Optimization of Extraction Conditions for Total Flavonoids from *Fructus Aurantii Immaturus* and Its Anti-UVB Radiation Activity

Xu CHANG[1], Yanhui WANG[1], Ximing LIU[1], Yunge MA[2], Yunhao LIU[1], Xiaobo CHEN[1], Mengyao SUN[1], Liyan LI[1]*

1. Medical School, Huanghe Science and Technology College, Zhengzhou 450063, China; 2. College of pharmacy, Henan University, Kaifeng 450046, China

Abstract [**Objectives**] Optimum extraction conditions of total flavonoids from *Fructus Aurantii Immaturus* (TFFAI) and its resistance activity to ultraviolet radiation were investigated in present research. [**Methods**] The optimal extraction conditions of TFFAI were determined by single factor and orthogonal experiments, and the survival rate of TFFAI on HaCaT cells irradiated with UVB rays was investigated. It's antioxidant capacity was determined by ABTS. [**Results**] The results showed that the highest yield of TFFAI was obtained with 70% ethanol at a solid-to-liquid ratio of 1∶50 (*w/v*) and 40 ℃ for 1.5 h by single-factor and orthogonal experiments. Total flavonoids (0.25 - 1.00 mg/ml) could significantly improve the survival rate of HaCaT cell line. Meanwhile, the maximum absorption peak of TFFAI was found at 283 nm, and *in-vitro* antioxidant experiment identified that TFFAI had a good clearance rate to ABTS. It suggestes that TFFAI could protect the cells from UVB damage by absorption of UVB rays and anti-oxidation. [**Conclusions**] TFFAI played a protective role on UVB irradiated cells through UVB ultraviolet absorption and antioxidant pathways.

Key words *Fructus Aurantss Immaturus*; Total flavonoids; Optimization of extraction conditions; UVB radiation resistance

DOI:10.19759/j.cnki.2164 - 4993.2023.04.024

Bitter orange is the dried young fruit of lime and its cultivated varieties or sweet orange in the family Rutaceae. The main active components of bitter orange are phenolic acids or flavonoids, volatile oils and alkaloids[1-4], which have the effects of liver protection, antioxidation, antibacterial, antitumor, hypoglycemic and antianxiety[5-10]. Among them, fat-soluble citrus biofanones are unique polymethoxyflavonoids (PMFs) of citrus, which have stronger biological activity than general flavonoids.

More and more people are suffering from skin photodamage caused by excessive ultraviolet radiation due to the aggravation of ozone layer destruction[5], and sunscreens are the most effective way to prevent skin from photodamage. However, in view of distruction disadvantages of traditional sunscreens, it is urgent to develop plant-based sunscreens with no toxic side effects. Many researchers have reported about plant origined UV filters, such as *Lonicera caerulea* L, *Bilberry fruit polyphenols*, *Codium fragile*, *green tea polyphenols*, *Sophora japonica* L, *blackberries*, *raspberries*, *Coffea Arabica*, *Ginkgo Biloba* L, *Vitis vinífera* L, *Amorphophallus*, *Litchi chinensis*, *Hylocereus polyrhizus*, *solanum nigrum*, *Guava-fruit*, *Perilla frutescens*, *etc.*[11-23], but the anti-UV ability of TFFAI has not been reported.

In present research, the anti-UVB irradiation ability of TFFAI was investigated in human epidermal cell model. It is meaningful for developing plant origined products as UVB fiter for sunscreens.

Received: April 27, 2023 Accepted: June 30, 2023

Supported by National Key Program of Innovation and Entrepreneurship Training for College Students (202211834021); Project Funds of Zhengzhou Science and Technology Bureau (ZZSZX202109).

Xu CHANG (2000 -), female, P. R. China, major: clinical medicine.

* Corresponding author. E-mail: liyanli0921@163.com.

Materials and Methods

Materials and Reagents

Fructus aurantii was purchased from the standardized demonstration area of ecological cultivation of *Fructus aurantii* in Hunan Province, and dried at 50 - 55 ℃, then crushed at 100 - 200 mesh. 98% hesperidin analytical standards were purchased from Aladdin. Human immortalized epidermal cell line HaCaT was purchased from Wuhan Procell Life Technology Co., LTD. Penicillin-streptomycin DMEM medium, trypsin-EDTA, and others were purchased from Life Science Co., LTD. Absolute ethanol and concentrated sulfuric acid were analytically pure.

Experimental Methods

Drawing of new hesperidin standard curve 0.01 g of the new hesperidin standard was prepared with 60% ethanol solution to concentrations of 10, 20, 30, 40, 50 and 60 μg/ml, respectively. The absorbance of each standard solution was measured by a UV-VIS spectrophotometer at the wavelength of 283 nm. The regression equation was obtained by drawing a standard curve with absorbance as the ordinate and concentration as the abscission.

Determination of TFFAI According to the maximum absorption at 283 nm of neohesperidin and TFFAI extract, the content of TFFAI extract was determined by ultraviolet spectrophotometry with neohesperidin as the standard. The egression equation was $C = 30.91A - 1.3814$ (correlation coefficient $R^2 = 0.9999$).

Formula for calculation of total flavonoid content:

$$P\ (\%) = \frac{c \times V_1 \times n}{W \times 10^6} \times 100$$

where c is the concentration of total flavonoids (μg/ml) calculated from the regression equation obtained from the new hesperidin standard curve; V_1 is the total volume (ml) of immature orange extract; n is dilution factor; W is the feed amount of immature orange (g).

Optimization of extraction conditions for TFFAI

Single factor experiment The basic conditions of single factor experiments were 60% of ethanol, solid-to-liquid ratio as 1 ∶ 20 (*w/v*), 60 ℃ and 1 h, and then the single variables such as ethanol concentration (50%, 60%, 70%, 80%, 90%), solid-to-liquid ratio (1 ∶ 10, 1 ∶ 20, 1 ∶ 30, 1 ∶ 40, 1 ∶ 50 g/ml), temperature (40, 50, 60, 70, 80 ℃), and time (0.5, 1.0, 1.5, 2.0, 2.5 h). Four single factors were used as variables to investigate effect on the extraction rate of TFFAI.

Orthogonal experiment According to the results of single factor experiments, solid-to-liquid ratio (A), ethanol concentration (B), time (C) and temperature (D) were used as the investigation levels (Table 1), and the extraction rate of TFFAI was used as the index to conduct an L_9 (3^4) orthogonal experiment (Table 2), and the results were verified by the experiment.

Table1 Factors and levels of orthogonal experiment for TFFAI extraction

Level	A (solid-to-liquid ratio) // g/ml	B (ethanol concentration) // %	C (time) h	D (temperature) ℃
1	1 ∶ 30	50	1.0	40
2	1 ∶ 40	60	1.5	50
3	1 ∶ 50	70	2.0	60

Qualitative analysis of the extract

The standard substance of TFFAI extract was scanned under UV using a UV-vis spectrophotometer in the wavelength range from 200 to 450 nm.

Cytotoxicity test of TFFAI on HaCaT cells

Human immortalized epidermal cells were cultured in MEM complete medium (37 ℃, 5% CO_2) containing 10% fetal bovine serum to 80% confluence and seeded into 96-well plates at a density of 5×10^4 cells/ml. On the next day, the medium containing serial concentrations of TFFAI (0.125 – 4.000 mg/ml) were changed to continue to culture for 24 h. Then, the culture medium containing 10% CCK-8 was changed for another 0.5 h, and the OD_{450} value was measured. Cell survival rate was calculated according to Cell survival rate = (*OD* drug group / *OD* normal control group) × 100%, and the cell survival rate of normal control group was set as 100%.

Protective effect of TFFAI on HaCaT cells irradiated by UVB

Cells grown to a confluence of 80% were seeded at a density of 5 × 104 cells/ml into a 96-well plate at 100 μl per well. The next day, the cells were divided into three groups: normal control group, UVB radiation group (30 – 120 mJ/cm^2), UVB radiation and TFFAI group (0.125, 0.25, 0.5, 1 mg/ml). After the cells were incubated with the medium containing serial concentrations of TFFAI for 2 h, the medium was removed and replaced with 30 μl of MEM medium for cell infiltration. To prevent cell damage caused by the heat generated by UV irradiation, the culture plate was placed on ice. After exposure to different doses of UVB (30, 60, 90, 120 mJ/cm^2) (UVB tube, 312 nm, irradiation intensity of 210 μw/cm^2), fresh medium was added to 100 μl, and the cells were further cultured for 24 h. After 0.5 h of incubation with 10% CCK-8 medium, the cells were cultured for 24 h. The OD_{450} value was determined. Cell viability was calculated.

Evaluation of antioxidant capacity of TFFAI (ABTS) *in vitro*

According to the determination method of Wang Hui *et al.*[24], ABTS working solution was prepared with slight changes, and an appropriate amount of TFFAI was weighed. Sample solutions with concentrations of 0.1 – 1.0 mg/ml were prepared with 60% ethanol solution, and then placed in a test tube with ABTS working solution at the amount of 1 ∶ 100. After mixing, the reaction was carried out at 30 ℃ for 10 min, and the absorbance at 734 nm was measured.

Statistical analysis

Experimental data were expressed as mean ± SEM. ANOVA and T test were used for data analysis. $P < 0.05$ was considered as significant difference, and $P < 0.01$ was considered as extremely significant difference.

Results and Discussion

New hesperidin standard curve

The new hesperidin standard curve (Fig. 1) was drawn, and the regression equation was $y = 0.032\,3x + 0.044\,8$, $R^2 = 0.999\,9$.

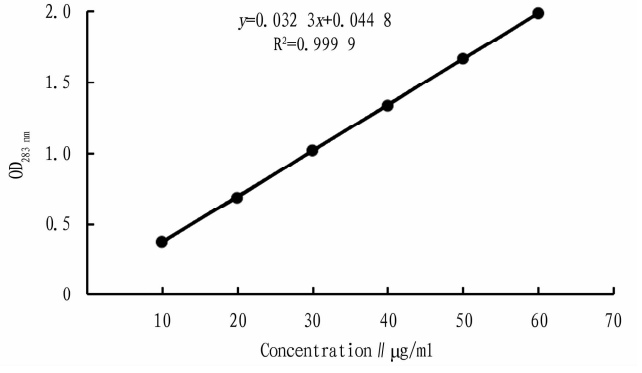

Fig. 1 Standard curve of neohesperidin

Single factor experiments on extraction of TFFAI

Effect of ethanol concentration on extraction yield of TFFAI

The effect of ethanol concentration on the extraction yield of TFFAI was tested under the conditions of solid-to-liquid ratio as 1 ∶ 20 (*w/v*), 50 ℃ and 1 h with only ethanol concentration changed, and the results are shown in Fig. 2. According to the figure, the extraction rate increased first and then decreased with the increase of ethanol concentration, and the extraction rate of TFFAI was the highest under the condition of ethanol concentration of 60%.

Effect of solid-to-liquid ratio on extraction yield of TFFAI

The effect of solid-to-liquid ratio on the extraction yield of TFFAI was tested at 60% ethanol concentration, 50 ℃ and 1 h, and the results are shown in Fig. 3. According to the figure, the extraction rate gradually increased with the increase of solid-to-liquid ratio and then decreased slightly. The increase of solvent amount is conducive to the full dissolution of the effective components, but too large solid-to-liquid ratio will cause the waste of solvent and energy, so the optimal solid-to-liquid ratio of 1 ∶ 40 (*w/v*) was determined.

Effect of temperature on extraction yield of TFFAI The effect of temperature on the extraction yield of TFFAI was tested under the conditions of ethanol concentration of 60%, solid-to-liquid

ratio of 1 ∶ 20 (*w*/*v*), and water bath time of 1 h. The results are shown in Fig. 4. According to the figure, with the increase of temperature, the extraction rate of TFFAI gradually increased first, then decreased and then increased, and the extraction rate of TFFAI was the highest at the temperature of 50 ℃.

Effect of extraction time on the extraction yield of TFFAI

The effect of extraction time on the extraction yield of TFFAI was tested at ethanol concentration of 60%, solid-to-liquid ratio of 1 ∶ 20 (*w*/*v*), and temperature of 50 ℃, and the results are shown in Fig. 5. According to the figure, the extraction rate at 1.5 h (9.7%) decreased slightly, and the extraction rate at 2.5 h (9.93%) increased again, but the range was not large. The optimal extraction time was 1.5 h from the perspective of energy saving.

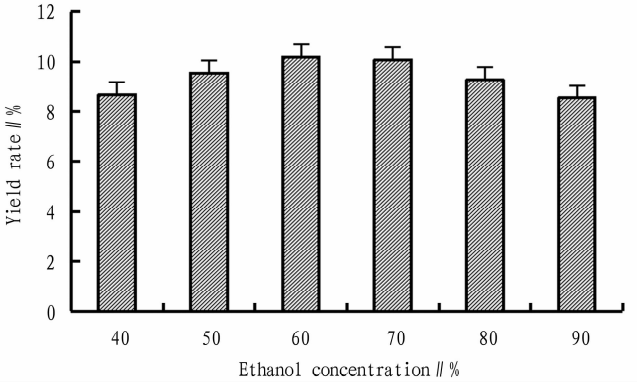

Fig. 2 Effect of ethanol concentration on extraction yield of TFFAI

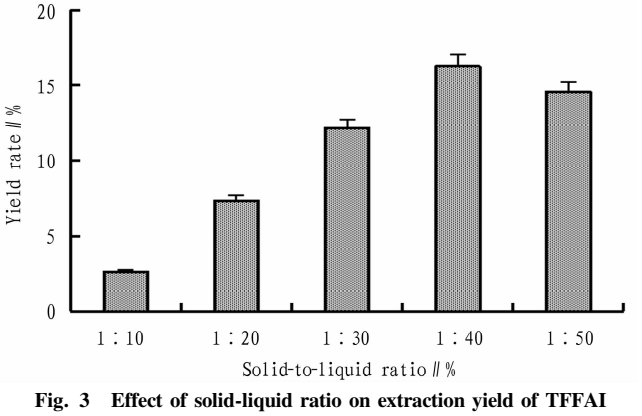

Fig. 3 Effect of solid-liquid ratio on extraction yield of TFFAI

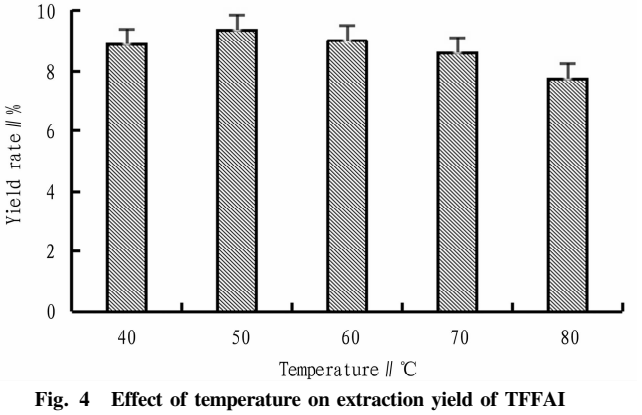

Fig. 4 Effect of temperature on extraction yield of TFFAI

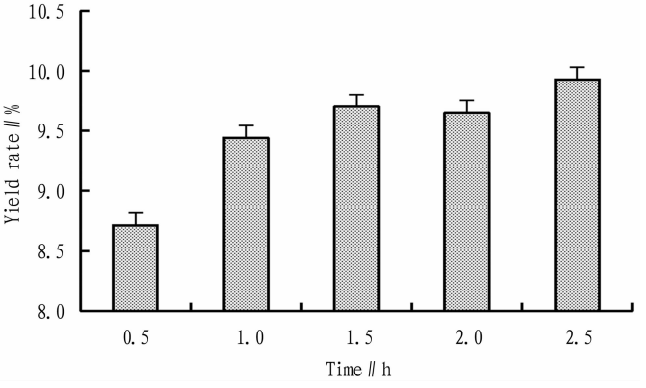

Fig. 5 Effect of time on extraction rate of TFFAI

Optimization of extraction condition of TFFAI

Orthogonal experimental design and results The $L_9(3^4)$ orthogonal test was designed on the basis of the single factor test results, and the results and range analysis are shown in Table 2.

Table 2 $L_9(3^4)$ Table of orthogonal experimental design and results of the orthogonal test

No.	A	B	C	D	Extract // %
1	1	1	1	1	9.68
2	1	2	2	2	9.34
3	1	3	3	3	9.69
4	2	1	2	3	13.84
5	2	2	3	1	13.15
6	2	3	1	2	12.84
7	3	1	3	2	17.09
8	3	2	1	3	16.49
9	3	3	2	1	18.73
K_1	28.71	40.61	39.01	41.56	
K_2	39.83	38.98	41.91	39.27	
K_3	52.31	41.26	39.93	40.02	
R	23.6	2.28	2.9	2.29	

According to the R value in Table 2, the order of the effects of solid-to-liquid ratio, ethanol concentration, time and temperature on the extraction rate of TFFAI was A > C > D > B, that is, solid-to-liquid ratio > time > temperature > ethanol concentration. The optimal extraction condition of TFFAI was $A_3C_2D_1B_3$, which was extracting with 70% ethanol at a solid-to-liquid ratio of 1 ∶ 50 (g/ml) at 40 ℃ for 1.5 h. The TFFAI extraction yield was 19.14% ±1.56% under these conditions.

Preparation of total flavonoids from *Fructus Aurantii Immaturus*

According to the best extraction conditions obtained under "Orthogonal experimental design and results", the TFFAI were extracted, and the combined extraction supernatant was collected, distilled and concentrated under reduced pressure, and freeze-dried in vacuum to obtain the dry powder of TFFAI.

Qualitative analysis of extract

The full UV scanning patterns of standard and immature orange extract sample solutions are shown in Fig. 6 and Fig. 7.

There were obvious peak at 283nm in UV scanning specturum of standard(Fig. 6) and the samplesolution (Fig. 7), indicating that the extract contained neohesperidin.

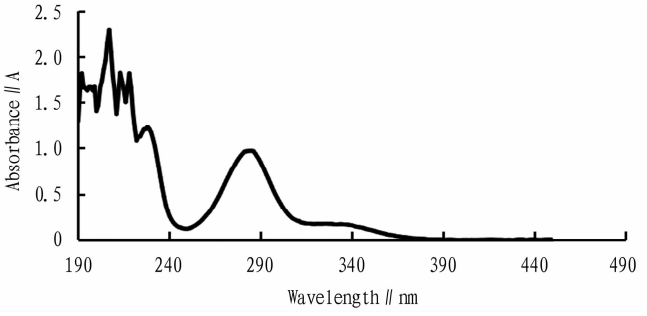

Fig. 6 UV scanning spectrum of standard solution

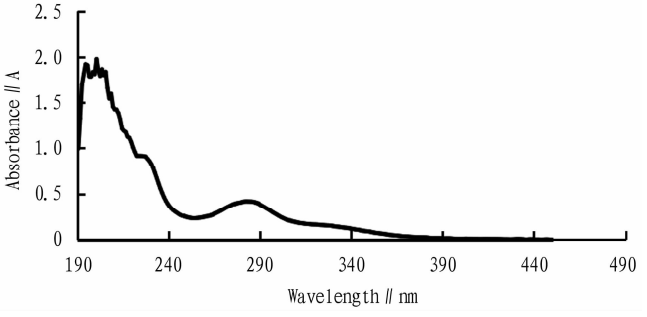

Fig. 7 UV scanning spectrum of sample solution

Evaluation of cytotoxicity of TFFAI

The viability of HaCaT cells was determined by CCK-8 assay after incubation of cells with TFFAI for 24 h, and the results are shown in Fig. 8. According to the figure, the cytotoxicity of TFFAI increased following with the increased concentration, so the maximum concentration of TFFAI in subsequent experiments was 1 mg/ml.

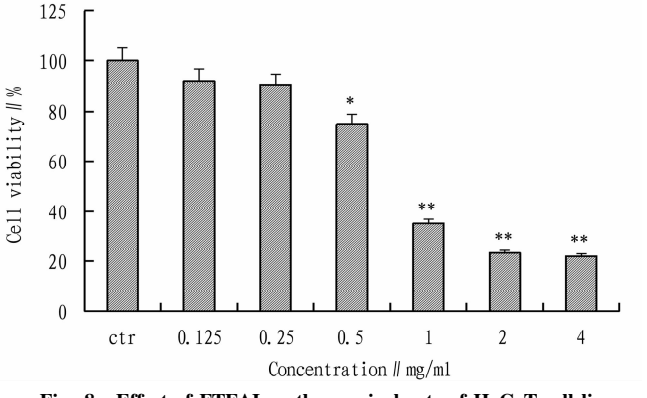

Fig. 8 Effect of FTFAI on the survival rate of HaCaT cell line

Evaluation of anti-UV radiation activity of TFFAI

The effect of TFFAI on the survival rate of HaCaT cells under UVB irradiation was shown in Fig. 9. According to the figure, the cell survival rate decreased significantly with the increase of UVB irradiation dose, and the cell survival rate was only 41.83% at 120 mJ/cm^2. However, pretreatment with TFFAI could significantly improve the cell survival rate, and the protective effect was more obvious with the increase of irradiation dose. 1mg/mL TFFAI could increase the cell survival rate from 46.42% to 69.23% in 90 mJ/cm^2 group. In addition, from the results of cytotoxicity, 1 mg/ml of TFFAI was still poisonous to the cells, and the cell survival rate was only 35.12%, but the UVB radiation on cell survival and UV scanning results showed that the TFFAI had strong ultraviolet absorption ability in UVB segment. The treated epidermal cells preferentially absorbed UVB rays when receiving ultraviolet radiation, reduced the damage of ultraviolet radiation to cells, and greatly improved the survival rate of cells. Therefore, the anti-ultraviolet radiation activity of TFFAI was confirmed.

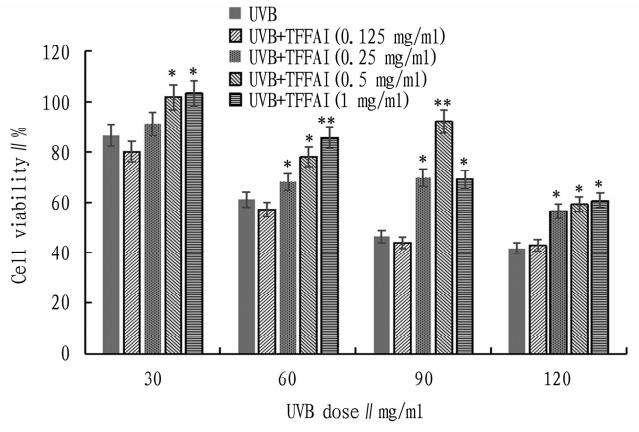

Fig. 9 Effects of TFFAI on the survival rate of UVB-irradiated HaCat cells, $^{*}P<0.05$, $^{**}P<0.01$, vs UVB group

Evaluation of antioxidant capacity of TFFAI *in vitro*

The antioxidant capacity of TFFAI was determined by ABTS clearance rate, and the results are shown in Fig. 10. The clearance rate of ABTs was 97.85% when TFFAI concentration was 0.8 mg/ml, and 100% when TFFAI concentration was 0.9 mg/ml. These results indicated that TFFAI had strong antioxidant capacity.

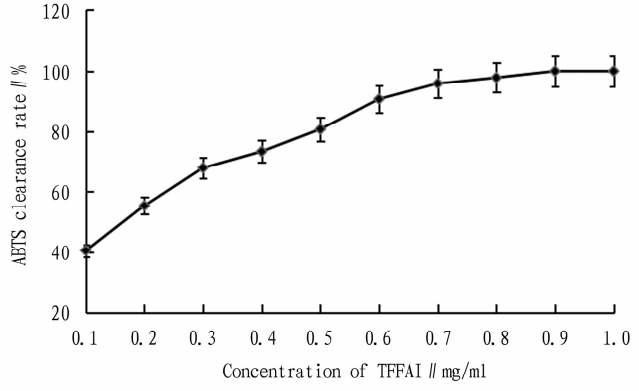

Fig. 10 Evaluation of antioxidant capacity of TFFAI (ABTS clearance rate)

In recent years, scholars have focused on extracting effective ingredients from natural resources that can reduce ultraviolet light damage and enhance skin protection, such as *Lonicera caerulea* L, *Bilberry fruit polyphenols*, *Codium fragile*, *green tea polyphenols*, *Sophora japonica* L, *blackberries*, *raspberries*, *Coffea Arabica*, *Ginkgo Biloba* L, *Vitis vinifera* L, *Amorphophallus*, *Litchi chinensis*, *Hylocereus polyrhizus*, *solanum nigrum*, *Guava-fruit*, *Perilla frutescens*, *etc.* [11-23]. Among them, polyphenols or flavonoids have excellent absorption ability to ultraviolet rays in the 280 – 320 nm band. Zhang *et al.* [25] also concluded that the maximum

absorption value of UVB zone of 30 common Chinese herbal medicine chemicals is mostly between 280 and 320 nm. Li *et al.* [30] summarized the research on UV-resistant components from plants in the past five years and found that most of them are flavonoids. The maximum UV absorption peak of total flavonoids in the present study was also in this range, indicating that it had UV absorption ability. Studies on total flavonoids in passion fruit, leve, kiwi fruit, jujube, green tea and other components[26-29] have found that they have strong antioxidant capacity, thereby reducing intracellular oxidative damage caused by UVB radiation and showing anti-UV radiation activity. By *in vitro* antioxidant experiment shows the optimal ABTS clearance level was obtained with 0.9 mg/ml TFFAI in this study, at the same time by cytology have confirmed that its cells and dermis of UVB radiation has a good protective effect. These tips for health food of the code is sunscreen function development and application of Chinese medicine has a good potentiality. This study provides a certain research basis for the screening and development of plant sunscreens and clinical drug candidates for the treatment of skin photodamage. The specific mechanism of the protection of TFFAI against UV light damage needs to be further studied.

Conclusion

In this study, the yield of TFFAI was the highest (19.14%) under the conditions of solid-to-liquid ratio 1∶50 (*w/v*), 1.5 h, 40 ℃ and 70% ethanol concentration. Cytological experiments showed that TFFAI pretreatment could significantly improve the survival rate of UVB-irradiated cells, 1 mg/ml TFFAI could increase 22.81% of the survival rate on 90mJ/cm^2 dose group. These results indicate that TFFAI has good anti-UVB radiation activity. Meanwhile, TFFAI had the strongest absorption peak at 283 nm, and has a good clearance ability to ABTS. Therefore, TFFAI may played a protective role on UVB-irradiated cells through both UVB ray absorption and antioxidant pathways.

References

[1] JABRI KAROUI I, MARZOUK B. Characterization of bioactive compounds in Tunisian bitter orange (*Citrus aurantium* L.) peel and juice and determination of their antioxidant activity[J]. BioMed Res Int, 2013 (2013): 345415.

[2] GONZáLEZ-MAS MC, RAMBLA JL, LÓPEZ-GREASA MP, *et al.* Volatile compounds in citrus essential oils: A comprehensive review[J]. Front Plant Sci, 2019(10): 1-18.

[3] SUNTAR I, KHAN H, PATEL S, *et al.* An overview on *Citrus aurantium* L.: Its functions as food ingredient and therapeutic agent[J]. Oxid Med Cell Longev., 2018(2018): 7864269.

[4] SINGH B, SINGH JP, KAUR A, *et al.* Insights into the chemical composition and bioactivities of citrus peel essential oils[J]. Food Res Int, 2021(143): 110231.

[5] WU JZ, HUANG GR, Li YJ, *et al.* Flavonoids from Aurantii Fructus Immaturus and Aurantii Fructus: Promising phytomedicines for the treatment of liver diseases [J]. Chinese Medicine, 2020(15): 89-106.

[6] DIVYA PJ, JAMUNA P, JYOTHI LA. Antioxidant properties of fresh and processed *Citrus aurantium* fruit[J]. Cogent Food Agric, 2016(2): 1184119.

[7] KARABIYIKLI S, DE˘GIRMENCI H, KARAPINAR M. Inhibitory effect of sour orange (*Citrus aurantium*) juice on *Salmonella typhimurium* and *Listeria monocytogenes*[J]. LWT Food Sci Technol, 2014(55): 421-425.

[8] LIM SW, LEE D R, CHOI B K, *et al.* Protective effects of a polymethoxy flavonoids-rich *Citrus aurantium* peel extract on liver fibrosis induced by bile duct ligation in mice[J]. Asian Pac J Trop Med, 2016 (9): 1158-1164.

[9] KIM GS, PARK HJ, WOO JH, *et al.* *Citrus aurantium* flavonoids inhibit adipogenesis through the Akt signaling pathway in 3T3-L1 cells[J]. BMC Complement Altern Med, 2012(12): 31.

[10] CHAVES NETO GC, BRAGA JEF, ALVES MF, *et al.* Anxiolytic effect of *Citrus aurantium* L. in Crack Users. Evid. based complement[J]. Evid Based Complement Alternat Med, 2017(2017): 7217619.

[11] LEE C, PARK GH, AHN EM, *et al.* Protective effect of *Codium fragile* against UVB-induced pro-inflammatory and oxidative damages in HaCaT cells and BALB/c mice[J]. Fitoterapia, 2013(86): 54-63.

[12] VOSTALOVA J, ZDARILOVA A, SVOBODOVA A. *Prunella vulgaris* extract and rosmarinic acid prevent UVB-induced DNA damage and oxidative stress in HaCaT keratinocytes[J]. Arch Dermatol Res, 2010 (302): 171-181.

[13] EIMETS CA, SINGH D, TUBESING K, *et al.* Cutaneous photoprotection from ultraviolet injury by green tea poly-phenols[J]. J Am Acad Dermatol, 2001(44): 425-432.

[14] LI LY, HUANG T, LAN C, *et al.* Protective effect of polysaccharide from *Sophora japonica* L. flower buds against UVB radiation in a human keratinocyte cell line (HaCaT cells)[J]. Journal of Photochemistry & Photo-biology, B: Biology, 2019(191): 135-142.

[15] CEFALI LC, FRANCO JG, NICOLINI GF, *et al.* *In vitro* antioxidant activity and solar protection factor of blackberry and raspberry extracts in topical formulation[J]. J Cosmet Dermatol, 2019, 18(2): 539-544.

[16] CHO YH, BAHUGUNA A, KIM HH, *et al.* Potential effect of compounds isolated from *Coffea arabica* against UV-B induced skin damage by protecting fibroblast cells[J]. J Photochem Photobiol B, 2017 (174): 323-332.

[17] CEFALI LC, ATAIDE JA, FERNANDES AR, *et al.* Evaluation of *in vitro* solar protection factor (SPF), antioxidant activity, and cell viability of mixed vegetable extracts from *Dirmophandra mollis* Benth, *Ginkgo Biloba* L, *Ruta Graveolens* L, and *Vitis vinifera* L[J]. Plants, 2019, 8 (11): 453-465.

[18] CEFALI LC, ATAIDE JA, SOUSA IMO. *In vitro* solar protection factor, antioxidant activity, and stability of a topical formulation containing Benitaka grape (*Vitis vinifera* L.) peel extract[J]. Nat Prod Res, 2020, 34 (18): 2677-2682.

[19] THIESEN LC, BRETZKE PE, BITTENCOURT CMDS, *et al.* *Litchi chinensis* leaf extract provides high *in vitro* photoprotection associated to a natural mineral clay[J]. Photodermatol Photoimmunol Photomed, 2020, 36(1): 61-62.

[20] VIJAYAKUMAR R, ABB GANI SS, ZAIDAN UH, *et al.* Exploring the potential use of *Hylocereus polyrhizus* peels as a source of cosmeceutical sunscreen agent for its antioxidant and photoprotective properties[J]. Evid Based Complement Alternat Med, 2020(2020): 7520736.

[21] SALEEM MA, NAZIR A, NAZIR F, *et al.* Comparison of UV protection properties of cotton fabrics treated with aqueous and methanolic extracts of *Solanum nigrum* and *Amaranthus viridis* plants[J]. Photodermatol Photoimmunol Photomed, 2019, 35(2): 93-99.

[22] MOTA MD, COSTA RYS, GUEDES AAS, *et al.* *Guava-fruit* extract can improve the UV-protection efficiency of synthetic filters in sun cream formulations[J]. J Photochem Photobiol B, 2019(201): 111639.

[23] CHOI HJ, SONG BR, KIM JE, *et al.* Therapeutic effects of cold-pressed perilla oil mainly consisting of linolenic acid, oleic acid and linoleic acid on UV-induced photoaging in NHDF cells and SKH-1 hairless mice[J]. Molecules, 2020(25): 989-1007.

(Continued on page 118)

application value of various model algorithms, and puts forward the difficulties and future prospects that may be encountered in the establishment and application of models. In addition, in the modeling practice of species distribution models, the rationality and accuracy of model results are closely related to the correct selection of prediction factors and modeling methods, the interaction between time and space scales and between environmental and geographical factors, and the impact of model extrapolation. Therefore, scholars need to understand the supporting framework of models as much as possible, clarify the way to build models, and correctly select model algorithms. Future research should attach great importance to important historical and geographical factors that lead to current species distribution patterns, and possible role of species ecological niche evolution. In the modeling process, the genealogical biogeography and landscape genetics theories are combined, and the natural processes such as biological interaction are reasonably introduced into the model framework. Meanwhile, a large amount of geographic data (such as remote sensing data) has been effectively utilized, greatly improving the accuracy of models. Therefore, with the development of various models and the improvement of their accuracy, the application of species distribution models in the study of various biological habitats and the prediction of changes in biological habitats caused by climate change will be further promoted, providing a reference for the establishment of biological reserves.

References

[1] JIANG X, NI J. Species-climate relationships of 10 desert plant species and their estimated potential distribution range in the arid lands of northwestern China[J]. Chinese Journal of Plant Ecology, 2005, 29(1): 98 -107. (in Chinese).

[2] ZHAI TQ, LI XH. Climate change induced potential range shift of the crested ibis based on ensemble models [J]. Acta Ecologica Sinica, 2012, 32(8): 2361 -2370. (in Chinese).

[3] ZHANG L, LIU SR, SUN PS, *et al.* Comparative evaluation of multiple models of the effects of climate change on the potential distribution of Pinus massoniana[J]. Chinese Journal of Plant Ecology, 2011, 35(11): 1091 -1105. (in Chinese).

[4] YEE THOMAS, MITCHELL ND. Generalized additive models in plant ecology[J]. Journal of Vegetation Science, 1991(2): 587 -602.

[5] HIRZEL AH, HAUSSER J, CHESSEL D, *et al.* Ecological niche factor analysis: How to compute habitat-suitability maps without absence data [J]. Ecology, 2002(83): 2027 -2036.

[6] ZHU Y, KANG MY. Application of ordination and GLM/GAM in the research of the relationship between plant species and environment[J]. Chinese Journal of Ecology, 2005, 24(7): 807 -811. (in Chinese).

[7] HOLZMANN I, AGOSTINI I, DEMATTEO K, *et al.* Using species distribution modeling to assess factors that determine the distribution of two parapatric howlers (*Alouatta* spp.) in South America[J]. International Journal of Primatology, 2014, 36(1): 1 -15.

[8] WALKER PA, COCKS KD. HABITAT: A procedure for modeling a disjoint environmental envelop for a plant or animal species[J]. Global Ecology and Biogeography, 1991(1): 108 -118.

[9] HEIKKINEN RK, LUOTO M, VIRKKALA R, *et al.* Biotic interactions improve prediction of boreal bird distributions at macroscales[J]. Global Ecology and Biogeography, 2010, 16(6):754 -763.

[10] LIU C, NEWELL G, WHITE M. On the selection of thresholds for predicting species occurrence with presence-only data[J]. Ecology and Evolution, 2016, 6(1): 337 -348.

[11] PATSIOU TS, CONTI E, ZIMMERMANN NE, *et al.* Topo-climatic microrefugia explain the persistence of a rare endemic plant in the Alps during the last 21 millennia[J]. Global Change Biology, 2014, 20(7): 2286 -2300.

[12] SVENNING JC, EISERHARDT WL, NORMAND S, *et al.* The influence of paleoclimate on present-day patterns in biodiversity and ecosystems[J]. Annual Review of Ecology Evolution and Systematics, 2015, 46(1): 551 -572.

[13] FORDHAM DA, SALTRé F, BROWN SC, *et al.* Why decadal to century timescale paleoclimate data is needed to explain presentday patterns of biological diversity and change[J]. Global Change Biology, 2018(24): 1371 -1381.

[14] MEIER ES, EDWARDS TC, KIENAST F, *et al.* Co-occurrence patterns of trees along macro-climatic gradients and their potential influence on the present and future distribution of Fagus sylvatica L[J]. Journal of Biogeography, 2011, 38(2): 371 -382.

[15] VEGA GC, PERTIERRA LR, OLALLA-TÁRRAGA MÁ. MERRAclim, a high-resolution global dataset of remotely sensed bioclimatic variables for ecological modelling[J]. Scientific Data, 2017(4): 170078.

Editor: Yingzhi GUANG Proofreader: Xinxiu ZHU

(Continued from page 114)

[24] WANG H, ZHOU Y. The method of ABTS assay for screening and evaluating antioxidant[J]. Guangzhou Chemical Industry, 2012, 40 (22): 41 -43.

[25] ZHANG H, ZHANG G, CHEN J. Study on the sunproof potential for thirty kinds of chemical constituents from Chinese herb medicine[J]. China Pharmacist, 2010, 13(7): 935 -938. (in Chinese).

[26] ZHANG XL, LI SY, SUN JN, el al. Optimization of extraction process and antioxidant activity of total flavonoids from passion fruit peel by response surface methodology[J/OL]. Feed Research, 2022(16): 83 -88. (in Chinese).

[27] HU XH, LONG JF, YANG SZ, *et al.* Study on toal flavonoids content and antioxidant activity of *Allium tuberosum* in three regions of southeast Guizhou[J]. Journal of Kaili University, 2022, 40(3): 67 -74. (in Chinese).

[28] LI JH, WENG GY, ZHU M, *et al.* Microwave-assisted extraction and antioxidant activities of total flavonoids from kiwi fruit residues[J]. Food Research and Development, 2022, 43(12): 79 -85. (in Chinese).

[29] ZAHRA ABOTORABIL, MOHSEN KHORASHADIZADEH, MINA ARAB, *et al.* Jujube and green tea extracts protect human fibroblast cells against UVB-mediated photo damage and MMP-2 and MMP-9 production[J]. Avicenna Journal of Dhytpmedicine, 2020, 10(3): 287 -296.

[30] LI LY, LAN C, HUANG T, *et al.* Natural products and extracts from plants as natural UV filters for sunscreens: A review [J]. Animal Model and Experiment Medicine, 2022(00): 1 -13.

Editor: Yingzhi GUANG Proofreader: Xinxiu ZHU