INTERNATIONAL CONGRESS ON NATURAL PRODUCTS RESEARCH
Kraków 2024, July 13-17

LIÈGE université
Center for Interdisciplinary Research on Medicines

Discovering original antiplasmodial compounds through mass-guided exploration of the alkaloid diversity in the *Strychnos* genus

Olivier Bonnet[a,*], Mehdi A. Beniddir[b], Pierre Champy[b], Charline Leroy[a], Carla Hamann[a], Allison Ledoux[a], Alembert Tiabou Tchinda[a,c], Luc Angenot[a], Michel Frédérich[a]

[a] CIRM, Department of Pharmacognosy, University of Liège, Avenue Hippocrate 15, 4000 Liège, Belgium.
[b] Équipe « Chimie des Substances Naturelles », BioCIS, CNRS, Université Paris-Saclay, 17 avenue des Sciences, 91400 Orsay, France.
[c] Laboratory of Phytochemistry, Center for Research on Medicinal Plants and Traditional Medicine, Institute of Medicinal Research and Medicinal Plants Studies, Yaoundé P.O. Box 13033, Cameroon.

Introduction

Malaria

In 2022, 249 million cases and 608,000 deaths[1,2]

Parasitic disease transmitted by mosquitoes of *Anopheles* genus[2]

Caused by 6 parasites of *Plasmodium* genus: *P. falciparum*, *P. ovale*, *P. vivax*, *P. knowlesi*, *P. malariae*, and, the emergence of a new species, namely *P. simium*[3]

Increasing resistance to marketed antimalarial treatments[1,2]

Remains a major public health problem[1,2]

Search for new active compounds in the plant kingdom

Strychnos

Belongs to **Loganiaceae** family, and includes about **200 tropical and subtropical species**[4]

Plants well known for their **tetanizing and curarizing properties**[5,6]

A **variety of traditional uses** (snakebites, arthritis, asthma, diarrhea, fever, malaria, and many others)[2,6,7]

Contains **alkaloids**, mainly of the monoterpene indole type, some of which showed **promising antiplasmodial activities** (*e.g.* strychnogucine B, isostrychnopentamine, and sungucine)[5,6]

Objective

Unveiling and identify **original antiplasmodial metabolites** from various species of the ***Strychnos*** genus using **metabolomic tools**, as well as **bio- and mass-guided fractionation**

Methodology, results, and conclusions

Application n°1

Unidentified metabolites with **masses superior to 900 *m/z*** were observed in **alkaloidic and methanolic crude extracts from *S. usambarensis* leaves** (African species)

Antiplasmodial activities: IC_{50} = **3.388 ± 2.655 µg/mL**, and 18.50 ± 2.659 µg/mL for **methanolic** and alkaloidic extracts, respectively (Tests on 3D7 strain of *Plasmodium falciparum,* n=3)

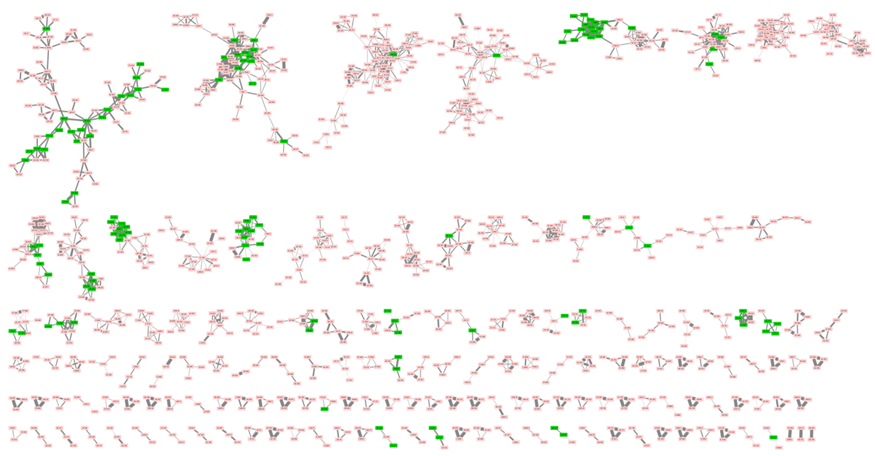
Targeting the metabolites with masses superior to 900 *m/z* using mass-guided fractionations

Isolation of the metabolite with a mass at 944 *m/z* by successively applying various purification methodologies:

1) **Preparative HPLC**:
PFP column; MeOH/Water + 0.1% formic acid
2) **Open column**:
Sephadex LH20®; MeOH
3) **HPLC-DAD**:
PFP column; MeOH/Water + 0.1% formic acid
4) **Open column**:
Sephadex LH20®; MeOH

Isolation of a metabolite with **a mass at 944 *m/z***, which is currently undergoing **structural elucidation**.

Compounds annotated using databases (GNPS, MIADB, …)



Generation of a molecular network from LC-HRMS/MS data of 44 crude extracts, and **screening of antiplasmodial activities** (Tests on 3D7 strain of *Plasmodium falciparum*, n=3)

Application n°2

Detection of **strychnine** in **7 species never described as strychnine producers** in the literature (trunk barks of *S. tricalysioides*, *S. camptoneura*, *S. congolana*, *S. boonei*, *S. densiflora*, *S. tchibangensis*, and leaves of *S. usambarensis*).

Additional TLC, HPLC-DAD, NMR, and UPLC-MS/MS analyses confirmed these identifications, and that **strychnine** was present in **trace amounts, except in *S. densiflora* trunk barks**.

SCAN ME
For more information about this study

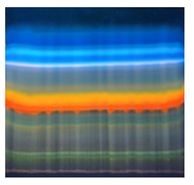
Proof of concept for metabolite exploration by **molecular networking**.

Application n°3

Selection of **dichloromethane (DCM) crude extract** (extraction with ammonia and dichloromethane) from ***S. longicaudata* trunk barks** (African species)

Antiplasmodial activities: IC_{50} = **4.944 ± 2.508 µg/mL**, and 13.73 ± 2.008 µg/mL for **dichloromethane** and methanolic extracts, respectively (Tests on 3D7 strain of *Plasmodium falciparum,* n=3)

Using bio- and mass-guided fractionations, unidentified metabolites were isolated by preparative HPLC and TLC, as well as open column



Stationary phases

PFP column	Silica gel 60	Silica gel 60

Mobile phases

MeOH/Water + 0.1% formic acid	MeOH/Sodium acetate 25%/Acetone (6.5:3.5:2 V/V/V)	DCM/MeOH (14:1, and 12:3 V/V)

Identification of **alstonine** (major compound) moderately active against malaria **+** Structural elucidation in progress of **8 unidentified compounds with promising antiplasmodial activities**.

Prospects

- **Interpretation of NMR and MS/MS data to determine the chemical structures of isolated compounds;**
- **Determination of IC_{50} values for the metabolite with a mass at 944 *m/z* contained in the leaves of *S. usambarensis*;**
- **Isolation of new metabolites from the other active fractions;**
- **Exploration of the other identifications suggested by the molecular network of the 44 crude extracts from *Strychnos* species.**

Contact

SCAN ME
Olivier.Bonnet@uliege.be
SCAN ME

References

[1] World Health Organization, 2024. World malaria report 2023. Available at https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2023 (Accessed July 02, 2024).
[2] Bonnet, O., Beniddir, M. A., Champy, P., Degotte, G., Mamede, L., Desdemoustier, P., Ledoux, A., Tchinda, A. T., Angenot, L., Frédérich, M, 2022. Unveiling antiplasmodial alkaloids from a cumulative collection of *Strychnos* extracts by multi-informative molecular networks. Frontiers in Molecular Biosciences, 9:967012.
[3] Brasil, P., Zalis, M. G., de Pina-Costa, A., Siqueira, A. M., Júnior, C. B., Silva, S., Areas, A. L. L., Pelajo-Machado, M., de Alvarenga, D. A. M., da Silva Santelli, A. C. F., Albuquerque, H. G., Cravo, P., Santos de Abreu, F. V., Peterka, C. L., Zanini, G. M., Suárez Mutis, M. C., Pissinatti, A., Lourenço-de-Oliveira, R., de Brito, C. F. A., de Fátima Ferreira-da-Cruz, M., Culleton, R., Daniel-Ribeiro, C. T., 2017. Outbreak of human malaria caused by *Plasmodium simium* in the Atlantic Forest in Rio de Janeiro: a molecular epidemiological investigation. The Lancet Global Health, 5(10), 1038-1046.
[4] World Flora Online, 2024. *Strychnos* L. Available at: https://www.worldfloraonline.org/taxon/wfo-4000036956 (Accessed July 02, 2024).
[5] Frédérich M., Hayette M. P., Tits M., De Mol P., Angenot L., 1999. *In vitro* activities of *Strychnos* alkaloids and extracts against *Plasmodium falciparum*. Antimicrobial Agents and Chemotherapy, 43(9), 2328-2331.
[6] Philippe G., De Mol P., Zèches-Hanrot M., Nuzillard J. M., Tits-Hanrot M., Angenot L., Frédérich M., 2003. Indolomonoterpenic alkaloids from *Strychnos icaja* roots. Phytochemistry, 62(4), 623-629.
[7] Dr. Duke's Phytochemical and Ethnobotanical Databases, US Dpt of agriculture. Available at: https://phytochem.nal.usda.gov/phytochem/search/list (Accessed July 02, 2024).

Acknowledgments
Etienne D. is greatly acknowledged for her help and her technical support.

CIRM ULiège
WEBSITE: www. cirm.uliege.be
CENTER FOR INTERDISCIPLINARY RESEARCH ON MEDICINES