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 dichromethane 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

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.

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.

Ethanol 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 ethyl acetate and dichloromethane crude extracts 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-isosanshoool showed moderate activity for a pure compound (113 ± 1.5 µg/mL).

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]), 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 antimalarial 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 (113 ± 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.

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

**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 (IC50 0.004 µg/mL).

IC50 values were calculated by linear regression.

**Supporting information**

A chromatogram as well as 1H and 13C NMR and EI-MS data of hydrosolvent 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 (IC50 0.004 µg/mL).

Each extract was tested in triplicate on three different plates (n = 3). IC50 values were calculated by linear regression.

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**Conflict of Interest**

The authors declare no conflict of interest.