Anticancer properties of sea buckthorn extracts

and variation in content of ursolic acid among sea buckthorn cultivars grown in Sweden

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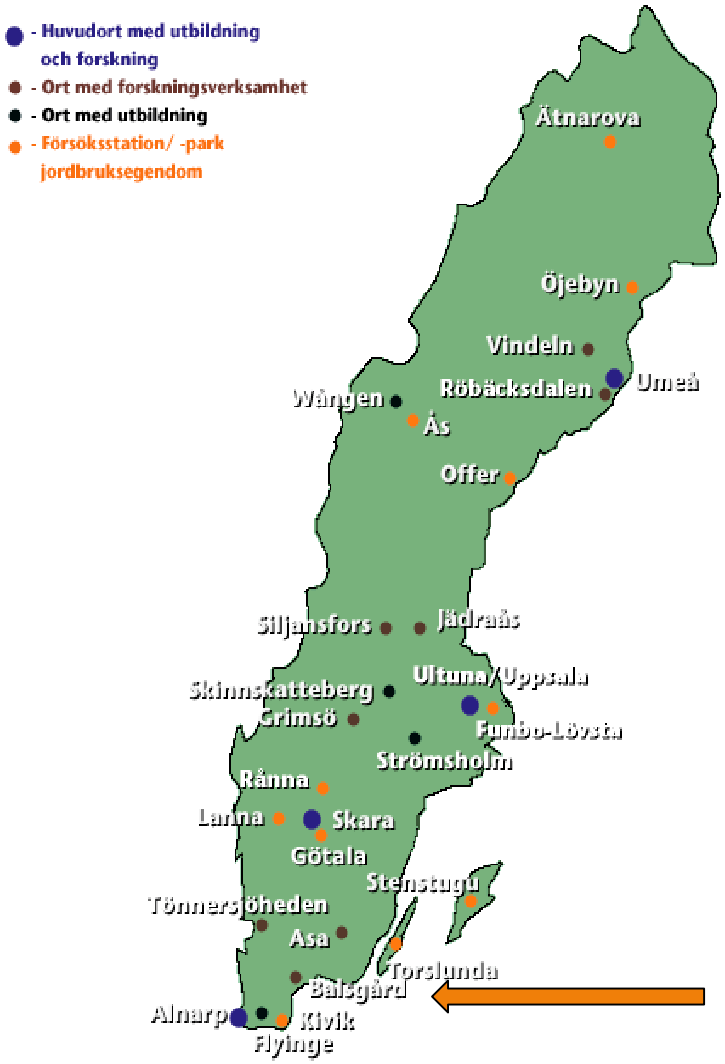
Balsgård – division of horticultural plant breeding



- Research on genetics and breeding of fruits and berries
- Plant breeding of apple (since 1945), black currants (since 1950) and sea buckthorn (since 1985)
- Vast collections of apple, pear, plum, cherries, chaenomeles, currants, sea buckthorn, rose hips, cowberries
- Centre of innovative beverages (2010)

Breeding and selection goals for sea buckthorn in Sweden

- Adaptation to local climate (hardiness, early ripening)
- Sweet taste (high sugar, low acidity)
- Absence of rancidity at full (over) maturity
- Consistent and high yield
- Suitability for cut and freeze harvesting

BHi 10726

Strong growth, green foliage
Few medium thorns
Circular to oblong fruits of medium size
Dense fruit clusters
Distinctive aroma and medium acidity
Early to medium ripening
Very rich yield

BHi 32415

Weak growth, gray foliage
Few long sharp thorns
Oblong to elliptic fruits of medium size
Semi sweet fruits at full ripeness
No rancidity, dense fruit clusters
Early to medium ripening
Rich yield

SLU

BHi 727115

Medium growth, green-gray foliage, few thorns of medium size
Ovate to oblong fruits of medium size
Early ripening
Semi sweet at full maturity, no rancidity
Semi dense fruit clusters
Rich yield

BHi 72587

Weak to medium growth, green-gray foliage, few thorns of medium size
Very large ovate to oblong fruits
Very early ripening
Long fruit stalks
Easy to pick by hand
Medium yield

Antiproliferative effects of sea buckthorn (*Hippophae rhamnoides* L.) extracts on human colon and liver cancer cell lines

Participants

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Objectives

- (1) to compare the antiproliferative and apoptotic effects of different sea buckthorn extracts on human colon cancer cells (the Caco-2 cell line) and human liver cancer cells (the Hep G2 cell line).

- (2) to determine whether the antiproliferative activities of sea buckthorn extracts are associated with the content of different bioactive compounds.

Background: Some previous anti-cancer studies on sea buckthorn

- Decreased proliferation of colon cancer and breast cancer cells. Correlation with vitamin C and carotenoids. (Olsson et al. J Agr Food Chem 2004)
- Quercetin, kaempherol and myricetin induced apoptosis of leukemia cells as well as decreased cell proliferation. (Hibasami et al. Int J Mol Med 2005)
- Antiproliferative and apoptotic effects on forestomach and skin cancer. (Padmavathi et al. Nutr Cancer 2005)
- Isorhamnetin can cause apoptosis in liver cancer cells. (Teng et al. Pharmacol Res 2006)
- Inhibition of stomach, prostate, intestine and breast cancer cell lines by sea buckthorn juice. (Boivin et al. Anticancer Res 2007)

Materials and methods

Plant material:

- BHi 10726 (for sequential and solvent mixture extraction)
- 24 cultivars and advanced selections (for screening of ursolic acid)

Cells:

- Colon cancer cells (Caco-2)
- Liver cancer cells (Hep G2)

Extraction procedures:

- Sequential extraktion successively using n-heptane, ethyl acetate, 96% ethanol, and water.
- Extraction by a solvent mixture of ethanol and acidified water (1:1).

Materials and methods

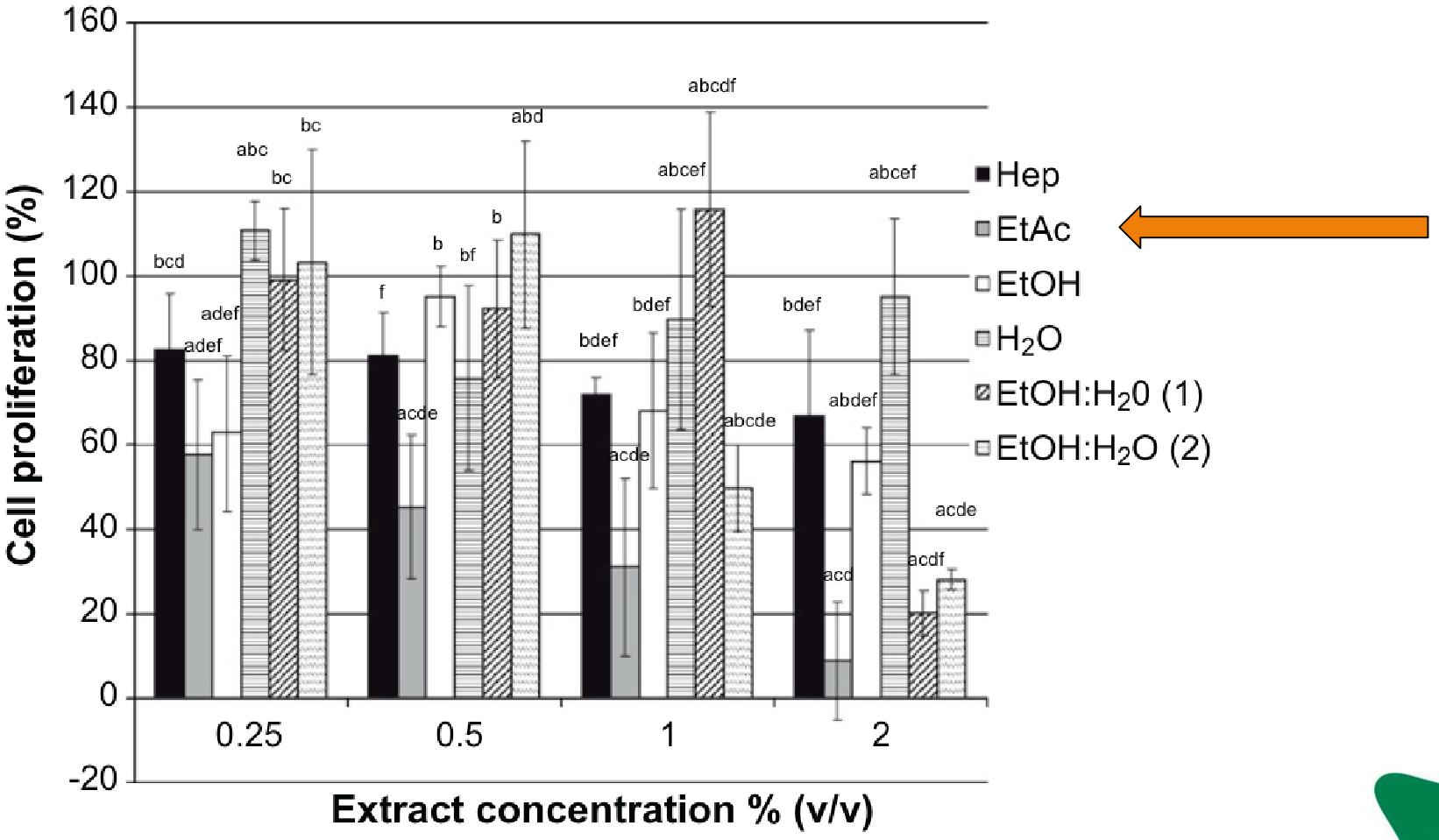
Cell analyses:

- Cell viability
- Cell proliferation
- Cell apoptosis

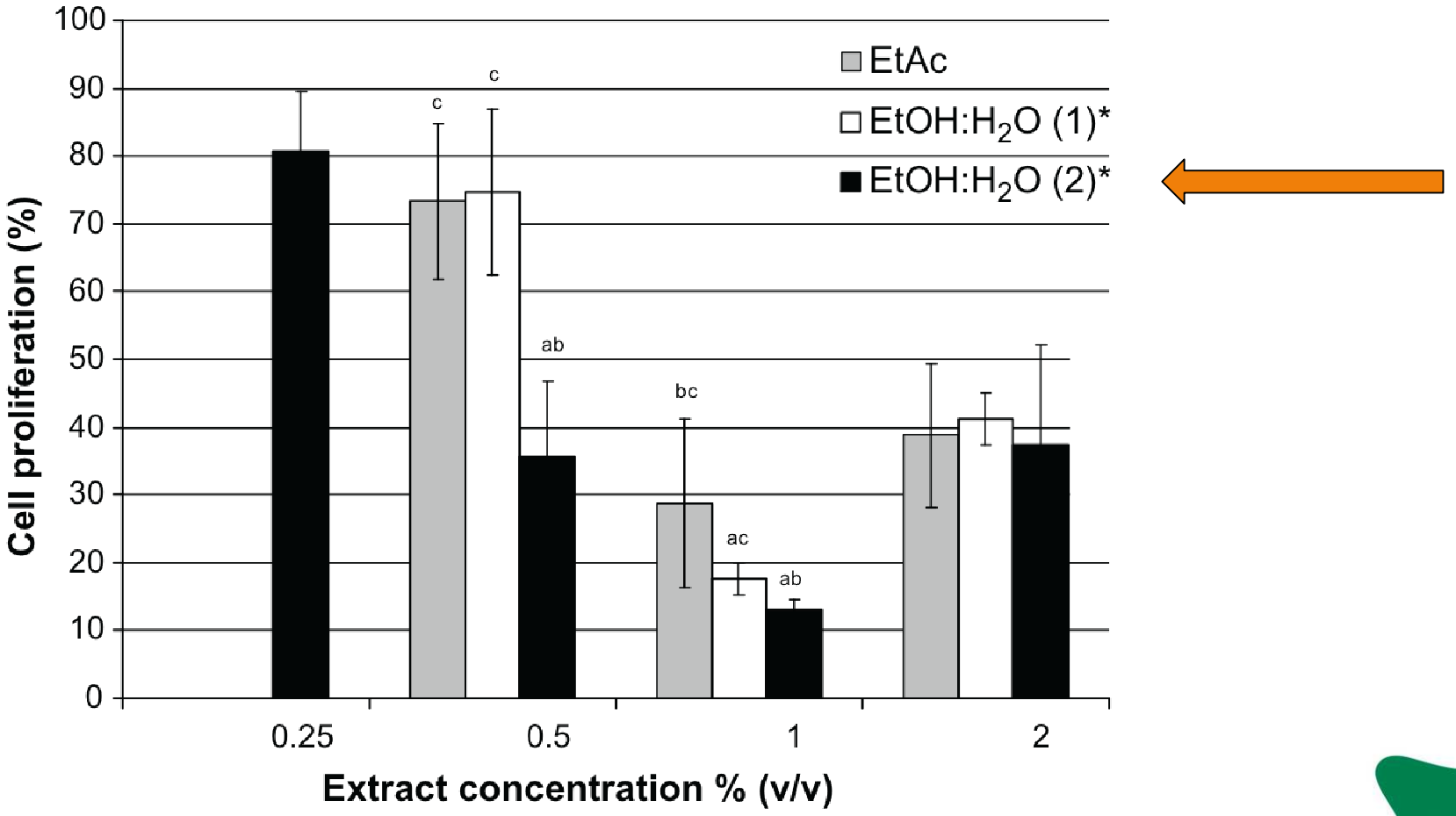
Chemical analyses:

- Total phenols
- Phenolic compounds (rutin, isorhamnetin-3-*O*-rutinoside, kaempferol-3-glucoside, isorhamnetin-3-*O*-rutinoside and proanthocyanidins)
- Ursolic acid

Results: Effects of different extracts of sea buckthorn on colon cancer cells



Results: Effects of different extracts of sea buckthorn on liver cancer cells



Results: Contents of different sequential extracts and ethanol:water extracts of sea buckthorn

Extract[a]	TP	PC	RT	IH-3R	IH-3G	KF-3G	UA
Heptane	362 ± 9	–	–	–	–	–	–
Ethyl acetate	1234 ± 50	998 ± 53	18.3 ± 0.4	149 ± 3	126 ± 2	4.5 ± 0.1	946 ± 17
Ethanol	2632 ± 18	2365 ± 68	79.6 ± 2.8	490 ± 16	250 ± 8	8.9 ± 0.3	92 ± 2
Water	456 ± 20	683 ± 50	6.2 ± 0.1	31 ± 0.4	6 ± 5	0.6 ± 0.02	4 ± 2
Ethanol:water (1)	3737 ± 56	2342 ± 148	79.8 ± 4.1	498 ± 24	282 ± 13	9.9 ± 0.5	43 ± 6
Ethanol:water (2)	4213 ± 42	6836 ± 525	96.2 ± 13.6	549 ± 29	300 ± 16	11.8 ± 2.2	20 ± 16

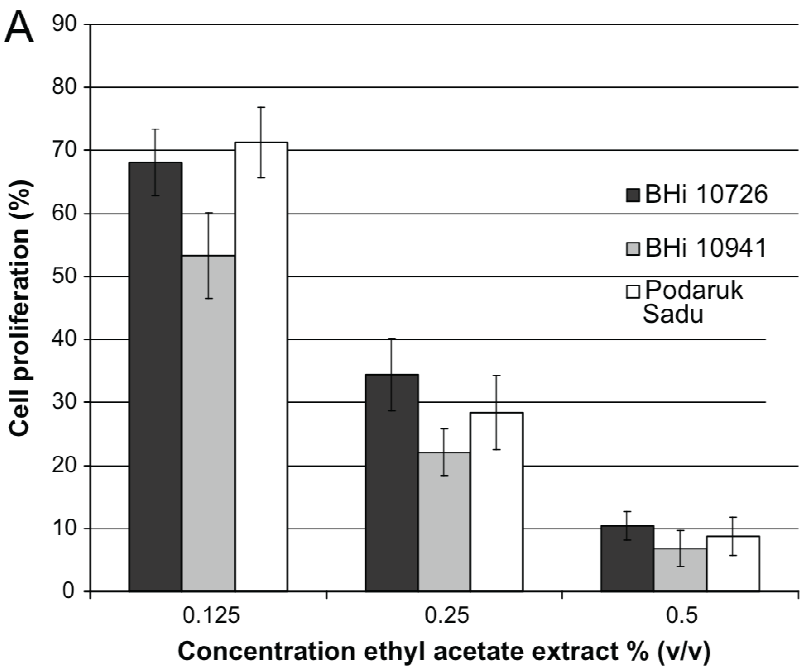
[a] TP = total phenolics; PC = proanthocyanidine; RT = rutin; IH-3R = isorhamnetin-3-O-rutinoside; IH-3G = isorhamnetin-3-O-glucoside; KF-3G = kaempferol-3-glucoside; UA = ursolic acid.

Cultivar	Average (µg/g DW)
Podaruk Sadu	3421
Gibrid Pertjik	3327
Trofimovskaja	2930
Nr 100	2915
Solnjetjnaja	2786
Moskovitskaja	2741
Prozratjnaja	2733
BHi 72579	2688
Krasnoplodnaja	2596
BHi 72782	2573
BHi 10742	2447
Ljublitelskaja	2400
BHi 10747	2244
BHi 72668	2223
Nr 81	2195
BHi 31843	2179
BHi 10726	2110
BHi 727102	2105
Krasnaja	2063
BHi 31701	1895
BHi 72588	1877
BHi 10941	1790
Nr 150	1736
BHi 10933	1416

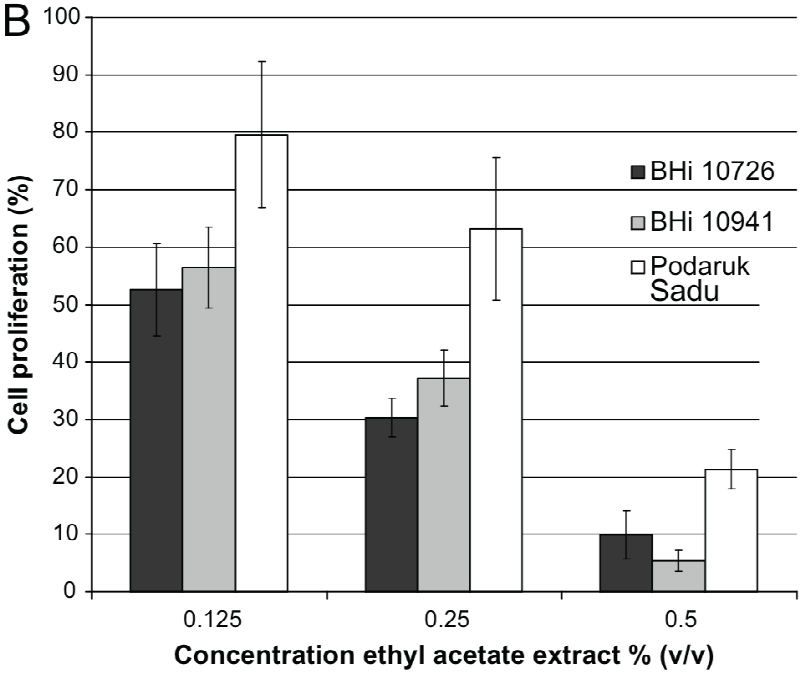
Results: Ursolic acid in different sea buckthorn cultivars and selections

Average: 2400 µg/g DW

Results: Inhibition of cancer cell proliferation by ethyl acetate extracts of different sea buckthorn cultivars



Colon cancer cells



Liver cancer cells

Results: Composition of ethyl acetate extracts of different sea buckthorn cultivars

Composition of ethyl acetate extracts of three different sea buckthorn cultivars, given as µg/g d.w. ± SD.

Cultivar	TP[a]	PC	RT	IH-3R	IH-3G	KF-3G	UA
BHi 10941	754 ± 16	865 ± 48	14.0 ± 0.6	201 ± 8	288 ± 12	9.9 ± 0.3	1307 ± 14
Podaruk Sadu	789 ± 16	1129 ± 85	21.9 ± 0.7	209 ± 6	426 ± 13	11.1 ± 0.4	1903 ± 40
BHi 10726	886 ± 18	929 ± 116	15.9 ± 0.6	227 ± 7	335 ± 10	11.9 ± 0.5	1213 ± 27

[a] TP = total phenolics; PC = proanthocyanidine; RT = rutin; IH-3R = isorhamnetin-3-O-rutinoside; IH-3G = isorhamnetin-3-O-glucoside; KF-3G = kaempferol-3-glucoside; UA = ursolic acid.

Conclusions

- The strongest inhibitory effect was found in ethyl acetate extract for colon cancer cells and in the ethanol:water extract for liver cancer cells.
- The antiproliferative effects were in both cases dose-dependent and were in the case of the ethyl acetate extract associated with an increase in apoptosis.
- The ethyl acetate fraction contained high levels of ursolic acid and rather low amounts of phenols.
- The ethanol:water extracts contained high levels of phenols, but little ursolic acid.
- The dose dependent effects observed were not solely caused by a single component and different extracts were most efficient for different cells. This points out the advantage of using whole extracts rather than isolated compounds when evaluating the physiological relevance of fruits and berries.