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INTRODUCTION

Obesity is the most common metabolic disease in developed nations. There are various therapeutic approaches to treating obesity, including suppression of food intake, increased thermogenesis, accelerated lipolysis and inhibition of lipogenesis. It has been suggested that inhibition of digestion and absorption of dietary fat is a key factor in treating of obesity so the application of a pancreatic lipase inhibitor was widely interesting. The only pancreatic lipase inhibitor currently appoved for longterm treatment of obesity is Orlistat. The use of Orlistat frequently results in adverse events including flatus, oily stools, fecal urgency or fecal incontinence, and abdominal pain (Harold E. Bays, 2004). Therefore, there is a need for more lipase inhibitors or medicinal products that are safe and effective. Previously we screened the anti-lipase activity of fifteen Thai herbs belonging to different families such as Compositae, Lauraceae, Labiatae, Rutaceae and Zingiberaceae.

Qurercus infectoria (Fagaceae) is a small tree or a shrub mainly present in western Asia and Southern Europe. The galls of *Q. infectoria* have been known to possess medicinal properties, such as astringent, anti-inflammatory, antiviral, antidiabetic, larvicidal, antibacterial, antiulcerogenic and gastroprotective activities The main constituents found in the galls of *Q. infectoria* are tannin (50-70%) and small amount of free defined as water–soluble polyphenolic compounds that have theability to precipitate proteins (Umachigia SP, *et al.*, 2008). In this study we investigated the lipase inhibitory activity of *Q. infectoria* extract by using the BALB-DTNB method (Kurooka and Kitamura, 1978) and also the levels of tannin and phenolic contents.

MATERIALS AND METHODS

Preparation of herbal extracts The galls of *Q. infectoria* were obtained from the local market and were crushed to small pieces before pulverized into powder. The extraction was carried out by using 95% ethanol in a percolator at room temperature. The extract was filtered and concentrated at 45°C under reduced pressure in a rotary vacuum evaporator.

Determination of total tannin content The total tannin content of the *Q. infectoria* extract was determined using the Folin- Ciocalteu reagent. The reaction mixture contained: 100 µl of diluted *Q. infectoria* extract, 500 µl of freshly prepared diluted Folin Ciocalteu reagent and 1 ml of 20 % sodium carbonate. Mixtures were kept in dark at ambient conditions for 1 h to complete the reaction. The absorbance at 760 nm was measured. Gallic acid was used as standard (Concentration of 0.05, 0.1, 0.2, 0.4, 0.8 and 1.0 mg/ml of tannic acid were prepared in ethanol). The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, thereby producing a blue color upon reaction. All determination was performed in triplicate and the total phenolic content was expressed as mg/g tannic acid equivalents (TAE).

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Statistical analysis The determinations were conducted in triplicate and results were expressed as mean ± standard error.

Preparation of pancreatic lipase enzyme The 0.2 g. of rat pancreas was mixed with 10 ml of 0.9% normal saline, spun at 7,000 rpm/min for 10min. and then the supernatant was kept under -80°C until next experiment.

Assay for lipase inhibitory activity Solution of 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 5 and 1 mg/ml of ethanolic extract of galls of *Q. infectoria* was prepared before enzyme assay. The positive control

(Xenical®:Orlistat 120 mg) solution was prepared by dissolving in phosphate buffer saline (PBS) and adjust to 0.5 mg/ml final concentration.

The 10 µl of each preparation were added to 1 ml of pancreatic lipase enzyme and then incubated the reaction at room temperature for 15 min. The active extracts were determined by using a commercial lipase assay kit (BioAssay Systems. USA) according to the manufacturer's instructions. The activity of the negative control was also checked with and without inhibitor. 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, a is the negative control without inhibitor. B is the activity of the enzyme with inhibitor and b is the negative control with inhibitor.

RESULTS

The dried powder of galls of *Q. infectoria* yielded 88.21% (w/w) of ethanolic extract. Standard curve for the determination of total tannin and total phenolic content was prepared by using different concentrations of tannic acid and gallic acid, respectively. The results have been presented in table 1. The total tannin and total phenolic contents of *Q. infectoria* extract were 625.83±2.08 mg of TAE/g and 655.86±2.22 mg of GAE/g,respectively. The anti-lipase activity of various concentrations this extract were shown in Table 2. where lipase inhibition is expressed in percentage (%). The extract at 10,50,100,200 mg/ml concentration showed no inhibition in lipase activity while the extract at the concentration of 600-1000 mg/ml showed moderate anti-lipase activity at 20.33-35.36% inhibition.

Table 1 The total tannin and total phenolic contents of *Q. infectoria* extract

	Total tannin Content (mg of TAE/g of extract)	Total Phenolic Content (mg of GAE/g of extract)
Ethanolic extract of *Q. infectoria*	625.83±2.08	655.86±2.22

The values are shown as mean±s.d. (n = 3).

Table 2 Anti-lipase activity of *Q. infectoria* extract

No.	Concentration of *Q. infectoria* extract	% Inhibition
1.	10 µg/ml	No Inhibition
2.	50 µg/ml	No Inhibition
3.	100 µg/ml	No Inhibition
4.	200 µg/ml	No Inhibition
5.	300 µg/ml	5.00
6.	400 µg/ml	18.52
7.	500 µg/ml	6.54
8.	600 µg/ml	20.33
9.	700 µg/ml	25.24
10.	800 µg/ml	27.46
11.	900 µg/ml	27.13
12.	1000 µg/ml	35.36
13.	Oristat (5 µg/ml)	54.44

DISCUSSION

Tannins are defined as naturally occurring polyphenolic compounds of high molecular weight to form complexes with the proteins. It has been reported that tannin has an inhibitory effect on pancreatic lipase activity and could be applied in the treatment of obesity (Lei F. *et al.*, 2007). In this study, ethanolic extract of *Q. infectoria* had high concentration of total tannin and total phenolic contents and inhibited lipase activity at the concentration over than 600 µg/ml. Therefore, the anti-lypase activity of *Q. infectoria* extract might be due to the high amounts of total tannin. The inhibitory effect of this crude

extract is much weaker than orlistat because the crude extract contains, not only active substances, but also non-active components.

CONCLUSION

The results of the current study showed that *Q. infectoria* extract has lipase inhibitory activity and has potential for the treatment of obesity. Further studies are needed to verify the inhibitory activities in animal models and also the safty evaluation.

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