Antitrypanosomal Activity of Triterpenoids and Sterols from the Leaves of *Strychnos spinosa* and Related Compounds

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Fractionation of an antitrypanosomal lipophilic leaf extract from *Strychnos spinosa* led to the isolation of eight triterpenoids and sterols in this plant part for the first time. Two of these were found to possess in vitro antitrypanosomal activity, namely, saringosterol (**14**) and 24-hydroperoxy-24-vinylcholesterol (**15**), with IC_{50} values of 7.8 ± 1.2 and 3.2 ± 1.2 μM, respectively. The latter compound was isolated from a plant source for the first time. A comparative study on the antitrypanosomal activity of the isolated triterpenoids and sterols and some related compounds has indicated that the presence of an oxygenated function at C-28 or an oxygenated side chain at C-17 seems to be important for the antitrypanosomal activity of triterpenoids and sterols, respectively.

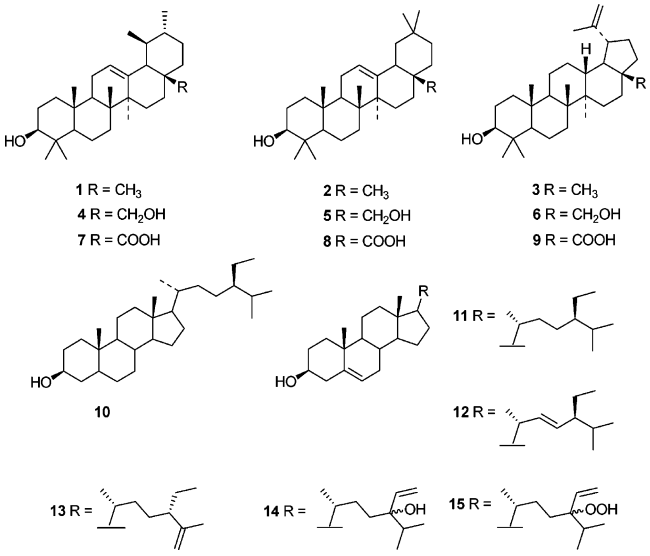
African trypanosomes (e.g., *Trypanosoma brucei* subspecies) are protozoal parasites responsible for human African trypanosomiasis (sleeping sickness) and nagana in cattle, and cause major health and economic problems in rural sub-Saharan Africa. Sleeping sickness currently affects about 70 000 people, but data are lacking for some regions. An estimated 60 million people are at risk of contracting this disease, which is fatal if left untreated. However, only a few drugs, with serious side effects, are currently registered to treat African trypanosomiasis, and the efficacy of these seems to be declining.[1] Therefore, there is an urgent need for new, safe, effective, and cheap antitrypanosomal medicines as well as for new leads with original mechanisms of action.[1]

In countries where sleeping sickness occurs, plants have been used traditionally for centuries and are still widely employed to treat this illness and other parasitic diseases. It is estimated that at least two-thirds of the world's population relies on traditional medical remedies due to the limited availability and affordability of pharmaceutical products.[2] A decoction obtained from the leaves of *Strychnos spinosa* Lam. (Loganiaceae) is used traditionally to treat African trypanosomiasis.[3] Accordingly, in a previous study our group showed that the leaf dichloromethane extract of this species has promising antitrypanosomal activity in vitro.[4] However, there is not much information on the constituents of the leaves of *S. spinosa*: three indolomonoterpenoid alkaloids (akagerine, 10-hydroxyakagerine, and kribine) have been isolated, and the presence of sterols and flavonols has been reported.[5,6]

Therefore, it was decided to undertake the bioassay-guided fractionation of this dichloromethane extract of *S. spinosa* leaves. Several active fractions were obtained, with none of them containing alkaloids, but triterpenoids and sterols were detected in some of the most active ones. A combination of different chromatographic techniques allowed the identification of four triterpenoids (**1**−**4**) and four sterols (**11**, **13**−**15**) from the leaves of this plant.

To determine their antitrypanosomal potency, these compounds were tested on *Trypanosoma brucei brucei* bloodstream forms cultured in vitro, as well as on mammalian cells (J774) to evaluate their selectivity. The analogous effects of some commercially available related triterpenoids and sterols were evaluated in order to establish preliminary structure−activity relationships.

Fractionation of the dichloromethane extract of *S. spinosa* leaves by high-speed countercurrent chromatography (HSCCC) gave 14 fractions, which were pooled on the basis of their TLC profiles. Eight of these were at least 4 times more active than the crude extract (fractions 1, 2, 4, 5, 6, 7, 11, 13, with IC_{50} values ranging from 2.7 to 5.2 μg/mL), indicating that a number of antitrypanosomal compounds were present, but none of them contained alkaloids that reacted with Dragendorff reagent on TLC. We chose to focus on the major triterpenoids and sterols in two of the active fractions (fractions 11 and 13) and isolated these compounds from the dichloromethane extract using different purification techniques.



Altogether, eight compounds were purified, namely, α- and β-amyrin (**1** and **2**), lupeol (**3**), uvaol (**4**), β-sitosterol (**11**),

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Table 1. In Vitro Antitrypanosomal Activity and Cytotoxicity of Triterpenoids and Sterols

	IC_{50}[a] (average ± standard deviation)				
	Trypanosoma brucei. brucei[b]		J774[c]		SI[d]
compound	μg/mL	μM	μg/mL	μM	
α-amyrin (**1**)	48.9 ± 3.1	114.5 ± 7.2	54.8 ± 1.9	128.5 ± 4.4	1.1
β-amyrin (**2**)	54.2 ± 0.7	126.9 ± 1.5	>100	>234.3	>1.8
lupeol (**3**)	19.3 ± 1.6	45.3 ± 3.7	>100	>234.3	>5.2
uvaol (**4**)	12.3 ± 0.7	27.8 ± 1.5	69.0 ± 3.5	155.8 ± 7.8	5.6
erythrodiol (**5**)	5.3 ± 0.3	12.0 ± 0.6	22.3 ± 0.3	50.3 ± 0.7	4.2
betulin (**6**)	4.0 ± 1.5	8.9 ± 3.3	29.7 ± 3.5	67.1 ± 7.9	7.4
ursolic acid (**7**)	1.0 ± 0.2	2.2 ± 0.3	6.0 ± 0.4	13.1 ± 0.9	6.0
oleanolic acid (**8**)	2.9 ± 0.4	6.4 ± 0.8	59.6 ± 1.2	130.5 ± 2.6	20.6
betulinic acid (**9**)	14.9 ± 2.9	32.6 ± 6.2	20.1 ± 0.3	44.0 ± 0.7	1.3
stigmastanol (**10**)	>100	>240	>100	>240	
β-sitosterol (**11**)	>100	>241.1	>100	>241.1	
stigmasterol (**12**)	55.5 ± 4.19	134.6 ± 10.2	>100	>242.3	>1.8
clerosterol (**13**)	53.6 ± 3.2	129.8 ± 7.7	>100	>242.3	>1.9
saringosterol (**14**)	3.3 ± 0.5	7.8 ± 1.2	>100	>233.3	>30.3
24-hydroperoxy-24-vinylcholesterol (**15**)	1.4 ± 0.5	3.2 ± 1.2	7.3 ± 0.05	16.4 ± 0.1	5.2
suramin	0.059 ± 0.004	0.041 ± 0.003	nd[e]	nd[e]	
colchicine	nd[e]	nd[e]	0.084 ± 0.017	0.210 ± 0.043	

[a] Values indicate 50% inhibitory concentrations. [b] *Trypanosoma brucei brucei* bloodstream forms. [c] Murine macrophages. [d] Selectivity index: $IC_{50(J774)}/IC_{50(Tbb)}$. [e] Not determined.

clerosterol (**13**), saringosterol (**14**), and 24-hydroperoxyvinylcholesterol (**15**). For these last two compounds, a mixture of both epimers (24*R*/*S*) was obtained. Structures were determined by a combination of MS and NMR experiments and by comparison with literature data.[7−13] Identity with reference compounds was also confirmed by LC-MS and GC-MS for α- and β-amyrin (**1** and **2**), lupeol (**3**), uvaol (**4**), β-sitosterol (**11**), and clerosterol (**13**).[14] As no or only few retention and mass spectrometric fragmentation data are available for several of these compounds, these are given in Tables S1 and S2 (Supporting Information). It must be noted that 24-hydroperoxy-24-vinylcholesterol (**15**) was not detectable by GC-MS (probably because of a thermal degradation of the peroxide function). These eight compounds were identified for the first time in the leaves of *S. spinosa*.

Additionally, this is the first report on the presence of uvaol (**3**), clerosterol (**13**), and saringosterol (**14**) in the genus *Strychnos* and the first report on the presence of 24-hydroperoxy-24-vinylcholesterol (**15**) in a plant.[8,15−18]

All isolated compounds were tested on *Trypanosoma brucei brucei* trypomastigotes cultured in vitro as well as on J774 murine cells to evaluate the selectivity of their antitrypanosomal effects (selectivity index = SI). As some triterpenoids and sterols are already known to exhibit interesting antitrypanosomal activity,[19] several commercially available related compounds were also tested, namely, ursolic acid (**7**) (ursane type), erythrodiol (**5**) and oleanolic acid (**8**) (oleanane type), betulin (**6**) and betulinic acid (**9**) (lupane type), and stigmastanol (**10**) and stigmasterol (**12**) (sterols), in order to establish preliminary structure−activity relationships. The activities and selectivity indices for these compounds are given in Table 1.

Some of the major compounds of active fractions [i.e., α- and β-amyrins (**1** and **2**), β-sitosterol (**11**)] of the dichloromethane extract of *S. spinosa* did not possess interesting antitrypanosomal activity. This implies either that some minor compounds should have a very high activity or that a synergistic effect should be considered. Among the investigated sterols, stigmastanol (**10**) and β-sitosterol (**11**), which have a saturated side chain, were inactive on the bloodstream forms of *T. b. brucei* and on the murine macrophages (IC_{50} > 100 μM). However, stigmasterol (**12**) and clerosterol (**13**), which both have a double bond in their side chain, had an effect on *T. b. brucei*, even though quite weak. An oxygenated side chain (hydroxyl or a hydroperoxyl group) present in the sterol dramatically increased the resultant antitrypanosomal activity (IC_{50} = 3−8 μM). An introduced hydroxyl group induced no toxic effects on the murine macrophages grown in vitro (IC_{50} > 100 μM) (SI > 30), unlike a hydroperoxyl group (IC_{50} = 16.4 μM) (SI 5.2).

β-Sitosterol (**11**), as well as other phytosterols, is known to exhibit a variety of pharmacological activities, including hypocholesterolemic and immunomodulating properties. Some phytosterols have been shown to be cytotoxic or inhibitors of the growth of selected cell lines but also to be ineffective against others.[20] The low toxicity of saringosterol (**14**) for normal cells has already been reported in an in vitro study of its antitubercular activity. In the same study, the two isomers were separated and the 24*R* isomer was shown to be 8 times more active than the 24*S* isomer.[21] This suggests that the antitrypanosomal activity obtained for the epimeric mixture could be higher for one of the stereoisomers, if they were to be separated.[9,21]

The in vitro cytotoxicity for various cancer cell lines of the sterol peroxide 24-hydroperoxy-24-vinylcholesterol (**15**) has also been documented. The reported IC_{50} values were in the range 0.7−19 μM depending on the cell line tested (murine leukemic cells and human lung cancer, colon cancer, and leukemia cells).[17] Nevertheless, this is the first time that the antitrypanosomal activity of saringosterol (**14**) and its hydroperoxy derivative (**15**) has been documented.

This is also the first report on the antitrypanosomal activity of five of the nine triterpenoids analyzed in this study, whereas β-amyrin (**2**), uvaol (**4**), ursolic acid (**7**), and oleanolic acid (**8**) have already been tested on trypanosomes. β-Amyrin (**2**) was shown to be inactive on *T. b. rhodesiense* bloodstream trypomastigotes.[22] Taketa et al. did not detect any activity for uvaol (**4**) on *T. b. brucei* bloodstream forms (IC_{50} > 32 μM),[23] while a weak activity was found in the present work. Ursolic acid (**7**) and oleanolic acid (**8**) have exhibited toxicity for the South American trypanosome *T. cruzi*, inducing 50% lysis at 21 and 80.4 μM, respectively, on *T. cruzi* blood trypomastigotes.[24] In contrast, the acetyl (on the C-3 hydroxyl group) and methyl ester (on the C-28 carboxyl group) derivatives were inactive. It was concluded that the presence of the two polar groups in both **7** and **8** is important for their antitrypanosomal effects. Ursolic acid (**7**) and oleanolic acid (**8**) also restricted the movement of *T. cruzi* epimastigotes (vector form), while betulinic acid (**9**) was practically inactive.[25,26]

Among the triterpenoids evaluated, ursolic acid (**7**) (ursane skeleton) was the most potent on *T. b. brucei* blood trypomastigotes with an IC_{50} value of 2.2 μM, similar to the value reported by Taketa et al.[23] Its in vitro selectivity index was small (SI = 6). In contrast,

oleanolic acid (**8**) (oleanane skeleton) was 3 times less potent on trypanosomes, but its activity was more selective (SI = 20.6). Betulinic acid (**9**), the third triterpene (lupane skeleton) with a C-28 carboxyl group, was much less active and showed no selectivity for the trypanosomes. However, when comparing the antitrypanosomal activity of the three triterpenes with a C-28 hydroxymethyl group, the triterpene with the lupane-type skeleton (**6**) was the most trypanocidal, followed by the oleanane-type skeleton (**5**) and the ursane-type triterpene (**4**). They were all 4 to 7 times more toxic to trypanosomes than to mammalian cells. Among the triterpenes with a C-28 methyl substituent, only lupeol (**3**) exhibited a low but selective activity on trypanosomes, in the same range of concentration as its derivative with a C-28 carboxyl group, while α- and β-amyrin (**1** and **2**) were inactive.

When considering the antitrypanosomal activity of the nine triterpenoids evaluated, consistent with the results of Cunha et al. on oleanolic acid and ursolic acid derivatives,[24] the presence of an oxygen-containing moiety at C-17 favors the resultant in vitro antitrypanosomal activity. The order of preference for the substitution on C-17 (C-28 carbon type) was $COOH > CH_2OH > CH_3$ for the ursane- or oleanane-type skeleton and $CH_2OH > COOH \geq CH_3$ for the lupane-type triterpenes included in this study.

Pentacyclic triterpenoid compounds are also characterized by an extremely diverse profile of biological activities including anti-inflammatory,[27] hepatoprotective,[28] antiviral (including anti-HIV),[29] antiparasitic,[30] and antitumor properties.[29,31] Especially with regard to the latter property, there is an increasing number of publications on these pentacyclic triterpenes as well as on other natural triterpenoids.[31] Betulinic acid (**9**) is undergoing preclinical development for the treatment of malignant melanoma.[29,31]

The precise mechanisms of action of oleanolic (**7**), ursolic (**8**), and betulinic acids (**9**) have not been elucidated, but they have been shown to induce apoptosis and to inhibit several enzymes, including human topoisomerases I and II.[32−34] It seems that their carboxylic acid functionalities are essential for the significant inhibitory activity of these DNA-manipulating enzymes.[32] Since the topoisomerases of kinetoplastids such as African trypanosomes have been identified as interesting potential drug targets,[35,36] it would be of interest to study the effect of those pentacyclic triterpenes on trypanosomal topoisomerases.

The present comparative study on the antitrypanosomal activity of isolated triterpenoids and sterols and related compounds has indicated that the presence of either an oxygenated side chain or an oxygenated function at C-28 seems to be important for their resultant activities. It can be noted that the sterols and triterpenes already reported for their activity on African trypanosomes[20,28] also have modified oxygenated side chains or an oxygenated function on C-28.

Experimental Section

General Experimental Procedures. ^1H and ^{13}C NMR spectra were recorded using a Bruker 300 NMR spectrometer in $CDCl_3$ at 300 MHz for ^1H and 75 MHz for ^{13}C NMR. For saringosterol (**14**) and 24-hydroperoxy-24-vinylcholesterol (**15**), ^1H, ^{13}C, COSY, HSQC, and HMBC spectra were recorded using a Bruker DRX-400 spectrometer in $CDCl_3$ at 400 MHz for ^1H and 100 MHz for ^{13}C NMR.

Fractionations by HSCCC were carried out on a Kromaton III (SEAB) (Villejuif, France) system, column length 200 m, internal diameter 2.5 mm, rotation speed 400 rpm, flow rate 2 mL/min. Medium-pressure liquid chromatography (MPLC) was conducted on silica gel (Merck Lichroprep Si 60 15−25 μm) on an Omnifit glass column (OM 6427 15 × 750 mm) with a flow rate of 1 mL/min. Vacuum-liquid chromatography (VLC) was carried out on Merck Lichroprep Si 60 (0.063−0.2 mm). Merck silica gel F_{254} TLC plates (mobile phases: toluene−EtOAc−MeOH, 80:18:2, and toluene−EtOAc−HCOOH, 8:2:1; detection: anisaldehyde−sulfuric acid reagent) were used for TLC, and C_{18} silica gel F_{254} aluminum foil-backed particles of 11 μm, 0.2 mm sorbent bed thickness, and 0.5 mL/min flow rate, for OPLC (optimum-performance laminar chromatography) (Bionisis-OSN50, Le Plessis Robinson, France) separations.

Compounds were analyzed by HPLC-MS with a LC Thermo Separation Products (TSP, San José, CA) system equipped with a P1000XR pump and a TSP AS 3000 autosampler. Separation of analytes was performed on a C_{18} Prevail column (150 × 2.1 mm i.d., particle size: 3 μm) (Alltech), using a linear gradient from 75% methanol−25% water (1% acetonitrile) to 100% methanol in 20 min and maintained for 20 min. The flow rate was 0.2 mL/min, and the column temperature was kept at 30 °C. The injection volume was 10 μL. Mass spectra were acquired using an LCQ mass spectrometer, equipped with an APCI source (Finnigan-MAT, San José, CA) in the positive-ion mode. The following APCI inlet conditions were applied: heated vaporization temperature: 390 °C, heated capillary temperature: 150 °C, sheath gas: 45 psi, auxiliary gas: 20 psi, and discharge current: 5 μA. Collision-induced dissociation (CID) spectra were recorded at relative collision energy of 33%. Solutions were made in absolute ethanol, methanol, and/or chloroform (HPLC grade) and filtered with DynaGard filters (Microgon Inc.) before LC injection.

The gas chromatograph used was a TRACE GC 2000 series (ThermoQuest, Italy), equipped with an autosampler AS2000 (ThermoQuest) interfaced to a Trace MS mass spectrometer (ThermoQuest), operating in the electron-impact mode.

Chromatographic separations were performed on a capillary column (DB-XLB; column length 15 m × 0.25 mm with a 0.25 μm film thickness) from J&W Scientific (Agilent Technologies, Santa Clara, CA). Helium was used as carrier gas at a flow rate of 1.2 mL/min. Samples (1 mL, in ethanol) were injected in the split mode (split ratio 1:9). The injector temperature was set at 250 °C, and the oven was programmed from 50 to 320 °C (10 °C/min); this last temperature was maintained for 3 min. The electron energy was 70 eV, and the ion source was at 250 °C. Samples were analyzed in a full-scan mode (250−650 amu).

The sterol standards used were α-amyrin (with HPLC, Extrasynthèse), β-amyrin (with HPLC, Extrasynthèse), β-sitosterol (95%, Sigma), lupeol (with HPLC, Extrasynthèse), clerosterol (Herbstandard), and uvaol (with HPLC, Extrasynthèse). Suramin and colchicine were purchased from Sigma.

Plant Material. Leaves of *S. spinosa* were collected in the savannas of Zou Province (Savane de Dan, near Abomey) in Benin in April 2002. The plant was identified by Prof. V. Adjakidjé (Université d'Abomey-Calavi, Benin). A voucher specimen was deposited at the Herbarium of the Belgian National Botanical Garden at Meise (BR S.P. 848106).

Extraction and Isolation. Plant extracts were prepared by percolating aliquots of 50 g of dried and powdered leaves with dichloromethane for 24 h at room temperature. The quantity of solvent used for each extraction (500 mL) was at least 10 times the amount of plant material used (50 g). The extracts were evaporated to dryness under reduced pressure. The solvent-free extracts were mixed and stored frozen. A total of 1.075 kg was extracted, with yields varying from 5% to 7%.

Fractionation of 3.5 g of the dichloromethane extract by HSCCC with the lower phase of the mixture heptane−acetonitrile−CH_2Cl_2 (10:7:3) as mobile phase in the head-to-tail mode gave 14 fractions. Fractions were pooled following their TLC profiles. F13 (133 mg) was submitted to MPLC with a step gradient of toluene−EtOAc, 98:2 (F13-1 F13-6), toluene−EtOAc, 95:5 (F13-7), and MeOH (F13-8). Fraction 13-2 (33.6 mg) afforded a mixture of α- and β-amyrin (**1** and **2**), while fraction 13-4 was pure β-sitosterol (**14**) (19.2 mg).

An aliquot (13.4 g) of the dichloromethane extract was fractionated by VLC on a funnel packed with 270 g of silica gel by a gradient elution of solvents of increasing polarity (*n*-hexane; *n*-hexane with increasing proportions of $CHCl_3$; $CHCl_3$; $CHCl_3$ with increasing proportions of EtOAc; EtOAc; EtOAc with increasing proportions of MeOH; MeOH; 300 mL for each step). Altogether, 23 fractions were obtained based on their TLC profiles. Fraction VLC-6 (5.2 g) was submitted to HSCCC using the same experimental conditions as above. The most active fraction, VLC 6-14 (84 mg), was further purified by an MPLC with toluene−EtOAc (98:2) as solvent and led to the isolation of saringosterol (**14**) (3.2 mg), clerosterol (**13**) (4.1 mg), and lupeol (**3**) (3.2 mg).

Portions (8 g) of the dichloromethane extract were dissolved in 750 mL of hexane and extracted by 5 × 250 mL of 80% aqueous methanol. The polar phase was washed by 5 × 250 mL of *n*-hexane, and both phases were evaporated to afford 0.6 g of a polar fraction and 7.4 g of a *n*-hexane fraction (H). Then, 1.3 g of the polar fraction was

fractionated by HSCCC with hexane−EtOAc−MeOH−H_2O (3:2:3:2) as solvent system, using the upper phase as mobile phase in the tail-to-head mode, to afford 16 fractions. Fraction 4, one of the most active fractions, was purified by an MPLC separation with toluene−EtOAc (99:1) as mobile phase, to obtain 1.7 mg of practically pure uvaol (**4**). The same procedure was repeated to isolate enough of this compound for structure determination, and its final purification was achieved by OPLC with MeOH−HCOOH (99:1) as mobile phase to obtain 7.4 mg of uvaol (**4**). Hexane fraction H was submitted to the same HSCCC and MPLC procedures as fraction VLC 6 followed by OPLC under the same conditions as described for uvaol, to afford 6.6 mg of 24-hydroperoxy-24-vinylcholesterol (**15**).

Cell Culture. *Trypanosoma brucei brucei* (strain 427) bloodstream forms and J774 cells (cancer murine macrophage-like cell line) were grown in vitro in HMI 9 medium[4] and RPMI 1640 with l-glutamine medium, respectively. The media were supplemented with 10% heat-inactivated fetal calf serum. All cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2.

Antitrypanosomal Activity and Cytotoxicity Assays. The antitrypanosomal activity of all fractions and pure compounds and the cytotoxicity of the pure compounds were assessed against *T. b. brucei* bloodstream forms and mammalian cells using the Alamar Blue assay, as previously reported.[4] IC_{50} values were calculated by linear interpolation and are given as mean values ± standard deviations calculated from at least four experiments. Suramin and colchicine were used as positive controls. Selectivity indices were calculated as follows: $IC_{50(J774)}/IC_{50(Tbb)}$.

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Supporting Information Available: Tables of GC-MS and LC-MS data on triterpenoids and sterols. This information is available free of charge via the Internet at http://www.pubs.acs.org.

References and Notes

(1) Barrett, M. P. *Lancet* **2006**, *367*, 1377−1378.
(2) Tagboto, S.; Townson, S. *Adv. Parasitol.* **2001**, *50*, 199−295.
(3) Neuwinger, H. D. *African Traditional Medicine: a Dictionary of Plant Use and Applications*; Medpharm: Stuttgart, 2000.
(4) Hoet, S.; Opperdoes, F.; Brun, R.; Adjakidjé, V.; Quetin-Leclercq, J. *J Ethnopharmacol.* **2004**, *91*, 37−42.
(5) Kerharo, J.; Adam, J. G. *La Pharmacopée Sénégalaise Traditionnelle: Plantes Médicinales et Toxiques*; Vigot Frères: Paris, 1974.
(6) Oguakwa, J. U.; Galeffi, C.; Nicoletti, M.; Messana, I.; Patamia, M.; Marini-Bettolo, G. B. *Gazz. Chim. Ital*. **1980**, *110*, 97−100.
(7) Goad, L. J.; Akihisa, T. *Analysis of Sterols*; Blackie Academic & Professional: London, 1997.
(8) Guyot, M.; Davoust, D. *Tetrahedron Lett.* **1982**, *23*, 1905−1906.
(9) Catalan, C. A. N.; Kokke, W. C. M. C.; Duque, C.; Djerassi, C. *J. Org. Chem*. **1983**, *48*, 5207−5214.
(10) Kurata, K.; Taniguchi, K.; Shiraishi, K.; Suzuki, M. *Phytochemistry* **1990**, *29*, 3678−3680.
(11) Sheu, J.-H.; Sung, P.-J. *J. Chin. Chem. Soc*. **1991**, *38*, 501−503.
(12) Dehmlow, E. V.; Van Ree, T.; Jakupovic, J.; Take, E.; Künsting, H. J. *J. Chem. Res., Synop*. **1998**, 252−253.
(13) Goad, L. J. In *Methods in Plant Biochemistry. Vol*. 7. *Terpenoids*; Charlwood, B. V., Banthorpe, D. V., Eds.; Academic Press: London, 1991; pp 369−434.
(14) Rozenberg, R.; Ruibal-Mendieta, N. L.; Petitjean, G.; Cani, P.; Delacroix, D. L.; Delzenne, N. M.; Meurens, M.; Quetin-Leclercq, J.; Habib-Jiwan, J.-L. *J. Cereal Sci*. **2003**, *38*, 189−197.
(15) Ktari, L.; Guyot, M. *J. Appl. Phycol*. **1999**, *11*, 511−513.
(16) Tang, H.-F.; Yi, Y.-H.; Yao, X.-S.; Xu, Q.-Z.; Zhang, S.-Y.; Lin, H.-W. *J. Asian Nat. Prod. Res*. **2002**, *4*, 95−101.
(17) Sheu, J.-H.; Wang, G.-H.; Sung, P.-J.; Chiu, Y.-H.; Duh, C.-Y. *Planta Med.* **1997**, *63*, 571−572.
(18) Sheu, J.-H.; Wang, G.-H.; Sung, P.-J.; Duh, C.-Y. *J. Nat. Prod.* **1999**, *62*, 224−227.
(19) Hoet, S.; Opperdoes, F.; Brun, R.; Quetin-Leclercq, J. *Nat. Prod. Rep*. **2004**, *21*, 353−364.
(20) Quilez, J.; Garcia-Lorda, P.; Salas-Salvado, J. *Clin. Nutr*. **2003**, *22*, 343−351.
(21) Wachter, G. A.; Franzblau, S. G.; Montenegro, G.; Hoffmann, J. J.; Maiese, W. M.; Timmermann, B. N. *J. Nat. Prod.* **2001**, *64*, 1463−1464.
(22) Bringmann, G.; Hamm, A.; Günther, C.; Michel, M.; Brun, R.; Mudogo, V. *J. Nat. Prod.* **2000**, *63*, 1465−1470.
(23) Taketa, A. T. C.; Gnoatto, S. C. B.; Gosmann, G.; Pires, V. S.; Schenkel, E. P.; Guillaume, D. *J. Nat. Prod.* **2004**, *67*, 1697−1700.
(24) Cunha, W. R.; Martins, C.; Ferreira, D. D.; Crotti, A. F. M.; Lopes, N. P.; Albuquerque, S. *Planta Med.* **2003**, *69*, 470−472.
(25) Abe, F.; Yamauchi, T.; Nagao, T.; Kinjo, J.; Okabe, H.; Higo, H.; Akahane, H. *Biol. Pharmacol. Bull.* **2002**, *25*, 1485−1487.
(26) Saeidnia, S.; Gohari, A. R.; Uchiyama, N.; Ito, M.; Honda, G.; Kiuchi, F. *Chem. Pharm. Bull.* **2004**, *52*, 1249−1250.
(27) del Carmen Recio, M.; Giner, R. M.; Manez, S.; Rios, J. L. *Planta Med.* **1995**, *61*, 182−185.
(28) Liu, J. *J Ethnopharmacol*. **2005**, *100*, 92−94.
(29) Baglin, I.; Mitaine-Offer, A.-C.; Nour, M.; Tan, K.; Cave, C.; Lacaille-Dubois, M.-A. *Mini-Rev. Med. Chem.* **2003**, *3*, 525−539.
(30) Steele, J. C. P.; Warhurst, D. C.; Kirby, G. C.; Simmonds, M. S. J. *Phytother. Res.* **1999**, *13*, 115−119.
(31) Setzer, W. N.; Setzer, M. C. *Mini-Rev. Med. Chem.* **2003**, *3*, 540−556.
(32) Mizushina, Y.; Ikuta, A.; Endoh, K.; Oshige, M.; Kasai, N.; Kamiya, K.; Satake, T.; Takazawa, H.; Morita, H.; Tomiyasu, H. *Biochem. Biophys. Res. Commun.* **2003**, *305*, 365−373.
(33) Syrovets, T.; Büchele, B.; Gedig, E.; Slupsky, J. R.; Simmet, T. *Mol. Pharmacol.* **2000**, *58*, 71−81.
(34) Chowdhury, A. R.; Mandal, S.; Mittra, B.; Sharma, S.; Mukhopadhyay, S.; Majumder, H. K. *Med. Sci. Monit.* **2002**, *8*, BR254-65.
(35) Das, A.; Dasgupta, A.; Sengupta, T.; Majumder, H. K. *Trends Parasitol.* **2004**, *20*, 381−387.
(36) Deterding, A.; Dungey, F. A.; Thompson, K.-A.; Steverding, D. *Acta Trop.* **2005**, *93*, 311−316.

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