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Original Research Article

Tyrosinase Inhibitory Activity and Thermostability of the Flavonoid Complex from *Sophora japonica* L (Fabaceae)

Jeng-Shiow Lai[1, 2], Chih-Chien Lin[1]* and Tsung-Ming Chiang[3]
[1]Department of Cosmetic Science, Providence University, No. 200, Sec. 7, Taiwan Boulevard, Shalu, Taichung City, 43301, [2]Department of Beauty Science, Chienkuo Technology University, No. 1 Chiehshou North Road, Changhua City 500, [3]Panion & BF Biotech Inc., 16 F, No. 3, Yuan. Chiu St., Nan Gang Dist., Taipei 115, Taiwan, ROC.

For correspondence: **Email: chchlin@pu.edu.tw; **Tel:** +886-4-26328001 ext 15409; **Fax:** +886-4-26311167.*

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Abstract

Purpose: *To investigate the tyrosinase inhibitory activity and thermostability of weak acid-treated Sophora japonica L. flavonoid complex (SJ-FC) in different solutions.*
Methods: *The flavonoid complex of S. japonica was isolated and treated with weak acid to generate SJ-FC. The anti-tyrosinase activities of SJ-FC and well-known tyrosinase inhibitors were compared by mushroom tyrosinase activity assay. The thermostabilities of SJ-FC and other inhibitors in different solution environments for long-term storage were also investigated.*
Results: *The results indicate that SJ-FC has potent tyrosinase inhibitory activity, and at a concentration of 0.1 %, SJ-DC has a tyrosinase inhibitory activity equal to that of 1 % ascorbic acid or hydroquinone. In addition, SJ-FC in both propylene glycol (PG) and glycerol solutions exhibited obvious tyrosinase inhibitory activity. Ascorbic acid and arbutin, two other tyrosinase inhibitors, exhibit < 60 % of their initial activity in both PG and H_2O solutions after 6 months of storage. However, SJ-FC stored in PG and H_2O solutions retained almost 100 % of its activity over a 6-month period.*
Conclusion: *SJ-FC is an effective and stable anti-tyrosinase agent and may be used as a function agent in medicines, foods and cosmetics.*

Keywords: *Flavonoid, Sophora japonica L., Tyrosinase inhibitor, Thermostability*

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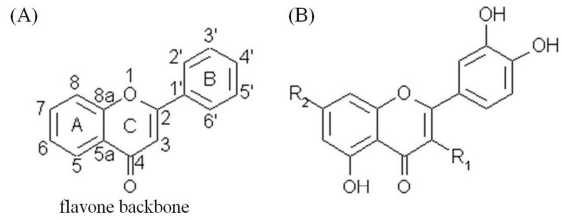
INTRODUCTION

The flowers and flower buds of *Sophora japonica* L. (Fabaceae), widely cultivated in Asia, and also referred to as *Styphnolobium japonicum* (L.) Schott., are famous traditional Chinese medicines [1]. *S. japonica* has antioxidant [2,3], anti-inflammatory [4] and anti-platelet activities [5] and can prevent weight gain in high-fat diet-induced obese mice [6]. In addition, *S. japonica* contains both anti-hemorrhagic and anti-hemostatic substances. *S. japonica* reduces the damage caused by cerebral infarction, partly because of its antioxidant and anti-inflammatory activities [1,7].

Flavonoids are secondary metabolites in plants and are of interest to the pharmaceutical, nutritional and cosmetic industries because of their biological activities [8,9]. The primary components of *S. japonica* include flavones, tetraglycosides, isoflavones, isoflavone tetraglycosides, triterpene glycosides, phospholipids, alkaloids, amino acids and polysaccharides [10]. However, the major components of the flowers and flower buds of *S. japonica* are rutin, quercetin and rutin-7-O-rhamnoside [11]. The structures are presented in Figure 1. Thus, some researchers have proposed that these flavonoid glycosides and

aglycones are responsible for the most important biological activities of *S. japonica* flowers, including antioxidant and anti-inflammatory activities [11,12].



Compounds	R1	R2
(1) Rutin	rutinoside	OH
(2) Quercetin	OH	OH
(3) Rutin-7-O-rhamnoside	rutinoside	rhamnoside

Figure 1: Chemical structure of **(A)** flavone backbone and **(B)** major flavonoids isolated from *Sophora japonica* L.

Many plant extracts, some rich in flavonoid aglycones and others rich in flavonoid glycosides, have antioxidant and anti-inflammatory activities. Studies have demonstrated that de-glycosylation may increase the absorption of dietary flavones *in vivo*, enhancing the antioxidant activities of these compounds or modulating inflammatory responses [13,14]. Therefore, adjusting the compositions of flavonoid aglycones and glycosides from natural sources can improve the desired biological functions.

Tyrosinase (EC 1.14.18.1) is the key enzyme catalyses the first two rate-limiting steps of biosynthesis of melanin [15,16]. Therefore, tyrosinase is a fine target for inhibition in the search for different types of skin-lightening agents. In practical uses, however, due to the safety and stability concerns, just few tyrosinase inhibitors are used as skin-lightening agents. Thus, the search for new and safe tyrosinase inhibitors is an important issue for the medical and cosmetic industries [16,17].

To our knowledge, there is only one study that showed that a rare compound, *N*-feruloyl-*N'-cis*-feruloyl-putrescine, from *S. japonica* can inhibit a cellular tyrosinase in human epidermal melanocytes [18]. The anti-tyrosinase activity of flavonoid mixtures from the flowers of *S. japonica* has not been reported. Therefore, in this study, we investigated the anti-tyrosinase activity of a weak acid-treated *S. japonica* flavonoid complex (SJ-FC) in different environments. In addition, the thermostabilities of SJ-FC and two other well-known anti-tyrosinase agents, ascorbic acid and arbutin, were compared.

EXPERIMENTAL

Materials and chemicals

Sophora japonica L. [*Styphnolobium japonicum* (L.) Schott.] was obtained from a local market in Taichung City (Taichung, Taiwan). Glycolic acid, ascorbic acid, hydroquinone, mushroom tyrosinase, L-tyrosine, propylene glycol (PG), glycerol and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). The α-arbutin and β-arbutin were purchased from Pentapharm (Basel, Switzerland). Deionized distilled water (ddH_2O) for solutions was obtained from a Milli-Q system (Millipore, Bedford, MA, USA).

Preparation of the flavonoid complex

Fresh flowers of *S. japonica* were washed with pure water and air-dried for 5 days at room temperature. The dehydrated *S. japonica* was homogenized by homogeneizer (Ultra-Turrax T18, IKA, Stauffen, Germany) and extracted with 50% ethanol at 25 °C for 30 minutes. The ratio of plant material to liquid was 1: 20 (w/w). The supernatant was centrifuged for 15 min at 3000 rpm and then filtered to remove debris. The filtered flavonoid mixture was then freeze-dried and treated with the weakly-acidic solution (0.05M sulfuric acid in 95 % ethanol) at 40 °C for 4 h to generate the *S. japonica* flavonoid complex (SJ-FC) [19]. Sulfuric acid was removed by addition of calcium hydroxide and then the prepared SJ-FC was freeze-dried prior to use.

Tyrosinase activity assay

To assay the test sample for inhibition of tyrosinase activity, 60 µL of test samples in H_2O, propylene glycol or glycerol at different concentrations was mixed with 100 µL of L-tyrosine (1 mM) in phosphate-buffered saline pH 6.8, propylene glycol or glycerol. Then, 40 µL of mushroom tyrosinase solution (100 units/mL) was added to the mixture, which was then incubated for 25 min at 37°C. The absorbance was measured spectrophotometrically at 475 nm, and the inhibition of dopachrome formation was calculated as % inhibition [20]. For the blank group, the complete analytical procedure was followed, including all chemicals and solvents, but no sample was added. The reduction of mushroom tyrosinase activity to a half-maximal inhibitory concentration value is defined as IC_{50}.

Thermostability analysis

Solutions of 0.1% ascorbic acid, arbutin and SJ-FC in PG or H_2O (pH 7) were placed in glass

bottles and kept in the dark. These samples were maintained at room temperature (25 °C) in an incubator. At predetermined intervals (from 0 to 180 days), the solutions were analyzed using the previously described tyrosinase inhibition assay to determine the stability of the inhibitory activities of these compounds.

Statistical analysis

The quantitative data for the present study were analyzed using Student's *t*-tests and are presented as mean ± SEM for three independent assays.

RESULTS

Preparation of SJ-FC

The yield of flavonoids from *S. japonica* flowers was about 0.502% (w/w, 5.02g SJ-FC from 1000g dried flowers of *S. japonica*).

Tyrosinase inhibitory activity of various tyrosinase inhibitors and SJ-FC

First, we compared the anti-tyrosinase activity of SJ-FC (0.1 %) with those of several tyrosinase inhibitors (1 %), including glycolic acid, β-arbutin, α-arbutin, ascorbic acid and hydroquinone in H_2O solution. The results are shown in Figure 2. It is clear that all anti-tyrosinase agents have inhibitory activities against mushroom tyrosinase. In addition, except for glycolic acid, all tested inhibitors at a 1 % concentration have inhibitory activities greater than 70 % relative to the control (blank, no sample was added). The inhibition of glycolic acid was approximately 37 %. Ascorbic acid and hydroquinone at a concentration of 1 % and SJ-FC at a concentration of 0.1 % had the greatest tyrosinase inhibitory activity (Figure 2).

The calculated half-maximal inhibitory concentration (IC_{50}) value of SJ-FC for tyrosinase activity in H_2O solution is 3.12 mg/mL (Table 1). Therefore, we can suppose that SJ-FC is a potent tyrosinase inhibitory agent.

Tyrosinase inhibitory activity of SJ-FC in PG and glycerol solutions

The results in Figure 3 show that SJ-FC at concentrations of 0.01 to 0.1 % inhibits tyrosinase in both polyol environments. Moreover, 0.1% SJ-FC suppressed tyrosinase almost completely in PG (Figure 3A) and glycerol (Figure 3B) solutions. Although the inhibition of SJ-FC at higher concentrations in both solutions did not differ significant, the results indicated that SJ-FC in PG has greater tyrosinase inhibitory activity (higher than 60% inhibition) at lower concentrations (0.01 % SJ-FC, Figure 3A and 3B). PG is a thus better polyol solution for the anti-tyrosinase function of SJ-FC.

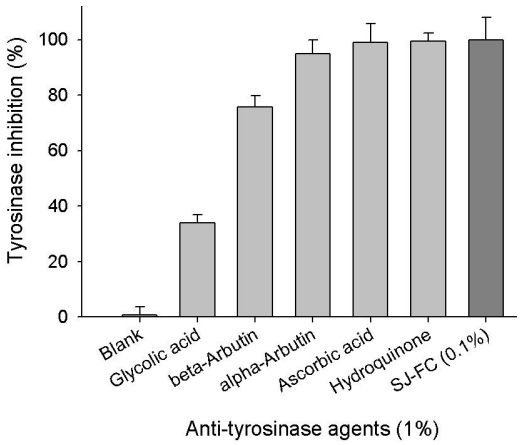


Figure 2: Tyrosinase inhibitory activities of various tyrosinase inhibitors (1 %) and SJ-FC (0.1 %) in H_2O solution.

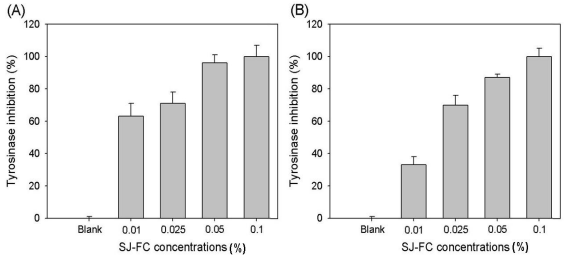


Figure 3: (A) Tyrosinase inhibitory activity of SJ-FC in PG solutions; (B) tyrosinase inhibitory activity of SJ-FC in glycerol solutions.

Stability of ascorbic acid, arbutin and SJ-FC in PG and aqueous solutions

The results of this assay are presented in Figures 4A, B and C. The results indicate that ascorbic acid decomposes in both PG and H_2O solutions. When the storage time is greater than 6 months, the inhibitory activity of ascorbic acid was decreased to < 60 % of its initial value (Figure 4A).

A similar result is presented in Figure 4B for arbutin. Arbutin's inhibitory function in PG and H_2O solutions decreased to 39% and 21%, respectively, of its initial activity after 6 months of storage. In addition, the stability of arbutin in PG is better than that in H_2O solution (Figure 4B).
Figure 4C shows the stability of SJ-FC in PG and H_2O solutions. Over the 6-month period storage, SJ-FC in PG and H_2O solutions retained almost all of its original activity, higher than 97 % inhibition. This result indicates that SJ-FC is a stable tyrosinase inhibitor in both PG and H_2O solutions.

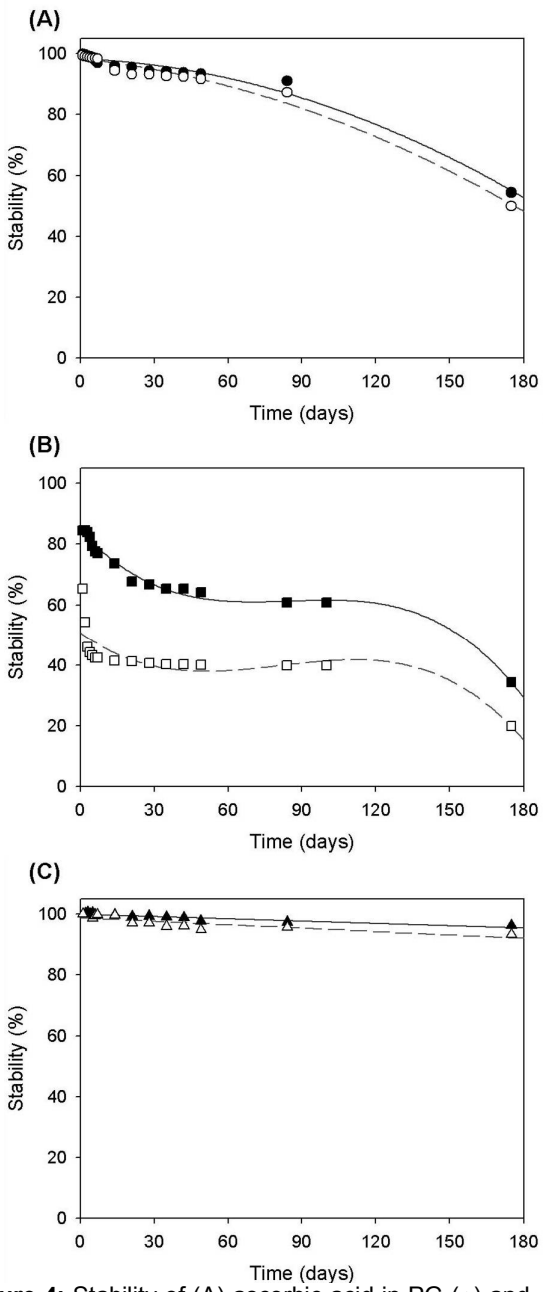


Figure 4: Stability of (A) ascorbic acid in PG (●) and H_2O (○) solutions; (B) arbutin in PG (■) and H_2O (□) solutions; (C) SJ-FC in PG (▲) and H_2O (Δ) solutions.

DISCUSSION

The main components of SJ-FC are rutin, quercetin and rutin-7-O-rhamnoside. We used the weakly-acidic solution treatment of its flavonoid mixture to obtain SJ-FC that has several functional constituents, including flavone glycosides and the aglycones and glycosides formed by hydrolysis of the flavone glycosides. Moreover, we suppose that weakly-acidic solution treatment also provides a procedure with broken of some unstable bonds to produce a relative stable flavonoid complex [19]. Additionally, the anti-tyrosinase activity of several important flavonoids, including rutin and quercetin, had been established by some previous studies [21-23]. For this reason, we can assume that the anti-tyrosinase activity of SJ-FC is given by these important components. Our results also demonstrate that SJ-FC is an effective and stable tyrosinase inhibitor.

This result also concludes that SJ-FC has potent anti-tyrosinase activity. The activity of SJ-FC is better than that of other common used tyrosinase inhibitors. Moreover, α-arbutin exhibits higher anti-tyrosinase activity than β-arbutin (Figure 2). This observation is similar to that of an earlier study showing that α-arbutin has greater tyrosinase inhibitory activity than β-arbutin [24]. When used as an anti-tyrosinase agent in medicines, foods and cosmetics, this inhibitor might exert its activity in aqueous environments, such as aqueous solutions, polyol-containing solutions and various types of emulsions. In addition, formulations used in medicines, foods and cosmetics frequently include PG and glycerol. Therefore, if SJ-FC is a functional inhibitor when dissolved in PG and glycerol, it can be used in more applications in the future. The results also indicate that SJ-FC in both PG and glycerol solutions has good tyrosinase inhibitory activity (Figure 3).

The stability of active ingredients for medicines, foods and cosmetics is an important factor in their use. Some potent anti-tyrosinase agents, such as kojic acid, arbutin and deoxyarbutin, are unstable in aqueous environments [25-27]. Thus, we compared the stability of SJ-FC with the stabilities of two well-known and frequently used tyrosinase inhibitors, ascorbic acid and arbutin, using a tyrosinase inhibitory activity-based method in PG and H_2O solutions. Our results indicate that SJ-FC is a potent and stable anti-tyrosinase agent. This result suggests that SJ-FC can act as a stable tyrosinase inhibitor in many types of environments (formulations) that involve long-term storage. Hence, this feature of SJ-FC will facilitate its use as ingredient in many applications. Moreover, based on these results, we can state that ascorbic acid and arbutin are very unstable in both PG and H_2O environments (Figures 4A and B).

CONCLUSION

SJ-FC is a potent tyrosinase inhibitory agent which, at a concentration of 0.1 %, has greater tyrosinase inhibitory activity than 1 % ascorbic acid or hydroquinone. In addition, SJ-FC in both PG and glycerol solutions has potent tyrosinase inhibitory activity. However, at lower

concentrations (0.01% SJ-FC), PG is a better polyol solution for the anti-tyrosinase function of SJ-FC. Ascorbic acid and arbutin exhibit < 60 % of their initial activity after storage in either PG and H_2O solutions for 6 months. However, SJ-FC stored in PG and H_2O solutions retains almost 100% of its activity over the 6-month period. Therefore, SJ-FC is a potent and stable anti-tyrosinase agent and may have various applications for medicines, foods and cosmetics in the future.

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