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# A new phenylpropanoid glycoside from *Psorospermum tenuifolium* Kotschy (Hypericaceae)

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**ABSTRACT**

A new phenylpropanoid glycoside, namely psorospermoside (**1**) was obtained from the *Psorospermum tenuifolium* bark extract together with eleven known compounds. Their structures were elucidated using spectroscopic and spectrometric methods including 1D, 2D-NMR and ESI-MS, as well as by comparison of their data with those reported in the literature. All the isolated compounds were assessed for their cytotoxicity effect on the human cervix carcinoma cell line KB3-1. Emodin (**2**) and its congener 2-geranylemodin (**3**) displayed significant cytotoxicity with $IC_{50}$ values of 11.4 µM and 19 µM, respectively. Furthermore, the chemophenetic significance of the isolated compounds was also discussed.

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## 1. Introduction

Plants belonging to the genus *Psorospermum* (Hypericaceae family) are small trees or shrubs widely disseminated in the tropical regions of Africa, Madagascar and South America (African plant database, 2019). Several species are used as traditional medicines in many African countries for the treatment of skin ailments, leprosy and subcutaneous wounds (Milne-Redhead, 1993). Furthermore, decoctions of leaves and stem barks of some species are used in case of fever (Ssegawa and Kasenene, 2007), diarrhea (Tabuti et al., 2003) or syphilis (Kerharo and Adam, 1964). Several species have been phytochemically and pharmacologically investigated for their secondary metabolites and their biological activities. As expected from plants of the Hypericaceae family, the phenolic anthraquinones and xanthones were the most encountered classes of metabolites from the chemical studies on the genus *Psoropermum* (Epifano et al., 2013). However, pharmacological investigations have not yet been extensively carried out on extracts and compounds from *Psoropermum* plants. Little bioactivity reported in the literature refers to antifungal (Zubair et al., 2011), cytotoxic (Amonkar et al., 1981; Leet et al., 2008; Pouli and Marakos, 2009), antibacterial (Tchakam et al., 2012), or antiplasmodial (Lenta et al., 2008; Jansen et al., 2010) effects. *Psorospermum tenuifolium* Kotschy is one of the 55 species and can be encountered in Cameroon or Nigeria where it has attracted considerable attention for its healing properties in the treatment of skin diseases (Epifano et al., 2013). *P. tenuifolium* have been little investigated for its phytochemicals and biological potencies. In the ongoing study, the plant was chemically and biologically investigated for potential cytotoxic metabolites. Twelve compounds were

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obtained from stem barks of *P. tenuifolium*, including one new phenylpropanoid glycoside and eleven known compounds. Their effects in cytotoxicity assay were evaluated on the cell line KB3-1 and emodin (**2**) exerted the most significant potency.

## 2. Experimental

### 2.1. General experimental procedures

Optical rotation indices were determined in methanol on a JASCO DIP-3600 digital polarimeter (JASCO, Tokyo, Japan) using a 10 cm cell. UV spectra were recorded on a Hitachi UV 3200 spectrophotometer in MeOH and IR spectra on an Alpha Platinum-ATR (Bruker, Rheinstetten, Germany). ESI-HR mass spectra were measured on Agilent Techn. 6220 TOF LCMS mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) and EI-MS on a Finnigan MAT 95 spectrometer (70 eV) (Thermo Fischer Scientific, Darmstadt, Germany) with perfluorokerosene as reference substance for ESI-HR-MS. The $^{1}$H- and $^{13}$C-NMR spectra were recorded at 500 MHz and 125 MHz, respectively, on Bruker DRX 500 NMR spectrometers (Bruker, Rheinstetten, Germany) in Pyridine-$d_5$. Chemical shifts are reported in δ (ppm) using tetramethylsilane (TMS) (Sigma-Aldrich, Munich, Germany) as internal standard, while coupling constants (*J*) were measured in Hz. Column chromatography was carried out on silica gel 230-400 mesh, Merck, (Merck, Darmstadt, Germany) and silica gel 70-230 mesh (Merck). Thin layer chromatography (TLC) was performed on Merck precoated silica gel 60 F254 aluminum foil (Merck) and spots were detected using diluted sulfuric acid spray reagent before heating. All reagents used were of analytical grade.

### 2.2. Plant material

The stem bark of *P. tenuifolium* (Hypericaceae) was collected in April 2017, in the village Nkol-Afamba (GPS coordinates: Latitude 3°51′32″N, Longitude 11°39′53″E), near Yaounde, Centre Region of Cameroon. The plant material was authenticated with the help of Mr. Victor Nana, a well-known botanist of the National Herbarium of Cameroon, where a voucher specimen was deposited and registered under the number 43860 HNC.

### 2.3. Extraction and isolation

The air-dried and powdered stem barks (~3 kg) of *P. tenuifolium* were consequently extracted three times with the mixture of dichloromethane/methanol (1/1, v/v) for 72 h, 48 h and 24 h, respectively. After filtration and evaporation of solvent under reduced pressure, 172.46 g of crude extract were obtained, dissolved in water and successively partitioned with *n*-hexane (Hex), ethyl acetate (EA), and n-butanol (BuOH) to obtain three solvent-soluble fractions labelled **A** (7.58 g), **B** (58.05 g), **C** (23.65 g), respectively, as well as the remaining water soluble fraction **D** (74.21 g). The fraction **B** was subjected to a silica gel column chromatography eluting with a stepwise gradient of petroleum ether/ dichloromethane (3:1 → 1:3, v/v), followed by petroleum ether–ethyl acetate (7:3 → 0:1, v/v) to afford twelve sub-fractions labelled $F_1$-$F_{12}$, along with eight compounds including 2-geranylemodin (**3**) (9 mg), 3-*O*-geranylemodin (**4**) (34 mg), 2-prenylemodin (**5**) (6 mg), bianthrone A1 (**7**) (17 mg), bianthrones A3 (**8**) (26 mg), lupeol (**9**) (4 mg), and catechin (**11**) (7 mg). The sub-fraction $F_9$ (9.12 g, PE/EA 3:2) was further purified by column chromatography on silica gel with a gradient of ethyl acetate in petroleum ether (9:1 → 3:2, v/v) to obtain emodin (**2**) (8 mg), vismione D (**6**) (4 mg), and betulinic acid (**10**) (26 mg); while the most polar sub-fractions $F_{11}$ (3.01 g, PE/EA 2:3), $F_{12}$ (6.83 g, PE/EA 1:4) and fraction **C** (BuOH) were combined and submitted to a column chromatography on silica gel using the gradient of methanol in ethyl acetate (0 to 20%) to yield psorospermoside (**1**) (4 mg) and daucosterol (**12**) (14 mg).

### 2.4. Spectroscopic data of compound **1**

Psorospermoside (**1**): $C_{24}H_{28}O_{11}$ white amorphous powder (MeOH); HR-ESI-MS: *m/z* 515.1521 $[M + Na]^+$ (calcd for $C_{24}H_{28}O_{11}Na$, 515.1524); $\alpha_D^{20}$ +5.8 (*c* 0.5, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 312 (1.72), 226 (1.59), 212 (2.17) nm; IR $(KBr)_{vmax}$ 3317, 2919, 2850, 1699, 1599, 1122, 1019, 832, 519 $cm^{-1}$; $^{1}$H NMR (500 MHz, pyridine-$d_5$) (Table 1) $^{13}$C NMR (125 MHz, pyridine-$d_5$) (Table 1).

### 2.5. Cytotoxicity assay

Cytotoxic activity screening of the isolates was done on KB-3-1 cells which were cultivated as a monolayer in DMEM (Dulbecco's modified Eagle medium) with glucose (4.5 $g.L^{-1}$), L-glutamine, sodium pyruvate and phenol red, supplemented with 10% foetal bovine serum (FBS). The cells were maintained at 37 °C and 5.3% $CO_2$-humidified air. On the day before the test, the cells (70% confluence) were detached with trypsin-ethylenediamine tetraacetic acid (EDTA) solution (0.05 %; 0.02% PBS) and placed in sterile 96-well plates in a density of 10000 cells in 100 µL medium per well. The dilution series of the compounds were prepared from stock solutions in DMSO of concentrations of 1 mM or 10 mM. The stock solutions were diluted with culture medium (10% FBS) at least 50 times. Some culture medium was added to the wells to adjust the volume of the wells to the wanted dilution factor. The dilution prepared from stock solution was added to the wells. Each concentration was tested in six replicates. Dilution series were prepared by pipetting liquid from well to well. The control contained the same concentration of DMSO as the first dilution. After incubation for 72 h at 37 °C and 5.3% $CO_2$-humidified air, 30 µL of an aqueous resazurin solution (175 µM) was added to each well. The cells were incubated at the same conditions for 6 h. Subsequently, the fluorescence was measured. The excitation was effected at a wavelength of 530 nm, whereas the emission was recorded at a wavelength of 588 nm. The $IC_{50}$ values were calculated as a sigmoidal

**Table 1**
$^{1}H$ (500 MHz) and $^{13}C$ (125 MHz) NMR assignments of 1 in Pyridine-$d_5$

| Units | Position | 1[a] | | HMBC |
|---|---|---|---|---|
| | | $\delta_C$ | $\delta_H$ (mult., *J* in Hz) | - |
| A | 1 | 102.9 | 5.79 (1H, d, 8.0) | C-2, C-3, C-1'' |
| | 2 | 75.7 | 6.03 (1H, dd, 1.6, 8.0) | C-1, C-3, C-4, C-9' |
| | 3 | 76.3 | 4.43 (1H, m) | C-1, C-2, C-4, C-5 |
| | 4 | 71.5 | 4.34 (1H, m) | C-2, C-3, C-5, C-6 |
| | 5 | 78.6 | 3.96 (1H, ddd, 2.4, 4.9, 9.4) | C-3, C-4, C-6 |
| | 6 | 62.3 | 4.33 (1H, m)<br>4.42 (1H, m) | C-4, C-5 |
| B | 1' | 125.9 | - | - |
| | 2'/6' | 130.1 | 7.52 (2H, d, 7.9) | C-2', C-4', C-6', C-7' |
| | 3'/5' | 116.6 | 7.12 (2H, d, 7.9) | C-1', C-3', C-5' |
| | 4' | 161.1 | - | - |
| | 7' | 144.5 | 7.94 (1H, d, 15.9) | C-1', C-2', C-6', C-8', C-9' |
| | 8' | 115.7 | 6.67 (1H, d, 15.9) | C-1', C-7', C-9' |
| | 9' | 166.5 | - | - |
| C | 1'' | 134.5 | - | - |
| | 2'', 6'' | 153.7 | - | - |
| | 3'', 5'' | 104.5 | 6.90 (2H, brs) | C-1'', C-2'', C-6'', C-7'' |
| | 4'' | 139.6 | - | - |
| | 7'' | 64.1 | 4.88 (2H, s) | C-3'', C-4'', C-5'' |
| | CH3O-2'', 6'' | 56.2 | 3.71 (6H, s) | C-2'', C-6'' |

[a]The chemical shifts are in δ values (ppm) from TMS.

dose response curve using GRAPHPAD PRISM 4.03. The $IC_{50}$ values equal the drug concentrations, at which vitality is 50%.

## 3. Results and Discussion

### 3.1. Phytochemical study

The crude extract of *P. tenuifolium* stem bark was subjected to repeated silica gel column chromatography to afford twelve secondary metabolites including one new compound **1** and eleven known compounds characterized as emodin (**2**) (Buchalter, 1969), 2-geranylemodin (**3**) (Delle Monache et al., 1987; Tiani et al., 2013), 3-*O*-geranylemodin (**4**) (Lenta et al., 2008; Tiani et al., 2013), 2-prenylemodin (**5**) (Lenta et al., 2008), vismione D (**6**) (Tiani et al., 2013; Botta et al., 1985), bianthrone A1 (**7**) (Botta et al., 1985), bianthrones A3a/3b (**8**) (Tini et al., 2013; Botta et al., 1985), lupeol (**9**) (Jain and Bari, 2010), betulinic acid (**10**) (Bisoli et al., 2008), catechin (**11**) (Davis et al., 1996) and daucosterol (**12**) (Mouffok et al., 2012) (Fig. 1). Their structures were confirmed by comparison of the spectral data of our compounds with those reported in the literature. Compound **1** was obtained as a white amorphous powder showing $[M+Na]^+$ ion peak at *m/z* 515.1521 (calcd. for $C_{24}H_{28}O_{11}Na$, 515.1524) on its HR-ESI-MS. The $^{1}H$ NMR spectrum (Table 1) of compound **1** showed

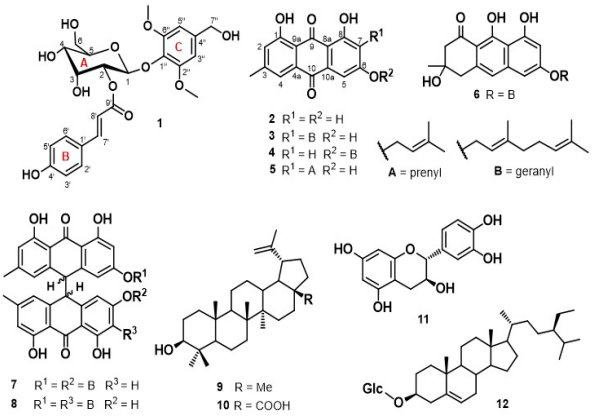


**Fig. 1.** Structures of compounds isolated from *P. tenuifolium*.

AA'BB'-type proton signals at δ 7.52 (d, *J* = 7.9 Hz, 2H) and δ 7.12 (d, *J* = 7.9 Hz, 2H), as well as an AA' system at δ 6.90 (brs, 2H). This observation suggested the presence of a 1,4-disubstituted and a 1,2,3,5-tetrasubstituted benzene rings in **1**. Additionally, we also observed signals corresponding to an α,β-unsaturated carbonyl group at δ 6.67 and 7.94 (d, *J* = 15.9 Hz, 1H each), two methoxy signals at δ 3.71 (6H, s), one highly deshielded oxymethylene signal at δ 4.88 (2H, s) and one signal at δ 5.79 (d, *J* = 8.0 Hz, 1H) corresponding to an anomeric proton. The large $^{3}J_{H}$-1,H-2 coupling constant suggested a β-glycosidic

linkage in **1**. All these findings were in accordance with the $^{13}C$ NMR spectrum (Table 1) in which signals of 24 carbon atoms were observed including signals of a glucosyl unit at δ 102.9, 78.6, 76.3, 75.7, 71.5 and 62.3; signals of an α,β-unsaturated carbonyl group at δ 166.5, two methoxy groups at δ 56.2 (x 2), one oxymethylene at δ 64.1, as well as fourteen $sp^2$ carbon signals in the range of δ 104.5-161.1. These spectroscopic data indicated that compound **1** is a phenylpropanoid glycoside containing one glucopyranosyl (unit A), one *trans-p*-coumaroyl (unit B) and one 1,2,3,5-tetrasubstituted benzene (unit C) groups. Careful analysis of the $^1H$ NMR spectrum indicated signals of six protons at δ 6.03 (1H, dd, 1.6, 8.0, H-2), 5.79 (1H, d, 8.0, H-1), 4.43 (1H, m, H-3), 4.42 (1H, m, H-6b), 4.34 (1H, m, H-4), 4.33 (1H, m, H-6a), 3.96 (1H, ddd, 2.4, 4.9, 9.4, H-5) assignable to the glucopyranose moiety. The COSY spectrum exhibited the correlations between H-1/H-2, H-2/H-3, H-3/H-4, H-4/H-5 and a cluster of correlation spots corresponding to correlations between the protons H-3, H-4 and H-6a/b. All these evidence easily allowed to build and assign the glucose scaffold from C-1 to C-6. The deshielded proton signal observed at δ 6.03 (H-2) indicated an esterification of C-2 (connection with unit B), while the carbon signal at *δ* 102.9 (C-1) suggested another substitution at C-1 (connection with unit C). The connections between units A-C were established on the basis of the HMBC correlations. Consequently, a long-range cross-peak was observed between the highly deshielded proton signal of unit A at *δ* 6.03 (H-2) and the carbon signal at *δ* 166.5 (C-9′) of unit B, supporting the connection C-2/C-9′ between units A and B. The HMBC correlations from δ 6.90 (H-3′′/H-5′′) to δ 64.1 (C-7′′) and from δ 3.71 ($OCH_3$-2′′/$OCH_3$-6′′) to δ 153.7 (C-2′′/C-6′′), allowed to set up the unit C (1,2,3,5-tetrasubstituted benzene group) as a 4′′-(hydroxymethyl)-2′′,6′′-dimethoxyphenolate moiety. It appears evident that the only position to link unit C is at position 1′′. This proposition was confirmed by the observed HMBC long range correlation from the anomeric proton at δ 5.79 (H-1) to the carbon signal at δ 134.5 (C-1′′) establishing the connection C-1/C-1′′ between units A and C. Based on all this evidence, the structure of new compound **1** was elucidated as shown in (Fig. 1) and given the trivial named psorospermoside.

### 3.2. Cytotoxicity assay

The cytotoxicity evaluation of the isolated compounds **1**-**12** was carried out on KB-3-1 cell line with griseofulvin as reference ($IC_{50}$ = 17-21 µM). The results obtained (Fig. 3, Table 2) showed that emodin (**2**, $IC_{50}$ = 11.4 µM, Fig. 2A) and 2-geranylemodin (**3**, $IC_{50}$ = 19 µM, Fig. 2B) are the most active compounds with potencies close to the used standard griseofulvin. However, the crude extract of *P. tenuifolium* showed not cytotoxicity and this observation may therefore indicate that the activity is supported by the minor compounds in the extract of *P. tenuifolium*.

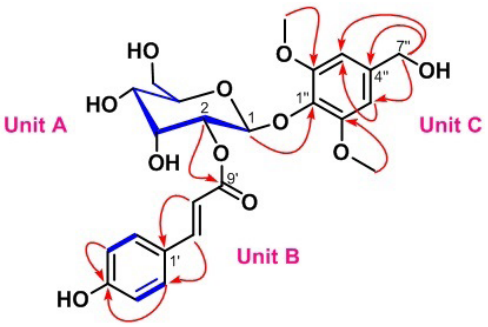


**Fig. 2.** Key COSY (blue) and HMBC (red) correlations of **1**.

**Table 2**
Cytotoxic potencies of compounds **1**-**12** on KB3-1 cell lines.

| KB3-1 cell lines | |
|---|---|
| Compound | $IC_{50}$ (µM) |
| Crude extract | n.a |
| 1 | n.a |
| 2 | 11.4 |
| 3 | 19.0 |
| 4 | >100 |
| 5 | >100 |
| 6 | n.a |
| 7 | >100 |
| 8 | >500 |
| 9 | n.a |
| 10 | n.a |
| 11 | n.a |
| 12 | n.a |
| Griseofulvin | 17-21 |

n.a: not active.

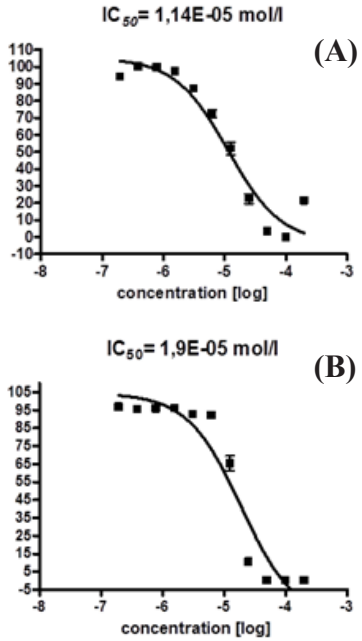


**Fig. 3.** Cytotoxic activity on KB3-1 cell lines: (**A**) emodin (**2**); (**B**) 2-geranylemodin (**3**).

### 3.3. Chemophenetic significance

During this phytochemical investigation of the bark of *Psorospermum tenuifolium*, twelve compounds (**1**-**12**, Fig. 1) have been isolated including one new phenylpropanoid glycoside (**1**), four anthraquinones (**2**-**5**), one anthrone (**6**), two bianthrones (**7**-**8**), two lupane-type triterpenoids (**9**-**10**), one flavonoid (**11**) and one steroid glycoside (**12**). Especially, emodin (**2**) and its derivatives (**3**-**5**) as well as bianthrone A3 (**8**) are reported for the first time from the species *P. tenuifolium* but were already reported from other species of the genus *Psorospermum*. However, only vismione D (**6**) and bianthrone A1 (**7**) were previously isolated from *P. tenuifolium* (Delle Monache et al., 1987b). These observations provide important evidence on the plant taxonomy. Indeed, anthraquinone and anthrone derivatives represent the most encountered classes of metabolites in the family Hypericaceae (Happi et al., 2020) and especially in the genus *Psorospermum* (Epifano et al., 2013). Therefore, they can be considered as chemomarkers for the genus *Psorospermum*. Previous works on *P. tenuifolium* led to the isolation of a lupane-type triterpenoid betuline (Delle Monache et al., 1987a,b), whereas its derivative lupeol acetate where obtained from *P. androsaemifolium* (Poumale et al., 2011). In the same way, the isolation of lupeol (**9**) and betulinic acid (**10**) is more comprehensible and provides additional information to enrich the chemistry of the genus *Psorospermum*. Furthermore, lupane-type triterpenoids might represent a new significant chemotaxonomic finding for the Hypericaceae plants. Compound **1** is reported for the first time from the family Hypericaceae, whereas the flavonoid catechin (**11**) and the steroid daucosterol (**12**) are commonly obtained from high plants.

## 4. Concluding remarks

In this study, twelve compounds including one new phenylpropanoid glycoside trivially named psorospermoside (**1**) were obtained from the stem bark of *P. tenuifolium* and characterized using spectroscopic and spectrometric methods as well as comparison of their data with those of the known compounds reported in the literature. Several of the isolated compounds were previously reported from the genus *Psorospermum* and confirmed the chemotaxonomy of *P. tenuifolium*. However, we observed significant cytotoxic effects of compounds **2** and **3** on human cancer cell lines KB3-1 in comparison with the standard griseofulvin. The results suggested that the minor compounds in the crude extract of *P. tenuifolium* are responsible of its potential cytotoxicity and these chemicals might help to design *P. tenuifolium* as a potential crude drug in the treatment of tumours.

## Conflict of interest

All authors declare no conflict of interest.

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