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In vitro and *in vivo* antiplasmodial activity of extracts and isolated constituents of *Alstonia congensis* root bark

R. Cimanga Kanyanga [a,d], S. Nsaka Lumpu [a], M. Tshodi Ehata [a], B. Mbamu Maya [a], C. Kikweta Munduku [a], F. Makila Bool-Miting[b], Paul Cos [c], Louis Maes [c], Arnold J. Vlietinck [d], Vassiliki Exarchou [d], Emmy Tuenter [d], Luc Pieters [d]

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

[b] *Departement of Pharmacology and Pharmacovigilance, Faculty of Pharmaceutical Sciences, University of Kinshasa, P.O. Box 212, Kinshasa XI, Democratic Republic of Congo*

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

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

Corresponding author: Dr. R. Cimanga Kanyanga, Natural Products & Food Research and Analysis (NatuRA), Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, B-2610, Antwerp, Belgium.

Email: kanyanga.cimanga@uantwerpen.be; rkcimanga@gmail.com

Abstract

Ethnopharmacological relevance: An aqueous decoction of root bark of *Alstonia congensis* Engl. (Apocynaceae) is used in several African countries to treat various ailments including malaria.

Materials and methods: Extracts of different polarity and isolated constituents were tested *in vitro* for their antiplasmodial activity against the chloroquine-resistant strain *Plasmodium falciparum* K1 and the chloroquine-sensitive strain *P. falciparum* NF54A19A, as well as for their cytotoxic effects againt MRC-5 cells (human lung fibroblasts). Extracts and fractions were evaluated *in vivo* against the chloroquine-resistant strain *P. yoelii* N67 and the chloroquine-sensitive strain *P. berghei berghei* ANKA.

Results: The aqueous extract, the 80% methanol extract and the alkaloid-enriched extract exhibited strong antiplasmodial activity against *P. falciparum* K1 with IC_{50} values < 10 µg/ml and against *P. falciparum* NF54 A19A with IC_{50} values < 0.02 µg/ml. *In vivo* against *P. yoelii* N67, at the highest oral dose of 400 mg/kg body weight, all extracts produced 70 - 73% chemosuppression, while against *P. berghei berghei*, more than 75% chemosuppression was observed. With regard to the isolated constituents, boonein was inactive *in vitro* against *P. falciparum* K-1 (IC_{50} > 64 µM), while echitamine, 6,7-seco-angustilobine B and β-amyrin exhibited moderate activity (IC_{50} < 30 µM). Against *P. falciparum* NF54 A19A, boonein was inactive (IC_{50} > 64 µM), while echitamine, 6,7-secoangustilobine and β-amyrin showed moderate IC_{50} values of 11.07, 21.26 and 40.70 µM, respectively.

Conclusion: These results demonstrated that all extracts from *A. congensis* root bark possessed antiplasmodial activity *in vitro* and *in vivo*. They can be used as raw materials for the preparation of ameliorated remedies for the treatment of uncomplicated malaria. The observed antiplasmodial activity may be due in part to the presence of indole alkaloids.

Keywords: *Alstonia congensis*; Apocynaceae; antiplasmodial activity; malaria

1. Introduction

Malaria is one the most prevalent and serious protozoan tropical diseases caused by *Plasmodium* species such as *P. falciparum*, *P. ovale*, *P. malariae*, *P. viva*x and *P. knowlesi*. *P. falciparum* accounts for the majority of infections and causes the most severe symptoms (Saifi et al., 2013). Many efforts have been made to eliminate this disease, which causes millions of clinical cases worldwide each year, and annually approximately one million people die from malaria. The World Health Organization (WHO) estimated that more than one billion people are at high risk of transmission, half of which live in endemic, mostly African, countries. The emergence and rapid spread of multi-resistant strains of *Plasmodium*, especially *P. falciparum*, becomes a major and serious problem for prophylaxis and treatment in the world. Antimalarial drugs such as chloroquine, sulfadoxine / pyrimethamine and others are no longer effective in most endemic areas, mainly in developing countries. Therefore, WHO has recommended the use of therapeutics based on combinations of artemisinin and derivatives with other drugs. However, in some countries *P. falciparum* is already resistant to artemisinin combination therapies (ACTs) (Noedl et al., 2008; WHO, 2015a; 2015b).

Because a large part of the world's population relies on plants as their primary sources of medicinal agents, it is not surprising to find in many countries a well-established system of traditional medicine. The recognition and validation of traditional medicine is important and could lead to the discovery of new plant-derived drugs, such as quinine isolated from *Cinchona* species and artemisinin isolated from *Artemisia annua* L., since medicinal plants are well known as important sources of bioactive natural metabolites. Many compounds belonging to a range of chemical classes were isolated from various medicinal plants belonging to different botanical families and were reported to exhibit antiplasmodial activity against *Plasmodium* strains *in vitro* and *in vivo*. Some of them can be considered as lead compounds for the discovery of new antimalarial drugs with new modes of action (Caniato

and Puricelli, 2003; Bero et al., 2009; Xu and Pieters, 2013; Memvanga et al., 2015; Vlietinck et al., 2015).

Alstonia congensis Engl. is a medicinal plant belonging the Apocyanceae family, and is used in traditional medicine in many African countries. Each plant part, such as leaves, stem bark and root bark, has a therapeutic value. The methanol extract of the leaves was evaluated *in vivo* for its antiplasmodial activity against *P. berghei* infected mice. At an oral dose of 200 mg/kg body weight, it was found to produce 74% chemosuppression (Awe and Opeke, 1990). However, in the present investigation, root bark was investigated. An aqueous decoction of root bark is traditionally used to treat uncomplicated malaria and various other ailments (Neuwinger, 2000; Kambu, 1990). We have already reported the antiprotozoal screening and the cytotoxicity of extracts and fractions from the leaves, stem bark and root bark of *Alstonia congensis* (Lumpu et al., 2013). In the present work, the *in vitro* and *in vivo* antiplasmodial activity of the aqueous and methanol 80% extracts and its fractions, as well as the alkaloid-enriched extract of *A. congensis* root bark, was evaluated against chloroquine-resistant and chloroquine-sensitive *Plasmodium* strains, as well as the *in vitro* activity of some isolated constituents.

2. Materials and methods

2.1. General experimental procedures

Optical rotation was determined on a Jasco P-2000 polarimeter (Easton, MD, USA) with Spectramanager software. NMR spectra were recorded on a Bruker DRX-400 instrument (Rheinstetten, Germany) operating at 400 MHz for ^{1}H and at 100 MHz for ^{13}C. An Agilent QTOF 6530 mass spectrometer (Santa Clara, CA, USA) with MassHunter version B.06

software was used to perform accurate mass measurements (ESI+ mode, resolution 20,000). Calibration was done externally and the samples were measured after direct infusion.

2.2. Plant material

Root bark of *Alstonia congensis* Engl. (Apocynaceae) was collected in Kinshasa / Ndjili (DR Congo) in October 2015. The plant was identified by Mr. B. Nlandu Lukebabio of the Institut National d'Etudes et de Recherhes en Agronomie (INERA), Faculty of Sciences, Department of Biology of the University of Kinshasa, DR Congo. A voucher specimen No. NL010215ACRB was deposited in the herbarium of this institute. Plant material was dried at room temperature and reduced to powder using an electric blender.

2.3. Extraction, fractionation and isolation of constituents

Samples of 1000 g of powdered root bark were macerated separately with distilled water and methanol 80% (each 2000 ml) for 24 h. After filtration, each marc was percolated exhaustively with the corresponding solvent. Macerate and percolate obtained from each solvent were separately combined and evaporated under reduced pressure yielding the corresponding dried extracts denoted as AC-1 (718.24 g) for the aqueous extract and AC-2 (825.90 g) for the methanol 80% extract. An amount of 60 g of AC-1 was dissolved in 300 ml HCl 0.5% and filtered on Whatman paper No. 1. The filtrate was subjected to liquid / liquid partition as shown in Fig. 1, yielding subfractions as shown.

The alkaloid-enriched extract was prepared by the acid / base method described by Harborne (1998) using 20 g of extract AC-2, and denoted as AC-3 (12.54 g). An amount of 6 g of AC-3 was submitted to column chromatography on silica gel (Davisil LG 60 A 40-63 µm, Merck, Germany), eluted with $CHCl_3$ / MeOH : 5-100% MeOH. Several fractions of 10 ml were

collected and analysed by TLC on silica gel plates (layer thickness 0.25 mm, Merck, Germany) using $CHCl_3$ / MeOH 9:1 as mobile phase. They were combined according to their chromatographic profile as follows: fractions 1-9 (60 mg), 10-20 (68 mg), 22-34 (116 mg), 36-38 (20 mg), 39-76 (178 mg), 76-96 (98 mg) and 97-136 (408 mg). Fractions 10-20 were submitted to preparative TLC on silica gel plates (layer thickness 1 mm, Merck, Germany) using $CHCl_3$ / MeOH 9:1 as mobile phase resulting in the isolation of compound **1** (23 mg). Fractions 22-34 were also submitted to preparative TLC using EtOAc / MeOH / H_2O :15 :5 :3 as mobile phase, resulting in the isolation of compound **2** (24 mg). In the same way fractions 39-67 and 76-96 resulted in the isolation of compound **3** (46 mg) and **4** (32.14 mg). All compounds were identified by NMR and mass spectrometric methods, and by measuring their optical rotation. Spectral data are added as Supporting Information.

2.4. In vitro antiplasmodial testing

The *in vitro* antiplasmodial activity against *P. falciparum* NF54 A19A (Institute of Tropical Medicine, Antwerp, Belgium) of extracts, fractions and isolated constituents was performed according to Desjardin et al. (1979). The activity against the chloroquine and pyrimethamine-resistant *P. falciparum* strain K1 was evaluated using the lactate dehydrogenase procedure previously described by Makler et al. (1994) with some modifications (Cos et al., 2006; Mesia et al., 2008; Tuenter et al., 2016). In both tests, the parasites were cultured in RPMI-160 medium supplemented with 2% P/S solution, 0.37 mM hypoxanthine, 25 mM HEPES, 25 mM $NaHCO_3$ and 10% O^+ human serum together with 4% human O^+ erythrocytes. The antiplasmodial assay was performed in 96-well tissue culture plates in triplicate. The IC_{50} values were derived from dose-response curves.

2.5. Cytotoxicity assay

The cytotoxicity of extracts, fractions and isolated compounds was evaluated on MRC-5 cells (human lung fibroblasts) cultured in MEM medium, supplemented with 20 mM L-glutamine, 16.5 mM $NaHCO_3$, 5% foetal calf serum and 2% P/S solution according to the MTT procedure as previously described (Cos et al., 2006; Mesia et al., 2008; Tuenter et al., 2016). The 50% cytotoxic concentration (CC_{50}) for each sample was derived from the dose-response curves. A selectivity index (SI), corresponding to the ratio between cytotoxic and antiparasitic concentrations, was calculated as $SI = CC_{50} / IC_{50}$.

2.6. In vivo antiplasmodial activity

The aqueous extract (AC-1), the 80% methanol extract (AC-2) and its fractions and the alkaloid-enriched extract (AC-3) were assessed for their *in vivo* activity in a 4-day suppressive test against *Plasmodium berghei berghei* ANKA and *Plasmodium yoelii* N67 infections in mice as previously described (Tona et al., 2001). Both *Plasmodium* strains were obtained from the Institute of Tropical Medicine of Antwerp, Belgium. White adult Swiss (OF1) mice, with a mean body weight of 23.2 ± 2.1 g from INRB (Institut de Recherche Biomédicale, Kinshasa, DR Congo) were divided in eleven groups. Group I (n = 3) orally received 5 ml saline solution 0.9% (negative control), while group II (n = 3) received in the same way 10 mg/kg/b.w chloroquine diphosphate (positive control). Groups III to XI (6 mice each) were inoculated intraperitoneally with 1×10^7 of erythrocytes infected with *P. berghei berghei* from a donor mouse and treated with 200 and 400 mg/kg/b.w of each selected extract or fraction dissolved in saline solution 0.9%. From day 0 to day 4, a thin film was made from a tail-blood sample from each mouse and stained with Giemsa so that the level of parasitaemia (%) could be evaluated (as half the number of schizonts with at least three nuclei each, counted in 200 erythrocytes). On day 4, the mean in each group of mice was

determined and the % chemosuppression for the given dose of each sample was calculated as: [(*A* – *B*)/*A*] x 100, where *A* was the mean parasitaemia in the negative control group and *B* the mean parasitaemia in the test group.

2.7. Statistical analysis

All data are expressed as mean ± standard error of the mean. Results were subjected to one way analysis of variance (ANOVA) followed by Student's t test for multiple comparisons. Values of $P < 0.05$ were considered as significant.

3. Results and discussion

3.1. In vitro antiplasmodial activity of extracts and fractions

The aqueous extract, the 80% methanol extract and its fractions, and the alkaloid-enriched extract were evaluated for their potential antiplasmodial activity against chloroquine-sensitive *P. falciparum* NF54 A19A. The following criteria were adopted: IC_{50} < 10 µg/ml: strong activity; 10 ≤ IC_{50} < 20 µg/ml: good activity; 20 ≤ CI_{50} < 30 µg/ml: moderate activity; 30 ≤ IC_{50} < 50 µg/ml: weak activity; IC_{50} ≥ 50 µg/ml: inactive. Results (Table 1) indicated that the aqueous (AC-1), 80% methanol (AC-2), alkaloid-enriched (AC-3) extracts, and the chloroform-soluble fraction AC-2.1 (rich in steroids and terpenes), the aqueous (acid) soluble fraction AC-2.2 (rich in alkaloids and phenolic compounds) and the chloroform (alkaline) soluble fraction AC-2.2.1 (rich in alkaloids) displayed strong antiplasmodial activity against this *Plasmodium* strain with IC_{50} values < 0.02 µg/ml. Against the chloroquine-resistant *P. falciparum* K1 strain, it was observed that the 80% methanol, the alkaloid-enriched extracts, and the 80% methanol AC-2.1.2, the aqueous (acid) AC-2.2 and chloroform (alkaline) AC-2.2.1 fractions exhibited strong antiplamsodial activity with IC_{50}

values of 4.03 ± 0.07, 3.01 ± 0.05, 3.70 ± 0.08, 2.04 ± 0.0 2 and 2.17 ± 0.14 µg/ml, respectively. All tested samples did not show cytotoxicity against MRC-5 cells (CC_{50} > 64 µg/ml), resulting in a high selectivity.

3.2. In vivo antiplasmodial activity of extracts and fractions

Results of the *in vivo* antiplasmodial activity of extracts and fractions from *A. congensis* root bark are shown in Table 2 and 3. It was found that all extracts and fractions could significantly decrease parasitaemia in infected mice caused by *P. berghei berghei* ANKA and *P. yoelii* N67. With regard to *P. berghei berghei* ANKA, at the highest oral dose of 400 mg/kg bw, the aqueous, 80% methanol and alkaloid-enriched extracts caused a reduction of parasitaemia of 84.68 ± 0.41, 86.23 ± 0.11% and 88.12 ± 0.10%, respectively. Activities of all extracts were significantly different at both oral doses ($p < 0.05$). The highest activity was observed for the alkaloid-enriched extract AC-3, followed by the 80% methanol extract AC-2 (Table 3). All fractions prepared from the 80% methanol AC-2 extract were able to significantly reduce parasitaemia in infected mice in dose-dependent manner. At the highest oral dose of 400 mg/kg bw, all fractions produced more than 68% chemosuppresssion. However, all fractions were less active than the parent extract.

Against *P. yoelii* N67, the aqueous, 80% methanol and alkaloid-enriched extracts produced 78.20 ± 0.41, 84.23 ± 0.14 and 85.63 ± 0.15% chemosuppression, respectively. At the highest oral dose of 400 mg/kg bw, all fractions caused more than 65% reduction of the parasitaemia The most active fraction was AC-2.2.1, producing 81.39 ± 0.08% chemosuppression, but again the fractions were not more active than the parent extract.

3.3. Structure elucidation and in vitro activity of isolated constituents

In view of the pronounced *in vitro* and *in vivo* activities of the alkaloid-enriched extract AC-3and the alkaloid-containing fraction AC-2.2.1, compared to other fractions, the AC-3 extract was selected in order to purify its main constituents.

The ^{1}H and ^{13}C-NMR data of compound **1** were in good agreement with the assignments reported for β-amyrin, a common triterpene (Mahato and Kundu, 1994) (Fig. 2) (^{13}C NMR assignments are given as Supporting Information, Table S1). Typical ^{1}H-NMR signals were observed at δ_H 5.20 (t, H-12) and 3.24 (m, H-3).

The low number of ^{1}H- and ^{13}C-NMR signals observed for compound **2** suggested a relatively small molecule, and it was identified as boonein. An ester functionality was detected, based on the ^{13}C-NMR signal at δ_C 174.8 ppm, linked to a -CH_2- group at 66.9 (t) ppm (4.17 / 4.28 ppm in ^{1}H-NMR). Two additional –CH_2- groups were present at δ_C 29.5 and 41.1 (δ_H 2.83 and 1.4-1.5, repectively, four –CH- groups at δ_C 33.9, 75.2, 44.0 and 47.2, corresponding to ^{1}H-NMR signals at 2.06, 4.1, 2.2 and 2.63 ppm, respectively. The signal at δ_C 75.2 / δ_H 4.1 suggested an oxygenated carbon. In addition, also a methyl group was detected at δ_C 13.7 / δ_H 1.20. Literature search showed that these spectral data were in complete agreement with those reported by Marini-Bettolo et al. (1983), who isolated this compound from *Alstonia boonei*. This was confirmed by measurement of the specific optical rotation (+18.5, *c* 0.5, MeOH). ^{1}H- and ^{13}C-NMR assignments are given as Supporting Information, Table S2. Mass spectrometry confirmed a protonated molecular ion at *m/z* 171.10, and a Na+-adduct at *m/z* 193.08.

The ^{1}H- and ^{13}C-NMR spectra of compound **3** suggested an indole alkaloid, which was identified as echitamine. A 4-proton spin system was observed at δ_H 7.74 (d), 6.75 (td), 7.10

(td) and 6.73 (d), assigned to H-9 to H-12. In the HSQC spectrum these signals were correlated to peaks at δ_C 126.7, 119.5, 128.8 and 110.7. Other characteristic signals occurred at δ_C 173.2 and 51.9, suggesting a COOMe group, and at 64.5 (t), suggesting a $–CH_2OH$ functionality. An *N*-methyl group was observed at δ_C 49.6, corresponding to a ^{1}H-NMR signal at 3.29 ppm. Literature search showed that the ^{1}H- and ^{13}C-NMR spectra were in complete agreement with those reported for echitamine (Keawpradub et al., 1994; the same numbering was used). ^{1}H- and ^{13}C-NMR assignments are given as Supporting Information (Table S3). The structure was confirmed by accurate mass measurements (Supporting Information).

Also the ^{1}H- and ^{13}C-NMR spectra of compound **4** suggested an indole alkaloid, and based on literature data it could be identified as 6,7-seco-angustilobine B. A 4-proton spin system was observed at δ_H 7.56 (d), 7.13 (t), 7.19 (t) and 7.35 (d), assigned to H-9 to H-12. These signals corresponded in a 2-dimensional HSQC spectrum to peaks at δ_C 120.5, 120.2, 122.3 and 110.9, respectively. Other characteristic signals occurred at δ_C 173.6 and 52.8, suggesting a COOMe group, and at 71.0 (t) and 69.2 (t), suggesting $–CH_2–$ functionalities linked to an oxygen atom. An *N*-methyl group was observed at δ_C 44.98, corresponding to a ^{1}H-NMR signal at 3.38 ppm. Literature search showed that the ^{1}H- and ^{13}C-NMR spectra were in complete agreement with 6,7-seco-angustilobine B (Yamauchi et al., 1990). ^{1}H- and ^{13}C-NMR assignments are given as Supporting Information, Table S4). Mass spectrometry confirmed a protonated molecular ion at *m/z* 341.30.

This is the first report of boonein from *Alstonia congensis*. Both alkaloids have been isolated before from the leaves, root or stem bark of *A. congensis* (Monsieur and Van Bever, 1955; Caron et al., 1989), *A. glaucescens* (Keawpradup et al., 1994), *A. boonei* (Adotey et al., 2012) and/or *A. scholaris* (Yamauchi et al., 1990; Kam et al., 1997). β-amyrin is an ubiquitous triterpene, isolated from many other medicinal plants (Mahato and Kundu, 1994),

as well as from *A. congensis* (Monsieur and Van Bever, 1955) and *A. boonei* (Okoye et al., 2014).

The isolated compounds were tested *in vitro* against the chloroquine-resistant strain *P. falciparum* K1 and the chloroquine-sensitive strain *P. falciparum* NF54 A19A, as well as for their cytotoxic effects againt MRC-5 cells (human lung fibroblasts) (Table 4). Boonein was inactive *in vitro* against *P. falciparum* K-1 (IC_{50} > 64 µM), while echitamine, 6,7-seco-angustilobine and β-amyrin exhibited moderate activity (IC_{50} <30 µM). Against *P. falciparum* NF54 A19A, boonein was inactive (IC_{50} > 64 µM), while echitamine, 6,7-secoangustilobine and β-amyrin showed moderate IC_{50} values of 11.07, 21.26 and 40.70 µM, respectively (Table 2). On the other hand, no cytotoxicity was observed, and with the exception of boonein, compounds **1**, **3** and **4** can be considered as reletively selective. The antiplasmodial activity against *P. falciparum* K1 (IC_{50} 25 µg/ml) of β-amyrine was previously reported (De Almeida Alves et al., 1997). To our knowledge, this is the first report on the antiplasmodial activity of the indole alkaloids echitamine and 6,7-seco-angustilobine B against both *Plasmodium* strains.

Taking account of the high reduction of the levels of parasitaemia in mice infected with *P. bergehei berghei* ANKA and *P. yoelii* N67, root bark extracts of *A. congensis* may be considered as potential raw materials for the preparation of ameliorated antimalarial herbal medicines to be used by the local people.

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Table 1. *In vitro* antiplasmodial activity (IC_{50}, µg/ml), cytotoxicity (CC_{50}, µg/ml), and selectivity index (SI) of extracts and fractions from *A. congensis* root bark.

Sample	MRC-5 cells (CC_{50}) (µg/ml)	*Pf* NF54 A19A (IC_{50}) (µg/ml)	*Pf* K1 (IC_{50}) (µg/ml)	SI *Pf* NF54A19A	SI *Pf* K1
AC-1	> 64	< 0.02	5.84 ± 0.04	> 3200	> 1096
AC-2	> 64	< 0.02	2.04 ± 0.12	> 3200	> 31.4
AC-2.1	> 64	< 0.02	5.66 ± 0.12	> 3200	> 11.4
AC-2.1.1	> 64	5.33 ± 0.15	8.66 ± .24	> 1200	> 7.4
AC-2.1.2	> 64	7.45 ± 0.05	4.03 ± 0.07	> 850	>15.8
AC-2.2	> 64	< 0.02	3.01 ± 0.05	> 3200	> 21.3
AC-2.2.1	> 64	< 0.02	3.70 ± 0.08	> 3200	> 17.3
AC-2.2.2	> 64	6.45 ± 0.07	> 64	> 9.9	n.a.
AC-3	> 64	< 0.02	2.17 ± 0.14	> 3200	> 29.5
Quinine	> 64	0.46 ± 0.06	0.25 ± 0.07	> 139.1	> 256.0
Tamoxifen	10.48 µM	-	-	-	-

Pf K1 : *Plasmodium falciparum* K1 ; *Pf* NF54 A19A : *Plasmodium falciparum* NF54 A19A ; n.a. : not applicable ; SI : Selectivity Index

AC-1: aqueous extract, AC-2: 80% methanol extract, AC-2.1 to AC-2.2.2 : chloroform petroleum ether, 80% methanol, aqueous acid, chloroform/OH^-, residual aqueous/OH^- fractions from the partition of the 80% methanol extract AC-2 extract, AC-3: alkaloid-enriched extract.

Table 2. *In vivo* antiplasmodial activity of extracts and fractions from *A. congensis* root bark against *P. berghei berghei* ANKA (% chemosuppression).

Extract / fraction	Oral Dose (mg/kg bw)	% Parasitaemia	% Chemosuppression
AC-1	200	4.91 ± 0.04	80.43 ± 0.12
	400	4.05 ± 0.11	84.68 ± 0.41
AC-2.1	200	8.14 ± 0.05	69.22± 0.11
	400	7.22 ± 0.01	72.70 ± 0.01
AC-2.1.1	200	9.32± 0.14	64.76 ± 0.11
	400	8.82 ± 0.21	66.65 ± 0.04
AC-2.1.2	200	8.03 ± 0.04	69.64 ± 0.14
	400	7.06 ± 0.07	73.30 ± 0.07
AC-2.2	200	6.89 ±0.12	73.95 ± 0.11
	400	6.24 ±0.04	76.40 ± 0.15
AC-2.2.1	200	6.65 ± 0.17	74.85 ± 0.31
	400	6.05 ± 0.15	77.12 ± 0.31
AC-2.2.2	200	9.21 ± 0.14	65.17 ± 0.14
	400	8.44 ± 0.04	68.09 ±0.11
AC-2	200	4.21± 0.31	84.08 ± 0.20
	400	3.64 ± 0.22	86.23 ± 0.11
AC-3	200	3.96	85.02 ± 0.17
	400	3.14	88.12 ± 0.10
Chloroquine	10	0.55	97.92 ± 0.22
Quinine	10	0.61	97.87 ± 0.14
Negative control	5 ml distilled water	26.45	0.00 ±0.00

AC-1: aqueous extract; AC-2: 80% methanol extract; AC-2.1 to AC-2.2.2 : chloroform, petroleum ether, 80% methanol, aqueous (acid), chloroform/OH^-, residual aqueous/OH^- fractions from the partition of 80% methanol extract AC-2; AC-3: alkaloid-enriched extract

Table 3. *In vivo* antiplasmodial activity of extracts and fractions from *A. congensis* root bark against *P. yoelii* N67.

Extract / fraction	Oral Dose (mg/kg bw)	% Parasitaemia	% Chemosuppression
AC-1	200	6.83 ± 0.02	76.06 ± 0.22
	400	6.22 ± 0.11	78.20 ± 0.41
AC-2	200	5.03 ± 0.31	82.37 ± 0.22
	400	4.50 ± 0.22	84.23± 0.14
AC-2.1	200	7.85 ± 0.05	72.49 ± 0.01
	400	7.15 ± 0.01	74.94 ± 0.03
AC-2.1.1	200	9.22± 0.14	67.69 ± 0.21
	400	8.45 ± 0.21	70.39 ± 0.04
AC-2.1.2	200	8.83 ± 0.04	69.06 ± 0.14
	400	8.35 ± 0.07	70.74 ± 0.07
AC-2.2	200	6.32 ±0.12	77.85 ± 0.11
	400	5.86 ±0.04	79.46 ± 0.19
AC-2.2.1	200	5.90 ± 0.17	79.32 ± 0.03
	400	5.31 ± 0.15	81.39 ± 0.08
AC-2.2.2	200	10.25 ± 0.14	64.08 ± 0.11
	400	9.15 ± 0.04	67.94 ± 0.09
AC-3	200	4.15 ± 0.07	85.45 ± 0.07
	400	4.10 ± 0.05	85.63 ± 0.15
Chloroquine	10	1.25 ± 0.02	95.62 ± 0.1 2
Quinine	10	1.10 ± 0.01	96.14 ± 0.04
Negative control	5 ml distilled water	28.54 ± 0.03	0.00 ± 0.00

AC-1: aqueous extract; AC-2: 80% methanol extract; AC-2.1 to AC-2.2.2 : chloroform, petroleum ether, 80% methanol, aqueous (acid), chloroform/OH^-, residual aqueous/OH^- fractions from the partition of 80% methanol extract AC-2; AC-3: alkaloid-enriched extract

Table 4. *In vitro* antiplasmodial activity (IC_{50}, µg/ml), cytotoxicity (CC_{50}, µg/ml), and selectivity index (SI) of isolated compounds **1** – **4**.

Compound	MRC-5 cells (CC_{50}) (µM)	*Pf* NF54 A19A (IC_{50}) (µM)	*Pf* K1 (IC_{50}) (µM)	SI *Pf* NF54 A19A	SI *Pf* K1
β-amyrin (**1**)	> 64	40.70 ± 0.16	20.57 ±0.02	> 1.6	> 3.1
Boonein (**2**)	> 64	> 64	> 64	n.a.	n.a.
Echitamine (**3**)	> 64	11.07 ± 0.10	28.24 ± 0.12	> 5.8	> 2.3
6,7-Seco-angustilobine B (**4**)	> 64	21.26 ± 0.32	25.02 ± 0.15	> 3.0	> 2.6
Quinine	> 64	0.46 ± 0.06	0.25 ± 0.07	> 139.1	> 256
Tamoxifen	10.48 µM	-	-	-	-

Pf K1 : *Plasmodium falciparum* K1 ; *Pf* NF54 A19A : *Plasmodium falciparum* NF54 A19A ; n.a. : not applicable ; SI : Selectivity Index

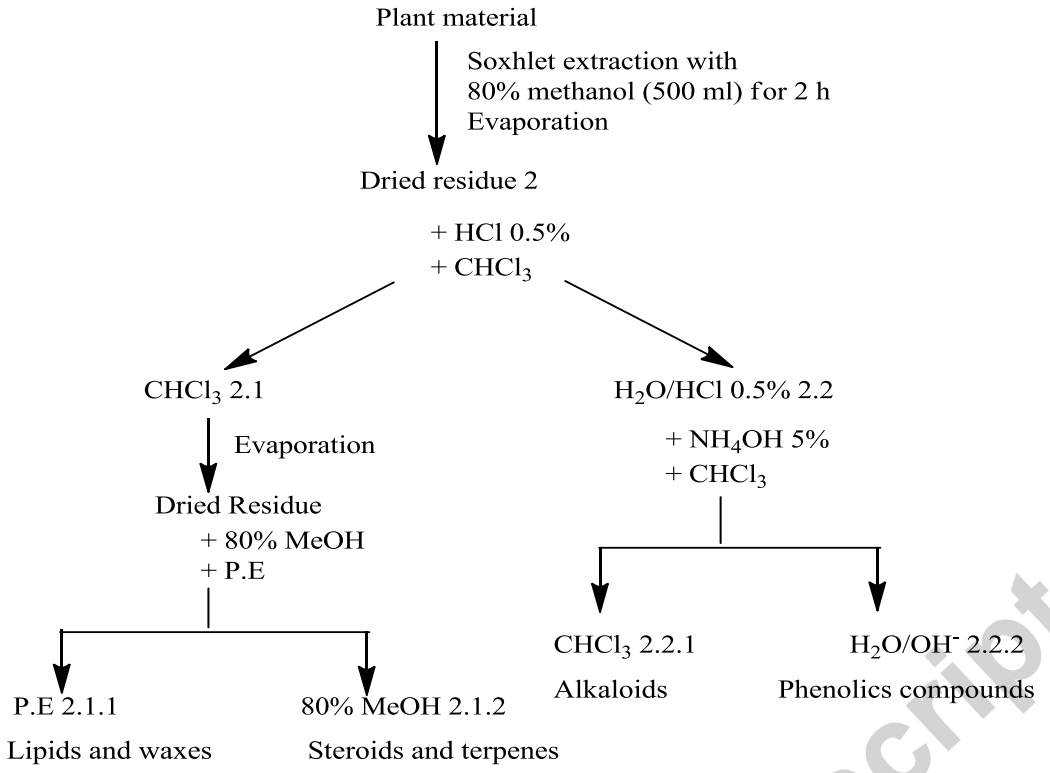


Figure 1. Fractionation of the methanol 80% extract AC-2

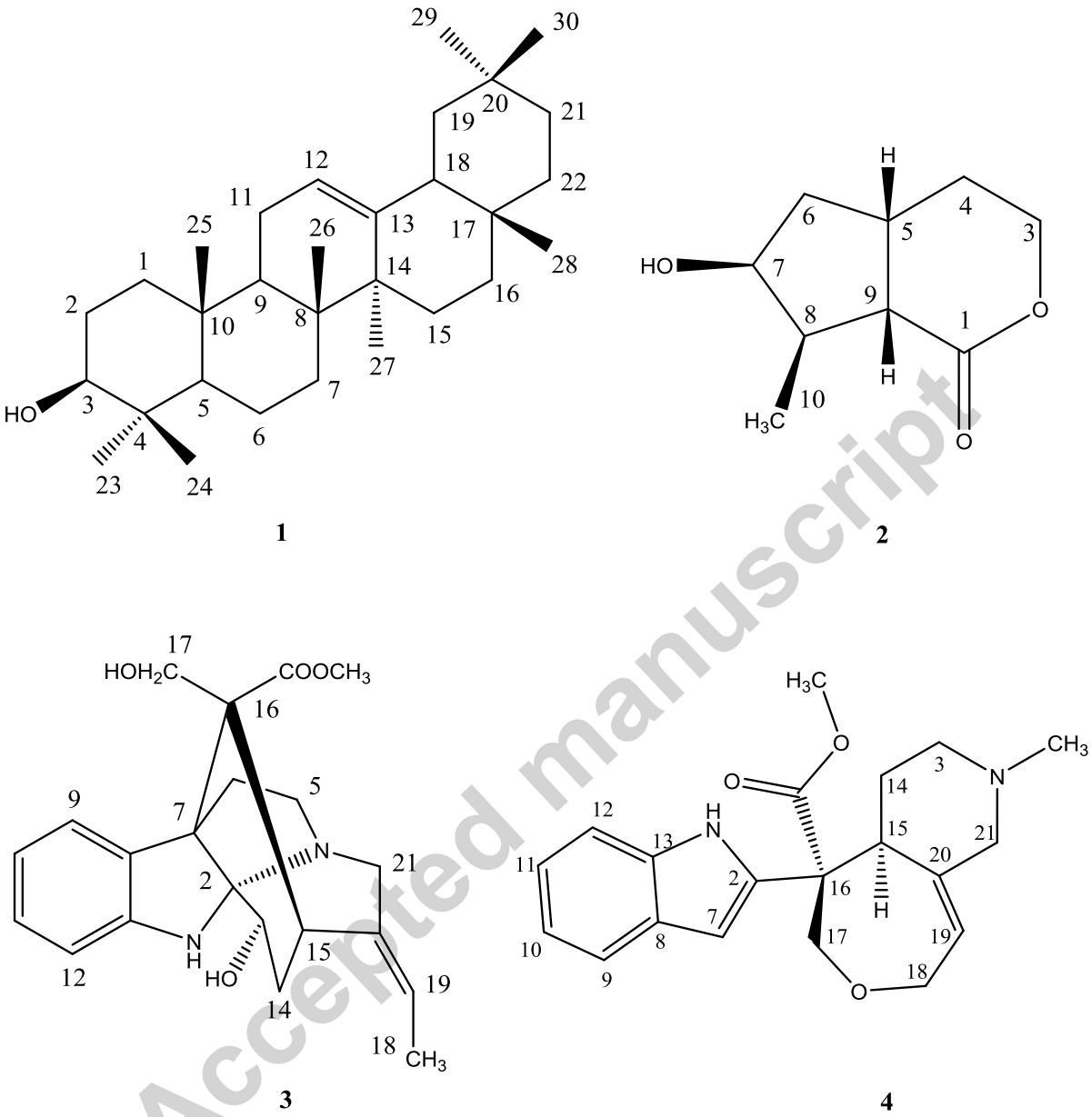
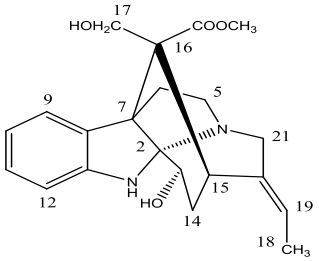
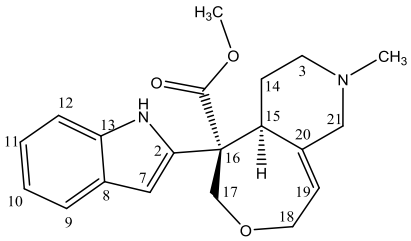


Figure 2. Antiplasmodial compounds isolated from the root bark of *Alstonia congensis*: β-amyrin (**1**), boonein (**2**), echitamine (**3**) and 6,7-seco-angustilobine B (**4**)





Graphical abstract