Analysis on Antibacterial, Detumescent and Antioxidant Activity of Huoshan Large-leaf Yellow Tea

Jinwu CHEN[1], Wen HU[1], Yimeng LI[1], Jiaojiao WANG[1], Yafei LIU[1], Yi LI[2], Lulu QI[1]*

1. School of Biology and Food Engineering, Hefei Normal University, Hefei 230601, China; 2. School of Life Sciences, Anhui University, Hefei 230039, China

Abstract [**Objectives**] To provide experimental basis for the effective development and utilization of Huoshan large-leaf yellow tea resources and the screening of safe and effective active ingredients of large-leaf yellow tea. [**Methods**] The active substances of Huoshan large-leaf yellow tea were extracted by hot-water extraction, and the freeze-dried powder of Huoshan large-leaf yellow tea was obtained by freeze drying. The antibacterial activity of the extract was preliminarily confirmed using the Oxford cup method, and its antimicrobial spectrum was analyzed using 14 strains. A xylene-induced mouse auricle swelling test was carried out to detect the swelling inhibition rate of the extract and analyze its *in-vitro* detumescent activity. Then, the antioxidant activity of the extract was identified through a DPPH free radical scavenging capacity test and a ferric reducing antioxidant power assay. [**Results**] The extract had significant inhibitory effects on various bacteria. The extract could effectively inhibit the growth of *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus hirae*, *Acinetobacter baumannii*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and other strains. The diameter of the inhibition zone increased with the increase of sample concentration. The extract had a significant inhibitory effect on auricle swelling induced by xylene in mice. When the concentration of the drug reached 1.0 mg/mL, its inhibition rate on mouse auricle swelling reached 55.2% ($P<0.01$), slightly lower than the swelling inhibition rate of the aspirin group (66.52%, $P<0.01$). The results of the antioxidant test showed that large-leaf yellow tea extract also had strong activity. Within the concentration range of 0.1 - 1.0 mg/mL, its DPPH radical scavenging rate increased with the increase of sample concentration. Within the concentration range of 0.1 - 1.0 mg/mL, its DPPH radical scavenging rate increased with the increase of sample concentration. When the concentration reached 1.0 mg/mL, the scavenging rate reached 69.75%. The Fe^{3+}-reduction capacity of the extract also increased with the increase of sample concentration within the concentration range of 0.1 - 2.5 mg/mL. When the concentration was 2.5 mg/mL, the reducing power of the extract reached 1.43 ± 0.04. However, its DPPH free radical scavenging rate and reducing power were slightly lower than the capacity of V_C at the same concentration. [**Conclusions**] The extract of Huoshan large-leaf yellow tea obtained by hot-water extraction had strong activity in many aspects, including inhibiting the growth of various microbes, subsiding swelling *in vitro* and resisting oxidation. These experimental results provide certain guiding significance for the basic research of Huoshan large-leaf yellow tea extract, as well as experimental data support for the subsequent development of functional foods and drugs of Huoshan large-leaf yellow tea.

Key words Huoshan large-leaf yellow tea, Antibacterial activity, Detumescence, Antioxidant activity

1 Introduction

In recent years, with the rise of natural health plants, research on large-leaf yellow tea has gradually become a hot topic. Its ingredients are constantly identified, and the medicinal functions of its active ingredients are deeply explored[1]. Large-leaf yellow tea is a type of yellow tea, as well as a light fermented tea. Specifically, large-leaf yellow tea produced in Huoshan has the best quality. The production process of large-leaf yellow tea is similar to that of green tea, but during its frying process, it requires "heaping up for yellowing", which will make the dry matter components undergo violent reactions such as oxidation and degradation under the influence of damp and heat, which will further lead to a sharp decrease in chlorophyll content and changes in the structure of catechin and soluble sugar[2-4]. These changes ultimately formed the material foundation of yellow leaves and tea soup.

Many studies have shown that tea generally contains tea polyphenols, tea polysaccharides, theaflavin and other active substances, which have blood sugar-lowering, blood lipid-lowering, anti-tumor and other effects on the human body[5-7]. For example, tea polyphenols, which are abundant in plants, are a natural product with unique physiological and pharmacological activity. Mhatre Susmit *et al.*[8-9] revealed the inhibitory effect of tea polyphenols on COVID-19, and they believed that there might be a binding site on the surface of COVID-19 that interacted with tea polyphenols. Tea polysaccharides are another renewable resource with great practical value and extensive sources. Tea polysaccharides are acidic glycoproteins, which have pharmacological effects such as hypoglycemic, hypolipidemic and antioxidant effects, combined with a large number of mineral elements. Chen Hao *et al.*[10-11] believe that tea polysaccharides have a certain effect in inhibiting liver injury and reducing fat accumulation in liver tissue. Theaflavin is orange yellow, and can increase the luster and taste of tea soup. Meanwhile, it also has enzyme activity-inhibiting and antiviral effects, and can be applied in food and medicine[12-13]. In this study, Huoshan large-leaf yellow tea was selected as the raw material to explore the antibacterial, detumescent and antioxidant activity of Huoshan large-leaf yellow tea ex-

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* Corresponding author. E-mail: ddl6766@163.com

tract. The experimental results indicated that the extract of Huoshan large-leaf yellow tea had strong activity in these aspects, which provides certain guidance for the basic research of tea extract and experimental data support for the subsequent development of functional foods and drugs of Huoshan large-leaf yellow tea.

2 Materials and methods

2.1 Materials

2.1.1 Strains and experimental animals. *Escherichia coli* (ATCC10536, 25922), *Staphylococcus aureus* (ATCC25923, 653), *Enterococcus hirae* (ATCC10541), *Acinetobacter baumannii* (ATCC19606), *Klebsiella pneumonia* (ATCC4352), *Pseudomonas aeruginosa* (ATCC27853), *Bacillus subtilis* (ATCC6633), *Saccharomyces cerevisiae* (ATCC9763), *Saccharomyces diastaticus* (ATCC28338), *Streptomyces filamentosus* Okami and *Umezawa* (ATCC19753) and *Aspergillus niger* (ATCC16404) were all preserved in the laboratory. Healthy Kunming mice weighing approximately (20 ± 2) g were provided by Experimental Animal Center of Anhui Medical University.

2.1.2 Reagents. Huoshan large-leaf yellow tea (Anhui Province Baoerzhongxiu Tea Industry Co., Ltd.); 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) (TCI (Shanghai) Development Co., Ltd.); potassium ferricyanide (Beijing Solarbio Science&Technology Co., Ltd.); peptone, tryptone, yeast extract, D-glucose, agar powder and ferric chloride (Sangon Biotechnch (Shanghai) Co., Ltd.).

2.1.3 Experimental instruments. Oxford cups (Shanghai Jinghong Electronic Technology Development Co., Ltd.); vertical rotary evaporator IKA RV10 (IKA Works Guangzhou); freeze dryer LGJ-10 (Beijing Songyuan Huaxing Technology Development Co., Ltd.); full-wavelength microplate reader SpectraMax M2e (Molecular Devices Shanghai Corporation); UV-VIS spectrophotometer UV-3200 (Shanghai Mapada Instruments Co., Ltd.); electric-heating constant-temperature incubator HPX-9082MBE (Shanghai Boxun Industrial Co., Ltd.).

2.2 Methods

2.2.1 Preparation of tea extract. First, 10 g of Huoshan large-leaf yellow tea was weighed and ground with a mortar. After adding 200 mL of ultrapure water, the sample was extracted with hot water in a 80 ℃ water bath for 20 min. Next, the soaking liquid was centrifuged at 5 000 r/min for 20 min to obtain clear tea soup. After centrifugation, the tea residue was extracted twice according to the above method. Next, 600 mL of extract was concentrated with a rotary evaporator to about 60 mL, which was then perform freeze-dried in vacuum for 10 h to obtain a freeze-dried powder of Huoshan large-leaf yellow tea soup, which was stored in a refrigerator at −20 ℃ for later use.

2.2.2 Analysis on antimicrobial activity of tea extract. Referring to references [14-15], the Oxford cup method was used to detect the antimicrobial activity of Huoshan large-leaf yellow tea extract. After activating *E. coli* (ATCC25922) and *S. aureus* (ATCC 25923) in LB solid medium, monoclonal clones were picked and inoculated in LB liquid medium, and cultured in a shaking incubator at 37 ℃ overnight to achieve a viable bacterial count of approximately 1.0×10^6 cfu/mL for use.

Above 0.5 mL of each of the two strains mentioned was put on an LB solid plate, and then evenly coated with a sterile coating rod. After the bacterial liquid was dried, sterilized Oxford cups (with an outer diameter of 8 mm) were placed on each plate in an equilateral triangle. An appropriate amount of freeze-dried powder of Huoshan large-leaf yellow tea extract was dissolved in ultrapure water, and the obtained liquid was filtered with a 0.22 μm filter. Next, the filtrate was diluted to solutions with concentrations of 10, 25, 50, 100, 250, 500, 750, and 1 000 μg/mL. One control group of sterile water (adding 100 μL of sterile water) was set in each plate. Other two Oxford cups were the experimental groups, which were added with 100 μL of corresponding extract aqueous solution with the same concentration. Three parallel plates were set for each concentration, and the diameter of the inhibition zone after incubation was measured at 37 ℃ for 24 h. When the diameter of the inhibition zone was greater than or equal to 10 mm, it was determined that the bacteria were susceptible to the concentration of the extract.

2.2.3 Study on the antimicrobial spectrum of tea extract. The Oxford cup method was adopted to test the antimicrobial activity of the extract against multiple microbial strains, and the experimental steps were based on the above method. The concentration of the extract aqueous solution was 1 mg/mL. *E. coli* (ATCC10536, 25922), *S. aureus* (ATCC25923, 653), *E. hirae* (ATCC10541), *A. baumannii* (ATCC19606), *K. pneumonia* (ATCC4352), *P. aeruginosa* (ATCC27853) and *B. subtilis* (ATCC6633) were cultured on LB medium at 37 ℃ for 24 h. *S. cerevisiae* (ATCC9763) and *S. diastaticus* (ATCC28338) were cultured in YPD medium at 30 ℃ for 48 h. *S. filamentosus* Okami and *Umezawa* (ATCC19753) and *A. niger* (ATCC16404) were cultured in PDA medium at 28 ℃ for 72 h. After sufficient cultivation time, the strains were observed and measured to record the inhibition zone diameters.

2.2.4 Analysis of *in-vitro* detumescent activity of tea extract. Referring to the xylene-induced auricle swelling test in mice[16], 50 healthy Kunming mice, 18 − 22 g, half male and half female, were randomly divided into five groups, with 10 mice in each group, namely the control group (physiological saline group), high-dose Huoshan large-leaf yellow tea extract group, low-dose Huoshan large-leaf yellow tea extract group, positive drug control group (aspirin), and ethanol group. Each mouse was given 0.2 mL of physiological saline in the saline group, 75% ethanol in the ethanol group, 0.2 mL of 1 mg/mL aqueous extract in the high-dose Huoshan large-leaf yellow tea extract group, 0.2 mL of 0.4 mg/mL aqueous extract in the low-dose group, and 0.2 mL of 0.4 mg/mL aspirin solution in the aspirin group. Each solution was applied to both sides of the mouse's right auricle three times a day for 5 d consecutively, to induce inflammation, leaving the left ear

as the control check. After 1 h of inflammation induction, the mice were euthanized by cervical dislocation. Two ears were cut along the baseline of the auricle, and each mouse's ear areas were calculated. Round ear pieces with a diameter of 6 mm were made with a puncher at the same area of the left and right ears, and weighed to calculate the swelling degree and swelling inhibition rate.

$$\text{Degree of auricle swelling (mg)} = M_r - M_l \quad (1)$$

where M_r is the weight of the right ear, and M_l is the weight of the left ear.

$$\text{Inhibition rate of auricle swelling (\%)} = [(C_0 - C_t)/C_0] \times 100\% \quad (2)$$

where C_0 represents the average degree of auricular swelling in the blank control group, and C_t is the average degree of auricular swelling in the treatment group.

2.2.5 Determination of antioxidant activity of tea extract. (i) Determination of DPPH free radical scavenging capacity. The DPPH scavenging capacity of Huoshan large-leaf yellow tea extract was determined by the DPPH colorimetric method[17]. The freeze-dried powder of Huoshan large-leaf yellow tea extract was accurately weighed and dissolved in ultrapure water to obtain test solutions with concentration gradients of 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL, respectively, and an appropriate amount of V_C was accurately weighed and dissolved in ultrapure water to give a V_C control solution. Next, 2 mL of one test solution and 2 mL of 0.1 mmol/L DPPH solution were added to a sample tube, denoted as A_1; 2 mL of DPPH and 2 mL of anhydrous ethanol were added to a blank tube, denoted as A_0; 2 mL of Vc and 2 mL of DPPH solution were added to a positive control tube in sequence, and the obtained solution was stored in dark for 30 min, and measured for its absorbance at 517 nm, with distilled water as the blank. To eliminate the influence of pigments, a sample reference group, which consisted of 2 mL of anhydrous ethanol and 2 mL of corresponding test solution, was set up, and the absorbance A_2 was measured. Each test was repeated three times to take an average value. The DPPH free radical scavenging rate (K) was calculated according to following formula:

$$K(\%) = [A_0 - (A_1 - A_2)]/A_0 \times 100\% \quad (3)$$

(ii) Determination of ferric reducing antioxidant power. According to the potassium ferricyanide method of Choochote *et al.*[18-19], the antioxidant activity of Huangda large-leaf yellow tea extract was evaluated according to its reduction capacity to Fe^{3+}. First, 1 mL of extract aqueous solution, 2.5 mL of phosphate buffer solution with concentration of 0.2 mol/L (pH = 6.6) and 2.5 mL of 1% potassium ferricyanide solution were mixed well, and the mixed solution was kept in a 50 ℃ water bath for 20 min, and then quickly put into ice to cool. Next, 1 mL of 10% trichloroacetic acid was added to terminate the reaction. After thoroughly mixing, 2.5 mL of the reaction solution was added and mixed well with 2.5 mL of sterile water and 0.5 mL of 0.1% $FeCl_3$, obtaining a solution, which was stood for 10 min, and measured for its absorbance value at 700 nm, denoted as A_1. Sterile water was used as a control, denoted as A_0. The concentration gradients of the sample were set as 0.1, 0.25, 0.5, 1, and 2.5 mg/mL, and V_C served as the positive control. The total reduction rate was calculated according to following formula:

$$\text{Total reducing power} = A_1 - A_0 \quad (4)$$

2.2.6 Statistical analysis of data. The data were represented by Mean ± SEM, and histograms were plotted using Graphpad Prism 9.0. The differences between groups were compared by *t*-tested using SPSS 22.0, with $P < 0.05$ indicating a statistically significant difference.

3 Results and analysis

3.1 Antimicrobial activity of tea extract A preliminary study was conducted on the antimicrobial activity of Huoshan large-leaf yellow tea extract using the Oxford cup method. The experimental results are shown in Table 1. From the diameter of the inhibition zone, it could be observed that when the sample concentration was 50 μg/mL, it exhibited an inhibitory effect on *S. aureus* [with a diameter of (10.68 ± 0.26) mm, greater than 10 mm], and when the concentration increased to 100 μg/mL, it had an inhibitory effect on *E. coli* [with a diameter of (11.17 ± 0.23) mm]. As the concentration of the extract increased, its inhibition zone against both bacteria also increased. These results indicated that the tea extract had good inhibitory effects on *E. coli* and *S. aureus*. And its inhibitory effect on *S. aureus* was stronger than that on *E. coli*.

Table 1 Antimicrobial activity of Huoshan large-leaf yellow tea extract mm

Strains	Inhibition zone diameters								
	0	10	25	50	100	250	500	750	1 000
Escherichia coli	8.07 ± 0.03	8.17 ± 0.06	8.40 ± 0.04	9.70 ± 0.11	11.17 ± 0.23	13.12 ± 0.21	14.72 ± 0.30	16.35 ± 0.34	17.85 ± 0.26
Staphylococcus aureus	8.17 ± 0.05	8.42 ± 0.16	9.52 ± 0.17	10.68 ± 0.26	12.98 ± 0.15	13.92 ± 0.29	15.32 ± 0.36	17.85 ± 0.35	19.90 ± 0.25

Note: 0, 10, 25, 50, 100, 250, 500, 750, 1 000: The concentration of the Huoshan large-leaf yellow tea extract in aqueous solution. Units: μg/mL.

3.2 Study on the antimicrobial spectrum of large-leaf yellow tea extract Previous studies have shown that tea has a broad antimicrobial spectrum, especially for bacteria in the intestines and digestive tract. In this study, the Oxford cup method was adopted to analyze the antimicrobial effect of Huoshan large-leaf yellow tea extract on a total of 14 strains of 12 microbes, including 9 bacterial strains and 4 fungal strains. The results are shown in Table 2, and the extract showed strong inhibitory effects on all bacteria in the study. However, the inhibitory effects on fungi such as mold and yeast were not significant. The order of inhibitory effects on different bacteria was *S. aureus* > *B. subtilis* > *E. coli* > *K. pneumonia* > *E. hirae* > *P. aeruginosa* > *A. baumannii*.

Table 2 Antimicrobial spectrum of Huoshan large-leaf yellow tea extract

Group	Strains	Inhibition zone diameters // mm	Antimicrobial effect
Bacteria	*Escherichia coli* ATCC10536	17.23 ± 0.26	+ + +
	E. coli ATCC25922	16.98 ± 0.28	+ + +
	Staphylococcus aureus ATCC25923	18.82 ± 0.31	+ + +
	S. aureus ATCC6538	18.30 ± 0.21	+ + +
	Enterococcus hirae ATCC10541	13.75 ± 0.20	+ +
	Acinetobacter baumannii ATCC19606	12.23 ± 0.40	+ +
	Klebsiella pneumonia ATCC4352	15.82 ± 0.27	+ + +
	Pseudomonas aeruginosa ATCC27853	13.22 ± 0.40	+ +
	Bacillus subtilis ATCC6633	17.57 ± 0.35	+ + +
Fungus	*Saccharomyces cerevisiae* ATCC9763	9.05 ± 0.23	–
	S. diastaticus ATCC28338	8.80 ± 0.33	–
	Streptomyces filamentosus Okami and *Umezawa* ATCC19753	9.10 ± 0.33	–
	Aspergillus niger ATCC16404	9.27 ± 0.21	–

Note: " + + + " Inhibition zone diameters (d) ≥15.00 mm," + + " 10.00 mm ≤d < 15.00 mm," – " d < 10.00 mm.

3.3 *In-vitro* detumescent activity of Huoshan large-leaf yellow tea extract From Table 3, it can be seen that the extract of Huoshan large-leaf yellow tea could significantly reduce the degree of auricle swelling induced by xylene in mice. When administered at concentrations of 0.4 and 1.0 mg/mL, the inhibitory rates on mouse auricle swelling were 32.13% and 55.2%, respectively, and there were significant differences compared with the saline group (0.4 mg/mL: $P < 0.05$, 1.0 mg/mL: $P < 0.01$). However, its swelling inhibition power was lower than that of the positive control group aspirin. The results showed that the extract of Huoshan large-leaf yellow tea could significantly inhibit auricle swelling induced by xylene in mice, and had a certain effect of swelling *in vitro*. The swelling inhibition rate increased with the increase of drug concentration.

Table 3 Effects of Huoshan large-leaf yellow tea extract on auricle swelling induced by xylene in mice (Mean ± SEM, $n = 10$)

Group	W_r // mg	W_l // mg	Auricle swelling // mg	Inhibition rate of auricle swelling // %
Control	6.33 ± 0.15	4.12 ± 0.11	2.21 ± 0.21	–
Ethyl alcohol	6.02 ± 0.14	4.01 ± 0.07	2.01 ± 0.19	9.05
0.4 mg/mL tea extract	5.64 ± 0.11	4.14 ± 0.10	1.50 ± 0.16 *	32.13
1.0 mg/mL tea extract	5.04 ± 0.13	4.05 ± 0.08	0.99 ± 0.11 **	55.20
Aspirin	4.87 ± 0.09	4.06 ± 0.09	0.74 ± 0.1 **	66.52

Note: * Compared with the control group, * $P < 0.05$; ** $P < 0.01$.

3.4 Determination of antioxidant activity of tea extract

3.4.1 Determination of DPPH free radical-scavenging capacity. From Fig. 1, it can be seen that both Huoshan large-leaf yellow tea extract and positive control V_C had strong scavenging capacities against DPPH free radicals. Within the concentration range of 0.1 – 1.0 mg/mL, the scavenging rate of DPPH free radicals was positively correlated with drug dosage. The higher the concentration of the extract, the stronger the DPPH free radical-scavenging rate. When the concentration reached 1.0 mg/mL, the scavenging rate of the extract on DPPH free radicals could reach 69.75%. However, at the same mass concentration, the DPPH free radical scavenging rate of the extract was slightly lower than that of the positiv e-control V_C.

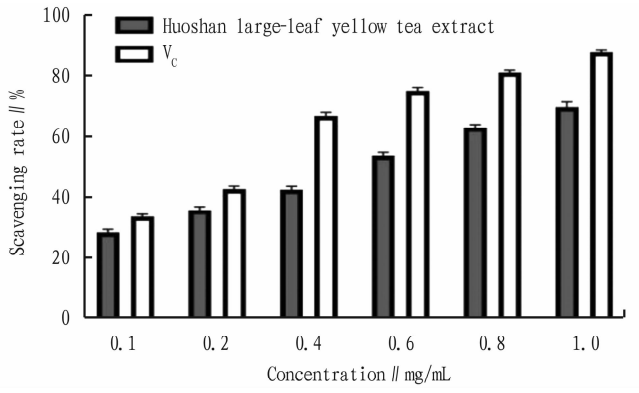

Fig. 1 Antioxidant activity of Huoshan large-leaf yellow tea extract on DPPH free radicals

3.4.2 Determination of ferric reducing antioxidant power. With V_C as a positive control, the reducing capacity of Huoshan large-leaf yellow tea extract with different concentrations was determined. As shown in Fig. 2, within the concentration range of 0.1 – 2.5 mg/mL, the capacity of the extract to reduce Fe^{3+} was positively correlated with its concentration, and its reducing power increased with the increase of sample concentration. At a concentration of 2.5 mg/mL, the reducing power of the extract reached 1.43 ± 0.04, slightly lower than the positive control V_C (2.06 ± 0.07).

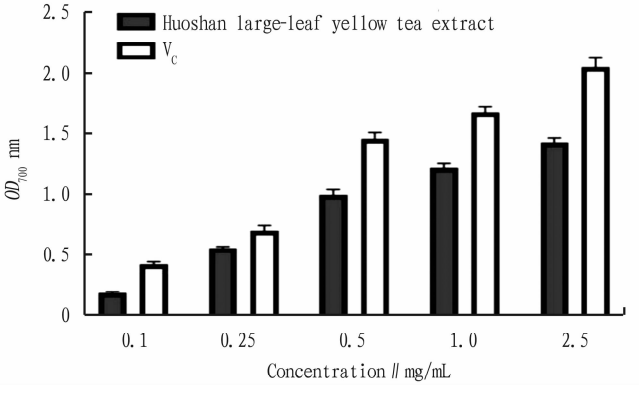

Fig. 2 Ferric reducing antioxidant power (FRAP) of Huoshan large-leaf yellow tea extract

4 Conclusions

Under the experimental conditions of this study, Huoshan large-leaf yellow tea extract had significant inhibitory effects on various microbes. The results of the Oxford cup test showed that the extract could effectively inhibit the growth of *E. coli*, *S. aureus*, *E. hirae*, *A. baumannii*, *K. pneumonia*, *P. aeruginosa*, *B. subtilis* and other strains. These microbes in the intestines and digestive tract may cause intestinal infections, urinary tract infections, meningitis, *etc.* The inhibitory effects of Huoshan large-leaf yellow

tea extract on these microbes will contribute to the development of anti-infective drugs.

In the xylene-induced auricle swelling test, the extract had a significant inhibitory effect on swelling. When the concentration reached 1.0 mg/mL, its inhibition rate on mouse auricle swelling reached 55.2%, only slightly lower than the swelling inhibition rate of the aspirin group (66.52%). The results indicated that the large-leaf yellow tea extract had certain anti-inflammatory and swelling-subsiding effects.

Large-leaf yellow tea extract also had strong antioxidant activity. Its scavenging effect on DPPH free radicals was good, and the scavenging effect increased with the increase of extract concentration. Free radicals, which are ubiquitous, can lead to aging, arteriosclerosis, cancer, *etc.* [20]. At present, there is no way to completely eliminate them, so we can only consider how to reduce the harm of free radicals. This study can provide a certain theoretical basis for the elimination of free radical hazards by large-leaf yellow tea extract. Meanwhile, large-leaf yellow tea extract also had a certain reducing power on ferric ions. The content of iron in human body is very small, so it needs to be ingested from food. The lack of iron can cause iron-deficiency anemia, lead poisoning, *etc.*, and the common existence of ferric ions in food is not easy to be absorbed by the human body. Only by reducing it to ferrous ions can it be absorbed by the human body. And ferrous ions can effectively combine with oxygen, and if it becomes ferric ions, the ability to carry oxygen will be greatly reduced[21]. The reduction effect of large-leaf yellow tea extract on iron ions can reduce ferric ions to ferrous ions, which to some extent promotes the absorption of iron element by the human body and increases blood oxygen content. In this study, the efficacy of Huoshan large-leaf yellow tea extract was analyzed, providing relevant experimental data for the comprehensive utilization and development of Huoshan large-leaf yellow tea.

References

[1] ZHU XF, LIU ZQ, WAN XC, *et al.* Comparison of simultaneous distillation and extraction and headspace-solid phase microextraction for analysis of aroma components of Huoshan large-leaf yellow tea by gas chromatography-mass spectrometry[J]. Food Science, 2020, 41(4): 214 – 221.

[2] GUO XY, HO CT, SCHWAB W, *et al.* Effect of the roasting degree on flavor quality of large-leaf yellow tea[J]. Food Chemistry, 2021(347): 129016. (in Chinese).

[3] ZHOU J, WU Y, LONG PP, *et al.* LC-MS-Based metabolomics reveals the chemical changes of polyphenols during high-temperature roasting of large-leaf yellow tea[J]. Journal of Agricultural and Food Chemistry, 2019, 67(19): 5405 – 5412. (in Chinese).

[4] HUA JJ, JIANG YW, YUAN HB, *et al.* Review on the changes of biochemical components and the influencing factors in piling process of yellow tea[J]. Journal of Tea Science, 2015, 35(3): 203 – 208. (in Chinese).

[5] ZHAI XT, HU YM, PEI ZY, *et al.* Insights into the key odorants in large-leaf yellow tea (*Camellia sinensis*) by application of the sensomics approach[J]. Journal of Agricultural and Food Chemistry, 2023, 71(1): 690 – 699.

[6] ZHOU J, ZHANG L, MENG QL, *et al.* Roasting improves the hypoglycemic effects of a large-leaf yellow tea infusion by enhancing the levels of epimerized catechins that inhibit alpha-glucosidase[J]. Food & Function, 2018, 9(10): 5162 – 5168.

[7] XU N, CHU J, DONG RR, *et al.* Yellow tea stimulates thermogenesis in mice through heterogeneous browning of adipose tissues[J]. Molecular Nutrition & Food Research, 2021, 65(2): 2000864.

[8] MHATRE S, SRIVASTAVA T, NAIK S, *et al.* Antiviral activity of green tea and black tea polyphenols in prophylaxis and treatment of COVID-19: A review[J]. Phytomedicine, 2021(85): 153286.

[9] MHATRE S, NAIK S, PATRAVALE V, *et al.* A molecular docking study of EGCG and theaflavin digallate with the druggable targets of SARS-CoV-2[J]. Computers in Biology and Medicine, 2021(129): 104137.

[10] CHEN H, HUANG YZ, ZHOU CC, *et al.* Effects of ultra-high pressure treatment on structure and bioactivity of polysaccharides from large leaf yellow tea[J]. Food Chemistry, 2022(387): 132862.

[11] ZHOU CC, HUANG YZ, CHEN JL, *et al.* Effects of high-pressure homogenization extraction on the physicochemical properties and antioxidant activity of large-leaf yellow tea polysaccharide conjugates[J]. Process Biochemistry, 2022(122): 87 – 94.

[12] YAMAZAKI T, SAGISAKA M, IKEDA R, *et al.* The human bitter taste receptor hTAS2R39 is the primary receptor for the bitterness of theaflavins[J]. Bioscience Biotechnology and Biochemistry, 2014, 78(10): 11753 – 1756.

[13] TIAN P, XIE WN, DING HY, *et al.* Theaflavin promoting apoptosis of mouse mammary tumor cells through phosphatidylinositol 3-kinase/protein kinase b signaling pathway[J]. Chinese Journal of Animal Nutrition, 2023, 35(2): 1288 – 1297. (in Chinese).

[14] YAN XM, WEI J, XU JY, *et al.* Study on antibacterial activities of tea polyphenols and tea saponin and their blends[J]. Science & Technology of Food Industry, 2014, 35(22): 159 – 161,171. (in Chinese).

[15] LIU SY, ZHANG QQ, LI H, *et al.* Comparative assessment of the antibacterial efficacies and mechanisms of different tea extracts[J]. Food, 2022, 11(4): 620.

[16] DU L, HAN X, GAO XH, *et al.* Preparation of curcumin microemulsion gel and its anti-inflammatory and anti-bacterial effects *in vitro*[J]. Journal of Shanxi Medical University, 2023, 54(2): 244 – 248. (in Chinese).

[17] ZHANG YX, YAN TC, ZHANG J, *et al.* Effect of antioxidants on main constituent and inoxidizability in hanfu apple cider fermenting process[J]. Journal of Shenyang Agricultural University, 2019, 50(6): 684 – 693. (in Chinese).

[18] CHOOCHOTE W, SUKLAMPOO L, OCHAIKUL D. Evaluation of antioxidant capacities of green microalgae[J]. Journal of Applied Phycology, 2014, 26(1): 43 – 48.

[19] ZHANG ZL, MIN YJ, HUANG RS, *et al.* Comparison of dried *Dendrobium huoshanense* and *D. officinale* in component content and antioxidant activity[J]. Natural Product Research and Development, 2020(32): 1104 – 1110, 1155. (in Chinese).

[20] AKBARI B, BAGHAEI-YAZDI N, BAHMAIE M, *et al.* The role of plant-derived natural antioxidants in reduction of oxidative stress[J]. Biofactors, 2022, 48(3): 611 – 633.

[21] MCNULTY R, KUCHI N, XU E, *et al.* Food-induced methemoglobinemia: A systematic review[J]. Journal of Food Science, 2022, 87(4): 1423 – 1448.