Phytochemistry Letters 28 (2018) 19–23

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Isolation and structure elucidation of two antiprotozoal bisbenzylisoquinoline alkaloids from *Triclisia gilletii* stem bark

R. Cimanga Kanyanga[a,b], C. Kikweta Munduku[a], S. Nsaka Lumpu[a], M. Tshodi Ehata[a], F. Makila Bool-Miting[c], O. Kambu Kabangu[a], B. Mbamu Maya[a], P. Cos[d], L. Maes[d], A.J. Vlietinck[b], E. Tuenter[b], K. Foubert[b], L. Pieters[b,*]

[a] *Department of Medicinal Chemistry and Pharmacognosy, Laboratoy of Pharmacognosy and Phytochemistry, University of Kinshasa, P.O. Box 212, Kinshasa, XI, Democratic Republic of the Congo*

[b] *Natural Products & Food Research and Analysis (NatuRA), Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, B-2610, Antwerp, Belgium*

[c] *Department of Pharmacology and Pharmaco-Vigilance, Faculty of Pharmaceutical Sciences, University of Kinshasa, P.O. Box 212, Kinshasa, XI, Democratic Republic of the Congo*

[d] *Laboratory of Microbiology, Parasitology and Hygiene (LMPH), Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, Universiteitsplein 1, B-2610, Antwerp, Belgium*

ARTICLE INFO

Keywords:
Triclisisia gilletii
Menispermaceae
Stem bark
Antiplasmodial activity
Malaria
Bisbenzylisoquinoline alkaloids
(–)-Phaeanthine
(–)-Pycmanilline

ABSTRACT

An aqueous decoction of stem bark of *Triclisia gilletii* (De Wild.) Staner (Menispermaceae) is used in several African countries to treat various diseases including malaria. The aqueous extract and the total alkaloid extract were evaluated *in vitro* for their antiplasmodial activity against the chloroquine-resistant *Plasmodium falciparum* strain K-1, the chloroquine-sensitive strain NF54 A19A, and *in vivo* in mice infected with *P. berghei berghei* and *P. yoelii* N67. Both extracts were active *in vitro* with IC_{50} values of 0.75 and 0.25 µg/ml against *P. falciparum* K1, respectively; and 1.15 and < 0.02 µg/ml against *P. falciparum* NF54 A19A, respectively. With regard to the *in vivo* activity, at the highest oral dose of 400 mg/kg body weight, the aqueous and the total alkaloid extracts produced 73.0% and 80.7% chemosuppression against *P. berghei berghei*, respectively, while against *P. yoelii* N67, a chemosuppression of 70.1%, and 78.4%, respectively, was observed. Two bisbenzylisoquinoline alkaloids were isolated from the total alkaloid extract, *i.e.* (–)-pycmanilline and (–)-phaeanthine in a yield of 0.20% and 0.40%, respectively. They were active *in vitro* against *P. falciparum* K-1 (IC_{50} 1.6 ± 0.3 and 0.8 ± 0.3 µM, respectively), and *P. falciparum* NF54 A19 A (IC_{50} 0.07 ± 0.01 and 0.03 ± 0.01 µM, respectively). Also against other protozoa IC_{50} values in the micromolar range were observed. (–)-Pycmanilline is reported for the first time.

1. Introduction

Malaria, a tropical disease caused by *Plasmodium* species, is one of the most important health problems in numerous regions in the world. In 2015, WHO estimated *P. vivax* to be responsible for 13.8 million cases, half of which in Africa. Malaria due to *P. vivax* is very difficult to control and its incidence decreases slowly compared to that of *P. falciparum* where these parasites coexist. It is well know that the use of chloroquine and antifolates (sulfadoxine-pyrimethamine) for antimalarial treatment is no longer effective in most endemic areas in the world. Therapeutics based on the combination of artemisinin and derivatives with other antiplasmodial drugs seem to be a better solution in overcoming the resistance of malaria parasites (WHO, 2015a,b). However, clinical resistance to some of these combinations has been reported in countries such as Cambodia (Noedl et al., 2008) and DR Congo (PNLP, 2005, 2007) suggesting the development of resistance by *P. falciparum*. There is a high need to look for new antimalarial agents from natural sources that are inexpensive, affordable and easily available to people mainly in developing countries. People in endemic areas have started for a long time to look for antimalarial remedies from natural sources by using medicinal plants according to daily practices of practitioners. The isolation and structure elucidation of bioactive compounds from medicinal plants based on traditional use seems to be a very promising approach for the discovery of new antimalarial drugs (Xu and Pieters, 2013; Vlietinck et al., 2015).

* Corresponding author.
E-mail address: luc.pieters@uantwerpen.be (L. Pieters).

https://doi.org/10.1016/j.phytol.2018.09.008
Received 25 June 2018; Received in revised form 27 August 2018; Accepted 3 September 2018

Triclisia gilletii (De Wild.) Staner (Menispermaceae) is a medicinal plant currently used in traditional medicine in DR Congo. Particularly, an aqueous decoction of stem bark is used for the treatment of malaria, fever, as a galactogen, analgesic, against haemorrhoids, vaginitis, intestinal worms, icterus, cutaneous eruptions, ulcers and asthenia. Kikueta et al. (2013) have reported the *in vitro* antiplasmodial activity of the aqueous extract, the 80% methanol extract and its fractions, and the total alkaloid extract from *T. gilletii* stem bark against the chloroquine-resistant strain *P. falciparum* K-1 and a Congolese chloroquine-sensitive strain, as well as *in vivo* antiplasmodial activity in mice infected with *P. berghei berghei*. However, active constituents had not been isolated. Whereas the genus *Triclisia* is well known for the presence of bisbenzylisoquinoline alkaloids, in a recent study Tiam et al. (2017) have reported a series of terpenes, flavonoids and nonacosan-10-ol from aerial parts of *T. gilletii* from Cameroon. In the present study, the *in vitro* and *in vivo* activity of the aqueous decoction and the total alkaloid extract of *T. gilletii* stem bark from DR Congo was confirmed, followed by the isolation and structure elucidation of two active constituents.

2. Experimental

2.1. General experimental procedures

Optical rotations were determined on a Jasco P-2000 spectrometer (Easton, MD, USA) with Spectramanger software. Nuclear magnetic resonance (NMR) spectra were recorded in CD_3OD on a Bruker DRX-400 instrument (Rheinstetten, Germany), operating at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR spectra. An Agilent QTOF 6530 mass spectrometer (SantaClara, CA, USA) with Mass Hunter version B.06 software was used to perform accurate mass measurements. The mass spectrometer was operated in the ESI^+ mode at a resolution of 20,000. Calibration was done externally and the samples were measured after direct infusion.

2.2. Plant material

Stem bark of *T. gilletii* was collected in Kinshasa in October 2015 and identified by Mr. Landu Lukebiabo B. of the Institut National de Recherche en Agronomie (INERA), Faculty of Sciences, Department of Biology, University of Kinshasa. A voucher specimen (NL102015TGRB) was deposited in the herbarium of this institute. The collected plant material was dried at room temperature and reduced to powder in an electronic blender.

2.3. Preparation of extracts

100 g of powdered stem bark of *T. gilletii* was mixed with 500 ml distilled water and heated on a hot plate for 15 min. After cooling and filtration on filter paper Whatman N° 1, the filtrate was evaporated *in vacuo* giving a dried extract denoted as AE-1 (42.34 g). For the extraction of the alkaloids, 500 g powdered stem bark was macerated and percolated with 80% MeOH. After filtration, the filtrate was evaporated *in vacuo* yielding a dried extract denoted as ME-1 (187.21 g), 10 g of which was dissolved in 300 ml distilled water and filtered. The filtrate was alkalinized with 30 ml NH_3 10% and extracted with $CHCl_3$ yielding a dried extract denoted ME-1.1 (4.85 g), responding positively to Dragendorff's reagent for alkaloids.

2.4. Isolation of alkaloids

An aliquot of 20 g of extract ME-1.1 was dissolved in 20 ml $CHCl_3$ and submitted to column chromatography on silica gel (Davisil, LG60A 60–200 µ, Grace, Germany), eluted with a gradient 0–100% $CHCl_3$/MeOH. Several fractions of 10 ml were collected and analysed by TLC on silica gel plates 60 F_{254} (layer thickness 0.25 mm, Merck, Germany) using $CHCl_3$/MeOH 9:1 as mobile phase. They were combined according to their chromatographic profile in several fractions: F1: 7.24 g, F2: 3.05 g, F3: 4.075 g and F4: 2. 28 g. Fraction 2 was submitted to preparative chromatography on silica gel plates (layer thickness 1 mm, Merck, Germany) using EtOAc/MeOH/H_2O (15:3:2) as a mobile phase, resulting in the isolation of chromatographically pure compound **1** (41 mg; 0.20%). Fraction 4 was also submitted to preparative chromatography in the same conditions resulting in the isolation of chromatographically pure compound **2** (81 mg; 0.40%). These two isolated compounds reacted positively with Dragendorff's reagent and $CeSO_4$/H_2SO_4 for alkaloids.

Table 1
^{13}C-NMR assignments of (–)-pycmanilline (**1**) and (–)-phaeanthine (**2**) (ppm, multiplicity) (100 MHz, CD_3OD) (ppm, multiplicity).

Carbon No	(–)-Pycmanilline (**1**) δ_C	(–)-Phaeanthine (**2**) δ_C
1	62.17, d	63.05, d
3	45.44, t	45.27, t
4	24.16, t	23.61, t
4a	131.50, s	129.20, s
5	111.64[a],d	107.34, d
6	153.83[b], s	153.09, s
7	141.39, s	139.31, s
8	146.11[c], s	149.33, s
8a	124.60, s	123.80, s
α	40.67, t	42.57, t
9	135.30, s	136.51, s
10	124.10, d	116.75, d
11	144.51, s	150.28, s
12	151.64, s	148.74, s
13	113.42, d	113.73, d
14	128.04, d	124.41, d
1'	166.46, s	64.52, d
3'	49.50, t	45.69, t
4'	28.19, t	25.93, t
4'a	132.00, s	129.00, s
5'	111.68[a], d	113.29, d
6'	153.06[b], s	150.96, s
7'	147.37[c], s	145.23, s
8'	113.92, d	121.44, d
8'a	122.18, s	128.80, s
α'	174.51, s (COOH)	37.49, t
9'	135.19, s	135.70, s
10'	132.14, d	133.88, d
11'	115.71, d	122.88[a], d
12'	162.18, s	155.11, s
13'	115.71, d	122.83[a], d
14'	132.14, d	131.83, d
N^2-Me	42.73, q	43.02, q
$N^{2'}$-Me	35.30, q	42.44, q
O-Me (C-7)	61.22, q	60.68, q
O-Me	56.41, q	56.09, q
	56.50, q	56.37, q
	56.56, q	56.78, q

[a, b, c]Assignments bearing the same superscript may be reversed in the same column.

(–)-Pycmanilline (**1**): $[\alpha]_D = -35.4°$ (*c* 0.5, CH_3OH); ^{1}H NMR (CD_3OD): characteristic signals: δ 7.87 (2H, br d, J = 8.6 Hz, H-10', H-14'), 7.08 (1H, s, H-8′), 7.05 (1H, dd, J = 8.8 Hz, 2.0 Hz, H-14), 6.96 (2H, br d, J = 8.6 Hz, H-11′, H-13′), 6.84 (1H, d, J = 2.0 Hz, H-10), 6.75 (1H, s, H-5′), 6.71 (1H, d, J = 8.8 Hz, H-13), 6.72 (1H, s, H-5), 3.85 (3H, s, O-Me), 3.83 (3H, s, O-Me), 3.73 (3H, s, O-Me), 3.61 (3H, s, O-Me), 3.03 (3H, s, N'-Me), 2.26 (3H, s, N-Me); ^{13}C NMR: see Table 1. HRMS *m/z* 669.2712 $[M+H]^+$ (calcd. for $C_{38}H_{41}N_2O_9$, 669.2812), corresponding to a molecular formula of $C_{38}H_{40}N_2O_9$ (MW 668.74).

*(–)-Phaeanthine (**2**)*: $[\alpha]_D = -187.3°$ (*c* 0.8, CH_3OH); ^{1}H NMR (CD_3OD): characteristic signals δ: 7.30 (1H, dd, J = 8.2 Hz, 2.2 Hz, H-14′), 6.93 (1H, dd, J = 8.2 Hz, 2.6 Hz, H-13′), 6.81 (1H, d, 8.2 Hz, H-13), 6.75 (1H, dd, J = 8.2 Hz, 1.9 Hz, H-14), 6.64 (1H, dd, J = 8.3 Hz, 2.5 Hz, H-11′), 6.55 (1H, s, H-5′), 6.44 (1H, d, J = 1.9 Hz, H-10), 6.31 (1H, s, H-5), 6.25 (1H, dd, J = 8.3 Hz, 2.2 Hz, H-10′), 5.90 (1H, s, H-8′),