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Screening for anti-pancreatic lipase properties of 28 traditional Thai medicinal herbs

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ABSTRACT

Objective: To screen the effect of 28 medicinal plants on inhibition of pancreatic lipase and evaluate the phytochemical contents of extracts.
Methods: The ethanolic extracts of 28 traditional Thai herbal medicines were assayed for their *in vitro* activities against porcine pancreatic lipase using *p*-nitrophenyl butyrate as a substrate. Quantitative estimation of flavonoids, phenolics, and alkaloids was done.
Results: Extracts from four herbs, *Memecylon edule* Roxb., *Garcinia vilersiana* Pierre, *Cryptolepis elegans* Wall. and *Phyllanthus chamaepeuce* Ridl., at a concentration of 100 μg/mL, strongly inhibited porcine pancreatic lipase by 90.97%, 92.04%, 94.64% and 95.38%, respectively. There was a significant positive correlation between phenolic content and inhibition activity. Inhibition activity was significantly correlated with flavonoid and with alkaloid contents.
Conclusions: From this result, it could be concluded that herbs represent a rich of anti-pancreatic lipase compounds, in particular, *Cryptolepis elegans* Wall. and *Phyllanthus chamaepeuce* Ridl. It is suggested that the phytochemical compounds from these plants may be applied for the prevention and treatment of obesity or hyperlipidemia.

1. Introduction

Obesity is becoming a worldwide epidemic, resulting in a major risk factor for coronary heart diseases including diabetes mellitus, metabolic syndrome, stroke, and some cancers [1]. Globally, around 39% of adults aged 18 and over were overweight in 2014 and 13% of them were clinically obese [2]. Therefore, prevention and treatment of obesity become an important factor for a healthy condition. The reduction of nutrient digestion and absorption by developing of enzyme inhibitors without altering major mechanism in gastrointestinal system became the most important strategies in the treatment of obesity [3,4]. The major source of unwanted calories is dietary lipids, therefore, lipid metabolism play a major role in maintaining energy homeostasis [5]. The identification and characterization of several enzymes involved in lipid metabolism have yielded a rich pool of potential targets for drugs to treat obesity and other metabolic disorders [6]. Pancreatic lipase is the key enzyme for dietary fat digestion and absorption. Therefore, inhibition of this enzyme would be in effect to reduce lipid absorption from intestine and lead to a consequence suppress of weight gain. Orlistat, a specific drug for inhibiting pancreatic lipase that reduces dietary fat absorption by 30%, has been approved for clinical use [4,7,8]. However, Orlistat can result in adverse side effects, such as fecal incontinence, flatulence, and steatorrhea [9,10]. Therefore, the investigation to find new safety medication for anti-obesity is still needed. The significant progress of the development of anti-obesity from medicinal plants has provided potential therapeutic targets for obesity [11,12]. It has been recently reported that natural compounds from plants and other organisms have been approved as anti-pancreatic lipase activities. For example, ethanolic extract from *Terminalia paniculata* bark [13], polyphenols from Oolong tea [14], *Abroma augusta* extract [15], pomegranate leaves ethanol extract [16], and other components from other kinds of herbs. However, it remains necessary to search for more effective lipase inhibitors from traditional herbs. In this study, we investigated the ethanolic

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extracts of 28 traditional Thai herbal medicines for their *in vitro* activities against porcine pancreatic lipase using *p*-nitrophenyl butyrate (*p*-NPB) as a substrate.

2. Materials and methods

2.1. Materials

Porcine pancreatic lipase, *p*-NPB, morpholinepropanesulphonic acid, quercetin, colchicine and gallic acid were purchased from Sigma Aldrich. The fresh leaves of 28 plants were collected from Plant Genetic Conservation Forest, Ubon Ratchathani Rajabhat University, Ubonratchathani Province, Thailand. All plant species were identified and authenticated by Mr. Prakorb Boonma, Senior Plant Taxonomist, Ubon Ratchathani Rajabhat University, Thailand.

2.2. Preparation of plant extracts

The leaves were dried in hot air oven at 50 °C for 48 h and grounded into fine powder. A total of 50 g 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.

2.3. Porcine pancreatic lipase inhibition assay

Lipase activity was measured using *p*-NPB as a substrate. The method was modified from the previously described by Kim *et al*. [17]. Briefly, an enzyme buffer was prepared by the addition 30 μL of solution of porcine pancreatic lipase (2.5 mg/mL in 10 mmol/L morpholinepropanesulphonic acid and 1 mmol/L ethylenediamine tetraacetic acid, pH 6.8) to 850 μL of Tris buffer (100 mmol/L Tris–HCl and 5 mmol/L $CaCl_2$, pH 7.0). Then, either 100 μL of the plant extracts (100 μg/mL) or Orlistat was added and incubated for 15 min at 37 °C. Ten microliter of substrate (10 mmol/L *p*-NPB in dimethyl formamide) was then added and incubated for 30 min at 37 °C. Lipase activity was determined by measuring the hydrolysis of *p*-NPB to *p*-nitrophenol at 405 nm using an ELISA reader (Biochrome, England). The inhibitory activity (I) was calculated according to the following formula:

$$I\% = \left(1 - \frac{B-b}{A-a}\right) \times 100$$

where A is the activity of the enzyme without inhibitor, and a is the negative control without inhibitor; B is the activity of the enzyme with inhibitor, and b is the negative control with inhibitor.

2.4. The half maximal inhibitory concentration (IC_{50}) determination

The IC_{50} value of extracts was determined at a concentration of 500.0, 250.0, 125.0, 100.0, 25.0, 12.5 and 5.0 μg/mL. Orlistat was used as a positive control. IC_{50} value was calculated by the following formula:

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

formula:where Low_{Inh}%/$High_{Inh}$% signify % inhibition directly below/above 50% inhibition, and Low_{Conc}/$High_{Conc}$ are the corresponding concentrations of extract.

2.5. Determination of total phenolic content

According to a previously described protocol [18], Folin–Ciocalteu reagent was used to determine the total phenolic content of extracts. Absorbance was measured at 725 nm. All tests were performed 6 times. The phenolic content was calculated based on a gallic acid standard curve.

2.6. Determination of total flavonoid content

Total flavonoid content was determined according to a previously discussed method [18] using quercetin as a standard. The absorbance was measured at 510 nm. The flavonoid content was calculated based on a quercetin standard curve.

2.7. Quantification of alkaloid content

Quantification of alkaloid content for extracts was carried out using a method described earlier [19]. The absorbance was taken at 500 nm and all tests were performed 6 times. The alkaloid content was evaluated based on the colchicine standard curve.

2.8. Statistical analysis

Statistical analysis of the data was performed using the SPSS 16.0 program. The comparison between Orlistat control and extract group was conducted using the Mann–Whitney *U* test, and the correlations between parameters were determined using the Spearman's rank test.

3. Results

A total of 28 extracts were prepared from leaf part of the traditional Thai medicinal herbal medicines and were tested at a concentration of 100 μg/mL for porcine pancreatic lipase inhibition (Table 1).

Table 1
Lipase inhibitory effects of 28 selected traditional Thai medicinal herbs. %.

Scientific name	Family	Inhibition
Artocarpus lakoocha Roxb.	Moraceae	12.68 ± 1.10*
Azadirachta indica A. Juss.	Meliaceae	34.85 ± 2.70*
Belamcanda chinensis (L.) DC.	Iridaceae	6.58 ± 1.30*
Brucea javanica (L.) Merr.	Simaroubaceae	22.83 ± 3.20*
Canarium subulatum Guill.	Burseraceae	−26.84 ± 1.70*
Canthium berberidifolium Geddes	Rubiaceae	−5.76 ± 0.70*
Congea siamensis Fletcher	Verbenaceae	70.00 ± 1.30
Cratoxylum formosum (Jack) Dyer T.	Guttiferaes	67.47 ± 1.30
C. elegans	Asclepiadaceae	94.64 ± 1.10
Dillenia ovata Wall.	Dilleniaceae	5.97 ± 0.60*
Diospyros filipendula Pierre ex Lecomte	Ebenaceae	59.15 ± 0.90*
Eurycoma longifolia Jack	Simaroubaceae	1.04 ± 1.20*
Ficus foveolata Wall.	Moraceae	6.99 ± 1.30*
Garcinia cowa Roxb. ex DC.	Guttiferae	14.91 ± 2.40*

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