

# Optimization of *Gymnopilus penetrans* collection for the discovery of antimalarials : correlation between metabolic profile and harvesting location

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## INTRODUCTION

247 million malaria cases were estimated in 2021 with 625,000 deaths estimated. The emergence of resistance treatments is of great concern. As a result it is urgent to discover new antiplasmodial compounds. [1] Recently the CIRM Laboratory of Pharmacognosy discovered that *Gymnopilus penetrans* had promising antimalarial potential. Its active constituents are metabolites present in very limited quantities. [2] The hypothesis that the production of metabolites by this fungus depends on the geographical area and the properties of the environment in which it develops has therefore been put forward.

## OBJECTIVE

Discussing the importance of *Gymnopilus penetrans* collecting location and proposing priority harvest sites if proven relevant.



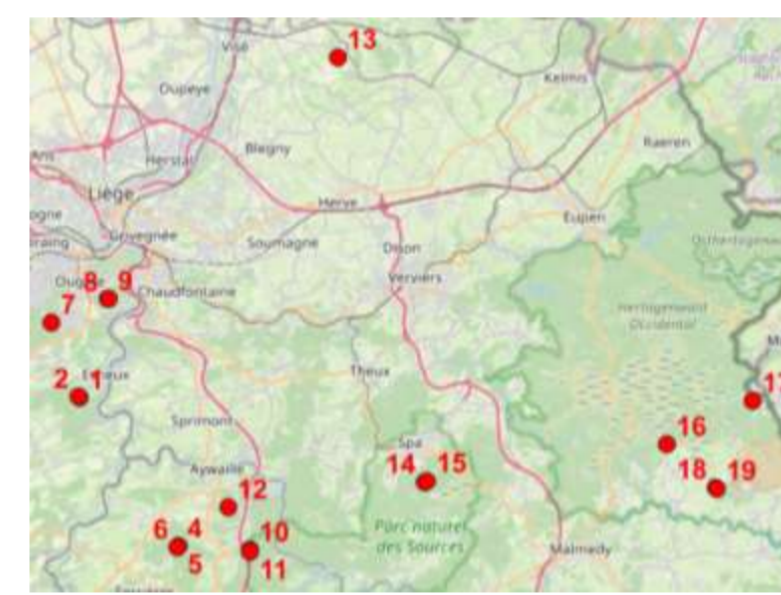
Figure 1 : *Gymnopilus penetrans*

## METHODS

### 1. Collection

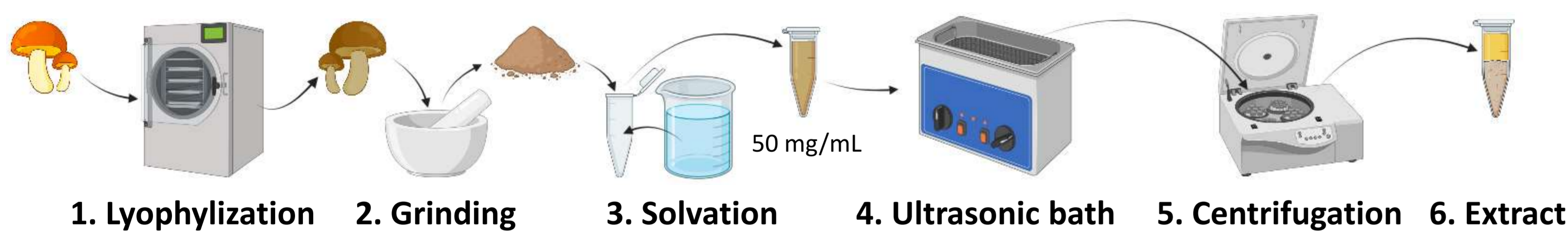
19 samples of *G. penetrans* were collected in different woods in the province of Liège. Afterwards they were frozen at -80°C.

**Data collected in the field** : date, place, GPS coordinates (with OsmAnd Maps®), substrate, altitude.



### 2. Extraction

**Solvent** : methanol

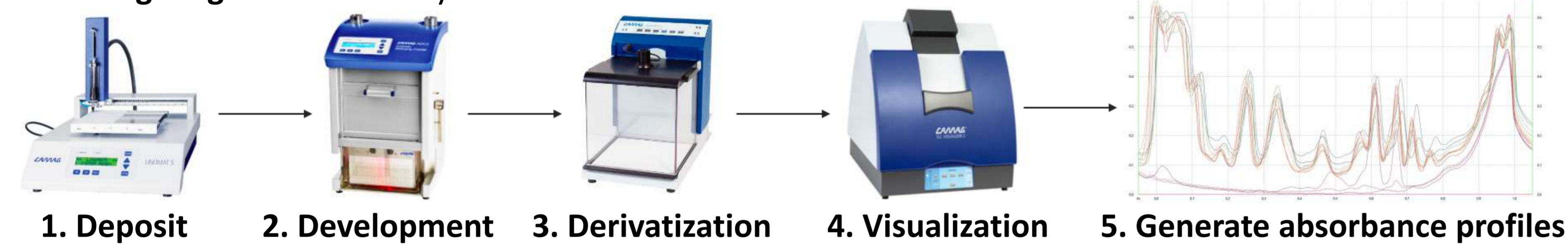


### 3. HPTLC

**Stationary phase** : HPTLC Silica gel 60 F<sub>254</sub>

**Mobile phase** : dichloromethane/methanol (5:1) and (2:1)

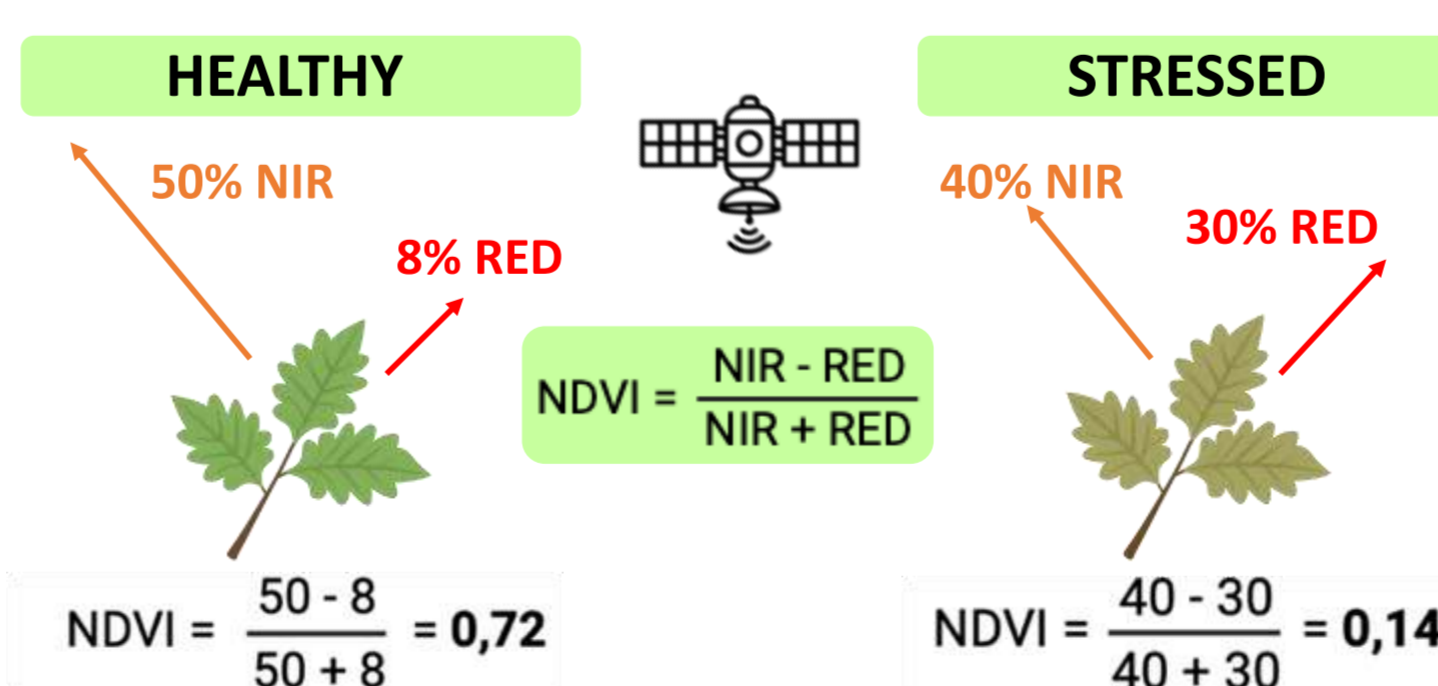
**Revealing reagent** : anisaldehyde



For each of the 19 samples, the absorbance of the compounds of interest previously isolated was measured and then compared with NDVI, altitude, substrate and harvest date.

### 4. Environmental analysis

NDVI (Normalised Difference vegetation Index) is the ratio that compares reflectance in the Red and Near Infrared bands. It varies from -1 to +1. A high NDVI indicates healthy vegetation. This index was calculated using SNAP software and multispectral satellite imagery (via the Sentinel-2 satellite of the European Copernicus program).



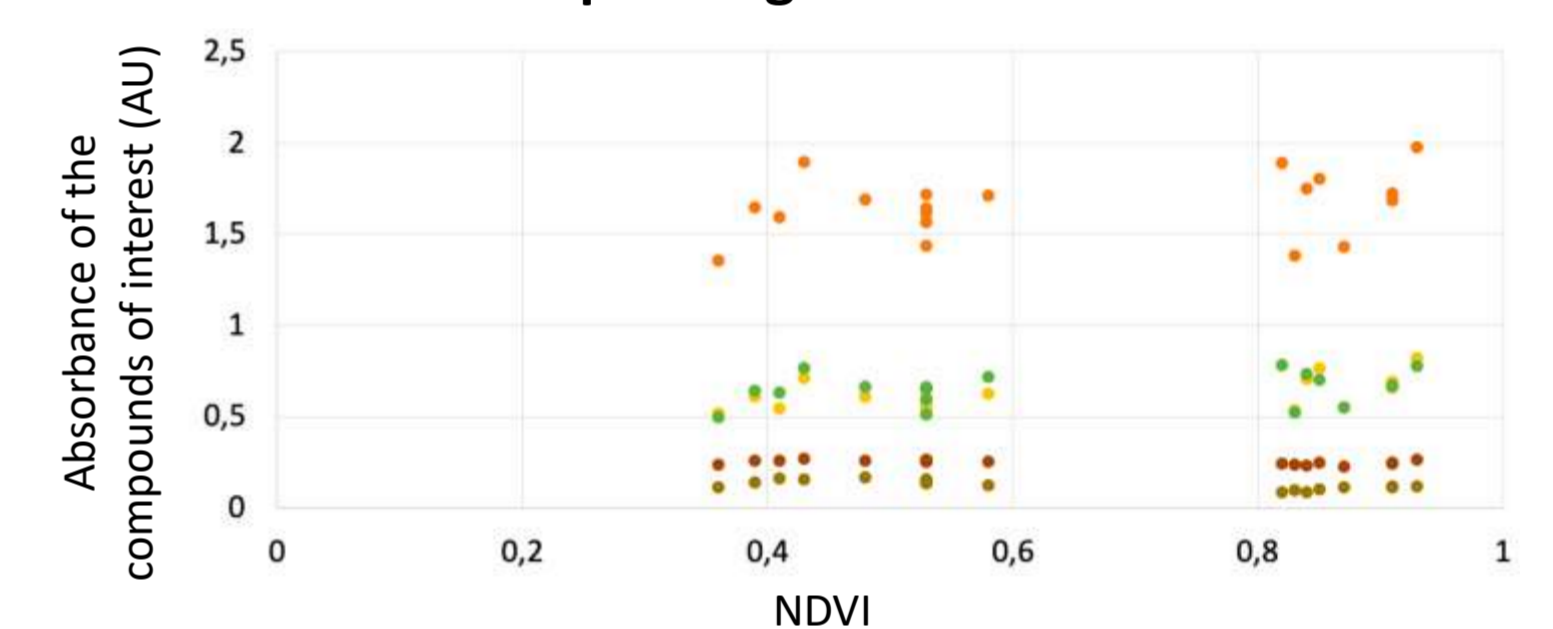
## RESULTS AND DISCUSSIONS

Compounds W, X, Y and Z are the active substances which had been previously isolated.

● W + X + Y + Z ● W ● X ● Y ● Z ● Y + Z

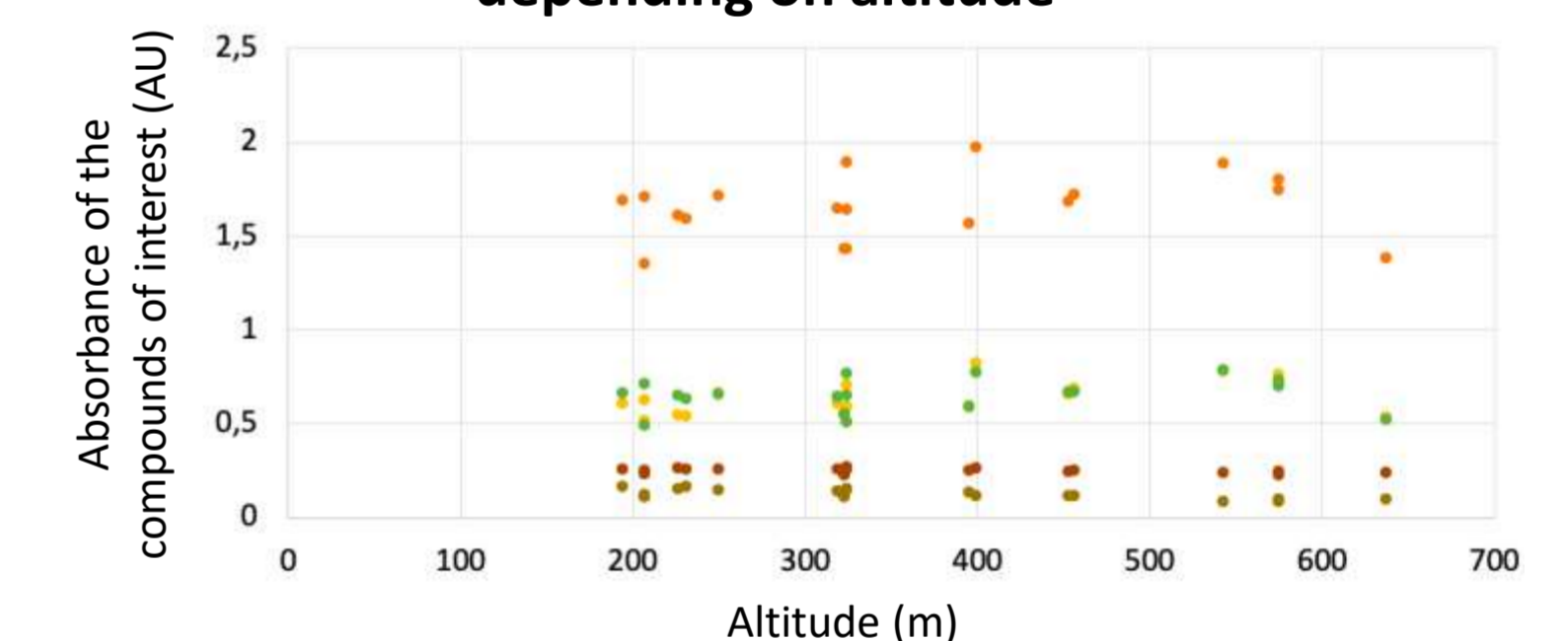
### 1. NDVI

Evolution of the absorbance of the compounds of interest depending on NDVI



### 2. Altitude

Evolution of the absorbance of the compounds of interest depending on altitude

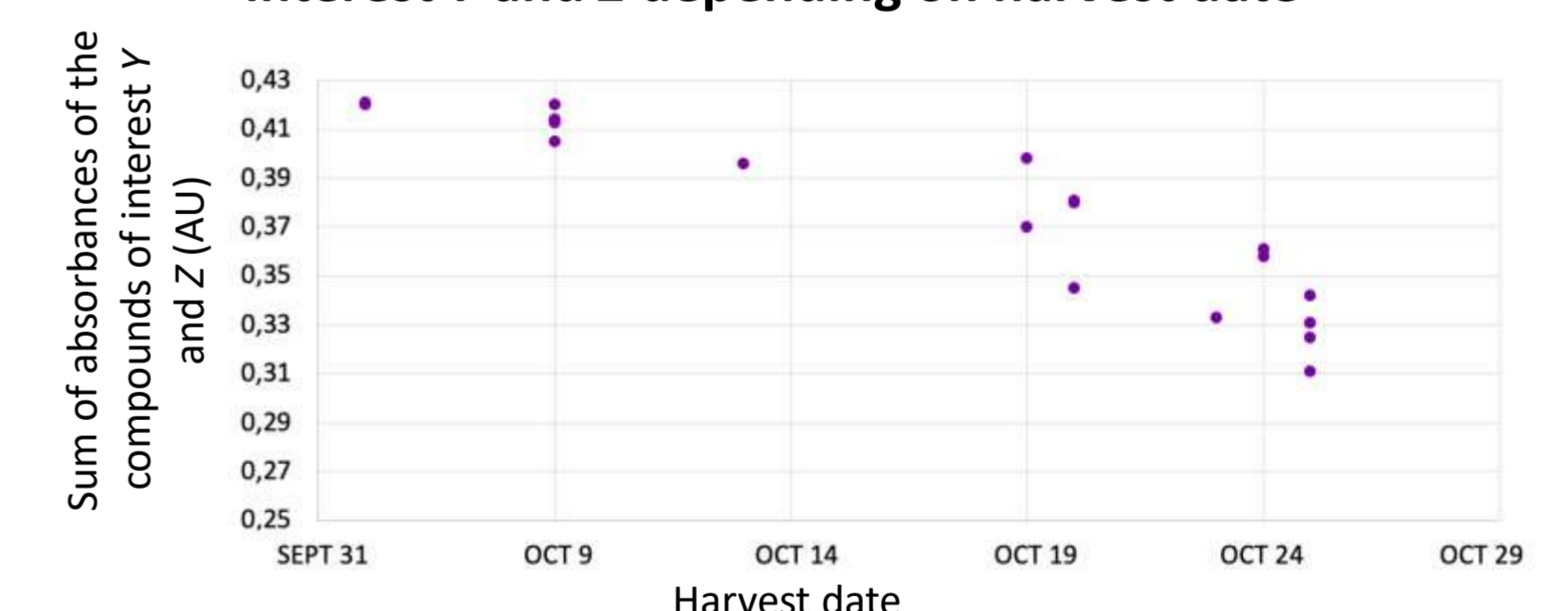


### 3. Substrate

For each substrate, the absorbances were in the same range.

### 4. Harvest date

Evolution of the sum of absorbances of the compounds of interest Y and Z depending on harvest date



The concentration of metabolites Y and Z could diminish with temperature and humidity.

## CONCLUSION AND PERSPECTIVES

There were variations in absorbances of the compounds of interest among the 19 samples, but there was not any relationship with NDVI, altitude nor substrate. However, it would be more interesting to harvest *G. penetrans* at the start of its collection season. Following this work, activity tests against *P. falciparum* could be carried out on the different extracts and on the compounds of interest in order to determine if there is a relationship between the activity and the metabolic profile or a relationship between the activity and the harvest environment. The results presented here suggest that the activity would be the same for each sample. If it is confirmed, samples of *G. penetrans* can be collected from any areas and then pooled together to obtain more fungal material. Fractionation can then be finalized in order to identify the active compounds.

## ACKNOWLEDGMENTS

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- (1) World malaria report 2022. Geneva: World Health Organization; 2022.
- (2) Clain, C.; Gils, M.; Ledoux, A.; Desdemoustier, P.; Turki, C.; Jérusalem, M.; Frédérick, M.; Castillo, G. Antiplasmodial Activity and Chemical Composition of Belgian Mushrooms, 2019.