Antioxidant and tyrosinase inhibition of ethanol extract from leaves and peel of *Passiflora edulis var. flavicarpa*

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ABSTRACT

In this work, the ethanol extracts from leaves and peel of *Passiflora edulis var. flavicarpa* (yellow passion fruit) were determined total phenolic content, antioxidant and tyrosinase inhibition property. The dried materials were ground, defatted by hexane and then extracted with ethanol (cold extraction). The leaves and peel extracts showed total phenolic content as 14.58 ± 0.26 and 13.93 ± 0.89 mg GAE/g extract when determined by Foline-Ciocalteu method. The extracts were determined antioxidant activity by $ABTS^{\bullet+}$ scavenging assay compared with ascorbic acid. The results indicated that inhibition concentration at 50% (IC_{50}) of ascorbic acid, the leaves extract and the peel extract were 2.72 ± 0.14, 183.20 ± 23.92 and 194.45 ± 15.40 µg/mL, respectively. Vitamin C equivalent antioxidant capacity (VCEAC) of the leaves extract and the peel extract were 14.85 and 13.99 mg/g extract, respectively. While, inhibitory effect of the extracts on tyrosinase was determined by DOPA oxidase assay. In the concentration of 100 µg/mL of the leaves extract and the peel extract showed the inhibition activity as 7.26% and 3.64%, respectively. Both extracts exhibited same antioxidant activity with modulate level. On the other hand, 100 µg/mL of both extracts showed low tyrosinase inbhbitory effect.

Keywords: *Passiflora deulis* var. *flavicarpa*, yellow passion fruit, phenolic, antioxidant, tyrosinase inhibition.

INTRODUCTION

Many efforts to find develop novel and more potent tyrosinase inhibitors, antioxidants without side effects become increasingly important in the food and the cosmetic products. Plants may present a rich source of such compounds, especially phenolic compounds, which can potentially be developed as alternative promising agents. The plant phenolic compounds, exhibit antioxidant and tyrosinase inhibition properties (Karim *et al*. 2014; Suh *et al*. 2014). In addition to the edible portion of whole fruits and vegetables, their non-edible portions could also be good sources of bioactive compounds, such as polyphenols, that could be supplemented in the diet. Interestingly, the byproducts of tropical fruits contain high levels of bioactive compounds (vitamins, minerals, polyphenolic antioxidants and dietary fiber), which may have positive health effects. There have been reported that the byproducts of cultivation or food industry such as leaves, fruit peels and seeds may potentially possess antioxidant and/or

tryrosinase inhibitory properties (Thitilertdecha *et al*. 2008; Maisuthisakul and Gordon, 2009; Fu *et al*. 2014).

Fruit of Yellow passion fruit (*Passiflora edulis var. flavicarpa*) is intended for the production of juices and soft drinks. However, the cultivation and the juice production generate several agricultural byproducts. Leaves and peel (albedo) of yellow passion fruit are byproduct. The aims of this study were to ascertain the phenolic content, the antioxidant property and the tyrosinase inhibitory effect of ethanol extract from leaves and peel from yellow passion fruit. The results confirm the possibility of using yellow passion fruit leaves and peel byproduct as source of natural ingredient.

MATERIALS AND METHOD

General

Ascorbic acid, Mushroom tyrosinase (EC 1.14.18.1) and L-DOPA (L-3,4-dihydroxyphenylalanine) were got from Sigma Chemical Co. ABTS [2, 2'-Azino-bis (3-ethylbenzthiazoline-6-sulphonic)] and gallic acid were purchased from Fluka (Germany). Potassium persulfate and Folin-Ciocalteu's Phenol reagent were obtained from Merck (Germany). All other chemicals were of analytical grade without further purification.

Ethanol extraction

Leaves and peel of *P. edulis var. flavicarpa* was collected from Chiang Mai province, Thailand, in December, 2014. The air-dried, powdered of the samples were defatted with n-hexane to obtain defatted samples. The samples were re-extracted thrice with 250 mL/50g sample of ethanol for 24 h at room temperature. The extract was combined, and ethanol was evaporated to absolute dryness at 50°C under reduced pressure. The crude extracts were stored at -20°C until use.

Determination of the total phenolic contents

The amounts of phenolic compounds in the extracts were determined with minor modification method of Waterman and Mole (1994) using Foline-Ciocalteu method and gallic acid was used as the standard phenolic compound. The extract solution (5 mg/mL) in appropriate solvent (0.25 mL) was mixed with 0.5 mL of Folin-Ciocalteu's phenol reagent and 2.75 mL of deionized water. After 5 min, 0.5 mL of 25% w/v sodium carbonate solution was added to the mixture. Subsequently, the shaken mixture was allowed to stand for 1 h at room temperature, after which the absorbance was read at 760 nm. Gallic acid was used for constructing the standard curve (20-100 µg/mL, $Y = 0.0068X + 0.0017$, $R^2 = 0.999$) and the results were expressed as mg gallic acid equivalents (GAE)/g sample, as mean of three replicates.

Determination of antioxidant activity

Antioxidant activity was determined by ABTS radical cation decolorization assays described by Re *et al*. (1999) with minor modification. Briefly, ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate in a ratio of 7:10 and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm and then 2.7 mL of this solution was mixed with 0.3 mL of the extract at different concentrations, and ascorbic acid was used for comparison. A control, containing 2.7 mL of $ABTS^{\bullet+}$ solution and 0.3 mL of ethanol was prepared. The mixture was incubated at room

temperature for 10 min and then the absorbance was measured at 734 nm. Each assay was conducted as three separate replicates. The ability to scavenge the $ABTS^{\bullet+}$ was calculated as percent $ABTS^{\bullet+}$ scavenging using the following equation:

$$\% \ ABTS^{\bullet+} \text{ scavenging} = [(A_C - A_S)/A_C] \times 100$$

Where A_C was the absorbance of the control and A_S was the absorbance of the mixture containing extracts. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of percent $ABTS^{\bullet+}$ scavenging against extract concentration. IC_{50} of reference antioxidant compounds, ascorbic acid, was used for comparison to IC_{50} of the extracts.

Determination of tyrosinase inhibition activity

The tyrosinase inhibition activity of the extract was measured by using L-DOPA as a substrate according to the modified method of Gupta and Masakapalli (2013). All test samples were first dissolved in DMSO at 10 mg/mL and diluted to 500 µg/mL with 20% DMSO. First, 50 µL of tyrosinase solution (10 units/mL) was mixed with 450 µL of 0.02 M phosphate buffer (pH 6.8) and 200 µL of 20% DMSO (A) or the test sample (B). After the mixture was pre-incubate at room temperature for 2 min, 300 µL of 5 mM L-DOPA was added to the mixture and measured absorbance at 475 nm. The reaction mixture was allowed to stand for 10 min at room temperature, and measured the absorbance again. The percentage of inhibition was calculated according to the following equation:

$$\% \text{ inhibition} = [(A_{10}-A_0)-(B_{10}-B_0)] \times 100/(A_{10}-A_0)$$

Where A_0 and A_{10} were the absorbance of the control at the beginning and after 10 min of the reaction. While, B_0 and B_{10} were the absorbance of the mixture containing extracts.

RESULTS AND DISCUSSION

The ethanol extracts and total phenolic contents

In this study, phenolic content, antioxidant activity and tyrosinase inhibition activity of *P. edulis var. flavicarpa* leaves and peel extracts were determined. The total phenolic contents of the extracts were determined by Foline-Ciocalteu method. The extraction yields and phenolic contents are presented in Table 1.

TABLE 1: Extraction yields and total phenolic content of the ethanol extracts from *P. edulis var. flavicarpa*

Extracts	Percentage yield	Total phenolic (mg GAE/g extract)
Leaves	17.86 ± 0.93	14.58 ± 0.26
Peel	6.82 ± 0.48	13.93 ± 0.89

Percentage yield of the leaves extract (17.86) was higher than the peel extract (6.82) maybe because the peel (albedo) contained more fiber that could not been extracted by ethanol. While, the total phenolic content in both extracts were almost same. Lopez-Vargas *et al* (2013) reported that yellow passion fruit albedo DMSO extract contained total phenolic content as 1.86 mg GAE/g. From this study the leaves and peel extracts contained total phenolic content as 14.58 and 13.93 mg GAE/g extract or 2.60, 0.95 mg GAE/g dry sample, respectively. Total phenolic content could be influenced by geographical origin, cultivar, harvesting, storage time and drying (Babbar *et al.* 2011). In addition, Naczk and Shahidi (2006) mentioned that the recovery of polyphenols from plant materials is influenced by the solubility of the phenolic compounds in the

solvent used for the extraction process. Furthermore, solvent polarity plays a key role in increasing phenolic solubility.

Antioxidant activity of the ethanol extracts

The leaves and peel extracts were determined for their antioxidant activities by using the $ABTS^{\bullet+}$ decolorization method. Ascorbic acid was used as reference compound. The extracts and ascorbic acid increased in antioxidative activity with increasing concentration as showed in Fig. 1.

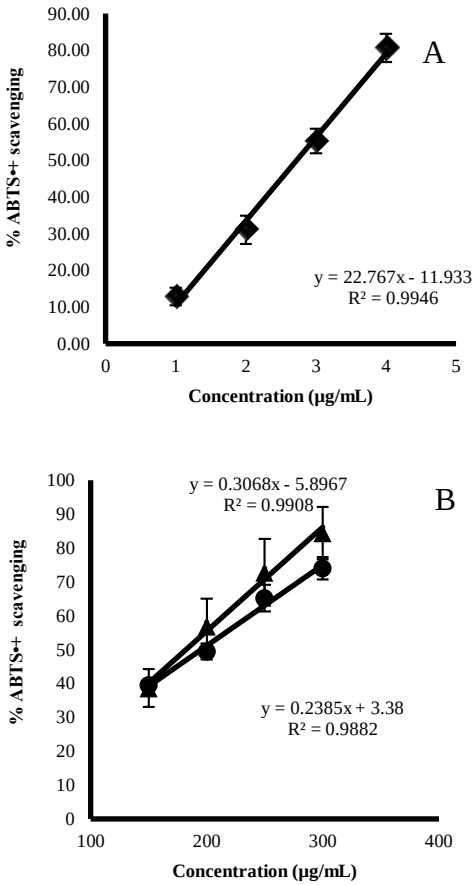

Fig. 1 Antioxidant activity of ascorbic acid (A) and the ethanol extracts (B) from leaves (▲) and peel (●)

TABLE 2: Antioxidant activity of ascorbic acid and the ethanol extracts of *P. edulis var. flavicarpa*

Sample	IC_{50} (µg/mL)	VCEAC (mg/g extract)
Ascorbic acid	2.72 ± 0.14	-
Leaves extract	183.20 ± 23.92	14.85
Peel extract	194.45 ± 15.40	13.99

The results were calculated as an inhibition concentration at 50% (IC_{50}) as showed in Table 2. Both extracts exhibited lower activity than ascorbic acid. In addition, the leaves and peel extracts showed almost same activity with IC_{50} as 183.20 and 194.45 µg/mL, respectively. Silva *et al.* (2013) reported that aqueous extract of *Passiflora edulis* showed total phenolic content as 8.3 mg GAE/g. Antioxidant potential with IC_{50} (DPPH method) as 1.10 mg/mL, which may be mainly associated with the presence of phenolic compounds. Moreover, Rudnicki *et al.* (2007) also reported that the antioxidant activities of leaves of *P. alata* and *P. edulis* extracts were significant correlated with polyphenol contents. The sixed major flavonoid compounds isolated from the leaves of *P. edulis var. flavicarpa*, lucenin-2, vicenin-2, isoorientin, isovitexin, luteolin-6-C-chinovoside, and luteolin-6-C-fucoside (Li *et al.* 2011). In this study also found the relationship between the total phenolic content and antioxidant activity in both the leaves and peel extracts. Indicating that, the phenolic compounds might be the major contributors to the antioxidant activities of these extracts.

Tyrosinase inhibition activity of the ethanol extracts

Tyrosinase is the rate limiting enzyme for production of melanin and other pigments via the oxidation of L-tyrosine. It uses a redox active copper as cofactor within its active site to oxidize arene rings and is involved in two distinct reactions which occur in a processive fashion: the hydroxylation of a monophenol followed by the conversion of the product o-diphenol to the corresponding o-quinone. The

o-quinone product then spontaneously converts to melanin (Briganti *et al*. 2003). Tyrosinase is also able to oxidize a great number of phenols and catechols to form ortho-quinones. The ethanol extracts were evaluated tyrosinase inhibitory property by DOPA oxidase assay. At low concentration the extracts could not inhibit tyrosinase enzyme, while at 100 µg/mL of the leaves and peel extracts contained the inhibition activity as 7.26% and 3.64%, respectively. These tyrosinase inhibition activities were very low. The phenolic compounds from both of the extracts could inhibit only free radical reaction but could not inhibit tyrosinase enzyme.

CONCLUSION

The yellow passion fruit leaves and peel ethanol extracts contained antioxidant property but don't have good tyrosinase inhibition property. Since passion fruit byproducts obtained from leaves and peel (albedo) are available in large quantities as a byproduct of cultivation and/or juice production. It could be used as natural ingredient in function food or cosmetic product because of its antioxidant activities.

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.

Briganti, S., Camera, E., & Picardo, M. (2003). Chemical and instrumental approaches to treat hyperpigmentation. Pigment Cell Research. 16, 101-110.

Fu, R., Zhang, Y., & Chen, G. 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.

Gupta, S. D., & Masakapalli, S. K. (2013). Mushroom tyrosinase inhibition activity of *Aloe vera L*. gel from different germplasms. *Chinese Journal of Natural Medicines, 11(6),* 616-620.

Karim, A. A., Azlan, A., Ismail, A., Hashim, P., Gani, S. S., Zainudin, B. H. & Abdullah, N. A. (2014). Phenolic composition, antioxidant, anti-wrinkles and tyrosinase inhibitory activities of cocoa pod extracct. *BMC Complementary and Alternative Medicine, 14,* 381-393.

Li, H., Zhou, P., Yang, Q., Shen, Y., Deng, J., Li, L., & Zhao, D. (2011). Comparative studies on anxiolytic activities and flavonoid compositions of *Passiflora edulis 'edulis'* and *Passiflora edulis 'flavicarpa'*. *Journal of Ethnopharmacology, 133,* 1085-1090.

Lopaz-Vargas, J. H., Fernandez-Lopez, J., Perez-Alvarez, J. A., & Viuda-Martos, M. (2013). Chemical, physic-chemical, technological, antibacterial and antioxidant properties of dietary fiber powder obtained from yellow passion fruit (*Passiflora edulis var. flavicarpa*) co-products. *Food Research International, 51,* 756-763.

Maisuthisakul, P., & Gordon, M. H. (2009). Antioxidant and tyrosinase inhibitory activity of mango kernel by product. *Food Chemistry, 117(2),* 332-341.

Naczk, M., & Shahidi, F. (2006). Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *Journal of Pharmaceutical and Biomedical Analysis, 41,* 1523–1542.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization Assay. *Free Radical Biology & Medicine, 26,* 1231-1237.

Rudnicki, M., Oliveira, M. R., Pereira, T. V., Reginatto, F. H., Dal-Pizzol, F., &

Moreira, J. C. F. (2007). Antioxidant and antiglycation properties of *Passiflora alata* and *Passiflora edulis* extracts. *Food Chemistry, 100,* 719-724.

Silva, J. K. D., Cazarin, B. B. B., Colomeu, T. C., Batista, G., Meletti, L. M. M., Paschoal, J. A. R., Bogusz, S., Furlan, M. F., Reyes, F. G. R., Augusto, F., Marostica, & M. R., Zollner, R. L. (2013). Antioxidant activity of aqueous extract of passion fruit (*Passiflora edulis*) leaves: In vitro and in vivo study. *Food Research International, 53,* 882-890.

Suh, S. S., Hwang, J., Park, M., Park H. S., & Lee, T. K. (2014). Phenol content, antioxidant and tyrosinase inhibitory activity of mangrove plants in Micronesia. *Asian Pacific Journal of Tropical Medicine, 7(7),* 531-535.

Thitilertdecha, N., Teerawutgulrag, A., Rakariyatham, N. (2008). Antioxidant and antibacterial activities of *Nephelium lappaceum* L. extracts. *LWT-Food Science and Technology, 41,* 2029-2035.

Waterman, P. G., & Mole, S. (1994). Analysis of phenolic plant metabolites. (p. 84). Oxford: Blackwell Scientific Publications.