Available online at www.sciencedirect.com
ScienceDirect
Phytomedicine 15 (2008) 367–372
Phytomedicine
www.elsevier.de/phymed

In vitro antiplasmodial activity of extract and constituents from *Esenbeckia febrifuga*, a plant traditionally used to treat malaria in the Brazilian Amazon

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Abstract

Esenbeckia febrifuga (Rutaceae) is a plant traditionally used to treat malaria in the Brazilian Amazon region. Ethanol extract of stems displayed a good antiplasmodial activity against *Plasmodium falciparum* strains W-2 (IC_{50} 15.5 ± 0.71 μg/ml) and 3 D7 (IC_{50} 21.0 ± 1.4 μg/ml). Two coumarins (bergaptene 1 and isopimpinellin 2), five alkaloids (flindersiamine 3, kokusaginine 4, skimmiamine 5, γ-fagarine 6 and 1-hydroxy-3-methoxy-*N*-methylacridone, 7), besides a limonoid (rutaevine 8), have been isolated for the first time from this species. Antiplasmodial activity of compounds 3, 5–8 has been evaluated *in vitro* against *P. falciparum* strains (W-2 and 3D7) and the furoquinolines 5 and 6 were the most potent displaying IC_{50} values < 50 μg/ml; flindersiamine (3) showed a weak activity while alkaloid 7 and rutaevine (8) were inactive ($IC_{50} > 100$ μg/ml).

Keywords: *Esenbeckia febrifuga*; Rutaceae; Antiplasmodial activity

Introduction

Malaria is one of the major parasitic diseases in the tropical and subtropical regions of the world and its aetiological agents are protozoans of the genus *Plasmodium*. It is responsible for over 1 million deaths each year and approximately 3.2 billion people, living in 107 countries, are presently at risk. Over 80% of malaria deaths occur in Africa and 15% in Asia. In the Americas, 14% of the population is at risk although the mortality is relatively low in this region. The emergence of chloroquine-resistant strains of *P. falciparum*, the most deadly species of malaria parasites, the resistance of vectors (*Anopheles* spp.) to insecticides, in combination with poverty and lack of good quality health care, are the main causes for the increase of malaria morbidity and mortality (WHO, 2005).

In general, Brazil reports approximately 40% of the total number of malaria cases in the Americas, of which almost 99% occurs in the Legal Amazon Region, where 12% of the population of the country lives. An increase in the number of cases began in the 1980s and a peak of 610,878 cases has been reported in 2000. An improvement in the epidemiological situation in 2006 has been related to the Plan for Intensification of Control Measures in the Amazon (PICAM), initiated in 2000.

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0944-7113/$ - see front matter © 2008 Elsevier GmbH. All rights reserved.
doi:10.1016/j.phymed.2008.02.001

In 2003, a National Program for Malaria Control (PNCM) was created by the Ministry of Health, an effort to strengthen the health services to provide conditions for rapid diagnosis, adequate treatment of the cases, control of vectors, fast detection of outbreaks and thus push on the measures to control malaria in the country (WHO, 2005). In 2001, a malaria network, the RAVREDA (Rede Amazônica de Vigilância da Resistência às Drogas Antimaláricas), was created and has gathered several American malarious countries, including Brazil. Besides monitoring the antimalarial drug resistance in the region, this network is also evaluating the susceptibility of the *Anopheles* vectors to insecticides. The Malaria Laboratory at Evandro Chagas Institute, state of Pará, under the coordination of Dr. Marinete M. Póvoa, is participating in this programme for evaluation of parasites' drug resistance, diagnosis methods and quality, entomology and control of malaria vectors (MS/SVS, 2007).

There is a consensus that new drugs to treat malaria are urgently needed. Many approaches to antimalarial drug discovery are available (Ridley, 2002; Rosenthal, 2003; Fidock et al., 2004). Investigation of plant-derived compounds is a valid strategy and this approach can benefit from traditional knowledge of populations from malarious regions. Natural products afforded two of the most important currently available drugs to treat malaria falciparum, quinine and artemisinin. The first one, a quinoline alkaloid, was isolated from *Cinchona* species used for treatment of fevers and/or malaria by South America Peruvian Indians and has been a template for the synthesis of chloroquine, the most widely used antimalarial drug. Artemisinin is responsible for the antimalarial activity of *Artemisia annua*, a species of millenar traditional use in China. The development of artemisinin derivatives has been a major advance in the chemotherapy of malaria (Wright, 2005).

It is estimated that 80% of the world's population depends on herbal remedies for treatment of diseases. Indeed, in malaria endemic areas, plant remedies are still widely used but mostly without assurance of their efficacy. Validation of traditionally used plants to treat malaria is important and requires clinical trials (Wright, 2005; Willcox and Bodeker, 2004) which must be preceded by phytochemical and toxicological studies that are necessary to guarantee efficacy and safety of herbal preparations (phytomedicines). Furthermore, knowledge of active compounds of a medicinal plant is important for development of standardized preparations for pre-clinical and clinical assays.

The genus *Esenbeckia* Kunth. (family Rutaceae, subfamily Rutoideae) includes ca. 30 species native to the tropical Americas (Dreyer et al., 1972). Previous chemical studies on species of this genus revealed the presence of typical rutaceous metabolites like coumarins, alkaloids, flavonoids, limonoids and terpenoids (Dreyer et al., 1972; Dreyer, 1980; Bevalot et al., 1984; Oliveira et al., 1996; Rios et al., 2002; Trani et al., 2004).

Esenbeckia febrifuga (A. St.-Hil.) A. Juss. ex Mart., popularly known in Brazil as "quina-do-mato" and "tres folhas", is used for the treatment of fever and/or malaria by inhabitants of the Brazilian Amazon region. An aqueous bark/stalk extract of this species has been previously assayed *in vivo* against *Plasmodium berghei*-infected mice, at a dose of 1.0 g/kg, and was shown to be partly active, causing 43% inhibition of parasite multiplication (Carvalho et al., 1991; Brandão et al., 1992).

In this paper we report on the phytochemistry of *E. febrifuga* and the *in vitro* evaluation against *P. falciparum* of an ethanol extract from stems of this species, as well of five out of the eight compounds isolated (Fig. 1). The susceptibilities were assessed against both chloroquine-sensitive (CQS) (3D7) and chloroquine-resistant (CQR) (W-2) strains of *P. falciparum*.

Experimental section

Isolation of chemical constituents: Stems of a tree growing at Campus Pampulha–UFMG, Belo Horizonte, state of Minas Gerais, Brazil, were collected and dried at 50 °C, in an oven with circulating air. A voucher specimen is deposited at the BHCB–UFMG (number 3825). Powdered stems (1.3 kg) were exhaustively extracted by percolation with EtOH; the combined extracts were concentrated in a rotavapor and dried under vacuum to afford 92 g of the crude extract. Chromatography of this extract (75 g), on a silica gel column (Merck 60; 0.040–0.063 mm) eluting initially with hexane–chloroform (80:20) and then increasing the proportions of chloroform followed by chloroform, then chloroform with increasing proportions of methanol, and finally with methanol, led to fractions which were combined according to the similarity on TLC (Merck q60 G) profiles. Repetition of the chromatographic separations and crystallization led to the isolation of compounds **1**–**8** (Fig. 1) which were identified by analysis of their spectrometric data. Mass spectra were recorded at 70 eV on a Kratos MS80 RFA (Manchester, UK). NMR spectra were recorded on chloroform-d (compounds **1**–**7**) and on DMSO-d_6 (**8**) on a Bruker AM-360 (360.136 MHz) and on a JEOL GSX 400 N (3999.65 MHz), at Fakultät fur Chemie und Pharmazie der Ludwig-Maximillians-Universität, Munich, Germany.

Antiplasmodial assay: Parasite strains were kept in continuous cultures in human erythrocytes suspended in RPMI 1640 supplemented with 10% human serum according to the method described by Trager and Jensen (1976). The antiplasmodial activity of the extract and test compounds was performed in 96-well tissue culture plates as described by Rieckman (1980) with