

Phytochemical Profile and Biological Activity Evaluation of *Zanthoxylum heterophyllum* Leaves against Malaria

Allison Ledoux¹, Hinerava Maraetefau¹, Olivia Jansen¹, Delphine Etienne¹, Joëlle Quetin-Leclercq², Patricia Clerc³, Jacqueline Smadja³, Michel Frédérich¹

¹ Laboratoire de Pharmacognosie, University Liege, Center for Interdisciplinary Research on Medicines (CIRM), Liège, Belgium

² Pharmacognosy Research Group, Louvain Drug Research Institute, Université Catholique de Louvain, Brussels, Belgium

³ Laboratoire de Chimie des Substances Naturelles et des Sciences des Aliments (LCSNA), Université de la Réunion, Saint Denis, France

Abstract

The aim of this study was to evaluate the antiplasmodial properties of *Zanthoxylum heterophyllum*, an endemic plant from the Mascarene Islands. *In vitro* antiplasmodial activity of ethyl acetate and dichloromethane crude extracts obtained from leaf samples collected on Reunion Island was evaluated on the *Plasmodium falciparum* 3D7 chloroquine-sensitive strain using a colorimetric method. The major active compound was identified by chromatographic and spectroscopic methods. The best antiplasmodial activity was obtained for the ethyl acetate extract (15 µg/mL < IC₅₀ < 50 µg/mL). The major compound was identified as a sanshool derivative, an alkylamide compound that has moderate antimalarial activity (IC₅₀ = 11.3 µg/mL). This is the first report of the presence of a sanshool derivative in *Z. heterophyllum*. The moderate antiplasmodial activity of hydroxy-γ-isosanshool was demonstrated for the first time.

Key words

Zanthoxylum heterophyllum · Rutaceae · malaria · antiplasmodial activity · Réunion Island · sanshool

Supporting information available online at <http://www.thieme-connect.de/products>

According to the last World Malaria Report [1], there were an estimated 627 000 malaria deaths worldwide in 2012. Malaria is caused by a parasite *Plasmodium* sp. and transmitted by *Anopheles* mosquitoes. The problem of parasite resistance towards available medicines such as chloroquine is increasing. Natural products could play an important role in the discovery of new antimalarial drugs. Indeed, the vegetal kingdom is an important source of new pharmacological active compounds, especially for the search of new antimalarial drugs as reviewed by Bero and al. [2]. *Zanthoxylum heterophyllum* Sm. is an endemic plant from Reunion Island, which belongs to the Rutaceae family [3]. In the traditional pharmacopoeia of the island, *Z. heterophyllum*, commonly named “poivrier des hauts”, is used for the treatment of backaches [4]. As far as we know, pharmacological properties of this plant have not been studied before, except in a paper where methanol leaf and stem extracts showed free radical scavenging properties [5]. Nevertheless, some *Zanthoxylum* species are known to have antiplasmodial properties, such as *Zanthoxylum chalybeum* [6] and *Zanthoxylum zanthoxyloides* [7].

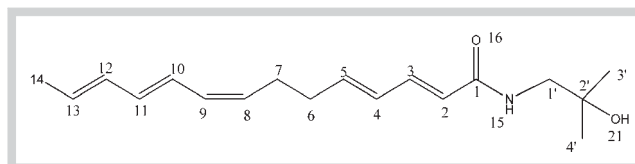


Fig. 1 Chemical structure of hydroxy-γ-isosanshool.

Results and Discussion

The major compound present in the ethyl acetate crude extract was identified as a sanshool derivative. Some sanshool derivatives were already described in *Zanthoxylum* sp., such as *Zanthoxylum piperitum* [8] and *Zanthoxylum integrifolium* [9]. By comparison of our NMR and MS data with literature data, it was identified as hydroxy-γ-isosanshool (Fig. 1), described by Chen et al. [9]. Ethyl acetate and dichloromethane crude extracts and hydroxy-γ-isosanshool (purity 90.58%) were tested *in vitro* against the *Plasmodium falciparum* 3D7 strain. In line with WHO guidelines and previous results from our team (Jansen et al. [10], Jonville et al. [11]), antiplasmodial crude extract activity was classified as follows: IC₅₀ ≤ 15 µg/mL, promising activity; IC₅₀ = 15–50 µg/mL, moderate activity; IC₅₀ > 50 µg/mL, weak activity; and at a level that cannot explain the existence of antiplasmodial activity in the plant: IC₅₀ > 100 µg/mL, inactivity.

The dichloromethane crude extract showed weak activity (77.8 ± 7.3 µg/mL), the ethyl acetate extract showed moderate activity (38.0 ± 11.3 µg/mL), and hydroxy-γ-isosanshool showed moderate activity for a pure compound (11.3 ± 1.5 µg/mL).

This is the first time that phytochemical and biological investigations are described for *Z. heterophyllum* and that hydroxy-γ-isosanshool, the major compound of the ethyl acetate extract, is described as an antiplasmodial compound. Our results indicate that this endemic plant has some potentialities as an antimalarial drug and that hydroxy-γ-isosanshool may play an important role in this activity.

Materials and Methods

Plant material: The leaves of *Z. heterophyllum* were collected on Reunion Island at Langevin and were identified by E. Boyer, Department of Biology, Université de la Réunion. A voucher specimen of the plant was deposited at the Université de La Réunion with the number RUN022F.

The leaves were oven-dried at 40 °C, ground following a standard process and then stored in a powder flask in an air-conditioned room.

Extraction and isolation: Dichloromethane and ethyl acetate crude extracts were obtained by macerating 5 g of dried leaves powder three times with 50 mL of solvent, under shaking for 30 min. After each maceration, the preparation was filtered and the residue was extracted under the same conditions. Filtrates obtained by each solvent were mixed and evaporated under reduced pressure.

The ethyl acetate crude extract was purified by preparative HPLC on a C-18 column using a binary solvent system with a flow rate of 30 mL/min: solvent A, acetonitrile, and solvent B, an HPLC grade aqueous solution of trifluoroacetic acid 0.05% (0–29 min, 10% A; 30–39 min, 40% A; 40–44 min, 60% A; 45–55 min, 80%

A). The preparative HPLC used was a Varian PrepStar 218 coupled with a DAD detector set at 408 nm (DAD ProStar 335 UV/Visible) and equipped with a fraction collector (440LC).

The purity of the major isolated compound was estimated on HPLC/UV/DAD using Hypersil ODS (C-18) columns (58 μ m, 4.6 \times 250 mm) with the same binary solvent system as described above, with a flow rate of 1 mL/min.

Identification: The major compound of the ethyl acetate fraction was identified by NMR and mass spectrometry. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance II 500 with TCI cryoprobe (^1H at 500 MHz and ^{13}C at 125 MHz) in CD_3OD . 2D experiments were performed using standard Bruker microprograms. ESI-MS was obtained on a Micromass Q-TOF microspectrometer in positive electrospray.

Antiplasmodial assays: Continuous culture of the *P. falciparum* chloroquine-sensitive (3D7) strain was maintained following the method of Trager and Jensen [12]. The strain was obtained from MR4 (MRA 102, ATCC, Manassas, Virginia, USA).

Each extract was dissolved in DMSO (Sigma) at a concentration of 10 mg/mL. The *P. falciparum* culture was placed in contact with a set of eight twofold dilutions of each extract in medium (final concentrations ranging from 0.8 to 100 $\mu\text{g}/\text{mL}$ and final DMSO concentration $\leq 1\%$) on two columns of a 96-well microplate for 48 h, as described by Jansen and al. [10]. Parasite growth was estimated by the determination of plasmodial lactate dehydrogenase activity as previously described [13]. Artemisinin (98%, Sigma-Aldrich) was used as a positive control (IC_{50} 0.004 $\mu\text{g}/\text{mL}$). Each extract was tested in triplicate on three different plates ($n = 3$). IC_{50} values were calculated by linear regression.

Supporting information

A chromatogram as well as ^1H and ^{13}C NMR and EI-MS data of hydroxy- γ -isosanshool are available as Supporting Information.

Acknowledgements

This work was financially supported by the project FEDER BIOMOLTCN (Activités Thérapeutiques, Cosmétologiques et Nutraceutiques de molécules issues de la Biodiversité terrestre, marine et microbienne de la zone Sud-Ouest de l'Océan Indien) and partially by the F.R.S.-FNRS (grant N° T.0190.13). The authors wish to thank J.N. Wauters for his technical assistance and J.C. van Heugen for recording MS spectra. We thank MR4 for providing us malaria parasites contributed by D. Carucci.

Conflict of Interest

The authors declare no conflict of interest.

References

- 1 World Health Organization. World Malaria Report 2013. Geneva: WHO; 2013: 256
- 2 Bero J, Frédérick M, Quetin-Leclercq J. Antimalarial compounds isolated from plants used in traditional medicine. *J Pharm Pharmacol* 2009; 61: 1401–1433
- 3 Coode M. 65. Rutacées. Flore des Mascareignes: la Réunion, Maurice, Rodrigues. Mauritius: The Sugar Industry Research Institute; 1979: 1–37
- 4 Lavergne R. Tisaneurs et Plantes Médicinales Indigènes – l'île de la Réunion. Saint Denis: Editions Orphie; 1990
- 5 Poullain C, Girard-Valenciennes E, Smadja J. Plants from Reunion Island: evaluation of their free radical scavenging and antioxidant activities. *J Ethnopharmacol* 2004; 95: 19–26
- 6 Muganga R, Angenot L, Tits M, Frédérick M. Antiplasmodial and cytotoxic activities of Rwandan medicinal plants used in the treatment of malaria. *J Ethnopharmacol* 2010; 128: 52–57
- 7 Adebayo JO, Krettli AU. Potential antimalarials from Nigerian plants: a review. *J Ethnopharmacol* 2011; 133: 289–302
- 8 Jang KH, Chang YH, Kim DD, Oh KB, Oh U, Shin J. New polyunsaturated fatty acid amides isolated from the seeds of *Zanthoxylum piperitum*. *Arch Pharm Res* 2008; 31: 569–572
- 9 Chen IS, Chen TL, Lin WY, Tsai IL, Chen YC. Isobutylamides from the fruit of *Zanthoxylum integrifoliolum*. *Phytochemistry* 1999; 52: 357–360
- 10 Jansen O, Angenot L, Tits M, Nicolas JP, De Mol P, Nikiéma JB, Frédérick M. Evaluation of 13 selected medicinal plants from Burkina Faso for their antiplasmodial properties. *J Ethnopharmacol* 2010; 130: 143–150
- 11 Jonville MC, Kodja H, Strasberg D, Pichette A, Ollivier E, Frédérick M, Angenot L, Legault J. Antiplasmodial, anti-inflammatory and cytotoxic activities of various plant extracts from the Mascarene Archipelago. *J Ethnopharmacol* 2011; 136: 525–531
- 12 Trager W, Jensen JB. Human malaria parasites in continuous culture. *Science* 1976; 193: 673–675
- 13 Kenmogne M, Prost E, Harakat D, Jacquier MJ, Frédérick M, Sondengam LB, Zèches M, Waffo-Téguo P. Five labdane diterpenoids from the seeds of *Aframomum zambesiacum*. *Phytochemistry* 2006; 67: 433–438

received December 5, 2014

revised December 5, 2014

accepted February 4, 2015

Bibliography

DOI <http://dx.doi.org/10.1055/s-0035-1545821>

Planta Med Lett 2015; 2: e10–e11

© Georg Thieme Verlag KG Stuttgart · New York ·
ISSN 2199-157X

Correspondence

Prof. Dr. Frédérick Michel

University of Liège
Laboratory of Pharmacognosy
Department of Pharmaceutical Sciences
Avenue de l'Hôpital 1
4000 Liège
Belgium
Phone: + 32 43 66 43 30
Fax: + 32 43 66 43 32
M.Frederich@ulg.ac.be

License terms

