Thai Journal of Pharmaceutical Sciences (TJPS)

Journal homepage: http://www.tjps.pharm.chula.ac.th

The influence of six Thai edible plants on anti-oxidant activities and cholesterol esterase inhibition

Sakunpak A*, Pathompak P, Charoonratana T and Chankana N

Faculty of Pharmacy and Sino-Thai Traditional Medicine Research Center (Cooperation between Rangsit University, Harbin Institute of Technology, and Heilongjiang University of Chinese Medicine), Rangsit University, Pathum Thani 12000, Thailand

Keywords: Thai edible plant; anti-oxidant activities; cholesterol esterase

Objectives: The present studies were aimed to evaluate *in vitro* anti-oxidative activities and cholesterol esterase inhibition of six Thai edible plants.
Methods: Anti-oxidant activity was evaluated by using DPPH free radical scavenging activity and reducing power by $FeCl_3$. The ethanolic extracts of six Thai edible plants were tested. Gallic acid, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were used as positive control. Moreover, pancreatic cholesterol esterase enzyme inhibitory activity of six edible plants was determined and orlistat was used as positive control.
Results: Ethanolic extract of *C. sphaerica* showed the highest free radical scavenging activity and reducing power. The various extract possessed significant anti-oxidant activities when ($P < 0.05$) compared with the standard. The pancreatic cholesterol esterase activity demonstrated strongly inhibited by ethanolic extract of *C. cambodiana*.
Conclusion: Six Thai edible plant extracts were evaluated for ant-oxidant activities and cholesterol esterase inhibition. *C. sphaerica* has exhibited significantly anti-oxidant activity more than another extract when tested with DPPH and FRAP methods. Ethanolic extract of *C. cambodiana* was strongest inhibited cholesterol esterase.

*Corresponding author: Faculty of Pharmacy and Sino-Thai Traditional Medicine Research Center (Cooperation between Rangsit University, Harbin Institute of Technology, and Heilongjiang University of Chinese Medicine), Rangsit University, Pathum Thani 12000, Thailand; Tel +66(02) 997-2222 (1501)
E-mail: apirak.s@rsu.ac.th

Introduction

Hypercholesterolemia is a major cause of atherosclerosis and coronary heart disease. Epidemiological evidences have clarified strong relations between serum cholesterol contents and hypercholesterolemia. High content of low density lipoprotein-cholesterol (LDL-C) and low content of high density lipoprotein (HDL-C) in blood circulation are realized to be implicated risk factors in hypercholesterolemia. However, amount of serum LDL-C extremely influence on clausal disease more than other factors[1]. Two mechanisms are concerned for treatment in high serum cholesterol patients. First mechanism is the inhibition of enzyme in the cholesterol metabolism. This mechanism has widely used in many cardiovascular drugs. Second mechanism is anti-oxidant activity, especially free radicals from lipid peroxidation. This mechanism is a new alternative target for hypercholesterolemia treatment. Anti-oxidants prevent free radicals by interacting directly to free radical or breaking down the chain reaction. In the presence of free radicals. These molecules interact with biomolecules rapidly and affect to protein, lipid and DNA damage[2-3]. The imbalance of free radical production and destruction stimulates the oxidative stress in cells. Resulting, the macromolecules are destroyed and increased the risk of cardiovascular diseases[4]. Many reasons support the positive correlation between amount of serum LDL and very low-density lipoprotein (VLDL), and free radicles production. They demonstrate that these lipoproteins are oxidized by free radicals produced in oxidative stress condition[5]. In this study, anti-oxidant activities and cholesterol esterase inhibition of Thai indigenous vegetables were evaluated for potential edible plants to be used as cholesterol-reducing agents.

Materials and Methods

***Chemicals and reagents*:** 2,2-diphenyl-1-picrylhydrayzl, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), cholesterol esterase from porcine pancreas (Lot.No. 26745), 6-hydroxy-2,5,7,8-tetramethylchroman (Trolox), orlistat, *p*-nitrophenyl butyrate (*p*-NPB), taurocholic acid sodium salt hydrate, and 2,4,6-tripyridyl-s-triazine (TPTZ) were purchased from Sigma-Aldrich (USA). Ferric chloride anhydrous was supplied from Carlo Erba Reagents (France). Ferrous sulfate $7H_2O$ was obtained from Ajax Finechem (Australia). All solvents were analytical grade purchased from Carlo Erba (Milano, Italy).

***Plant materials*:** Six of fresh Thai indigenous vegetables were collected from Phattalung Province, Thailand, in June

2013. The vouchers of each specimen were deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand (Table 1). Dried samples were ground in mortar to make a powder for analysis.

Table 1 Plant uses

Family	Botanical names	Voucher number	Part use
Guttiferae	*Garcinia cowa* Roxb.	SKP 083 07 03 01	leaves
Lecythidaceae	*Careya sphaerica* Roxb.	SKP 022 03 19 0	leaves
Polygonaceae	*Polygonum odoratum* Lour.	SKP 151 16 15 01	leaves
Euphorbiaceae	*Glochidion perakense* Hook.f.	SKP 071 07 23 01	leaves
Euphorbiaceae	*Antidesma ghaesembilla* Gaertn.	SKP 183.1 01 07 01	leaves
Rutaceae	*Clausena cambodiana* Guill.	SKP 166 03 03 01	leaves

***Plant extraction*:** Six ethanolic plant extracts were prepared as follows; 1 g of dried plant powder was sonicated with ethanol 10 ml for an hour. The extract was filtered and extracted in ethanol two more times. The filtrate were pooled and concentrated with a rotary evaporator. The extracts were stored in a well-close container and protected from light.

Anti-oxidant activities

***DPPH radical scavenging activity*:** DPPH solution was performed according to the method of Kumar *et al*., (2011) with some modifications[6]. Briefly, 0.15 M of DPPH was freshly prepared in ethanol for DPPH solution. The crude extracts of edible plant were dissolved in ethanol to prepare a stock solution (4 mg/ml). After, 50 µl of the serial dilution of ethanolic plant extract was added into 96-well plate and followed by 150 µl of DPPH solution. The mixture was incubated at room temperature for 90 min in the dark. The absorbance was measured at 517 nm. DPPH radical scavenging activity was expressed in the efficiency of extract to decrease DPPH color to 50% (EC_{50}). The anti-oxidative curves were plotted between concentration of extract on the x-axis and %DPPH free radical scavenging activity on the y-axis to determine the EC_{50}. Gallic acid, BHA and BHT were used as a reference standard. Measurements were performed in triplicate.

$$\text{DPPH radical scavenging activity}\,(\%) = \frac{\left[A_0 - (A_1 - A_S)\right]}{A_0} \times 100$$

Where, A_0 is the absorbance of the control DPPH solution
A_1 is the absorbance of the sample in DPPH solution after 90 min
A_s is the absorbance of the sample in ethanol

Ferric reducing activity power (FRAP) assay: This method based on the reduction of colourless ferric complex (Fe^{3+}-TPTZ) to blue-colored ferrous complex (Fe^{2+}-TPTZ) by the reducing agent at low pH. FRAP assay was performed as described by Benzie and Strain (1996) with some modifications[7]. In brief, FRAP reagent was prepared daily by mixing 10 volumes of 450 mM acetate buffer, pH 3.6 with 1 volume of 15 mM TPTZ in 40 mM HCl and 1 volume of 30 mM ferric chloride. FRAP reagent was pre-warm at 37 °C in water bath for 30 min. The crude extracts were prepared in ethanol at concentration of 0.1 mg/ml. 20 µl of ethanolic extracts were aliquot into 96-well plate and added 200 µl of FRAP reagent. The mixture was incubated at 37 °C for 30 min and continuously measured the absorbance at 593 nm. FRAP and TEAC (Trolox equivalent anti-oxidant capacity) values of samples were determined by comparing with the standard curve of ferrous sulfate and trolox, respectively. The measurements were performed in triplicate.

***Inhibition of pancreatic cholesterol esterase activity*:** Cholesterol esterase enzyme inhibitory activity was evaluated in the presence of bile salt and *p*-nitrophenyl butyrate (*p*-NPB). The crude ethanol extracts were prepared in DMSO as a stock solution (4 mg/ml) and diluted into 0.4 mg/ml with ultrapure water. The final concentration of DMSO in reaction was 2%. According to Adisakwattana (2012) method with some modifications[8], reaction mixed was prepared by mixing 1 volume of 1.0 mM taurocholic acid sodium salt, with 1 volume of 0.5 mM *p*-NPB in 100 mM sodium phosphate buffer, pH 7.0 and 1 volume of 100 mM NaCl. The stock solution of porcine cholesterol esterase (5.7 µg/ml) was dissolved in 100 mM sodium phosphate buffer, pH 7.0. The reaction was started by adding 50 µl of each crude extract and cholesterol esterase into 96-well plate, and incubated at 25 ºC for 5 min. After, 150 µl of reaction mixed was added then continuously incubated at 25 ºC for 5 min. The absorption values were determined by measuring the optical density at 405 nm. Orlistat was used as positive control. All measurements were made in triplicate. The percentage of enzyme inhibition was calculated according to the following formula:

$$\%\ \text{Inhibition} = \frac{\left[A_0 - (A_1 - A_S)\right]}{A_0} \times 100$$

Where, A_0 is the absorbance of the control reaction (enzyme + reaction mixed)
A_1 is the absorbance of the sample in reaction (sample + enzyme + reaction mixed)
A_s is the absorbance of the sample (sample + buffer)

***Statistical analysis*:** Results were expressed as mean ± standard deviation (SD) of three independent experiments for each sample. One-way analysis of variance (ANOVA) was performed. The $p < 0.05$ was considered significant.

Results

***DPPH radical scavenging activity*:** DPPH assay was used to determine the radical scavenging activity of six vegetable extracts. The results (Table 2) showed that the vegetable extracts revealed widely different anti-oxidative activities, ranging from 20-1,700 µg/ml. *C. sphaerica* showed the highest anti-oxidative activity with EC_{50} value of 21.28 ± 0.41 µg/ml, whereas the EC_{50} of gallic acid and BHA exhibited at values of 6.14 ± 0.12 and 15.58 ± 0.04 µg/ml, respectively. *P. odoratum* and *A. ghaesembilla* were markedly in high anti-oxidative activity group by presented the EC_{50} value nearby BHT.

***Ferric reducing activity power*:** Ferric reducing power of extracts was illustrated in table 2 with two groups of anti-oxidative properties. The high anti-oxidant activity included *C. sphaerica* and *P. odoratum*. Among these groups, *C. sphaerica* exhibited the highest FRAP and TEAC values of 917.6 ± 41.1 µM and 374.0 ± 16.8 µM, respectively. While on the contrary, the low anti-oxidative activity, *G. cowa* manifested the lowest reducing power of 11.2 ± 2.7 µM and 5.8 ± 1.1 µM for FRAP and TEAC values.

Table 2 DPPH radical scavenging activity and ferric reducing activity power of six Thai edible plants

Plant samples	DPPH radical scavenging activity	Ferric reducing activity power	
	EC_{50} ± SD (mg/ml)	FRAP ± SD (µM)	TEAC ± SD (µM)
C. sphaerica	21.28 ± 0.41	917.6 ± 41.33	374.03 ± 16.79
P. odoratum	23.63 ± 0.28	642.74 ± 24.33	262.36 ± 9.88
G. perakense	30.75 ± 1.13	523.77 ± 13.55	214.03 ± 5.51
A. ghaesembilla	34.87 ± 0.57	430.44 ± 26.94	176.11 ± 10.94
C. cambodiana	66.80 ± 0.68	245.05 ± 8.44	100.08 ± 3.43
G. cowa	1764.76 ± 44.41	11.21 ± 2.70	5.80 ± 1.10
Gallic acid	6.14 ± 0.12	-	-
BHA	15.58 ± 0.04	-	-
BHT	30.04 ± 0.51	-	-

Inhibition of pancreatic cholesterol esterase activity: The pancreatic cholesterol esterase activity demonstrated strongly inhibited by ethanol extract of *C. cambodiana* in the presence of taurocholate (Figure 1). *C. cambodiana* revealed 97.1 ± 0.3% inhibition of cholesterol esterase which higher than *G. cowa*, the lowest inhibitory value, 2-fold. Orlistat showed cholesterol esterase inhibition value of 85.7 ± 0.1%. *C. cambodiana*, *P. odoratum* and *A. ghaesembilla* displayed approximately inhibitory value as standard. Therefore, they were observed as the high enzyme inhibition.

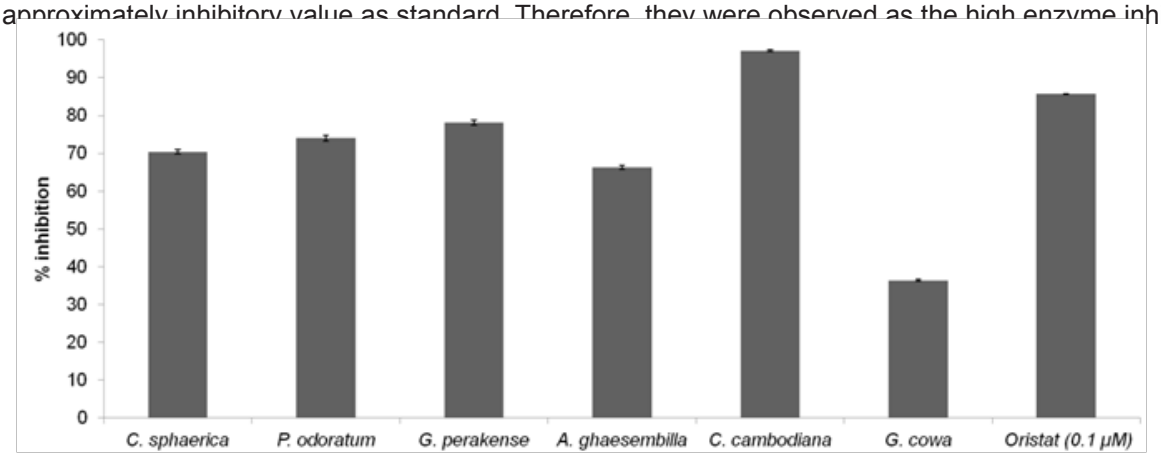

Figure 1 The inhibition of pancreatic cholesterol esterase activity

Discussion

In present study, the ethanolic extract of six Thai traditional vegetables was selected for evaluated the correlation between the anti-oxidative activities and the inhibition of cholesterol esterase activity. The results of FRAP and DPPH assay indicated that the extracts expressed the same tendency of anti-oxidative properties. The inhibitory activity of cholesterol esterase slightly related to anti-oxidative activities. Previous studied revealed the positive correlation of the anti-oxidative property and inhibition of cholesterol esterase of natural compounds. The flavonoids and phenolics in leaves of tea demonstrated hyperlipidemia lowing property. In the presence of these compounds in extract, it exhibited the

anti-oxidant activities, and likewise the inhibition of cholesterol esterase activity[5]. In addition, some kinds of isocoumarin acted as the irreversible inhibitor of cholesterol esterase by covalent bond formation to catalyzing site of cholesterol esterase.

Conclusion

Six Thai edible plant extracts were evaluated for anti-oxidant activities and cholesterol esterase inhibition. *C. sphaerica* has exhibited significantly anti-oxidant activity more than another extract when tested with DPPH and FRAP methods. Ethanolic extract of *C. cambodiana* was strongest inhibited pancreatic cholesterol esterase.

Acknowledgements

The authors wish to thank the Faculty of Pharmacy and Sino-Thai Traditional Medicine Research Center (Cooperation between Rangsit University, Harbin Institute of Technology, and Heilongjiang University of Chinese Medicine), Rangsit University, Pathum Thani, Thailand, for all chemicals and instruments. This research was partly funded by the Research Institute of Rangsit University, Pathum Thani, Thailand (Grant No. 73/55).

References

1. Elahi, M. M., Kong, Y. X., Matata, B. M. Oxidative stress as a mediator of cardiovascular disease. *Oxid* Med Cell Longev. 2009; 2: 259-269.
2. Madamanchi, N.R., Vendrov, A., & Runge, M. S. Oxidative stress and vascular disease. Arterioscler Thromb Vasc Biol. 2005; 25: 29-38.
3. Adisakwattana, S., Ruengsamran, T., Kampa, P., Sompong, W. In vitro inhibitory effects of plant-based foods and their combinations on intestinal α-glucosidase and pancreatic α-amylase. BMC Complement Altern Med. 2012; 12: 1-8.
4. Forbes, J.M., Coughlan, M.T., Cooper, M.E. Oxidative Stress as a Major Culprit in Kidney Disease in Diabetes. Diabetes. 2008; 57: 1446-1454.
5. Araujo, F.B., Barbosa, D.S., Hsin, C.Y., Maranhão, R.C., Abdalla, D.S.P. Evaluation of oxidative stress in patients with hyperlipidemia. Atherosclerosis. 1995; 117: 61-71.
6. Kumar, P., Sivashanmugam, T., Umamaheswari, M., Subhadradevi, V., Jagannath, P. Cholesterol esterase enzyme inhibitory and antioxidant activities of leaves of Camellia sinensis (L.) Kuntze. using in vitro models. Int J Pharm Sci Rev Res. 2011; 2(11): 2923-2928.
7. Benzie, I.F., Strain J.J. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. Anal Biochem. 1996; 15: 70-76.
8. Heidrich, J.E., Contos, L.M., Hunsaker, L.A., Deck, L.M., Vander Jagt, D.L. Inhibition of pancreatic cholesterol