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Antiplasmodial, anti-complement and anti-inflammatory *in vitro* effects of *Biophytum umbraculum* Welw. traditionally used against cerebral malaria in Mali

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ABSTRACT

Ethnopharmacological relevance: *Biophytum umbraculum* Welw. (Oxalidaceae) is a highly valued African medicinal plant used for treatment of cerebral malaria, a critical complication of *falciparum* malaria.
Aim of the study: To provide additional information about traditional use of *B. umbraculum* and to test plant extracts and isolated compounds for *in vitro* activities related to cerebral malaria.
Materials and methods: The traditional practitioners were questioned about indication, mode of processing/application, dosage and local name of *B. umbraculum*. Organic extracts and some main constituents of the plant were investigated for anti-malaria, anti-complement activity and inhibition of NO secretion in a RAW 264.7 cell line.
Results: Treatment of cerebral malaria was the main use of *B. umbraculum* (fidelity level 56%). The ethyl acetate extract showed anti-complement activity (ICH_{50} 5.7 ± 1.6 μg/ml), inhibition of macrophage activation (IC_{50} 16.4 ± 1.3 μg/ml) and *in vitro* antiplasmodial activity (IC_{50} K1 5.6 ± 0.13 μg/ml, IC_{50} NF54 6.7 ± 0.03 μg/ml). The main constituents (flavone *C*-glycosides) did not contribute to the activity of the extract.
Conclusion: Inhibition of complement activation and anti-inflammatory activity of *B. umbraculum* observed in this study might be possible targets for adjunctive therapy in cerebral malaria together with its antiplasmodial activity. However, clinical trials are necessary to evaluate the activity due to the complex pathogenesis of cerebral malaria.

1. Introduction

The annual herb *Biophytum umbraculum* Welw. (Oxalidaceae) (syn. *Biophytum petersianum* Klotzsch) commonly found in tropical and subtropical Africa and Asia, is a highly valued medicinal plant used against cerebral malaria (CM) in African countries (Burkill, 2000; Gronhaug et al., 2008; Lye et al., 2008). CM is one of the most severe complications of *Plasmodium falciparum* infection and accounts for a significant proportion of malaria mortality (Higgins et al., 2011). The overall incidence of CM was found to be 1.12% in endemic areas of Africa (Idro et al., 2010), however, children < 5 years have a much higher risk of developing this condition (Ikome et al., 2002). The fatality rate associated with CM is particularly high in African children, 18%, even when optimal anti-malarial treatment is received (Higgins et al., 2011). Besides, up to 25% of the individuals that recover from CM will suffer from neurological and cognitive deficits. This suggests that strategies directed to adjuvant therapy should be considered to reduce mortality and complications of CM (Grau and Craig, 2012; Higgins et al., 2011; Ngoungou et al., 2007).

The pathogenesis of CM is complex. Current theories emphasize that excessive and dysregulated inflammation, endothelial activation and upregulation of coagulation pathways are the three main processes involved. Trying to counteract these processes, several conventional adjunctive therapies have been tested in clinical trials the last five decades (John et al., 2010). Inhibitors of cytoadherence, immunomodulators, arginine/NO supplementation

Abbreviations: CM, cerebral malaria; EtOAc, ethyl acetate; MeOH, methanol; BuOH, butanol; DCM, dichloromethane; SI, Selectivity Index; RAW 264.7, murine macrophage cell line; NO, Nitric Oxide

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and neuroprotective agents have been tested, however, sadly without clear beneficial outcome (Jain et al., 2013).

Traditional medicine has given us, among others, the successful anti-malarial drugs quinine and artemisinin (Phillipson and Wright, 1991). Potentially, traditional medicine could also provide us with new leads for treating CM. So far, *Azadirachta indica* A. Juss (Meliaceae) and *Curcuma longa* L. (Zingiberaceae) have shown some promising results as adjuvant therapy due to their immunomodulating properties (Bedri et al., 2013; Jain et al., 2013; Mimche et al., 2011). Plant extracts and natural products have been shown to also act as inhibitors of the complement system (Kulkarni et al., 2005). This system plays a key role in CM, initiating and augmenting the innate immune responses, including inflammation, endothelial activation, opsonization and coagulation. Together, these mechanisms can result in blood-brain barrier dysfunction and neuronal injuries and thus contribute to the clinical characteristics of CM (Biryukov and Stoute, 2014; Silver et al., 2010). Complement activation has recently been shown to correlate with disease severity (Berg et al., 2015; Goto and Sanchez, 2013).

The high consensus reported here for the use of *B. umbraculum* against CM might indicate the presence of active substances important for reducing the inflammation seen in cerebral malaria. This study therefore aims to (i) acquire more information about how the plant material is prepared and administered, (ii) carry out a preliminary screening of extracts and pure compounds from *B. umbraculum* that can contribute to improved knowledge about the traditional use against cerebral malaria.

2. Materials and methods

2.1. Interviews with traditional practitioners in Mali

Structured interviews took place in Bamako, Siby and Dioïla in Mali in February 2011. A total of 38 traditional practitioners traditional practitioners were interviewed; 25 men and 13 women. The traditional practitioners were asked about local name, indications for use, which plant part that was employed, how the plant material was prepared, and how the preparation was administered and dosed. Interviews were carried out individually in the local language, mostly Bambara, taking 15–20 min. The local botanist ensured that the traditional practitioners were talking about the correct plant. Before being interviewed, the traditional practitioners were informed about why the interviews were carried out and how the information would be used. The biodiversity rights for indigenous people are protected trough the Nagoya convention.

2.2. Plant material, extracts and isolated compounds

Aerial parts of *B. umbraculum* were collected in Blendio, Mali (11°36′48″N, 6°20′40″W). The plant name has been checked against www.theplantlist.org, 16.10.2015. A voucher specimen has been deposited under no. 2653 in the herbarium at the Department of Traditional Medicine, Bamako. The plant material was extracted as described previously (Pham et al., 2013). Briefly, dried and powdered flowering aerial parts (305 g) were extracted at room temperature with dichloromethane (DCM, yield 1.7%), followed by methanol (MeOH, yield 5.8%). The MeOH extract was suspended in distilled water and successively partitioned with ethyl acetate (EtOAc) and butanol (BuOH) resulting in an EtOAc extract (yield 1.5%), a BuOH extract (yield 1.7%) and an aqueous residue (1.7%). The previously reported natural products cassiaoccidentalin A, isovitexin and isoorientin, Fig. 1, (estimated yield from EtOAc extract were 1.7%, 1.4% and 0.3%, respectively) were isolated from the EtOAc extract by reverse phase (C18) flash chromatography and revers phase preparative thin layer chromatography (Pham et al., 2013). Commercial samples of isovitexin (≥ 98% Sigma) and isoorientin (≥ 98% Sigma) were used in the *in vitro* assays. Based on H1 NMR the purity of cassiaoccidentalin A was ≥ 95% (spectra not shown).

2.3. Antiplasmodial activity in vitro

Antiplasmodial activity of the extracts and pure compounds was evaluated as described by Snyder et al. (2007). This method is based on radioisotope incorporation of [^{3}H] hypoxanthine in parasitized erythrocytes. Briefly, *Plasmodium falciparum* strain NF54 and the chloroquine resistant strain K1 was cultivated in human erythrocytes in RPMI 1640 medium (Gibco) supplemented with 0.5% ALBUMAX® II, 25 mM Hepes, 25 mM $NaHCO_3$, 0.36 mM hypoxanthine, 100 μg/ml neomycine (Sigma). DMSO was used as vehicle. Infected erythrocytes, 100 μl per well with 2.5% hematocrit and 0.3% parasitemia, were added to each well containing test samples (0.31, 0.25, 1.25, 2.5, 5.0, 10.0 μg/ml) in 100 μl medium in

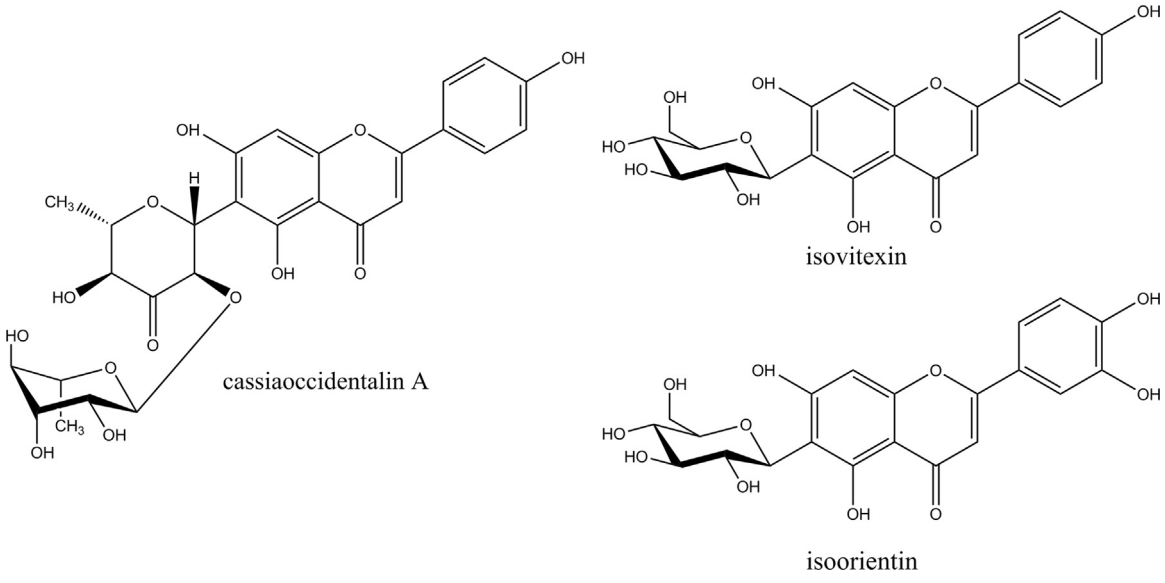

Fig. 1. Chemical structures of the three flavone-C-glycosides present in *B. umbraculum*.