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Constituents of *Erythrina caffra* Stem Bark Grown in Egypt

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Abstract – A new 3-indolyl propionate alkaloid; (–) abrine **(1)**, was isolated from the alcohol extract of *Erythrina caffra* stem bark together with the alkaloid (–) hypaphorine **(2)**. Phytochemical investigation of the chloroform extract led to the isolation of one fatty alcohol; 1-octacosanol, isolated for the first time from genus *Erythrina*, two dienoid alkaloids; erythraline and erysodine, and three isoflavonoids; 3*S* (+) 2'-*O*-methylphaseollidinisoflavan **(3)**, (6a*R*, 11a*R*)- 3-hydroxy-10-dimethylallyl-9-methoxypterocarpan, known as sandwicensin **(4)** and 3*R* (–) erythbidin A **(5)**. It is worth mentioning that this is the first report for the isolation of these isoflavonoids from *E. caffra*. The absolute configuration of 3*S* (+) 2'-*O*-methylphaseollidinisoflavan **(3)** was not previously reported. The isolated isoflavonoids showed promising antibacterial activity against *Staphylococcus aureus*.
Keywords – *Erythrina caffra*, alkaloids, isoflavanoids, *Staphylococcus aureus*

Introduction

Genus *Erythrina* is a member of family Fabaceae, subfamily Papilionideae. It comprises over 108 species of orange or red flowered trees, shrubs and herbaceous plants. The genus is found throughout the tropical and sub-tropical warm regions of the world. (Raven, 1974). *Erythrina* species are used traditionally to treat infections, malaria, inflammation, jaundice, anemia, dysentery (Saidu *et al*., 2000) female infertility, stomach pain, and gonorrhea (Lee *et al.,* 2009). Several species of *Erythrina* are used for their tranquilizer effects (Garín-Aguilar *et al.,* 2000; Vasconcelos *et al.,* 2007; Ferreira de Lima *et al.,* 2006). Biological testing confirmed most of the traditional uses of *Erythrina* species. *Erythrina caffra* bark is traditionally used to treat sores, wounds, arthritis, sprains and aches (Verschaeve and Van Staden, 2008), while the leaves are used as paste for urinary complaints and venereal diseases and as infusion for earache (Pillay *et al.,* 2001). The ethyl acetate extract of *E. caffra* bark showed antibacterial and cyclooxygenase inhibition activities (Majinda *et al.,* 2005; Pillay *et al.,* 2001).

Previous phytochemical investigations on the *Erythrina* species have revealed that flavonoids (El-Masry *et al.,* 2002) and alkaloids (Amer, 1991) are the principle secondary metabolites of the genus. Pterocarpans, one of the largest subclasses of isoflavonoids, are widely found in the genus *Erythrina*. More than 38 new pterocarpans were isolated during the period between the years 2000-2009 (Zaatout, 2009).

Previous phytochemical work on *E. caffra* examined the alkaloidal fraction and resulted in the isolation of a number of alkaloids. However, literatures lack any information on the flavonoids constituents of *E. caffra*. That is may be due to difficulty facing scientist in isolation of the bioactive compounds (Pillay *et al.,* 2001). In this work we attempted to further study the constituents of *E. caffra* stem bark.

Experimental

General – Melting points were determined in open capillary tubes using Thermosystem FP800 Mettler FP80 central processor supplied with FP81 MBC cell apparatus, and were uncorrected. UV spectra were obtained in methanol and with different shift reagents on a Unicum Heyios-α UV-Visible Spectrophotometer. Optical rotations were recorded on a Jasco P-2000 Polarimeter. CD curves were recorded on J-815CD Spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-500 (Central Lab at the College of Pharmacy, King Saud University) Spectrometer operating at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR. The chemical shift values are reported in δ (ppm) relative to the internal

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standard TMS or residual solvent peak, the coupling constants (*J*) are reported in Hertz (Hz). 2D-NMR experiments (COSY, HSQC and HMBC) were obtained using standard Bruker program. HRESIMS were measured using a JEOL JMS-HX-110 instrument. ESIMS were measured using E.I. Finnigan model 4600 quadrupole system. Centrifugal preparative TLC (CPTLC) using Chromatotron (Harrison Research Inc. model 7924): 4 mm silica gel P254 disc.

Plant material – The stem bark of *E. caffra* Thumb. was collected from Alexandria (Cultivation garden) in September, 2008. The plant was identified by the Late Prof. Nabil El. Hadidi, Department of Botany, Faculty of Science, Cairo University and a voucher specimen (#EC-14) were kept in the Department of Pharmacognosy, Alexandria University, Alexandria, Egypt.

Extraction and isolation – Fresh sliced stem bark of *E. caffra* (2 kg) was extracted to exhaustion with $CHCl_3$ (12 L) at room temperature. The solvent was evaporated under reduced pressure at 45 °C to give 10 g of viscous residue. The bark was then extracted with 12 L of 70% EtOH at room temperature. Ten grams of dry alcohol extract were obtained after evaporation of solvent under reduced pressure at 45 °C.

Fractionation of the EtOH extract – The dry EtOH extract (10 g) was dissolved in 300 ml EtOH/H_2O mixture 2 : 1 and successively extracted with hexane (3 × 300 ml) and EtOAc (3 × 250 ml). The hexane fraction (0.8 g) gave *β*-sitosterol and *β*-amyrin after purification on silica gel column.

The EtOAc fraction (2 g) was chromatographed over silica gel column (100 g, 3 cm i.d.). Elution started with 5% MeOH in $CHCl_3$ with gradual increase in the ratio of MeOH. Twenty nine fractions, 150 ml each, were collected, screened by TLC using $CHCl_3$: MeOH (7 : 3) and similar fractions were combined. Fractions eluted with 18% methanol gave 24 mg of **1** by crystallization from MeOH. Fractions eluted with 20% methanol were subjected to CPTLC using 20% MeOH in $CHCl_3$ to give 3 mg of **2**.

Fractionation of the $CHCl_3$ extract – The dry chloroform extract (10 g) was fractionated using silica gel column (300 g, 5 cm i.d.) and elution was carried out using $CHCl_3$ followed by $CHCl_3$/MeOH mixtures in a gradient system. Fifty fractions, 250 ml each, were collected, screened by TLC using $CHCl_3$: MeOH (7 : 3) and similar fractions were combined.

Fractions eluted with 5% MeOH in $CHCl_3$ were subjected to CPTLC using 5% MeOH in $CHCl_3$ give 10 mg of white solid identified as 1-Octacosanol.

Fractions eluted with 8% MeOH in $CHCl_3$ were chromatographed over silica gel column (30 g, 2 cm i.d.). Elution was carried out using 5% MeOH in $CHCl_3$ followed by CPTLC using 5% MeOH in $CHCl_3$ to give 30 mg of the alkaloid erythraline previously isolated from the same plant (Amer *et al.*, 1993).

Fractions eluted with 10% MeOH in $CHCl_3$, were further purified over silica gel column (50 g, 2 cm diameter) eluting with 5% MeOH in $CHCl_3$ followed by CPTLC using 5% MeOH in $CHCl_3$ to give 10 mg of **3** and 20 mg of **4**.

CPTLC (5% MeOH in $CHCl_3$) of fractions eluted with 13% MeOH in $CHCl_3$ gave 15 mg of the alkaloid erysodine previously isolated from the same plant (Amer *et al.*, 1993) after repeated crystallization from EtOAc/hexane mixture.

Similarly, fractions eluted with 15% MeOH in $CHCl_3$ were subjected to CPTLC (5% MeOH in $CHCl_3$) to give 8 mg of **5**.

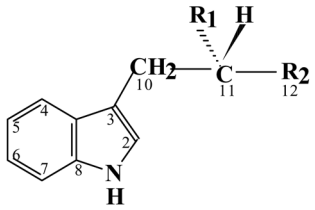
(–) Abrine (1) – White crystals, m.p. 248 - 250 °C (MeOH); UV λ_{max}, nm: (MeOH) 219, 279; $[\alpha]^{25}$ –13.25° (*c* 0.02, MeOH); ^{1}H- and ^{13}C-NMR data (Table 1); HRFABMS *m/z* 219.1133 $[M+H]^+$ (calc. for 219.1134 $[C_{12}H_{14}N_2O_2+H]^+$).

(–)Hypaphorine (2) – Amorphous residue, UV λ_{max}, nm: (MeOH) 219, 280; $[\alpha]^{25}$ –4.75° (*c* 0.02, MeOH); ^{1}H-NMR ($CDCl_3$): δ_H 7.21 (IH, s, H-2), 7.35 (1H, br.d, *J* = 8.0 Hz, H-4), 7.12 (1H, br.t, *J* = 8.0 Hz, H-5), 7.06 (1H, br.t, *J* = 8.0 Hz, H-6), 7.65 (1H, br.d, *J* = 8.0 Hz, H-7), 3.46 (2H, t, *J* = 5.5 Hz, H-10), 3.93 (1H, dd, *J* = 5.5, 9.0 Hz, H-10), 3.33 (9H, s, C<u>H</u>$_3$) 8.57(NH, indolic); ^{13}C-NMR ($CDCl_3$): δ_C 125.1 (C-2), 109.1 (C-3), 112.4 (C -4), 122.6 (C-5), 120.0 (C-6), 119.0 (C-7), 138.1 (C-8), 128.3 (C-9), 24.8 (C-10), 80.7 (C-11), 171.6 (C-12), 52.8 (<u>C</u>H$_3$);

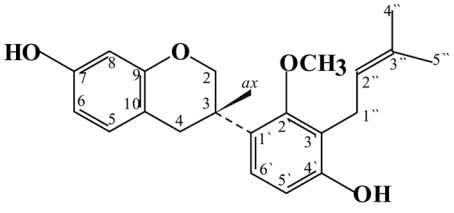
Table 1. ^{1}H- and ^{13}C-NMR spectral data (δ ppm) of HCl salt of 1[a]

Position	^{1}H-NMR[b]	^{13}C-NMR	HMBC
2	7.27 (1H, s)	125.6	C-3, C-8, C-9
3	–	107.2	
4	7.40 (1H, d, *J* = 7.0)	112.6	
5	7.15 (1H, br.d, *J* = 6.5)	122.9	
6	7.08 (1H, br.d, *J* = 6.5)	120.4	
7	7.63 (1H, d, *J* = 7)	119.1	
8	–	138.3	
9	–	128.3	
10	3.53 (2H, br.s)	26.7	C-2, C-3, C-11, C-12
11	4.28 (1H, br.s)	62.6	C-3, C-10, C-12, CH_3
12	–	170.9	
CH_3	2.73 (3H, s)	32.9	C-11

[a]Data were collected in CD_3OD
[b]*J* values in parenthesis in Hz.



1: **R1=** $NH(CH_3)$ **R2=** COOH
2: **R1=** $^{+}N(CH_3)_3$ **R2=** COO^{-}



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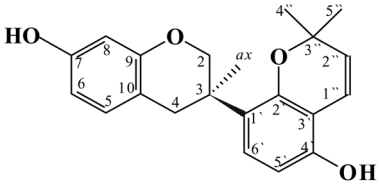

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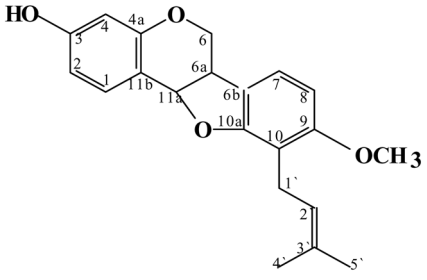

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HRFABMS *m/z* 247.1443 $[M+H]^+$ (calc. for 247.1447 $[C_{14}H_{18}N_2O_2+H]^+$).

3*S* (+) 2'-*O*-Methylphaseollidinisoflavan (3) – Yellowish white residue. UV λ_{max}, nm: (MeOH) 282; (NaOMe) 245(sh), 291; ($AlCl_3$) 281, 314, 359; ($AlCl_3$/HCl) 281, 313, 361; (NaOAc) 282, 328(sh); $[\alpha]^{25}$ +115.9° (*c* 0.02, MeOH). CD Δε (MeOH; *c* 1.0): +10.86 (258), –4.86 (264), –9.14 (279), –11.43 (284), –10.86 (288); ^{1}H-NMR ($CDCl_3$): δ_H 4.29 (1H, dd, *J* = 2.5, 9.0 Hz, H-2_{eq}), 3.97 (1H, t, *J* = 10.5 Hz, H-2_{ax}), 3.55 (1H,m, H-3 $_{ax}$), 2.90 (2H, br.dd, *J* = 10.5, 14.5 Hz, H-4), 6.98 (1H, d, *J* = 8.0 Hz, H-5) 6.42 (1H, dd, *J* = 8.0, 2.5 Hz, H-6), 6.40 (1H, br.s, H-8), 6.67 (1H, d, *J* = 8.5 Hz, H-5'), 6.91 (1H, d, *J* = 8.5 Hz, H-6'), 3.48 (2H, d, *J* = 6.0 Hz, H-1"), 5.28 (1H, br.s, H-2"), 1.87 (3H, s, H-4"), 1.79 (3H, s, H-5"), 3.75 (3H, s, O<u>C</u>H_3); ^{13}C-NMR ($CDCl_3$): δ_C 70.7 (C-2), 31.4 (C-3), 31.9 (C-4), 130.4 (C-5), 107.9 (C-6), 154.8 (C-7), 103.3 (C-8), 155.1 (C-9), 114.8 (C-10), 120.8 (C-1'), 156.9 (C-2'), 126.4 (C-3'), 154.9 (C-4'), 112.3 (C-5'), 125.9 (C-6'), 23.6 (C-1"), 121.8 (C-2"), 135.2 (C-3"), 17.9 (C-4"), 25.7 (C-5"), 62.3 (O<u>C</u>H_3); ESIMS *m/z* (rel. int.): 703 ($2M + Na^+$, 100), 698 ($[2M + H_2O]^+$, 87), 358 ($[M + H_2O]^+$, 58), 363 ($[M + Na]^+$, 15), 341 ($[M + H]^+$,100).

(6a*R*, 11a*R*)-Sandwicensin (3-hydroxy-10-dimethylallyl-9-methoxypterocarpan) (4) – Yellow residue. UV λ_{max}, nm: (MeOH) 232(sh) 281, 287; (NaOMe) 248, 288; ($AlCl_3$) 232(sh), 281, 287, 314, 359; ($AlCl_3$/HCl) 232(sh), 281, 287, 313, 361; (NaOAc) 280, 287(sh); $[\alpha]^{25}$ –192.1 (*c* 0.02, MeOH). CD Δε (MeOH; *c* 1.0): +47.6 (230), –57(246), +52.4 (270), –17.3 (277), +16.9 (284), +65.3 (295); ^{1}H-NMR ($CDCl_3$): δ_H 7.43 (1H, d, *J* = 8.5 Hz, H-1), 6.57 (1H, dd, *J* = 8.5, 2.5 Hz, H-2), 6.43 (1H, br. s, H-4), 4.25 (1H, dd, *J* = 5, 11 Hz, H-6), 3.68 (1H, t, *J* = 11 Hz, H-6), 3.55 (1H, m, H-6a), 7.04 (1H, d, *J* = 8.5 Hz, H-7), 6.44 (1H, d, *J* = 6.5 Hz, H-8), 5.48 (1H, d, *J* = 6.5 Hz, H-11a), 3.31 (2H, m, H-1'), 5.26 (1H, m, H-2'), 1.78 (3H, s, H-4'), 1.68 (3H, s, H-5'), 3.83(3H, s, O<u>C</u>H_3); ^{13}C-NMR ($CDCl_3$): δ_C 132.4 (C-1), 109.6 (C-2), 156.9 (C-3), 103.2 (C-4), 156.6 (C-4a), 66.5 (C-6), 40.0 (C-6a), 119.3 (C-6b), 121.5 (C-7), 103.0 (C-8), 158.6 (C-9), 113.1 (C-10), 158.5 (C-10a), 77.9 (C-11a), 113.1 (C-11b), 22.9 (C-1'), 122.3 (C-2'), 131.4 (C-3'), 25.7 (C-4'), 17.7 (C-5'), 55.9 (O<u>C</u>H_3); ESIMS *m/z* (rel. int.): 356 ($[M+H_2O]^+$, 52), 339 ($[M + H]^+$,75), 283 ($[M-C_4H_7]^+$, 38).

3*R* (–) Erythbidin A (5) – Yellow crystals, m.p. 200 - 202 °C (MeOH); UV λ_{max}, nm: (MeOH) 227 (sh) 283; (NaOMe) 236 (sh), 289, 331; ($AlCl_3$) 228 (sh), 280, 313, 359; ($AlCl_3$/HCl) 228 (sh), 280, 311, 359; (NaOAc) 284, 360 (sh); $[\alpha]^{25}$ –17.1 (*c* 0.02, MeOH). CD Δε (MeOH; *c* 1.0): –4.17 (265), +1.04 (271), –6.77 (279), +7.29 (287); ^{1}H-NMR ($CDCl_3$): δ_H 4.16 (1H, dd, *J* = 7.5, 14.5 Hz, H-2_{eq}) 4.04 (1H, t, *J* = 10.0 Hz, H-2_{ax}), 3.54 (1H, m, H-3 $_{ax}$), 2.98 (1H, dd, *J* = 11.0, 15.5 Hz, H-4), 2.87 (1H, m, H-4), 6.96 (1H, d, *J* = 8.0 Hz, H-5), 6.42 (1H, dd, *J* = 8, 2.5 Hz, H-6), 6.39 (1H, d, *J* = 2.5 Hz, H-8), 6.31 (1H, d, *J* = 8.5 Hz, H-5'), 6.82 (1H, d, *J* = 8.5 Hz, H-6'), 6.67 (1H, d, *J* = 10.0 Hz, H-1"), 5.63 (1H, d, *J* = 10.0 Hz, H-2"), 1.45 (3H, s, H-4"), 1.44 (3H, s, H-5"); ^{13}C-NMR ($CDCl_3$): δ_C

Table 2. MIC and MBC values of the tested isoflavonoids against *Staphylococcus aureus* ATCC6538P

Compound	MIC (mg/ml)	MBC (mg/ml)
3S (+) 2'-*O*-methylPhaseollidinisoflavan **(3)**	0.125	0.5
(6a*R*, 11a*R*)- Sandwicensin **(4)**	0.0625	0.5
3R (–) Erythbidin A **(5)**	0.0625	0.25

70.1 (C-2), 31.8 (C-3), 30.3 (C-4), 130.3 (C-5), 107.8 (C-6), 155.2 (C-7), 103.2 (C-8), 154.9 (C-9), 114.8 (C-10), 121.1 (C-1'), 151.5 (C-2'), 109.5 (C-3'), 150.3 (C-4'), 107.4 (C-5'), 126.9 (C-6'), 116.6 (C-1"), 129.2 (C-2"), 76.0 (C-3"), 27.7 (C-4" and C-5"); ESIMS *m/z* (rel. int.): 671 ($[2M + Na]^+$, 100), 666 ($[2M + H_2O]^+$, 65), 347 ($[M + Na]^+$, 17), 325.0 ($[M + H]^+$, 62).

1-octacosanol – White amorphous solid; ^{1}H-NMR ($CDCl_3$): δ_H 3.64 (2H, t, J = 7.0 Hz, H-1), 1.57 (2H, m, H-2), 1.28 (50 H, br.s, H-3 to H-27), 0.88 (3H, t, J = 7.0 Hz, H-28); ^{13}C-NMR ($CDCl_3$): δ_C 63.1 (C-1), 32.8 (C-2), 22.7-31.9 (C-3-C-27), 14.0 (C-28); ESIMS *m/z* (rel. int.): 392 $[M-H_2O]^+$, 364, 97, 83, 57.

Discussion

HRFABMS of **1** shows a pseudomolecular ion peak $[M + H]^+$ at *m/z* 219.1133 for the molecular formula $C_{12}H_{14}N_2O_2$. The UV spectrum of **1** in MeOH shows λ_{max} at 219 and 279 nm, characteristic for a mono-substituted indole skeleton (Kondo *et al.,* 1994). This fact was further confirmed from ^{1}H-NMR spectrum (Table 1) which exhibits signals for four coupled aromatic sp^2 protons at δ_H 7.40, 7.15, 7.08 and 7.63 (H-4, H-5, H-6 and H-7, respectively). Moreover, ^{1}H-NMR spectrum showed a singlet for an olefinic proton at δ_H 7.27 typical of the indolic H-2, thus the site of substitution on the indole ring is deduced to be at C-3 (Tasdemir *et al.,* 2002). NMR spectra of **1** exhibit an extra proton singlet integrated for three methyl protons at δ_H 2.73 correlated to a carbon signal at δ_C 32.9 in an HSQC experiment typical for an aliphatic *N*-methyl function at the amino group of the side chain, and not on the indole nitrogen (Tasdemir *et al.,* 2002). The ^{1}H-NMR spectra also showed a methylene protons signals at δ_H 3.53 (2H, br.s) and one methine proton at δ_H 4.28 (1H, br.s) which were correlated with carbon signals at δ_C 26.7 (C-10) and 62.6 (C-11) in the HMQC experiment respectively. A quaternary carbon at δ_C 170.9 (C-12) was assigned to a carbonyl group. HMBC experiment confirmed the presence of {-CH_2-CH-NHCH_3-COOH} side chain connected at C-3 of the indole nucleus (Tasdemir *et al.,* 2002; Kinjo *et al.,* 1991). The data of **1** were closely similar to those reported for 2-methylamino-3-(3-indolyl) propionic acid commonly known as abrine (Kinjo *et al.,* 1991; Ma *et al.,* 1991). The (L) stereochemistry of the single chiral center (C-11) was determined from the downfield shift of the aliphatic methine (δ_H 4.28, H-11) comparing with that of L-tryptophan (Pouchert and Behnke, 1993). These data together with the negative optical rotation ($[\alpha]^D$ –13.25) lead to establish **1** as the L (–) form of abrine, a new enantiomer isolated for the first time from the nature.

HRFABMS of **2** showed a pseudomolecular ion peak $[M + H]^+$ at *m/z* 247.1443 for the molecular formula $C_{14}H_{18}N_2O_2$. Both NMR and UV data showed a close similarity to **1**. The significant difference from **1** was the presence of a singlet at δ_H 3.33 integrated for nine protons, characteristic for $N(CH_3)_3$ in the ^{1}H-NMR spectrum. The data of **2** were consistent with those reported for 2-trimethylamino-3-(3-indolyl) propionate commonly known as hypaphorine (Kinjo *et al.,* 1991; Ma *et al.,* 1991). The (L) stereochemistry of the single chiral center (δ_H 3.93, C-11) is determined from the downfield shift of the aliphatic methine comparing with that of L-tryptophan (Pouchert and Behnke, 1993). Hypaphorine was previously isolated from different *Erythrina* species (Amer, 2001; Ozawa *et al.,* 2008). However, this is the first report for the isolation of hypaphorine from *E. caffra.*

The UV spectrum of **3** in MeOH showed only one λ_{max} at 282 nm, characteristic for isoflavan skeleton (Harborne, 1988). This fact was confirmed from the ^{1}H-NMR spectrum which exhibits the complex ABMXX` system [–O–$H_A$$CH_B$-$CH_M$-$H_X$$CH_{X'}$] for the five aliphatic protons of the isoflavan heterocyclic ring. This system is assigned for H-2_{ax} and H-2_{eq} (δ_H 3.97 and 4.29), H-3_{ax} (δ_H 3.55) and 2H-4 (δ_H 2.90), correlated to carbons at (δ_C 70.7, 31.4 and 31.9) characteristic of isoflavan skeletons (Kumar *et al.,* 1989). The axial position of H-3 is deduced from the coupling constants with 2H-2; $J_{2eq,3ax}$ = 2.5 Hz, $J_{2ax,3ax}$ = 10.5 Hz. In the ^{1}H-NMR spectrum of **3** the ABX system at δ_H 6.98, 6.42 and 6.40 is assigned for H-5, H-6 and H-8 of ring A, respectively. The two *ortho* coupled aromatic protons at δ_H 6.67 and 6.91 assigned for H-5' and H-6', respectively (Tanaka *et al.,* 1998), indicating a 2', 3', 4' trisubstituted B-ring. The NMR spectra of **3** also showed a methoxyl signal at δ_H 3.75 (δ_C 62.3) and characteristic signals for a prenyl group appearing as *gem*-dimethyl signals at δ_H 1.87 (3H, s, H-4") & δ_H 1.79 (3H, s, H-5"), one methine proton at δ_H 5.28 (1H, br.s, H-2") and two methylene protons at δ_H 3.48 (2H, d, J = 6.0 Hz, H-1") along with their carbon signals at δ 23.6, 121.8, 135.2, 17.9 and 25.7 corresponding to C-1", C-2", C-3", C-4" and

C-5'', receptively. The four oxygenated quateranary aromatic carbons in the ^{13}C-NMR of **3** were assigned to C-7, C-9, C-2' and C-4' on biogenetic basis (Preston, 1975). The chemical shift of the methoxyl carbon at δ_C 62.3 was typical for methoxyl flanked by di-*ortho* substituents (Rottman and James, 1985). Consequently, the methoxyl group was assigned to C-2' position while the prenyl group was assigned to C-3'. These assignments were further confirmed from the HMBC experiment were the aliphatic protons at δ_H 3.48 (H-1'') showed correlation with the carbon at δ_C 156.9 (C-2') which is attached to the methoxyl carbon. ESI-MS of **3** showed a pseudomolecular ion peak $[M + H]^+$ at *m/z* 341 consistent with the molecular formula $C_{21}H_{24}O_4$. The absolute configuration at C-3 was determined from the CD spectrum, where the negative cotton effect in the 260 - 300 nm region indicates the (3*S*) configuration (Kumar *et al.,* 1989; Kurosawa *et al.,* 1978). The data of **3** are identical with those reported in literature for 2'-*O*-methylphaseollidinisoflavan (Preston, 1975) previously isolated from *E. x bidwillii* (Tanaka *et al.,* 1998). It worth to mention that, this is the first report for the absolute configuration of **3** and the first report for its isolation from *E. caffra*.

The UV and NMR data of **5** indicated a close relation and similar substitution pattern with **3**. However, NMR data lack the methoxyl group present in **3** and the prenyl signals were replaced by a dimethyl pyran ring signals. These facts were supported by ESI-MS that showed a pseudomolecular ion peak $[M + H]^+$ at *m/z* 325 consistent with the molecular formula $C_{20}H_{20}O_4$. The formation of the dimethyl pyran ring can occur by coupling of C-3' with either C-2' or C-4' hydroxyl. The two possible structures are known. The fact that **5** failed to produce any color with Gibb's reagent (King, 1957; Kumar *et al.,* 1989) indicates undoubtfully that cyclization took place between C-3' and C-2' hydroxyl. The above discussion enables the identification of **5** as erythbidin A, previously isolated from *E. x bidwillii* (Tanaka *et al.,* 1998). The absolute configuration at C-3 was determined from the CD spectrum as (3*R*) configuration, due to the positive cotton effect in the 260-300 nm region (Kumar *et al.,* 1989; Kurosawa *et al.,* 1978). This is the first report for isolation of **5** from *E. caffra*.

The NMR spectra of **4** showed close similarity to those of **3** in the aromatic region and the prenyl signals. ESI-MS of compound **4** shows a pseudomolecular ion peak at *m/z* 339 deducing the molecular formula to be $C_{21}H_{22}O_4$, two unites less than **3**. These data suggested a cyclization between hydroxyl group and a non-oxygenated carbon leading to a petrocarpan skeleton closely related to **3**. The UV λ_{max} at 281 and 287 nm were in favour of pterocarpan skeleton (Harborne, 1988). This fact is supported by ^{1}H- and ^{13}C-NMR spectra where only four aliphatic protons appear; 2H-6 at δ_H 3.68, 4.25 (δ_C 66.5), H-6a at δ_H 3.55 (δ_C 40.0), H-11a at δ_H 5.48 (δ_C 77.9). The positions of the methoxyl and the prenyl group at C-9 and C-10, respectively were determined by the HMBC experiment and were consistent with the substitutions at **3** and **5**. The H-1' aliphatic protons of the prenyl group at δ_H 3.31 showed 2-bonds correlation with C-10 (δ_C 113.1) and 3-bonds correlation with C-9 (δ_C 158.6). The methoxyl protons at δ_H 3.83 show 3-bonds correlation with C-9 (δ_C 158.6) and 4-bonds correlation with C-8 (δ_C 103.0). The data of **4** were similar to those reported for 3-hydroxy-10-dimethylallyl-9-methoxypterocarpan known as sandwicensin. The CD spectrum of compound **4**, which shows a positive cotton effect at 295 nm, and the negative optical rotation ($[\alpha]_D$ –192.1) determine the (6a*R*, 11a*R*)-*cis* configuration. sandwicensin was previously isolated from the genus *Erythrina* (Tanaka *et al.,* 1998; Khaomek *et al.,* 2004,; Sato *et al.,* 2003), but this is the first report for its isolation from *E. caffra*.

Extracts of the stem bark *E. caffra* showed antibacterial activity (Pillay *et al.,* 2001). In our previous work, seven prenylated flavonoids were isolated from the stem bark extract of *E. lysistemon*. Three of them showed promising antibacterial activity against resistant *Staphylococcus aureus* strains (Zaatout, 2002). Sandwicensin (**4**) showed *in vitro* anti-HIV activity (Veitch, 2007) and it also showed potent antibacterial activity against MRSA (Djiogue *et al.,* 2009). These findings motivated us to explore the antimicrobial activity of the three isolated flavonoids. Three parameters were determined to evaluate the antimicrobial activity against *Staphylococcus aureus* ATCC6538P, *Pseudomonas aeruginosa* ATCC9027, *Candida albicans* ATCC2091 and *Escherichia coli* ATCC8739. Inhibition zones were determined using cup plate technique (NCCLS, 2002). Only significant inhibition zones were obtained against *Staphylococcus aureus*. Both the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined for **3 - 5** (Table 2) using broth dilution technique (Fernandes *et al.,* 1985). The three isoflavonoids showed promising activity against *Staphylococcus aureus*.

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