

Sesquiterpene lactone-containing extracts from two chicory cultivars show different anthelmintic activity *in vitro* against *Ostertagia ostertagi*



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Background:

- Mechanisms behind *in vivo* anthelmintic (AH) effects of **forage chicory** are poorly understood
- Bioactive plant compounds like **sesquiterpene lactones** (SL) are believed to play a role
- Lacking evidence of direct activity of well-characterised SL-containing extracts towards parasitic nematode stages and against cattle nematodes

Objective:

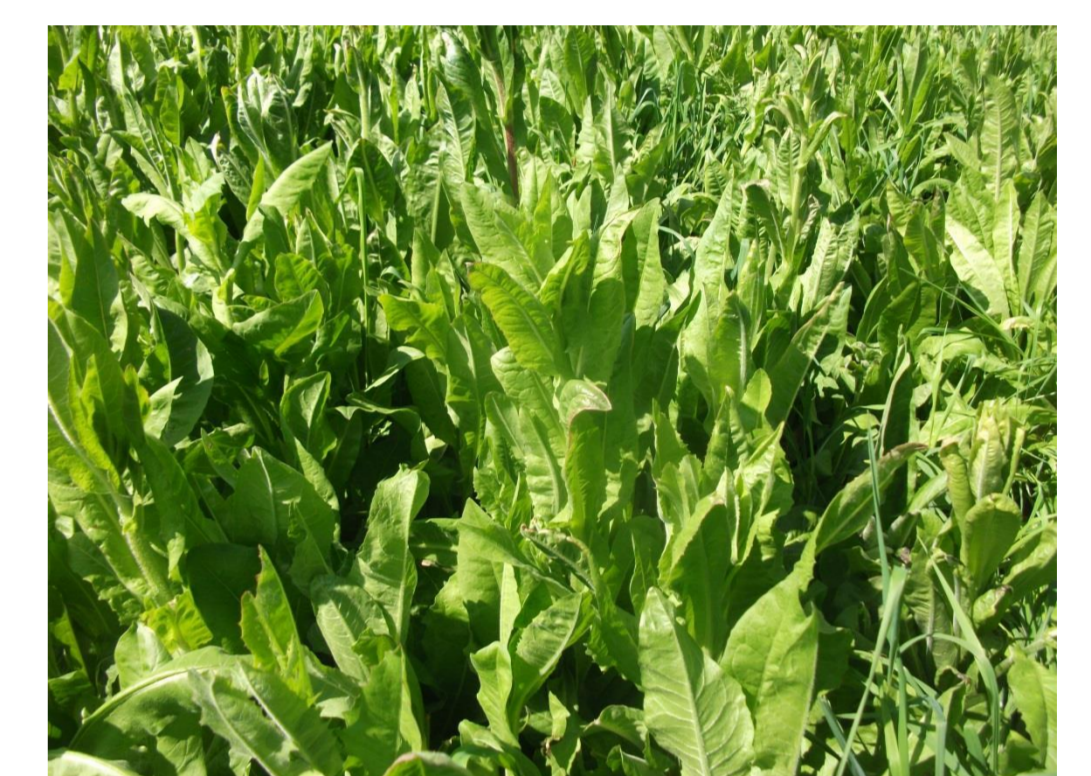
- To test the direct inhibitory activity of SL from two forage chicory cultivars on free-living and parasitic stages of the pathogenic cattle nematode *Ostertagia ostertagi*

Methods:

- Leaves from chicory cultivars 'Spadona' and 'Puna II' were freeze-dried, extracted with methanol/water and SL purified by solid-phase extraction
- Chemical profiling of the extracts by liquid chromatography (LC)
- AH activities of extracts (in DMSO) were tested with *O. ostertagi* L1 (larval feeding inhibition assay), L3 (larval exsheathment inhibition assay) and adult worms (adult motility inhibition assay)



Chicory cultivar 'Spadona'



Chicory cultivar 'Puna II'

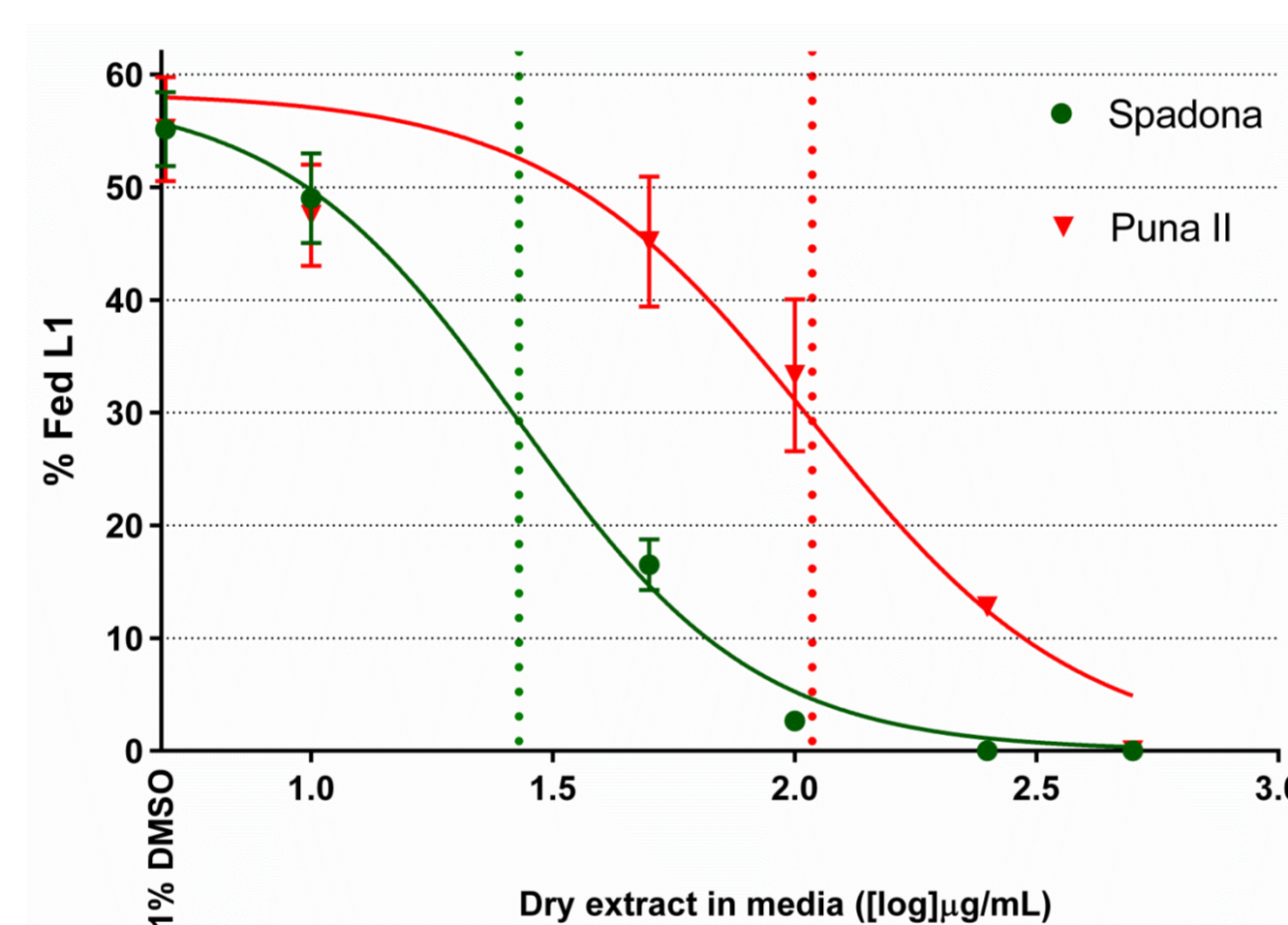
Results: Larval feeding inhibition assay (LFIA)

- Both extracts demonstrated a dose-dependent inhibition of larval feeding
- Spadona extract was 4-fold more potent than Puna II extract ($P < 0.0001$)

EC₅₀ values obtained in the LFIA with SL-containing extracts from chicory cv. Spadona and cv. Puna II

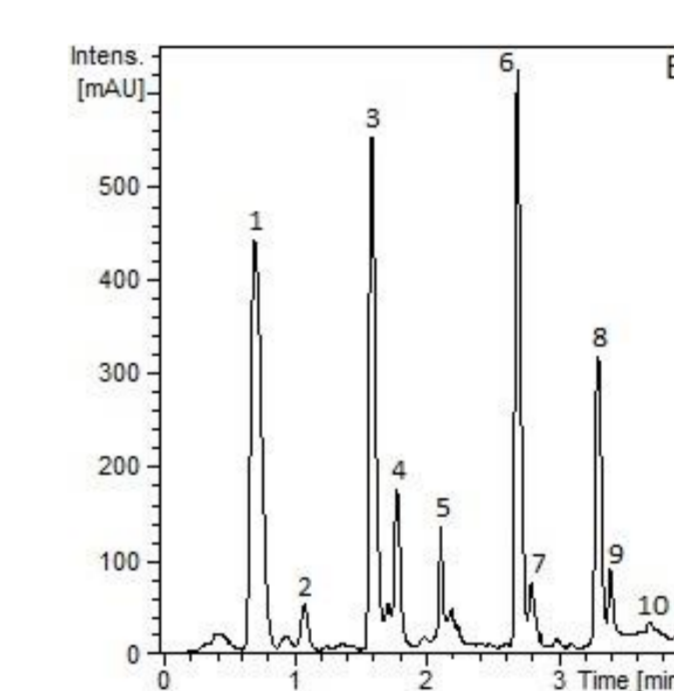
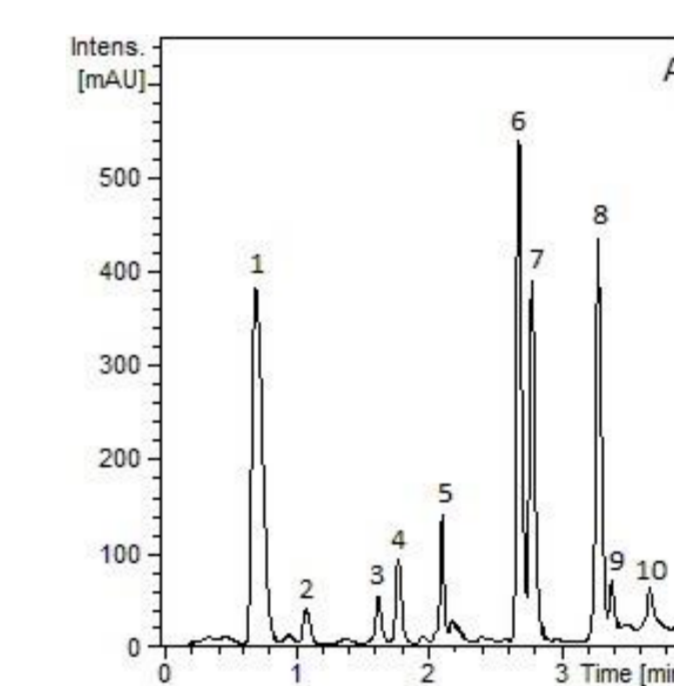
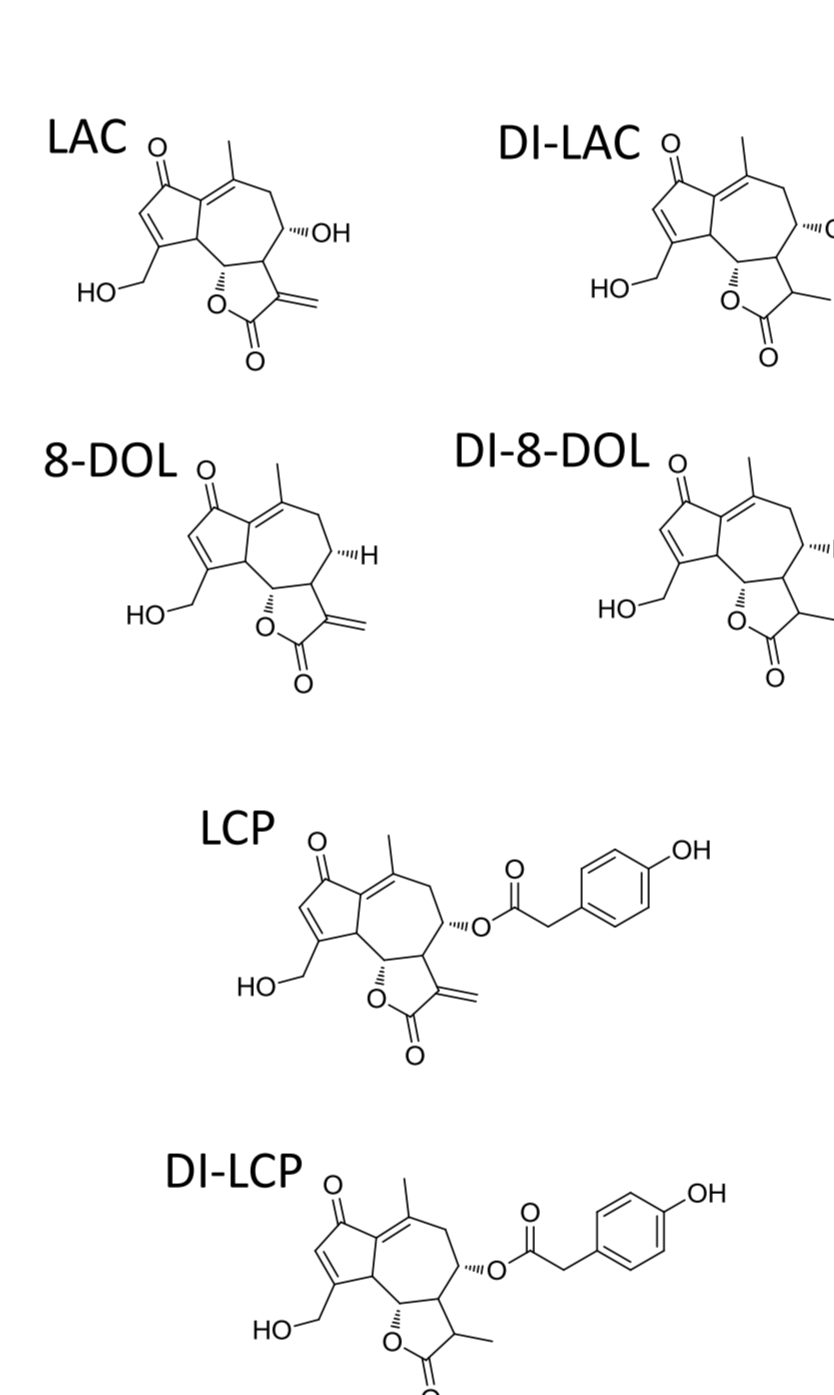
Chicory extract	Spadona	Puna II
EC ₅₀ (µg dry extract/mL)	27***	109
95 % CI	22-32	81-146.2
R ²	0.98	0.92

CI = confidence interval; R² = goodness-of-fit; *** $P < 0.0001$



Dose-response curves obtained in the LFIA with SL-containing extracts from chicory 'Spadona' and 'Puna II'

Results: Chemical profile SL-containing extracts by LC



SL detected in extracts from chicory 'Spadona' and 'Puna II'

LC chromatograms of extracts from chicory 'Spadona' (A) and 'Puna II' (B)

Chemical characterisation by LC of extracts from chicory 'Spadona' (A) and 'Puna II' (B)

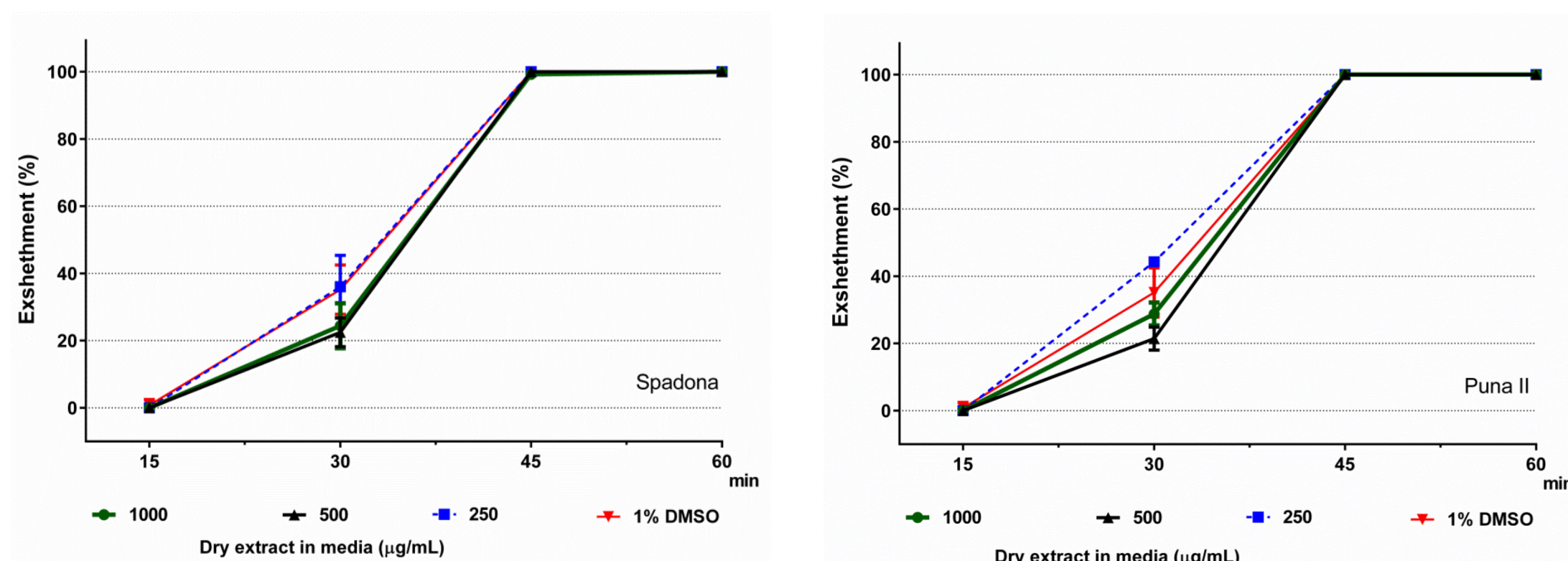
Chicory extract		Spadona	Puna II
Peak	Compound	µg mg ⁻¹ dry extract	µg mg ⁻¹ dry extract
1	U-1	143.0	137.7
2	U-2	9.6	17.7
3	DI-LAC	10.7	105.3
4	LAC	19.7	37.3
5	U-3	25.7	30.5
6	8-DOL	105.7	108.6
7	DI-8-DOL	76.5	20.4
8	DI-LCP	87.8	65.2
9	LCP	11.1	19.5
10	U-4	22.3	13.5
Total SL		311.5	356.3
Unknown		200.5	199.5

U: Unknown; SL: sesquiterpene lactones; LAC: Lactucin; DI-LAC: 11, 13-dihydro-lactucin; 8-DOL: 8-deoxylactucin; DI-8-DOL: 11, 13-dihydro-8-deoxylactucin; LCP: Lactucopicrin; DI-LCP: 11, 13-dihydro-lactucopicrin

Results:

Larval exsheathment inhibition assay (LFIA)

- Extracts from neither of the two chicory cultivars interfered with the exsheathment of *O. ostertagi* L3 at any of the tested concentrations



Exsheathment kinetics in *O. ostertagi* L3 incubated with SL-containing extracts from chicory 'Spadona' and 'Puna II' or 1% DMSO (negative controls)

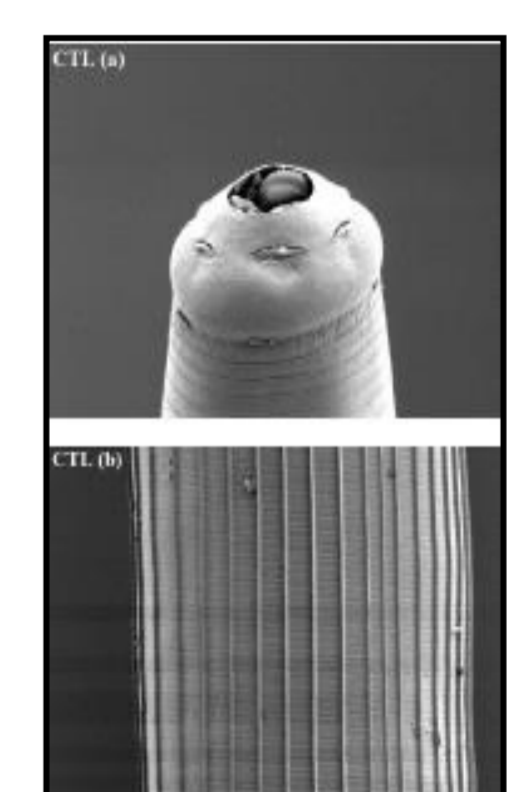
Results: Adult motility inhibition assay (AMIA)

- Both extracts demonstrated a dose-dependent inhibition of worm motility
- Spadona extract showed a significantly higher potency and exerted faster worm paralysis than Puna II extract at all time points ($P < 0.0001$)
- No morphological damage was observed in the cuticle of chicory exposed worms (scanning electron microscopy)

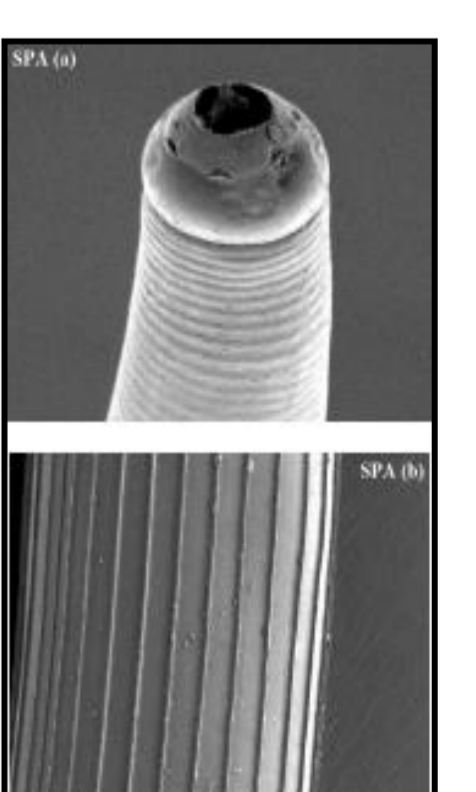
EC₅₀ values obtained in AMIA with SL-containing extracts from chicory 'Spadona' and 'Puna II'

Incubation time	6 hours		24 hours		48 hours	
	Spadona	Puna II	Spadona	Puna II	Spadona	Puna II
EC ₅₀ (µg dry extract/mL)	167***	449	80***	228	36***	82
95 % CI	148-191	406-497	71-92	199-262	32-40	72-93
R ²	0.96	0.95	0.97	0.95	0.98	0.96

CI = confidence interval; R² = goodness-of-fit; *** $P < 0.0001$



Adult *O. ostertagi* exposed to 1% DMSO (negative control)



Adult *O. ostertagi* exposed to Spadona-extract (1000 µg/mL)

Conclusions:

- SL-containing extracts from forage chicory induced direct and dose-dependent inhibitory effects against feeding and motility of *O. ostertagi* L1 and adults, resp., but not on the exsheathment of L3
- Distinct AH activity and SL-profiles were detected in extracts from two chicory cultivars. This may help to identify the most active anti-parasitic compound(s)

Further research:

- Mechanisms of AH action of SL-containing extracts from chicory?
- Are different AH activities between cultivars preserved *in vivo*?
- Are SL the only anti-parasitic compounds in forage chicory?

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