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PHENOLIC METABOLITES FROM *ERYTHRINA* SPECIES*

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Key Word Index—*Erythrina sigmoidea*; *E. eriotricha*; Leguminosae; esters of ferulic and isoferulic acid; prenylated isoflavanones; pterocarpans, flavanones; biological activities.

Abstract—From the stem and root bark of *Erythrina sigmoidea* and *E. eriotricha*, one new prenylated flavanone, named sigmoidin L, and two new esters of ferulic and isoferulic acid, erythrinassinates C and D, were isolated together with eight known compounds, including one ester of isoferulic acid [3β-*O*-(*E*)-isoferuloyl oleanolic acid], one flavanone (sigmoidin A), four isoflavones (scandenone, 6,8-diprenylgenistein), flemiphilippinin B and 8-prenyldaidzein and two pterocarpans (gangetinin and calopocarpin). Their structures were elucidated by analysis of spectral data and chemical evidence. The *in vitro* anitmicrobial spectrum and potencies of the isolated compounds are also reported. © 1997 Elsevier Science Ltd

INTRODUCTION

In the previous papers [1–5], we reported a series of flavanoids and isoflavonoids from the stem and root bark of some *Erythrina* species found in Cameroon. In a continuing study of these plants, one new prenylated flavanone, named sigmoidin L (**1**) and two new esters: one of isoferulic acid, *n*-tetradecanyl isoferulate (**3**) and another of ferulic acid, *n*-hexacosanyl ferulate (**4**), were isolated from the stem and root bark of *Erythrina sigmoidea* and *E. eriotricha* along with eight known compounds including: 3β-*O*-(*E*)-isoferuloyl oleanolic acid (**5**), sigmoidin A (**6**), scandenone (**7**), 6,8-diprenylgenistein (**8**), fleminphilippinin B (**9**), 8-prenyldaidzein (**10**), gangetinin (**11**) and calopocarpin (**12**). The present paper describes the isolation and structural elucidation as well as the antimicrobial activity of these compounds.

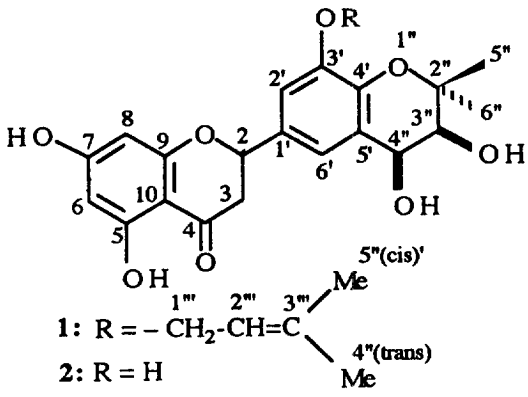
RESULTS AND DISCUSSION

The finely powdered stem bark of *E. sigmoidea* was extracted with *n*-hexane, ethyl acetate and methanol, successively. The ethyl acetate extract, when assayed for antimicrobial activity, gave a wide inhibition zone against the Gram positive bacterium, *Staphylococcus aureus*. This extract was repeatedly subjected to silica gel column chromatography to afford one novel compound, named sigmoidin L (**1**) and two known compounds, identified as scandenone (**7**) and 6,8-diprenylgenistein (**8**) by direct comparison with authentic samples and literature data [6, 7]. On the other hand, bioactivity-guided fractionation of the CH_2Cl_2 extracts of the root bark of *E. sigmoidea* and *E. eriotricha*, by silica gel column chromatography followed by recrystallization as described in the Experimental, led to the isolation of two esters of ferulic and isoferulic acid, erythrinassinates C (**3**) and D (**4**), in addition to seven known compounds including: 3β-*O*-(*E*)-isoferuloyloleanolic acid (**5**) [8], sigmoidin A (**6**) [1], flemiphilippinin B (**9**) [9], 8-prenyldaidzein (**10**) [10], gangetinin (**11**) [11] and calopocarpin (**12**) [12]. The known compounds were identified by comparison of their spectral data with literature values [1, 8–12]. The ^{13}C NMR spectral data of 3β-*O*-(*E*)-isoferuloyloleanolic acid (**5**), flemiphilippinin B (**9**), gangetinin (**11**) and calopocarpin (**12**) are reported here for the first time. Their assignments were made on the basis of known related compounds as well as the multiplicities in J_{mod}, HMBC and HMQC spectra.

Compound **1**, sigmoidin L, was obtained as colourless needles and reacted positively to the $FeCl_3$ reagent. The $[M]^+$ at *m/z* 456.1760 in the high resolution EI-mass spectrum corresponds to $C_{25}H_{28}O_8$ (calcd 456.1783). The IR spectrum of **1** showed absorption bands for free hydroxyl (3425), chelated hydroxyl (3215), conjugated carbonyl (1645), olefine (1580) and ether (1278, 1267 cm^{-1}) functionalities. In

* Part 34 in the series 'Erythrina studies'.

† Author to whom correspondence should be addressed.



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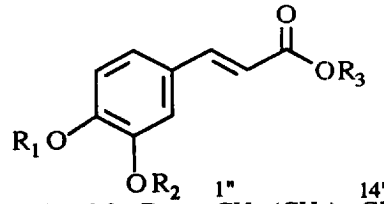
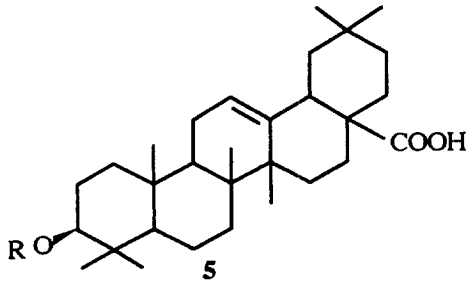
2: R = H



3: R_1= H; R_2= Me; R_3= – CH_2 $(CH_2)_{12}CH_3$

4: R_1= Me; R_2 = H; R_3 = $-CH_2$ $(CH_2)_{24}$ CH_3

13: R_1 = H; R_2 = Me; R_3 = $C_{28}H_{57}$



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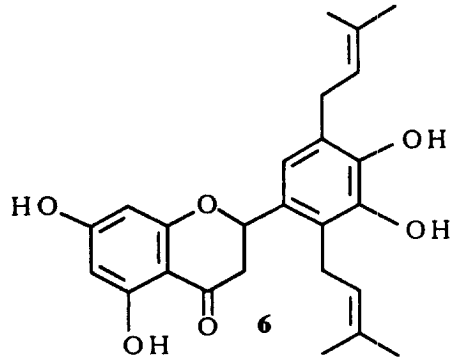
R= isoferuloyl moiety



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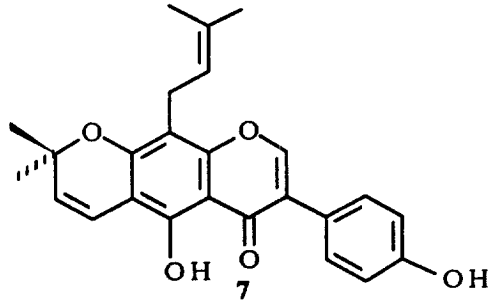

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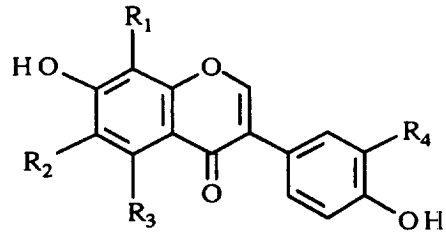
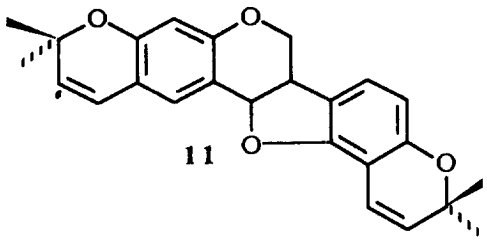


8 : R_1=R_2= isoprenyl; R_3=OH; R_4=H

9 : R_1= R_2= isoprenyl; R_3= OH; R_4=O Me

10 : R_1= isoprenyl; R_2=H; R_3=H; R_4=H



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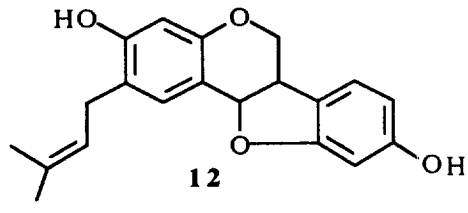

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its ^{1}H NMR spectrum (Table 1), signals characteristic of a flavanone were found. The AMX spin system at δ 2.67 (*dd*, J = 17.2 and 3.2 Hz), δ 3.16 (*dd*, J = 12.9 and 17.2 Hz) and δ 5.37 (*dd*, J = 3.2 and 12.8 Hz) are typical for H-3eq., H-3ax and H-2 respectively in the flavanone skeleton. This was confirmed by the HETCOR spectrum in which the two former protons correlated with a carbon at δ 43.3 ppm and the latter (δ 5.37) exhibited a cross peak with a carbon at δ 80.2 ppm. The UV spectrum of **1** showed a maximum absorption band at λ_{max} (MeOH) 289 nm and the aluminium chloride and sodium acetate induced bathchromic shifts indicating that sigmoidin L was a 5,7-dihyroxyflavanone [13].

Table 1. ^{1}H (300.0 MHz, $CDCl_3$, δ ppm) and ^{13}C-NMR (75.45 MHz, $CDCl_3$, δ ppm) spectral data of sigmoidin L (**1**) and G (**2**)

Atoms	Sigmoidin L (**1**)		Sigmoidin G (**2**)	
	^{1}H [multiplicity, J (Hz)]	^{13}C	^{1}H [multiplicity, J (Hz)]	^{13}C
2	5.37 (1H, *dd*, 3.2 and 12.8)	80.2	5.35 (1H, *dd*, 3.1 and 12.9)	80.2
3ax	3.16 (1H, *dd*, 12.9 and 17.2)	43.2	3.17 (1H, *dd*, 12.9 and 17.1)	43.1
3eq.	2.67 (1H, *dd*, 17.2 and 3.2)	43.2	2.65 (1H, *dd*, 17.2 and 3.1)	43.1
4	—	180.2	—	180.1
5	—	164.6[a]	—	164.3[a]
6	5.94 (1H, *d*, 2.3)	96.7[b]	5.95 (1H, *d*, 2.1)	96.7[b]
7	—	165.1[a]	—	165.0[a]
8	5.88 (1H, *d*, 2.3)	95.8[b]	5.89 (1H, *d*, 2.1)	95.8[b]
9	—	166.9[a]	—	167.7[a]
10	—	100.3	—	100.1
1′	—	130.9	—	131.7[c]
2′	7.16 (1H, *d*, 2.0)	113.1	7.11 (1H, *d*, 1.5)	113.2
3′	—	129.1	—	129.0[c]
4′	—	146.2	—	146.0[d]
5′	—	145.1	—	144.2[d]
6′	6.91 (1H, *d*, 2.0)	118.0	6.85 (1H, *d*, 1.5)	117.7
2″	—	69.8	—	69.7
3″	3.56 (1H, *d*, 10.9)	76.7	3.54 (1H, *d*, 11.0)	76.6
4″	4.50 (1H, *d*, 10.9)	80.0	4.51 (1H, *d*, 11.0)	80.0
5″	1.23 (3H, *s*)	19.7	1.24 (3H, *s*)	19.6
6″	1.52 (3H, *s*)	26.9	1.53 (3H, *s*)	27.0
1‴	4.35 (2H, *d*, 8.0)	65.4	—	—
2‴	5.38 (1H, *t*, 8.0)	138.0	—	—
3‴	—	133.9	—	—
4‴	1.80 (3H, *s*)	25.8	—	—
5‴	1.69 (3H, *s*)	17.8	—	—
5-OH	12.00 (1H, *s*, exch. in D_2O)	—	11.44 (1H, *s*, exch. in D_2O)	—

[a–d] assignments may be reversed within the same column.

In fact, the ^{1}H NMR spectrum of (**1**) was closely related to that of sigmoidin G (**2**), a compound previously isolated from the same source [4], except that a set of signals corresponding to an additional prenyloxy group was observed at δ 1.80 (3H, *s*), 1.69 (3H, *s*), 4.35 (2H, *d*, $J = 8.0$ Hz) and δ 5.38 ppm (1H, *d*, $J = 8.0$ Hz). Further support for the presence of a prenyloxy substituent came from the EI mass spectrum of (**1**) which showed, besides a molecular ion at *m/z* 456, intense ion peaks at *m/z* 85 at *m/z* 371 ($[M]^+$-85) due to the loss of the prenyloxy moiety (C_5H_9O) from the molecular ion.

Comparison of the ^{1}H NMR chemical shifts and coupling constants, as well as ^{13}C and 2D NMR (^{1}H–^{1}H COSY, HMQC and HMBC) data with those previously reported for closely sigmoidin G (**2**), allowed the assignments of ^{1}H and ^{13}C NMR signals (Table 1) of **1** as well as the location of prenyloxy substituent. The location at the C-3′ position on ring B of the prenyloxy moiety, was confirmed by a NOE difference experiment. An enhancement of the H-2′ (28%) signal was observed when the allylic proton H-1‴ at δ 4.35 ppm was irradiated. This was further supported by the significant retro-Diels–Alder fragmentation peaks at *m/z* 153, 303, 231 and 72. The relative *trans* configuration of the vicinal diol grouping in the pyran ring, was indicated by the coupling constant ($J = 11.3$ Hz) between carbonyl hydrogens and by the fact that no NOE enhancement was observed between the two hydrogens suggesting that they have a diaxial-like relationship. Thus sigmoidin L **1** is 5,7,4″,5″-tetrahydroxy-3′-(3‴-methylbutoxy-2‴-ene)-6″,6″-dimethyl [2″,3″,4′,5′]flavanone.

Compound (**3**), unknown in the literature, was recrystallized from ethyl acetate–hexane mixture as an amorphous solid, mp 76°. The presence of bands at 3460 and 1605 cm^{-1} in its IR spectrum and a positive response to the methanolic $FeCl_3$ colour test, suggested that **3** was a phenol. Its molecular formula, $C_{24}H_{38}O_4$ ($[M]^+$ 390.2762, calcd 390.2769), was deduced by high resolution EI mass spectrum. This formula indicated six degrees of unsaturation. The presence of double bonds was indicated by IR bands at 1625, 1580 cm^{-1} while a carbonyl band of an ester was shown at 1710 cm^{-1} and a long chain band at 725 cm^{-1}. The UV spectrum of **3** showed absorption bands at λ_{max} (MeOH) 238 ($\log\varepsilon$ 4.05) and 325 nm ($\log\varepsilon$ 4.06) which is very similar to that of erythrinassinate A (**13**), an ester of ferulic acid previously isolated from three *Erythrina* species [14, 15]: *Erythrina excelsa*, *E. senegalensis* and *E. glauca*. In the ^{1}H NMR spectrum in $CDCl_3$, the presence of a *trans* double bond was confirmed by signals at δ 6.26 and 7.60 ppm with a coupling constant of $J = 16.0$ Hz. A

typical ABX spin system at δ 6.93 (*d*, $J = 8.6$ Hz), 7.06 (*dd*, $J = 8.6$ and 2.2 Hz) and 7.02 ppm (*d*, $J = 2.2$ Hz) established the presence of three aromatic protons with *ortho*, *ortho*/*meta* and *meta* coupling, respectively. The presence of one methoxyl group and one hydroxyl group was also shown by a three protons singlet at δ 3.80 ppm and one proton broad signal exchangeable with D_2O. Furthermore, the two protons triplet at δ 4.15, the 24H broad signal at δ 1.10–1.39 and the three protons triplet at δ 0.88 ppm were consistent with the presence of an *n*-tetradecanyl long chain moiety. Compound **3** is therefore a long chain ester of ferulic or isoferulic acid. This was supported by the ^{13}C-NMR spectrum (see Experimental) which showed signals at δ 167.3 (C-3′) due to the carbonyl group of an ester function and δ 144.6 (C-1′) and 109.3 ppm (C-2′) due to a side chain C–C double bond. Further confirmation of this skeleton came from the mass spectrum of (**3**) which showed, besides the molecular ion, significant fragment peaks at *m/z* 177 and 194, both characteristic of a methoxy- and hydroxy-substituted cinnamic moiety [16]. The positions occupied by hydroxyl and methoxyl groups on aromatic ring were established by a NOE difference experiment which resulted in the enhancement (25%) of the doublet at δ 7.02 ppm (H-2) when the methoxyl group at δ 3.80 ppm was irradiated. This indicated that the H-2 is in close spatial proximity with respect to the methoxyl group. Thus, the hydroxyl is attached at the C-4 position in the aromatic ring. From the above spectroscopic studies, erythrinassinate C (**3**) was deduced to be a *n*-tetradecanyl ferulate.

Compound (**4**), mp 68–70°, obtained as colourless powder, gave a positive reaction with the methanolic $FeCl_3$ test. The molecular ions at *m/z* 558.4643 (calcd 558.4648) in the high resolution mass spectrum correspond to the molecular formula $C_{36}H_{62}C_4$ containing six unsaturations. IR, UV, ^{1}H and ^{13}C NMR spectroscopic data of erythrinassinate D (**4**) are almost superimposable with those of erythrinassinate C (**3**) indicating that the two compounds are very similar. The major difference between them was found in their NOE difference spectra. When the methoxyl group, which in the ^{1}H NMR of compound **4** resonates at δ 3.85 ppm, was irradiated, an enhancement (24%) of the H-5 signal at δ 6.82 ppm was observed indicating the close spatial proximity of H-5 and methoxy group. This result showed clearly that in structure **4**, the methoxyl group is located at C-4 position and the hydroxl at C-3 position in the aromatic ring. Moreover, the molecular ion at *m/z* 558 in the mass spectrum of **4** and the 48H broad signal at δ 1.08–1.38 ppm in its ^{1}H NMR were consistent with the presence of a *n*-hexacosanyl group. Thus, erythrinassinate D (**4**) is *n*-hexacosanyl isoferulate.

From Table 2, it appears that all these compounds are inactive against the Gram negative bacterium, *Escherichia coli*. Their activities against Gram positive organisms were found to be less than those of the known antibiotics erythromycin or tetracycline. Although the activity of each compound is moderate, their total quantity is quite large and they may therefore account not only for the potency of the crude extract but can also serve to rationalize the folkloric use of the *Erythrina* plants for the control of certain infections.

EXPERIMENTAL

Plant material. The stem and root barks of two *Erythrina* species: *E. sigmoidea* Hua and *E. eriotricha* Harms used in this study were collected at Foumban (Western province of Cameroon in June 1988 and 1992) and at Meiganga (Adamaoua Province of Cameroon in June 1987 and February 1994), respectively. Voucher specimens documenting the collection are deposited at the National Herbarium, Yaounde-Cameroon.

Extraction and isolation. The dried ground stem bark (5 kg) of *E. sigmoidea* was successively extracted with hexane, EtOAc and MeOH. A part of residue (30 g), obtained after evapn of the EtOAc extract, was fractionated over silica gel. Elution with a $CHCl_3$–MeOH mixt. of increasing polarity afforded a total of 53 frs (*ca.* 250 ml) collected and mixed on the basis of TLC. Frs 10–30 eluted with a mixt. of $CHCl_3$–MeOH (3:2) were evapd and the residue (4.8 g) obtained, was subjected to repeated CC on silica gel using a mixt. of

Table 2. Antimicrobial potency of isolated compounds *in vitro*

Compounds	Microorganisms* tested (MIC, μg ml^{-1}) 1	2	3	4
Erytrinassinate C (**3**)	>100	>100	>100	>100
Erytrinassinate D (**4**)	>100	>100	>100	>100
Sigmoidin A (**6**)	20	>100	>50	20
Scandenone (**7**)	12.5	>100	12.5	6.50
6,8-Diprenyl genistein (**8**)	6.25	>100	6.25	6.25
8-Prenyl daidzein (**10**)	10.5	–100	12.5	10.5
Tetracycline	0.1	6.25	>100	0.2
Erythromycin	0.2	>50	0.78	—

* Microorganisms: 1 = *Staphylococcus aureus* 209P; 2 = *Escherichia coli* RL65; 3 = *Mycobacterium smegmatis* ATCC 607; 4 = *Bacillus subtilis*.

$CHCl_3$–MeOH as elution gradient to yield scandenone (**7**) (900 mg), 6,8-diprenylgenistein (**8**) (600 mg) and sigmoidin L (**1**) (50 mg).

The dried and pulverized root bark (10.0 kg) of the same plant were treated in the same manner as described above for the stem bark. The residue (60 g) obtained after evapn of the hexane extract, was CC over silica gel, eluted with hexane, hexane–EtOAc and EtOAc mixts. A total of 150 frs (*ca* 200 ml per fr.) were collected and combined on the basis of TLC. Frs 3–7 eluted with a mixt. of hexane–EtOAc (31:1) yielded gangetinin (**11**) as white crystals (91 mg). From frs 56–77 obtained with mixt. of hexane–EtOAc (19:1), white powder (2.5 g) of erythrinassinate C (**3**) pptd.

On the other hand, the purification of a part (200 g) of the residue, obtained after evapn of the EtOAc extract of the root bark of *E. sigmoidea* Hua, by repeated CC over silica gel eluted with a mixt. of cyclohexane–EtOAc, led to the isolation of calopocarpin (**12**) (18 mg).

The dried ground stem bark (10 kg) of *Erythrina eriotricha* was successively extracted with hexane, $CHCl_3$ and MeOH. Concn of the $CHCl_3$ extract under red. pres. gave a brown sticky oil (200 g). A part of the residue (100 g) was chromatographed over silica gel eluted with a hexane–EtOAc mixt. of increasing polarity. A total of 200 frs (*ca.* 50 ml) were collected and combined on the basis of TLC.

From frs 10–12 eluted with a mixt. of hexane–EtOAc (17:3), erythrinassinate D (**4**) pptd as a colourless powder (15 mg). Frs 13–30 eluted with hexane–EtOAc (3:1) were subjected to repeated CC over silica gel eluted with a mixt. of cyclohexane–EtOAc to afford as white crystals: sigmoidin A (**6**) (10 mg), flemiphilippinin B (11 mg) (**9**) and 3-*β*-*O*-(*E*)-isoferuloyl oleanolic acid (**5**) (8 mg).

The dried ground root bark of the same plant (9 kg) were treated in the same manner as stated above for the stem bark. A part of the residue (100 g), resulted from the evapn of the $CHCl_3$ extract, was subjected to repeated CC over silica gel eluted with mixt. of cyclohexane–EtOAc of increasing polarity to give 8-prenyl daidzein (**10**) as an amorphous yellow powder (40 mg).

Sigmoidin (*L*) (**1**). Colourless needles, mp 205°; $[\alpha]_D^{25}$ −21.8 (MeOH; *c* = 0.04); HRMS *m/z*: 456.1760 (calcd. for 456.1783); UV λ_{max}^{MeOH} nm (log ε): 289 (3.54); +NaOAC: 298 (3.58); +$AlCl_3$+HCl: 299 (3.25); IR; ν_{max}^{KBr}: 3425, 3215, 1645, 1580, 1385, 1365, 1278 and 1267, 1182 and 1160 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), and ^{13}C NMR (75.45 MHz) see Table 1; EIMS *m/z* (rel. int.): 456 $[M]^+$ (5), 400 (4), 370 (5), 371 (60), 339 (27), 303 (22), 231 (18), 180 (8), 153 (100), 91 (15), 85 (37) and 72 (32).

Erythrinassinate C (**3**). Amorphous powder; mp 76°; HRMS *m/z* 390.2762 (calcd for $C_{24}H_{38}O_4$: 390.2769); UV λ_{max}^{MeOH} (log ε): 328 (4.06), 295 (3.98) and 237 nm (4.01); IR ν_{max}^{KBr}: 3450, 1710, 1660, 1625, 1510, 1480, 1280, 1160 and 725 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.88 (3H, *t*, *J* = 7.3 Hz, Me), 1.10–1.39 [24H, *bs*, $(CH_2)_{12}$], 3.80 (3H, *s*, 3-OMe), 4.15 (2H, *t*, *J* = 7.3 Hz, OCH_2CH_2R), 5.82 (1H, *b*, exchangeable D_2O, OH), 6.26 (1H, *J* = 16.0 Hz, =CH, H-2′), 6.93 (1H, *d*, *J* = 8.6 Hz, H-5), 7.02 (1H, *d*, *J* = 2.2 Hz, H-2), 7.06 (1H, *dd*, *J* = 8.6 and 2.2 Hz, H-6), 7.60 (1H, *d*, *J* = 16.0 Hz, =CH, H-1′); ^{13}C NMR (75.45 MHz, $CDCl_3$, δ ppm): 14.1 (C-14″), 22.7 (C-13″), 26.0 (C-3″), 28.8 (C-2″), 29.3 (C-4″ and C-11″), 29.7 (C-5″ → C-10″), 31.9 (C-12″), 55.9 (3-OMe), 64.6 (C-1″), 109.3 (C-2′), 114.7 (C-5), 115.7 (C-2), 123.0 (C-6), 127.1 (C-1), 144.6 (C-1′), 147.9 (C-3, C-4), 167.3 (C-3′). EIMS *m/z* (rel. int.): 390 $[M]^+$ (100), 194 (81), 177 (70), 57 (58).

Erythrinassinate D (**4**). Colourless amorphous powder; mp 68–70°; HRMS *m/z* 558.4643 (calcd for $C_{24}H_{38}O_4$: 558.4648); UV λ_{max}^{MeOH} (log ε): 325 (4.04), 294 (3.96) and 235 nm (4.01); IR ν_{max}^{KBr}: 3480, 1725, 1625, 1510, 1280, 1160 and 720 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 0.84 (3H, *t*, *J* = 7.1 Hz, Me), 1.08–1.38 [48H, *bs*, $(CH_2)_{12}$], 3.85 (3H, *s*, 4-OMe), 4.15 (2H, *t*, *J* = 7.1 Hz, OCH_2CH_2R), 5.82 (1H, *b*, exchangeable D_2O, OH), 6.22 (1H, *J* = 15.9 Hz, =CH, H-2′), 6.82 (1H, *d*, *J* = 8.4 Hz, H-5), 6.98 (1H, *d*, *J* = 2.1 Hz, H-2), 7.00 (1H, *dd*, *J* = 8.4 and 2.1 Hz, H-6), 7.56 (1H, *d*, *J* = 15.9 Hz, =CH, H-1′); ^{13}C NMR (75.45 MHz, $CDCl_3$, δ ppm): 13.9 (C-26″), 22.6 (C-25″), 25.9 (C-3″), 28.6 (C-2″), 29.3 (C-4″ and C-23″), 29.6 (C-5″ → C-2″), 31.8 (C-24″), 55.9 (4-OMe), 64.6 (C-1″), 109.7 (C-2′), 114.9 (C-5), 115.7 (C-2), 123.4 (C-6), 127.3 (C-1), 144.8 (C-3, C-4 and C-1′), 169.6 (C-3′); EIMS *m/z* (rel. int.): 558 $[M]^+$ (100), 194 (80), 177 (65), 57 (55).

3-*β*-*O*-(E)-*Isoferuloyl oleanolic acid* (**5**). Mp 255°, IR, UV, ^1H NMR and MS spectra data matched well with the literature values [8]. ^{13}C NMR (125.0 MHz, $CDCl_3$, δ ppm): 38.2 (C-1), 25.5 (C-2), 80.8 (C-3), 37.3 (C-4), 55.1 (C-5), 18.0 (C-6), 32.7 (C-7), 39.2 (C-8), 47.5 (C-9), 37.3 (C-10), 22.8 (C-11), 121.8 (C-12), 144.0 (C-13), 41.6 (C-14), 27.8 (C-15), 22.5 (C-16), 46.3 (C-17), 41.1 (C-18), 47.7 (C-19), 30.6 (C-20), 33.8 (C-21), 32.6 (C-22), 28.6 (C-23), 16.5 (C-24), 15.3 (25), 18.0 (C-26), 25.6 (C-27), 180.4 (C-28), 33.9 (C-29), 23.5 (C-30), 126.2 (C-1′), 129.9 (C-2′ and C-6′), 116.0 (C-3′ and C-5′), 159.6 (C-4′), 144.8 (C-1″), 115.2 (C-2″) and 169.6 (C-3″).

Flemiphilippinin B (**9**). Mp 155° (lit. [9] 154–156°), IR, UV ^1H NMR and MS spectral data matched well with published ones [9]; ^{13}C NMR (125.0 MHz, $CDCl_3$ δ ppm): 152.6 (C-2), 123.1 (C-3), 181.3 (C-4), 157.6 (C-5), 105.4 (C-6), 159.6 (C-7), 105.8 (C-8), 153.3 (C-9), 110.2 (C-10), 123.3 (C-1′), 114.5 (C-2′), 146.4 (C-3′), 1456.0 (C-4′), 114.4 (C-5′), 121.8 (C-6′), 29.8 (C-1″), 121.6 (C-2″), 135.5 (C-3″), 25.8 (C-4″), 17.9 (C-5″), 29.7 (C-1‴), 121.3 (C-2‴), 134.1 (C-3‴), 25.8 (C-4‴), 17.8 (C-5‴) and 56.0 (3-OMe).

Gangetinin (**11**). Mp 224°, IR, UV, ^1H NMR and MS spectral data are identical to those published in the literature [11]; ^{13}C NMR (100.0 MHz, $CDCl_3$, δ ppm): 128.4 (C-1), 116.3 (C-2), 156.3 (C-3), 104.7 (C-4), 155.3 (C-4a), 66.5 (C-6), 43.7 (C-6a), 119.1 (C-6b),

123.7 (C-7), 108.6 (C-8), 153.7 (C-9), 106.3 (C-10), 154.6 (C-10a), 78.8 (C-11a), 112.9 (C-11b), 76.6 (C-2′), 129.6 (C-3′), 116.5 (C-4′), 27.7 (C-5′), 29.0 (C-6′), 76.0 (C-2″), 129.1 (C-3″), 116.3 (C-4″), 27.8 (C-5″) and 27.9 (C-6″).

Calopocarpin (**12**). Mp 180°, IR, UV, ^{1}H NMR and MS spectral data are identical to those published in the literature [12]; ^{13}C NMR (100.0 MHz, $CDCl_3$, δ ppm): 132.0 (C-1), 122.4 (C-2), 158.8 (C-3), 98.1 (C-4), 161.3 (C-4a), 66.7 (C-6), 39.9 (C-6a), 118.9 (C-6b), 107.8 (C-7), 103.1 (C-8), 155.1 (C-9), 98.1 (C-10), 156.4 (C-10a), 78.9 (C-11a), 112.1 (C-11b), 27.9 (C-1′), 123.4 (C-2′), 131.8 (C-3′), 25.6 (C-4′) and 17.5 (C-5′).

Sigmoidin A (**6**) [1], scandenone (**7**) [7], 6,8-diprenylgenistein (**8**) [6], 8-prenyldaidzein (**10**) [10] were identified by comparison of their physical data (mp, IR, UV, ^{1}H NMR and ^{13}C NMR and MS) with those published in the literature.

Antimicrobial activity screening. The Minimal Inhibition Concentration (MIC) was defined as the lowest concn of antimicrobial agents in the agar medium resulting in complete inhibition of visible growth. The MIC of the tested compounds were determined by the agar-streak dilution technique against representative Gram-positive and negative organisms. The results obtained are given in Table 2.

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