In vitro antiprotozoal action of 7 spices, medicinal plants and essential oils against 3 stages of Eimeria tenella

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Growing societal concerns to replace coccidiostats in poultry farms have led to the use of alternatives such as plant bio-actives. To investigate the mode of action of natural molecules, we designed this *in vitro* study to screen anti-protozoal activity of 4 spices or medicinal plants described by non-volatile active molecules (MedP) and 3 essential oils (EO) against 3 different stages of *E. tenella*.

MATERIAL AND METHODS

- **Natural products tested**:
  - **MedP**: Fabacea powder, Urticaceae extract, Punicaceae extract, Acanthaceae extract
  - **EO**: Lilaceae EO, Lamiaceae EO, Lauraceae EO
- **Negative control**: H_20, soybean hemicellulose, DMSO
- **Protozoal strain**: *Eimeria tenella* recombined strain
- 3 experimental steps after cytotoxicity assay (colorimetric MTS test, $1,5.10^4$ MDBK seed, incubation 5h at 37°C):
  1. Direct anti-protozoite activity test: incubation 41°C for 1h. Killed sporozoites were stained with Evans Blue Dye. Each sample tested in triplicate.
     Viability rate with fluorescence microscopy as % viability = number of live parasite in tested sample/ number of live parasite in negative control*100
  2. Invasion inhibition test: 96 microplates: 1.5×10^4 MDBK cells, 1.5×10^4 *E. tenella* sporozoites expressed enzymatic activity; substrate added for 2h at 37°C, 4 replicates per tested sample.
     % invasion = absorbance (550 nm) tested sample/ absorbance (550 nm) negative control*100
  3. Sporulation rate test: 5.10^5 non-sporulated oocysts (in 2% potassium dichromate solution), incubation for 72h at 26°C; each sample tested in triplicate.
     % sporulation = number of sporulated oocysts in tested sample/number of sporulated oocyst in negative control*100
- **Statistical test**: Mann-whitney test compared to negative control for each test ($p<0.05$).

RESULTS AND DISCUSSION

The 4 MedP samples (Fabacea powder, Urticaceae extract, Punicaceae extract, Acanthaceae extract) were less cytotoxic (lethal dose 10% (LD10) than the 3 EO samples (Lilaceae EO, Lamiaceae EO, Lauraceae EO) (MedP diluted at 10^{-2}, EO diluted at 10^{-4}-10^{-6}).

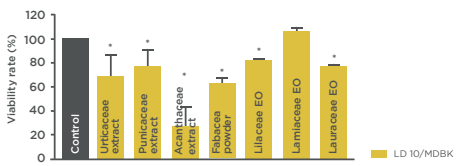

Fig.1 DIRECT ANTI-PROTOZOAL ACTIVITY AGAINST *E. TENELLA* SPOROZOITES (*: p<0.05 ; **:p<0.01; ***: p<0.001)

MedP samples showed direct anti-protozoal activity against *E. tenella* stages: sporozoite viability rates were between 76.2% and 27% (p=0.05). Lilaceae EO and Lauraceae EO exhibited 81% and 77% (p=0.05) sporozoite viability rates, respectively (Fig. 1).

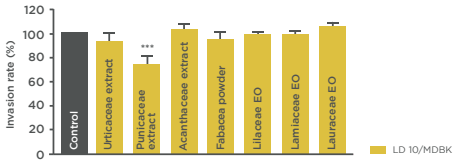

Fig.2 MDBK CELL INVASION BY *E. TENELLA* (*: p<0.05; **: p<0.01; ***: p<0.001)

Punicaceae extract showed a reduction of 32% of cell invasion.

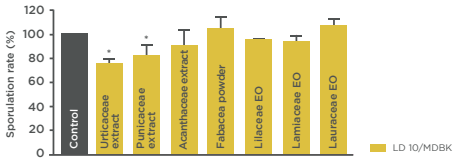

Fig.3 SPORULATION INHIBITION OF *E. TENELLA* (*: p<0.05; **: p<0.01; ***: p<0.001)

Acanthaceae extract and Urticaceae extract showed a sporulation inhibition of *E. tenella* oocysts (p=0.05) (Fig. 3). These medicinal plant extracts exhibited anti-protozoal activity against the different *E. tenella* stages contrary to the tested EOs which target only sporozoites. These results were in accordance with studies reporting several modes of action of medicinal plant extracts, both direct anti-protozoal activity and protective cell activity **[1]**.

CONCLUSION

Medicinal plant extracts exhibited an anti-protozoal activity against the different *E. tenella* stages. EOs only target sporozoites in this study. These findings help to understand how plant bio-actives could act to reduce *E. tenella* pathogenicity. Further studies could be carried out to test *in vitro* and/or *in vivo* potential synergies between different natural compounds.

REFERENCE

[1] WUNDERLICH et al. (2014), « Towards Identifying Novel Anti-Eimeria Agents: Trace Elements, Vitamins, and Plant-Based Natural Products ». Parasitology Research 113, no 10: 3547-56

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