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# Lignan Derivatives from *Fraxinus rhynchophylla* and Inhibitory Activity on Pancreatic Lipase

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**Abstract** – Pancreatic lipase digests dietary fats by hydrolysis, which is a key enzyme for lipid absorption. Therefore, reduction of fat absorption by the inhibition of pancreatic lipase is suggested to be a therapeutic strategy for obesity. We previously reported coumarins and secoiridoids of *Fraxinus rhynchophylla* as inhibitory constituents on adipocyte differentiation. Further investigation on *F. rhynchophylla* led to the isolation of lignan derivatives such as lignans (**1 - 10**), sesquilignans (**11 - 14**) and coumarinolignans (**15 - 17**). Among them, coumarinolignans and sesquilignans were first reported from *Fraxinus* species. Among the constituents isolated, sesquilignans showed the significant inhibition on pancreatic lipase, whereas lignans and coumarinolignans exerted weak effects.
**Keywords** – *Fraxinus rhynchophylla*, pancreatic lipase, sesquilignan, coumarinolignan, obesity

## Introduction

Obesity is becoming one of the serious threats to global health in modern days. Obesity is no longer considered only a cosmetic problem but associated with several pathological disorders, including diabetes, hypertension, atherosclerosis and cancer (Kopelman, 2000). Due to these serious problems in health, World Health Organization (WHO) has prompted to promote strategies to prevent and control its progress (Brug and Crawford, 2009). Obesity arises from chronic imbalance between energy intake and energy expenditure, which results in excessive total fat mass. Energy intake starts from fat absorption through digestion of fat into monoglycerides and fatty acids, catalyzed by lipases. Absorbed fat is further accumulated into adipose tissue, which is called adipocyte differentiation. Therefore, inhibition of fat absorption and/or fat accumulation by the disturbance of lipase and adipocyte differentiation is suggested to be important therapeutics in obesity.

Pancreatic lipase, a key enzyme for lipid absorption, is responsible for the hydrolysis of 50-70% of total dietary fats (Birari and Bhutani, 2007). Reduction of fat absorption by the inhibition of pancreatic lipase is known to be beneficial for the regulation of obesity (Yun, 2010). Orlistat, a specific pancreatic lipase inhibitor, has been clinically used for the prevention of obesity (Ballinger and Peikin, 2002). Recently, there is a growing interest in searching for pancreatic lipase inhibitors from natural products with lower adverse effects. Several natural products including saponins and flavonoids have been reported to have pancreatic lipase inhibitory activity (Xu *et al*., 2005; Nakai *et al*., 2005; Li *et al*., 2007; Lee *et al*., 2010).

*Fraxinus rhynchophylla* Dence (Oleaceae) is a traditional medicinal plant widely used in Asia. The stem barks of *F. rhynchophylla* have been used as an antibacterial, analgesic and anti-inflammatory agent. In addition, diuretic, anti-coagulant, antiallergic effects have been reported by pharmacological studies (Kim *et al*., 1999; Bae 1999).We previously investigated the inhibitory activity of *F. rhynchophylla* on adipocyte differentiation using 3T3-L1 preadipocyte cell lines. Our previous study suggests that coumarins and secoiridoids of *F. rhynchophylla* are effective in inhibition of adipocyte differentiation (Shin *et al*., 2010; Choi *et al*., 2011). In a continuation of our research on *F. rhynchophylla*, further seventeen lignan derivatives were isolated and the structures were identified. In the present, we report the isolation of constituents from *F. rhynchophylla* and inhibitory effects on pancreatic lipase activity.

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## Experimental

**General experimental procedures** – NMR spectra were recorded on a Bruker DRX 500 MHz NMR spectrometer using $CDCl_3$, $CD_3OD$ and DMSO-$d_6$ as solvents. EI-mass spectra were obtained on VG Autospec Ultima (Micromass, Manchester, UK) mass spectrometers. Semi-preparative HPLC was performed using a Waters HPLC system equipped with Waters 600 Q-pumps, a 996 photodiode array detector, and Waters Empower software using Gemini-NX ODS-column (5 μm, 10 × 150 mm). Silica gel (70 - 230 mesh, Merck, Germany) and Sephadex LH-20 (25 - 100 μm, Amersham Biosciences, Sweden) were used for open column chromatography (CC). Thin-layer chromatography (TLC) was performed on a precoated silica gel 60 $F_{254}$ (0.25 mm, Merck, Germany). All other chemicals and reagents were analytical grade.

**Plant material** – The stem barks of *F. rhynchophylla* were purchased from local herbal Market, Chungbuk, Korea in December 2008. They were identified by the herbarium of College of Pharmacy at Chungbuk National University, where a voucher specimen was deposited (CBNU200812-FR).

**Extraction and isolation** – The stem barks of *F. rhynchophylla* (6.0 kg) were extracted 3 times with 80% MeOH, which yielded the methanolic extract (595 g). The methanolic extract was then suspended in $H_2O$ and partitioned successively with *n*-hexane, $CHCl_3$, EtOAc and *n*-BuOH. The $CHCl_3$ fraction (45.2 g) was subjected to column chromatography over Sephadex LH-20 with MeOH to give 11 fractions (C1-C11).Compounds **15** and **16** were obtained by recrystallization of C5 and C7, respectively, using MeOH. C6 was further subjected to CC over Sephadex LH-20 eluted with $CH_2Cl_2$:MeOH to give 3 subfraction (C61-C63). Compound **8** was isolated from C61 by semipreparative HPLC. Compounds **1**, **6**, **11**, **12** and **17** were isolated from C62 by semipreparative HPLC. Compound **2** was isolated from C9 by semipreparative HPLC. Compounds **7**, **9**, **13** and **14** were isolated from C10 by semipreparive HPLC. The EtOAc fraction (50 g) was subjected to silica gel column chromatography with a mixture of $CHCl_3$-MeOH to give 7 fractions (E1-E7). E6 was further subjected to Sephadex LH-20 column chromatography using MeOH to give 5 subfractions (E61-E65). The *n*-BuOH fraction was subjected to HP-20 column chromatography eluted with the mixture of MeOH and $H_2O$ to give 6 fractions (B1-B6). Compounds **4**, **5** and **10** were isolated from B2, B3 and B4, respectively, by semipreparative HPLC after Sephadex LH-20 column chromatography using MeOH. The purity of each compound was verified > 95% by HPLC.

**Assessment of pancreatic lipase activity** – Pancreatic lipase inhibitory activity was evaluated using previously reported methods with a minor modification (Nakai*et al*, 2005; Lee *et al*., 2010). Briefly, enzyme solution was prepared by the reconstitution of porcine pancreatic lipase (Sigma, St. Louis, MO) in 0.1 M Tris-HCl buffer (pH 8). Then, test sample was mixed with enzyme buffer, and incubated for 15 min at 37 ºC. After incubation, 10 mM *p*-nitrophenylbutyrate (*p*-NPB) was added and the enzyme reaction was allowed to proceed for 15 min at 37 ºC. Pancreatic lipase activity was determined by measuring the hydrolysis of *p*-NPB to *p*-nitrophenol at 405 nm using a microplatereader. Lipase activity was expressed as the percentage decrease in the OD when incubated with the test compounds. Orlistat (Sigma, St. Louis, MO) was used as a positive control.

**Statistical analysis** – The evaluation of statistical significance was determined by the Student's *t*-test with a value of $p < 0.05$ or less considered to be statistically significant.

## Results and discussion

The methanolic extract was further fractionated into *n*-hexane, $CHCl_3$, EtOAc and *n*-BuOH fractions. Among them, $CHCl_3$ and EtOAc fraction showed significant inhibitory activity on pancreatic lipase at the concentration of 100 μg/ml and 300 μg/ml (Table 1). Further fractionation and separation of $CHCl_3$, EtOAc and *n*-BuOH fraction by several chromatographic methods yielded seventeen lignan derivatives such as lignans (**1 - 10**),

**Table 1.** Effects of total methanolic extract and each fraction of *F. rhynchophylla* on pancreatic lipase activity

| Fraction | Pancreatic lipase activity (%)[a] | |
|---|---|---|
| | 100 μg/ml | 300 μg/ml |
| Control | 100.0 ± 3.1 | 100.0 ± 3.1 |
| Total extract | 90.7 ± 5.0 | 74.0 ± 4.9 |
| *n*-hexane fraction | 96.7 ± 5.4 | 77.0 ± 5.3 |
| $CHCl_3$ fraction | 78.2 ± 4.3* | 68.0 ± 5.2* |
| EtOAc fraction | 73.3 ± 5.1* | 53.9 ± 7.1** |
| *n*-BuOH fraction | 87.7 ± 4.9 | 77.5 ± 6.1 |
| Aqueous fraction | 100.4 ± 3.5 | 87.7 ± 6.5 |

[a] Relative pancreatic lipase activity (%) was calculated as (activity of compound with substrate – negative control of compound without substrate) / (activity without compound with substrate – negative control of without compound and substrate) × 100.
*$p < 0.05$, **$p < 0.01$compared with differentiated control.

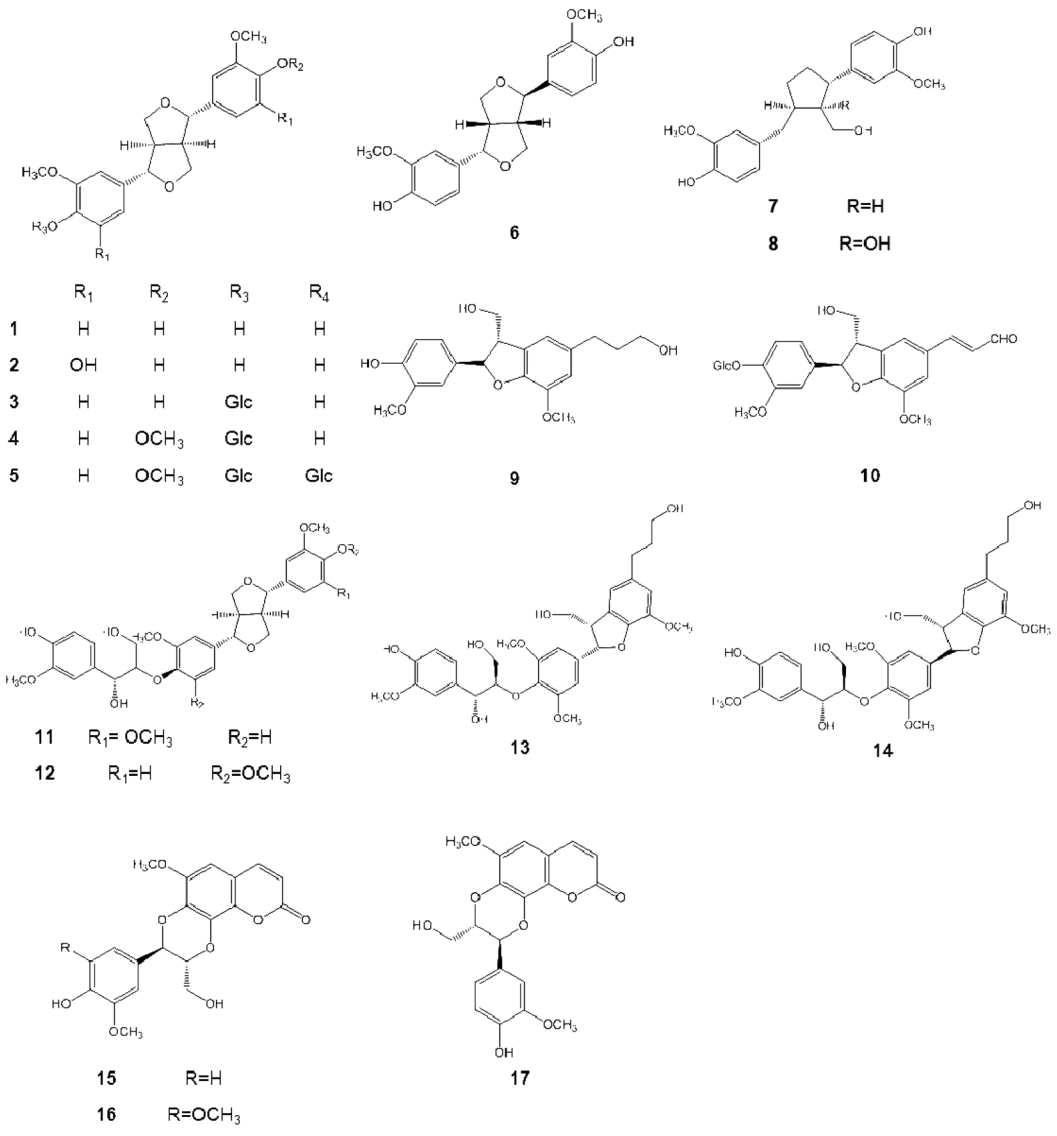


**Fig. 1.** Chemical constituents isolated from the stem barks of *F. rhynchophylla.*

sesquilignans (**11 - 14**) and coumarinolignans (**15 - 17**). All the constituents were identified as pinoresinol (**1**), 8-hydroxypinoresinol (**2**), pinoresinol 4-*O*-β-D-glucoside (**3**), syringaresinol 4-*O*-β-D-glucoside (**4**), syringaresinol 4, 4′-*O*-β-D-diglucoside (**5**), epipinoresinol (**6**), lariciresinol (**7**), olivil (**8**), dihydrodehydrodiconiferyl alcohol (**9**), balanophonin 4-*O*-β-D-glucoside (**10**), ficusesquilignan (**11**), hedyotol C (**12**), acernikol (**13**), dihydrobuddlenol B (**14**), cleomiscosin A (**15**), cleomiscosin C (**16**) and cleomiscosin B (**17**) (Fig. 1), by the direct comparison of their physicochemical and spectroscopic data with those of previously reported (Achenbach *et al*., 1983; Yoshinari*et al*., 1990; Li and Kuo, 2000; Schumacher *et al*., 2002; Morikawa *et al*, 2003; Xie *et al*., 2003; Ouyang *et al*., 2007; Ranjan and Sahai, 2009). To our best knowledge, compounds with the skeletons of sesquilignans (**11-14**) and coumarinolignans (**15-17**) are first reported from *Fraxinus* species. In addition, compounds **7-10** were first reported from this plant.

All isolates were evaluated for their inhibitory effects on pancreatic lipase activity using porcine pancreatic lipase *in vitro.* Among the compounds isolated, two sesquilignan (**13 - 14**) also showed significant inhibition, followed by other sesquilignans (**11 - 12**) and lignan (**9**). However, other lignans (**1 - 8**, **10**) and coumarinolignans (**15 - 17**) exerted little effects on pancreatic lipase activity at the concentration tested in our study.

Natural products contain diverse constituents, which

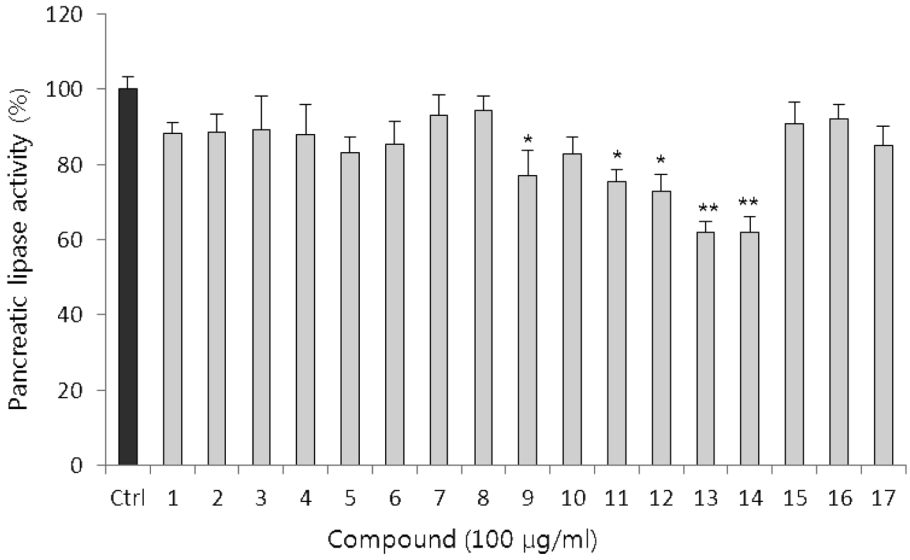


**Fig. 2.** Effect of compounds **1 - 17** from *F. rhynchophylla* on pancreatic lipase activity. Pancreatic lipase activity was measured using 4-nitrophenylbutyrate as a substrate. Relative lipase activity (%) was calculated as (activity of compound with substrate – negative control of compound without substrate) / (activity without compound and with substrate – negative control without compound and substrate) × 100. Results are expressed as the mean ± S.D. of three independent experiments, each performed using triplicate wells. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with control.

allow multiple activities. Synergic action of these mechanisms is preferred for the most effective way to treatment of obesity. Therapeutics for obesity can be developed by various ways such as lipase inhibition, suppression on food intake, stimulation of energy expenditure, inhibition on adipocyte differentiation and regulation on lipid metabolism (Yun, 2010). We previously demonstrated the inhibitory activity of coumarins and secoiridoids of *F. rhynchophylla* on adipocyte differentiation (Shin *et al*., 2010; Choi *et al*., 2011). Our present study shows the inhibitory activity of sesquilignans of *F. rhynchophylla* on pancreatic lipase. Therefore, we suggest that *F. rhynchophylla*, which contains coumarins, secoirioids and sesquilignans, might be beneficial to the treatment of obesity by the combinatorial action of these constituents.

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