In vitro antiprotozoal activity and cytotoxicity of extracts and isolated constituents from **Greenwayodendron suaveolens**

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D. Musuyu Muganza[a], B. Fruth[b,c], J. Lami Nzunzu[a], E. Tuenter[d], K. Foubert[d], P. Cos[e], L. Maes[e], R. Cimanga Kanyanga[a,d], V. Exarchou[d], S. Apers[d], L. Pieters[d,*]

[a] Faculty of Pharmaceutical Sciences, University of Kinshasa, P.O. Box 212, Kinshasa XI, Democratic Republic of Congo

[b] Ludwig Maximilian University of Munich, Faculty of Biology/ Department Biology II, Großhaderner Straße 2, D - 82152 Planegg-Martinsried, Germany

[c] Centre for Research and Conservation / KMDA, Koningin Astridplein 20-26, B – 2018, Antwerp, Belgium.

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

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

Correspondence

D. Musuyu Muganza

Faculty of Pharmaceutical Sciences, University of Kinshasa

P.O. Box 212, Kinshasa XI, Democratic Republic of Congo

cdmuganza@hotmail.com

Abstract

Ethnopharmacological relevance: The Nkundo people (Nkundo area of Bolongo, Mai-Ndombe district, Bandundu Province, DR Congo) use various plant parts of the tree *Greenwayodendron suaveolens* (Engl. & Diels) Verdc. (syn. *Polyalthia suaveolens* Engl. & Diels) (Annonaceae) against malaria, but its antiprotozoal constituents are not known.

Materials and methods. The crude 80% ethanol extract from the fruits, leaves, root bark and stem bark and 16 fractions were assessed *in vitro* for their antiprotozoal activity against *Trypanosoma brucei brucei*, *T. cruzi*, *Leishmania infantum* and the chloroquine and pyrimethamine-resistant K1 strain of *Plasmodium falciparum* (*Pf*-K1). Their cytotoxic effects were evaluated against MRC-5 cells. Active constituents were isolated by chromatographic means, identified using spectroscopic methods, and evaluated in the same assays.

Results: The root bark extract showed the highest activity against *P. falciparum* K1 (IC_{50} 0.26 µg/mL) along with the stem bark alkaloid fraction (IC_{50} 0.27 µg/mL). The root bark alkaloid fraction had a pronounced activity against all selected protozoa with IC_{50} values < 1 µg/mL. The 90% methanol fractions of the different plant parts showed a pronounced activity against *P. falciparum* K1, with IC_{50} values ranging between 0.36 µg/mL and 0.69 µg/mL. Four constituents were isolated: the triterpenes polycarpol, and dihydropolycarpol, the latter one being reported for the first time from nature, and the alkaloids polyalthenol and N-acetyl-polyveoline. They were active to a various degree against one or more protozoa, mostly accompanied by cytotoxicity. The highest selectivity was observed for *N*-acetyl-polyveoline against *P. falciparum* K1 (IC50 2.8 µM, selectivity index 10.9).

Conclusions: These results may explain at least in part the traditional use of this plant species against parasitic diseases such as malaria in DR Congo.

Keywords: Antiprotozoal activity; Cytotoxicity; *Greenwayodendron suaveolens*; *Polyalthia suaveolens*; *Plasmodium falciparum*; malaria

1. Introduction

Parasitic diseases are among the most important causes of mortality and provoke large economic problems in developing countries. They have become a major public health problem mainly because of the development of resistance by most causative parasitic species against the common drugs. Therefore, it has become an urgent necessity to discover new therapeutic agents against these parasitic diseases, and to evaluate if traditional medicine can make a contribution in the search for new therapeutic approaches. In developing countries, both rural and urban populations are greatly dependent on medicinal plants for the treatment of different ailments including parasitic diseases.

In the frame of the project "Cuvette Centrale as reservoir of medicinal plants in DRCongo" (Fruth, 2011), ethnobotanical and ethnopharmacological investigations were conducted in the Nkundo area of Bolongo (Mai-Ndombe district, Bandundu Province, Democratic Republic of the Congo). It was found that the Nkundo people use various plant parts of *Greenwayodendron suaveolens* (formerly *Polyalthia suaveolens*, a 35-40 m high tree) alone or in mixtures with other plants to treat various diseases, including parasitic diseases such as uncomplicated malaria and helminthiasis (Mato, 2005; Musuyu Muganza, 2006). In general, this plant is used as an aqueous decoction *per os* and/or as enema against backaches, sexual weakness, headaches, malaria, loss of appetite, snakebite, pelvic pains, hepatitis, epilepsy, insanity, rheumatism, constipation, stomach pains, toothache, etc. (Tsabang et al., 2012; Fruth et al., 2010; Musuyu Muganza, 2006; Wieckhorst, 2002; Betti, 2002; Neuwinger, 2000; White & Abernethy, 1996; Bouquet, 1969). The fruits are edible and are also appreciated in their season by bonobos (*Pan paniscus*), chimpanzees (*Pan troglodytes*), gorillas (*Gorilla* sp.), other small primates and birds that all count as great seeds dispersers (Beaune et al., 2013; Beaune, 2012; Hohmann et al. 2006; Cousins and Huffman, 2002; White & Abernethy, 1996). The leaves are also reported by some Nkundo hunters to be occasionally consumed by some species of monkeys in spite of their bitterness (own unpublished data). In

some regions of Central African Republic and Cameroun, the leaves of this forest tree, also called “tobacco of ancestors” or “forest tobacco”, are used and appreciated as cigarettes (Oishi and Hayashi, 2014; Roulette et al., 2014; Roulette, 2010). As other Annonaceae family members, the genus *Greenwayodendron* (formerly *Polyalthia*) is known to be rich in metabolites such as alkaloids, acetogenins, phenolics, sterols and terpenes (Waterman, 1986).

In a previous study it was found that the stem bark total alkaloid fraction of *G. suaveolens* exhibited an IC_{50} value of 4.08 µg/mL against the *P. falciparum* K1 strain (Akendengué et al., 2005). Mesia et al. (2008) have reported an IC_{50} <1 µg/mL with SI >64 for a stem bark 80% MeOH extract of *G. suaveolens* against the chloroquine-sensitive Ghana strain of *P. falciparum*. Boyom et al. (2011) found an IC_{50} <10 µg/mL for a 95% EtOH extract from leaves, and an IC_{50} of 5.66 µg/mL for stem bark against the W2 strain of *P. falciparum*. In a preliminary screening of 33 plant species from DR Congo, antiprotozoal activities were observed for a root bark extract of *G. suaveolens*, most importantly against *Leishmania infantum* with an IC_{50} value of 8.0 µg/mL (Musuyu Muganza et al., 2012), confirming earlier results by Lamidi et al., 2005. In the present work, the systematic *in vitro* evaluation of the antiprotozoal activity of extracts and fractions from the fruits, leaves, root bark and stem bark as well as isolated constituents of *Greenwayodendron suaveolens* was carried out.

2. Materials and methods

2.1. Plant material

The plant material including fruits, leaves, root bark and stem bark was collected in March 2011 at the research site Luikotale (South 02°45.610', East 20°22.723') (Hohmann and Fruth, 2003) in the south-western part of the southern block of the Salonga National Park (Bandundu province, DR Congo). The plant was identified by B. Nlandu of the Institut National d’Etudes

et de Recherches en Agronomie (INERA / Kinshasa) and by J.P. Habari of the Department of Biology (Faculty of Sciences, University of Kinshasa). Voucher specimens PROCUV/MPI 1215 and PROCUV/MPI 2743 of the plant have been deposited in the herbarium of this institute. The plant materials were air-dried in the shade and reduced to powder (IKA®A11 basic, Germany).

2.2. Preparation of crude extracts and fractions

Four crude 80% EtOH total extracts were prepared from the powder of each plant part. An amount of 50 g powder was macerated with 500 mL of 80% EtOH for 48 h under permanent shaking. Then each mixture was filtered and evaporated under reduced pressure (40-45 °C) yielding dried extracts denoted as PSFR-3.1 (9.06 g), PSLE-3.1 (4.04 g), PSRB-3.1 (5.68 g) and PSSB-3.1 (4.03 g) for fruits, leaves, root bark and stem bark, respectively. An amount of 3 g of each dry extract was subjected to liquid-liquid partition as reported before (Fig. S1, Supplementary Material) (Ieven et al., 1979; Kikueta et al., 2013). In this fractionation scheme, each of the four total extracts was suspended in distilled water, acidified with 2% HCl until pH 3-5 and exhaustively washed with dichloromethane (DCM). This dichloromethane (DCM) phase was evaporated to dryness under reduced pressure at 40-45 ºC. The aqueous acidic phase was basified (pH = 9) with 10% ammonia and extracted again with DCM to afford enriched alkaloid fractions PSFR-3.2 (60.2 mg), PSLE-3.2 (154.1 mg), PSRB-3.2 (95.1 mg) and PSSB-3.2 (175.7 mg) for fruits, leaves, root bark and stem bark extracts, respectively). The aqueous phases were labeled as alkaline aqueous fractions PSFR-3.3 (679.5 mg), PSLE-3.3 (832.1mg), PSRB-3.3 (611.7 mg) and PSSB-3.3 (714.3 mg). The acidic DCM extract was dissolved in 90% MeOH and quantitatively extracted with petroleum ether, yielding the petroleum ether fractions PSFR-3.4 (412.6 mg), PSLE-3.4 (706.4), PSRB-3.4 (558.9 mg) and PSSB-3.4 (609.6 mg) and the 90% MeOH fractions PSFR-3.5 (656.8 mg), PSLE-3.5 (747.6 mg), PSRB-3.5 (874.7 mg) and PSSB-3.5 (812.1 mg). All fractions were

dried under reduced pressure at 40-45 ºC, apart from the alkaline aqueous fractions that were lyophilized.

2.3. Phytochemical screening and TLC analysis

The crude extracts and fractions were submitted to a phytochemical screening using different color reactions and TLC on silica gel plates 60 F_{254} (layer thickness 0.25 mm, Merck). Two mobile phases (a polar and a non-polar one) and different reagents were used for the detection of some major phytochemical groups such as alkaloids (Dragendorff reagent), flavonoids (Neu reagent), tannins (Fast Blue Salt B reagent), terpenes and steroids (Liebermann-Burchard reagent) (Wagner and Bladt, 1996).

The four 90% MeOH fractions (PSFR-3.5, PSLE-3.5, PSRB-3.5 and PSSB-3.5) from all the tested plant parts were analysed by TLC (Silica gel 60 F_{254}). The mobile phase was $CHCl_3$-EtOAc (4:6), and spots were visualized under UV lamp and sprayed with the Liebermann or Dragendorff reagent.

2.4. Isolation and structure elucidation

The isolation of different constituents was done following the scheme presented in Fig. S2 (Supplementary Material). Fraction PSRB-3.5 (5.0 g) was subjected to open column chromatography (5 x 90 cm) on silica gel (Davisil® LC60A, 60-200 µm) with methylethylketone / toluene (2:3) as mobile phase. The collected fractions (10 mL each) were monitored by TLC (silica gel 60F_{254}, Merck) using the same eluent; the sulfuric acid / vanillin reagent, followed by heating to 110˚C during 5 min, was used for visualization. Fractions with similar TLC profiles were combined and evaporated to dryness to give 12 recombined fractions (I-XII). Fraction II (0.46 g) was subjected to further column chromatography on silica gel (2 x 40 cm; 5 mL fractions) yielding 3 other recombined subfractions (IIa, IIb and IIc). Preparative thin-layer chromatography (PTLC) of IIc (103 mg) on silica gel plates

(Analtech, layer thickness 2 mm, Sigma-Aldrich) with the same mobile phase yielded compound **1** (9.6 mg), which corresponded to the main band on the plate. Fraction III (1.2 g) was fractionated with repeated silica gel column chromatography (5 x 90 cm; 10 mL fractions) and gave 5 other recombined subfractions (IIIa, IIIb, IIIc, IIId and IIIe); subfraction IIId (551 mg) was further fractionated on a Sephadex LH-20 column (diameter 2.5 cm; adsorbent weight 50 g) using MeOH as eluent (95 fractions of 5 mL each) to give 5 further subfractions (IIId1, IIId2, IIId3, IIId4 and IIId5). From both subfractions IIId1 (17 mg) and IIId2 (367.7 mg), compound **2** (8.9 mg and 24.6 mg, respectively) was isolated using PTLC in the same conditions. Likewise, compound **3** (21 mg) was also obtained by separation of IIId4 and IIId3 by PTLC. Fraction IV (127 mg) was fractionated on a Sephadex LH-20 column (diameter 2 cm; adsorbent weight 35 g) using MeOH as eluent. A total of 50 fractions (2 mL each) was obtained and recombined in 4 subfractions IVa (13 mg), IVb (43 mg), IVc (22 mg) and IVd (36 mg) according to their TLC profile. Unfortunately, these profiles did not display the presence of any major compounds. Fraction V (750 mg) was further fractionated on a Sephadex LH-20 column (diameter 2.5 cm; adsorbent weight 50 g) using MeOH as eluent. A total of 55 fractions (2 mL each) were obtained and recombined in 3 subfractions (Va, Vb and Vc) according to their TLC profile. Subfraction Vb was separated by PTLC (using the same conditions as above) yielding compound **4** (101.3 mg) as the main component.

NMR spectra were recorded in deuterated chloroform ($CDCl_3$) on a Bruker DRX-400 spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany) operating at 400.13 MHz for ^{1}H and at 100.61 MHz for ^{13}C.

Accurate mass spectra of compounds **3** and **4** were obtained by direct infusion using a QTOF 6530 mass spectrometer (Agilent Technologies) equipped with an electrospray ionisation (ESI). For the detection of compounds **1** and **2**, an amMS Q Exactive™ (Thermo Fisher

Scientific) was used with Atmospheric Pressure Chemical Ionization (APCI). For both instruments full scan data were acquired in positive ion mode.

Isolated constituents were identified by spectroscopic methods as the triterpenes polycarpol (**1**) and dihydropolycarpol (**2**), and the alkaloids polyalthenol (**3**) and *N*-acetyl-polyveoline (**4**).

Dihydropolycarpol (2). ^{1}H NMR ($CDCl_3$): δ 5.84 (1H, d, J=6.0 Hz, H-7), 5.30 (1H, d, J=6.3 Hz, H-11), 4.26 (1H, dd, J=9.5 Hz, 5.8 Hz, H-15), 3.23 (1H, dd, J=11.3 Hz, 4.4 Hz, H-3), 1.15 (6H, overlapping signal, H-26, H-27), 1.02 (3H, s, H-30), 1.01 (3H, s, H-19), 0.96 (3H, s, H-32), 0.90 (3H, s, H-31), 0.90 (3H, d, J=4.8 Hz, H-21), 0.60 (3H, s, H-18). ^{13}C NMR ($CDCl_3$): δ 35.7 (C-1), 27.8 (C-2), 78.9 (C-3), 38.7 (C-4), 49.0[a] (C-5), 22.9 (C-6), 121.3 (C-7), 140.9 (C-8), 146.1 (C-9), 37.4 (C-10), 116.1 (C-11), 38.5 (C-12), 44.3 (C-13), 52.0 (C-14), 74.8 (C-15), 40.1 (C-16), 49.1[a] (C-17), 15.9[b] (C-18), 22.9 (C-19), 36.0 (C-20), 18.5 (C-21), 36.8 (C-22), 20.5 (C-23), 40.3 (C-24), 29.7 (C-25), 25.0 (C-26, C-27), 28.2 (C-30), 15.8[b] (C-31), 17.2 (C-32) (numbering according to Jung et al., 1990) (assignments bearing the same superscript may be interchanged).
HR-APCI-MS *m/z* 425.37693 $[M\text{-}H_2O+H]^+$ (calcd. for $C_{30}H_{49}O$, 425.37779); *m/z* 407.36666 $[M\text{-}2H_2O+H]^+$ (calcd. for $C_{30}H_{49}$, 407.367228), corresponding to a molecular formula for dihydropolycarpol of $C_{30}H_{50}O_2$ (MW 442).

Compounds **1**, **3** and **4**: see Supplementary Material.

2.5. Evaluation of the biological activities

Antiprotozoal activity testing (on *Trypanosoma brucei brucei* (*Tbb*), *T. cruzi* (*Tc*), *Leishmania infantum* (*Li*) and chloroquine and pyrimethamine-resistant K1 strain (*PF*-K1) of *Plasmodium falciparum*) and cytotoxicity testing (on MRC-5 cell lines, i.e. human lung fibroblasts) were carried out according to previously described procedures and expressed as IC_{50} values (Cos et al., 2006; Mesia et al., 2008). For the evaluation of the *in vitro* antiprotozoal screening of extracts and fractions the following criteria were adopted: $IC_{50} \leq 5$ µg/mL: pronounced activity; $5 < IC_{50} \leq 10$ µg/mL: good activity; $10 < IC_{50} \leq 20$ µg/mL: moderate activity; $20 <$

$IC_{50} \leq 40$ µg/mL: low activity; $IC_{50} > 40$ µg/mL: inactive. The selectivity index (SI), the ratio of the 50% cytotoxicity concentration (CC_{50}) on the IC_{50} value was calculated for each tested sample in order to evaluate its selectivity. For reasons of cost effectiveness, the first antiprotozoal screening was based on single measurements; pure compounds were tested in triplicate against *P. falciparum*, being the main target, and also the new compound dihydropolycarpol was tested in triplicate.

3. Results and discussion

Phytochemical screening of the crude 80% EtOH extracts of the different parts of *G. suaveolens* and their respective fractions revealed the presence of alkaloids, polyphenols (tannins and flavonoids), steroids and terpenes as presented in Table 1.

All the 80% ethanol crude extracts obtained from the fruits, leaves, root bark and stem bark, and their different fractions were evaluated *in vitro* for their putative antiprotozoal activity against *Trypanosoma brucei brucei*, *T. cruzi*, *Leishmania infantum* and the chloroquine and pyrimethamine resistant K1 strain of *Plasmodium falciparum*. Their cytotoxicity against MRC-5 cell lines was also assessed. IC_{50} values and the corresponding selectivity indices (SI) values are presented in Table 2.

Comparing the 4 crude 80% EtOH extracts, the root bark extract PSRB-3.1 was most active against *Pf*-K1 (IC_{50} 0.26 µg/mL), although rather cytotoxic (SI 29.62). The stem bark extract PSSB-3.1 was found to be the most active against both *Tbb* and *Tc* with respective IC_{50} values of 2.01 µg/mL (SI = 17.85) and 2.00 µg/mL (SI = 17.94), whereas the root bark extract PSRB-3.1 was found to display the most important activity, but not selective, against *Li* (IC_{50} = 8.11 µg/mL, SI = 0.95). According to Musuyu Muganza et al. (2012), the root bark aqueous extract of *P. suaveolens* displayed a comparable activity against *Li* but with a better selectivity (IC_{50} 8.00 µg/mL, SI = 4.4). Lamidi et al. (2005) observed a potent antileishmanial activity of the MeOH stem bark crude extract against promastigotes (IC_{50} = 1.8 µg/mL) and

intracellular amastigotes (IC_{50} 8.6 µg/mL), and of the 50% MeOH extract (IC_{50} = 9.8µg/mL) on promastigotes.

The alkaloid fraction of the stem bark PSSB-3.2 showed pronounced and selective activity against *Pf*-K1 (IC_{50} 0.27 µg/mL, SI >237.04), which was more active than reported by Akendengué et al. (2005), who observed an IC_{50} value of 4.08 µg/mL against the same strain. The root bark extract PSRB-3.2 showed a broad activity against *Tbb* (IC_{50} 0.50 µg/mL, SI >128), *Tc* (IC_{50} 0.25 µg/mL, SI >256), *Li* (IC_{50} 0.63µg/mL, SI >101.59) and *Pf*-K1 (IC_{50} 0.75 µg/mL, SI >85.33). With regard to the 90% MeOH fractions, the best activity was observed for the root bark fraction PSRB-3.5 against *Pf*-K1, having an IC_{50} of 0.36 µg/mL (SI 81.58).

Previous phytochemical investigations of the genus *Greenwayodendron* (formerly *Polyalthia*) revealed bioactive constituents such as alkaloids, acetogenins, phenolics, terpenoids. In particular, the species *G. suaveolens* has been shown to contain indolosesquiterpenes, triterpenes, and different types of alkaloids (Cavé et al., 1978; Okorie 1980; Okorie, 1981; Hocquemiller et al., 1981; Hasan et al., 1982; Kunesch et al., 1985; Yoo et al., 2005; Lamidi et al., 2005; Nyasse et al., 2006; Ngantchou et al., 2010; Williams et al., 2010).

Further separation of fraction PSRB-3.5 by successive open column chromatography on silicagel and Sephadex LH-20 yielded 4 compounds (**1** - **4**) (Fig. S2). Compound **1** was isolated from fraction II (subfraction IIc), compounds **2** and **3** from subfractions IIId1-4, and compound **4** from subfraction Vb. Fraction I seemed to contain lipids and waxes; F-VI to XII were devoid of major substances according to their TLC profiles, which displayed numerous minor substances.

The ^{1}H- and ^{13}C-NMR spectra of compound **1** showed the typical features of a triterpene, more in particular containing three double bonds as evidenced by the ^{13}C-NMR

lines at δ 116.1, 121.3 and 124.9 (all CH-groups), and δ 131.2, 140.9 and 146.1 (all quaternary carbons). Two signals could be assigned to oxygen-substituted carbons, i.e. at δ 74.8 and δ 78.9. The NMR spectral data were in complete agreement with those published for the steroid polycarpol, or lanosta-7,9(11),24-triene-3β,15α-diol by Gomes Pereira et al. (2003), who isolated it from *Duguetia glabriuscula* R. E. Fr. (R. E. Fr.) (Annonaceae) (Fig. 1). Originally polycarpol had been obtained from *Polyalthia oliveri* Engl. by Hamonnière et al. (1977), and since then from various other sources. However, this rare triterpene seems to be exclusive for the Annonaceae family (Jung et al., 1990).

The ^{1}H- and ^{13}C-NMR spectral data of compound **2** were very similar to those observed for **1**, but only 4 ^{13}C-NMR lines were present at δ 116.1, 121.3 (both CH-groups), 140.9 and 146.1 (both quaternary carbons). This easily led to the conclusion that the double bond at position 24 in the side-chain was saturated, which was confirmed by detailed analysis of the NMR spectra. The β-configuration of the hydroxy group in position C-3 was established by the coupling pattern observed in the 1H-NMR spectrum (dd, J = 11.3 Hz and 4.4 Hz, as reported before for polycarpol. Likewise, also the hydroxy group in position C-15 had the same configuration (α) as in polycarpol, based on the similar coupling pattern (dd, J = 9.5 and 5.8 Hz). Therefore, compound **2** could unambiguously be identified as lanosta-7,9(11)-diene-3β,15α-diol or dihydropolycarpol. To the best of our knowledge this is a new compound that has not been reported before.

Based on their ^{1}H- and ^{13}C-NMR spectral data compounds **3** and **4** were identified as the known indolosesquiterpene alkaloids polyalthenol (**3**) and *N*-acetyl-polyveoline (**4**). Polyalthenol (**3**) was originally isolated from stem bark of *Polyalthia oliveri* (Leboeuf et al., 1976) and had also been reported before from roots of *Polyalthia* (*Greenwayodendron*) *suaveolens* (Williams et al., 2010). *N*-acetyl-polyveoline (**4**) has already been isolated from

the stem bark of *Polyalthia suaveolens* by Ngantchou et al., 2010. All structures were confirmed by high-resolution mass spectrometry.

Antiprotozoal activities of the isolated compounds are presented in Table 3. Polycarpol **1** displayed the most important activity against *T. cruzi* (IC_{50} = 1.4 µM; SI = 2.0) even greater than the reference benznidazol (IC_{50} = 2.1 µM); it also exhibited the highest activity against *L. infantum* (IC_{50} = 3.17 µM), being 3 times more potent than miltefosine: IC_{50} = 9.02 µM). However, pronounced cytotoxicity (IC_{50} 2.8 µM) resulted in poor selectivity indices. Polycarpol has been isolated from *Pipostigma preussi* (Annonaceae) by Ngantchou et al. (2009), who observed an IC_{50} against *T. brucei* of 5.1 µM, and a moderately cytotoxic effect against mammalian MRC-5 cells with a CC_{50} of 54.6 µM. Jung et al. (1990) have observed significant but unselective cytotoxicities in various human tumor cell lines, including A-549 (ED_{50} = 0.54 µg/mL), HT-29 (ED_{50} = 0.13 µg/mL) and MCF-7 (ED_{50} = 0.22 µg/mL).

Dihydropolycarpol (**2**) was less active than polycarpol (1), but since it was about 7 times less cytotoxic, at least SI values ranging from 2 to 8 could be obtained.

Polyalthenol (**3**) showed good activities against all selected protozoa with IC_{50} values ranging between 2.6 and 8.4 µM, but again there was a lack of selectivity since a relatively high cytotoxicity was observed (IC_{50} = 5.8 µM). This compound is known for its antibacterial activity with MIC_{90} = 4 µg/ mL against MRSA (Methicillin Resistant- *Staphylococcus aureus*) (Williams et al., 2010), and its synthesized analogues for their anticancer activity (Marcos et al., 2012; 2014); but no previous data could be found concerning the antiprotozoal activity of polyalthenol.

N-acetyl-polyveoline (**4**) showed good activity towards *Pf*-K1 (IC_{50} = 2.8 µM) accompanied by a low cytotoxicity (IC_{50} = 29.8 µM; SI = 10.9). Ngantchou et al. (2010) found N-acetyl-polyveoline inactive against *Trypanosoma brucei* and this finding is in

accordance with our results. Kouam et al. (2014) also found this compound in the stem bark of *Polyalthia oliveri* and they reported a moderate antiplasmodial activity (IC_{50} = 29.1 µM) against erythrocytic stages of chloroquine-sensitive *Plasmodium falciparum* NF54 strain and low cytotoxicity (IC_{50} = 42.7 µM) on the rat skeletal myoblast (L6) cell line.

The four 90% MeOH fractions (PSFR-3.5, PSLE-3.5, PSRB-3.5 and PSSB-3.5) from all the tested plant parts were analysed by TLC together with the isolated constituents. It was found that compounds **1** (polycarpol) and **3** (polyalthenol) were notably present in PSRB-3.5 and PSSB-3.5, whereas they were not or at least much less present in fruit and leaves (PSFR-3.5 and PSLE-3.5). Compound **4** (*N*-acetyl-polyveoline) was more abundant in fruit (PSFR-3.5) but less in PSRB-3.5 and PSSB-3.5; it could not be detected in PSLE-3.5.

Sesquiterpenyl indoles are known as a small group of natural compounds occurring in plants, fungi and bacteria (Ding et al., 2011; Zhang et al., 2012) especially from endophytes (Ding et al., 2010) associated with mangroves, though very interesting due to their molecular complexity and wide variety of biological properties, such as antibacterial (Zhang et al., 2012) anticancer or even anti-HIV (Ding et al., 2010).

Ngantchou et al. (2010) found a good anti-*Tbb* activity for a mixture of 2 alkaloids (polysin and its epimer greenwayodendrin-3-one) isolated from the stem bark. The pronounced activity of the root bark alkaloid fraction can therefore be more likely justified by the presence of some active alkaloids in this fraction.

When comparing the IC_{50} values of the extracts and fractions listed in Table 2 with the IC_{50} values of the isolated constituents listed in Table 3, it appears they have mostly the same order of magnitude. This may be due to the presence of other, yet unidentified active principles, or more likely to a synergistic effect of their constituents.

4. Conclusion

These results confirm the traditional knowledge concerning the antiparasitic activities of *Greenwayodendron suaveolens,* which can be considered as a very promising potential source for antiprotozoal substances especially against *Plasmodium*, and support the great potential of this plant species, all parts of which are used in ethnomedicine. Bioactive fractions can be found in each of the four tested plant parts suggesting valuable biological benefits for all the consumers i.e. both humans and animals. The hypothesis that non-human primates among others eat the bitter leaves and the fruits for possible medicinal benefit can be considered. Four compounds have been isolated, identified and tested on the selected protozoa, i.e. the triterpenes polycarpol and dihydropolycarpol, and the alkaloids polyalthenol and *N*-acetyl-polyveoline. All of them were active to a various degree against one or more protozoa, mostly accompanied by cytotoxicity. The highest selectivity was observed for *N*-acetyl-polyveoline against *P. falciparum* K1.

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Conflict of interest

Authors declare that there are no conflicts of interests.

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Table 1: Results of the phytochemical screening

Phytochemical groups/ Samples	PSFR-1	PSLE-1	PSSB-1	PSRB-1	PSFR-2	PSLE-2	PSSB-2	PSRB-2	PSFR-3.1	PSFR-3.2	PSFR-3.3	PSFR-3.4	PSFR-3.5	PSLE-3.1	PSLE-3.2	PSLE-3.3	PSLE-3.4	PSLE-3.5	PSRB-3.1	PSRB-3.2	PSRB-3.3	PSRB-3.4	PSRB-3.5	PSSB-3.1	PSSB-3.2	PSSB-3.3	PSSB-3.4	PSSB-3.5
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+
Flavonoids	+	+	+	+	+	+	+	+	+	-	+	-	-	+	-	+	-	-	+	-	+	-	-	+	-	+	-	-
Tannins	+	+	+	+	+	+	+	+	+	-	+	-	-	+	-	+	-	-	+	-	+	-	-	+	-	+	-	-
Terpenes & Steroids	+	+	+	+	+	+	+	+	+	-	-	+	+	+	-	-	+	+	+	-	-	+	+	+	-	-	+	+

+: present; -: absent

Table 2: Antiprotozoal and cytotoxic activity of different crude extracts and fractions from different parts of *Greenwayodendron suaveolens*.

Samples	Antiprotozoal activity IC_{50} (µg/mL)				Cytotoxicity IC_{50} (µg/mL)	MRC-5 Selectivity Index (SI)			
	Tbb	***Tc***	***Li***	***Pf*-K1**	**MRC-5 cells**	**MRC-5 / *Tbb***	**MRC-5 / *Tc***	**MRC-5 / *Li***	**MRC-5 / *Pf*-K1**
PSFR-3.1	33.71	34.27	>64.00	19.77	>64.00	>1.90	>1.87	-	>3.24
PSFR-3.2	33.19	14.79	24.05	16.49	34.56	1.04	2.34	1.44	2.10
PSFR-3.3	>64.00	>64.00	>64.00	5.44	>64.00	-	-	-	>11.76
PSFR-3.4	8.23	7.49	24.05	3.12	30.45	3.70	4.07	1.27	9.76
PSFR-3.5	2.11	3.58	40.32	0.69	>64.00	>30.33	>17.88	>1.59	>92.75
PSLE-3.1	8.36	27.86	43.07	1.15	>64.00	>7.66	>2.30	>1.49	>55.65
PSLE-3.2	33.45	31.17	34.56	3.17	>64.00	>1.91	>2.05	>1.85	>20.19
PSLE-3.3	>64.00	>64.00	>64.00	19.03	>64.00	-	-	-	>3.36
PSLE-3.4	8.11	8.33	8.00	12.00	32.00	3.95	3.84	4.00	2.67
PSLE-3.5	8.50	8.06	32.46	0.56	37.26	4.38	4.62	1.15	66.54
PSRB-3.1	8.17	7.38	8.11	0.26	7.70	0.94	1.04	0.95	29.62
PSRB-3.2	0.50	0.25	0.63	0.75	>64.00	>128.00	>256.00	>101.59	>85.33
PSRB-3.3	>64.00	>64.00	>64.00	12.70	>64.00	-	-	-	>5.04
PSRB-3.4	8.23	7.88	27.27	1.89	22.88	2.78	2.90	0.84	12.11
PSRB-3.5	2.04	2.11	7.51	0.36	29.37	14.40	13.92	3.91	81.58
PSSB-3.1	2.01	2.00	24.05	1.04	35.87	17.85	17.94	1.49	34.49
PSSB-3.2	8.57	28.28	20.32	0.27	>64.00	>7.47	>2.26	>3.15	>237.04
PSSB-3.3	>64.00	>64.00	>64.00	>64.00	>64.00	-	-	-	-
PSSB-3.4	8.17	2.00	5.04	1.80	17.31	2.12	8.66	3.43	9.62
PSSB-3.5	2.20	2.05	6.82	0.41	7.47	3.40	3.64	1.10	18.22

PSFR: *Greenwayodendron suaveolens* fruits; **PSLE:** *Greenwayodendron suaveolens* leaves; **PSRB:** *Greenwayodendron suaveolens* root bark; **PSSB:** *Greenwayodendron suaveolens* stem bark;

3.1: 80% crude ethanol extract, **3.2:** Dichloromethane fraction rich in alkaloids, **3.3:** Alkaline aqueous fraction rich in salts & hydrophilic substances; **3.4:** Petroleum ether fraction rich in lipids and waxes; and **3.5:** 90% methanol fraction rich in steroids and terpenes from each selected plant part;

***Tbb*: *Trypanosoma brucei brucei*, *Tc*: *Trypanosoma cruzi*, *Li*: *Leishmania infantum*, MRC-5:** Human Fetal Lung Fibroblast Cells; ***Pf*-K1:** chloroquine and pyrimethamine resistant K-1 strain of *Plasmodium falciparum*.

Table 3: Antiprotozoal and cytotoxic activity of different substances isolated from the root bark of *Greenwayodendron suaveolens*.

Tested substances	**Antiprotozoal activity IC_{50} (µM)**				**MRC-5 cells Cytotoxicity CC_{50} (µM)**	**MRC-5 Selectivity Index (SI)**			
	Tbb	***Tc***	***Li***	***Pf*-K1**		**MRC-5 / *Tbb***	**MRC-5 / *Tc***	**MRC-5 / *Li***	**MRC-5 / *Pf*-K1**
Polycarpol (**1**)	8.1	1.4	3.2	3.0 ± 3.3	2.8 ± 2.0	<1	2.0	<1	<1
Dihydropolycarpol (**2**)	8.1 ± 0.1	2.4 ± 0.1	8.0 ± 3.0	10.3 ± 0.5	19.4 ±2.2	2.4	8.1	2.4	1.9
Polyalthenol (**3**)	8.2	8.4	8.1	2.6 ± 3.3	5.8 ± 0.8	<1	<1	<1	2.2
N-acetyl-polyveoline (**4**)	>32	8.4	> 32	2.8 ± 3.5	29.8 ± 4.1	<1	3.5	<1	10.6
Tamoxifen					10.48				
Benznidazole		2.13							
Miltefosine			9.02						
Suramine	0.03								
Chloroquine				0.08					

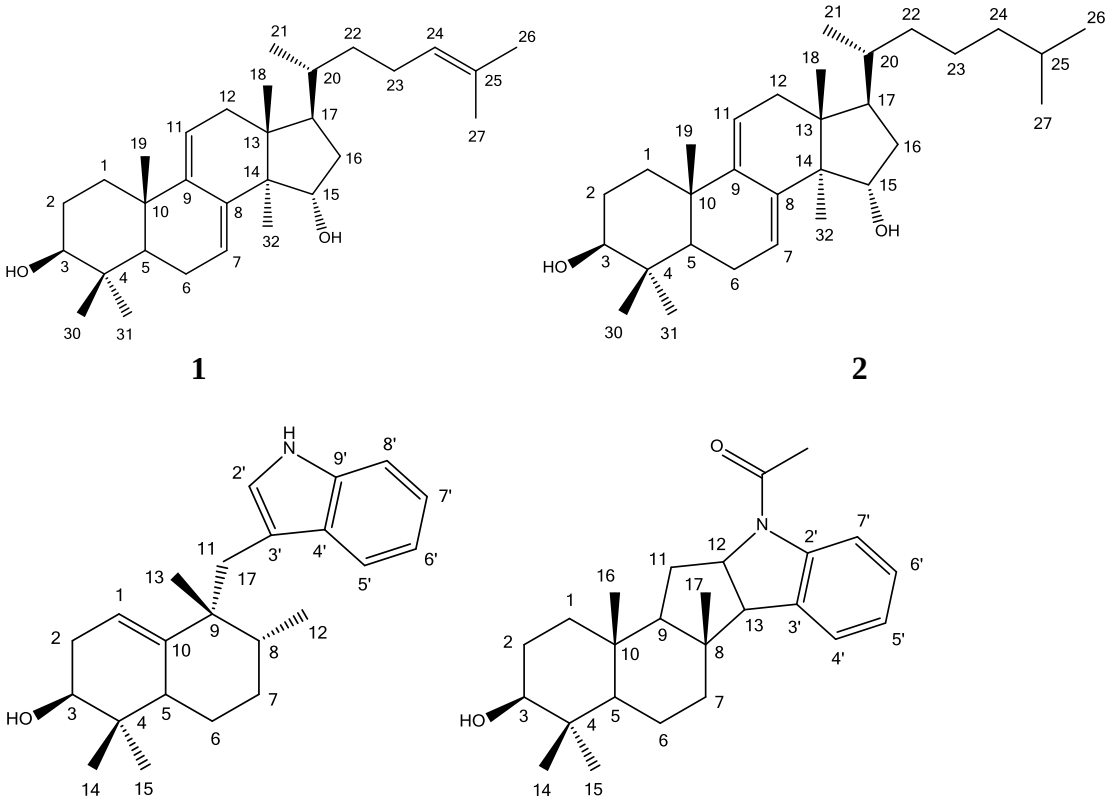

Fig. 1: Structure of isolated constituents: polycarpol (**1**), dihydropolycarpol (**2**), polyalthenol (**3**) and *N*-acetyl-polyveoline (**4**).