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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 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 Congo*

[d] *Laboratory of Microbiology, Parasitology and Hygiene (LMPH), Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, Universiteitsplein1, B-2610, Antwerp, Belgium*

Corresponding author:

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

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 A19A (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.

Keyswords: *Triclisisia gilletii*; Menispermaceae; stem bark; antiplasmodial activity; malaria; bisbenzylisoquinoline alkaloids; (–)-phaeanthine; (–)-pycmanilline

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).

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.

(–)-Pycmanilline (**1**): $[\alpha]_D$ = -35.4 ° (*c* 0.5, CH_3OH); ^1H 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); ^1H 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'), 3.89 (1H, dd, J = 10.9 Hz, 5.5 Hz, H-1'), 3.78 (3H, s, O-Me), 3.72 (1H, d, J= 9.5 Hz, H-1), 3.62 (3H, s, O-Me), 3.25 (3H, S, -O-Me), 3.04 (3H, s, -O-Me), 2.52 (3H, s, N'-Me), 2.17 (3H, s, N-Me); ^{13}C NMR: see Table 1. HRMS *m/z* 623.3125 $[M+H]^+$ (calcd. for $C_{38}H_{43}N_2O_6$, 623.3116), corresponding to a molecular formula of $C_{38}H_{42}N_2O_6$ (MW 622.76).

2.5. In vitro antiprotozoal activity and cytotoxicity

Extracts and fractions from *T. gilletii* stem bark were evaluated against *P. falciparum* strain NF54 A19A (*Pf*- NF54 A19A) sensible to chloroquine using the method previously described by Desjardins et al. (1979). Antiprotozoal activity against *P. falciparum* K1 (*Pf*-K1) (chloroquine-resistant), *Trypanosoma brucei brucei*, *T. brucei rhodesiense*, *T. cruzi* and *Leishmania infantum*, as well as cytotoxicity against MRC-5 cells (human lung fibroblasts),

were determined as published before (Musuyu Muganza et al., 2016; Kikueta et al., 2013; and references cited therein).

2.6. In vivo antiplasmodial activity (4-day test)

Suppressive activity during early infection was evaluated using a 4-day schizonticidal test (Peters and Robinson, 1999; Elufioye and Agbedahunsi, 2004). Briefly, mice (22.41 ±0.05 g body weigth (bw)) were inoculated separately on the first day (day 0) with *P. berghei berghei* ANKA (sensitive to chloroquine) and *P. yoelii* N67 (resistant to chloroquine). Mice were randomly divided into nine groups as follows: group I and II (3 mice each) orally received 5 ml water and 10 mg/kg bw of chloroquine and constituted the negative and the positive control group, respectively. Groups III to IX (10 mice each), infected separately with *P. berghei berghei* and *P. yoelii* N67, orally received 200 or 400 mg/kg bw of each extract or fraction. The test samples were orally administered daily for four consecutive days (day 0-3) between 9.00 and 11.00. Twenty four hours after administration, blood smears were made from tail blood, stained with Giemsa stain, and evaluated microscopically. Parasitaemia was calculated by dividing the number of parasitised erythrocytes by the total number of erythrocytes.

Chemosuppression (%) was calculated using the following formula: Ac-At/Ac x 100

where Ac is the average parasitaemia in the negative control group and At the average parasitaemia in the treated group.

3. Results and discussion

The *in vitro* activity, cytotoxicity and selectivity of the extracts of *T. gilletii* stem bark is presented in Table 2. The aqueous extract AE-1, which corresponds to the traditional preparation, showed IC_{50} values around 1 µg/ml against both strains, with a selectivity index (SI) 5 – 10. However, the total alkaloid extract ME-1.1 was more active against both

Plasmodium strains, showing an IC_{50} of 0.25 µg/ml and < 0.02 µg/ml against the chloroquine-resistant and chloroquine-sensitive strains, respectively. Since against MRC-5 cells the IC_{50} was only 4.11 µg/mL, a good selectivity was obtained. Results of the *in vivo* evaluation of the extracts against *P. berghei berghei* ANKA and *P. yoelii* N67 are summarised in Tables 3 and 4, respectively. Both extracts were active against both strains, and again the total alkaloid extract was more active than the aqueous extract, with chemosuppressions up to 80% and above.

Based on the results of the *in vitro* and *in vivo* evaluation, the total alkaloid extract was selected for isolation of its active constituents. Two compounds were obtained. The ^{1}H and ^{13}C-NMR of compound **1** were in first approximation in relatively good agreement with those reported for 6,7-seco-isotetrandrine (Schmeda-Hirschman et al., 1996), but the typical aldehyde signal was lacking. Instead, a carboxylic acid moiety was present. Therefore, it was concluded that compound **1** was seco-isotetrandrine carboxylic acid, reported before as pycmanilline from *Pycnarrhena manillensis* (Regalado et al., 1987) (Fig. 1). However, for pycmanilline, a positive specific optical rotation of +33 ° was reported, whereas for compound **1** a negative value of -35.4 ° was measured. It could be concluded that compound **1** is (–)-pycmanilline, a bisbenzylizoquinoline alkaloid reported for the first time, whereas its known enantiomer is (+)-pycmanilline.

The ^{1}H and ^{13}C-NMR for compound **2** were in good agreement with those reported for (+)-tetrandrine (Lin et al., 1993). (+)-Tetrandrine has a 1*S*,1*S'*-configuration; its enantiomer with a 1*R*,1*R'*-configuration is known as (–)-phaeanthine. Detailed ^{13}C-NMR investigations have shown that C-α and C-α' show a chemical shift around 42 ppm and 38 ppm, respectively, in the 1*S*,1*S*' and an 1*R*,1*R'* series of bisbenzylizoquinoline alkaloids, whereas both are found around 38 ppm in case of 1*R*,1*S'*- (isotetrandrine) and 1*S*,1*R'*-configuration (peinamine derivatives) (Koike et al. 1979). Since for compound **2** the signals for C-α and C-α' are found

at 42.57 and 37.49 ppm respectively, it belongs to the 1*S*,1*S'* or 1*R*,1*R'*-series. Based on the negative specific optical rotation, it could be concluded that compound **2** is (–)-phaeanthine (Fig. 1). The structure was confirmed by accurate mass measurement. To our knowledge, these two bisbenzylisoquinoline alkaloids are isolated for the first time from *T. gilletii* stem bark. Whereas the bisbenzylisoquinoline structure is less obvious in *seco*-compounds such as pycmanilline, they are clearly biogenetically derived from bisbenzylisoquinolines, by oxidative cleavage of the C-α' – C-1' bond, converting C-α' to a carboxylic acid moiety, and C-1' to a carbonyl group (Regalado et al., 1987; Schmeda-Hirschmann et al., 1996).

Both alkaloids were evaluated *in vitro* against a panel of protozoa (Table 5). Both alkaloids isolated from the total alkaloid extract showed a pronounced antiplasmodial activity, especially against the chloroquine-sensitive strain NF54 A19A (IC_{50} values < 0.1 µM), but also against the chloroquine-resistant strain IC_{50} values of 1.6 µM and 0.8 µM were observed for (–)-pycmanilline (**1**) and (–)-phaeanthine (**2**), respectively. Taking into account the relatively low cytotoxicity, a selectivity index (SI) of almost 594 and 373 could be calculated for **1** and **2**, respectively, against *Pf* NF54 A19A. Against *Pf*-K1, SI values were only 26 and 14, respectively. Although compound **2** showed an IC_{50} against *Li* of 4.3 µM, it was accompanied by cytotoxicity against the macrophages (PMM) used in the assay. Also against the three *Trypanosoma* species investigated, IC_{50} values in the micromolar range were observed. In general, (–)-phaeanthine (**2**) was more active than (–)-pycmanilline (**1**), but it is also more cytotoxic.

Different bisbenzylisoquinoline alkaloids were isolated from various medicinal plants belonging to the Annonaceae, Beriberidaceae, Menispermaceae, Monimiaceae and Ranunculaceae. They were reported to exhibit antiplasmodial activity against different strains of *P. falciparum* with IC_{50} < 10 µg/ml (Marshall et al., 1994; Angerhofer et al., 1999; Mambu et al., 2000, Iwasa et al., 2001; Otshudi et al., 2005; Murebwayire et al., 2008). Also

antitrypanosomal activity has been reported (Hoet et al., 2004). (–)-Phaeanthine was reported to exhibit strong antiplasmodial activity against the *P. falciparum* W2 strain (resistant to chloroquine) and the *P. falciparum* D6 strain (sensible to chloroquine) ($300 < IC_{50} < 500$ nM), but was cytotoxic against KB cell lines (CC_{50} = 10 800 nM) (Angerhofer et al., 1999). Our results are in good agreement with Ekong et al. (1991) and Marshall et al. (1994) concerning the activity of (–)-phaeanthine against the *P. falciparum* K-1 chloroquine-resistant strain. To our knowledge, this is the first report of the antiplasmodial activity of (–)-phaeanthine against the *P. falciparum* NF 54 chloroquine -sensible strain, and of the antiplasmodial activity of (–)-pycmanilline.

4. Conclusions

Based on the observed *in vitro* activity, both isolated constituents may contribute at least in part to the *in vivo* activity in mice, and explain the traditional use of stem bark extracts of *Tricilisia gilletii*. As commonly observed in medicinal plant research, the activity of the extracts cannot completely be explained by the IC_{50} values of the isolated constituents. This may be due to the occurrence of other, unidentified active constituents, and/or by synergistic effects. The traditional preparations should be administered in high amounts or for a long time to avoid failure or relapse. This kind of treatment is usually applicable in traditional medicine where healers recommend the consumption of large quantities of their remedies for many days or weeks to treat a definite disease.

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References

Angerhofer, C.K., Guinaudeau, H., Wongpanich, V., Pezzuto, J.M., Cordell, G.A., 1999. Antiplasmodial and cytotoxic activity of natural bisbenzyisoquinoline alkaloids. J. Nat. Prod. 62, 59-66.

Desjardins, R.E., Canfield, C.J., Haynes, D., Chulay, J.D., 1979. Quantitative assessment of antimalarial activity *in vitro* by a semiautomated microdilution technique. Antimicrob. Agents Chemother.16, 710-718.

Ekong, R., Partridge, S.J., Anderson, M.M., Kirby, G.C., Warhurst, D.C., Russell, P.F., Philippson, J.D., 1991. *Plasmodium falciparum* effects of phaeanthine, a naturally-occurring bisbenzylisoquinoline alkaloid, on chloroquine-resistant and chloroquine-sensitive parasites *in vitro*. Ann. Trop. Med. Parasitol. 85, 205-213.

Elufoye, T.O., Agbedahunsin, J.M., 2004. Antimalarial activities of *Tithonia diversifolia* (Asteraceae) and *Crossopteryx febrifuga* (Rubiaceae) on mice *in vivo*. J. Ethnopharmacol. 93, 167-171.

Hoet, S., Opprdoes, F., Brun, R., Quetin-Leclerq, J., 2004. Natural products against African trypanosomes: a step towards new drugs. Nat. Prod. Rep. 21, 353-364.

Iwasa, K., Moriyasu, M., Tachibana, Y., Kim, H-S., Wataya, Y., Wiegrebe, W., Bastow, K.F., Cosentimo, L.M., Kozuka, M., Lee, K-H., 2001. Simple isoquinoline and benzylizoquinoline alkaloids as potential antimicrobial, antimalarial, cytotoxic, and anti-HIV agents. Bioorg. Med. Chem. 9, 2871-2884.

Kikueta, C.M., Kambu, O.K., Mbenza, A.P., Mavinga, S.T., Mbamu, B.M., Cos, P., Maes, L., Apers, S., Pieters, L., Cimanga, R.K., 2013. *In vitro* and *in vivo* antimalarial activity

and cytotoxicity of extracts and fractions from the leaves, root-bark and stem-bark of *Triclisia gilletii*. J. Ethnopharmacol. 149, 438-442.

Koike, L., Marsaioli, A.J., Rúveda, E.A, Reis, F., 1979. Stereochemical aspects and ^{13}C-NMR spectroscopy of the berbamine class of bisbenzylizoquinoline alkaloids. Tetrahedron Lett. 39, 3765-3678.

Lin, L.-Z., Shieh, H.-L., Angerhofer, C.K., Pezzuto, J.M., Cordell, G.A., Xue, L., Johnson, M.E., Ruangrungsi, N., 1993. Cytotoxic and antimalarial bisbenzylisoquinoline alkaloids from *Cyclea barbata*. J. Nat. Prod. 56, 22-29.

Mambu, L., Martin, M., Razafimahefa, D., Ramanitrahasimbola, D., Rasoanaiva, P. Frappier, F., 2000. Spectral characterization and antiplasmodial activity of bisbenzylisoquinoline isolated from *Isolona ghesquiereina*. Planta Med., 66: 537-540.

Marshall, S.J., Russell, P.F., Wright, C.W., Anderson, M.M., Phillipson, J.D., Kirby, G.C., Warhurst, D.C., Schiff, P.L., 1994. *In vitro* antiplasmodial, antiamoebic, and cytotoxic activities of a series of bisbenzylisoquinoline alkaloids. Antimicrob. Agents Chemother. 38, 96-103.

Murebwayire, S., Frédérich, M., Hannaert, V., Jonville, M., Duez, P. 2008. Antiplasmodial and antitrypanosomal activity of *Triclisia sacleuxii* (Pierre) Diels. Phytomed. 15, 728-733.

Musuyu Muganza, D., Fruth, B., Nzunzu Lami, J., Tuenter, E., Foubert, K., Cos, P., Maes, L., Cimanga Kanyanga, R., Exarchou, V., Apers, S., Pieters, L. 2016. *In vitro* antiprotoza**o**l activity and cytotoxicity of extracts and isolated constituents from Greenwayodendron suaveolens. J. Ethnopharmacol. 193, 510-516.

Noedl, H., Se, Y., Schaecher, K. Smith, B.L., Socheat, D., Fukuda, M.M. 2008. Evidence of artemisinin-resistant malaria in western Cambodia. New Engl. J. Med. 359, 2619-2620.

Otshudi, A.L., Apers, S., Pieters, L., Claeys, M., Pannecouque, C., De Clercq, L., Van Zeebroeck, A., Lauwers, S., Frédérich, M., Foriers, A., 2005. Biologically active bisbenzylisoquinoline alkaloids from the root-bark of *Epinetrum vilosum*. J. Ethnopharmacol., 102, 89-94.

Peters, W., Robinson, B.L. 1999. The chemotherapy of rodent malaria. Ann. Trop. Med. Parsitol. 93, 325-339.

PNLP, 2005. Echecs thérapeutiques à l'artésunate-amodiaquine (ASAQ) et à l'artémether-lumefantrine (Coartem) chez les enfants de moins de 5 ans avec paludisme à *Plasmodium falciparum* en République Démocratique du Congo. Programme National de Lutte contre le Paludisme. Ministère de la Santé Publique, Kinshasa/Ngaliema, RD Congo.

PNLP, 2007. Faire reculer le paludisme, Plan stratégique 2007-2011. Ministère de la Santé de la République Démocratique du Congo. Programme National de Lutte contre le Paludisme. Ministère de la Santé Publique, Kinshasa/Ngaliema, RD Congo.

Regalado, J.C., Cao, C-y., Fu, E., Lin, F-t., Lin, M-c., Wong, L.K., Paul, L. Schiff, Jr. 1987. Phaeanthine 2'a-N-oxide and pycmanilline, new bisbebzylizoquinoline alkaloids from *Pycnarrhena manillensis*. Heterocycles, 26: 2573-2578.

Schmeda-Hirschmann, G., Dutra-Behrens, M., Habermehl, G., Jakupovic, J. 1996. Seco-isotetrandrine from *Laurelia sempervirens*. Phytochemistry, 41: 339-341.

Tiam, E.R., Ngono Bikobo, D.S., Zintchem A.A.A., Mbabi Nyemeck, N. 2nd, Moni Ndedi, E.D.F., Betote Diboué, P.H., Nyegue, M.A., Atchadé, A.T., Pegnyemb, D.E., Bochet, C.G., Koert, U. 2017. Secondary metabolites from *Triclisia gilletii* (De Wild) Staner (Menispermaceae) with antimycobacterial activity against *Mycobacterium*

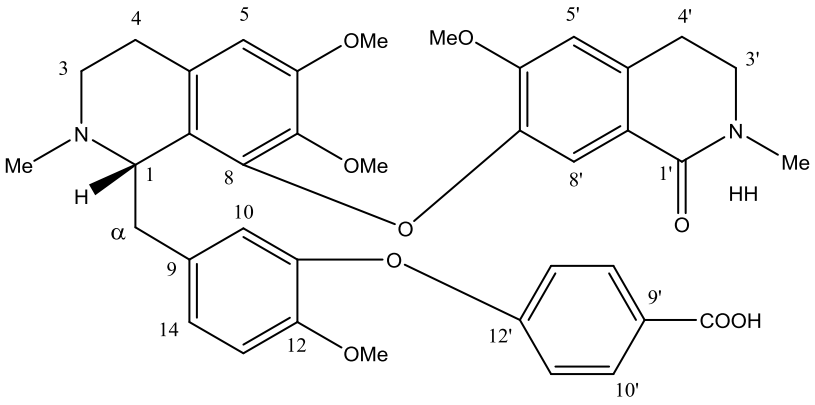
tuberculosis. Nat. Prod. Res. 2017 Nov 16:1-9 (https://doi.org/10.1080/14786419.2017.1402324).

Vlietinck, A.J., Pieters, L., Apers, S., Cimanga, K., Mesia, K., Tona, L. 2015. The value of Central-African traditional medicine for lead finding: Some case studies. J. Ethnopharmacol. 174, 607-617

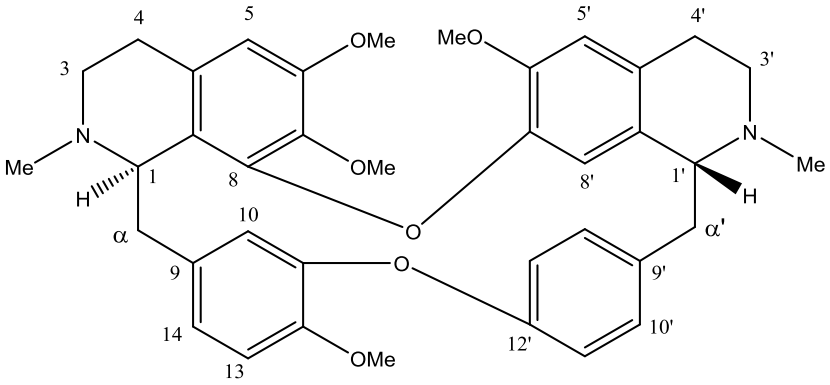
WHO. 2015a. La stratégie technique de lutte contre le paludisme 2016-230. Available from : http://www.who.int/malaria/areas/global_technical_strategy/fr/

WHO. 2015b. Rapport sur le paludisme dans le monde 2015. Available from : http://www.who.int/malaria/publications/world-malaria-report-2015/report/en/

Xu, Y.J., Pieters, L. 2013. Recent developments in antimalarial natural products isolated from plants. Mini Rev. Med. Chem. 13, 1056-1072.



(–)-Pycmanilline (**1**)



(–)-Paeanthine (**2**)

Figure 1. Structure of (–)-pycmanilline (**1**) and (–)-phaeanthine (**2**)

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.

Table 2: *In vitro* antiplasmodial activity, cytotoxicity (IC_{50} ± SEM, µg/ml) and selectivity index (SI) of *T. gilletii* stem bark extracts

Sample	MRC-5	*Pf*-K1	*Pf*-NF54 A19A	*Pf*-K1 (SI)	*Pf* NF54 A19A (SI)
AE-1	7.33 ± 0.25	0.75± 0.14	1.15 ± 0.23	9.77	6.37
ME-1.1	4.11 ± 0.11	0.25 ± 0.02	< 0.02	16.44	> 205.50

Pf: *Plasmodium falciparum*; AE-1: Aqueous extract; ME-1.1: Total alkaloid extract

Table 3. *In vivo* antiplasmodial activity of *T. gilletii* stem bark extracts against *P. berghei berghei*

Extract	Oral treatment (mg/kg bw)	% Parasitaemia	% Chemosuppression
AE-1	200	8.10 ± 0.50	70.43 ± 0.60
	400	7.40 ± 1.10	73.00 ± 1.40
ME-1.1	200	6.05 ±0.20	78.00 ± 0.60
	400	5.35 ± 1.40	80.74 ± 1.10
Chloroquine	10	0.40 ± 0.10	98.54 ± 0.80
Artesunate	5	0.00 ±0.00	100.00 ± 0.00
Negative control	5 ml water	27.40 ± 1.30	0.00 ±0.00

AE-1: Aqueous extract; ME-1.1: Total alkaloid extract

Table 4. *In vivo* antiplasmodial activity of T. *gilletii* stem bark extracts against *P. yoelii* N67

Extracts	Oral treatment (mg/kg bw)	% Parasitaemia	% Chemosuppression
AE-1	200	9.15 ± 0.20	69.80 ± 0.32
	400	9.05 ± 0.14	70.13 ± 0.51
ME-1.1	200	6.05 ± 0.02	80.03 ± 0.21
	400	5.45 ± 0.07	82.01 ± 0.57
Chloroquine	10	1.15 ± 0.10	96.20 ± 0.80
Artesunate	5	0.00 ±0.00	100.00 ± 0.00
Negative control	5 ml water	30.30 ± 1.30	0.00 ±0.00

AE-1: Aqueous extract; ME-1.1: Total alkaloid extract

Table 5 : Antiprotozoal and cytotoxic activity of compounds **1** and **2** (IC_{50} ± SEM, µM)

Test compound	Antiprotozoal activity						PMM	MRC-5 cells
	Tb	***Trh***	***Tc***	***Li***	***Pf*-K1**	***Pf*-NF54 A19A**		
(–) Pycmanilline (**1**)	30.1 ± 1.4	5.4 ± 1.6	15.6 ± 0.8	27.9 ± 2.8	1.6 ± 0.3	0.05 ± 0.01	>64.0	41.6 ± 11.3
(–)-Phaeanthine (**2**)	6.3 ± 0.4	1.8 ± 0.2	3.7 ± 0.8	4.3 ± 1.2	0.8 ± 0.3	0.03 ± 0.01	8.0	11.2 ± 3.5
Positive controls:								
Tamoxifen								10.48
Benznidazole			2.4					
Miltefosine				6.8				
Suramine	0.03							
Chloroquine					0.08	< 0.03		

Tb: *Trypanosoma brucei brucei*

TRh: *Trypanosoma brucei rhoidiense*

Tc: *Trypanisoma cruzi*

Li: *Leishmania infantum*

Pf: *Plasmodium falciparum*

PMM: Primary mouse peritoneal macrophages