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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 flavonols (Dai & 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 dry 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.

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

Plant part	Solvent	Abbreviation	Extraction yield (%)
Stem	Ethanol	SE	20.09
	Methanol	SM	42.60
	Acetone	SA	11.18
Flower	Ethanol	FE	31.70
	Methanol	FM	44.92
	Acetone	FA	21.56
Leaf	Ethanol	LE	21.39
	Methanol	LM	28.53
	Acetone	LA	11.19

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% Na_2CO_3 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 $AlCl_3$ colorimetric method (Zhishen, Mengcheng, & Jianming, 1999). To 500 µL of the different extracts, 2 mL of DW plus 15 µL of 5% $NaNO_2$ were added and the mixture was then shaken. After incubation at room temperature for 6 min, 150 µL of 10% $AlCl_3$, 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

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 TYR solution (0.5 mg mL^{-1} or 500 units mL^{-1}) 100 µL of the extract was added plus 1,800 µL of phosphate buffer (0.2 M, pH 7.0) and incubated in a water bath at 30 °C for 15 min. The final concentration of the extract was 0.5 mg mL^{-1}. Thereafter, 50 µL of 10 mM L-DOPA solution was added and the absorbance at 475 nm was immediately monitored using the UV-vis spectrophotometer at 0 and 6 min. The same reaction mixture having the extract replaced by the equivalent amount of 5% DMSO served as the control. Ascorbic acid (AA) was used as the reference compound. The percentage inhibition of TYR activity was calculated using this equation: $[(\Delta A_{control}-\Delta A_{sample})/\Delta A_{control}] \times 100$, where $\Delta A_{control}$ is the change of absorbance at 475 nm of the control and ΔA_{sample} is the change of absorbance at 475 nm with the extract.

2.7 DPPH radical scavenging assay

The radical scavenging activity was determined using the DPPH method (Brand-Williams, Cuvelier, & Berset, 1995). A fixed volume (0.2 mL) of the crude extract was mixed with 1 mL of 0.1 mM DPPH (final concentration of the extract was 1.67 mg mL^{-1}) and stored in the dark at room temperature for 30 min. Absorbance was measured at 517 nm using the UV-vis spectrophotometer. Controls were prepared as in the tested group except that the plant solution was replaced with the extraction solvent. AA was used as the reference standard. The DPPH free radical scavenging activity was calculated using the following equation: DPPH scavenging activity (%) = $[(A_0-A_1)/A_0] \times 100$ where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

2.8 Reducing power assay

The reducing power of the extracts was determined by their Fe^{3+} reducing ability according to the method of Yen and Chen (1995). The extracts (0.2 mL) were mixed with 0.5 mL of phosphate buffer (0.2 M, pH 6.8) and 0.5 mL of 1% potassium ferricyanide. The mixture was incubated in a water bath at 50 °C for 20 min. After incubation, 0.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3,000 rpm for 10 min. The supernatant (1.5 mL) was mixed with 1.5 mL of DW plus 0.1 mL of 0.1% ferric chloride. The final concentration of the extract was 0.38 mg mL^{-1}. The absorbance was measured 10 min later at 700 nm using the UV-vis spectrophotometer. AA was used as the reference standard. A blank was prepared without adding the extract or standard. The increasing percentage in reducing power was calculated using the equation: $[(A_{sample}-A_{blank})/A_{blank}] \times 100$, 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 (%) = $[1-(A-B)/(C-D)] \times 100$, 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 + buffer + 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

Table 2. Total phenolic (TPC) and flavonoid contents (TFC) of various extracts from *I. balsamina*.

Samples	TPC (mg GAE g^{-1})	TFC (mg QE g^{-1})
SE	292.08±10.61 e	242.96±4.32 g
SM	321.03±24.23 e	219.17±1.08 h
SA	177.79±8.71 f	529.76±2.84 f
FE	1,688.27±30.02 a	1,080.05±8.67 b
FM	1,596.08±38.59 b	884.83±1.34 d
FA	1,692.46±31.39 a	1,139.97±4.49 a
LE	1,301.60±19.47 c	879.42±1.01 d
LM	1,094.74±8.27 d	856.41±4.93 e
LA	1,302.36±27.18 c	929.31±2.41 c

Sample abbreviations are provided in Table 1. Data are expressed as mean±SE (n=3). Means with different letters in the same column are significantly different (P<0.05).

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, 20 09). 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; Sakunphueak & Panichayupakaranant, 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.

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 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

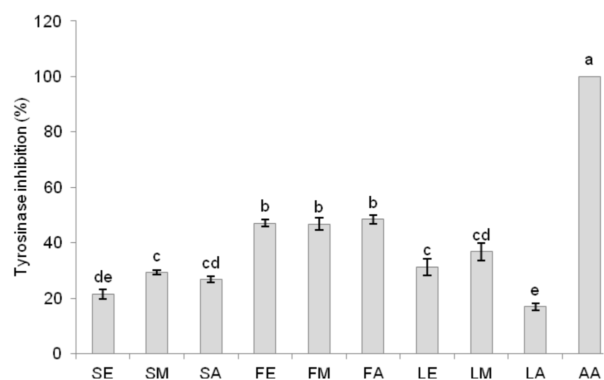

Figure 1. Tyrosinase inhibition activity of *I. balsamina* extracts. Sample abbreviations are provided in Table 1. Ascorbic acid (AA) was the positive control. Data are expressed as mean±SE (n=3). Different letters above bars indicate significant difference (P<0.05).

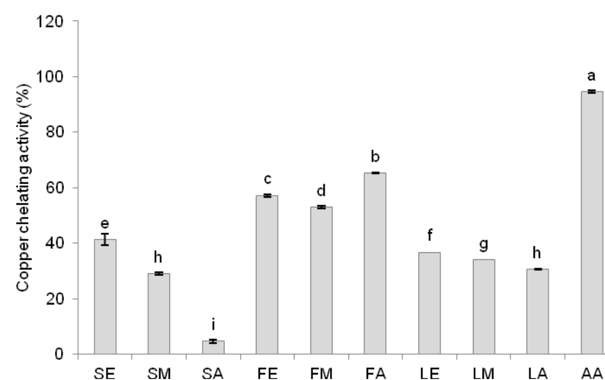

Figure 2. Copper-chelating activity of *I. balsamina* extracts. Sample abbreviations are provided in Table 1. Ascorbic acid (AA) was the positive control. Data are expressed as mean±SE (n=3). Different letters above bars indicate significant difference (P<0.05).

Table 3. Correlation coefficients between responses studied.

	Phenolic compound	Flavonoid	Anti-tyrosinase	Copper chelating	Radical scavenging	Reducing power
Phenolic compound	1	0.929***	0.632***	0.760***	0.934***	0.956***
Flavonoid		1	0.542**	0.560**	0.837***	0.879**
Anti-tyrosinase			1	0.716***	0.776***	0.806***
Copper chelating				1	0.818***	0.807***
Radical scavenging					1	0.975***
Reducing power						1

, *, correlation significant at P<0.01 and P<0.001 level, respectively.

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 phenols 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.

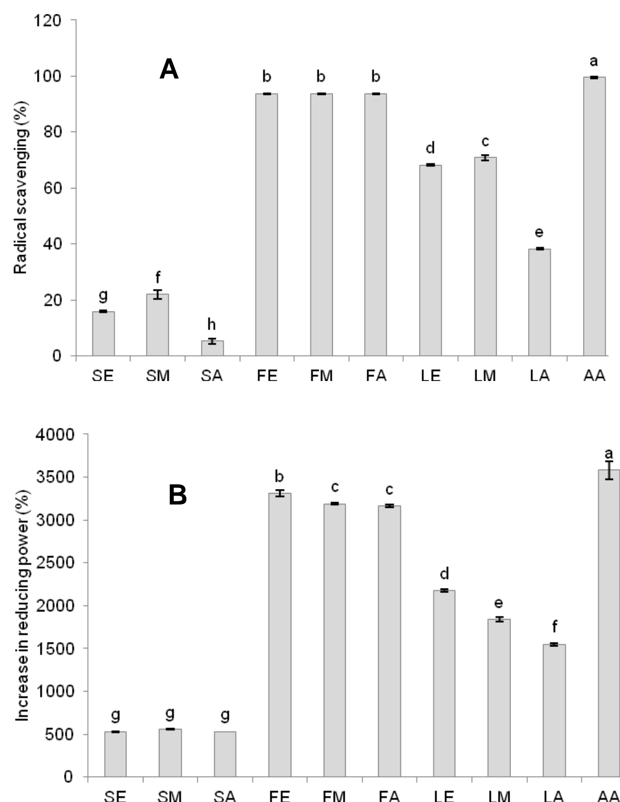

Figure 3. Antioxidant activities of *I. balsamina* extracts, (A) DPPH scavenging activity and (B) reducing power activity. Sample abbreviations are provided in Table 1. Ascorbic acid (AA) was the positive control. Data are expressed as mean±SE (n=3). Different letters above bars indicate significant difference (P<0.05).

4. Conclusions

The results revealed good potential for ethanol, methanol, and acetone extracts from *I. balsamina* for TYR inhibition and antioxidant activities at 10 mg mL^{-1}. The flower extracts exhibited greater potential activity than the stem and leaf extracts, which generally indicated that the flower extracts are more suitable for this application. The extracts also had high amounts of TPC and TFC which showed a strong correlation with the anti-TYR and antioxidant activities. To our knowledge, this is the first report to present the anti-TYR

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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References

Babbar, N., Oberoi, H. S., Uppal, D. S., & Patil, R. T. (2011). Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. *Food Research International*, *44*, 391–396. doi:10.1016/j.foodres.2010.10.001

Boulekbache-Makhlouf, L., Medouni, L., Medouni-Adrar, S., Arkoub, L., & Madani, K. (2013). Effect of solvents extraction on phenolic content and antioxidant activity of the byproduct of eggplant. *Industrial Crops and Products*, *49*, 668–674. doi:10.1016/j.indcrop.2013.06.009

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, *28*, 25–30. Retrieved from https://doi.org/10.1016/S0023-6438(95)80008-5

Briganti, S., Camera, E., & Picardo, M. (2003). Chemical and instrumental approaches to treat hyperpigmentation. *Pigment Cell Research*, *16*, 101–110. doi:10.1034/j.1600-0749.2003.00029.x

Chang, T. S. (2009). An updated review of tyrosinase inhibitors. *International Journal of Molecular Sciences*, *10*, 2440–2475. doi:10.3390/ijms10062440

Chua, L. S. (2016). Untargeted MS-based small metabolite identification from the plant leaves and stems of *Impatiens balsamina*. *Plant Physiology and Biochemistry*, *106*, 16–22. doi:10.1016/j.plaphy.2016.04.040

Dai, J., & Mumper, R. J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, *15*, 7313–7352. doi:10.3390/molecules15107313

Ding, Z. S., Jiang, F. S., Chen, N. P., Lv, G. Y., & Zhu, C. G. (2008). Isolation and identification of an anti-tumor component from leaves of *Impatiens balsamina*. *Molecule*, *13*, 220–229. doi:10.3390/molecules13020220

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y. H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis*, *22*, 296–302. doi:10.1016/j.jfda.2013.11.001

Farnsworth, N. R., & Bunyapraphatsara, N. (1992). *Thai medicinal plant: recommended for primary health care system*. Bangkok, Thailand: Prachachon.

Fu, R., Zhang, Y., Guo, Y., & Chen, F. (2014). Antioxidant and tyrosinase inhibition activities of the ethanol-insoluble fraction of water extract of *Sapium sebiferum* (L.) Roxb. leaves. *South African Journal of Botany*, *93*, 98–104. doi:10.1016/j.sajb.2014.04.003

Hua, L., Peng, Z., Chia, L. S., Goh, N. K., & Tan, S. N. (2001). Separation of kaempferols in *Impatiens balsamina* flowers by capillary electrophoresis with electrochemical detection. *Journal of Chromatography A*, *909*, 297–303. doi:10.1016/S0021-9673(00)01102-X

Imam, M. Z., Nahar, N., Akter, S., & Rana, M. S. (2012). Antinociceptive activity of methanol extract of flowers of *Impatiens balsamina*. *Journal of Ethnopharmacology*, *142*, 804–810. doi:10.1016/j.jep.2012.06.004

Ishiguro, K., & Oku, H. (1997). Antipruritic effect of flavonol and 1,4-naphthoquinone derivatives from *Impateins balsamina* L. *Phytotherapy Research*, *11*, 343–347. doi:10.1002/(SICI)1099-1573(199708)11:5<343::AID-PTR103>3.0.CO;2-4

Kang, S-N., Goo, Y-M., Yang, M-R., Ibrahim, R. I. H., Cho, J-H., Kim, I-S., & Lee, O-H. (2013). Antioxidant and antimicrobial activities of ethanol extract from the stem and leaf of *Impatiens balsamina* L. (Balsaminaceae) at different harvest times. *Molecules*, *18*, 6356–6365. doi:10.3390/molecules18066356

Karadag, A., Ozcelik, B., & Saner, S. (2009). Review of methods to determine antioxidant capacities. *Food Analytical Methods*, *2*, 41–60. doi:10.1007/s12161-008-9067-7

Kim, Y. J., & Uyama, H. (2005). Tyrosinase inhibitors from natural and synthetic source: structure, inhibition mechanism and prospective for the future. *Cellular and Molecular Life Sciences*, *62*, 1707–1723. doi: 10.1007/s00018-005-5054-y

Krishnaiah, D., Sarbatly, R., & Nithyanandam, R. (2011). A review of the antioxidant potential of medicinal plant species. *Food and Bioproducts Precessing*, *89*, 217–233. doi: 10.1016/j.fbp.2010.04.008

Liang, C., Lim, J. H., Kim, S. H., & Kim, D. S. (2012). Dioscin: a synergistic tyrosinase inhibitor from roots of *Smilax china*. *Food Chemistry*, *134*, 1146-1148. doi: 10.1016/j.foodchem.2012.03.003

Lin, J. W., Chiang, H. M., Lin, Y. C., & Wen, K. C. (2008). Natural products with skin-whitening effects. *Journal of Food and Drug Analysis*, *16*, 1–10.

Ma, M., Wlaschek, M., Tantcheva-Poór, I., Schneider, L. A., Naderi, L., Razi-Wolf, Z., . . . Scharffetter-Kochanek, K. (2001). Chronological ageing and photoageing of the fibroblasts and the dermal connective tissue. *Clinical and Experimental Dermatology*, *26*, 592–599. doi: 10.1046/j.1365-2230.2001.00905.x

Oku, H., & Ishiguro, K. (2001). Antipruritic and antidermatitic effect of extract and compounds of *Impatiens balsamina* L. in atopic dermatitis model NC mice. *Phytotherapy Research*, *15*, 506–510. doi:10.1002/ptr.964

Oku, H., & Ishiguro, K. (2002). Cyclooxygenase-2 inhibitory 1,4-naphthoquinones from *Impatiens balsamina* L. *Biological and Pharmaceutical Bulletin*, *25*, 658–660. doi:10.1248/bpb.25.658

Razali, N., Mat-Junit, S., Abdul-Muthalib, A. F., Subramaniam, S., & Abdul-Aziz, A. (2012). Effects of various solvents on the extraction of antioxidant phenolics from the leaves, seeds, veins and skins of *Tamarindus indica* L. *Food Chemistry*, *131*, 441–448. doi:10.1016/j.foodchem.2011.09.001

Sahreen, S., Khan, M. R., & Khan, R. A. (2010). Evaluation of antioxidant activities of various solvent extracts of *Carissa opaca* fruits. *Food Chemistry*, *122*, 1205–1211. doi:10.1016/j.foodchem.2010.03.120

Saiga, A., Tanabe, S., & Nishimura, T. (2003). Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment. *Journal of Agricultural and Food Chemistry*, *51*, 3661–3667. doi:10.1021/jf021156g

Sakunphueak, A., & Panichayupakaranant, P. (2012). Comparison of antimicrobial activities of naphthoquinones from *Impatiens balsamina*. *Natural Product Research*, *26*, 1119–1124. doi:10.1080/14786419.2010.551297

Shiino, M., Watanabe, Y., & Umezawa, K. (2001). Synthesis of *N*-substituted *N*-nitrosohydroxylamines as inhibitors of mushroom tyrosinase. *Bioorganic & Medicinal Chemistry*, *9*, 1233–1240. doi:10.1016/S0968-0896(01)00003-7

Shivakumara, Wahengbam, S., Rana, N. K., Kundu, S., Bole, S., & Vedamurthy, A. B. (2014). Phytochemical screening and biological activities of *Impatiens balsamina* L. seeds. *International Journal of Fundamental and Applied Sciences*, *3*, 22–26.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*, 144–158.

Solano, F., Briganti, S., Picardo, M., & Ghanem, G. (2006). Hypopigmenting agents: an updated review on biological, chemical and clinical aspects. *Pigment Cell Research*, *19*, 550–571. doi:10.1111/j.1600-0749.2006.00334.x

Su, B. L., Zeng, R., Chen, J. Y., Chen, C. Y., Guo, J. H., & Huang, C. G. (2012). Antioxidant and antimicrobial properties of various solvent extracts from *Impatiens balsamina* L. stem. *Journal of Food Science*, *77*, C614–C619. doi:10.1111/j.1750-3841.2012.02709.x

Yen, G. C., & Chen, H. Y. (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. *Journal of Agriculture and Food Chemistry*, *43*, 27–32. doi:10.1021/jf00049a007

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, *64*, 555–559. doi:10.1016/S0308-8146(98)00102-2