

BarkCure

December 2018

www.barkcure.no

Newsletter no.2

Project facts:

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

NOK 11 mill. funding

Project period:
June 2017-October 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

Dear reader

We are now one and a half years into the BarkCure project and we are on the track. All the bark material that we are to use in the project is now collected from the sawmills and in storage. We have six master batches all together, two from pine and four from spruce. Condensed tannins have been extracted from all master batches using three different types of solvents. The extracts are currently being used in different types of laboratory tests to evaluate their efficacy against gastro-intestinal parasites. Preliminary results indicate that there are promising effects, and our

PhD student Berit Marie Blomstrand presented some of these results at an international conference in Spain this autumn. Based on results from the ongoing laboratory tests, we will scale up the extraction of the most promising extracts this coming spring. These extracts will be tested in sheep infected with gastro-intestinal nematodes. This animal experiment will be run at Scotland's Rural College, one of our partners in Edinburgh next summer.

We also communicate our research activities and results on our project web page www.barkcure.no

Best regards
Håvard Steinshamn
Project leader

Karl-Christian Mahnert



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Value chain bark/ selection of bark material

The Norwegian Institute of Wood Technology performs the debarking process creating the value chain. One important question is what would be the minimum price per ton of bark that the industry partners have to realize for production of condensed tannins (CT) in bark to be competitive in comparison to the utilization of bark today (gardening products, energetic recovery). This will be investigated by interviewing key personnel and industrial partners.

In 2018, the task has been to estimate the wood percentage and moisture content in the bark material produced during the previous summer and winter. Preliminary results indicate a lower moisture content in the bark collected last winter (no water spray) and a lower wood percentage in the summer bark (exception Master Batch II: intermittent production) (figure 1).



Drum debarking (Photo: logprollc.com)



Rotary debarking (Photo: <http://www.directindustry.com>)

MB	Wood species	Debarking method	Moisture content [% of dw]		Wood percentage [% of dw]	
			Summer (A)	Winter (B)	Summer (A)	Winter (B)
I	Spruce	Ring	268	234	19	23
II	Spruce	Drum	200	303	17	25
III	Pine	Ring	363	164	6	34

Figure 1. Moisture content and wood percentage



Mixing of master batch. Photo: Karl-Christian Mahnert

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Chemical characterization, isolation and fractionation of bark CT

Extracts of CT have been prepared from bark 'Master batches' collected by The Norwegian Institute of Wood Technology. The summer-batches were received in August 2017, and the winter-batches in April 2018.

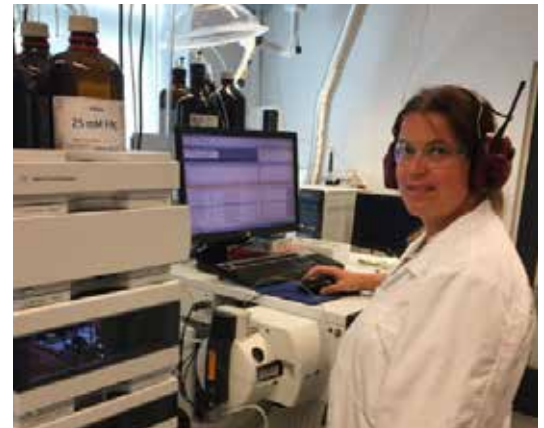
For analyses and characterisation of CTs, the bark was dried and milled before extraction with water and water-miscible solvents. As expected, higher CT-yields were obtained when solvents containing methanol or acetone were used, compared to water only. However, the yield in water is of interest with respect to potential industrial production, as water extraction will be cheaper than using solvents.

Quantitative analyses were performed using spectrophotometric assays and chromatographic methods, after degradation of the complex polymers to monomers. The overall CT-yields were highest from spruce bark, however, the CT-content of the dried extracts was highest from pine, which is in accordance with preliminary results from *in vitro* assays (see below). For spruce, the bark from rotary-debarking had higher CT-content than the bark from drum-debarking.

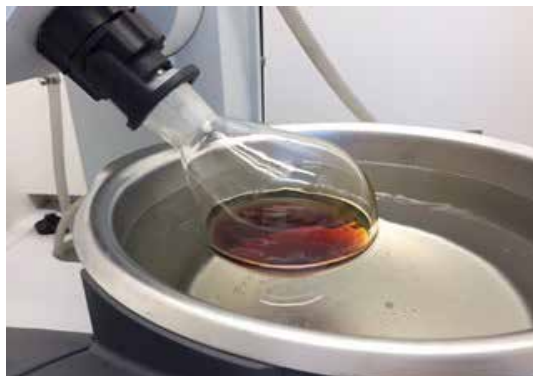
Photos: Janne Beate Øiaas

Extracts from the summer batch (9 extracts) for *in vitro* evaluation were delivered in January 2018, while extracts from the winter batch were ready in June 2018.

Further characterisation of the CT-extracts by chromatographic methods and mass spectrometry (LC-MS) is on-going and indicates different ratios between the two main classes of monomers in spruce and pine.



Characterisation of CT-extracts, Janne Beate Øiaas



Isolation and fractionation of bark CT's



Spruce, drum debarking



Pine, rotary debarking



Spruce, rotary debarking

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Biological activity of bark CT extracts against sheep nematodes

In 2018 we started to test the biological efficacy of bark CT extracts on selected ovine nematodes *in vitro*.

So far, nine bark extracts from the summer batch (sampled during autumn 2017) were tested (spring 2018). The nine bark extracts from the winter batch (sampled in March-April 2018) are now being tested. Efficacy against *Teladorsagia circumcincta* has been assessed with an egg hatch test and a larval motility test with a real time cell analyser. The same methods will be applied to test the extracts against *Trichostrongylus colubriformis* (planned February 2019).

Preliminary results show that pine extracts have high efficacy independent of extraction method, with the acetone extract giving the highest egg hatch inhibition (EH% <10). Water and methanol extracts of spruce had little or no effect (EH% >90) (figure 2). Preliminary results from the larval motility test with real time cell analysis show that methanol and acetone pine extracts demonstrated a reduction in larval motility in all concentrations. Water extracts and methanol pine 1 extract showed motility reducing efficacy in the two highest concentrations.

Preliminary conclusion:

CT extracted from Pine bark, by means of methanol and acetone, demonstrated the highest anthelmintic efficacy in both methods tested. There seems to be a positive correlation between anthelmintic efficacy and the CT content of the extracts.



Teladorsagia circumcincta larva. Photo: Berit M. Blomstrand



Berit M. Blomstrand: - Lab work at SRUC. Photo: Sokratis Ptochos

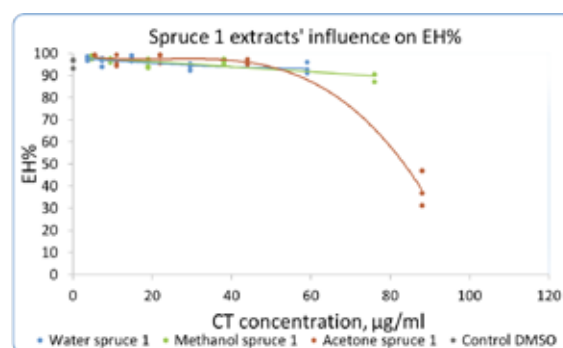
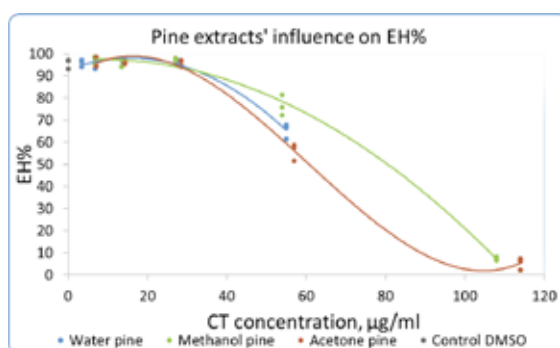


Figure 2. Two extracts influence on nematode egg hatch (EH%)

Biological activity of bark CT extracts against cattle nematodes and sheep protozoa

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The plan for next summer is to test *in vitro* anthelmintic efficacy of selected bark extract against cattle nematodes and antiprotozoal efficacy against sheep *Eimeria spp* field isolates.

The extracts from both the summer and the winter batch will be tested on different life cycle stages of cattle nematodes, and we will use different methods for the different stages. The life cycle of gastro-intestinal nematode is illustrated in figure 3, where the different stages, L1-L5, are indicated.

These methods used will be *Larval feeding inhibition assay* (L1), *Larval migration assay* (L3) and *Egg hatch assay* (egg-L1). These tests are due ultimo January 2019.

This autumn we have assessed the *in vitro* anti-protozoal efficacy of all the 18 bark extracts on *Cryptosporidium parvum*, a protozoan causing diarrhea in both humans and animals. We have tested the ability of the extract to inhibit the intracellular growth of *C. parvum* (figure 4). Thus far the results indicate that the acetone and methanol pine extracts show a promising efficacy, while the water extracts show no growth inhibition.

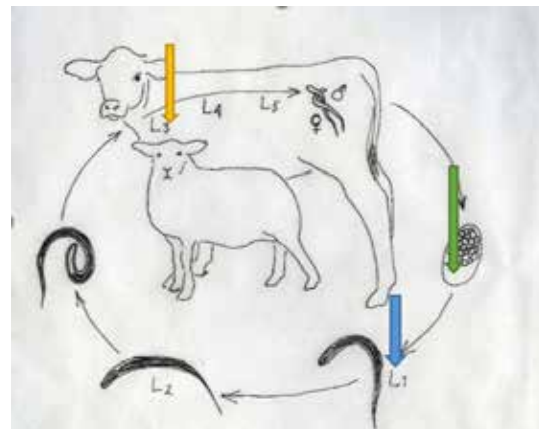


Figure 3. Life cycle of gastro intestinal nematodes. Ill: Anne de Boer

Furthermore, we will collect *Eimeria spp* field isolates and purify them. Thereafter, the cell cultures will be infected, and different extracts will be tested for their ability to inhibit sporozoite invasion into, and growth inhibition of *Eimeria* in the cells.

The results from the *in vitro* tests of the bark extracts will determine the experimental design for assessment in sheep, which will take place in Scotland in July 2019.

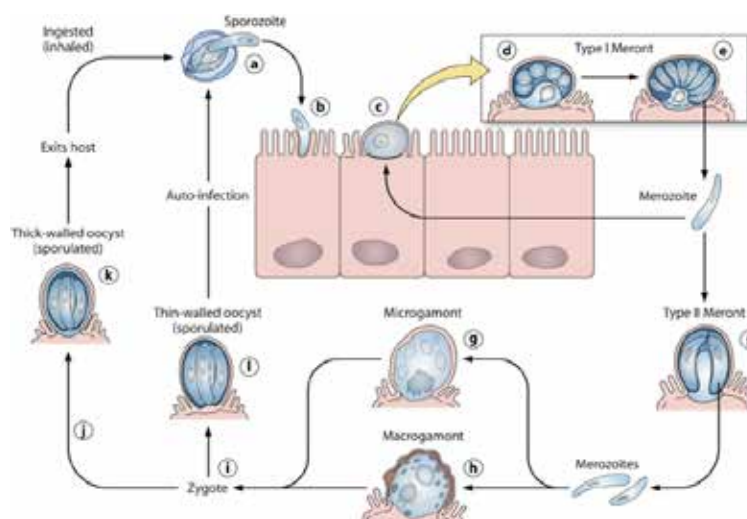


Figure 4. Life cycle of *Cryptosporidium parvum* (www.pixsharp.com)



Research partners:



Industry Partners:



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