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## Deliverable No 4.2: Internal report on a comparative study where the anthelmintic activity of heather extracts collected across EU is tested in vitro

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## I. Executive summary

Gastrointestinal Nematode (GIN) parasitism is the main health challenge small ruminants face with detrimental consequences on their health, welfare, and economic returns. Gastrointestinal parasite control is usually achieved with the use of anthelmintic drugs. Use of anthelmintics is strictly regulated in the organic sector. However, due to the global spread of anthelmintic resistance and their impact on the environment, the organic sector is seeking for non-chemical complements or alternatives. Examples of alternatives used by farmers include grazing management strategies, selective breeding for resistance and protein supplementation for improved productivity and immunity. The primary focus of our group is to investigate the anthelmintic efficacy of bioactive plants as an alternative for the control of GIN. Bioactive plants contain plant secondary metabolites (PSM), which are compounds not essential for plant growth or reproduction, but are thought to play a role in plant defences. One group of PSM are the condensed tannins and they have been associated with significant anthelmintic activity. The perennial shrub heather is rich in condensed tannins and is abundant across northern Europe. The objective of our work was to quantify the anthelmintic efficacy of heather extracts. To do so, we have performed a comparative analysis of extracts obtained from two heather species – *Calluna vulgaris* and *Erica cinerea* – collected in five different European countries, across two seasons and tested against two GIN species.

*Calluna vulgaris* samples were collected in the UK (Scotland), Germany, Norway, Switzerland, and Spain, where a second heather species (*Erica cinerea*) was also collected. Extracts were tested against the egg hatching and the larvae motility of the GIN species *Teladorsagia circumcincta* (abomasal) and *Trichostrongylus colubriformis* (intestinal). Our results show that heather extracts from different countries and seasons had a significant impact on egg hatching and larvae motility. In addition, *T. circumcincta* eggs were more susceptible compared to *T. colubriformis*. Our results indicate that heather could be a contributor to parasite control management in small ruminants. The variation in efficacy may be explained by differences in environmental factors impacting heather growth, the presence and levels of condensed tannins, and their interactions with the parasite species. Demonstrating anthelmintic activity in living animals and identifying the sources of variation in anthelmintic efficacy is fundamental in optimising heather as a viable alternative in the control of GIN.



## 2. Introduction

The use of anthelmintics in organic farming is regulated. However, its limited use is permitted due to gastrointestinal nematodes threatening the health and welfare of animals worldwide. There is a requirement for a reduction of contentious inputs such as anthelmintics in organic farming. The project focuses on developing alternatives for sustainable parasite control to reduce anthelmintic use and consequently slow down the rise in resistance. One of such alternatives is the use of bioactive plants; this study focuses on the perennial shrub heather (*Ericaceae* family) as it is a plant rich in condensed tannins (CT) which have been shown to have anthelmintic properties. The plant is also highly abundant across Europe, so it could be a readily available control method with geographical location not limiting its use.

The objective to this study was to conduct a comparative analysis of heather acetic extracts from five European countries across two seasons against two parasite species, as well as comparing the extracts of two types of heather species from Spanish samples. To identify effects of heather on certain parasite mechanisms, we used two *in vitro* assays: Egg Hatch Assay (EHA) and larval motility test using Real Time Cell Essay (RTCA) to determine both ovicidal activity and motility effects of the heather extracts. To investigate the active compounds within the heather samples, we carried out further EHA tests using the condensed tannin inhibitor polyvinyl polypyrrolidone (PVPP) as well as chemical analysis of all heather samples to determine the polyphenols present, including condensed tannin type, size and ratio of subunits.

## 3. Materials and Methods

Heather samples from five European countries were collected and sent to SRUC for drying and extraction processes. The samples were mostly picked between January and March for the winter samples, and March to May for the spring samples. The same collection and processing protocol was followed by heather pickers in all countries.

All heather samples were analysed to determine their polyphenol content, including type and amount of CTs, and tested *in vitro* with the aim of quantifying their impact on egg hatching and larval motility. An egg hatch test and a larval motility test, as described by Athanasiadou et al, 2021 were performed to quantify heather extract efficacy against two ovine nematodes, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*.

## 4. Results and conclusions

Results showed the heather extracts reduced egg hatching of both nematode species in a dose dependent manner ( $p < 0.001$ ) and reduced larval motility at the highest concentration of  $200 \mu\text{g/ml}$  ( $p < 0.05$ ). The anthelmintic efficacy of the heather varied by season, country of origin and the species of heather tested on both egg hatching and larval motility. Variation was also seen on the susceptibility of the two parasite species egg hatching rate, with *T. circumcincta* eggs being more susceptible to heather extracts compared to *T. colubriformis* eggs. These variations in extract activity were not always consistent between the two *in vitro* tests, indicating that the active compounds may target different parasite life stages.

Incubations of extracts at the highest concentration with PVPP caused egg hatching percentage to become significantly different from those incubated without PVPP ( $p < 0.001$ ) and not differ from controls ( $p > 0.05$ ). This indicated that at least some part of the activity observed was attributed to condensed tannins, although not all of it. Polyphenol content was somewhat associated with activity, but the activity could not be attributed to a single polyphenol.



Our results show that heather extracts had a significant impact on egg hatching and larvae motility and the anthelmintic efficacy of the extracts varied as a function of season, country of origin and heather species. Our results indicate that heather could be a contributor to parasite control management in small ruminants. The variation in efficacy may be explained by differences in environmental factors impacting heather growth, the presence and levels of condensed tannins and other polyphenols, and their interactions with the parasite species. Demonstrating anthelmintic activity in living animals and identifying the sources of variation in anthelmintic efficacy is fundamental in optimising heather as a viable alternative in the control of GIN.

## 5. Reference

Athanasiadou S, Almvik M, Hellström J, Madland E, Simic N and Steinshamn H (2021) Chemical Analysis and Anthelmintic Activity Against *Teladorsagia Circumcincta* of Nordic Bark Extracts In vitro. *Front. Vet. Sci.* 8:666924.

## 6. Dissemination activities related with the Deliverable

This deliverable is part of an ongoing manuscript for a scientific peer-reviewed publication. It will be submitted to a scientific journal in due time (likely submission date is December 2021), and, once published (gold open access), will be linked to this deliverable. The details contained in this study are still confidential until online publication by the journal.