



Tropical Fruit Peel Extracts as Potential Agents for Treatment of Obesity and Inhibitor of Tyrosinase Activity

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Introduction

Fruit wastes are generated in large quantities in Thailand due to the high consumption and industrial manufacturing of the edible parts of fruits, non edible parts such as peels and other fruit residues are become waste products. An inappropriate management of these waste products can cause environmental serious problems. One of the most benefit methods to solve this problem is to investigate its biological activities. Therefore, the development to use fruit wastes as sources of bioactive compounds, nutraceuticals, and cosmetics active ingredients may be of valuable economic consideration and make good use of natural resources.

Pancreatic lipase is regarded as the safest target to develop anti-obesity medicine.¹ This enzyme is the key enzyme for dietary fat digestion and absorption. Orlistat, a specific drug for pancreatic lipase inhibitor that reduces dietary fat absorption by 30% has been approving for clinical use². However, Orlistat can result in adverse side effects, such as fecal incontinence, flatulence, and steatorrhea³. Therefore, the investigation to find new safety medication for anti-obesity is still needed.

Inhibition of tyrosinase, the key enzyme in melanin biosynthesis, is regarded as clinical useful for the treatment of hyperpigmentation. Tyrosinase inhibitor also can be applied in cosmetics for whitening purposes⁴.

The search for bioactive ingredients in natural products has demonstrated that plant extracts containing high anti-oxidant activity and anti-oxidative associated compounds may be related to tyrosinase and pancreatic lipase inhibitory activities.^{5,6} The previous studies have been described that fruit peel extracts exhibit high antioxidant potency and phenolic contents.^{7,8} This study therefore aims to evaluate the anti-pancreatic lipase and anti-tyrosinase activities of peels of 14 tropical fruits, to provide an inexpensive and readily sources of bioactive compounds for using as anti-obesity and cosmetic whitening agents.

Methods

Fruit peel samples

Total 14 species of tropical fruit peels that use in this study were Santol (*Sandoricum koetjape* (Burm.f.) Merr.), Salak (*Salacca zalacca* (Gaertn.) Voss), Tamarind (*Tamarindus indica* L.), Rambutan (*Nephelium lappaceum* L.), Mangosteen (*Garcinia mangostana* L.), Longkong (*Lansium domesticum* Corr.), Longan (*Dimocarpus longan* Lour.), Dragon fruit (*Hylocereus undatus* (Haw) Britt. Rose.), Burmese grape (*Baccaurea ramiflora* Lour.), Pomelo (*Citrus maxima* (Burm.) Merr.), Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), Pineapple (*Ananas comosus* (L.) Merr.), Mandarin orange (*Citrus reticulata* Blanco) and Cantaloupe (*Cucumis melo* L. var. *cantaloupensis*). All samples were collected from local market in Ubonratchathani during April-May 2016.

Preparation of plant extracts

Fruit peel samples were dried in hot air oven at 50°C for 48 hrs. and ground into fine powder. Fifty gram of powder was extracted in 95% ethanol and concentrated at 55°C in a rotary vacuum evaporator (Heidorf, Germany). The obtained extracts were stored at -20°C until use.

Porcine pancreatic lipase inhibitory assay

Lipase activity was measured using *p*-nitropheny butyrate (*p*-NPB) as a substrate. The method was modified from the previously described by Kim et al.⁹. Briefly, an enzyme buffer was prepared by the addition 30 ul of solution of porcine pancreatic lipase (10 mg/ml in 10 mM morpholinepropanesulphonic acid and 1 mM EDTA, pH 6.8) to 850 ul of Tris buffer (100 mM Tris-HCl and 5 mM CaCl₂, pH 7.0). Then, either 100 ul of the plant extracts (1 mg/ml) or Orlistat was added and incubated for 15 min at 37°C. Ten microliter of substrate (10 mM *p*-NPB in dimethyl formamide) was then added and incubated for 30 min at 37°C. Lipase activity was

determine by measuring the hydrolysis of *p*-NPB to *p*-nitrophenol at 405 nm using an ELISA reader (Biochrome, England). The inhibitory activity was calculated according to the following formula:

$$\% \text{ Inhibition} = [1 - (B - b / A - a)] \times 100$$

where A is the activity of the enzyme without inhibitor, a is the negative control without inhibitor, B is the activity of the enzyme with inhibitor, and b is the negative control with inhibitor. Orlistat was used as a positive control. All samples were done in 6 repeated.

Tyrosinase inhibitory assay

Quantitative antityrosinase activity was determined spectrophotometrically. The method was modified from the previously described.^{10,11} 2 mM of L-tyrosine was dissolved in 50 mM phosphate buffer, 1 mg/ml of extracts and purified compounds were dissolved in 1% DMSO. Kojic acid was used as a positive control. 70 μ l of extracts (1 mg/ml) and standard sample were mixed with 30 mL of tyrosinase (167 U/L) in triplicate in a 96-well microtiter plate, and were incubated at 37°C for 15 minutes. After incubation, 110 μ l of 2 mM L-tyrosine was added and incubated at 37°C for 15 minutes. Tyrosinase activity was determined by measuring the change in absorbance at 492 nm using an ELISA reader (Biochrome, England). The inhibitory activity was calculated according to the following formula:

$$\% \text{ Inhibition} = [(Abs_{(control)} - Abs_{(sample)}) / Abs_{(control)}] \times 100$$

Kojic acid was used as a positive control. All samples were done in 6 repeated.

The half maximal inhibitory concentration (IC₅₀) determination

The half maximal inhibitory concentration or IC₅₀ value was determined in extracts at a concentration of 1,000-6.25 μ g/ml. IC₅₀ value was calculated by the following formula:

$$IC_{50} = (50\% - LOW_{Inh\%}) / (High_{Inh\%} - LOW_{Inh\%}) \times (High_{Conc} - LOW_{Conc}) + LOW_{Conc}$$

Results

Porcine pancreatic lipase and tyrosinase inhibitory activities of fruit peel extracts

Fourteen extracts were prepared from fruit peels and were tested at a concentration of 1 mg/ml for porcine pancreatic lipase and tyrosinase inhibition (Table 1). Among 14 fruit peel extracts, 5 plants were found to have strong inhibitory activity of >50% against porcine pancreatic lipase: 78.06% with mangosteen, 66.74% with mandarin orange, 59.66% with rambutan, 54.70% with santol and 51.17% with longan. There were 6 fruit peel extracts showed moderate effect against pancreatic lipase: 34.89% with Burmese grape, 34.47% with salak, 36.30% with longkong, 30.64% with pomelo and cantaloupe and 25.69% with tamarind. Two fruit peel extracts showed strong inhibition on tyrosinase activity: 85.98% with salak and 71.06% with tamarind. There were several herbs showed moderate effect on inhibition against tyrosinase: 47.76% with longkong, 46.75% with rambutan, 32.92% with santol, 23.78% with longan and 23.08% with mangosteen.

Table 1 Anti-pancreatic lipase and anti-tyrosinase of fruit peel extracts (1 mg/ml)

No.	Fruit peels	% Inhibition (\pm SD)	
		Pancreatic lipase	Tyrosinase
1.	Santol	54.70 \pm 0.15	32.92 \pm 2.45
2.	Salak	34.47 \pm 2.47	85.98 \pm 3.26
3.	Tamarind	25.69 \pm 2.36	71.06 \pm 1.24
4.	Rambutan	59.66 \pm 1.54	46.75 \pm 1.32
5.	Mangosteen	78.06 \pm 2.47	23.08 \pm 1.58
6.	Longkong	36.30 \pm 0.12	47.76 \pm 2.45
7.	Longan	51.17 \pm 1.18	23.78 \pm 2.84
8.	Dragon fruit	5.17 \pm 2.67	-1.75 \pm 0.54
9.	Burmese grape	34.89 \pm 2.81	-1.87 \pm 1.24
10.	Pomelo	30.64 \pm 0.25	-6.05 \pm 0.98
11.	Watermelon	-9.05 \pm 0.19	-3.86 \pm 1.64
12.	Pineapple	-4.74 \pm 1.98	-1.56 \pm 2.54
13.	Mandarin orange	66.74 \pm 3.16	-2.52 \pm 2.39
14.	Cantaloupe	30.64 \pm 0.97	-1.31 \pm 0.57

IC₅₀ of selected crude extracts for porcine pancreatic lipase and tyrosinase inhibitory activities

The different concentration of some selected fruit peel extracts were measured for IC₅₀ at concentration of 1000-6.25 μ g/ml (Figure 1). For anti-pancreatic lipase activity, fruit peel extracts of santol, rambutan, mangosteen and Mandarin orange had IC₅₀ values of 435.25, 324.58, 147.32 and 187.25 μ g/ml, respectively. Orlistat had IC₅₀ value of 9.6 μ g/ml. For anti-tyrosinase activity, fruit peel extracts of salak and tamarind had IC₅₀ values of 172.24 and 215.13, respectively. Whereas, kojic acid had IC₅₀ value of 8.2 μ g/ml.

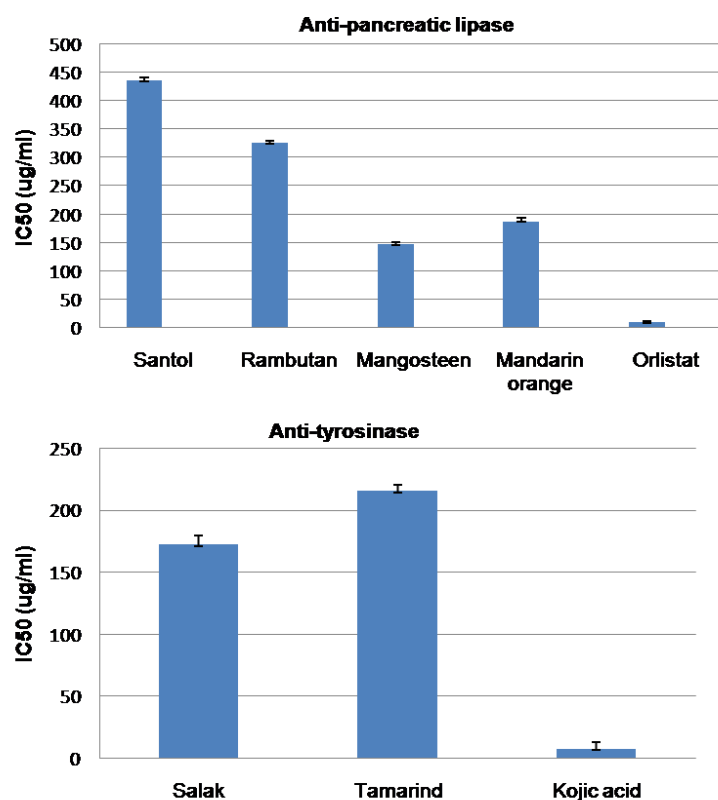


Figure 2 The half maximal pancreatic lipase (above) and tyrosinase (below) inhibitory concentration (IC₅₀) of selected fruit peel extracts.

Discussion

Fruit wastes are one of the main sources of municipal waste. In this study, we demonstrated the potential uses of fruit peels as anti-obesity agent and inhibitor of tyrosinase activity. We found that mangosteen, mandarin orange, rambutan, santol and longan peel extracts showed strong inhibition against pancreatic lipase. We also found that peel extracts of salak and tamarind, showed strong inhibition against tyrosinase activity.

The previous studies have been described that fruit peel extracts exhibit antioxidant potency and phenolic content indicated that phenolics could be one of the main contributors to the anti-oxidant of fruit peels.^{7,8} The strong evidences of positive correlation between phenolic contents, antioxidant activity and inhibitory effects against pancreatic lipase and tyrosinase were investigated^{12,13,14}. These may be implied that antioxidant properties and high in phenolic contents of fruit peel extracts are the key factors for pancreatic lipase and tyrosinase *in vitro*. However, the further investigation both *in vitro* and *in vivo* should be performed to elucidate the bioactive compounds, to clarify the molecular mechanism and to verify the main effective phytochemicals in these candidates which are responsible for the inhibition of pancreatic lipase and tyrosinase activity. These suggested that these fruit peels seem to be the potential candidates as the inhibitor of pancreatic lipase and tyrosinase for using in the food and cosmetic industries.

Conclusion

In this study, we investigated anti-pancreatic lipase and anti-tyrosinase activities of fruit peel extracts from 14 species. 5 of 14 fruit peel extracts showed the strong inhibition against pancreatic lipase activity including mangosteen, mandarin orange, rambutan, santol and longan. We found that peel extracts of salak and tamarind, showed strong inhibition against tyrosinase activity. These results indicated that non-edible part of fruit could be inexpensive and readily available sources of anti-pancreatic lipase and tyrosinase activities for using in the food and cosmetic industries.

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