

Extracts of Edible Plants Inhibit Pancreatic Lipase, Cholesterol Esterase and Cholesterol Micellization, and Bind Bile Acids

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Summary

The application of edible plants with more effective ability to inhibit fat digestion and absorption has recently been explored for possible treatment of hyperlipidaemia. The aim of the present study is to investigate the effect of nine edible plants on the inhibition of pancreatic lipase and pancreatic cholesterol esterase activities, as well as the inhibition of cholesterol micelle formation, and bile acid binding. Our findings have shown strong pancreatic lipase inhibitory activity and the inhibition of cholesterol micellization by mulberry leaf extract. Safflower extract was the most potent inhibitor of pancreatic cholesterol esterase. In addition, cat's whiskers and safflower extracts had a potent bile acid binding activity. It is suggested that a daily intake of these edible plants may delay postprandial hypertriglycerolaemia and hypercholesterolaemia, and therefore may be applied for the prevention and treatment of hyperlipidaemia.

Key words: edible plants, pancreatic lipase, cholesterol micellization, pancreatic cholesterol esterase, bile acid binding

Introduction

Hyperlipidaemia is a group of metabolic disorders characterized by the elevated levels of triglycerides and cholesterol in the blood. The prevalence of hyperlipidaemia has dramatically increased worldwide due to a modern lifestyle and an increase of consumption of a high-fat diet (1). It is well known that a new attempt to reduce the absorption of free fatty acids is by delaying triglyceride digestion with the inhibition of pancreatic lipase (2). Pancreatic cholesterol esterase plays a pivotal role in hydrolyzing dietary cholesterol esters (3). The hydroly-

sis of cholesterol esters in the lumen of the small intestine is catalyzed by pancreatic cholesterol esterase, which liberates free cholesterol. Moreover, it enhances the incorporation of cholesterol into the mixed micelle and aids transport of free cholesterol to the enterocyte (4). Inhibition of cholesterol esterase is expected to limit the absorption of dietary cholesterol, resulting in delayed cholesterol absorption (5). Consequently, the principal steps in the absorption of dietary cholesterol are emulsification, hydrolysis of the ester bond by a pancreatic esterase, micellar solubilization, and absorption in the proximal jejunum (6). It has recently been reported that the

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reduction of cholesterol absorption by inhibiting cholesterol micellization in the intestinal lumen is a new target site of intervention for the treatment of hyperlipidaemia (7). In addition, binding bile acids by forming insoluble complexes in the intestine and increasing their faecal excretion have been hypothesized as a possible mechanism of lowering plasma cholesterol level. This consequently reduces the bile acid pool. As a result, greater amount of cholesterol is converted to bile acids to maintain a steady level in the circulation (8). One of the most important strategies in the prevention and treatment of hyperlipidaemia includes delaying fat digestion and absorption through gastrointestinal mechanisms such as the inhibition of pancreatic lipase, pancreatic cholesterol esterase activities as well as the inhibition of cholesterol micellization, and bile acid binding (9). The result of this action is an attenuated postprandial hypertriacylglycerolaemia and hypercholesterolaemia, and consequently, reduced risk of the progression of micro- and macrovascular complications including microangiopathy, cardiovascular, and cerebrovascular diseases (10). For example, the long-term efficacy of the administration of pancreatic lipase inhibitor has been reflected in the improvements in blood pressure, insulin resistance, weight loss, and serum lipid levels (11). Furthermore, the long-term administration of bile acid sequestrants to hypercholesterolemic patients resulted in overall reductions in total cholesterol and low-density lipoprotein (LDL) cholesterol accompanied by a 19 % reduction in the incidence of coronary heart disease (12). It has been reported that the inhibition of cholesterol absorption by reducing the solubility of cholesterol micelles can lower the production of very low-density lipoprotein (VLDL) cholesterol in the liver and decrease LDL cholesterol concentration in the blood (13). Also, the inhibition of pancreatic cholesterol esterase reduces cholesterol absorption and LDL cholesterol in humans (14).

Consumption of edible plants could be a more effective method for the prevention or treatment of hyperlipidaemia. Many edible plants present an exciting opportunity for the development of newer therapeutics for biologically active antihyperlipidaemic agents from natural resources, especially the reduction of fat digestion and absorption (15–19). Edible plants such as mulberry, Beijing grass, sweetleaf, pennywort, ginkgo, safflower, cat's whiskers, senna, and jiaogulan are consumed as herbal tea and dietary supplements worldwide. It is be-

lieved that they are able to delay postprandial hypertriacylglycerolaemia and hypercholesterolaemia in obese patients. Previously, these edible plants have been investigated for their antihyperglycemic activities through pancreatic α -amylase, and intestinal α -glucosidase inhibitory activities as well as antiglycation (20). However, antihyperlipidemic effects of these edible plants through the inhibition of lipid digestion and absorption are not well known.

Therefore, the aim of the present investigation is to evaluate the effects of nine edible plants on the inhibition of pancreatic lipase and pancreatic cholesterol esterase activities, as well as the inhibition of cholesterol micellization, and bile acid binding.

Materials and Methods

Chemicals

p-Nitrophenylbutyrate (*p*-NPB), oleic acid, phosphatidylcholine, glycodeoxycholic acid, taurodeoxycholic acid, taurocholic acid, porcine cholesterol esterase and porcine pancreatic lipase were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Cholesterol test kits were purchased from HUMAN GmbH Co. (Wiesbaden, Germany). Total bile acid kit was purchased from Bio-Quant Co. (San Diego, CA, USA). All other chemical reagents used in this study were of analytical grade.

Preparation of extracts

The list of edible plants used in this study is given in Table 1. Extraction from plants (30 g) was done with distilled water (500 mL) at 90 °C for 2 h. The samples were filtered through 70-mm Whatman filter paper. The extracts were then centrifuged at 8000 rpm for 10 min. The aqueous solution was lyophilized by using a freeze drier (20).

Phytochemical analysis

Total phenolic content was determined according to Sriplang *et al.* (21). Each sample (50 μ L) was mixed with 50 μ L of Folin-Ciocalteu reagent followed by 50 μ L of Na₂CO₃ (10 % by mass per volume). The absorbance was then measured at 760 nm after incubation at 30 °C for 60 min. Total phenolic content was expressed as mg of gallic acid equivalent per g of dry mass of the extract (Table 2).

Table 1. The list of edible plants used in this study

Plant samples			
Common name	Scientific name	Family	Used part
Beijing grass	<i>Murdannia loriformis</i>	Commelinaceae	whole plant
sweetleaf	<i>Stevia rebaudiana</i>	Asteraceae	leaves
pennywort	<i>Centella asiatica</i>	Mackinlayaceae	leaves
safflower	<i>Carthamus tinctorius</i>	Asteraceae	flowers
ginkgo	<i>Ginkgo biloba</i>	Ginkgoaceae	leaves
cat's whiskers	<i>Orthosiphon aristatus</i>	Lamiaceae	whole plant
senna	<i>Cassia angustifolia</i>	Fabaceae	leaves
jiaogulan	<i>Gynostemma pentaphyllum</i>	Cucurbitaceae	whole plant
mulberry	<i>Morus alba</i>	Moraceae	leaves

Table 2. Phenolic content and the IC₅₀ values of the edible plant extracts needed to inhibit pancreatic lipase and cholesterol esterase

Sample	IC ₅₀ values		
	w (total phenolics) mg/g	γ (pancreatic lipase) mg/mL	γ (pancreatic cholesterol esterase) mg/mL
Beijing grass	(12.2±2.6) ^a	0.11±0.01	>3
sweetleaf	(34.8±0.3) ^a	0.53±0.02	>3
pennywort	(37.0±2.4) ^a	0.12±0.01	>3
safflower	(63.5±4.8) ^a	0.56±0.04	1.70±0.15
ginkgo	(37.5±7.6) ^a	0.05±0.01	>3
cat's whiskers	(33.9±2.6) ^a	0.13±0.01	>3
senna	(65.4±12.4) ^a	0.81±0.03	2.57±0.21
jiaogulan	(80.1±6.0) ^a	0.58±0.05	>3
mulberry	130.9±12.9	0.01±0.01	>3

Results are expressed as mean values±S.E.M. (standard error of the mean), $N=3$; ^atotal phenolic content (expressed as mg of gallic acid equivalent per g of dry mass of the extract) of edible plant extracts had previously been reported by Adisakwattana *et al.* (20)

Pancreatic lipase inhibition

Pancreatic lipase activity was slightly modified according to a previously described method (22). A volume of 25 μ L of the sample solution dissolved in distilled water and 25 μ L of the pancreatic lipase solution (50 U/mL) were mixed in the well of a microtiter plate. Then, a volume of 50 μ L of oleate ester of fluorescent 4-methylumbelliferone (4-MUO) solution (0.1 mM) dissolved in phosphate-buffered saline (PBS) was added to initiate the enzyme reaction. After incubation at 37 °C for 20 min, 100 μ L of 0.1 M sodium citrate (pH=4.2) were added to stop the reaction. The amount of 4-MUO released by the lipase was measured using a fluorescence microplate reader at an excitation wavelength of 320 nm and an emission wavelength of 450 nm. Orlistat was used as positive control in this study.

Pancreatic cholesterol esterase inhibition

The pancreatic cholesterol esterase inhibition was measured spectrophotometrically at 25 °C (23). The extracts were incubated with mixtures containing 5.16 mM taurocholic acid, 0.2 mM *p*-NPB in 100 mM sodium phosphate buffer, 100 mM NaCl, pH=7.0. The reaction was initiated by adding porcine pancreatic cholesterol esterase (1 μ g/mL). After incubation for 5 min at 25 °C, the absorbance of the mixtures was measured at 405 nm. Simvastatin was used as positive control in this study.

Cholesterol micellization

Artificial micelles were used as a model system for *in vitro* cholesterol solubilization, which contains predominantly uniform particles based on sodium taurocholate, egg lecithins, cholesterol, and oleic acid to reflect the natural mixed micelle. They were prepared according to a previously used method (7) with minor modifications. In brief, the mixtures (2 mM cholesterol, 1 mM oleic acid, and 2.4 mM phosphatidylcholine) were dissolved in methanol and dried under nitrogen before adding 15 mM PBS containing 6.6 mM taurocholate salt, at pH=7.4. The emulsion was sonicated twice for 30 min using a sonicator. The micelle solution was incubated overnight at 37 °C. The extracts (final concentration at

10 mg/mL) or equivalent PBS used as control were added to the mixed micelle solution and incubated for further 2 h at 37 °C. The mixture was then centrifuged at 16 000 rpm for 20 min. The supernatant was collected for the determination of cholesterol by using total cholesterol test kits. Gallic acid was used as positive control.

Bile acid binding

The bile acid binding assay was slightly modified according to the previously described method (24). Taurocholic acid, glycodeoxycholic acid and taurodeoxycholic acid were used as bile acids in this experiment. Briefly, the extracts (final concentration at 1 mg/mL) were incubated with each bile acid (2 mM) in 0.1 M phosphate-buffered saline (PBS), pH=7, at 37 °C for 90 min. The mixtures were filtered through 0.2- μ m filter to separate the bound from the free bile acids and frozen at -20 °C until the analysis was carried out. The bile acid concentration was analyzed spectrophotometrically at 540 nm by using bile acid analysis kit. Cholestyramine was used as a positive control in this study.

Statistical analyses

The IC₅₀ values were calculated from plots of log concentration of inhibitor concentration *vs.* percentage inhibition curves by using the SigmaPlot v. 10.0 software (Systat Software Inc., San Jose, CA, USA). Values were expressed as mean±standard error of the mean (S.E.M) for $N=3$.

Results

IC₅₀ values of edible plant extracts for pancreatic lipase inhibition

The results in Table 2 show the IC₅₀ values of nine edible plant extracts needed to inhibit pancreatic lipase. All extracts markedly inhibited pancreatic lipase activity in dose-dependent manner. The findings showed that mulberry extract was the most effective pancreatic lipase inhibitor (IC₅₀=(0.01±0.01) mg/mL), whereas senna extract was the least potent inhibitor among the tested extracts

($IC_{50}=(0.81\pm 0.03)$ mg/mL). However, all extracts were less potent than orlistat (control) in inhibiting pancreatic lipase ($IC_{50}=(1.34\pm 0.13)$ μ g/mL).

Pancreatic cholesterol esterase inhibition by edible plant extracts

At concentration of 2 mg/mL, safflower and senna extracts markedly inhibited pancreatic cholesterol esterase activity by 55 and 45 %, respectively, whereas other extracts inhibited this enzyme activity by about 10–22 % (data not shown). The results in Table 2 show that safflower and senna extracts demonstrated a potent inhibitory activity against pancreatic cholesterol esterase with the IC_{50} values of (1.70 \pm 0.15) and (2.57 \pm 0.21) mg/mL, respectively. However, they were less potent than simvastatin ($IC_{50}=(0.08\pm 0.01)$ μ g/mL), which was used as control.

Inhibition of cholesterol micellization by edible plant extracts

The results in Table 3 show the percentage of inhibition of cholesterol micellization by edible plant extracts at concentration of 10 mg/mL. Among the 9 edible plants, mulberry and sweetleaf strongly inhibited cholesterol micellization with values >50 %. Other extracts showed moderate inhibitory activity (9–39 %), whereas safflower extract had no inhibitory effect on the formation of cholesterol micelles. Comparably, gallic acid (0.2 mg/mL) used as control markedly inhibited the formation of cholesterol micelles about (27.26 \pm 2.17) %.

Bile acid binding by edible plant extracts

The percentage of bile acid binding by the edible plant extracts (1 mg/mL) is shown in Table 3. The results show that cat's whiskers and safflower had the highest ability to bind glycodeoxycholic acid and taurodeoxycholic acid, respectively, to a degree of 53 %. Cholestyramine (1 mg/mL) bound (75.5 \pm 2.3) and (42.7 \pm 3.6) % of glycodeoxycholic and taurodeoxycholic acid, respectively. Taurodeoxycholic acid was slightly bound by all extracts that exhibited binding capacity around 20–38 %, nearly the same as the binding degree of cholestyramine (27.7 \pm 2.4) %.

Discussion

This study was designed to investigate the effect of nine edible plants on the inhibition of pancreatic lipase, cholesterol micellization, pancreatic cholesterol esterase, and bile acid binding. Some edible plants had previously been reported to have antihyperlipidemic properties. For example, flavonoids from mulberry leaves demonstrate hypolipidemic effect in Triton WR-1339-induced hyperlipidemic mice (25). In addition, administration of mulberry leaf extract increased adipocytokine expression and decreased the expression of tumour necrosis factor- α (TNF- α), monocyte chemotactic protein-1 (MCP-1) and macrophage markers in white adipose tissue of db/db mice, resulting in reduced risk factor for atherosclerotic cardiovascular disease (26). The supplementation of pennywort extract significantly decreased serum triglyceride level, body and liver mass of the rats exposed to 0.1 % hydrogen peroxide-induced oxidative stress (27). The long-term treatment of ginkgo leaf extract suppressed the elevation of serum cholesterol and lactate dehydrogenase level in rats fed high-fat diet (28). Cholesterol-lowering mechanism of ginkgo extract may be due to its inhibition of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase activity, reduction of cholesterol influx into the cell, and activated expression of cholesterologenic genes involved in cholesterol metabolism, such as sterol-responsive element-binding protein 2 (SREBP2) (29). It has been reported that daily administration of cat's whiskers aqueous extract significantly decreased plasma triglyceride concentration in streptozotocin (STZ)-induced diabetic rats (21). In addition, chronic treatment with jiaogulan extract for 3–5 weeks reduces postprandial hypertriglycerolaemia induced by olive oil in the Zucker fatty rats (30). Consequently, jiaogulan has been identified as a novel liver X receptor- α (LXR- α) activator that selectively enhances ATP-binding cassette transporter 1 (ABCA1) and apoE gene expression, leading to the control of cholesterol homeostasis (31). Furthermore, consumption of sweetleaf extract reduces the levels of cholesterol, triglyceride, LDL-C in hypercholesterolemic women (32). It has been shown that treatment with senna leaf extract has a lipid-lowering effect in rats with experimentally induced, alcohol-related liver damage

Table 3. The effect of edible plant extracts on the inhibition of cholesterol micellization and bile acid binding

Sample	Cholesterol micellization inhibition/%	Bile acid binding/%		
		Glycodeoxycholic acid	Taurocholic acid	Taurodeoxycholic acid
Beijing grass	18.01 \pm 1.44	30.31 \pm 6.88	28.70 \pm 2.08	6.52 \pm 0.88
sweetleaf	52.94 \pm 4.84	35.27 \pm 7.06	29.42 \pm 2.31	3.93 \pm 2.11
pennywort	27.26 \pm 3.09	15.72 \pm 7.06	28.95 \pm 5.24	29.91 \pm 3.92
safflower	0.54 \pm 0.90	30.56 \pm 3.13	27.32 \pm 0.93	53.66 \pm 2.32
ginkgo	27.26 \pm 3.09	34.90 \pm 6.84	28.34 \pm 1.33	24.12 \pm 2.12
cat's whiskers	37.41 \pm 2.93	53.66 \pm 2.32	35.67 \pm 1.02	20.45 \pm 5.13
senna	27.02 \pm 4.03	39.96 \pm 2.93	20.34 \pm 1.92	3.33 \pm 0.17
jiaogulan	9.76 \pm 1.84	29.43 \pm 6.88	32.55 \pm 1.66	8.90 \pm 1.83
mulberry	57.61 \pm 6.00	25.35 \pm 5.81	34.64 \pm 2.16	14.80 \pm 3.14

Results are expressed as mean values \pm S.E.M., $N=3$

(33). Oral administration of aqueous senna extract causes a significant suppression in blood cholesterol and triglyceride levels in STZ-induced neonatal diabetic rats (34). In this study, antihyperlipidemic mechanisms of edible plant extracts by inhibiting the activity of pancreatic lipase and cholesterol esterase, by inhibiting cholesterol micellization and by bile acid binding are given. The findings indicate that the selected edible plant extracts, such as mulberry and ginkgo, showed promising inhibitory activities against pancreatic lipase. After testing the potential mechanism at the site of bile acid binding, it was concluded that the extracts from cat's whiskers and safflower showed the highest ability to bind glycodeoxycholic acid and taurodeoxycholic acid, respectively. In addition, safflower and senna extracts demonstrated a potent inhibitory activity against pancreatic cholesterol esterase. As mentioned above, these *in vitro* findings may suggest antihyperlipidemic activities of these edible plant extracts based on their interference with lipid digestion and absorption through inhibition of pancreatic lipase and cholesterol esterase activities as well as inhibition of cholesterol micellization and bile acid binding. In addition, it can be hypothesized that daily intake of these edible plants may delay the increase of postprandial hypertriacylglycerolaemia and hypercholesterolaemia, and consequently prevent hyperlipidaemia, reduce the risk of an individual in developing micro- and macrovascular complications including coronary heart disease (CHD), cardiovascular, and cerebrovascular diseases. Further *in vivo* studies on animal model must be conducted in order to confirm this hypothesis.

Published research reports that polyphenolic compounds show the ability to inhibit pancreatic lipase activity (8–11,35) and the formation of cholesterol micelles (36). Kahlon and Smith (37) investigated the effect of fruit extract on bile acid binding in *in vitro* model, suggesting that bile acid binding by fruit may be related to their polyphenolic content. It is interesting to note that mulberry, ginkgo and pennywort leaves are rich in polyphenols such as catechin, epicatechin and quercetin (38–40). Evidence from *in vitro* studies reveals that these polyphenols inhibit the intestinal digestion and absorption of dietary lipids by inhibition of pancreatic lipase activity and cholesterol micellization. For instance, catechin, epicatechin (41), and quercetin (42) exhibit a good inhibitory activity against pancreatic lipase. Furthermore, an *in vitro* study shows that catechins reduce micellar solubility of cholesterol (43). According to these reports, it can be hypothesized that polyphenolic compounds, especially catechin, epicatechin and quercetin from edible plants may be the main contributors to the inhibition of pancreatic lipase and cholesterol micellization.

Conclusions

Results of the study show that the 9 edible plant extracts tested consist of a wide range of polyphenols, and delay fat digestion and absorption through gastrointestinal mechanisms such as inhibition of pancreatic lipase and pancreatic cholesterol esterase activities as well as the inhibition of cholesterol micellization, and bile acid binding. Mulberry seems to be a potential candidate as

the inhibitor of pancreatic lipase and cholesterol micellization, whereas cat's whiskers and safflower show the highest ability to bind glycodeoxycholic and taurodeoxycholic acid, respectively. Safflower and senna extracts demonstrate good inhibitory activity against pancreatic cholesterol esterase. Consequently, some edible plants may have great potential as dietary supplements or nutraceutical foods with antihyperlipidaemic properties.

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