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Original Research Article

Chemical constituents from the leaves and liana of *Salacia nitida* (Benth.) N.E.Br. (Celastraceae) and their antimicrobial activities

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ABSTRACT

One 4'-hydroxy-2,4,6-trimethoxybenzophenone (**1**) was isolated from the liana and leaves of *Salacia nitida* (Benth.) N.E.Br., together with *n*-hexacosane (**2**), 29-hydroxyfriedelane (**3**), 3β-friedelinol (**4**), *n*-hexacosan-1-ol (**5**), *n*-octacosan-1-ol (**6**), mangiferin (**7**), *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**8**), friedelin (**9**), 30-hydroxyfriedelin (**10**), salaspermic acid (**11**), 22*β*-*epi*-maytenfolic acid (**12**), orthosphenic acid (**13**), maltose (**14**), D-mannitol (**15**), cangoronine (**16**), 7-hydroxyfriedelane-1,3-dione (**17**), tingenone (**18**), pristimerin (**19**), α-amyrin acetate (**20**), β-sitosterol (**21**), stigmasterol (**22**), 21-hydroxyfriedelan-3-one (**23**), abruslactone A (**24**) and 2α-hydroxypopulnonic acid (**25**). The structures of the isolated compounds were established by means of spectroscopic analysis. In addition, the structure of (**1**) was confirmed by its X-ray diffraction. Compounds (**1**), (**7**), (**10**)-(**11**), (**13**), (**16**)-(**19**) and (**25**) were evaluated for their antimicrobial activities. Compound (**18**) showed a significant activity against *Staphylococcus aureus* (MIC=23.8 μM), while compounds (**11**) and (**19**) exhibited moderate inhibiting effect against *Staphylococcus aureus* (MIC=53.8 μM) and *Candida glabrata* (MIC=105.9 μM), respectively.

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1. Introduction

Plants have been the basic source of sophisticated traditional medicine systems for thousands of years and have been instrumental to early pharmaceutical drug discovery and industry (Elujoba et al., 2005). The significance of traditional medicine has gained vital importance worldwide and its practices are continuing because of its biomedical benefits as well as the cultural belief of some populations in many parts of the world (Ganesan and Xu, 2017). In collaboration with the traditional medicine practitioners and local indigenous people, an appreciable level of studies has been done on medicinal plants. These studies included the ethnobotanical surveys and the extraction of active ingredients in plants (Dikaso et al., 2006; Bankeu et al., 2017; Mohammadhosseini et al., 2017). *Salacia* L. species (Family: Celastraceae), are widely distributed in tropical regions and have been used for thousands of years in traditional medicine for the treatment of several ailments including malaria, rheumatism, asthma, fever, menorrhagia, diabetes and skin diseases (Warrier et al., 1994). *S. nitida* (Benth.) N.E.Br. is a woody climber of which two varieties have been distinguished: *S. nitida* (Benth.) N.E.Br. var. *nitida* and *S. nitida* var. *bipindensis* (Loes.) Hallé (Hallé, 1990). *S. nitida* (Benth.) N.E.Br. is a

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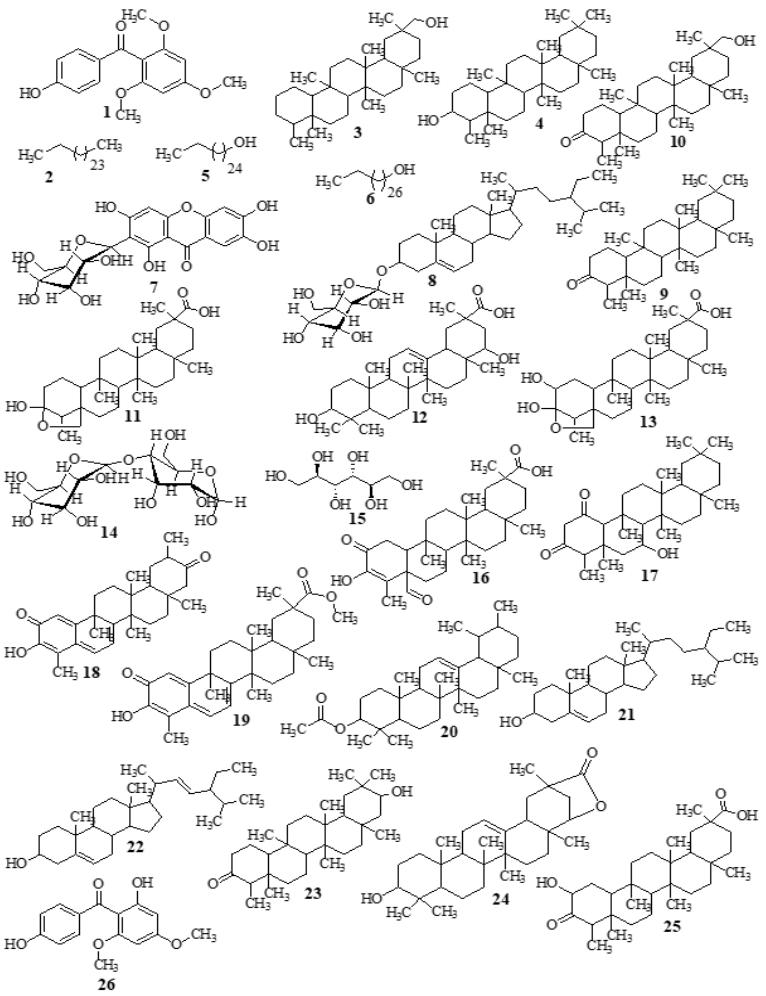


Fig. 1. Chemical structures of compounds (1)-(26).

liana of 3-30 m long and 6 cm diameter widely distributed in Cameroon, Congo, Gabon, Liberia, Ivory Coast, Sierra Leone, Nigeria, Ghana and Democratic Republic of Congo rained forests (Hallé, 1990). It is also distributed in Sri Lanka, South-West India, Thailand, Philippines, Java and South Africa (Dooka and Ezejiofor, 2017). The roots of *S. nitida* are used in the South Eastern part of Nigeria for the treatment of malaria and typhoid fever (Ogbonna et al., 2008; Nwiloh et al., 2016). Previous phytochemical investigations of *Salacia* L. species resulted in the isolation of a wide range of secondary metabolites such as anthocyanidins, catechins, phenolic acids, quinones, triterpenoids, gutta-percha, xanthones, stilbenes, eudesmane-type sesquiterpenes and megastigmane glycosides (Kawazoe et al., 1997; Kishi et al., 2003; Carvalho et al., 2005; Mba'ning et al., 2011). The phytochemical screening of the ethanolic extract of root bark of *S. nitida* (Benth.) N.E.Br. revealed the presence of alkaloids, tannins, saponins, phenols, anthocyanins and flavonoids (Nwiloh et al., 2016). The pharmacological study of its roots and leaves demonstrated its antiplasmodial (Ogbonna et al., 2008; Nwiloh et al., 2019), antidiabetic (Dooka and Ezejiofor, 2017; Zawua and Kagbo, 2018) and cytoprotective (Dooka and Ezejiofor, 2017) properties.

Even though a preliminary phytochemical screening was carried out on the root bark of *S. nitida* (Benth.) N.E.Br., no attempt has been made so far to isolate compounds from any part of this plant. Therefore, the purpose of the present study was to isolate and characterize its compounds. We report herein on the isolation and structural elucidation of a benzophenone, 4′-hydroxy-2,4,6-trimethoxybenzophenone (**1**), alongside with twenty-four known compounds (Fig. 1) from the CH_2Cl_2-MeOH (1:1 v/v) extracts of the leaves and liana of *S. nitida* (Benth.) N.E.Br. To the best of our knowledge, the isolation of a benzophenone is reported here for the first time from the genus *Salacia*. Compound (**1**) is also reported here for the first time from a natural source.

2. Experimental

2.1. General experimental procedures

Melting point was measured on a Gallenkamp Melting Point Apparatus. IR spectrum was recorded on an IR Prestige-21 Fourier Transform IR spectrometer (Shimadzu). A single crystal was examined on a Rigaku Supernova diffractometer using CuKα (λ=1.54184 Å) radiation. ^{1}H and ^{13}C NMR spectra were recorded on

a Bruker Ultrashield spectrometer operating at 400 MHz (^{1}H) and 100 MHz (^{13}C), respectively; with TMS as internal standard. Chemical shifts are reported as δ values. Probes were dissolved in 0.5 mL $CDCl_3$, Acetone-*d6* or DMSO-*d6*. HR-ESIMS were recorded on a Bruker Compact Q-TOF mass spectrometer equipped with Dionex UltiMate 3000 UHPLC and electrospray ionization (ESI). For the direct infusion MS, the spectrometer was operated in positive and negative modes (mass range: 100-1500, with a scan rate of 1.00 Hz) with automatic gain control to provide high-accuracy mass measurements within 1 ppm deviation using Na formate as calibrant. The spray voltage was 4.5 kV with a capillary temperature of 200 ºC. The flow rate of sample was 180 µL/h and nitrogen was used as sheath gas (4 L/min). Silica gel 230-400 mesh (Merck), silica gel 70-230 mesh (Merck) and Sephadex LH-20 were used for column chromatography, while pre-coated aluminum silica gel 60 F_{254} sheets were used for TLC with different mixtures of *n*-hexane-ethyl acetate, and dichloromethane-methanol solvent systems as eluents. Spots were visualized with UV light (254 and 365 nm) or using vanillin reagent (1 g of vanillin in 70 mL ethanol 96% + 10 mL conc. sulfuric acid). Medium pressure purifications were performed using a CombiFlash apparatus (Teledyne Isco, Lincoln, NE, USA) fitted with a RP Silica column (Teledyne Isco). Ethyl acetate, n-hexane, dichloromethane and methanol were used for both column and medium pressure chromatographies. All these solvent were of analytical grade.

2.2. Plant material

S. nitida (Benth.) N.E.Br. was collected in March 2016 at the locality of Nko'o Long (25 Km along the Kribi-Ebolowa road, 2º56'14N and 9º54'27E, 18 m altitude) in the South region of Cameroon and identified by Mr. Nana Victor, botanist at the National Herbarium where a voucher specimen has been deposited (Nº 43647/HNC).

2.3. Extraction and purification

The plant material was chopped, air-dried and powdered. Powders of the stem (2.7 kg) and leaves (4.9 kg) were extracted at room temperature with a mixture of dichloromethane-methanol (1:1 v/v) (10 L and 15 L, respectively, 72 h each repeated two times). Solvents were evaporated under reduced pressure and yielded 116.5 g and 115.8 g of extracts, respectively.

The leaves extract was partitioned with n-hexane and methanol (1:1 v/v) at room temperature to afford 60.3 g of a methanol-soluble residue. A portion of 50 g of this extract was fractionated by column chromatography over silica gel (230-400 mesh, Merck, 600 g), eluting with mixtures of *n*-hexane-ethyl acetate (85:15, 75:25, 6:4, 4:6, 2:8 and 1:0 v/v) and ethyl acetate-methanol (9:1, 75:25, 5:5 and 1:0 v/v) solvent systems. 215 fractions (300 mL of each) were collected and combined on the basis of TLC profiles into five main fractions, F1-F5. Fraction F1 (9.7 g) was subjected to column chromatography over silica gel (70-230 mesh, 291 g), eluting with a mixture of *n*-hexane-ethyl acetate (1:0, 95:5, 9:1, 85:15 and 8:2 v/v) to yield (**2**) (3.4 mg), (**3**) (4.8 mg), (**4**) (2.1 mg) and (**5**) (6.1 mg). The column chromatography of fraction F2 (7.4 g) over silica gel (222 g) with a mixture of *n*-hexane-ethyl acetate (9:1, 85:15, 8:2, 75:25 and 7:3 v/v) afforded (**6**) (3.2 mg). Fraction F3 (6.1 g) eluted with the mixture of *n*-hexane-ethyl acetate (7:3, 6:4, 5:5, 4:6, 3;7 and 25:75 v/v) in a column fitted with silica gel (183 g) afforded (**1**) (3.5 g). Fraction F4 (8.3 g), after a silica gel (249 g) column elution with mixtures of *n*-hexane-ethyl acetate (5:5, 35:65, 25:75, 1:9 and 0:1 v/v) and ethyl acetate-methanol (95:5, 9:1 and 85:15 v/v) afforded (**7**) (2.7 g) and (**8**) (2.2 mg). Fraction F5, a dark gum, afforded no compound.

The extract of the liana (112.7 g), was also subjected to a column chromatography over silica gel (1350 g) and eluted with mixtures of *n*-hexane-ethyl acetate (1:0, 95:5, 9:1, 85:15, 8:2, 75:25, 65:45, 5:5, 4:6, 2:8 and 1:0 v/v) and ethyl acetate-methanol (9:1, 8:2, 7:3, 5:5, 4:6 and 1:0 v/v). 116 fractions of 300 mL each were collected. Compound (**9**) (102.5 mg) was obtained from fractions 9 to 14 (Fr 9-14) while eluting with *n*-hexane-ethyl acetate (85:15). Compound (**10**) (4.3 mg) crystallized from Fr 27 and 28 with *n*-hexane-ethyl acetate (75:25) as eluent. The elution of the combined Fr 49-51 and Fr 55-56 (3.1 g sample mixture and 90 g silica) with the mixture of *n*-hexane-ethyl acetate (4:6) afforded (**11**) (35.1 mg) and (**12**) (2.5 mg), respectively. Compound (**13**) (61.5 mg) crystallized from Fr 65-67 during elution with the mixture of *n*-hexane-ethyl acetate (25:75). The major component (**7**) (381.3. mg) crystallized during extraction and was also obtained from Fr 85-92 using ethyl acetate-methanol (9:1) as solvent. Compounds (**14**) (14.2 mg) and (**15**) (192.9 mg) were isolated from Fr 93-102 and Fr 103-107, respectively, with eluting solvents ethyl acetate-methanol (7:3) and ethyl acetate-methanol (5:5), respectively. Fr 31-40 combined (1.2 g) and purified by medium pressure liquid chromatography CombiFlash fitted to a RP silica column (30 g, 26.4 mL) with the eluting systems *n*-hexane-ethyl acetate (75:25) for 5 min and (65:35) for 20 min at the flow rate of 20 mL/min afforded (**16**) (15.6 mg). Derived fractions were purified by Sephadex LH-20 using methanol as solvent to afford (**17**) (13.4 mg) and (**18**) (11.1 mg). The medium pressure liquid chromatography of Fr 20-26 (2.3 g), as previously described, yielded (**19**) (276.1 mg), (**20**) (31.7 mg), (**21**) and (**22**) (2.0 mg), and (**23**) (2.6 mg). Using the same technique, Fr 27-30 (1.9 g) yielded (**24**) (15.1 mg). Fractions Fr 47-62 (6.3 g) were subjected to a CombiFlash chromatography system, fitted to a RP Silica column (30 g, 26.4 mL). The gradient of *n*-hexane-ethyl acetate (9:1 for 15 min, 8:2 for 10 min, 7:3 for 10 min, 6:4 for 10 min, 5:5 for 5 min and 0:1 for 5 min) was

Table 1
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of compounds **1** (in Acetone-*d6*) and **26** (in $CDCl_3$).

Position	1		26	
	δ ^{1}H (m, *J* in Hz)	δ ^{13}C	δ ^{1}H (m, *J* in Hz)	δ ^{13}C
C=O	-	192.7	-	197.5
1	-	112.4	-	105.8
2	-	159.3	-	165.2
3	6.33 (s)	91.7	6.18 (d, 2.4)	93.7
4	-	162.6	-	165.9
5	6.33 (s)	91.7	5.98 (d, 2.4)	91.4
6	-	159.3	-	161.6
1′	-	131.9	-	133.9
2′	7.67 (d, 8.4)	132.4	7.55 (d, 8.4)	114.1
3′	6.88 (d, 8.4)	115.9	6.83 (d, 8.4)	131.1
4′	-	163.1	-	158.7
5′	6.88 (d, 8.4)	115.9	6.83 (d, 8.4)	131.1
6′	7.67 (d, 8.4)	132.4	7.55 (d, 8.4)	114.4
2-OCH_3	3.69 (s)	56.1	-	-
4-OCH_3	3.89 (s)	55.8	3.87 (s)	55.6
6-OCH_3	3.69 (s)	56.1	3.54 (s)	55.2
4′-OH	9.10 (s)	-	-	-

applied with the flow rate of 20 mL/min. This process afforded compounds (**1**) (18.1 mg), and (**25**) (70.6 mg).

2.4. Spectroscopic data of compound (**1**)

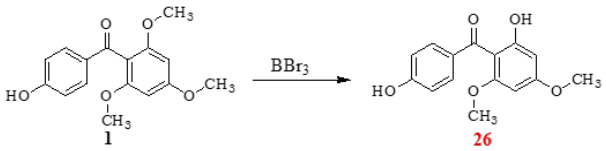
Colorless crystals; mp 162-163 °C; IR (KBr) ν_{max} 3447 cm^{-1} (OH), 1651 cm^{-1} (C=O) and 1513-1617 cm^{-1} (aromatic ring); ^{1}H NMR (Acetone-*d6*, 400 MHz) and ^{13}C NMR (Acetone-*d6*, 100 MHz) see Table 1; HR-ESIMS: $[M+H]^+$, *m/z* 289.1158 (calcd 289.1071 for $C_{16}H_{17}O_5$).

2.5. X-ray structure determination

A single crystal of compound (**1**) (Fig. 2) was obtained and examined. The crystal was kept at 100.01(10) K during data collection. Using Olex2 (Dolomanov et al., 2009), the structure was solved with the ShelXD (Sheldrick, 2008) structure solution program using Dual Space and refined with the ShelXL (Sheldrick, 2015) refinement package using Least Squares minimization. Crystal data (Table 2) for (**1**): empirical formula $C_{16}H_{16}O_5$ (*M*=288.29 g/mol), monoclinic, space group $P2_1/n$ (no. 14), *a*=14.3272(5) Å, *b*=8.0795(3) Å, *c*=24.9909(13) Å, *β*=104.342(5), *V*=2802.7(2) Å^3, *Z*=8, *T*=100.01(10) K, μ(CuKα)=0.847 mm^{-1}, *Dcalc*=1.366 g/cm^3, 10736 reflections measured (6.508≤2Θ≤144.21), 5462 unique (R_{int}=0.0268, R_{sigma}=0.0352) which were used in all calculations. The final R_1 was 0.0509 for 4545 reflections with *I*>2σ(*I*) and wR_2 was 0.1301 for all data. Crystallographic data reported in this paper have been deposited with the Cambridge Crystallographic Data Centre number CCDC 1850044.

2.6. Demethylation reaction of (**1**)

A solution of boron tribromide (0.8855 mmol) in dichloromethane (1:10 v/v) was slowly added to a cooled (-78 °C) solution of (**1**) [102 mg (0.3542 mmol) in 9.8 mL CH_2Cl_2 and 0.2 mL MeOH] under argon.



The cooling bath was removed and the resulting dark colored solution was slowly warmed up to room temperature and stirred for 48 h. The dark colored suspension was poured into icy water and filtered to remove the dark colored solid. The aqueous layer was separated out and extracted with chloroform twice. The combined organic extracts were dried with Na_2SO_4 to a mixture that was further separated (Perruchon, 2004). The separation was achieved using a medium pressure liquid chromatography CombiFlash fitted to a RP silica column (30 g, 26.4 mL) with the eluting systems *n*-hexane-ethyl acetate (9:1) for 10 min, (8:2) for 10 min and (7:3) for 10 min at a flow rate of 20 mL/min. A yellowish oily compound **26** (39.2 mg) was obtained.

2.7. Evaluation of the antimicrobial activity

Antimicrobial susceptibility of some compounds isolated in the present work were evaluated against important yeasts and bacteria pathogens: *Candida albicans* ATCC 64548, *Candida glabrata* ATCC 90030, *Candida parapsilosis* ATCC 22019, *Criptococcus gatti* ATCC 32269, *Paracoccidioides brasiliensis* Pb18, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 14502, *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecium* VRE16. *P. brasiliensis* Pb18 was isolated from mice as described by Castilho et al. (2014) and *E. faecium* VRE16 was an ST412 strain isolated from a perianal swab collected from a male patient as described by De Mello et al. (2016). All other microorganisms are of the American Type of Culture Collection. Minimal inhibitory concentration (MIC) was performed using the reference method for broth dilution recommended by the Clinical and Laboratory Standards Institute (CLSI, 2008; CLSI, 2015). Experiments were performed in 96-well plates using Sabouraud broth for yeasts, and Mueller Hinton broth for bacteria. The compounds

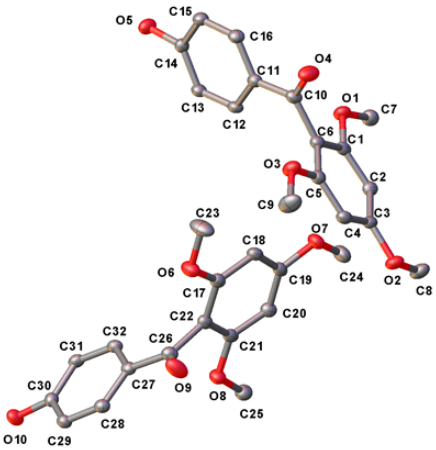


Fig. 2. ORTEP view of compound (**1**).

Table 2
Crystal data and structure refinement for compound **1**.

Empirical formula	**$C_{16}H_{16}O_5$**
Formula weight	288.29
Temperature/K	100.01(10)
Crystal system	monoclinic
Space group	$P2_1/n$
a/Å	14.3272(5)
b/Å	8.0795(3)
c/Å	24.9909(13)
α/°	90
β/°	104.342(5)
γ/°	90
Volume/Å^3	2802.7(2)
Z	8
ρ_{calc}g/cm^3	1.366
μ/mm^{-1}	0.847
F(000)	1216.0
Crystal size/mm^3	0.222 × 0.151 × 0.015
Radiation/Å	CuKα (λ = 1.54184)
2Θ range for data collection/°	6.508 to 144.21
Index ranges	-17 ≤ h ≤ 17, -7 ≤ k ≤ 9, -30 ≤ l ≤ 24
Reflections collected	10736
Independent reflections	5462 [R_{int} = 0.0268, R_{sigma} = 0.0352]
Reflections with *I* > 2σ(*I*)	4545
Data/restraints/parameters	5462/0/387
Goodness-of-fit on F^2	1.091
Final R indexes [*I* > 2σ(*I*)]	R_1 = 0.0509, wR_2 = 0.1233
Final R indexes [all data]	R_1 = 0.0627, wR_2 = 0.1301
Largest diff. peak/hole / e Å^{-3}	0.27/-0.25

were diluted at concentrations 100-fold higher than the final concentrations in 100% dimethyl sulfoxide (Mallinkrodt), followed by further dilution (1:50) in an appropriate culture medium. The concentrations of all targed compounds ranged from 1.00 to 250 µg.mL^{-1}. Each well was filled with 195 µL of serial broth dilutions containing the targed compounds and inoculated with 5 µL of a fresh culture of each organism (10^5 CFU). Plates were incubated for 48 h at 37 ºC for yeasts and for bacteria 24 h at 35 ºC. The MIC was defined as the lowest concentration that prevented any discernible growth, or at least 90% reduction in the growth relative to growth of the untreated control. Fluconazole and ampicilin were used as positive controls for yeasts and bacteria, respectively and a pure culture broth was the negative control.

3. Results and Discussion

3.1. Structure elucidation of compound (**1**)

Compound (**1**) was obtained as colorless crystals. Its molecular formula $C_{16}H_{16}O_5$, with 9 double bonds equivalence, was determined from the NMR data and its positive HR-ESIMS which showed the pseudo-molecular ion peak $[M+H]^+$ at *m/z* 289.1158 (calcd 289.1071 for $C_{16}H_{17}O_5$). The IR spectrum showed the presence of hydroxyl group (3447 cm^{-1}), carbonyl group (1651 cm^{-1}) and aromatic ring (1513-1617 cm^{-1}).

The ^{1}H NMR spectrum revealed a singlet of one hydroxyl proton at δ_H 9.10 (4′-OH), two doublets of an A_2B_2 aromatic ring pattern at δ_H 7.67 (2H, *J*=8.4 Hz, H-2′/6′) and δ_H 6.88 (2H, *J*=8.4 Hz, H-3′/5′), one singlet of two protons of a 1,2,4,6-tetrasubstituted aromatic ring at δ_H 6.33 (H-3/5) and two singlets of three methoxy groups at δ_H 3.89 (3H, 4-OMe) and δ_H 3.69 (6H, 2/6-OMe).

The ^{13}C NMR spectrum displayed signals of 16 carbons sorted in combination with the HSQC experiment into seven quaternary carbons including one carbonyl at δ_C 192.7, six aromatic methine carbons and three methoxy carbons.

The HMBC spectrum showed correlations (Fig. 3) between the hydroxyl proton at δ_H 9.10 (4′-OH) and the carbons at δ_C 115.9 (C-3′/5′) and 163.1 (C-4′); the proton of the A_2B_2 system at δ_H 7.67 (H-2′/6′) and the carbons at δ_C 115.9 (C-3′/5′), 131.9 (C-1′), 163.1 (C-4′) and 192.7 (C=O). According to these correlations, the hydroxyl group is located on a *para*-substituted benzene ring showing the A_2B_2 spin system. Other correlations were observed between the singlet of two protons at δ_H 6.33 (H-3/5) and the carbons at δ_C 112.4 (C-1), 159.3 (C-2/6), 162.6 (C-4) and 192.7 (C=O). The correlations observed between the singlet of a methoxy group at δ_H 3.89 (4-OMe) and the carbon at δ_C 162.6 (C-4); and also between the singlet of two methoxy groups at δ_H 3.69 (2/6-OMe) and the carbon at δ_C 159.3 (C-2/6) indicated that all the methoxy groups are located on the 1,2,4,6-tetrasubstituted aromatic ring. These observations were further confirmed with a demethylation reaction carried out on compound (**1**). In fact, the ^{1}H NMR of the reaction product (**26**) compared to that of compound (**1**) showed two singlets of methoxy groups at δ_H 3.54 (6-OMe) and 3.87 (4-OMe) and two doublets of one proton each at δ_H 5.98 (*J*=2.4 Hz, H-5) and 6.18 (*J*=2.4 Hz, H-3). HMBC correlations (Fig. 3) between the methoxy group at δ_H 3.54 and the carbon at δ_C 161.6 (C-6), the methoxy group at δ_H 3.87 and the carbon at δ_C 165.9 (C-4) and the protons at δ_H 5.98 and 6.18 and the carbons at δ_C 161.6 (C-6) and 165.9 (C-4) clearly showed that the two remaining methoxy groups are on the same aromatic ring. Consequently, the three methoxy groups in compound (**1**) are located on the same aromatic ring. All these findings corroborated with the X-ray analysis of this compound (Fig. 2) (see Experimental Section and Table 2). Compound (**1**) was then assigned as 4′-hydroxy-2,4,6-trimethoxybenzophenone, previously obtained as synthetic derivative (Bertil et al., 1973; Gohil et al., 2010) and reported here for the first time from a natural source. This is also the first report of characterization of benzophenones from the Salacia genus and to the best of our knowledge from the Celastraceae family. Benzophenones are precursors of xanthones. In fact, the central step in the xanthone biosynthetic pathway is the formation of the C13 skeleton, key precursors of which may be polyhydroxybenzophenones (El-seedi et al., 2010). Mangiferin, is the only xanthone already isolated from Celastraceae and is one of the chemotaxonomic marker in the roots, stems or aerial parts of *Salacia* L. species (Karunanayake and Sirimanne, 1985; Mba'ning

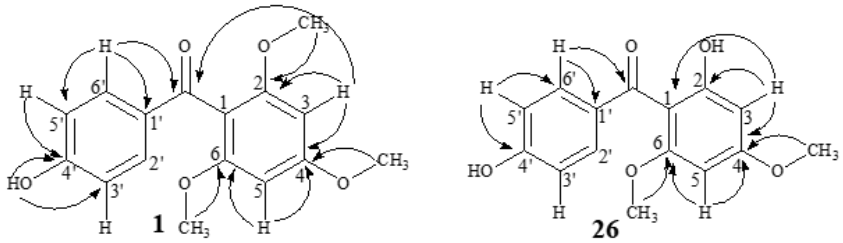


Fig. 3. Key HMBC correlations of compounds (**1**) and (**26**).

et al., 2011; Sellamuthu et al., 2012; Basu et al., 2013; Deepak et al., 2015; Singh et al., 2018). Compound (**1**) might be an intermediate compound during the biosynthesis of mangiferin.

3.2. Identification of compounds (**2-25**)

Twenty-four other isolated compounds were known and identified as *n*-hexacosane (**2**) (Rukaiyat et al., 2015), 29-hydroxyfriedelane (**3**) (Patra and Chaudhuri, 1987), 3*β*-friedelinol (**4**) (Duarte et al., 2009), *n*-hexacosan-1-ol (**5**) (Kainsa and Singh, 2016), *n*-octacosan-1-ol (**6**) (Thippeswamy et al., 2008), mangiferin (**7**) (Kim et al., 2006), *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**8**) (Peshin and Kar, 2017), friedelin (**9**) (Duarte et al., 2009), 30-hydroxyfriedelin (**10**) (Duarte et al., 2009), salaspermic acid (**11**) (Hu et al., 2014), 22*β*-*epi*-maytenfolic acid (**12**) (Silva et al., 2002), orthosphenic acid (**13**) (Hu et al., 2014), maltose (**14**) (Colson et al., 1975), D-mannitol (**15**) (Chuluunbaatar et al., 2017), cangoronine (**16**) (Hu et al., 2014), 7-hydroxyfriedelane-1,3-dione (**17**) (Joshi et al., 1973), tingenone (**18**) (Gunatilaka et al., 1989), pristimerin (**19**) (Itokawa et al., 1991), *α*-amyrin acetate (**20**) (Okoye et al., 2014), *β*-sitosterol (**21**) and stigmasterol (**22**) (Chaturvedula and Prakash, 2012), 21-hydroxyfriedelin (**23**) (Setzer et al., 2000), abruslactone A (**24**) (Chang et al., 1982; Silva et al., 2002) and 2*α*-hydroxypopulnonic acid (**25**) (Estrada et al., 1994).

3.3. *In vitro* antimicrobial activity

Compounds (**1**), (**7**), (**10-11**), (**13**), (**16-19**) and (**25**) were tested for their antifungal and antibacterial potencies against 5 yeasts comprising *C. gatti*, *C. parapsilosis*, *C. glabrata*, *C. albicans* and *P. brasiliensis*, 2 Gram-negative bacteria involving *E. coli* and *P. aeruginosa* and 2 Gram-positive bacteria containing *E. faecium* and *S. aureus*. The results are depicted in Table 3. Generally, the antimicrobial activity is considered to be significant if MIC<25 µM (Cos et al., 2006). Except for compound (**10**), all the tested compounds exhibited antimicrobial activity against at least two microbes with MIC values ranged from 23.8 to 595.2 µM. Compound (**18**) showed the highest activity (MIC=23.8 µM) against *S. aureus*. On the other hand, compounds (**19**) and (**11**) exhibited moderate inhibiting effect against *S. aureus* (MIC=53.8 µM) and *C. glabrata* (MIC=105.9 µM), respectively. Furthermore, compound (**1**) exhibited weak activities against *C. albicans*, *C. glabrata* and *E. coli* with MIC values of 347.2, 520.8 and 520.8 µM, respectively. Mangiferin (**7**), the major metabolite of the plant showed weak activities against *C. albicans* and *C. glabrata* with a MIC value of 355.5 µM against both microbes. The overall results showed that *S. aureus*, had the highest susceptibility to tingenone (**18**) and pristimerin (**19**). This corroborated the previous reported results for the two compounds against the same bacteria (Rodrigues et al., 2012; Ezem et al., 2015).

Table 3

Antimicrobial activity of some isolated compounds.

	Compounds (MIC in µg/ml [µM])										Positive control	
	1	**7**	**10**	**11**	**13**	**16**	**17**	**18**	**19**	**25**	**Flu**	**Amp**
C.a.	100 [347.2]	150 [355.5]	-	100 [211.9]	-	150 [309.9]	100 [219.3]	100 [238.1]	200 [431.0]	150 [317.8]	15 [49.0]	*
C.g.	150 [520.8]	150 [355.5]	-	50 [105.9]	150 [307.4]	250 [516.5]	150 [328.9]	100 [238.1]	100 [215.5]	-	10 [32.6]	*
C.p.	-	-	-	-	-	-	-	-	100 [215.5]	150 [317.8]	20 [65]	*
Cr.g.	-	-	-	-	150 [307.4]	-	-	-	150 [323.2]	150 [317.8]	15 [49.0]	*
P.b.	-	-	-	-	150 [307.4]	-	-	-	-	150 [317.8]	15 [49.0]	*
E.c.	150 [520.8]	-	-	-	-	-	-	200 [476.1]	-	-	*	4 [11.5]
S.a.	-	-	-	-	-	-	200 [438.6]	10 [23.8]	25 [53.8]	-	*	4 [11.5]
P.a.	-	250 [592.4]	-	-	-	-	-	250 [595.2]	-	-	*	1 [2.9]
E.f.	-	250 [592.4]	-	-	-	-	-	250 [595.2]	-	-	*	>200 [573.1]

(-): >250; Flu: Fluconazole; Amp: Ampicilin; *: Not applicable; *C.a.*: *C. albicans* ATCC64548; *C.g.*: *C. glabrata* ATCC90030; *C.p.*: *C. parapsilosis* ATCC22019; *Cr.g.*: *C. gatti* ATCC 32269; *P.b.*: *P. brasiliensis* Pb18; *E.c.*: *E. coli* ATCC25922; *S.a.*: *S. aureus* ATCC25923; *P.a.*: *P. aeruginosa* ATCC14502; *E.f.*: *E. faecium* VRE16.

4. Concluding remarks

The chemical analysis of the CH_2Cl_2-MeOH (1:1) extracts of the leaves and liana of *S. nitida* (Benth.) N.E.Br. led to the isolation of twenty-five compounds including one benzophenone isolated for the first time from a natural source. Nine of the ten tested compounds exhibited an antimicrobial activity to at least two micro-organisms. Tingenone (**18**) exhibited a significant inhibiting effect against *S. aureus* while pristimerin (**19**) and salaspermic acid (**11**) exhibited moderate effects against *S. aureus* and *C. glabrata*, respectively. The present investigation shows that *S. nitida* (Benth.) N.E.Br. may constitute an important source of bioactive molecules.

Conflict of interest

The authors declare that there is no conflict of interest.

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