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Pancreatic lipase inhibitory constituents from *Morus alba* leaves and optimization for extraction conditions

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ABSTRACT

The leaves of *Morus alba* (Moraceae) have been traditionally used for the treatment of metabolic diseases including diabetes and hyperlipidemia. Thus, inhibitory effect of *M. alba* leaves on pancreatic lipase and their active constituents were investigated in this study. Twenty phenolic compounds including ten flavonoids, eight benzofurans, one stilbene and one chalcones were isolated from the leaves of *M. alba*. Among the isolated compounds, morachalcone A (**20**) exerted strong pancreatic lipase inhibition with IC_{50} value of 6.2 μM. Other phenolic compounds containing a prenyl group showed moderate pancreatic lipase inhibition with IC_{50} value of <50 μM. Next, extraction conditions with maximum pancreatic lipase inhibition and phenolic content were optimized using response surface methodology with three-level-three-factor Box–Behnken design. Our results suggested the optimized extraction condition for maximum pancreatic lipase inhibition and phenolic content as ethanol concentration of 74.9%; temperature 57.4 °C and sample/solvent ratio, 1/10. The pancreatic lipase inhibition and total phenolic content under optimized condition were found to be 58.5% and 26.2 μg GAE (gallic acid equivalent)/mg extract, respectively, which were well matched with the predicted value.

Metabolic disorders including diabetes, hyperlipidemia, atherosclerosis and obesity have close relation to lipid metabolism. Excessive fat is accumulated in adipose tissues and excessive free fatty acids released from adipose tissues, in turn, contribute metabolic disorders. Increased fat also cause oxidative stress and inflammation, which make metabolic disorders worse. Therefore, regulation of fat intake and accumulation is required for the prevention and treatment of metabolic disorders.[1,2] Due to these serious problems in health, diverse attempt has been tried to reduce fat absorption. Pancreatic lipase (PL) is a key enzyme for lipid absorption by the hydrolysis fat into glycerol and fatty acid.[3] Therefore, PL inhibition results in the reduction of fat absorption and is beneficial for the regulation of metabolic disorders.

Morus alba (Moraceae), also known as mulberry, is a deciduous tree that is widely distributed in Asia including Korea. All parts of this tree including roots, fruits, twigs and leaves are of great significance in traditional medicine. Among them, the leaves of *M. alba* has been used in traditional medicine for the treatment of metabolic disorders such as diabetes, hyperlipidemia and high blood pressure.[4] Anti-allergic and anti-melanogenesis activity of *M. alba* leaves also have been reported.[5–8]

Traditionally, herb decoctions are prepared by extraction with water. However, many factors such as extraction solvent, extraction time, extraction temperature and solid–liquid ratios affect the composition of extract as well as its biological activity.[9,10] Therefore, optimization of extraction condition is essential for maximum efficacy. Optimization of extraction condition can be effectively achieved by response surface methodology, especially in case of optimizing several variables. Response surface methodology derives optimal condition efficiently by taking into several factors simultaneously. Therefore, it is fast and reasonable way for optimization.[11–14]

Benzofurans and flavonoids are reported as major constituents of *Morus* species.[15,16] In addition, beneficial role of *M. alba* leaves in the regulation of metabolic disorders have been investigated.[7,17,18] Related to PL, the extract and chlorogenic acid of *M. alba* has been reported to inhibit PL activity.[19,20] In the present study, we tried to investigate PL inhibitory constituents of *M. alba* leaves. Extraction condition such as ethanol concentration, extraction temperature and sample/solvent ratio was also optimized using response surface methodology for maximum PL inhibition and phenolic content.

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We have tried to search for PL inhibitory compounds from *M. alba* leaves. The methanolic extract was fractionated into *n*-hexane, CH_2Cl_2, EtOAc and *n*-BuOH fraction. Further fractionation of CH_2Cl_2 and EtOAc-soluble fractions resulted in the isolation of twenty compounds.[21] The structures of isolated compounds were determined by spectroscopic analysis and the comparison of literature values as ten flavonoids, kaempferol (**1**), quercetin (**2**), isorhamnetin (**3**), norartocarpetin (**4**), kuwanon C (**5**), astragalin (**6**), quercetin-3-*O*-glucopyranoside (**7**), kaempferide 3-*O*-glucoside (**8**), 7,2′,4′-trihydroxyflavanone (**9**), steppogenin (**10**), and eight benzofurans, moracin M (**11**), wittifuran E (**12**), 2-(3,5-dihydroxyphenyl)-5,6-dihydroxybenzofuran (**13**), moracin N (**14**), moracin C (**15**), albafuran A (**16**), moracin X (**17**), morunigrol C (**18**), one stilbene, oxyresveratrol (**19**) and one chalcone, morachalcone A (**20**) (Fig. 1).[15,16,22–34] The isolated compounds were tested for their PL inhibitory activity.[35] Among the isolated compounds, morachalcone A (**20**) showed strong inhibition against PL with IC_{50} of 6.2 μM. Compounds **5**, **14**, **15** and **18** exerted moderate PL inhibition with IC_{50} <32.0 μM. As mentioned above, phenolic compounds isolated from *M. alba* leaves in this study can be divided further into flavonoid, benzofuran, stilbene and chalcone. Flavone aglycones (**1**–**5**) showed moderate PL inhibition (32.4–72.5% inhibition at 100 μg/ml) and prenylated flavonoid (**5**) showed the most potent inhibition (72.5% inhibition at 100 μg/ml). However, flavone glycosides (**6**-**8**) showed weak inhibition (<12.6% inhibition at 100 μg/ml) in our assay system. Benzofurans (**11**–**18**) inhibited PL activity but exerted differential effects depending on functional groups. Addition of hydroxyl groups increased inhibitory activity as observed in compounds **12** and **13** (55.9% and 59.1% inhibition, respectively, at 100 μg/ml) compared to compound **11** (21.5% inhibition at 100 μg/ml). Addition of prenyl group to benzofuran also increased PL inhibition as observed in compounds **14** and **15** (61.7% and 64.8% inhibition, respectively, at 100 μg/ml) compared to compound **11** (21.5% inhibition at 100 μg/ml). Taken together, phenolic compounds are suggested to be active constituents for PL inhibitory effect of *M. alba* leaves. Analysis of structure activity relationship also suggested the importance of a prenyl moiety for PL inhibition, as observed in compounds **5**, **14**, **15**, **18** and **20** (see Table 1).

To maximize PL inhibition and phenolic content, extraction condition was optimized using response surface methodology.[36] As shown in Table 2, Box–Behnken design (BBD) with three-level-three-factor was employed for three extraction variables, such as extraction solvent, extraction temperature and extraction ratio. The ranges of these variables were selected on the basis of preliminary single factor experiment as extraction solvent (X_1, ethanol volume 0–100%), extraction temperature (X_2, 20–60 °C) and sample/solvent ratio (X_3, 1/5–1/15, w/v) and

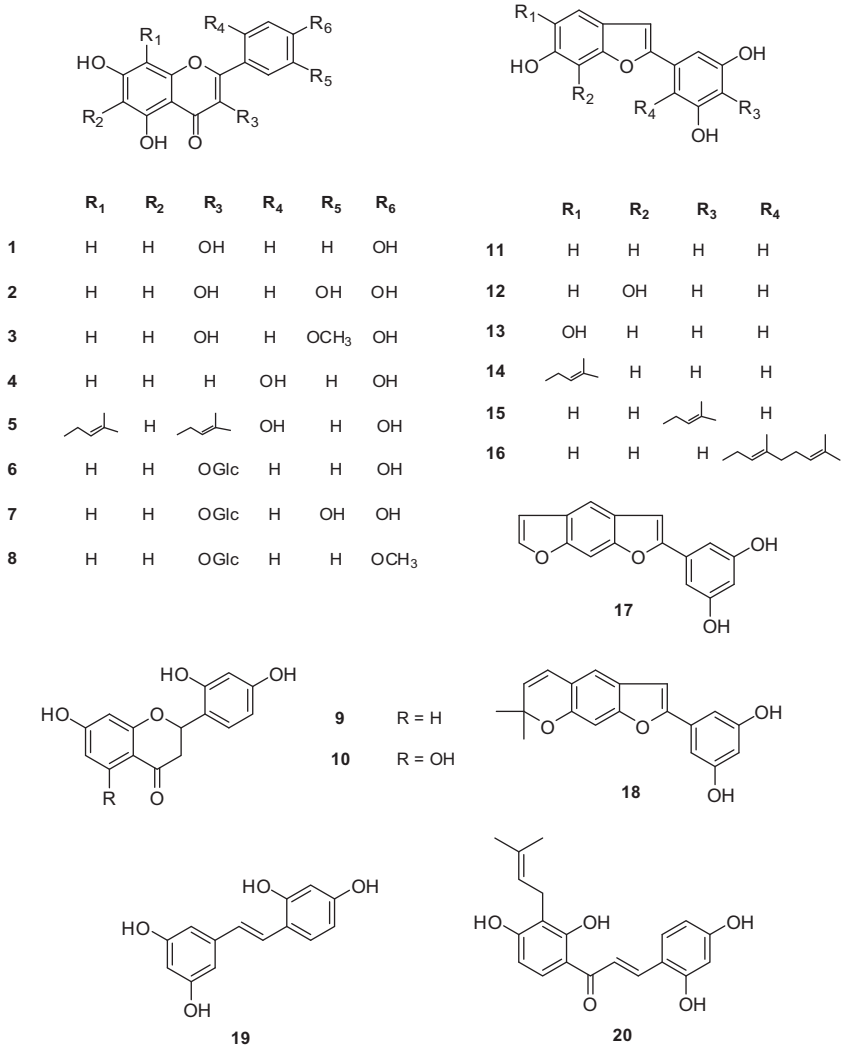

Figure 1. Chemical structures of compounds **1**–**20** from *M. alba* leaves.