



# Trends in Phytochemical Research (TPR)

Journal Homepage: <http://tpr.iau-shahrood.ac.ir>



Original Research Article

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

BRICE M. MBA'NING<sup>1,✉</sup>, JOËL E. T. ATEBA<sup>1</sup>, ANGELBERT F. AWANTU<sup>2</sup>, LUCIANA S. AMARAL<sup>3</sup>, GERVAIS M. HAPPI<sup>4</sup>, BEATE NEUMANN<sup>5</sup>, GEORG STAMMLER<sup>5</sup>, BRUNO N. LENTA<sup>4</sup>, SILVÈRE A. NGOUELA<sup>1</sup>, IRAN MALAVAZI<sup>6</sup>, ETIENNE TSAMO<sup>1</sup>, NORBERT SEWALD<sup>7</sup> AND EDSON RODRIGUES-FILHO<sup>3</sup>

<sup>1</sup>Department of Organic Chemistry, Faculty of Science, University of Yaoundé 1, Po. Box 812, Yaoundé, Cameroon

<sup>2</sup>Department of Chemistry, Faculty of Science, University of Bamenda, Po. Box 39, Bambili, Cameroon

<sup>3</sup>Departamento de Química, Universidade Federal de São Carlos, CP 676, 13.565-905 SP, Brazil

<sup>4</sup>Department of Chemistry, Higher Teacher Training College, University of Yaoundé 1, Po. Box 47, Yaoundé, Cameroon

<sup>5</sup>Inorganic chemistry, Department of Chemistry, Faculty of Chemistry, Bielefeld University, University str. 25, Bielefeld, Germany

<sup>6</sup>Departamento de Genética e Evolução, Universidade Federal de São Carlos, CP 676, 13.565-905 SP, Brazil

<sup>7</sup>Department of Chemistry, Organic and Bioorganic Chemistry, Bielefeld University, Po. Box 100131, D-33501 Bielefeld, Germany

### 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**), 3 $\beta$ -friedelinol (**4**), *n*-hexacosan-1-ol (**5**), *n*-octacosan-1-ol (**6**), mangiferin (**7**),  $\beta$ -sitosterol-3-*O*- $\beta$ -D-glucopyranoside (**8**), friedelin (**9**), 30-hydroxyfriedelin (**10**), salaspermic acid (**11**), 22 $\beta$ -*epi*-maytenfolic acid (**12**), orthosphenic acid (**13**), maltose (**14**), D-mannitol (**15**), cangoronine (**16**), 7-hydroxyfriedelane-1,3-dione (**17**), tingenone (**18**), pristimerin (**19**),  $\alpha$ -amyrin acetate (**20**),  $\beta$ -sitosterol (**21**), stigmasterol (**22**), 21-hydroxyfriedelan-3-one (**23**), abruslactone A (**24**) and 2 $\alpha$ -hydroxypropulnonic 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  $\mu$ M), while compounds (**11**) and (**19**) exhibited moderate inhibiting effect against *Staphylococcus aureus* (MIC=53.8  $\mu$ M) and *Candida glabrata* (MIC=105.9  $\mu$ M), respectively.

### ARTICLE HISTORY

Received: 06 February 2019

Revised: 12 May 2019

Accepted: 16 May 2019

ePublished: 15 June 2019

### KEYWORDS

Antimicrobial activity  
Benzophenone  
Leaves and liana  
NMR spectroscopy  
*Salacia nitida* (Benth.) N.E.Br.  
X-ray diffraction

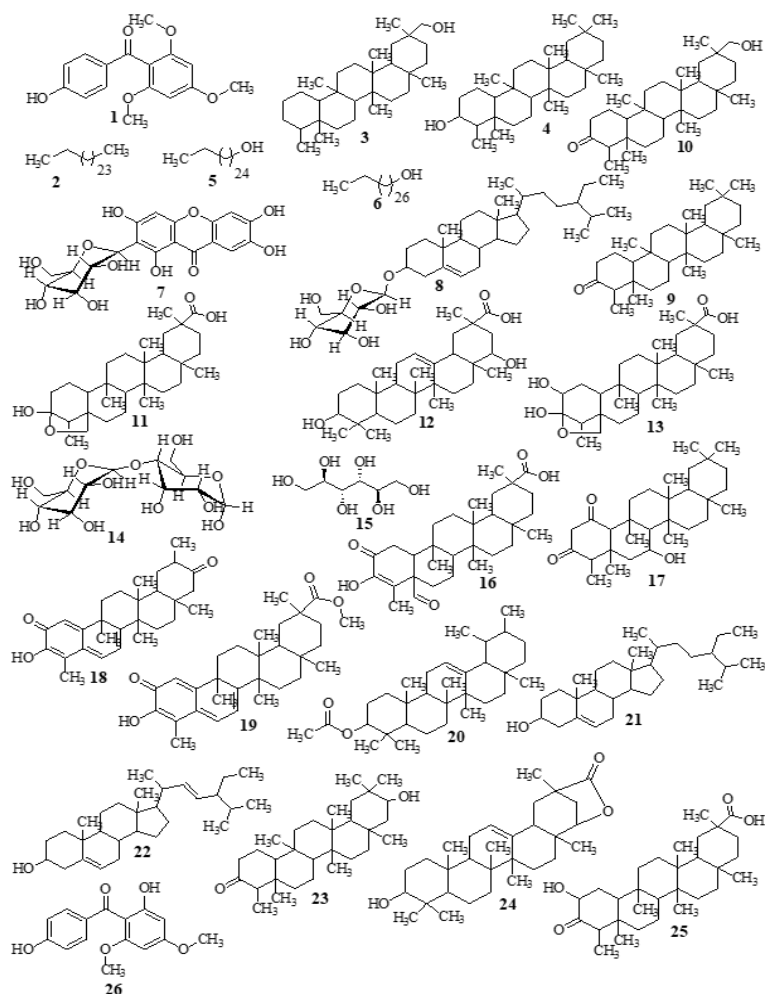
© 2019 Islamic Azad University, Shahrood Branch Press, All rights reserved.

### 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

✉ Corresponding author: Brice M. Mba'ning  
Tel: +237 675 11 98 05; Fax: +237 675 11 98 05  
E-mail address: [brice\\_mbaning@yahoo.fr](mailto:brice_mbaning@yahoo.fr)



**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, xanthenes, stilbenes, eudesmane-type sesquiterpenes and megastigmane glycosides (Kawazoe et al., 1997; Kishi et al., 2003; Carvalho et al., 2005; Mba'ninge 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  $\text{CH}_2\text{Cl}_2$ -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  $\text{CuK}\alpha$  ( $\lambda=1.54184 \text{ \AA}$ ) radiation.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on

a Bruker Ultrashield spectrometer operating at 400 MHz ( $^1\text{H}$ ) and 100 MHz ( $^{13}\text{C}$ ), respectively; with TMS as internal standard. Chemical shifts are reported as  $\delta$  values. Probes were dissolved in 0.5 mL  $\text{CDCl}_3$ , Acetone-*d*6 or DMSO-*d*6. 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 °C. The flow rate of sample was 180  $\mu\text{L}/\text{h}$  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<sub>254</sub> 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, 2°56'14N and 9°54'27E, 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 (N° 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**

<sup>1</sup>H (400 MHz) and <sup>13</sup>C (100 MHz) NMR data of compounds **1** (in Acetone-*d*<sub>6</sub>) and **26** (in CDCl<sub>3</sub>).

| Position           | 1                             |                   | 26                            |                   |
|--------------------|-------------------------------|-------------------|-------------------------------|-------------------|
|                    | δ <sup>1</sup> H (m, J in Hz) | δ <sup>13</sup> C | δ <sup>1</sup> H (m, J in Hz) | δ <sup>13</sup> 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 <sub>3</sub> | 3.69 (s)                      | 56.1              | -                             | -                 |
| 4-OCH <sub>3</sub> | 3.89 (s)                      | 55.8              | 3.87 (s)                      | 55.6              |
| 6-OCH <sub>3</sub> | 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**)

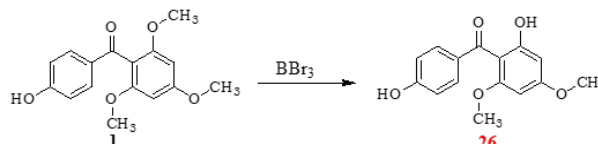
Colorless crystals; mp 162-163 °C; IR (KBr)  $\nu_{\max}$  3447 cm<sup>-1</sup> (OH), 1651 cm<sup>-1</sup> (C=O) and 1513-1617 cm<sup>-1</sup> (aromatic ring); <sup>1</sup>H NMR (Acetone-*d*<sub>6</sub>, 400 MHz) and <sup>13</sup>C NMR (Acetone-*d*<sub>6</sub>, 100 MHz) see Table 1; HR-ESIMS: [M+H]<sup>+</sup>, *m/z* 289.1158 (calcd 289.1071 for C<sub>16</sub>H<sub>17</sub>O<sub>5</sub>).

#### 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<sub>16</sub>H<sub>16</sub>O<sub>5</sub> (*M*=288.29 g/mol), monoclinic, space group P2<sub>1</sub>/n (no. 14), *a*=14.3272(5) Å, *b*=8.0795(3) Å, *c*=24.9909(13) Å,  $\beta$ =104.342(5), *V*=2802.7(2) Å<sup>3</sup>, *Z*=8, *T*=100.01(10) K,  $\mu$ (CuK $\alpha$ )=0.847 mm<sup>-1</sup>, *D*<sub>calc</sub>=1.366 g/cm<sup>3</sup>, 10736 reflections measured (6.508≤2 $\theta$ ≤144.21), 5462 unique (*R*<sub>int</sub>=0.0268, *R*<sub>sigma</sub>=0.0352) which were used in all calculations. The final *R*<sub>1</sub> was 0.0509 for 4545 reflections with *I*>2 $\sigma$ (*I*) and *wR*<sub>2</sub> 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**)

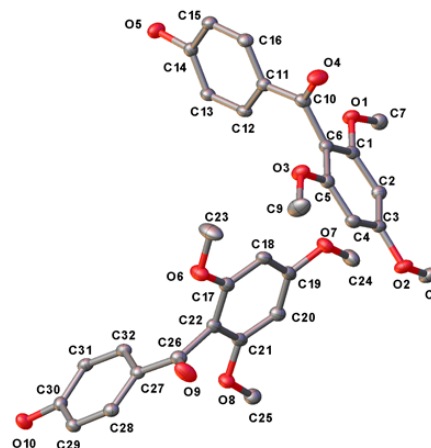
A solution of boron tribromide (0.8855 mmol) in dichloromethane (1:10 v/v) was slowly added to a cooled (-78 °C) solution of (**1**) [102 mg (0.3542 mmol) in 9.8 mL CH<sub>2</sub>Cl<sub>2</sub> and 0.2 mL MeOH] under argon.



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<sub>2</sub>SO<sub>4</sub> 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, *Cryptococcus 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 <sub>16</sub> H <sub>16</sub> O <sub>5</sub>                |
|---|---|
| Formula weight                              | 288.29  |
| Temperature/K                               | 100.01(10)  |
| Crystal system                              | monoclinic  |
| Space group                                 | P2 <sub>1</sub> /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 <sup>3</sup>                       | 2802.7(2)   |
| Z   | 8   |
| ρ <sub>calc</sub> /cm <sup>3</sup>          | 1.366   |
| μ/mm <sup>-1</sup>                          | 0.847   |
| F(000)                                      | 1216.0  |
| Crystal size/mm <sup>3</sup>                | 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 <sub>int</sub> = 0.0268, R <sub>sigma</sub> = 0.0352] |
| Reflections with I > 2σ(I)                  | 4545  |
| Data/restraints/parameters                  | 5462/0/387  |
| Goodness-of-fit on F <sup>2</sup>           | 1.091   |
| Final R indexes [I > 2σ(I)]                 | R <sub>1</sub> = 0.0509, wR <sub>2</sub> = 0.1233             |
| Final R indexes [all data]                  | R <sub>1</sub> = 0.0627, wR <sub>2</sub> = 0.1301             |
| Largest diff. peak/hole / e Å <sup>-3</sup> | 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 targeted compounds ranged from 1.00 to 250 μg.mL<sup>-1</sup>. Each well was filled with 195 μL of serial broth dilutions containing the targeted compounds and inoculated with 5 μL of a fresh culture of each organism (10<sup>5</sup> CFU). Plates were incubated for 48 h at 37 °C for yeasts and for bacteria 24 h at 35 °C. 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 ampicillin 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**)

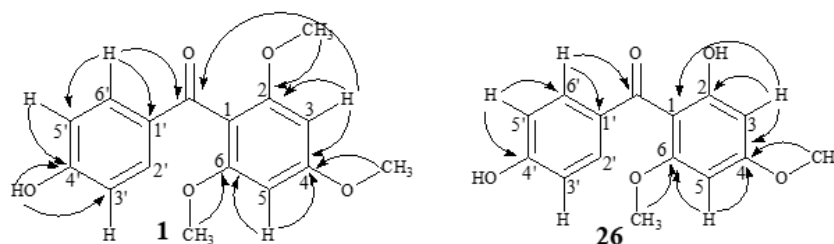
Compound (**1**) was obtained as colorless crystals. Its molecular formula C<sub>16</sub>H<sub>16</sub>O<sub>5</sub>, with 9 double bonds equivalence, was determined from the NMR data and its positive HR-ESIMS which showed the pseudo-molecular ion peak [M+H]<sup>+</sup> at m/z 289.1158 (calcd 289.1071 for C<sub>16</sub>H<sub>17</sub>O<sub>5</sub>). The IR spectrum showed the presence of hydroxyl group (3447 cm<sup>-1</sup>), carbonyl group (1651 cm<sup>-1</sup>) and aromatic ring (1513-1617 cm<sup>-1</sup>).

The <sup>1</sup>H NMR spectrum revealed a singlet of one hydroxyl proton at δ<sub>H</sub> 9.10 (4'-OH), two doublets of an A<sub>2</sub>B<sub>2</sub> aromatic ring pattern at δ<sub>H</sub> 7.67 (2H, J=8.4 Hz, H-2'/6') and δ<sub>H</sub> 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<sub>H</sub> 6.33 (H-3/5) and two singlets of three methoxy groups at δ<sub>H</sub> 3.89 (3H, 4-OMe) and δ<sub>H</sub> 3.69 (6H, 2/6-OMe).

The <sup>13</sup>C NMR spectrum displayed signals of 16 carbons sorted in combination with the HSQC experiment into seven quaternary carbons including one carbonyl at δ<sub>C</sub> 192.7, six aromatic methine carbons and three methoxy carbons.

The HMBC spectrum showed correlations (Fig. 3) between the hydroxyl proton at δ<sub>H</sub> 9.10 (4'-OH) and the carbons at δ<sub>C</sub> 115.9 (C-3'/5') and 163.1 (C-4'); the proton of the A<sub>2</sub>B<sub>2</sub> system at δ<sub>H</sub> 7.67 (H-2'/6') and the carbons at δ<sub>C</sub> 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<sub>2</sub>B<sub>2</sub> spin system. Other correlations were observed between the singlet of two protons at δ<sub>H</sub> 6.33 (H-3/5) and the carbons at δ<sub>C</sub> 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<sub>H</sub> 3.89 (4-OMe) and the carbon at δ<sub>C</sub> 162.6 (C-4); and also between the singlet of two methoxy groups at δ<sub>H</sub> 3.69 (2/6-OMe) and the carbon at δ<sub>C</sub> 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 <sup>1</sup>H NMR of the reaction product (**26**) compared to that of compound (**1**) showed two singlets of methoxy groups at δ<sub>H</sub> 3.54 (6-OMe) and 3.87 (4-OMe) and two doublets of one proton each at δ<sub>H</sub> 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<sub>H</sub> 3.54 and the carbon at δ<sub>C</sub> 161.6 (C-6), the methoxy group at δ<sub>H</sub> 3.87 and the carbon at δ<sub>C</sub> 165.9 (C-4) and the protons at δ<sub>H</sub> 5.98 and 6.18 and the carbons at δ<sub>C</sub> 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 $\beta$ -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),  $\beta$ -sitosterol-3-*O*- $\beta$ -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 $\beta$ -epimaytenfolic 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),  $\alpha$ -amyrin acetate (**20**) (Okoye et al., 2014),  $\beta$ -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 $\alpha$ -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  $\mu$ M (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  $\mu$ M. Compound (**18**) showed the highest activity (MIC = 23.8  $\mu$ M) against *S. aureus*. On the other hand, compounds (**19**) and (**11**) exhibited moderate inhibiting effect against *S. aureus* (MIC = 53.8  $\mu$ M) and *C. glabrata* (MIC = 105.9  $\mu$ M), 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  $\mu$ M, 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  $\mu$ M 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 $\mu$ g/ml [ $\mu$ M]) |                |           |                |                |                |                |                |                |                | Positive control |                 |
|--------------|--|----------------|-----------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|------------------|-----------------|
|              | <b>1</b>                                 | <b>7</b>       | <b>10</b> | <b>11</b>      | <b>13</b>      | <b>16</b>      | <b>17</b>      | <b>18</b>      | <b>19</b>      | <b>25</b>      | Flu              | Amp             |
| <i>C.a.</i>  | 100<br>[347.2]                           | 150<br>[355.5] | -         | 100<br>[211.9] | -              | 150<br>[309.9] | 100<br>[219.3] | 100<br>[238.1] | 100<br>[431.0] | 150<br>[317.8] | 15<br>[49.0]     | *               |
| <i>C.g.</i>  | 150<br>[520.8]                           | 150<br>[355.5] | -         | 50<br>[105.9]  | 150<br>[307.4] | 250<br>[516.5] | 150<br>[328.9] | 100<br>[238.1] | 100<br>[215.5] | -              | 10<br>[32.6]     | *               |
| <i>C.p.</i>  | -  | -              | -         | -              | -              | -              | -              | -              | 100<br>[215.5] | 150<br>[317.8] | 20<br>[65]       | *               |
| <i>Cr.g.</i> | -  | -              | -         | -              | 150<br>[307.4] | -              | -              | -              | 150<br>[323.2] | 150<br>[317.8] | 15<br>[49.0]     | *               |
| <i>P.b.</i>  | -  | -              | -         | -              | 150<br>[307.4] | -              | -              | -              | -              | 150<br>[317.8] | 15<br>[49.0]     | *               |
| <i>Ec.</i>   | 150<br>[520.8]                           | -              | -         | -              | -              | -              | -              | 200<br>[476.1] | -              | -              | *                | 4 [11.5]        |
| <i>S.a.</i>  | -  | -              | -         | -              | -              | -              | 200<br>[438.6] | 10<br>[23.8]   | 25<br>[53.8]   | -              | *                | 4 [11.5]        |
| <i>P.a.</i>  | -  | 250<br>[592.4] | -         | -              | -              | -              | -              | 250<br>[595.2] | -              | -              | *                | 1 [2.9]         |
| <i>E.f.</i>  | -  | 250<br>[592.4] | -         | -              | -              | -              | -              | 250<br>[595.2] | -              | -              | *                | >200<br>[573.1] |

(-): >250; Flu: Fluconazole; Amp: Ampicillin; \*: 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; *Ec.*: *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<sub>2</sub>Cl<sub>2</sub>-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 microorganisms. 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.

#### Acknowledgement

The authors are grateful to the TWAS-CNPq (The World Academy of Science-Conselho Nacional de Desenvolvimento Científico e Tecnológico) for the fellowship awarded to B. M. M. (Award n° 190644/2015-0). The authors also wish to acknowledge the German Academic Exchange Service (DAAD) for the financial support to the Yaoundé-Bielefeld Graduate School of Natural Products with Antiparasite and Antibacterial Activities (YaBiNaPA, project n° 57316173).

#### References

Bankeu, K.J.J., Dawé, A., Mbiantcha, M., Feuya, T.G.R., Ali, I., Tchuenmogne, T.M.A., Mehreen, L., Lenta, N.B., Ali, M.S., Ngouela, S.A., 2017. Characterization of bioactive compounds from *Ficus vallis-choudae* Delile (Moraceae). *Trends Phytochem. Res.* 1(4), 235-242.

Basu, S., Pant, M., Rachana, R., 2013. *In vitro* antioxidant activity of methanolic-aqueous extract powder (root and stem) of *Salacia oblonga*. *Int. J. Pharm. Pharm. Sci.* 5 (3), 904-909.

Bertil, H., Hans, F., Bo Fredholm, B., Torsten, P., Sten, V., 1973. Secondary phosphoric acid esters and their salts. *Ger. Offen.* 148 pp. Patent DE2240229 A1.

Carvalho, P.R.F., Silva, D.H.S., Bolzani, V.S., Furlan, M., 2005. Antioxidant quinonemethide triterpenes from *Salacia campestris*. *Chem. Biodivers.* 2(3), 367-372.

Castilho, D.G., Chaves, A.F., Xander, P., Zelanis, A., Kitano, E.S., Serrano, S.M., Tashima, A.K., Batista, W.L., 2014. Exploring potential virulence regulators in *Paracoccidioides brasiliensis* isolates of varying virulence through quantitative proteomics. *J. Proteome Res.* 13(10), 4259-4271.

Chang, H.M., Chiang, T.C., Thomas, C.W.M., 1982. Isolation and structure elucidation of abruslactone A: A new oleanene-type triterpene from the roots and vines of *Abrus precatorius* L. *J. Chem. Soc. Chem. Commun.* 20, 1197-1198.

Chaturvedula, V.S.P., Prakash, I., 2012. Isolation of stigmasterol and  $\beta$ -sitosterol from the dichloromethane extract of *Rubus suavissimus*. *Int. Curr. Pharm. J.* 1(9), 239-242.

Chuluunbaatar, E., Sodnomtseren, P., Chimedtseren, C., Batsuren, G., Dulamjav, B., 2017. Isolation of two flavonoids and mannitol from *Lagotis integrifolia* (Willd.) Schischk (Scrophulariaceae). *Cent. Asian J. Med. Sci.* 3(2), 167-172.

CLSI, 2008. Reference method for broth dilution antifungal. Susceptibility testing of filamentous fungi; Approved Standard-Second Edition. CLSI document M38-A2. Wayne, PA: Clinical and Laboratory Standards Institute, 28(16).

CLSI, 2015. Methods for dilution antimicrobial. Susceptibility tests for bacteria that grow aerobically; Approved Standard-Tenth Edition. CLSI document M07-A10. Wayne, PA: Clinical and Laboratory Standards Institute, 35(2).

Colson, P., Slessor, K.N., Jennings, H.J., Smith, C.P., 1975. A carbon-13 nuclear magnetic resonance study of chlorinated and polyol analogs of glucose and related oligomers. *Can. J. Chem.* 53(7), 1030-1037.

Cos, P., Vlietinck, A.J., Berghe, D.V., Maes, L., 2006. Anti-infective potential of natural products: How to develop a stronger *in vitro* 'proof-of-concept'. *J. Ethnopharmacol.* 106, 290-302.

De Mello, S.S., Tyne, D.V., Dabul, A.N.G., Gilmore, M.S., Camargo, I.L.B.C., 2016. High-Quality Draft Genome Sequence of the Multidrug-Resistant Clinical Isolate *Enterococcus faecium* VRE16. *Genome Announc.* 4(5), e00992-16.

Deepak, K.G.K., Giri, P.R., Kishor, P.B.K., Suekha, C., 2015. *Salacia* as an ayurvedic medicine with multiple targets in diabetes and obesity. *Ann. Phytomed.* 4(1), 46-53.

Dikaso, D., Makonnen, E., Debella, A., Abede, D., Urga, K., Makonnen, W., Melaku, D., Assefa, A., Makonnen, Y., 2006. *In vitro* anti-malarial activity of hydroalcoholic extracts from *Asparagus africanus* Lam in mice infected with *P. berghei*. *Ethiop. J. Health Dev.* 20(2), 117-121.

Dolomanov, O.V., Bourhis, L.J., Gildea, R.J., Howard, J.A.K., Puschmann, H., 2009. OLEX2: A Complete structure solution, refinement and analysis program. *J. Appl. Crystallogr.* 42(2), 339-341.

Dooka, B.D., Ezejiofor, A.N., 2017. Antidiabetic and cytoprotective effects of ethanolic extract of *Salacia nitida* root on alloxan-induced diabetic rats. *IOSR J. Pharm. Biol. Sci.* 12(1), 87-93.

Duarte, L.P., Silva de Miranda, R.R., Rodrigues V.S.B., Silva, G.D.F., Vieira Filho, S.A., Knupp, V.F., 2009. Stereochemistry of 16 $\alpha$ -hydroxyfriedelin and 3-oxo-16-methylfriedel-16-ene established by 2D NMR spectroscopy. *Molecules* 14, 598-607.

El-seedi, H.R., El-Barbary, M.A., El-Ghorab, D.M.H., Bohlin, L., Borg-Karlson, A.-K., Goransson, U., Verpoorte, R., 2010. Recent insights into the biosynthesis and biological activities of natural xanthenes. *Curr. Med. Chem.* 17, 854-901.

Elujoba, A.A., Odeleye, O.M., Ogunyemi, C.M., 2005. Traditional medicine development for medical and dental primary health care delivery systems in Africa. *Afr. J. Trad. Complement. Altern. Med.* 21(1), 46-61.

Estrada, R., Cardenas, J., Esquivel, B., Rodriguez-Hahn, L., 1994. D:A-Friedo-oleanane triterpenes from the roots of *Acanthothamnus aphyllus*. *Phytochemistry* 36(3), 747-751.

Ezem, S.N., Akpuaka, M.U., Ajiwe, V.I.E., 2015. Isolation of quinomethides-tingenone and pristimerin from the whole root



- of *Salacia oliveriana* (Celastraceae). American J. Chem. Appl. 2(6), 120-128.
- Ganesan, K., Xu, B., 2017. Ethnobotanical studies on folkloric medicinal plants in Nainamalai, Namakkal District, Tamil Nadu, India. Trends Phytochem. Res. 1(3), 153-168.
- Gohil, V.M., Sheth, S.A., Nilsson, R., Wojtovich, A.P., Lee, J.H., Perocchi, F., Chen, W., Clish, C., Ayata, C., Brookes, P.S., Mootha, V.K., 2010. Nutrient-sensitized screening for drugs that shift energy metabolism from mitochondrial respiration to glycolysis. Nat. Biotechnol. 28(3), 249-257.
- Gunatilaka, A.A.L., Fernando, C., Kikuchi, T., Tezuka, Y., 1989. <sup>1</sup>H and <sup>13</sup>C NMR analysis of three quinone-methide triterpenoids. Magn. Reson. Chem. 27, 803-811.
- Hallé, N., 1990. Célastracées (Hippocratéoidées), in: MESIRES (Ed.), Flore du Cameroun 32. MESIRES, Yaoundé, Cameroun, pp 110-111.
- Hu, P.-Y., Zhang, D., Pu, S.-B., Li, Z.-L., Zhou, H.-H., 2014. Chemical constituents from the roots of *Tripterygium wilfordii*. Asian J. Chem. 26(14), 4344-4346.
- Itokawa, H., Shiota, O., Ikuta, H., Morita, H., Takeya, K., Itaka, Y., 1991. Triterpenes from *Maytenus ilicifolia*. Phytochemistry 30(11), 3713-3716.
- Joshi, B.S., Kamat, V.N., Viswanat, N., 1973. Triterpenes of *Salacia prinoidea* DC. Tetrahedron 29(10), 1365-1374.
- Kainsa, S., Singh, R., 2016. Flavan-3-ol isomers isolated from *Euphorbia thymifolia*. Pharmacogn. Commun. 6(1), 28-33.
- Karunanayake, E.H., Sirimanne, S.R., 1985. Mangiferin from the root barks of *Salacia reticulata*. J. Ethnopharmacol. 13(2), 227-228.
- Kawazoe, K., Shimogni, N., Takaishi, Y., Rao, K.S., Imakura, Y., 1997. Four stilbenes from *Salacia lehmbachii*. Phytochemistry 44(8), 1569-1573.
- Kim, C.Y., Mi-Jeong, A., Kim, J., 2006. Preparative isolation of mangiferin from *Anemarrhena asphodeloides* rhizomes by centrifugal partition chromatography. J. Liq. Chromatogr. Relat. Technol. 29, 869-875.
- Kishi, A., Morikawa, T., Matsuda, H., Yoshikawa, M., 2003. Structures of new friedelane- and norfriedelane-type triterpenes and polyacylated eudesmane-type sesquiterpene from *Salacia chinensis* Linn. (*S. prinoidea* DC., Hippocrateaceae) and radical scavenging activities of principal constituents. Chem. Pharm. Bull. 51 (9), 1051-1055.
- Mba'ning, B.M., Lenta, B.N., Ngouela, S., Nounougou, D.T., Tantangmo, F., Talontsi, F.M., Tsamo, E., Laatsch, H., 2011. Salacetal, an oleanane-type triterpene from *Salacia longipes* var. *camerunensis*. Z. Naturforsch. 66b, 1270-1274.
- Mohammadhosseini, M., Sarker, S.D., Akbarzadeh, A., 2017. Chemical composition of the essential oils and extracts of *Achillea* species and their biological activities: A review. J. Ethnopharmacol. 199, 257-315.
- Nwiloh, B.I., Uwakwe, A.A., Akaninwor, J.O., 2016. Phytochemical screening and GC-FID analysis of ethanolic extract of root bark of *Salacia nitida* L. Benth. J. Med. Plants Stud. 4(6), 283-287.
- Nwiloh, B.I., Uwakwe, A.A., Akaninwor, J.O., 2019. Biochemical effects of ethanolic extract from root bark of *Salacia nitida* L. Benth in *Plasmodium berghei*-malaria-infected mice. A. J. Physiol. Biochem. Pharmacol. 9(1), 1-8.
- Ogbonna, D.N., Sokari, T.G., Agomuoh, A.A., 2008. Antimalarial activity of some selected traditional herbs from South Eastern Nigeria against *Plasmodium* species. Res. J. Parasitol. 3(1), 25-31.
- Okoye, N.N., Ajaghaku, D.L., Okeke, H.N., Ilodigwe, E.E., Nworu, C.S., Okoye, F.B.C., 2014. Beta-amyirin and alpha-amyirin acetate isolated from the stem bark of *Alstonia boonei* display profound anti-inflammatory activity. Pharm. Biol. 52(11), 1478-1486.
- Patra, A., Chaudhuri, S.K., 1987. Assignment of carbon-13 nuclear magnetic resonance spectra of some friedelanes. Magn. Reson. Chem. 25(2), 95-100.
- Perruchon, S., 2004. Synthèses et étude des relations structure-fonction des flavonoides. Thèse de Doctorat, Université de Rennes 1, Rennes, France, P.139.
- Peshin, T., Kar, H.K., 2017. Isolation and characterization of  $\beta$ -sitosterol-3-O- $\beta$ -D-glucoside from the extract of the flowers of *Viola odorata*. Br. J. Pharm. Res. 16(4), 1-8.
- Rodrigues, V.G., Duarte, L.P., Silva, G.D.F., Silva, F.C., Góes, J.V., Takahashi, J.A., Pimenta, L.P.S., 2012. Evaluation of antimicrobial activity and toxic potential of extracts and triterpenes isolated from *Maytenus imbricata*. Quim. Nova 35(7), 1375-1380.
- Rukaiyat, M., Garba, S., Labaran, S., 2015. Antimicrobial activities of hexacosane isolated from *Sanseveria liberica* (Gerome and Labroy) plant. Adv. Med. Plant Res. 3(3), 120-125.
- Sellamuthu, P.S., Arulselvan, P., Munappan, B.P., Kandasamy, M., 2012. Effect of mangiferin isolated from *Salacia chinensis* regulates the kidney carbohydrate metabolism in streptozotocin-induced diabetic rats. Assian Pac. J. Trop. Biomed. 2(3), S1583-S1587.
- Setzer, W.N., Setzer, M.C., Peppers, R.L., McFerrin, M.B., Meehan, E.J., Chen, L., Bates, R.B., Nakkiew, P., Jackes, B.R., 2000. Triterpenoid constituents in the bark of *Balanops australiana*. Australian J. Chem. 53(9), 809-812.
- Sheldrick, G.M., 2008. A short history of SHELX. Acta Crystallogr. A 64(1), 112-122.
- Sheldrick, G.M., 2015. Cristal structure refinement with SHELXL. Acta Crystallogr. C Struc. Chem. 71(1), 3-8.
- Silva, G.D.F., Duarte, L.P., Vieira Filho, S.A., Doriguetto, A.C., Mascarenhas, Y.P., Ellena, J., Castellano, E.E., Cota, A.B., 2002. Epikatonc acid from *Austroplenckia populnea*: structure elucidation by 2D NMR spectroscopy and X-ray crystallography. Magn. Reson. Chem. 40(5), 366-370.
- Singh, A.K., Raj, V., Keshari, A.K., Rai, A., Kumar, P., Rawat, A., Maity, B., Kumar, D., Prakask, A., De, A., Samanta, A., Bhattacharya, B., Saha, S., 2018. Isolated mangiferin and naringerin exert antidiabetic effect via PPAR<sub>γ</sub>/GLUT4 dual agonistic action with strong metabolic regulation. Chem. Biol. Interact. 25, 280:33-44.
- Thippeswamy, G., Sheela, M.L., Salimath, B.P., 2008. Octacosanol isolated from *Tinospora cardifolia* downregulates VEGF gene expression by inhibiting nuclear translocation of NF- $\kappa$ B and its DNA binding activity. Eur. J. Pharmacol. 588(2-3), 141-150.
- Warrier, P.K., Nambiar, V.P.K., Ramankutty, C., 1994. Indian Medicinal Plants - a Compendium of 500 Species, Orient Longman Private Ltd.: 3-6-752, Himayatnagar, Hyderabad 500 029 (A.P.), India, p 47.
- Zawua, C.I., Kagbo, H.D., 2018. Anti-diabetic properties of the root extracts of *Salacia nitida* Benth on alloxan induced diabetic rats. European J. Med. Plants 24(3), 1-15.