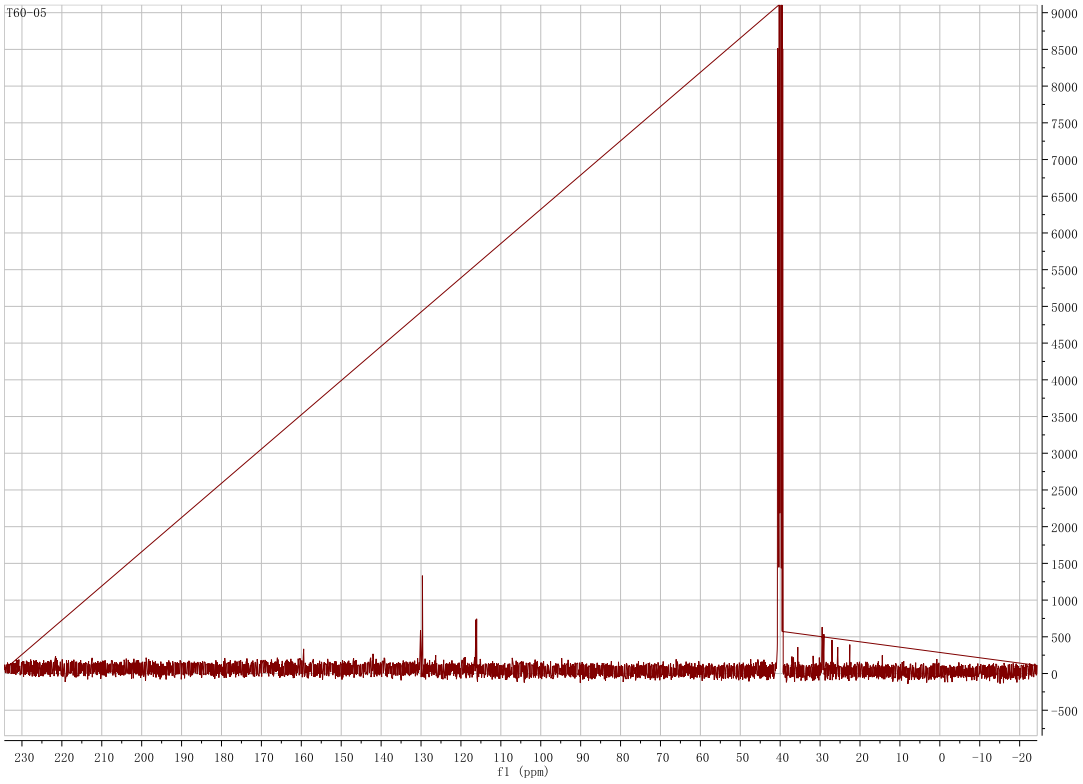
Mass spectrum in negativeion mode




$^{1}$H NMR (400 MHz, DMSO)




$^{13}$C-NMR (100 MHz, DMSO)


T60-05
9000
8500
8000
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)