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Anti-inflammatory Metabolites of *Agrimonia pilosa* Ledeb. and Their Mechanism

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The anti-inflammatory (INF) compounds (**1-15**) were isolated from *Agrimonia pilosa* Ledeb. (APL) by activity-guided isolation technique. The isolated compounds (**1**–**15**) were identified as quercetin-7-O-rhanmoside (**1**), apigenin-7-O-glycoside (**2**), kaempferol-7-O-glycoside (**3**), apigenin-7-O-[6″-(butyl)-glycoside] (**4**), querceitn (**5**), kaempferol (**6**), apigenin (**7**), apigenin-7-O-[6″-(pentyl)-glycoside] (**8**), agrimonolide (**9**), agrimonolide-6-O-glucoside (**10**), desmethylagrimonolide (**11**), desmethylagrimonolide-6-O-glucoside (**12**), luteolin (**13**), vitexin (**14**) and isovitexin (**15**). Flavonoids, compound **2**, **3**, **11**, and **14-15** have been found in APL for the first time. Furthermore, two novel flavone derivatives, compound **4** and **8**, have been isolated inceptively in plant. In the no cytotoxicity concentration ranges of 0-20 μM, nitric oxide (NO) production level of **1-15** was estimated in LPS-treated Raw 264.7 macrophage cells. The flavone aglycones, **7** (apigenin, IC_{50} = 3.69±0.34 μM), **13** (luteolin, IC_{50} = 4.62±0.43 μM), **6** (kaempferol, IC_{50} = 14.43±0.23 μM) and **5** (quercetin, IC_{50} = 19.50±1.71 μM), exhibited excellent NO inhibitory (NOI) activity in dose-dependent manner. In the structure activity relationship (SAR) study of apigenin-derivatives (APD), apigenin; Api, apigenin-7-O-glucoside; Api-G, apignenin-7-O-[6"-(butyl)-glycoside]; Api-BG and apignenin-7-O-[6"-(pentyl)-glycoside]; Api-P, from APL on INF activity was investigated. The INF mediators level such as NO, INF-cytokines, NF-KB proteins, iNOS and COX-2 were sharply increased in Raw 264.7 cells by LPS. When pretreatment with APD in INF induced macrophages, NOI activity of Api was most effective than other APD with IC_{50} values of 3.69±0.77 μM. And the NOI activity was declined in the following order: Api-BG (IC_{50} = 8.91±1.18 μM), Api-PG (IC_{50} = 13.52±0.85 μM) and API-G (IC_{50} = 17.30±0.66 μM). The NOI activity of two novel compounds, Api-PG and Api-BG were lower than their aglycone; Api, but more effective than Api-G (NOI: Api-PG and Api-BG). And their suppression ability on INF cytokines such as TNF-α, IL-1β and IL-6 mRNA showed the similar tendency. Therefore, the anti-INF mechanism study of Api-PG and Api-BG on nuclear factor-kappa B (NF-κB) pathway, representative INF mechanism, was investigated and Api was used as positive control. Api-BF was more effectively prevent the than phosphorylation of pIκB kinase (p-IKK) and p65 than Api-PG in Raw 264.7 cells. In contrast, Api-PG and Api-BG were not reduced the phosphorylation of inhibitor of kappa B alpha (IκBα). Moreover, pretreatment with Api-PG and Api-BG, dose-dependently inhibited LPS-induced expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) mRNAs and proteins in macrophage cells, and their expression were correlated with their NOI activity. Therefore, APL can be utilized to health promote agent associated with their AIN metabolites.

Key words: *Agrimonia pilosa* Ledeb., Anti-inflammatory, Flavonoids, Nuclear factor-kappa B, iNOS, COX-2