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DIOSPYRONE, A NEW COUMARINYLBINAPHTHOQUINONE FROM *DIOSPYROS CANALICULATA* (EBENACEAE): STRUCTURE AND ANTIMICROBIAL ACTIVITY

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ABSTRACT. A new binaphthoquinone bearing two 4-hydroxy-5-methylcoumarin-3-yl units has been isolated from the stem bark of *Diospyros canaliculata* De Wildeman in addition to five known compounds: lupenone, betulinic acid, gerberinol, plumbagin and canaliculatin. Their structures were established on the basis of 1D (^{1}H, ^{13}C and DEPT) and 2D (^{1}H-^{1}H COSY, HMQC and HMBC) NMR experiments. Some of the above compounds showed a significant antimicrobial activity against bacteria and yeasts.

KEY WORDS: Diospyrone, *Diospyros canaliculata*, Ebenaceae, Bacteria, Yeasts

INTRODUCTION

The genus *Diospyros* (Syn. *Persimmon*) with more than 350 species is the most important of the family Ebenaceae. About 130 species have so far been studied and compounds such as terpenoids, coumarins and naphthoquinones have been isolated [1]. *Diospyros canaliculata* is a tree found throughout the forest zone of West and Central Africa [2]. A bark extract is poisonous and blistering, causing gangrene round the wound, and is a common ingredient in Guere (Ivory Coast) arrow poisons [3]. The purification of the stem bark extracts of *D. canaliculata* furnished the known compounds lupenone (**1**), betulinic acid (**2**), gerberinol (**3**), plumbagin (**4**), canaliculatin (**5**) and a new disubstituted binaphthoquinone named diospyrone (**6**). We report herein the structure elucidation of the above compounds as well as their antimicrobial activities.

RESULTS AND DISCUSSION

The methanol extract of the stem bark of *Diospyros canaliculata* De Wildeman afforded, after repeated chromatographic techniques, five compounds among which two triterpenes **1** and **2**, two naphthoquinone derivatives **4** and **5**, the coumarin **3** and a new disubstituted binaphthoquinone **6**.

Compounds **1-5** have been isolated and identified from their spectroscopic data as well as from Co-TLC with authentic samples available in our laboratory as lupenone, betulinic acid, gerberinol, plumbagin and canaliculatin, respectively. Their spectral data are available for consultation. The above compounds were previously isolated from many other *Diospyros* species such as *D. maritime* [4], *D. kaki* [5], *D. crassiflora*, *D. monbuttensis* and *D. mollis* [1]. It

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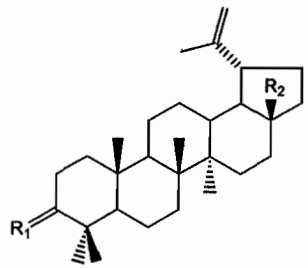
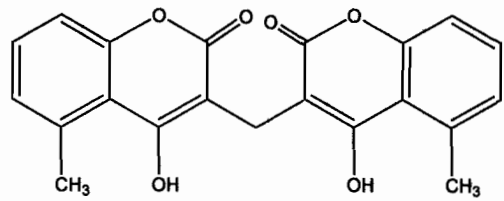
is noteworthy that, plumbagin (**4**) and canaliculatin (**5**) have been previosly isolated from *D. Canaliculata* by Waterman *et al.* [6, 7].



1 $R_1 = O$ $R_2 = CH_3$
2 $R_1 = \beta$-OH $R_2 = COOH$



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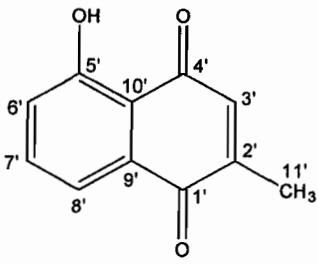

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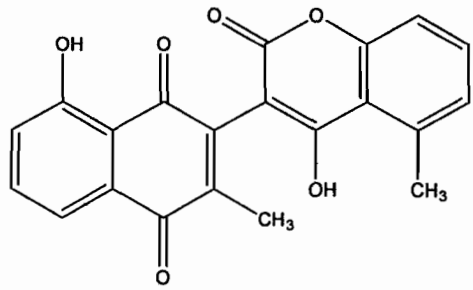

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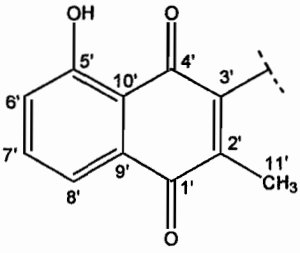
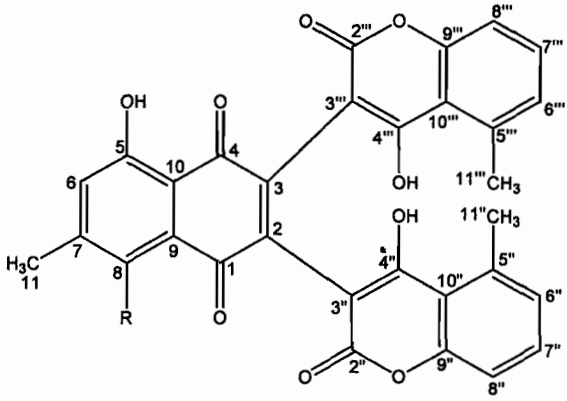


3'-plumbaginyl



6 R = 3'-plumbaginyl
7 R = H

Compound **6** was obtained as an amorphous yellow powder in methanol and was sparingly soluble in chloroform and acetone. The UV spectrum of **6** showed absorption maxima at λ_{max} 213, 283, 341, 390 and 411 nm (log ε 2.32, 2.45, 2.53, 2.59, 2.61, respectively) typical of an 4-oxycoumarin and a juglone derivatives [2, 8]. The IR spectrum showed absorptions at ν_{max} 2858-3401 (OH), 1555-1660 (broard, olefinic and conjugated carbonyl functions) cm^{-1}. The

HR-FABMS (positive mode) exhibited an $[M+H]^+$ ion peak at *m/z* 723 in keeping with the molecular formula $C_{42}H_{26}O_{12}$ containing thirty degrees of unsaturations and suggested a strongly conjugated electronic system in **6**.

Table 1. ^{13}C NMR chemical shifts of compound **4** and **6** in $CDCl_3$ and D_3C-CO-CD_3.

Atoms N°	6			4		
	δ_C (ppm)		DEPT	δ_C (ppm)		DEPT
	$CDCl_3$	D_3C-CO-CD_3		$CDCl_3$	D_3C-CO-CD_3	
1	182.1	183.9	C			
1'	184.2	184.5	C	184.6	185.2	C
2	145.2*	146.5*	C			
2'	146.3*	147.7*	C	149.6	150.7	C
3	143.9*	144.3*	C			
3'	143.8*	143.9*	C	135.4	136.0	CH
4	187.2	189.3	C			
4'	188.8	190.6	C	190.2	191.5	C
5	162.0	162.8	C			
5'	161.6	162.2	C	161.2	161.9	C
6	125.8	125.3	CH			
6'	124.1	124.4	CH	124.1	124.5	CH
7	147.3	147.9	C			
7'	136.3	137.4	CH	136.0	137.2	CH
8	127.5	128.2	C			
8'	119.3	119.6	CH	119.2	119.5	CH
9	130.5	131.8	C			
9'	132.5	132.9	C	132.1	133.2	C
10	114.5	115.3	C			
10'	115.0	115.4	C	115.2	116.0	C
11	20.7	20.6	CH_3			
11'	14.0	13.8	CH_3	16.4	16.3	CH_3
2"	165.3 or	166.4 or	C			
2"'	164.8	165.8	C			
3"	99.2 or	100.5 or	C			
3"'	98.3	99.5	C			
4"	159.1 or	161.0 or	C			
4"'	159.5	160.6	C			
5"	139.4 or	139.2 or	C			
5"'	139.1	138.9	C			
6"	128.4 or	128.6 or	CH			
6"'	128.3	128.5	CH			
7"	132.7 or	133.5 or	CH			
7"'	132.6	133.0	CH			
8"	115.3 or	116.2 or	CH			
8"'	115.1	115.6	CH			
9"	154.5 or	155.5 or	C			
9"'	154.4	155.4	C			
10"	115.2 or	115.8 or	C			
10"'	115.0	115.5	C			
11"	23.4 or	23.5 or	CH_3			
11"'	23.5	23.6	CH_3			

*The assignment of C_2 and $C_{2'}$, C_3 and $C_{3'}$ may be interchanged.

Its ^{13}C NMR spectrum in $CDCl_3$ revealed the presence of 42 carbon atoms among which six carbonyls and four sp^3 carbon atoms of the methyl groups. The 32 remaining carbon atoms

shifted in the range δ_C 99.5 to 160.6, indicating their olefinic and/or aromatic nature. Among the six carbonyls, two were those of the α,β-unsaturated lactones (δ_C 165.8 and 166.4) and four of α,β-unsaturated ketonic functions (δ_C 183.9, 184.5, 189.3 and 190.6). The 24 remaining degrees of unsaturation should be made of sp^2 aromatic, olefinic and rings systems. The DEPT experiment revealed 14 carbon atoms attached to a total of 22 hydrogen atoms: 4 methyl (δ_C 13.8, 20.6, 23.5 and 23.6) and 10 methine groups (Table 1). Thus it clearly appeared that **6** contains 22 quaternary carbon atoms among which six were linked to oxygen atoms in view of their deshielded chemical shifts at δ_C 155.4, 155.5, 161.0, 160.6, 162.2 and 162.8. The presence of the above two lactone functions in **6** also indicated that among the six oxygenated quaternary carbon atoms, four were linked to the hydroxyl groups and then, justified the presence of 26 hydrogen atoms.

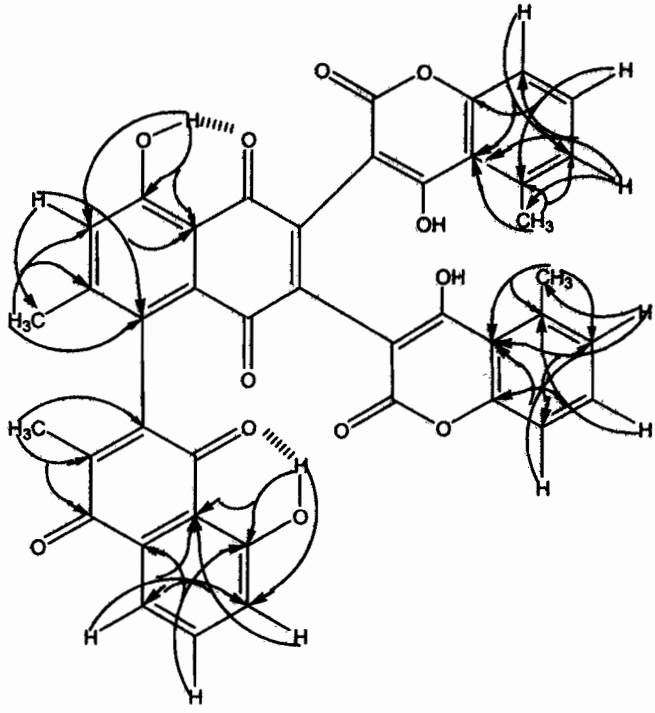
Interpretation of ^1H and ^{13}C NMR spectral data using 2D experiments (^1H-^1H COSY, HMQC and HMBC) led to a total assignment of signals of **6**. The analysis of ^1H NMR spectral data of **6** through ^1H-^1H COSY connectivities revealed the presence of ten aromatic proton signals which were distributed in four system units: three 1,2,3-trisubstituted benzene units and an isolated proton system. The three 1,2,3-trisubstituted benzene units were further shown to be made of two 5-methyl-4-oxycoumarinyl and a juglonyl moieties and the fourth one could be identified as a 7-methyljuglonyl unit. A deep analysis of the different signal systems led to their total characterisation. In fact, the signal at δ_H 7.16 (H-6") which appeared as a doublet (J = 8 Hz) due to the coupling with H-7" at δ_H 7.48 (t, J = 8 Hz) and linked to the carbon atom at δ_C 128.6 (C-6"). From the HMBC experiment, H-6" further showed three ^3J correlations with δ_C 23.5 (CH_3-11"), 115.8 (C-10") and 115.2 (C-8") (Scheme 1). Also, the H-8" signal at δ_H 7.10 (d, J = 8 Hz) showed two ^3J correlations with C-6" and C-10".

Table 2. ^1H-NMR signals for compound **4** and **6** in $CDCl_3$ and D_3C-CO-CD_3.

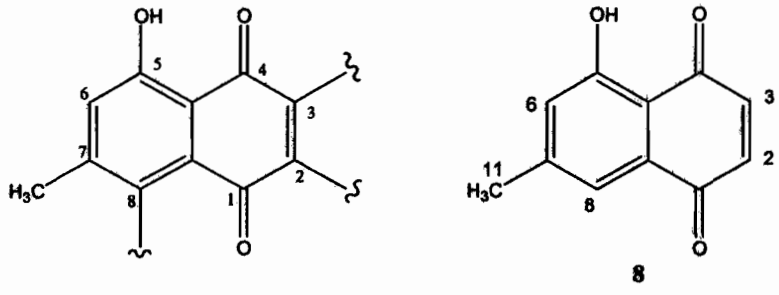
Atoms	**6**		**4**	
N°	$CDCl_3$	D_3C-CO-CD_3	$CDCl_3$	D_3C-CO-CD_3
6	7.33 s	7.45 s		
11	2.23 s	2.34 s		
5-OH	12.29 s	12.50 s		
3'			6.75 (q, J = 2 Hz)	6.94 (q, J = 2 Hz)
6'	7.24 (dd, J = 2 and 8 Hz)	7.30 (dd, J = 1 and 8 Hz)	7.19 (dd, J = 2 and 8 Hz)	7.30 (dd, J = 1 and 8 Hz)
7'	7.60 (t, J = 8 Hz)	7.76 (t, J = 8 Hz)	7.55 m	7.75 (t, J = 8 Hz)
8'	7.68 (dd, J = 2 and 8 Hz)	7.66 (dd, J = 1 and 8 Hz)	7.55 m	7.60 (dd, J = 1 and 8 Hz)
11'	1.88 s	1.85 s	2.15 (d, J = 2 Hz)	2.19 (d, J = 2 Hz)
5'-OH	11.85 s	11.95 s	11.90 s	12.00 s
6"	7.11 br s or	7.16 (d, J = 8 Hz) or		
6"'	7.09 br s	7.05 (d, J = 8 Hz)		
7"	7.44 (t, J = 8 Hz) or	7.48 (t, J = 8 Hz) or		
7"'	7.37 (t, J = 8 Hz)	7.39 (t, J = 8 Hz)		
8"	7.04 (d, J = 8 Hz) or	7.10 (d, J = 8 Hz) or		
8"'	7.10 (d, J = 8 Hz)	7.01 (d, J = 8 Hz)		
11"	2.75 s or	2.78 s or		
11"'	2.67 s	2.64 s		

The above signals constituted the first 1,2,3-trisubstituted benzene ring system of the two 5-methyl-4-oxycoumarin units. The two remaining three adjacent aromatic proton systems were : δ_H 7.05 (d, J = 8Hz, H-6"'), 7.01 (d, J = 8 Hz, H-8"'), 7.39 (t, J = 8 Hz, H-7"') and δ_H 7.30 (dd, J = 2 and 8 Hz, H-6'), 7.76 (t, J = 8 Hz, H-7'), 7.66 (dd, J = 2 and 8 Hz, H-8') and could be

respectively assigned to the other 5-methyl-4-oxycoumarinyl and the juglonyl units. Observed data (Tables 1 and 2) of the juglonyl unit were similar to those of plumbagin (**4**) [1, 9, 10] in which H-3' proton is absent. Further inspection of the ^{1}H NMR spectrum of **6** revealed the presence of one aromatic singlet proton at δ_H 7.45 from ^{1}H-^{1}H COSY experiment and showed three ^{3}J correlations with carbon atoms at δ_C 20.6 (C-11), 115.3 (C-10) and 128.2 (C-8) from the experiment HMBC (Scheme 1). The isolated proton belonged to the fourth proton system which exhibited data very similar to those of 7-methyl juglone (**8**) [1, 10]. Therefore, the presence of one proton (H-6) instead of four in **8** corroborated the absence of H-2, H-3 and H-8 protons (see Scheme 2) which consequently were substituted by two 5-methyl-4-oxycoumarinyl and the plumbaginyl units as described above.



Scheme 1. HMBC correlations of compound **6**.



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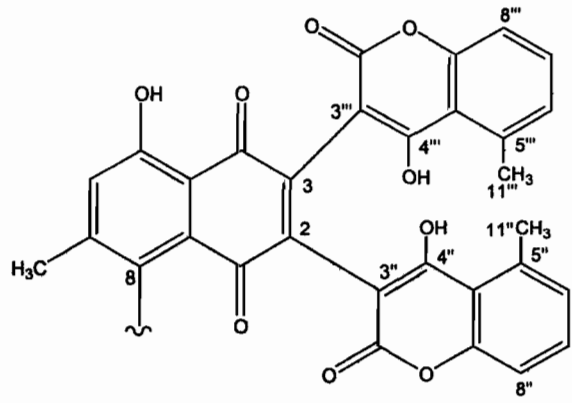
Scheme 2. 2, 3 and 8-substituted positions on unit **8** in compound **6**.



Scheme 3. 8-substituted position on unit 7 in compound **6**.

According to the remaining C-8 substituted position, the absence of H-3' proton on the plumbaginyl unit confirmed the C-3'-C-8 linkage (Scheme 3). In addition, the works of Terence and co-workers on juglone derivatives corroborated the presence of an arene-quinone bond as it is found in several binaphthoquinones [11] and clearly justified the C-3'-C-8 linkage in compound **6**. The HR-FABMS fragmentation pattern was in good agreement with the structure **6**. At *m/z* 176 an ion peak attributed to a 5-methyl-4-oxycoumarinyl fragment was observed. Also, another important ion peak at *m/z* 383 was seen which was a typical internal cleavage of quinonoid and coumarol rings [12, 13].

In view of the above evidence, the structure of **6** was found to be a coumarinylbinaphthoquinone and named diospyrone, which to our knowledge has not yet been described in the literature.

Compound **4** and **6** showed a strong and large spectrum antimicrobial activity (Table 3). The strongest antifungal activities with MIC lower than that of the reference antibiotics were observed with compound **4** against *Candida krusei* and compound **6** against *C. albicans* and *C. krusei* whereas the most important antibacterial effect exhibited by **4** was noted on *Proteus vulgaris*, and that of **6** was observed on *Salmonella typhi* and *Staphylococcus aureus* with their antimicrobial activity greater than that of gentamycin.

Table 3. Minimal inhibition concentration* (MIC) as determinined for two compounds isolated from *Diospyros canaliculata*, gentamycin and nystatin. *

Tested microrganisms	MIC (µg/mL) of the compounds		
	4	**6**	GM/N**
Bacteria			
Escherichia coli	78.12	19.53	10
Klebsiella pneumoniae	156.25	78.12	10
Proteus vulgaris	9.76	312.5	5
Salmonella typhi	39.06	4.88	5
Staphylococcus aureus	19.53	9.76	10
Streptococcus faecalis	39.06	19.53	10
Yeasts			
Candida albicans	39.06	4.88	30
Candida krusei	19.53	4.88	30

*Results of the MIC recorded as mean of triplicated experiments. **GM: gentamycin; N: nystatin.

EXPERIMENTAL

General. ^{1}H NMR spectra were recorded at 300 and 500 MHz in $CDCl_3$ and 400 MHz in acetone-d_6, ^{13}C NMR spectrum were measured at 75 and 125 MHz in $CDCl_3$ and 100 MHz in acetone-d_6. Chemical shifts were given on a δ_H (ppm) scale with tetramethylsilane as an internal standard; melting points (°C) were uncorrected. Thin layer chromatography (TLC) was performed on a precoated Kieselgel 60 F_{254} plates (Merck) and column chromatography (CC) was carried out with silica gel 60 particle size 0.063-0.200 mm (70-230 Mesh ASTM).

Plant material. The stem bark of *Diospyros canaliculata* De Wildeman was collected from *Mount Fébé* in the Central Province of Cameroon in May 1998. A Voucher Specimen (9653 SRFCam) is deposited at the Cameroon National Herbarium, Yaoundé.

Extraction, isolation and characterization. The dried and chipped stem bark (1.5 kg) of *Diospyros canaliculata* was macerated in methanol for three days. The methanol extract was evaporated to dryness and the residue (300 g) was fractionated with hexane and ethyl acetate to give respectively 25 g and 60 g of extracts. The hexane extract (20 g) was chromatographed on a silica gel column eluted with hexane-ethyl acetate in increasing polarity and the fractions were further purified by column chromatography to yield the following compounds : **4** (50 mg), **1** (10 mg) and **3** (25 mg). The ethyl acetate extract (50 g) was chromatographed with hexane-ethyl acetate gradient and the fractions were repurified by column chromatography to yield compounds **2** (75 mg), **5** (10 mg) and **6** (35 mg).

Gerberinol (**3**) white crystals; m.p. 266-267 °C. UV (λ_{max} nm, log ε): (254, 3.98). EIMS (rel. int. %) *m/z* 364 $[M]^+$ (100), 134 $[C_8H_6O_2]^+$ (96), 176 $[C_{10}H_8O_3]^+$ (97), 188 $[C_{11}H_8O_3]^+$ (98.4). ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.10 (2H, d, J = 8 Hz, H-6); 7.39 (2H, t, J = 8 Hz, H-7); 7.21 (2H, d, J = 8 Hz, H-8); 3.78 (2H, s, CH_2); 2.79 (6H, s, $2CH_3$) and 11.81 (2H, s, 2 OH). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 168.4 (C-2); 102.8 (C-3); 167.6 (C-4); 138.5 (C-5); 128.2 (C-6); 131.6 (C-7); 115.1 (C-8); 153.6 (C-9); 115.3 (C-10); 23.3 (CH_3); 20.1 (CH_2).

Plumbagin (**4**) orange crystals; m.p. 75-76 °C. UV (λ_{max} nm, log ε): (254, 3.98). EIMS (rel. int. %) *m/z* 188 $[M]^+$ (100), 173 (59.72) $[M\text{-}CH_3]^+$, 131 (63.58) $[M\text{-}C_3H_5O]^+$, 120 (61.84) $[M\text{-}C_4H_4O]^+$, 92 (62.86) $[M\text{-}C_5H_4O_2]^+$. ^{1}H NMR (300 MHz, $CDCl_3$): Table 2. ^{13}C NMR (75 MHz, $CDCl_3$): Table 1.

Canaliculatin (**5**) yellow solid crystals; m.p. > 300 °C. UV (λ_{max} nm, log ε): (254, 3.98). EIMS, (rel. int. %) m/z 362 $[M]^+$ (92.98) , 135 (100). ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.36 (dd, J = 2 and 8 Hz, H-6); 7.77 (t, J = 8 Hz, H-7); 7.61 (dd, J = 2 and 8 Hz, H-8); 7.17 (d, J = 8 Hz, H-6'); 7.53 (t, J = 8 Hz, H-7'); 7.27 (d, J = 8 Hz, H-8'); 2.01 (s, CH_3-11); 2.71 (s, CH_3-11') and 11.83 (s, 5'-OH). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 183.7 (C-1), 149.0 (C-2) 137.6 (C-3), 187.6 (C-4), 159.8 (C-5), 123.7 (C-6), 136.4 (C-7), 118.5 (C-8), 131.9 (C-9), 114.7 (C-10), 14.0 (CH_3-11), 164.3 (C-2'), 97.7 (C-3'), 160.0 (C-4'), 138.0 (C-5'), 127.6 (C-6'), 131.8 (C-7'), 114.2 (C-8'), 154.1 (C-9'), 114.9 (C-10'), 23.1 (CH_3-11').

Diospyrone (**6**). Amorphous yellow powder, UV (λ_{max} nm, log ε): (213, 2.32); (283, 2.45); (341, 2.53); (390, 2.59) and (411, 2.61). IR ν_{max} cm^{-1}: 2858-3401(OH), 1555-1660 (broad, olefinic and carbonyl functions). HR-FABMS *m/z* (rel. int. %) 723 (70) $[M+H]^+$, 176 (58), 383 (100); ^{1}H NMR (400 MHz in acetone-d_6 and 500 MHz in $CDCl_3$): Table 2. ^{13}C NMR (100 MHz in acetone-d_6 and 125 MHz in $CDCl_3$): Table 1.

Antimicrobial assay

The antimicrobial activity of compounds **4** and **6** was studied using the microdilution assay on a total of 8 microbial cultures belonging to six aerobic bacterial species (*Escherichia coli* LMP0101U, *Staphylococcus aureus* LMP0206U, *Proteus vulgaris* LMP0103, *Klebsiella pneumoniae* LMP0210U, *Streptococcus faecalis* LMP0207U, and *Salmonella typhi* LMP0209U) and two *Candida* species (*Candida albicans* LMP0204U and *Candida krusei* LMP0311U). These strains were clinically isolated from the urogenital discharges of patients in the *Centre Pasteur du Cameroun* health institution and monitored in the Laboratory of the Applied Microbiology and Molecular Pharmacology (LMP) of the University of Yaoundé I. The strains were activated at 37 °C for 24 hours on nutrient agar (NA), sabouraud glucose agar (SGA) (fungi).

The antimicrobial activity was evaluated on the basis of the minimal inhibition concentration (MIC). The inocula of microorganisms were prepared from 12 h broth culture and the suspensions were adjusted to 0.5 Mc Farland turbidity. The tested compounds (**4** and **6**) were first dissolved in dimethylsulfoxide (DMSO) 10% to the highest dilution (625 µg/mL) and serial twofold dilutions were made in a concentration ranging from 2.44 µg/mL to 625 µg/mL in the 96 wells microplate containing nutrient broth. MIC values of the tested compounds against the above pathogens were determined according to the microdilution method [14]. Gentamycin (bacteria) and nystatin (yeasts) diluted prior in water were used as reference antibiotics. The results are presented in Table 3.

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