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식물추출물의 Human-ACAT 저해활성 검색

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Screening for Human ACAT-1 and ACAT-2 Inhibitory Activity of Edible Plant Extracts

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ABSTRACT : Cholesterol acyltransferase (ACAT) catalyzes the acylation of cholesterol to cholesteryl ester with long chain fatty acids and ACAT inhibition is a useful strategy for treating hypercholesterolemia or atherosclerosis. Inhibitory effects on ACAT of the MeOH extracts prepared from 163 edible plants were evaluated. 15 species out of 163 species exhibited higher than 50% of inhibition on the hACAT-1 and 9 species exhibited higher than 50% of inhibition on the hACAT-2 activity at their concentration of 100 μg/mL.

Key Words **:** Edible Plant, Acyl-CoA: Cholesterol Acyltransferase (ACAT), Hypercholesterolemia, Atherosclerosis

INTRODUCTION

Inhibition of Acyl-CoA: cholesterol acyltransferase (ACAT, E.C.2.3.1.26), which catalyzes the acylation of cholesterol to cholesteryl esters with long chain fatty acids, is a very attractive target for the treatment of hypercholesterolemia and atherosclerosis (Brown *et al*., 1975). It was found to be present as two isoforms in mammals (Anderson *et al*., 1998; Coses *et al*., 1998), ACAT-1 and ACAT-2, with different tissue distribution and membrane topology (Joyce *et al*., 2000). However, most ACAT inhibitors, which were screened by rat liver microsomal ACAT, have problems associated with low oral bioavailability and adrenal and/or hepatic toxicity in clinical trials (Dominick *et al*., 1993; Matsuo *et al*., 1996). ACAT-1 plays a critical role in foam cell formation in macrophages, whereas ACAT-2 is in charge of the cholesterol absorption process in intestinal mucosal cells (Rudel *et al*., 2001). These findings were consistent with the following results that atherosclerosis lesions were reduced at ACAT-1 mice, whereas ACAT-2 mice have limited cholesterol absorption in the intestine, and decreased cholesterol ester content in the liver and plasma lipoproteins (Accad *et al*., 2000). Therefore, ACAT-1 or ACAT-2 may be effective for the development of a useful hypercholesterolemic or anti-atherogenic agent (Sliskovic *et al*., 2002).

Recently, many researchers have studied ACAT-1 and ACAT-2 inhibitory activity for plant extracts or plant-derived compounds. Im *et al*. investigated lupane-type triterpenoids isolated from *I. macropoda*, lupeol and betulin, to exert an inhibitory effect against ACAT activity (Im *et al*., 2006). And Kim *et al*. reported triterpenoids from the flower of *Campsis grandiflora* showed inhibitory effects on hACAT-1 and hACAT-2 (Kim *et al*., 2005).

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As a part of our screening studies to search new hypercholesterolemia and atherosclerosis materials, 163 edible plant extracts were evaluated for Inhibitory effects on the human ACAT-1 (hACAT-1) and human ACAT-2 (hACAT-2) expressed and characterized from Hi5 cells by recombinant baculoviruses (Cho *et al*., 2003).

MATERIALS AND METHODS

1. Plant materials and cell line

The 163 edible plants, which were permitted as food materials by Korea Food & Drug Administration, were purchased at an agricultural and fishery products market located in Suwon, Korea. The plants were taxonomically identified with respect to morphology by Prof. Dae-Keun Kim, College of Pharmacy, Woosuk University, Jeonju, Korea. Voucher specimens (KHU0501001~163) are reserved at the Laboratory of Natural Products Chemistry, KyungHee University, Suwon, Korea. Hi5 cells were obtained from Gibco-BRL (Grand Islands, NY).

2. Preparation of plant extracts

Each of the dried plants (50 g) were powdered and extracted with 80% MeOH (0.5 L × 2) for 24 hr at room temperature. The fresh materials (500 g) were cut or sliced and extracted with 100% MeOH (1 L × 2) for 24 hr at room temperature. The extract solution was evaporated under reduced pressure at 45℃ and then completely dried by vacuum centrifugal evaporator. The completely dried MeOH extracts were used as samples for activity screening tests.

3. ACAT activity assay

The microsomal fractions of Hi5 cells containing baculovirally expressed hACAT-1 and hACAT-2 were used as sources of the enzyme. The activities of the hACAT-1 and hACAT-2 were measured according to the method of Brecher and Chan (Brecher & Chan, 1980), with slight modification (Jeong *et al*., 1995). The reaction mixture, containing 4 μL of microsomes (8 ㎎/mL protein), 20 ìL of 0.5 M potassium phosphate buffer (pH 7.4) with 10 mM dithiothreitol, 15 μL of bovine serum albumin (fatty acid-free, 40 ㎎/mL), 2 μL of cholesterol in acetone (20 ㎍/mL, added last), 41 μL of water, and 10 μL of test sample in a total volume of 92 μL, was preincubated for 20 min at 37℃ with brief vortexing and sonication. The reaction was initiated by the addition of 8 μL of [1-^{14}C] oleoyl-CoA solution (0.05 μCi, final conc. 10 μM). After 25 min of incubation at 37℃, the reaction was stopped by the addition of 1 mL of isopropanol : heptane (4 : 1; v/v). A mixture of 0.6 mL of heptane and 0.4 mL of 0.1 M potassium phosphate buffer (pH 7.4) with 2 mM dithiothreitol was then added to the terminated reaction mixture. The above solution was mixed and allowed to phase separation under gravity for 2 min. Cholesterol oleate was recovered in the upper heptane phase (total volume 0.9-1.0 mL). The radioactivity in 100 μL of the upper phase was measured in a liquid scintillation viral with 3 mL of scintillation cocktail using a liquid scintillation counter. Background values were obtained by preparing heat inactivated microsomes or normal insect cell lysate microsomes, usually background value was 200-250 cpm, while 8000 cpm of ACAT reaction. The hACAT activity was expressed as a defined unit, cholesteryl oleate pmol/ min/㎎ protein.

RESULTS AND DISCUSSION

For the development of a useful hypercholesterolemic or anti-atherogenic agent, 163 kinds of edible plant extracts were examined for hACAT-1 inhibitory activity (Table 1). As the result of this experiment, 15 species exhibited higher than 50% of inhibition on the hACAT-1 at their concentration of 100 ㎍/mL. Eleven plant extracts-*Angelica gigas, Chlorella vulgaris, Cinnamomum cassia, Coriolus versicolor, Commiphora molmol*, *Eugenica caryophyllata Laurus nobilis, Myristica fragrans, Oenothera erythrosepala, Perilla frutescent,* and *Zanthoxylum schinifolium*- showed inhibitory activity on the hACAT-1 higher than 50% inhibition at the concentration of 100 ㎍/mL. And four plant extracts-*Capsella bursa-pastoris* (92.1 ± 0.6%)*, Piper nigrum* (97.3 ± 0.5%), *Rosmarinus officinalis* (94.3 ± 0.3%), and *Elletaria cardamomum* (93.2 ± 0.1%)- showed strong inhibition on the hACAT-1 at the same concentration. The previously described 15 plant extracts showing hACAT-1 inhibition effect were also evaluated for hACAT-2 inhibition effect (Table 1). The 9 extracts showed higher than 50% inhibition activity on the hACAT-2 at the concentration of 100 ㎍/mL as the followings; *C. bursa-pastoris, C. molmol, E. cardamomum, E. caryophyllata, L.*

Table 1. Inhibitory activities (%) of MeOH extracts from edible plants on hACAT-1 and hACAT-2†.

Scientific name	Korean name	Plant Part	hACAT-1 (100 ㎍/㎖)	hACAT-2 (100 ㎍/㎖)
Acanthopanax sessiliflorus	Ogapi	roots‡	38	–
Acanthopanax sessiliflorus	Ogapi	stems‡	21	–
Actinidia arguta	Darae	Fruits§	–3	–
Adenophora triphylla	Jandae	roots‡	4	–
Agaricur bisporus	Yangsongibeoseot	whole‡	–15	–
Allium cepa	Yangpa	bulbs§	–8	–
Allium chinense	Lakgyo	bulbs§	0	–
Allium fistulosum	Pa	whole§	–4	–
Allium sativum	Maneul	bulbs§	–3	–
Allium schoenoprasmum	Golpa	whole§	9	–
Ananas comosus	Pineapple	fruits§	–7	–
Angelica gigas	Danggwi	roots‡	29	–
**Angelica gigas*	Danggwi	leaves§	65.3 ± 0.3	31.1 ± 0.6
Angelica keiskei	Sinseoncho	leaves§	36	–
Apium graveolens	Celery	leaves§	–3	–
Arachis hypogaea	Tang-kong	seeds‡	1	–
Aralia elata	Dureup	aerial parts§	–4	–
Artemisia princeps	Ssug	aerial parts§	37	–
Asparagus officinalis	Asparagus	stems§	17	–
Aster scaber	Chwi	leaves§	18	–
Auricularia auricula-judae	Mogibeoseot	whole‡	9	–
Brassica campestris	Yuchae	seeds‡	2	–
Brassica juncea	Gat	aerial parts§	0	–
Brassica cernua	Gyeoja	aerial parts§	21	–
Brassica oleracea	Kale	aerial parts§	45	–
Brassica oleracea	Yangbaechu	aerial parts§	–3	–
Brassica campestris ssp rapa	Sunmu	roots§	3	–
Brassica rapa	Baechu	aerial parts§	–6	–
Camellia sinensis	Nokcha	leaves‡	38	–
**Capsella bursa-pastoris*	Naengi	aerial parts§	92.1 ± 0.6	61.6 ± 0.1
Capsicum annuum	Gochu	fruits‡	7	–
Capsicum annuum	Paprica	fruits§	3	–
Capsicum annuum	Pimang	fruits§	–3	–
Capsosiphon fulvescens	Maesaengi	whole§	–4	–
Carica papaya	Papaya	fruits§	–2	–
Carthamus tinctorius	Honghwa	seeds‡	26	–
Carya illinoensis	Pecan	fruits‡	6	–
Cassia obtusifolia	Gyeolmyeongja	seeds‡	3	–
Castanea crenata	Bam	fruits§	–5	–
Chaenomelis sinensis	Mogwa	fruits§	–1	–
**Chlorella vulgaris*	Chlorella	whole‡	66.2 ± 0.7	45.5 ± 0.4
Cichorium endivia	Endive	aerial parts§	6	–
Cichorium intybus	Chicory	leaves§	–12	–
**Cinnamomum cassia*	Gyeji	twigs‡	57.0 ± 0.3	40.7 ± 0.3

†Plant extracts showing inhibitory activity higher than 50% on hACAT-1 were subjected to screening for inhibitory activity on hACAT-2.
‡The dried plants (50 g) were powdered and extracted with 80% MeOH .
§The fresh plants (500 g) were cut or sliced and extracted with 100% MeOH.
*The data are presented as the mean ± standard deviation of three replications.

Table 1. continued.

Scientific name	Korean name	Plant Part	hACAT-1 (100 $\mu g/m\ell$)	hACAT-2 (100 $\mu g/m\ell$)
Cinnamomum cassia	Gyepi	barks‡	32	–
Citrullus vulgaris	Subak	flesh§	3	–
Citrullus vulgaris	Subak	skin of fruits§	–1	–
Citrullus vulgaris	Subak	seeds§	–5	–
Citrus limon	Lemon	fruits§	–3	–
Citrus paradisii	Jamong	fruits§	–7	–
Citrus sinensis	Oragne	fruits§	–8	–
Citrus unshiu	Milgam	fruits§	13	–
Coix lacrymajobi	Yulmu	seeds‡	–3	–
Colocasia antiquorum	Toran	corms§	–9	–
**Commiphora molmol*	Molyak	barks‡	77.4 ± 0.5	58.5 ± 0.1
**Coriolus versicolor*	Gureumbeoseot	whole‡	60.5 ± 0.1	42.3 ± 0.8
Corylus heterophylla	Gaeam	fruits‡	6	–
Cucumis melo	Chamoe	fruits§	1	–
Cucumis melo	Melon	fruits§	–10	–
Cucumis sativus	Oi	fruits§	–4	–
Cucurbita moschata	Hobak	fruits§	–1	–
Daucus carota	Danggeun	roots§	2	–
Dioscorea batatas	Ma	roots§	–12	–
Diospyros Kaki	Gam	fruits§	4	–
Durio zibethinus	Durian	fruits§	–8	–
**Elletaria cardamomum*	Sodugu	fruits§	81.1 ± 0.5	59.9 ± 0.1
Eucommia ulmoides	Duchung	barks§	12	–
**Eugenica caryophyllata*	Jeonghyang	cloves‡	93.2 ± 0.1	51.4 ± 0.7
Euphoria longana	Yongan	fruits§	–5	–
Ficus carica	Muhwagwa	leaves‡	32	–
Flammulina velutipes	Paengibeoseot	whole‡	–5	–
Foeniculum vulgare	Hoehyang	fruits§	40	–
Fortunella mararita	Geumgyul	fruits§	–7	–
Fragaria ananassa	Ttalgi	fruits§	–5	–
Ganoderma lucidum	Yeongjibeoseot	whole‡	1	–
Garcinia mangostana	Mangosteen	fruits§	–1	–
Ginkgo biloba	Eunhaeng	seeds‡	–1	–
Glycine max	Baektae	seeds‡	1	–
Glycine max	Daedu	seeds‡	–8	–
Glycine max	Geomjeongkong	seeds‡	3	–
Glycyrrhiza uralensis	Gamcho	roots‡	33	–
Gossypium indicum	Mokhwa	seeds‡	4	–
Helianthus annuus	Haebaragi	seeds‡	4	–
Hizikia fusiforme	Tot	whole§	1	–
Hordeum vulgare	Bori	seeds‡	23	–
Ilex paraguayensis	Mate	leaves‡	0	–
Ixeris dentata	Sseumbagui	whole§	–2	–
Jasminum grandiflorum	Jasmine	leaves‡	21	–

†Plant extracts showing inhibitory activity higher than 50% on hACAT-1 were subjected to screening for inhibitory activity on hACAT-2.
‡The dried plants (50 g) were powdered and extracted with 80% MeOH .
§The fresh plants (500 g) were cut or sliced and extracted with 100% MeOH.
*The data are presented as the mean ± standard deviation of three replications.

Table 1. continued.

Scientific name	Korean name	Plant Part	hACAT-1 (100 $\mu g/m\ell$)	hACAT-2 (100 $\mu g/m\ell$)
Juglans regia	Hodu	seeds‡	2	–
Lactuca sativa	Sangchu	aerial parts§	–4	–
Lactuca sativa	Yangsangchi	aerial parts§	–1	–
Laminaria japonica	Dasima	whole§	11	–
**Laurus nobilis*	Wolgyesu	leaves§	74.4 ± 0.2	51.0 ± 0.4
Lenttinula edodes	Pyogobeoseot	whole§	–4	–
Ligularia fischeri	Gomchwi	leaves§	46	–
Lycium chinense	Gugija	fruits‡	–7	–
Macadamia ternifolia	Macadamia	fruits§	–10	–
Malva verticillata	Auk	leaves‡	6	–
Mangifera indica	Mango	fruits§	–4	–
Mentha arvensi	Bakha	leaves§	28	–
Momordicae grosvenori	Nahangwa	fruits§	–7	–
Morus alba	Odi	fruits§	–4	–
Musa paradisiaca	Bannana	fruits§	4	–
**Myristica fragrans*	Yukdugu	fruits§	78.1 ± 0.8	58.5 ± 0.2
Nelumbo nucifera	Yeongeun	rhizomes§	1	–
Nephelium lappaceum	Rambutan	fruits§	6	–
**Oenothera erythrosepala*	Dalmajikkot	seeds‡	84.5 ± 0.7	63.2 ± 0.3
Olea europaea	Olive	leaves§	35	–
Oryza sativa	Heukhyangmi	seeds‡	11	–
Oryza sativa	Hyunmi	seeds‡	2	–
Oryza sativa	Ssal	seeds‡	–2	–
Panicum miliaceum	Gijang	seeds‡	–9	–
**Perilla frutescens*	Chajogi	leaves§	68.3 ± 0.1	36.6 ± 0.5
Persea americana	Avocado	fruits§	25	–
Petroselinum crispum	Parsley	aerial parts§	12	–
Phaseolus angularis	Pat	seeds‡	8	–
Phaseolus radiatus	Nokdu	seeds‡	–1	–
Phaseolus vulgaris	Gangnangkong	seeds‡	10	–
Pinus densiflora	Solip	leaves§	36	–
Pinus koraiensis	Jat	fruits‡	3	–
**Piper nigrum*	Huchu	fruits‡	97.3 ± 0.5	77.0 ± 0.4
Pistachia vera	Pistachio	fruits§	5	–
Pisum sativum	Wandu	seeds‡	–3	–
Plantago asiatica	Jilgyeongi	leaves§	28	–
Platycodon grandiflorum	Doraji	roots§	0	–
Pleurotus ostreatus	Neutaribeoseot	whole‡	5	–
Polygonatum odoratum	Dunggeulrae	leaves‡	–10	–
Poncirus trifoliata	Taengja	fruits§	32	–
Porphyra tenera	Gim	whole§	39	–
Prunus amygdalus	Almond	seeds‡	–8	–
Prunus armeniaca	Salgu	fruits§	–9	–
Prunus Avium	Cherry	fruits§	16	–
Prunus mume	Maesil	fruits§	–3	–

†Plant extracts showing inhibitory activity higher than 50% on hACAT-1 were subjected to screening for inhibitory activity on hACAT-2.
‡The dried plants (50 g) were powdered and extracted with 80% MeOH .
§The fresh plants (500 g) were cut or sliced and extracted with 100% MeOH.
*The data are presented as the mean ± standard deviation of three replications.

Table 1. continued.

Scientific name	Korean name	Plant Part	hACAT-1 (100 ㎍/㎖)	hACAT-2 (100 ㎍/㎖)
Prunus salicina	Jadu	fruits§	–6	–
Pteridium aquilinum	Gosari	aerial parts§	19	–
Pueraria lobata	Chilk	roots§	7	–
Punica granatum	Seokryu	fruits§	6	–
Quercus acutissima	Dotori	fruits‡	23	–
Quercus acutissima	Sangsuri	leaves§	–1	–
Rosa banksiae	Jangmi	flowers‡	7	–
**Rosmarinus officinalis*	Rosmary	leaves‡	94.3 ± 0.3	79.1 ± 0.6
Rubus coreanus	Bokbunja	Fruits‡	37	–
Schisandra chinensis	Omija	Fruits‡	10	–
Secale cereale	Homil	seeds‡	–3	–
Sesamum indicum	Chamkkae	seeds‡	8	–
Sesamum indicum	Geomeunkkae	seeds‡	10	–
Setaria italica	Jo	seeds‡	12	–
Solanum melongena	Gaji	fruits§	8	–
Sorghum bicolor	Susu	seeds‡	–6	–
Spinacia oleracea	Sigeumchi	leaves§	2	–
Tricholoma matsutake	Songibeoseot	whole‡	–13	–
Triticum aestivum	Mil	seeds‡	–1	–
Ulva lactuca	Galparae	whole§	34	–
Umbilicaria escuenta	Seokibeoseot	whole‡	–3	–
Undaria pinnatifida	Miyeok	whole§	5	–
Vigna sinensis	Dongbu	seeds§	–9	–
Vitis vinifera	Podo	fruits§	–2	–
Wasabia japonica	Gochunaengi	leaves§	2	–
**Zanthoxylum schinifolium*	Sancho	fruits§	78.6 ± 0.7	45.9 ± 0.2
Zea mays	Chal Oksusu	seeds‡	–6	–
Zea mays	Mae Oksusu	seeds‡	7	–
Zingiber officinale Roscoe	Saenggang	rhizomes§	–7	–
Zizyphus jujuba	Daechu	fruits‡	–10	–

†Plant extracts showing inhibitory activity higher than 50% on hACAT-1 were subjected to screening for inhibitory activity on hACAT-2.
‡The dried plants (50 g) were powdered and extracted with 80% MeOH .
§The fresh plants (500 g) were cut or sliced and extracted with 100% MeOH.
*The data are presented as the mean ± standard deviation of three replications.

nobilis, M. fragrans, O. erythrosepala, P. nigrum, and *R. officinalis* exhibited hACAT-2 inhibition effect with 61.6, 58.5, 59.9, 51.4, 51.0, 58.5, 63.2, 77.0 and 79.1% at the concentraton, respectively. *P. nigrum* and *R. officinalis* showed very high inhibition activity on both of human ACAT-1 and ACAT-2.

The plant extracts significant exhibiting hACAT inhibitory activities (more than 75.0% inhibition) were examined for the activity reported in the literature. *C. bursa-pastoris* was reported as an inhibitor of tumor and bacteriocide (Selenu *et al*., 2005). *C. molmol* has been studied of immunomodulatory effects (El-Ashmawy *et al*., 2006) and it reported to have cytotoxic and anti-inflammatory effects on human gingival fibroblasts cells (Tipton *et al*., 2003). *E. cardamomum* has been found effective for a treatment of postoperative nausea and vomiting (De Pradier, 2006). Numerous papers have been published on *E. caryophyllata*, which are widely cultivated globally and their potential health effects including hypertension, hyperlipidemia, arteriosclerosis (Zhang, 2007), antioxidant activity (Lee *et al*., 2002) and anticoagulation and anticancer activities (Han *et al*., 2007). In particular, Eugenol is the major component responsible for the biological activities of *E. caryophyllata* (Bainard *et al*., 2006; Raghavenra *et al*., 2006; Ogata, 2004). *L. nobilis* was reported to contain cytotoxic sesquiterpenes (Barla *et al*., 2007) and antioxidant activity (Conforti *et al*., 2006). *O. erythrosepala* was reported to exhibit antitumor activity (Miyamaoto *et al*., 1993). *P. nigrum* also has been studied very well about hACAT inhibition activity (Rho *et al*., 2007) and hypertension (Haze *et al*., 2002). Rho *et al.* reported the isolation and structural elucidation of six alkylamides, and inhibitory effects of these compounds on ACAT, which was prepared from microsomes of the liver of rat. And *R. officinalis* extracts were known to relax smooth muscles of intestine, and have hepatoprotective and antitumerogenic activity (Al-Sereiti *et al*., 1999). The most important constituents of *R. officinalis* are caffeic acid and its derivates such as rosmarinic acid, which have been reported to have antioxidant effect (Frankel, 1999), inflammatory response in the pathogenesis of atherosclerosis (Naito *et al*., 2003) and inhibitory effect on LDL oxidation (Fuhrman *et al*., 2000). *Z. schinifolium* was reported to have apoptogenic activity against human acute leukemia Jurkat T cells (Jun *et al*., 2007) and human hepatoma cells (Paik *et al*., 2005).

In this study, the extracts from 163 edible plants, which were permitted as food by Korea Food & Drug Administration, were screened for inhibitory activity on hACAT-1 and hACAT-2, and 15 species showed significant inhibitory activities. In conclusion, the findings of this study suggest that the methanolic extract of 15 species, may prove useful in the treatment of hypercholesterolemia and atherosclerosis.

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