Preliminary screening of 44 plant extracts for anti-tyrosinase and antioxidant activities

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Abstract: In order to find new tyrosinase inhibitors and antioxidant materials, we investigated 44 plants, which were evaluated for the anti-tyrosinase and antioxidant activities. The mushroom tyrosinase inhibition assay and 2, 2-Diphenyl-1- picrylhydrazyl (DPPH) radical scavenging assay were conducted to evaluate these activities. Among all tested plant extracts, *Morus alba* L. (positive control), *Rhodiola crenulata* (Hook. f. et Thoms.) H. Ohba, *Momordica charantia* L., *Cuminum cyminum* L. *et al.* exhibit higher tyrosinase inhibition. *Rhodiola crenulata* (Hook. f. et Thoms.) H. Ohba, *Rosa rugosa* Thunb. and *Eugenia caryophyllata* Thunb. perform the highest antioxidant activity, similar to vitamin C (the positive control). A low positive correlation is found in the DPPH radical scavenging and tyrosinase inhibition assay. Considering these factors, the extracts of *Rhodiola crenulata* (Hook. f. et Thoms.) H. Ohba, *Alpinia officinarum* Hance and *Zanthoxylum bungeanum* Maxim. exhibit high anti-tyrosinase and antioxidant activities and could be used in the cosmetic industry. Further studies are warranted to characterize the compounds responsible for the anti-tyrosinase and antioxidant properties of these plant extracts.

Keywords: Anti-tyrosinase, antioxidant, DPPH, screening, skin-whitening.

INTRODUCTION

Melanin plays an influential role in phenotypic appearance, protection of the skin against ultraviolet (UV) rays. However, much exposure in the sunlight or diseases will promote the formation of melanin. On one hand, the increase of melanin formation has an influence on the appearance of skin. On the other hand, the excessive melanin formation and the accumulation of melanin in the skin may cause hyperpigmentation of disorders (Ha *et al*., 2005; Kim *et al*., 2005) such as melasma, freckles and geriatric pigment spots. Tyrosinase (EC 1.14.18.1), a copper-containing enzyme, is the key enzyme of the formation of melanin. First, the hydroxylation of L-tyrosine to L-3, 4-dihydroxyphenylalanine (L-DOPA) is catalyzed by tyrosinase. Second, the oxidation of L-DOPA to L-DOPA-quinone is catalyzed (Lim *et al.*, 2009). The quinine is a highly reactive compound. It can be transformed into melanin in a series of non-enzymatic reactions (Baurin *et al*., 2002).

The white skin has always been the pursuit of Asian women. Developing tyrosinase inhibitors can not only meet the demand of skin-whitening agents, but also lay foundation of drugs for hyperpigmentation. Over the past years, increasing attention has been paid to plant extracts for developing modern medicine and cosmetic products. Especially, in China, the concern of the Chinese edible herbs is increasing. Many researchers recommend an increase of the use of plant-based natural medical product (Tlili *et al.*, 2011). Several chemicals isolated from plant origin have been tested as cosmetics and pharmaceuticals to prevent overproduction of melanin as whitening agents. A lot of factors may affect skin whitening. It's suggested that antioxidant properties have played an important role in inhibiting melanin synthesis (Ling *et al.*, 2008).

Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Antioxidant activity is a very important pharmacological property. Many pharmacological functions such as antiaging, antimutagenicity, anticarcinogenicity and skin whitening originate from this property (Takahiro *et al*., 2010). Numerous spices (Ramos *et al.*, 2003; Rubio *et al.*, 2013) and medicinal herbs (Zheng and Wang, 2001) have been tested in an effort to identify novel and potential antioxidants. Studies of natural antioxidants are coming to front to researchers for use in foods or medicinal materials to replace synthetic antioxidants. Particularly, recent studies are focused on natural products including vegetables and wild plant sources (Young *et al.*, 2012).

Though many antioxidant preparations and skin whitening products are currently available, the demand for novel whitening agents is always increasing. Some potentially dangerous bleach is often combined with. Developing new skin whitening agents from natural sources is an available method to overcome the side effects of using agents that contain potentially dangerous compounds. Therefore, it is important to find novel and safe skin depigmenting agents.

In order to meet the growing demand of natural sources and find novel, safer depigmenting agents, 44 plant

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extracts were screened. In the study, the tyrosinase inhibition and antioxidant activities of the plant extracts were measured by mushroom tyrosinase inhibition assay and DPPH radical scavenging assay, respectively.

MATERIALS AND METHODS

Plant materials

Zanthoxylum bungeanum Maxim. (#13012) was collected in Tianshui, Gansu Province, China. *Elaeagnus angustifolia* L. (#13017), *Juglans regia* L. (#13018), and *Ginkgo biloba* L. (#13019) were collected at campus of Lanzhou University of Technology, Gansu Province. *Zanthoxylum armatum* DC. (#13030) was collected in Nanning, Guangxi Province, China. *Perilla frutescens* (L.) Britt. (#13041), *Fritillaria ussuriensis* Maxim. (#13042), *Eugenia caryophyllata* Thunb. (#13043), *Crataegus pinnatifida* Beg. (#13044), *Carthamus tinctorius* L. (#13045), *Rhodiola crenulata* (Hook. f. et Thoms.) H. Ohba (#13046), *Cynomorium songaricum* Rupr. (#13047), *Trigonella foenum-graecum* L. (#13048), *Cuminum cyminum* L. (#13049), *Morus alba* L. (#13050), *Atractylodes macrocephala* Koidz (#13051), *Angelica sinensis* (Oliv.) Diels. (#13052), *Foeniculum vulgare* Mill. (#13053), *Illicium verum* (#13054), *Glycyrrhiza uralensis* Fisch. (#13055), and *Puerariae Lobata* (Willd.) Ohwi (#13056) were purchased from the Yellow River herb market in Lanzhou, Gansu Province. The other plants were gotten from the Vanguard supermarket in Lanzhou, Gansu Province. All plants were identified by Pro. Yi Cai (Guangxi University of Chinese Medicine in China). The voucher specimens of plants were deposited at the Herbarium of School of Life science and Engineering, Lanzhou University of Technology.

Chemicals

DPPH, L-tyrosine, L-DOPA, mushroom tyrosinase were purchased from Sigma. The ethanol, the sodium dihydrogen phosphate, and the disodium hydrogen phosphate were analytical grade from Fuyu Chemical Co., Ltd.

Preparation of plants

After obtained, the plant materials were left to dry at room temperature. The dried plant materials were pulverized and subsequently extracted with 80% ethanol. Fifteen grams of plant material were extracted at 80□ during 90min in a 180mL solution of 80% ethanol. The extract was centrifuged and the residue was extracted in 90mL solvent under the same conditions. After centrifuged, the extract was combined. The extracts were concentrated under a vacuum in a rotary evaporator at 50□. The residual water in the extract was removed by lyophilization to obtain dried powder. The lyophilized powders were reserved in the refrigerator before use.

Tyrosinase inhibition assay

The tyrosinase inhibitory activity was measured as described by Baurin *et al.* (2002) with minor modifications. The plant extracts were prepared at 3 different concentrations (1, 2, 4mg/mL) by 80% (v/v) ethanol. One hundred microliters of 0.5mmol/L L-DOPA solution, 100μL of 10mmol/L L-tyrosine, 3.5mL of 50 mmol/L phosphate buffers and 200μL of the test sample solution of the different concentrations, were mixed. Then, 50μL of mushroom tyrosinase (1600U/mL) was added. The mixture was then incubated at 37□ for 5min, and the absorbance was measured at 473nm. The absorbance of the same mixture without tyrosinase and samples was used as the blank control. In order to eliminate the influence of the sample, the sample control was set. The sample control was contained samples and all above agents except the tyrosinase. The inhibition percent of tyrosinase activity was calculated as follows: inhibition % =1-(B-C) /A ×100. Where A= absorbance of blank control at 473nm, B= absorbance at 473nm with a test sample and C=absorbance of sample control at 473nm.

DPPH radical scavenging assay

The DPPH radical scavenging assay was measured by the modified method of Yılmaz *et al*. (2013). DPPH is a stable free radical which when dissolved in ethanol exhibits blue-violet color. The loss of color indicates radical scavenging activity (Zhao *et al*., 2006) and the objective is to evaluate the antioxidant activity of extracts.

The DPPH solution was made up at 0.0025mg/mL. It was dissolved with 80% (v/v) ethanol. First, 2.0mL of ethanol solution containing samples at different concentrations was mixed with 2.0mL of DPPH solution. This was the sample group. The assay also should contain the blank control and sample control. The blank control contained all regents without sample, while the sample control contained all regents except the DPPH solution. The mixture was then incubated for 30min at room temperature. The mixture was scanned at full wavelength for the maximum absorption wavelength. And then, the wavelength was determined at 520nm. Following that, the absorbance at 520nm was measured and the inhibition (%) of DPPH radical formation was calculated with the following equation: inhibition (%) = 1- (C-B)/A×100, in which A was the absorbance at 520nm of the control group contained all reagents without the sample; B was the absorbance at 520 nm of the sample group contained all reagents without DPPH; C was the absorbance at 520 nm of the sample group (Jiang *et al.*, 2013; Chun *et al*., 2005).

STATISTICAL ANALYSIS

The PASW statistics 18 (formerly SPSS Statistics) is used to analyze the relationship of mushroom tyrosinase inhibition assay and DPPH radical scavenging assay. The correlation analysis is done by a Pearson correlation coefficient (*r*). Differences with a confidence interval of $P<0.05$ were considered significant.

RESULTS

Tyrosinase inhibition assay

All plant extracts were screened for tyrosinase inhibitory activity. The assay was carried out at three different concentrations: 1, 2 and 4mg/mL. Table 1 summarizes the yield of the plant extracts and the results of mushroom tyrosinase inhibition of all tested plant extracts. In this

Table 1: The results of the extraction rates and tyrosinase inhibition of all plant extracts

No.	Plant	Part	Extraction rate/%	Inhibition rates of tyrosinase (%)		
				1mg/mL	2mg/mL	4mg/mL
1	Elaeagnus angustifolia L.	Branches	7.14	24.95	43.13	48.76
2	Perilla frutescens (L.) Britt.	Branches and leaves	5.13	15.02	16.93	33.75
3	Fritillaria ussuriensis Maxim	Bulb	11.79	15.94	16.63	17.34
4	Eugenia caryophyllata Thunb.	Flower buds	39.43	20.62	24.92	26.94
5	Jugtans regia	Flowers	33.17	17.99	28.89	36.07
6	Dendranthema morifolium Ramat.	Flowers	29.63	38.98	47.54	54.37
7	Carthamus tinctorius L.	Flowers	31.64	5.06	23.32	61.60
8	Zanthoxylum bungeanum Maxim.	Fruit	27.46	49.94	56.47	61.81
9	Crataegus pinnatifida Beg.	Fruit	47.63	30.78	37.40	44.54
10	Momordica charantia L.	Fruit	31.11	54.98	63.62	65.01
11	Lycium barbarum L.	Fruit	44.44	10.59	14.85	43.21
12	Ziziphus jujuba Mill.	Fruit	36.92	8.83	24.40	26.63
13	Elaeagnus angustifolia L.	Fruit	48.15	6.75	15.03	30.47
14	Juglans regia L.	Green husk	29.38	8.74	27.55	30.73
15	Elaeagnus angustifolia L.	Leaves	15.56	20.99	28.82	43.73
16	Mentha haplocalyx Briq.	Leaves	28.15	4.01	5.53	51.72
17	Cynomorium songaricum Rupr.	Pedicel	30.96	5.68	6.47	12.44
18	Citrus reticulata Blanco	Peel	31.33	-3.20	7.59	27.17
19	Cinnamomum cassia Presl	Bark	17.57	23.75	35.44	46.29
20	Zingiber officinale Rosc.	Rhizome	20.64	21.19	25.65	35.00
21	Dioscorea opposita Thunb.	Root tuber	19.14	3.17	12.58	32.45
22	Rhodiola crenulata (Hook. f. et Thoms.) H. Ohba	Roots	20.43	49.23	65.13	75.04
23	Morus alba L.	Roots	10.36	72.73	98.78	98.91
24	Trigonella foenum-graecum L.	Seed	21.65	60.70	63.59	69.44
25	Cuminum cyminum L.	Seed	14.23	46.14	71.34	80.78
26	Piper longum L.	Ear	20.71	42.45	42.14	20.95
27	Amomum tsao-ko Crevost et Lemarie	Fruit	14.14	42.84	11.24	3.55
28	Amomum kravanh Pierre ex Gagnep.	Fruit	5.52	46.07	42.11	31.12
29	Atractylodes macrocephala Koidz.	Rhizome	27.04	16.17	4.49	0.54
30	Angelica sinensis (Oliv.) Diels	Roots	42.31	25.80	13.66	0.80
31	Cistanche deserticola Y. C. Ma	Stem	43.70	30.90	17.91	-2.88
32	Nelumbo nucifera Gaertn.	Seed	20.50	36.59	32.73	29.74
33	Jasminum sambac (L.) Ait.	Flowers	25.71	29.79	61.90	52.46
34	Rosa rugosa Thunb.	Flowers	32.26	15.22	40.54	13.80
35	Foeniculum vulgare Mill.	Fruit	21.99	16.64	18.43	12.94
36	Ginkgo biloba L.	Fruit	10.43	19.25	35.20	16.32
37	Illicium verum	Fruit	27.04	15.02	19.91	13.25
38	Laurus nobilis L.	Leaves	21.43	0.13	8.18	1.36
39	Juglans regia L.	Leaves	36.30	14.45	19.45	15.07
40	Alpinia officinarum Hance	Rhizome	19.50	47.85	58.96	21.54
41	Glycyrrhiza uralensis Fisch.	Roots	24.93	64.59	76.65	64.72
42	Pueraria lobata (Willd.) Ohwi	Roots	10.36	0.78	13.22	3.13
43	Helianthus tuberosus L.	Tubers	35.71	8.04	11.20	8.93
44	Zanthoxylum armatum DC.	Branches	16.17	-8.07	-10.73	-37.24

Table 2: The IC_{50} values of all plant extracts

No.	Plant	Part	IC_{50} (mg/mL)
1	Rhodiola crenulata (Hook. f. et Thoms.) H. Ohba	Roots	0.0042
2	Eugenia caryophyllata Thunb.	Flower bulb	0.0059
3	Rosa rugosa Thunb.	Flowers	0.0073
4	Cinnamonmum cassia Presl	Bark	0.0087
5	Alpinia officinarum Hance	Rhizome	0.0096
6	Zanthoxylum bungeanum Maxim.	Fruit	0.0135
7	Jugtans regia	Leaves	0.0161
8	Illicium verum	Fruit	0.0170
9	Cistanche deserticola Y. C. Ma	Stem	0.0173
10	Elaeagnus angustifolia L.	Branches	0.0174
11	Cynomorium songaricu Rupr.	Pedicel	0.0199
12	Amomum tsao-ko Crevost et Lemarie	Fruit	0.0203
13	Juglans regia L.	Flowers	0.0210
14	Zingiber officinale Rosc.	Rhizome	0.0226
15	Perilla frutescen(L.) Britt.	Branches and leaves	0.0285
16	Dendranthema morifolium Ramat.	Flowers	0.0303
17	Laurus nobilis L.	Leaves	0.0326
18	Glycyrrhiza uralensis Fisch.	Roots	0.0335
19	Elaeagnus angustifolia L.	Leaves	0.0352
20	Juglans regia L.	Green husk	0.0520
21	Cuminum cyminum L.	Seed	0.0705
22	Zanthoxylum armatum DC.	Branches	0.0734
23	Piper longum L.	Ear	0.0770
24	Crataegus pinnatifida Beg.	Fruit	0.0799
25	Dioscorea opposita Thumb.	Root tuber	0.0855
26	Foeniculum vulgare Mill.	Fruit	0.0884
27	Morus alba L.	Roots	0.0942
28	Jasminum sambac(L.) Ait.	Flowers	0.0972
29	Momordica charantia L.	Fruit	0.1061
30	Pueraria lobata (Willd.) Ohwi	Roots	0.1094
31	Elaeagnus angustifolia L.	Fruit	0.1117
32	Amomum kravanh Pierre ex Gagnep.	Fruit	0.1293
33	Mentha haplocalyx Briq.	Leaves	0.1433
34	Trigonella foenum-graecum L.	Seed	0.1887
35	Atractylodes macrocephala Koidz.	Rhizome	0.1932
36	Lycium barbarum L.	Fruit	0.2565
37	Citrus reticulata Blanco	Peel	0.2650
38	Carthamus tinctorius L.	Flowers	0.3399
39	Fritillaria ussuriensis Maxim	Bulb	0.3889
40	Angelica sinensis (Oliv.) Diels	Roots	0.4862
41	Nelumbo nucifera Gaertn.	Seed	0.8644
42	Ginkgo biloba L.	Fruit	1.1388
43	Ziziphus jujuba Mill.	Fruit	1.2232
44	Helianthus tuberosus L.	Tubers	1.3961

table, the tyrosinase inhibition values are presented as percentage (%).

As shown in table 1, the first 25 plant extracts produce a concentration-dependent increase in tyrosinase inhibition. On the contrary, the inhibition rates of the following 7 extract (From No. 26 to No. 32), such as the stem of *Cistanche deserticola* Y.C. Ma, the fruit of *Amomum tsao-ko* Crevost et Lemarie, *et al*., are decreased by the increasing concentrations. It's indicated the concentration-dependent decrease in the tyrosianse inhibition. For the next 11 extracts (From No. 33 to No. 43) in the column, the inhibitions are the highest at the concentration of 2mg/mL, which indicates that these extracts have the highest inhibition at the tested medium concentration. With the continuously increasing of

Table 3: The correlation of DPPH and tyrosinase inhibition

		DPPH	TI-1	TI-2	TI-4	TI-H
DPPH	Pearson Correlation					
	Sig. (2-tailed)					
	N	44				
TI-1	Pearson Correlation	-0.181				
	Sig. (2-tailed)	0.24				
	N	44	44			
TI-2	Pearson Correlation	-0.16	0.869**			
	Sig. (2-tailed)	0.299	0			
	N	44	44	44		
TI-4	Pearson Correlation	-0.165	0.631**	0.790**		
	Sig. (2-tailed)	0.285	0	0		
	N	44	44	44	44	
TI-H	Pearson Correlation	-0.216	0.798**	0.888**	0.903**	
	Sig. (2-tailed)	0.158	0	0	0	
	N	44	44	44	44	44

concentration, their inhibition rates on tyrosinase were decreased，or even the formation of melanin is activated. The last plant extract, the branches of *Zanthoxylum armatum* DC. (No. 44) exhibits activation for tyrosinase.

Of all the extracts assayed, the value of tyrosinase inhibition rates of 9 plant extracts (contained positive control, *Morus alba* L.) are demonstrated higher than 50% at 2mg/mL. These 9 extracts exhibit strong anti-tyrosinase activity. They are the roots of *Glycyrrhiza uralensis* Fisch., the roots of *Rhodiola crenulata* (Hook. f. et Thoms.) H. Ohba, the roots of *Morus alba* L., the fruit of *Momordica charantia* L., the fruit of *Zanthoxylum bungeanum* Maxim., the seed of *Trigonella foenum-graecum* L., the seed of *Cuminum cyminum* L., the rhizome *Alpinia officinarum* Hance, and the flowers of *Jasminum sambac* (L.) Ait. At the concentration of 1mg/mL, the value of tyrosinase inhibition rates of 4 plant materials (contained positive control, *Morus alba* L.) are higher than 50%.

DPPH radical scavenging assay

In this experiment, each of the 44 plant materials was screened at 5 different concentrations. The IC_{50} value is the half maximal (50%) inhibitory concentration. The IC_{50} value was calculated according to the inhibition rates of the 5 concentrations. The IC_{50} values of all tested plant extracts are in table 2.

As shown in table 2, the IC_{50} values of *Rhodiola crenulata* (Hook. f. et Thoms.) H. Ohba, *Rosa rugosa* Thunb., *Eugenia caryophyllata* Thunb., *Cinnamonmum cassia* Presl and *Alpinia officinarum* Hance are 0.0042, 0.0073, 0.0059, 0.0087 and 0.0096, respectively. They are close to vitamin C (the positive control, IC_{50} = 0.0040). It revealed that these 5 plant extracts show high antioxidant activity. Among the 5 plant extracts, *Rhodiola crenulata* (Hook. f. et Thoms.) H. Ohba exhibits the highest antioxidant activity.

The IC_{50} values of the fruit of *Zanthoxylum bungeanum* Maxim, the fruit of *Amomum tsao-ko* Crevost et Lemarie, the fruit of *Crataegus pinnatifida* Beg., the fruit of *Foeniculum vulgare* Mill., the fruit of *Illicium verum*, the stem of *Cistanche deserticola* Y.C. Ma, the branches of *Elaeagnus Angustifolia* L., the branches of *Zanthoxylum armatum* DC., the pedicel of *Cynomorium Songaricum* Rupr., the flowers of *Dendranthema morifolium* Ramat., the flowers of *Jasminum sambac* (L.) Ait., the flowers of *Juglans regia* L., the rhizome of *Zingiber officinale* Rosc., the branches and leaves of *Perilla frutescens* (L.) Britt., the leaves of *Elaeagnus Angustifolia* L., the leaves of *Juglans regia* L., the leaves of *Laurus nobilis* L., the roots of *Glycyrrhiza uralensis* Fisch., the roots of *Morus alba* L., the green husk of *Juglans regia* L., the seed of *Cuminum cyminum* L., the ear of *Piper longum* L., the root tuber of *Dioscorea opposita* Thunb. are less than 0.1000, which indicates the relatively high antioxidant activity of the 23 plants. The IC_{50} values of the fruit of *Momordica charantia* L., the fruit of *Elaeagnus Angustifolia* L., the fruit of *Amomum kravanh* Pierre ex Gagnep., the fruit of *Lycium barbarum* L., the roots of *Angelica sinensis* (Oliv.) Diesl, the roots of *Puerariae Lobata* (Willd.) Ohwi, the leaves of *Mentha haplocalyx* Briq., the rhizome of *Atractylodes macrocephala* Koidz, the peel of *Citrus reticulata* Blanco, the flowers of *Carthamus tinctorius* L., the bulb of *Fritillaria ussuriensis* Maxim., the seed of *Trigonella foenum-graecum* L.and the seed of *Nelumbo nucifera* Gaertn. are between 0.1 and 0.9, which shows these 13 plant extracts are of certain antioxidant activity. While the fruits of *Ginkgo biloba* L., the fruit of *Zizyphus jujuba* Mill, and the tubers of *Helianthus tuberosus* L. evidence low antioxidant activity.

DISCUSSION

Tyrosinase inhibition assay

Of all the tested plant extracts, the inhibition and activation for tyrosinase can be found. *Morus alba* L. extract exhibits the highest inhibition of tyrosinase with 72.73% at 1mg/mL. This result is agreed with previous works (Lee *et al*., 1997).

Among all the plant extracts, 7 plant extracts including the rhizome of *Zingiber officinale* Rosc., the root tuber of *Dioscorea opposita* Thunb., the leaves and fruit of *Elaeagnus angustifolia* L., the seed of *Trigonella foenum-graecum* L., the flowers of *Carthamus tinctorius* L. and the peel of *Citrus reticulata* Blanco are the positive linear correlation (fig. 1). It can be found that the linear correlation may occur within certain range of concentration, when the concentration is beyond the range, it may be not linear correlation. For example, the second plant extract, when the concentration increases from 1mg/mL to 2mg/mL, it shows a proportional linear doubling of activity with doubling in concentration. But at 4mg/mL, the tyrosinase inhibition rate increased little.

But the concentration-dependent decrease in the tyrosianse inhibition is probably because the targets of tyrosinase have been approximated or saturated at the lowest concentration. With the increasing of concentration, the tyrosinase inhibition of extracts is decreased.

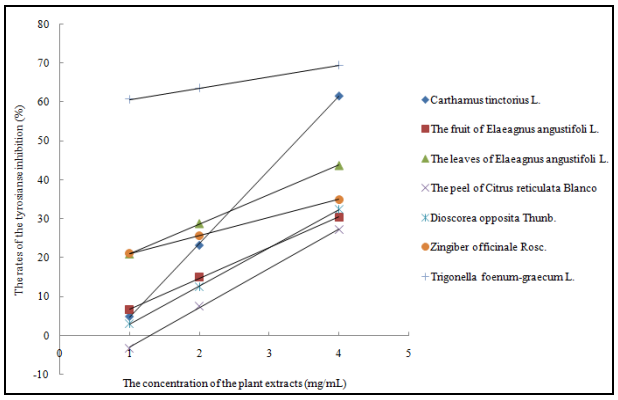

Fig. 1 The relationship between the rates of tyrosinase inhibition and the concentration of several plant extracts presenting linear relation

Except *Morus alba* L., of these 9 plants, *Rhodiola crenulata* (Hook. f. et Thoms.) H. Ohba, *Momordica charantia* L., *Cuminum cyminum* L., *Alpinia officinarum* Hance and *Glycyrrhiza uralensis* Fisch. have been reported to have the capability of inhibition of tyrosinase. *Rhodiola crenulata* (Hook. f. et Thoms.) H. Ohba exhibits high tyrosinase inhibition in accordance with the previous work (Zhao et al., 2009). Other plants also have been demonstrated to have the capability of anti-tyrosinase activity (Liu and Liu, 2007; Lu *et al*., 2007). As far as can be determined from a survey of the literature, the 4 other plant extracts which demonstrate a capability to inhibit tyrosinase, are described for the first time in this skin depigmenting property. The activities of these 4 plants show their cosmetic potential.

Except 3 plants, the peel of *Citrus reticulata* Blanco, *Cistanche deserticola* Y.C. Ma and *Zanthoxylum armatum* DC., all the plant materials exhibit tyrosinase inhibition in different extents. The peel of *Citrus reticulata* Blanco, *Cistanche deserticola* Y.C. Ma represent both activation and inhibition of tyrosinase. The peel of *Citrus reticulata* Blanco performs weak activation of tyrosinase at the lowest concentration. While *Cistanche deserticola* Y.C. Ma exhibits activation of tyrosinase only at the highest concentration. It remains to be studied that whether the cause of the bidirectional role in tyrosinase is a single active substance or diverse active substances (Fu *et al*., 2003). The branches of *Zanthoxylum qrmatum* DC. present only activation of tyrosinase. Further study can be conducted in these 3 plant materials to develop the drug of activating tyrosinase. They have the potential of treating the vitiligo.

DPPH radical scavenging assay

Reactive oxygen species (ROS) including superoxide anion free radical, hydroxyl radical, hydrogen peroxide, nitrogen dioxide, nitric oxide radical, are generated by normal metabolic oxidative damage to cells. ROS can stimulate the production of melanin pigment cells, and promote the generation of hyper pigmentation. The DPPH radical scavenging assay is a useful method for antioxidant research. Scavenging free radicals can inhibit the oxidation reaction of the melanin formation process, and is a way of skin whitening.

The result of DPPH radical scavenging assay showed the tested plant extracts exhibit the radical scavenging activity in different degrees. The IC_{50} value of *Rhodiola crenulata* (Hook. f. et Thoms.) H. Ohba is close to vitamin C (the positive control, IC_{50} = 0.0040). *Rhodiola crenulata* (Hook. f. et Thoms.) H. Ohba exhibits the highest antioxidant activity.

The relationship between DPPH and tyrosinase inhibition

Table 3 shows the correlation of DPPH and tyrosinase inhibition. The results of DPPH scavenging assay are the IC_{50} values while the results of tyrosinase inhibition assay are tyrosinase inhibition rates. Therefore, the negative numbers of Pearson correlation indicate the positive correlation of DPPH radical scavenging assay and tyrosinase inhibition assay. It's indicated the low positive correlation of DPPH radical scavenging and tyrosianse inhibition. It's suggested that the scavenging of oxygen free radicals is not the only probable mechanism of skin

whitening. There are other mechanisms of skin whitening, such as metabolic whitening, anti-inflammatory whitening. Therefore, some plant extracts exhibit good antioxidant activity, while they are with low or without tyrosinase inhibitory activity, for example *Dioscorea opposita* Thunb..

Concerning the extraction rate and the both activities, the flower bulb of *Eugenia caryophyllata* Thunb. (Extraction rate, 39.43%) and the fruit of *Crataegus pinnatifida* Beg. (Extraction rate, 47.63%) exhibit much higher anti-tyrosinase and antioxidant activities. Owing to the high extraction rate, these two extract were more promising.

Among all tested plant extracts, there are some exhibit higher anti-tyrosinase and antioxidant activities. Except *Morus alba* L. (the positive control), 3 plant extracts from *Rhodiola crenulata* (Hook. f. et Thoms.) H. Ohba, *Alpinia officinarum* Hance and *Zanthoxylum bungeanum* Maxim. exhibit better antioxidant and anti-tyrosinase activities. Especially, *Rhodiola crenulata* (Hook. f. et Thoms.) H. Ohba had been also confirmed to have the potential of delaying aging and skin-whitening (Gong *et al*., 2006). Further study can be done about these plant extracts to develop skin-whitening products.

CONCLUSION

According to the correlation of DPPH and tyrosianse inhibition, there is no direct relation between antioxidant activity and skin whitening, and the antioxidant activity isn't only one of whitening mechanisms. So, free radical scavenging activities, especially DPPH radical isn't the possible key target to screening for whitening agents. It is significant to find novel and safe skin depigmenting agents from natural sources to meet this growing demand currently. Many plant extracts exhibit the activity of skin whitening in our study. For example *Rhodiola crenulata* (Hook. f. et Thoms.) H. Ohba, *Zanthoxylum bungeanum* Maxim, *Morus alba* L., *et al*.. These plant extracts could be used in the cosmetic products. Subsequent experiments can be done to study the substances and the mechanism of the inhibitory in future. The isolation and the structural elucidation of the active constituents from the plants will provide useful guidance in the development of skin depigmenting agents.

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