Introduction

The Glycyrrhiza genus consists of about 30 species and is widely distributed all over the world. In China, three species of Glycyrrhiza are used as licorice and recorded in the Chinese Pharmacopoeia as gancao: Glycyrrhiza uralensis, Glycyrrhiza glabra, and Glycyrrhiza inflata. G. uralensis is the most widely used species and constitutes more than 90% of total licorice production (Zhang & Ye, 2009). As a tonifying herbal medicine, licorice appears extensively as a component herb in approximately 60% of all traditional Chinese medicine (TCM) prescriptions because it produces the synergistic therapeutic effects when combined with other herbal medicines (Wang & Yang, 2007). In addition, licorice is commonly used in healthcare products and as a food additive because of its sweet taste.

Licorice extracts including active constituents of triterpenoids and flavonoids have the skin health activities of skin-whitening, sun screening, and anti-inflammatory, and antioxidative (Xu & Bu, 2001; Ma et al., 2003; Fu et al., 2013; Chen et al., 2014). Flavonoids including chalcones, isoflavones, flavonones, flavononols, isoflavones, and arylcoumarins (Lee et al., 2007) in licorice have drawn considerable interest for their structural diversity and the pharmacological activities (Yokota et al., 1998; Simmler et al., 2013). Recently, fermented active constituents have been increasingly utilized as functional cosmetic materials. During fermentation, some precursors in plant extracts are...
hydrolyzed, while some new active constituents are synthesized, which lead to the increased biological activities (Chen et al., 2007). For example, fermented liquorice by Aspergillus oryzae HC-12 significantly improve the pharmaceutical effects of anti-inflammation and abirritation compared to the unfermented liquorice (Li et al., 2010).

The present paper wished to investigate in vitro skincare activities, and the main chemicals in different extracts and fermenting liquorice. The antioxidant, tyrosinase inhibition activities of the 75% ethanol extract, petroleum extract fraction (PeF), ethyl acetate fraction (EaF), n–butanol extract fraction (nBF), and water extract fraction (WtF) of fermented liquorice (FL) and non–fermented liquorice (NFL) were evaluated. The inhibitory effects of FL and NFL on matrix metalloproteinase–1 (MMP–1) level were also investigated using human dermal fibroblasts (HDFs). In order to elucidate the main chemical constituents contributed to the activities, total flavonoid, polyphenol, and polysaccharide contents were determined by UV spectrophotometry, 8 main chemicals of the different fractions, FL, and NFL were quantitatively analyzed by the established HPLC/DAD method.

**Material and Methods**

1. **Chemicals and regents**  
   The radix of *G. uralensis* was collected from Gansu province, Saccharomyces cerevisiae (2,3880) was purchased from China General Microbiological Culture Collection Center (CGMCC), 1,1-Diphenyl–2–picrylhydrazyl radical (DPPH), mushroom tyrosinase, and Folin–Ciocalteu reagent were obtained from Biodee Biotechnology Co., Ltd. (Beijing, China). Matrix metalloproteinase–1 (MMP–1) was purchased from (CUSABIO, No, 121032323), Gallic acid (GAE) and L–DOPA were purchased from Sigma–Aldrich (USA). HPLC–grade methanol was purchased from Merck (Germany), NaNO2, HCl, Na2CO3, AlCl3 and NaOH were purchased from Beijing Chemical Plant (Beijing, China) . Ultra–pure water (18.2 MΩ) was prepared with a Milli–Q water purification system (Millipore, USA), Liquiritigenin (1), isoliquiritigenin (2), liquiritiside (3), isoliquiritiside (4), glabridin (5), glycyrrhizic acid (6), glycyrrhetinic acid (7), and licochalcone A (8), rutin (9) were purchased as reference standards from Beijing Simianti Tech. Co., Ltd. (China). All of the reference compounds were determined to be greater than 98% pure by HPLC analysis. All other reagents were of analytical grade.

2. **Extraction method and preparation of fermented liquorice**  
   Air–dried samples (0.1 kg) were sieved and extracted with 1.5 L of 75% ethanol (v/v) by ultrasonic extraction for 60 min. The extraction was then concentrated and successively partitioned between water and organic solvents to obtain 4 fractions: petroleum (PeF), ethyl acetate (EaF), n–butanol (nBF), and water (WtF). The concentrated extraction was freeze dried and was diluted with water (NFL). Next, 50 mL of each diluted extract was poured into a 250 mL D–flask (pH was adjusted to 9,0) and sterilized for 15 min. The diluted extracts were then added with 1,0 mL inoculated Saccharomyces cerevisiae (1 × 10^6 CFU/mL) and incubated at 30℃ for 40 h with mild shaking. The fermenting liquor (FL) was sterilized for 30 min, filtered and freeze dried to the following chemical and bioactivity assays.

3. **Cell culture**  
   Human dermal fibroblasts (HDFs) and B16F10 mouse melanoma cells were purchased from the Cell Bank of the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. The cells were cultured in DMEM supplemented with 10% FBS (BioWhittaker, Walkersville, USA), 1% penicillin streptomycin (Gibco BRL, USA), at 37℃ in a humidified atmosphere containing 5% CO₂.

4. **DPPH radical scavenging activity**  
   Rates of radical scavenging by the samples were measured by the DPPH method (Rahman et al., 2010). For each sample, an aliquot of 0.5 mL at different concentrations was added to 1,0 mL DPPH solution (100 µM) for 30 min. Methanol was used as a blank solution. The decrease in absorbance at 517 nm was measured. The optical absorption module uses a UV silicon photodiode. DPPH radical scavenging activity was expressed as a percentage (%) of the original absorbance as follows:  
   \[ \text{DPPH radical scavenging activity} = \frac{(A_{\text{DPPH}} – A_{\text{AS}})}{A_{\text{DPPH}}} \times 100 \]  
   Where AS is the absorbance of the solution after the sample extract has been added, whereas ADPPH is the absorbance of the DPPH solution.

5. **Mushroom tyrosinase assay**  
   The effects of the extracts on free mushroom tyrosinase activity were determined spectrophotometrically as described...
previously (Mosmann et al., 1983) with minor modifications, Tyrosinase activity was determined using L-DOPA as a substrate. In brief, 250 μL of 250 units/mL of mushroom tyrosinase in 0.1M phosphate buffer (pH 6.8), 400 μL of the samples at various concentrations and 350 μL of 0.1 M phosphate buffer (pH 6.8) were added to each well of a 96-well plate (TPP, Switzerland) and mixed. The assay mixture was preincubated at 37°C for 10 min. Next, 400 μL of 2.5 mM L-DOPA in 0.1 M phosphate buffer (pH 6.8) was added to each well and incubation was continued for 25 min at 37°C. The amount of dopachrome formed in the reaction mixture was determined against the blank solution at 475 nm on a microplate reader (Infinite® 200 PRO Tecan Sunrise, Switzerland). The percentage of tyrosinase activity was calculated as follows:

Tyrosinase activity (%)=[(A−B)/(C−D)]×100

where A is the absorbance of the reaction mixture containing the test sample and mushroom tyrosinase; B is the absorbance of the blank sample containing the test sample, but without mushroom tyrosinase; C is the absorbance of the reaction mixture without the test sample and with mushroom tyrosinase; and D is the absorbance of the well without the test sample or mushroom tyrosinase (L-DOPA alone).

6. Cell viability assay

Cell viability and proliferation were evaluated using the 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Lee et al., 2006), B16F10 cells (Purchased from China Agricultural Microbial Culture Collection and Management Center) were pretreated with the samples at various concentrations. After incubation for 48 h, MTT solution (final concentration: 5 mg/mL) was added. Next, the cells were incubated at 37°C for 3 h. Finally, the absorbance of each sample was measured on a microplate reader at 570 nm to obtain the percentage of viable cells.

7. Assay of cellular tyrosinase activity

Cellular tyrosinase activity was measured using a previously described method (Hosoi et al., 1985) with some modifications. Six-well plates (TPP, Switzerland) were seeded with B16F10 melanoma cells (2×10⁵ cells/well in 3 mL medium). The plates were incubated overnight in a humidified CO₂ incubator with a 5% CO₂ atmosphere at 37°C to allow cells to adhere. Next, the cells were exposed to increasing doses of the test extracts or arbutin for 48 h. After the treatment, the cells were washed with sodium phosphate buffered saline (pH 6.8) and lysed. The lysates were clarified by centrifugation (5424: Eppendorf, Germany) at 12,000 rpm for 30 min at 4°C. The protein concentration of each sample was determined using bovine serum albumin as the standard. The reaction mixture, consisting of 40 μg protein (adjusted to 100 μL with 0.1 M PBS (pH 6.8) and 50 μL L-DOPA (1%), was added to each well of a 96-well plate. After incubation at 37°C for 1 h, the absorbance of each well was measured at 475 nm using a microplate reader. Tyrosinase activity was calculated by the following formula:

Tyrosinase activity (%)=OD475sample/OD475control × 100

8. Measurement of melanin content

Melanin content was measured as described previously with some modifications (Chang et al., 2002), B16F10 melanoma cells were seeded (2×10⁵ cells/well in 3 mL medium) in 6-well culture plates and incubated overnight to allow cells to adhere. The cells were exposed to various concentrations of the test extracts or arbutin for 48 h. At the end of the treatment, the cells were washed with PBS and lysed with 1 M NaOH containing 10% DMSO for 30 min at 80°C. The absorbance was measured at 475 nm using a microplate reader, Melanin content was calculated by the following formula:

Melanin content (%)=OD475sample/OD475control × 100

9. Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assays (ELISA) were performed to measure MMP−1 level, HDFs were cultured in 6-well plates (5×10⁵/mL cells) with DMEM containing 10% fetal bovine serum for 24 h, after which the medium was replaced with serum-free medium containing the test samples. After incubation for 24 h, the supernatant was collected from each well and MMP−1 level was measured using an ELISA kit (CUSABIO, China).

10. Total flavonoid content

The total flavonoid content of the extracts was determined using a previously reported colorimetric method (Singleton et al., 1999). Briefly, 0.4 mL of NaNO₂ (5%, v/v) was added to 1.0 mL of each appropriately diluted sample. To this mixture, 0.4 mL of AlCl₃ (10%, v/v) was added after 6 min, Six minutes later, 4.0 mL of NaOH (4%, v/v) and ethanol (60%, v/v) was added to 10 mL of the resulting solution. The absorbance was measured at 508 nm using a microplate reader (TECAN, Shanghai, China).
after 15 min. A standard curve was constructed using rutin. Total flavonoid content was expressed as mg of rutin equivalent per g of the samples. The samples were analyzed in triplicate.

11. Total polyphenol content

The total polyphenol content of the extracts was measured over 6 days by Folin–Ciocalteu colorimetry (Fang et al., 2011). Folin–Ciocalteu reagent was prepared by diluting the commercial reagent concentrate with water (1:2). The supernatant of each sample (0.1 mL) was added to 0.1 mL methanol/0.3% HCl (6:4, v/v), after which the resulting solution was added to 10% Na₂CO₃ (2.0 mL). Folin–Ciocalteu reagent (0.1 mL) was added to the mixture, which was then kept in the dark for 30 min. The samples were shaken thoroughly and the absorbance was measured at 750 nm a microplate reader. The standard curve was constructed using gallic acid (mg GAE g⁻¹).

12. Total polysaccharide content

The total polysaccharide content of the extracts was determined by the improved phenol–sulfuric acid method (Fang et al., 2011). Measurements were taken at a wavelength of 490 nm using a microplate reader (SpectraMax 190; Molecular Devices LLC, USA). The total polysaccharide contents of the PeF, EaF, nBF, and WtF fractions were calculated as glucose/g using the calibration curve. The samples were analyzed in triplicate.

13. Quantitation of the main constituents by HPLC/DAD

A small portion of different samples were passed through a 0.45 μm filter, after which a 5.0 μL volume was injected for HPLC/DAD quantitative analysis. Chromatographic separation was carried out using an Agilent 1260LC series system (Agilent Technologies, USA) equipped with an online vacuum degasser, quaternary pump, autosampler, thermostatted column compartment, and diode array detector (DAD). Agilent Technologies Chemstation software for LC (B.02.01) was used. HPLC separation was performed using an Agilent ZORBAX SB–C18 column (4.6 mm × 250 mm, 5 μm). The detection wavelength was set to 254 nm. The mobile phase consisted of

Figure 1. The representative chromatogram of standards and different extractions.

(A) standards; (B) PeF extract; (C) EaF extract; (D) nBF extract; (E) WtF extract; (F) 75% licorice extract; (G) fermenting licorice extract; (1) liquiritigenin; (2) isoliquiritigenin; (3) liquiritoside; (4) isoliquiritoside; (5) glabridin; (6) glycyrrhizic acid; (7) glycyrrhethinic acid; (8) licochalcone A.
water/acetic acid (99.8:0.2, v/v) (A) and acetonitrile (B). The gradient program consisted of 20%–30% (B) for 0–10 min, 30%–50% (B) for 10–25 min, and 50%–90% (B) for 25–35 min. The flow rate was 1.0 mL min⁻¹. The column temperature was set to 30°C. Peak assignments were made for each single-compound injection by comparing their retention times and characteristic absorption spectra from the DAD with those of the authentic standards. The HPLC/DAD method was used to simultaneously determine 8 compounds in the extract fractions, FL, and NFL. The content of each compound of interest was calculated using an appropriate standard curve. Representative chromatograms of the samples are shown in Figure 1.

14. Data analysis
The statistical significance of the differences between the mean measurements of each treated group and that of the control group were determined using Dunnett’s t-test. Results of p<0.05 were considered statistically significant. The data was also analyzed by one-way analysis of variance (ANOVA) using SPSS 17.0 (IBM Corp., USA).

Results and Discussion

1. DPPH radical scavenging activity
The antioxidant capacities of the 75% ethanol extract of G. uralensis, as well as those of the EaF, nBF, WtF, PeF, FL, and NFL are shown in Figure 2. The DPPH radical scavenging activities of the EaF (IC₅₀=200 μg/mL) and PeF (IC₅₀=370 μg/mL) were significantly higher than those of the total extract (IC₅₀=630 μg/mL), nBF, and WtF. While the yield of the PeF (0.5%) from the total extract was significantly lower than that of the EaF (20.4%). The DPPH radical scavenging activity of the FL (IC₅₀=192 μg/mL) was significantly higher than that of the NFL (IC₅₀=298 μg/mL).

Figure 2. DPPH free radical scavenging activity of different fractions of licorice, fermented licorice and non-fermented licorice.
(A) Different fractions of licorice; (B) Fermented and non-fermented licorice. Results are mean±SD (n=3). TE, total extraction; Pef, petroleum fraction; EaF, ethylacetate fraction; nBF, n-butanol fraction; WtF, water fraction.

Figure 3. Tyrosinase inhibitory activity of licorice, fermented licorice, and unfermented licorice.
(A) Different components of licorice; (B) Fermented licorice and unfermented licorice. The result is mean±SD (n=3). TE, total extraction; Pef, petroleum fraction; EaF, ethylacetate fraction; nBF, n-butanol fraction; WtF, water fraction.
2. Mushroom tyrosinase inhibition activity

Tyrosinase is an important enzyme in the process of melanin synthesis. The inhibitory activities of the EaF and PeF were significantly greater than those of the nBF and WtF (Figure 3).

The extraction yield of the PeF (0.5%) from the 75% extraction was very low, it was concluded that most of the active constituents were extracted into the EaF. In addition, the mushroom tyrosinase inhibition activities of the FL and NFL solutions were not significantly different.

3. Evaluation of the cellular effects

The cell viability of B16F10 cells exposed to the EaF and nBF at concentrations of 50, 100, 200 and 300 μg/mL was tested. As the detection concentrations of EAF and NBF increase, the cell viability of B16F10 melanoma cells gradually decreased, and when the EAF detection concentration was 200 μg/mL, the cell viability decreased to 79.5%; When the concentration of NBF detection was 600 μg/mL, the cell viability decreased to 56.0%.

The EaF showed obvious inhibition of cellular tyrosinase and melanogenesis in B16F10 melanoma cells with concentrations of 50, 100, and 200 μg/mL. EaF significantly reduced tyrosinase activity in B16F10 cells in a dose-dependent manner, with activity of 39.7% at a concentration of 50 μg/mL, 10.4% at 100 μg/mL, and 10.7% at a concentration of 250 μg/mL. Compared with arbutin, EaF at the same concentration produces less melanin and has a better inhibitory effect on tyrosinase activity. At concentrations of 200 and 300 μg/mL, nBF showed no significant inhibitory effect on cell tyrosinase or melanin synthesis (Table 1). The effects of the test samples on the B16F10 cells were ranked similarly to their effects on mushroom tyrosinase and DPPH radical scavenging; therefore, the EaF was determined to be the most active fraction on skin-whitening activities. In HDFs, FL showed more significant inhibition on MMP-1 level in comparison with that of NFL (Table 2).

MMP-1 is considered to be a key cellular regulator that plays a prominent role in the breakdown of the dermal extracellular matrix, namely of collagen types I and III, during the photoaging process, which results in collagen deficiency and leads to wrinkling. Inhibitors of the major collagen-degrading enzymes like MMP-1 could be useful anti-aging agents (Lu et al., 2013). The results of these experiments show that FL has potential as a raw material for anti-aging cosmetics.

### Table 1. Effects of arbutin, fractions ethyl acetate n-butanol, on cellular tyrosinase activity, melanin content and cell viability in B16F10 melanoma cells

<table>
<thead>
<tr>
<th>Samples</th>
<th>Concentration (mg mL(^{-1}))</th>
<th>Cell viability (%)</th>
<th>Cellular melanin content (%)</th>
<th>Cellular tyrosinase activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>100.0±4.5</td>
<td>100.0±3.5</td>
<td>100.0±2.3</td>
</tr>
<tr>
<td>Arbutin</td>
<td>0.1%</td>
<td>72.2±9.8</td>
<td>68.9±4.9</td>
<td>40.3±2.2</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>0.05</td>
<td>102.4±6.2</td>
<td>79.4±1.3</td>
<td>39.7±1.1</td>
</tr>
<tr>
<td>fraction</td>
<td>0.1</td>
<td>90.2±0.2</td>
<td>30.3±6.7</td>
<td>10.4±2.2</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>79.5±6.6</td>
<td>11.5±2.0</td>
<td>10.4±1.5</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>0.2</td>
<td>101.0±1.3</td>
<td>110.1±1.7</td>
<td>132.2±4.0</td>
</tr>
<tr>
<td>fraction</td>
<td>0.3</td>
<td>80.7±3.5</td>
<td>108.3±19.3</td>
<td>103.0±0.9</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>56.0±1.2</td>
<td>102.2±4.6</td>
<td>78.2±2.7</td>
</tr>
</tbody>
</table>

Data are presented as means±SD of three independent experiments. The decreased results showed significant differences with \(^*\) \(p<0.05\) and \(^**\) \(p<0.01\) compared with the control. Relative activity (\%) = \(\%\) of sample versus control.

### Table 2. MMP-1 expression and cell viability induced by FL and NFL in nHDF cells

<table>
<thead>
<tr>
<th>Samples</th>
<th>Concentration (mg mL(^{-1}))</th>
<th>Cell viability (%)</th>
<th>MMP-1 (ng mL(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>100.0±4.5</td>
<td>10.06±2.68</td>
</tr>
<tr>
<td>Fermented licorice (FL)</td>
<td>0.075</td>
<td>96.46±8.28</td>
<td>4.46±1.36</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td>101.32±2.86</td>
<td>3.59±1.46</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>82.92±7.39</td>
<td>2.27±0.49</td>
</tr>
<tr>
<td>Non-fermented licorice (NFL)</td>
<td>0.075</td>
<td>126.99±12.20</td>
<td>8.22±3.11</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td>140.67±22.15</td>
<td>6.04±1.19</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>128.20±8.97</td>
<td>4.47±0.71</td>
</tr>
</tbody>
</table>

Duncan’s multiple range test was used to analyzed the differences of the groups \((p<0.05, ~^**p<0.01\) compared with the control group).
4. Total flavonoid, protein, and polysaccharide contents
The contents of total flavonoids, total polyphenols, and total polysaccharides in the extracts are shown in Table 3.

Flavonoids, phenolic acids, and polysaccharides have been isolated from licorice (Xie et al., 2009). In order to elucidate the main bioactive constituents responsible for the activity of licorice, total flavonoid content, total polyphenol content, and total polysaccharide content were determined. The EaF showed the highest total flavonoid content and total polyphenol content. In addition, FL showed total flavonoid content and total polyphenol content obviously higher than those of NFL. However, the total polysaccharide content of licorice was low. Previous reports have indicated that total flavonoids from licorice have anti-tumor (Zhao et al., 2006), antioxidant (Cui et al., 2007), anti-aging (Ye et al., 2004), skin-whitening, and anti-chloasma effects (Wang et al., 2003). We further quantified the main chemicals in the PeF, EaF, nBF, WtF, FL, and NFL by HPLC/DAD.

5. Quantitation of the main constituents of different fractions, FL and NFL

Liquiritigenin (1), isoliquiritigenin (2), liquiritoside (3), isoliquiritoside (4), glabridin (5), glycyrrhizic acid (6), glycyrrhetinic acid (7), and licochalcone A (8) were quantified in each fraction, FL, and NFL (Table 4).

Obvious differences were observed among the PeF, EaF, nBF, and WtF. The assayed chemicals were mainly distributed in the EaF and nBF. With the exception of glycyrrhetinic acid (7), all of the chemicals were detected in the EaF. Flavonoids liquiritigenin, isoliquiritigenin, liquiritoside, isoliquiritoside, glabridin, and licochalcone A in the EaF were determined to be the main contributors to the skin−whitening activities of the EaF, Glabridin (5, 46.9 mg g−1) and licochalcone A (8, 64.9 mg g−1) were the main chemical constituents of the EaF, Glabridin (5) and licochalcone A (8) have been reported to possess a wide range of biological activities. Glabridin (5) is widely used in cosmetics and has antioxidant (Ables et al., 2007), melanin synthesis inhibitory, and anti−inflammatory (Yokota et al., 1998) activities, Licochalcone A (8) also has antioxidant and anti−inflammatory properties, which may be mediated by inhibition of PGs, COX−1, and COX−2 (Kolbe et al., 2006). Licochalcone

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Table 3. The contents of total ingredients from of different fractions and FL (µg/mg)

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total flavonoids (µg/mg)</th>
<th>Total polyphenols (µg/mg)</th>
<th>Total polysaccharides (µg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total extraction</td>
<td>19.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>136.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>128.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Petroleum fraction</td>
<td>80.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>147.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>226.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>412.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>43.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>n-Butanol fraction</td>
<td>25.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>206.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>96.0&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Water fraction</td>
<td>-4.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>156.0&lt;sup&gt;e&lt;/sup&gt;</td>
<td>151.2&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fermented licorice</td>
<td>575.3&lt;sup&gt;e&lt;/sup&gt;</td>
<td>850.9&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.3&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Non-fermented licorice</td>
<td>407.7&lt;sup&gt;f&lt;/sup&gt;</td>
<td>763.0&lt;sup&gt;f&lt;/sup&gt;</td>
<td>3.0&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The data was analyzed by one-way analysis of variance (ANOVA). Different characters in the same column means they have significant difference between the samples (p<0.05).

Table 4. The contents of 8 chemical constituents in different extractions (µg/mg)

<table>
<thead>
<tr>
<th>No.</th>
<th>Chemical constituents</th>
<th>Petroleum fraction</th>
<th>ethyl acetate fraction</th>
<th>n-butanol fraction</th>
<th>Water fraction</th>
<th>FL</th>
<th>NFL</th>
<th>Change rate of Fermentation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Liquiritigenin</td>
<td>nd</td>
<td>7.4</td>
<td>2.4</td>
<td>nd</td>
<td>44.89</td>
<td>48.27</td>
<td>171</td>
</tr>
<tr>
<td>2</td>
<td>Isoliquiritigenin</td>
<td>1.2</td>
<td>2.5</td>
<td>4.0</td>
<td>9.4</td>
<td>110.11</td>
<td>175.00</td>
<td>38.6</td>
</tr>
<tr>
<td>3</td>
<td>Liquiritoside</td>
<td>1.6</td>
<td>19.5</td>
<td>104.7</td>
<td>2.0</td>
<td>285.72</td>
<td>405.59</td>
<td>46.9</td>
</tr>
<tr>
<td>4</td>
<td>Isoliquiritoside</td>
<td>0.5</td>
<td>8.1</td>
<td>22.4</td>
<td>0.2</td>
<td>25.69</td>
<td>174.35</td>
<td>258</td>
</tr>
<tr>
<td>5</td>
<td>Glabridin</td>
<td>6.1</td>
<td>46.9</td>
<td>nd</td>
<td>nd</td>
<td>37.94</td>
<td>nd</td>
<td>-95.0</td>
</tr>
<tr>
<td>6</td>
<td>Glycyrrhizic acid</td>
<td>22.1</td>
<td>3.7</td>
<td>74.9</td>
<td>131.2</td>
<td>1609.66</td>
<td>2480.68</td>
<td>36.8</td>
</tr>
<tr>
<td>7</td>
<td>Glycyrrhetinic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Licochalcone A</td>
<td>3.8</td>
<td>64.9</td>
<td>nd</td>
<td>nd</td>
<td>49.42</td>
<td>nd</td>
<td>-97.1</td>
</tr>
</tbody>
</table>

nd, not detected; FL, fermented licorice; NFL, non-fermented licorice.
A (8) also inhibited PGE release from HaCaT cells treated with UV radiation (Song et al., 2015). The results described above suggest that glabridin (5) and licochalcone A (8) were the main contributors to the antioxidant, tyrosinase inhibitory, and melanin synthesis inhibitory activities of the EaF. Therefore, the EaF, with high concentrations of glabridin (5) and licochalcone A (8), may have good anti-inflammatory properties.

Obvious differences were observed between FL and NFL. The glabridin (5) and licochalcone A (8) contents were significantly decreased after fermentation, while compounds liquiritigenin (1), isoliquiritigenin (2), liquiritoside (3), isoliquiritoside (4), and glycyrrhizic acid (6) were enriched after fermentation. It could be concluded that the enriched compounds maybe the main contributors to the samples against MMP-1 level. The content of isoliquiritigenin (2), which has been reported to have anti-tumor (Hsu et al., 2004), anti-oxidant (Chin et al., 2007), and anti-inflammation activities (Kwon et al., 2007), was increased by 38.6% in FL solution. The content of glycyrrhizic acid (6), an anti-inflammatory compound, was increased by 36.8% in FL (Takei et al., 2007). During the process of fermentation, S. cerevisiae expressed some enzymes, including phenylalanine ammonia-lyase (PAL), cinnaamate-4-hydroxylase (C4H), 4-coumarate: coenzyme A ligase (4CL), chalcone synthase (CHS), chalconereductase (CHR), chalconeisomerase (CHI), and flavonoid glycosyltransferase (FGT), which participated in the biosynthesis of compounds 1–4 (Figure 4).

The fermented extract of G. uralensis has significant activities on antioxidant and MMP-1 level, and is a promising raw material for anti-aging medicines and cosmetics.

### Conclusion

Licorice is widely used in cosmetic industries, EaF fraction containing high levels of flavonoids showed significant inhibitory effects on melanin synthesis and was a promising plant material on skin-whitening utilization. In the same time, it could be concluded that the anti-aging activities of licorice could be improved after fermentation. These results wish to offer empirical evidence supporting utilization of licorice in medicines and cosmetics intended to enhance skin health.

### Author’s contribution

GM and HZ contributed equally to this work, ZG and LL designed all experimental investigations, and developed the process for fabricating the fermentation combinations, GS collected SAXS data, XZ and XG assisted with experimental design. XG and HZ oversaw the project, and contributed to all aspects of analysis and experimental design. ZG wrote the manuscript with assistance from LL and MG.

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References


Activities of Licorice and Fermenting Licorice


국문초록
감초 및 발효감초의 다양한 추출물의 In Vitro 생물학적 활성 및 관련 화학물질

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목적: 본 논문은 감초와 발효감초(FL) 추출물의 주요 화학성분과 이들의 in vitro 피부미용 활성 및 유효성분의 차이를 정성적 및 정량적으로 분석하고자 한다. 또한 감초의 효능에 대한 발효의 영향을 평가한다. 방법: 발효 추출물과 무발효추출물의 DPPH 자유 라디칼 소거 활성, 버섯 티로시나제 활성, B16F10 세포의 티로시나제 활성 및 멜라닌 합성 억제 활성을 평가하였다. 글리시리진, 이소글리시리진, 리퀴리토사이드, 이소리퀴리티게닌 및 글리시리진 케톤 A를 포함한 에틸 아세테이트 성분(EaF)이 멜라닌 합성에 미치는 영향을 확인하기 위해 in vitro 시험을 진행하였다. 또한 FL의 인간 진피 섬유아세포(HDF)에서 MMP-1의 발현을 확인하였다. 결과: 글리시리진, 이소글리시리진, 리퀴리토사이드, 이소리퀴리티게닌은 FL에서 개선되었으며, FL의 항산화 및 MMP-1 저해 활성이 유의하게 증가하였다. 결론: 발효 후 감초의 항노화 효과가 크게 향상되었으며, 이는 발효 과정이 감초의 효능 향상에 좋은 효과가 있음을 나타냅니다.

핵심어: 감초, 발효감초, 스킨케어활성, HPLC/DAD, MMP-1
中文摘要

甘草和发酵甘草中不同提取物的体外生物活性及相关化学物质

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目的：对不同甘草和发酵甘草（FL）提取物中主要化学物质的差异及其体外护肤活性和活性成分进行定性和定量分析。评价发酵对甘草功效的影响。方法：评价不同提取物和不同提取物对B16F10细胞的DPPH自由基清除活性、蘑菇酪氨酸酶活性、酪氨酸酶活性和黑色素合成抑制活性。在体外测试了在体外测试了甘草甜素、异甘草甜素、甘草苷、异甘草苷和甘草甜素酮A等乙酸乙酯组分（EaF）对黑色素合成的影响。进一步评估了真皮成纤维细胞（HDF）中基质金属蛋白酶-1（MMP-1）水平。结果：甘草甜素、异甘草甜素、甘草苷、异甘草苷在FL中得到改
善，FL的抗氧化和MMP-1抑制活性显著提高。结论：发酵后甘草抗衰老效果显著提高，说明发酵过程对提高甘草药效有较好的效果。

关键词：甘草，发酵甘草，护肤活性，HPLC/DAD，MMP-1