The effects of condensed tannins against cattle nematodes

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Anthelmintic plants as a novel approach for controlling parasitic nematodes in ruminants

Plant secondary metabolites (PSM): glycosides, saponins, lactones, polyphenols

the polyphenolic compounds group called

condensed tannins
Tannin-containing plants with anthelmintic activity against sheep and goat nematodes

Sulla
 (*Hedysarum coronarium*)

Birdsfoot trefoil
 (*Lotus corniculatus*)

Big trefoil
 (*Lotus pedunculatus*)

Sainfoin
 (*Onobrychis viciifolia*)

Sedicea 1espedeza
 (*Lespedeza cuneata*)
EU Marie Curie Project ”HealthyHay”

- Research of sainfoin (*Onobrychis viciifolia*) – improvement of breeding, investigation of chemical composition, nutritional, environmental and antiparasitic benefits of sainfoin
- Aim: *in vitro* effects of tannin-containing plant extracts against cattle nematodes
Material and Methods

• 3 plant extracts:
  • *Onobrychis viciifolia*
  • *Lotus pedunculatus*
  • *Lotus corniculatus*

• Chemical analysis of plant extracts:
  • PC/PD ratio, CT content, mDP, cis/trans ratio

• 2 cattle nematodes:
  • *Ostertagia ostertagi*
  • *Cooperia oncophora*

• 2 *in vitro* assays:
  • Larval feeding inhibition assay (LFIA)
  • Larval exsheathment assay (LEA)
Scheme of larval feeding inhibition assay

100 L1 larvae

plant extract

incubation 2 h

E. coli labelled with FITC

incubation 20 h

inverted fluorescence microscope

Unfed larva

Fed larva

larvae on slide
Scheme of larval exsheathment assay

1. Incubation of ensheathed L3 in extract (3 h)
2. Washing with PBS
3. Transferring of larve into wells containing hypochlorite sodium
4. Stop reaction by adding Lugol solution after 15, 30, 45, and 60 min
5. Counting of exsheathed and ensheathed larvae under microscope
Concentrations of extract:
2.5, 10, 40, 160 µg/ml
Lev = Levamisole (40 µg/ml) as positive control
PBS as negative control
160 µg/ml of extract+PVPP (50 mg/ml)

O.v. = O. viciifolia
L.p. = L. pedunculatus
L.c. = L. corniculatus
### EC$_{50}$ in LFIA and tannin content and composition of three plant extracts

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>C. oncophora</th>
<th>O. ostertagi</th>
<th>CT</th>
<th>mDP</th>
<th>PC/PD</th>
<th>cis/trans ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC$_{50}$ (95% CI) (µg/ml)</td>
<td>EC$_{50}$ (95% CI) (µg/ml)</td>
<td>mean (g CT/100g ext.)</td>
<td>mean (%)</td>
<td>PC : PD mean (%)</td>
<td>cis : trans mean (%)</td>
</tr>
<tr>
<td><strong>Onobrychis viciifolia</strong></td>
<td>8.6 (5.17-14.43)</td>
<td>7.7 (4.83-12.05)</td>
<td>12.5</td>
<td>27.6</td>
<td>33.4 : 66.6</td>
<td>81.6 : 18.4</td>
</tr>
<tr>
<td><strong>Lotus pedunculatus</strong></td>
<td>2.6 (2.22-3.09)</td>
<td>2.6 (2.39-2.89)</td>
<td>19.4</td>
<td>26.4</td>
<td>19.3 : 80.7</td>
<td>79.1 : 20.9</td>
</tr>
<tr>
<td><strong>Lotus corniculatus</strong></td>
<td>12.8 (9.74-16.96)</td>
<td>16.1 (13.61-19.50)</td>
<td>9.4</td>
<td>18.2</td>
<td>61.4 : 38.6</td>
<td>62.4 : 37.6</td>
</tr>
</tbody>
</table>
Results: LEA

L. pedunculatus

% of exsheathed larvae

0 20 40 60 80 100

15 30 45 60 Time (min)

- - - PBS ▲ 2400 µg/ml ▲ 1200 µg/ml ▲ 600 µg/ml

O. vicilifolia

% of exsheathed larvae

0 20 40 60 80 100

15 30 45 60 Time (min)

- - - PBS ▲ 2400 µg/ml ▲ 1200 µg/ml ▲ 600 µg/ml

PVPP

% of exsheathed larvae

0 20 40 60 80 100

15 30 45 60 Time (min)

- - - PBS ▲ O. vicilifolia ▲ L. pedunculatus ▲ L. compositus

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Discussion

- all three plant CT extracts act against L1 and L3 of *O. ostertagi* and *C. oncophora*
- different sensitivity of LFIA and LEA
- ranking according to effect in LFIA:
  1. *L. pedunculatus*
  2. *O. viciifolia*
  3. *L. corniculatus*
- anthelmintic effect associated with CT content and PC/PD ratio
- need of *in vivo* experiment in cattle
- combination of pasture management and plant anthelmintics or chemical anthelmintics = successful control of GIT nematodes in future
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For more information, please, visit: http://sainfoin.eu