BarkCure

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www.barkcure.no

Newsletter no.3

Project facts:

BarkCure is funded by the Norwegian Research Council through the BIONÆR programme.

NOK 11 mill. funding

Project period:
June 2017-December 2020.

Project owner: Norwegian Centre of Organic Agriculture (NORSØK)

Project leader:
Håvard Steinshamn, NIBIO

In front: Kristin Sørheim, NORSØK, Sokratis Ptochos, SRUC, Berit Marie Blomstrand, NORSØK, Spiridoula Athanasiadou, SRUC. Back: Håvard Steinshamn, NIBIO, Ian Woolsey, Veterinærinstituttet, Karl-Christian Mahnert, Norsk Treteknisk Institutt, Stig Milan Thamsborg, UCPH, Heidi Enemark, Veterinærinstituttet, Inga Marie Aasen, SINTEF. Photo: Anita Land

We are now approaching the final stage of the project. During the course of the project, we have encountered some challenges and difficulties. It became obvious early last year that due to lack of pilot plant facilities for doing large-scale tannin extraction, it was not possible to produce sufficient quantities of extract to be used in the planned sheep trial. We had to change approach and decided to use mouse as a model animal. Due to this change, we had to prolong the project and were granted additionally six months by the Norwegian Research Council.
The mouse experiment was successfully carried out despite the outbreak of covid-19 and lockdown in the UK. This is thanks to extraordinary efforts by our partner, SRUC, as Berit Marie Blomstrand, our PhD student was forced to leave the UK and go home to Norway.

Our partner, the Norwegian Veterinary Institute, encountered similar challenge with lockdown and the peak of work with keeping donor animals and running laboratory experiments. However, they managed to run the planned experiments. We had a successful meeting with the industry partners in Oslo in October 2019. In the last six months of the project, our focus will be on writing scientific publications and disseminate project results to different target groups. We also communicate our research activities and results on our project web page www.barkcure.no

Best regards
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Chemical characterization, isolation and fractionation of bark CT

Larger quantities of bark extracts were produced in 2019 for the animal feeding experiments. Bark of Norwegian spruce (Picea abies) was collected in winter (March 2019) by NORSØK.

The stems were debarked using a drum debarker device, and the bark was partly dried to approximately 40 % dry matter by air drying. Bark from pine (Pinus sylvestris) was collected (by Treteknisk) at the associate partner Bergene Holm in March 2018.

Before extraction, the bark was milled to chips of 1-3 cm size. Extract from pine for the mouse feeding was prepared in ordinary laboratory equipment, using acetone as solvent. Spruce extract for sheep feeding was prepared by water extraction from 235 kg bark.

The extraction was carried out using SINTEF Ocean's mobile pilot plant, which includes mills, a 1 m3 tank, centrifuges, and an evaporator. Only the tank and the evaporator were used for the bark extraction. Approximately 1300 l extract was produced, which was evaporated to 82 l before freeze drying to approximately 6 kg dried extract.

The dry weight yields of both extracts were the same as previously obtained in preparation of extracts for in vitro assays, but the CT-yields were lower, probably due to grinding to smaller particles (1-2 mm) in the small-scale extractions.

Pictures showing different stages of the extraction process
Photo: Inga M. Aasen

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Biological activity of bark condensed tannins (CT) extracts against nematodes

The anthelmintic efficacy of 18 bark extracts has been tested with in vitro methods against the cattle gastrointestinal nematode Ostertagia ostertagi.

An animal experiment has been carried out to provide free-living life stages of O. ostertagi, for use in in vitro studies. Two bull calves (Norwegian Red Cattle, NRF) were monospecificaly infected with oral administration of the 3rd stage larvae (L3, infective form of the nematode) under the experimental permission of the Norwegian Food Safety Authority.

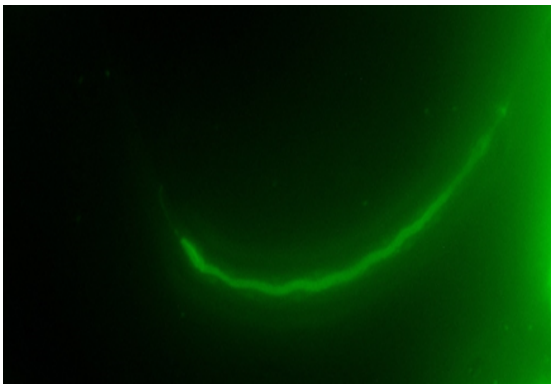
The 18 bark extracts (3 bark sources × 3 extraction methods × 2 bark collecting seasons) containing CTs in various concentrations were tested in vitro on various life stages of the nematode (i.e. egg, 1st stage larva (L1), L3).

The two bull calves, three months of age (Norwegian Red Cattle) were monospecificaly infected with Ostertagia ostertagi for the propagation of the parasite. Photo: Sokratis Ptochos

So far, we have tested the efficacy of the bark extracts on inhibiting the hatching procedure of the eggs to L1 and the inhibiting efficacy on L1 feeding at serially diluted concentrations of the extracts.

The Egg Hatching Assay and the Larval Feeding Assay have shown so far that there are anthelmintic properties in the compounds of the bark extracts and further analysis will reveal if this result is also CT content concentration correlated. The last in vitro test will be complete by the summer 2020 with which we will determine the paralytic effect of the extracts on the L3 by evaluating the inhibiting efficacy of the extracts on larval migration through fine mesh (Larval Migration Assay).

This initial in vitro screening of all the bark extracts on cattle nematodes will identify those with anthelmintic properties and drive us through the selection of those that can be further investigated for their potential use as alternative anthelmintic treatments in this animal species.



Ostertagia ostertagi L1 fed with fluorescent E. coli during the Larval Feeding Inhibition Assay. The bright green line indicates the intestinal content of the larva inside the larval body that is seen in this photo as a pale halo. Photo: Sokratis Ptochos.

Anthelmintic efficacy of BarkCure extracts tested against gastrointestinal nematodes (GIN) of sheep in vitro

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In vitro studies comparing the anthelmintic efficacy of different bark extracts against GIN eggs are now complete. The results show a significant inhibition of egg hatching in the presence of certain bark extracts The efficacy of the extracts depended on the bark species, the season sampled, the solvent used during the extraction process and the dose applied.

Eggs from the GIN species Trichostrongylus colubriformis were more susceptible than Teladorsagia circumcincta to a greater range and concentrations of bark extracts. Ultimately, pine bark sampled in winter and extracted with acetone demonstrated the greatest anthelmintic efficacy (up to 100%) at the lowest concentrations.

Regression analyses showed that condensed tannin (CT) content alone does not explain all the variation observed (Fig. 1). Additional analyses to determine CT structure and other compounds present in the bark extracts with possible anthelmintic efficacy are currently underway.

In vitro studies exploring the anthelmintic activity of the bark extracts against third stage GIN larvae are currently on hold due to COVID-19 pandemic.

Results to date have not shown any significant reduction in larval motility in the presence of bark extracts at the concentrations tested. If this outcome is confirmed it will give insights into the possible ways of incorporating bark extracts in sustainable parasite control strategies.

Image of GIN larvae hatching from egg.
Photo: F. Shepherd.

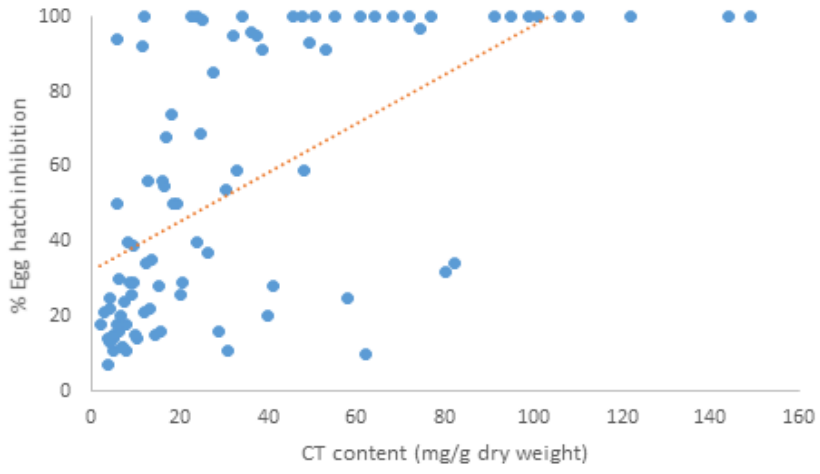

Fig. 1. Regressions analyses comparing T. colubriformis egg hatch assay results against the CT content of all the bark extracts tested. R2 = 0.387.

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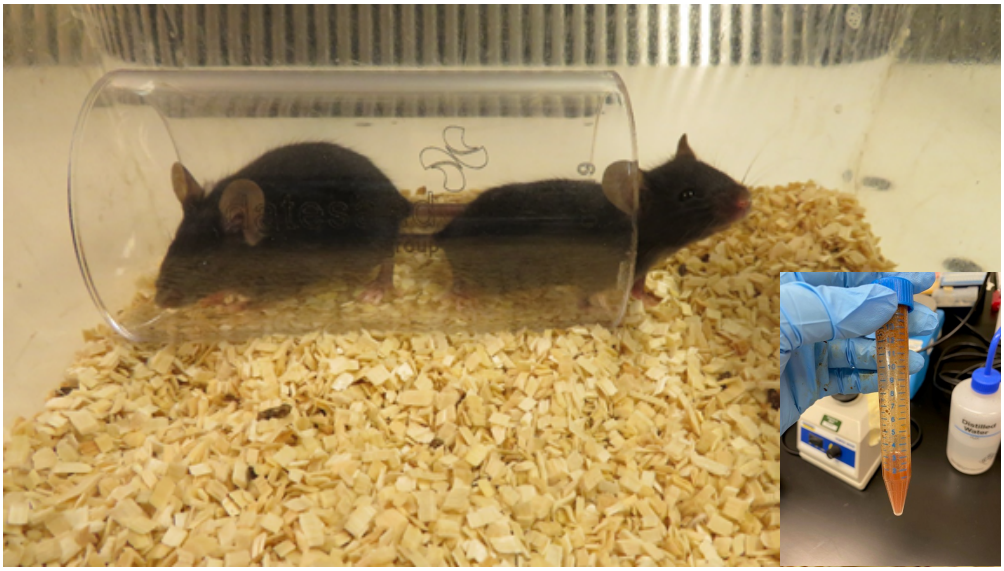
Anthelmintic efficacy of BarkCure extracts tested against gastrointestinal nematodes (GIN) of sheep in vitro

In vivo anthelmintic efficacy of bark extract on Heligmosomoides bakeri in two different mice lines



From the mouse experiment in Scotland. Excrement sample in picture to the right Photo: B.M. Blomstrand

March 3rd, 2020, the in vivo mouse trial started at Scotland's Rural College (SRUC) in Edinburgh, where the aim was to test the anthelmintic efficacy of acetone pine extract against the intestinal nematode Heligmosomoides bakeri.

We infected two mouse lines (BALB/c and C56BL/6, resistant and susceptible to the parasite, respectively) with H. bakeri (day 0) and treated the mice with bark extract in three different concentrations (0 mg/kg, low concentration and high concentration extract) for three days (day 19-21).

Feed intake and body weight were measured twice a week throughout the experiment, and faecal samples were taken at four time points the last two weeks.

After five weeks (day 28), the mice were euthanised and dissected. At necropsy, samples from spleen, small intestines and the mesenteric lymph nodes were taken and stored in RNAlater for later processing.

Furthermore, measurements of carcass weight and faecal egg count were collected for evaluation of the efficacy of the bark extracts against H. bakeri.

Despite the challenge of COVID-19 and the closedown of borders and universities, we managed to complete the trial.

We are now working on the results statistically to evaluate the antiparasitic efficacy of the bark extract.

Biological activity of bark extracts against Eimeria spp.

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The project group in the BC Project also got funding from the Regional Research Funding Body to test extract from bark from Norwegian spruce (Picea abies) against Eimeria infections in an animal experiment, and we will give a brief summary of this work.

From the lamb experiment at Tingvoll. Photo: Peggy Haugnes

Naïve lambs (n=24), aged between 18 and 21days, were randomly distributed by gender and weight into three experimental groups.

8 lambs were inoculated with Eimeria oocysts without any treatment, 8 lambs were inoculated with Eimeria oocysts and treated orally with bark extract for 12 days, and 9 lambs were not inoculated with parasites or given CT containing bark extract.

The amount of bark extract administered per lamb and day corresponded to 0,05% of BMW. The bark extract had a significant effect on the development of Eimeria spp. in the lambs measured by faecal excretion of oocysts per gram faeces ($p<0,001$).

Analysis of the faecal samples revealed a significantly lower faecal score (measure of faecal consistency from 1-5, where 1 is normal) in the untreated group on day 8 after infection, but 14 days and 19 days post treatment the treated group had less diarrhoea and significantly lower faecal score ($p<0.001$) than the untreated group.

The weight gain was significantly lower for the group that was infected and treated with bark extract than for the group that was infected but not treated and the control group, measured from birth until the end of the experiment ($p<0.01$, paired t-test).

The weight gain was significantly lower for the group that was infected and treated with bark extract than for the group that was infected but not treated and the control group, measured from birth until the end of the experiment ($p<0.01$, paired t-test). The weight gains in the control group and the untreated group (EIN) were not significantly different.

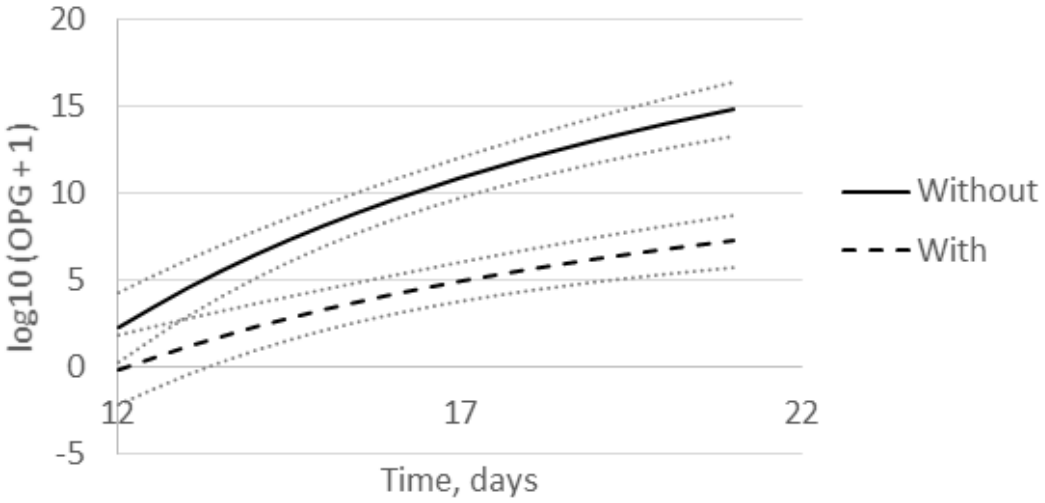

Figure 2: Faecal excretion of Eimeria oocysts per g faeces (log10(OPG+1)) in lambs without (Without) and with (With) oral bark extract treatment. The dotted lines indicate the 95% confidence interval.

Research partners:

NORSØK · NIBIO Norsk institutt for bioøkonomi · Treteknisk · SINTEF · SRUC Scotland's Rural College · Københavns Universitet · Veterinærinstituttet

The Research Council of Norway

Industry Partners:

ANIMALIA · BERGENE HOLM AS · Fellesskapet · NSG · Norske Skog · NORGES BONDELAG

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