SATUREJA HORTENSIS L. AND *CALENDULA OFFICINALIS* L., TWO ROMANIAN PLANTS WITH *IN VIVO* ANTIPARASITIC POTENTIAL ON DIGESTIVE PARASITES OF PIGS

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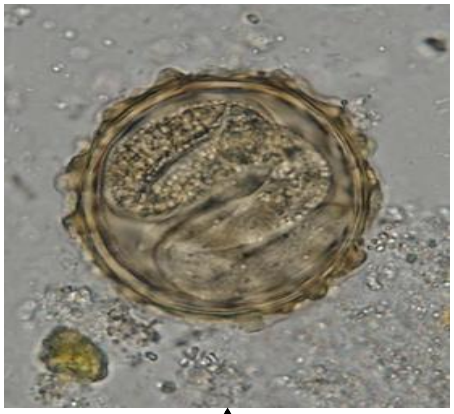
Introduction

- Parasitic diseases have a considerable effect on pig production, causing economic losses due to high morbidity and mortality.
- Due to continuously increasing drug resistence in parasites and prohibited use of antiparasitic medications in organic pig farming practices, phytotherapy could represent a valid, biologically available and cost effective alternative for parasite control.
- The use of phytotherapeutic remedies has notably increased over the past decade due to their biodegradability, decreased toxicity, environmentally friendliness, and to some extent their antiparasitic effect.

Fig. 1. Picture showing a free-range (low-input) farm.

Aims

- **The present study was designed to assess the antiparasitic potential of *Calendula officinalis, and Satureja hortensis* on naturally occurring gastrointestinal parasites of swine in two free-range (low-input) farms from Transylvania**.



Materials and methods

1. Biochemical analyses of medicinal plants

- High performance liquid chromatography coupled with mass spectrometry (HPLC/MS) was used for the analysis of biologically active compounds present in the plant extracts. All the procedures were performed at the Iuliu Haţieganu University of Medicine and Pharmacy, in Cluj-Napoca.

2. Experimental design and swine husbandry

- For each farm and plant:
  - 3 control groups
    - 10 weaners, 10 fatteners and 10 sows
  - 3 experimental groups
    - 10 weaners, 10 fatteners and 10 sows
    - received ***C. officinalis*** in a dosage of **140 mg/kg bw/day** and ***S. hortensis*** in a dosage of **100 mg/kg bw/day** for 10 consecutive days

3. Assessment of antiparasitic efficacy

Faecal egg count reduction test: **FECR (%) = 100 x (1–[T2/T1] x [C1/C2])**

- **T1** and **T2** are the mean pre- and post-treatment faecal egg counts (FEC) of a treated group
- **C1** and **C2** are the mean pre- and post-treatment FEC of control group

Materials and methods

- 720 faecal samples were collected from weaners, fatteners, and sows.
- Coproparasitological examination methods: flotation (Willis, McMaster), active sedimentation, modified Ziehl-Neelsen stained fecal smear, modified Blagg technique and oocysts/eggs cultures.

Fig. 2. Materials required for the coproparasitological methods.

Results

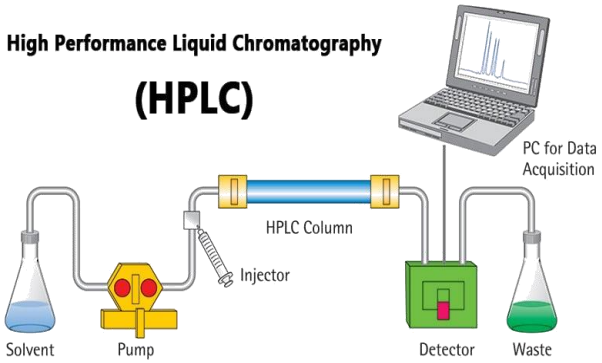


Table 1. The HPLC/MS analysis of chemical compounds in alcoholic plant extracts (10%)

Chemical class	**Chemical compound**	**Plant species and plant part used for extract preparation and the results of HPLC-MS analysis**	
		***Calendula officinalis* L.**	***Satureja hortensis* L.**
		aerial part	**aerial part**
Polyphenols (µg/mL)	Chlorogenic acid	220.767	<LOQ
	Caffeic acid	-	<LOQ
	p-coumaric acid	-	1.464
	Ferulic acid	-	0.557
	Isoquercitrin	38.877	6.515
	Rutoside	18.819	<LOQ
	Quercitrin	<LOQ	0.365
	Quercetol	-	0.394
	Luteolin	-	6.621
	Apigenin	-	2.442
	Syringic acid	1.51	2.28
	Protocatechuic acid	0.67	0.95
	Vanillic acid	0.44	0.65

Results

Chemical class	Chemical compound	Plant species and plant part used for extract preparation and the results of HPLC-MS analysis	
		Calendula officinalis L.	*Satureja hortensis* L.
		aerial part	aerial part
Tocopherols (ng/mL)	α-tocopherol	61.6	86.8
	γ-tocopherol	248.9	89.0
	Δ-tocopherol	9.3	13.2
Sterols (µg/mL)	Ergosterol	0.500	1.420
	Stigmasterol	72.888	14.215
	B-sitosterol	241.997	313.315
	Campesterol	1.635	6.140
Methoxylated flavones (ng/mL)	Jaceosidin	-	8820.76
	Hispidulin	-	2483.00
	Acacetin	-	12691.97

HPLC/MS—high performance liquid chromatography coupled with mass spectrometry; "-"—Not found; <LOQ—identified based on MS spectra but not determined quantitatively, below limit of quantification.

Results

The examination revealed parasitic infections with *Balantioides coli*, *Eimeria* spp., *Cryptosporidium* spp., *Ascaris suum*, *Trichuris suis*, and *Oesophagostomum* spp.

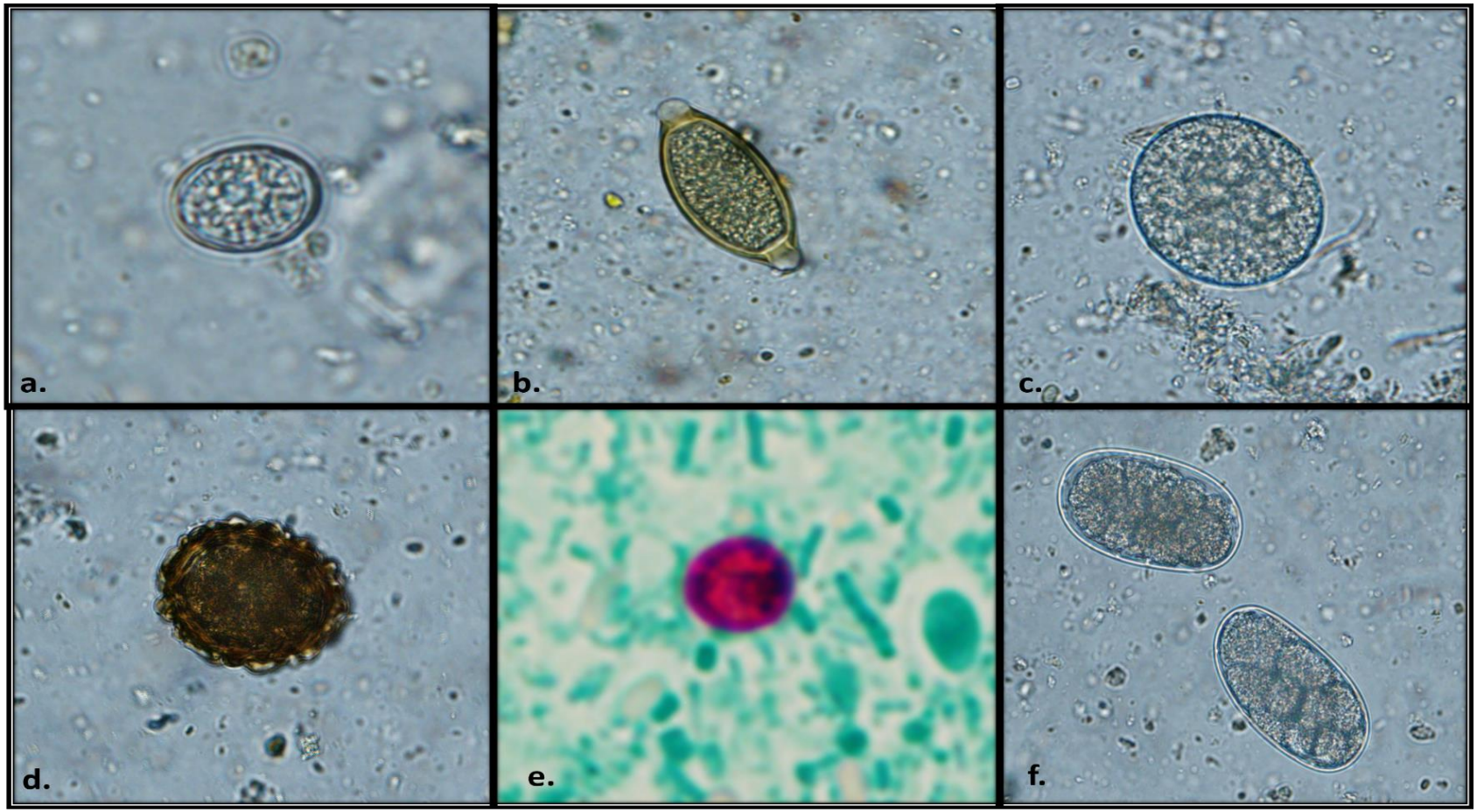


Fig. 3. Coproparasitological examination results: **a**- *Eimeria spp.* oocyst, **b**- *T. suis egg*, **c**- *B. coli* cyst, **d**- *A. suum* egg, **e**- *Cryptosporidium* spp. oocyst and **f**- *Oesophagostomum spp.* egg.

Results

Table. 2 Percentage of faecal egg/oocyst/cyst count reduction (%) recorded on days 14, and 28 post-treatment in F1 and F2 farms (using FECR formula)

Parasite	*C. officinalis* (14)						*C. officinalis* (28)					
	Weaners		Fatteners		Sows		Weaners		Fatteners		Sows	
	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
A. suum	-	-	**15.2**	10.3	-	49.9	-	-	54.2	34.9	-	**79.9**
T. suis	-	-	-	**8.2**	-	-	-	-	-	**20.3**	-	-
Oesophagostomum spp.	-	**60.5**	-	-	-	**28.6**	-	32.9	-	-	-	45.8
Eimeria spp.	91.8	42.5	**95.5**	75.9	-	74.9	72.5	57.1	88.9	**30.0**	-	76.5
B. coli	72.0	**90.9**	73.1	**53.6**	84.9	69.8	74.7	69.2	58.3	61.1	76.1	58.2
Parasite	***S. hortensis* (14)**						***S. hortensis* (28)**					
	Weaners		Fatteners		Sows		Weaners		Fatteners		Sows	
	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
A. suum	-	-	70.8	77.1	**91.1**	88.7	-	-	77.1	81.2	72.1	**59.7**
T. suis	-	-	**80.5**	84.0	-	-	-	-	**90.3**	87.1	-	-
Oesophagostomum spp.	-	-	-	-	80.2	**69.2**	-	-	-	-	**100**	83.7
Eimeria spp.	78.2	68.7	76.3	89.7	**25.1**	70.3	66.8	80.3	46.8	83.8	80.9	**94.1**
B. coli	80.1	88.4	**63.5**	74.7	70.2	70.5	83.6	**86.5**	72.2	71.2	70.7	74.6

"-"= was not diagnosed;

Conclusions

- The present experiment was conducted between April and June 2022, on two low-input (free-range) farms, located in the Transylvania area, involving pigs of the Bazna and Mangalitza breeds.
- Both plant powders at the previously mentioned doses for 10 consecutive days, showed promising *in vivo* antiparasitic activity.
- *C. officinalis* had a strong antiprotozoal activity and mildly antihelmintic effects while *S. hortensis* was very effective against both helminths and protozoa infections.
- The antiparasitic efficacy can be attributed to the presence of polyphenols, sterols, tocopherols and flavonoids.
- The current study is the first report about the antiparasitic effects of *C. officinalis* and *S. hortensis* against digestive parasites of pigs, from Romania.

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Thank you for your attention!