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Isolation of anti-inflammatory compounds from Sambucus ebulus leaves through in vitro activity-guided fractionation

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The *in vitro* anti-inflammatory effects of subextracts (*n*-hexane, chloroform, ethyl acetate, *n*-butanol, remaining water) of the methanol extract of the leaves of *Sambucus ebulus* L. (Adoxaceae) were investigated for their inhibitory activities on the activation of Nuclear factor kappa B (NF-κB) on lipopolysaccharide induced Raw 264.7 cells. The *n*-hexane, chloroform and ethyl acetate subextracts inhibited NF-κB activation at 50, 100 and 100 µg/mL concentrations, respectively. Two flavonoid mixtures [quercetin-3-*O*-β-D-glucopyranoside, quercetin-3-*O*-β-D-galactopyranoside], two flavonoids [isorhamnetin-3-*O*-β-D-glucopyranoside (*1*), isorhamnetin-3-*O*-rutinoside (*2*)] were isolated from ethyl acetate subextract. 10-*O*-acetylpatrinoside (*3*) and a new iridoid [Sambulin B (*4*)] was obtained from chloroform and *n*-hexane subextracts respectively. Structures were elucidated by NMR and MS. The compounds exerted inhibitions between 30 – 80% on NF-κB. Flavonoids were applied to cells at 25, 50, 75 and 100 µg/mL concentrations. Sambulin B was applied at 6,25, 12,5, 25 and Sambulin A applied at 12,5, 25 and 50 µg/mL concentrations. The effects on nitric oxide (NO), prostaglandine E_2 (PGE_2), tumor necrosis factor (TNF*α*) and interleukins (IL-1*α*, IL-1*β*, IL-2, IL-6) were investigated by Griess and ELISA. The effects on iNOS, COX-2 protein levels and phosphorylation levels of mitogen activated protein kinases and I kappa B alpha (IκB*α*) were examined by Western Blotting. *1*, *2*, *3* and 4 inhibited NO productions (between 59 – 84%), iNOS levels were decreased. 2, 3 and 4 exerted inhibitions on PGE_2 between 39 – 84%, COX-2 protein levels were decreased. *1* and *2* prevented p38/IκB*α* phosphorylations while 3 inhibited JNK/p38. Compound 4 inhibited JNK phosphorylation. All compounds (except mixtures) inhibited TNF*α* more than 29% and only 4 inhibited IL-6.

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