In vitro antiparasitic activity of chemically characterized extracts from *Lotus uliginosus* (cv. INIA E-TANIN) and *Maytenus ilicifolia* (Congorosa), against gastrointestinal nematodes of sheep and cattle, in Uruguay

Olivaro, C.[1], Reyno, R.[2], Lara, S.[2,3], De Souza, G.[2], Escobal, M.[1], Mederos, A[2*1] Centro Universitario Regional Noreste

[1]Universidad de la República, Tacuarembó, Uruguay; [2] Instituto Nacional de Investigación Agropecuaria, Ruta 5 Km 386, Tacuarembó; [3] Facultad de Veterinaria, Universidad de la República, Uruguay

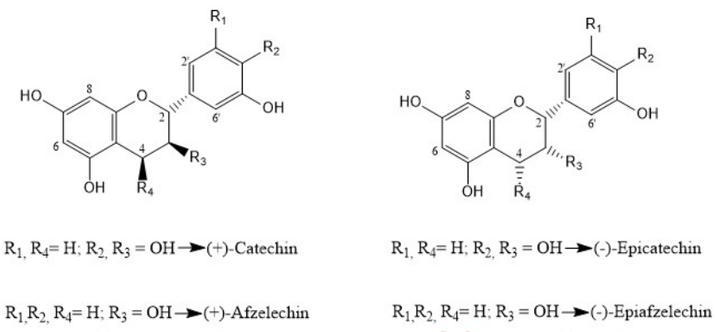
METHODS

- The phenolic rich extracts of *Lotus uliginosus* cv INIA E-Tanin (E-Tanin) or *Maytenus ilicifolia* (MI) were investigated by liquid chromatography coupled with mass spectrometry.
- The antiparasitic activity of E-Tanin extract was investigated using the Egg Hatch Test (EHT) and Larval Migration Inhibition Assay (LMIA) using gastrointestinal nematode (GIN) eggs from fresh feces and infective larvae (L3) from sheep.
- The antiparasitic activity of MI extract was investigated using the EHT using GIN eggs from fresh feces from cattle.
- Logistic models were constructed, and effective concentrations (EC50 and EC95) were estimated using the GraphPad Prism 7.1 software.

RESULTS

STRUCTURE OF CONDENSED TANNINS

- According to the chromatographic and spectral data (MS^1 and MS^2), free flavan-3-ols and flavonols, flavonol-O-glycosides, and condensed tannins (CT) were identified as the main compounds in the analyzed extracts.
- Flavonoid glycosides with quercetin and kaempferol as aglycones were identified in all extracts.
- CT were found formed by (epi) catechin (procyanidins) and (epi) gallocatechin. (prodelphinidins) units in *Lotus uliginosus* extract.



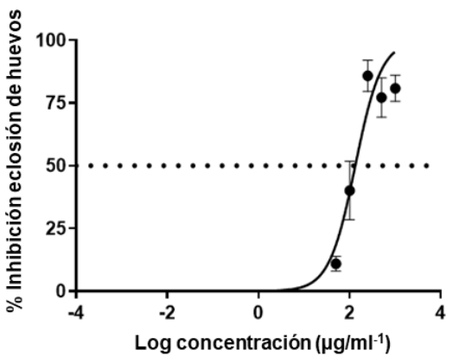
General structure of flava-3-oles

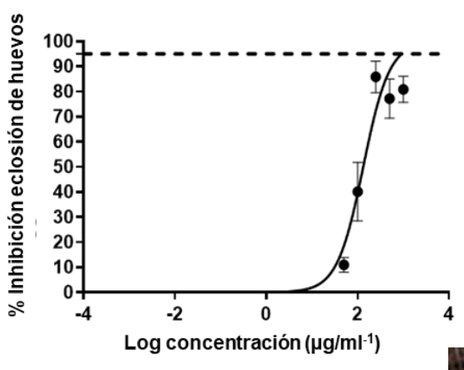
- In both extracts of *Maytenus ilicifolia* were identified CT formed by (epi) catechin, (epi) gallocatechin, and (epi) afzelechin units.
- The phenolic fingerprints of both extracts of *Maytenus ilicifolia* by LC-MS were similar, except for the presence of (epi) gallocatechin and the tannins containing it, which were found in branches but not in leaves, according to the analytic methods used..

ANTIPARASIT ACTIVITY

***Lotus uliginosus* INIA E-Tanin**

The EC_{50} and EC_{95} from E-Tanin rich extract were 133µg/ml (95% CI 109-162) and 924µg/ml (95% CI 527-1621) (R^2=0,82) respectively.





Maytenus ilicifolia

Total phenolics (TP), total tannins (TT), and condensed tannins (CT) content from Lotus uliginosus INIA E-tannin (E-Tanin) and leaves and branches of *Maytenus ilicifolia* and their phenolic-rich extracts.

Sample	TP (g TAE/100g)	TT (g TAE/100g)	CT (gLEU/100g)
E-Tanin rich extract	24.9	14.6	12.4
MI extract leaves rich extract	42.2	39.7	25.2
MI extract branches rich extract	29.0	20.3	19.1

g TAE/100 g: g tannic acid equivalent/100 g dry plant material or phenolic-rich extract.
g LEU/100 g: g leucocyanidin equivalent/100 g dry plant material or phenolic-rich extract

LMIA results for the E-Tanin rich extract lethal concentration (LC) 50 and 95 and their range

LC	mg/ml	Range		R^2
50	7.7	5.1	12.1	0.63
95	1267	163	9838	0.63

Cattle GIN egg hatching inhibition (mean percentage ± SE) caused by *Maytenus ilicifolia* branches and leaves extracts, without and with addition of polyvinylpyrrolidone (PVPP) and the lethal concentrations ($EC_{50,}$ EC_{95} mg/ml) for each of the extracts .

Concentration (mg/ml)	Branches	Branches+PVPP	Leaves	Leaves+PVPP
7.8	100.0 ±0^{az}	98.4±0.6^{az}	83.9±1.3^{az}	60.1±6.6^{by}
3.9	99.8±0.15^{az}	100.0±0^{az}	87.2±0.5^{bz}	62.2±2.7^{cy}
1.95	100.0±0^{az}	99.7±0.2^{az}	34.8±2.8^{by}	8.2±0.8^{cx}
0.97	100.0±0^{az}	99.2±0.3^{a}	37.7±6.1^{by}	12.1±1.7^{cx}
0.49	98.1±0.3^{az}	97.1±0.5^{az}	29.1±2.2^{byx}	16.3±0.6^{cx}
0.25	97.5±1.3^{az}	91.5±0.3^{az}	23.5±1.7^{byx}	17.4±1.0^{bx}
0.12	77.8±0.9^{ay}	64.8±10^{ay}	11.8±0.9^{bx}	20.7±1.2^{bx}
0.058	44.1±1.4^{ax}	25.4±3.1^{bx}	11.1±1.6^{cx}	18.0±1.5^{bcx}
Negative control	8.575±1.2^{w}	8.573±1.2^{x}	8.753±1.2^{x}	8.573±1.2^{x}
Positive control	100.0±0^{z}	100.0±0^{z}	100.0±0^{z}	100.0±0^{z}
EC_{50}	0.065	0.092	0.89	3.4
EC_{95}	0.25	0.33	30.18	2563

MAIN CONCLUSIONS

- ✓ . The E-Tanin rich extract showed Good antiparasitic activity, affecting mainly eggs development with scarse effect on the L3 stages.
- ✓ The MI branches rich extract had high effect inhibiting egg hatching at low. We did not have a significant effect with or without PVPP. This could be due to the high CT content from total phenol compounds.
- ✓ Additional studies are needed to determine which compounds contained in the extracts are the most bioactive or if there are synergistic or antagonistic effects between them.