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

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

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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 < IC_{50} < 50 µg/mL). The major compound was identified as a sanshool derivative, an alkylamide compound that has moderate antimalarial activity (IC_{50} = 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 activitiy · 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].

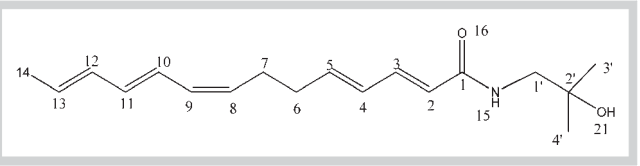

Fig. 1 Chemical structure of hydroxy-γ-isosanshool.

Results and Discussion

The major compound present in the ethyl acetate crude extract was identified as a sanshool derivative. Some sanshool derivates were already described in *Zanthoxylum* sp., such as *Z.anthoxylum piperitum* [8] and *Z.anthoxylum integrifoliolum* [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: IC_{50} ≤ 15 µg/mL, promising activity; IC_{50} = 15–50 µg/mL, moderate activity; IC_{50} > 50 µg/mL, weak activity; and at a level that cannot explain the existence of antiplasmodial activity in the plant: IC_{50} > 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 potenzialities 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 µm, 4.6 × 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. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance II 500 with TCI cryoprobe (^{1}H 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 µg/mL and final DMSO concentration ≤ 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 µg/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 ^{1}H and ^{13}C NMR and EI-MS data of hydroxy-γ-isosanshool are available as Supporting Information.

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Conflict of Interest

The authors declare no conflict of interest.

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