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 antimalarial properties of Zanthoxylum heterophyllum, an endemic plant from the Mascarene Islands. In vitro antimalarial 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 antimalarial 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 (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 report of the presence of a sanshool derivative in Z. heterophyllum. The moderate antimalarial activity of hydroxy-γ-isosanshool was demonstrated for the first time.

Key words
Zanthoxylum heterophyllum · Rutaceae · malaria · antimalarial activity · Réunion Island · sanshool

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

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 [10, Jonville et al. [11]], antimalarial 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 antimalosomal 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 antimalarial 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 RUNO22F.

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. 1H and 13C NMR spectra were recorded on a Bruker Avance II 500 with TCI cryoprobe (1H at 500 MHz and 13C at 125 MHz) in CD3OD. 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 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 (IC50 0.004 µg/mL).

**Supporting information**

A chromatogram as well as 1H and 13C NMR and EI-MS data of hydroy-y-isosanshool are available as Supporting Information.

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**Conflict of Interest**

The authors declare no conflict of interest.