Pachypodostyflavone, a New 3-methoxy Flavone and Other Constituents with Antifilarial Activities from the Stem Bark of *Duguetia staudtii*

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Bibliography

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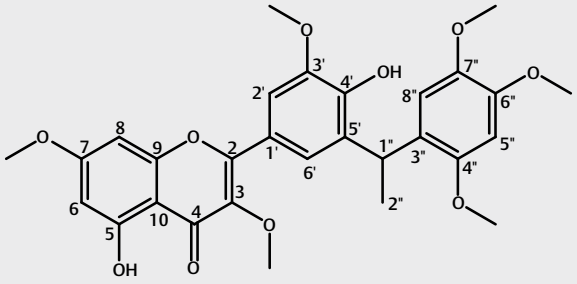
ABSTRACT

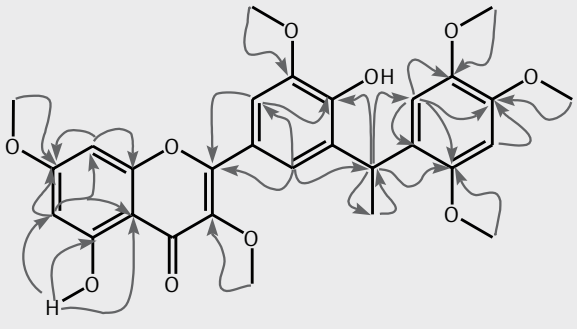
A new flavone derivative named pachypodostyflavone (**1**), along with 8 known compounds (**2–9**) and a mixture of *β*-sitosterol and stigmasterol were isolated from the stem bark of *Duguetia staudtii* (Annonaceae), based on a bioassay-guided fractionation. Their structures were determined using high-resolution mass spectrometry and NMR spectroscopic data, as well as by comparison with the literature values of their analogs. Selected isolated compounds were evaluated for their *in vitro* antifilaricidal activities on *Onchocerca ochengi* microfilariae and adult worms. Inhibition of motility was evaluated spectroscopically on microfilaria and adult male worms. Viability was determined on adult female worms by the MTT/Formazan assay. Auranofin at 10 µM and 2 % DMSO were used as positive and negative controls, respectively. Compounds **1** and **7** showed potent anti-onchocerca activities with 100 % activity at 250 µg/mL on both *O. ochengi* adult male and female worms, while compound **5** displayed 100 % activity at 30 µg/mL.

Introduction

Duguetia staudtii (Engl. & Diels) Chatrou (formerly known as *Pachypodanthium staudtii*) belongs to the Annonaceae family, and it is a large-bole tree measuring up to 40 m in height with a straight cylindrical diameter of ca. 70 cm and long narrow leaves. It is widely distributed throughout the West and Central African regions [1], ranging from Sierra Leone to Zaire and Cameroon in the dense evergreen forest [2]. In folk medicine, various parts of this plant are used for the treatment of several human ailments including chest pain, bronchitis, gastrointestinal troubles, edemas, and cancer [3, 4]. Previous chemical studies of plants of the genus *Duguetia* resulted in the discovery of a variety of alkaloids [5–8], aromatic compounds [9], flavonoids [10], bisnorlignans [1], 2,4,5-trimethoxystyrene [11], and triterpenoids [12]. Some of these compounds exhibited interesting biological activities including potent antivirals [1], in addition to anti-inflammatory and urease inhibitor activities that were recently reported [12]. Concerning our previous chemotaxonomic studies on this plant species, we also reported that lignans and flavonoids could be considered as chemotaxonomic markers for the genus. In continuation of our search for bioactive compounds from Cameroonian medicinal plants [13–15], we have investigated the bark of *D. staudtii* for its minor secondary metabolites. Herein, we report the bioassay-guided fractionation, as well as the structural elucidation of a new flavone and the antifilarial activities of the selected isolated compounds. Onchocerciasis remains one of the serious illnesses particularly in sub-Saharan Africa, and it is among the neglected tropical diseases [16]. The only currently approved drug, ivermectin, which, unfortunately, is only microfilaricidal has serious side effects on humans co-infected with high loads of Loa loa [17].



▶ **Fig. 1** Structure of the new compound (**1**).



▶ **Fig. 2** Key HMBC correlations of compound **1**.

Results and Discussion

The CH_2Cl_2/MeOH (1:1, *v*/*v*) extract of the stem bark of *D. staudtii* afforded a residue that was subjected to repeated column chromatography to give several fractions that were further purified to yield pachypodostyflavone (**1**) (▶ **Fig. 1**), along with 8 known compounds including 5-hydroxy-7,3′,4′-trimethoxyflavone (**2**) [18], pachypodol (**3**) [19], pachypostaudin B (**4**) [1], costunolide (**5**) [20], *β*-amyrin (**6**) [21], isocorypalmine (**7**) [8], govanine (**8**) [22], and octacosanoic acid (**9**) [23], in addition to a mixture of *β*-sitosterol and stigmasterol [24]. The known secondary metabolites were characterized by comparison with physical and NMR data reported in the literature. However, the sesquiterpene lactone, costunolide (**5**) was isolated for the first time from the Annonaceae family. Thus this study provides additional information on chemotaxonomic markers on the Annonaceae family.

Compound **1** was obtained as an orange solid (dec. 181–183 °C). Its molecular formula $C_{29}H_{30}O_{10}$ was established from the positive ion mode HR-ESI-MS, which showed the quasi-molecular ion peak $[M+Na]^+$ at *m*/*z* 561.1737 (calcd. for $C_{29}H_{30}O_{10}Na$, 561.1731) (**Fig. S1**). The IR spectrum showed characteristic vibration bands for hydroxyl group (3660 cm^{-1}), a conjugated carbonyl group (1738 cm^{-1}), and aromatic double bonds (1655 and 1595 cm^{-1}), while the UV maxima absorption bands at λ_{max} 354 and 269 were suggestive of a flavone skeleton [25]. The ^1H NMR spectrum (▶ **Table 1**; **Fig. S2**) exhibited a signal of a chelated hydroxyl group (δ_H 12.69), the resonances of aromatic protons observed in the deshielded region (δ_H 7.70–6.40), and the signals of 2 sets of aliphatic protons in the up-field region at δ_H 4.85–1.60 which included 6 sharp 3-proton singlets at δ_H 3.98–3.80 for methoxyl groups. The ^{13}C NMR (▶ **Table 1**; **Fig. S3**) displayed 29 carbon signals that were sorted by DEPT and HSQC experiments into 1 methyl, 6 methoxyl, 7 methines, and 15 quaternary carbons, including a characteristic flavone carbonyl group at δ_C 178.7 [26]. However, the aromatic proton signals were sorted based on their coupling constants into 3 separate benzene ring systems as follows: (a) The meta-coupled aromatic proton signals at δ_H 6.42 and 6.37 (1H each, *d*, 2.2 Hz, H-6, H-8) set the presence of a tetra-substituted benzene ring, characteristic for A-ring of flavones with the oxygenation at positions 5 and 7 [27]. Thus, the chelated hydroxyl group was attached at C-5 of the flavone skeleton, as illustrated by HMBC cross-peaks (▶ **Fig. 2**) observed between the proton signal at δ_H 12.69 (5-OH) with the carbon signals at δ_C 165.4 (C-7), 162.0 (C-5), 106.0 (C-10), and 97.7 (C-6). (b) The presence of another tetra-substituted benzene ring was also set by the meta-coupled aromatic proton signals at δ_H 7.69 and 7.52 (1H each, *d*, 2.0 Hz, H-6', H-2'), characteristic for B-ring in the flavone unit. The flavone moiety was further confirmed by HMBC correlations (▶ **Fig. 2**) observed between the proton signal at δ_H 7.69 (H-6') with the carbon signals at δ_C 156.4 (C-2), 146.0 (C-4'), 108.5 (C-6'), and 31.2 (C-1") and also between the proton at δ_H 7.52 (H-2') with the carbon signals at δ_C 156.4 (C-2), 146.0 (C-4'), and 121.4 (C-2'). (c) The 1,2,4-trioxygynated

▶ **Table 1** 1H and 13C NMR data of compound 1 ($CDCl_3$).

C and H no.	^{1}H (500 MHz)	^{13}C (125 MHz)	HMBC
	δ_H (*mult*., *J* in Hz)	δ_C type	H→C
2	–	156.4 C	
3	–	146.5 C	
4	–	178.7 C	
5	–	162.0 C	
6	6.37 (*d*, 2.2)	97.7 CH	C-7; C-10; C-8
7	–	165.4 C	
8	6.42 (*d*, 2.2)	92.2 CH	C-7; C-9
9	–	156.7 C	
10	–	106.0 C	
1’	–	121.3 C	
2’	7.52 (*d*, 2.0)	108.5 CH	C-2
3’	–	138.8 C	
4’	–	146.0 C	
5’	–	132.3 C	
6’	7.69 (*d*, 2.0)	121.4 CH	C-2; C-2’; C-1”
1”	4.84 (*q*, 7.2)	31.2 CH	C-4’; C-4”; C2”
2”	1.63 (*d*, 7.2)	19.8 CH_3	C-1”
3”	–	125.2 C	
4”	–	150.9 C	
5”	6.56 (*s*)	98.2 CH	C-4”
6”	–	148.2 C	
7”	–	143.2 C	
8”	6.84 (*s*)	112.5 CH	C-3”; C-4”; C-6”; C-7”
3-OCH_3	3.98 (*s*)	56.2 CH_3	C-3
7-OCH_3	3.90 (*s*)	55.8 CH_3	C-7
3’-OCH_3	3.81 (*s*)	60.0 CH_3	C-3’
4”-OCH_3	3.83 (*s*)	56.8 CH_3	C-5”
6”-OCH_3	3.89 (*s*)	56.1 CH_3	C-6”
7”-OCH_3	3.83 (*s*)	56.8 CH_3	C-7”
5-OH	12.69 *s*)	–	C-5; C-6; C-10
4’-OH	6.41 (*s*)	–	C-4’; C-5’; C-3’

1-phenylethyl group (a styrene derivative moiety) was deduced from the signals of 2 aromatic proton singlets at δ_H 6.84 and δ_H 6.56 (1H each, H-8”, H-5”), along with 2 sets of aliphatic protons at δ_H 4.84 (1H, *q*, 7.2 Hz, H-1”) and 1.63 (3H, *d*, 7.2 Hz, H-2”), which were further supported in the ^{13}C NMR and HSQC spectra with resonances at δ_C 112.5 (C-8”), 98.2 (C-5”), 31.2 (C-1”), and 19.8 (C-2”), respectively. Thus, the proton signal at δ_H 4.84 (H-1”) displayed HMBC correlations with the carbon signals at δ_C 150.9 (C-4”), 146.0 (C-4’), 132.3 (C-5’), 125.2 (C-3”), 121.4 (C-2’), 112.5 (C-8”), and 19.8 (C-2”), which therefore suggested that the linkage was *via* the C-5’ position of B-ring of the flavone unit. Additionally, both proton signals at δ_H 6.84 (H-8”) and 6.56 (H-5”) displayed HMBC cross peak correlations with the carbon signals at δ_C 150.9 (C-4”), 148.2 (C-6”), 143.2 (C-7”), 125.2 (C-3”), and 31.2 (C-1”), which further confirmed the presence of the 1,2,4-trioxygynated 1-phenylethyl group. Furthermore, the ^{1}H NMR spectrum in combination with the ^{13}C NMR and HSQC spectra displayed 6 sharp 3-proton singlets at $\delta_{H/C}$ 3.98/56.2, 3.90/55.8, 3.89/56.1, 3.83/56.9, 3.83/56.8, and 3.81/60.0, which suggested the presence of 6 methoxyl groups. These methoxyl groups were respectively attached at C-3 (146.5), C-7 (165.4), C-6” (148.2), C-4” (150.9), C-7” (143.2), and C-3’ (138.8) as illustrated by HMBC correlations (▶ **Fig.2**). Based on the above evidence, the structure of **1** was elucidated as 5-hydroxy-[4-hydroxy-3-methoxy-5-(1-(2,4,5-trimethoxyphenyl)ethyl)]flavone and assigned a trivial name of pachypodostyflavone (▶ **Fig. 1**). The proposed structure was fully supported (see ▶ **Table 1**) by HMBC, DEPT, and COSY spectra. Key HMBC correlations of **1** are illustrated in ▶ **Fig. 2**.

The CH_2Cl_2/MeOH crude extract of *D. staudtii* at 250 µg/mL was 100 % active on microfilariae (mf) (*i. e.*, it completely inhibited the mf motility at this concentration) (see ▶ **Table 2**). The same result was also observed with the extract on *Onchocerca ochengi* adult female worms (*i. e.*, the extract completely killed the worms at 250 µg/mL). These results led to the fractionation of the crude extract as described below in the extraction and isolation section.

At 250 µg/mL, the soluble-methanol extract B and fraction A_1 showed 100 % activity on both the mf and adult male worms, whereas those activities were not determined for extract B.

Since fraction A_1 showed 100 % activity on the mf and adult male worms, it was further fractionated to obtain pure compound **5**, which also showed in primary screens 100 % activity at 250 µg/mL, on both adult male and female worms. Interestingly, this compound was further assessed at 30 µg/mL against the positive control (auranofin) for its antifilarial activity, and it was found to possess 100 % activity. Therefore, further modification of its structure might lead to the development of new antifilarial drugs. Compounds **1** and **7**, which were also evaluated, showed potent anti-onchocerca activities with 100 % activity at 250 µg/mL on both *O. ochengi* adult male and female worms, whereas compound **3** was moderately active on the worms as compared to the positive control, auranofin (50 % activity) (see ▶ **Table 2**). Therefore, this study supports *D. staudtii* as a source of new antifilarial secondary metabolites.

Materials and Methods

General experimental procedures

HR-ESI-MS spectra were recorded on a QTOF-MS Spectrometer (QTOF Bruker) equipped with a HESI source. The spectrometer operated in positive mode (mass range: 100–1500, with a scan rate of 1.00 Hz) with automatic gain control to provide high-accuracy mass measurements within 0.40 ppm deviation using Na Formate as calibrant. The ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, on Bruker DRX 500 NMR spectrometers (Bruker Corporation) in $CDCl_3$ or CD_3OD. Chemical shifts (δ) were reported in ppm using tetramethylsilane (TMS) (Sigma-Aldrich) as an internal standard, while coupling constants (*J*) were measured in Hertz (Hz). Column chromatography (width 5.5–8.5 cm; depth 25.4 cm) was carried out on silica gel 230–400 mesh and 70–230 mesh (Merck). IR spectra were recorded with an Alpha spectrometer (Bruker) by attenuated total reflection (ATR) technique on a diamond crystal. TLC was performed on Merck precoated silica gel 60 F_{254} aluminum foil (Merck), and spots were detected using diluted sulfuric acid (50 % [v/v]) spray reagent before heating. The

▶ **Table 2** *Onchocerca ochengi* microfilariae primary screen.

Sample codes and compounds	Conc. (µg/mL)	% inhibition of mf motility										Remarks
		Time (h)										
		24		48		72		96		120		
E_1	250	100	100	100	100	100	100	100	100	100	100	✓
E_2	250	100	100	100	100	100	100	100	100	100	100	✓
E_3	250	100	100	100	100	100	100	100	100	100	100	✓
A_1	250	100	100	100	100	100	100	100	100	100	100	✓
1	250	100	100	100	100	100	100	100	100	100	100	✓
3	250	0	0	50	50	50	50	50	50	50	50	~
5	250	50	50	100	100	100	100	100	100	100	100	✓
7	250	0	0	25	25	50	50	50	50	50	50	~
Neg. control	30	0	0	0	0	0	0	0	0	0	0	✓
Pos. control (Amocarzine)	30	50	50	50	50	50	50	100	100	100	100	✓
Pos. control (Auranofin)	10	50	50	50	50	50	50	100	100	100	100	✓

E_1 = DCM/MeOH extract; E_2 = soluble-DCM extract; E_3 = soluble-MeOH extract; A_1 = n-Hex fraction; ✓ = active; ~ = moderately active.

molecular composition of the isolated compounds was identified by exact mass determinations. All reagents used were of analytical grade. Melting points were measured using "Melting Point Meter" type MPM-H2, N° 0310148.

Plant material

The stem bark of *D. staudtii* Engl & Diels was collected in the Dja forest at Lomié-Bertoua (GPS coordinates provided by system WGS8: altitude 665 m, latitude N 4°34'38", longitude E 13°41'04") in the East region of Cameroon, in July 2016. The botanical identification was done by Mr. Victor Nana, a botanist at the National Herbarium of Cameroon where a voucher specimen was deposited under the number 52711/HNC.

Extraction and bioguided isolation

The air-dried and powdered stem bark (~5.9 kg) of *D. staudtii* was extracted 2 times (20 L each) with a mixture of dichloromethane/methanol (1:1, *v/v*) at room temperature for 72 and 24 h, respectively. The extract was filtered, and evaporation of the solvent under vacuum afforded a brown crude residue (382 g). This extract showed 100% activity at 250 µg/mL on mf, as described in the bioassay section below. The same result was also observed with the extract on *O. ochengi* adult female worms. Therefore, a part of the crude residue (380 g) was successively fractionated by a vacuum liquid chromatography (VLC) with dichloromethane (DCM) and MeOH to give soluble-DCM (**A**, 210 g) and methanol (**B**, 171 g) extracts. These 2 extracts **A** and **B** were also separately assessed at 250 µg/mL for their antifilarial activity on *O. ochengi* mf, and this resulted in 100% activity on mf of both extracts.

The DCM fraction of the stem barks was once more subjected to VLC over silica gel (Merck, 230–400 mesh) eluting with n-Hex/EtOAc (ranging from 0 to 100% of EtOAc, *v/v*), EtOAc, and EtOAc/MeOH, in increasing order of polarity. Sixty 1000 mL-fractions were collected and combined according to their TLC profiles to give 8 main fractions (A_{1-8}). The study of these fractions led to the isolation of 13 compounds that were fully characterized.

Part of extract **A** (207.0 g) was subjected to flash silica gel (230–400 mesh) column chromatography (width 5.5–8.5 cm; depth 25.4 cm) using a stepwise gradient of n-Hex/EtOAc (ranging from 0 to 100% of EtOAc, *v/v*). Afterward, a total of 150 fractions (fr_1–fr_{150}) of ca. 500 mL each was collected and combined based on TLC analysis to yield 8 main fractions (A_1–A_8). These fractions were also separately assessed for their antifilaricidal activity on any of the 3 parasite stages (mf, *O. ochengi* adult male and female worms) used in the bioassay for further fractionation. Fraction A_1 (fr_1–fr_{24}: 28.0 g; ~2000 mL) obtained with pure n-Hex as eluent was 100% active at 250 µg/mL on the 3 parasite stages. Therefore, it was later subjected to a silica gel column chromatography (CC) and eluted with n-Hex to give a yellow crystal **5** (125 mg; mp: 109–111 °C). Fraction A_2 (fr_{25}–fr_{39}: 30.0 g; ~3500 mL) obtained with n-Hex/EtOAc (9:1–8:2, *v/v*) was chromatographed over silica gel CC and eluted with a gradient of n-Hex/EtOAc (9.75:0.25–7.5:2.5, *v/v*) to yield a white amorphous powder **6** (5.4 mg), a white powder **9** (18.4 mg), and a mixture of sterols (210.8 mg).

Fraction **3** (fr_{40}–fr_{61}: 25.8 g; ~4000 mL, n-Hex/EtOAc 7:3, *v/v*) was subjected to silica gel column chromatography and eluted with a mixture of n-Hex/EtOAc in increasing order of polarity to yield a fluorescent yellow crystal **3** (10.2 mg; m.p.: 167–169 °C), compound **4** (75.0 mg), and compound **8** (6.5 mg). Fraction A_4 (fr_{62}–fr_{76}: 31.0 g, ~5000 mL, n-Hex/EtOAc 6:4, *v/v*) was eluted over silica gel CC with the same solvent system to afford compound **6** (7.0 mg). Fraction A_5 (fr_{77}–fr_{86}: 25.0 g, ~3500 mL n-Hex/EtOAc 1:1, *v/v*) was found to be a complex mixture of compounds and therefore was not further investigated. Fraction A_6 (fr_{87}–fr_{103}: 32.0 g, n-Hex/EtOAc 4:6) was purified over silica gel CC and eluted with a gradient of n-Hex/EtOAc (7:3, *v/v*) to afford an orange needle-shaped crystal **1** (77.7 mg) and a yellow power **2** (8.5 mg). Fractions A_7 (fr_{104}–fr_{124}: 19.0 g, ~1500 mL; n-Hex/EtOAc 3:7) and A_8 (fr_{125}–fr_{150}:

13.0 g, ~5000 mL; pure EtOAc) were gummy and were not further investigated.

Part of the soluble-methanol extract **B** (169.0 g) was also subjected to flash silica gel column chromatography, using a gradient of EtOAc in n-hexane, then a mixture of EtOAc/MeOH of increasing order of polarity. Afterward, 78 fractions of ca. 500 mL each were collected and combined based on TLC analysis into 5 main fractions (B_1–B_5). Only fraction B_1 (fr_1–fr_{32}: 38.0 g) obtained with n-Hex/EtOAc (6:4, *v*/*v*) was chromatographed over silica gel CC and eluted with a gradient of n-Hex/EtOAc (7:3–0:10, *v*/*v*) to afford a yellow amorphous powder **7** (7.5 mg).

Pachypodostyflavone (**1**): Orange solid ($CHCl_3$); dec. 181–183 °C; $[\alpha]_D^{20}$ 0 (*c* 0.5; CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 229 (1.08), 269 (0.73), 293 (0.56), 354 (0.85) nm; IR (KBr) ν_{max} 3660, 1738, 1655, 1595, 1489, 1345, 1205, 1038 cm^{-1}; ^1H and ^{13}C NMR data, see ▶ **Table 1**; HR-ESI-MS: *m*/*z* 561.1737 $[M + Na]^+$ (calcd. for $C_{29}H_{30}O_{10}Na$, 561.1731).

Antifilarial assay

Preparation of mammalian cells

LLC-MK2 cells obtained from American Type Culture Collection (ATCC) were proliferated in a T-25 culture flask (Corning) in CCM at 37 °C in 5 % CO_2 humidified air. The cells were grown in 96-well plates until they became fully confluent and served as feeder layers for the mf assays.

Isolation of O. ochengi worms and culture conditions

O. ochengi was used for *in vitro* assays as it is the closest known relative to *O. volvulus*. The worms were isolated as previously described by Cho-Ngwa et al. [28]. RPMI-1640 supplemented with L-glutamine, 5 % newborn calf serum, and 2 × antibiotic-antimycotic (penicillin/streptomycin amphotericin B) was the culture medium. For adult worm assays, the worm masses were incubated overnight in a 2 mL culture medium in 12-well culture plates at 37 °C and 5 % CO_2 in humidified air. It is noteworthy that male worms usually emerge from the worm masses while female worms remain in them.

For the mf assays, the highly motile mf that emerged from the skin slivers were concentrated by centrifugation (400 *g*, 10 min), re-suspended, and distributed into wells (15 mf/100 µL of CCM/well) of 96-well culture plates containing fully confluent LLC-MK2 cell layer. The viability and sterility of cultures were monitored for 24 h before the addition of extracts, compounds, and/or control compounds.

Screening on O. ochengi worms

Primary screens were done to eliminate inactive fractions. For the adult worm assay, the worms were treated in triplicates with either fractions at 250 µg/mL in 4 mL of CCM or auranofin (Origin: Enzo Life Science. Purity 99.9 %), and amocarzine (Origin: Ciba-Geigy Limited. Purity 99.9 %) at 10 µM (serving as positive control) or 2 % DMSO (negative control). Pure compounds were tested at 30 µg/mL. The viability of worms was assessed after an incubation period of 5 days. It is noteworthy that 2 % DMSO was shown to be safe for worms.

For the mf assays, primary screens of fractions screens were done in duplicates at 250 µg/mL to eliminate inactive fractions. The mf was incubated and viability assessed microscopically daily for 5 days. Pure compounds were tested at 30 µg/mL.

Adult male worms' viability was assessed by evaluation of worm motility using an inverted microscope and viability scores ranging from 100 % (complete inhibition of motility), 75 % (only head or tail of worm shaking occasionally), 50 % (whole worm motile, but sluggishly), 25 % (only little change in motility), to 0 % (no observable change in motility) were assigned. Also, adult female worm viability was assessed biochemically by visual estimation of the percentage inhibition of formazan (blue color) formation following incubation of the worm masses in 500 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide dissolved in ICM (MTT solution, 0.5 mg/mL) for 30 min [29]. Viability scores assigned ranged from 100 %, parasite killing (no blue formazan coloration seen), 90 %, 75 %, 50 %, 25 %, to 0 % (entire worm appears blue as in negative control).

Mf viability scores were assigned based on percentage motility, using the following key: 100 % (all mf immotile), 75 % (only head or tail of mf shaking, occasionally), 50 % (the whole body of mf motile but sluggishly or with difficulties), 25 % (almost vigorous motility), and 0 % (vigorous motility).

A fraction/compound was considered active if there was ≥ 90 % inhibition of male worm motility or formazan formation; moderately active if there was 50–89 % inhibition of male worm motility or formazan formation and inactive if there was < 50 % inhibition of male worm motility or formazan formation. All experiments were repeated at least once to confirm activity.

Conclusion

The bioguided phytochemical study of the stem bark of *D. staudtii* (Annonaceae) yielded a new flavone derivative (**1**) and 8 known compounds, including the first-ever isolated sesquiterpene lactone named costunolide (**5**) from the plant family. However, compounds **1** and **5** showed potent anti-onchocerca properties with 100 % activity at 250 and 30 µg/mL, respectively, on both *O. ochengi* adult male and female worms. Therefore, this study supports *D. staudtii* as a source of new antifilarial secondary metabolites.

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Conflict of Interest

All the authors in this article have made significant contributions to the preparation of this manuscript. We certify that all results presented in this manuscript are authentic. Therefore we declare no conflict of interest.

References

[1] Ngadjui BT, Lontsi D, Ayafor JF, Sondengam BL. Pachypophyllin and pachypostaudins A and B: three bisnorlignans from *Pachypodanthium staudtii*. Phytochemistry 1989; 28: 231–234

[2] Dieter N. African Ethnobotany: Poisons and Drugs: Chemistry, Pharmacology and Toxicology. CRC press; 1996: 57–59

[3] Bouquet A. "Féticheurs et Médecines traditionnelles du Congo-Brazzaville, vol. 36 Mémoire" O.R.S.T.O.M., Paris; 1969 vol. 36 1969;

[4] Burkill HM. 2nd Ed. The Useful Plants of West Tropical Africa, vol. 1 Royal Botanic Garden, Kew; 1985

[5] Cavé A, Kunesch N, Leboeuf M, Bevalot F, Chiaroni A, Riche C. Alkaloids of Annonaceae.25. Staudine, a new isoquinoline alklaoids from *Pachypodanthium staudtii* Engl. and Dields. J Nat Prod 1980; 43: 103–111

[6] Sarpong K, Santra DK, Kapadia GJ, Wheeler JW. Alkaloidal constituents of *Pachypodanthium staudtii*. J Nat Prod 1977; 40: 616–617

[7] Bevalot F, Leboeuf M, Bouquet A, Cavé A. Annonacea alkaloids: alkaloids from the bark of the stem and roots of *Pachypodanthium confine* Engl. and Diels. Ann Pharm Fr 1977; 35: 65–72

[8] Bevalot F, Lebœuf M, Cavé A. Alkaloids of the Annonaceae part 21 alkaloids of Pachypodanthium staudtii. Plantes Med Phytother 1977; 11: 315–322

[9] Bevalot F, Leboeuf M, Cavé A. Pachysontol, a new aromatic compound from Pachypodanthium staudtii Engl. And Dields. Annonaceae. "Comptes Rendus Herbdomadaires des Seances de l'Academie des Sciences Series C" 19782 86: 405–408

[10] Leboeuf M, Cavé A, Bhaumik PK, Mukherjee B, Mukherjee R. The phytochemistry of the Annonaceae. Phytochemistry 1982; 21: 2783–2813

[11] Waterman PG. Chemical studies on Annonaceae- 2,4,5-trimethoxystyrene from *Pachypodanthium staudtii*. Phytochemistry 1976; 15: 347–347

[12] Ngouonpe WA, Mbobda WAS, Happi MG, Mbiantcha M, Tatuedom KO, Ali MS, Lateef M, Tchouankeu JC, Kouam SF. Natural products from the medicinal plant *Duguetia staudtii* (Annonaceae). Biochem Syst Ecol 2019; 83: 22–25

[13] Mountessou BYG, Tchamgoue J, Dzoyem JP, Tchuenguem RT, Surup F, Choudhary MI, Green IR, Kouam FS. Two xanthones and two rotameric (3→8) biflavonoids from the Cameroonian medicinal plant *Allanblackia floribunda* Oliv. (Guttiferae). Tetrahedron Lett 2018; 59: 4545–4550

[14] Tchamgoue J, Hafizur Md R, Tchouankeu JC, Kouam FS, Adhikari A, Hameed A, Green IR, Choudhary MI. Flavonoids and other constituents with insulin secretion activity from *Pseudartria hookeri*. Phytochem Lett 2016; 17: 181–186

[15] Happi MG, Kouam SF, Talontsi FM, Lamshöft M, Zühlke S, Bauer JO, Strohmann C, Spiteller M. Antiplasmodial and cytotoxic triterpenoids from the bark of the Cameroonian medicinal plant *Entandrophragma congoënse*. J Nat Prod 2015; 78: 604–614

[16] WHO First WHO report on neglected tropical diseases: Working to overcome the global impact of neglected tropical diseases 2010; 1–150

[17] Cupp EW, Mackenzie CD, Unnasch TR. Importance of ivermectin to human onchocerciasis: past, present, and the future. Res Rep Trop Med 2011; 2: 81–92

[18] Ersöz T, Harput ÜŞ, Saracoğlu İ, Çaliş İ. Phenolic compounds from *Scutellaria pontica*. Turk J Chem 2002; 26: 581–588

[19] Kim K, Kim JH, Jeong S, Choi YW, Choi HJ, Kim CY, Kim YM. Pachypodol, a methoxyflavonoid isolated from *Pogostemon cablin* Bentham exerts antioxidant and cytoprotective effects in HepG2 cells: possible role of ERK-Dependent Nrf2 activation. Int J Mol Sci 2019; 20: 1–14

[20] Rasul A, Parveen S, Ma T. Costunolide: a novel anti costunolide: a novel anti-cancer sesquiterpene lactone cancer sesquiterpene lactone cancer sesquiterpene lactone. Bangladesh J Pharmacol 2012; 7: 6–13

[21] Fingolo CE, Santos T de S, Filho MDMV, Kaplan MAC. Triterpene esters: natural products from *Dorstenia arifolia* (Moraceae).. Molecules 2013; 18: 4247–4256

[22] Mehra K, Garg HS, Bhakuni DS, Khanna NM. Alkaloids of *Corydalis govaniana* Wall.: Isolation & structure of three new tetrahydroprotoberberine alkaloids, corygovanine, govadine & govanine & of a known phtalideisoquinoline base, bicuculline. Indian J Chem 14B: 1976; 844–848

[23] Khan R, Khanam Z, Khan AU. Isolation and characterization of n-octacosanoic acid from *Viburnum foetens*: a novel antibiofilm agent against *Streptococcus Mutans*. Med Chem. Res 2012; 21: 1411–1417

[24] Jamal AK, Yaacob WA, Din LB. A chemical study on *Phyllanthus columnaris*. Eur J Sci Res 2009; 28: 76–81

[25] Gao Y, Su Y, Yan S, Wu Z, Zhang X, Wang T, Gao X. Hexaoxygenated flavonoids from Pteroxygonum giraldii. Nat Prod Commun 2010; 5: 223–226

[26] Kitanaka S, Takido M. Demethyltorosaflavones C and D from *Cassia nomame*. Phytochemistry 1992; 31: 2927–2929

[27] Queiroz EF, Ioset J-R, Ndjoko K, Guntern A, Foggin CM, Hostettmann K. On-line identification of the bioactive compounds from Blumea gariepina by HPLC-UV–MS and HPLC-UV–NMR, combined with HPLC-micro-fractionation. Phytochem Anal 2005; 16: 166–174

[28] Cho-Ngwa F, Abongwa M, Ngemenya MN, Nyongbela KD. Selective activity of extracts of *margaritaria discoidea* and *homalium Africanum* on *Onchocerca ochengi*. BMC Complement Altern Med 2010; 10: 1–7

[29] Cho-Ngwa F, Daggfeldt A, Titanji VPK, Gronvik K. Preparation and characterisation of specific monoclonal antibodies for the detection of adult worm infections in onchocerciasis. Hybridoma 2005; 24: 283–290