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Furfuran lignans and a flavone from *Artemisia gorgonum* Webb and their *in vitro* activity against *Plasmodium falciparum*

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ABSTRACT

The chemical composition of the aerial parts of the Cape Verdean endemic shrub *Artemisia gorgonum* Webb (Asteraceae) was careful investigated, which led to the isolation and identification of six known furfuran lignans: eudesmin (**1**), magnolin (**2**), epimagnolin A (**3**), aschantin (**4**), kobusin (**5**), sesamin (**6**) and a flavone: artemetin (**7**). Compounds **1**–**7** were evaluated *in vitro* for their cytotoxicity in a screening panel consisting of various mammalian tumor cell lines, for their antimalarial activity against chloroquine-resistant *Plasmodium falciparum* (FcB1 strain) and for their cytotoxicity against murine normal cells (CFU-GM). While no promising cytotoxicity against human tumor cells were noticed, marginal potency and selectivity was found for compounds **1**–**5** against murine colon 38. Besides, compounds **2**–**7** showed mild antiplasmodial activities, **6** and **7** being the most active compounds (IC_{50} 3.37 and 3.50 μg/ml respectively) without noticeable toxicity on mammalian normal cells. This is the first report of antiplasmodial activity for furfuran lignans and the first isolation of **1**–**7** from *Artemisia gorgonum*.

1. Introduction

Malaria is one of tropical diseases responsible for one million deaths and 500 million new cases annually. Malaria is a particularly devastating disease in sub-Saharan Africa, where about 90% of cases and deaths occur, but is also a serious public health problem in certain regions of South East Asia and South America. Currently, no single drug is effective for treating multi-drug resistant malaria, and effective combination therapy includes costly artemisinin derivatives, or mixtures with older drugs, so the treatment of malaria has been becoming increasingly problematic in virtually all malarious regions of the world (Santos-Ciminera et al., 2007; Tjitra et al., 2008). It makes the search for new antimalarial molecules a pressing need. The vast majority of the existing antimalarial chemotherapeutic agents are based on natural products, among them artemisinin (White, 2008), and medicinal plants continue providing excellent molecular templates in the search for antimalarial drugs (Kaur et al., 2009). Many species belonging to the genus *Artemisia* (Asteraceae) have proved to be a rich source of novel chemical structures with biomedical potential (Tan et al., 1998). For example, the endemic Cape Verdean *Artemisia gorgonum* Webb, better known as 'losna' by the natives, is an aromatic plant largely used in local folk medicine as a treatment for symptoms associated with fever (Gomes et al., 1995).

Our first phytochemical investigations of *Artemisia gorgonum* revealed the presence of a high number of bioactive sesquiterpene lactones in the CH_2Cl_2 extract (Ortet et al., 2008), whereas a total of 111 volatile compounds were identified in the essential oil of this plant exhibiting moderate antiplasmodial and strong antioxidant capacities (Ortet et al., 2010). Because some other compounds with distinct UV and MS spectra were detected in the HPLC–DAD–MS profiles of other fractions, we decided to go further into the characterization of the secondary metabolome of this plant, with the hope to discover additional bioactive metabolites. Herein, we report the isolation and structure identification of seven known aromatic compounds, as well as their *in vitro* inhibitory activity against chloroquine-resistant *Plasmodium falciparum*, FcB1 strain. Furthermore, these compounds were evaluated *in vitro* for their

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cytotoxicity in a screening panel consisting of various mammalian tumor cell lines (L1210, Colon 38, H-116 and H-125M), and murine normal cells (CFU-GM).

2. Materials and methods

2.1. General experimental techniques

Electrospray ionisation (ESI) mass spectra were obtained with a Bruker Esquire 3000 Plus spectrometer in the positive or negative mode. NMR experiments were performed on a Bruker Avance 500 MHz spectrometer. Chemical shifts (δ in ppm) were referenced to the carbon (δ_C 77.16) and residual proton (δ_H 7.26) signals of $CDCl_3$. Preparative HPLC was performed on a Waters equipment with a 600E pump, an autoinjector 417 and Photodiode Array Detector 996 coupled with a SEDEX 55 Evaporative Light Scattering Detector (ELSD). TLC was performed with Kieselgel 60 F_{254} (Merck glass support plates) and spots were detected after spraying with 10% H_2SO_4 in EtOH reagent and heating.

2.2. Plant material

The leaves and flowers of *Artemisia gorgonum* were collected in Fogo, Cape Verde islands in March 2006. Taxonomical identification was performed by Izildo Gomes. A voucher specimen was deposited in the Herbarium of INIDA, Santiago, Cape Verde.

2.3. Extraction and isolation

The air-dried aerial parts of *Artemisia gorgonum* (140 g) were extracted at r.t. with EtOH (2× 0.5 l) for 10 days. The combined extract was concentrated *in vacuo* to give 38 g of a residue. 20 g of the residue were then partitioned between CH_2Cl_2 and H_2O (4× 250 ml) to afford, after solvent removal, the organic fraction (7.3 g). A portion of the CH_2Cl_2 fraction was fractionated by flash chromatography over silica gel (100 g) with solvent mixtures of increasing polarities: cyclohexane (100%, 0.5 l); cyclohexane/CH_2Cl_2 (8:2, 0.5 l); cyclohexane/CH_2Cl_2 (5:5, 0.5 l); CH_2Cl_2 (0.5 l); CH_2Cl_2/EtOAc (5:5, 0.5 l); EtOAc (0.5 l); EtOAc/MeOH (5:5, 0.5 l); MeOH (0.5 l) to obtain eight fractions (**F1**–**F8**, respectively). Evaporation of the CH_2Cl_2/EtOAc 5:5 fraction (**F5**) led to 1.9 g of residue, which was fractionated by column chromatography over silica gel with a gradient solvent system: cyclohexane (350 ml); cyclohexane/CH_2Cl_2 (5:5, 400 ml); CH_2Cl_2 (400 ml); CH_2Cl_2/EtOAc (9:1, 200 ml); CH_2Cl_2/EtOAc (8:2, 400 ml); CH_2Cl_2/EtOAc (7:3, 200 ml); CH_2Cl_2/EtOAc (6:4, 200 ml); CH_2Cl_2/EtOAc (5:5, 400 ml); EtOAc (400 ml); EtOAc/MeOH (8:2, 400 ml); EtOAc/MeOH (5:5, 400 ml); MeOH (200 ml) to afford 185 fractions of 25 ml each, which were grouped into 26 fraction after analyzing them on TLC. The fraction number 16 was evaporated *in vacuo* and subsequently purified by semi-preparative reverse-phase HPLC (C_{18}, Luna Phenomenex, 250 mm × 10 mm, 5 μm) in an isocratic mode of MeOH/H_2O (45:55, 3 ml min^{-1}) to yield compounds **1**–**4** (2.7, 3.6, 2.9 and 2.5 mg, respectively). Evaporation of the CH_2Cl_2 fraction (**F4**) led to 0.8 g of residue, which was subjected to semi-preparative reverse-phase HPLC (C_{18}, Luna Phenomenex, 250 mm × 10 mm, 5 μm) with a gradient of H_2O/CH_3CN (flow 3 ml min^{-1}, from 95:5 to 20:80 in 40 min) to afford pure compounds **5** and **6** (6.4 and 17.2 mg, respectively). On the other hand, a portion of the CH_2Cl_2 fraction obtained from the crude extract was fractionated by RP-C_{18} flash chromatography (elution with a decreasing polarity gradient of H_2O/MeOH from 1:0 to 0:1, then MeOH/CH_2Cl_2 from 1:0 to 0:1 and finally CH_2Cl_2/n-hexane from 1:0 to 0:1). The MeOH (100%, 3.2 g) fraction was then subjected to semi-preparative reverse-phase HPLC (C18, Luna Phenomenex, 250 mm × 10 mm, 5 μm) with a gradient of H_2O/CH_3CN (flow 3 ml min^{-1}, from 55:45 to 20:80 in 35 min) to yield pure compound **7** (6.9 mg).

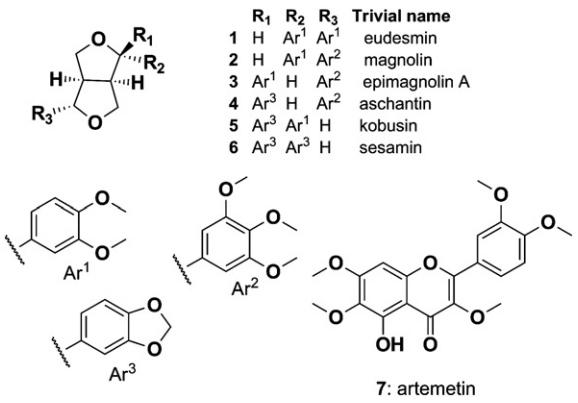

Fig. 1. Structures of apolar metabolites **1**–**7** isolated from *Artemisia gorgonum*.

2.4. Antimalarial assay

The antimalarial activity of compounds **1**–**7** was evaluated *in vitro* against *Plasmodium falciparum* FcB1 strain, following a reported method (Desjardins et al., 1979) and chloroquine was used as standard (Ortet et al., 2008).

2.5. Cytotoxicity assay

The cytotoxicity of the compounds **1**–**7** was evaluated *in vitro* against a variety of murine and human tumor and normal cells (Sinha et al., 2008). Briefly, 15 μl of each sample was dropped onto a 6.5 mm filter disk and allowed to dry. The disk was placed at the edge of a tissue culture dish containing one of the cell lines studies, the cell being embedded in an agar matrix. Seven days later, the cytotoxicity was defined in terms of a zone value measured from the edge of the filter disk to the point of colony formation on the dish. The assay not only provides an index of the potency of the compound but also defines whether there is any solid tumor selectivity upon which further preclinical development is based.

3. Results and discussions

The EtOH extract of leaves and flowers of *Artemisia gorgonum* was partitioned between CH_2Cl_2/H_2O. The CH_2Cl_2 extract exhibited antimalarial activity (IC_{50} = 3.6 μg/ml), which was considered promissory according present endpoint criteria for medicinal plants extracts (Huber and Koella, 1993; Soh and Benoit-Vical, 2007). After several separation steps, the most bioactive fractions were deeper studied until yielding seven pure metabolites which were identified as eudesmin (**1**) (Latip et al., 1999), magnolin (**2**) (Seo, 2010), epimagnolin A (**3**) (Seo, 2010), aschantin (**4**) (Ahmed et al., 2002), kobusin (**5**) (Latip et al., 1999), sesamin (**6**) (Kuropka and Glombitza, 1987) and artemetin (**7**) (Arriaga-Giner et al., 1983), respectively based on spectroscopic analyses and comparison with reported NMR data (Fig. 1). This is the first report of metabolites **1**–**7** in *Artemisia gorgonum*.

These compounds were evaluated for inhibition of *in vitro Plasmodium falciparum* growth and the results are provided in Table 1. Except for eudesmin (**1**) and magnolin (**2**), that were considered inactive, all compounds showed antiplasmodial activity, being sesamin (**6**) and artemetin (**7**) the most actives, with IC_{50} values of 3.37 and 3.50 μg/ml, respectively; followed by the lignans, epimagnolin A (**3**), aschantin (**4**), and kobusin (**5**) which exhibited similar IC_{50} and IC_{90}.