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Antiplasmodial anthraquinones and hemisynthetic derivatives from the leaves of *Tectona grandis* (Verbenaceae)

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ABSTRACT

Chemical investigation of the methanol extract of the leaves of *Tectona grandis* led to the isolation of one new anthraquinone derivative, grandiquinone A (3-acetoxy-8-hydroxy-2-methylanthraquinone) (**1**), along with nine known compounds: 5,8-dihydroxy-2-methylanthraquinone (**2**), hydroxysesamone (**3**), 3-hydroxy-2-methylanthraquinone (**4**), quinizarine (**5**), betulinic acid (**6**), ursolic acid (**7**), tectograndone (**8**), corosolic acid (**9**) and sitosterol 3-*O*-β-D-glucopyranoside (**10**). Compounds **2** and **3** were isolated for the first time from the leaves of this plant, while **5** has never been reported from the genus *Tectona*. Hydroxysesamone (**3**) and tectograndone (**8**) were subjected to cyclisation and acetylation reactions to afford two hemisynthetic derivatives, 6,9-dihydroxy-2,2-(dimethyldihydropyrano)-3,4-dihydro-2*H*-benzo[*g*]chromene-5,10-dione (**11**) and acetyltectograndone (**12**) respectively, which are reported here for the first time. The ethyl acetate-soluble portion, some of the isolated compounds and hemisynthetic derivatives were evaluated for their antiplasmodial activity against the multidrug-resistant Dd2 strain of *Plasmodium falciparum*. Compound **3** showed a prominent activity, while **2**, **8**, **9**, **11** and **12** showed significant *in vitro* anti-malarial activity. Compound **1** was weakly active in this test. The structures of the compounds were elucidated by spectroscopic methods and comparison of the data with the literature.

1. Introduction

Tectona grandis Linn. is a large tree from southeast Asia which grows up to 50 m in height. It is commonly known as sagwan or teak and is the most important of the three species that belong to the genus *Tectona* (Macias et al., 2008). This plant is widely used in Asian countries in the treatment of diabetes, lipid disorders, ulcers, inflammation, bronchitis, cancer, skin diseases, malaria and tuberculosis (Rajuri et al., 2010; Warrier, 1994). In Cameroon, it is locally used in the treatment of fever. Previous phytochemical investigation on *T. grandis* has led to the isolation of triterpenoids, flavonoids (Ragasa et al., 2008a), chromomoric acid derivatives (Ragasa et al., 2008b), anthraquinones (Sumthong et al., 2006, 2008), naphthoquinones (Pradeep and Pahup, 2004; Lacret et al., 2011), anthraquinone-naphthoquinones (Aguinaldo et al., 1993; Lacret et al., 2011), apocarotenoids (Macias et al., 2008), lignans (Lacret et al., 2012). Some of these metabolites showed anti-mycobacterial, antifungal and allelopathic activities (Sumthong et al., 2006, 2008; Pradeep and Pahup, 2004; Aguinaldo et al., 1993; Macias et al., 2008; Lacret et al., 2011). In the course of our search for potent biological antiplasmodial compounds from Cameroonian medicinal plants (Zofou et al., 2011a), we carried out the chemical investigation of the leaves of the title plant and report herein the isolation and structure elucidation of a new anthraquinone named grandiquinone A (**1**), as well as the antiplasmodial activity of the ethyl acetate-soluble fraction and some isolated constituents. The hemisynthesis of one naphthoquinone and one anthraquinone-naphthoquinone were carried out and the evaluation of the antiplasmodial activity of the obtained derivatives is also reported.

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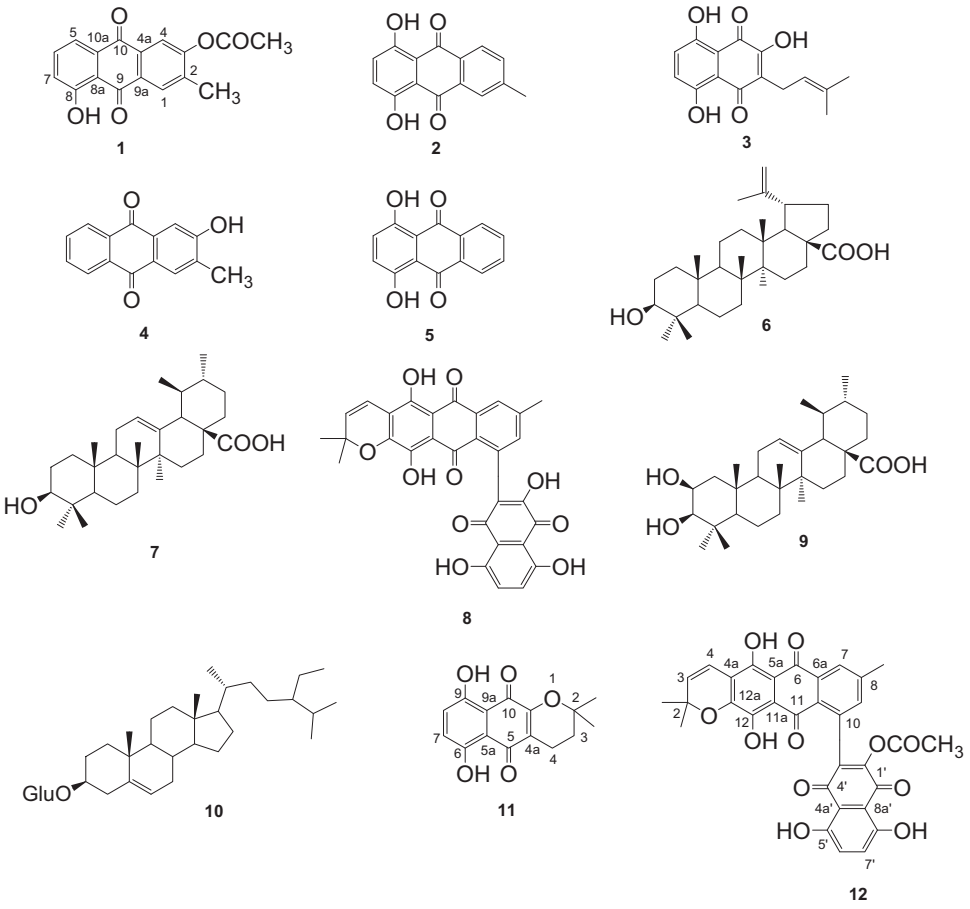

Fig. 1. Chemical structures of compounds isolated from *T. grandis* (**1**–**10**) and of cyclised and acetylated derivatives (**11**–**12**).

2. Results and discussion

The ethyl acetate-soluble fraction of the leaves of *T. grandis* was fractionated by silica gel column chromatography followed by gel permeation over Sephadex LH-20 to afford the new anthraquinone derivative, grandiquinone A (**1**), along with the known 5,8-dihydroxy-2-methylanthraquinone (**2**) (Hua et al., 2004), hydroxysesamone (**3**) (Hasan et al., 2001), 3-hydroxy-2-methylanthraquinone (**4**) (Da Silva et al., 2008), quinizarine (**5**) (Hua et al., 2004), betulinic acid (**6**) (Shukla et al., 2010), ursolic acid (**7**) (Shukla et al., 2010), tectograndone (**8**) (Aguinaldo et al., 1993), corosolic acid (**9**) (Mohammed et al., 1991), and sitosterol 3-*O*-*β*-D-glucopyranoside (**10**) (Singh et al., 2010). Two hemisynthetic compounds, 6,9-dihydroxy-2,2-(dimethyldihydropyrano)-3,4-dihydro-2*H*-benzo[*g*]chromene-5,10-dione (**11**) and acetyl tectograndone (**12**) were newly reported. Structures of the compounds (Fig. 1) were elucidated by 1D and 2D NMR spectroscopy, ESI and EI-MS and comparison of the data with those reported in the literature.

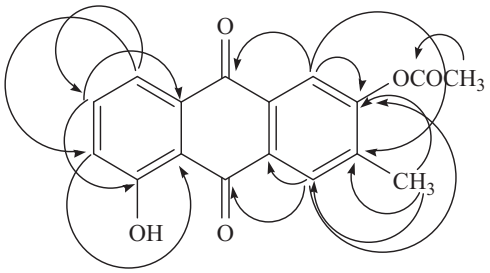

Fig. 2. HMBC (^{1}H–^{13}C) correlations for **1**.

Compound **1** was obtained as an orange powder from petroleum ether. The molecular formula was determined as $C_{17}H_{12}O_5$ by HR-ESI-TOF-MS, which showed the pseudo-molecular ion peak at *m/z* 297.0762 (calcd. 297.0763 for $[M+H]^+$), in conjunction with NMR data, indicating 12 degrees of unsaturation. Its UV spectrum showed maxima at λ_{max} 216, 226, 262, 336 and 407 nm. The IR spectrum exhibited absorption bands at ν_{max} 3047, 1757 and 1670 cm^{-1}, indicating the existence of hydroxyl, acetyl and ketone functionalities respectively. The ^{1}H NMR spectrum (Table 1) exhibited two different aromatic proton spin systems: three protons of a 1,2,3-substituted aromatic ring at δ 7.82 (1H, dd,

Table 1
^{1}H and ^{13}C NMR data for compound **1** (500 and 125 MHz, in $CDCl_3$), δ in ppm, *J* in Hz.

Position	δ_H	δ_C	HMBC (H → C)
1	8.20, 1H, s	130.5	C-3, C-9, C-9a
2	–	137.9	–
3	–	154.4	–
4	7.94, 1H, s	121.1	C-2, C-3, C-4a, C-10
4a	–	130.8	
5	7.82, 1H, dd (12.0, 6.0)	119.6	C-6, C-7
6	7.67, 1H, t (12.0)	136.7	C-8, C-10a
7	7.31, 1H, dd (12.0, 6.0)	124.5	C-5, C-8a
8	–	162.6	
8a	–	116.0	
9	–	188.0	
9a	–	133.6	
10	–	181.6	
10a	–	133.4	
CH_3OC=O	–	168.3	
$OCOCH_3$	2.40, 3H, s	20.7	
2-CH_3	2.37, 3H, s	16.8	C-2, C-3, C-1
8-OH	12.59, 1H, s	–	

Table 2
Antiplasmodial activities of isolated and modified compounds from the ethyl acetate-soluble fraction of the leaves of *T. grandis*.

Substance	IC_{50} on Dd2 *Plasmodium falciparum* IC_{50} (μg/mL)	CC_{50} on LLC-MK2	SI LLC-MK2/Dd2
Grandiquinone A (**1**)	6.24 ± 1.21	ND	ND
5,8-Dihydroxy-2-methylanthraquinone (**2**)	1.63 ± 0.04	125.24 ± 25.45	76.83
Hydroxysesamone (**3**)	0.82 ± 0.06	97.66 ± 52.96	119.1
Tectograndone (**8**)	1.57 ± 0.17	77.14 ± 21.40	49.13
Corosolic acid (**9**)	1.61 ± 0.14	161.96 ± 73.97	100.6
6,9-Dihydroxy-2,2-(dimethyldihydropyrano)-3,4-dihydro-*2H*-benzo[*g*]chromene-5,10-dione (**11**)	1.24 ± 0.43	73.94 ± 6.14	56.63
Acetyltectograndone (**12**)	1.06 ± 0.39	134.13 ± 53.96	126.54
Ethyl acetate-soluble fraction	35.15 ± 3.39	>1000	>28.57
Chloroquine	0.100 ± 0.042	ND	ND

IC_{50}, drug concentration causing 50% inhibition of parasite growth and multiplication; CC_{50}, cytotoxic concentration causing 50% inhibition of mammalian cell growth and multiplication; SI, selectivity index = CC_{50}/IC_{50}. IC_{50} and CC_{50} values are means and standard deviations obtained from six different replicate tests.
Dd_2 is a chloroquine resistant strain of *P. falciparum*. Crude extract TGF_{EA} (ethylacetate-soluble fraction) was considered weakly active (for crude extracts, $IC_{50} < 5$ μg/mL is considered very active, 5 μg/mL ≤ IC_{50} ≤ 10 μg/mL as active, 10 μg/mL ≤ IC_{50} ≤ 25 μg/mL as moderately active, 25 μg/mL ≤ IC_{50} ≤ 50 μg/mL as weakly active and $IC_{50} > 50$ μg/mL as inactive.
Compounds with $IC_{50} < 0.06$ μg/mL were considered as very active, those with 0.06 ≤ IC_{50} ≤ 5 μg/mL as active, 5 μg/mL ≤ IC_{50} ≤ 10 μg/mL as weakly active while those with $IC_{50} > 10$ μg/mL were considered as inactive (Mahmoudi et al., 2006).
Selective index (SI) = CC_{50} on LLC-MK2 cells/IC_{50} on *P. falciparum*. $CC_{50} < 1.0$ μg/mL (highly toxic); CC_{50} 1.0–10.0 μg/mL (moderately toxic);.
CC_{50} 10.0–30.0 μg/mL (mildly toxic) and $CC_{50} > 30$ μg/mL (non-toxic) (Malebo et al., 2009). SI > 10 (non- toxic) and SI < 10 (toxic) (Sarr et al., 2011).

J = 6.0 Hz and 12.0 Hz, H-5), 7.67 (1H, t, *J* = 12.0 Hz, H-6) and 7.31 (1H, dd, *J* = 6.0 Hz and 12.0 Hz, H-7), and two *para* aromatic protons at δ 8.20 (s, H-1) and 7.94 (s, H-4). This spectrum also displayed resonances of two methyl groups attached to sp^2 carbons at δ 2.40 (3H, s, 3-$OCOCH_3$) and δ 2.37 (3H, s, 2-CH_3). One chelated hydroxyl group was observed at δ 12.59 (1H, s, 8-OH). The ^1H–^1H COSY spectrum showed unambiguously the connectivity H-5–H-6–H-7, in agreement with the three-substituted aromatic ring pattern. These spectroscopic data suggested the presence of a 1,4-anthraquinone system in the molecule. The ^{13}C NMR spectrum and the APT experiment, showed 17 carbon atoms consisting of two methyl groups, five methines and ten quaternary carbons including two oxygenated aromatic carbon signals at δ 154.4 (C-3) and δ 162.6 (C-8), one ester carbonyl group at δ 168.3 (3-$OCOCH_3$) and two conjugated carbonyl groups at δ 181.6 (C-10) and 188.0 (C-9), further supporting the existence of the anthraquinone nucleus. By comparison of the ^1H and ^{13}C NMR data with those of the known compound 3,8-dihydroxy-2-methylanthraquinone (Shukla et al., 2010), **1** was identified as an anthraquinone with an acetyl group. Pertinent correlations in the HMBC spectrum (Table 1) between H-1 (δ 8.20) and C-3 (δ 154.4), C-9 (δ 188.0) and C-9a (δ 133.6) as well as between H-4 (δ 7.94) and C-10 (δ 181.6) indicated the location of the acetyl group (δ 168.3, C=O) at C-3 of the anthraquinone nucleus. The location of the methyl group at C-2 was further confirmed by HMBC spectrum which showed a ^3J correlation between H-4 (δ 7.94) and C-2, and also with the singlet nature of the signals of H-1 and H-4 in the ^1H NMR spectrum. The remaining correlations observed in the HMBC spectrum confirmed the proposed structure (Fig. 2). Compound **1** was thus characterized as the new derivative 3-acetoxy-8-hydroxy-2-methylanthraquinone (**1**), trivially named grandiquinone A (**1**) which is the acetylated derivative of tectone isolated from the leaves of *T. grandis* (Shukla et al., 2010).

In addition to 3-acetyltectone (grandiquinone A) (**1**), nine known compounds (**2**–**10**), were isolated. To the best of our knowledge, 5,8-dihydroxy-2-methylanthraquinone (**2**), hydroxysesamone (**3**) and quinizarine (**5**) were reported for the first time from *T. grandis*. Quinizarine (**5**), a well known synthetic hydroxyanthraquinone, has been so far isolated only from *Cassia* genus. It is then reported for the first time in the genus *Tectona* (Lee, 2003; Yang et al., 2003). The cyclisation of hydroxysesamone (**3**) and acetylation of tectograndone (**8**) gave two new derivatives, 6,9-dihydroxy-2,2-(dimethyldihydropyrano)-3,4-dihydro-2*H*-benzo[*g*]chromene-5,10-dione (**11**) and acetyltectograndone (**12**), respectively. Their structures were established by comparison of their NMR data with those of related compounds reported in the literature (Aguinaldo et al., 1993; Hasan et al., 2001; Fujiwara et al., 1998; Matsumoto et al., 1985).

The ethyl acetate-soluble fraction, five of the isolated compounds (**1**–**3**, **8**, **9**) and hemisynthetic derivatives **11** and **12** were tested for their antiplasmodial activity against the multidrug-resistant Dd2 strain of *Plasmodium falciparum* and their effects on the LLC-MK2 monkey kidney epithelial cell line. The antimalarial profiles as well as the selectivity in the biological activity are presented in Table 2. Despite its relatively low antiplasmodial activity (IC_{50} of 35.15 μg/mL), this extract was shown to contain promising antimalarial constituents, among which hydroxysesamone (**3**) was the most prominent, with an IC_{50} of 0.82 μg/mL. 5,8-Dihydroxy-2-methylanthraquinone (**2**), tectograndone (**8**), and corosolic acid (**9**) exhibited a significant activity (1.57 μg/mL ≤ IC_{50} ≤ 1.63 μg/mL), while grandiquinone A (**1**) showed a weak activity (IC_{50} 6.24 μg/mL). Interestingly, the hemisynthetic derivatives 6,9-dihydroxy-2,2-(dimethyldihydropyrano)-3,4-dihydro-2*H*-benzo[*g*]chromene-5,10-dione (**11**) and tectograndone acetate (**12**) also displayed significant activity with the IC_{50} values of 1.24 μg/mL and 1.06 μg/mL, respectively. Natural and synthetic quinones have shown significant antimalarial activity against chloroquine-resistant *Plasmodium* parasites (Sittie et al., 1999; Eyong et al., 2006; Fotie, 2006; Osman et al., 2010). Previous structure–activity studies demonstrated that an aldehyde group at C-2 and a phenolic hydroxy group at C-3 on the skeleton enhance the activity of anthraquinones against the growth of *P. falciparum* (Sittie et al., 1999; Osman et al., 2010). The presence of a methyl group at C-2 together with phenolic hydroxy group at C-3 seems to be also important for this activity. 3-Hydroxy-2-methylanthraquinone (**4**) for example exhibited strong inhibition against the 3D7 strain of *P. falciparum* with an IC_{50} value of 0.34 μM (Osman et al., 2010). Newbouldiaquinone A, a naphtoquinone-anthraquinone coupled *via* an ether bridge than a C–C bond was reported to display moderate antimalarial activity (Eyong et al., 2006). It is well known that lapachol, a hydroxyprenylated naphtoquinone structurally related to hydroxysesamone (**3**), is highly effective against the prevention of *P. falciparium* (Andrade-Neto et al., 2004). Our results showed that the product of cyclisation, 6,9-dihydroxy-2,2-(dimethyldihydropyrano)-3,4-dihydro-2*H*-benzo[*g*]chromene-5,10-dione (**11**) was about 2 times less active than its precursor **3**. The prenyl group present in ring B of **3** is likely to be responsible for the anti-malarial property of this

molecule. Thus, cyclisation of the isoprenyl group may have led to a drastic reduction of the activity of **11**. Further investigations are therefore highly needed to identify the target and fully elucidate the mechanism of action of this compound. With regards to their specificity, all the six compounds tested showed no significant cytotoxicity with their CC_{50} values ranging from 73.94 to 161.96 μg/mL. They all also proved to be highly selective for the *Plasmodium* parasite with SI ranging from 46 to 126. These cytotoxicity values obtained from the monkey kidney cells could suggest their potential as safer therapy and therefore their potential as lead anti-malarial candidates.

3. Experimental

3.1. General experimental procedures

Melting points of the isolated compounds were determined using an Electrothermal IA9000 Series digital melting point apparatus (Bibby scientific, Great Britain). MS detection was carried out using Micromass ESI-Q-TOF II instrument using ESI ionization in the positive mode (Waters). EIMS spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluorkerosine as reference substance for HR-ESI-TOF-MS (Japan). IR spectra were recorded on a Shimadzu FTIR-8400S spectrophotometer (Japan). UV spectra were recorded on a Shimadzu UV-160A spectrometer (Japan) in absolute ethanol (Scharlau) and alkaline ethanol. The NMR spectra were measured on Bruker 300 MHz, 500 MHz and 600 MHz NMR Avance II spectrometers equipped with cryoprobe, with TMS as an internal reference. Chemical shifts were recorded in δ (ppm) and the coupling constants (J) are in hertz. Silica gel 60 F_{254} (70–230; Merck; Darmstadt, Germany) was used for column chromatography. Precoated silica gel Kieselgel 60 F_{254} plates (0.25 mm thick) were used for TLC, and spots detected by spraying with 50% H_2SO_4 followed by heating at 100 °C.

3.2. Plant material

The leaves of *T. grandis* were collected at Limbe, South-West Region of Cameroon in August 2009. Authentification was performed by Mr Victor Nana who compared it with a voucher specimen (No. 61993 HNC) at the Cameroon National Herbarium, Yaoundé.

3.3. Extraction and isolation

Dried leaves of *T. grandis* (3 kg) were extracted with methanol (25 L) for 72 h at room temperature to yield a crude extract (117 g) after evaporation under vacuum. This extract was dissolved in H_2O (1 L) and extracted with EtOAc (3 × 1 L) to give an EtOAc-soluble fraction (72 g) after evaporation under vacuum. Seventy grams of this fraction were subjected to silica gel column chromatography eluted with *n*-hexane–EtOAc (10:0, 95:5, 90:10, 8:2, 1:1, 0:10) and EtOAc–MeOH (9:1, 8:2, 1:1, 0:10) to afford 28 fractions of 600 mL each. These fractions were combined on the basis of their TLC profiles (using mixtures of *n*-hexane–EtOAc 85:15, 70:30, 30:70) to give five major fractions A–E (A: 1–11; B: 12–14; C: 15–17; D: 18–22; E: 23–28). Fraction A (6 g) was subjected to column chromatography over silica gel with a gradient of *n*-hexane–EtOAc to give compounds **2** (10 mg) and **3** (40 mg). Fraction B (20 g) was separated by column chromatography over silica gel using *n*-hexane–EtOAc mixtures of increasing polarity to yield compound **1** (2 mg). Fraction C (10 g) was subjected to column chromatography on Sephadex LH-20 using *n*-hexane–dichloromethane–methanol (7:4:0.5) to afford four subfractions (C1–C4). Subfractions C1 (0.5 g) and C2 (1.0 g) were further chromatographed on silica gel columns, eluting with mixtures of *n*-hexane–EtOAc (100:0, 95:5, 90:0, and 80:20) to yield compounds **4** (5 mg) and **6** (50 mg), respectively. The mixture crystallized from subfraction C3 (52.5 mg) was purified by column chromatography on silica gel, eluting with *n*-hexane–EtOAc (9:1) to give compound **7** (10 mg). Fraction D (13.5 g) was subjected to silica gel column chromatography eluted with *n*-hexane–EtOAc mixtures of increasing polarity to afford five subfractions (D1–D5). Successive chromatography of subfraction D2 (100 mg) over Sephadex LH-20 (*n*-hexane–dichloromethane–methanol, 7:4:0.5) and silica gel (*n*-hexane–CH_2Cl_2–Me_2CO, 10:7:3.5) yielded **9** (15 mg). Compounds **5** (3 mg) and **8** (72 mg) were obtained from the purification of subfraction D5 (5.0 g) over silica gel using *n*-hexane–CH_2Cl_2 (6:4) as solvent system. Fraction E (1 g) was chromatographed over a silica gel column eluting with a gradient of CH_2Cl_2–EtOAc (10:0, 95:5, 90:10, 80:20, 70:30, 1:1, 0:10) to yield **10** (30 mg).

3.4. Compound *1*

3.4.1. 3-Acetoxy-8-hydroxy-2-methylanthraquinone (*1*)

Orange powder; mp 167–168 °C; UV (MeOH), λ_{max} (log ε): 216 (3.11), 226 (3.05), 262 (3.51), 336 (2.19), 407 (2.49) nm; IR (KBr) 3047, 2956, 1757, 1670, 1635, 1596, 1299 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 500 and 125 MHz resp.) see Table 1; HR-ESI-MS (pos. mode) m/z 297.0762 $[M+H]^+$ (calcd. for $C_{17}H_{13}O_5$: 297.0763); ESI-MS (pos. mode) m/z (rel. int.) 255 $[M-OCOCH_3]^+$, 100), 297 ($[M+H]^+$, 94), 298 ($[M+2H]^+$, 23), 319 ($[M+Na]^+$, 17).

A solution of hydroxysesamone (**3**) (18 mg) in formic acid (2.0 mL) was heated on a steam-bath for 2 h. The mixture was poured into ice and the solid formed was crystallized from *n*-hexane–AcOEt to give **11** as red amorphous powder (15 mg).

Tectograndone (**8**) (30 mg) was dissolved in pyridine (3 mL) and acetic anhydride (3 mL) and stirred at room temperature for 24 h. Ten milliliters of water were added to the mixture and stirred for 30 min. Extraction with CH_2Cl_2 and purification over a silica gel column with *n*-hexane–AcOEt (7:3) as solvent gave acetyltectograndone (**12**) (10 mg).

3.4.2. 6,9-Dihydroxy-2,2-(dimethyldihydropyrano)-3,4-dihydro-2H-benzo[g]chromene-5,10-dione (*11*)

Red powder; mp 155–156 °C; IR (KBr) 2972, 2947, 1600, 1670, 1461, 1288, 829 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ (ppm) 12.75 (1H, s, 6-OH), 12.25 (1H, s, 9-OH), 7.20 (1H, d, J = 9.3, H-8), 7.15 (1H, d, J = 9.3, H-7), 2.61 (2H, t, J = 13.0, H-4), 1.83 (2H, t, J = 13.0, H-3), 1.44 (6H, s, 11-CH_3/12-CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 187.7 (C-10), 182.7 (C-5), 157.8 (C-9), 156.6 (C-6), 155.1 (C-10a), 130.1 (C-8), 127.9 (C-7), 120.8 (C-4a), 111.4 (C-5a), 110.6 (C-9a), 78.7 (C-2), 31.2 (C-3), 26.5 (C-11/C-12), 16.4 (C-4); ESI-MS m/z (rel. int): 297 $[M+Na]^+$ (100), 441 (9), 571 $[2M+Na]^+$ (2).

3.4.3. Acetyltectograndone (*12*)

Amorphous solid; ^1H NMR (600 MHz, $CDCl_3$): δ (ppm) 13.79 (1H, s, 5-OH), 12.97 (1H, s, 12-OH), 12.54 (1H, s, 8′-OH), 11.60 (1H, s, 5′-OH), 8.28 (1H, s, H-7), 7.68 (1H, d, J = 9.6, H-6′), 7.31 (1H, s, H-9), 7.25 (1H, d, J = 9.6, H-7′), 6.74 (1H, d, J = 10.0, H-4), 5.75 (1H, d, J = 10.0, H-3), 2.52 (3H, s, 8-Me), 2.21 (3H, s, 2′-CH_3OCO), 1.53 (3H, s, 2-Me), 1.52 (3H, s, 2-Me); ^{13}C NMR (150 MHz, $CDCl_3$): δ 187.5 (C-4′), 187.0 (C-1′), 185.0 (C-6), 182.7 (C-11), 168.0 ($OCOCH_3$), 158.0 (C-8′), 157.6 (C-5′), 156.1 (C-5), 151.4 (C-12a), 149.3 (C-12), 145.4 (C-8), 138.3 (C-9), 135.5 (C-2′), 132.5 (C-10), 132.0 (C-3), 131.9 (C-6′), 131.1 (C-10a), 129.9 (C-7), 128.0 (C-7′), 126.4 (C-3′), 126.1 (C-6a), 116.8 (C-4a), 115.4 (C-4), 112.9 (C-11a), 111.0 (C-4′a), 79.4 (C-2), 28.7 (2-CH_3), 28.6 (2-CH_3), 22.1 (8-CH_3); ESI-MS m/z (rel. int): 316 (5), 413 (100), 441 (20), 585 (5), 605 (15), 631 (10), 647 (46), 669 (5), 803 (81).

3.5. In vitro *antiplasmodial assay*

The culture of *P. falciparum* strains was carried out as previously described by using the method of Trager and Jensen with some modifications (Zofou et al., 2011b). The ethyl acetate extract, compounds **1**, **2**, **3**, **8**, **9**, **11** and **12** were evaluated *in vitro* for their activity against the multidrug-resistant Dd2 strain of *P. falciparum*. Drug sensitivity assay was carried out using the parasite lactate dehydrogenase assay, as previously reported (Zofou et al., 2011b).

3.6. *Cytotoxicity test for crude extract and compounds*

The cytotoxicity of pure compounds was investigated against LLC-MK2 (ATCC, USA) monkey kidney epithelial cells according to the procedure described (Malebo et al., 2009; Mosmann, 1983; Zofou et al., 2011b).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytol.2014.01.010.

References

Andrade-Neto, V.F., Goulart, M.O.F., Filho, J.F.S., Silva, M.J., Pinto, M.C.F.R., Pinto, A.V., Zalis, M.G., Carvalho, L.H., Krettli, A.U., 2004. Antimalarial activity of phenazines from lapachol, β-lapachone and its derivatives against *Plasmodium falciparum* in vitro and *Plasmodium berghei* in vivo. Bioorg. Med. Chem. Lett. 14, 145–1149.

Aguinaldo, A.M., Ocampo, O.P.M., Bowden, B.F., Gray, A.I., Peter, G., 1993. Tectograndone, an anthraquinone-naphtoquinone pigment from the leaves of *Tectona grandis*. Phytochemistry 4, 933–935.

Da Silva, V.C., Geraldo de Carvalho, M., Alves, A.N., 2008. Chemical constituents from leaves of *Palicourea coriaceae* (Rubiaceae). J. Nat. Med. 62, 356–357.

Eyong, K.O., Folefoc, G.N., Kuete, V., Beng, V.P., Krohn, K., Hussain, H., Nkengfack, A.E., Saeftel, M., Sarite, S.R., Hoerauf, A., 2006. Newbouldiaquinone A: a naphthoquinone-anthraquinone ether coupled pigment, as a potential antimicrobial and antimalarial agent from *Newbouldia laevis*. Phytochemistry 67, 605–609.

Fotie, J., 2006. Quinones and malaria. Antiinflamm. Antiallergy Agents Med. Chem. 5, 357–366.

Fujiwara, A., Mori, T., Iida, A., Ueda, S., Hano, Y., Nomura, T., Tokuda, H., Nishino, H., 1998. Antitumor-promoting naphthoquinones from *Catalpa ovata*. J. Nat. Prod. 61, 629–632.

Hasan, A.F.M.F., Furumoto, T., Begum, S., Fukui, H., 2001. Hydroxysesamone and 2,3-epoxysesamone from roots of *Sesamum indicum*. Phytochemistry 58, 1225–1228.

Hua, D.H., Havens, J., Perchellet, E.M., Wang, Y., Lou, K., Perchellet, J.P., Iwamoto, T., 2004. Synthesis and *in vitro* antitumor activity of substituted anthracene-1,4-diones. Tetrahedron 60, 10155–10163.

Lacret, R., Varela, R.M., Molinillo, J.M.G., Nogueiras, C., Macias, F.A., 2011. Anthratectone and naphtotectone, two quinones from bioactive extracts of *Tectona grandis*. J. Chem. Ecol. 37, 1341–1348.

Lacret, R., Varela, R.M., Molinillo, J.M.G., Nogueiras, C., Macias, F.A., 2012. Tectonoelins, new norlignans from bioactive extract of *Tectona grandis*. Phytochem. Lett. 5, 382–386.

Lee, H.S., 2003. Inhibitory effects of quinizarin isolated from *Cassia tora* seeds against human intestinal bacteria and aflatoxin B_1 biotransformation. J. Microbiol. Biotechnol. 13, 529–536.

Macias, F.A., Lacret, R., Varela, R.M., Nogueiras, C., Molinillo, J.M.G., 2008. Bioactive apocarotenoids from *Tectona grandis*. Phytochemistry 69, 2708–2715.

Malebo, H.M., Tanja, W., Cal, M., Swaleh, S.A.M., Omolo, M.O., Hassanali, A., 2009. Anti-plasmodial, anti-trypanosomal, anti-leishmanial and cytotoxicity activity of selected Tanzanian medicinal plants. Tanzan J. Health Res. 11, 226–234.

Mahmoudi, N., de Julian-Ortiz, J.V., Cicerone, L., Galvez, J., Mazier, D., Danism, M., Derouin, F., Garcia-Domenech, R., 2006. Identification of new antimalarial drugs by linear discriminant analysis and topological virtual screening. J. Antimicrob. Chemother. 57, 489–497.

Matsumoto, T., Ichihara, A., Yanagiy, M., Yuzawa, T., Sannai, A., Oikawa, H., Sakamura, S., Eugster, C.H., 1985. Two new syntheses of the pyranojuglone pigment α-caryopterone. Helv. Chim. Acta 68, 2324–2331.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.

Mohammed, Y., Javed, K., Hasnain, M., 1991. α-Amyrin derivatives from *Corchorus depressus*. Phytochemistry 30, 1989–1992.

Osman, C.P., Ismail, N.H., Ahmad, R., Ahmat, N., Awang, K., Jaafar, F.M., 2010. Anthraquinones with antiplasmodial activity from the roots of *Rennellia elliptica* Korth. (Rubiaceae). Molecules 15, 7218–7226.

Pradeep, K.G., Pahup, S., 2004. A naphthoquinone derivative from *Tectona grandis* (Linn). J. Asian Nat. Prod. Res. 6, 237–240.

Ragasa, C.Y., Lapina, M.C., Lee, J.J., Mandia, E.H., Rideout, J.A., 2008a. Secondary metabolites from *Tectona philippinensis*. Nat. Prod. Res. 9, 820–824.

Ragasa, C.Y., Tepora, M.M., Espinelli, D.H., Mandia, E.H., Rideout, J.A., 2008b. Chromomoric acid derivatives from *Tectona philippinensis*. J. Nat. Prod. 71, 701–705.

Rajuri, A., Rao, K.N.V., Banji, D., Chaithanya, R.K., 2010. A review on *Tectona grandis* Linn: chemistry and medicinal uses (family: Verbenaceae). Herbal Tech Industry 1, 6–9.

Sarr, S.O., Perrotey, S., Fall, I., Ennahar, S., Zhao, M., Diop, Y.M., Candolfi, E., Marchioni, E., 2011. *Icacina senegalensis* (Icacinaceae), traditionally used for the treatment of malaria, inhibits *in vitro Plasmodium falciparum* growth without host cell toxicity. Malar. J. 10, 85, http://dx.doi.org/10.1186/1475-2875-10-85.

Shukla, N., Kumar, M., Akanksha, A.G., Rahuja, N., Singh, A.B., Srivastava, A.K., Rajendran, S.M., Maurya, R., 2010. Tectone, a new antihyperglycemic anthraquinone from *Tectona grandis* leaves. Nat. Prod. Commun. 5, 427–431.

Singh, N., Shukla, N., Singh, P., Sharma, R., Rajendran, S.M., Maurya, R., Palit, G., 2010. Verbascoside isolated from *Tectona grandis* mediates gastric protection in rats via inhibiting proton pump activity. Fitoterapia 81, 755–761.

Sittie, A.A., Lemmich, E., Olsen, C.E., Hviid, L., Kharazmi, A., Nkrumah, F.K., Christensen, S.B., 1999. Structure–activity studies: in vitro antileishmanial and antimalarial activities of anthraquinones from *Morinda lucida*. Planta Med. 65, 259–261.

Sumthong, P., Damveld, R.A., Choi, Y.H., Arentshorst, M., Ram, A.F.J., Van den hondel, C.A.M.J.J., Verpoorte, R., 2006. Activity of quinones from Teak (*Tectona grandis*) on fungal cell wall stress. Planta Med. 72, 943–944.

Sumthong, P., Romero-Gonzalez, R.R., Verpoorte, R., 2008. Identification of anti-wood rot compounds in Teak (*Tectona grandis* L. f) Sawdust extract. J. Wood Chem. Technol. 28, 247–260.

Yang, Y.C., Lim, M.Y., Lee, H.S., 2003. Emodin isolated from *Cassia obtusifolia* (Leguminosae) seed shows larvicidal activity against three mosquito species. J. Agric. Food Chem. 51, 7629–7631.

Warrier, P.S., 1994. Indian Medicinal Plants, 1st ed. Orient Longman Pvt. Ltd., New Delhi, India, pp. 245–248.

Zofou, D., Kowa, T.K., Wabo, H.K., Tane, P., Titanji, V.P.K., 2011a. *Hypericum lanceolatum* (Hypericaceae) as a potential source of new anti-malarial agents: a bioassay-guided fractionation of the stem bark. Malar. J. 10, 1–7.

Zofou, D., Tene, M., Ngemenya, M.N., Tane, P., Titanji, V.P.K., 2011b. *In vitro* antiplasmodial activity and cytotoxicity of extracts of selected medicinal plants used by traditional healers of Western Cameroon. Malar. Res. Treat. 2011 , http://dx.doi.org/10.4061/2011/561342, Article ID: 561342, 6 pages.