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SHORT COMMUNICATION

Anti-inflammatory effects of mulberry (*Morus alba* L.) root bark and its active compounds

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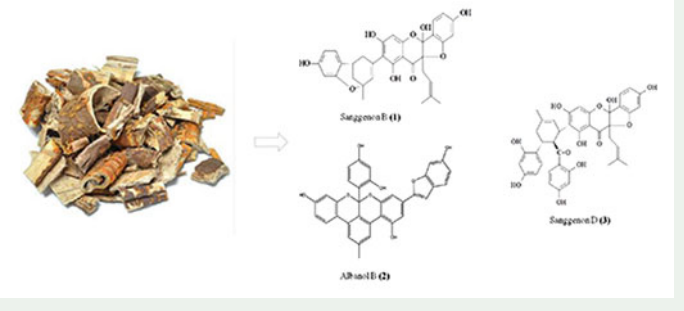
ABSTRACT

Mulberry (*Morus alba* L.) root bark (MRB) was extracted using methanol and the extracts were subjected to tests of anti-inflammatory effects. The ethyl acetate fraction demonstrated the best anti-inflammatory effects. Purified compounds, sanggenon B, albanol B and sanggenon D, showed inhibitory effects on NO production in LPS-stimulated RAW264.7 cells and albanol B demonstrated the best anti-inflammatory effects. Regarding the underlying molecular mechanisms, further investigations showed that treatments with Albanol B reduced production of pro-inflammatory cytokines and decreased expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). These results would contribute to development of novel anti-inflammatory drugs from MRB.



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KEYWORDS
Mulberry root bark; anti-inflammatory; Diels-Alder type adducts; albanol B

1. Introduction

Mulberry (*Morus alba* L.) root bark (MRB) is traditionally used in Asian countries as herbal medicine, which demonstrates antiviral, anti-phlogistic, cardioprotective,

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diuretic, antidepressant and analgesic functions (Lim et al. 2015; Zheng et al. 2017), inhibitory effects on digestive enzymes (pancreatic lipase, α-amylase and α-glucosidase) and 3T3-L1 adipocyte differentiation (Wu et al. 2015), anti-inflammatory, lipolytic and cardioprotective effects (Chen et al. 2013; Li et al. 2017; Cao et al. 2018). These studies suggested that a bunch of bioactive compounds might exist in MRB. Moreover, constituents of MRB might vary among production places. Further studies on MRB from other production places might find new bioactive compounds.

Eo et al. (2014) reported anti-inflammatory effects of MRB originated from Korea. However, the bioactive compounds were not detected. In the present study, we investigated anti-inflammatory effects of n-hexane, dichloromethane, ethyl acetate, n-butanol and water extracts of MRB collected from Korea and identified the bioactive compounds. The underlying molecular mechanisms were also explored. These results might be useful to discover novel anti-inflammatory drugs from MRB.

2. Results and discussion

2.1. Anti-inflammatory activities of MRB fractions

Various *Morus* plants have demonstrated anti-inflammatory activity. For example, ethanolic extract of *Morus indica* (Balasubramanian et al. 2005), betulinic acid, β-sitosterol and germanicol isolated from *Morus nigra* (Padilha et al. 2010) and Diels–Alder type adducts purified from *Morus macroura* (Dai et al. 2004) displayed anti-inflammatory activity. In the present study, effects of MRB fractions were examined on viability of RAW264.7 cells and established nontoxic dose ranges. Finally, 50-200 μg/mL of the n-hexane, n-butanol and water fractions, and 5–20 μg/mL of the dichloromethane and ethyl acetate fractions were confirmed safe and then applied in the subsequent experiments (Figure S1A, Table S1). Among the five fractions, the ethyl acetate fraction was the most effective (EC50 = 7.10 μg/mL). Treatment with 2, 5, and 10 μg/mL ethyl acetate fraction resulted in approximately 13.67%, 46.51%, and 63.99% reduction of NO production in the LPS-stimulated cells, respectively (Figure S1B, Table S1).

2.2. Bioactive compounds in ethyl acetate fraction

Based on analysis of ^{1}H NMR and ^{13}C NMR spectroscopic data (Hano et al. 1985), the structures of purified compounds were identified as sanggenon B (**1**), albanol B (**2**), and sanggenon D (**3**) (Figure 1). All of these three compounds displayed significant inhibitory effects on NO production and none significant cytotoxicity. Albanol B demonstrated the best anti-inflammatory effect. Treatments with 1, 2, and 5 μg/mL albanol B reduced the LPS-stimulated NO production by 3.69%, 14.27%, and 52.58%, respectively (Figure S2). The EC50 value of sanggenon B, albanol B, and sanggenon D was 6.49, 4.8, and 14.85 μg/mL, respectively. Among them, albanol B showed the lowest EC50, representing the highest anti-informatory activity. However, treatments with 10 μg/mL sanggenon B, 10 μg/mL albanol B, and 50 μg/mL sanggenon D showed toxicity to RAW264.7 cells (Figure S2).

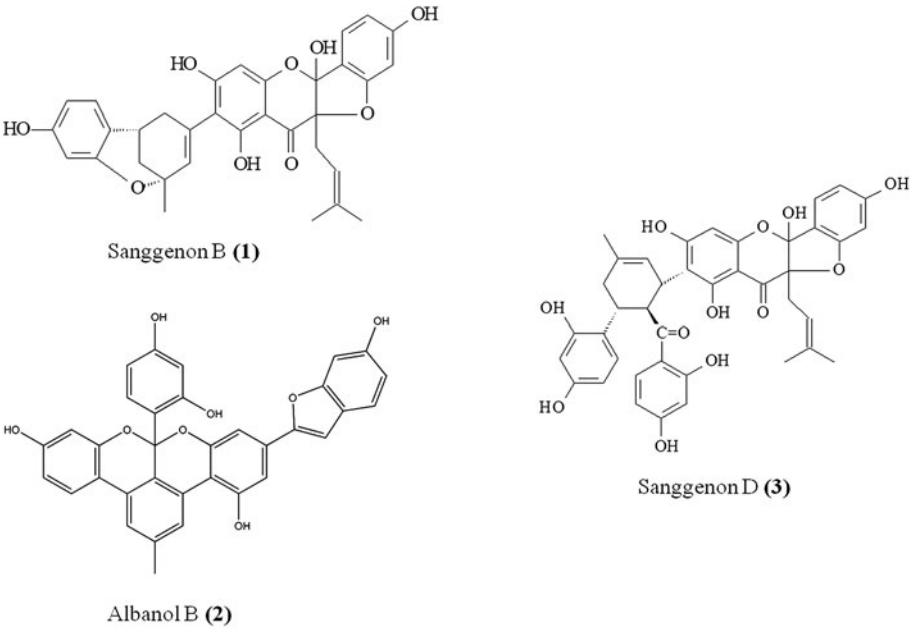

Figure 1. Chemical structures of sanggenon B (1), albanol B (2) and sanggenon D (3).

2.3. Molecular mechanisms underlying anti-inflammatory activity of albanol B

TNF-α and interleukin 6 (IL-6) are critical pro-inflammatory cytokines that play key roles in inflammation process (Mannel and Echtenacher 2000). In the present study, treatments with albanol B significantly suppressed level of TNF-α and IL-6. Treatments with 1, 2, and 5 μg/mL albanol B reduced the TNF-α level by 55.30%, 63.10% and 67.33%, respectively. Similarly, treatment with 5 μg/mL albanol B significantly downregulated level of IL-6, but treatments with 1 and 2 μg/mL albanol B did not (Figure S3).

Nuclear factor-kappa B (NF-κB) signaling pathway is a multi-component pathway that regulates the expression of hundreds of genes and various processes, including inflammation (Courtois and Gilmore 2006). iNOS and COX-2 are two downstream genes of NF-κB and compounds that interfere with both iNOS and COX-2 are potential inhibitors of NF-κB (Seong et al. 2016). In the present study, albanol B reduced the expression levels of iNOS and COX-2 in a dose-dependent manner (Figure S4). The results of qRT-PCR analyses showed that treatment with albanol B significantly suppressed the mRNA level of both iNOS and COX-2 (Figure S5). Taken together, these results suggested that anti-inflammatory effects of albanol B might be attributed to its inhibitory effects on levels of NO, TNF-α and IL-6 through blocking iNOS and COX-2 expression.

3. Conclusions

In summary, three Diels-Alder type adducts, sanggenon B, albanol B and sanggenon D, were purified from the extracts, which might be the anti-inflammatory constituents in MRB extracts. Albanol B showed the best anti-inflammatory activity, which inhibited expression of iNOS and COX-2 and suppressed production of pro-inflammatory

cytokines and mediators in LPS-induced RAW264.7 cells. These findings suggested that MRB could be a potential natural source of anti-inflammatory drugs.

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Disclosure statement

All authors declare no conflict of interest.

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