Study on the relation of structure and antioxidant activity of isorhamnetin, quercetin, phloretin, silybin and phloretin isonicotinyl hydrazone

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ABSTRACT

Background: Some flavonoids compounds from the Chinese Traditional Medicine such as isorhamnetin, quercetin, phloretin, silybin have excellent antioxidant activity. Phloretin isonicotinyl hydrazone which was a new compound synthesized by our team, was expected to have more antioxidant activity than phloretin. **Materials and Methods:** The experiment examined the scavening free radicals activity and inhibition of lipid peroxidation activity of quercetin, isorhamnetin, phloretin, silybin and phloretin isonicotinyl hydrazone, with the methods including scavening1,1-diphenyl-2-picrylhydrazyl(DPPH) radical,2,2-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) radical and inhibition of Sprague Dawley (SD) rats liver mitochondria lipid peroxidation induced by Fe2+ / Vc system in vitro. **Results:** The experiment results showed that the five flavonoids had excellent antioxidant activity with the suitable concentration. In the experiment of scavening DPPH, ABTS radicals and inhibiting lipid peroxidation, the IC50 of isorhamnetin was respectively 24.61μmol/L,14.54μmol/L, 6.67μmol/L. The IC50 of quercetin was respectively 3.07μmol/L, 3.64μmol/L, 6.67μmol/L. The IC50 of phloretin was respectively 7.69μmol/L, 4.54μmol/L, 12.50μmol/L. The IC50 of phloretin isonicotinyl hydrazone was respectively 1.92 μmol/L, 2.27μmol/L, 2.08μmol/L. The IC50 of silybin was respectively 96.15μmol/L, 7.10μmol/L,104.16μmol/L.

Conclusion: The experiment results showed that quercetin, isorhamnetin, phloretin, silybin and phloretin isonicotinyl hydrazone had excellent antioxidant activity. **This was the first time to study** the antioxidant activity of phloretin isonicotinyl hydrazone. Moreover, for isorhamnetin, quercetin, phloretin, silybin, **this was the first time to study the activity of inhibition of SD rats liver mitochondria lipid peroxidation induced by Fe2+ / Vc system in vitro**. In a word, the results of experiments showed that the molecular had more phenolic hydroxyl groups, the ability of scavenging DPPH, ABTS radicals free radical was stronger. While phloretin isonicotinyl hydrazone which was added a hydrazone group, increased the ability of scavenging DPPH, ABTS free radical and inhibiting lipid peroxidation than phloretin. Quercetin is the typical structure flavonoid, phloretin is the two-hydrogen-chalcone flavonoid with the open loop structure after hydrogenation. Silybin is the lignan flavonoid compound which has the ABCDE five rings, the structure is much more complicated. The different molecular structures of quercetin , phloretin and silybin lead to the different antioxidant activity.

Key words: antioxidant activity, lipid peroxidation, quercetin, silybin and phloretin isonicotinyl hydrazone.

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INTRODUCTION

Five kinds of flavonoids with different molecular structures were chosen to study the antioxidant activity, including quercetin, isorhamnetin, phloretin, silybin and phloretin isonicotinyl hydrazone. The experiment examined the antioxidant activity with the Methods including scavenging DPPH radical ,ABTS radical and inhibition of lipid peroxidation in the liver in vitro. This was the first time to study the antioxidant activity of phloretin isonicotinyl hydrazone. Moreover, for isorhamnetin, quercetin, phloretin, silybin, this was the first time to study the activity of inhibition of SD rats liver mitochondria lipid peroxidation in vitro.

Free radicals mainly including various forms of reactive oxygen, whose oxidation are very intense, will do great harm to human body. Free radicals can result in the DNA oxidation, modification, and even rupture. Free radicals can result in the protein and enzyme oxidation,changing its structure and function.Free radicals can also result in highly unsaturated fatty acid oxidation to generate lipid peroxide in phospholipid molecules of cell membranes, which generate biological membrane injury. Organization cell aging is also closely related with more and more free radicals in the body.

Liver mitochondria lipid peroxidation in vitro, is an excellent model for studying antioxidant activity. Fe^{2+} / Vc system can startup liver mitochondria lipid peroxidation in vitro. Malondialdehyde (MDA) is the most important stability product of lipid peroxidation. Thiobarbituric Acid Reactive Substance (TBARS) is the product of color reaction between MDA and thiobarbituric acid (TBA).By measuring the content of product TBARS , the study will indicate the extent of the lipid peroxidation.

Isorhamnetin is called 3, 4', 5, 7- four hydroxyl-3 'Oxygen methyl flavonoid or quercetin-3 'armour ether, whose molecular formula is $C_{16}H_{12}O_7$. Quercetin is called 3, 3 ', 4 ', 5, 7-five hydroxyl flavonoid , whose molecular formula is $C_{15}H_{10}O_7$. Today many experiments already proved that isorhamnetin and quercetin had a very broad range of physiological and pharmacological activities, such as dilating coronary vascular, lowering blood fat, anti-aggregating platelet and protecting heart, kidney and liver, removing the oxygen free radicals, etc. In recent years, many experiments proved that isorhamnetin and quercetin could significantly inhibit the growth of many kinds of cancer cells such as Eca-109 cells[1],lewis lung cancer [2],human breast cancer cells[3,4], monoblastic leukemia [5], BEL-7402 cells [6], stomach cancer A549 cells[7], colon cancer cells [8], human oral squamous carcinoma cells (SCC-25) [9], pancreatic tumor cells [10], etc. Isorhamnetin and quercetin are the main flavonoids from *Sarcopyramis bodinieri* var.*delicata* [11-13], which is belonged to *Melastomata- ceae*, widely used as rare and valuable Chinese herbal medicine.It is mainly distributed in jiang xi, fu jian, tai wan, etc.

Phloretin whose molecular formula is $C_{15}H_{14}O_{5,}$ is the two-hydrogen -chalcone flavonoid, existing in the peel and root skin of apple, pear and vegetables juice etc. Phloretin can fade melanin, make the skin whitening, its effect is better than kojic acid and arbutin. Phloretin are commonly used as new type of whitening agent in cosmetics. In addition, phloretin also has rich biological activities, such as antioxidant[14-18], antitumor, falling blood sugar, protecting blood vessels etc. Phloretin can also enhance adipocyte differentiation and adiponectin expression in 3T3-L1 cells[19], suppress stimulated expression of endothelial adhesion molecules and reduce activation of human platelets[20], protect from the ultraviolet-induced photodamage in human skin[21], inhibit of oxidation of aqueous emulsions of omega-3 fatty acids and fish oil[22].H. P. Vasantha Rupasinghe et al. found that phloretin and phloretin glucoside could suppress the oxidation induced by oxygen free radical of omega 3 fatty acids and fish oil in the emulsion system. So phloretin would be a natural antioxidant in food to polyunsaturated fatty acid . [23]'The research of Bashir M. Rezk et al. showed that phloretin was a kind of effective antioxidant to clear peroxidation - nitroso -anion and inhibit lipid peroxidation.[24]

Phloretin Isonicotinyl Hydrazone whose molecular formula is $C_{21}H_{19}N_3O_5$, is a new compounds synthesized by our study team. Acyl hydrazone compounds have many unique structure features: (1) they contain many coordination atoms,which have strong coordination ability; (2) they have coordination atoms such as nitrogen, oxygen and sulfur atoms etc. which are similar to biological environment; (3) their molecules have greater conjugated system,which have large second harmonic generation (SHG) coefficient.Acyl hydrazone compounds have many pharmacological activities,such as anti-bacteria(include anti-tuberculosis bacteria), anti-inflammation, anti-virus, anti-oxidation, anti-cancer, anti-tumour, etc. These findings inspired high research enthusiasm of many chemists and biologists. [25-28]

Silybin whose molecular formula is $C_{25}H_{22}O_{10}$, is the lignan flavonoid extracted from compositae plants *Silybum marianum (L.) Gaertn.* Silybin has significant antioxidant activity[29,30], can also promote anticancer effect[31], and effect on the development of mammary tumors in HER-2/neu transgenic mice.[32] Silybin can also antiproliferate gynaecological malignancies[33], decrease glucose uptake by inhibiting GLUT proteins[34]. Silybin was used widely as clinical medicine to treat acute or chronic hepatitis, liver cirrhosis, toxic liver damage and liver fibrosis [35-36]. In recent years, the studies also found that silybin had significant inhibitory effect on many kinds of epithelial tumors such as lung cancer, prostate cancer, and colon cancer, bladder cancer , skin cancer ,etc.

MATERIALS AND METHODS

Chemicals and reagents

Quercetin, isorhamnetin, DPPH, TBA, ABTS were purchased from Sigma Chemical Company (St. Louis,

MO, USA). Phloretin and silybin were obtained from Huike Chemical Company (Purity is more than 96%, shanxi province, china). Phloretin isonicotinyl hydrazone is a new compound synthesized by our team, whose purity is more than 95%.

Sodium dihydrogen phosphate, disodium phosphate, ferrous sulfate, potassium sulfateb, $K_2S_2O_8$ and all solvents were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

C3606 organization mitochondria separation reagent kit was purchased from Biyuntian company (Shanghai, China). SD rats were bought from medical school test animals center of Nanchang University.

Visible spectra measurements were done using UV-2450 spectrophotometer (Shimadzu, Japan). HPLC chromatography were done using Hitachi L-2000 HPLC chromatography instrument (Hitachi company, Japan), The IR profiles were done using

FTIR Nicolet 5700 Fourier transform infrared spectrometer (Nicolet company,USA);

The synthesis and characterization of phloretin isonicotinyl hydrazone

20 mmol phloretin (5.4854 g) and 21 mmol isoniazid (2.8802 g) were weighed respectively in 250 ml three-neck-flask, and then 5 ml ethanol was added to dissolve them, then 50 ml toluene and 0.5 g p-toluene sulfonic acid were joined. The time of backflow –dividing water-reaction was 48 h. The synthesis route was showed in figure 3 .The reaction liquid was separated 3 times by 40 to 70 μ m silica gel column chromatograp hy. The elution agents was made up of 20:1 ethyl acetate / methanol. Thin layer chromatography (TLC)was a good way of track detection. We collected the second component, steamed the solvent , and vacuum-dried the component to constant weight.

HPLC chromatography conditions of phloretin isonicotinyl hydrazone were the followings. Hitachi L-2000 HPLC chromatography instrument and Autima reversed-phase chromatography column were used. The mobile phase was composed of 45%acetonitrile and 55% water. The volume of every sample was 10 μ L. The detected wavelength was 310 nm.

The samples for IR spectrum were prepared by the KBr method. Within the scope of 400- 4000 cm $^{-1}$, the IR spectrum of phloretin isonicotinyl hydrazone was measured by the FT-IR infrared spectrometer.

DPPH free radical-scavenging capacity

With reference to the method of Lee YL, et al., [37,38] the reaction system was amended as the following steps. In the tube, we joined in turn 1 ml tested samples in different concentration and 0.5 ml 0.6 mmol/L DPPH methanol solution, and then added ethanol to 5 ml final volume. The reaction lasted 30 min in room temperature and dark environment. We determinated parallelly three times the absorb light value at 517nm. Each sample was measured in triplicate and averaged. The activity was calculated according to the following formula:

$$\text{DPPH scavenging activity (\%)} = \frac{A_C - A_S}{A_C} \times 100\%$$

where A_C is the absorbance value of the control and A_S is the absorbance value of the added test samples solution.

ABTS free radical-scavenging capacity

With reference to the method of Re, R, et al. with some modifications, [39] the reaction system was amended as the following steps. ABTS was dissolved in water to make a concentration of 7 mmol/L. ABTS $^+$ was produced by reacting the ABTS stock solution with 2.45 mmol/L potassium persulfate ($K_2S_2O_8$) and allowing the mixture to stand in the dark at room temperature for 12-16 h before using. For the test of samples, the $ABTS^+$ stock solution was diluted with methanol to an absorbance of 0.70 ± 0.02 at 734 nm.

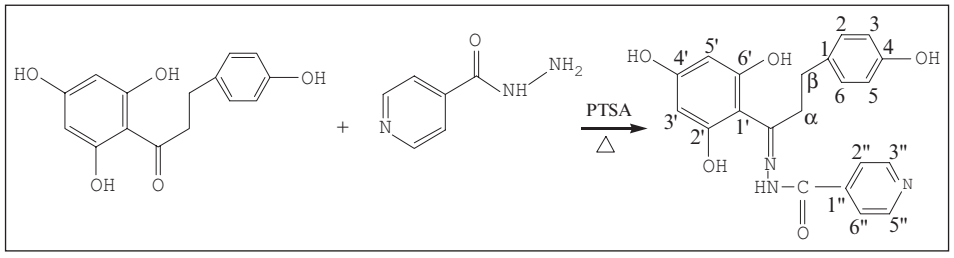

Figure 1. Synthesis of phloretin isonicotinyl hydrazone

After the addition of 5 ml of diluted $ABTS^+$ to 0.5 ml of diluted samples, the absorbance reading was taken 6 min after the initial mixing. The activities of the samples were evaluated by comparison with a control (containing 5 ml of $ABTS^+$ solution and 0.5 ml of ethanol). Each sample was measured in triplicate and averaged. This activity is given as percentage of $ABTS^+$ scavenging that is calculated by the following formula:

$$ABTS^+ \text{ scavenging activity } (\%) = \frac{A_C - A_S}{A_C} \times 100\%$$

where A_C is the absorbance value of the control and A_S is the absorbance value of the added test samples solution.

INHIBITION OF LIVER MITOCHONDRIA LIPID PEROXIDATION IN VITRO

Preparation of SD rats liver mitochondria [40-42]

The liver of SD rats was obtained by breaking the cervical of rats, opening the abdominal cavity, taking out the liver , washing it 2 times with cold PBS, and using scissors to cut the liver tissue into very small pieces. Then we added 10 multiples volume cold mitochondria separation reagent A into the liver pieces, homogenated it 10 times on the ice. Then the liver homogenate was centrifuged in the condition of 600 rpm, 4 °C,5 minutes. Then we moved the upper liquid to another centrifugal tube, and centrifuged it in the condition of 11000 rpm, 4 °C,10 minutes. The precipitation was the liver mitochondria, which was stored in -20 °C.

Preparation of the BSA standard protein standard curve

0.5 mg/ml BSA standard protein liquid were taken 0.5, 0.4, 0.3, 0.2, 0.1 ml to PBS solution diluted to 0.5 ml. So we got respectively the BSA standard protein solutions with the concentration of 0.5, 0.4, 0.3, 0.2, 0.1 mg/ml. In the above solution ,we added 4.5 ml BCA solution, put the solution in 37 °C for 20 min, and determined the absorbency at 562 nm. The BSA standard protein standard curve was made of protein concentration and absorbency.

The experiment's result showed that the benefice relationship was good in 0.1 ~ 0.5 mg/ml range of the standard protein concentration. The regression equation is y = 1.536 × 0.0532, R2 = 0.9953.

The measure of liver mitochondrial protein content

The protein content of mitochondria suspending liquid can be measured by the method of Coomassie G-250 blue staining. The reaction liquid was made of 0.1 ml liver mitochondria suspending liquid in PBS and 5 ml Coomassie G-250.After 2 min , The absorb light value of the reaction liquid was measured at 595 nm, with the reference of Coomassie G-250. The protein content of mitochondria liquid was calculated by the absorption value and BSA standard curve. 0.7 mg/ml mitochondrial protein concentration was appropriate.

Inhibition of liver mitochondria lipid peroxidation in vitro

In the tubes, we added in turn respectively 0.5ml more than 5 kinds of concentration gradient antioxidant drug, 1ml mitochondria levitation liquid, 0.25 ml 0.1mmol/l Fe^{2+}, and 0.25 ml 1mmol/l Vitamin C. The positive control group was added 0.5 ml 0.05 mol/l PBS buffer liquid, not the 0.5 ml antioxidant drug solution. The blank group was added only 1 ml 0.05 mol/l PBS buffer liquid and 1 ml mitochondrial levitation liquid.

We placed the test tubes in concussion incubation machine in 37°C for 1 hour, then added 2 ml solution of 20% CCl_3COOH and 2.5% hydrochloric acid, 10 min later added 2 ml solution of 0.67% TBA and 0.3% NaOH. We placed the test tubes in the 95°C water for 30 min, then centrifuged them 3500rpm for 10 min, measured the OD value at 532 nm. The experiment was repeated 3 times, the result was the average.

$$\text{Lipid peroxidation inhibition activity } (\%) = \frac{A_C - A_S}{A_C} \times 100\%$$

where A_C is the absorbance value of the positive control group and A_S is the absorbance value of the added test samples solution.

RESULTS AND DISCUSSION

The ^1H NMR and ^{13}C NMR data of phloretin isonicotinyl hydrazone

^1H NMR (DMSO-d_6,600 MHz) δ: 2.76 (2H, t, J=7.8 Hz, H-β), 3.19 (2H, t, J = 7.8 Hz, H-α), 5.77(2H, s, H-3', 5'), 6.66 (2H, dd, J=2.4, 1.8 Hz, H-2, 6), 7.10 (2H, dd, J=2.4, 1.8 Hz, H-3, 5), 7.82 (2H, dd, J=2.4, 1.8 Hz, H-2'', 6''), 8.79-8.81 (3H, m, H-3'', 5'', N-NH), 9.13 (1H, s, OH-4), 10.74 (1H, d, J = 1.2 Hz, OH-2'), 12.25 (2H, s, OH-4', 6')[13]

C NMR (DMSO-d_6,150 MHz) δ: 203.7(s, -C=N), 165.2(s, -C=O), 164.4(s, C-4', 6'), 156.0(s, C-2'), 155.8(s, C-4), 150.9(d, C-3", 5"), 140.0(s, C-1"), 132.2(s, C-1), 129.6(d, C-2, 6), 121.7(d, C-2", 6"), 115.5(d, C-3, 5), 103.4(s, C-1'), 90.5(d, C-3', 5'), 45.8(e, C-β), 30.1(e, C-α)

The HPLC profile and IR profile of phloretin isonicotinyl hydrazone were showed in Figure 2 and Figure 3. The UV spectra, elementary analysis, and thermo-gravimetric analysis of phloretin and phloretin isonicotinyl hydrazone were showed in Nanchang university master's degree thesis, whose title was Synthesis, Characterization and Bioactivity Evaluation of Rare Earth Complexes with Phloretin, Phloretin –Isonicotinoyl Hydrazone.

DPPH free radical-scavenging capacity

From figure 4 and figure 5, it was known that isorhamnetin, quercetin , phloretin, phloretin isonicotinyl hydrazone and silybin had significant activity of scavenging DPPH free radical with the suitable concentration. Their final concentration IC_{50} of scavenging DPPH free radical was respectively 32μmol/L, 4μmol/L, 10μmol/L, 2.5μmol/L L, 125μmol/L. The activity order from strong to the weak was phloretin isonicotinyl hydrazone, quercetin , phloretin,isorhamnetin, and silybin.

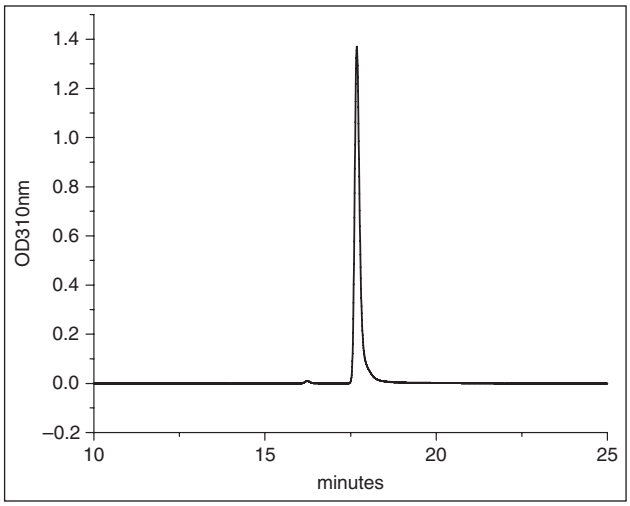

Figure 2. The HPLC profile of phloretin isonicotinyl hydrazone

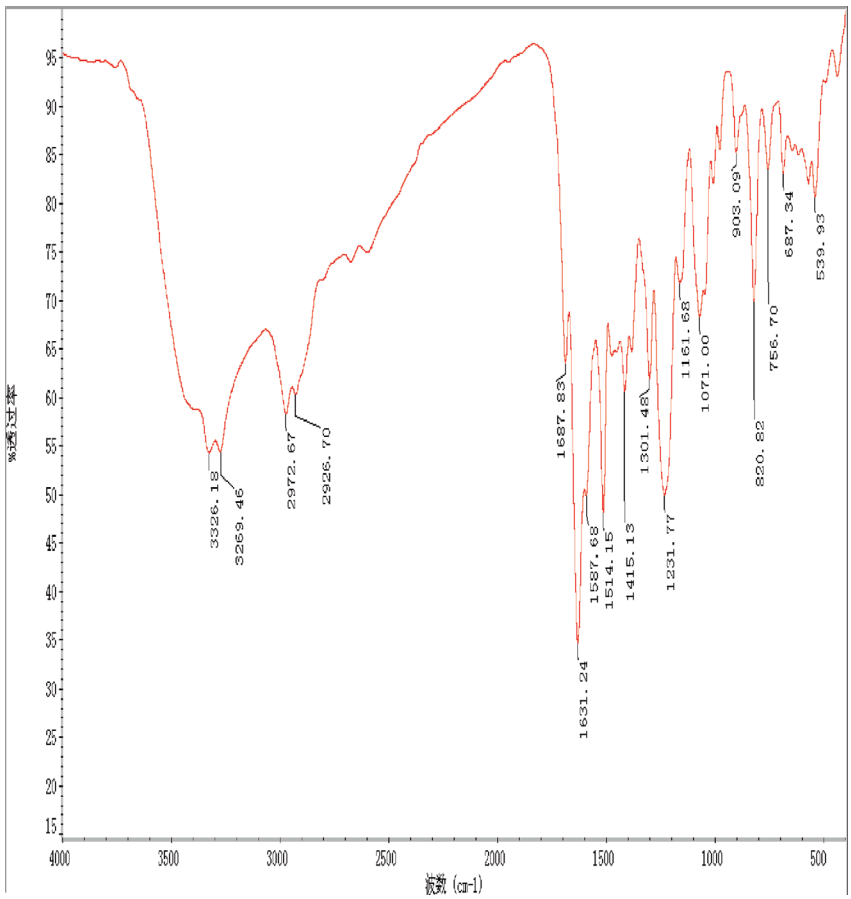

Figure 3. The IR profile of phloretin isonicotinyl hydrazone

The experimental results showed that with the same concentration, the activity of quercetin of scavenging DPPH free radical was stronger than the activity of isorhamnetin. For example, with the 4μmol/L concentration, the ratio of scavenging DPPH radicals of isorhamnetin was 10.44%, while the ratio of quercetin was 50.13%.The molecular structure of quercetin and isorhamnetin are very similar. The only different structure is that the 3 ' Carbon atoms of isorhamnetin is linked with Oxygen methyl, while the 3 ' Carbon atoms of quercetin is linked with hydroxyl. With the different molecular structure , quercetin and isorhamnetin have different activity of scavenging DPPH free radical.

As we expected, the experimental results showed that with the same concentration, the activity of phloretin isonicotinyl hydrazone of scavenging DPPH free radical was stronger than the activity of phloretin. For example, with the 10μmol/L concentration, the ratio of scavenging DPPH radicals of phloretin was 44.47%, while the ratio of phloretin isonicotinyl hydrazone was 76.97%.

In a word, the result of experiment showed that the molecular had more phenolic hydroxyl groups, the ability of scavenging DPPH free radical was stronger. While phloretin isonicotinyl hydrazon which was added a hydrazone group, increased the ability of scavenging DPPH free radical than phloretin.

The activity of scavenging DPPH radicals from strong to the weak was in turn quercetin , phloretin and silybin. Their final concentration IC_{50} of scavenging DPPH free radical was respectively 4μmol/L, 10μmol/L, 125μmol/L. The molecular structure of quercetin , phloretin and silybin are very different. Quercetin is the typical structure flavonoid, phloretin is the two-hydrogen -chalcone flavonoid with the open loop structure after hydrogenation. Silybin is the lignan flavonoid compound which has the ABCDE five rings, the structure is much more complicated. The different molecular structures of quercetin , phloretin and silybin lead to theAbout the experiment of scavening DPPH radicals, because there were some differences in the reseach system, the IC50 of quercetin, isorhamnetin, phloretin and silybin were not the same results. For example, the IC_{50} of quercetin was respectively 5.5μmol/L[44] and 4.5μmol/L[45]. The IC_{50} of isorhamnetin was 26.7 μg/ml or 72.75μmol/L[46]. The IC_{50} of phloretin was 50μmol/L[47]. The IC_{50} of silybin was 327μmol/L[48].

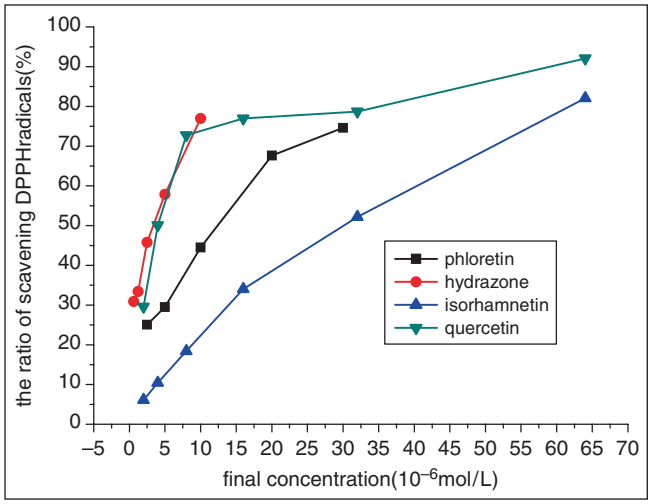

Figure 4. The relationship between final concentration and the ratio of scavening DPPH radicals.

ABTS free radical-scavenging capacity

From figure 5 and figure 6, it was known that isorhamnetin, quercetin , phloretin, phloretin isonicotinyl hydrazone

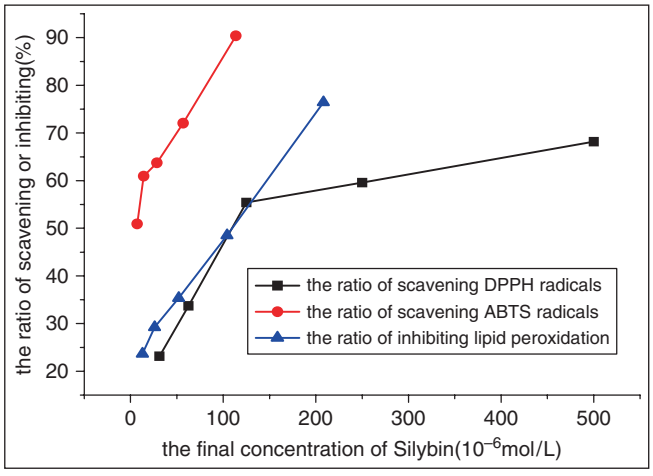

Figure 5. The relationship between final concentration of Silybin and the ratio of scavening DPPH, ABTS radicals or inhibiting lipid peroxidation.

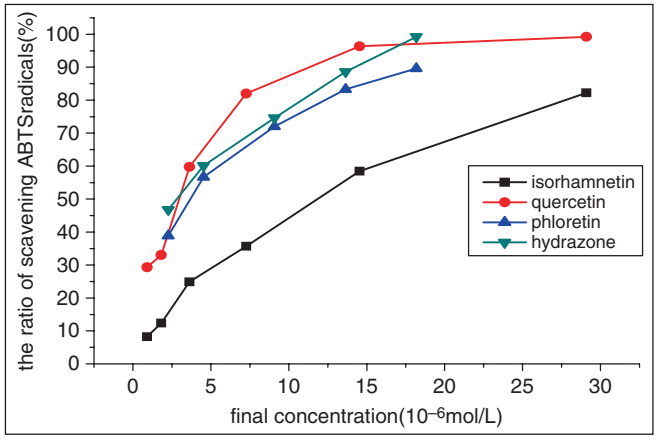

Figure 6. The relationship between final concentration and the ratio of scavening ABTS radicals.

and silybin had significant activity of scavenging ABTS free radical with the suitable concentration. Their final concentration IC_{50} of scavenging ABTS free radical was respectively 14.54μmol/L, 3.64μmol/L, 4.54μmol/L, 2.27μmol/L, 7.10μmol/L. The activity order from strong to the weak was phloretin isonicotinyl hydrazone, quercetin , phloretin, silybin and isorhamnetin.

The experimental results showed that with the same concentration, the activity of quercetin of scavenging ABTS free radical was stronger than the activity of isorhamnetin. For example, with the 14.54 μmol/L concentration, the ratio of scavenging ABTS radicals of isorhamnetin was 58.46%, while the ratio of quercetin was 96.35%.The molecular structure of quercetin and isorhamnetin are very similar. The only different structure is that the 3 ' Carbon atoms of isorhamnetin is linked with Oxygen methyl, while the 3 ' Carbon atoms of quercetin is linked with hydroxyl. With the different molecular structure , quercetin and isorhamnetin have different activity of scavenging ABTS free radical.

As we expected, the experimental results showed that with the same concentration, the activity of phloretin isonicotinyl hydrazone of scavenging ABTS free radical was stronger than the activity of phloretin. For example, with the 2.27μmol/L concentration, the ratio of scavenging ABTS radicals of phloretin was 38.90%, while the ratio of phloretin isonicotinyl hydrazone was 46.80%.

The activity of scavenging ABTS radicals from strong to the weak was in turn quercetin , phloretin and silybin. Their final concentration IC_{50} of scavenging ABTS free radical was respectively 3.64μmol/L, 4.54μmol/L, 7.10μmol/L.The molecular structures of quercetin , phloretin and silybin are very different. The different molecular structures of quercetin , phloretin and silybin lead to the different activity of scavenging ABTS radicals.

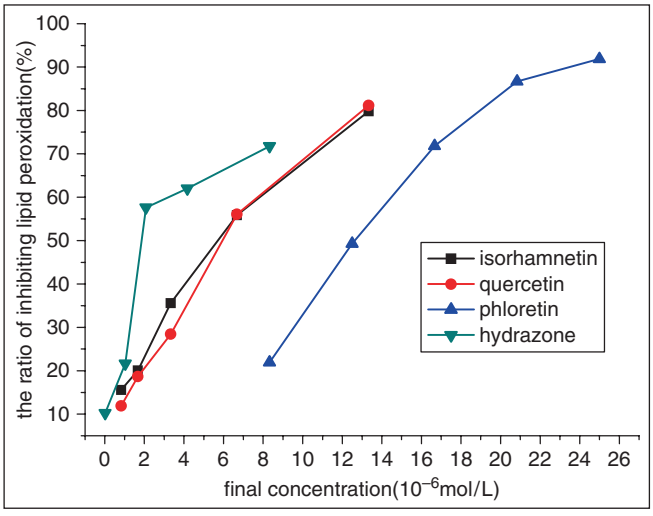

Figure 7. The relationship between final concentration and the ratio of inhibiting lipid peroxidation.

Inhibition of liver mitochondria lipid peroxidation in vitro

From figure 5 and figure 7, it was known that isorhamnetin, quercetin , phloretin, phloretin isonicotinyl hydrazone and silybin had significant activity of inhibiting lipid peroxidation with the suitable concentration. Their final concentration IC_{50} of inhibiting lipid peroxidation was respectively 6.67μmol/L, 6.67μmol/L, 12.5μmol/L, 2.08μmol/L, 104.16μmol/L. The activity order from strong to the weak was phloretin isonicotinyl hydrazone, quercetin , phloretin and silybin. With the same concentration, the activity of quercetin are very similar to the activity of isorhamnetin.

As we expected, the experimental results showed that with the same concentration, the activity of phloretin isonicotinyl hydrazone of inhibiting lipid peroxidation was stronger than the activity of phloretin. For example, with the 8.33μmol/L concentration, the ratio of inhibiting lipid peroxidation of phloretin was 21.92%, while the ratio of inhibiting lipid peroxidation of phloretin isonicotinyl hydrazone was 71.76%.

The activity of inhibiting lipid peroxidation from strong to the weak was in turn quercetin , phloretin and silybin. Their final concentration IC_{50} of inhibiting lipid peroxidation was respectively 6.67μmol/L, 12.5μmol/L, 104.16μmol/L.The molecular structures of quercetin , phloretin and silybin are very different. The different molecular structures lead to the different activity of inhibiting lipid peroxidation.

Lipid peroxidation is the oxidation degradation chain reaction process of the unsaturated fatty acid , including three stages which are start, extensions and termination. In the extensional process of lipid peroxidation ,there will generate many kinds of free radicals, such as oxygen free radicals, fat-oxygen free radical and fat free radicals. In the termination stage , there will generate many kinds of small molecules ,such as the malondialdehyde (MDA) .The products can do harm to many kinds of cell functions. There is a close relationship between the damage and many kinds of diseases.[43] It may be beneficial to enhance properly antioxidation products.

CONCLUSION

The experiment results showed that the five kinds of flavonoids have excellent antioxidant activity with the

suitable concentration. In the experiment of scavening DPPH, ABTS radicals and inhibiting lipid peroxidation, the IC_{50} of isorhamnetin was respectively 24.61μmol/L, 14.54μmol/L, 6.67μmol/L. The IC_{50} of quercetin was respectively 3.07μmol/L, 3.64μmol/L, 6.67μmol/L. The IC_{50} of phloretin was respectively 7.69μmol/L, 4.54μmol/L, 12.50μmol/L. The IC_{50} of phloretin isonicotinyl hydrazone was respectively 1.92 μmol/L, 2.27μmol/L, 2.08μmol/L. The IC_{50} of silybin was respectively 96.15μmol/L, 7.10μmol/L, 104.16μmol/L.

In a word, the result of experiment showed that the molecular had more phenolic hydroxyl groups, the ability of scavenging DPPH, ABTS radicals free radical was stronger. While phloretin isonicotinyl hydrazon which was added a hydrazone group, increased the ability of scavenging DPPH, ABTS free radical and inhibiting lipid peroxidation than phloretin. Quercetin is the typical structure flavonoid, phloretin is the two-hydrogen-chalcone flavonoid with the open loop structure after hydrogenation. Silybin is the lignan flavonoid compound which has the ABCDE five rings, the structure is much more complicated. The different molecular structures of quercetin , phloretin and silybin lead to the different antioxidant activity.

This was the first time to study the antioxidant activity of phloretin isonicotinyl hydrazone which is a new compound synthesized by our team and is expected to have more antioxidant activity than phloretin. Moreover, for isorhamnetin, quercetin, phloretin, silybin, this was the first time to study the activity of inhibition of SD rats liver mitochondria lipid peroxidation in vitro.

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