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## Trypanocidal activity of diarylheptanoids from *Schrankia leptocarpa* DC

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### Abstract

*Schrankia leptocarpa* is a medicinal species used traditionally in Benin to treat malaria. A previous study showed interesting antimalarial activity against two strains of *Plasmodium falciparum*. Phytochemical investigation of *S. leptocarpa* DC. (Mimosaceae) led to the isolation of two diarylheptanoids (1–2), three phenolic acids (3–5), one p-coumaric acid derivative (6) and three flavonoids (7–9). The structures of the isolated compounds were established by combination of spectroscopic methods. All isolated compounds were tested in vitro for antiprotozoal activity against *P. falciparum*, *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, and *Leishmania donovani*. Compounds 1 and 2 showed trypanocidal and antiplasmodial activities in the micromolar range. Compound 1 showed also strong submicromolar trypanocidal activity against *T. b. rhodesiense* with an IC<sub>50</sub> value of = 0.79 ± 0.3 μM.

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### 1. Introduction

*Schrankia leptocarpa* DC. (Mimosaceae) is a straggling perennial herb native of tropical South America and introduced into West Africa, where the species are traditionally used in folk medicine. In Benin, the aerial part is used in decoction to treat eruptive fevers and hypertension (Adjanooun et al., 1989). Alkaloids have been described in the fruit of the close related species *Schrankia uncinata* (Smolenski et al., 1973). No phytochemical studies have been reported to date on this species.

Actually, malaria is the most crucial problem of public health in African sub-Saharan countries. 74% of the population lives in area of strong endemic disease and 18% in epidemic area. About 600 million persons are exposed to malaria, and because of the devastating nature of the illness and the failure of the most affordable therapeutic agents there is an urgent need to develop new drugs or vaccines for the treatment, prevention and management of the disease (Asase et al., 2010; Waako et al., 2005).

Human African trypanosomiasis (HTA) is a parasitic disease which affects humans and animals, caused by protozoa of the species *Trypanosoma brucei* and transmitted by the tsetse fly. The disease is endemic in some regions of sub-Saharan Africa, covering about 36 countries and 60 million people. About 48,000 people died of it in 2008 (Boseley, 2009). The treatment and control of the disease was based on the prophylactic and

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therapeutic use of trypanocides. Regrettably, their intensive use over decades leads to drug resistance which grows to a major problem (Peregrine, 1994).

The increasing global spread of drug resistance to most of the available and affordable antiprotozoal drugs is a major concern and requires innovative strategies to combat the disease. There is an urgent need for new chemotherapeutic compounds, which are easy to administer and store, and which are of low cost (Asokan et al., 2011).

In a continuing search for antiprotozoal compounds from traditionally used medicinal plants, we have previously investigated a crude methanolic extract of the aerial part of *S. leptocarpa* which was found to be active in vitro against *Plasmodium falciparum* K1 multidrug resistant strain (Weniger et al., 2004).

In this study, we report the isolation and structure elucidation of two diarylheptanoids (1–2), three phenolic acid (3–5), one *p*-coumaric acid derivate (6) and three flavonoids (7–9). All isolated compounds were evaluated for their in vitro antiprotozoal activities against human pathogens responsible of malaria, visceral leishmaniasis, Chaga's disease and sleeping sickness.

## 2. Materials and methods

### 2.1. Plant material

Entire plant of *S. leptocarpa* DC. (Mimosaceae) was collected in October 2008 in the area of Sèmè-Kpodji, Ouémé department (Southern Benin). Botanical identification of the plant was performed by taxonomists from the Herbarium National of Abomey-Calavi University in Benin. A voucher specimen (Houngnon 954b) was deposited at the same Herbarium. The collected entire plant was air-dried, powdered, and subjected to extraction with methanol.

### 2.2. Extraction and isolation

Dry powdered aerial parts of *S. leptocarpa* (750 g) were extracted with 2 L methanol using a Soxhlet extractor. The methanolic extract was taken to dryness under vacuum, yielding 75.6 g. It was afterwards dissolved in water (750 ml) and then partitioned with cyclohexane (250 ml x 4), methylene chloride (400 ml x 3), ethyl acetate (400 ml x 3) and methanol (400 ml x 3). Each extract was taken to dryness under vacuum and the residues were stored at room temperature. The methylene chloride was purified on column chromatography (Si gel 60, 0.063–0.200; Merk) using a mixture of increasing solvent polarity (methylene chloride, ethyl acetate and finally methanol) to obtain three subfractions named Sbf1, Sbf2 and Sbf3. The sub-fractions Sbf2 and Sbf3 obtained were purified again by preparative liquid chromatography (Gilson VP 250/21, Nucléodur 100–10 C18ec, Macherey Nagel, UV detection 205 nm) by gradient elution (flow rate 10 ml/min) using water–methanol (50:50 to 100:00 v/v) with 0.01% formic acid as mobile phase, to obtain compounds 1 (17.2 mg) and 2 (8.7 mg).

The ethyl acetate extract (2.5 g) was dissolved in methanol and purified by preparative liquid chromatography (Gilson VP 250/21, Nucléodur 100–10 C18ec, Macherey Nagel, UV detection

254 nm) by gradient elution (flow rate 10 ml/min) using acidified water (0.01% formic acid) and methanol (80:20 to 00:100 v/v) as mobile phase, to obtain compounds 3 (18.2 mg), 4 (23.4 mg) and 5 (8 mg).

The methanol extract (10 g) was first cleaned using Sephadex LH-20 exclusion chromatography (Sephadex LH20, 25–100  $\mu$ m, Merck), according to the method described by Houghton and Raman (1998). To remove tannins, the methanol extract was first dissolved in 100 ml of methanol/water (1:1, v/v) and 50 ml of methylene chloride are added to the obtained mixture. After homogenization, two phases (liquid and precipitate) are formed. The mobile phase was concentrated and cleaned using Sephadex LH-20 column (diameter: 3 cm; Sephadex height: 35 cm) and the mixture of methanol and methylene chloride (80:20, v/v) as mobile phase. The tannin free methanol extract was afterwards purified using preparative liquid chromatography (Gilson VP 250/21, Nucléodur 100–10 C18ec, Macherey Nagel, UV detection 280 nm) by gradient elution (flow rate 10 ml/min) using acidified water (0.01% formic acid) and methanol (95:05 to 00:100 v/v, 65 min) as mobile phase to obtain twelve (12) fractions among which four correspond to compounds 6 (9.5 mg), 7 (29.3 mg), 8 (6.2 mg) and 9 (9.7 mg).

Structural determination of the isolated compounds was carried out by spectrophotometric methods. NMR spectra (1D and 2D) were recorded in MeOH-*d*<sub>4</sub> using a Bruker 400 Mhz spectrometer. The chemical shifts ( $\delta$ ) are reported in parts per million (*ppm*) relative to tetramethylsilane (TMS  $\delta=0$ ). The coupling constants (*J*) are given in Hz. COSY, HSQC and HMBC experiments were recorded with gradient enhancements using sine-shaped gradient pulses. The raw data were transformed and the spectra evaluated with the standard Bruker NMRtec NMRnotebook software. FT-IR experiment was achieved with, SmartOrbit ATR, Thermo Nicolet 380 (Thermo Electron Corporation). LC/HR-ESI-MS, were recorded on a HPLC Agilent 1200RRLC equipped with an Agilent 6520 Accurate Mass Q-TOF spectrometer, HPLC was also used for UV spectra. The Mass spectra ESI-MS were recorded with a spectrometer. The nine isolated compounds were identified by comparing their spectroscopic data with those of reported values. Our spectroscopic data were in accordance with the published data in the literature.

### 2.3. Biological assays

#### 2.3.1. Antiplasmodial activity

Antiplasmodial activity was evaluated against the 3D7 sensitive strain and the K1 resistant strain of *P. falciparum*. Quantitative assessment of *in vitro* antimalarial activity against the K1 resistant strain was determined by means of the microculture radioisotope technique based on the method previously described by Desjardins et al. (1979) and modified by Ridley et al. (1996). The assay uses the uptake of [<sup>3</sup>H]hypoxanthine by parasites as an indicator of viability. Continuous *in vitro* cultures of asexual erythrocytic stages of *P. falciparum* were maintained following the methods of Trager and Jensen (1976). Compounds were tested against K1 strain (multidrug pyrimethamine/chloroquine resistant strain) (Thaithong and Beale, 1981). Initial concentration of each compound was 30  $\mu$ g/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 compound at 37 °C, [<sup>3</sup>H] hypoxanthine (Amersham 115 Int., Buckinghamshire, UK) was added to each well and the incubation was continued for another 24 h at the same temperature. IC<sub>50</sub> was calculated by linear interpolation between the two drug concentrations above and below 50% (Huber and Koella, 1993). Chloroquine and artemisinin were used as positive references. The values are means of two independent assays. Each assay was run in duplicate.

Continuous cultures of asexual erythrocytic stages of an 3D7 chloroquine sensitive strain of *P. falciparum* were maintained following the procedure of Trager and Jensen (1976) and as described previously (Frederich et al., 2002). Parasite growth was estimated by determination of lactate dehydrogenase activity as described by Delhaes et al. (1999) and Makler et al. (1993) and slightly modified. Briefly, in a new microtiter plate, a 20 µl subsample of the contents of each well was mixed with 100 µl of a substrate solution containing 1 mg lithium L-lactate (Sigma), 0.2 mg 3-acetyl pyridine adenine dinucleotide (APAD, Sigma), 0.2 µl Triton X-100 (Sigma), 10 µg saponine (Merck) in TRIS buffer (pH 8, Sigma). After incubation for 20 min, 20 µl of a mix of nitroblue tetrazolium (NBT, 2 mg/ml in TRIS pH 8 buffer, SIGMA) and phenazine ethosulfate (PES, 0.1 mg/ml in TRIS pH 8 buffer, Sigma) was added to each well. After another 30 min of incubation, the formation of the reduced form of APAD was measured at 595 nm. Each test sample was applied in a series of eight fourfold dilutions (final concentrations ranging from 20 to 0.0012 µg/ml) and was tested in triplicate. Artemisinin (Sigma, Bornem, Belgium) and chloroquine diphosphate (Sigma C6628), were used as antimalarial references.

### 2.3.2. Antitrypanosomal activity

The assays were performed according to the procedures described by Freiburghaus et al. (1996). The compounds were dissolved in 10% DMSO, and working stock solutions of 1 mg/mL in serum containing culture medium were prepared. Diluted compounds (100 µL) were pipetted in duplicate into the first row of a 96-well microtiter plate (Costar, Corning, NY, USA). With complete culture medium, three-fold serial dilutions were prepared. After the addition of *Trypanosoma brucei rhodesiense* bloodstream form trypanosomes from axenic culture, the concentrations of the compounds ranged from 500 to 0.07 µg/mL. The total number of trypanosomes in each well was  $2 \times 10^2/100 \mu\text{l}$ . The plate was then incubated for 72 h at 37 °C in 5% CO<sub>2</sub>. Two hours before the end of the incubation 10 µl of Alamar blue solution was added. Fluorescence was measured after 2 h of incubation with the dye Alamar blue in a fluorescence plate reader at 530 nm excitation and 590-nm emission wavelength (Cytofluor 2300, Millipore, Bedford, MA, USA) (Räz et al., 1997). IC<sub>50</sub> values were calculated from the sigmoidal inhibition curve. The values are means of two independent assays. Each assay was run in duplicate.

### 2.3.3. *Trypanosoma cruzi* assay

Rat skeletal myoblasts (L-6 cells) were seeded in 96-well microtiter plates at 2000 cells per well per 100 µL in RPMI

1640 medium with 10% FBS and 2 mM glutamine. After 24 h, 5000 trypomastigotes of *T. cruzi* were added in each well (100 µL) with or without a serial drug dilution. The plates were incubated at 37 °C in 5% CO<sub>2</sub> for 4 days. After 96 h, the minimum inhibitory concentration (MIC) was determined microscopically. For measurement of the IC<sub>50</sub>, the substrate CPRG/Nonidet was added to the wells. The color reaction that developed during the following 2–4 h was read photometrically at 540 min. IC<sub>50</sub> values were calculated from the sigmoidal inhibition curve. The values are means of two independent assays. Each assay was run in triplicate.

### 2.3.4. Leishmanicidal activity

Fifty microliters of culture medium, a 1:1 mixture of SM medium (Cunningham, 1977) and SDM-79 medium (Brun and Schönenberger, 1979) at pH 5.4 supplemented with 10% heat-inactivated FBS, was added to each well of a 96-well microtiter plate (Costar). Serial drug dilutions in duplicates were prepared covering a range from 30 to 0.041 µg/mL. Then, 10<sup>5</sup> axenically grown *Leishmania donovani* amastigotes (strain MHOM/ET/67/L82) in 50 µL medium were added to each well and the plate incubated at 37 °C under a 5% CO<sub>2</sub> atmosphere for 72 h. Ten microliters of resazurin solution (12.5 mg resazurin dissolved in 100 mL distilled water) were then added to each well and incubation continued for a further 2–4 h. The plate was then read in a Spectramax Gemini XS microplate fluorometer (Molecular Devices, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and emission wavelength of 588 nm (Räz et al., 1997). Fluorescence development was measured and expressed as percentage of the control. Data were transferred into the graphic program Softmax Pro (Molecular Devices), which calculated IC<sub>50</sub> values. The values are means of two independent assays. Each assay was run in triplicate.

## 3. Results and discussion

The phytochemical analysis of the aerial parts of *S. leptocarpa* led to the isolation of nine (9) compounds identified as 1,7-bis-(4-hydroxyphenyl)-3-hepten-5-one (**1**), 5-hydroxy-1,7-bis(4-hydroxyphenyl)heptan-3-one (**2**), Methyl-1-chlorogenate (**3**), Ethyl-1-chlorogenate (**4**), Caffeic acid (**5**), p-coumarylagmatine (**6**), Quercetin 3-rhamnosyl-(1–2) [rhamnosyl-(1–6)-glucoside or Manghasline (**7**), Kaempferol 3-(2,6-dirhamnosyl)glucoside) or Mauritanin (**8**) and Quercetin 3-neohesperidoside (**9**) (see Fig. 1).

Compound **1** was previously isolated from *Betula platyphylla* var. *japonica* (Fuchino et al., 1996) and *Alnus maximowiczii* (Motoo et al., 1995), **2** from *Alpinia officinarum* (Matsuda et al., 2009) and *Alpinia blepharocalyx* (Ali et al., 2001), **3**, **4**, and **5** identified from *Eremochloa ophiuroides* and *Helianthus annuus* (Lee et al., 2010; Hiroshi and Shingo, 1972), **6** from *Hordeum vulgare* and barley (Lee et al., 1997; Stoess, 1965). The three flavonoids (**7–9**) were also previously isolated. Compound **7** from *Vasconcellea pubescens* and *Styphnolobium japonicum* (Simirgiotis et al., 2009; Kite et al., 2007), **8** from *Acalypha indica* (Nahrstedt et al., 2006) and **9** from *Ficus pumila* (Abraham et al., 2008). To the best of our knowledge, compounds **1–9** are described for the first time from this genus.

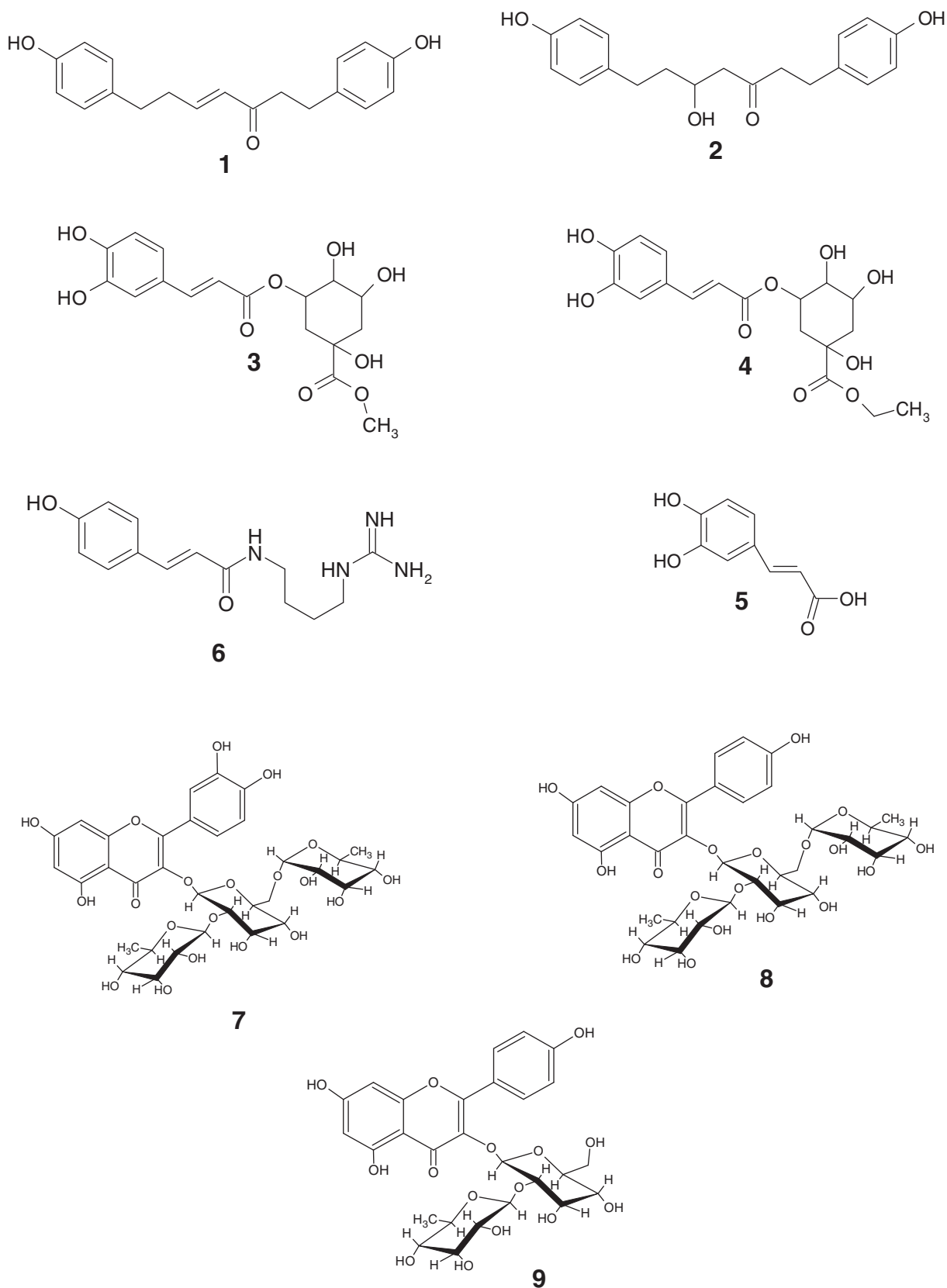


Fig. 1. Isolated compounds from *S. leptocarpa*.

Isolated compounds were tested *in vitro* for their antiprotozoal activities against *P. falciparum* K1 multidrug-resistant strain, *P. falciparum* 3D7 chloroquine-sensitive strain, *T. b. rhodesiense*

STIB 900 strain, *T. cruzi* Tulahuen C4 strain, and *L. donovani* MHOM-ET-67/L82 strain (Table 1). The most active compounds were the two isolated diarylheptanoids (1–2), the three phenolic

Table 1  
Inhibitory effect of compounds isolated from *S. leptocarpa*.

Compounds	Antiprotozoal activity IC <sub>50</sub> (μM)				
	<i>T.b. rhodensiense</i>	<i>T. cruzi</i>	<i>L. donovani</i>	<i>P. falciparum</i>	
				K1	3D7
1	0.79±0.17	27.67±3.18	9.27±1.78	7.93±1.34	18.14±1.65
2	7.03±1.43	78.41±6.94	51.88±13.49	7.73±0.40	37.52±1.34
3	>90	>90	>90	>5	>50
4	31.47±2.12	>90	>90	>5	>50
5	34.65±0.37	>90	>90	>5	>50
<i>Control</i>					
Melarsoprol	0.005				
Benznidazole		1.69			
Miltefosine			0.47		
Chloroquine				0.19	0.039
artemisinin				0.007	–

Data shown are values from duplicate experiments.

*P. falciparum* K1 resistant strain; *P. falciparum* 3D7 sensitive strain; *L. donovani* MHOM/ET/67/L82 strain axenic amastigotes; *T. brucei rhodensiense* STIB 900 strain trypomastigotes; *T. cruzi* Tulahuen C4 strain.

acids derivatives showing no to weak activity (IC<sub>50</sub>>10 μM). 1–2 showed trypanocidal and antiplasmodial activities in the sub-micromolar to the micromolar ranges against *T. b. rhodensiense* (0.79±0.17 μM and 7.03±1.43 μM) and *P. falciparum* K1 multiresistant strain (7.93±1.34 and 7.73±0.4 μM). The most active compound being 1 (IC<sub>50</sub>=0.79±0.17 μM).

Previous work showed antileishmanial (Alves et al., 2003; Araujo et al., 1998) and antitrypanosome (Kamnaing et al., 2003) activities for diarylheptanoids. Our results are in accordance with those obtained previously and provide scientific evidence supporting the use of *S. leptocarpa* in folk medicine.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.sajb.2012.06.011>.

## References

- Abraham, L.C.N., Tako, M., Hanashiro, I., Tamaki, H., 2008. Antioxidant flavonoid glycosides from the leaves of *Ficus pumila* L. Food Chemistry 109, 415–420.
- Adjahoun, E.J., Adjakidjè, V., Ahyi, M.R.A., Akeassi, L., Akoegninou, A., d'Almeida, J., 1989. Médecine traditionnelle et pharmacopée. Contribution aux études ethnobotaniques et floristiques en République du Bénin. Agence de Coopération Culturelle et Technique, Paris, p. 339.
- Ali, M.S., Yasuhiro, T., Suresh, A., Arjun, H.B., Shigetoshi, K., 2001. Six new diarylheptanoids from the seeds of *Alpinia blepharocalyx*. Journal of Natural Products 64, 289–293.
- Alves, L., Vignólio, M., Marcuzzo, D.C.C., Lea, C.F., Leonor, L., 2003. *In vitro* antiproliferative effects of several diaryl derivatives on *Leishmania* Spp. Biological & Pharmaceutical Bulletin 26, 453–456.
- Araujo, C.A.C., Elegrio, L.V., Leon, L.L., 1998. Antileishmanial activity of compounds extracted and characterized from *Centrolobium sclerophyllum*. Phytochemistry 49, 751–754.
- Asase, A., Akwetey, G.A., Achel, D.G., 2010. Ethnopharmacological use of herbal remedies for the treatment of malaria in the Dangme West District of Ghana. Journal of Ethnopharmacology 129, 367–376.
- Asokan, B., Abdul, A.R., Naveen, K.K., Dinkar, S., 2011. *In vitro* antimalarial activity of medicinal plant extracts against *Plasmodium falciparum*. Parasitology Research 108, 15–22.
- Boseley, S., 2009. New treatments raise hope of cutting sleeping sickness deaths". The Guardian. May 15.
- Brun, R., Schönenberger, M., 1979. Cultivation and *in vitro* cloning or procyclic culture forms of *Trypanosoma brucei* in a semi-defined medium. Acta Tropica 36, 289–292.
- Cunningham, I., 1977. New culture medium for maintenance of tsetse tissues and growth of trypanosomatids. The Journal of Protozoology 24, 325–329.
- Delhaes, L., Lazaro, J.E., Gay, F., Thellier, M., Danis, M., 1999. The microculture tetrazolium assay: another colorimetric method of testing *Plasmodium falciparum* chemosensitivity. Annals of Tropical Medicine and Parasitology 93, 31–40.
- Desjardins, R.E., Canfield, C.J., Haynes, J.D., Chilay, J.D., 1979. Quantitative assessment of antimalarial activity *in vitro* was determined by a semi-automated microdilution technique. Antimicrobial Agents and Chemotherapy 16, 710–718.
- Frederich, M., Jacquier, M.J., Thepnier, P., DeMol, P., Tits, M., Philippe, G., Delaude, C., Angenot, L., Zeches-Hanrot, M., 2002. Antiplasmodial activity of alkaloids from various *Strychnos* species. Journal of Natural Products 65, 1381–1386.
- Freiburghaus, F., Kaminsky, R., Nkunya, M.H.H., Brun, R., 1996. Evaluation of African medicinal plants for their *in vitro* trypanocidal activity. Journal of Ethnopharmacology 55, 1–11.
- Fuchino, H., Konishi, S., Satoh, T., 1996. Chemical evaluation of *Betula* species in Japan.II. Constituents of *Betula platyphylla* var. *japonica*. Chemical & Pharmaceutical Bulletin 44, 1033–1038.
- Hiroshi, A., Shingo, M., 1972. Identification of auxin-active substances as Ethyl Chlorogenate and indolyl-3-acetic acid in immature seeds of *Helianthus annuus*. Agricultural and Biological Chemistry 36, 42–46.
- Houghton, P.J., Raman, A., 1998. Laboratory handbook for the fractionation of natural extracts. Chapman and Hall, London.
- Huber, W., Koella, J.C., 1993. A comparison of the three methods of estimating EC<sub>50</sub> in studies of drug resistance of malaria parasite. Acta Tropica 55, 257–261.
- Kamnaing, P., Apollinaire, T., Eric, A., Tanifum, M.H.K., Tchuendem, P.T., Johnson, F., Ayafor, O.S., Donna, R., Maurice, M.I., Brian, S., Cyrus, B.,

2003. Trypanocidal diarylheptanoids from *Aframomum letestuanum*. *Journal of Natural Products* 66, 364–367.
- Kite, G.C., Stoneham, C.A., Veitch, N.C., 2007. Flavonol tetraglycosides and other constituents from leaves of *Styphnolobium japonicum* (Leguminosae) and related taxa. *Phytochemistry* 68, 1407–1416.
- Lee, J., Thomas, V., Jurgen, S., Benno, P.M.L., 1997. Methyljasmonate-induced accumulation of Coumaroyl conjugates in barley leaf segments. *Phytochemistry* 44, 589–592.
- Lee, E.M., Seung, S.L., Byung, Y.C., Jae-Young, C.C., So, R.A., Soo, J.J., Tae, H.K., 2010. Pancreatic lipase inhibition by C-Glycosidic flavones isolated from *Eremochloa ophiuroides*. *Molecules* 15, 8251–8259.
- Makler, M., Ries, J., Williams, J., Bancroft, J., Piper, R., Gibbins, B., Hinrichs, D., 1993. Parasite lactate dehydrogenase as an assay for *Plasmodium falciparum* drug sensitivity. *The American Journal of Tropical Medicine and Hygiene* 48, 739–741.
- Matsuda, H., Souichi, N., Yoshimi, O., Seikou, N., 2009. Melanogenesis inhibitors from the rhizomes of *Alpinia officinarum* in B16 melanoma cells Yoshikawa. *Bioorganic & Medicinal Chemistry* 17, 6048–6053.
- Motoo, T., Akemi, H., Kazushi, H., Yoshinori, A., 1995. Diarylheptanoids, flavonoids, stilbenoids, sesquiterpenoids and a phenanthrene from *Alnus maximowiczii*. *Phytochemistry* 40, 1263–1264.
- Nahrstedt, A., Hungeling, M., Petereit, F., 2006. Flavonoids from *Acalypha indica*. *Fitoterapia* 77, 484–486.
- Peregrine, A.S., 1994. Chemotherapy and delivery systems: haemoparasites. *Veterinary Parasitology* 54, 223–248.
- Räz, B., Iten, M., Grether, Y., Kaminsky, R., Brun, R., 1997. The Alamar Blue assay to determine drug sensitivity of African trypanosomes (*T. b. rhodesiense* and *T. b. gambiense*) *in vitro*. *Acta Tropica* 68, 139–147.
- Ridley, R.G., Werner, H., Hugues, M., Catherine, J., Arnulf, D., Raffaello, M., Synese, J., Wolfgang, F.R., Alberto, G., Maria, A.G., Heinrich, U., Werner, H., Sodsri, T., Wallace, P., 1996. 4-Aminoquinolone analogs of chloroquine with shortened side chains retain activity against chloroquine-resistant *Plasmodium falciparum*. *Antimicrobial Agents and Chemotherapy* 40, 1846–1854.
- Simirgiotis, M.J., Peter, D.S.C., Schmeda-Hirschmann, G., 2009. Identification of phenolic compounds from the fruits of the mountain papaya *Vasconcellea pubescens* A. DC. grown in Chile by liquid chromatography-UV detection-mass spectrometry. *Food Chemistry* 115, 775–784.
- Smolenski, S.J., Silinis, H., Farnsworth, W.R., 1973. Alkaloids screening III. *Lloydia* 36, 359–389.
- Stoess, A., 1965. Antifungal factors in barley. III. Isolation of p-coumaroylagmatine. *Phytochemistry* 4, 973–976.
- Thaithong, S., Beale, G.H., 1981. Resistance of 10 Thai isolates of *Plasmodium falciparum* to chloroquine and pyrimethamine by *in vitro* tests. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 75, 271–273.
- Trager, W., Jensen, J.B., 1976. Human malaria parasites in continuous culture. *Science* 193, 673–675.
- Waako, P.J., Gumedde, B., Smith, P., Folb, P.I., 2005. The *in vitro* and *in vivo* antimalarial activity of *Cardiospermum halicacabum* L. and *Momordica foetida* Schumch. Et Thonn. *Journal of Ethnopharmacology* 99, 137–143.
- Weniger, B., Lagnika, L., Vonthron-Sénécheau, C., Adjobimey, T., Gbenou, J., Moudachirou, M., Brun, R., Anton, R., Sanni, A., 2004. Evaluation of ethnobotanically selected Benin medicinal plants for their *in vitro* antiplasmodial activity. *Journal of Ethnopharmacology* 90, 279–284.