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Expanding chemical space from *Strychnos variabilis*: Late-stage Functionalization Strategies with Emphasis on Photochemistry

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- *Strychnos variabilis* De Wild., a small deciduous tree found near Brazzaville and Kinshasa, contains minimal indoline alkaloids.
- Our lab isolated numerous rare flavonoids, including flavonol glycosides, over three decades ago.
- These flavonoids, especially in glycosidic form, are known for their enhanced antiviral activity.

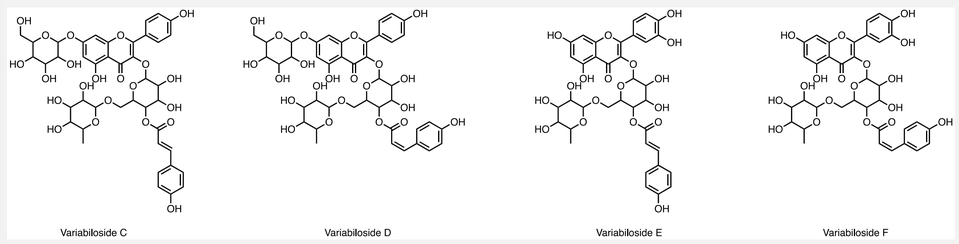

Figure 1: Structure of variabilosides C-F.

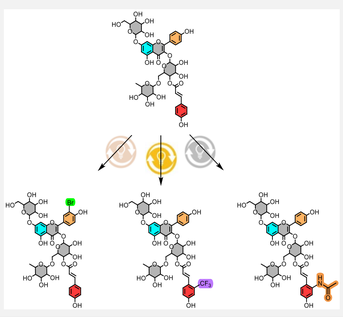

Figure 2: On a flavonoid scaffold, use of different photocatalyst/reactants gives access to specific introduction of moieties of interest.

- In drug discovery, late-stage functionalization (LSF) is a new strategy which gives access to chemoselective transformations on complex molecules without the need of protecting groups. LSF targets C–H bonds for generating new analogs, optimizing lead compounds, blocking metabolization, diversifying scaffolds, and tritium labeling.
- Amongst the available LSF methodologies, photocatalysis is based on light-driven single-electron-transfer processes and is compatible with sophisticated molecules, as demonstrated by a growing number of papers and reviews. Using specific photocatalysts and reactant enable selective introduction of moieties of interest in drug discovery, such as trifluoromethyl, hydroxyl or acetamide group, for instance.

- The dendrograms generated from our analysis reveal that the activity of the crude extract can be primarily attributed to the presence of variabilosides C and D. These compounds exhibit a high protection rate, demonstrating their significant contribution to the observed activity.
- Additionally, kaempferol 3-O-robinobioside and quercetine 3-O-robinobioside were found to moderately participate in the activity, with equivalent impact. Their activity highlights their role in the overall effectiveness of the extract.
- On the other hand, variabilosides E and F, which differ from C and D only in the genin (quercetine instead of kaempferol), exhibited very low activity levels.

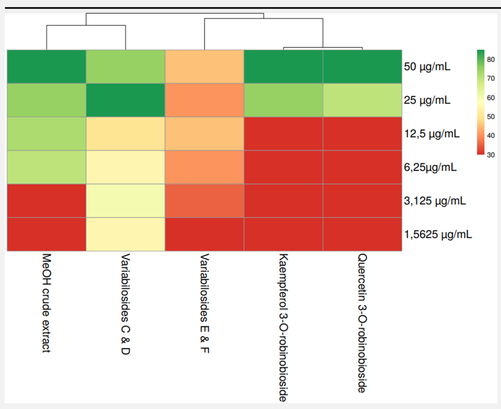

Figure 3: Heat map showing the percentage of living cells on day 5 post-infection of variabilosides C-F and the crude extract, with clustering of compounds/extract (red = low rate of living cells – green = high rate of living cells expressed in %).

Columns are clustered using Euclidean distances and Ward method.

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Objectives & strategies

Adopting late-stage functionalization (LSF) strategy through photochemistry is applied on *S. variabilis* extracts to chemodiversify the compounds, with a focus on flavonoids.

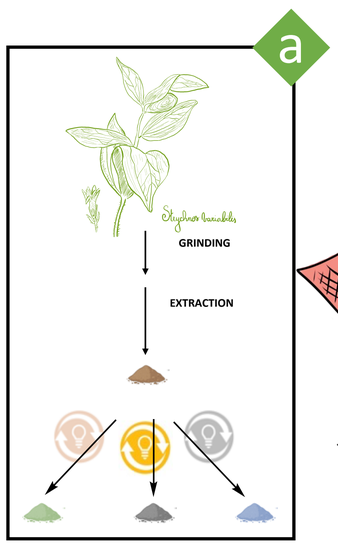

Figure 4: Application of LSF through photochemistry using specific photocatalysts/reagents on different *Strychnos* extracts investigated in our laboratory.

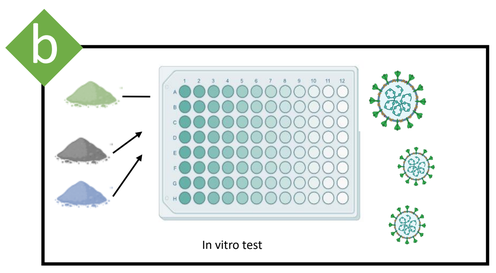

Figure 5: Exploration of FLS via RPC using a series of suitable catalysts (substrate=pure compound isolated from *S. variabilis*)

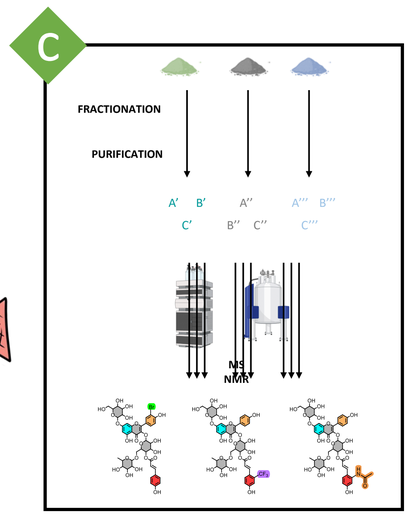

Figure 6: In parallel, pharmacological evaluation and structural identification of new products after fractionation.

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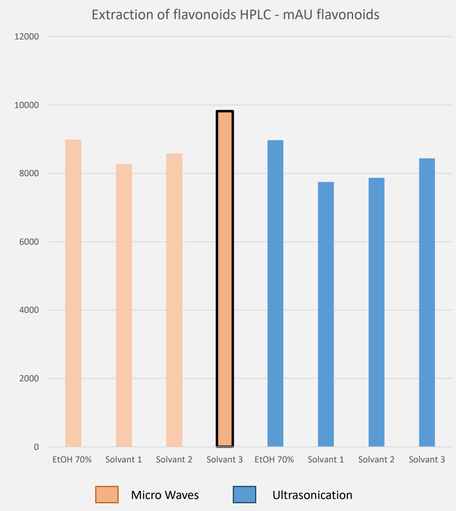
Flavonoids extraction optimization:

We have optimized the extraction of flavonoids using different solvents with two techniques (ultrasonication and microwave extraction). Using HPLC-UV, we determined that the best extraction was achieved with microwave.



Crude extract obtained after solvent evaporation under vacuum was dissolved in DMSO

Photochemodiversification

Reactions were performed on the extract using cross-coupling reactions with adaptive dynamic homogeneous catalysis. We applied the technique of adaptive dynamic homogeneous catalysis (AD-HoC) with nickel, typically used for general C(sp^2)–(hetero)atom coupling reactions under visible-light-driven redox reaction conditions, to the total plant extract.

Experimental conditions

- Extract : 500 mg of plant powder and 10 mL of solvent 3.
- **Catalyst**: 3 mL of MeOH plant extract (50mg/mL). We hypothesize that the chlorophyll content could potentially eliminate the need for an additional catalyst
- **Nucleophile additive**: No addition. We hypothesize that the molecule present in the extract could play this role.
- **Nickel concentration** : 0,1 mM
- Blue light (440 nm) using PR160L440 Kessil LED lamp.

In this preliminary work, different conditions were tested, changing the duration, the potency of the blue light and the solvent nature.

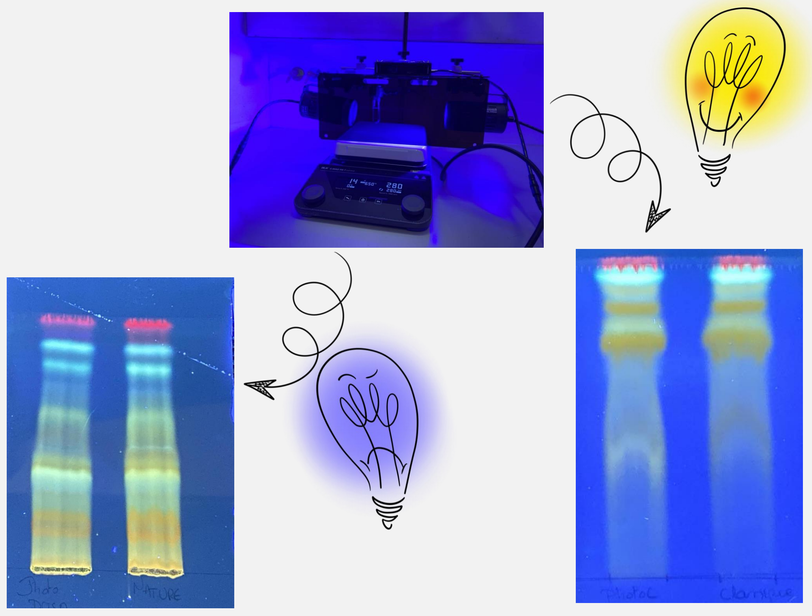


Figure 7: TLC for the chemodiversified extract vs initial extract sample

Based on TLC profiles, some of the tested conditions were linked with subtle variations of the chemical composition of the extracts.
However, the parallel pharmacological evaluation did not succeed to discriminate the activity of the modified extracts compared to the extracts before the chemodiversification.

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Communication of negative results in scientific research is usually considered as not crucial. However, this 'rule' is changing, especially with the rise of artificial intelligence. Such communication prevents the unnecessary duplication of efforts and saves time and resources for other researchers (you don't have to thank us ☺). Additionally, negative results contribute to the completeness of scientific knowledge. They also help improving methodologies and highlight limitations in experimental designs. Sharing these findings promotes transparency and accelerates overall scientific progress.
While it looks attractive, from a chemical point of view, to elucidate the structural variation obtained after certain conditions (as observed after TLC), such efforts may be regarded as useless considering how pharmacological activity has been conserved.
Surprisingly, flavonoids were found stable under 440 nm light irradiation.

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