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Bioassay-guided fractionation of elderflower extract revealed potential anti-diabetic compounds

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Black elder (*Sambucus nigra* L.)

Black elder (Caprifoliacea) is commonly used for making preserves, juice, and wine and are believed to have health-beneficial effects. Preparations of elder have been used in traditional medicine to treat e.g. colds, influenza, inflammation, and diabetes. Most studies on the bioactivity of elder focus on anthocyanins in berries, which have shown to exhibit antiviral, immune modulating, and antioxidant activities [1]. Few studies have dealt with the health-promoting effects of elderflowers, although they produce many potential bioactive metabolites such as flavonoids and phenolic acids. However, recently it was found that aqueous extracts of elderflowers exhibit insulin like and insulin-releasing actions *in vitro*. The metabolites responsible for the observed effect was not identified and major elderflower metabolites such as quercetin 3-*O*-rutinoside, lupeol, and β-sitosterol did not individually stimulate insulin secretion [2].

Methanolic extract of elderflowers

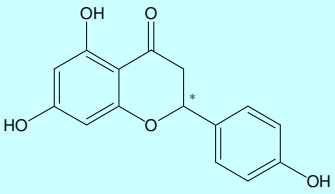
C_{18}-RP Flash CC (Ø70) with MeCN-H_2O gradient

PHENOLIC ACIDS

FLAVONOIDS

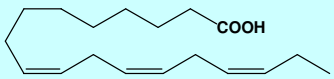
Fractions I + J

C_{18}-RP semi-prep HPLC with MeCN-H_2O gradient



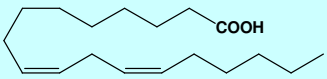
(±)-Naringenin

Fraction K



α-Linolenic acid 18:3 (9c, 12c, 15c)

Fraction L



Linoleic acid 18:2 (9c, 12c)

Experimental work

3 kg of elderflowers (cv. Haschberg) was mascerated and extracted twice overnight with 8 L methanol. The dried extract was separated by RP flash column chromatography to give 12 fractions. Fractions B+C contained phenolic acids, primarily 3-, 4-, and 5-*O*-caffeoylquinic acid. Fractions D-H contained mostly the phenolics quercetin 3-*O*-rutinoside, kaempferol 3-*O*-rutinoside, isorhamnetin 3-*O*-rutinoside, and 1,5-di-*O*-caffeoylquinic acid. In fractions I+J the flavanone naringenin was the major constituent. Fractions K and L were dominated by α-linolenic acid and linoleic acid. All metabolites were purified by RP semi-preparative HPLC and identified by HPLC-DAD, LC-MS, and standard addition.

Bioactivity and perspectives

In a screening of plant extracts for potential anti-diabetic effects the extracts of elderflowers were found to contain compounds with bioactivities similar to those of partial peroxisome proliferator-activated receptor gamma (PPARγ) agonists [3]. Bioassay-guided chromatographic fractionation of the elderflower extract yielded four bioactive fractions (marked with red) and the major metabolites in these were naringenin, α-linolenic acid, and linoleic acid. Bioactivity was assessed using a PPARγ transactivation assay. Fatty acids are well-known activators of PPARγ, but naringenin is not and will have to be further tested to establish its potential as an anti-diabetic compound. Moreover, large difference in the content of bioactive compounds and other metabolites of elderflower varieties was found, indicating the importance of choosing the optimal elder varieties in order to develop effective functional foods/herbal products for prevention/treatment of type 2 diabetes.

References
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