Improvement Effect of *Ficus vasculosa* Ethanol Extract on D-galactose-Induced Mice Aging

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Abstract

This study was to investigate antioxidant activities of the ethanol extract from young edible leaves of *Ficus vasculosa* in vitro and in vivo. *Ficus vasculosa* ethanol extract (FVEE) showed significantly higher reducing power and α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenge activity than vitamin C (*P* < 0.05). FVEE also showed an activity to resist the D-galactose-induced aging in mice assessed by serum and tissue levels of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). Total serum and tissue oxidative status, total antioxidant response, glutathione (GSH) and malondialdehyde (MDA) levels have been also measured. Pretreatment with FVEE at 200 mg/kg·body weight significantly increased enzyme activity of SOD and CAT in serum and hepatic tissue (*P* < 0.05), as well as significantly increased enzyme activity of SOD in kidney (*P* < 0.05). Furthermore, high concentration of FVEE pretreatment significantly increased the level of GSH in serum, hepatic tissue and kidney (*P* < 0.05), meanwhile significantly decreased MDA production in hepatic tissue and kidney (*P* < 0.05). In addition, the phytochemical investigation discovered six previously described compounds from FVEE, naringenin (1), vanillic acid (2), 9, 16-dioxo-10, 12, 14-octadeca-trienoic acid (3), 2, 6-dimethoxy-1, 4-benzoquinone (4), apigenin (5) and norartocarpetin (6), and all compounds were isolated from this plant for the first time. Among the various compounds found, the rare highly unsaturated fatty acid 9, 16-dioxo-10, 12, 14-octadeca-trienoic acid (3) has been identified, which had been isolated only once before from *F. vasculosa*. Evaluation of the antioxidant activity of isolated compounds showed naringenin (1) to be the most active. According to our research, FVEE present very high antioxidant activity in vitro due to the presence of several compounds known for their antioxidant activity such as flavonoid and phenolic acid. In vivo, the ethanol extract had improvement effects against D-galactose-induced aging by reducing oxidative stress.

Keywords

*Ficus vasculosa*, D-galactose, aging, bioactive compounds

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Aging and related diseases are becoming major public health problems worldwide. The role of oxidative stress plays in the pathophysiology of age-related human diseases, such as neurodegenerative diseases, heart attack, stroke, diabetes, and cancer is well documented. Natural products from fruits and plants are proved to have antioxidant properties by neutralizing excessive free radicals, reducing inflammation and oxidative stress. Considering health risks of synthetic antioxidants, especially cancer, there is increasing interest in replacing them with natural ones, which are generally of botanical origin.

The genus *Ficus* belongs to the Moraceae family and comprises about 1000 species around the world, which are distributed in the tropical to subtropical areas, and 98 species occur in China. *Ficus* species are traditionally used as food and medicine plant to promote human health for about 10 000 years. A few of *Ficus* species young leaves, including *Ficus vasculosa*, are consumed as wild vegetables by the ethnic groups in Xishuangbanna, Southwest China, as well as an important source of biologically active compounds of nutritional value. There is only one previous investigation of *F. vasculosa*, which was carried out to analyze the in vitro antioxidant properties of young leaves of the plant. These findings suggest that the young edible leaves might be a potential source of...
natural antioxidants. However, there is no literature about the in vivo antioxidant activity and phytochemical investigation of these plants.

*Ficus vasculosa* is mainly distributed in the monsoon forest of Guangdong, Hainan, Guangxi, Yunnan and Guizhou, China. Our previous study for the first time to isolate one triterpene from the ethyl acetate (EtOAc) phase of *F. vasculosa* ethanol extract (FVEE) and many other constituents in the EtOAc fraction was still not fully investigated. The aging model using mice induced by D-galactose is related to free radical and the accumulation of waste substances in metabolism. The previous research confirmed that the oxidative biomarkers in blood, brain, and liver were consistent with natural aging animals and proved the reliability of the mimetic aging model. Therefore, the present study was conducted to evaluate the improving effect of FVEE on D-galactose-induced aging mice. Additionally, some bioactive compounds present in the EtOAc fraction of FVEE were isolated and identified (Figure 1).

The in vitro α,α-diphenyl-β-picrylhydrazyl (DPPH) scavenging activity of FVEE was shown in Figure 1a. FVEE exhibited a great activity to resist DPPH radicals in a dose-dependent manner. Following the FVEE treatment, the absorbance of the reaction solution was increased (Figure 1b). A higher absorbance of the reaction solution indicated greater reducing power. These results were similar to a study that reported by Shi et al. This observation of reducing power activity suggested FVEE could reduce the most Fe ions, which exhibited a good reducing capability in vitro. Natural products with metal chelating potential could have beneficial effects on metal catalyzed biochemical reactions such as DNA fragmentation and auto-oxidative glycation and glycoxidation reactions.

In ferrous ion (Fe) chelating assay, FVEE also showed a good activity to chelate Fe in vitro (Figure 1c). Furthermore, the DPPH radical scavenging activities and reducing power in FVEE group were significantly better than vitamin C (P < 0.05).

The effect of intragastric administration of FVEE on serum levels of antioxidant capability of D-galactose-induced aging mice is summarized in Table 1. There is a significant decrease in serum SOD, CAT, and GSH-Px (P < 0.05) and an increased level of MDA (P < 0.05) in model group, as compared with the control group. CAT, GSH-Px, and GSH were significantly increased (P < 0.01) by the high dose of FVEE (FVEE-200) and VC, while SOD was only significantly increased (P < 0.01) in the FVEE-200 group. FVEE-200 group showed significantly higher antioxidant capability in increasing the serum enzyme activity of SOD, CAT, and GSH than that of FVEE. The bars with different letters are significantly different (P < 0.05).
in the VC treatment group (P < 0.05). Furthermore, both FVEE and VC treatments were able to reduce the lipid peroxidation-related MDA generations in D-galactose-induced aging mice. There was no statistical difference between FVEE-50 and FVEE-200 groups in terms of SOD and GSH-Px activities increase and MDA level decrease.

The effect of FVEE on hepatic oxidative status of D-galactose-induced aging mice is shown in Table 2. Model group showed significant decrease in hepatic SOD, CAT, GSH-Px, and GSH (P < 0.05) and significant increase in MDA (P < 0.05) as compared with the control group. Significant increase in hepatic SOD, CAT, GSH-Px, and GSH (P < 0.01) were observed in FVEE-50, FVEE-200, and VC groups as compared with the model group. SOD, CAT, and GSH in FVEE-50 and FVEE-200 groups were significantly higher than that in VC treated aging mice (P < 0.05). However, there was no significant difference between FVEE groups and VC group in terms of GSH-Px increase. There was no statistical difference between FVEE-50 and FVEE-200 groups in terms of SOD, CAT, GSH-Px, and GSH activities increase. In addition, high dose of FVEE (FVEE-200) also significantly reduced MDA generation in liver of aging mice (P < 0.05).

SOD, CAT, GSH-Px, and GSH (P < 0.05) in model group was significantly lower than that in control group, while MDA level in model group was significantly higher than that in control group (Table 3). FVEE and VC groups exhibited a significant increase in kidney SOD (P < 0.01) with concomitant decrease in kidney MDA (P < 0.01) as compared with the model group. Additionally, FVEE group showed a significantly increase in the kidney SOD and GSH as compared with the model group, which was better than that in VC group (P < 0.05). Furthermore, FVEE-50 and FVEE-200 groups were equally efficient on CAT, GSH-Px, and GSH activities and MDA level except SOD.

SOD, CAT, and GSH-Px are the important antioxidant enzymes, increasing their expression and function can effectively defend oxidation and prevent aging. In this respect, in the present study, the FVEE treatment groups showed an increase of SOD, CAT, and GSH-Px in serum, hepatic, and kidney as compared with the untreated group (model group), indicating the FVEE treatment had antioxidant effect and the protective activity on the D-galactose-induced aging in mice. GSH is the major free thiol in most living cells and is the key antioxidant in animal tissues. As an oxidative stress marker, high levels of MDA can lead to lipid peroxidation. In this study, the high dose of FVEE treatment group (FVEE-200) exhibited significantly higher GSH levels and lower MDA levels in serum, hepatic, and kidney than that in the untreated group (model group), suggesting the FVEE treatment might reduce D-galactose-induced oxidative damage.

The isolated compounds (Figure 2) from FVEE were identified as naringenin, vanillic acid, 9,16-dioxo-10,12,14-octadeca-trienoic acid, apigenin, and norartocarpetin, respectively, by spectral analysis (1H-nuclear magnetic resonance [NMR], 13C-NMR, DEPT, 2D NMR, and high resolution mass spectrometry [HRMS]), as well as by comparison with literature data. Compounds 1-6 were obtained from this plant and compound

### Table 1. Effect of FVEE on the Blood Level of Antioxidant Capability in D-Galactose Induced Aging Mice.

| Groups  | SOD (U/mL) | CAT (U/mL) | GSH-Px (mol/mL) | GSH (mg/L) | MDA (nmol/mL) |
|---------|------------|------------|----------------|------------|---------------|
| Control | 727.72 ± 97.04 | 21.00 ± 3.67 | 358.33 ± 38.56 | 1.94 ± 0.55 | 17.58 ± 3.28 |
| Model   | 459.88 ± 128.4* | 8.47 ± 5.21* | 134.63 ± 8.28* | 1.71 ± 0.56 | 32.87 ± 3.32* |
| VC-100  | 627.30 ± 127.97 | 35.68 ± 8.94* | 284.88 ± 14.65* | 0.78 ± 0.00*# | 20.51 ± 3.23* |
| FVEE-50 | 818.22 ± 48.84*# | 10.05 ± 5.71*# | 234.15 ± 18.40*# | 1.55 ± 1.10 | 20.98 ± 1.61*# |
| FVEE-200 | 1978.61 ± 439.73*# | 218.83 ± 35.45*# | 319.02 ± 16.61 nga | 3.69 ± 0.98*#▲ | 16.32 ± 4.50*# |

CAT, catalase; FVEE, F. vasculosa ethanol extract; GSH, glutathione; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase.

Values are expressed as mean ± standard deviation (n = 10). VC-100: mice treated with vitamin C (100 mg/kg/day); FVEE-50: mice treated with low dose of FVEE (50 mg/kg/day); FVEE-200: mice treated with high dose of FVEE (200 mg/kg/day). *P < 0.05 vs control; ▲P < 0.01 vs model; ♦P < 0.05 FVEE groups vs VC group.

### Table 2. Effect of FVEE on the Hepatic Level of Antioxidant Capability in D-Galactose Induced Aging Mice.

| Groups  | SOD (U/mL) | CAT (U/mL) | GSH-Px (mol/mL) | GSH (mg/L) | MDA (nmol/mL) |
|---------|------------|------------|----------------|------------|---------------|
| Control | 149.99 ± 17.5 | 37.39 ± 6.12 | 68.49 ± 5.72 | 12.75 ± 2.06 | 0.10 ± 0.11 |
| Model   | 100.72 ± 4.17* | 11.98 ± 0.07* | 2.07 ± 1.62* | 10.68 ± 0.67* | 3.59 ± 0.27* |
| VC-100  | 60.46 ± 0.29*# | 7.58 ± 0.11*# | 3.63 ± 0.77*# | 7.08 ± 0.30*# | 2.88 ± 0.38* |
| FVEE-50 | 130.98 ± 1.96*# ▲ | 15.33 ± 0.80*# ▲ | 3.37 ± 1.41*# ▲ | 13.81 ± 0.31*# ▲ | 3.13 ± 0.38* |
| FVEE-200 | 150.03 ± 3.02*# ▲ | 16.54 ± 0.11*# ▲ | 3.64 ± 0.77*# ▲ | 14.63 ± 0.71*# ▲ | 2.52 ± 0.23* |

CAT, catalase; GSH, glutathione; GSH-Px, glutathione peroxidase; FVEE, F. vasculosa ethanol extract; MDA, malondialdehyde; SOD, superoxide dismutase.

Values are expressed as mean ± standard deviation (n = 10). VC-100: mice treated with vitamin C (100 mg/kg/day); FVEE-50: mice treated with low dose of FVEE (50 mg/kg/day); FVEE-200: mice treated with high dose of FVEE (200 mg/kg/day). *P < 0.05 vs control; ▲P < 0.01 vs model; ♦P < 0.05 FVEE groups vs VC group.
3 was isolated from the family Moraceae for the first time. Except compound 3, all compounds were also evaluated for their antioxidant activity using the DPPH assay (Table 4). Among the isolated compounds, the most significant activity was detected in naringenin 1 and apigenin 5, with the DPPH radical scavenging activities of 77.30% and 66.74% at the concentration of 100 µg/mL, respectively. Previous studies have confirmed the antioxidant potential of these compounds, in vivo or in vitro. Naringenin29 was reported to have antioxidant and hepatoprotective effects on carbon tetrachloride induced hepatic damage in mice. Another study30 has confirmed that vanillic acid and 2,6-dimethoxy-1,4-benzoquinone had scavenging activities against DPPH free radical. Apigenin 31 was found to have protective effect against hydrogen peroxide-induced damage in osteoblastic cells and may be useful for the treatment of oxidative-related bone disease. Norartocarpetin32 exhibited strong scavenging activities against 2,2-azobis-3-ethylbenzthiazoline-6-sulphonic acid radical.

In conclusion, FVEE had a good antioxidant property in vitro due to an important flavonoids and phenolic compounds. In vivo, the extracts had improvement effects against D- galactose-induced aging by reducing oxidative stress. The fractionation and investigation of FVEE afforded the identification of six known antioxidant compounds. Concluding, it seems that the F. vasculosa leaves rich in bioactive compounds can be a good source of antioxidants for the human diet. This study may give rise to further research on the potential of its commercial exploitation in nutraceutical industry.

**Experimental**

**General**

Thin layer chromatography analyses were carried out on silica gel GF254 plates (Qingdao Marine Chemical Ltd.; China). Column chromatography (CC) was performed by using silica gel (100-200 mesh, Qingdao Marine Chemical Ltd.; Qingdao, China) and Sephadex LH-20 (Amersham Biosciences, Sweden). NMR spectra of 1H-NMR (600 MHz) and 13C-NMR (150 MHz) were obtained on a Bruker AV-600 spectrometer using tetramethylsilane as an internal reference. Electrospray ionization (ESI)-MS was measured on an API 2000 LC/MS/MS instrument, and HR-ESI-MS data were obtained on LTQ Orbitrap Discovery mass spectrometer.

**Table 3. Effect of FVEE on the Kidney Level of Antioxidant Capability in D-Galactose Induced Aging Mice.**

| Groups | SOD (U/mL) | CAT (U/mL) | GSH-Px (mol/mL) | GSH (mg/L) | MDA (nmol/mL) |
|--------|------------|------------|-----------------|-------------|--------------|
| Control | 19.31 ± 4.81 | 5.71 ± 1.18 | 144.14 ± 14.99 | 15.32 ± 3.31 | 4.12 ± 0.43 |
| Model  | 5.43 ± 1.57* | 1.87 ± 0.28* | 47.85 ± 11.92* | 2.51 ± 0.54* | 8.57 ± 2.05* |
| VC-100 | 9.278 ± 1.14* | 3.63 ± 0.84* | 53.60 ± 7.31* | 2.76 ± 0.98* | 4.82 ± 0.89* |
| FVEE-50 | 14.48 ± 1.92* | 4.30 ± 1.49* | 46.95 ± 1.06* | 7.15 ± 2.76* | 5.17 ± 0.37* |
| FVEE-200 | 17.88 ± 1.37#▲ | 4.93 ± 1.25# | 49.87 ± 0.95* | 9.03 ± 2.12* | 4.76 ± 0.74# |

CAT, catalase; FVEE, F. vasculosa ethanol extract; GSH, glutathione; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase. Values are expressed as mean ± standard deviation (n = 10). VC-100: mice treated with vitamin C (100 mg/kg/day); FVEE-50: mice treated with low dose of FVEE (50 mg/kg/day); FVEE-200: mice treated with high dose of FVEE (200 mg/kg/day). *P < 0.05 vs control; #P < 0.01 vs model; ▲P < 0.05 FVEE groups vs VC group.

**Table 4. Radical Scavenging Activity of the Compounds Isolated From FVEE.**

| Samples | DPPH radical scavenging activity % (in 100 µg/mL) |
|---------|------------------------------------------|
| Compound 1 | 77.30 ± 3.06 |
| Compound 2 | 24.56 ± 2.29 |
| Compound 3 | NT |
| Compound 4 | 58.41 ± 7.18 |
| Compound 5 | 66.74 ± 4.40 |
| Compound 6 | 37.53 ± 2.76 |
| VC | 91.83 ± 2.04 |

DPPH, α,α-diphenyl-β-picrylhydrazyl; FVEE, F. vasculosa ethanol extract; NT, not tested.
**Plant Material**

The young leaves of *F. vasculosa* were collected from Shiwan Mountains (Guangxi, China) in October 2013 and were identified by Mr Ye-cong Zhong at Guangxi institute of forestry survey and design, Nanning, China. Fresh leaves were air-dried in shade and pulverized with a household food processor.

**Extraction**

Leaf powder (5 kg) was extracted repeatedly with 90% aqueous ethanol of (3 × 10 L) at room temperature. The resulting extracts were pooled and evaporated under reduced pressure using a rotary evaporator EYELA-1 (Tokyo Rikakikai Co., Japan). The residues were collected and stored at 4°C.

**In Vitro Antioxidant Activity Assay**

DPPH radicals scavenging activity was evaluated by the method described by Hatano et al. An aliquot of 0.1 mL of DPPH radical (Sigma-Aldrich, USA) in ethanol was added to a test tube with 0.5 mL of sample solution (in a final concentration 0.625, 1.25, 2.5, 5, and 10 mg/mL for extracts and 100 µg/mL for compounds) in ethanol. Ethanol was used instead of the sample as a control. VC was used as a positive control. The mixture was shaken vigorously and then left at room temperature for 30 minutes in the dark. The absorbance of the reaction mixture was then measured at 517 nm using a microplate reader ELx808 (BioTek Instruments Inc.; Winooski, VT, USA). VC was employed as a reference.

The reducing power was tested according to the method described by Siddharaju et al. A Sample solution (1.5 mL) (in a final concentration 1.25, 2.5, 5, 10, and 20 mg/mL for extracts and 100 µg/mL for compounds) was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of aqueous potassium ferricyanide (1%). Afterward, the mixture was first incubated at 50°C for 20 minutes, and then 2.5 mL of 10% trichloroacetic acid was added. Following these treatment, the mixture was centrifuged at 3000 rpm for 10 minutes; 2.5 mL of the upper layer of the solution was mixed with 2.5 mL of distilled water and 0.5 mL of FeCl₃ solution (1%) for 10 minutes. The absorbance was read at 700 nm using a UV752N spectrophotometer (Shanghai Precision Science Instrument Co Ltd, Shanghai, China).

Ferrous ion chelating activity was measured according to the method described by Lee et al. The reaction mixture contained 500 µL of sample solution (in a final concentration 1.25, 2.5, 5, 10, and 20 mg/mL for extracts and 100 µg/mL for compounds), FeCl₂ (0.6 mM, 100 µL), and 900 µL methanol. The control contained all the reaction reagents except the samples. The mixture was thoroughly shaken and left at room temperature for 5 minutes. Following treatment, 100 µL of ferrozine (5 mM) solution was mixed with the reaction reagents, shaken, and kept at room temperature for 10 minutes. The absorbance of the Fe²⁺-ferrozine complex was measured at 562 nm using a UV752N spectrophotometer.

**Experimental Animals**

The experiments were carried out on male KM mice (18-22 g) provided by experimental animal central of Guilin medical university. All of the animals were first housed with a standard condition (25 ± 1°C; 12 hours light/dark cycle) in an SPF grade animal room of school of public health in Guilin medical university and provided with appropriate food and water. All the procedures involving animals were approved by the Animal Ethics Committee of Guilin medical university, the approval number of the study: SCXK(Gui) 2013-0001.

**Experimental Design**

The mice were randomly divided into 5 groups (10 mice/group): control group (saline), model group (D-galactose-induced group), D-galactose-induced + VC group, as well as D-galactose-induced + FVEE low and FVEE-high groups. The negative control group were intraperitoneally injected with saline, and the other groups were administered a daily subcutaneous injection of D-galactose (120 mg/kg/day) at the neck consecutively for 8 weeks. The Control and Model groups were treated with saline. The VC group was administered with VC (100 mg/kg/day). In FVEE-low and FVEE-high groups, they were given FVEE at doses of 50 mg/kg/day (FVEE-50) and 200 mg/kg/day (FVEE-200), respectively. At the end of the experiments, all of the mice were euthanized using CO₂ and sacrificed; blood was collected from the inferior vena cava using a vacuum blood collection tube, then centrifuged (3000 × g for 10 minutes at 4°C) and stored at −80°C until further study. The liver and kidney of the mice were collected and dissected on ice. All the tissue samples were snap-frozen in liquid nitrogen and stored at −80°C until further analysis.

**Biochemical Analyses**

The activities of SOD, CAT, and GSH-Px, as well as the GSH and MDA levels, were measured with a commercial kit (Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) according to the manufacturer’s instructions.

**Fractionation, Isolation, and Identification of Compounds From FVEE**

The 90% aqueous ethanol extract was further extracted sequentially with petroleum ether and followed by EtOAc, to give petroleum ether-soluble fraction, EtOAc-soluble fraction, and water-soluble fraction, respectively. The EtOAc-soluble fraction was separated using silica gel CC and eluted with petroleum ether-EtOAc (9:1→7:3), petroleum ether-EtOAc-MeOH (5:5:1→5:5:3) and MeOH to yield 5 fractions (A-E). Fraction B was subjected to CC on silica gel, eluting with petroleum ether-acetone (9:1→1:1), to give 4 sub-fractions (B1–B4). Compound 1 (12.0 mg) was isolated from fraction B3 by Sephadex LH-20 (Amersham Biosciences, Sweden) CC eluted with MeOH. Fraction C was subjected to CC on silica gel, eluting with...
CHCl₃-MeOH (95:5→1:1), to afford 7 sub-fractions (C1–C7). Fraction C1 was subjected to CC on silica gel (eluted with CHCl₃-MeOH, 97:3) and was further separated by Sephadex LH-20 CC (eluted with MeOH) to yield compounds 2 (27.6 g) and 3 (6.5 mg). Compound 4 (21.9 mg) was isolated from fraction C2 by silica gel column, eluting with petroleum ether-acetone (7:3). Fraction C3 was also subjected to CC on silica gel, eluting with CHCl₃-MeOH (8:2) to afford compound 5 (72.0 mg). Fraction C4 was applied to CC on silica gel with petroleum ether-acetone (6:4) as eluent and then on Sephadex LH-20 CC eluted with MeOH to yield compound 6 (57.3 mg). All compounds were identified by means of spectral data (1D, 2D NMR and HRMS) and direct comparison with the respective literature data.

Statistical Analysis

Results were expressed as means and standard deviations. The statistical analyses were carried out using SPSS 20.0 software. The level of significance was set at $P < 0.05$.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References

1. Bhat AH, Dar KB, Ances S, et al. Oxidative stress, mitochondrial dysfunction and neurodegenerative diseases; a mechanistic insight. Biomed Pharmacother. 2015;74:101-110.
2. Ho E, Karimi Galougahi K, Liu CC, Bhindi R, Figtree GA. Biological markers of oxidative stress: applications to cardiovascular research and practice. Redox Biol. 2013;1(1):483-491.
3. Kotur-Stevuljevic J, Bogavac-Stanojevic N, Jelic-Ivanovic Z, et al. Oxidative stress and paraoxonase 1 status in acute ischemic stroke patients. Atherosclerosis. 2015;241(1):192-198.
4. Rochette L, Zeller M, Cortin Y, Diabetes VC. Oxidative stress and therapeutic strategies. Biochim Biophys Acta. 2014;1840:2709-2729.
5. Nourazarian AR, Kangari P, Salmaninejad A. Roles of oxidative stress in the development and progression of breast cancer. Asian Pac J Cancer Prev. 2014;15(12):4745-4751.
6. Zhu J, Yi X, Zhang J, Chen S, Wu Y, et al. Chemical profiling and antioxidant evaluation of Yangxinsi Tablet by HPLC–ESI-Q-TOF-MS/MS combined with DPPH assay. J Chromatogr B. 2017;1060:262-271.
7. Bast A, Haenen GRMM. The toxicity of antioxidants and their metabolites. Environ Toxicol Pharmacol. 2002;11(3-4):251-258.
8. Editorial committee of Chinese academy of sciences: Ficus Linn. In: Flora republi-ciae popularis sinicae. Beijing: Science Press; 1998:Vol. 23. 66.
9. Zhao TZ, Yang DR, Xun JH. The role and comprehensive value of fig tree in tropical rainforests of Xishuangbanna. Froest Res. 2001;14:441-445.
10. Zhang L. Present situation and development prospect of fig plant resources in Xishuangbanna Yunnan. Chin Wild Plant Resour. 2002;23:15-17.
11. Xu YK, Liu HM, Xiao CF, et al. The nutrient contents of six species and its evaluation as woody vegetables. J Wuhan Bot Res. 2005;28:95-99.
12. Shi Y-X, Xu Y-K, Hu H-B, Na Z, Wang W-H, et al. Preliminary assessment of antioxidant activity of young edible leaves of seven Ficus species in the ethnic diet in Xishuangbanna, Southwest China. Food Chem. 2011;128(4):889-894.
13. Editorial committee of Chinese academy of sciences: Ficus Linn. In: Flora republi-ciae popularis sinicae. Beijing: Science Press; 1998:Vol. 23. 117-118.
14. Wei QW, Chen XL, Zhao YY, et al. Bioactivities and chemical constituents of extracts from Ficus vasculosa leaves. Nat Prod Res Dev. 2016;28:1055-1059.
15. Ho S-C, Liu J-H, Wu R-Y. Establishment of the mimetic aging effect in mice caused by D-galactose. Biogerontology. 2003;4(1):15-18.
16. Kaviani E, Rahmani M, Kaedi A, et al. Protective effect of atorvastatin on D-galactose-induced aging model in mice. Behav Brain Res. 2017;334:55-60.
17. Iman F, Sara H, Ayat K, et al. Metformin ameliorates the age-related changes of D-galactose administration in ovariectomized mice. Food Clin Pharmacol. 2018;32(4):392-399.
18. Fatemi I, Khalouei A, Kaedi A, et al. Protective effect of metformin on D-galactose-induced aging model in mice. Iran J Basic Med Sci. 2018;21(1):19-25.
19. Bourichte H, Karnaouf N, Belhadj H, et al. Free radical, metal-chelating and antibacterial activities of methonolic extract of Capparis spinosa buds. Adv Environ Biol. 2013;5:281-287.
20. Zhao X, GJ L, YY H, et al. Improvement effects and mechanism research of polyphenol extracts from Kudingcha on carbon tetrachloride induced hepatic damage in mice. Sci Technol Food Ind. 2018;39:289-295.
21. Edén-Ínals M, Sunal E, Kanhag B. Age-related changes in the glutathione redox system. Cell Biochem Funct. 2002;20(1):61-66.
22. Castorina C, Campisi A, Di Giacomo C, et al. Lipid peroxidation and antioxidant enzymatic systems in rat retina as a function of age. Neurochem Res. 1992;17(6):599-604.
23. Chen HL, Dong XP, Zhang M, et al. Study on the chemical constituents of Viola jedoensis. Chin Tradit Herb Drugs. 2010;41:874-877.
24. Wang XM, Zhang Q, Rena KSM, et al. Chemical constituents in whole plant of Cynomorium songaricum. Chin Tradit Herb Drugs. 2011;42:458-460.
25. Wang LS, XM M, Guo YJ, et al. Study on the chemical constituents of Pteria fulfillment. Chin J Chin Mater Med. 2004;29:58-61.
26. Wu TS, Yang CC, PL W , et al. A quinol and steroids from the leaves and stems of *Rhinacanthus nasutus*. *Phytochemistry*. 1995;40:1247-1249.

27. Du ZL, Yin ZQ, Wang L, et al. Coumarins and flavonoids from leaves of *Broussonetia papyrifera*. *Nat Prod Res Dev*. 2008;20:630-632.

28. Xu L, Ji CJ, Tan NH, et al. Isolation and identification of norar-tocarpetin in mulberry. *Food Sci*. 2010;31:101-103.

29. Hermenean A, Ardelean A, Stan M, et al. Antioxidant and hepatoprotective effects of naringenin and its β-cyclodextrin formulation in mice intoxicated with carbon tetrachloride: a comparative study. *J Med Food*. 2014;17(6):670-677.

30. Guo LX, Ma YM, Qiao K, et al. Chemical constituents from *Akebia trifoliate* and their antioxidant activities. *Chinese Tradit Pat Med*. 2017;39:338-342.

31. Jung WW. Protective effect of apigenin against oxidative stress-induced damage in osteoblastic cells. *Int J Mol Med*. 2014;33(5):1327-1334.

32. Abbas GM, Abdel Bar FM, Baraka HN, Gohar AA, Lahloub M-F. A new antioxidant stilbene and other constituents from the stem bark of *Morus nigra* L. *Nat Prod Res*. 2014;28(13):952-959.

33. Hatano T, Kagawa H, Yasuhara T, Okuda T. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. *Chem Pharm Bull*. 1988;36(6):2090-2097.

34. Siddhuraju P, Mohan PS, Becker K. Studies on the antioxidant activity of Indian Laburnum (Cassia fistula L.): a preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. *Food Chem*. 2002;79(1):61-67.

35. Lee J, Koo N, Min DB. Reactive oxygen species, aging, and antioxidative nutraceuticals. *Compr Rev Food Sci Food Saf*. 2004;3(1):21-33.