Interreg

Cofinancé par l'Union Européenne
Kofinanziert von der Europäischen Union

Grande Région | Großregion

ExtraBark

LIÈGE université
Gembloux
Agro-Bio Tech

Laboratory of Chemistry of Natural Molecules

From Bark to Bio-Protection: EXTRABARK Unlocking Solutions for Plant Protection

Leonel TAGUIMJEU, Manon GENVA and Marie-Laure FAUCONNIER

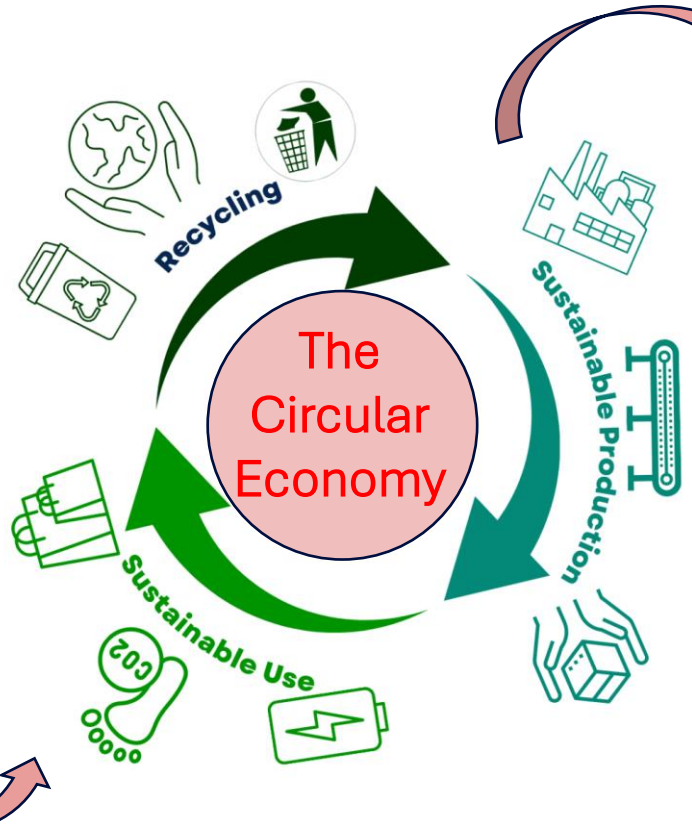
ISCP
INTERNATIONAL SYMPOSIUM ON CROP PROTECTION

May 19 – Ghent, Belgium

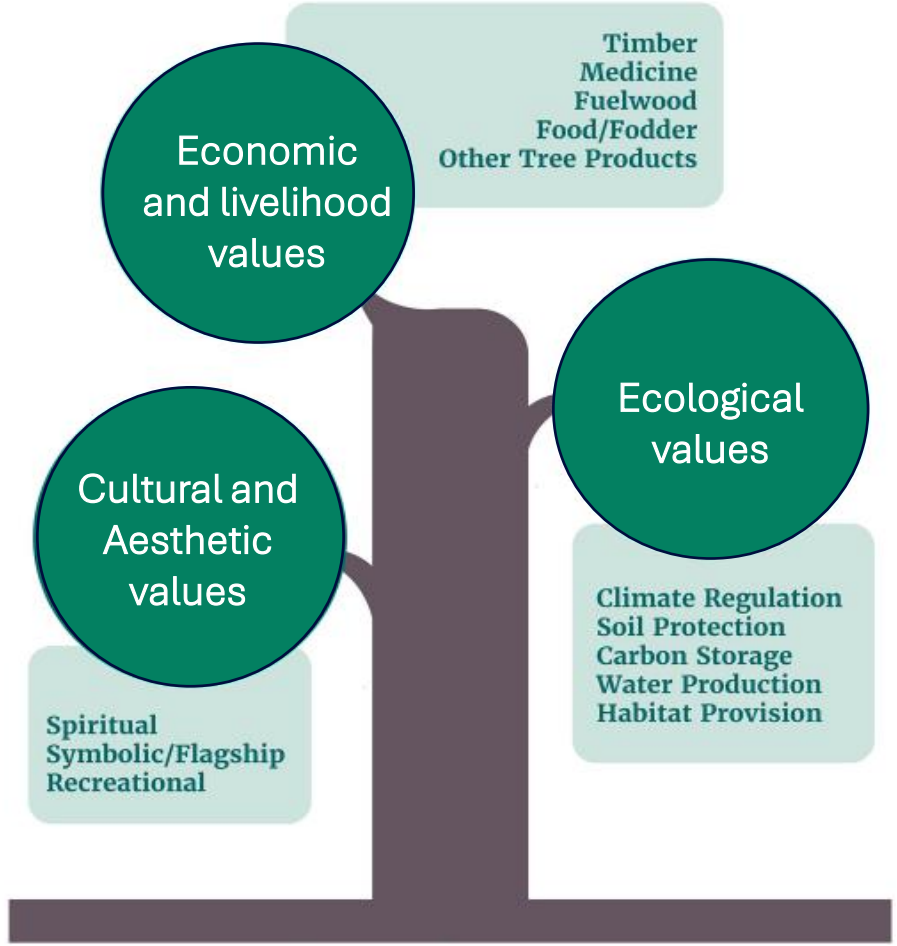
Bark: From Low-Value to Valuable Resource

➔ Low-value

Mainly fuel or mulch



➔ Functional and economic value



Challenges of Chemical Pesticides

Harmful effects on humans

The impact on soil quality and climate change

➔ In this context, identifying new bio-based resources for sustainable plant protection has become a major challenge.

ExtraBark

Valorization of bark (wood industry co-products) through the extraction of high-value compounds for the development of plant and wood protection products as sustainable alternatives to synthetic chemicals.

What Led to the Emergence of the EXTRABARK Project?

- Search for natural, bio-based, and renewable alternatives through the extraction of natural molecules.
- The bark resource in the Greater Region is extremely abundant.
- Despite this significant resource, bark valorization pathways remain limited, and there are very few specialized stakeholders capable of meeting this demand.

➔ EXTRABARK wants to address this gap by exploring the implementation of a complete bark valorization value chain.

The Project

- EXTRABARK objective

Extract, characterize, and valorize biocidal secondary metabolites from regional forest bark to develop more environmentally friendly bio-based solutions.

- The investigated tree species

Selection of 10 priority tree species available in the region.

Fir

Beech

Oak

Douglas fir

Scots pine

Spruce

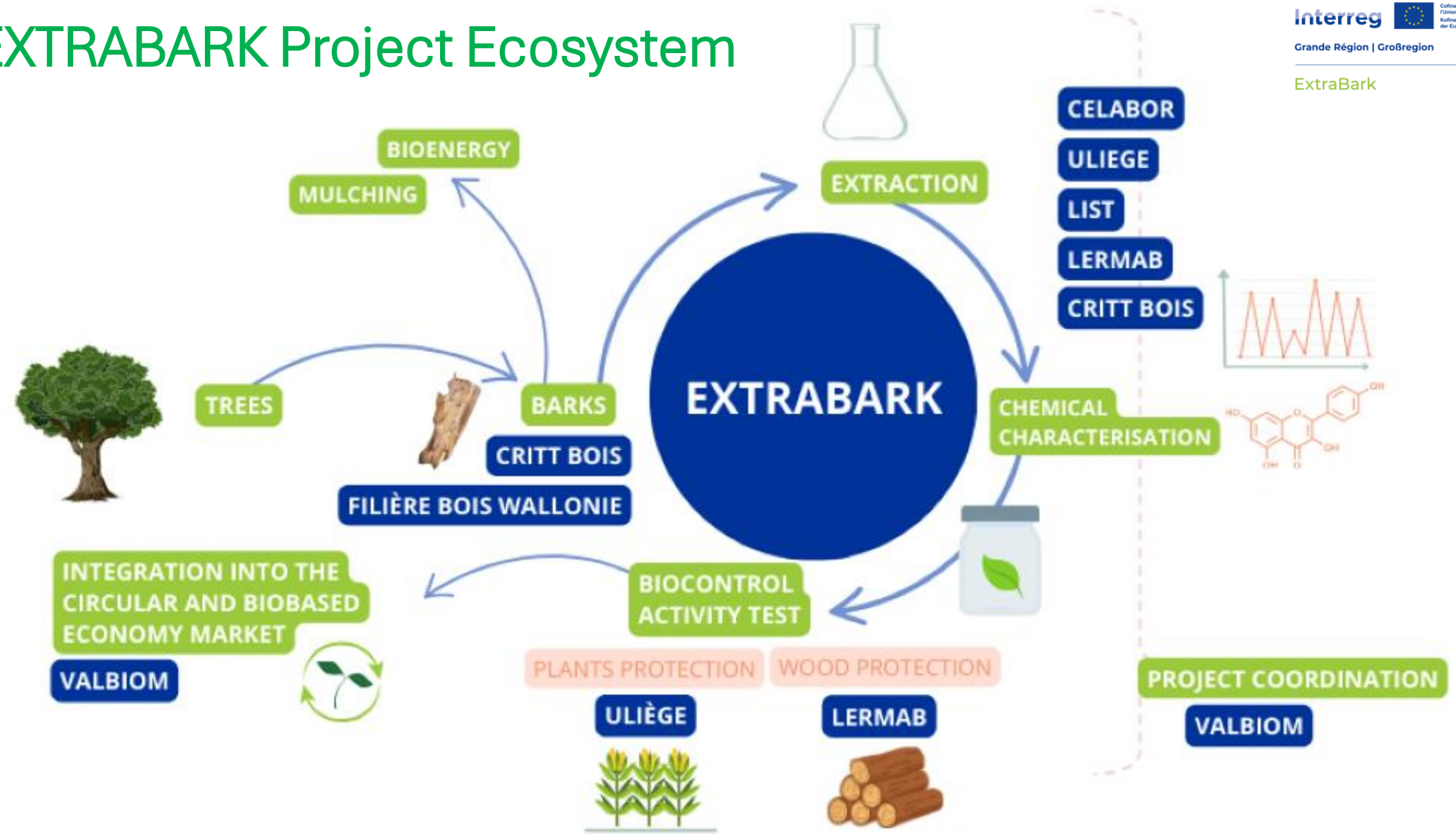
Maritime pine

Larch

Locust

Poplar

The EXTRABARK Project Ecosystem



Four Eco-Friendly Extraction Processes Employed

1 HYDRODISTILLATION

Volatile compounds
Essential oil

2 SUPERCRITICAL CO_2

Solvent-free extraction
Lipophilic molecules

Ground bark

3 AQUEOUS MACERATION

Sustainable process
Water-soluble compounds

4 HYDROETHANOLIC MACERATION

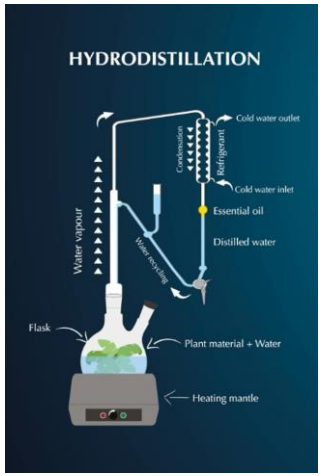
Bio-based solvent
Broad spectrum of molecules

GC–MS AND LC–MS/MS ANALYSES

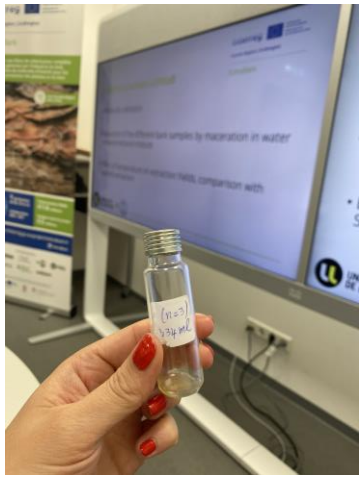
Essential Oils (EO): Extraction by Hydrodistillation

Crushing

(1kg x 3)



Clevenger



Essential oil
Low EO yields (< 0.2%)

Analysis by GC-MS:
- 3 injections (repetitions)
- 2 colums (HP5 – MS and DB Wax)

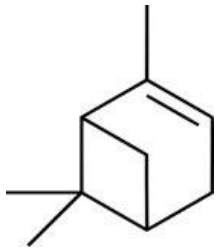
- Hydrodistillation: very simple, inexpensive, and direct contact with plant material.
- GC-MS analysis, why 2 colums?

- Better separation and resolution ;
- Confirm peak identification ;
- Improve the reliability and accuracy of the results.

Essential oils (EO): Chemical Composition

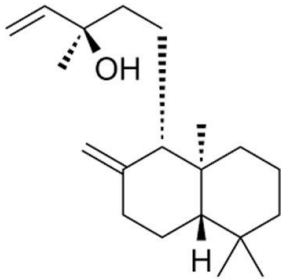
- **Mainly:** Terpenes (mono, sesqui and diterpenes)
- **But also:** Alcohols, esters, acids, and ketones.
- Main compound of EXTRABARK's EO

Spruce: 36 compounds



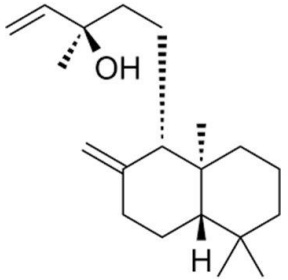
α-Pinene (21.74 %)

Larch: 30 compounds



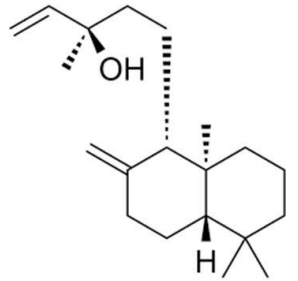
Manool (44.89 %)

Black locust: 13 compounds



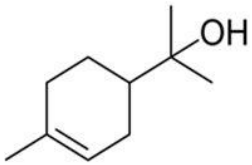
Manool (34.09 %)

Fir: 34 compounds



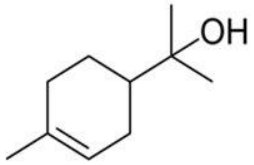
Manool (29.38 %)

Douglas: 20 compounds



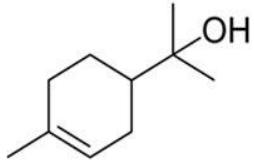
α-terpineol (26 %)

Scots pine: 18 compounds



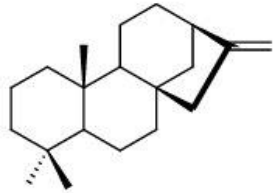
α-terpineol (33.66 %)

Maritime pine: 38 compounds



α-terpineol (21.17 %)

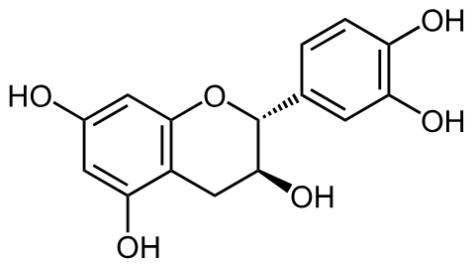
French oak: 29 compounds



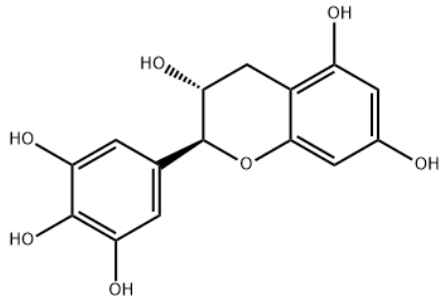
Phyllocladene (28.61 %)

Chemical Characterization of Polar (aqueous and hydroethanolic) Extracts

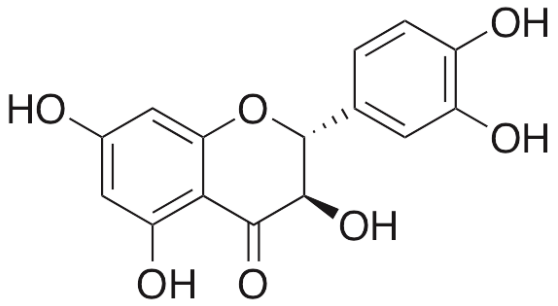
- Mainly: Flavonoids, polyphenols and stilbenes.



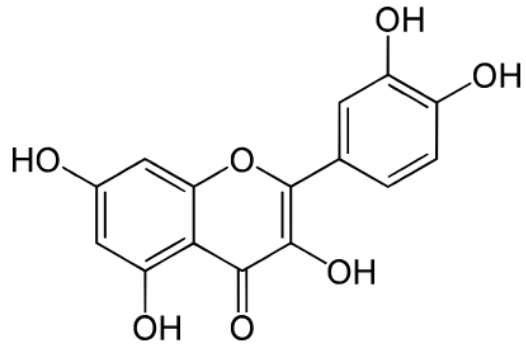
Catechin



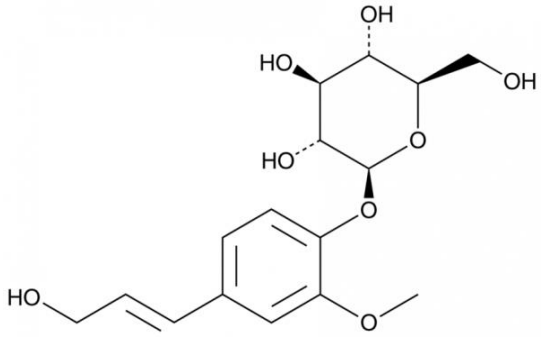
Gallocatechin



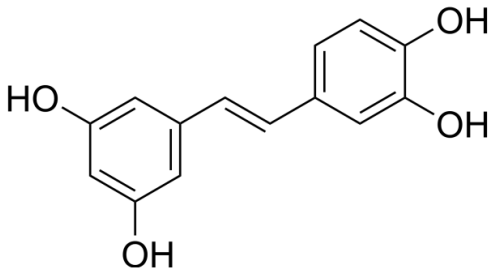
Taxifoline



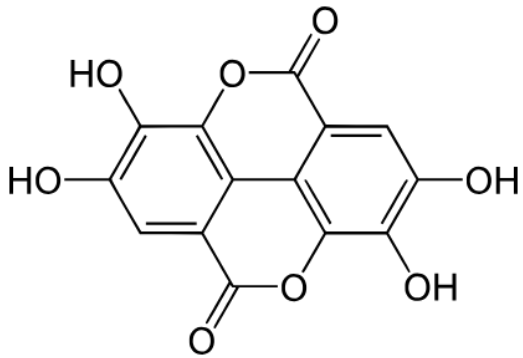
Quercetine



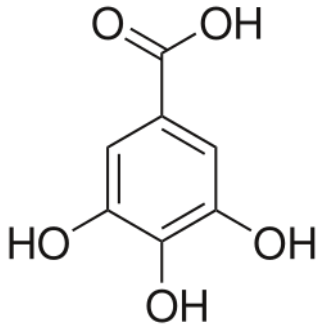
Coniferin



Piceatannol



Ellagic acid



Gallic acid

Biological Properties – Plant Protection

From four types of extracts to three types of bioassays

1. Herbicides against monocot (*Lolium perenne*) and dicot (*Trifolium pratense*) weeds as an alternative to glyphosate.

2. Fungicides against major crop fungal diseases (*Aspergilus flavus, Fusarium verticillioides, Phytophthora infestans*).

3. Insecticides for stored product pests (*Sitophilus granarius*).

Herbicide Activity (Phytotoxicity)

Methods

- **Plant species tested:** a dicotyledon (***Trifolium pratense***) and a monocotyledon (***Lolium perenne*** L.).

- **Growth stages assessed:** cotyledons and first leaves.

- **Five different solutions were prepared for plant spraying:**

Conditions	Modalities
1	**Negative control**: water (blank) + 0.5 % (*v/v*) of ethanol + 0.5 % (*v/v*) Tween 20
2	**Positive control**: pelargonic acid at 3.43 %
3	**Positive control**: round up based on acetic acid 61g/L
4	3% (*v/v*) tested sample + 0.5 % (*v/v*) of ethanol + 0.5 % (*v/v*) Tween 20
5	**Negative control**: water (blank)

- Determine the viability percentage at the targeted growth stage:

➔ A higher viability percentage therefore indicates lower phytotoxicity.

Herbicide Activity (Phytotoxicity)

8 mL of each condition sprayed per test.

Figure 1: Spraying of the pots

Condition 1: water + 0.5 % (*v/v*) of ethanol + 0.5 % (*v/v*) Tween 20

Condition 5: water (blank)

Day 0 (before spraying)

Day 7 (after spraying)

Herbicide Activity (Phytotoxicity)

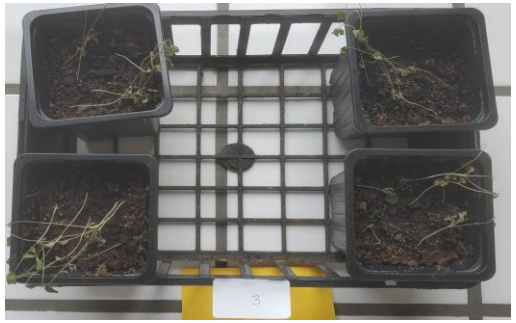
- 8 mL of each condition sprayed per test

Figure 1: Spraying of the pots

Condition 2: pelargonic acid at 3.43 %

Condition 3: round up based on acetic acid 61g/L

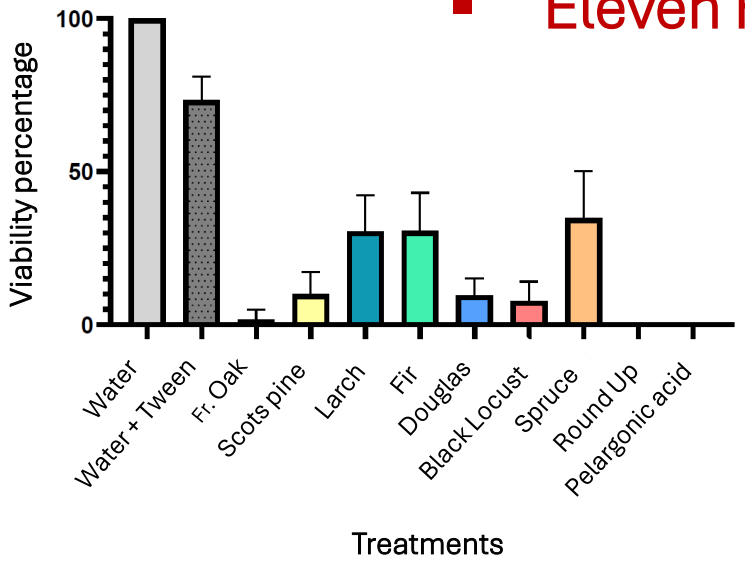
- General remark

Day 0 (before spraying)	Day 7 (after spraying)
	
	

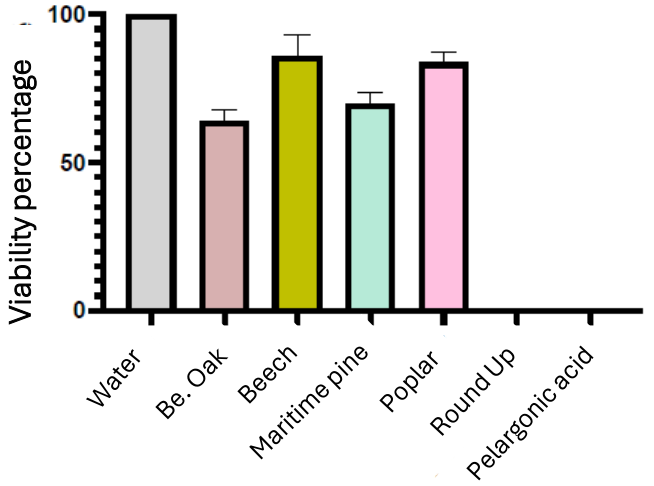
The negative and positive controls had the desired effects.

Herbicidal Potential of Polar Extracts – *T. pratense*

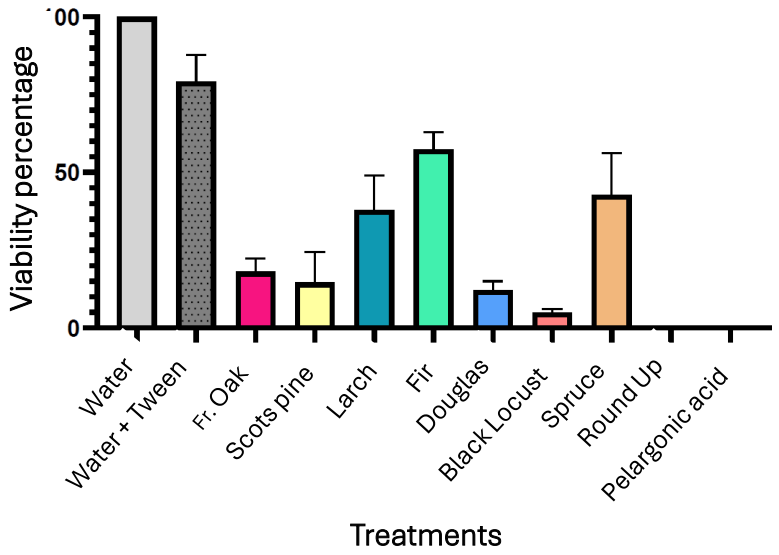
- Eleven Hydroethanolic Extracts (1:1)

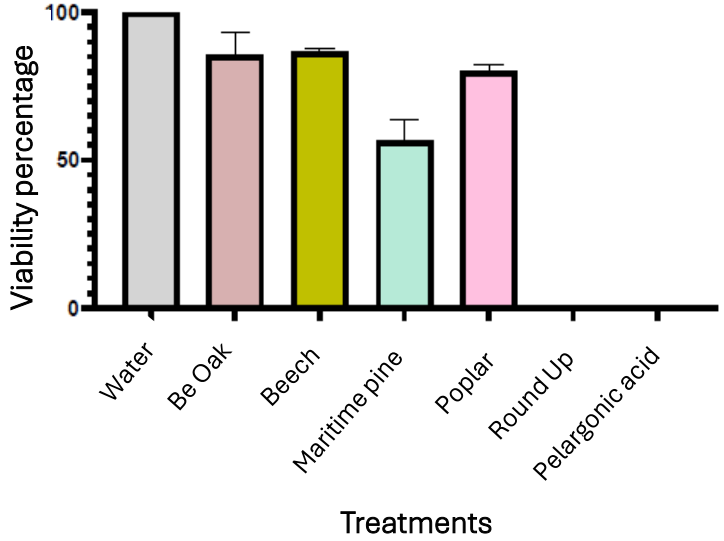


- Cotyledons



- First leaves

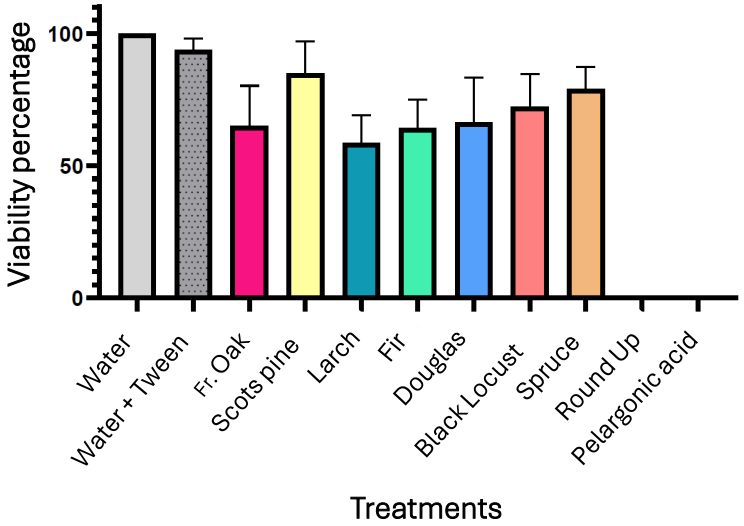


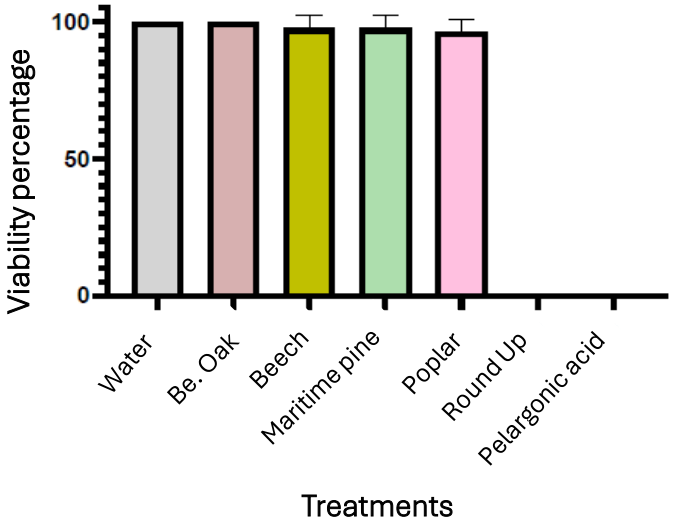


Herbicidal Potential of Polar Extracts – *L. perenne*

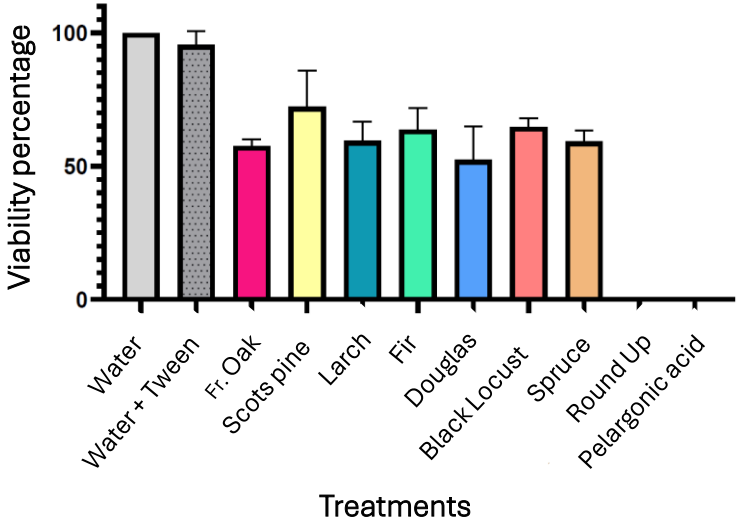
- Eleven Hydroethanolic Extracts (1:1)

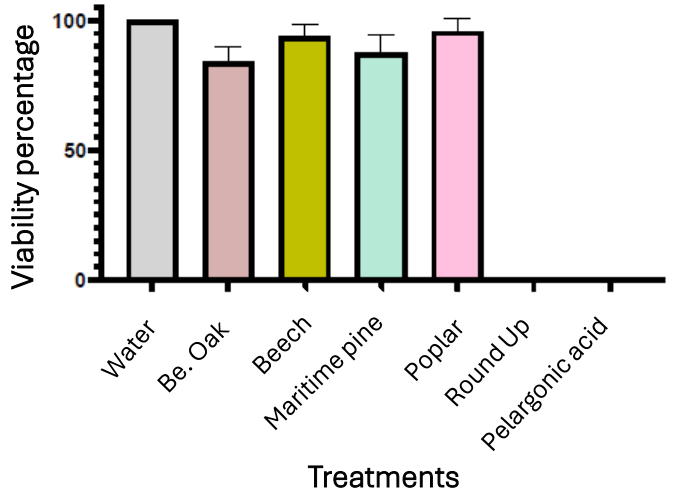
- Cotyledons





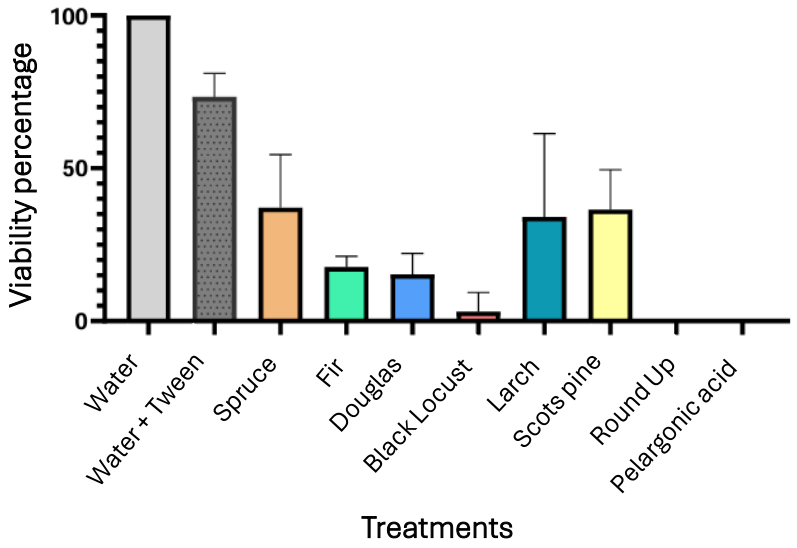
- First leaves

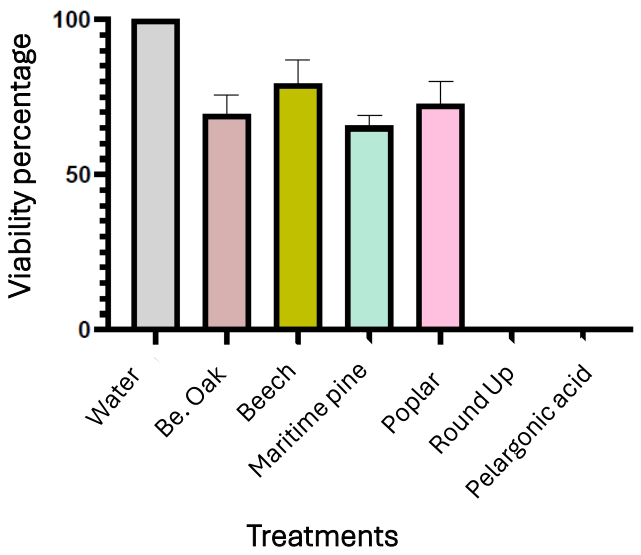




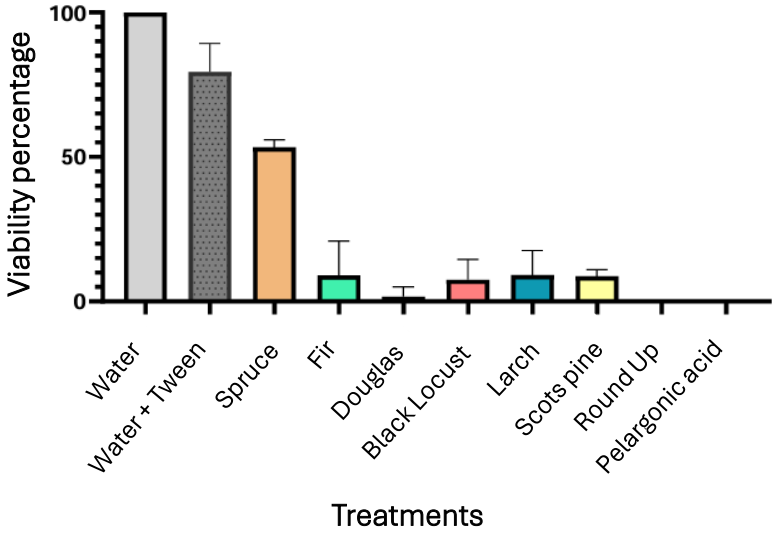
Herbicidal Potential of Polar Extracts – *T. pratense*

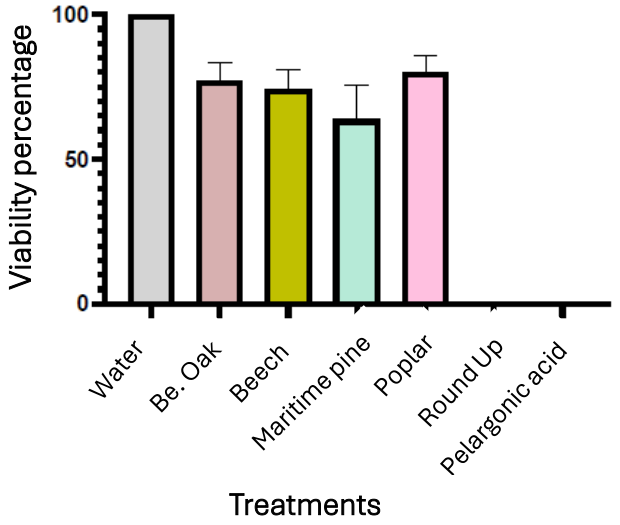
- Ten Aqueous Extracts
  - Cotyledons





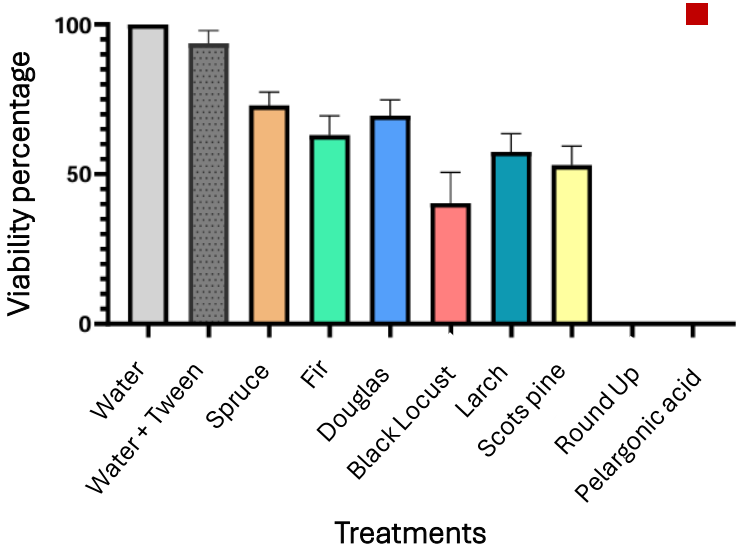
  - First leaves

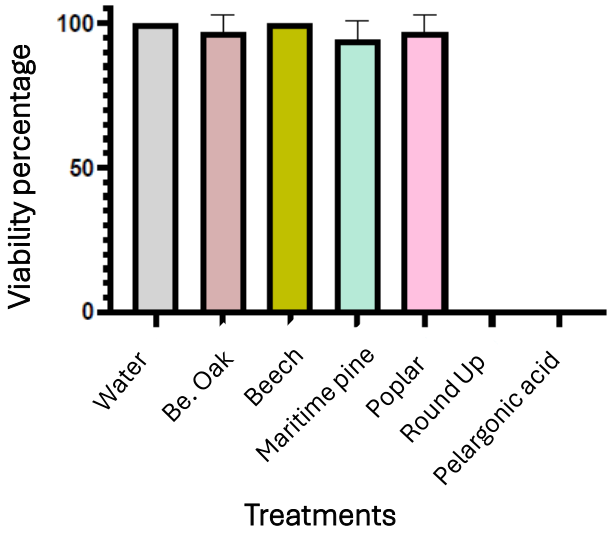




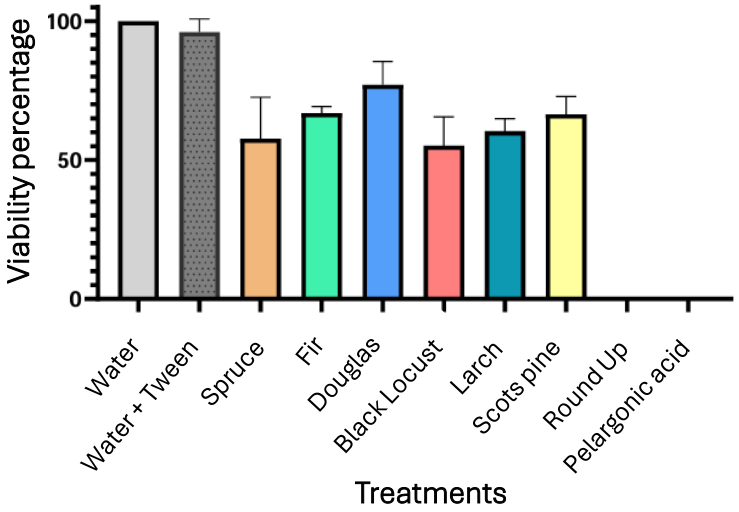
Herbicidal Potential of Polar Extracts – *L. perenne*

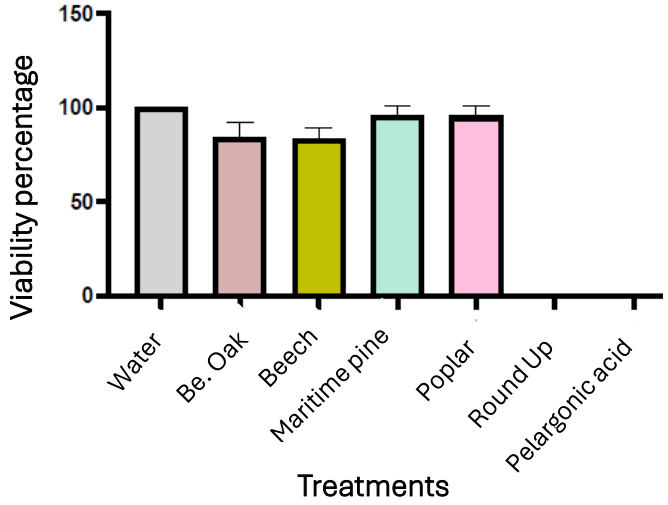
- Ten Aqueous Extracts
  - Cotyledons



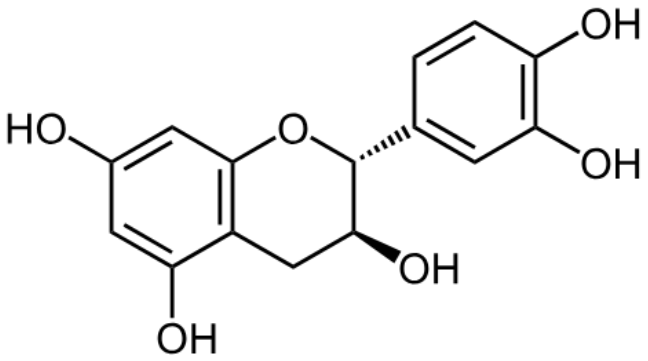


  - First leaves

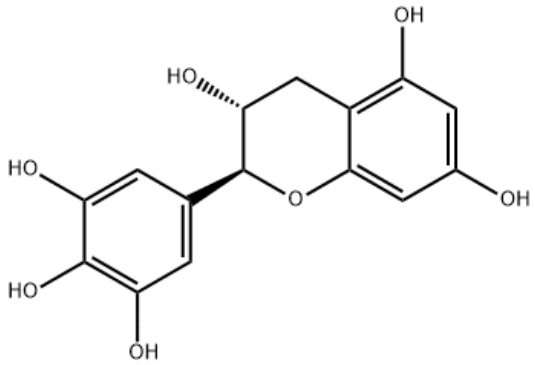




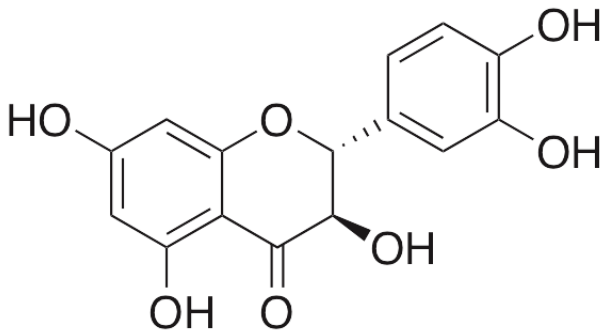
Herbicidal Potential of Polar Extracts



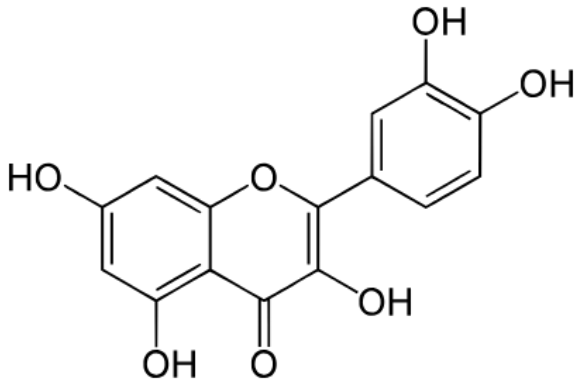
Catechin



Gallocatechin



Taxifoline



Quercetine

Discover Plants

Review

Flavonoids in plant-environment interactions and stress responses

Jitendra R. Patil[1] · Kuldeep J. Mhatre[2] · Kushi Yadav[3] · Lal Sahab Yadav[4] · Sudhakar Srivastava[5] · Ganesh Chandrakant Nikalje[1]

Received: 15 May 2024 / Accepted: 5 December 2024
Published online: 27 December 2024

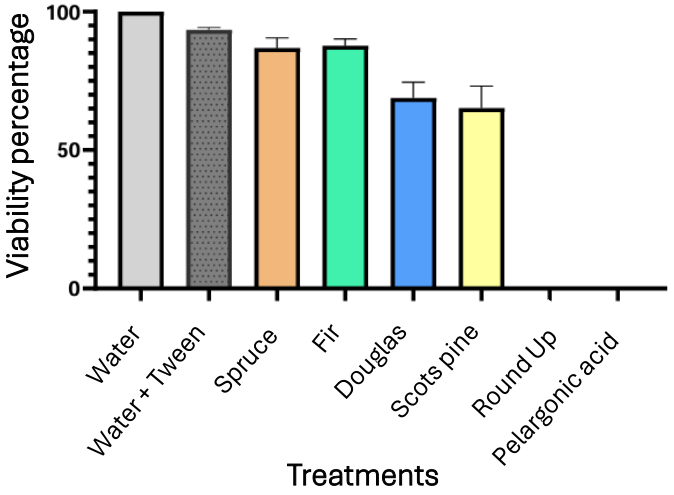
(Patil *et al.*, 2024)

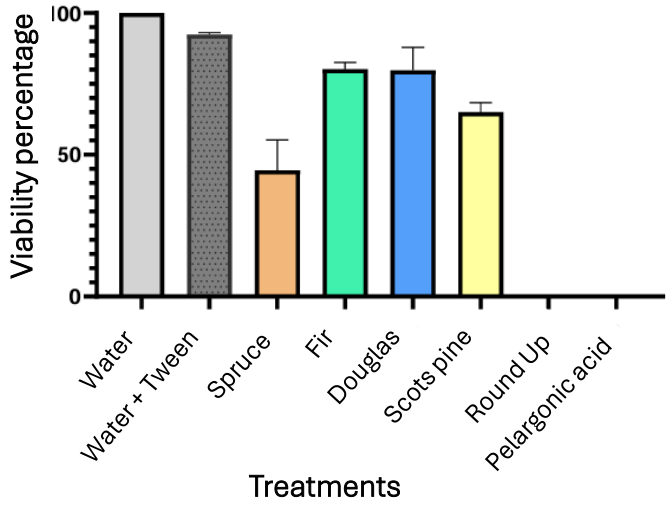
➔ Flavonoids could be responsible for the herbicidal potential observed in the aqueous and hydroethanolic extracts.

Herbicidal Potential of Apolar Extracts

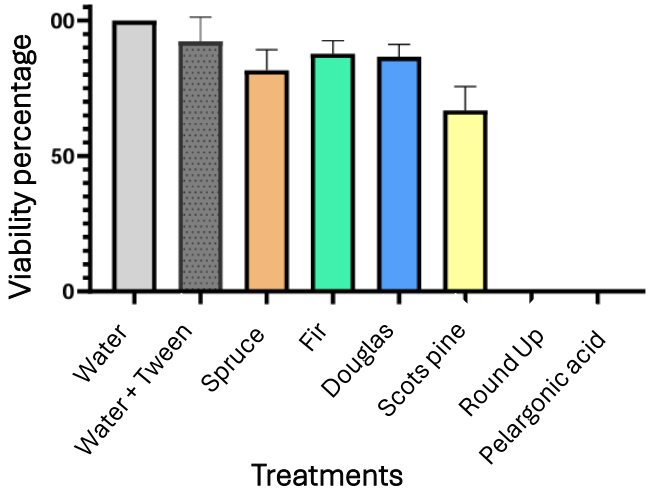
- Four essential oils
  - Cotyledons
  - First leaves

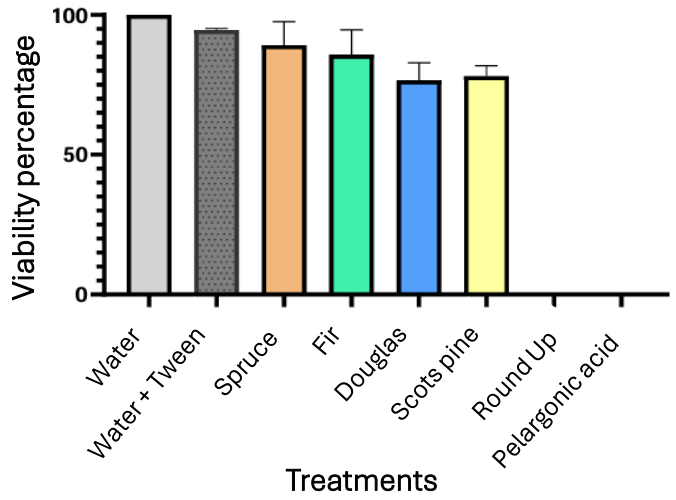
Trifolium pratense





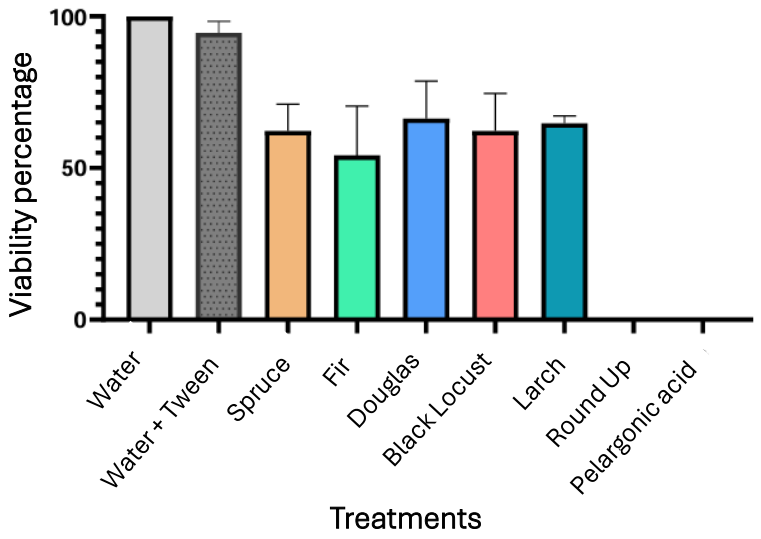
Lolium perenne

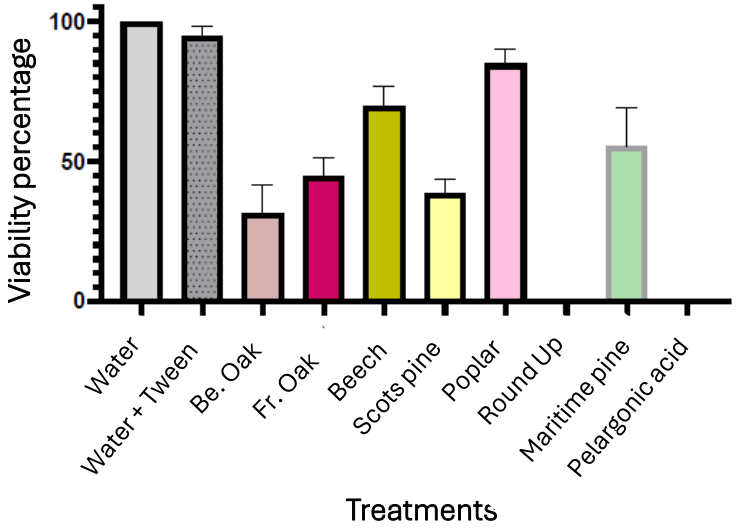




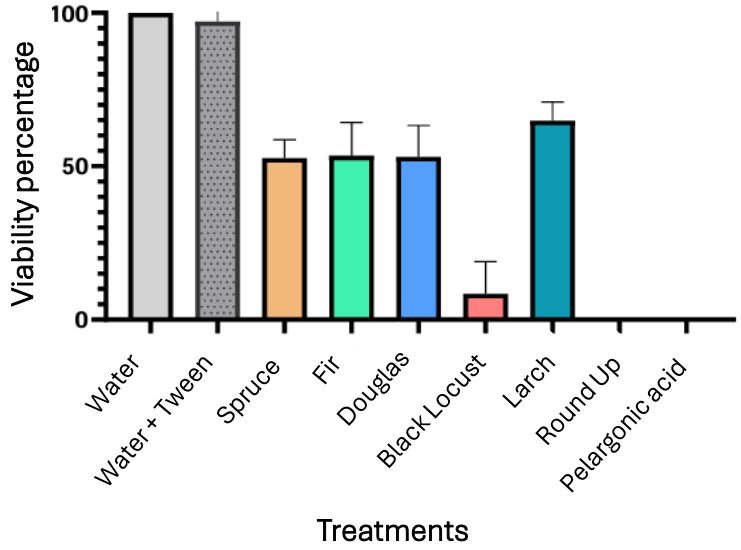
Herbicidal Potential of Apolar Extracts – *T. pratense*

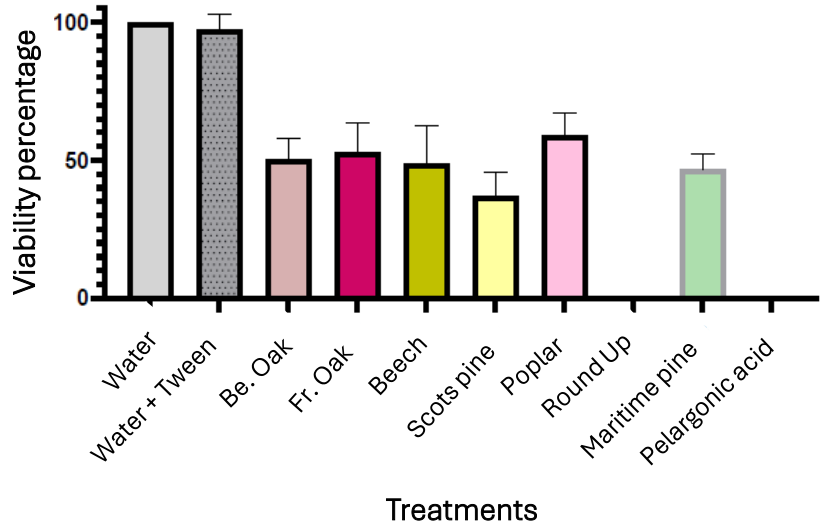
- Eleven CO_2 extracts
  - Cotyledons





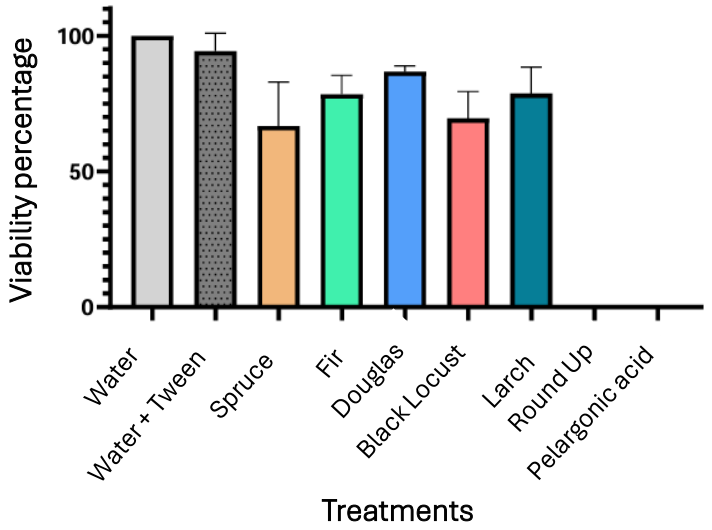
  - First leaves

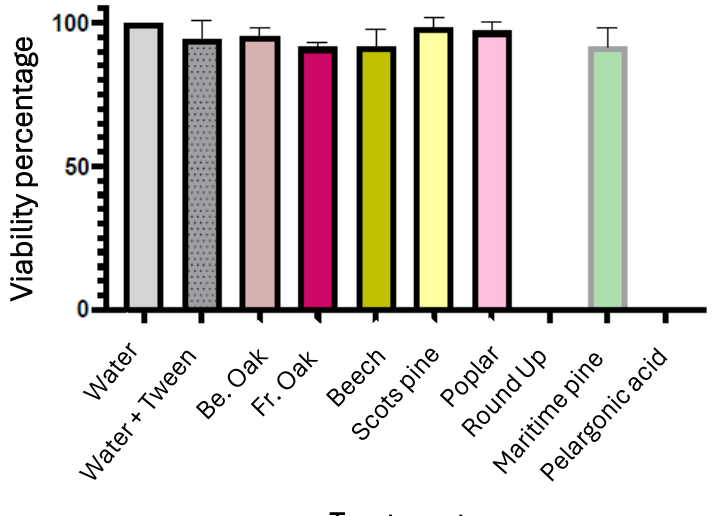




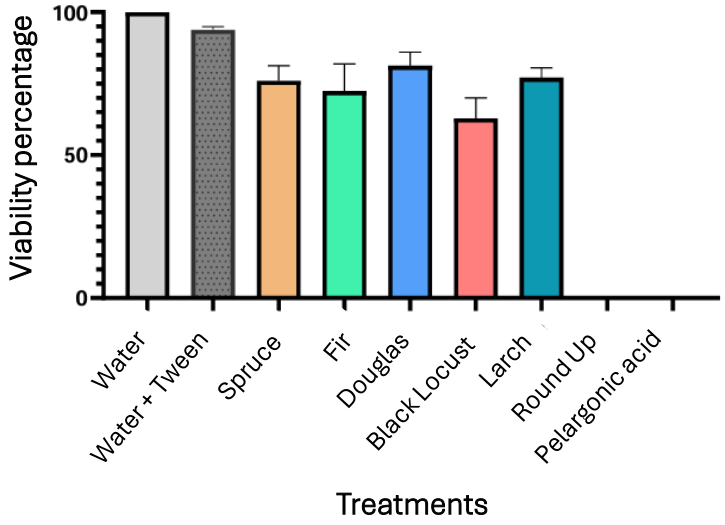
Herbicidal Potential of Apolar Extracts – *L. perenne*

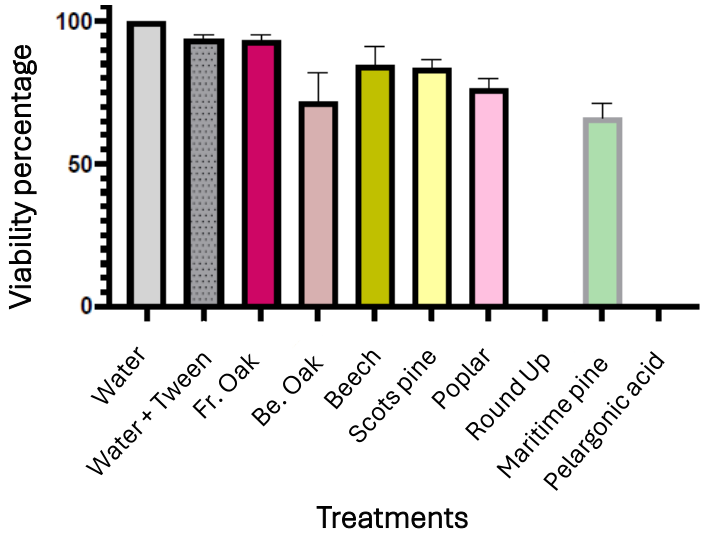
- Eleven CO_2 extracts
  - Cotyledons





  - First leaves





Fungicide Activity

- Methods

- Fungal strains: *Aspergillus flavus* and *Fusarium verticillioides*;

- Non-volatile extracts were evaluated by direct contact, whereas essential oils were tested both by fumigation and direct contact;

- Culture medium: PDA (Potato Dextrose Agar);

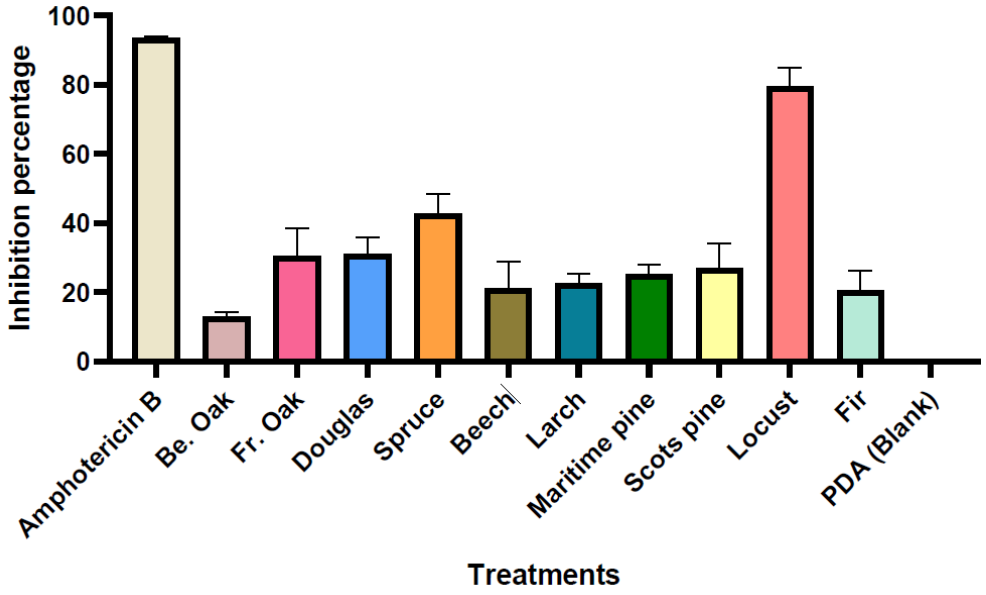
- Samples were tested at 3% (*w/v*);

- Amphotericin B was used as the positive control.

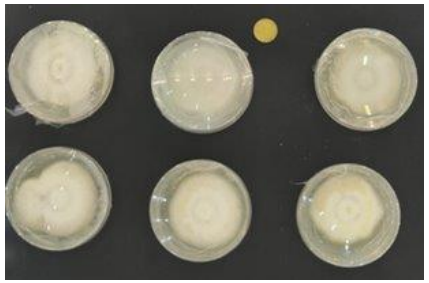
Fungicide Activity

- Hydroethanolic Extracts (1:1)

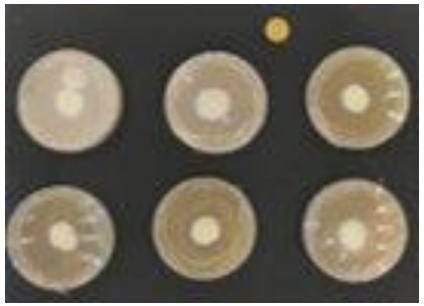
Percentage inhibition of *Fusarium verticillioides* infection mycelial growth by hydroethanolic extracts



The hydroethanolic extract of locust showed strong antifungal activity, with 80% inhibition of the growth of *F. verticillioides* infection.

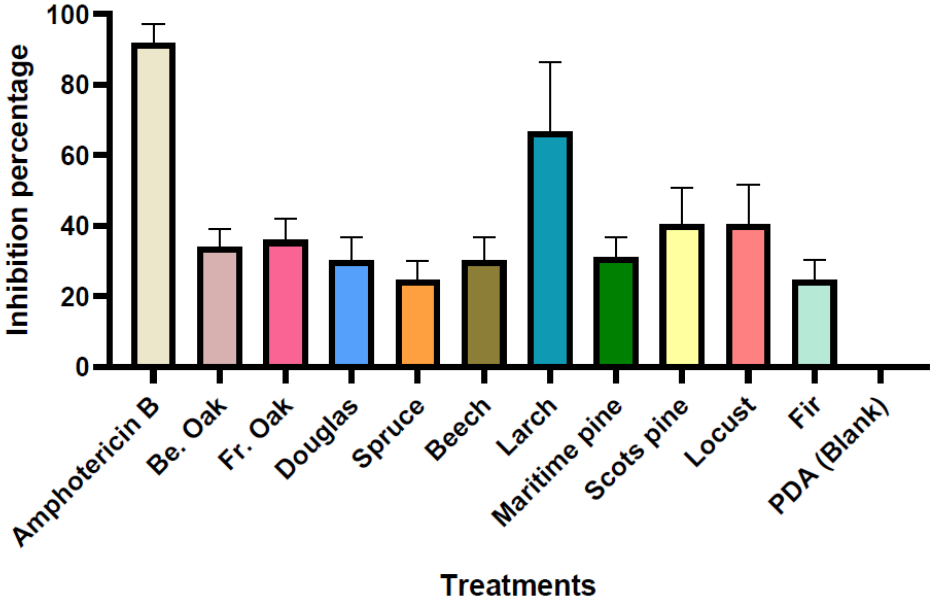


Blank



Locust: 80% inhibition

Percentage inhibition of *Aspergillus flavus* infection mycelial growth by hydroethanolic extracts



The hydroethanolic extract of larch showed significant antifungal activity, with 66.65% inhibition of the growth of *A. flavus* infection.

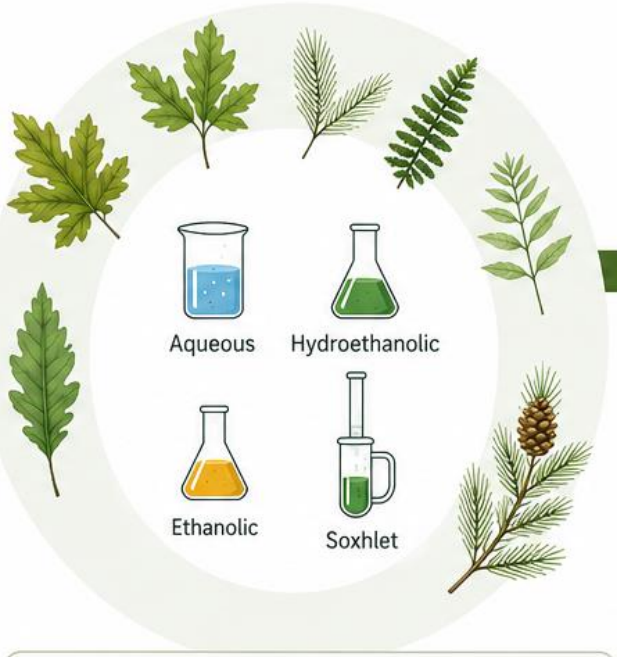
Fungicide Activity

	F. verticillioides	*A. flavus*
Black locust (aqueous)	65% Significant antifungal activity	–
Poplar (aqueous)	–	62% Significant antifungal activity
CO_2 extracts (all samples)	× Ineffective against both strains	× Ineffective against both strains
Scots pine (EO)	~ Moderate effect	+ Significant antifungal activity
Maritime pine (EO)	= Fungistatic effect (inhibits growth, not lethal)	= Fungistatic effect (inhibits growth, not lethal)

+ Significant antifungal activity
~ Moderate effect
= Fungistatic effect
× Ineffective

- **Screening**

40 extracts screened:
10 species, 4 extraction methods



- **3 Selection Criteria**

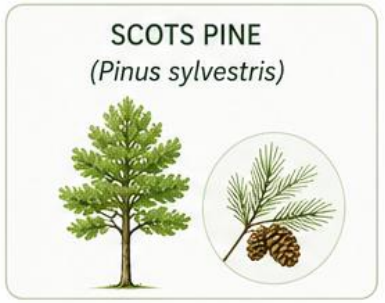
Extraction yields:
An extract with a low extraction yield can never become an economically viable solution.

Efficacy
VS commercial standards
(glyphosate and pelargonic acid, two commercial herbicides)

Resource availability

- **Selected for SCALE-UP: 2 extracts**

- **For in-depth laboratory-scale assays**



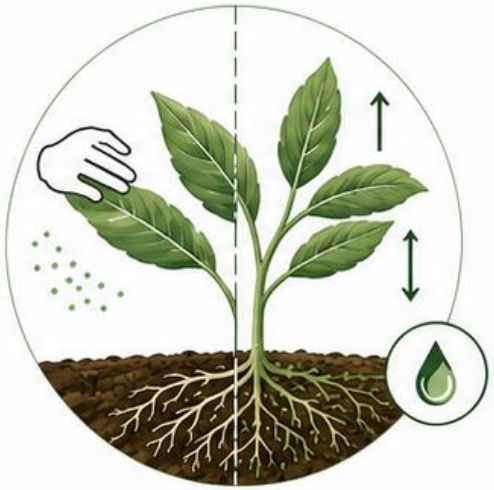
From Lab to Field

Scale-Up of Eco-Extraction and Targeted Biocidal Activities under Real-World Conditions

- Scale-up underway at CELABOR

Perspectives

Insecticidal assays of the different types of extracts against *Sitophilus granarius* (ongoing).

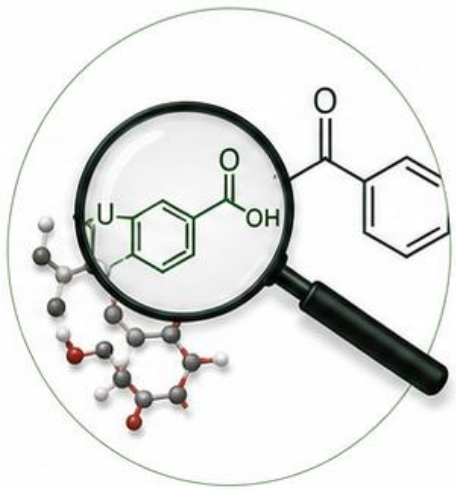


2

Study of the mode of action of the most promising extracts.

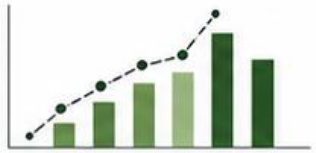
Contact activity

Systemic activity



3

Identifying the molecules responsible for the observed activities.



4

Field trials

Conclusion

- Turning forestry by-products into sustainable crop protection solutions may contribute to shaping a more resilient and environmentally friendly agriculture.
- Several aqueous and hydroethanolic extracts exhibited promising herbicidal and fungicidal activities, varying according to tree species and extraction process;
- These results highlight the potential of bark as a source of bio-based molecules with biocidal activity, while the project's integrated approach, supported by the diversity of the partners involved, enables bark valorization strategies that reconcile industrial constraints with agricultural needs.

Prof. Dr. Marie-Laure Fauconnier

Dr. Manon Genva

LIÈGE université
Gembloux
Agro-Bio Tech

Thanks !!!

pltaguimjeu@uliege.be

PROJECT PARTNERS

Financial partners

Methodological partners

Avec le soutien du Fonds européen de développement régional, de la Wallonie et du Ministère luxembourgeois de l'Environnement, du Climat et de la Biodiversité du Grand-Duché

THANK YOU FOR YOUR ATTENTION

Follow the latest news from the project

https://interreg-gr.eu/project/extrabark-fr/

ExtraBark