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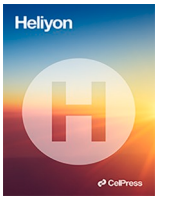

Research article

Exploring the potential of white birch sap: A natural alternative to traditional skin whitening agents with reduced side effects

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ABSTRACT

Common tyrosinase (TYR) inhibitors used in cosmetics, such as hydroquinone, kojic acid, and arbutin, can cause side effects including erythema, skin peeling, and dryness. Therefore, the development of natural whitening agents that offer excellent permeability, minimal irritation, and high safety has become a primary focus in the field of TYR inhibitors. In this study, we demonstrate that White birch sap (WBS), within a safe concentration range, effectively reduces TYR activity and melanin content in both B16F10 mouse melanoma cells and zebrafish larvae. Importantly, WBS exhibits minimal irritation to neutrophils in fluorescent zebrafish and does not affect the behavior of adult zebrafish. Furthermore, WBS downregulates the gene expression levels of microphthalmia-associated transcription factor, TYR, tyrosinase-related protein-1, and tyrosinase-related protein-2 in B16F10 cells. In conclusion, our research confirms that WBS, a naturally derived substance, offers high safety and mild effects, making it a promising candidate for a skin-whitening agent.

1. Introduction

The demand for cosmetic ingredients, including skin lighteners, sunscreens, acne treatments, and skin moisturizers, has surged in recent years [1]. Concurrently, skin whitening has emerged as a growing trend in the cosmetics market [2]. The crucial step to skin whitening is to inhibit the production of melanin, and enzymes are one of the key factors affecting all biochemical reactions in the body. Hence, inhibiting the action of key enzymes in the melanin production process of cells is considered to be an effective way to prevent melanin production and reduce the amount of melanin.

Microphthalmia-associated transcription factor (MITF), a principal protein controlling melanin synthesis, upregulates tyrosinase (TYR), tyrosinase-related protein-1 (TYRP-1) and tyrosinase-related protein-2 (TYRP-2) to promote the synthesis of melanin [3]. The

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copper-containing metal enzyme TYR, also known as polyphenol oxidase, is widely found in different organisms [4] and is the first and only rate-limiting step in the catalytic production of melanin [5,6]. Therefore, the development of tyrosinase inhibitors could be a viable approach for creating whitening products or treat hyperpigmentation [7]. In recent years, various tyrosinase inhibitors have been developed for skin whitening and skin pigmentation regulation in the pharmaceutical and cosmetic industries [8–10]. However, commonly used tyrosinase inhibitors such as kojic acid [11], hydroquinone [12] and arbutin [13] have significant drawbacks. For instance, kojic acid storage is unstable, skin permeability and skin irritation, the use of cosmetics is limited [14]. Arbutin chemically unstable is considered a precursor to hydroquinone which increases cancer risk by causing DNA damage [15,16], and is associated with many adverse reactions including contact dermatitis, irritation, erythema and leukoderma [17]. Consequently, natural whitening agents with excellent permeability, little irritation and high safety [18] have become the main research direction of tyrosinase inhibitors at present. In this context, we develop a neglected plant into natural skin whitening agent which is more effective and safter than common skin whitening chemicals.

White birch sap (WBS) is a colorless or yellowish liquid containing a variety of nutrients required by organisms, which is extracted from the birch tree and widely applied in the food and beverage industry. WBS has excellent water mobility [19]. The WBS in this experiment contained vitamin C, total phenols, 13 amino acids, 5 mineral nutrients and 7 essential trace elements. WBS can be utilized to prevent hepatitis, skin rashes, intestinal worms and scurvy. In addition, its cosmetic applications are mainly for hair growth and freckles removal [20]. However, there is still a gap in the research of its skin whitening function and biocompatibility. The zebrafish is widely recognized as a valuable model organism for drug screening due to its high throughput capabilities and cost-effectiveness [21]. Moreover, the transparency of zebrafish embryos allows for direct visualization of melanin in the entire body compartment. Therefore, we employed zebrafish in this study to investigate the depigmentation effect of WBS.

In this study, zebrafish were intended for embryo toxicity detection, determined the safe concentration range of WBS, evaluated its whitening effect with *in vitro* and *in vivo* tests, and tested skin irritation whit neutrophils fluorescent zebrafish. To this end, we confirm that WBS is a safe and effective skin whitening agent, showing promise as a safe and effective whitening ingredient in cosmetics. The study design is summarized in Fig. 1.

2. Materials and methods

2.1. Preparation of embryo medium and WBS solution

The configuration of embryo medium is 20 L as an example: 75 g sodium chloride, 200 mL 0.5%w/w potassium chloride, 200 mL 1%w/w calcium chloride and 200 mL 0.25%w/w sodium hydroxide were mixed together, enough double distilled water was added to dissolve them in a thermostat water bath, and then double distilled water was added to achieve a volume of 20 L. Finally, 26 mL of

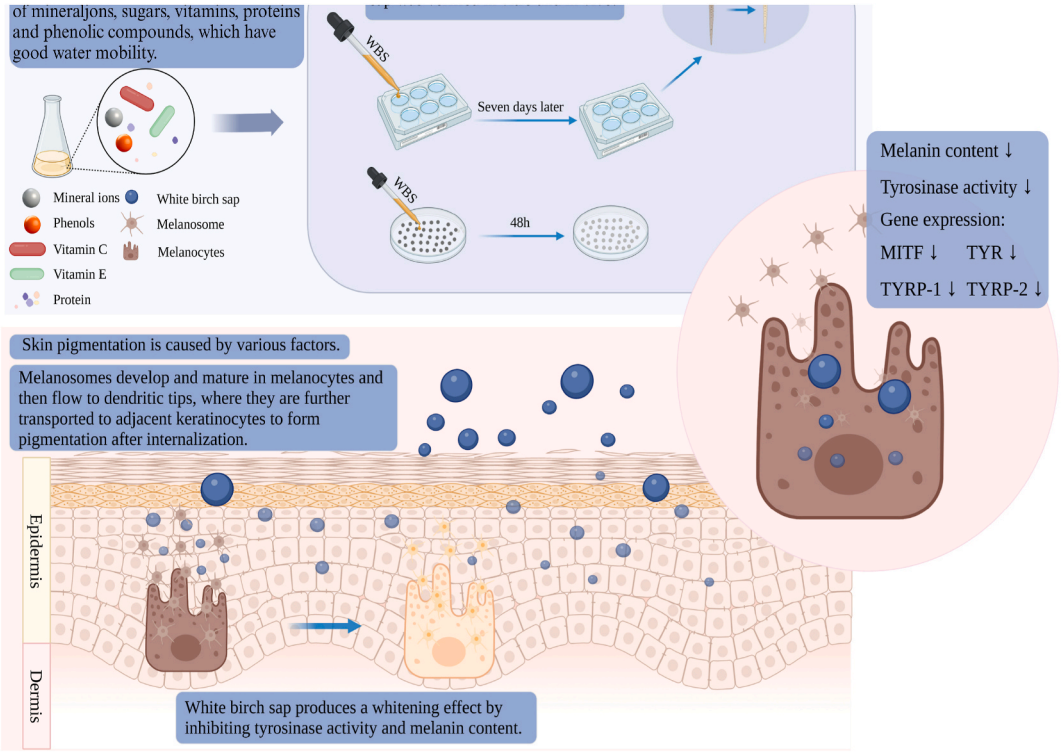


Fig. 1. Study design for the analysis of the whitening effect of white birch sap.

methylene blue solution was added to the solution to produce the embryo medium.

WBS freeze-dried powder was purchased from Shaanxi Moore Biotechnology Co., Ltd. (Shanxi, China), sourced from the Lesser Hinggan Mountains in Northeast China. WBS freeze-dried powder was dissolved in embryo medium or PBS to prepare WBS solution with different concentrations and then treated aseptically through aqueous filtration membrane for subsequent experiment. In order to better describe the characteristics of WBS, we observed and recorded the appearance of WBS freeze-dried powder and WBS solution. The mean dynamic light scattering (DLS) and zeta potential (Litesizer 500, Anton Paar Trading Co., Ltd. Shanghai, China) of WBS solution were determined to understand the dispersion coefficient of WBS in PBS. In addition, we analyzed the material composition of WBS. We analyzed the vitamin C content using the 2, 6-dichlorophenol titration method, using a digital titrator (DE-M, Pland, Germany) for titration readings. Total phenol content was analyzed using Folinphenol colorimetry and measured using a UV–visible spectrophotometer (UV-3600, Shimadzu, Japan). The amino acid composition was analyzed by high performance liquid chromatography (HPLC). A Shimadzu LC-20AD HPLC system equipped with a C18 column (4.6 × 250 mm, 5 μm particle size) was used. The mobile phase A consisted of 0.1 mol/L sodium acetate solution (pH 6.50): ethyl ether = 93:7, and the mobile phase B consisted of water:acetonitrile = 20:80. The flow rate was 1.0 mL/min and the column temperature was 40 °C. Inductively coupled plasma mass spectrometr (ICP-MS) was used to determine the mineral composition of WBS. The ICP-MS (ICP-OES730, Agilent, USA) instrument settings were as follows: the plasma gas flow rate was 15 L/min, the auxiliary gas flow rate was 1.5 L/min, and the atomizer flow rate was 0.75 L/min. All tests were repeated three times, and monitoring data were obtained under normal operating conditions of the instrument.

2.2. Maintenance of zebrafish

Wild-type zebrafish (purchased from Zebrafish resource center, Wuhan, China) were housed in a five-storey single-row Aquatic System (Haisheng Marine Biological Equipment Co., Ltd. Shanghai, China) with a 14 h light:10 h dark cycle at a temperature of 28.5 ± 1 °C. Breeding, mating and embryo collection methods of zebrafish as described by predecessors, collected embryos were placed in incubators [22,23].

2.3. In vivo toxicity study

The experimental process of zebrafish embryo co-culture was referred to previous studies [24,25], and appropriate modifications were made. In brief, after 6–8 h post fertilization (hpf) embryos were placed into 6-well plates (30 embryos per well) in groups of three wells containing 10 mL embryo medium. The compound to be tested is dissolved in the embryo medium, added to each hole, and the culture medium was changed every morning and evening. Embryos incubated with WBS (50, 100, 200, 300 μg/mL) and arbutin (250 μg/mL) purchased from Sigma-Aldrich (St. Louis, MO, USA) were used to observe the hatchability rate of zebrafish larvae on day 3 and the total mortality rate over 7 days. At the same time, after the 48 h culture period, live embryos were anaesthetised in tricaine methanesulfonate solution (0.03%w/w) and fixed in 3%w/w methylcellulose. Images were then taken with a type microscope for phenotypic evaluation of anti-melanosis.

2.4. Behavioral testing

To further evaluate the safety of WBS, we performed an experimental analysis of the behavior of adult fish. Behavioral testing was performed using the novel tank test (NTT), which was used to assess the anxiety-like response [26]. Briefly, the NTT was a trapezoidal tank (1.5 L, 15 height × 28 top/22 bottom × 7 width cm) that was essentially divided into top and lower halves [27]. The fish was transferred to the tank and kept for 15 min to adapt to the new environment. The experimental group was immersed in WBS (300 μg/mL) for ten days (20 min/day). Ten zebrafish were used in each group. In our experiment, each fish was placed in novel tank and video was recorded immediately for 5 min. The videos were analyzed using Fish Track (Zebrafish Analysis Software, XinRuan Information Technology Co., Ltd. Shanghai, China).

2.5. Enzyme-linked immunosorbent assay (ELISA)

Tyrosinase activity and melanin content of zebrafish were determined using an ELISA kit purchased from Shenzhen Zike Biotechnology Co., Ltd. (Shenzhen, China). The sample solutions for the determination of tyrosinase activity and melanin content were treated in the same way as described by the reagent vendor. Specifically, about 120 zebrafish larvae were taken from each group and homogenized in an ice bath with 0.9%w/w normal saline after rinsing with PBS three times and draining the PBS solution. The sample homogenate was separated by centrifugation at 2500 rpm for 10 min. The supernatant after centrifugation was collected for the detection of tyrosinase activity and melanin content, and the corresponding results were calculated by using an enzyme marker at the wavelength of 450 nm.

2.6. Cell cultures and MTT assay

B16F10 murine melanoma cells (Henan, China) were grown in RPMI 1640 (Cellgro Mediatech, Inc., Manassas, VA) medium containing 10%w/w foetal bovine serum (FBS, Gibco) with 100 U/mL penicillin and 100 μg/mL streptomycin in a humidified atmosphere with 5%v/v CO_2 at 37 °C. Human keratinocyte (HaCat) cells (purchased from American Type Culture Collection) were

cultured in Dulbecco's modified Eagle's medium (DMEM) and the same conditioned medium used to culture B16F10 cells. The 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to evaluate the effects of WBS on cell viability.

2.7. Determination of melanin content

The method for the determination of melanin content in B16F10 cells was as described previously [28], and slightly modified. B16F10 cells (6.8×10^4 cells/mL, 2.0 mL) were cultured in 6-well plates for 24 h, and then incubated with WBS (50–300 μg/mL) and arbutin (250 μg/mL) solutions of different concentrations for 48 h. The cells were collected and washed twice with PBS, then dissolved in 200 μL 1 N NaOH (containing 10%w/w dimethyl sulfoxide) at 70 °C for 2 h. The cell lysates were added to 96-well plates, and the absorbance values were measured at 470 nm using a microplate reader to determine the melanin content of each lysate. Melanin content was calculatedby comparing the sample to the blank control group, with the percentage of the blank control group being considered the melanin content of the sample.

2.8. Tyrosinase activity measurement

As previously stated [29,30], tyrosinase activity was estimated by measuring the oxidation rate of L-DOPA purchased from McLean (Shanghai, China), with minor modifications. In brief, murine-derived B16F10 melanoma cells were incubated for 48 h with different concentrations of WBS (50–300 μg/mL) and arbutin (250 μg/mL) solutions. The cells were collected and cleaned twice with PBS, and then 1 mL RIPA lysis buffer (Beyotime Biotechnology Co., Ltd. Shanghai, China) was added for lysis at 4 °C for 1 h. The supernatant was obtained by centrifugation at 10,000 *g* and 4 °C for 15 min. The total protein content was determined with BCA kit (purchased from Solebaol Technology Co., Ltd. Beijing, China). The lysate was adjusted to ensure uniform total protein concentrations for all samples by adding the lysate buffer. 80 μL of the above mixture was taken into 96-well plates and added with 80 μL of 0.1%w/w L-DOPA (prepared with 0.1 M, pH = 6.8 phosphoric acid buffer, the reaction mixture should be used in 2 h after preparation), incubated at 37 °C for 10 min, and the reaction was detected at 492 nm wavelength. Data were expressed as a percentage of the control value.

2.9. Skin irritation of zebrafish by WBS

3 dpf neutrophils fluorescent zebrafish (cms Tg/+) were divided into 6-well plates with three zebrafish in each group and three parallel wells in each group. Control group, WBS (50–300 μg/mL) group and arbutin (250 μg/mL) group were divided into untreat and different concentrations. The test product was dissolved into embryo medium and ingested into the zebrafish. After skin absorption for a period of time (24 h, 48 h and 72 h), transgenic zebrafish with neutrophils fluorescent were directly observed with a microscope after anesthesia with 0.02%w/w tricaine, and images were obtained for subsequent analysis [31]. Compared with the model control group, the green spot was neutrophils. The more green fluorescence, the more neutrophils, the stronger the skin irritation.

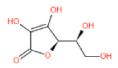
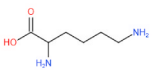
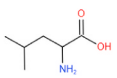
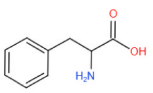
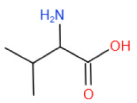
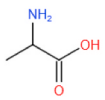
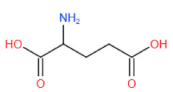
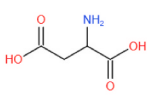
2.10. Quantitative real-time polymerase chain reaction (qRT-PCR)

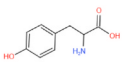
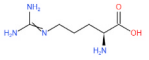
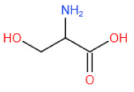
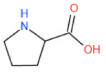
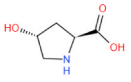
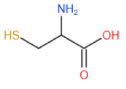
B16F10 cells used for RNA extraction were cultured in the same way as 2.7. After the treated cells were removed from the culture medium and collected, they were washed with PBS. To extract total RNA, Trizol (Takara, Japan) was used to lysate the cells, according to the manufacturer's instructions. Following that, 1 μL total mRNA was reverse transcribed to cDNA using MonScriptTm RTIll Super Mix (Mona Biotechnology Co., LTD., Suzhou, China) in accordance with the manufacturer's protocol, followed by preparation of fivefold dilutions of the cDNA samples. Finally, a 1 μL final cDNA sample was prepared for qRT-PCR using the CFX Connect (Bole Life Medicine Products (Shanghai) Co., Ltd. Shanghai, China) and the following primers: MITF: 5′-CTGATGGACGATGCCCTCTC-3′ and 5′-TCCGTTTCTTCTGCGCTCAT-3′; TYR: 5′-CTCTGGGCTTAGCAGTAGGC-3′ and 5′- GCAAGCTGTGGTAGTCGTCT-3′; TYRP-1: 5′-CCCCTAGCCTATATCTCCCTTTT-3′ and 5′-TACCATCGTGGGGATAATGGC-3′; TYRP-2: 5′-GTCCTCCACTCTTTTACAGACG-3′ and 5′-ATTCGGTTGTGACCAATGGGT-3′; β-actin： 5′-GGCTGTATTCCCCTCCATCG-3′ and 5′-CCAGTTGGTAACAATGCCATGT-3′ equipped with Taq SYBR green fluorescent dyes (Mona Biotechnology Co., LTD., Suzhou, China). Relative gene expression was normalized to the mRNA expression of the housekeeping gene β-actin and calclated by using the $2-\Delta\Delta Ct$ method [32].

2.11. Western blot analysis

Cells were lysed in strong RIPA lysis buffer (Beyotime, P0013B) at 4 °C for 1 h then each supernatant fraction was retained after centrifugation at 12,000×*g* for 30 min at 4 °C. Supernatant proteins in the extract was determined by the Coomassie brilliant blue method. After the protein content in each sample was balanced, the protein was denatured by heating for 10 min in a 100 °C water bath. Proteins were separated by electrophoresis on 10%w/v PAGE gels (YEASEN, 20326ES62) then transferred onto PVDF membranes (Beyotime, FFP24). The PVDF membrane was placed into Tris-buffered saline plus Tween-20 (TBST) (Beyotime, ST673-100 mL) sealing solution containing 5%w/v skimmed milk powder and sealed for 1 h then incubated with TYRP-1 (Sanying, 16672-1-AP), TYRP-2 (Abcam, ab221144), MITF (Boster, A00269-3) and GAPDH (Boster, BM3874) primary antibodies overnight at 4 °C. Next, membranes were washed with TBST then incubated with HRP-conjugated secondary antibodies (Boster, BA1054) at room temperature for 2 h. After washing the TBST film, it was placed into BeyoECL Moon (Beyotime, P0018FS) for exposure and fixation. The integral optical density of the protein bands was measured by ImageJ image acquisition and analysis system software, and the GAPDH protein bands were detected in each sample as an internal reference.

Table 1
Vitamin C, total phenols, amino acids and trace elements in white birch sap.

Substances		Chemical structure formula	Content of substance
Vitamin C (mg/100g)			17.56
Total phenol (mg/100g)			0.2
Essential amino acids (mg/100mL)	Lysine		0.1
	Leucine		0.1
	Phenylalanine		1.7
	Valine		0.1
Non-essential amino acids (mg/100mL）	Alanine		0.2
	Glutamic acid		0.4
	Aspartic acid		0.9

	Tyrosine kinase		0.1
	Arginine		0.1
	Serine		0.2
	Proline		0.1
	Hydroxyproline		0.1
	Cysteine		0.2
Mineral nutrition (mg/L）	Potassium	K	5.0529
	Sodium	Na	3.4549
	Calcium	Ca	3.8242
	Magnesium	Mg	1.2013
	Phosphorus	P	0.2340
Essential trace elements for human body (mg/L）	Zinc	Zn	0.1249
	Cobalt	Co	0.1310
	Molybdenum	Mo	0.1469
	Iron	Fe	0.3207
	Strontium	Sr	0.8067
	Manganese	Mn	4.3067
	Silicon	Si	6.8074
Harmful elements (mg/L）	Lead	Pb	Not detected
	Arsenic	As	Not detected
	Cadmium	Cd	Not detected

2.12. Statistical analysis

All statistical analyses were performed using GraphPad Prism (GraphPad Software Inc. San Diego, California, USA). All data were expressed as group means ± SEM or as box plots indicating the median. Parametric tests, such as the two-tailed unpaired *t*-test and ordinary oneway analysis of variance (ANOVA) followed by Tukey's post hoc test or Dunnett's multiple comparison test, were used to analyze the mean differences between groups. Significance levels indicated in all figures were as follows: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

3. Results

3.1. Characterization of powders and solutions of WBS

WBS freeze-dried powder was light yellow powder (Fig. S1A); 1×10^4 μg/mL WBS solution was light yellow and clear (Fig. S1B). The zeta potential indicated the stability of the system and the higher the absolute value (positive or negative) of the zeta potential, the more stable the system [33]. In this study, the WBS solution showed negative zeta potential, as shown in Fig. S1D. We measured the mean DLS of the WBS solution as 219.4 nm (Fig. S1C) and the mean zeta potential as −17.6 mV (Fig. S1D), and the results showed that the solution had good dispersion and stability. WBS contains vitamin C, total phenols, 13 kinds of amino acids, 5 kinds of mineral nutrients and 7 essential trace elements, as shown in Table 1.

3.2. Effects of different WBS concentrations on hatching and survival rate of zebrafish larvae

The safe concentration range of WBS was determined by co-culturing WBS with zebrafish embryos. Specifically, fertilized zebrafish eggs were selected and transferred to embryo culture medium containing WBS (0, 100, 200, 300, 400, 500, 600 μg/mL) for incubation and reproduction for 7 days. Hatching rates were counted on day 3, and the results showed that the parameter was almost unaffected in the concentration range of 0–400 μg/mL. In addition, incubation rate significantly decreased when the concentration was increased to the range of 400–600 μg/mL. In addition, no zebrafish larvae normally hatched at concentrations above 600 μg/mL (Fig. 2A). On day 7, the survival rate of zebrafish larvae was analyzed, and the results showed that the survival rate of zebrafish larvae exposed to embryo medium containing WBS concentration of 0–300 μg/mL had no significant difference compared with the control group. Notably, zebrafish larval survival rates significantly decreased with increasing concentrations in the range of 300–600 μg/mL, and remained almost above 50% at 400 μg/mL, but decreased to almost 0% after exposure to 500 μg/mL (Fig. 2B). The unhatched embryos at 48 h and hatched embryos at 72 h of the WBS (300 μg/mL) and arbutin (250 μg/mL) against zebrafish embryos were shown in Fig. S2. WBS (300 μg/mL) had no toxicity to the development of zebrafish and had milder effects and less irritation than arbutin (250 μg/mL).

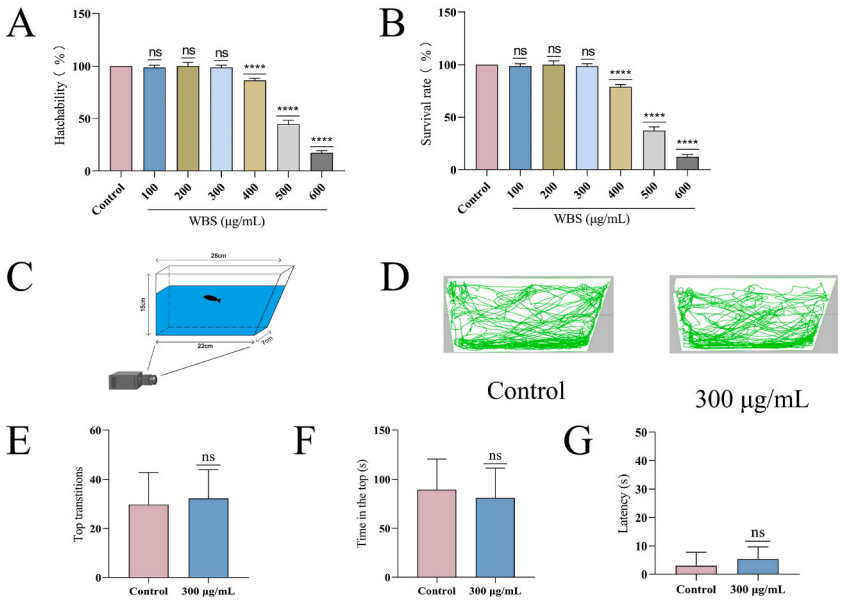


Fig. 2. Analysis of hatching rate and survival rate of zebrafish larvae. Hatching rates of zebrafish larvae with different concentrations were counted on day 3 (**A**), and survival rates of zebrafish larvae exposed to different concentrations were counted on day 7 (**B**), compared with controls. The structural drawing of the novel tank (**C**); Zebrafish trajectory diagram in novel tank test (**D**); Number of (**E**) top transitions for WBS (300 μg/mL) immersion groups; There was no significant difference between (**F**) the time spent on the top and (**G**) the latency to enter the top. Significant differences between the control and WBS exposed groups are indicated by an asterisk. The data are expressed as the mean ± S.E.M. and were analyzed by one-way. Significance was defined as *$p<0.05$, **$p<0.01$, ***$p<0.001$ and ****$p<0.0001$.

According to the above data, the concentration of 0–300 μg/mL was used for follow-up experiments.

3.3. WBS had no effect on the behavior of adult zebrafish

NTT was used to assess the effects of WBS (300 μg/mL) exposure on the behavior of adult zebrafish. Upon entering a new environment, such as a novel tank, zebrafish attempted to seek safety by diving to the bottom of the tank. Thus, the proportion of time spent at the bottom of the tank was considered a quantifiable measure of its level of stress or anxiety. The structure diagram of the novel tank and the zebrafish trajectory diagram in the novel tank are shown in Fig. 2C and D. As shown in Fig. 2, through data analysis, there was no significant difference in the number of top transitions (Fig. 2E), the time spent on the top (Fig. 2F) and the time of latency to enter the top (Fig. 2G) between WBS (300 μg/mL) and control. The results showed that acute exposure to WBS (300 μg/mL) had no effect on the behavior of adult zebrafish. The safety of WBS is further demonstrated.

3.4. Quantitative measurement of tyrosinase activity and melanin content in zebrafish embryos

In order to verify whether WBS treatment can affect skin pigmentation, zebrafish was used as an *in vivo* model to detect tyrosinase activity and melanin content. Compared with the control group, WBS significantly inhibited melanin production in zebrafish in a dose-dependent manner (Fig. 3A). It is worth noting that WBS inhibited melanin production throughout the melanin-producing compartment in zebrafish, and WBS (300 μg/mL) inhibited melanogenesis better than arbutin (250 μg/mL) (Fig. 3B). Treatment with WBS (300 μg/mL) significantly reduced tyrosinase activity to 67.45% of the untreated control level, and the effect was similar to that observed in the arbutin group (Fig. 3C). The length of the scale is 500 μm. These results indicate that WBS can significantly inhibit pigment accumulation *in vivo*.

3.5. Cytocompatibility of WBS

The safety of cosmetics is a serious issue. For example, hydroquinone (an effective skin lightener) contributes to several pathological conditions, such as erythema, dryness and skin desquamation, and has a potential cancer risk. Kojic acid, for example, is a potent tyrosinase inhibitor that induces contact dermatitis and is potentially genotoxic. To assess the safety of WBS, cell viability assays were performed on B16F10 cells and HaCat cells. WBS had no effect on cell viability at 50–500 μg/mL. Thus, WBS did not produce cytotoxicity to HaCat cells (Fig. 4A) and B16F10 cells (Fig. 4B) at concentrations of 50–500 μg/mL.

3.6. WBS reduced melanin content and tyrosinase activity in B16F10 cells

WBS in the concentration range of 50–300 μg/mL was used to evaluate the effects of melanin content and tyrosinase activity in B16F10 cells. As shown in Fig. 4C and D, B16F10 cells were treated with 50 μg/mL WBS, and there was no significant change in melanin content and tyrosinase activity. After treating B16F10 cells with 100 μg/mL WBS, there was no significant change in melanin content but tyrosinase activity decreased to 87.72% of the control group After 200 μg/mL WBS treatment, the melanin content and

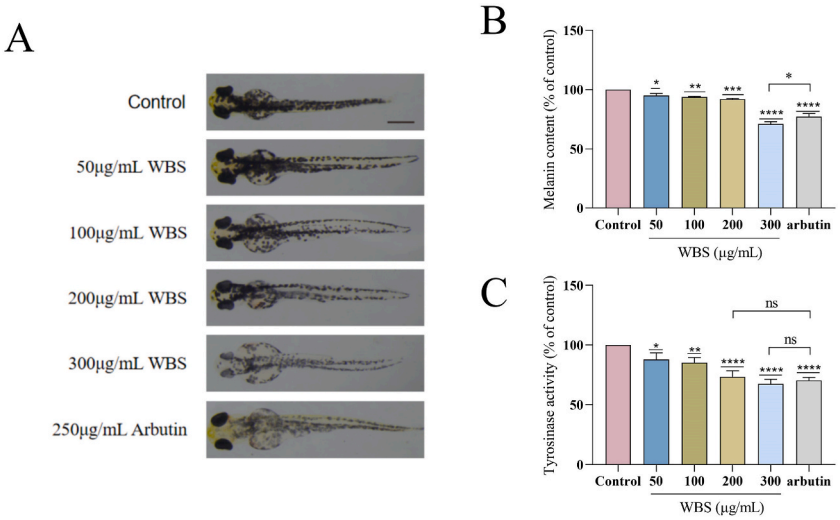


Fig. 3. The characterization of melanin content in zebrafish treated with each WBS concentration (50–300 μg/mL) and arbutin (250 μg/mL) (**A**). After WBS treatment, the melanin content of zebrafish decreased (**B**), and WBS significantly inhibited tyrosinase activity in a dose-dependent manner (**C**). The data are expressed as the mean ± S.E.M. and were analyzed by one-way. Significance was defined as $*p<0.05$, $**p<0.01$, $***p<0.001$ and $****p<0.0001$.

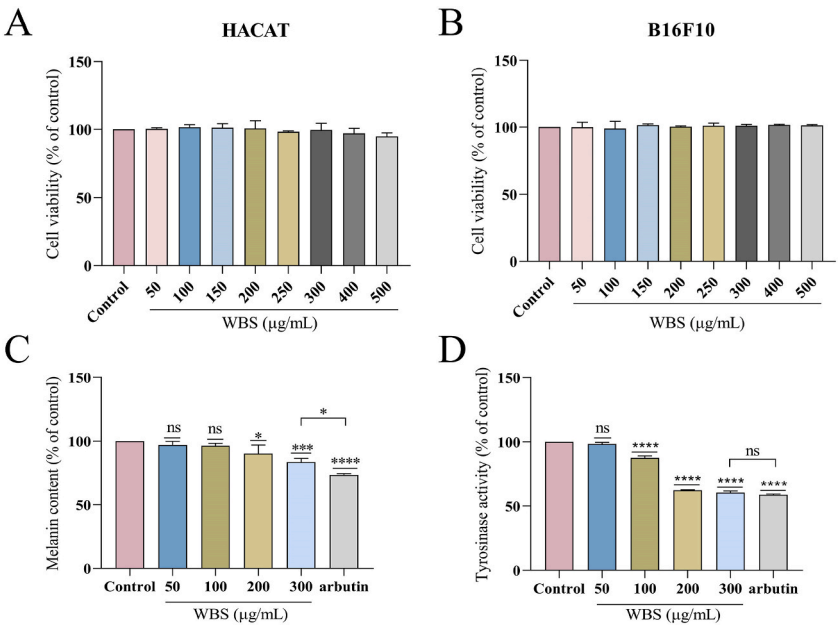


Fig. 4. The cell activities of B16F10 (A) and HaCaT (B) and the melanin content of B16F10 (C) and HaCaT (D) after white birch sap treatment. The data are expressed as the mean ± S.E.M. and were analyzed by one-way. Significance was defined as *$p<0.05$, **$p<0.01$, ***$p<0.001$ and ****$p<0.0001$.

tyrosinase activity decreased to 87.81% and 62.32% of the control group, respectively. After 300 μg/mL WBS treatment, the melanin content and tyrosinase activity decreased to 82.27% and 60.54% of the control group, respectively. The effect of 300 μg/mL WBS on tyrosinase activity was similar to that of 250 μg/mL arbutin, but the inhibitory effect on melanin content was slightly lower than arbutin. Therefore, these findings together confirm that WBS can effectively inhibit melanin production in B16F10 cells. The morphology of HaCat cells treated with WBS (300 μg/mL) and arbutin (250 μg/mL) did not change significantly (Fig. S3A), but that of B16F10 cells treated with arbutin (250 μg/mL) reduced the proliferation rate and affected the morphology of B16F10 cells (Fig. S3B). Compared with arbutin (250 μg/mL), WBS (300 μg/mL) has fewer stimulation on B16F10 cells and has higher safety.

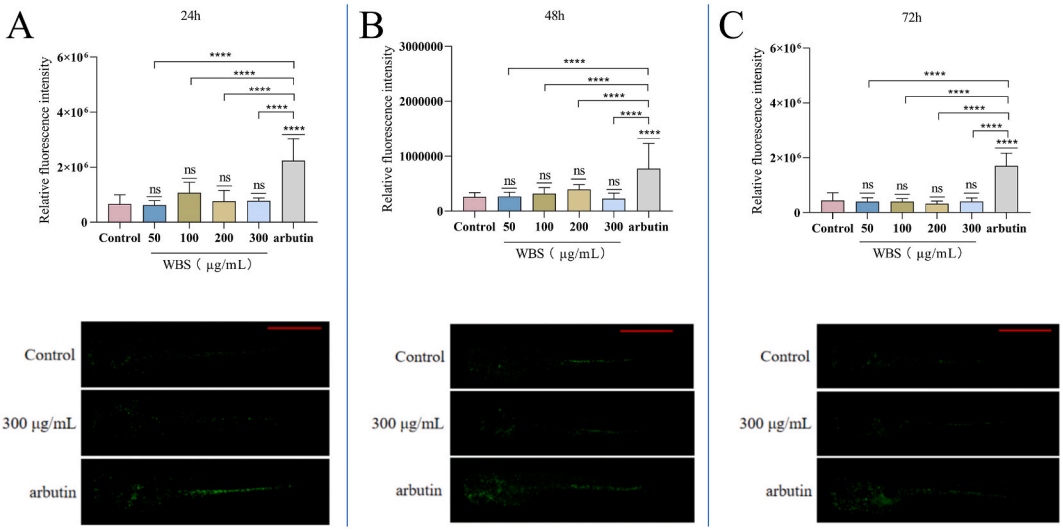


Fig. 5. At 24 h (A), 48 h (B) and 72 h (C), there were no significant difference in the fluorescence intensity of neutrophils fluorescent zebrafish treated with WBS (300 μg/mL) and control group, but there was significant difference in the fluorescence intensity of neutrophils fluorescent zebrafish treated with arbutin (250 μg/mL). The data are expressed as the mean ± S.E.M. and were analyzed by one-way. Significance was defined as *$p<0.05$, **$p<0.01$, ***$p<0.001$ and ****$p<0.0001$.

3.7. WBS had little effect on skin irritation of neutrophils fluorescent zebrafish

The irritating effect of irritant components on skin is mainly achieved through the pathway of inducing inflammatory response [34]. The generation of inflammatory response will make the body produce immune response. Various immune cells migrate and gather to the site of epidermal inflammation under the action of chemokines, among which neutrophils have the strongest sensitivity and specificity [35]. Neutrophils are a major component of the innate immune response and are the most abundant type of circulating cell in humans and zebrafish [36]. Transgenic lines of labeled zebrafish neutrophils that respond to wounds and bacterial infections provide an important tool for *in vivo* imaging [37].

The changes in fluorescence intensity of neutrophils fluorescent zebrafish exposed to WBS (300 μg/mL) and arbutin solution (250 μg/mL) are shown in Fig. 5 (A-C). The schematic diagram of zebrafish treated with WBS (50, 100, and 200 μg/mL) could be viewed in Fig. S4. The length of the scale is 1 mm. Within the safe concentration range, there was no significant difference in the fluorescence intensity of WBS exposed zebrafish compared with control group at 24 h (Figs. 5A), 48 h (Fig. 5B) and 72 h (Fig. 5C), but there was a significant difference in the fluorescence intensity of WBS exposed zebrafish compared with control group. These results suggest that WBS is mild and has no irritating effect on skin within the safe concentration range (50–300 μg/mL).

3.8. Effects of WBS on the expression of key mediators of melanogenesis

Melanogenesis is the physiological process of melanin synthesis in melanocytes located in the base layer of the epidermis to protect the skin from UV damage [38]. Melanin is synthesized in the melanosomes of melanocytes and transported to the surrounding keratinocytes, where it forms a melanin cap that limits UV penetration into the epidermis and dermis [39]. TYR, TYRP-1 and TYRP-2 are three key enzymes of melanin synthesis. Melanogenesis is mediated by a complex series of signaling pathways, each associated with a master regulator called MITF, which controls the expression of genes such as TYR, TYRP-1, and TYRP-2. To explore the effect of WBS in inhibiting melanin synthesis, we examined the effect of WBS and arbutin on the gene expression of four key mediators in B16F10 cells. qRT-PCR analysis show that WBS could effectively down-regulate the mRNA expression levels of MITF, TYR, TYRP-1 and TYRP-2 (Fig. 6A–D). At the same time, as shown in Fig. 6 (E-H), the results of Western blot analysis show that WBS inhibits the protein expression of TYRP-1/GAPDH, TYRP-2/GAPDH, and MITF/GAPDH in a dose-dependent manner. The original Western blot can be found in Figs. S5–8. Together, these findings indicated that WBS decreased the expression of MITF at the gene level, which in turn inhibited the gene expression of TYR, TYRP-1 and TYRP-2 produced by the three melanin species.

4. Conclusions and discussion

WBS is considered to be one of the most profitable non-wood forest products and is used as an ingredient in food and drink as well as being used to make fresh drinks [40]. A previous study showed that WBS has differentiation-inducing properties on keratinocytes cultured *in vitro*, controlling the production of moisture-retaining and barrier related factors [41]. In this study, we used the

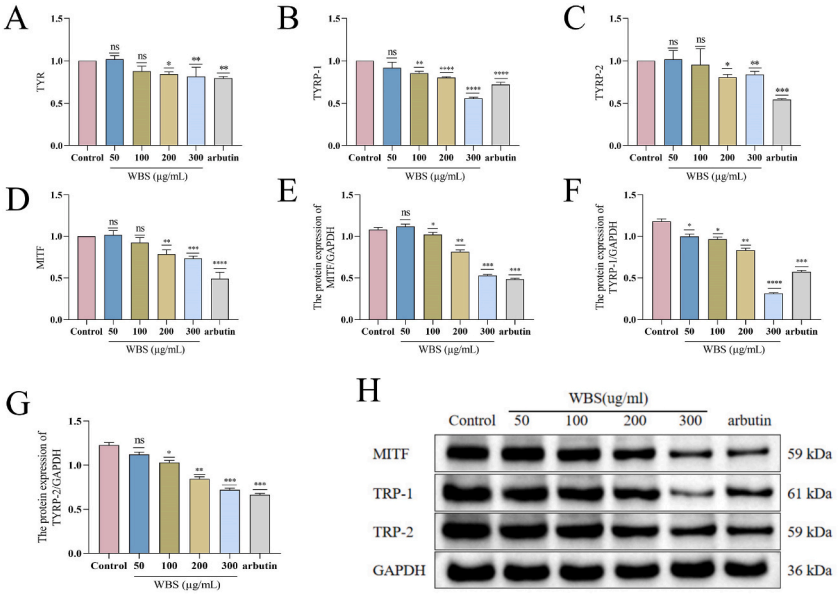


Fig. 6. WBS inhibited the expression of TYR (A), TYRP-1 (B), TYRP-2 (C) and MITF (D) in B16F10 cells. Levels of MITF (E), TYRP-1 (F) and TYRP-2 (G) proteins in B16F10 cells treated with WBS as determined by western blotting. GAPDH was a loading control (H). The data are expressed as the mean ± S.E.M. and were analyzed by one-way. Significance was defined as $*p<0.05$, $**p<0.01$, $***p<0.001$ and $****p<0.0001$.

freeze-dried powder of WBS to realize the controllability of the solution concentration of WBS, and studied the inhibition effect of different concentrations of WBS on melanin synthesis. We directly observed the distribution of melanin and the darkening results of pigment by using the unique advantage of whole-body transparency of young zebrafish. In the zebrafish model, WBS inhibited melanin production and tyrosinase activity in a dose-dependent manner, and inhibited melanin production throughout the body compartments. Certainly, skin pigmentation is a complex biological process that can be caused by a variety of factors (UV irradiation, drug effects, dermatological diseases, etc.) (Fig. 7A) [42]. The process of melanin synthesis is illustrated in Fig. 7C. Melanin is synthesized and stored in melanosomes, which matures to the tip of the dendrite and is further transported to adjacent keratinocytes via the protein MLPH forms a tripartite complex with Ras-related proteins and Myosin-Va to form pigmentation [43]. The effect of pigmentation is related to the transport of melanosomes and their distribution in the perinuclear area. Melanin can be divided into eumelanin and pheomelanin. Melanin is first generated by the hydroxylation of phenylalanine to L-tyrosine or directly from L-tyrosine, and then by hydroxylation to L-3, 4-dihydroxyphenylalanine (L-DOPA). Under the action of TYR, L-DOPA is further oxidized to L-DOPA quinone. In the absence of cysteine, dopa quinone is converted to dopamine by intramolecular addition and spontaneously decomposes to 5, 6-dihydroxyindole (DHI), forming eumelanin polymers, or through the conversion of TYRP-2, 5, 6-dihydroxyindole-2-carboxylic acid (DHICA) is catalyzed by TYRP-1 to indole-5, 6-quinone carboxylic acid. Finally, true melanin accumulates. In the presence of cysteine, dopa quinone reacts with cysteine to form cysteine dopamine, which is further converted to quinoline and ultimately polymerized to form melanin. The content of melanin also depends on the expression of tyrosinase and the activity of tyrosinase [44]. Our results indicate that WBS can effectively inhibit the activity of tyrosinase, down-regulate the expression of tyrosinase, and ultimately lead to the reduction of melanin pigment. Further understanding of the regulatory pathways of melanin production may contribute to identify more specific drug action targets for effective control of pigmentation disorders. In melanocytes, microscopy-associated transcription factors influence TYR, TYRP-1, and TYRP-2 to regulate melanin production [45]. At the same time, many cytokines activate signaling pathways by interacting with receptors on melanin membranes, including phosphatidylinositol 3-kinase/Akt pathway, cyclic adenosine/protein kinase A pathway, mitogen activated protein kinase pathway, Endothelin-1-mediated signal-grade pathway and Wnt

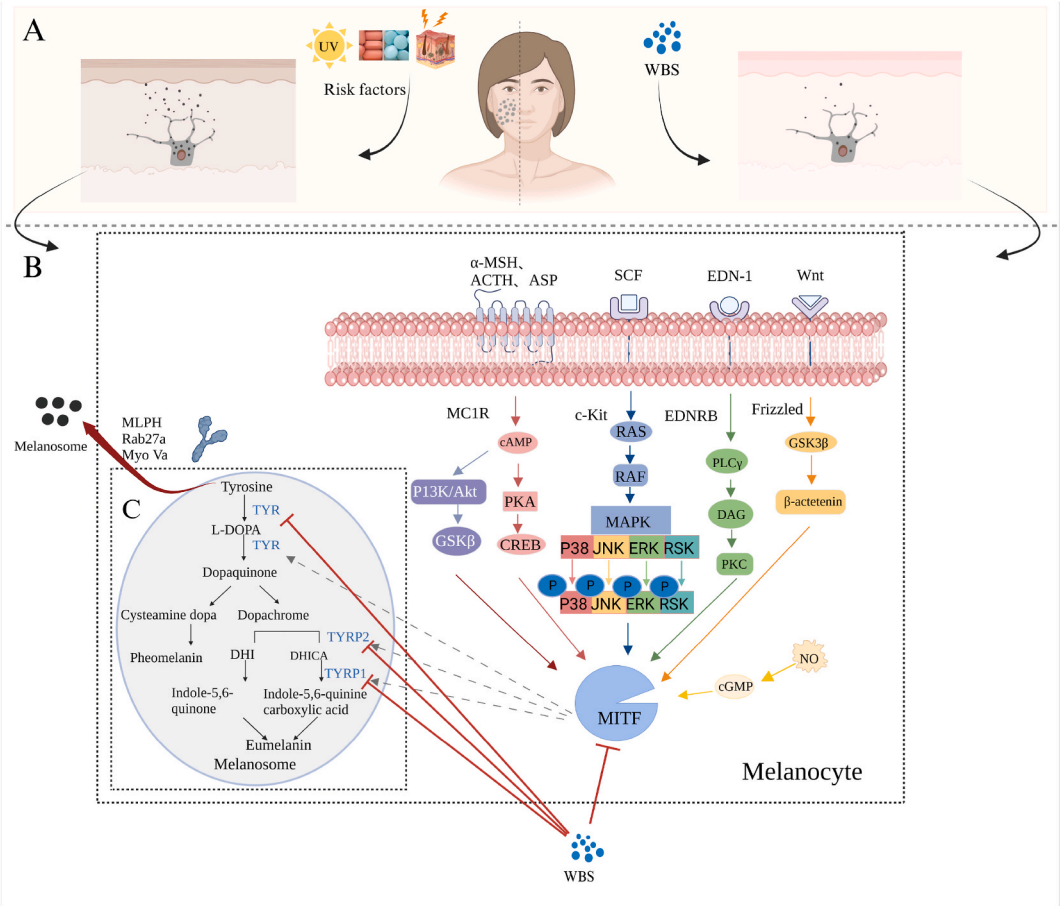


Fig. 7. WBS reduces skin pigmentation by inhibiting MITF, TYR and TYRP-1 and the underlying mechanism. (**A**) WBS can reduce skin pigmentation. (**B**) Cytokines activate signaling pathways (phosphatidylinositol 3-kinase/Akt pathway, cyclic adenosine/protein kinase A pathway, mitogen activated protein kinase pathway, Endothelin-1-mediated signal-grade pathway and Wnt pathway), and regulated melanogenesis through MITF. (**C**) Processes of melanin production and transfer.

pathway. And regulate the production process of melanin through MITF (Fig. 7B) [3]. Here, we discussed the inhibitory effect of WBS on tyrosinase activity and the effect on melanin content. However, whether WBS has certain effects on several signaling pathways regulating transcription factors related to microphthalmia and related transporters of melanosomes still needs to be further investigated.

Clearly, when considering the practical use of tyrosinase inhibitors, it is crucial that they do not have a toxic effect on humans. According to this study, WBS had no toxicity towards mouse melanoma B16F10 and human keratinocyte HaCat cells. Furthermore, WBS has not been shown to be toxic to embryonic development or skin irritation in neutrophil fluorescent zebrafish. This suggests that WBS, as a naturally derived whitening agent, has high biocompatibility and low irritability. The content of vitamin C in WBS is 17.56 mg/100g. It is well known that vitamin C can inhibit skin pigmentation and play a whitening role by inhibiting TYR activity and reducing melanin content [46]. Many polyphenols have good whitening and antioxidant effects [47], and the total phenolic content in WBS is 2 mg/kg, which has potential whitening effect, but the specific whitening effect and phenolic substances need to be further studied. Meanwhile, WBS contains four essential amino acids, nine non-essential amino acids, five mineral nutrients and seven essential trace elements can provide nutrients for the skin. Mechanistically, our results suggest that WBS inhibits the synthesis of melanin by inhibiting MITF as well as downstream TYR, TYRP-1 and TYRP-2 (Fig. 7B). Nevertheless, while the whitening activity of WBS is well established *in vivo* and *in vitro*, it is challenging to guarantee its effectiveness on human skin due to the complex factors influencing whitening effects. Therefore, in the future it's necessary to study the whitening effects and safety in humans before including them in whitening cosmetics.

Data availability statement

Videos is available at figshare, accession numbers: 10.6084/m9. figshare.23,832,816. The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

Ethical approval for the animal study in this study was granted by Wenzhou University Research Ethics Committee. Reference number WZU-2023-079.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26715.

References

[1] K. Kim, H.-I. Jeong, I. Yang, S.-J. Nam, K.-M. Lim, Acremonidin E produced by *Penicillium* sp. SNF123, a fungal endophyte of *Panax ginseng*, has antimelanogenic activities, J. Ginseng Res. 45 (2021) 98–107, https://doi.org/10.1016/j.jgr.2019.11.007.

[2] J. Kim, S.-C. Hong, E.H. Lee, J.W. Lee, S.-H. Yang, J.-C. Kim, Preventive effect of *M. cochinchinensis* on melanogenesis via tyrosinase activity inhibition and p-PKC signaling in melan-A cell, Nutrients 13 (2021) 3894, https://doi.org/10.3390/nu13113894.

[3] F. Liu, L. Qu, H. Li, J. He, L. Wang, Y. Fang, X. Yan, Q. Yang, B. Peng, W. Wu, L. Jin, D. Sun, Advances in biomedical functions of natural whitening substances in the treatment of skin pigmentation diseases, Pharmaceutics 14 (2022) 2308, https://doi.org/10.3390/pharmaceutics14112308.

[4] X. Zhang, G. Bian, P. Kang, X. Cheng, K. Yan, Y. Liu, Y. Gao, D. Li, Recent advance in the discovery of tyrosinase inhibitors from natural sources via separation methods, J. Enzym. Inhib. Med. Chem. 36 (n.d.) 2104–2117. https://doi.org/10.1080/14756366.2021.1983559..

[5] K. Mou, W. Pan, D. Han, X. Wen, F. Cao, Y. Miao, P. Li, *Glycyrrhizin* protects human melanocytes from H2O2-induced oxidative damage via the Nrf2-dependent induction of HO-1, Int. J. Mol. Med. 44 (2019) 253–261, https://doi.org/10.3892/ijmm.2019.4200.

[6] T. Pillaiyar, M. Manickam, V. Namasivayam, Skin whitening agents: medicinal chemistry perspective of tyrosinase inhibitors, J. Enzym. Inhib. Med. Chem. 32 (2017) 403–425, https://doi.org/10.1080/14756366.2016.1256882.

[7] J.Y. Lee, J. Lee, D. Min, J. Kim, H.-J. Kim, K.T. No, Tyrosinase-targeting gallacetophenone inhibits melanogenesis in melanocytes and human skin- equivalents, Int. J. Mol. Sci. 21 (2020) 3144, https://doi.org/10.3390/ijms21093144.

[8] L. Zhang, G. Tao, J. Chen, Z.-P. Zheng, Characterization of a new flavone and tyrosinase inhibition constituents from the twigs of *Morus alba L*, Molecules 21 (2016) 1130, https://doi.org/10.3390/molecules21091130.

[9] D. Roggenkamp, N. Dlova, T. Mann, J. Batzer, J. Riedel, M. Kausch, I. Zoric, L. Kolbe, Effective reduction of post-inflammatory hyperpigmentation with the tyrosinase inhibitor isobutylamido-thiazolyl-resorcinol (Thiamidol), Int. J. Cosmet. Sci. 43 (2021) 292, https://doi.org/10.1111/ics.12694.

[10] H. Lu, K. Yang, L. Zhan, T. Lu, X. Chen, X. Cai, C. Zhou, H. Li, L. Qian, G. Lv, S. Chen, Optimization of flavonoid extraction in *Dendrobium officinale* leaves and their inhibitory effects on tyrosinase activity, Int. J. Anal. Chem. (2019) 7849198, https://doi.org/10.1155/2019/7849198, 2019.

[11] M. Saeedi, M. Eslamifar, K. Khezri, Kojic acid applications in cosmetic and pharmaceutical preparations, Biomed. Pharmacother. 110 (2019) 582–593, https://doi.org/10.1016/j.biopha.2018.12.006.

[12] S. T, A. N. F, A. Fr, Hydroquinone: myths and reality, Clin. Exp. Dermatol. 46 (2021), https://doi.org/10.1111/ced.14480.

[13] Y.C. Boo, Arbutin as a skin depigmenting agent with antimelanogenic and antioxidant properties, Antioxidants 10 (2021) 1129, https://doi.org/10.3390/antiox10071129.

[14] C.L. Burnett, W.F. Bergfeld, D.V. Belsito, R.A. Hill, C.D. Klaassen, D.C. Liebler, J.G. Marks, R.C. Shank, T.J. Slaga, P.W. Snyder, F.A. Andersen, Final report of the safety assessment of Kojic acid as used in cosmetics, Int. J. Toxicol. 29 (2010), https://doi.org/10.1177/1091581810385956, 244S–73.

[15] F.J. Enguita, A.L. Leitão, Hydroquinone: environmental pollution, toxicity, and microbial answers, BioMed Res. Int. 2013 (2013) 542168, https://doi.org/10.1155/2013/542168.

[16] H. Zhou, J.K. Kepa, D. Siegel, S. Miura, Y. Hiraki, D. Ross, Benzene metabolite hydroquinone up-regulates chondromodulin-I and inhibits tube formation in human bone marrow endothelial cells, Mol. Pharmacol. 76 (2009) 579–587, https://doi.org/10.1124/mol.109.057323.

[17] Y.-N. S, F. K, O. A, U. A, S. O. J, T. D, Probable allergic contact dermatitis from hydroquinone presenting as leukomelanoderma: report of two cases, J. Dermatol. 44 (2017), https://doi.org/10.1111/1346-8138.14011.

[18] D.J. Newman, G.M. Cragg, Natural products as sources of new drugs from 1981 to 2014, J. Nat. Prod. 79 (2016) 629–661, https://doi.org/10.1021/acs.jnatprod.5b01055.

[19] D. Keita, G. Léger, N. Bordenave, Rheological and water binding properties of xanthan, guar and ultra-finely milled oatmeal in white birch sap: influence of sap minor constituents, Food Res. Int. 147 (2021) 110478, https://doi.org/10.1016/j.foodres.2021.110478.

[20] S. Rastogi, M.M. Pandey, A. Kumar Singh Rawat, Medicinal plants of the genus *Betula*–traditional uses and a phytochemical-pharmacological review, J. Ethnopharmacol. 159 (2015) 62–83, https://doi.org/10.1016/j.jep.2014.11.010.

[21] P. Zhang, S. He, S. Wu, Y. Li, H. Wang, C. Yan, H. Yang, P. Li, Discovering a multi-component combination against vascular dementia from danshen-honghua herbal pair by spectrum-effect relationship analysis, Pharmaceuticals 15 (2022) 1073, https://doi.org/10.3390/ph15091073.

[22] J. Wang, Y. Li, K. Lai, Q. Zhong, K.A. Demin, A.V. Kalueff, C. Song, High-glucose/high-cholesterol diet in zebrafish evokes diabetic and affective pathogenesis: the role of peripheral and central inflammation, microglia and apoptosis, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 96 (2020) 109752, https://doi.org/10.1016/j.pnpbp.2019.109752.

[23] R. Geisler, N. Borel, M. Ferg, J.V. Maier, U. Strähle, Maintenance of zebrafish lines at the European zebrafish resource center, Zebrafish 13 (Suppl 1) (2016) S19–S23, https://doi.org/10.1089/zeb.2015.1205.

[24] D. Sun, D. Xu, C. Yang, J. Chen, M.B. Shahzad, Z. Sun, J. Zhao, T. Gu, K. Yang, G. Wang, Inhibition of Staphylococcus aureus biofilm by a copper-bearing 317L-Cu stainless steel and its corrosion resistance, Mater. Sci. Eng. C 69 (2016) 744–750, https://doi.org/10.1016/j.msec.2016.07.050.

[25] K.-S. Hwang, J.Y. Yang, J. Lee, Y.-R. Lee, S.S. Kim, G.R. Kim, J.S. Chae, J.H. Ahn, D.-S. Shin, T.-Y. Choi, M.A. Bae, A novel anti-melanogenic agent, KDZ-001, inhibits tyrosinase enzymatic activity, J. Dermatol. Sci. 89 (2018) 165–171, https://doi.org/10.1016/j.jdermsci.2017.11.004.

[26] D. Batir-Marin, M. Boev, O. Cioanca, C. Mircea, A.F. Burlec, G.J. Beppe, A. Spac, A. Corciova, L. Hritcu, M. Hancianu, Neuroprotective and antioxidant enhancing properties of selective *equisetum* extracts, Molecules 26 (2021) 2565, https://doi.org/10.3390/molecules26092565.

[27] L. Wang, J. Ma, W. Wu, Y. Fang, F. Liu, Q. Yang, X. Hu, X. Gu, Z. He, D. Sun, L. Jin, X. Zhang, Effect of aerobic exercise as a treatment on type 2 diabetes mellitus with depression-like behavior zebrafish, Life Sci. 300 (2022) 120578, https://doi.org/10.1016/j.lfs.2022.120578.

[28] J. Liu, R. Jiang, J. Zhou, X. Xu, Z. Sun, J. Li, X. Chen, Z. Li, X. Yan, D. Zhao, Z. Zheng, L. Sun, Salicylic acid in *ginseng* root alleviates skin hyperpigmentation disorders by inhibiting melanogenesis and melanosome transport, Eur. J. Pharmacol. 910 (2021) 174458, https://doi.org/10.1016/j.ejphar.2021.174458.

[29] Y. Lee, S. Kumar, S.H. Kim, K.-Y. Seong, H. Lee, C. Kim, Y.-S. Jung, S.Y. Yang, Odorless glutathione microneedle patches for skin whitening, Pharmaceutics 12 (2020) 100, https://doi.org/10.3390/pharmaceutics12020100.

[30] Q. Abbas, Z. Ashraf, M. Hassan, H. Nadeem, M. Latif, S. Afzal, S.-Y. Seo, Development of highly potent melanogenesis inhibitor by *in vitro, in vivo* and computational studies, Drug Des. Dev. Ther. 11 (2017) 2029–2046, https://doi.org/10.2147/DDDT.S137550.

[31] Y. Liu, Q. Chen, Y. Li, L. Bi, S. Lin, H. Ji, D. Sun, L. Jin, R. Peng, Hydrogen sulfide-induced oxidative stress mediated apoptosis via mitochondria pathway in embryo-larval stages of zebrafish, Ecotoxicol. Environ. Saf. 239 (2022) 113666, https://doi.org/10.1016/j.ecoenv.2022.113666.

[32] K.-C. Wu, Y.-C. Hseu, Y.-C. Shih, G. Sivakumar, J.-T. Syu, G.-L. Chen, M.-T. Lu, P.-C. Chu, Calycosin, a common dietary isoflavonoid, suppresses melanogenesis through the downregulation of PKA/CREB and p38 MAPK signaling pathways, Int. J. Mol. Sci. 23 (2022) 1358, https://doi.org/10.3390/ijms23031358.

[33] L. Liu, J. Zeng, B. Sun, N. Zhang, Y. He, Y. Shi, X. Zhu, Ultrasound-assisted mild heating treatment improves the emulsifying properties of 11S globulins, Molecules 25 (2020) 875, https://doi.org/10.3390/molecules25040875.

[34] B.R. You, J.-M. Yoo, S.Y. Baek, M.R. Kim, Anti-inflammatory effect of aged black garlic on 12-O-tetradecanoylphorbol-13-acetate-induced dermatitis in mice, Nutr. Res. Prac. 13 (2019) 189–195, https://doi.org/10.4162/nrp.2019.13.3.189.

[35] E.A. Harvie, A. Huttenlocher, Neutrophils in host defense: new insights from zebrafish, J. Leukoc. Biol. 98 (2015) 523–537, https://doi.org/10.1189/jlb.4MR1114-524R.

[36] B. Hamza, E. Wong, S. Patel, H. Cho, J. Martel, D. Irimia, Retrotaxis of human neutrophils during mechanical confinement inside microfluidic channels, Integr. Biol. Quant. Biosci. Nano Macro 6 (2014) 175–183, https://doi.org/10.1039/c3ib40175h.

[37] C. Zhang, C. Li, X. Jia, K. Wang, Y. Tu, R. Wang, K. Liu, T. Lu, C. He, In vitro and in vivo anti-inflammatory effects of polyphyllin VII through downregulating MAPK and NF-κB pathways, Molecules 24 (2019) 875, https://doi.org/10.3390/molecules24050875.

[38] H. Tagashira, A. Miyamoto, S. Kitamura, M. Tsubata, K. Yamaguchi, K. Takagaki, G. Imokawa, UVB stimulates the expression of Endothelin B receptor in human melanocytes via a sequential activation of the p38/MSK1/CREB/MITF pathway which can Be interrupted by a French maritime pine bark extract through a direct inactivation of MSK1, PLoS One 10 (2015) e0128678, https://doi.org/10.1371/journal.pone.0128678.

[39] T. Hoshino, M. Matsuda, Y. Yamashita, M. Takehara, M. Fukuya, K. Mineda, D. Maji, H. Ihn, H. Adachi, G. Sobue, Y. Funasaka, T. Mizushima, Suppression of melanin production by expression of HSP70, J. Biol. Chem. 285 (2010) 13254–13263, https://doi.org/10.1074/jbc.M110.103051.

[40] I. Svanberg, R. Sõukand, Ł. Łuczaj, R. Kalle, O. Zyryanova, A. Dénes, N. Papp, A. Nedelcheva, D. Šeškauskaitė, I. Kołodziejska-Degórska, V. Kolosova, Uses of tree saps in northern and eastern parts of Europe, Acta Soc. Bot. Pol. 81 (2012) 343–357, https://doi.org/10.5586/asbp.2012.036.

[41] M. Moriyama, E. Naru, Y. Misaki, A. Hayashi, No. 3 Vol. 42, No. 2, pp. 94–101, 2008 Effect of Birch (*Betula* platyphylla Sukatchev var. japonica Hara) sap on cultured human epidermal keratinocyte differentiation, Int. J. Cosmet. Sci. 31 (2009) 159–160, https://doi.org/10.1111/j.1468-2494.2008.00466_3.x.

[42] H.-C. Tang, Y.-C. Chen, Identification of tyrosinase inhibitors from traditional Chinese medicines for the management of hyperpigmentation, SpringerPlus 4 (2015) 184, https://doi.org/10.1186/s40064-015-0956-0.

[43] D.J. Mathew, J.M. Sánchez, C. Passaro, G. Charpigny, S.K. Behura, T.E. Spencer, P. Lonergan, Interferon tau-dependent and independent effects of the bovine conceptus on the endometrial transcriptome, Biol. Reprod. 100 (2019) 365–380, https://doi.org/10.1093/biolre/ioy199.

[44] J. Zheng, R.-J. Zhang, Y.-M. Chen, X. Ye, Q.-X. Chen, D.-Y. Shen, Q. Wang, Synthesis of caffeic acid ester morpholines and their activation effects on tyrosinase, Process Biochem. 62 (2017) 91–98, https://doi.org/10.1016/j.procbio.2017.07.004.

[45] J.-W. Kim, H.-I. Kim, J.-H. Kim, O.-C. Kwon, E.-S. Son, C.-S. Lee, Y.-J. Park, Effects of ganodermanondiol, a new melanogenesis inhibitor from the medicinal *mushroom Ganoderma lucidum*, Int. J. Mol. Sci. 17 (2016) 1798, https://doi.org/10.3390/ijms17111798.

[46] H.M. Kim, K.-A. Byun, S. Oh, J.Y. Yang, H.J. Park, M.S. Chung, K.H. Son, K. Byun, A mixture of topical forms of polydeoxyribonucleotide, vitamin C, and niacinamide attenuated skin pigmentation and increased skin elasticity by modulating nuclear factor erythroid 2-like 2, Molecules 27 (2022) 1276, https://doi.org/10.3390/molecules27041276.

[47] T. Pillaiyar, M. Manickam, V. Namasivayam, Skin whitening agents: medicinal chemistry perspective of tyrosinase inhibitors, J. Enzym. Inhib. Med. Chem. 32 (2017) 403–425, https://doi.org/10.1080/14756366.2016.1256882.