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Effect of *Clerodendranthus spicatus* (Thunb.) C. Y. Wu on the exercise ability of D-galactose-induced oxidative aging mice

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Abstract

The *Clerodendranthus spicatus* (Thunb.) C. Y. Wu extract (CSTCYWE) was administered to D-galactose-induced aging mice to investigate the effect and mechanism of CSTCYWE on exercise endurance in aging mice. The effect of CSTCYWE on the improvement of exercise capacity in aging mice was evaluated by detecting serum biochemical indicators, pathological changes in tissues, and expression by qPCR. The composition of CSTCYWE was analyzed by HPLC. The experiment showed that CSTCYWE increased the running time and the exhaustive swimming time of aging mice to improve their exercise endurance. CSTCYWE also reduced the levels of BUN, BLA, and MDA and increased the levels of HG, MG, SOD, and GSH-Px in the serum of aging mice. Pathological observation found that CSTCYWE alleviated the liver and kidney tissue lesions and damage caused by aging. Meanwhile, CSTCYWE could down-regulate the relative mRNA expressions of nNOS, iNOS, TNF-α, and syncytin-1 and up-regulate the relative mRNA expressions of Cu/Zn-SOD, Mn-SOD, and CAT in the mouse liver tissue and skeletal muscle tissue. The results of component analysis showed that CSTCYWE contained caffeic acid, hypericin, isoquercetin, dihydroquercetin, rosmarinic acid, baicalin, luteolin, and baicalein. These active ingredients enable CSTCYWE to act on aging mice and improve their exercise capacity.

Keywords: *Clerodendranthus spicatus* (Thunb.) C. Y. Wu; D-galactose; oxidative; exercise; mRNA.

Practical Application: *Clerodendranthus spicatus* (Thunb.) C. Y. Wu can be developed as a functional food, but there are few studies on its functional components and mechanism. This study verified its potential active role in enhancing the exercise ability of aging people, which is conducive to its development and utilization.

1 Introduction

With the increase in age, the brain function gradually declines and the exercise ability also declines simultaneously, and it is accompanied by frequent fatigue. Aging is an irreversible phenomenon of human life. Usually, it is considered to be a gradual degenerative change in the health of human organisms. Along with the aging process, the human body's exercise capacity is significantly reduced and the cardiopulmonary function is also reduced. At the same time, there will be reduced muscle activity and other phenomena (Lanza & Nair, 2009). A study has shown that long-distance runners have higher telomerase activity in white blood cells than ordinary people, and that aging may be delayed in cases with good exercise ability. In addition, they also had slower heart rates and lower blood pressure and cholesterol levels, directly demonstrating that exercise capacity correlates with aging (Bloomer et al., 2009). Good exercise ability can enhance the function of the cardiovascular system, increase cardiac excitability, enhance myocardial contractility, dilate coronary arteries, and improve blood circulation and myocardial oxygen utilization. At the same time, good exercise ability can also promote reduction in blood lipids, delay hardening of arteries, and reduce the incidence of cardiovascular disease (Hill et al., 2007). At the same time, with an increase in age, osteoporosis exerts an impact on the exercise ability, thus affecting the exercise of the elderly. Then exercise and fitness become an effective method to prevent and treat osteoporosis. Exercise can improve bone blood circulation, strengthen bone metabolism, and improve bone elasticity and toughness, thereby delaying the aging process of bone cells (Gao et al., 2020). In addition, fatigue caused by aging may be related to various factors, such as exhaustion of energy substances in the body, accumulation of metabolites, and disturbance of the internal environment. Exercise can improve the fatigue state of the body by regulating the function of the human body, keep the human body alive, and provide the ability to persist in exercise, which is also an important means of delaying aging (Katzman et al., 2017).

The aging process of organisms is the result of accumulation of free radicals continuously produced by the body's tissue cells. Free radicals can cause DNA damage, which can lead to mutations and induce the development of various diseases (Singh et al., 2016). Free radicals are intermediate products of normal metabolism, and their reaction ability is very strong, which can oxidize various substances in cells and damage biofilms (Iwansyah et al., 2021; Seon et al., 2021). It can also cross-link macromolecules, such as proteins and nucleic acids, affecting their normal functions (Fan et al., 2017). Aging causes

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decline in the antioxidant enzyme activity in the body and the non-enzymatic antioxidant capacity, resulting in the increase in free radicals in the body, which will interfere with the normal metabolic process of the human body, cause fatigue, reduce the exercise capacity, and reduce the quality of life of the elderly. In order to maintain the normal operation and healthy state of the body itself and resist aging of the body caused by various reactive oxygen species, there are various antioxidant substances in the body that can inhibit the production of various reactive oxygen species. Preventive antioxidants are the first line of defense against oxidation in the human body, mainly through various antioxidant enzymes in the body, such as superoxide dismutase (SOD), catalase (CAT), and glutathione. Glutathione peroxidase (GSH-Px) can inhibit the production of various free radicals and reactive oxygen species, thereby slowing aging (Yu et al., 2015). Therefore, it is very important to identify measures that have strong antioxidant capacity, and can delay aging and enhance exercise capacity. It may be an effective way to improve the aging situation through active substances to increase exercise capacity.

Clerodendranthus spicatus (Thunb.) C. Y. Wu is a plant distributed in Asia and Oceania. Existing studies have shown that it has a good protective effect on the kidneys and can be effective against a variety of kidney diseases (Wang et al., 2016). It can also play a biologically active role as a healthy tea by acting as an antibacterial and anti-inflammatory agent. In this study, a mouse model of aging was established to test the effect of *Clerodendranthus spicatus* (Thunb.) C. Y. Wu extract (CSTCYWE) on improving the exercise capacity, thereby inhibiting the effect of aging; and at the same time, the mechanism of CSTCYWE was verified by molecular biological methods. The findings provide a theoretical basis for further research and utilization of *Clerodendranthus spicatus* (Thunb.) C. Y. Wu.

2 Materials and methods

2.1 Experimental sample

Freeze-dried *Clerodendranthus spicatus* (Thunb.) C. Y. Wu (Kunming Xuanqing Biotechnology Co., Ltd, Kunming, Yunnan, China) was extracted with 20 times the amount of 70% ethanol and filtered, and then the extract was collected. Then the filter residue was extracted again by the same method, and the two extracts were combined and then rotary evaporation was performed to obtain CSTCYWE.

2.2 Establishment of a mouse model of aging

ICR male mice (male, 6 weeks old, n = 50, body weight: 20 ± 2 g) were purchased from the Experimental Animal Center of Chongqing Medical University. Mice were housed at a temperature of 25 ± 2 °C and a relative humidity of 50 ± 5% on a 12-hour light/dark cycle and were given unrestricted access to standard mouse chow and drinking water, with litter changes every two days, for one week of adaptive feeding. Mice were randomly divided into the following 5 groups: normal group, model group, Vc group, CSTCYWE-L group, and CSTCYWE-H group. The whole experiment was carried out for 10 weeks. During this period, mice were provided with standard feed and drinking water. From the first week, except for the normal group, the other groups of mice were intraperitoneally injected with D-galactose (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) at a dose of 100 mg/(kg·d) for 6 weeks. Starting from the seventh week, mice in the normal group and the model group were given 0.9% normal saline daily at a dose of 0.1 mL/10 g; mice in the Vc group were given a daily dose of 200 mg/kg of vitamin C (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) solution; mice in the CSTCYWE-L group and CSTCYWE-H group were administered the CSTCYWE solution daily at doses of 100 mg/kg and 200 mg/kg, respectively, for 4 weeks (Cao et al., 2017). After another 4 weeks, relevant tests were performed and the mice were euthanized, and blood and tissues were collected for experiments (Figure 1).

2.3 Running test

After the 10-week experiment, the mouse wheel was set at 20 r/min and the mice were forced to run on the wheel (YH-CS, Wuhan Yihong Technology Co., Ltd, Wuhan, Hubei, China). The mice were given an electroshock when they stopped running, and they were electrocuted 5 times in a row till they were exhausted, and the running time of mice was recorded and compared (Yi et al., 2021).

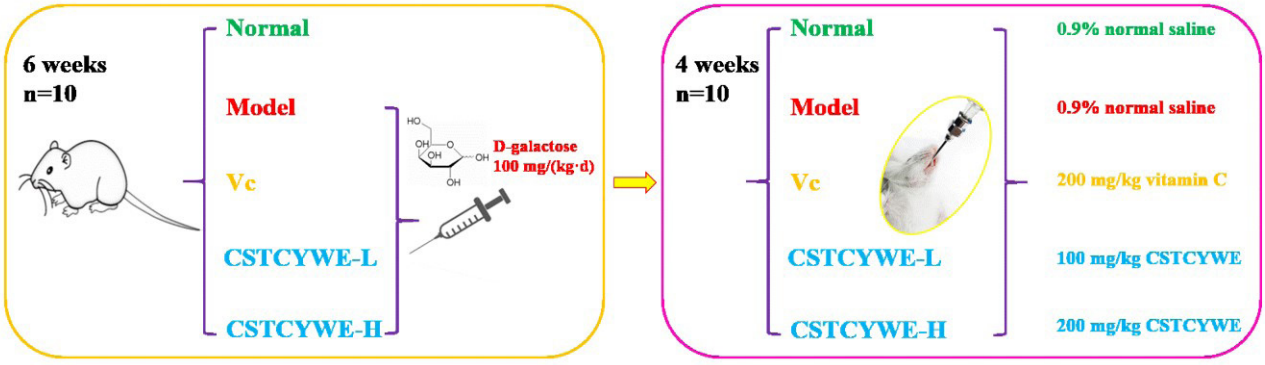

Figure 1. Experimental design.

2.4 Negative gravity swimming test

After the 10-week experiment, the mice were placed in a constant temperature water tank with a water temperature of 28 ± 2 °C and a water depth of 20 cm. Mice were exhausted if they could not rise to the surface for more than 10 s, and the swimming time of mice was recorded and compared (Yi et al., 2021).

2.5 Tissue H&E staining analysis

The liver or kidney tissues of mice were washed using normal saline, and 0.5 cm^2 of the tissues were removed and fixed in formalin solution (10% concentration). Liver or kidney tissues were dehydrated in an ethanol gradient, soaked in xylene and ethanol for 30 min to clarify the tissue, embedded in paraffin, cut into sections of about 2 μm with a microtome, and mounted on glass slides. H&E dyes were used for tissue staining, followed by observation of the morphological changes under a light microscope (BX43, Olympus, Tokyo, Japan).

2.6 Mouse serum index detection

The obtained mouse blood was centrifuged at 4000 rpm for 10 min at 4 °C; and then the mouse serum was separated and collected, and it was stored at -80 °C for later use. Serum levels of BUN, BLA, MDA, HG, MG, SOD, and GSH-Px were determined using appropriate biochemical kits according to the protocol recommended by the reagent manufacturer (Shanghai Enzyme Link Biotechnology Co., Ltd, Shanghai, China).

2.7 Real-time quantitative PCR detection

In this study, messenger RNA (mRNA) expression in mouse liver tissue and skeletal muscle tissue was determined by the SYBR green method. About 100 mg of liver tissue and skeletal muscle tissue of mice were cut into pieces, and the total RNA in liver tissue and skeletal muscle tissue was extracted using Trizol reagent (Invitrogen, New York, NY, USA). The concentration of RNA was determined using a microspectrophotometer (Nano-100, Hangzhou Allsheng Instruments Co.,Ltd, Hangzhou, Zhejiang, China). The cDNA template was obtained by reverse transcription using the Revert Aid First Strand cDNA Synthesis Kit. Then, it was amplify by SteponePlus real-time PCR system (Thermo Fisher Scientific, Waltham, MA, USA) with a system of 10 μL SYBRGreen PCR Master Mix, 1 μL upstream primer and 1 μL downstream primer, 1 μL cDNA template (Thermo Fisher Scientific, Waltham, MA, USA), and 7 μL DEPC (17). The PCR conditions were as follows: pre-denaturation at 95 °C for 3 min; denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, extension at 72 °C for 15 s, for 40 cycles; the final dissolution curve was performed at 95 °C for 30 s, 60 °C for 30 s, and it was completed at 95 °C for 15 s. Finally, the relative expression level of each gene was calculated by the $2^{-\Delta\Delta CT}$ method (Yi et al., 2021), where CT was the cycle threshold, for which GAPDH was used as the internal reference gene. Table 1 shows the primer sequence information used in this study.

2.8 HPLC

CSTCYWE and standard samples were carried out with methanol, and the samples were passed through a 0.22-micron organic filter membrane and then transferred to a brown liquid phase vial for measurement. The chemical composition of CSTCYWE was then determined by HPLC under the following liquid chromatography conditions: The chromatographic column was Accucore C_{18} column (5 μm, 4.6 × 250 mm); mobile phase was A: 0.5% acetic acid water, fluidity B: acetonitrile; flow rate was 0.5 mL/min; column temperature was 30 °C; detection wavelength was 359 nm, the injection volume was 5 μL, and pre-equilibration was performed for 10 min (Bakkaloglu et al., 2021).

2.9 Data analysis

Three or more parallel experiments were performed for all indicators in the experiment, and the results were expressed as the average value. Meanwhile, differences between data were assessed by one-way ANOVA using Duncan's multiple range test (MRT, $P < 0.05$, IBM SPSS 22 statistical software) (Kaska et al., 2021).

3 Results and discussion

3.1 Effects of CSTCYWE on exercise endurance in aging mice

Table 2 shows that compared with the normal group, the running time and the exhaustive time of weight-bearing swimming in the mouse model of aging were significantly shortened ($P < 0.05$), indicating that aging caused a significant decrease in the exercise capacity and exercise endurance of mice. When

Table 1. Primer sequence in this experiment.

Gene	Forward sequence	Reverse sequence
Cu/Zn-SOD	5′-AACCAGTTGTGTTGTGAGGAC-3′	5′-CCACCATGTTTCTTAGAGTGAGG-3′
Mn-SOD	5′-CAGACCTGCCTTACGACTATGG-3′	5′-CTCGGTGGCGTTGAGATTGTT-3′
CAT	5′-GGAGGCGGGAACCCAATAG-3′	5′-GTGTGCCATCTCGTCAGTGAA-3′
NF-κB p65	5′-GAGGCACGAGGCTCCTTTTCT-3′	5′-GTAGCTGCATGGAGACTCGAACA-3′
nNOS	5′-TCGTCCAACTTCTGGGCTCTT-3′	5′-CCTTCTCTTCCTCCCCTCTCTTC-3′
iNOS	5′-CAAAGGCTGTGAGTCCTGCAC-3′	5′-ACTTTGATCAGAAGCTGTCCC-3′
TNF-α	5′-ATGGGGGGCTTCCAGAA-3	5′-CCTTTGGGGACCGATCA-3′
Syncytin-1	5′-GTTAACTTTGTCTCTTCCAGAATCGA-3′	5′-CATCAGTACGTGGGCTAGCA-3′
GAPDH	5′-TGACCTCAACTACATGGTCTACA-3′	5′-CTTCCCATTCTCGGCCTTG-3′

the mice were treated with CSTCYWE by gavage, the exercise tolerance of mice was increased, and the increase in exercise tolerance showed a gradual upward trend with an increase in the CSTCYWE dose ($P < 0.05$). At the same concentration, the effect of CSTCYWE was better than that of Vc. It can be seen that CSTCYWE can resist the aging of mice caused by D-galactose, and it can significantly improve the exercise ability of aging mice ($P < 0.05$).

As the body ages, its vitality declines. Free radicals in the body increase, and free radicals are associated with pathological damage, and they also affect motor function, resulting in decreased exercise capacity and fatigue. It can reduce the damage caused by free radicals to the body's cell membrane, ensure the integrity of the body's cell membrane, maintain the normal operation of the oxidative respiratory chain and the normal structure and function of mitochondria, maintain exercise ability, and improve body function (Vitale et al., 2013). Both running endurance and weight-bearing exhaustive swimming test are experimental methods to verify the exercise ability; and the improvement in exercise ability is the most powerful macroscopic manifestation of the body's anti-fatigue ability, and it is also an effective manifestation of the body's resistance to aging (Ogura et al., 2011). In this study, CSTCYWE directly reflects the effect of improving the exercise ability of aging mice by improving the ability of mice to run and swim with weight.

Table 2. Effects of *Clerodendranthus spicatus* (Thunb.) C. Y. Wu on exercise endurance of aging mice.

Group	Running time (s)	Load exhaustion swimming time (s)
Normal	52.63 ± 1.13^{a}	313.54 ± 14.25^{a}
Model	35.61 ± 1.36^{e}	195.42 ± 18.93^{e}
Vc	41.05 ± 1.23^{c}	254.71 ± 12.10^{c}
CSTCYWE-L	38.02 ± 0.79^{d}	218.91 ± 10.88^{d}
CSTCYWE-H	45.37 ± 1.41^{b}	278.12 ± 10.69^{b}

After analysis, a-e means that there are significant differences between the two groups with different superscripts ($P < 0.05$).

3.2 Histopathological evaluation in mice

The histomorphology of mouse liver tissue is shown in Figure 2. The hepatic lobules of mice in the normal group had a clear and complete structure, and the hepatocytes were arranged radially with the central vein in the center. In the model group, the structure of hepatic lobules was basically complete, liver cells were neatly arranged, scattered cells were decreased in size, the cytoplasmic density was increased, the nucleoplasm was condensed, the nuclear membrane nucleolus was broken, and apoptotic bodies were occasionally seen. The hepatic lobule structure in the CSTCYWE-H group was basically intact, meanwhile in the CSTCYWE-H group and Vc group, the size of cells was occasionally reduced and the nucleoplasm was condensed.

The observation of mouse kidney tissue showed that the kidney tissue of the model group showed a certain degree of lesions, a considerable number of glomeruli appeared irregular in shape, some glomeruli had ruptured, and inflammatory cells had infiltrated between tissues (Figure 3). The glomerulus and cell structure of mice in the normal group were intact, and both CSTCYWE and Vc could reduce the damage to the renal tissue and reduce the damage in the kidney group. Among them, CSTCYWE-H had the best effect, and it could promote

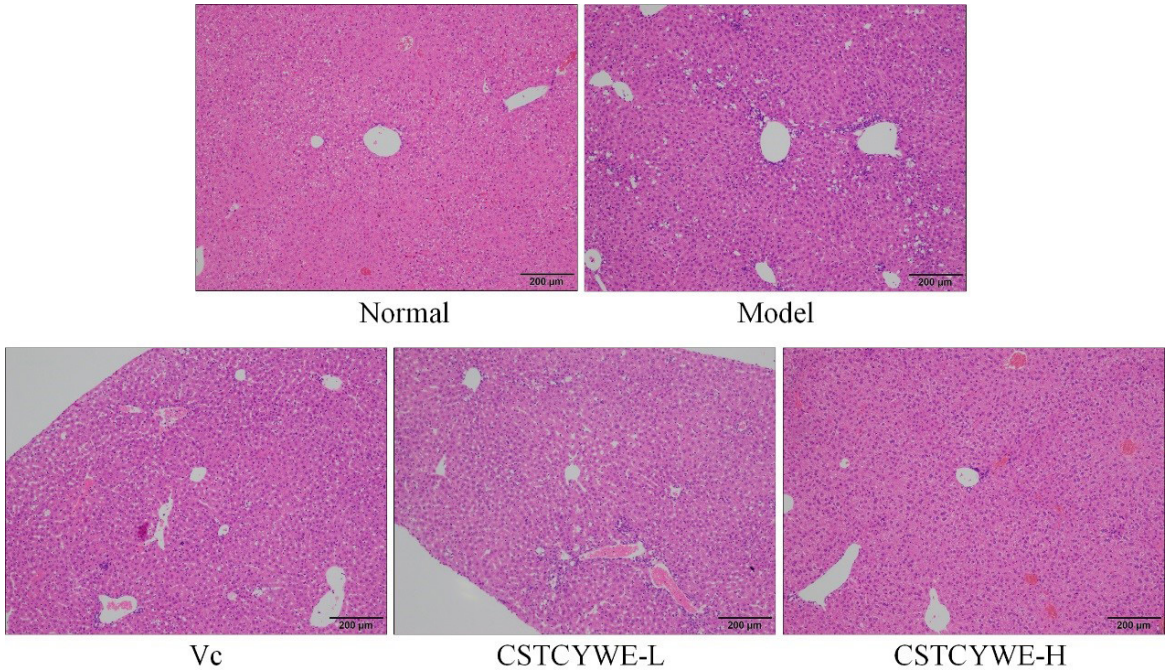

Figure 2. H&E pathological observation of liver tissue in aging mouse.

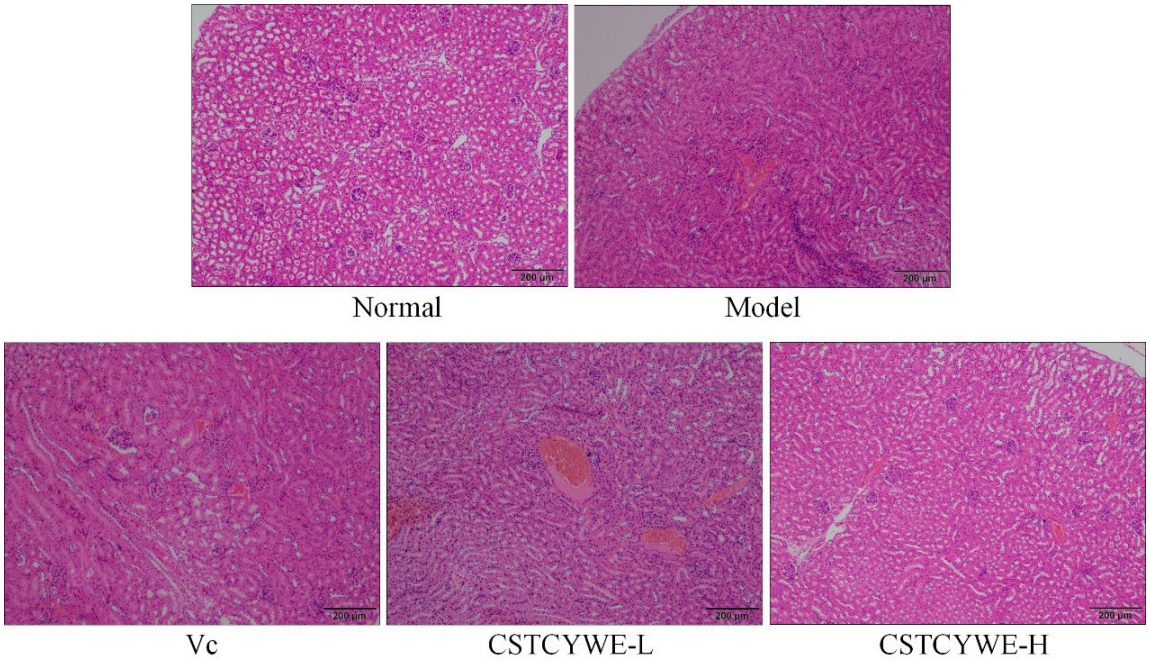

Figure 3. H&E pathological observation of renal tissue in aging mouse.

the tissue morphology of kidney tissue as close as that in the normal group.

After the body ages, the liver, the central organ of oxidation, can sensitively reflect the degree of oxidation of the organism, and it is also a place where free radicals and lipid peroxides are easily generated. The damage to liver tissue cells is closely related to the toxic reaction of free radicals, and the increase in free radicals caused by aging can be clearly manifested in the degree of pathological changes in the liver tissue (Zhou et al., 2016). At the same time, with aging of the body, the kidneys will gradually shrink, the kidney tissue will have lesions, and the secretion of prostaglandins will decrease, resulting in a decrease in vasoconstriction and blood flow (Izquierdo et al., 2012). In the aging state, vasoconstriction of the liver and kidneys decreases, resulting in a decrease in blood supply to various organs of the body, which directly reduces the exercise capacity (Wille et al., 2014). The pathology examination in this study observed that CSTCYWE can reduce tissue decline and lesions in the liver and kidney, and one of its possible effects is to improve the motor function.

3.3 BUN, BLA, MDA, HG, MG, SOD, and GSH-Px levels in mouse serum

As shown in Table 3, the serum levels of BUN, BLA, and MDA in the model group were significantly higher than those in the other groups, while the levels of HG, MG, SOD, and GSH-Px were significantly lower than those in the other groups ($P < 0.05$). After the effects of CSTCYWE and Vc, the serum levels of BUN, BLA, and MDA in the CSTCYWE group and Vc group were lower than those in the model group, while the levels of HG, MG, SOD, and GSH-Px were higher than those in the model group; the above serum levels in the CSTCYWE-H group were the closest to those in mice of the normal group.

Prolonged exercise in aging conditions prevents the body from metabolizing sugars and fats, thereby increasing the rate of protein and amino acid metabolism and significantly increasing the content of BUN (Hsu et al., 2016). In the energy supply system, the body will be in a state of hypoxia during strong exercise, and the body will produce more muscle lactic acid, which will gradually cause the body to be in a state of fatigue. Muscle lactate will further penetrate into the blood to form blood lactate, and BLA can be used as an important indicator for judging the body's anti-fatigue state (Dimitrov et al., 2012). The contents of HG and MG in the body also determine the body's exercise endurance and anti-fatigue ability. When the content of MG in the body decreases, the energy-supplying substances in the muscles decrease, and MG undergoes glycolysis for energy supply. At this time, a large amount of lactic acid will be produced, which will weaken the exercise ability of muscles (Petersen et al., 2004; Shiose et al., 2012).

After exercising excessively, the production of free radicals in the body will increase greatly, and this phenomenon is more obvious in the aging state. The human body itself has a variety of antioxidant enzymes, such as CAT, GSH-Px and SOD, which scavenge free radicals (Maleki et al., 2018). GSH-Px is an important enzyme that catalyzes the decomposition of hydrogen peroxide in the body, and its role is to remove hydrogen peroxide and lipid hydroperoxide, which can protect the integrity of cell membrane structure and function (Yoon & Park, 2014). SOD is the only enzyme whose substrate is oxygen free radicals among thousands of enzymes in aerobic organisms, and it plays a crucial role in the balance of oxidation and anti-oxidation in the body. This enzyme can disproportionate oxygen free radicals to generate

Table 3. The BUN, BLA, MDA, HG, MG, SOD, and GSH-Px serum levels in aging mouse.

Group	BUN (mmol/L)	BLA (mmol/L)	MDA (mmol/mL)	HG (mg/mL)	MG (mg/mL)	SOD (U/mL)	GSH-Px (μmol/mL)
Normal	7.62 ± 0.45^{e}	21.03 ± 0.69^{e}	6.52 ± 0.41^{e}	11.02 ± 0.23^{a}	1.71 ± 0.08^{a}	336.75 ± 10.83^{a}	205.63 ± 8.32^{a}
Model	18.36 ± 0.61^{a}	38.33 ± 0.75^{a}	17.74 ± 0.62^{a}	7.11 ± 0.25^{e}	0.92 ± 0.04^{e}	121.07 ± 6.57^{e}	104.53 ± 5.60^{e}
Vc	12.51 ± 0.48^{c}	28.33 ± 0.35^{c}	10.32 ± 0.46^{c}	8.12 ± 0.22^{c}	1.31 ± 0.04^{c}	236.41 ± 11.08^{c}	142.69 ± 7.85^{c}
CSTCYWE-L	15.71 ± 0.39^{b}	31.25 ± 0.57^{b}	14.30 ± 0.55^{b}	7.63 ± 0.23^{d}	1.13 ± 0.05^{d}	167.85 ± 12.63^{d}	120.68 ± 6.91^{d}
CSTCYWE-H	10.30 ± 0.44^{d}	24.48 ± 0.39^{d}	8.06 ± 0.45^{d}	9.88 ± 0.31^{b}	1.50 ± 0.05^{b}	296.14 ± 8.91^{b}	177.20 ± 9.32^{b}

After analysis, a-e means that there are significant differences between the two groups with different superscripts ($P < 0.05$).

oxygen and hydrogen peroxide and effectively scavenge superoxide anion free radicals, thereby reducing the generation of more toxic hydroxyl radicals and protecting the cells. It is free in the body. It is an important antioxidant enzyme within the radical scavenging system (Mphahlele et al., 2021). If the number of free radicals produced by the body far exceeds the defense capacity of antioxidant enzymes in the body, the unsaturated lipids in the tissue cell membrane will be damaged by free radicals and the fluidity of the membrane and the function of cells will be reduced, resulting in lipid peroxidation, whoses product is MDA. MDA can seriously damage the structure of cell membranes, leading to cell swelling and necrosis, and it is often used to indirectly reflect the changes in the body's free radical metabolism and the degree of tissue peroxidative damage (Aschner et al., 2021). Aging can lead to increased levels of lipid peroxides in the body. Antioxidant enzymes in the body are an important line of defense to protect the tissue cells from excessive free radical damage. By detecting the activity of antioxidant enzymes in body tissues, it can reflect the body's ability to resist oxidative damage and the degree of fatigue (Chen & Bahia, 2021). In this study, CSTCYWE also showed an intervention effect on the levels of BUN, BLA, HG, MG, MDA, SOD, and GSH-Px in aging mice, and it played a role in improving the exercise capacity of aging mice.

3.4 mRNA expressions of Cu/Zn-SOD, Mn-SOD, CAT, nNOS, iNOS, and TNF-α in mouse liver tissue

Figure 4 shows that the mRNA expression levels of Cu/Zn-SOD, Mn-SOD, and CAT in the liver tissue of mice in the model group were the lowest, and the levels of Cu/Zn-SOD, Mn-SOD, and CAT in mice in the normal group were the highest. At the same time, the expressions of nNOS, iNOS, and TNF-α in mice in the model group were the strongest, and the above expressions in mice in the normal group were the weakest. Compared with the model group, CSTCYWE and Vc could up-regulate the expressions of Cu/Zn-SOD, Mn-SOD, and CAT and down-regulate the expressions of nNOS, iNOS, TNF-α, and CSTCYWE-H in mice treated with D-galactose (the model group). It had the strongest regulatory effect, which could promote the expressions of Cu/Zn-SOD, Mn-SOD, CAT, nNOS, iNOS, and TNF-α as close as those in the normal group.

3.5 mRNA expressions of Cu/Zn-SOD, Mn-SOD, CAT, nNOS, iNOS, TNF-α, and syncytin-1 in mouse skeletal muscle tissue

As can be seen in Figure 5 the mRNA expression levels of Cu/Zn-SOD, Mn-SOD, and CAT in the skeletal muscle tissue of mice in the model group were the lowest, and the corresponding expression levels in the normal group were the highest. Compared with the model group, CSTCYWE and Vc could up-regulate the expressions of Cu/Zn-SOD, Mn-SOD, and CAT in skeletal muscle tissue, and CSTCYWE-H was the most up-regulated. On the contrary, the mRNA expression levels of nNOS, iNOS, TNF-α, and syncytin-1 in mouse skeletal muscle tissue were the highest in the model group and the lowest in the normal group. The mRNA expressions of nNOS, iNOS, TNF-α, and syncytin-1 in the CSTCYWE-H group were only higher than those in the normal group, and they were significantly ($P < 0.05$) lower than those in the model group, Vc group, and CSTCYWE-L group.

The role of CAT is to catalyze the decomposition of hydrogen peroxide to generate water, which can remove the products of peroxidative stress in red blood cells, peroxisomes, and mitochondria, thereby blocking the damage caused by reactive oxygen species to cells and inhibiting oxidative stress (Vergani et al., 2011). Cu/Zn-SOD and Mn-SOD are isomers of SOD in the body. Cu/Zn-SOD is another SOD free radical scavenger with Cu^{2+} and Zn^{2+} as active centers in the cytoplasm; Mn-SOD is present in the cytoplasm, it is an SOD free radical scavenger with Mn4+ as an active center in mitochondria (Leung et al., 2008). When the body ages, the exercise capacity decreases; and after exercise, the body will experience oxidative stress and generate free radicals. CAT, Cu/Zn-SOD, and Mn-SOD can inhibit free radicals in the body and can play a role in reducing fatigue and improving the exercise capacity (López-Cruz et al., 2010). The experimental results of this study showed that the generation of free radicals was increased in the liver tissue and skeletal muscle tissue of aging mice, and a series of oxidative stress reactions occurred. CSTCYWE can effectively enhance the activities of CAT, Cu/Zn-SOD, and Mn-SOD, thereby improving the ability of mice to eliminate free radicals and enhance the ability of mice to protect against oxidative stress. Ultimately, it can protect the body and improve the exercise ability of aging mice.

NOS can be divided into 3 types, namely endothelial-NOS (eNOS), which mainly exists in vascular endothelial cells, nNOS, which mainly exists in the central and enteric nerve cells, and cell-inducible iNOS, which mainly exists in macrophages (Fujii et al., 2016). Nitric oxide (NO) is continuously produced in the skeletal muscle, the content of NO in the resting state is relatively low, and the production of NO is higher in the process of skeletal muscle contraction. nNOS is distributed on the membrane of fast-twitch muscle fibers, and studies have shown that the expression of nNOS is significantly up-regulated

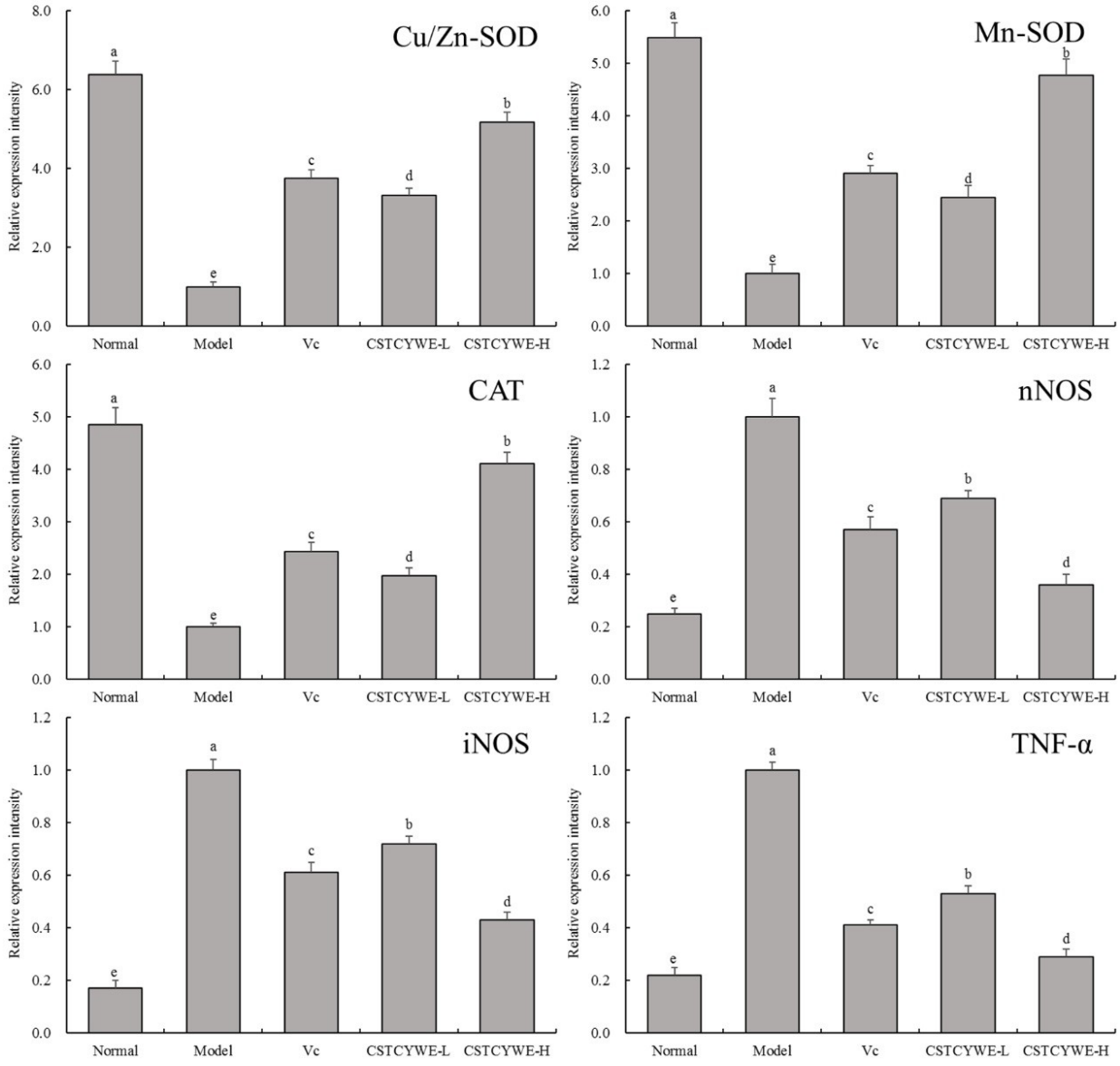

Figure 4. The Cu/Zn-SOD, Mn-SOD, CAT, nNOS, iNOS, and TNF-α mRNA expressions in mouse liver tissue. After analysis, a-e means that there are significant differences between the two groups with different superscripts ($P < 0.05$).

after exhaustive exercise, while the expression of nNOS is decreased after recovery from exercise exhaustion (Kim et al., 2019). The expression of iNOS is induced by endotoxin and various cytokines, such as TNF-α, mainly in the hepatocytes, macrophages, and neutrophils. iNOS is one of the three subtypes of NOS that catalyzes more NO production, and NO is widely involved in various pathological processes in oxidative aging and immune responses and is an important mediator of aging (Park et al., 2018; Yano et al., 2010). TNF-α is a multifunctional cytokine secreted by the monocytes and macrophages, which can promote the secretion of reactive oxygen species, such as O_2^-, H_2O_2, and NO, by macrophages and control the secretion of these oxidative active substances, which can promote the secretion of reactive oxygen species by the macrophages. Thus, it can reduce the degree of oxidative damage and delay aging (Grandi et al., 2016). Therefore, the expression levels of iNOS, nNOS, and TNF-α can reflect the exercise capacity and degree of fatigue. The Syncytin-1 gene is located on chromosome 7 of the human genome, and the encoded envelope glycoprotein Syncytin-1 plays a role in regulating immunity by mediating the growth and differentiation of trophoblast cells. High expression of Syncytin-1 in the skeletal muscle may result in damage to the motor neurons (Oluwole et al., 2007). High expression of syncytin-1 in the skeletal muscle can induce the activation of spinal cord lumbar enlargement-related glial cells, resulting in the accumulation of oxygen free radicals and mitochondrial damage. Motor neuron disease patients with high Syncytin expression are activated by oxidative stress, and it is speculated that abnormal expression of Syncytin is related to muscle denervation (Uygur et al., 2019). In this study, CSTCYWE could significantly down-regulate the expressions of iNOS, nNOS, TNF-α, and Syncytin-1, and it showed that CSTCYWE

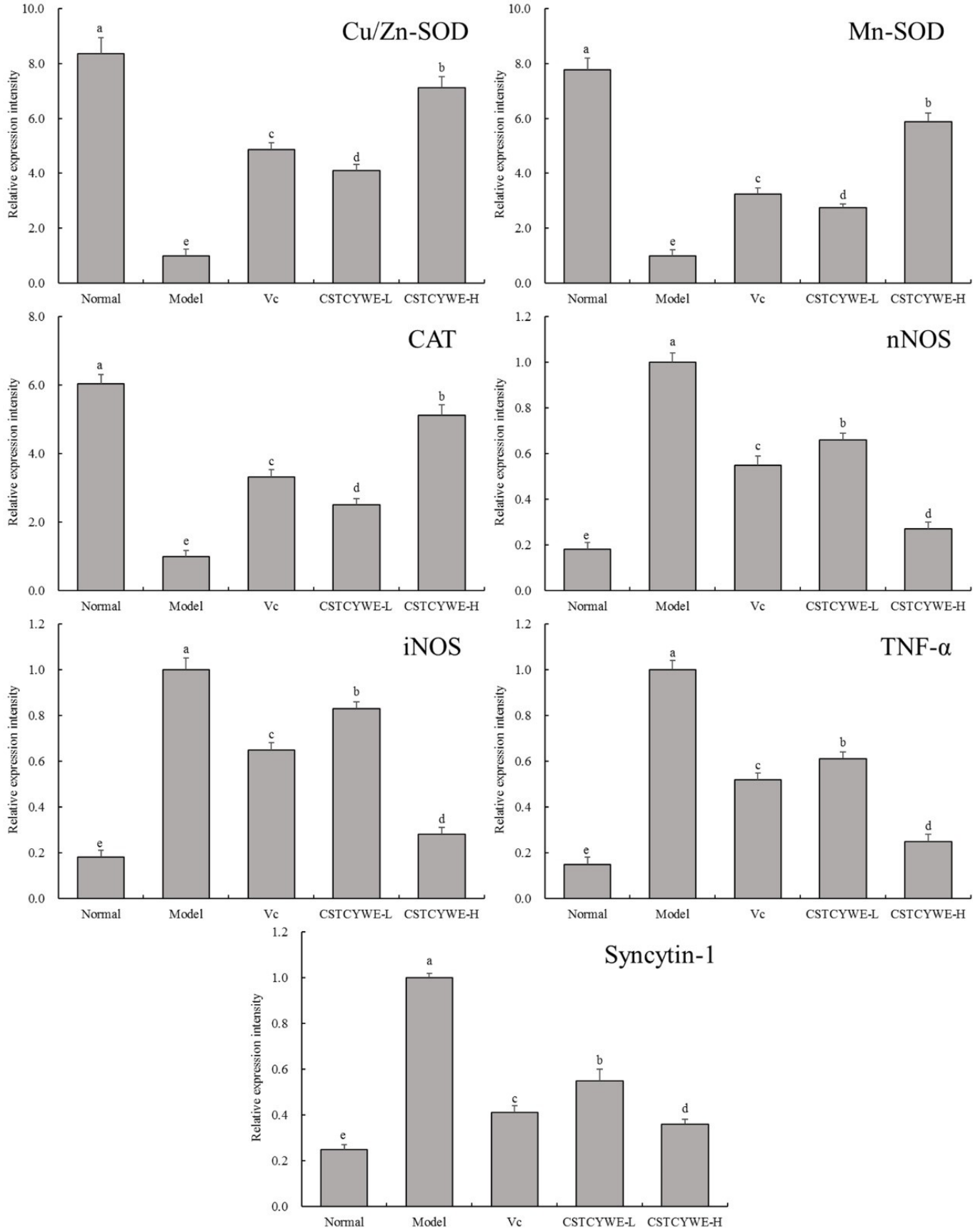

Figure 5. The Cu/Zn-SOD, Mn-SOD, CAT, nNOS, iNOS, TNF-α, and syncytin-1 mRNA expressions in mouse skeletal muscle tissue. After analysis, a-e means that there are significant differences between the two groups with different superscripts ($P < 0.05$).

alleviated exercise-induced fatigue and oxidative stress, improved the ability of mice to scavenge free radicals, and enhanced the exercise ability of mice.

3.6 Composition of CSTCYWE

The composition of CSTCYWE was detected by HPLC, and the results showed that CSTCYWE contained 8 compounds, namely caffeic acid, hypericin, isoquercetin, dihydroquercetin, rosmarinic acid, baicalin, luteolin, and baicalein (Figure 6). Isoquercetin content was the highest, and dihydroquercetin, rosmarinic acid, and baicalein contents were also higher.

Caffeic acid, hypericin, isoquercetin, dihydroquercetin, rosmarinic acid, baicalin, luteolin, and baicalein are compounds with good antioxidant activity. All of them have a certain inhibitory effect (Li et al., 2013; Kazemi et al., 2012; Cruz-Zúñiga et al., 2016; Chen & Deuster, 2009; Tepe et al., 2007; Waisundara et al., 2011; Zhang et al., 2013; Cho et al., 2011). Isoquercetin is closely related to the exercise capacity, and animal experiments have

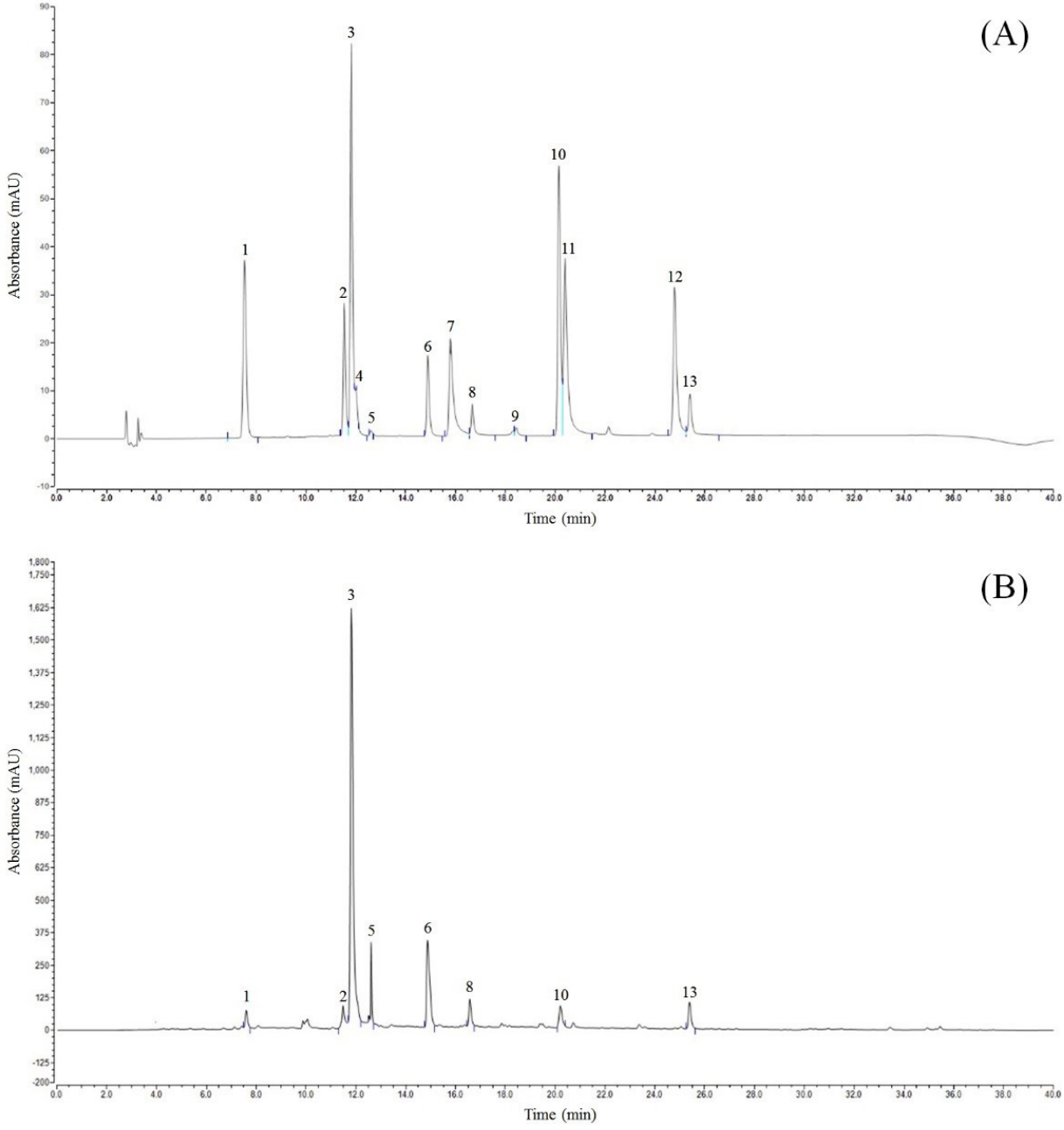

Figure 6. Composition analysis of *Clerodendranthus spicatus* (Thunb.) C. Y. Wu extract (CSTCYWE). (A) standard chromatogram and (B) CSTCYWE chromatogram. 1: caffeic acid. 2: hypericin. 3: isoquercetin. 4: ferulic acid. 5: dihydroquercetin. 6: rosmarinic acid. 7: myricetin. 8: baicalin. 9: new orange peel dihydrochalcone. 10: luteolin. 11: quercetin. 12: kaempferol. 13: baicalein.

shown that it has anti-aging and endurance-enhancing effects (Zhang et al., 2013); it has also been observed to reduce fatigue and improve motor performance in cyclists (Cho et al., 2011). As the core ingredient of CSTCYWE, it plays a central role in CSTCYWE-induced improvement in the exercise capacity of aging mice, and the other active ingredients also help to improve the exercise capacity through their antioxidant and anti-aging effects.

4 Conclusion

In this study, the antioxidative effect of CSTCYWE and the ability to improve the exercise ability were verified through the established mouse model of aging. The experimental results showed that CSTCYWE can reduce the level of oxidative stress in mice to resist aging, and it can also improve the activity of muscle tissue, thereby improving the anti-fatigue ability and exercise ability of aging mice. In conclusion, this study explored the effect of CSTCYWE on the recovery of exercise capacity in aging mice and expounded its mechanism, which provides a reference for the future development of food-derived antioxidants for anti-fatigue and improvement of exercise function in the elderly. At the same time, the change of bone in the aging process of the elderly is also a very important aspect. Therefore, it is necessary to increase the use of animal models (Eor et al., 2020; Lee et al., 2020) to test the effect of CSTCYWE on animal bones in the aging state in future research, and to verify the effect of the CSTCYWE more comprehensively.

Abbreviations

HPLC: high performance liquid chromatography. qPCR: quantitative polymerase chain reaction. BUN: blood urea nitrogen. BLA: blood lactic acid. MDA: malondialdehyde. HG: hepatic glycogen. MG: muscle glycogen. SOD: superoxide dismutase. GSH-Px: glutathione peroxidase. nNOS: neuronal nitric oxide synthase. iNOS: inducible nitric oxide synthase. TNF-α: tumor necrosis factor-alpha. Cu/Zn-SOD: copper/zinc-SOD. Mn-SOD: manganese-SOD. CAT: catalase.

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