In vitro effect of antioxidant activities on Chinese herbal medicine extracts

Chiu, Miin[1]*; Yan, Ren-Ting [1]; Wang, Yu-Shu [1]
Department of Applied Cosmetology, National Tainan Junior College of Nursing, Tainan City, Taiwan

* Chiu, Miin : No.78, Sec. 2, Minzu Rd., West Central Dist., Tainan City, Taiwan;
886-958833626, chiumiin@ms28.hinet.net.

Abstract

The objective of this study is to develop the new raw materials from Chinese herbal medicines (CHMs) that can be used in cosmetic industry. Four clearing heat-fire Chinese herbal medicines - *Richardia scabra* (Rs)、*Taxillus chinensis* (Tc)、*Arundo donax* (Ad) and *Anemarrhena asphodeloides* (Aa) were chosen to carry out the biological activities evaluations of free radical-scavenging ability, ferric reducing antioxidant power (FRAP) , total phenolic content (TPC) and total flavonoid content (TFC). Results showed that the order of scavenging free radical ability in four aqua extracts is BHA > Rs > Tc > Vit.C ≈ Aa. Tc contains slightly higher than 100 mg GAE /g aqua extract and 150 mg QCT /g aqua extract, therefore, it presents a strong reducing ability. Conclusion: *Taxillus chinensis* (Tc) is worth to be selected as the best raw material in manufacturing anti-aging skincare products because of having high extraction ratio (13.5%), strong antioxidative ability and high reducing ability.

Keywords: antioxidant activities, Chinese herbal medicine.

Introduction

In recent years, consumers have paid more and more attention to "natural", "environmental protection" and "plant extraction", so that there is a lot of space for development in this regard for cosmetic industry. We make a comprehensive survey, many products on the market are claimed with a natural and environmentally friendly concept, but

most of the antioxidants used in medicines, food, cosmetics, beverages and even feed are synthetic, these synthetic antioxidants are harmful to health or have potential risks to the environment. Chinese herbal medicines in Taiwan were widely used during the impact of COVID-19, and many of them had strong antioxidant power. We take this as a thought to study the antioxidant power of Chinese herbal medicines, hoping to develop natural antioxidants from Chinese herbal medicines as a development innovation, low-cost, environmentally friendly and safe raw materials for cosmetics and skin care products.

Materials and Methods

The materials used in this study include Chinese herbal medicines and chemicals which are described as follows:

Chinese herbal medicine: Richardia scabra (Rs)、Taxillus chinensis (Tc)、Arundo donax (Ad) and Anemarrhena asphodeloides (Aa).

Chemicals: 95% Ethanol, DPPH (2,2-diphenyl-1-picrylhydrazyl), BHA (Butylated hydroxy-anisole), PBS (Phosphate Buffer Saline), Potassium hexacyanoferrate(III), Iron(III) chloride hexahydrate, TCA (trichloroacetic acid), Folin-Ciocalteus phenol reagent, Sodium carbonate, Gallic acid, Sodium nitrite, aluminum chloride, Sodium hydroxide, Quercetin, etc. are purchased from Xinxin Chemical and Jingming Chemical respectively And friends and trading companies.

Instruments: electronic balance scale coarse scale:(Sartorius/SA07-15US12R, fine scale: SHIMADZU /SA-121A2F-1), electromagnetic heating stirrer (Thermo/ SP88857100), pH meter (model: EUTECH pH-510), pulverizer (Model: RT-04), Rotary Decompression Concentrator (Brand Yamato, Model: RE 200), Manifold Type Freeze Dryer (Brand: UNISS, Freeze-Drying Host Model: FDM-5-50ºC , Vacuum Helper Pu model: VP-200), spectrophotometer (Perkin Elmer® precisely/Lambda 25).

Methods

(1) Sample preparation

In this study, aqua extracts were obtained by microwave and alcohol extracts came from 95% ethanol soak solution.

(2) Determination of pH

Use a pH meter to measure and record the pH value of all extracts.

(3) Antioxidant activity evaluation of all extracts

(i) Free radical scavenging activity assay

The determination of free radical scavenging ability was based on the method described by Shimada et al. and modified. BHA (Butylated hydroxyanisole) was used as the standard substance, and the alcohol substitute sample was used as the blank control group.

Weigh 50 mg of extract to 100 mL, add 1 mL of DPPH to 4 mL of sample, mix thoughtly for 2 hours. Measuring the absorbance at the wavelengths of 517.8 nm for aqueous extraction and extraction, and calculate the free radical scavenging ability of DPPH by the formula. The lower the absorbance value, the stronger the hydrogen supply capacity of the sample and the stronger the ability to scavenge free radicals.

Capability to capture DPPH radical (%)=[1-(A_{517nm}.sample / A_{517nm}.blank)]×100

(2) Ferric reducing antioxidant power (FRAP) assay

Referring to the method of Shi and Dalal, the calibration line was made with BHA as the standard product, and the sample was replaced by RO water as the blank control group.

Weigh 0.025g of the extract to quantify to 25mL, and take 1mL of sample, add 1mL of pH6.6 PBS and 1mL of 1% red blood salt, mix well, and heat to 50°C for 30 minutes in a water bath. After cooling, add 1 mL of 10% TCA, centrifuge at 3000 RPM for 10 minutes, take 2 mL of the supernatant clear liquid, add 2 mL of deionized water and 0.4 mL of 0.1% ferric chloride, and mix for 10 minutes. The maximum absorption wavelength is 700.0 nm for aqueous extraction and wine extraction. Measured at 683.9 nm, and calculated its reducing power by the formula, the higher the absorbance, the stronger the reducing power of the sample.

(3) Total Phenol content

According to the method of Woisky and Salatino, the calibration curve was made with Gallic acid as the standard.

Weigh 25mg of extract to 25mL, and take 0.5mL sample, add 2.5mL 10% phenol reagent (Folin-Ciocalteus phenol reagent) and 2.0mL 10% sodium carbonate solution (Sodium carbonate) and mix well. After 30 minutes of coloring, the maximum absorption wavelength was measured at 760 nm for aqueous extraction and 760 nm for wine extraction.

(4) Total flavonoid content assay

In this experiment, Quercetin was used as a standard to make calibration lines. Referring to the method of Christel with slight modification, 50 mg of the extract was weighed and quantified to 100 mL. Take 0.5mL sample, add 2mL deionized water and 0.15mL 5% sodium nitrite (Sodium Nitrite) and let stand for 5 minutes, add 0.15mL 10% aluminum chloride (aluminium chloride), mix and let stand for 5 minutes, then add 1.2mL Deionized water and 1mL of 1M sodium hydroxide were mixed for 10 minutes, and the color was measured at the maximum absorption wavelength of 355.8 nm for aqueous

extracts and 324.0 nm for alcohol extracts, and the measurement was completed within 15 minutes.

Results

The extraction ratio, pH and various forms of antioxidant power were evaluated and tested for Rs、Tc、Ad and Aa. The results were as the following:

(1) The extraction ratio

The extraction rates of all Chinese herbal medicines after microwave extraction and alcohol soak extraction were summarized in Table 1. The results showed that the extraction ratio of aqueous extract was slightly higher than that of ethanol extract. Both ethanol and aqueous extracts of Tc were more than 10%. The highest extraction ratio of aqueous extract was Anemarrhena asphodeloides. The highest extraction ratio of ethanol extracts was Tc.

Table 1 Extraction ratio of four Chinese herbal medicine (%)

	Rs	Tc	Ad	Aa
Aqua extracts	7.14	13.35	13.3	33.77
Alcohol extracts	7.42	27.11	1.92	7.75

(2) pH value of all extracts

The pH of Chinese herbal medicine aqueous extracts and ethanol extracts was measured, and the results are shown in Table 2. pH range of aqua extracts is 5.86 ± 0.37 (at 23.0±1.8°C), pH range of alcohol extracts is 5.63 ± 0.18 (at 22.9±1.6°C).

Table 2 The pH value of Chinese herbal extracts

	Rs	Tc	Ad	Aa
Aqua extracts	5.45	5.73	5.93	6.32

Alcohol extracts	5.45	5.63	5.57	5.87

(3) DPPH free radical scavenging efficacy

Spectrophotometer was used to measure the change of absorbance value after DPPH radical reacted with the sample to observe the antioxidant capacity of the sample. From Table 3 showed that scavenging efficacy of ethanol extracts are higher than aqueous extracts; the Tc and Rs ethanol extracts are similar to BHA; the order of free radical removal ability for aqua extracts were BHA> Rs > Tc > Vit.C ≈ Aa.

Table 3 DPPH free radical scavenging capacity of 4CHMs extracts (%)

	BHA	Vit. C	Rs	Tc	Ad	Aa
Aq. extracts	96.2	74.9	79.5	78.3	69.5	73.5
Alc. extracts	94.6	92.2	93.7	94.6	70.5	86.9

(4) FRAP reducing power assessment

The results of the reducing power of the samples obtained by the redox reaction between iron ions and antioxidants (as shown in Table 4) showed that aqua extract of Tc contains the highest content of total phenols and total flavonoids, so it presented strong reducing power (about the same as vitamin C).

Table 4 Determination of reducing power of 4 CHMs extracts (BHA mg/g)

	BHA	Vit. C	Rs	Tc	Ad	Aa
Aq. extracts	126.7	117.5	44.1	110.0	18.3	14.5
Alc. extracts	128.3	117.9	54.8	110.1	12.7	28.4

(5) Determination of total phenolic content

The Folin-Ciocalteau reagent will react with the reducing hydroxyl group on the polyphenolic compound to form a blue substance, which is used to detect the content of the

polyphenolic compound in the sample. As can be seen from the results presented in Table 5, alcohol extracts are higher than aqueous extracts, and all are highest in Tc.

Table 5 Total phenol content of 4 CHMs extracts (GAE mg/g)

	Rs	Tc	Ad	Aa
Aq. extracts	16.3	104.5	3.8	6.8
Alc. extracts	20.7	102.0	6.4	13.4

(6) Determination of total flavonoids content

The total flavonoid content in the extract was converted from the Quercetin standard curve, and the unit was Quercetin Equivalent (QCT) mg/g. The results were shown in Table 6. Ethanol extracts are higher than aqueous extracts, and Tc was the highest.

Table 6 Total flavonoid content of 4 CHMs extracts (QCT mg/g)

	Rs	Tc	Ad	Aa
Aq. extracts	54.8	150.1	10.1	41.8
Alc. extracts	60.8	132.1	11.6	35.1

Discussion

In this study showed that the antioxidant power of Taxillus chinensis is enough to replace vitamin C, and it is a new resource among antioxidants. It can be widely used in skin care products or cosmetics that require “natural”, Contributed to environmental protection.

Conclusion

1. Among Chinese herbal medicines, the ethanol and aqueous extracts of Tc more than 10%, and the highest extraction ratio was the aqueous extracts of Aa.
2. The pH value of all Chinese herbal medicines was weakly acidic, ranging from 5.45 to 6.32.

3. The free radical scavenging ability of Chinese herbal medicine alcoholic extracts were higher than that of aqueous extracts. The ability of Tc and Rs ethanol extracts was similar to that of BHA.
4. The reducing power of Chinese herbal medicines aqueous extract of Tc had the highest content of total phenols and total flavonoids, so it has a strong reducing power (about the same as vitamin C).
5. In terms of antioxidant content, combined with the detection results of total phenols and total flavonoids that the ethanol extract was higher than the aqueous extract, and the highest total content was found in Tc.
6. Based on the above results, Tc not only had the highest extraction ratio but also its antioxidant capacity and antioxidant content are both on the top position, and its pH value is between 4.6 and 6.5. In conclusion, Tc is an excellent antioxidant raw material that can be added to cosmetics and skin care products and can also be used as a reference material when developing anti-aging products in the future.

Acknowledgments

Thanks to National Tainan Junior College of Nursing strong support.

Conflict of Interest Statement

NONE.

References

1. Hui-M;Chia-C; Calvin Yu-C; Hsin-Y;Wen-Y; Chang-H; Shi-Z;Shih-L; Chien-Y;Tin-Y, (2010), Application of bioactivity database of Chinese herbal medicine on the therapeutic prediction, drug development, and safety evaluation.Journal of Ethnopharmacology，132(2): 429~437.
2. Qiuhong Wang;Haixue Kuang;Yang Su;Yanping Sun;Jian Feng;Rui Guo;Kelvin

Chan (2013)，Naturally derived anti-inflammatory compounds from Chinese medicinal plants.Journal of Ethnopharmacology, 146(1): 9~3.

3. Pei-Gen Xiao; Shan-Tian Xing; Li-Wei Wang, (1993), Immunological aspects of Chinese medicinal plants as antiageing drugs. Journal of Ethnopharmacology, 38(2~3): 159~165。
4. Kon-Young Ji;Ki Mo Kim;Yun Hee Kim;A-Rang Im;Joo Young Lee;Boram Park;MinKyun Na;Sungwook Chae. (2019), The enhancing immune response and anti-inflammatory effects of Anemarrhena asphodeloides extract in RAW 264.7 cells. Phytomedicine 59: 152789.
5. Xiao-Yu Song;Feng-Ying Han;Jing-Jie Chen;Wei Wang;Yan Zhang;Guo-Dong Yao;Shao-Jiang Song (2019), Timosaponin AIII, a steroidal saponin, exhibits anti-tumor effect on taxol-resistant cells in vitro and in vivo. Steroids, 146: 57-64.
6. Moonkyu Kang; Inho Jung; Jonghyun Hur; Soon Han Kim; Jeong Hun Lee; Ji-Yun Kang; Kyoung Chul Jung; Kyoung Soo Kim; Myung Chul Yoo; Dong-Suk Park; Jae-Dong Lee; Yong-Baik Cho (2010), The analgesic and anti-inflammatory effect of WIN-34B, a new herbal formula for osteoarthritis composed of Lonicera japonica Thunb and Anemarrhena asphodeloides BUNGE in vivo. Journal of Ethnopharmacology.131(2): 485-496。
7. Pan Yingming; Liang Ying; Wang Hengshan; Liang Min.(2004), Antioxidant activities of several Chinese medicine herbs. Food Chemistry, 88(3): 347-350。
8. Feng Zhang; Qi Yang; Lian-Na Sun; Shou-Hong Gao; Xia Tao; Wan-Sheng Chen (2014), Fingerprint analysis of Zhimu-Huangbai herb pair and simultaneous determination of its alkaloids, xanthone glycosides and steroidal saponins by HPLC-DAD-ELSD, Chinese Journal of Natural Medicines, 12(7): 525-534

9. Suzuki S., Sato H., Shimada H., Takashima N. (1993), Arakawa M. Comparative Free Radical Scavenging Action of Angiotensin-Converting Enzyme Inhibitors with and without the Sulfhydryl Radical, Pharmacology, 47, 61- 65.
10. Shi X., Dalal NS., Jain AC. (1991), Antioxidant behaviour of caffeine: Efficient scavenging of hydroxyl radicals, Food and Chemical Toxicology, 29(1) , 1-6.
11. Woisky RG., Salatino A. (1998), Analysis of propolis: some parameters and procedures for chemical quality control, Journal of Apicultural Research, 37(2), 99-105.
12. Christel Q.D., Gressier B., Vasseur J., Dine T., Brunet C., Luyckx M., Cazin M., Cazin J.C., Bailleul F. and Trotin F. (2000), Phenolic compounds and antioxidant activities of buckweat hulls and flour. J. Ethnopharmacol, 72, 35-42.