



Improving the robustness of laying hens and piglets against parasitic and bacterial infections

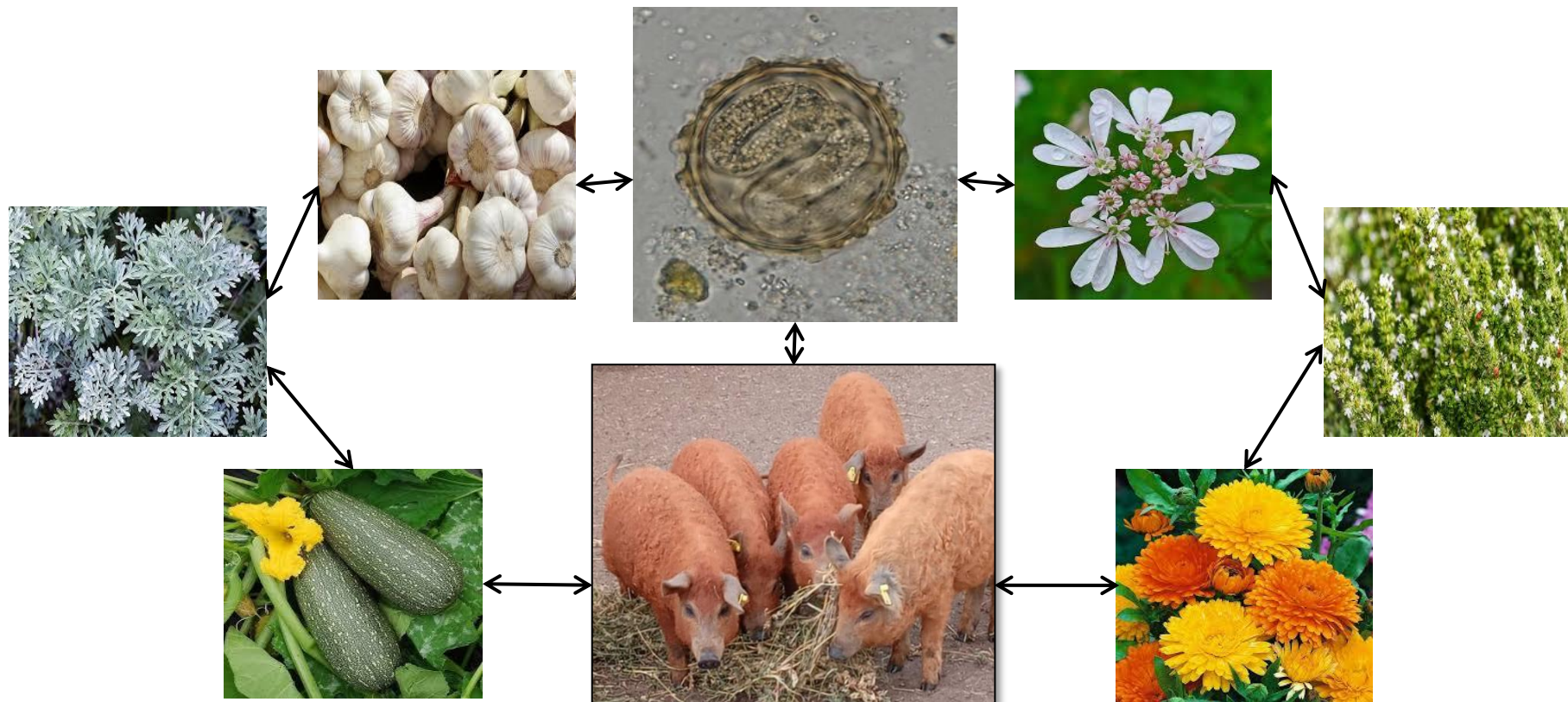
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Introduction

- Parasitic diseases have a considerable effect on pig production, causing economic losses due to high morbidity and mortality.
- Due to continuously increasing drug resistance 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.



- The primary objective of this research was to identify a plant-based formula that exhibits effectiveness in combating pig parasitoses without interfering with their welfare and health.
- The present studies were designed to assess, *in vitro* and *in vivo*, the antiparasitic potential of *Allium sativum*, *Artemisia absinthium*, *Cucurbita pepo*, *Coriandrum sativum*, *Calendula officinalis*, and *Satureja hortensis* on naturally occurring gastrointestinal parasites of swine in two free-range (low-input) farms from Transylvania, and also to evaluate the parasitic prevalence in the same farms.





Prevalence of Swine Gastrointestinal Parasites in Two Free-Range Farms from Nord-West Region of Romania.

The effects of *Allium sativum* L., *Artemisia absinthium* L., *Cucurbita pepo* L., *Coriandrum sativum* L., *Satureja hortensis* L. and *Calendula officinalis* L. on the embryogenesis of *Ascaris suum* eggs during an in vitro experimental study

The *in vitro* anticoccidial activity of Some Herbal Extracts against *Eimeria* spp. oocysts isolated from piglets.

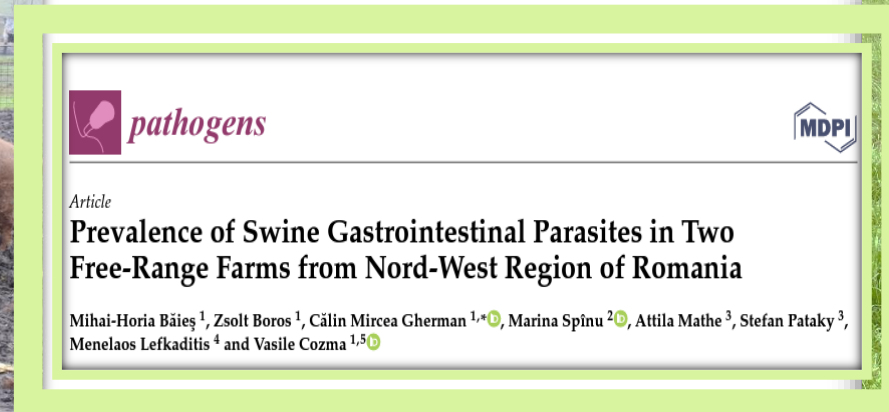
***In vivo* assessment of the antiparasitic effects of *Allium sativum* and *Artemisia absinthium* against gastrointestinal parasites in swine, from low-input farms, in NW of Romania.**

The effects of *Coriandrum sativum* L. and *Cucurbita pepo* L. against gastrointestinal parasites in swine: An *in vivo* study.

***Satureja hortensis* L. and *Calendula officinalis* L., two Romanian plants with *in vivo* antiparasitic potential on digestive parasites of pigs.**

Starting point: Prevalence of Swine Gastrointestinal Parasites in Two Free-Range Farms from Nord-West Region of Romania

- Parasitic diseases cause significant economic losses in swine industry.
- The number of free-range swine farms in Romania has increased in the last decades.
- This study provided essential information on Transylvania's distribution of gastrointestinal parasites in pigs.
- Different species of gastrointestinal parasites are present in most pigs reared in free-range farms in the study area (*Eimeria spp.*, *Cryptosporidium spp.*, *Oesophagostomum spp.*, *Trichuris suis*, *Ascaris suum*, *Strongyloides ransomi* and *Balantidium coli*).
- Information of great value to farmers, policymakers, and researchers alike, leading to safer and healthier pork production for public consumption.
- Control strategies are needed to raise awareness among pig farmers about the impact of these parasites on the productivity and health of pigs as well as on human health.

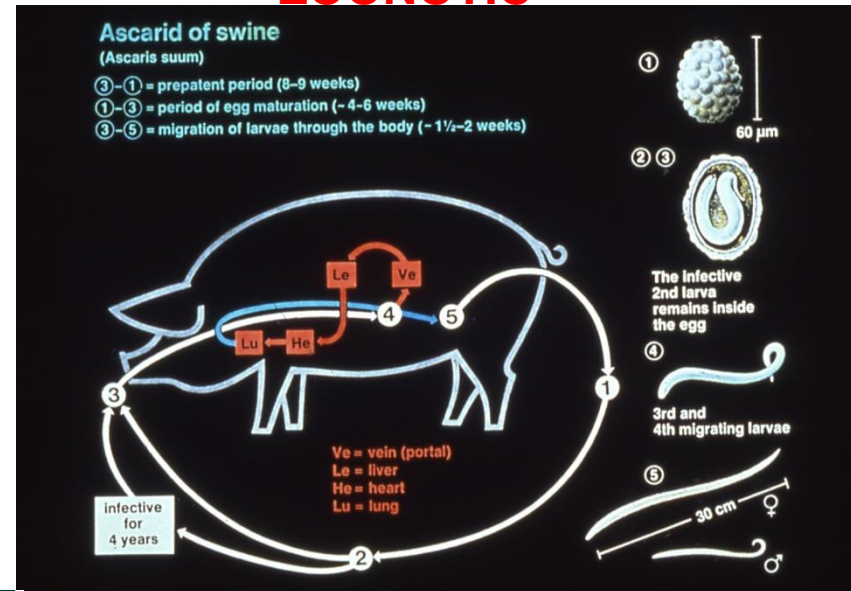


2. The effects of *Allium sativum* L., *Artemisia absinthium* L., *Cucurbita pepo* L., *Coriandrum sativum* L., *Satureja hortensis* L. and *Calendula officinalis* L. on the embryogenesis of *Ascaris suum* eggs during an in vitro experimental study.

Background & Aim

- ❖ *Ascaris suum* is present in traditionally managed herds and on industrialized farms, especially in old fatteners and sows.
- ❖ Increasing resistance against antihelmintics redirected the research towards alternative, traditional therapies, medicinal plants included.
- ❖ This study comparatively evaluated the *in vitro* effects of *Allium sativum*, *Artemisia absinthium* L., *Cucurbita pepo*, *Coriandrum sativum*, *Satureja hortensis* L. and *Calendula officinalis* on inhibition of *A. suum* egg hatching and larval development.

ZOONOTIC



Piglets: - catarrhal enteritis, obstruction of the small intestine, volvulus (a bowel obstruction in which a loop of bowel has abnormally twisted on itself) with gangrene of intestinal wall;

- the cadavers are weak, showing anemia, rickets, hyperkeratosis, jaundice, liver degeneration and cirrhosis;
- larval stages induce - pulmonary oedema, bronchial pneumonia, hemorrhage, liver necrosis.

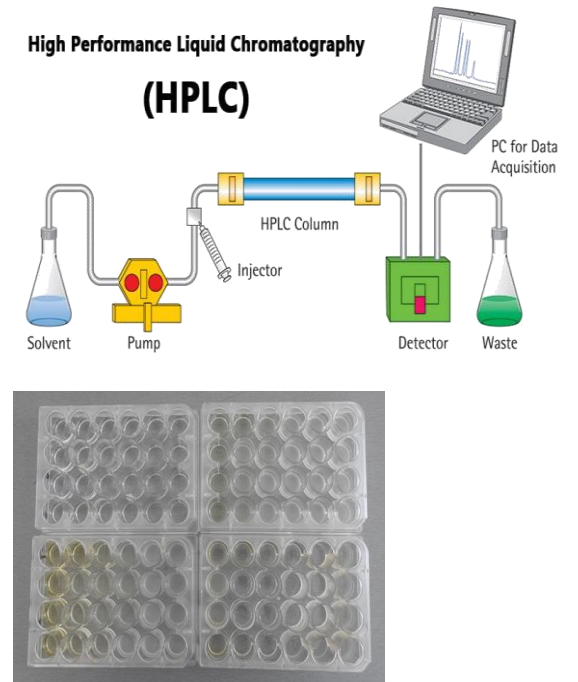
Materials and methods

1. Chemical 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 Hațieganu University of Medicine and Pharmacy, in Cluj-Napoca.

2. Experimental design

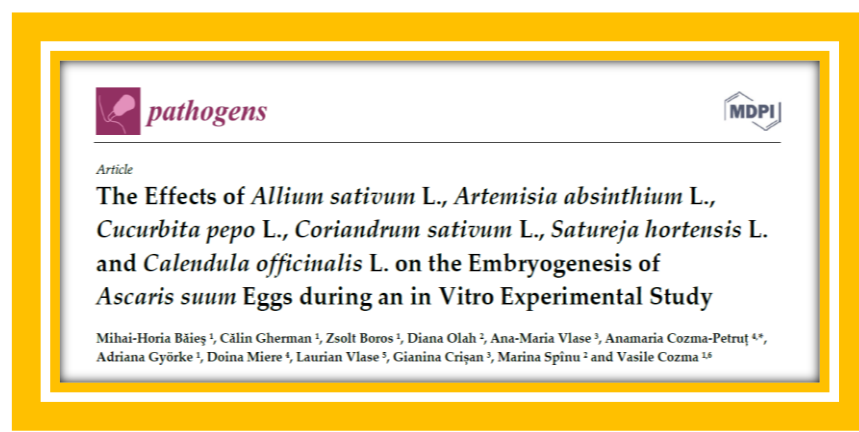
- ❖ *A. suum* eggs were collected from randomly sampled of traditionally maintained swine faeces. In 3 ml cell culture plates, the **egg suspension** (ES, 8×10^3 /ml) was divided in two control (C) (1C - 1ml ES + 1 ml distilled water, 2C- five plates of 1ml ES + 1ml ethanol of 70%, 35%, 17.5%, 8.75%, and 4.375%, respectively) and six experimental groups.
- ❖ The experimental (E, 1-6) groups included ES + **each alcoholic plant extract (10%, 5%, 2.5%, 1.25%, 0.625%)**. Both C and E were performed in quintuplicate.
- ❖ All groups were incubated at 27 °C for a total of 21 days, *A. suum* eggs being examined after 2, 14 (L1) and 21 (L2/L3) days.



Results

1. Analysis of plant extracts

A. absinthium had the highest polyphenol content **56.754 µg/mL**



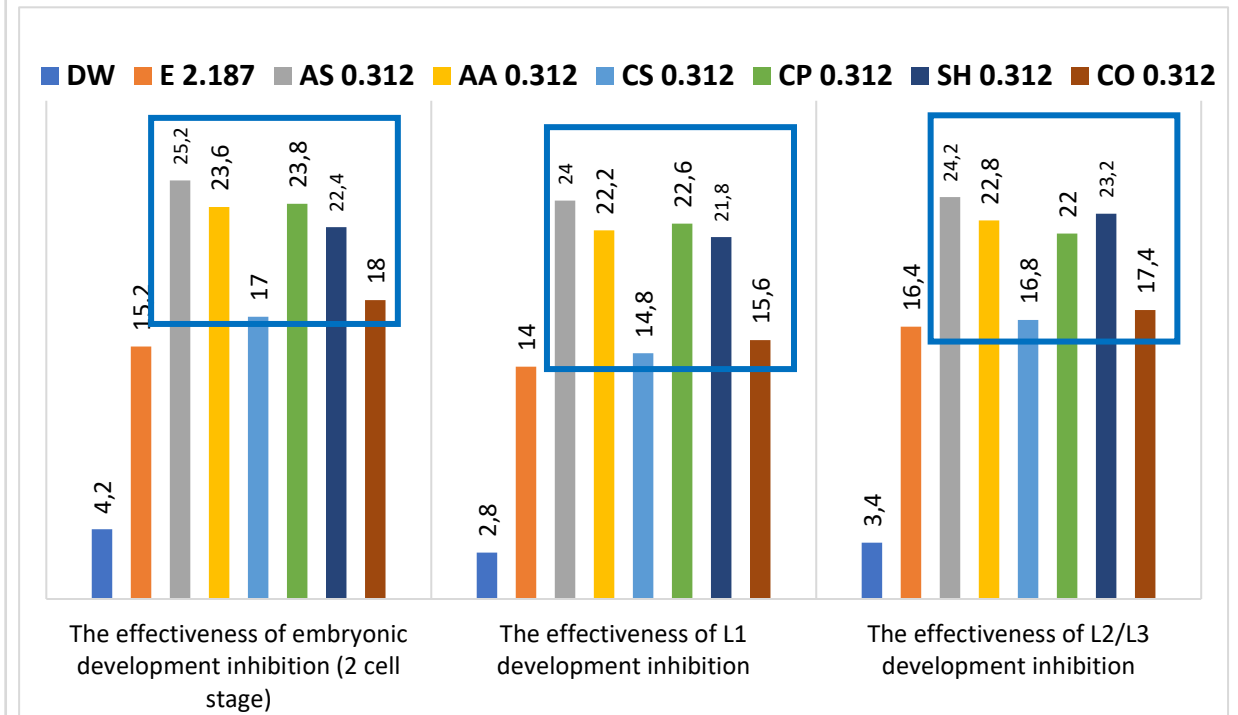
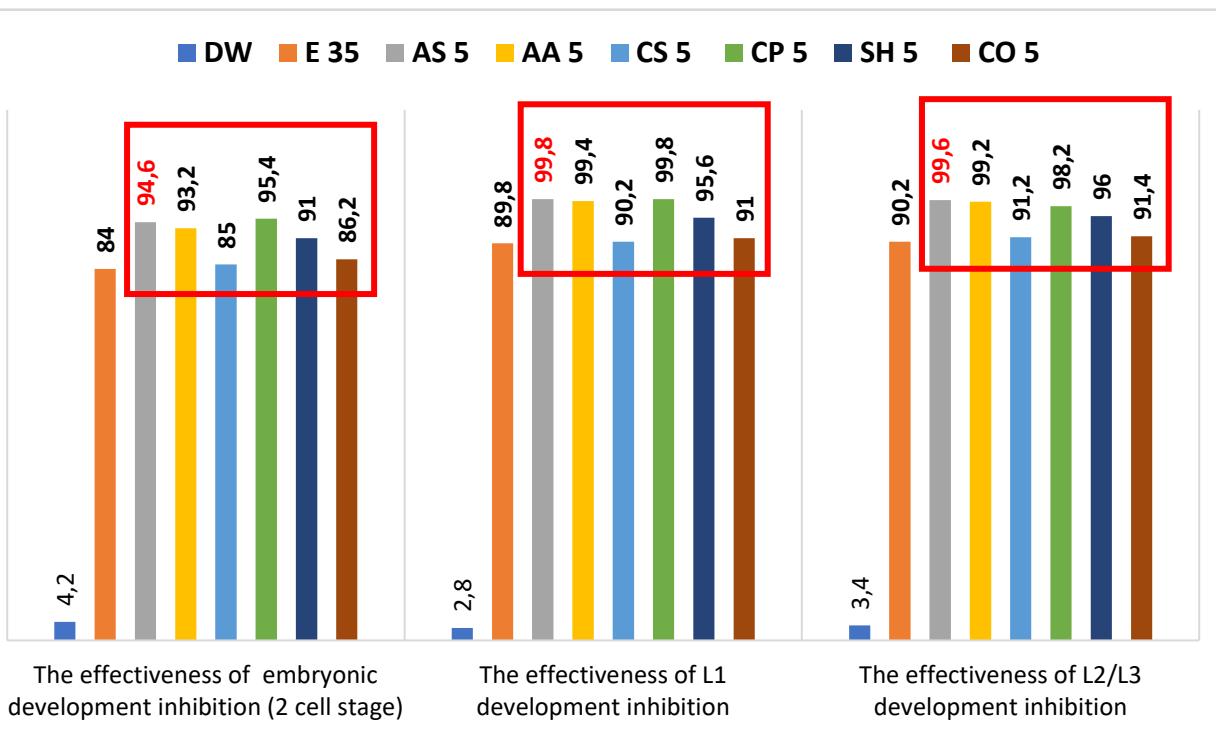
Bioactive Compounds		Vegetal Species and Plant Part Used for Extraction and HPLC-MS Analysis					
		<i>A. absinthium</i>	<i>S. hortensis</i>	<i>C. officinalis</i>	<i>A. sativum</i>	<i>C. sativum</i>	<i>C. pepo</i>
		herba	herba	herba	bulbus	fructus	semen
Tocopherols (ng/mL)	α-tocopherol	50.0	86.8	61.6	36.1	-	-
	γ-tocopherol	23.8	89.0	248.9	-	-	446.0
	Δ-tocopherol	5.0	13.2	9.3	-	-	23.2
Sterols (µg/mL)	Ergosterol	0.344	1.420	0.500	-	0.584	-
	Stigmasterol	34.831	14.215	72.888	-	9.675	22.024
	B-sitosterol	140.985	313.315	241.997	-	31.548	5.355
	Campesterol	3.329	6.140	1.635	-	1.780	0.358
Methoxylated flavones (ng/mL)	Jaceosidin	-	8820.76	-	-	-	-
	Hispidulin	3047.92	2483.00	-	-	-	-
	Eupatorin	976.53	-	-	-	-	-
	Casticin	15,384.14	-	-	-	-	-
	Acacetin	-	12691.97	-	-	-	-
Sesquiterpene lactones (ng/mL)	α-santonin	450.52	-	-	-	-	-
	Vulgarin	6499.39	-	-	-	-	-
Sulfoxide (µg/mL)	Aliin	-	-	-	14.726	-	-

HPLC/MS—high performance liquid chromatography coupled with mass spectrometry

Results

2. Analysis of Plant Extracts Activity

The efficacy of all tested plants, when compared to the control groups increased with concentration. Anti-embryogenic effects on the *A. suum* eggs were expressed by all plants, with more pronounced influence of the *A. sativum*, *A. absinthium*, *C. pepo* and *S. hortensis* extracts at all tested concentrations.



Percentage of embryogenesis inhibition at 5 % and 0.312 % concentrations: Distilled water (DW), Ethanol (E), *A. sativum* L. (AS), *A. absinthium* L. (AA), *C. sativum* L. (CS), *C. pepo* L. (CP), *S. hortensis* L. (SH), *C. officinalis* L. (CO).

3. The *in vitro* anticoccidial activity of some herbal extracts against *Eimeria* spp. oocysts isolated from piglets.

The aim of this study was to evaluate the effects of the alcoholic extracts from *Allium sativum* L. (garlic), *Artemisia absinthium* L. (wormwood), *Coriandrum sativum* L. (coriander), *Cucurbita pepo* L. (pumpkin), *Satureja hortensis* L. (summer savory), and *Calendula officinalis* L. (marigold) on the sporulation of *Eimeria suis* and *Eimeria deblickei* oocysts, isolated from piglets.

- Statistical analysis showed that all plant extracts were effective in inhibiting the sporulation of both *E. suis* and *E. deblickei* oocysts as well as destroying them
- The alcoholic extracts of *C. officinalis*, *A. absinthium*, and *C. sativum* were the most potent and obtained the lowest LC50 values.
- As our *in vitro* results demonstrated that the APEs at higher concentrations had a dual effect, both inhibitory and destructive, their use as disinfectants in livestock shelters seems encouraging.



Photos: Prof. A. Joachim ,Vienne



Article

The In Vitro Anticoccidial Activity of Some Herbal Extracts against *Eimeria* spp. Oocysts Isolated from Piglets

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Băieș MH, Györke A, Cotuțiu VD, Boros Z, Cozma-Petruț A, Filip L, Vlase L, Vlase AM, Crișan G, Spînu M, Cozma V. *The In Vitro Anticoccidial Activity of Some Herbal Extracts against Eimeria spp. Oocysts Isolated from Piglets*. *Pathogens*. 2023 Feb 6;12(2):258. doi: 10.3390/pathogens12020258.



Prevalence of Swine Gastrointestinal Parasites in Two Free-Range Farms from Nord-West Region of Romania.

The effects of *Allium sativum* L., *Artemisia absinthium* L., *Cucurbita pepo* L., *Coriandrum sativum* L., *Satureja hortensis* L. and *Calendula officinalis* L. on the embryogenesis of *Ascaris suum* eggs during an in vitro experimental study

The *in vitro* anticoccidial activity of Some Herbal Extracts against *Eimeria* spp. oocysts isolated from piglets.

In vivo studies

In vivo assessment of the antiparasitic effects of *Allium sativum* and *Artemisia absinthium* against gastrointestinal parasites in swine, from low-input farms, in NW of Romania.

The effects of *Coriandrum sativum* L. and *Cucurbita pepo* L. against gastrointestinal parasites in swine: An *in vivo* study.

Satureja hortensis L. and *Calendula officinalis* L., two Romanian plants with *in vivo* antiparasitic potential on digestive parasites of pigs.

Background & Aim

- ✓ Internal parasitic diseases of swine constitute one of the most important health issues in low-input livestock farming, affecting the welfare, reproduction performance and productivity of the infected animals.
- ✓ Phytotherapeutic remedies can be used for prophylaxis and therapy of digestive parasitosis and are a viable and sustainable alternative to chemical antiparasitics, but few of them have been subjected to scientific validation.
- ✓ Low-input swine farming in Romania adopted the traditionally use of the phytotherapy for controlling the pathogens in livestock.
- ✓ **The current studies aimed at evaluating, the *in vivo* antiparasitic activity of *Allium sativum*, *Artemisia absinthium* L., *Cucurbita pepo*, *Coriandrum sativum*, *Satureja hortensis* L. and *Calendula officinalis* powders against digestive parasites in swine, in two low-input farms from Transylvania area.**



Materials and methods

- ✓ 2160 faecal samples were collected from weaners, fatteners, and sows.
- ✓ Different coproparasitological methods, including flotation (Willis, McMaster), centrifugal sedimentation, Ziehl-Neelsen staining as modified by Henricksen, a modified Blagg technique, and faecal cultures (nematode larvae/protozoan oocyst cultures) were involved during testing.

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 Hațieganu 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

a) received *A. sativum* in a dosage of 180 mg/kg BW/day and *A. absinthium* in a dosage of 90 mg/kg BW/day for 10 consecutive days

b) received *C. sativum* in a dosage of 170 mg/kg BW/day and *C. pepo* in a dosage of 500 mg/kg BW/day for 10 consecutive days

c) 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: $FECR (\%) = 100 \times (1 - [T2/T1] \times [C1/C2])$

- **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



A. absinthium



C. officinalis



C. pepo

In vivo assessment of the antiparasitic effects of *Allium sativum* and *Artemisia absinthium* against gastrointestinal parasites in swine, from low-input farms, in NW of Romania

Results

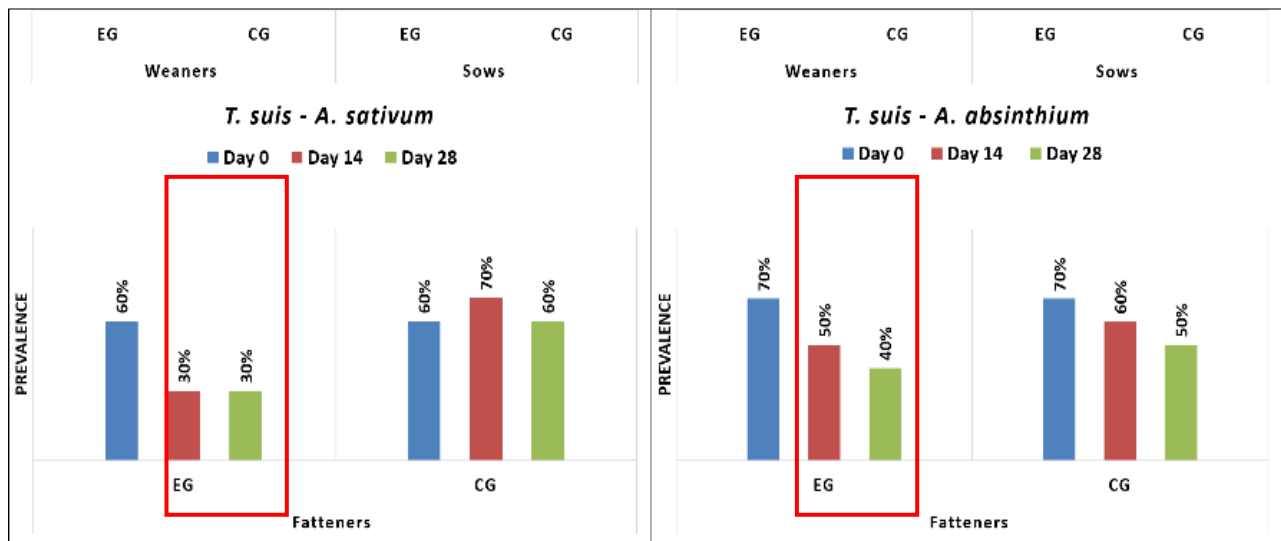
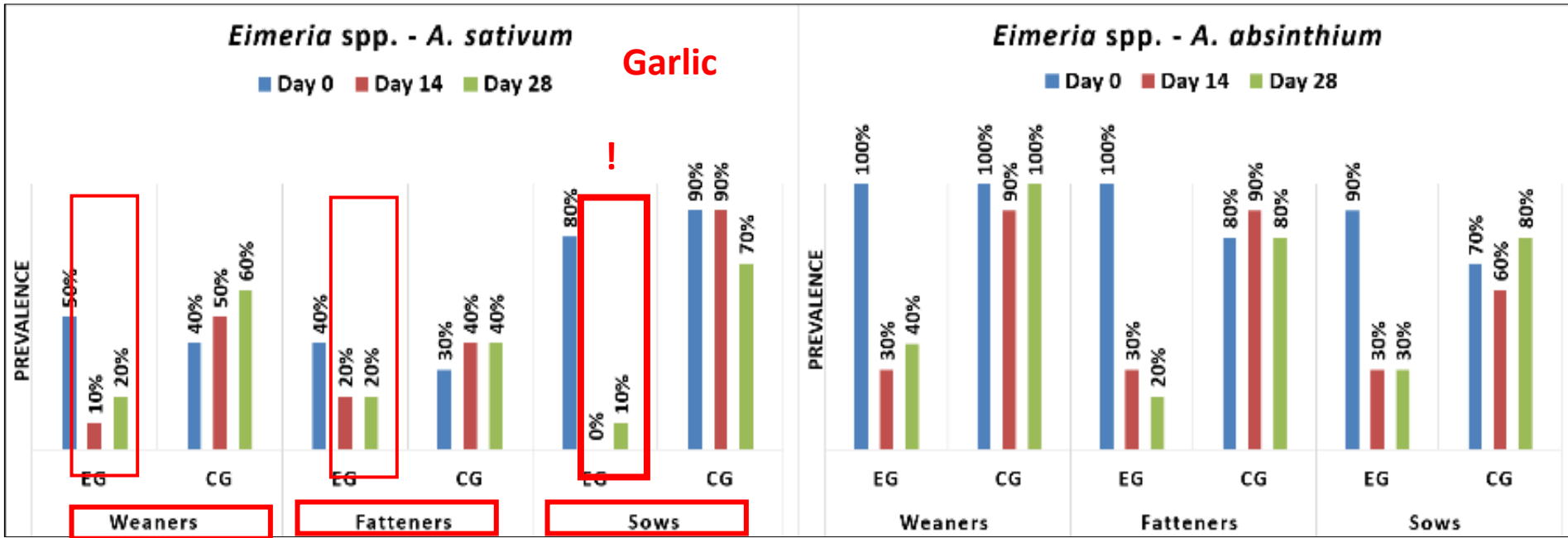
Bioactive compounds		Vegetal species and plant part used for extraction and HPLC-MS analysis	
		<i>Artemisia absinthium</i> L.	<i>Allium sativum</i> L.
		herba	bulbus
Polyphenols ($\mu\text{g/mL}$)	Chlorogenic acid	107.15	-
	Caffeic acid	-	1.221
	p-coumaric acid	0.621	-
	Ferulic acid	0.759	0.456
	Sinapic acid	-	0.228
	Vitexin	1.631	-
	Isoquercitrin	56.754	-
	Rutoside	3.826	-
	Quercitrin	1.113	-
	Quercetol	6.285	-
	Luteolin	1.159	-
	Kaempferol	3.666	-
	Apigenin	0.481	-
	Syringic acid	1.85	-
	Protocatechuic acid	1.32	-
Vanillic acid	1.98	-	

Bioactive compounds		Vegetal species and plant part used for extraction and HPLC-MS analysis	
		<i>Artemisia absinthium</i> L.	<i>Allium sativum</i> L.
		herba	bulbus
Tocopherols (ng/mL)	α -tocopherol	50.0	36.1
	γ -tocopherol	23.8	-
	Δ -tocopherol	5.0	-
Sterols ($\mu\text{g/mL}$)	Ergosterol	0.344	-
	Stigmasterol	34.831	-
	B-sitosterol	140.985	-
	Campesterol	3.329	-
Methoxylated flavones (ng/mL)	Jaceosidin	-	-
	Hispidulin	3047.92	-
	Eupatorin	976.53	-
	Casticin	15384.14	-
	Acacetin	-	-
Sesquiterpene lactones (ng/ml)	α -santonin	450.52	-
	Vulgarin	6499.39	-
Sulfoxide ($\mu\text{g/mL}$)	Aliin	-	14.726

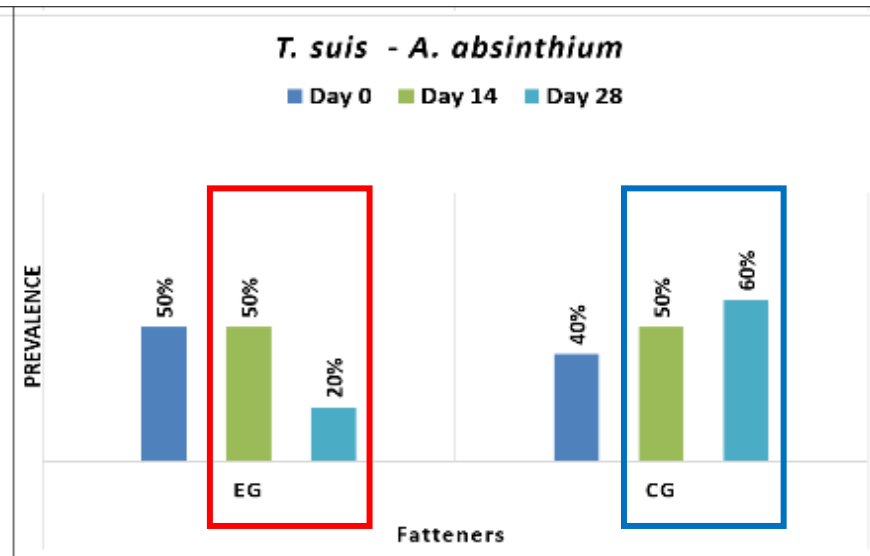
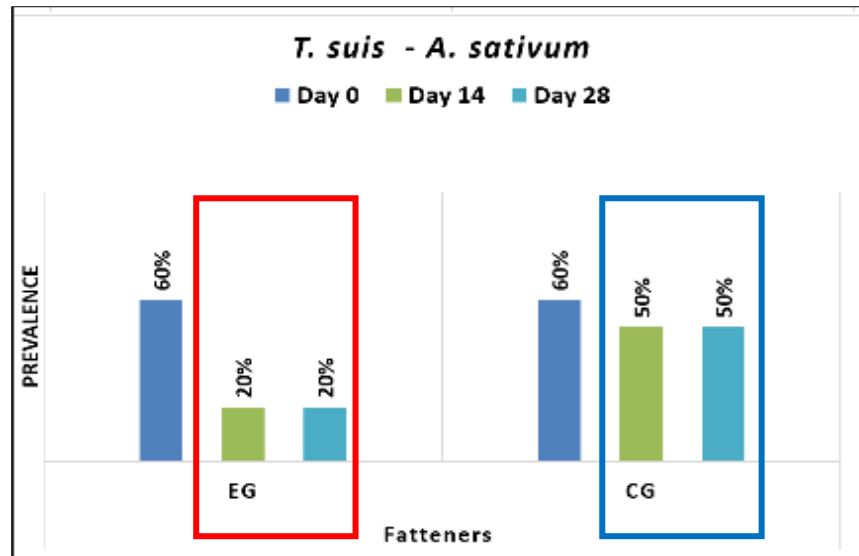
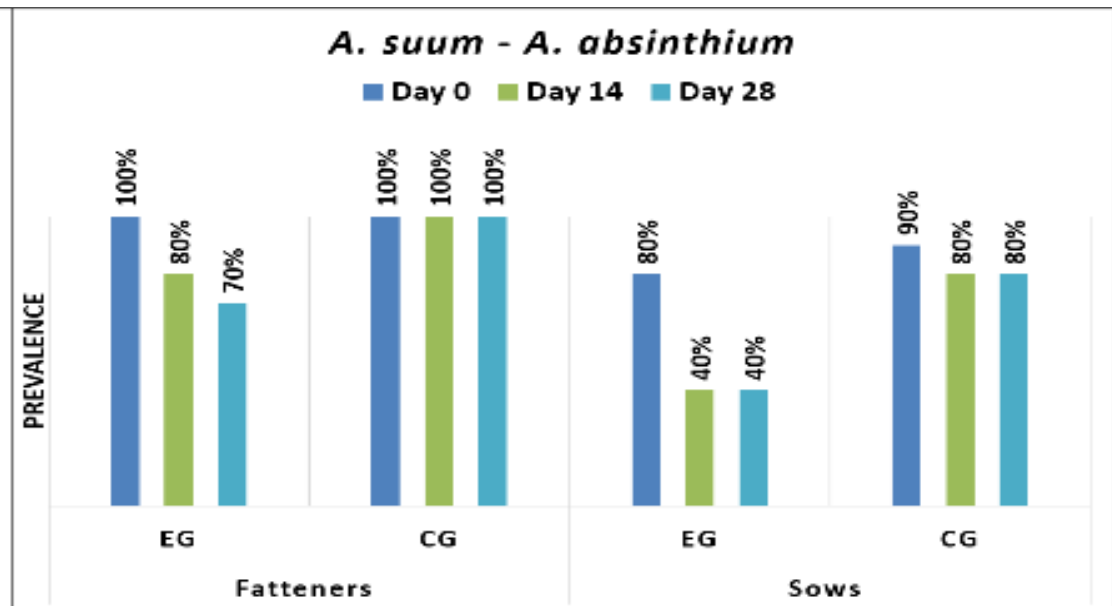
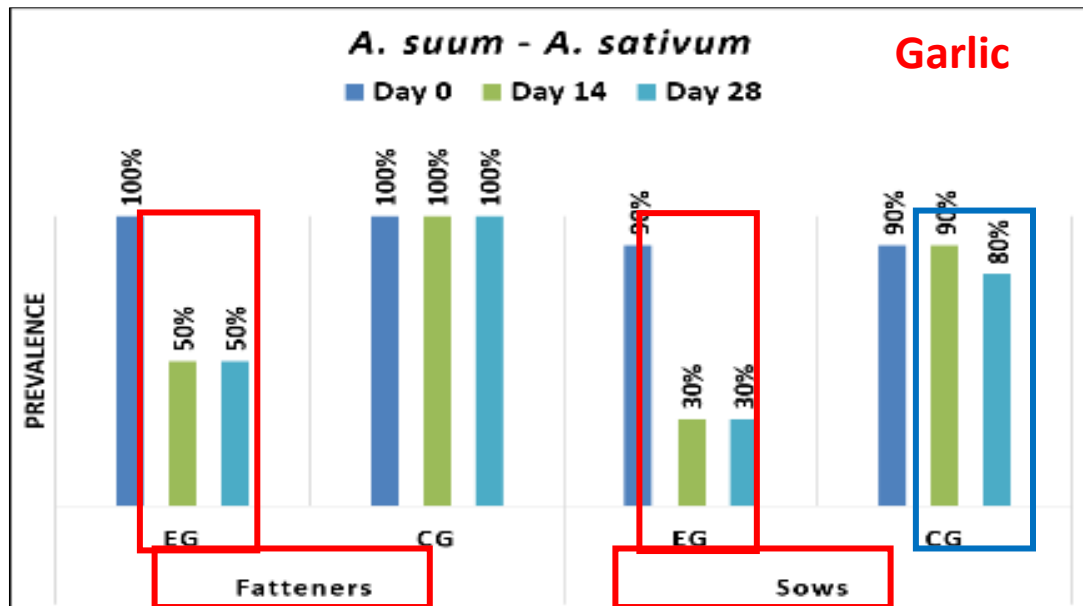


Results

The coproparasitological examination revealed co-infections of up to five species of gastrointestinal parasites, namely *Eimeria* spp., *Oesophagostomum* spp, *Trichuris suis*, *Ascaris suum*, *Cryptosporidium* spp., *S. ransomi* and *Balantioides coli*.



Prevalence (%) of investigated protozoa and nematodes on farm 1 by age group (EG = experimental group; CG = control group).



Prevalence (%) of investigated protozoa and nematodes on **farm 2** by age group (EG = experimental group; CG = control group).

Both plant powders at the previously mentioned doses for 10 consecutive days had a strong antiprotozoal and anthelmintic activity, with *A. sativum* being more effective, thus ***A. sativum* and *A. absinthium* have the potential of treating gastrointestinal parasitoses in swine.**

The effects of *Coriandrum sativum* L. and *Cucurbita pepo* L. against gastrointestinal parasites in swine: An *in vivo* study

Results

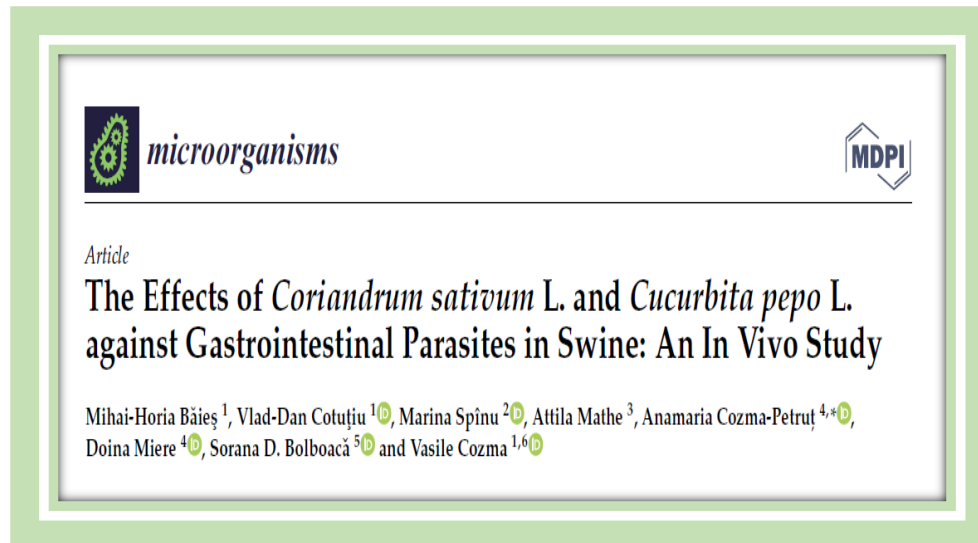


Coriandrum sativum L.



Cucurbita pepo L.

- **Both plant powders at the previously mentioned doses for 10 consecutive days, were efficient against gastrointestinal parasites in swine. coriander was more effective against protozoa while pumpkin showed better efficacy against helminths.**
- Considering all the constraints of Romanian livestock farming, these results are a beacon of hope for better management and welfare practices in the swine farming.
- In addition, to the best of our knowledge, this is the first ethnopharmacological report on the antiparasitic effects of *C. pepo* and *C. sativum* traditionally used in Romania for treating protozoa and nematode infections in swine.



Băieș MH, Cotuțiu VD, Spînu M, Mathe A, Cozma-Petruț A, Miere D, Bolboacă SD, Cozma V. The Effects of *Coriandrum sativum* L. and *Cucurbita pepo* L. against Gastrointestinal Parasites in Swine: An In Vivo Study. *Microorganisms*. 2023 May 6;11(5):1230. doi: 10.3390/microorganisms11051230.

III. *Satureja hortensis* L. and *Calendula officinalis* L., two Romanian plants with *in vivo* antiparasitic potential on digestive parasites of pigs

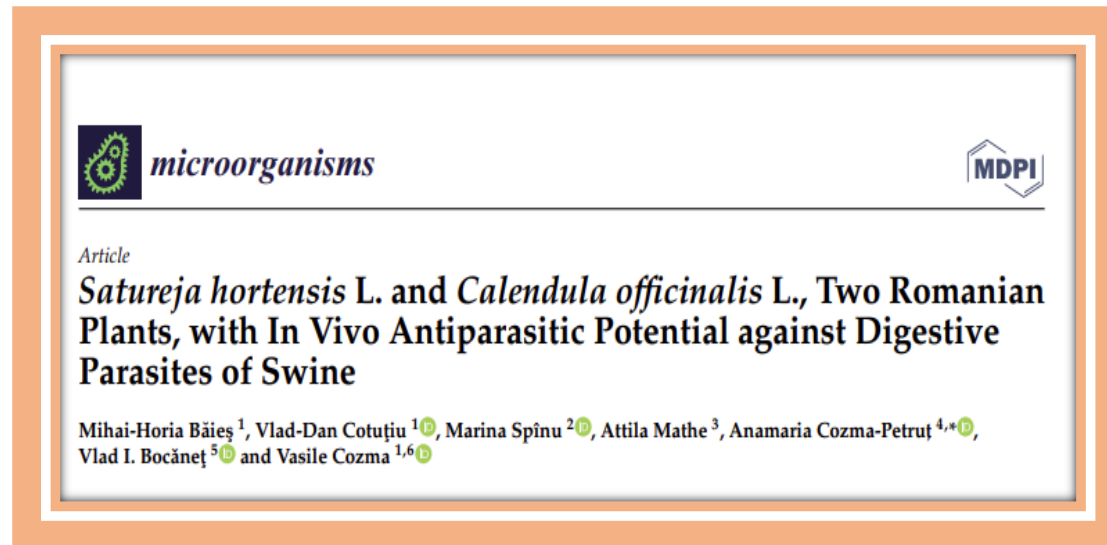


Calendula officinalis L.



Satureja hortensis L.

- 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.



Băieș MH, Cotuțiu VD, Spînu M, Mathe A, Cozma-Petruț A, Bocăneț VI, Cozma V. *Satureja hortensis* L. and *Calendula officinalis* L., Two Romanian Plants, with In Vivo Antiparasitic Potential against Digestive Parasites of Swine. *Microorganisms*. 2023 Dec 13;11(12):2980. doi: 10.3390/microorganisms11122980.



Thank you for your attention!