Antimicrobial and radical scavenging flavonoids from the stem wood of *Erythrina latissima*

Musa Chacha, Gomotsang Bojase-Moleta and Runner R.T. Majinda

Department of Chemistry, University of Botswana, Private Bag UB 00704, Gaborone, Botswana

Received 17 February 2004; revised 8 October 2004.
Available online 20 November 2004.

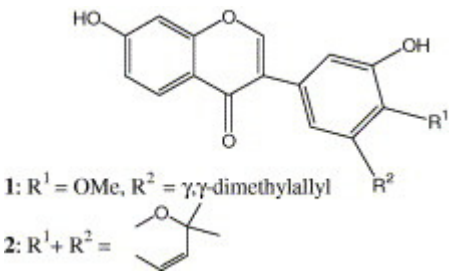
Abstract

From the stem wood of *Erythrina latissima*, two isoflavones and a flavanone were isolated and characterized as 7,3′-dihydroxy-4′-methoxy-5′-(γ,γ-dimethylallyl)isoflavone (erylatissin A), 7,3′-dihydroxy-6″,6″-dimethyl-4″,5″-dehydropyrano [2″,3″: 4′,5′]isoflavone (erylatissin B), (–)-7,3′-dihydroxy-4′-methoxy-5′-(γ,γ-dimethylallyl)flavanone (erylatissin C), respectively, in addition to 10 known flavonoids. Structures of these compounds were determined on the basis of their spectroscopic data. These compounds showed antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Candida mycoderma*. The isolated compounds also exhibited weak radical scavenging properties towards DPPH radical.

Graphical abstract

The stem wood of *Erythrina latissima* yielded two isoflavones, erylatissins A **1** and B **2**, and a flavanones (–)-7,3′-dihydroxy-4′-methoxy-5′-(γ,γ-dimethylallyl)flavanones **3**, in addition to ten known flavonoids. The isolated compounds showed antimicrobial activity against *Escherichia coli*,

Staphylococcus aureus, *Bacillus subtilis* and *Candida mycoderma*, as well as exhibited weak radical scavenging properties towards DPPH radical.



Keywords: *Erythrina latissima*; Fabaceae-Papilionoideae; Isoflavones; Flavanone; Erylatissins A–C; Antibacterial; Antifungal activity; Radical scavenging properties

Article Outline

1. Introduction

The genus *Erythrina* comprises over 110 species showing cosmopolitan distribution (Joubert, 1998). Previous investigations of a number of species have shown them to be good source of C-prenylated flavones, isoflavones, isoflavanones and pterocarpans (Barron and Ibrahim, 1996 and Dewick, 1994) and of nitrogenous compounds (Bisby et al., 1994).

Erythrina latissima E. Meyer (Fabaceae-Papilionoideae) is a tree 9–24 m tall, found in Botswana, Zimbabwe and South Africa and whose root and stem are burnt and used for dressing open wounds (Van Wyk and Van Wyk, 1998). Previous studies on the seeds have yielded *Erythrina* alkaloids (Wanjala and Majinda, 2000a and Wanjala and Majinda, 2000b), while the pods, root bark and stem bark yielded alkaloids, stilbenoids, lignans and flavonoids (Wanjala et al., 2002, Wanjala and Majinda, 2000a, Wanjala and Majinda, 2000b and Wanjala and Majinda, 2001). In this paper, we present results of an investigation of the stem wood.

2. Results and discussion

The chloroform extract of the stem wood of *Erythrina latissima* was subjected to a succession of chromatographic procedures, including silica gel chromatography, gel permeation chromatography using Sephadex LH-20 and preparative TLC to afford three pure isolates **1**, **2** and **3**.

The EI-MS spectrum of compound **1** showed a molecular ion peak at *m/z* 352.1 corresponding to the molecular formula $C_{21}H_{20}O_5$. The ^{13}C NMR spectra gave 21 carbon signals and the DEPT and HMQC spectra confirmed that eleven of these were protonated carbons. The DEPT spectrum further showed one methoxy, two methyls, one methylene and seven methine carbons. A singlet proton observed at δ_H 8.14 (H-2) with a corresponding carbon resonance at δ_C 153.1 (C-2) in the ^1H and ^{13}C spectra respectively indicated that compound **1** is an isoflavone. The nature and identity of the isoflavone was evident from the ^1H NMR spectrum

(Table 1) which showed the presence of a pair of *meta* coupled aromatic protons at δ_H 7.07 (*d*, *J* = 2.1 Hz) and δ_H 6.90, (*d*, *J* = 2.1 Hz) and an ABD aromatic spin system at δ_H 8.06, (*d*, *J* = 8.7 Hz), δ_H 7.00, (*dd*, *J* = 8.7, 2.1 Hz) and δ_H 6.91, (*d*, *J* = 2.1 Hz). The presence of a methoxy was evident from the sharp 3H signals at δ_H 3.82 ppm. The presence of a γ,γ-dimethylallyl (3-methylbut-2-enyl) group was evident from the two allylic methyl signals (δ_H 1.75, δ_H 1.72), one methylene doublet (δ_H 3.36, *J* = 7.2 Hz) and 1H allylic triplet (δ_H 5.32, *J* = 7.2). In addition, the EI-MS spectral fragmentation ions at *m/z* 137 (A_1) caused by *retro* Diels–Alder cleavage of the C-ring (Nkengfack et al., 1989) shows that ring A has only one hydroxyl group and no other substituents.

Table 1.

^{1}H (300 MHz) and ^{13}C (75.4 MHz) NMR chemical shifts for compounds **1** and **2** in acetone-d_6

Position	**1**		**2**	
	δ_H	**δ_C**	**δ_H**	**δ_C**
2	8.14, *s*	153.1	8.18, *s*	152.9
3		124.6		125.3
4		175.0		175.1
5	8.06, *d* (8.8)	127.9	8.08, *d* (8.8)	128.0
6	7.00, *dd* (8.7, 2.1)	115.4	7.01, *dd* (8.7, 2.2)	115.2
7		162.7		162.7
8	6.91, *d* (2.1)	102.7	6.92, *d* (2.2)	102.7
9		158.3		158.3
10		117.8		118.1
1′		128.7		121.8
2′	7.07, *d* (2.1)	115.7	7.06, *d* (2.0)	116.9

Position	1		2	
	δ_H	δ_C	δ_H	δ_C
3′		150.0		145.5
4′		146.0		140.0
5′		135.2		124.6
6′	6.91, *d* (2.1)	121.5	6.87, *d* (2.0)	118.3
1″	3.36, *d* (7.2)	28.6		
2″	5.32, *t* (7.2)	123.7		76.9
3″		131.9	5.77, *d* (9.8)	131.4
4″	1.75, *s*	17.4	6.43, *d* (9.8)	122.6
5″	1.72, *s*	25.4	1.46, *s*	27.5
6″			1.46, *s*	27.5
4′-OCH_3	3.82, *s*	60.1		

Assignments were confirmed by COSY, HMQC, HMBC and DEPT experiments.

The HMBC data showed a proton at δ_H 8.06 to have a long-range correlation with C-4 (δ_C 175.0), C-7 (δ_C 162.7) and C-9 (δ_C 158.3). This observation confirmed that an ABD aromatic spin system is on A-ring and hence *meta* coupled aromatic protons are on B-ring. The ^{13}C NMR chemical shift values (δ_C 146.0 and δ_C 150.0) of the sp^2 carbon atoms in B-ring requires oxygenation to occur on adjacent carbons viz at C-3′ and C-4′. Two *meta* coupled protons at δ_H 7.07 and δ_H 6.91 were assigned to H-2′ (δ_C 115.7) and H-6′ (δ_C 121.5) and hence the γ,γ-dimethylallyl group could only be placed at C-5′ (δ_C 135.2). The H-6′ proton (δ_H 6.91) showed an HMBC correlation with a carbon resonating at δ_C 150.0 (C-4′) and the same carbon showed another correlation with methoxy protons resonating at δ_H 3.82, showing that the methoxy group at C-4′ position. Further,

the carbon chemical shift value of the methoxy group (δ_C 60.1) showed it to be in a hindered position (i.e., flanked by two *ortho di* substituents) thus confirming the position of the methoxy group to be at C-4′ rather than C-3′. The rest of the structure was also confirmed through study of direct and long-range heteronuclear CH-coupling interactions. On the basis of these observations compound **1** was identified as 3′,7-dihydroxy-4′-methoxy-5′-(γ,γ-dimethylallyl)isoflavone (5-deoxy-5′-γ,γ-dimethylallylpratensein) and named erylatissin A.

The EI-MS of compound **2** showed a molecular ion at *m/z* 336.2 consistent with the molecular formula $C_{20}H_{16}O_5$ with NMR signals characteristic an isoflavone nucleus. A comparison of its ^1H and ^{13}C (Table 1) NMR spectra with those of **1** revealed identical oxygenation at C-7, C-3′ and C-4′. In compound **2**, however, signals due to 2,2-dimethylpyran (cyclised γ,γ-dimethylallyl unit) were observed instead of a normal γ,γ-dimethylallyl unit. The connectivity of the 2,2-dimethylpyran ring in ring B was deduced from HMBC data which showed a correlation between the vinylic protons at H-4″ with C-6′, C-5′ and C-4′, and H-5″ showed the expected ^3J correlation with C-5′ implying that the 2, 2-dimethylpyran group is connected in ring B at C-4′ and C-5′ carbons. Compound **2** was therefore identified as 7,3′-dihydroxy-6″, 6″-dimethyl-4″,5″-dehydropyrano [2″,3″:4′,5′] isoflavone (5-deoxysemilicoisoflavone B) and named erylatissin B.

The EI-MS of compound **3** showed a molecular ion peak at *m/z* 354.1 consistent with the molecular formula $C_{21}H_{22}O_5$. This was confirmed from ^{13}C NMR spectrum that showed twenty one carbon signals. A complete three proton spin system from the COSY spectrum, together with the three doublet of doublets in the ^1H NMR spectrum at δ_H 5.43 (H-2, *dd*, J = 12.9, 3.0 Hz), δ_H 3.00 (H-3a, *dd*, J = 12.7, 16.7 Hz) and δ_H 2.71 (H-3b, *dd*, J = 16.7, 3.0 Hz) indicates that ring C is saturated. Furthermore the α-*O*-substituted alkyl proton at δ_H 5.43 (H-2) indicates that compound **3** is a flavanone. Comparison of its ^1H and ^{13}C (Table 2) NMR with those of **1** revealed identical oxygenation pattern, with the methoxy group also at C-4′. The ^1H, ^{13}C, HMQC and HMBC spectra indicated the same

substitution pattern as in compound **1** in both rings A and B. All natural (–)-flavanones have been shown to have S-chirality at C-2, using circular dichroism spectroscopy (by observing the Cotton effect). The measured specific optical rotation for **3** was found to be –78° and thus from this value the absolute configuration at C-2 was assigned as 2S (Dewick, 1994). Compound **3** is thus identified (–)-7,3′-dihydroxy-4′-methoxy-5′-(γ,γ-dimethylallyl)flavanone (5-deoxysigmoidin B-4′-methyl ether) and named erylatissin C.

Table 2.

^{1}H (300 MHz) and ^{13}C (150 MHz) NMR chemical shifts for compound **3** in acetone-d_6

Position	δ_H	δ_C
2	5.43, 1H, *dd* (12.7, 3.0)	79.9
3a	3.00, 1H, *dd* (16.7,12.7)	44.2
3b	2.71, 1H, *dd* (16.7, 3.0)	44.2
4		190.0
5	7.73, 1H, *d* (8.6)	128.9
6	6.59, 1H, *dd* (8.6, 2.2)	110.8
7		165.3
8	6.45, 1H, *d* (2.2)	103.2
9		163.9
10		114.3
1′		135.6
2′	6.97, 1H, *d* (2.0)	112.9
3′		150.0
4′		146.2
5′		135.7

Position	δ_H	δ_C
6′	6.87, 1H, *d* (2.0)	119.1
1″	3.38, 2H, *d* (7.2)	28.7
2″	5.31, 1H, *t* (7.2)	123.4
3″		131.8
4″	1.75, 3H, *s*	17.4
5″	1.73, 3H, *s*	25.4
4′-OCH_3	3.82, 3H, *s*	60.2

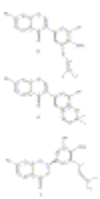
Assignments were confirmed by COSY, HMQC, HMBC and DEPT experiments.

The genus *Erythrina* is known to elaborate various flavonoids classes and a recent review (Majinda et al., 2004) showed reported 76 isoflavones, 40 pterocarpans, 34 flavanones, 27 isoflavanones, 6 isoflavans, 5 chalcones, 4 isoflav-3-enes and 3 flavonols. These metabolites, especially the isoflavones are fairly distributed in the genus. However, the isoflavones of the daidzein (5-deoxygenistein or 7,4′-dihydroxyisoflavone) derivatives to which erylatissins A **1** and B **2** belongs are relatively rare with only 10 having been so far reported. Six of these 10 are reported from *E. latissima*, with one or two each from *E. abyssinica* (1), *E. arborescens* (1), *E. eriotriocha* (1), *E. indica* (1), *E. lysistemon* (1), *E. orientalis* (1), *E. poeppigiana* (1) *E. sigmoidea* (2), *E. suberosa var glabrescence* (1) *E. variegata* (2) and *E. x.bidwillii* (1). The flavanones of the liquitirigenin (5-deoxyflavanone or 7,4′-dihydoxyflavanone) class to which erylatissin C **3** belongs are rare with only five so far reported from the genus. Liquitirigenin, the simplest member, was isolated from *E. lysistemon* but the rest of the members, abyssinones I–IV, have been reported in *E. abyssinica* (all four), *E. latissima* (abyssinones II and IV), and *E. sigmoidea* [abyssinone IV] (Majinda et al., 2004). It thus appear that while daidzein derivatives are fairly distributed in the genus with the greatest number found in *E. latissima*, the liquiritigenin

derivatives have so far been found in *E. abyssinica* and *E. latissima* and one report each in *E. lysistemon* and *E. sigmoidea*. The chemical types found in *E. abyssinica* and *E. latissima* seem to confirm the fact that the two are also morphologically very similar.

2.1. Antimicrobial and DPPH radical scavenging activity

The reported compounds were tested in vitro for their antimicrobial activities using rapid agar overlay or immersion bioautography (Rahalison et al., 1991 and Rios et al., 1988). All the tested compounds were less active against Gram-negative bacteria than both Gram-positive bacteria and fungi (Table 3). The compounds tested were isoflavones (**1**, **2**, **4**, **6**, **7**, and **11**), flavanones (**3** and **5**), pterocarpans (**8**, **9** and **13**) and chalcones (**10** and **12**). The most active compounds were **9**, **10** and **3** in that order. It seems that for chalcones the presence of a 3′-prenyl unit enhanced activity while for pterocarpans the presence of a 6a-hydroxy and/or a 9-methoxy groups were important for activity and for flavanones 3′,4′-hydroxylation and 5′-prenylation were essential for activity. The isolated compounds showed weak radical scavenging properties with compounds **8**, **10** and **4** being the most active. It seems for the pterocarpans both the presence of 9-hydroxy and a 10-prenyl group were important for radical scavenging activity while it appears the presence of a 9-methoxy and/or a 6a-hydroxy reduced activity of the same, while for chalcones it appears the presence of a 3′-prenyl and 4′-hydroxy enhanced activity. Furthermore, it appears that for isoflavones the presence a 4′-hydroxylation and less bulky substituents in B-ring enhanced activity and that hydroxylation at either 7 only or both 5,7 did not seem to affect activity.



Full-size image (31K)

Table 3.

The antibacterial, antifungal and radical scavenging activities of the compounds isolated from the root wood of *Erythrina latissima*

Compound	**Test organisms (minimum inhibitory amount of compound in µg)**				**DPPH assays**	
	E. coli	***S. aureus***	***B. subtilis***	***C. mycoderma***	**TLC (µg)**	**IC_{50} (µg/ml)**
1	5.00	0.10	0.10	0.02	0.5	780
2	NA	1.00	1.00	1.00	10	>1000
3	0.50	0.10	0.01	0.01	0.5	710
4	NA	1.00	5.00	0.05	0.1	380
5	10.00	0.50	0.50	0.01	0.5	630
6	NA	50.00	50.00	0.05	NT	NT
7	0.50	0.10	0.10	0.02	0.5	671
8	5.00	0.50	0.50	0.10	0.1	135
9	0.10	0.01	0.01	0.05	1.0	>1000
10	0.50	0.01	0.01	0.05	0.1	160
11	100.00	1.00	5.00	0.10	0.5	354
12	1.00	0.10	0.10	0.50	0.5	810
13	10.00	1.00	1.00	0.50	0.5	450
Reference	Chl	Chl	Chl	Miconazole	Qu < 0.05	Qu 7
Compounds	0.001	0.0001	0.0001	0.0001	Ga < 0.05	Ga 4
					Aa < 0.10	Aa 18

NA, no activity up to 100.00 µg; NT, not tested; Chl, chloramphenicol; Qu, quercetin; Ga, gallic acid; Aa, ascorbic acid. Results are recorded as means of three readings.

3. Experimental

3.1. General

M.ps uncorrected. UV: Shimadzu UV-2501PC spectrophotometer. IR: Perkin–Elmer 2000 FT-IR spectrometer. ^{1}H NMR, ^{13}C NMR, DEPT, COSY, HMQC, HMBC: Bruker Avance DPX 300 and DRX 600 spectrometers using standard pulse sequences and referenced to residual solvent signals. EI-MS: Finnigan MAT SSQ 7000 Single Quadrupole Instrument at 70 eV. CC: silica gel 60 (0.040–0.063 mm, Merck). Preparative and analytical TLC: silica gel 60 $PF_{254+366}$ (Merck). Visualisation of chromatograms: UV (254 and 366 nm) and Vanillin–sulphuric acid spray.

3.2. Plant material

Stem wood of *E. latissima* was collected in Mapoka Village, North East District, Botswana. The species was identified by Dr. L.M. Turton and a voucher specimen (E 0897) is deposited at the University of Botswana Herbarium.

3.3. Extraction and isolation

The dry and powdered stem wood (1.4 kg) was extracted with $CHCl_3$/MeOH (1:1) mixture and removal of solvent from the extract gave a brown residue (352.0 g). The extract was dissolved in water and partitioned successively between $CHCl_3$ and *n*-BuOH. The $CHCl_3$ extract (266.2 g) was adsorbed on 150.0 g of silica gel and applied to a normal column packed with 250.0 g of silica gel using $CHCl_3$. The column was eluted using the following solvent systems: $CHCl_3$/EtOAc (6:1)-fractions 1–5, $CHCl_3$/EtOAc (6:2)-fractions 6–8, $CHCl_3$/EtOAc (6:3)-fractions 9–12, EtOAc-fractions 13–19.

Fractions that showed similar components on TLC were combined together as follows: 4–11 and 14–19. The combined fraction 14–19 (30.0 g) was adsorbed in 30.0 g of silica gel and applied on a column packed with 80.0 g of silica gel using

$CHCl_3$/EtOAc (6:2) and run with the same solvent system to obtain 7,4′-dihydroxyisoflavone (daidzein), **4** (15.9 mg) (Dewick, 1994 and Yu et al., 2000).

The combined fraction 4–11 (108.2 g) was adsorbed in 100 g of silica gel and applied on a normal column packed with 200 g of silica gel using $CHCl_3$. Thirty nine fractions were collected and the solvent systems used to elute the column were $CHCl_3$ (100%)-fractions 1–18, $CHCl_3$/EtOAc (9:1)-fractions 19–27, $CHCl_3$/EtOAc (6:2)-fractions 28–36 and EtOAc (100 %)-fractions 37–39. These fractions, based on TLC, were combined as follows: fraction A (fractions 1–13), B (fractions 14–19), C (fractions 20–32) and D (fractions 33–39).

The combined fraction B was applied on the preparative TLC and eluted with toluene/EtOAc/AcOH (45:4:1) (×3 developments) to give 7,4′-dihydroxy-3′-γ,γ-dimethylallylflavanone (abyssinone II) **5** (123.6 mg) (Kamat et al., 1981).

The combined fraction C was passed through Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) to give fractions C-1, C-2 and C-3. Fraction C-1 was applied on preparative TLC eluted with toluene/EtOAc/AcOH (40:9:1) (×4 developments) to give 7,3′-dihydroxy-4′-methoxyisoflavone (calycosin) **6** (34.4 mg); 7,4′-dihydroxy-3′-γ,γ-dimethylallyl isoflavone(neobavaisoflavone) **7** (77.4 mg) (Nkengfack et al., 1994); 7,3′-dihydroxy-4′-methoxy-5′-prenylflavanone **3** (80.0 mg) and 7,3′-dihydroxy-4′-methoxy-5′-γ,γ-dimethylallyl isoflavone **1** (24.0 mg).

Fraction C-2 was applied on preparative TLC eluted with toluene/EtOAc/AcOH (40:9:1) [×3] to give band II and I. Both bands were re-applied on preparative TLC eluted with toluene/EtOAc/AcOH (45:4:1) [×5] to give compounds 7,3′-dihydro-6″, 6″-dimethyl-4″, 5″-dehydropyrano[2″,3″:4′,5′]isoflavone **2** (5.0 mg) and 3,9-dihydroxy-10-γ,γ-dimethylallylpterocarpan (phaseollidin) **8** (4.7 mg) (Mitscher et al., 1988) from band I and 3,6a-dihydroxy-9-methoxy-10-γ,γ-dimethylallylpterocarpan (cristacarpin) **9** (103.9 mg) (Fomum et al., 1986 and Tanaka et al., 1996) from band II.

Fraction C-3 was passed through Sephadex LH-20 (EtOAc) to give fraction C-3-1 and C-3-2. Fraction C-3-1 was applied on the preparative TLC eluted with toluene/EtOAc/AcOH to give 4,2′,4′-trihydroxy-3′-γ,γ-dimethylallylchalcone **10** (12.2 mg) (Asada et al., 1998). Fraction C-3-2 (20.0 g) was adsorbed in 20.0 g of silica gel and applied to a column packed with 50.0 g of silica gel using toluene/EtOAc/AcOH (40:9:1) and eluting with the same solvent system to give 5,7,4′-trihydroxyisoflavone (genistein) **11** (132.2 mg) (Asres et al., 1985) and fraction C-3-2-1. Fraction C-3-2-1 was applied on preparative TLC eluted with toluene/EtOAc/AcOH (45:4:1) to give 4,2′,4′-trihydroxychalcone **12** (50.1 mg) (Bohm, 1994) and 3,9-dihydroxypterocarpan (demethylmedicarpin) **13** (4.7 mg) (Dewick, 1994 and Huang and Liou, 1997).

3.3.1. 7,3′-Dihydroxy-4′-methoxy-5′-*γ*,*γ*-dimethylallylisoflavone (erylatissin A), 1

Brown paste. UV: λ_{max} MeOH nm (logε): 310 (sh), 249 (4.2); +NaOMe 334 (sh), 260; +$AlCl_3$ 309 (sh), 247; +$AlCl_3$/HCl 310, 249; +NaOAc 336 (sh), 265; +NaOAc/H_3BO_3 248. IR (KBr pellets): ν_{max} cm^{-1} 3414, 2923, 1624, 1509, 1290. EI-MS: *m/z* (rel. int.), 352 (60) $[M]^{+\bullet}$, 337 (38), 137 (100) (MF $C_{21}H_{20}O_5$).

3.3.2. 7,5′-Dihydroxy-6″,6″-dimethyl-4″,5″-dehydropyrano[2″,3″:4′,5′]isoflavone (erylatissin B), 2

Yellowish paste. UV: λ_{max} MeOH nm (logε): 312 (sh), 256 (4.10); +NaOMe 335 (sh), 275; +$AlCl_3$ 314 (sh), 256; +$AlCl_3$/HCl 313 (sh), 257; +NaOAc 334 (sh), 276; +NaOAc/H_3BO_3 256. IR (KBr pellets): ν_{max} cm^{-1} 3415, 2925, 1618, 1250. EI-MS: *m/z* (rel. int.) 336 (100) $[M]^{+\bullet}$, 199 (16), 137 (51) (MF $C_{20}H_{16}O_5$).

3.3.3. (–)-7,3′-Dihydroxy-4′-methoxy-5′-*γ*,*γ*-dimethylallylflavanone (erylatissin C), 3

Yellow solid, m.p. 56–59 °C, $[\alpha]_D$ –78° (MeOH, c 0.025). UV: λ_{max} MeOH nm (logε): 329 (sh), 279 (4.10); +NaOMe 337; +$AlCl_3$ 328 (sh), 279 (4.10); +$AlCl_3$/HCl 280; +NaOAc 335, +NaOAc/H_3BO_3 329. IR (KBr pellets): ν_{max} cm^{-1} 3414, 2924, 1618, 1249. EI-MS: *m/z* (rel. int.) 354 (93) $[M]^{+\bullet}$, 269 (100), 217 (15), 137 (46) (MF $C_{21}H_{22}O_5$).

3.4. Antibacterial and antifungal assays

The activity tests were done using the TLC bioautographic technique using the standard procedures by Rahalison et al., 1991; against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Candida mycoderma*. The standards used for comparison were chloramphenicol for bacteria and miconazole for fungus. Exact procedure is as previously reported (Bojase et al., 2002 and Wanjala et al., 2002). The experiments were run in triplicate.

3.5. DPPH assays

Reduction of DPPH (2,2-diphenyl-1-picrylhydrazyl or 2,2-diphenyl-1-(2,4,6-trinitrophenyl)-hydrazyl) radical [molecular formula $C_{18}H_{12}N_5O_6$, Mwt 394]. TLC autographic assay: after developing and drying, TLC plates (with amounts of sample ranging from 0.1 to 100 µg) were sprayed with 0.2% (2 mg/ml) of DPPH solution in methanol. The plates were examined half an hour after spraying. Active compounds appeared as yellow spots against a purple background (Cuendet et al., 1997, Cuendet et al., 2000 and Takao et al., 1994). The samples were done in triplicate and the mean value of the three was recorded.

One millilitre of 500 µM (0.2 mg/ml) DPPH in methanol was mixed with equal volumes of test compounds at various concentrations, mixed well and kept in the dark for 30 min. The absorbance at 517 nm was monitored in the presence of different concentrations of the samples. Blank experiment was also carried out, with just solvent and DPPH (i.e., 2 ml of 500 µM in methanol), to determine the absorbance of DPPH before interacting with the compounds. The amount of sample in µg/ml at which the absorbance at 517 nm decreases to half its initial value was used as the IC_{50} value of the compound (Naik et al., 2003). The samples were done in triplicate and the mean value of the three was recorded.

Acknowledgements

R.R.T.M. thank IFS (Grant No: F/2698/2) and UBRPC (Vote R475) for research grants. M.C. thank DAAD-NAPRECA for a scholarship.

References

Asada et al., 1998 Y. Asada, W. Li and T. Yoshikawa, Isoprenylated flavonoids from the hairy root cultures of *Glycyrrhiza glabra*, *Phytochemistry* **47** (1998), pp. 389–392. Abstract | **Article** | PDF (338 K) | View Record in Scopus | Cited By in Scopus (28)

Asres et al., 1985 K. Asres, P. Mascagini, M.J. O'Neill and J.D. Phillipson, Isoflavanoids from *Bolusanthus speciosus* (bolus) Harms Leguminosae, *Z. Naturforsch* **40C** (1985), pp. 617–620.

Barron and Ibrahim, 1996 D. Barron and R.K. Ibrahim, Isoprenylated flavonoids – a survey, *Phytochemistry* **43** (1996), pp. 921–982. Abstract | PDF (3169 K) | View Record in Scopus | Cited By in Scopus (59)

Bisby et al., 1994 F.A. Bisby, J. Buckingham and J.B. Harborne, Phytochemical Dictionary of the Leguminosae, Chapman and Hall, London (1994).

Bohm, 1994 B.A. Bohm In: J.B. Harborne, Editor, *The Flavonoids – Advances in Research Since 1986*, Chapman and Hall, University Press (1986), p. 391 411—413.

Bojase et al., 2002 G. Bojase, C.C.W. Wanjala, B.A. Gashe and R.R.T. Majinda, Antimicrobial flavonoids from *Bolusanthus speciosus*, *Planta Med.* **68** (2002), pp. 615–620. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (8)

Cuendet et al., 1997 M. Cuendet, K. Hostettmann and O. Potterat, Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*, *Helv. Chim. Acta* **80** (1997), pp. 1144–1152. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (165)

Cuendet et al., 2000 M. Cuendet, O. Potterat, A. Salvi, B. Testa and K. Hostettmann, A stilbene and dihydrochalcones with radical scavenging activities

from *Loiseleuria procumbens*, *Phytochemistry* **54** (2000), pp. 871–874. Abstract | **Article** | PDF (105 K) | View Record in Scopus | Cited By in Scopus (15)

Dewick, 1994 P.M. Dewick In: J.B. Harborne, Editor, *The Flavonoids – Advances in Research Since 1986*, Springer, New York (1994), p. 117.

Fomum et al., 1986 Z.T. Fomum, J.F. Ayafor, J. Wandji, W.G. Fomban and A.E. Nkengfack, Erythrinasinate, an ester from three *Erythrina species*, *Phytochemistry* **25** (1986), pp. 757–759.

Huang and Liou, 1997 K.F. Huang and L.F. Liou, Constituents of *Erythrina crista-galli*, *Chin. Pharm. J. (Taipei)* **49** (1997), pp. 305–314. View Record in Scopus | Cited By in Scopus (5)

Joubert, 1998 F.J. Joubert, Purification and properties of proteinase inhibitors from *Erythrina corallodendron* seeds, *Phytochemistry* **27** (1998), pp. 1297–1300.

Kamat et al., 1981 V.S. Kamat, F.Y. Chuo, I. Kubo and K. Nakanishi, Antimicrobial agents from an East African medicinal plant *Erythrina abyssinica*, *Heterocycles* **15** (1981), pp. 1163–1169.

Majinda et al., 2004 Majinda, R.R.T., Wanjala, C.C.W., Juma, B.F. 2004. Bioactive Non-Alkaloidal Constituents from the Genus *Erythrina*. In: Utta-ur-Rahman, (Ed.), Studies in Natural Products Chemistry, vol. 32. Elsevier, Amsterdam, in press.

Mitscher et al., 1988 L.A. Mitscher, S.K. Okute, S.R. Gollapudi, S. Drake and E. Anova, Antimicrobial pterocarpans of Nigerian *Erythrina mildbraedii*, *Phytochemistry* **27** (1988), pp. 3449–3452. Abstract | PDF (532 K) | View Record in Scopus | Cited By in Scopus (25)

Naik et al., 2003 G.H. Naik, K.I. Priyadarsini, J.G. Satav, M.M. Banavalikar, D.P. Sohoni, M.K. Biyani and H. Mohan, Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine, *Phytochemistry* **63** (2003), pp. 97–104. **Article** | PDF (228 K) | View Record in Scopus | Cited By in Scopus (40)

Nkengfack et al., 1989 A.E. Nkengfack, D.R. Sanson, M.S. Tempesta and Z.T. Fomum, Two new flavonoids from *Erythrina eriotriocha*, *J. Nat. Prod.* **52** (1989),

pp. 320–324. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (15)

Nkengfack et al., 1994 A.E. Nkengfack, T.W. Vouffo, J.C. Vardamides, J. Kouam, Z.T. Fomum, M. Meyer and O. Sterner, Phenolic metabolites from *Erythrina* species, *Phytochemistry* **46** (1994), pp. 573–578.

Rahalison et al., 1991 L. Rahalison, M. Hamburger, K. Hostettmann, M. Monod and E.A. Frenk, Bioautographic agar overlay method for the detection of antifungal compounds from higher plants, *Phytochem. Anal.* **2** (1991), pp. 199–203. **Full Text** via CrossRef

Rios et al., 1988 J.L. Rios, M.C. Recio and A. Villar, Screening methods for natural products with antimicrobial activity: a review of the literature, *J. Ethnopharmacol.* **23** (1988), pp. 127–149. Abstract | PDF (1449 K) | View Record in Scopus | Cited By in Scopus (155)

Takao et al., 1994 T. Takao, Kitatani F, N. Wanatabe, A. Yagi and K. Sakata, A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish, *Biosci. Biotechnol. Biochem.* **58** (1994), pp. 1780–1783. View Record in Scopus | Cited By in Scopus (135)

Tanaka et al., 1996 H. Tanaka, T. Tanaka and H. Etoh, A pterocarpan from *Erythrina orientalis*, *Phytochemistry* **42** (1996), pp. 1473–1475. Abstract | PDF (182 K) | View Record in Scopus | Cited By in Scopus (7)

Van Wyk and Van Wyk, 1998 B. Van Wyk and P. Van Wyk, *Field Guide to Trees of Southern Africa* **390**, Struik Publishers, Capetown (1998) p. 512.

Yu et al., 2000 D. Yu, X. Yang, J. Gao, L. Xu and S. Yang, Studies of chemical constituents of *Erythrina arborescens*, *Zhongguo Zhingyao Zazhi* **25** (2000), pp. 353–355.

Wanjala and Majinda, 2000a C.C.W. Wanjala and R.R.T. Majinda, Two novel glucodienoid alkaloids from *Erythrina latissima* seeds, *J. Nat. Prod.* **63** (2000), pp. 871–873. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (9)

Wanjala and Majinda, 2000b C.C.W. Wanjala and R.R.T. Majinda, A new isoflavanone from the stem bark of *Erythrina latissima*, *Fitoterapia* **71** (2000), pp.

400–405. Abstract | **Article** | PDF (108 K) | View Record in Scopus | Cited By in Scopus (3)

Wanjala and Majinda, 2001 C.C.W. Wanjala and R.R.T. Majinda, Isoflavone glycosides from the root wood of *Erythrina latissima*, *J. AOAC. Int.* **84** (2001), pp. 451–453. View Record in Scopus | Cited By in Scopus (3)

Wanjala et al., 2002 C.C.W. Wanjala, B.F. Juma, G. Bojase, B.A. Gashe and R.R.T. Majinda, Erythrinaline alkaloids and antimicrobial flavonoids from *Erythrina latissima*, *Planta Med.* **68** (2002), pp. 640–642. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (12)

Corresponding author. Tel: + 267 355 2492; fax: + 267 355 2836