A025 Constituents in evening primrose oil with radical scavenging, cyclooxygenase and neutrophil elastase inhibitory activity

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Evening primrose oil (EPO) is widely used as a dietary supplement. EPO reportedly has beneficial effects in rheumatic and arthritic conditions, atopic dermatitis, psoriasis and various other ailments (1), but the present clinical evidence for most uses need to be substantiated by further rigorous trials (2). Our interest in bioactive plant constituents in medicinal plants and herbal supplements led us to investigate the non-triglyceride fraction of EPO. Analysis of cold pressed, non-raffinated EPO, surprisingly revealed the presence of lipophilic radical scavengers. A highly enriched fraction of these compounds could be obtained from the oil by extraction with aqueous ethanol and subsequent liquid-liquid partioning with petroleum. LC-DAD-MS analysis showed that the fraction contained three aromatic compounds (**1-3**) with identical UV and ESI-MS spectra. The compounds were isolated by RP-HPLC and their structure established by chemical and spectroscopic means as the 3-O-*trans*-caffeoyl derivatives of betulinic, morolic and oleanolic acid, respectively. The morolic acid derivative was a new compound. The radical scavenging activity of the three esters, assessed in a microtitre-based assay with the stable DPPH radical, was comparable to that of ascorbic acid (IC_{50}'s 52 - 64 µM, and 94 µM, respectively). Compounds **1-3** also strongly inhibited human leucocytic elastase (IC_{50} 0.32 µM), and eicosanoid synthesis catalyzed by cyclooxygenase–1 (IC_{50} 0.12 µM), and -2 (IC_{50} 0.4 - 2.5 µM) *in vitro*. The IC_{50} values for the positive control diclofenac were 0.05 µM (COX-1) and 0.013 µM (COX-2). In contrast to cold pressed EPO, commercial samples of evening primrose oils contained only traces of these lipophilic antioxidants.

References: 1. Mahady, G.B.; Fong, H.H.S.; Farnsworth, N.R. (2001) Botanical Dietary Supplements: Quality, Safety and Efficacy; Swets and Zeitlinger, Lisse, Netherlands. **2.** Kleijnen J. (1994) Br. Med. J. 309: 824 – 825.

A026 Antiinflammatory flavonoids from the leaves of *Salix gracilistyla* Miq.

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The aerial parts of *Salix gracilistyla* (Salicaceae) has been used as an analgesic and fever remedy in Korean folk medicine. Several phenolic compounds were reported on *Salix* spp. However, there are no intensive chemical and biological works on the constituents of *S. gracilistyla*. This research was conducted to isolate the major components from the leaves of *S. gracilistyla* and to evaluate their anti-inflammatory activities *in vitro*. Six flavonoid derivatives were isolated by repeated chromatographic isolation of EtOAc soluble fraction. Their structures were elucidated as luteolin (**1**), luteolin 7-O-β-D-glucoside (**2**), apigenin 7-O-β-D-glucoside (**3**), chrysoeriol 7-O-β-D-glucoside (**4**), 6"-*p*-coumaroylapigenin 7-O-β-D-glucoside (**5**) and 4"-*p*-coumaroylapigenin 7-O-β-D-glucoside (**6**) by the analysis of spectroscopic evidences. To examine the effects of these compounds on the arachidonic acid cascade related enzymes, we used several *in vitro* assay systems. As results, two acylated flavonoids, 5 and 6, inhibited 5-lipoxygenase (5-LO) dependent LTC_4 production by BMMC in the presence of c-*Kit* ligand, IL-10 and LPS (IC_{50} = 7.4 µg/ml and 1.8 µg/ml). Furthermore, compound **6**, a new compound, inhibited cyclooxygenase-2 (COX-2) dependent delayed PGD_2 production (IC_{50} = 11.4 µg/ml).

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