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Antiplasmodial activity of *Mezoneuron benthamianum* leaves and identification of its active constituents

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ABSTRACT

Ethnopharmacological relevance: Decoctions of the leaves of *M. benthamianum* Baill. are used by traditional healers in Guinea to treat malaria and this use was validated by a preliminary clinical assay.
Aim of the study: To evaluate the *in vitro* antiplasmodial activity and to identify active compounds from extracts of *M. benthamianum* leaves.
Material and methods: Antiplasmodial activity of extracts, fractions and pure compounds was evaluated *in vitro* against a chloroquine-sensitive strain of *Plasmodium falciparum* (3D7) using the measurement of the plasmodial lactate dehydrogenase activity. Selectivity of extracts and purified compounds for *Plasmodium* parasites was evaluated by using WST-1 test on HeLa human cells. Compounds were isolated using normal phase silica gel column chromatography and prepHPLC and their structures elucidated using extensive spectroscopic analysis.
Results: Hydroethanolic extracts (70% v/v) of *M. benthamianum* leaves showed a moderate *in vitro* activity against *P. falciparum* 3D7, with IC_{50} in the range 22.5 – 32.6 µg/mL, depending on the batch; while a dark precipitate formed during ethanol evaporation showed higher activity (IC_{50} =6.5 µg/mL). The fractionation was performed on this most active fraction and was followed by *in vitro* antiplasmodial assay. Active compounds (5, 7, 8) belong to several phytochemical classes, contributing together to the global antiplasmodial activity of the hydroethanolic extract against *P. falciparum* parasite. This study finally allowed the isolation of three diterpenes including two new compounds named Mezobenthamic acids A (1) and B (2) and neocaesalpin H (3), as well as quercetin (4), kaempferol (7), resveratrol (6), gallic acid (9) and its ethylester (5), β-sitosterol glucoside (10) and 13b-hydroxy-pheophorbide a (8).
Conclusion: This study gives some concrete evidence to support the ethnopharmacological use of *Mezoneuron benthamianum* leaves extract in the management of malaria. The active compounds can be further studied for their antiplasmodial potential, as well as their suitability to be used as quality markers for the standardization of this herbal drug from the Guinean traditional pharmacopeia.

1. Introduction

Malaria, a parasitic disease caused by *Plasmodium* sp. and transmitted by *Anopheles* mosquitoes, remains a huge public health problem in Sub-Saharan African countries. Despite a significant reduction in the global incidence of malaria in the last 15 years, there were still an estimated 214 millions new malaria cases worldwide in 2015, causing an estimated 438,000 deaths, of which 90% occurs in Africa, mostly by under–five children. In West Africa, about 345 million people from 17 countries are living in area at risk for malaria, almost exclusively due to *P. falciparum* (WHO, 2015). In Guinea, malaria cases represent about 40% of medical consultations.

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The low accessibility of the local population to conventional medicines leads people to use traditional remedies to cure malaria. In this context, the plants used in traditional medicine deserve some attention because they can contain some compounds active against *Plasmodium* parasite and their study can be of first interest in the discovery of new antimalarials. Furthermore, there is an urgent need of new treatment, as the parasite is becoming resistant to several conventional drugs including a threat of resistance to artemisinins, the actual mainstay in world malaria fighting (Lubell et al., 2014). At the other hand, and particularly in sub-saharan countries, active and non toxic plants could also be valorized with success in standardized ITM (Improved Traditional Medicines) that constitute available and affordable treatment for local population (Willcox, 2011; Willcox et al., 2012).

Mezoneuron benthamianum Baill., also named *Caesalpinia benthamiana* (Baill.) Herend. and Zarucchi (Fabaceae family), is a shrub or woody climber growing in West Africa. This plant is used in traditional medicine for the management of several diseases such as ulcers, wounds, skin disease, in different countries (Osho, 2014). In Guinea, the leaves are used to treat malaria as attested by an ethnobotanical survey (Traoré et al., 2013) and the plant was selected in 2006 by the Research and Valorization Center on Medicinal Plants of Dubreka for preclinical ethnomedical investigations (unpublished data). Evaluated on two groups of 21 childrens (5–15 years, *versus* chloroquine), an hydroethanolic leaves extract showed promising results regarding to its efficacy (92% of reduction of parasitic load in ITM group at day 28, *versus* 91% for chloroquine) as well as its tolerance. Moreover, a methanolic leaf extract was already shown to display a good antiplasmodial activity (close to 5 µg/mL) with a good selectivity index (> 11) in an *in vitro* antiprotozoal screening (Traoré et al., 2014a).

Mezoneuron benthamianum is already known to contain gallic acid derivatives (Binutu and Cordell, 2000; Zamble *et al.*, 2008), cassane diterpenoids (Dickson et al., 2007, 2012), flavonoids, tannins and anthraquinones (Osho, 2014), while different pharmacological properties such as antibacterial (Binutu and Cordell, 2000; Dickson et al., 2006, 2007, 2012), anti-diarrhoeal, analgesic, antipyretic and anti-inflammatory (Mbagwu et al., 2007,2008), anticandidal (Scott et al., 2012) and antioxidant (Dickson et al., 2006 and 2007; Scott et al., 2012) activities are also described for some extracts of this plant.

The aim of this study is to give concrete evidence to support the ethnopharmacological use of *Mezoneuron benthamianum* leaves against malaria by testing some extracts on a *P. falciparum in vitro* model. A bioactivity-guided fractionation was undertaken on *Mezoneuron benthamianum* leaves extracts, which leaded to the identification of the compounds responsible for the antimalarial activity, the discovery of two new diterpenes, as well as the identification of quality markers for this herbal drug used in traditional medicine.

2. Materials and methods

2.1. Plant material

The leaves of *M. benthamianum* were harvested in Dubreka - Guinea in May 2005 and June 2008 (respectively batch 1 and 2). The plant was authenticated and voucher specimen deposited at the Department of Botanic of the Research and Valorization Center on Medicinal Plants of Dubreka (N° 27HK411) and at the Laboratory of Pharmacognosy – University of Liege. Only batch 1 was considered for extensive phytochemical study.

The plant name has been checked with www.theplantlist.org In this database, *Mezoneuron benthamianum* Baill. is listed as a synonym for *Caesalpinia benthamiana* (Baill.) Herend. and Zarucchi. (http://www.theplantlist.org/tpl1.1/record/ild-41661).

However, in two other well-known databases previously consulted (Tropicos and African plant database), *M. benthamianum* Baill. is recorded as the basionym / accepted name (http://www.tropicos.org/Name/50067342; http://www.ville-ge.ch/musinfo/bd/cjb/africa/details.php*langue=an & id=70999); while *Caesalpinia benthamiana* (Baill.) Herend. and Zarucchi is listed as a synonym / rejected name (http://www.tropicos.org/Name/50165960; http://www.ville-ge.ch/musinfo/bd/cjb/africa/details.php*langue=an & id=63913). Moreover, according to the recommended database "Medicinal Plant Names Service" from Kew Royal Botanic Gardens, (http://mpns.kew.org/mpns-portal/searchName*) both names are listed as "accepted" and as synonyms of each other.

Because of this unclear situation and in the aim to stay in accordance with previous studies of our team with this plant, we choose to keep the name *Mezoneuron benthamianum* for the present study.

2.2. In vitro *antiplasmodial activity*

Activity against *P. falciparum* chloroquine-sensitive 3D7 strains was assessed following the procedure already described in Frédérich et al. (2002). The parasites were obtained from Prof. Grellier (Museum d'Histoire Naturelle, Paris, France). Each compound, fraction and extract was applied in a series of eight 2-fold dilutions (final concentrations ranging from 0.8 to 100 µg/mL for an extract and from 0.08 to 10 µg/mL for a pure substance) on two rows of a 96-well microplate and were tested in triplicate (n =3). Parasite growth was estimated by determination of lactate dehydrogenase activity as described previously in Jonville et al. (2013). Artemisinin (98%, Sigma-Aldrich) was used as positive control.

2.3. Cytotoxicity assay

Human HeLa cells were maintained in continuous culture in a humid atmosphere at 37 °C and 5% CO_2 in DMEM medium (Bio-Whittaker-LONZA), supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine (200 mM), and penicillin (100 IU/mL)–streptomycin (100 µg/mL) (Pen-Strep) (Bio-Whittaker-LONZA). The assays followed the procedure already described in Jonville et al. (2013). The viability of the cells was determined by adding WST-1 (Roche) tetrazolium salt as a cytotoxicity indicator and by reading the absorbance at 450 nm with a scanning multiwell spectrophotometer after about 1 h. Camptothecin (95% HPLC, Sigma–Aldrich) was used as a positive control. The selectivity index (SI) value allows the evaluation of the selective activity of the extracts/pure compound against the parasite compared to its toxicity for human cells. The SI value is calculated as the ratio between cytotoxic IC_{50} values and 3D7 parasitic IC_{50} values.

2.4. Chromatographic experimental procedures

HPLC: Chromatographic elution was carried out at a flow rate of 0.55 mL/min using 0.1% (v/v) formic acid in water as solvent A and 0.1% (v/v) formic acid in acetonitrile as solvent B with a linear gradient from 10% B to 90% B in 20 min, and held for 5 min.

TLC and column chromatography: Analytical TLC was performed on precoated Si gel F_{254} (Merck 1.05735) plates. After development, the dried plates were examined under 254 nm and 366 nm UV light and sprayed with vanillic acid. LiChroprep Si 60 (43–60 µm, Merck 9336) and sephadex LH-20 (Sigma-Aldrich) were used for column chromatography.

Prep-HPLC: The Prep-HPLC used was a Varian PrepStar 218 coupled with a DAD detector set at 408 nm (DAD ProStar 335 UV/Vis) and equipped with a fraction collector (440LC). The column used was the LichroPrep 100 RP-18 (12 µm, Merck).