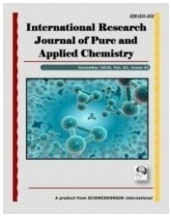

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Compounds from *Diospyros canaliculata* (*Ebenaceae*) and their Antiparasitic Activities

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Authors' contributions

This whole work was carried out in collaboration between all authors. Authors BNL and LMK collected the plant and made the extraction. Authors BNL, RFN, JN and FT wrote the protocol and performed the phytochemical analysis. Author MK performed the atiparasitic assays and author CA the spectroscopic analysis. Authors BNL and FT wrote the first draft of the manuscript. Author SN contributed in the literature searches. Authors ET and NS managed the supervision of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Discovering new lead compounds against parasitic diseases is a crucial step to ensuring a sustainable global pipeline for new effective drugs. This study focus on the isolation and the antiparasitic screening of secondary metabolites of the dichloromethane-methanol (1:1) extract of the stem bark of *Diospyros canaliculata* which showed antiprotozoal activity *in vitro* during preliminary screening.

Study Design: According to the literature, plants of the genus *Diospyros* are potential sources of antiparasitic secondary metabolites. As far as we know, extracts from *D. caniculata* have not yet been investigated for their antiparasitic activity.

Methodology: The air-dried and ground stem bark of *Diospyros canaliculata* was extracted at room temperature with a mixture of dichloromethane-methanol (1:1). The extract was concentrated to dryness under vacuum and the residue was subjected to repeated column chromatographic separation. The structures of the isolates were established by means of spectroscopic methods. These compounds were screened *in vitro* for their activity against four parasitic protozoa: *Plasmodium falciparum*, *Leishmania donovani, Trypanosoma brucei rhodesiense* and *Trypanosoma cruzi* and for their cytotoxic potential on mammalian cells.

Results: The dichloromethane-methanol (1:1) extract of the stem bark of *D. canaliculata* showed antiprotozoal activity *in vitro* during preliminary screening. Phytochemical investigation of this extract led to the isolation of a new coumarinyl naphtoquinone canaliculin (**1**) together with the known canaliculatin (**2**), betulin (**3**), plumbagin (**4**), ismailin (**5**), gerberinol (**6**) and betulinic acid (**7**). Compounds **2-7** were evaluated *in vitro* for their antiprotozoal activities against *P. falciparum* (NF_{54}), *L. donovani*, *T. b. rhodesiense and T. cruzi*. Plumbagin (**4**) exhibited good activity against all the tested strains with IC_{50} below 0,9 µg/mL while other tested compounds exhibited weak to moderate activity, generally with limited selectivity.

Conclusion: From the present study, we infer that the dichloromethane-methanol (1:1) extract of the stem bark of *D. canaliculata* contains antiparasitic compounds. Unfortunately, the antiparasitic potency of these compounds may reflect general toxicity, as shown by their low IC_{50} recorded against the mammalian cell line

Keywords: Diospyros canaliculata; Ebenaceae; coumarinyl naphtoquinone; antiparasitic activities.

1. INTRODUCTION

Diospyros is one of the most important genera of the *Ebenaceae* family with more than 350 species [1]. They are mostly trees and rarely shrubs well-known for their medicinal uses since ancient times in many traditional medicinal systems such as Ayurveda, traditional Chinese medicine and the African folklore [2]. Previous phytochemical investigations of plants of the genus *Diospyros* have revealed the presence of bioactive terpenoids, norbergerins, coumarins and naphtoquinones [2,3]. In addition, several plants of this genus are known to be sources of potent antiparasitic compounds [4]. In a continuing search for antiprotozoal compounds from Cameroonian medicinal plants, we have investigated the dichloromethane-methanol (1:1) extract of the stem bark of *Diospyros canaliculata* which showed antiprotozoal activity *in vitro* during preliminary screening. Herein, we report on the isolation and structure elucidation of a new coumarinyl naphtoquinone, canaliculin together with the antiparasitic activity of the known compounds canaliculatin (**2)** [1], betulin (**3**) [5], plumbagin (**4**) [1], ismailin (**5**) [6], gerberinol (**6**) [1], and betulinic acid (**7**) [5] (Fig.1). Except plumbagin (**4**) which showed good antiparasticic potency against all the tested strains with IC_{50} below 0,9 µg/mL, other tested compounds showed weak to moderate activity, and generally with limited selectivity.

2. MATERIALS AND METHODS

2.1 General

Melting points were determined on a Büchi-540 melting point apparatus. UV spectra were measured on a Spectronic Unicam Spectrophotometer. IR spectra were determined on Nicolet 380. LC-HRESI-MS (Liquid Chromatography- High Resolution Electrospray Ionization Mass Spectrometry) were run on Agilent Technologies 6520 Accurate Mass Q-TOF (Quadrupole Time-of-flight). ^{1}H and ^{13}C NMR spectra were run on AVANCE III 400 and 500, Bruker spectrometers equipped with 5 mm

BBFO+ ^{1}H and ^{13}C probes operating at 400 and 100 MHz respectively for ^{13}C direct detection and 500 and 125 MHz respectively for inverse two dimensional detection methods, with residual DMSO-d_6 as internal standard, respectively 2.5 in ^{1}H and 39.5 ppm in ^{13}C. Silica gel (Merck, 230–400 and 70–230 mesh) were used for column chromatography, while pre-coated aluminum silica gel 60 F254 sheets were used for TLC (Thin Layer Chromatography) with different mixtures of *n*-hexane, ethyl acetate, dichloromethane, and methanol as eluents; spots were visualized under UV lamps (254 and 365 nm) or by heating after spraying with 50% H_2SO_4 reagent.

2.2 Plant Material

The stem bark of *Diospyros canaliculata* was collected in April 2010 at Kribi in the South region of Cameroon. The plant was identified at the National Herbarium of Cameroon, where a voucher specimen (Nr.9653/SRFCam) has been deposited.

2.3 Extraction and Isolation

The stem bark of *D. canaliculata* was dried away from solar radiation between 20 and 25ºC. The air-dried and ground stem bark obtained (5 kg) was extracted at room temperature separately with dichloromethane-methanol (1:1) (2 x 5 L, 24 h each). The extract was concentrated under vacuum at room temperature to afford 120 g of brown crude extract. This extract was screened for its antiplasmodial activity against the NF54 strains of *Plasmodium falciparum* and exhibited antiplasmodial activity with IC_{50} value of 7.24 µg/mL. It was then subjected to repeated flash column chromatography over silica gel (230-400 mesh) eluted with *n*-hexane and *n*-hexane/ ethyl acetate mixtures of increasing polarity and resulted in the collection of 75 fractions of 400 mL each that were combined on the basis of TLC (*Thin layer chromatography)* analysis to yield three main fractions labelled F1 (7.2 g), F2 (11.3 g) and F3 (12.6 g). Fraction F1 (7.2 g) was subjected to CC (column chromatography) over silica gel (Merck, 70–230 mesh), eluting with *n*-hexane/ ethyl acetate mixtures (1:0 to 7:3) to yield canaliculin (**1**, 3.8 mg), canaliculatin (**2**, 300.0 mg), plumbagin (**4**, 1800 mg) and ismailin (**5**, 35.8 mg). Fraction F2 (11.3 g) was also chromatographed on a silica gel column (SiO_2, 70 - 230 mesh, eluting with a gradient of hexane/ ethyl acetate) and resulted in the collection of 160 fractions of 75 mL each, that were combined on the basis of TLC analysis. Further purification of subfractions 71-75 by CC over silica gel (Merck,70–230 mesh) eluting with an *n*-hexane/ethyl acetate mixtures (2:8 to 4:6) afforded betulin (**3**, 97.1 mg), canaliculatin (**2**, 37.8 mg), gerberinol (**6**, 25.0 mg) and betulinic acid (**7**, 59 mg). Fraction F3 (12.6 g) was a dark complex mixture that was not studied further.

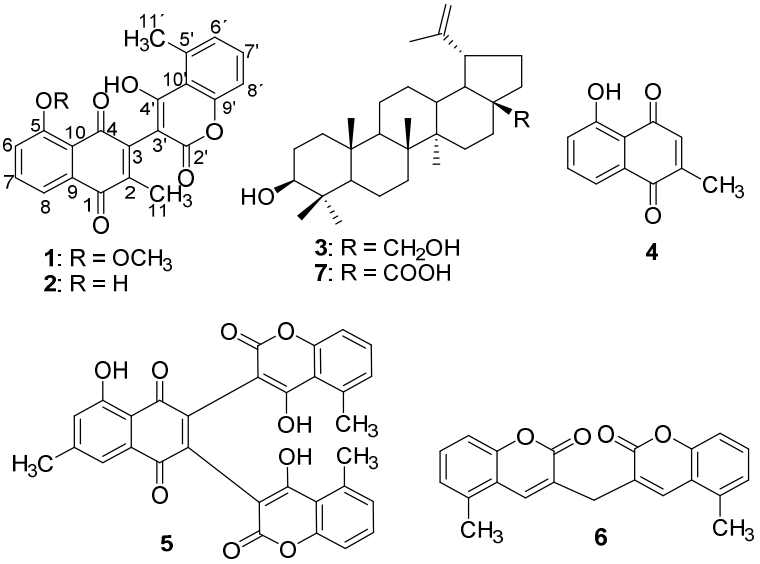

Fig. 1. Structures of isolated compounds

Canaliculin (1): Red powder; IR ($CDCl_3$) ʋmax 3447, 1651.0 and 1513-1617 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 1; HRESI-MS *m/z* $[M+H]^+$ at 377.1023 (Calcd 377.1019 for $C_{22}H_{17}O_6$).

Canaliculatin (2): Yellow powder; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; ESI-MS *m/z* 363 $[M+H]^+$

Betulin (3): White powder; ^1H-NMR ($CDCl_3$, 400 MHz): δ_H 4.68 (br s, H- 29a), 4.58 (br s, H-29b), 3.80 (d, *J* = 10.8 Hz, H-28a), 3.33 (d, *J* = 10.8 Hz, H-28b), 3.19 (1H, dd, *J* = 11,2 Hz, 5.0 Hz, H-3), 0.70 to 1.80 (6x CH_3). ESI-MS *m/z* 441 $[M-H]^-$

Plumbagin (4): Orange crystal; ^1H NMR ($CDCl_3$, 400 MHz): δ_H 2.15 (Me-2, d, *J* = 1.6 Hz), 6,71 (q, *J* = 1.6 Hz, H-3), 7.48-7.54 (2H; m), 7.15 (dd, q, *J* = 7.3 and 2.3 Hz), 11.9 (-OH). ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 115.3 (C-10), 119.4 (C-8), 124.3 (C-6), 132.2 (C-9), 135.6 (C-3), 136.2 (C-7), 149.6 (C-2), 161.3 (C-5), 184.9 (C1), 190.4 (C-4), 16.7 (C-11). HRESI-MS: $[M+H]^+$ at *m/z* 189.05506.

Ismailin (5): Amorphous powder; ^{13}C NMR (DMSO, 100 MHz):187.4, 181.3, 165.8, 160.5, 159.5, 159.4, 153.9, 147.6, 144.1, 142.9, 137.7, 132.1, 132.0, 127.8, 127.6, 122.7, 119.4, 114.6, 114.4, 113.5, 98.1, 97.4, 23.1, 23.0, 22.0; ESI-MS *m/z* 535 $[M-H]^-$.

Gerberinol (6): White powder; ^1H NMR (DMSO-d_6, 400 MHz): 7.47 (2H, t, *J* = 8.2 Hz, H-7, H-7′), 7.24 (2H, d, *J* = 8.2 Hz, H-8, H-8′), 7.17 (2H, d, *J* = 8.2 Hz, H-6, H-6´), 3.73 (2H, s, CH_2), 2.74 (6H, s, $2CH_3$).

Betulinc acid (7): Needle shaped crystals; ^1H NMR (400 MHz, $CDCl_3$): δ_H 4.70 (br s, H- 29a], 4.56 (br s, H-29b), 3.12 (dd, 1H, *J* = 10.8, 4.8Hz, H-3,), 2.97 (m, 1H), 1.64 (s, 3H, H-30), 0.73 (s, 3H), 0.95 (s, 3H), 0.91 (s, 3H), 0.94 (s, 3H), 0.80 (s, 3H). ESIMS *m/z* 455 $[M-H]^-$

2.4 Evaluation of the Antiparastic Activities of the Extract and Compounds 2-5

2.4.1 Effect against *Trypanosoma brucei rhodesiense*

The *Trypanosoma brucei rhodesiense* stock was isolated in 1982 from a human patient in Tanzania and after several mouse passages cloned and adapted to axenic culture conditions [7]. Minimum essential medium (50 µL) supplemented with 25 mM HEPES, 1g/L additional glucose, 1% MEM non-essential amino acids (100x), 0.2 mM 2-mercaptoethanol, 1mM sodium-pyruvate and 15% heat inactivated horse serum was added to each well of a 96-well microtiter plate. Serial drug dilutions of eleven 3-fold dilution steps covering a range from 100 to 0.002 µg/mL were prepared. Then $4x10^3$ blood stream forms of *T. b. rhodesiense* STIB 900 in 50 µL was added to each well and the plate incubated at 37ºC under a 5 % CO_2 atmosphere for 70 h. 10 µL Alamar Blue (resazurin, 12.5 mg in 100 mL double-distilled water) was then added to each well and incubation continued for a further 2-4 h [8]. Subsequently, the plates were analyzed with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wave length of 536 nm and an emission wave length of 588 nm. The IC_{50} values were calculated by linear regression [9] from the sigmoidal dose inhibition curves using Softmax Pro software (Molecular Devices Cooperation, Sunnyvale, CA, USA).

2.4.2 Effect against *Trypanosoma cruzi*

Rat skeletal myoblasts (L-6 cells) were seeded in 96-well microtitre plates at 2000 cells/well in 100 µL RPMI 1640 medium with 10% FBS and 2 mMl-glutamine. After 24 h the medium was removed and replaced by 100 µL per well containing 5000 trypomastigote forms of *T. cruzi* Tulahuen strain C2C4 containing the β-galactosidase (Lac Z) gene [10]. After 48 h the medium was removed from the wells and replaced by 100 µL fresh medium with or without a serial drug dilution of eleven 3-fold dilution steps covering a range from 100 to 0.002 µg/mL. After 96 h of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterility. The substrate CPRG/Nonidet (50 µL) was then added to all wells. A colored reaction occurred within 2-6 h and was evidenced photometrically at 540 nm. Data were analyzed with the graphic programme Softmax Pro (Molecular Devices), and the IC_{50} values were obtained by linear regression [9] from the sigmoidal dose inhibition curves.

2.4.3 Effect against *Leishmania donovani axenic amastigotes*

Amastigotes of the *L. donovani* strain MHOM/ET/67/L82 were grown in axenic culture at 37ºC in SM medium at pH 5.4 supplemented

with 10% heat-inactivated fetal bovine serum under an atmosphere of 5% CO_2 in air [11]. One hundred microlitres of culture medium with 10^5 amastigotes from axenic culture with or without a serial drug dilution were seeded in 96-well microtitre plates. Serial drug dilutions of eleven 3-fold dilution steps covering a range from 90 to 0.002 µg/ml were prepared. After 70 h of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterile conditions. 10 µL of Alamar Blue (12.5 mg resazurin dissolved in 100 mL distilled water) were then added to each well and the plates incubated for another 2 h [12]. The plates were afterwards analyzed with a Spectramax Gemini XS microplatefluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wave length of 536 nm and an emission wave length of 588 nm. Data were analyzed using the software Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA, USA). Decrease of fluorescence (= inhibition) was expressed as percentage of the fluorescence of control cultures and plotted against the drug concentrations. The IC_{50} values were calculated by linear regression from the sigmoidal inhibition curves [9].

2.4.4 Effect against *Plasmodium falciparum* (NF54)

In vitro activity against erythrocytic stages of *P. falciparum* was determined using a ^{3}H-hypoxanthine incorporation assay [13,14] using the drug sensitive NF54 strain [15] and the standard drug chloroquine (Sigma C6628). Compounds were dissolved in DMSO at 10 mg/ml and added to parasite cultures incubated in RPMI 1640 medium without hypoxanthine, supplemented with HEPES (5.94 g/L), $NaHCO_3$ (2.1 g/L, neomycin (100 U/mL), Albumax^R (5 g/L), and washed human red cells A^+ at 2.5% haematocrit (0.3% parasitaemia). Serial drug dilutions of eleven 3-fold dilution steps covering a range from 100 to 0.002 µg/mL were prepared. The 96-well plates were incubated in a humidified atmosphere at 37 C; 4% CO_2, 3% O_2, 93% N_2. After 48 h 50 µL of ^{3}H-hypoxanthine (= 0.5 µCi) were added to each well of the plate, which were then incubated for further 24 h under the same conditions. The plates were then harvested with a Betaplate™ cell harvester (Wallac, Zurich, Switzerland), and the red blood cells transferred into a glass fibre filter washed with distilled water. The dried filters were inserted into a plastic foil with 10 mL of scintillation fluid, and counted in a Betaplate™ liquid scintillation counter (Wallac, Zurich, Switzerland). IC_{50} values were calculated from sigmoidal inhibition curves by linear regression [9] using Microsoft Excel.

2.4.5 *In vitro* cytotoxicity with L6 cells

Assays were performed in 96-well microtiter plates, each well containing 100 µL of RPMI 1640 medium supplemented with 1% L-glutamine (200 mM) and 10% fetal bovine serum, and 4000 L-6 cells (a primary cell line derived from rat skeletal myoblasts) [16,17]. Serial drug dilutions of eleven 3-fold dilution steps covering a range from 100 to 0.002 µg/mL were prepared. After 70 hours of incubation the plates were inspected under an inverted microscope to ensure growth of the controls and sterile conditions. 10 µL of Alamar Blue was then added to each well and the plates incubated for another 2 hours. The plates were then analyzed with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wave length of 536 nm and an emission wave length of 588 nm. The IC_{50} values were calculated by linear regression [9] from the sigmoidal dose inhibition curves using SoftmaxPro software (Molecular Devices Cooperation, Sunnyvale, CA, USA).

3. RESULTS AND DISCUSSION

Coumpound **1** was obtained as a brick red powder. It reacts positively both with ferric chloride and Borntrager reagent, indicating its phenolic and quinone nature, respectively. The IR spectrum showed the presence of hydroxyl group (3447 cm^{-1}), carbonyl group (1651 cm^{-1}) and aromatic ring (1513-1617 cm^{-}1. Its molecular formula $C_{22}H_{16}O_6$, with 15 degrees of unsaturation, was deduced from its HRESI-MS spectrum which showed the pseudo-molecular ion peak $[M+H]^+$ at *m/z* 377.1023 (Calcd 377.1019 for $C_{22}H_{17}O_6$). The spectroscopic data of compound **1** pointed to a close similarity with those of canaliculatin (**2**) but with 14 mass units higher than that of this last compound. Indeed, the broad band decoupled ^{13}C NMR (DMSO-d_6, 125 MHz) spectrum of **1** (Table 1) revealed signals for 22 carbons which were sorted by DEPT and HSQC techniques into thirteen quaternary carbons, among them two carbonyls of a 1,4-naphtoquinone at δ_C 181.2 (C-1) and δ_C 177.9 (C-4) and four carbons of a coumarin moiety at δ_C 137.7 (C-5′), δ_C 159.6 (C-2′), δ_C 159.6 (C-4′) and δ_C 154.5 (C-9′) [6]; two methyls respectively at δ_C 12.7 (C-11) and 23.0 (C-11′); six methine groups and one methoxy group at δ_C

56.2 (C-12). The ^{1}H NMR spectrum of compound **1** (DMSO-d_6, 500 MHz, Table 1) exhibited signals for two 1,2,3-trisubstituted benzene ring spin systems [(δ_H 7.19 (1H, d, *J* = 7.8 Hz, H-6′), 7.29 (1H, d, *J* = 7.8 Hz, H-8′), 7.55 (1H, t, *J* = 7.8 Hz, H-7′) and (δ_H 6.91 (1H, d, *J* = 7.8 Hz H-8), 7.25 (1H, d, *J* = 7.8 Hz, H-6), 7.56 (1H, t, *J* = 7.8 Hz, H-7))], two methyl groups [δ_H 1.78 (3H, s, H-11) and δ_H 2.71 (3H, s, H-11′)], and a broad singlet of a free hydroxyl group at δ_H 11.71. Analysis of the ^{1}H NMR data, the ^{1}H-^{1}H COSY couplings and the HMBC correlations of compound **1** (Fig. 2), revealed some similarities with that of canaliculatin (**2**) [1]. The difference between these data was indeed the presence of the signal of the methoxy group (δ_H 3.98/δ_C 56.2) in compound **1** and the disappearance of the signal of the chelated phenolic hydroxyl group of compound **2**. Therefore, the location of the methoxy group was unambiguously deduced from the correlations observed in the HMBC spectrum between the methoxy protons at δ_H 3.92 and the carbon C-5 (δ_C 162.2). The location of the methoxy was also confirmed by the NOE correlation with the aromatic proton H-6 at δ_H 7.56. These data clearly indicated that compound **1** is a methylated derivative of canaliculatin, and thus is a new coumarinyl naphtoquinone with the structure as shown.

Compounds **2**-**5** were screened *in vitro* for their activity against four parasitic protozoa: *Plasmodium falciparum*, *Leishmania donovani, Trypanosoma brucei rhodesiense* and *Trypanosoma cruzi* [7-16]. The cytotoxic potential of these compounds on mammalian cells was also assessed and the selectivity index (SI: IC_{50} L6 cell/IC_{50} parasite) was calculated [17].

As shown in Table 2, the extract exhibited antiplasmodial activity against the NF54 strain of *P. falciparum in vitro*. Previous studies reported that extracts of several *Diospyros* species possess antiplasmodial activity [18]. The isolates also displayed *in vitro* antiplasmodial activity against the same strain of *P. falciparum* with IC_{50} values between 0.41 µg/mL and 50 µg/mL. The best inhibition against this protozoan parasite was displayed by plumbagin (**4**) (IC_{50} 0.408 µg/mL), but the compound showed also cytotoxicity against a mammalian (L-6) cell line. The antimalarial activity of plumbagin against $T_{9/94}$ line of *Plasmodium falciparum* was previously reported [19]. The low activity of betulin (**3**) against the NF54 strain corroborates with the result previously published which demonstrated no activity on *P. faciparum* K1 and T9-96 strains [20].

Concerning the antitrypanosomal assay, plumbagin (**4**) exhibited good potency against *T. b. rhodesiense* with IC_{50} of 0.067 µg/mL and some selectivity (SI 7). The compounds canaliculatin and ismailin showed moderate activity and a certain selectivity. None of the compounds tested showed activity against *T. cruzi* except plumbagin but its activity was not selective. On the other hand, except betulin (**3**), the extract and other tested samples showed moderate growth inhibitory activity against *L. donovani* but limited selectivity.

Plumbagin and canaliculatin possess a naphtoquinone skeleton and naphthoquinone metabolites have been reported to possess good antileishmanial, antiplasmodial and antitrypanosomal activities *in vitro* [4,8,21-23].

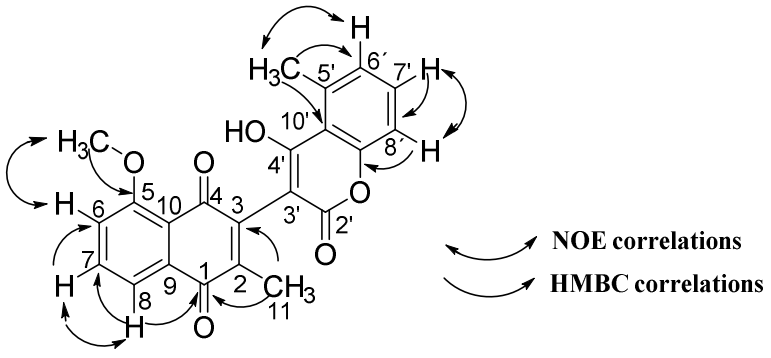

Fig. 2. Selected NOE and HMBC correlations observed in compound 1

Table 1. ^{1}H and ^{13}C NMR data of compounds 1 and 2

Positions	Compounds			
	1		2	
	δ_H[a,c] (m, *J* in Hz)	δ_C[a,b]	δ_H[d,e]	δ_C[d,f]
1	-	181.2	-	183.7
2	-	141.4		149.0
3	-	137.3	-	137.6
4	-	177.9	-	187.6
5	-	162.2	-	159.8
6	7.25 (d, *J* = 7.8 Hz)	114.7	7.39 (dd, *J* = 8,2 and 1.2 Hz)	123.7
7	7.56 (t, d, *J* = 7.8 Hz)	136.9	7.80 (t, *J* = 8,2 Hz)	136.4
8	6.91 (d, d, *J* = 7.8 Hz)	120.1	7.64 (dd, *J* = 7,8 and 1.2 Hz)	118.5
9	-	142.6	-	131.9
10	-	118.5	-	114.7
11	1.78 (s)	12.7	2.01 (s)	14.0
OCH_3	3.92 (s)	56.2	-	-
2′	-	159.6	-	164.3
3′	-	100.0	-	97.7
4′	-	159.6	-	160.0
5′	-	137.7	-	138.0
6′	7.19 (d, *J* = 7.8 Hz)	127.5	7.20 (d, *J* = 7.9 Hz)	127.6
7′	7.55 (t, *J* = 7.8 Hz)	131.8	7.55 (t, *J* = 8,2 Hz)	131.8
8′	7.29 (d, *J* = 7.8 Hz)	114.8	7.29 (d, *J* = 8,2 Hz)	114.2
9′	-	154.5	-	154.1
10′	-	114.5	-	114.9
11′	2.71 (s)	23.0	2.72 (s)	23.1

[a]Spectra were recorded in DMSO-d_6, [b]125 MHz, [c]400 MHz , [d]Spectra were recorded in $CDCl_3$, [e]400 MHz, [f]100 MHz

Table 2. *In vitro* antiprotozoal and cytotoxic activities of extract and some of the isolates

	IC_{50} (µg/mL)					Selectivity index			
Sample	***Plasmodium falciparum* NF54**	***Leishmania donovani***	***Trypanosoma b. rhodesiense***	***Trypanosoma cruzi***	***L6 cell line***	***P. falciparum* NF54**	***L. donovani***	***T. b. rhodesiense***	***T. cruzi***
Extract	7.24	2.99	1.77	24.9	8.35	1.2	2.8	4.7	0.34
Canaliculatin (**2**)	22.6	2.73	2.38	27.1	18.9	0.8	6.9	7.9	0.7
Betulin (**3**)	50	100	59.2	65.4	52.6	1.1	0.5	0.9	0.8
Plumbagin (**4**)	0.408	0.85	0.067	0.664	0.473	1.2	0.6	7	0.71
Ismailin (**5**)	28.6	5.89	6.42	62.4	56.60	2.0	9.5	8.7	0.9
*Chloroquine	0.006								
*Miltefosine		0.145							
*Melarsoprol			0.003						
*Benznidazole				0.531					
*Podophyllotoxin					0.006				

** Reference. The IC_{50} values are the means of two independent assays; the individual values vary less than a factor 2*

4. CONCLUSION

Seven compounds of which a new coumarinyl naphtoquinone (**1**) were isolated from the dichloromethane-methanol (1:1) extract of the stem bark of *D. canaliculata.* The results of the antiparasitic screening of these compounds confirm the antiprotozoal activities of crude extract. Unfortunately, the extract and the tested compounds may reflect general toxicity, as shown by their low ic_{50} recorded against the mammalian cell line.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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