Short Communication

Anti-tyrosinase and antioxidant activities of *Impatiens balsamina* L.

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Abstract

The ethanol, methanol, and acetone extracts of different parts of *Impatiens balsamina* L., were prepared and evaluated for anti-tyrosinase and antioxidant activities. Among the solvents of extraction, methanol was the most potent solvent. The tyrosinase inhibition assay suggested that the flower extracts have an anti-tyrosinase activity that was about 50% in relation to copper-chelating activity and better than leaves and stems extracts. Total phenolic and flavonoid contents found in the methanol flower extracts were 1,596.08±38.59 mg gallic acid equivalent g⁻¹ and 884.83±1.34 mg quercetin equivalent g⁻¹, respectively, which were much more than the leaves and stems. The total phenolic and flavonoid contents possessed the anti-tyrosinase activity with r=0.632 and 0.542, respectively. Nevertheless, the flower extract exhibited the greatest amount of antioxidant activity. In conclusion, the flower extract of *I. balsamina* could serve as a natural anti-tyrosinase compound to promote antioxidant activities and can be developed for further applications.

Keywords: antioxidant, *Impatiens balsamina* L., phytochemicals, tyrosinase inhibition

1. Introduction

Tyrosinase (TYR, EC 1.14.18.1), also known as polyphenol oxidase, is widely distributed in nature. It occurs as copper-containing oxidase which catalyzes two distinct reactions of melanin synthesis, the hydroxylation of L-tyrosine to 3-(3,4-dihydroxyphenyl)-L-alanine (L-DOPA) and the oxidation of L-DOPA to dopaquinone (Kim & Uyama, 2005; Lin, Chiang, Lin, & Wen, 2008). TYR is responsible for the pigmentation of human skin, resulting in epidermal hyperpigmentation that leads to various dermatological disorders such as melasma, freckles, age spots, and skin cancer (Kim & Uyama, 2005). TYR-inhibiting materials may be clinically applicable for the treatment of some dermatological disorders associated with melanin hyperpigmentation and they are important in cosmetic products to maintain skin whiteness (Shiino, Watanabe, & Umezawa, 2001).

Antioxidant activity is related to compounds capable of protecting a biological system against potentially harmful effects of processes or reactions involving reactive oxygen species (Karadag, Ozcelix, & Saner, 2009). Thus, taking supplements or food containing antioxidants is an alternative way of reducing oxidative damage.

Plants and their extracts present a rich source of antioxidant activity compounds, such as phenolic compounds and flavonoids, which can potentially be developed into alternative promising agents for food and pharmaceutical formulations (Krishnaiah, Sarbatly, & Nithyanandam, 2011). Apart from their antioxidant property, plant-based compounds usually have a whitening effect (Solano, Briganti, Picardo, & Ghanem, 2006). Plant phenolic compounds and especially flavonoid are correlated with antioxidant and TYR inhibition properties (Solano et al., 2006).

*Impatiens balsamina* L., also known as garden balsam, is an annual herbaceous plant of the Balsaminaceae family and is cultured as an ornamental garden plant (Su et al., 2012). For centuries, the aerial parts of this plant have been used in traditional Thai medicine (locally called Tien-Bann in Thai) to treat thorn or glass-puncture wounds, abscesses, ingrown nails and chronic ulcers caused by allergic reactions to detergents (Farnsworth & Bunyapraphatsara, 1992). The plant has also been used as a Chinese herbal medicine for articular rheumatism and bruises and as a folk remedy for itching.

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associated with insect bites (Ishiguro & Oku, 1997). Moreover, several studies reported the antioxidant activity of *I. balsamina* extract (Kang et al., 2013; Shivakumara et al., 2014; Su et al., 2012). Pharmacological studies have proven the antipruritic, antidermatitic and antinociceptive effects as well as cyclooxygenase-2 inhibitory activity of the flower extract (Imam, Nahar, Akter, & Rana, 2012; Oku & Ishiguro, 2001; 2002), anti-tumor activity of the leaf extract (Ding, Jiang, Chen, Lv, & Zhu, 2008), and anti-diabetic and anti-inflammatory activities of the seed extract (Shivakumara et al., 2014) as well as antimicrobial activity (Kang et al., 2013; Sakunphueak & Panichayupakaranant, 2012; Su et al., 2012). However, there is currently no report on the anti-TYR activity of this species.

The yield and biological activity of natural extracts are dependent on the solvent used for extraction. Polar solvents, i.e. ethanol, methanol, and acetone, are frequently used to recover polyphenol from plant material (Boulekbache-Makhlouf, Medouni, Medouni-Adrar, Arkoub, & Madani, 2013; Do et al., 2014). Ethanol is a good solvent for polyphenol extraction and is safe for human consumption, whereas methanol and acetone were found to be more efficient in the extraction of lower molecular weight polyphenols and higher molecular weight flavonoids (Daï & Mumper, 2010).

This study investigated the anti-TYR and antioxidant activities of different plant parts and solvent extracts prepared from *I. balsamina*. In addition, the total phenolic compound and flavonoid contents were assessed to highlight the correlation between these phytochemicals and the values of TYR inhibition and antioxidant. Moreover, the copper-chelating activity was determined to reveal the possible mechanism of TYR inhibition of the extracts. The aim of this study was to explore a new source of anti-TYR and antioxidant agents for cosmeceutical purposes.

### 2. Materials and Methods

#### 2.1 Chemicals

Folin-Ciocalteu reagent and quercetin were obtained from Merck (Germany). Gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), mushroom tyrosinase, L-DOPA, and pyrocatechol violet were purchased from Sigma-Aldrich (USA). Other reagents used in the experiments were of analytical grade.

#### 2.2 Plant materials

*I. balsamina* L. was cultivated in the Manorom District of Chai Nat Province in Thailand from December 2016 to March 2017. Three-month old plants were transferred to the greenhouse of the Department of Botany, Faculty of Liberal Arts and Science, Kasetsart University, Kamphaeng Saen, Nakhon Pathom Province, Thailand. The plants were harvested in March 2017 (2 weeks after transfer). The plants were identified and authenticated by a taxonomist from the Plant Varieties Protection Office, Department of Agriculture, Bangkok, Thailand and the voucher specimen (BK. No. 68977) was deposited in the Bangkok Herbarium (BK), Bangkok, Thailand.

#### 2.3 Crude extract preparation

The aerial parts, that consisted of the stems, flowers (purple color), and leaves, were separated, collected, and thoroughly cleaned. They were dried in a hot-air oven at 40 °C for 48 h. Each part was then ground separately into a fine powder. Ten grams of each dried sample were extracted separately by soaking in 250 mL of different selected solvents, i.e. ethanol, methanol or acetone, for 48 h with constant shaking at 90 rpm at room temperature under dark conditions. After 48 h, the infusions were filtered through Whatman No. 1 filter paper and the residue was re-extracted with an equal volume of the relevant solvent. After 48 h, the processes were repeated. The combined filtrates were evaporated to dryness under a vacuum using a rotary evaporator. The extract yield was calculated as the percentage of the dry extract based on the raw material yield by weight. Stock solutions of *I. balsamina* extracts were prepared at a concentration of 10 mg mL⁻¹ of ethanol, to give nine different extracts (Table 1). They were then stored at 4 °C until use.

#### 2.4 Determination of total phenolic compound content (TPC)

The TPC was determined by the Folin-Ciocalteu (FC) method (Singleton & Rossi, 1965) with minor modifications. Fifty µL of the different extract solutions were mixed with 250 µL of the FC reagent and kept at room temperature for 8 min. Thereafter, 750 µL of 20% Na₂CO₃ and then 950 µL of distilled water (DW) were added, mixed and incubated at 25 °C for 30 min. After incubation, the absorbance was taken using a UV-vis spectrophotometer (UV-1800, Shimadzu, Japan) at 765 nm. Gallic acid was used as a standard, and the results were expressed as milligrams of gallic acid equivalent (GAE) g crude extract⁻¹.

#### 2.5 Determination of total flavonoid content (TFC)

The TFC assay was performed using the AlCl₃ colorimetric method (Zhitao, Mengcheng, & Jianming, 1999). To 500 µL of the different extracts, 2 mL of DW plus 15 µL of 5% NaNO₂ were added and the mixture was then shaken. After incubation at room temperature for 6 min, 150 µL of 10% AlCl₃, 2 mL of 2 M NaOH, and 200 µL of DW were added to the mixture. The solution was mixed and incubated at 25 °C for 30 min. The absorbance was measured at 415 nm

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**Table 1.** Extraction yields of *I. balsamina* extracts using different solvents.

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Solvent</th>
<th>Abbreviation</th>
<th>Extraction yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem</td>
<td>Ethanol</td>
<td>SE</td>
<td>20.09</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>SM</td>
<td>42.60</td>
</tr>
<tr>
<td></td>
<td>Acetone</td>
<td>SA</td>
<td>11.18</td>
</tr>
<tr>
<td>Flower</td>
<td>Ethanol</td>
<td>FE</td>
<td>31.70</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>FM</td>
<td>44.92</td>
</tr>
<tr>
<td></td>
<td>Acetone</td>
<td>FA</td>
<td>21.56</td>
</tr>
<tr>
<td>Leaf</td>
<td>Ethanol</td>
<td>LE</td>
<td>21.39</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>LM</td>
<td>28.53</td>
</tr>
<tr>
<td></td>
<td>Acetone</td>
<td>LA</td>
<td>11.19</td>
</tr>
</tbody>
</table>
using the UV-vis spectrophotometer. Quercetin was used as the standard for calibration curve, and the results were expressed as milligrams of quercetin equivalent (QE) g crude extract\(^{-1}\).

2.6 TYR inhibition assay

The TYR inhibition effect was measured using L-DOPA as the substrate according to the method described by Liang, Lim, Kim, and Kim (2012) with some modifications. The extracts were evaporated to dryness and re-dissolved in 5% dimethyl sulfoxide (DMSO). To 100 µL of the mushroom L of the mushroom sample acts on a dry he control. Ascorbic acid (AA) was added to the control. The inhibition effect was measured using L-dopa (PV) was added (final concentration of the extract was 0.5 mg mL\(^{-1}\)). The absorbance was measured 10 min later at 700 nm using the UV-vis spectrophotometer. The absorbance of the control and A\(_{sample}\) was calculated using the following equation: 

\[
\text{TYR activity} = \frac{(A_{sample} - A_{blank})}{A_{control}} \times 100
\]

where A\(_{sample}\) is absorbance of the extract solution and A\(_{blank}\) is absorbance of the blank solution.

2.9 Copper-chelating assay

The copper-chelating activity was determined according to the method of Saiga, Tanabe, and Nishimura (2003). The extracts (0.5 mL) were mixed with 2 mL of 50 mM sodium acetate buffer (pH 6.0) and 200 µL of 2 mM CuSO\(_4\). After incubation at room temperature for 10 min, 200 µL of 2 mM pyrocatechol violet (PV) was added (final concentration of the extract was 1.72 mg mL\(^{-1}\)). The mixture was incubated at room temperature for 20 min. The absorbance was measured at 632 nm using the UV-vis spectrophotometer. AA was used as a reference agent. The copper-chelating activity was calculated using this formula: copper chelating activity (%) = \[1 - \frac{(A_{sample} - A_{blank})}{A_{control}}\] × 100, where A is the absorbance of the sample + CuSO\(_4\) + PV; B is the absorbance of the sample + buffer + PV; C is the absorbance of the buffer + CuSO\(_4\) + PV, and D is the absorbance of the buffer + CuSO\(_4\) + PV.

2.10 Statistical analysis

All the data were expressed as mean±standard error of three replications. Data were subjected to analysis of variance followed by Duncan’s multiple-range tests. P<0.05 was considered statistically significant using the R software program (version 3.0.2). Correlation between the parameters was established using Pearson’s correlation coefficient.

3. Results and Discussion

3.1 Extraction yields

The yields of various extracts on a dry-weight basis ranged from 11.18% to 44.92%. The methanol flower extract had the highest yield (Table 1). The extraction yields in descending order were methanol, ethanol, and acetone. This showed that methanol was the best solvent for extraction of compounds from the three parts of I. balsamina. Several researchers found that methanol was the most effective organic solvent regarding the extraction of bioactive compounds (Razali, Mat-Junit, Abdul-Muthalib, Subramaniam, & Abdul-Aziz, 2012; Sahreen, Khan, & Khan, 2010). Among the plant parts, the highest extraction yield was found in the flower, followed by the stem, whereas the leaf had the lowest yield (Table 1).

3.2 Total phenolic and total flavonoids contents

The TPC and TFC amounts varied significantly according to the plant part and the solvent used. In general, the amounts of TPC and TFC were the highest in the acetone extracts, followed by the ethanol and methanol extracts (Table 2). The TPC and TFC amounts in the flower and leaf extracts were clearly higher than in the stem extracts, with the highest amount of TPC and TFC being found in the acetone flower extract (Table 2). This result coincided with Chua (2016) and Kang et al. (2013) who reported that the amounts of TPC and TFC of I. balsamina leaf extract were higher than the stem extract.
The results of this study showed that none is the most popular depigmenting agent (Briganti, Camera, & Picardo, 2003). On the other hand, TYR inhibitory activity of I. balsamina in this study may be due to the accumulation of quinone in this plant.

Copper is the active site of TYR; therefore, a compound with copper-chelating activity may be advantageous for TYR inhibition (Babbar, Oberoi, Uppal, & Patil, 2011). In order to better understand the possible mechanism of the TYR inhibition of the extracts, their copper-chelating activity was investigated. The results showed that the flower extracts had higher copper-chelating activity than the leaf and stem extracts and the acetone flower extract had the highest activity (Figure 2). Pearson’s correlation analysis indicated a significant positive correlation (r=0.716) between TYR inhibitory activity and copper-chelating activities (Table 3). It might be claimed that the I. balsamina extracts inhibited TYR activity by their ability to chelate the copper in the active site that led to inactivation of the enzyme. The interaction with copper at the active site of TYR is one mechanism of action of TYR inhibitor (Briganti et al., 2003). The results of this study showed that copper-chelating activity was positively correlated with the phytochemical constituents of the extracts (Table 3). It suggested that phenolic compounds in the extract could bind to the active site of TYR and act as competitive inhibition. The inhibition of TYR activity might depend on the hydroxyl groups on the polyphenol compounds which may form hydrogen bonds with an enzyme active site that results in lower enzymatic activity (Briganti et al., 2003). Phenol and flavonoid extract. The accumulation of phenolic compounds in different plant organs may be associated with their role in the life of the plant. The high amounts of these compounds in the flower and leaf could be explained by their protection function against oxidative damage to the plant cell due to free radicals from sunlight (Chua, 2016). The role of the plant stem is mainly to deliver food from the leaves to the roots, flowers or fruits. As a result, there are relatively low amounts of TPC and TFC in the stem extract.

### 3.3 Anti-TYR activity

TYR is the rate-limiting enzyme for the production of melanin. The anti-TYR effect was determined by calculating the oxidation of L-DOPA to dopaquinone according to in vitro mushroom TYR assay. The various extracts prepared from I. balsamina demonstrated their ability to inhibit TYR activity (Figure 1). Among the stem, leaf, and flower parts, the flower extracts of all three solvents displayed the strongest anti-TYR activity with enzymatic inhibition nearly 50%, while the lowest inhibition was in the acetone leaf extract. A strong correlation occurred between TYR inhibition and TPC and TFC (r=0.5) (Table 3). The anti-TYR activity of the extracts could be attributed mainly to their high levels of TPC and TFC. Polyphenols are the largest group in TYR inhibition (Chang, 2009). Action mechanisms of phenolic compounds not only have good affinity for the TYR in order to prevent the formation of dopaquinone but also they can bind to TYR and reduce its catalytic activity (Chang, 2009). Some phenolic compounds such as kojic acid, coumarin, kaempferol, and quercetin are known to be potent TYR inhibitors (Kim & Uyama, 2005; Solano et al., 2006). Kaempferol and quercetin have been reported from the flower of I. balsamina (Hua, Peng, Chia, Goh, & Tan, 2001; Ishiguro & Oku, 1997). Moreover, naphthoquinones have been reported as a group of pharmacological active compounds in the flower and leaf of I. balsamina (Ishiguro & Oku, 1997; Oku & Ishiguro, 2001; 2002; Sukunphueak & Panichayuaparakaran, 2012). As already known, hydroquinone is the most popular depigmenting agent (Briganti, Camera, & Picardo, 2003). On the other hand, TYR inhibitory activity of I. balsamina in this study may be due to the accumulation of quinone in this plant.
agents usually inhibit enzyme TYR due to their abilities to chelate copper at the active site (Lin et al., 2008). The inhibition ability may be explained in terms of similarity between the dihydroxyphenyl group in L-DOPA and the α-keto group in flavonoids (Kim & Uyama, 2005).

### 3.4 Antioxidant activity

The standard methods of antioxidant evaluation, DPPH scavenging and reducing power assay, were employed to determine the antioxidant activity. The various extracts showed different patterns of antioxidant activity. The flower extracts of all three solvents exhibited the most activity, followed by the leaf extracts and the stem extracts in both radical scavenging (more than 80%) and reducing power (more than 3,000%) assays (Figure 3A and 3B). The antioxidant activity of the stem, leaf, and flower extracts of this species were also reported by Chua (2016), Kang et al. (2013) and Su et al. (2012). A strong correlation between both assays was obtained (Table 3). This indicated that the extracts possessed antioxidant mechanisms in the form of both free radical scavenging and reducing power. Therefore, it could be claimed that active compounds in these extracts were good electron donors and could terminate oxidation chain reaction by reducing the oxidized intermediate into the stable form.

The antioxidant activity demonstrated by the various extracts was in line with the findings for TPC and TFC which demonstrated a high correlation between these parameters (Table 3). The correlations indicated that phenol and flavonoid contributed to the antioxidant activity in the extracts. The antioxidant activity of phenol or polyphenols is mainly due to their chelating of redox-active metal ions, inactivating lipid free radical chains, and preventing hydroperoxide conversion into reactive oxyradicals, which allows them to act as reducing agents, hydrogen donors, and singlet-oxygen quenchers (Babbar et al., 2011; Sahreen et al., 2010). High degrees of correlation between anti-TYR activity and radical scavenging and reducing power activities were observed (Table 3). These findings suggested that the antioxidant activity mechanism may also be an important reason for TYR inhibitory activity. The strong antioxidant activity of I. balsamina extract is responsible, at least in part, for its treatment effect on skin diseases. Excessive UV radiation will induce various skin diseases while too much melanin will be produced (Fu, Zhang, Guo, & Chen, 2014). Ma et al. (2001) suggested that reactive oxygen species scavengers, as an antioxidant, may reduce hyperpigmentation.

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**Table 3. Correlation coefficients between responses studied.**

<table>
<thead>
<tr>
<th>Phenolic compound</th>
<th>Flavonoid</th>
<th>Anti-tyrosinase</th>
<th>Copper chelating</th>
<th>Radical scavenging</th>
<th>Reducing power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic compound</td>
<td>1</td>
<td>0.929***</td>
<td>0.760***</td>
<td>0.934***</td>
<td>0.956***</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>1</td>
<td>0.542**</td>
<td>0.560**</td>
<td>0.837***</td>
<td>0.879**</td>
</tr>
<tr>
<td>Anti-tyrosinase</td>
<td>1</td>
<td>0.716***</td>
<td>0.776***</td>
<td>0.818***</td>
<td>0.806***</td>
</tr>
<tr>
<td>Copper chelating</td>
<td>1</td>
<td>1</td>
<td>0.807***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radical scavenging</td>
<td>1</td>
<td>1</td>
<td>0.975***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reducing power</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**, ***: correlation significant at P<0.01 and P<0.001 level, respectively.
activity of *I. balsamina*. The possible mechanism of the TYR inhibition effect is its copper-chelating property. The results also showed little difference among the selected solvents in their anti-TYR and antioxidant activities. Based on the study’s findings, *I. balsamina* may be considered potentially useful as a source of natural anti-TYR and antioxidant compounds, which may be considered as skin-depigmentation agents following further clinical investigations.

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