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EXPERIMENTAL RESEARCH

Antiseptic property screening for 18 natural plants and efficiency–toxicity research of *Coptis chinensis* fermentation

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[Abstract] **Objective** This study aimed to compare the antibacterial performance of 18 natural plants against six types of microorganisms that tend to grow in cosmetics and select the best natural antiseptic herbs with low toxicity and a broad antibacterial spectrum. The safety performance and effects of the antioxidant, whitening, and reduced inflammation of *Coptis chinensis* fermentation were explored. **Methods** The inhibition performance of the alcoholic extracts for the 18 natural plants against six microorganisms was compared by using the cylinder plate method. The MTT assay was used to determine the cell viability of *C. chinensis* extract and fermentation products. DPPH, hydroxyl, and ABTS radical scavenging rates as well as iron reduction ability were used to evaluate the antioxidant efficacy. In addition, the activity inhibition rate of tyrosinase and hyaluronidase indicated the whitening and anti-inflammatory properties of *C. chinensis* extract and fermentation broth. **Results** Results showed that among the 18 natural plants, *C. chinensis* extract had the most prominent inhibition circles and the smallest minimum inhibitory concentration (MIC) value against six microorganisms. After fermentation, the IC_{50} value of *C. chinensis* alcoholic extract increased significantly with the cell survival rate, indicating its low biosafety. The free radical scavenging rate, tyrosinase inhibition rate, and hyaluronidase inhibition rate of fermented *C. chinensis* extract increased significantly, showing better antioxidant activity, whitening activity, and anti-inflammatory activity than alcohol extract. **Conclusion** Among the 18 natural plants, *C. chinensis* has the most substantial antibacterial ability. After fermentation by yeast, the cytotoxicity of *C. chinensis* decreases, and the antioxidant, whitening, and anti-inflammatory activities increase, indicating that microbial fermentation attenuates toxicity and enhances the effect of plant preservatives.

[Key words] Fermentation; Antibacterial; Antioxidant; Whitening; Anti-inflammatory; *Coptis chinensis*

1 Introduction

Preservatives are vital components in extending the expiration date and ensuring the safety of cosmetic products. Preservative systems in cosmetics include traditional chemical synthetic preservatives with natural preservatives obtained from plant/microbial sources[1]. Although artificial preservatives can inhibit the growth and reproduction of microorganisms, they are irritating and toxic. They can also easily lead to skin allergy, redness, and other skin problems[2]. Therefore, safe and hypoallergenic natural preservatives of plant/microbial origin have received wide

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attention from the daily cosmetic industry. Wild plants are safe and non-irritating; thus, they are greatly favored by cosmetic researchers, and they have shown great application potential as a chemical synthetic preservative[3]. Many plants contain various active antibacterial ingredients[4-7], including polyphenols, vitamin E, and other active substances that can scavenge free radicals[8]. After fermentation, the active ingredients are sublimated and extended, which can increase the content of the active ingredients of natural plants and reduce their toxic effects on organisms[9]. Li *et al*.[10] proved that Resina Draconis has an inhibitory effect on *Propionibacterium acnes* and *Staphylococcus aureus* through bacteriostatic experiments and antioxidant and whitening effects through *in vitro* experiments, which can be used as a natural raw material of cosmetics. The *in vitro* experiments also proved that it has antioxidant and whitening effects, which can be used as a natural ingredient in cosmetics. Liu[11] studied the excellent antibacterial effect of Triphala extract in cosmetics and demonstrated its efficacy in eliminating acne. In addition, many skin care cosmetics often contain natural *Ginkgo biloba*, *Aloe vera*, and *honeysuckle* extracts with anti-sensitivity, anti-inflammatory, antibacterial, and whitening effects[12-13]. Natural preservative products have been receiving considerable attention inside and outside the cosmetic industry. Compared with traditional preservatives, "natural" preservatives, such as essential oil and plant extract preservatives, have complex components and high contents of bioactive substances, including substances that can inhibit the growth of a variety of bacteria or fungi. At present, the effective antibacterial ingredient in plant extracts primarily includes alkaloids, flavonoids, plant peptides, lignin, tannins, amines, steroids, coumarins, lactones, saponins, phenolic compounds, anthraquinones, terpenoids, organic acids, and essential oils. "Natural" preservatives have a massive potential in the cosmetic market. The use of "natural" preservatives in the cosmetic industry is reasonable because they can address the scarcity of cosmetic preservatives, and they are also highly favored by most consumers. After reviewing many works of literature, this paper studied 18 kinds of natural plants and screen the antibacterial ability of their crude extracts.

In this paper, 80% ethanol solution was used for organic solvent extraction of 18 kinds of natural plants that had been preliminarily screened in literature, and the alcohol crude extract was obtained. Then, the antibacterial and anticorrosive ability of the crude extract was studied. In this paper, the minimum inhibitory concentration (MIC) measured by the Oxford cup method and two fold dilution method was used to compare the magnitude of the inhibitory performance of 18 natural plants against six microorganisms susceptible to breeding in cosmetics, and the combined circle of inhibition and MIC values was used to screen *Coptis chinensis* as the best antibacterial natural plant. In addition, human-immortalized epidermal cells (HaCaT) were used to determine the antibacterial performance of *C. chinensis via Pichia pastoris* KM71 (KM71) and *P. pastoris* EBY100 (Y100) fermentation samples to identify cytotoxicity. Such cells were also used to determine DPPH, hydroxyl and ABTS radical scavenging rate, iron reduction capacity of *C. chinensis* extract, and fermentation solution to assess antioxidant capacity and to determine the tyrosinase activity inhibition rate using L-tyrosine as substrate to assess whitening efficacy and hyaluronidase activity. Furthermore, the anti-inflammatory properties of *C. chinensis* extract and fermentation broth were evaluated on the basis of the inhibition rate of L-tyrosine.

2 Material

2.1 Main reagents

C. chinensis (C806210501), *Illicium verum* (200801), *Glycyrrhiza* (180602261), *Sophora flavescens* (C32821202), *Lonicera japonica*

(C308210901), *Foeniculum vulgare* (2020801), *Piper nigrum* (171205551), *Galla chinensis* (201001–02), *Taraxacum mongolicum* (201002), *Radix Aucklandiae* (201101), *Andrographis paniculata* (20052608), *Phryma leptostachya* (210901), *Mentha haplocalyx* (C074211201), *Prinsepia utilis* (11905134), *Thymus mongolicus* (200904), *Rosmarinus officinalis* (201120), *Schisandra chinensis* (C563110501), and *Houttuynia cordata* Thunb (C618211201) were purchased from Zhongshan Xian Yi Tang Traditional Chinese Medicine Pill Co., Ltd. and identified as authentic by Mr. Wang Yan of Guangdong Pharmaceutical University. *Candida albicans*, *Aspergillus niger*, *Bacillus subtilis*, *Escherichia coli*, *S. aureus*, and *Pseudomonas aeruginosa* were purchased from Guangdong Microbial Strain Storage Center. Anhydrous ethanol (Tianjin Damao Chemical Reagent Factory, lot no. 20270119), Nipagin methyl ester (Jiangsu Hongshi Biotechnology Co., Ltd., lot no. HS20200510); ferrous sulfate (Tianjin Yongda Chemical Reagent Co., Ltd., lot no. 20170425 and C12819522), hyaluronidase (Ron Chemical Reagent, lot no. C11220743), tyrosinase (Hefei Bomei Biological Company, lot no. 31B20972), and DPPH (Shanghai Maclean Biochemical Technology Co.) were used in this study. HaCaT cells were purchased from ATCC.

2.2 Main instruments

A VarioskanLUX multi-functional enzyme labeler (Thermo Fisher, USA), 5W-CT-1FD-type clean bench (Shanghai Shenan Medical Equipment Factory), DZX-50FAS vertical pressure steam sterilizer (Shanghai Shenan Medical Equipment Factory), FA/JA electronic balance (Shanghai Hengping Scientific Instruments Co., Ltd.), KQ-500DB desktop CNC Ultrasonic Cleaner (Kunshan Ultrasonic Instrument Co., Ltd.), and HPX-9082MBE Electrothermal Thermostat (Shanghai Boxun Industrial Co., Ltd. Medical Equipment Factory) were utilized in this study.

3 Methods

3.1 Extraction of natural plants

Eighteen natural plant raw materials, such as *C. chinensis*, were dried, crushed through a 60-mesh sieve, sealed, and stored. Next, 20 g of powder was taken into a 250 mL conical flask, added with 80% ethanol at a ratio of 1∶10 (m∶V), and extracted ultrasonically at 55 ℃ for 1 h. The extract was filtered under reduced pressure and concentrated. The volume of deionized water was fixed to 1 g/mL and stored in a sealed container.

3.2 Strain activation and preparation of bacterial solution

E. coli, *S. aureus*, *P. aeruginosa*, *B. subtilis*, *A. niger*, and *C. albicans* were inoculated using an inoculation ring. The six albicans were inoculated into the nutrient broth and mold liquid medium, placed in a constant-temperature water bath at 37 ℃, and activated in a shaker for 24 h.

3.3 MIC of plant extracts against the six species

Compared with plant alcohol extracts prepared by twofold dilution, the bacterial solution diluted with physiological saline was added and shaken well. The bacterial liquid medium was incubated at 37 ℃ for 24 h, and the fungal liquid medium was set at 28 ℃ for 48 h. Saline and nipagin methyl ester were used as negative and positive controls, respectively, to calculate the MIC of each plant extract.

3.4 Bacterial inhibition circle test of 18 natural plant extracts

The cylinder plate method was used to prepare the solid medium required for the test bacteria. The plant extracts were added into the Oxford cup as the sample group, and normal saline and methyl hypogene were used as the negative and positive controls, respectively. After 24 or 48 h culture, the mean value and standard deviation of the diameter of the inhibition zone were calculated.

3.5 Fermentation solution extraction treatment

C. chinensis was crushed through a 60-mesh sieve. 10 g of powde was filled with ethanol to 50 mL, and the bacterial solution of KM71 and Y100 was added into the culture bottle after 10 min of ultrasound. Next, 1 mL of methanol was added, and the culture was carried out in a shaker at 30 ℃ and 100 r/min for 3 days. Afterward, the concentration of ethanol was adjusted to 80%. Finally, 1 g/mL of *C. chinensis* fermentation extract was prepared by adding water to 10 mL.

3.6 Fermentation treatment of extraction solution

C. chinensis was crushed through a 60-mesh sieve, and 10 g of powder was collected. Next, 100 mL of 80% ethanol was added into a round-bottomed flask for 1-h ultrasonic extraction at 55 ℃, then filtered, spun, and filled with water to 50 mL. Then, 3 mL of KM71 and Y100 strains was added. Afterward, 1 mL of methanol was added and cultured in a shaker at 100 r/min and 30 ℃ for 3 days. The fermentation liquid was centrifuged before use.

3.7 Fermentation treatment of *C. chinensis*

C. chinensis was crushed through a 60-mesh sieve; 10 g of the powder was filled with sterilized deionized water to 50 mL, and 3 mL of KM71 and Y100 bacterial solution was added. Then, 1 mL of methanol was added and incubated in a shaker at 30 ℃ and 100 r/min for 3 days. The fermentation liquid was centrifuged before use.

3.8 Cell culture

Based on Duan's experimental cell culture method[14], HaCaT cells were digested with 0.25% trypsin to make a suspension, and the cell count was 5×10^4/mL. HaCaT cells were digested and inoculated in 96-well cell plates by adding 200 μL of cell suspension per well and placed in an incubator for 24 h. Normal cells were also set as the control. The cells were incubated at 37 ℃ for 24 h in an incubator with a 5% CO_2 volume fraction.

3.9 Cell viability determination

After 24 h, the supernatant DMEM culture was sucked out. Afterward, 200 μL of samples of different concentrations was added to each well, and the supernatant was discarded after incubation for 4 h. The medium containing 0.5 μg/mL of MTT was added with 200 μL of samples per well for 4 h, and the absorbance was measured at 490 nm to calculate cell viability. Cell viability was calculated as follows: Cell viability (%) = absorbance value of sample group/absorbance value of the control group.

3.10 Antioxidant efficacy of *C. chinensis* extract and fermentation solution

3.10.1 DPPH free radical scavenging rate

The corresponding concentration samples were mixed with 0.1 mol/L of DPPH and anhydrous ethanol solution at a volume ratio of 1∶1. The blank group was mixed with DPPH and anhydrous ethanol at the same volume of 1∶1 and reacted at room temperature under a dark condition for 30 min. The light absorption value was measured at 517 nm. The absorbance of the DPPH reaction solution with different concentrations of samples is denoted as A_1. The absorbance of the reaction solution between anhydrous ethanol and samples of different concentrations was denoted as A_2. The absorbance of DPPH and the anhydrous ethanol reaction solution was denoted as A_3. Then, the DPPH clearance rate of the sample was calculated as $[1 - (A_1 - A_2)/A_3] \times 100\%$.

3.10.2 Scavenging rate of hydroxyl radicals

One milliliter of the sample with corresponding concentration was collected and added to 1 mL of 3 mmol/L salicylic acid–ethanol solution,

1 mL of 3 mmol/L $FeSO_4$, and 1 mL of 2 mmol/L H_2O_2 in a water bath at 37 ℃ for 15 min for reaction. Afterward, the mixture was centrifuged at 6 000 r/min for 5 min to obtain the supernatant. The absorbance measured at 530 nm was denoted as A_1. H_2O_2 was replaced with an equal amount of ultra-pure water to detect its absorbance (A_{10}), and the sample was replaced with an equal amount of ultra-pure water to detect its absorbance (A_0). Hydroxyl free radical scavenging rate (%) = $[1 - (A_1 - A_{10})/A_0] \times 100\%$.

3.10.3 ABTS radical scavenging rate

Three milliliters of aqueous ABTS solution (12 mmol/L) and 3 mL of potassium persulfate solution (2.45 mmol/L) were homogeneously mixed and stabilized at room temperature for 12–16 h. The absorbance of potassium persulfate solution at 734 nm was adjusted to 0.700 ± 0.025 with DMSO as the dilution medium. The samples were added at different concentrations and shaken well to avoid light. ABTS + clearance (%) = $[1 - (A_1 - A_2)/A_0] \times 100\%$ (A_0 is the blank control group; A_1 is the absorbance value of the sample group and ABTS solution; A_2 is the absorbance value of the sample and solvent group).

3.10.4 Determination of total reducing power

1 mL of the sample, 1 mL of pH 6.8 phosphate-buffered solution, and 1 mL of 1% $K_2[Fe(CN)_6]$ were mixed well in a centrifuge tube and reacted at 50 ℃ for 20 min with ice bath cooling. Then, the mixture was added with 500 mL of 10% trichloroacetic acid and centrifuged at 6 000 r/min for 10 min. Another 1 mL of distilled water and 1 mL of 0.1% $FeCl_3$ were collected and reacted for 10 min. The absorbance at 700 nm was measured by taking the supernatant.

3.11 Tyrosinase inhibition of *C. chinensis* extract and fermentation solution

In accordance with a previously described method[15]: 1 mL of the corresponding concentration of the sample (pH 6.8 phosphate-buffered solution as blank) was substituted with phosphate-buffered solution and 625 μL of tyrosinase (200 U/mL) at 37 ℃ for 10 min. Then, 1 mL of 0.05% L-tyrosine solution was added and then placed in a water bath at 37 ℃ for 40 min, and the absorbance was measured at 475 nm. The inhibition rate of tyrosinase activity was calculated as 1− (C−D)/(A−B) (A is the absorbance value of the system without sample with enzyme; B is the absorbance value of the system without sample without enzyme; C is the absorbance value of the system with sample with enzyme; D is the absorbance value of the system with the selection without enzyme).

3.12 Hyaluronidase inhibition rate of *C. chinensis* extract and fermentation broth

Based on a previously described method[16], the solution to be tested (50 μL) was incubated with hyaluronidase solution (10 μL) for 10 min at 37℃ and then incubated for another 10 min after adding calcium chloride (12.5 mM, 20 μL). Afterward, sodium hyaluronate (50 μL) was added to the reaction mixture and continuously incubated for 40 min, followed by the addition of sodium hydroxide (0.9 M, 10 μL) and sodium borate to the reaction mixture. P-dimethylaminobenzaldehyde (50 μL, 67 mM) was added and incubated for 10 min at 37 ℃. In addition, the absorbance (λ) at 585 nm was measured. Hyaluronidase inhibition rate = $(A_0 - A_1)/A_0 \times 100\%$ [A_0 is the absorbance value of the blank control group (without a sample group), and A_1 is the absorbance value of the sample group].

4 Results

4.1 MIC (mg/mL) of 18 natural plant crude extracts against six bacteria

As shown in the above Table 1, the MIC of *Coptis chinensis* extract against six kinds of bacteria,

Table 1 Minimum inhibitory concentration (MIC) results for 18 types of *Chinese medicines*

Natural plant extracts	MIC (mg/mL)					
	S. aureus	*E. coli*	*P. aeruginosa*	*B. subtilis*	*C. albicans*	*A. niger*
C. chinensis	1.953	7.813	15.625	7.813	3.906	1.953
Illicium verum	1.953	31.250	62.500	62.500	7.813	15.625
Phryma leptostachya	15.625	62.500	62.500	62.500	7.813	31.250
Andrographis paniculata	31.250	125.000	125.000	125.000	62.500	125.000
Foeniculum vulgare	1.953	250.000	250.000	15.625	125.000	62.500
Aucklandia lappa	7.813	62.500	62.500	125.000	62.500	15.625
Sophora flavescens	31.250	31.250	31.250	7.813	7.813	62.500
Galla chinensis	7.813	15.625	15.625	15.625	62.500	250.000
Taraxacum mongolicum	62.500	31.250	31.250	62.500	125.000	62.500
Lonicera japonica	250.000	15.625	15.625	250.000	125.000	125.000
Glycyrrhiza	1.953	62.500	62.500	62.500	125.000	125.000
Mentha haplocalyx	62.500	62.500	62.500	31.250	7.813	62.500
Piper nigrum	500.000	250.000	250.000	250.000	250.000	250.000
Prinsepia utilis	31.250	125.000	125.000	62.500	125.000	62.500
Thymus mongolicus	62.500	125.000	125.000	125.000	250.000	62.500
Rosmarinus officinalis	125.000	125.000	125.000	125.000	250.000	12.500
Houttuynia cordata	31.300	62.500	62.500	62.500	31.300	15.600
Schisandra chinensis	25.000	50.000	50.000	25.000	12.500	25.000

including *S. aureus*, *E. coli*, *P. aeruginosa*, *B. subtilis*, *C. albicans*, *A. niger*, was lower than that of other extracts.

4.2 Circle of the inhibition of six kinds of bacteria by 18 types of plant extracts

The Oxford cup method was used to determine the antibacterial activity of the alcoholic extracts of 18 natural plants, including *C. chinensis* and Anise, against six test bacteria. The results are shown in Table 2, demonstrating that various natural plants have different antibacterial activities against different bacteria at the same concentration (1 mg/mL). *C. chinensis* extract has the best antibacterial activity against six kinds of bacteria, which has a wide antibacterial range and the strongest antibacterial comprehensive ability. It can be used as a good natural antibacterial agent in cosmetics.

Based on previous literature, *C. chinensis* contains berberine, flavonoids, and other active ingredients with good antioxidant ability. In addition, *C. chinensis* fermentation solution (KM71), *C. chinensis* fermentation (KM71) extract, *C. chinensis* extraction fermentation (KM71) solution obtained after microbial fermentation of two yeasts, *P. pastoris* KM71, and *P. pastoris* EBY100 liquid, *C. chinensis* fermentation solution (Y100), *C. chinensis* fermentation (Y100) extract, and *C. chinensis* extraction fermentation (Y100) with antibacterial, antioxidant, whitening, and anti-inflammatory ability were explored in this study.

4.3 Inhibition circles of six bacterial species using the ferments of *C. chinensis*

The Oxford cup method was used to determine the bacteriostasis of the six tested bacteria. The results given in Table 3 showed that the bacteriostatic zone of the fermentation liquid of *C. chinensis* was more significant than that of the extract of *C. chinensis* under the same concentration (1 g/mL) for different bacteria, indicating that the bacteriostatic ability of *C. chinensis* fermented products was enhanced.

4.4 Cytotoxicity experiments

The MTT method was used to detect the effects

Table 2 Circle of the inhibition of six types of bacteria by 18 types of herbal medicines

Natural plant extracts	Diameter of an antibacterial circle/mm					
	S. aureus	*E. coli*	*P. aeruginosa*	*B. subtilis*	*C. albicans*	*A. niger*
Coptis chinensis	26.350±0.48**	23.660±1.02**	24.930±1.23**	21.490±0.81**	28.330±0.48*	46.860±0.99**
Illicium verum	17.350±0.55**	17.280±0.29*	19.870±0.74**	15.310±0.75	16.500±0.27*	17.460±0.40*
Phryma leptostachya	15.680±0.75**	12.500±0.65**	15.560±0.67*	12.830±0.35**	25.870±1.50**	18.810±0.88**
Andrographis paniculata	13.870±0.58**	14.870±0.51**	15.930±1.05*	11.870±0.79**	8.180±0.53**	0
Foeniculum vulgare	12.060±0.98**	12.120±0.69**	19.180±1.16**	25.120±0.23**	0	12.310±0.37**
Aucklandia lappa	17.930±0.66**	12.680±0.25*	12.560±0.41*	15.120±0.58**	15.650±0.81**	17.060±0.21*
Sophora flavescens	14.430±0.32*	11.820±0.44*	15.060±0.98**	12.810±0.53**	18.050±0.13*	12.130±0.34*
Galla chinensis	18.250±0.26*	16.060±1.20**	27.500±0.65**	23.970±0.34*	23.970±0.34*	0
Taraxacum mongolicum	12.810±0.25*	12.370±0.74**	13.310±0.37*	11.180±0.53**	0	9.9300±0.11*
Lonicera japonica	15.180±0.53**	13.370±0.51*	21.450±0.42*	9.370±0.69**	0	0
Glycyrrhiza	15.620±1.38**	12.680±0.79**	13.900±0.42*	14.430±0.32*	0	0
Taraxacum mongolicum	15.750±0.38*	15.250±0.26*	17.000±0.70**	8.870±0.23*	18.100±0.22*	11.370±0.18*
Piper nigrum	10.250±0.80**	12.680±0.26*	14.680±0.37*	13.750±0.26*	0	0
Prinsepia utilis	15.260±0.47*	16.760±0.35*	12.070±0.40*	13.840±0.27*	13.910±0.37*	12.970±0.38*
Thymus mongolicus	16.000±0.57**	17.650±0.42*	12.740±0.41*	13.290±0.44*	16.960±0.28*	15.100±1.03**
Rosmarinus officinalis	15.030±0.49*	15.030±0.43*	12.140±0.46*	13.120±0.51**	15.010±0.38*	13.110±0.49*
Houttuynia cordata	15.250±0.55*	15.950±0.63**	13.760±0.50*	14.930±0.29*	16.160±0.41*	17.110±0.54**
Deionized water(Negativecontrol)	0	0	0	0	0	0
0.2% Nipagin methyl ester (Positive control)	17.700±0.41*	18.150±0.33*	16.020±0.28*	15.490±0.29*	17.980±0.37*	18.120±0.38*

Note: The diameter of the Oxford cup and inhibition circle is 8 and >8 mm, respectively, which have an antibacterial effect.

Table 3 Results of inhibition circles of six bacterial species by the ferment of *C. chinensis*

Natural plant extracts	Diameter of the antibacterial circle/mm					
	S. aureus	*E. coli*	*P. aeruginosa*	*B. subtilis*	*C. albicans*	*A. niger*
Fermentation (KM71)	33.170±0.87**	35.200±1.15**	29.990±1.80**	34.130±0.10*	33.370±0.24**	49.180±0.72**
Fermentation (Y100)	33.370±0.77**	35.410±0.41*	30.180±0.67**	31.360±0.57**	34.350±0.05*	49.990±0.80**
Fermentation (Y100) Extract	35.290±1.61**	35.550±0.50**	37.230±0.87*	35.570±0.39*	39.040±0.67**	47.140±0.53**
Fermentation (KM71) Extract	37.550±0.95**	32.810±0.04*	45.840±1.82*	33.390±0.52**	36.730±0.62**	49.110±0.30*
Extract Fermentation (Y100)	35.980±0.89**	34.533±0.87**	32.840±0.59**	39.900±0.54**	31.940±0.41*	49.600±0.93**
Extract Fermentation (KM71)	38.300±0.95**	34.920±0.95**	31.850±0.44*	32.830±0.50**	33.090±0.36*	45.100±0.20*
Deionized water (Negative control)	0	0	0	0	0	0
0.2%Nipagin methyl ester (Positive control)	17.700±0.41*	18.150±0.33*	16.020±0.28*	15.490±0.29*	17.980±0.37*	18.120±0.38*

Note: The diameter of the Oxford cup and inhibition circle is 8 and > 8 mm, respectively, which have an inhibition effect.

of *C. chinensis* extract and fermentation broth on the survival rate of HaCaT cells. The results given in Fig. 1 showed that different concentrations of extract and fermentation broth had specific effects on the viability of cells, and a specific concentration dependence was observed. That is, cytotoxicity increased with the sample concentration.

As shown in Table 4, IC_{50} of *C. chinensis* after fermentation by two microorganisms (KM71 and Y100) was higher than that of *C. chinensis* extract, indicating that the cytotoxicity of *C. chinensis* samples after fermentation was reduced. The IC_{50} value of

C. chinensis Extract fermentation > *C. chinensis* fermentation Extract > *C. chinensis* Fermentation.

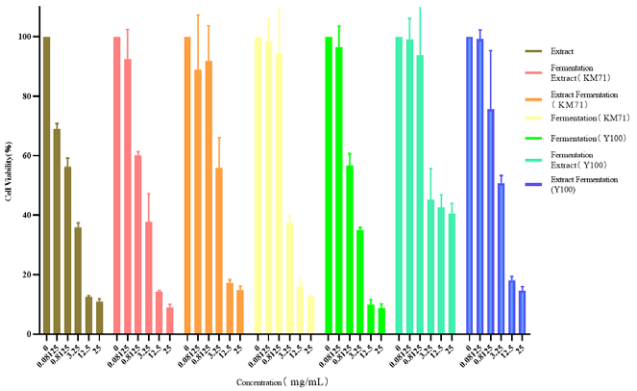

Fig. 1 Results of MTT experiments on HaCaT cells.

Table 4 IC_{50} value of *Coptis chinensis* extract or fermentation solution

Samples	IC_{50}/(mg·mL^{-1})
Extract	0.800
Fermentation (KM71)	0.868
Fermentation (Y100)	0.987
Fermentation extract (Y100)	1.848
Extract fermentation (Y100)	2.185
Fermentation extract (KM71)	1.605
Extract fermentation (KM71)	3.394

4.5 Results of the antioxidant efficacy of *C. chinensis* extract and fermentation broth

4.5.1 DPPH radical scavenging rate

The DPPH radical scavenging rate was tested in the concentration range of 0.004 to 0.782 mg/mL for extracts and ferments of *C. chinensis* based on the IC_{50} value of *C. chinensis*. As shown in Fig. 2, the DPPH scavenging rate of *C. chinensis* extract and ferment had a concentration-dependent relationship. The DPPH radical scavenging rate of *C. chinensis* extract was 39.25% at 0.782 mg/mL, and the DPPH radical scavenging rate of *C. chinensis* fermentation solution (KM71), *C. chinensis* fermentation (KM71) extract, *C. chinensis* extraction fermentation (KM71) solution, *C. chinensis* fermentation solution (Y100), *C. chinensis* fermentation (Y100) extract, and *C. chinensis* extraction fermentation (Y100) at the same concentration increased to 90.03%, 94.77%, 88.25%, 85.26%, 90.69%, and 70.99%, respectively, indicating that the antioxidant properties of *C. chinensis* fermentation broth, fermentation extract, and extract fermentation broth increased after microbial fermentation

4.5.2 Experimental data on the scavenging rate of hydroxyl radicals

The hydroxyl radical scavenging rate of each sample of *C. chinensis* was tested in the concentration range of 0.005–0.782 mg/mL for extracts and ferments.The results were exhibited in Fig. 3. The highest hydroxyl radical scavenging rate of 42.89% was observed for the quotes of *C. chinensis* in the cell-safe concentration range. After fermentation by two yeasts, KM71 and Y100, their similar concentrations of *C. chinensis* ferment (KM71), *C. chinensis* fermentation extract (KM71), *C. chinensis* fermentation extract (KM71) solution, *C. chinensis* ferment (Y100) solution,

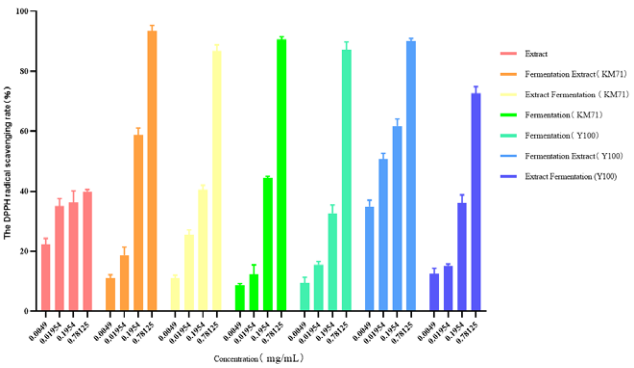

Fig. 2 DPPH radical scavenging rate of each sample of *Coptis chinensis*.

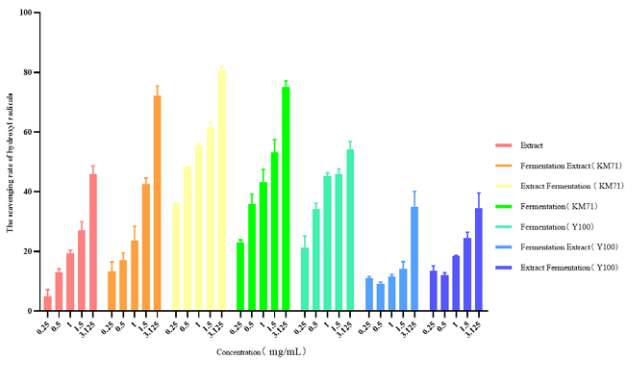

Fig. 3 Hydroxyl radical scavenging rate of each sample of *Coptis chinensis*.

C. chinensis fermentation extract (Y100), and *C. chinensis* fermentation extract (Y100) increased to 73.51%, 81.54%, 70.03%, 56.08%, 48.67%, and 48.09%, respectively, showing a significant increase in antioxidant properties after fermentation.

4.5.3 ABTS radical scavenging rate

The concentration range of 0.050–2.000 mg/mL was selected to study the ABTS radical scavenging rate. The results were given in Fig. 4. In the concentration range of 0.050–2.000 mg/mL, a quantitative-effect relationship was observed between the concentration of each sample of *C. chinensis* and ABTS radical scavenging rate, and the ABTS scavenging rate increased significantly with the increase of concentration. As shown in the above graph, the overall trend of the ABTS extreme scavenging rate of *C. chinensis* extract was lower than that of fermentation broth (Y100 and KM71) and extractive fermentation broth (Y100, KM71, Y100, and KM71) in the concentration range of 0.050–2.000 mg/mL, which indicated that *C. chinensis* was treated with yeast Y100 and KM71. All fermentation treatments showed improved antioxidant properties. In the low concentration range of 0.005–0.325 mg/mL, the ABTS scavenging rate was substantially increased after treatment with Saccharomyces cerevisiae Y100 and KM71. The ABTS radical scavenging rate of *C. chinensis* extract was 43.09% at a concentration of 0.325 mg/mL. By contrast, the fermentation broth Y100 and KM71; extract fermentation broth Y100; and the ABTS radical scavenging rate of the fermentation solution KM71 and fermentation extract Y100 and KM71 at these concentrations were 83.39%, 56.94%, 71.67%, 65.06%, 59.07%, and 1.03%, respectively, indicating that the ABTS scavenging rate of *C. chinensis* samples treated with KM71 and Y100 and their antioxidant properties were improved.

4.5.4 Total reducing power measurement results

As shown in Fig. 5, compared with the *C. chinensis* extract, the absorbance values of *C. chinensis* fermentation solution (KM71 and Y100) increased at the same concentration: *C. chinensis* fermentation extract > *C. chinensis* extraction fermentation solution > *C. chinensis* fermentation solution. The total reducing power of the sections after Y100 fermentation was higher than that of the quotes after KM71 fermentation. In the concentration range of 0.195–6.250 mg/mL, a quantitative relationship was observed between the concentration and total reducing power of all seven *C. chinensis* samples. The absorbance value of the absolute reducing power and antioxidant property increased with the increase of the concentration. At the concentration of 0.782 mg/mL, the absorbance value of *C. chinensis* extract was 0.013, whereas the absorbance of fermentation solutions Y100 and KM71, extraction fermentation solutions Y100 and KM71, and fermentation extracts Y100 and KM71 at this concentration were 1.346, 0.571, 0.642, 0.668,

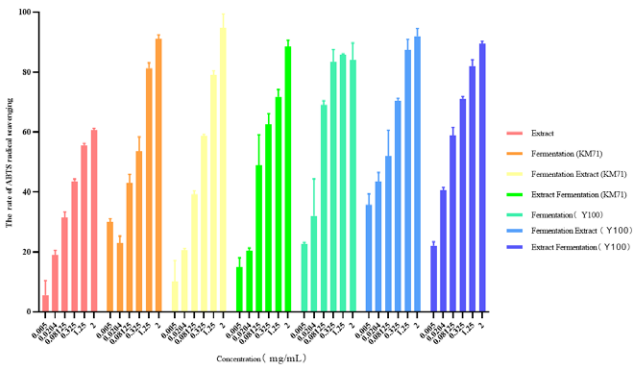

Fig. 4 ABTS free radical scavenging rate of each sample of *Coptis chinensis*.

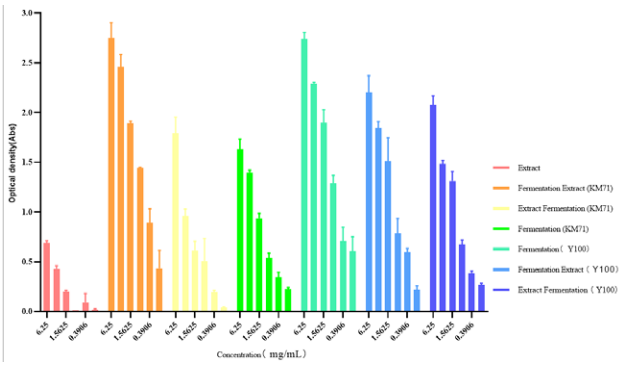

Fig. 5 Determination of the total reducing power of each sample of *Coptis chinensis*.

0.891, and 1.446, respectively, which indicated that the antioxidant properties of *C. chinensis* were improved after KM71 and Y100 treatments.

4.6 Results of the whitening efficacy of each *C. chinensis* sample

The results given in Fig. 6 showed the quantitative relationship between the concentration and tyrosinase inhibition rate of each sample of *C. chinensis*. With the increase of concentration, the tyrosinase inhibition rate increased, indicating that its whitening performance improved. In the high concentration range (5 and 10 mg/mL), the fermentation of KM71 increased the tyrosinase inhibitory activity of *C. chinensis* by 10% compared with that of the *C. chinensis* extract. After the fermentation of Y100, the highest concentration of 10 mg/mL increased the inhibition rate by 20%. Therefore, the whitening efficacy of *C. chinensis* was improved after microbial treatment.

4.7 Results of the anti-inflammatory efficacy of each *C. chinensis* sample

As is shown in Fig. 7, a quantitative-effect relationship was observed between the concentration and tyrosinase inhibition rate of all seven samples of *Flos daturae*. The hyaluronidase inhibition rate increased with the increase of concentration, thereby enhancing its anti-inflammatory properties. In addition, the inhibition rate of the hyaluronidase of Flos daturae extract at a concentration of 0.040 mg/mL was 47% after Y100 treatment with fermentation extracts Y100 and Y100, which increased by 30% and 22%, respectively. The hyaluronidase inhibition rate of fermentation extracts KM71 and KM71 and fermentation broth (KM71) after KM71 treatment increased by 16%, 19%, and 36%, respectively, which indicated that the anti-inflammatory activity of the samples obtained after KM71 and Y100 fermentation treatments was improved.

5 Discussion

China is a vast country, and it has more than 12 000 herbal medicines, which contain a variety of active ingredients, including flavonoids, alkaloids, phenols, and quinones, with significant antibacterial and bactericidal effects. Compared with chemical preservatives, herbal antibacterial has low drug resistance. Some of these herbs also exhibit antibacterial activity through synergistic effects when combined with antibiotics[17]. Most chemically synthesized products have sound antibacterial effects. In addition, the normal skin flora can be disrupted, thereby leasing to the occurrence of skin infections[18-19]. The search for natural, safe, and low-toxicity preservatives has become a significant challenge in the cosmetic industry. Thus, this study evaluated the antibacterial ability of 18 herbal extracts, including *C. chinensis*, *Licorice*, and *Fishycus*, against six microorganisms that tend to grow in cosmetics based on the MIC results and Oxford cup method[20]. Comparing the MIC values with the inhibition diameters of the

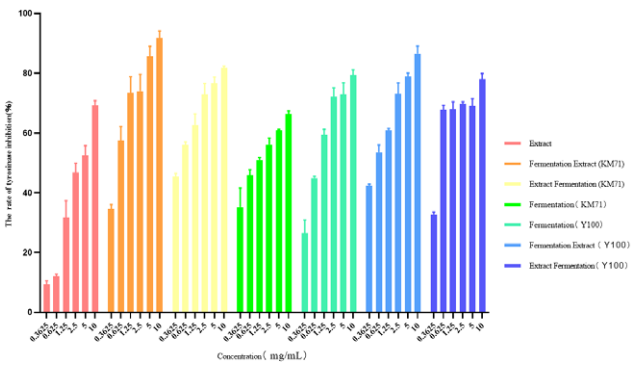

Fig. 6 Results of tyrosinase inhibition assay for each sample of *Coptis chinensis*.

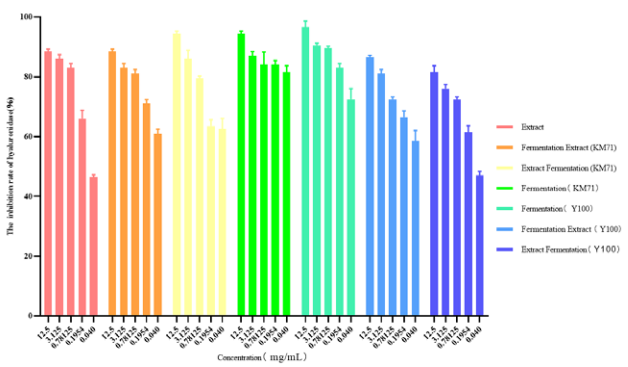

Fig. 7 Results of the anti-inflammatory efficacy of each *Coptis chinensis* sample.

Oxford cup method, the single natural plant with the best overall inhibition effect and the broadest inhibition spectrum was *C. chinensis* oil extract. The same herbal medicine processed by different methods showed distinct effects.

Microbial fermentation is a traditional method of processing herbal medication, which affects the abundance of relevant components in herbal medicines[21] and changes the content of active ingredients in herbal medicines while exhibiting different effects. During microbial fermentation of *C. chinensis* using the yeast *P. pastoris* KM71 and *P. pastoris* EBY100, the sequence of fermentation and extraction was adjusted to obtain *C. chinensis* extract and finally *C. chinensis* fermentation solution (KM71), *C. chinensis* fermentation extract (KM71), *C. chinensis* extraction fermentation solution (KM71), *C. chinensis* fermentation solution (Y100), *C. chinensis* fermentation extract (Y100), *C. chinensis* extract fermentation solution (Y100). The results of antibacterial experiments showed that fermentation concoction could improve the antibacterial effect while reducing the toxicity of *C. chinensis*. Combining the IC_{50} values and inhibition circle data of the six fermentation samples, the *C. chinensis* extract fermentation solution (KM71), which is the least toxic to cells, was considered as the preferred natural preservative. Meanwhile, 1.000–4.000 mg/mL was selected as the best application concentration in cosmetics. In addition, antioxidant, whitening, anti-inflammatory, and anti-aging properties are commonly used in the cosmetic industry to assess whether the product can improve health status and brightness of the skin[22-25]. The antioxidant, anti-inflammatory, and whitening efficacy of the fermented products of Flos daturae were higher than those of Flos daturae extracts, indicating that microbial fermentation reduces toxicity and increases plant efficacy of preservatives. Moreover, different fermentation strains and fermentation extraction processes can affect the efficacy of *C. chinensis* samples during microbial fermentation. However, their influence, rules, and deep mechanism remain to be explored.

6 Conflicts of interest

These authors have no conflict of interest to declare.

7 Acknowledgments

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