

## SUPPLEMENTARY MATERIAL

### Cytotoxic flavonoids and other constituents from the stem bark of *Ochna schweinfurthiana*.

Joseph T. Ndongo<sup>a</sup>, Mark E. Issa<sup>b</sup>, Angélique N. Messi<sup>c</sup>, Joséphine Ngo Mbing<sup>c\*</sup>, Muriel Cuendet<sup>b</sup>, Dieudonné E. Pegnyemb<sup>c</sup> and Christian G. Bochet<sup>d</sup>

<sup>a</sup> Department of Chemistry, Higher Teacher Training College, University of Yaoundé 1, P. O. Box 47, Yaoundé, Cameroon

<sup>b</sup> School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Quai Ernest-Ansermet 30, CH-1211 Genève 4, Switzerland

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

<sup>d</sup> Department of Chemistry, University of Fribourg, Chemin du Musée 9, CH-1700 Fribourg, Switzerland

\*Corresponding author. E-mail: [j\\_mbing@yahoo.com](mailto:j_mbing@yahoo.com)

## ABSTRACT

Seven flavonoids, hemerocallone (**1**), 6,7-dimethoxy-3',4'-dimethoxyisoflavone (**2**), amentoflavone (**4**), agathisflavone (**6**), cupressuflavone (**8**), robustaflavone (**9**), and epicatechin (**10**), together with three other compounds, lithospermoside (**3**),  $\beta$ -D-fructofuranosyl- $\alpha$ -D-glucopyranoside (**5**) and 3 $\beta$ -*O*-D-glucopyranosyl- $\beta$ -stigmaterol (**7**) were isolated from the ethyl acetate extract of the stem bark of *Ochna schweinfurthiana* F. Hoffm. All the compounds were characterized by spectroscopic and mass spectrometric methods, and by comparison with literature data. Cytotoxicity of the extracts and compounds against cervical adenocarcinoma (HeLa) cells was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Compounds **4** and **6** exhibited good cytotoxic activity, with IC<sub>50</sub> values of 20.7  $\mu$ M and 10.0  $\mu$ M, respectively.

*Key words:* *Ochna schweinfurthiana*, Ochnaceae, flavonoids, cytotoxic activity

## Experimental

### General experimental procedures

Thin layer chromatography (TLC) analyses were done using aluminium sheets coated with silica gel 60 F<sub>254</sub>. Flash column chromatography (FC) was carried out using Brunschwig silica gel 60 Å (32-63 mesh). Commercially available products were used without further purification. <sup>1</sup>H- and <sup>13</sup>C-NMR spectra were recorded with a FT 300 MHz or FT 360 MHz spectrometer with solvent residual signals used as a reference. Chemical shifts are given in ppm, coupling constants *J* are expressed in Hertz (multiplicity: *s* = singlet, *d* = doublet, *dd* = double of doublet, *t* = triplet, *dt* = double triplet, *q* = quadruplet, *m* = multiplet). IR spectra were recorded with a Fourier transform FTIR spectrometer. High resolution ESI mass spectra were measured with an ion-cyclotron FT/MS (4.7 T) spectrometer.

### Plant material

The stem bark of *O. schweinfurthiana* F. Hoffm. were collected in the North of Cameroon in December 2011. The identity of the plant material was confirmed at the Cameroonian National Herbarium in Yaoundé by Victor Nana, where a voucher specimen (HNC 40171) has been deposited.

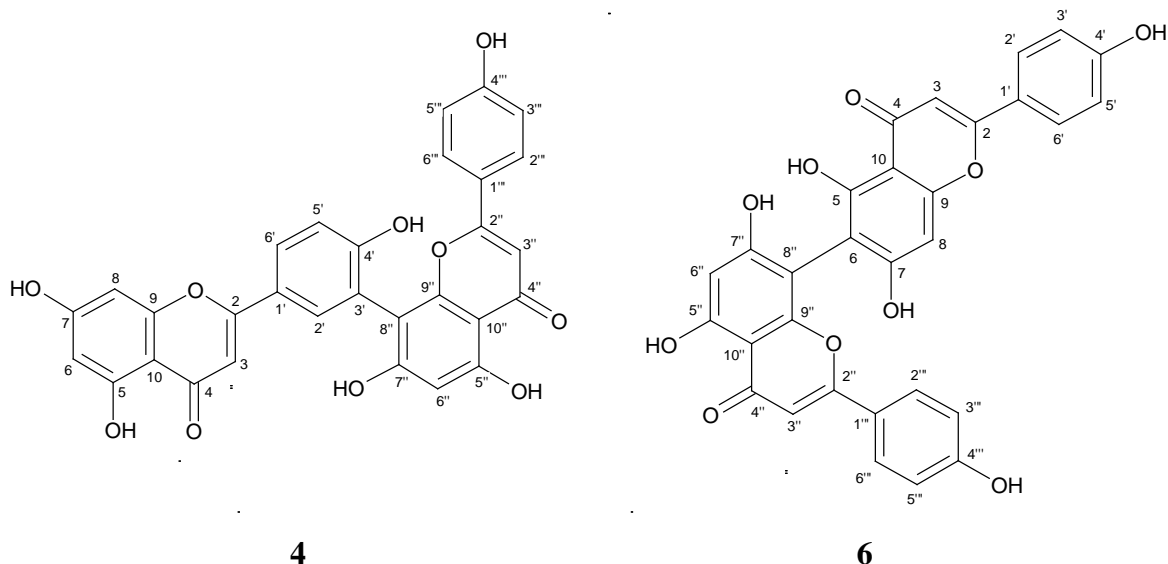
### Extraction and isolation

The EtOAc extract (30.6 g), obtained from partition of methanolic crude extract of the stem bark of *O. schweinfurthiana* resuspended in water, was separated using various chromatographic techniques. It was first subjected to normal-phase silica gel column and eluted with CH<sub>2</sub>Cl<sub>2</sub>/MeOH of increasing polarity (30:1 to 5:1, MeOH) to give seven fractions (Fr.1-Fr.7) on the basis of TLC composition. Fr.2 (2.1 g) was subjected to repeated CC on silica gel eluted with CH<sub>2</sub>Cl<sub>2</sub>/MeOH (10:1) to yield 6 mg hemerocallone (Zhang & Liang 2011) and 5 mg 6,7-dimethoxy-3',4'-dimethoxyisoflavone (Sree et al. 1986). Fr.3 (2.9 g) was also subjected to silica gel column chromatography, eluted with CH<sub>2</sub>Cl<sub>2</sub>-MeOH gradient and afforded a precipitate that were washed with CH<sub>2</sub>Cl<sub>2</sub> to give 125 mg lithospermoside (Sosa et al. 1977). The mother liquors from Fr.3 was applied to silica gel column chromatography, and eluted with (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 10:1) to yield 17 mg amentoflavone (Ndongo et al. 2010; Ying et al. 2010, Geiger et al. 1994). Fr.5 (3.8 g) was purified by CC with CH<sub>2</sub>Cl<sub>2</sub>/MeOH (10:1, 8:1, and 5:1) to yield 400 mg β-D-fructofuranosyl α-D-glucopyranoside (Fang et al. 2011), 11 mg agathisflavone (Ndongo et al. 2010; Shrestha et al. 2012; Geiger et al. 1994), and 7 mg 3β-O-D-glucopyranosyl-β-stigmasterol (Chaurasia & Wichtl 1987). Fr. 6 was first chromatographed

on a silica gel column (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 10:1 and 5:1) to give sub-fractions Fr.6A and Fr.6B, which were further purified using the same procedure as before to yield 8 mg cupressuflavone (Lin et al. 1989) and 10 mg robustaflavone (Lin and Chen 1974). Further purification of Fr.1 was conducted by repeated silica gel column chromatography and preparative TLC to give 6 mg epicatechin (Spek et al. 1984). The chemical structure of each isolated compound was elucidated on the basis of spectroscopic methods, including 2D NMR experiments, and confirmed by comparison with the literature.

*Amentoflavone* (**4**), Amorphous yellow powder, m.p. 231-232°, C<sub>30</sub>H<sub>18</sub>O<sub>10</sub>, ESI-MS: *m/z* 539.1 [M+H]<sup>+</sup>; <sup>1</sup>H-NMR (500 MHz, DMSO-d<sub>6</sub>): δ (ppm): 13.03 (1H, s, OH-5), 6.70 (1H, s, H-3), 6.25 (1H, d, *J* = 2.0 Hz, H-6), 6.32 (1H, d, *J* = 2.0 Hz, H-8), 6.81 (1H, d, *J* = 2.2 Hz, H-2'), 6.68 (1H, d, *J* = 8.7 Hz, H-5'), 7.61 (1H, d, *J* = 8.7; 2.2 Hz, H-6'), 13.36 (1H, s, OH-5''), 6.67 (1H, s, H-3''), 6.44 (1H, s, H-6''), 6.94 (2H, d, *J* = 8.7 Hz, H-2'''/6'''), 6.68 (2H, d, *J* = 8.7 Hz, H-3'''/5'''). <sup>13</sup>C-NMR (125 MHz, DMSO-d<sub>6</sub>): δ (ppm): 164.1 (C-2), 102.0 (C-3), 181.7 (C-4), 161.1 (C-5), 98.6 (C-6), 163.6 (C-7), 93.7 (C-8), 157.1 (C-9), 103.4 (C-10), 118.3 (C-1'), 131.1 (C-2'), 122.0 (C-3'), 162.7 (C-4'), 117.8 (C-5'), 126.6 (C-6'), 162.8 (C-2''), 102.2 (C-3''), 181.2 (C-4''), 160.2 (C-5''), 100.3 (C-6''), 161.4 (C-7''), 103.6 (C-8''), 154.4 (C-9''), 102.3 (C-10''), 121.5 (C-1'''), 127.9 (C-2'''/6'''), 115.3 (C-3'''/5'''), 160.4 (C-4''').

*Agathisflavone* (**6**), Amorphous yellow powder, C<sub>30</sub>H<sub>18</sub>O<sub>10</sub>, ESI-MS: *m/z* 539.2 [M+H]<sup>+</sup> <sup>1</sup>H-NMR (500 MHz, CD<sub>3</sub>OD): δ (ppm): 6.81 (1H, s, H-3), 6.68 (1H, s, H-8), 7.52 (2H, d, *J* = 8.8 Hz, H-2'/6'), 6.72 (2H, d, *J* = 8.8 Hz, H-3'/5'), 6.63 (1H, s, H-3''), 6.35 (1H, s, H-6''), 7.87 (2H, d, *J* = 8.7 Hz, H-2'''/6'''), 6.92 (2H, d, *J* = 8.7 Hz, H-3'''/5'''). <sup>13</sup>C-NMR (125 MHz, CD<sub>3</sub>OD): δ (ppm): 165.9 (C-2), 103.3 (C-3), 183.7 (C-4), 162.4 (C-5), 100.0 (C-6), 105.4 (C-7), 94.8 (C-8), 156.9 (C-9), 103.8 (C-10), 123.2 (C-1'), 129.1 (C-2'/6'), 116.8 (C-3'/5'), 164.4 (C-4'), 166.0 (C-2''), 105.1 (C-3''), 184.1 (C-4''), 162.3 (C-5''), 100.5 (C-6''), 164.3 (C-7''), 104.8 (C-8''), 158.9 (C-9''), 103.6 (C-10''), 123.3 (C-1'''), 129.4 (C-2'''/6'''), 116.9 (C-3'''/5'''), 164.8 (C-4''').



### Cell lines

The human cervical cancer cell line HeLa was obtained from the American Type Culture Collection (ATCC). HeLa cells were cultured in RPMI 1640 culture medium supplemented with 10 % fetal bovine serum (Life Technologies, Switzerland), 100  $\mu\text{g mL}^{-1}$  penicillin, 250  $\mu\text{g mL}^{-1}$  streptomycin (Life Technologies). The growth medium was changed every 2 or 3 days and the cells were maintained in a humidified atmosphere supplemented with 5 %  $\text{CO}_2$  at 37°C.

### Cell viability assay

Cell viability was measured by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay, which is based on the conversion of MTT to formazan crystals by mitochondrial dehydrogenases (Mosmann 1983). Briefly, HeLa cells were seeded in 96-well plates at 5,000 cells/well in 200  $\mu\text{L}$  culture medium. The cells were allowed to adhere for 24 hours under standard conditions then treated with increasing concentrations of compounds for 72 hours. After the incubation, the cells received 20  $\mu\text{L}$  of MTT solution for 2 hours. The media and MTT mixture were then aspirated and the formazan-containing cells were solubilised in 100  $\mu\text{L}$  of DMSO. Optical density of formazan was measured at 550 nm on a Biotek plate reader. The percentage of cell viability was calculated as the absorbance of each test well divided by the average absorbance of the vehicle control wells then multiplied by 100. The concentration that resulted in a 50% reduction in viability ( $\text{IC}_{50}$ ) was calculated from the log<sub>10</sub> concentration versus normalized response curves using the following equation:

$y = 100 / (1 + 10^{(\text{LogIC}_{50-x}) \cdot \text{hill slope}})$ , where  $y$  is the measured absorbance at 550 nm and  $x$  is the drug concentration.

## References

- Chaurasia N, Wichtl M. 1987. Sterols and steryl glycosides from *Urtica dioica*. J Nat Prod. 50:881–885.
- Fang R, Veitch NC, Kite GC, Howes MJ, Porter EA, Simmonds MS. 2011. Glycosylated constituents of *Iris fulva* and *Iris brevicaulis*. Chem Pharm Bull. 59:124–128.
- Lin YM, Chen FC. 1974. Robustaflavone from seed kernels of *Rhus succedanea*. Phytochemistry. 13:1617–1619.
- Lin YM, Chen FC, Lee KH. 1989. Hinokiflavone, a cytotoxic principle from *Rhus succedanea* and the cytotoxicity of the related biflavonoids. Planta Med. 55:166–168.
- Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63.
- Ndongo JT, Shaaban M, Ngo Mbing J, Ngono BD, Atchadé A de T, Pegnyemb DE, Laatsch H. 2010. Phenolic dimers and an indole alkaloid from *Campylospermum flavum* (Ochnaceae). Phytochemistry 71:1872–1878.
- Shrestha S, Park Ji-Hae, Lee Dae-Young, Cho Jin-Gyeong, Seo Woo-Duck, Kang Hee Cheol, Yoo Ki-Hyun, Chung In-Sik, Jeon Yong-Jin, Yeon Seung-Woo, Baek Nam-In. 2012. Cytotoxic and neuroprotective biflavonoids from the fruit of *Rhus parviflora*. J Korean Soc Appl Bio Chem. 55:557–562.
- Sosa A, Winternitz F, Wide R, Pavia AA. 1977. Structure of a cyanoglucoside of *Lithospermum purpureo-caeruleum*. Phytochemistry. 16:707–709.
- Spek AL, Kojic-Prodic B, Labadie RP. 1984. Structure of (-)-epicatechin: (2R,3R)-2-(3, 4-dihydroxyphenyl)-3, 4-dihydro-2H-1-benzopyran-3, 5, 7-triol, C<sub>15</sub>H<sub>14</sub>O<sub>6</sub>. Acta Cryst. C40, 2068–2071.
- Sree RMM, Venkata RE, Ward RS. 1986. Carbon-13 nuclear magnetic resonance spectra of isoflavone. Magn Reson Chem. 24:225–230.
- Ying J, Guogang Z, Enlong M, Hongmei Z, Jin G, Jie H. 2010. Amentoflavone and the extracts from *Selaginella tamariscina* and their anticancer activity. Asian J Traditional Med. 5:226–229.
- Zhang Z, Liang Y. 2011. Study on chemical constituents from the root of *Hemerocallis citrina*. Zhongyaocai. 34:1371–1373.