Research Article

IN VITRO ANTIPLASMODIAL AND *ANTILEISHMANIAL* ACTIVITIES OF FLAVONOIDS FROM *ANOGEISSUS LEIOCARPUS* (COMBRETACEAE)

Barthélemy Attioua[af*]; Latifou Lagnika[b]; Dodehe Yeo[c], Cyril Antheaume[d]; Marcel Kaiser[e], Bernard Weniger[f]; Annelise Lobstein[f]; Catherine Vonthron-Sénécheau[f]

[a] UFR des Sciences des Structures de la Matière et Technologie, Université de Cocody, 01 BP 582 Abidjan, Côte d'Ivoire.
[b] Laboratoire de Biochimie et de Biologie Moléculaire, Université d'Abomey-Calavi, 04 BP 0320 Cotonou, Bénin.
[c] Laboratoire de Pharmacodynamie Biochimique, Université de Cocody-Abidjan, Côte d'Ivoire.
[d] Service Commun d'Analyse RMN, Faculté de Pharmacie, Université De Strasbourg, 74, route du Rhin.
[e] Swiss Tropical and Public Health Institute Parasite Chemotherapy Socinstr. 57P.O. BoxCH-4002 Basel.
[f] Unité de Pharmacognosie, UMR UDS/CNRS 7200, Faculté de Pharmacie, Université Louis De Strasbourg, France.

*Corresponding author's E-mail: attioua@yahoo.fr

Accepted on: 21-08-2011; **Finalized on:** 20-11-2011.

ABSTRACT

Anogeissus leiocarpus Guill. & Perr. (Combretaceae) is an African indigenous tree. It has been of interest to researchers because it is used in Ivory Coast as *antimalaria* remedy. The *in vitro antiplasmodial* and *antileishmanial* activities of the leaf and it major constituents are investigated her for the first time. Chemical composition of ethyl acetate crude extract was analyzed by NMR (1D and 2D), LC–ESI-MS and HPLC reverse phase methods. Results demonstrated that ethyl acetate crude extract from *A. leiocarpus* presented in vitro *antiplasmodial* and *antileismanial* activities (IC_{50}: 10 and 25 µg/ml respectively). Eight flavonoids were isolated, among which *Procyanidin B2* (8) and *Quercetin* (3), that showed *antiplasmodial* activity with IC_{50} value of 5.3 and 6.6 µM respectively. With the *antileishmanial* activity, the best IC_{50} value was obtained with *Rutin* (5) (IC_{50}=1.6 µM). C*ytotoxicity* was also made. These findings demonstrate that the leaves of *A. eliocarpus* are a reach source of flavonoids with potential *antileismanial* and *antiplasmodial* properties. Investigations of this species are in progress for other medicinal properties.

Keywords: *Anogeissus, Leiocarpus*, Combretaceae, Isolaton, Flavonoids, *Antiplasmodial, Antileishmanial*.

INTRODUCTION

Leishmaniasis, a disease caused by a number of species of protozoan parasites belonging to the genus *Leishmania*, is regarded as a major public health problem that affects around 12 million people in 80 countries and causes morbidity and mortality mainly in Africa, Asia, and Latin America[1,2]. Historically, the chemotherapy of *leishmaniasis* has been based on the use of pentavalent antimonial drugs. Other medications, such as *pentamidine* and *amphotericin B*, have been used as alternative drugs. However, these medicines are not orally active, requiring long-term parenteral administration, not to mention that they lead to serious side effects[1,2]. Malaria is another important tropical disease which has the potential to affect nearly 40% of the world's population and is responsible for 1–2 million deaths each year[1,3]. Human malaria is endemic to 90 countries and is caused by protozoan parasites of the genus *Plasmodium*, mainly *Plasmodium falciparum*. The development of resistance to mainstay drugs like *chloroquine*, and controlled use of new *artemisinin* analogs have created an urgent need to discover new antimalarial agents. Recently, the clinical use of *artemisinin*, a sesquiterpene lactone isolated by *Artemisia annua*, for the treatment of malaria has prompted interest in the discovery of new pharmaceuticals of plant origin with *antiplasmodial* activity[1,4]. Nature remains an ever evolving source for compounds of medicinal importance among which flavonoids. The exact mechanism of antimalarial action of flavonoids is unclear but some flavonoids are shown to inhibit the influx of L-glutamine and myoinositol into infected erythrocytes[5]. *Exiguaflavanone A* and *exiguaflavanone B* from *Artemisia indica* exhibited in vitro *antiplasmodial* activities (IC_{50} = 4.6 and 7.0 µg/mL) respectively[6]. Many biflavones have been reported to possess moderate to good antimalarial activity like, sikokianin B and C [IC_{50} = 0.54 and 0.56 lg/ml] respectively isolated from *Wikstroemia indica*[7], [IC_{50} = 80.0 ng/mL] isolated from *Ochna integerrima*[8], and [IC50 = 6.7 µM] from *Garcinia livingstonei*[9]. *Anogeissus leiocarpus Guill. & Perr.* (synonym: *Anogeissus schimperi Hochst. ex Hutch & Dalziel*) (Combretaceae)) is a woody species commonly found in forest savannahs of West Africa[10,11]. Its Leaves are used in Nigeria and in Guinea as *antimalarial*[12,13]. *Combretaceae* family is a wide range of tannins, flavonoids, terpenoids and stilbenoids[14,15]. In this sense, as part of our ongoing biological studies on *A. leiocarpus* is the isolation of polyphenols and the evaluation of their *antiprotozoal* and *antileishmanial* activities; which has not been done before.

MATERIALS AND METHODS

General

The principal method used for compound isolation was column chromatography. Silica gel 60 (230-400 mesh, Merck) and Sephadex LH-20 were used as stationary phase. Purifications were realized on column chromatography in combination with recrystallising

method. Analytical TLC (Thin Layer chromatography) was performed on percolated silica gel 60 F_{254} plates (Merck) and detection was achieved by spraying with sulfuric vanillin, followed by heating 5min at 105°C. Nuclear magnetic resonance (NMR): 1D (^{1}H, ^{13}C, and DEPT-135) and 2D (^{1}H COSY) spectra were recorded on a Bruker AVANCE DMX-400. Molecular weight were determined using Liquid chromatography combined with electro spray by ionization and mass spectroscopy (LC–ESI-MS) at Finnigan-MAT P4000 HPLC–DAD system interface to a Finningan LCQTM Duo ion Trap mass spectrometer (Thermo Electron GmbH, Germany) equipped with an electrospray interface coupled to an integrated syringe pump system. Biological activities were evaluated *in vitro* against *Plasmodium falciparum K1 and Leishmania donovani.*

Plant material

The leaves of *Anogeissus leiocarpus* were collected between June and August 2001 in the Savannah region in Bouandougou near Seguela (Northern Ivory Coast). Botanical determination was performed by Pr. L. Aké Assi (Centre National de Floristique, Université de Cocody, Abidjan). Voucher specimen (n°185C) is deposited at the Herbarium of the Centre National de Floristique (CNF).

Extraction and isolation

Air dried plant material of *A. leiocarpus* (688.5 g) was ground (0.2mm sieve) and defatted with cyclohexane (3.2L) overnight at room temperature. The plant material residues was future extracted tree time for 12h with methylene chloride (3x3.2L) at 40°C, then tree time for 12h at room temperature with ethyl acetate (EtOAc) (3x2.5L). Each extract was taken to dryness under vacuum and the residue was stored at room temperature. The Methylene chloride and ethyl acetate extracts yielded 7.4g and 3.2g respectively. A part of the ethyl acetate crude extract (3.0g) was fractionated a first time on silica gel column chromatography (diameter (d)=2.5Cm, height (h)=15Cm), using the mixture EtOAc/MeOH (10:0 to 8.5:1.5 v/v) following a gradient of polarity. Three fractions were collected on the base of their TLC profile. Each fraction was later purified using Sephadex LH-20 exclusion chromatography, according to the method described by Houghton and Raman[16]. Fraction I (150mg) was purified using Sephadex LH-20 column chromatography (d=1.2Cm, h=10Cm). The elution solvent was the mixture MeOH/H_2O (8:2 v/v). Compound **1** (25mg) was obtained and recrystallized in EtOAc. Fraction II (1500mg) was fractionated by the same method using a column of 1.2Cm as diameter and 15Cm as height. The system MeOH/H_2O (7:3 v/v) was used as mobile phase. Compounds **2** (15mg), **3** (25mg), **4** (17mg), **5** (50mg) and **6** (35mg) were isolated. Fraction III was fractionated using the same method and compounds **8** (40mg) was obtained. Itch isolated compounds were analyzed by HPLC method, reverse phase before NMR analyses. The elution solvent was the mixture: **A** (H_2O with 0.1%TFA) and **C** (Acetonitrile). The column size was C18 RP. The Retention times (R_T) of every compounds are given below. **1** (R_T 45.12min), **2** (R_T 37.2min), **3** (R_T 38.3min), **4** (R_T 26.7min), **5** (R_T 25.3min), **6** (R_T 27.2min), **7** (R_T 39.1min), **8** (R_T 17.3min).

Catechin (**1**): Yellow amorphous powder (MeOH), LC–ESI-MS m/z: 290.0791 (calcul. for $C_{15}H_{14}O_6$ 290.0788). ^{1}H NMR (400MHz, CD_3OD): δ 5.05ppm (H-2, d, J=6.5 Hz), 4.49ppm, (H-3, ddd, 5.6; 7.7 and 2.1Hz), 2.83ppm (H-4α, dd, J= 5.6 and 16.0 Hz,), 2.58ppm (H-4β, dd, J=8.0 and 16Hz), 5.71ppm (H-6, d, J=2.0Hz), 5.75ppm (H-8, d, J=2.0 Hz), 6.58ppm (H-1', d, J=8.4 Hz) and 6.49ppm (H-2' and H-5', d, J=8.4Hz). ^{13}C NMR (100MHz, CD_3OD): δ 82.1ppm (C-2), 67.8ppm (C-3), 26.6ppm (C-4), 157.3ppm (C-5), 95.3ppm (C-6), 157.8ppm (C-7), 94.8ppm (C-8), 157.4ppm (C-9), 10.3.1ppm (C-10), 122.2ppm (C-1'), 117.4ppm (C-2'), 144.6 ppm(C-3'), 147.4ppm (C-4'), 115.2ppm (C-5') and 132.6ppm (C-6').

4H-1-Benzopyran-4-one, 7-[(6-deoxy-α-L-mannopyranosyl)oxy]-5-hydroxy-2-(4-hydroxy-3-methoxyphenyl) (**2**): Yellow amorphous powder (MeOH); LC–ESI-MS: m/z: 446.1191 (calcul. for $C_{22}H_{22}O_{10}$ 446.1136). ^{1}H NMR (400MHz, CD_3OD): δ 6.70ppm (H-3, s), 6.32ppm (H-6, s), 6.73ppm (H-8, s), 7.21ppm (H-1', *d, J=8.4Hz*), 6.84ppm (H-2', *d, J= 8.4Hz*), 6.78ppm (H-5', s), 3.83ppm (O-CH_3, s); ^{1}C NMR (100MHz, CD_3OD): δ 163.7ppm (C-2), 104.5ppm (C-3), 182.2ppm (C-4), 161.1ppm (C-5), 98.1ppm (C-6), 166.2ppm (C-7), 92.0ppm (C-8), 159.0ppm (C-9), 103.3ppm (C-10), 121.1ppm (C-1'), 112.2ppm (C-2'), 150.0ppm (C-3'), 147.2ppm (C-4'), 114.9ppm (C-5'), 122.9ppm (C-6'). The rhamnosyl group ^{1}H and ^{13}C NMR chemical shifts were at 5.87ppm (H-1'', m), 3.90ppm (H-2'', m), 3.49ppm (H-3'', m), 3.40ppm (H-4'', m), 3.85ppm (H-5'', m), 1.15ppm (H-6", *d, J=6.2Hz*) and 105.6ppm (C-1''), 73.3ppm (C-2''), 70.7ppm (C-3''), 73.5ppm (C-4''), 74.2ppm (C-5''), 17.0ppm (C-6').

Quercetin (**3**): Yellow amorphous powder (MeOH); LC–ESI-MS m/z: 302.0381 (calcul. for $C_{15}H_{10}O_7$ 302.0374). ^{1}H NMR (400MHz, CD_3OD): δ 5.92ppm (H-6, s), 6.22ppm (H-8, s), 6.71ppm (H-1', s), 6.94ppm (H-4', *d, J= 8.2Hz*), 7.17ppm (H-5', *d, J=8.2Hz*); ^{1}C NMR (100MHz, CD_3OD): δ 146.7ppm (C-2), 135.5ppm (C-3), 178.2ppm (C-4), 161.1ppm (C-5), 98.1ppm (C-6), 166.2ppm (C-7), 94.0ppm (C-8), 159.5ppm (C-9), 105.3ppm (C-10), 115.1ppm (C-1'), 145.2ppm (C-2'), 146.5ppm (C-3'), 115.2ppm (C-4'), 121.1ppm (C-5'), 122.4ppm (C-6').

Isoquercetin (**4**): Yellow amorphous powder (MeOH); LC–ESI-MS m/z: 286.0477, (calcul. for $C_{15}H_{10}O_6$ 286.0432). ^{1}H NMR (400MHz, CD_3OD): δ 5.92ppm (H-6, s), 6.22ppm (H-8, s), 6.71ppm (H-1', s), 6.94ppm (H-4', *d, J= 8.2Hz*), 7.17ppm (H-5', *d, J=8.2Hz*); ^{1}C NMR (100MHz, CD_3OD): δ 156.7ppm (C-2), 135.5ppm (C-3), 178.2ppm (C-4), 161.1ppm (C-5), 98.1ppm (C-6), 166.2ppm (C-7), 94.0ppm (C-8), 159.5ppm (C-9), 105.3ppm (C-10), 115.1ppm (C-1'), 145.2ppm (C-2'), 146.5ppm (C-3'), 115.2ppm (C-4'), 121.1ppm (C-5'), 122.4ppm (C-6'). The glucopyranosyl group's ^{1}H and ^{13}C NMR chemical shifts

were at 5.67ppm (H-1'', *d, J= 6.2Hz*), 3.79ppm (H-2'', m), 3.49ppm (H-3'', m), 3.40ppm (H-4'', m), 3.76ppm (H-5'', m), 3.79ppm (H-6''α, m), 3.54ppm (H-6''β, m) and 109.6ppm (C-1''), 75.9ppm (C-2''), 76.7ppm (C-3''), 71.5ppm (C-4''), 81.0ppm (C-5''), 62.3ppm (C-6').

Rutin (**5**): Yellow amorphous powder (MeOH); LC–ESI-MS m/z: 610.1503 (calcul. for $C_{27}H_{30}O_{16}$, 610.1412). ^{1}H NMR (400MHz, CD_3OD): δ 5.92ppm (H-6, s), 6.22ppm (H-8, s), 6.71ppm (H-1', s), 6.94ppm (H-4', *d, J= 8.2Hz*), 7.17ppm (H-5', *d, J=8.2Hz*); ^{1}C NMR (100MHz, CD_3OD): δ and 156.7ppm (C-2), 135.5ppm (C-3), 178.2ppm (C-4), 161.1ppm (C-5), 98.1ppm (C-6), 166.2ppm (C-7), 94.0ppm (C-8), 159.5ppm (C-9), 105.3ppm (C-10), 115.1ppm (C-1'), 145.2ppm (C-2'), 146.5ppm (C-3'), 115.2ppm (C-4'), 121.1ppm (C-5'), 122.4ppm (C-6'). The glucosyl group's ^{1}H and ^{13}C NMR chemical shifts were at 5.67ppm (H-1'', *d, J=6.1 Hz*), 3.79ppm (H-2'', m), 3.49ppm (H-3''α, m), 3.40ppm (H-4'', m), 3.96ppm (H-5'', m), 3.69ppm (H-6''β, m), 3.38ppm (H-6''β, m), and 109.6ppm (C-1''), 75.9ppm (C-2''), 76.7ppm (C-3''), 71.5ppm (C-4''), 81.0ppm (C-5''), 68.3ppm (C-6''). The rhamnosyl group shows shifts at 5.03ppm (H-1"', m), 3.74ppm 'H-2"', m), 3.50ppm (H-3"', m), 3.40ppm (H-4"', m), 3.75ppm (H-5"', m), 1.18ppm (H-6"' β, m), 3.54ppm (H-6"' β, m) and 112.3ppm (C"'-1), 73.2ppm (C-2"'), 772.5ppm (C-3"'), 73.2ppm (C-4"'), 74.1ppm (C-5"') and 17.1ppm(C-6"').

Vitexin (**6**): Yellow amorphous powder (MeOH); LC–ESI-MS m/z: 432.1003 (calcul. for $C_{21}H_{20}O_{10}$ 432.0980). ^{1}H NMR (400MHz, CD_3OD): δ 6.71ppm (H-3, s), 5.88ppm (H-6, s), 7.55ppm (H-1', *d, J=8.3Hz*), 6.65ppm (H-2', *d, J=8.3Hz*), 6.64ppm (H-4', *d, J= 8.2Hz*), 7.55ppm (H-5', *d, J=8.2Hz*); ^{1}C NMR (100MHz, CD_3OD): δ 163.7ppm (C-2), 104.5ppm (C-3), 182.2ppm (C-4), 161.1ppm (C-5), 98.1ppm (C-6), 163.2ppm (C-7), 107.0ppm (C-8), 159.5ppm (C-9), 105.3ppm (C-10), 130.1ppm (C-1'), 115.2ppm (C-2'), 156.5ppm (C-3'), 115.2ppm (C-4'), 130.1ppm (C-5'), 122.4ppm (C-6'). The D-glucopyranosyl group's ^{1}H and ^{13}C NMR chemical shifts were at 4.98ppm (H-1'', *d*), 3.79ppm (H-2'', m), 3.49ppm (H-3'', m), 3.40ppm (H-4'', m), 3.76ppm (H-5'', m), 3.79ppm (H-6''α, m), 3.54ppm (H-6''β, m) and 73.6 ppm (C-1''), 70.9ppm (C-2''), 76.7ppm (C-3''), 71.5ppm (C-4''), 81.0ppm (C-5''), 62.3ppm (C-6').

Kaempferol (**7**)**:** Yellow amorphous powder (MeOH); LC–ESI-MS m/z: 286.0441 (calcul. for $C_{15}H_{10}O_6$, 286.0432). ^{1}H NMR (400MHz, CD_3OD): δ 5.92ppm (H-6, s), 6.22ppm (H-8, s), 7.58ppm (H-1', *d, J=8.4Hz*), 6.66ppm (H-2', *d, J=8.4Hz*), 6.665ppm (H-4', *d, J= 8.2Hz*), 7.58ppm (H-5', *d, J=8.2Hz*); ^{13}C NMR (400MHz, CD_3OD): δ 146.7ppm (C-2), 135.5ppm (C-3), 178.2ppm (C-4), 161.1ppm (C-5), 98.1ppm (C-6), 166.2ppm (C-7), 94.0ppm (C-8), 159.5ppm (C-9), 105.3ppm (C-10), 129.1ppm (C-1'), 115.2ppm (C-2'), 156.5ppm (C-3'), 115.2ppm (C-4'), 129.1ppm (C-5'), 122.4ppm (C-6').

Procyanidin B2 (**8**): Yellow amorphous (MeOH), LC–ESI-MS m/z: 578.1341 (calcul. for $C_{30}H_{26}O_{12}$ 578.1332). A dimmer of compound **1**; two rings system (A and B); On the ring A, ^{1}H NMR (400MHz, CD_3OD): δ 5.05ppm (H-1, d), 4.88ppm (H-3, m), 4.12ppm (H-4, m), 5.83ppm (H-6, s), 5.86ppm (H-8, s), 6.93ppm (H-1', s), 6.72pp (H-4', *d, J=8.2Hz*), 6.75ppm (H-5', *d, J=8.2Hz*); ^{1}C NMR (100MHz, CD_3OD): δ 83.1ppm (C-2), 72.6ppm (C-3), 36.1ppm (C-4), 157.2ppm (C-5), 95.7ppm (C-6), 158.0ppm (C-7), 96.1ppm (C-8), 157.4ppm (C-9), 100.6ppm (C-10), 115.9ppm (C-1'), 145.1ppm (C-2'), 144.6ppm (C-3'), 116.1ppm (C-4'), 121.3ppm (C-5'), 131.0ppm (C-6'). On the ring B, ^{1}H NMR (400MHz, CD_3OD): δ 5.05ppm (H-1, *d, J=6.1Hz*), 4.48ppm (H-3, m), 2.81ppm (H-4α, m), 2.55ppm (H-4β, m), 5.93ppm (H-6, s), 6.93ppm (H-1', s), 6.72ppm (H-4', *d, J=8.2Hz*), 6.70ppm (H-5', *d, J=8.2Hz*); ^{13}C NMR (100MHz, CD_3OD): δ 81.9ppm (C-2), 67.6ppm (C-3), 28.1ppm (C-4), 154.2ppm (C-5), 95.8ppm (C-6), 157.0pp (C-7), 106.8ppm (C-8), 153.4ppm (C-9), 103.6ppm (C-10), 115.9ppm (C-1'), 145.7ppm (C-2'), 144.6ppm (C-3'), 116.1ppm (C-4'), 121.3ppm (C-5'), 131.0ppm (C-6').

Biological assay

Antiplasmodial assay: Quantitative assessment of *antiplasmodial* activity *in vitro* was determined by means of the microculture radioisotope technique based upon the method previously described by Desjardins *et al.*[17] and modified by Ridley et al.[18]. The assay uses the uptake of [3H] hypoxanthine by parasites as an indicator of viability. Continuous in vitro cultures of asexual erythrocytic stages of *Plasmodium falciparum* were maintained following the methods of Trager and Jensen[19]. Plant extracts were tested on K1 strain (multidrug *pyrimethamine/ chloroquine*-resistant strain[20]. Initial concentration of the plant extracts and the isolated compounds was 30 µg/ml diluted with two-fold dilutions to make seven concentrations, the lowest being 0.47 µg /ml. After 48 h incubation of the parasites with the extracts or the compound at 37°C, [3H] hypoxanthine (Amersham, UK) was added to each well and the incubation was continued for another 24h at the same temperature. The concentrations of the extract and the compound at which the parasite growth (=[3H]hypoxanthine uptake) was inhibited by 50% (IC_{50}) was calculated by linear interpolation between the two drug concentrations above and below 50%[21]. *Chloroquine* was used as positive controls and DMSO was employed as the negative (vehicle) control. The values are means of two independent assays; each assay was run in duplicate.

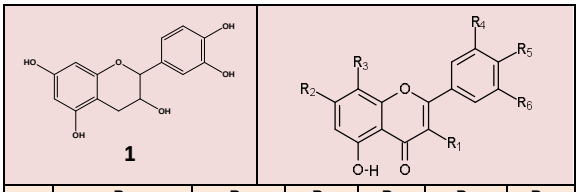
Antileishmanial assay*:* a transgenic cell line of *Leishmania donovani promastigotes* showing stable expression of luciferase was used as the test organism. Cells in 200µL of growth medium (L-15 with 10% FCS) were plated at a density of 2×106 cells per mL in a clear 96-well microplate. Stock solutions of the standards and test compounds/extracts were prepared in DMSO. Culture medium without cells and the controls were

incubated (at 26°C for 72h) simultaneously, in duplicate, at six concentrations of the test samples. An aliquot of 50µL was transferred from each well to a fresh opaque/black microplate, and 40 µL of Steadyglo reagent was added to each well. The plates were read immediately in a Polar Star galaxy microplate luminometer. IC_{50} value was calculated from dose-response inhibition graphs. *Miltefosine* was tested as standard *antileishmanial* agents.

Cytotoxicity assay: Cytotoxicity assay of the plant extracts was done following the method of Pagé *et al.*[22] with the modification of Ahmed *et al.*[23]. Cell line L6 (rat skeletal muscle myoblasts) were seeded in 96-well Costar microtiter plates at 2.2×10^5 cells/ml, 50µl per well in MEM supplemented with 10% heat inactivated fetal bovine serum (FBS). A three-fold serial dilution ranging from 500 to 0.07µg/ml of cruds extracts in test medium was added. Plates with a final volume of 100µl per well were incubated at 37°C for 72h in a humidified incubator containing 5% CO_2. Alamar Blue was added as viability indicator according to Ahmed *et al.*[23]. After an additional 2h of incubation, the plate was measured with a fluorescence scanner using an excitation wavelength of 536nm and an emission wavelength of 588nm (SpectraMax GeminiXS, Molecular Devices). IC_{50} values were calculated from the sigmoidal inhibition curve.

RESULTS AND DISCUSSION

The ethyl acetate crude extract prepared from the leaves of *A. leiocarpus,* presents an i*n vitro antiplasmodial* and *antileishmanial* activities with IC_{50} value of 10 and 25µg/ml respectively. Fractionation of this crude extract by a series of column chromatography, using Silica gel and Sephadex LH-20 successively as stationary phases, leaded to the isolation and identification of eight flavonoids (Figure 1).



	R_1	R_2	R_3	R_4	R_5	R_6
2	H	O-Rha	H	H	OMe	OH
3	OH	OH	H	OH	OH	H
4	O-Gluc	OH	H	OH	OH	H
5	O-Gluc-Rha	OH	H	OH	OH	H
6	H	OH	Rha	H	OH	H
7	OH	OH	H	H	OH	H

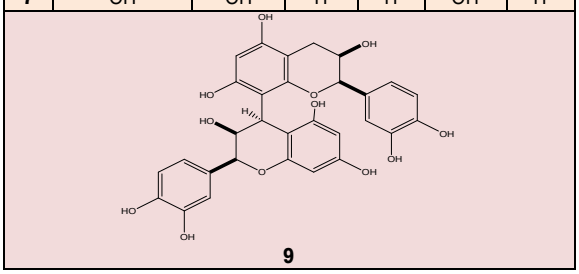


Figure 1: Structures of isolated compounds from *A. leiocarpus*: *Cathecin* (**1**), *4H-1-Benzopyran-4-one, 7-[(6-deoxy-α-L-mannopyranosyl)oxy]-5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)* (**2**), *Quercetin* (**3**) , *Isoquercetin* (**4**), *Rutin* (**5**), *Vitexin* (**6**), *Kaempferol* (**7**), and *Procyanidin B2* (**8**).

Identifications were based on LC–ESI-MS and NMR (^1H and ^{13}C) data. These spectral data of all the isolated compounds were in agreement with previously published data, thereby allowing for identification of *Catechin* (**1**)[24,25]; *4H-1-Benzopyran-4-one, 7-[(6-deoxy-α-L-mannopyranosyl)oxy]-5-hydroxy-2-(4-hydroxy-3-methoxy phenyl)* (**2**)[26]; *Quercetin* (**3**) [27, 28]; *Isoquercetin* (**4**)[29]; *Rutin* (**5**)[30]; *Vitexin* (**6**)[31]; *Kaempferol* (**7**) [32]; *Procyanidin B2* (**8**)[33]. These results are in accordance with literature data. Indeed, the *Combretaceae* family is recognized as a rich source of polyphenols[14]. Other flavonoids had been isolated from *A. leiocarpus* bark by Mehdi[34]. It is also known that ethyl acetate is the best solvent for phenolic compounds isolation. The *in vitro antileishmanial and antiplasmodial* activities of crude extract and the isolated compounds are summarized in Table 1.

Table 1: *In vitro* biological activity of the isolated flavonods against *Plasmodium falciparum k1, Leishmania donovani* and their citotoxicity

Compounds	L. don. Axen		P.falc. k1		Cytotox. L6
	IC_{50} (µM)	SI	IC_{50} (µM)	SI	IC_{50} µg/ml
Crude extract	25*	nd	10*	nd	>100
1	nd	nd	146.6	nd	>100
2	nd	nd	-	nd	>100
3	nd	nd	**6.61**	nd	68.1
4	64.3	2.8	29.2	6.3	52.3
5	**1.6**	13.1	32.77	1.0	20.9
6	nd	nd	nd	nd	>100
7	69.2	1.6	20.4	5.5	31.7
8	34.1	1.7	**5.3**	11.0	34.0
Miltefosine	0.199*				
Chloroquine			0.085*		
Podophyllotoxin					0.008*

SI (selectivity index)= IC_{50} Cytotoxcity/IC_{50} Parasites; **nd**=not determined; ***** IC_{50} value in µg/ml

Regarding the *in vitro antileishmanial* assay, compound **6** is the most active with IC_{50} value of 1.6 µM. A moderate activity was observed with compounds **4** (IC_{50}=64.3 µM), **9** (IC_{50}=34.0 µM) and (**7**) (IC_{50}=69.2 µM). There was no activity for compounds **1**, **2**, and **6**. With the *in vitro antiplasmodial* assay, the highest activity was observed with compounds **3** (IC_{50}=6.6 µM) and **8** (IC_{50}= 5.3 µM). A low activity was observed for compounds **4** (IC_{50}=29.2 µM), **5** (IC_{50} =32.7 µM) and **7** (IC_{50}=20.4µM). No activity was observed for compounds **1, 2,** and **6**. *In vitro antiplasmodial* activities of polyphenols in present study are comparable to those of other polyphenols obtained from other plant families[6,35,36]. The best *in vitro antiplasmodial* activity was obtained with *Quercetin* (**3**) (IC_{50}=6.6 µM). This IC_{50} value is in agreement with those obtained by Nobutoshi M *et al.*[37]. A comparison of the activities of *Quercetin* (**3**), *Isoquercetin* (**4**) and *Rutin* (**5**) suggests that the addition of one glycoside group or an addition of a system Glucose-Rhamnose at C-3 decreases considerably the *antiplasmodial* activity. Compared with other polyphenols such as *Kaempferol* (**7**), the *antiplasmodial* activity decreases to IC_{50}=20.4 µM when there is no hydroxyl group at C-2'. There is no more

activity when the D-glycosyl group is at C-8 for *Vitexin* (**6**) or when there is a rhamnosyl group at C-7 for compound **2**. We can notice as well as *Cathecin* (**1**) had no activity; what was in agreement with literature data[38]. Vitexin (**6**) presented no activity against both parasites (*L donovani and P. falciparum*) which is in confirming with Montenegro et al.[30] analyses. Previous studies on major components from ethyl acetate extract of *A. leiocarpus* leaves have shown that polyphenol present in this plant have high to low *antiplasmodial* activity. Our results are thus consistent with those reported by other investigators using other plant extracts[39]. Compounds **2, 4, 8** and **8** have not been studied before for their *in vitro antileishmanial* and *antiplasmodial* activities; what have been realized here.

CONCLUSION

Investigation of the ethyl acetate crude extract of *A. leiocarpus*'s *leaves has* leaded to the isolation of eight flavonoids. Their *in vitro antileishmanial* and *antiplasmodial* activities were evaluated. The most *in vitro antiplasmodial* activity were obtained with *Quercetin* (**3**) and *Procyanidin B2* (**8**). Concerning the *in vitro antileishmanial* activity, only *Rutin* (**5**) showed an excellent activity. Towards these results, we can say that fkavonoids from *A. leiocarpus* are not responsible for it *antileismanial* and *antimalarial* activities. We plane to investigate the methylene chloride crude extract.

Acknowledgements: The authors wish to thank the AUF (Agence Universitaire de la Francophonie) for it financial support, Cyril Antheaume and Patrick Wehrung for NMR and MS analyses respectively. Special thanks to retired Professor L. Aké Assi who kindly performed the botanical determinations.

Declaration of Interest: There are none.

REFERENCES

1. Rocha LG, Almeida JRGS, Macedo RO, Barbosa-Filho JM, A review of natural products with antileishmanial activity, *Phytomedicine.,* 12, 2005, 514.
2. Kayser O, Kiderlen AF, Croft SL., Novel Anti-Infective Compounds from *Marine Bacteria*, *Stud. Nat. Prod. Chem.,* 26, 2002, 779.
3. Andrade SF, Da Silva Filho AA, Resende DO, Silva MLA, Cunha WR, Nanayakkara NPD, *In vitro* antileishmanial, antiplasmodial and cytotoxic activities of phenolics and triterpenoids from *Baccharis dracunculifolia* D. C. (Asteraceae), *Z. Naturforsch C,* 63, 2008, 889.
4. Muhammad I, Dunbar DC, Khan SI, Tekwani BL, Bedir E, Takamatsu S, Screening of Plant Extracts for Searching Antiplasmodial Activity, *J. Na.t. Prod.,* 66, 2003, 962-967.
5. Elford BC, l-Glutamine influx in malaria-infected erythrocytes: a Target for antimalarials?, *Parasitology Today* 2, 1986, 309.
6. Chanphen R, Thebtaranonth Y, Yuthavong Y, Wanauppathamkul S, Antimalarial principles *from Artemisia indica*, *J. Nat. Prod.*, 61, 1998, 1146-1147.
7. Nunome S, Ishiyama A, Kobayashi M, Otoguro K, Kiyohara H, Yamada H, Omura S, "*In vitro* anti-malarial activity of biflavonoids from *Wikstoemia indica*", *Planta Med.*, 2004, 70-76.
8. Ichino C, Kiyohara H, Soonthornchareonnon N, Chuakul W, Ishiyama A, Sekiguchi H, Namatame M, Otoguro K, Omura S, Yamada H, Anti-malarial activity of biflavonoids from *Ochna integerrima*, *Planta Med.*, 72, 2006, 611-614.
9. Mbwambo ZH, Kapingu MC, Moshi MJ, Machumi F, Apers S, Cos P, Ferreria D, Marais JP, Vanden Berghe D, Maes L, Vlietinck A, Pieters L, Antiparasitic activity of some xanthones and biflavonoids from the root bark of Garcinia livingstonei, *J. Nat. Prod.,* 69, 2006, 369.
10. Oliver-Bever, Medicinal plants in tropical West Africa, Cambridge-University Press, first ed., Library of Cambridge, Cambridge, 1986.
11. Houéhanou T, Kindomihou V, Houinato M, Sinsin B, Dendrometrical characterization of a common Plant species (*Anogeissus leiocarpa* (DC.) Guill. & Perr.) in Pendjari Biosphere Reserve and in a surrounding land use area (Benin-West Africa), www.biota-africa.de.
12. Adjanohoun F, Ahyi MRA, Aké AL, Contribution to Ethnobotanical and Floristic Studies in Western Nigeria, CSTR-OUA, 1991, 220.
13. Bhat RB, Eterjere EO, Olapido VT, Ethnobotanical studiesfrom Central Nigeria, *Journal of Economic Botany,* 44, 1990, 382–390.
14. Eloff JN, Katerel DR, The biological activity and chemistry of the southern African *Combretaceae, J. Ethnopharmacol.,* 119, 2008, 686–699.
15. Martini ND, Katerere DRP, Eloff JN, Biological activity of five antibacterial flavonoids from *Combretum erythrophyllum* (Combretaceae), *J. Ethnopharmacol.,* 93, 2004, 207–212.
16. Houghton PJ, Raman A, Laboratory Handbook for the Fractionation of Natural Extracts, Chapman & Hall, London, 1998, 49.
17. Desjardins RF, Canfield CJ, Haynes CD, Chilay JD, Quantitative assessment of antimalarial activity in vitro was determined by a semi-automated microdilution technique, *Antimicrob. Agents Chemother.,*16, 1979, 710–718.
18. Ridley RG, Werner H, Hugues M, Catherine J, Arnulf D, Raffaello M, Synese J, Wolfgang FR, Alberto G, Maria AG, Heinrich U, Werner H, Sodsri T, Wallace P, 4-Aminoquinoline analogs of chloroquine with shortened side chains retain activity against chloroquine-resistant *Plasmodium falciparum*, *Antimicrob. Agents Chemother.,* 40, 1996, 1846–1854.
19. Trager W, Jensen JB, Human malaria parasites in continuous culture, *Science* 193, 1976, 673–675.
20. Thaithong S, Beale GH, Resistance of 10 Thai isolates of *Plasmodium falciparum* to chloroquine and pyrimethamine by in vitro tests, *Trans. R. Soc. Trop. Med. Hyg.,* 75, 1981, 271–273.
21. Huber W, Koella JC, A comparison of the three methods of estimating EC50 in studies of drug resistance of malaria parasite, *Acta Trop.,* 55, 1993, 257–261.

22. Pagé M, Noel C, A new fluorimetric assay for cytotoxicity measurements in vitro, *Int. J. of Oncol.,* 3, 1993, 473–476.

23. Ahmed SA, Gogal RM, Walsh JE, A new rapid and simple non-radioactive assay to monitor and determine the proliferation of lymphocytes: an alternative to [3H]thymidine incorporation assay, *J. Immunol. Methods* 170, 1994, 211–224.

24. Vuong QV, BG John, M. Nguyen, Roach DP, Extraction and isolation of *Catechins* from tea, *J. Sep. Sci.* 33 (21), 2010, 3415–3428.

25. Mutai C, Rukunga G, Vagias C, Roussis V, *In vivo* screening of antimalarial activity of *Acacia mellifera* (Benth) (Leguminosae) on Plasmodium berghei in mice, *African Journal of Traditional, Complementary and Alternative Medicines* 5(1), 2008, 46-50.

26. Tiwari RD, Singh J, Flavonoids from the leaves of *Cassia occidentalis*, *Phytochemistry* 16(7) (1977) 1107-1108

27. Gluchoff-Fiasson K, Fiasson JL, Favre-Bonvin J, Quercetin glycosides from *antarctic ranunculus species*, *Phytochemistry* 37(6), 1994, 1629-1636.

28. Montenegro H, Gonzalez J, Ortega-Barria E, Cubilla-Rios L, Antiprotozoal Activity of Flavonoid Glycosides Isolated from Clidemia sericea and Mosquitoxylon jamaicense, Pharmac*eutical Biology (Philadelphia, PA, United States) 45(5),* 2007, 376-380.

29. Mendez J, Bilia RA, Ivano M, Phytochemical investigations of *Licania* genus. Flavonoids and triterpenoids from *Licania pittieri*, *Pharmaceutica Acta Helvetiae* 70(3), 1995, 223-226.

30. Saleh NAM and Towers GHN., Rutin from *tetraplasandra meiandra*, *Phytochemistry* 10(4), 1971, 89.

31. Kang J, Li Z, Wu T, Jensen GS, Schauss AG, Wu X, Antioxidant capacities of flavonoid compounds isolated from *Acai pulp* (Euterpe oleracea Mart.), *Food Chemistry* , 2010, 33.

32. Xian-Kang F, Jie G, Dan-Ni Z, *Kaempferol* and *Quercetin* isolated from *Euonymus alatus* improve glucose uptake of 3T3-L1 cells without adipogenesis activity, *Life Sci.* 82 (11-12), 2008, 615-622.

33. Foo L Y and Karchesy JJ, Procyanidin dimers and trimers from *Douglas fir inner* bark, *Phytochemistry,* 28, (6), 1989, 1743-1747.

34. Mehdi C, Benayache S, Benayache F, N'Gom S, Koné M, Aton R, Weniger B, Lobstein A, Triterpenes and polyphenols from *Anogeissus leiocarpus* (Combretaceae), *Biochemical Systematics and Ecology,* 36, 2008, 59-62.

35. Jenett-Siems K, Siems K, Jakupovic J, Solis PN, Gupta MP, Mockenhaupt FP, Bienzle U, Eich E, Sipandinolide: a butenolide including a novel type of carbon skeleton from Siparuna andina, *Planta Med*., 66, 2000, 384.

36. Kraft C, Jenett-Siems K, Siems K, Gupta MP, Bienzle U, Eich E, Antiplasmodial activity of isoflavones from *Andira inermis*, *J. Ethnopharmacol*., 73, 2000, 131.

37. Nobutoshi M, Huq MM, Satoru T, Sawako I, Toshihiro H, MKotomasa MK, New Anti-Malarial Flavonol Glycoside from *Hydrangeae Dulcis Folium*, *Bioorg. Med. Chem. Lett.,* 11, 2001, 2445–2447.

38. Jean B, Njikam N, Johnson AF, Leonardo K, Bascoa PR, *In vitro* antimalarial activity of limonoids from *Khaya grandifoliola* C.D.C. (Meliaceae), *J. Ethnopharmacol.,* 69, 2000, 27–33.

39. Murakami H, Kawabuchi A, Matsumoto R, Ido T, Nakashima N, "A Multi-Mode-Driven Molecular Shuttle: Photochemically and Thermally-reactive Azobenzene Rotaxanes", *J. Am. Chem. Soc*., 127, 2005, 15891-15899.

****