IFSCC 2025 full paper (ABSTRACT N° IFSCC2025-397)

Dihydromyricetin: extraction, efficacy tests and its application in cosmetics

Xiaofang Tan [1,*], Xingqi Zhang [1], Wenfeng Xu [2] , Shiwei Li [2] , Ruixue Liu [1]

[1] Guangdong Bawei Biotechnology Corporation,Guangzhou,China
[2] Guangdong Youzhi Testing Technology Corporation,Guangzhou,China

1. Introduction

Ampelopsis grossedentata(Hand.-Mazz.)W.T.Wang(AG), has been used as herbal tea for a long time in China. It has a variety of ingredients including flavonoids, polyphenols, polysaccharides, steroids, terpenes, volatile components, amino acids and mineral elements, among them flavonoids and polyphenols are the main non-volatile active components [1]. Flavonoids in AG include dihydromyricetin(DMY), myricetin, myricitrin, quercetin, kaempferol, etc. DMY is the main flavonoids in AG, and its content in leaves is up to 10%~35% [2-3]. DMY is a natural dihydroflavonol compound, shows multiple health-benefiting activities including antioxidant, anti-inflammatory, immunomodulatory, hypoglycemic, anti-hypertension, lipid-lowering, and hepatoprotective effects [4-6].

At present, the antioxidant properties of AG and DMY(or used as extract from *AG*) have been applied in food industry, to increase food shelf life by reducing oxidative deterioration, and to replace synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butylhydroquinone (TBHQ) [7-8]. As natural antioxidants they can attend consumer's increasing desire for clean labels. They can also be used in cosmetics, but DMY monomer has not been in the list of used raw materials for cosmetics in China, and there are few efficacy reports on its application in cosmetics. In this paper, we studied the extraction process, identification, efficacy evaluation, and application of DMY from AG, developed a multi-functional and high active product of DMY to use in cosmetics.

2. Materials and Methods

2.1. Preparation of DMY

AG material was obtained from Hengdong County, Hunan Province, China. The dried leaves of AG was put into deionized water (the solid−liquid ratio was 1:10, g:ml), the mixture was heated at 90℃ for 1h and then filtered while it was hot, the filter residue was collected and extracted under the same conditions for 2 times, the filtrate was collected, combined and cooled to room temperature, then transferred to a refrigerator at 4℃ for 12h, centrifuged to

collect the precipitate. The precipitate was redissolved in ethanol at room temperature (g:ml, additive amount of ethanol was 2 times relative to the leaves), then removed insoluble matter by filtration, the filtrate was evaporated using a rotary evaporator to remove the ethanol to obtain paste. The paste was redissolved in 20% ethanol aqueous solution (g:ml, 3 times relative to the leaves) with 0.5% (w/w, relative to the leaves) active carbon, heated for 10min by stirring, then filtered while it was hot, the filtrate crystallized at room temperature for 12h, then filtered to get the filter cake, this process was repeatedly for 2-3 times. Dispersed the filter cake and dried them in a vacuum drying oven at 45℃ to obtain dry material of DMY [7-9].

2.2. Measurements and Identification

The DMY standard sample and DMY sample were identified by analytical techniques including HPLC, IR, MS, NMR.

2.3. *In vitro* efficacy tests

DPPH free radical scavenging test [10]: Prepared 0.05mg/mL of DPPH standard stock solution and 0.05mg/mL ascorbic acid standard solution with 75.00%(v/v) ethanol aqueous solution. The sample was dissolved to 0.50 mg/mL in deionized water. The sample solution and DPPH standard stock solution were mixed fully (1:1) and was incubated in darknesslet at 25℃ for 30minutes. Measured the absorbance at 517nm. The amount of DPPH scavenging capacity was calculated as follows: $[1 - (A_i - A_j)/A_c] \times 100\%$, where A_i , A_j and A_c represent the absorbances of the sample, BC (without DPPH) and NC (without sample), respectively.

ABTS free radical scavenging test [11]: Prepared 7mmol/L of ABTS and 2.45mmol/L potassium sulfate with distilled water, mixed the two solutions (1:1), and placed in dark at room temperature overnight (12~16 h). ABTS solution was diluted with phosphoric acid buffer (10mmol/L, pH7.4) , so that its absorbance was 0.700 at 734nm, that is ABTS working solution. Mixed the sample solution and ABTS working solution fully (1:9) and incubated in darkness at 25℃ for 30minutes. Measured the absorbance at 734nm.The amount of ABTS scavenging capacity was calculated as follows: $[1 - (A_i - A_j)/A_0] \times 100\%$, where A_i , A_j and A_0 represent the absorbances of the sample, BC (without ABTS) and NC (without sample), respectively.

Oil antioxidant test: By adding the samples to oil, and placing them at 65℃ for 28days, investigating the change of peroxide values, to observe the antioxidant activity of the samples on oils. The peroxide in oil reacted with potassium iodide (KI) under acidic conditions, releasing free iodine (I_2). By titrating with sodium thiosulfate ($Na_2S_2O_3$), the amount of iodine generated was used to calculate the peroxide value.

ROS free radical scavenging in zebrafish embryos: Healthy 24hpf zebrafish embryos were selected and treated with phenylthiourea (PTU) solution for 24h, then treated with the samples and incubated at 28℃ for 24h. ROS staining solution was added and the reaction was kept away from light at 28℃ for 1h. The fluorescence image was captured using a fluorescence microscope equipped with a color digital camera. The fluorescence intensity was quantified using ImageJ software.

Promotion of caudal fin regeneration in zebrafish embryos: Healthy zebrafish embryos without hatched were selected, removed the caudal fin of zebrafish embryos to establish injured model. Then treated with the samples and incubated for 48 hours. The image was captured using a stereoscope.

Promotion tests of Type I collagen in HFF-1 cells: Test samples were serially diluted and administered to HFF-1 cells, non-cytotoxic concentrations (cell viability > 90%, confirmed via MTT assay and morphological integrity) were selected for subsequent evaluation. In detection, cells were seeded into 96-well plates and incubated at 37°C/5%CO_2 for 24h, then DMEM medium containing the sample was added to each well, medium without samples was

blank control and medium containing TGF-β was set as positive control. The cells were cultured at 37℃ and 5% CO_2 for 24h. The content of human type I collagen protein was measured after cultivation according to the instructions of the ELISA kit. The promotion rate of Type I collagen was calculated as follows: (S/C-1) × 100%, where S and C represent the type I collagen protein level of sample group and blank control group.

LPS-induced inhibition of cellular inflammatory factor IL-6: Test samples were serially diluted and administered to RAW264.7 murine macrophages, non-cytotoxic concentrations (cell viability > 90%, confirmed via MTT assay and morphological integrity) were selected for subsequent anti-inflammatory evaluation. In detection, cells were seeded into 96-well plates and incubated overnight at 37°C/5% CO_2, then cells were treated with the sample(which was diluted with cell culture medium containing LPS to a non-toxic concentration), LPS-induced negative controls, and dexamethasone positive controls. Followed by 24~48 h incubation, IL-6 levels in centrifuged supernatants were detected using ELISA kit according to the manufacturer's instructions. The downregulation rate of IL-6 was calculated as follows: $(1-A_S/A_{NC}) \times 100\%$, where A_S and A_{NC} represent the IL-6 level of sample group and negative control group.

Collagenase inhibition assay: The sample was pre-incubated with collagenase in a Tris buffer (pH=7.5) at room temperature for 10minutes, followed by the addition of FALGPA substrate. After further incubation at 37℃ in dark area, the reaction was terminated. Measured the absorbance of the reaction system at 345nm. The inhibition rate was calculated as follows: $(1-E_S/E_{NC}) \times 100\%$, where E_S and E_{NC} represent the enzyme activity of sample group and negative control group.

Tyrosinase inhibition assay: The sample was pre-incubated with tyrosinase in a phosphate buffered saline at 37℃ for 10 minutes, followed by the addition of L-tyrosine. After further incubation at 37℃ for 30minutes, the reaction was terminated. Measured the absorbance of the reaction system at 475nm. The inhibition rate was calculated as follows: $(1-E_S/E_{NC}) \times 100\%$, where E_S and E_{NC} represent the enzyme activity of sample group and negative control group.

2.4. *In vivo* efficacy tests

After applying the samples on selected test area (facial skin) of adult volunteers for 28 days, the differences of before use(D0) and after 28 days using (D28) in the moisture content of the stratum corneum, skin elasticity, skin tightness in the test area and the corresponding control area were compared to evaluate the efficacy of the samples.

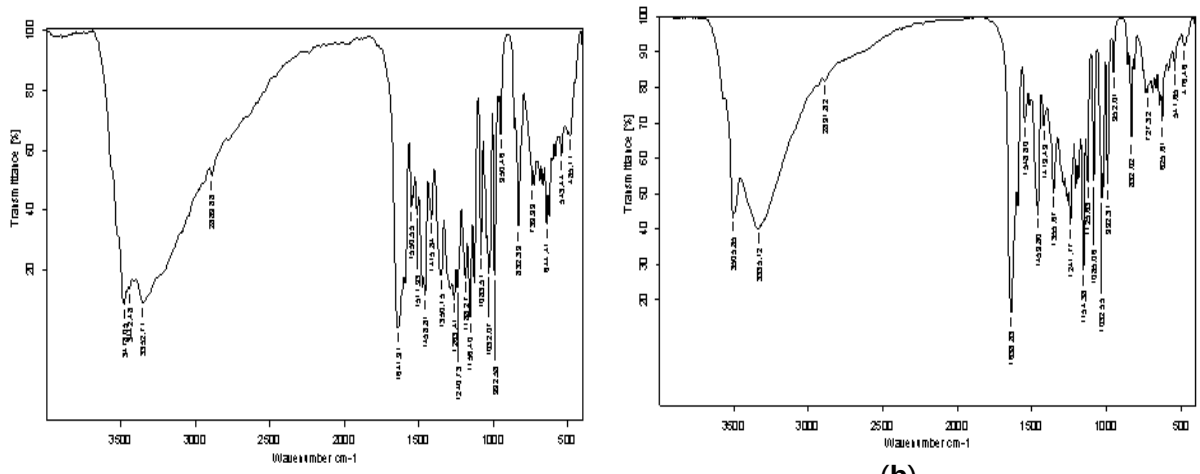
2.5. Statistical Analysis

All experiments were performed in triplicate. Statistical analyses were conducted using Minitab 17. Significance levels were defined as: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $P < 0.0001$; "ns" denotes nonsignificant differences ($p > 0.05$).

3. Results

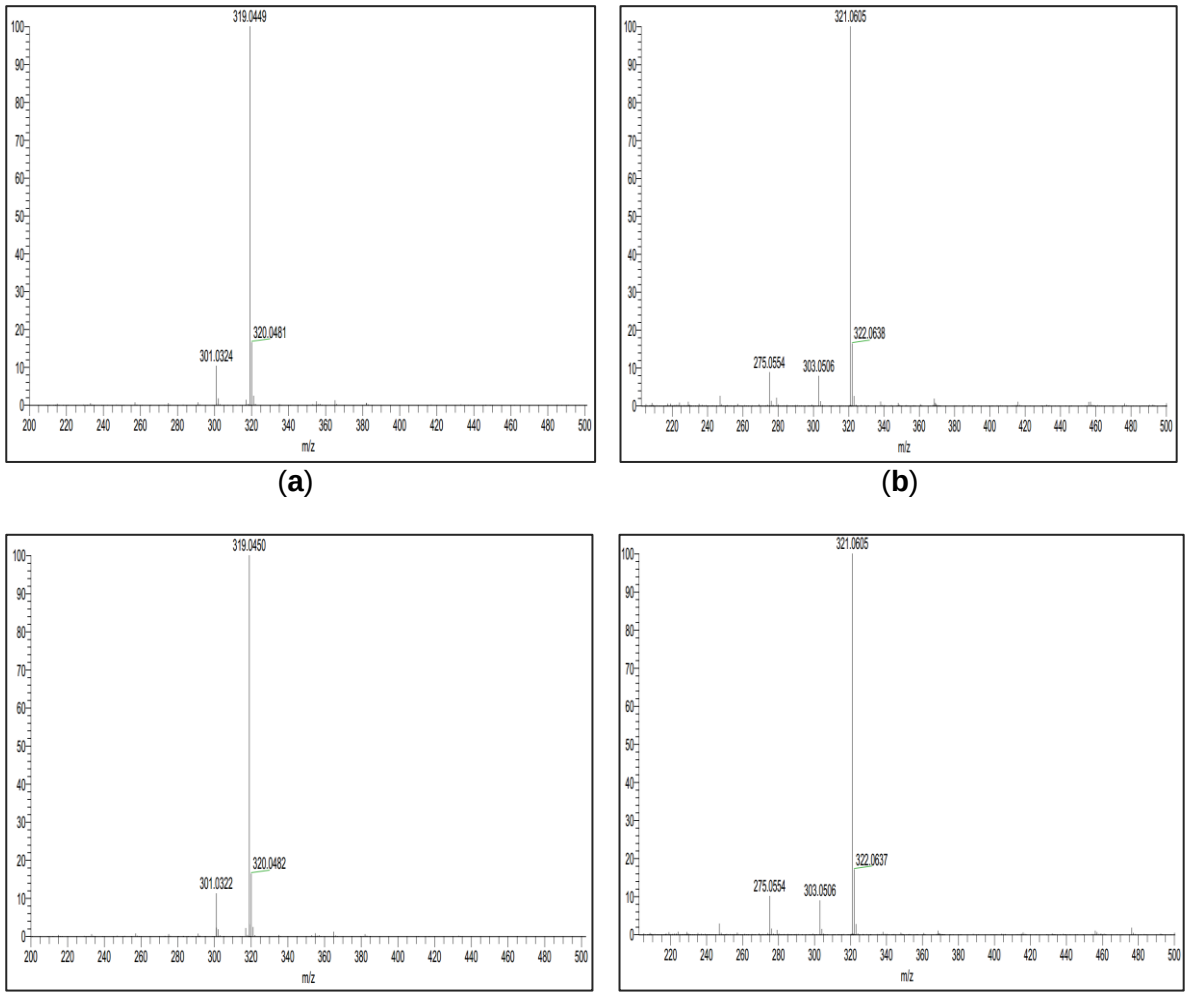
3.1. Identification

The DMY sample extracted from AG was an off white to light yellow powder, with the content of principal component DMY over 95%(three batches of samples were tested, the content were 95.29%, 96.22%, and 98.49%). The infrared absorption spectrum, mass spectrum, nuclear magnetic resonance spectrum, and spectral data of the sample were consistent with the DMY standard, that indicated the principal component of the sample was DMY.



(**a**) (**b**)

Figure 1. (a)Infrared absorption spectrum of the sample.(b)Infrared absorption spectrum of the DMY standard.



(**a**) (**b**)

(**c**) (**d**)

Figure 2. (a)Mass spectrum of the sample(negative ion scanning).(b)Mass spectrum of the sample(positive ion scanning).(c)Mass spectrum of the DMY standard(negative ion scanning).(d)Mass spectrum of the DMY standard(positive ion scanning).

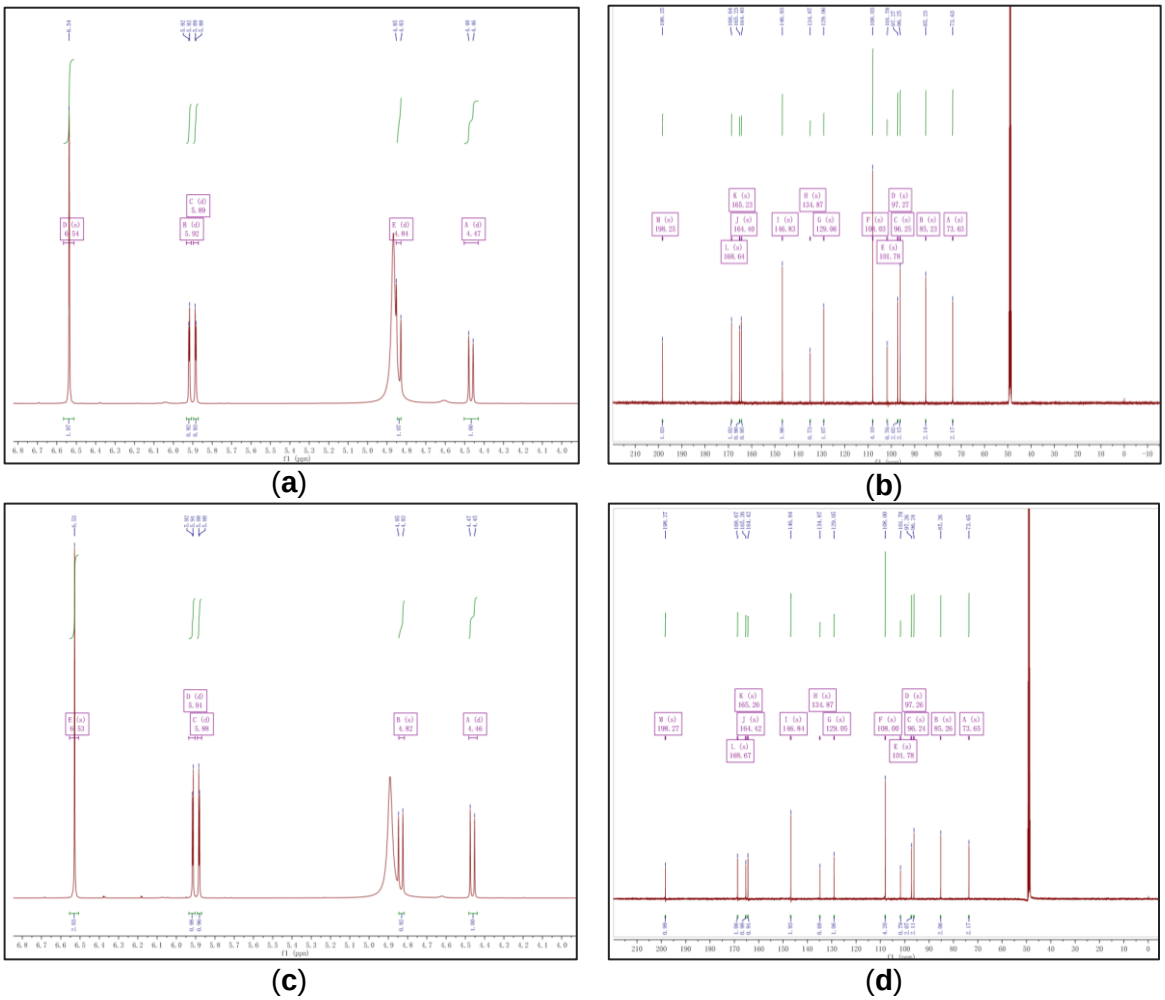


Figure 3. (a)^{1}H NMR spectrum of the sample.(b)^{13}C NMR spectrum of the sample.(c)^{1}H NMR spectrum of the DMY standard.(d)^{13}C NMR spectrum of the DMY standard.

3.2. *In vitro* efficacy tests

3.2.1. DPPH and ABTS free radical scavenging test

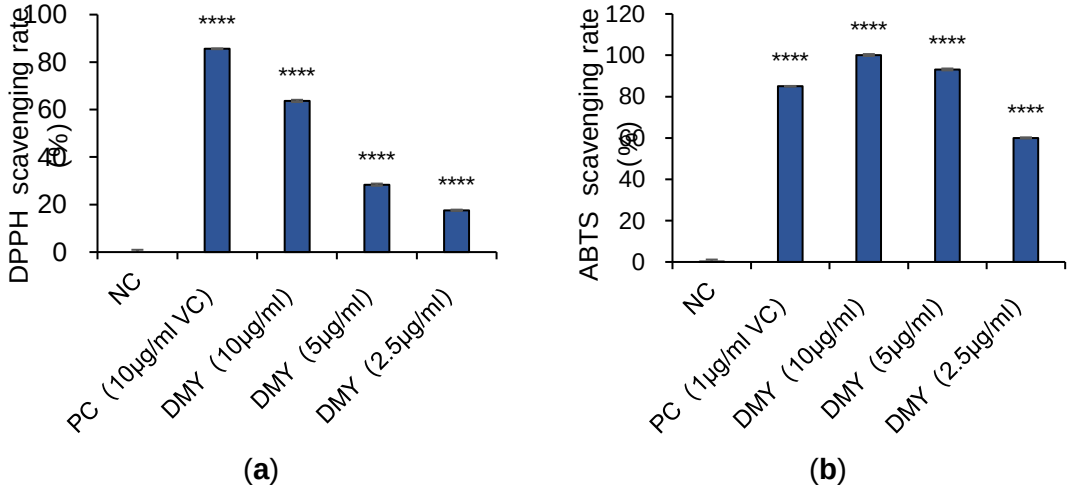


Figure 4. (a) DPPH free radical scavenging ability of the DMY sample.(b) ABTS free radical scavenging ability of the DMY sample.

The DMY sample from AG showed concentration based antioxidant activity. The minimum concentration tested showed significantly ($p < 0.0001$) radical scavenging activity(2.5 μg/mL),

the DPPH and ABTS free radical scavenging rate were 17.60% and 60.02%. Increasing the concentration of DMY, significantly enhanced ($p < 0.0001$) the DPPH and ABTS radical scavenging activity. The maximum DPPH and ABTS radical scavenging activity of DMY were determined to be 63.66% and 100% at 10 μg/mL, respectively.

3.2.2. Oil antioxidant test

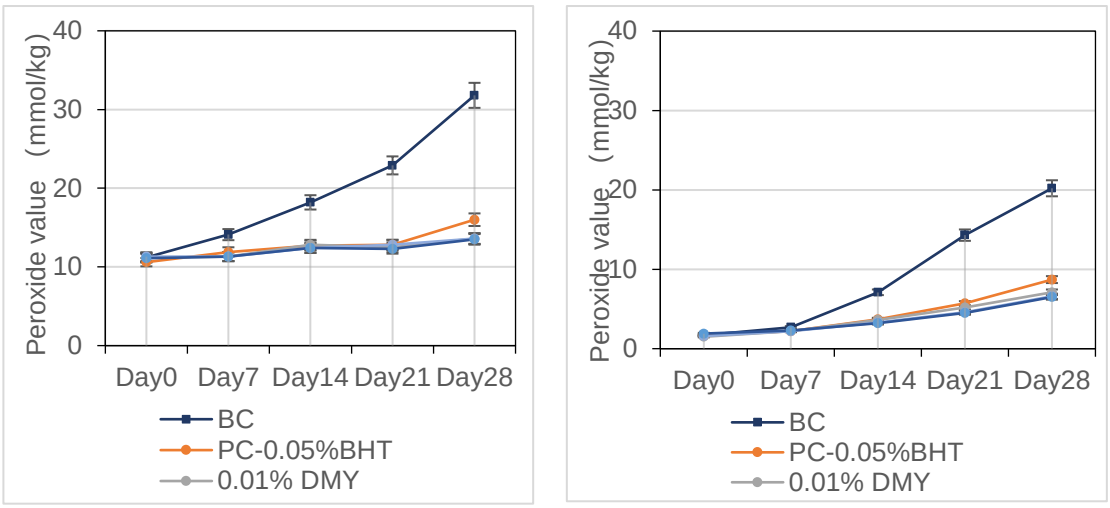


Figure 5. (a) The oxidation process of the camcha seed oil with antioxidant. (b).The oxidation process of olea europaea oil with antioxidant. No antioxidants added in the blank control(BC).

The peroxide value of camellia seed oil and olea europaea oil increased significantly at high temperatures, indicating that they were gradually oxidized and rancid. Adding 0.01% of the DMY sample in olea europaea oil and camcha seed oil, at 65℃ for 28days, the oxidation process was delayed by 65.69% and 88.35%, respectively, which were better than 0.05%BHT (62.16% and 72.30%), showed that DMY can effectively inhibit oil oxidation and can be used as antioxidant in cosmetics.

3.2.3. ROS free radical scavenging and promotion tests of caudal fin regenerationin in zebrafish embryos

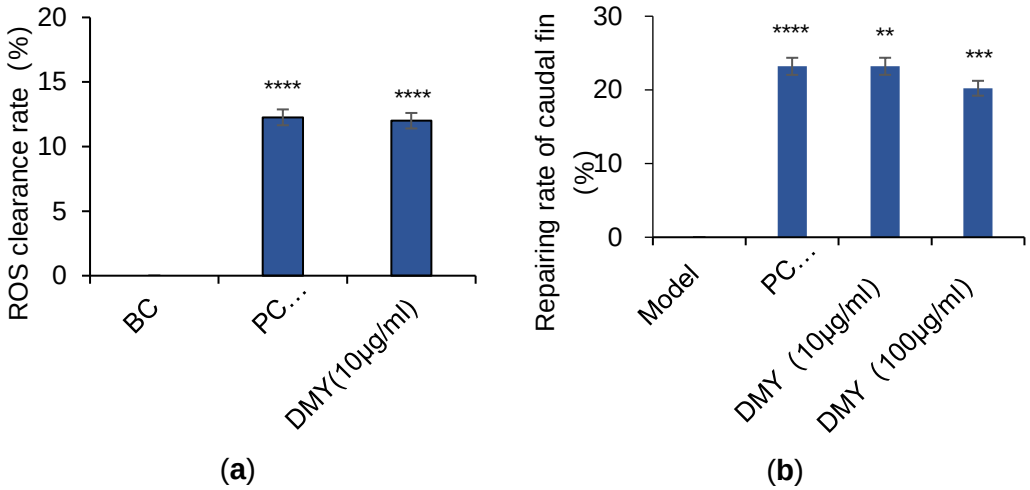
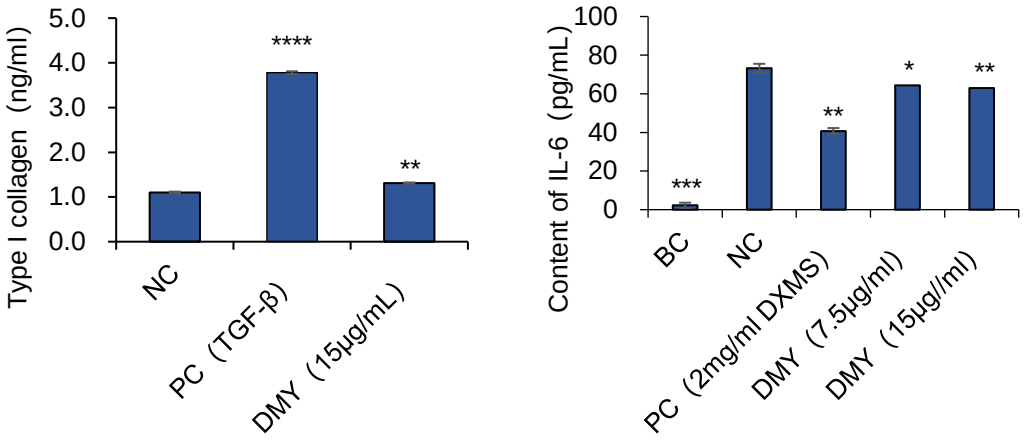


Figure 6. (a) ROS free radical scavenging ability of the DMY sample in zebrafish embryos.(b) Ability of the DMY sample to promote caudal fin regeneration in zebrafish embryos .N=20.

The survival rate of fish embryos in each test group was more than 90% after exposure, indicating that the test was valid. When the concentration of DMY was 10 μg/ml, the scavenging rate of reactive oxygen species (ROS) in zebrafish embryos was 12.00%, which was significantly different from the blank control ($P < 0.05$), better than the positive control glutathione(100μg/ml GSH). (Figure 6.(a))

When the concentration of the DMY sample was 10 μg/ml and 100 μg/ml, the promotion rate of zebrafish tail fin repairing was 23.20% and 20.22%, which was significantly different from the model control ($P < 0.05$). (Figure 6.(b))

3.2.4. Promotion tests of Type I collagen in HFF-1 cell and LPS-induced inhibition of cellular inflammatory factor IL-6



(**a**) (**b**)

Figure 7. (a) Promotion of Type I collagen in HFF-1 cells by the DMY sample.(b) LPS-induced inhibition of cellular inflammatory factor IL-6 by the DMY sample.

When the concentration of DMY was 15.9 μg/ml, the survival rate of HFF-1 cells was 92.43%, and there was no obvious cytotoxicity. Therefore, the concentration of 15 μg/ml was selected for the next experiment, at this concentration, the up-regulation rate of type I collagen was 19.09%, which was significantly different from that in the negative control group ($P < 0.05$). It showed that DMY can significantly promote the production of Type I collagen in HFF-1 cells. (Figure 7.(a))

When the concentration of DMY was 15.9 μg/ml, the survival rate of RAW264.7 cells was 95.82%, and there was no obvious cytotoxicity. Therefore, the concentration of 15 μg/ml and 7.5 μg/ml were selected for the next test. At this concentrations, the down-regulation rate of IL-6 inflammatory factor caused by LPS were 24.57% and 12.12%, which was significantly different from the negative control group ($P < 0.05$). It showed that DMY can significantly inhibit the release of inflammatory factor IL-6.(Figure 7.(b))

3.2.5. Collagenase inhibition assay and tyrosinase inhibition assay

When the concentration of DMY was 15 μg/ml, the inhibition rate of collagenase was 35.00%, the inhibition rate of tyrosinase was 35.23%,which were significantly different from the negative control groups ($P < 0.05$). The DMY sample had the ability to inhibit collagenase and tyrosinase activity.

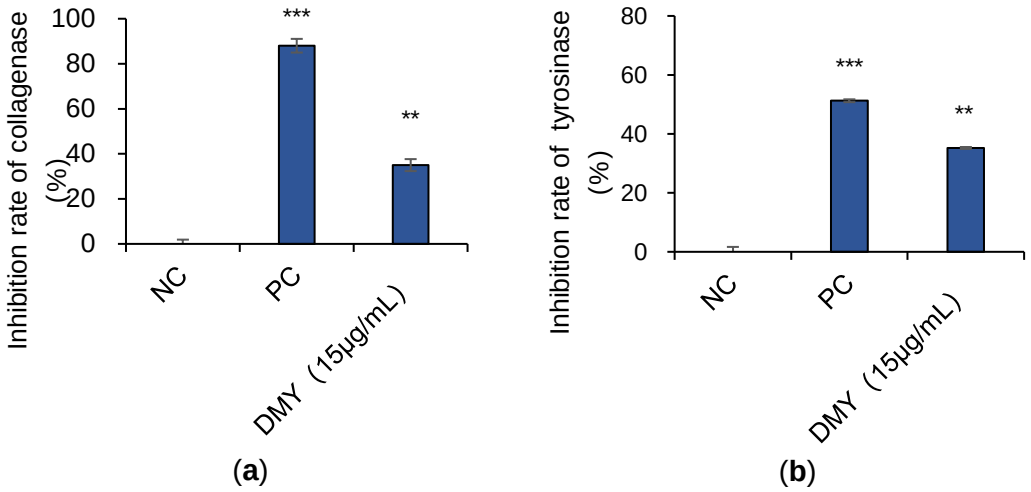


Figure 8. (a) The inhibition ability to collagenase of DMY. (b) The inhibition ability to tyrosinase of DMY.

3.3. *In vivo* efficacy tests

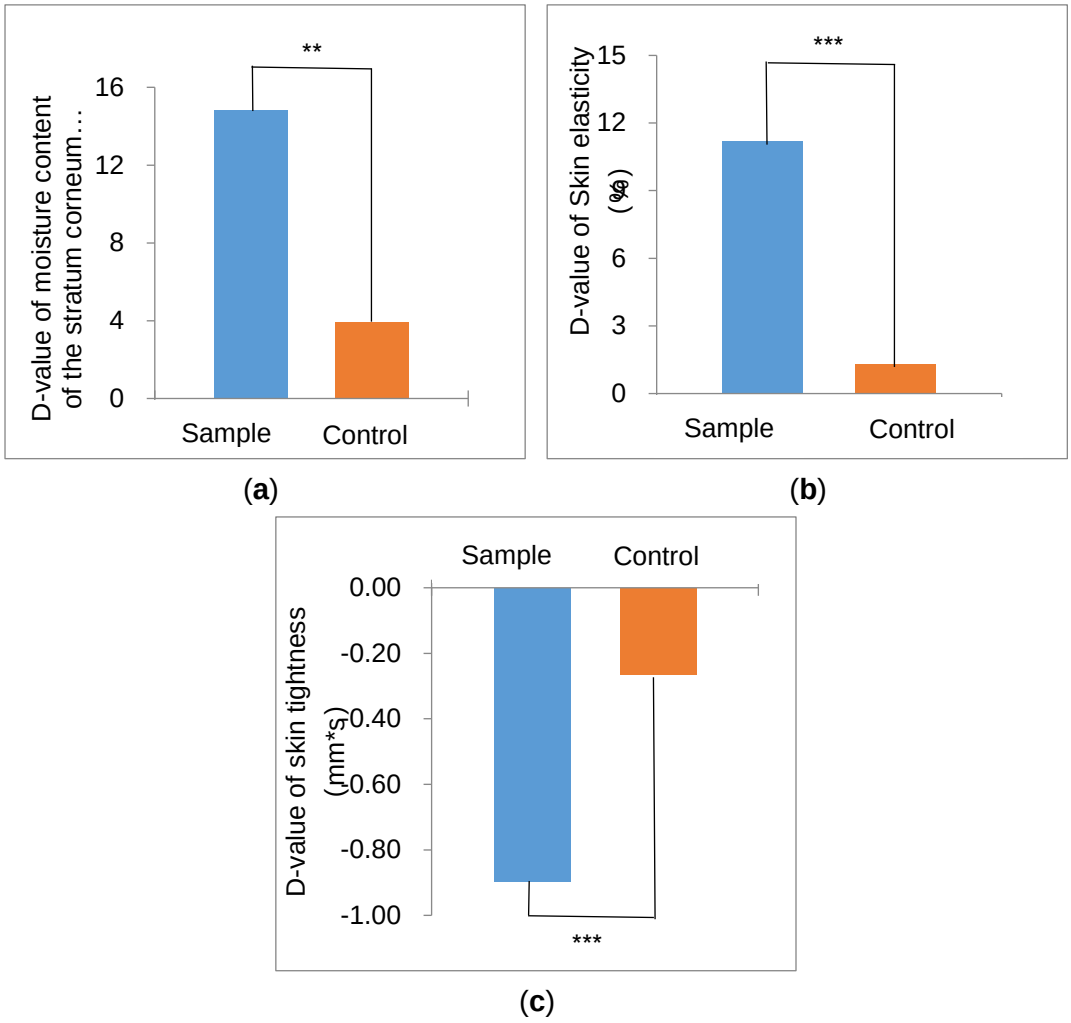


Figure 9. (a) Changes of before and after 28 days using (D28-D0) in the moisture content of the stratum corneum. (b) Changes of before and after 28 days using (D28-D0) in the skin

elasticity.(c) Changes of before and after 28 days using (D28-D0) in the skin tightness. The sample groups were emulsion with 0.5%(w/w) DMY, the control groups were the same emulsion without DMY. N=30.

Test of the emulsion with 0.5%DMY form AG on 30 Chinese volunteers indicated, after 28 days using, the moisture content of the stratum corneum was improved by 34.30%, the skin elasticity by 18.64%, and the skin tightness by 24.52%. Correspondingly, the improvment of self control were 8.95%, 2.21%, 7.14%, respectively. It showed that cosmetic formula with DMY form AG had good moisturizing, tightening, and anti-wrinkling effects.

4. Discussion

DMY is an important plant flavonoid with high health-benefiting activities. But the phenol hydroxyl structure makes it unstable, besides, the low solubility in water and poor membrane permeability limit its use in cosmetics. At present it is mainly used in food and medicine as capsules, or applied as an antioxidant in oil to extend shelf life.

In the above efficacy tests, we founded that effective concentration of DMY in vivo test was much higher than in vitro test. So improving stability, solubility and membrane permeability is the key to promote the application of DMY in cosmetics. Researchers have tried to use DMY in drug delivery systems such as inclusion complexes, nano-encapsule, microemulsion, co-crystals, phospholipid complexes, or acylation to provide higher solubility and bioavailability [13-18]. In order to improve stability and solubility of DMY, we tried to use dipropylene glycol, polysorbate-80, PEG-40 hydrogenated castor oil, isononyl isononanoate, and ppg-26-butanol polyether-26 with DMY to prepare microemulsion, but the use of the microemulsion was limited by pH of formula, which needed to be controlled not higher than pH4.5, and the maximum amount of DMY in the microemulsion was just 5%. So further research is needed to expand the use of DMY in cosmetics.

5. Conclusion

The sample extracted from AG was confirmed that the principal component was DMY, with the content over 95%. Used a variety of test methods to analyze and verify its efficacy, In vitro efficacy tests including DPPH, ABTS and ROS free radical scavenging in zebrafish embryos, oil antioxidant test, promotion tests of caudal fin regeneration in zebrafish embryos, promotion tests of Type I collagen in HFF-1 cell, LPS-induced inhibition of cellular inflammatory factor IL-6, inhibition test of collagenase and tyrosinase etc. In vivo efficacy tests include improving tests of skin elasticity, skin tightness and water content of stratum corneum. Based on these results we concluded that DMY has good anti-oxidation, anti-inflammatory, repairing, moisturizing, anti-wrinkling, potential whitening effects, so it has broad application prospects as a natural functional ingredient in cosmetics.

References

[1] Renata C.V, Carneiro, Liyun Ye, Naerin Baek, Gustavo H.A Teixeira, et al. Vine tea (Ampelopsis grossedentata): A review of chemical composition, functional properties, and potential food applications. Journal of Functional Foods. Volume 76, January 2021, 104317.

[2] Tian S, Zhang Y, Yang Y, Yang W, & Gong Y. Dihydromyricetin in ampelopsis grossedentata by reversed-phase high performance liquid chromatograpohy. Journal of Hunan Agricultural University, 2002, 28(1), 32–34.

[3] Gao W, Lee S. U, Li J, & Lee J. W. Development of improved process with treatment of cellulase for isolation of ampelopsin from dried fruits of ampelopsis grossedentata. Bioresources, 2016, 11(1), 2712–2722.

[4] Li H, Li Q, Liu Z, Yang K, Chen Z, Cheng Q, Wu L. The Versatile Effects of Dihydromyricetin in Health. Evid Based Complement Alternat Med. 2017;2017:1053617.

[5] Dan L, Yiqin M , Lijun D, Xin-An Z. Dihydromyricetin: A review on identification and quantification methods, biological activities, chemical stability, metabolism and approaches to enhance its bioavailability.Trends in Food Science & Technology. 91(2019) 586-597.

[6] Wang R, Pi J, Su XH, et al. Dihydromyricetin suppresses inflammatory responses in vitro and in vivo through inhibition of IKKb activity in macrophages[J]. Scanning, 2016, 38(6): 901-912.

[7] Caihua J, Mingxing Z, Weibo M, Jinghuan L, Siming Z, Shanbai X, Xuebo H. Evaluation of antioxidant properties of the different tissues of vine tea (Ampelopsis grossedentata) in stripped canola oil and sunflower oil. Vol. 0, Journal of Food Science.Iss. 0, 2020

[8] Baek N, Neilson A. P, Eigel W. N, O'Keefe S. F. Antioxidant properties of a dihydromyricetin-rich extract from vine tea (Ampelopsis grossedentata) in menhaden oil. Research & Reviews: Journal of Botanical Sciences, 2015, 4(3), 53-63.

[9] Hongchao H, Fan L, Mingjie W, Zhihuan F, et al. New Method for Extracting and Purifying Dihydromyricetin from Ampelopsis grossedentata. ACS Omega 2020, 5, 13955-13962.

[10] Sarkar P, Stefi R.V, Pasupuleti M, Paray B.A, Al-Sadoon M.K, Arockiaraj J. Antioxidant molecular mechanism of adenosyl homocysteinase from cyanobacteria and its wound healing process in fibroblast cells. Mol. Biol. Rep. 2020, 47, 1821–1834.

[11] Sannasimuthu A, Kumaresan V, Anilkumar S, Pasupuleti M, Ganesh M.R, Mala K, Paray B.A, Al-Sadoon M.K, Albeshr M.F, Arockiaraj J. Design and characterization of a novel Arthrospira platensis glutathione oxido-reductasederived antioxidant peptide GM15 and its potent anti-cancer activity via caspase-9 mediated apoptosis in oral cancer cells. Free Radic. Biol. Med. 2019, 135, 198-209.

[12] Kang M.C, Cha S.H, Wijesinghe W.A.J.P, Kang S.M, Lee S.H, Kim E.A, Song C.B, Jeon Y.J. Protective effect of marine algae phlorotannins against AAPH induced oxidative stress in zebrafish embryo. Food Chem. 2013,138, 950–955.

[13] Liu B, Ma Y, Yuan C, Su C, Hu L, Wang J. Characterization, stability and antioxidant activity of the inclusion complex of dihydromyricetin with hydroxypropyl-β-cyclodextrin. Journal of Food Biochemistry, 2012, 36(5), 634–641.

[14] Dalcin A, Santos C. G, Gündel S. S, Roggia I, Raffiffiffin R. P, Ourique A. F, et al. Anti biofifilm effffect of dihydromyricetin-loaded nanocapsules on urinary catheter infected by pseudomonas aeruginosa. Colloids Surface B Biointerfaces, 2017, 156, 282–291.

[15] Solanki S. S, Sarkar B, Dhanwani R. K. Microemulsion drug delivery system: For bioavailability enhancement of ampelopsin. Isrn Pharmaceutics, 2012(2), 108164.

[16] Wang C. G, Tong Q, Hou X, Hu S, Fang J, Sun C. C. Enhancing bioavailability of dihydromyricetin through inhibiting precipitation of soluble cocrystals by a crystallization inhibitor. Crystal Growth & Design, 2016, 16(9), 5030–5039.

[17] Liu B, Du J, Jie Z, Chen C, et al. Characterization and antioxidant activity of dihydromyricetin-lecithin complex. European Food Research and Technology, 2009(230(2)), 325-331.

[18] Cao S. L, Deng X, Xu P, Huang Z. X, Zhou J, Li X. H, et al. Highly efficient enzymatic acylation of dihydromyricetin by the immobilized lipase with deep eutectic solvents as cosolvent. Journal of Agricultural and Food Chemistry, 2017,65(10), 2084–2088.