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Potential of *Helleborus foetidus* extracts as phytopharmaceutical for integrative oncology

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Introduction & Aim

Anthroposophic medicine can be used to broaden therapeutic options and take into account the dynamic interplay of physical, biological, psychological, and spiritual factors in health and illness (Bartelme, 2020).

The aim of this study was to investigate the anti-cancerous potential of *Helleborus foetidus* extracts that could be used as an anthroposophic drug for integrative oncology treatment.

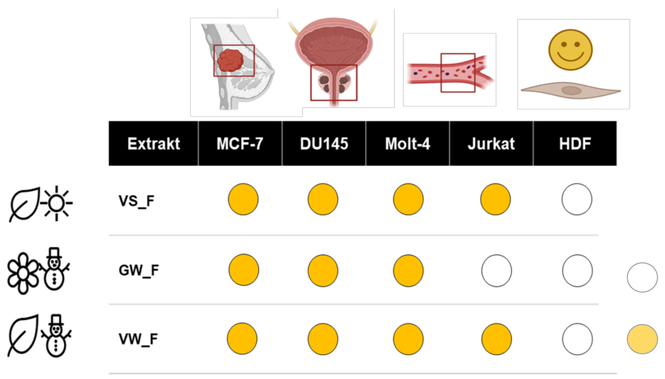
Materials & Methods

Human cells representing lymphoma (Jurkat), breast (MCF7) and prostate (DU145, PC-3) cancer, as well healthy cells (fibroblasts and keratinocytes) were treated for 48 h with aqueous *H. foetidus* extracts in 96 well plates. Mouse fibroblasts (BALB/3T3) were used as comparators. Cell viability was measured using the CCK-8 assay and effects on the cell cycle were determined by flow cytometry after staining the DNA with Hoechst 33342 (Fig 1).

For the identification of specific active constituents, compounds were isolated from a MeOH plant extract using liquid chromatography and their structures elucidated by MS and NMR analysis (Fig. 2). The toxicity of a subset of compounds (including several bufadienolides) was analyzed on MCF-7 and BALB/3T3 cells (Fig 3).

Results

A



B

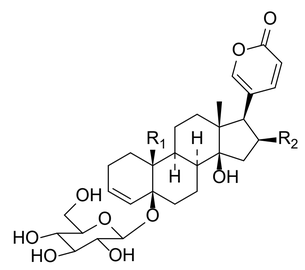
high viability → low viability

Extract	BALB/3T3	MCF-7	MDA-MB231	DU145	PC-3	HDF	NHEK	Molt-4	Jurkat
GWaNF	1050	53	298	<62	114	15	<6	>4000	45
GWcF	4717	58	513	45	91	22	<6	>4000	41
VWaNF	6329	<31	117	<62	25	3	<6	>4000	8
VWcF	6494	23	171	9	25	5	<6	>4000	11
GSaNF	54	36	106	17	19	9	<6	88	35
GScF	172	46	133	18	19	9	<6	397	56
VSaNF	1433	<31	213	<62	<21	4	<6	>4000	18
VScF	2591	37	327	18	25	7	<6	>4000	13

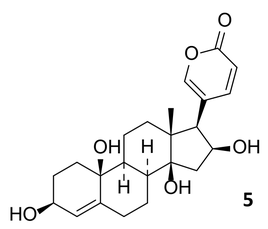
Figure 1: Water based plant extracts (harvested in summer or winter, from generative or vegetative parts). **A** Effects on cell cycle determined by flow cytometry . **B** Cytotoxicity to several cell lines expressed as IC50 in µg fresh plant/ml. Red stands for low viabilty and green for high viability.

Figure 2: Schematic representation of the isolation of the active compounds and their structures

1. Extraction (MeOH)
2. n-BuOH/H_2O Partition
3. Chromatographic separation (LH-20, HPLC)



	R_1	R_2
1	OH	OCHO
2	CHO	OCHO
3	CHO	H
4	OH	OH



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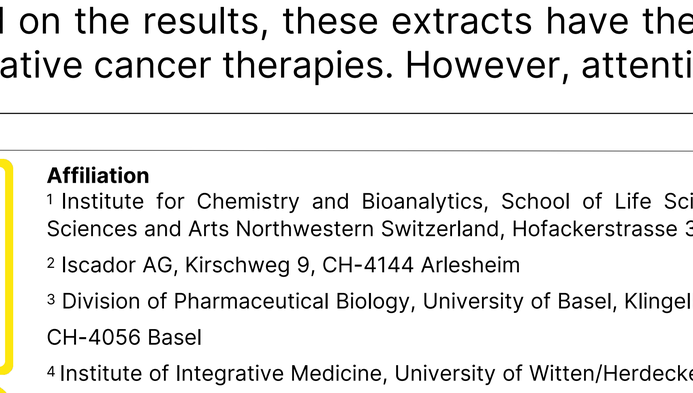
A



B

compound	Balb/3T3	MCF-7	MDA-MB-231	DU145	HDF	Jurkat	Molt-4
1	> 10'000	976	1'247	119	104	80	83
2	> 10'000	37	182	28	4	113	12
3	> 10'000	15	293	13	7	28	5
4	> 10'000	1'050	> 10'000	645	414	1'329	195
5	> 10'000	3'270	> 10'000	2'750	8'258	5'138	3'739
mix	> 10'000	90	591	40	27	87	73

Figure 3A Cytotoxicity of compounds isolated from H. foetidus, showing cytotoxic effect of bufadienolides (Bufadienolides 1-5) on MCF-7 cells. **B** IC50 (in nM) of bufadienolides (see Figure 2) on several cells lines. Red stands for low viability and green for high viability.

Discussion

The results show that the extracts were cytotoxic to the cancer cell lines. Plant extracts arrested the cycle of cancer cell lines (extending S or G2/M) and were highly cytotoxic to cancer cell lines and healthy human fibroblasts and keratinocytes, whereas mouse fibroblasts were more resistant. Analysis of the fractions obtained from the extracts demonstrated that *H. foetidus* contains numerous bufadienolides, which were the main drivers of the observed cytotoxicity (specifically for the human cells).

In **conclusion**, the use of a panel of cancer and healthy cells, coupled with analytical methods allowed us to identify the most potent substances present in *H. foetidus* extracts.
Based on the results, these extracts have the potential to be developed as an anthroposophic phytopharmaceutical drug to support integrative cancer therapies. However, attention will need to be paid to potentially harmful effects on healthy cells.

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Literatur:
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