Antimicrobial and antioxidant flavonoids from the root wood of *Bolusanthus speciosus*

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Abstract

Three new flavonoids-5,7,4′-trihydroxy-6-[1-hydroxy-2-methylbuten-2-yl]isoflavone (isogancaonin C), 7,2′-dihydroxy-4′-methoxy-isoflav-3-ene (bolusanthin III), 6,6′-dihydroxy-4′-methoxy-2-arylbenzofuran (bolusanthin IV), in addition to eight known flavonoids; derrone, medicarpan, genistein, wighteone, lupiwighteone, gancaonin C, 7-hydroxy-4′-methoxyisoflavone and 7,3′-dihydroxy-4′-methoxyisoflavone were isolated from the root wood of *Bolusanthus speciosus*. The compounds showed strong antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Candida mycoderma*. The isolated compounds also showed moderate to strong radical scavenging properties against DPPH radical with the highest activities shown by the 2-aryl-benzofuran, the isoflav-3-ene and 7,3′-dihydroxy-4′-methoxyisoflavone in decreasing order.
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1. Introduction

Bolusanthus speciosus Harms (Fabaceae), otherwise called Tree Wisteria, is a medium sized tree that grows between 10–13 m tall (Van Wyk and Van Wyk, 1998; Venter and Venter, 1996; Fabian and Germishhuizen, 1997). It occurs in bush veld often in heavy alkaline soils, and flowers in loose hanging racemes that are pale blue to violet and has flat pods (Van Wyk and Van Wyk, 1998). The tree is monotypic and endemic in subtropical South Africa, Botswana, Zimbabwe, Mozambique and Zambia. The dried inner bark is used to relieve abdominal pains, emetism and tuberculosis (Palgrave, 1997). Previous research by other workers on the seeds (Asres et al., 1985) yielded isoflavonoids and leaves (Asres et al., 1986) yielded alkaloids and that by our research group on the stem bark, and root bark revealed the presence of flavonoids (Bojase et al., 2001a, b, 2002). From chloroform and ethyl acetate extracts of the root wood we report three new flavonoids whose characterization is presented below. The antimicrobial and radical scavenging properties of the new and known compounds isolated are also described.

2. Results and discussion

Compound **1** was obtained as a creamy solid with a mp 259–260 °C, and gave an EI-MS molecular ion peak at *m/z* 354 consistent to molecular formula $C_{20}H_{18}O_6$. The ^1H NMR spectrum showed a singlet signal at δ 8.29 (δ_C 153.5) typical of an H-2 isoflavone proton. The ^1H NMR further revealed the presence of an AA′BB′ spin system that is comprised of protons at δ 7.36 (2H, *dd*, *J* = 2.0, 8.6 Hz) and 6.82 (2H, *dd*, *J* = 2.0, 8.6 Hz) assignable to H-2′ & H-6′ and H-3′ & H-5′ of ring B respectively. The ^1H NMR further showed the presence of a prenyl unit comprised of protons resonating at δ 3.27 (2H, *d*, *J* = 7.1 Hz), 5.39 (1H, *t*, *J* = 7.2 Hz), 3.75 (2H, *s*), and 1.72 (3H, *s*) assignable to H-1″, H-2″, H-4″ and H-5″ respectively. It was further established that C-4″ of a prenyl unit was hydroxylated and this was confirmed by DEPT 135 spectrum that showed a deshielded sp³ methylene carbon signal at δ 66.0. The ^1H NMR further showed a proton singlet signal at δ 6.45 and a chelated hydroxyl proton at δ 13.23. The proton at δ

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6.45 showed key HMBC correlations with C-6 (δ 111.7), C-7 (δ 162.9), C-9 (δ 156.2) and C-10 (δ 105.0) and was therefore assignable to H-8 of ring A of an isoflavone skeleton. The mass fragment at m/z 236, resulting from a *retro* Diels–Alder fragmentation (Mabry and Markham, 1975), confirmed that ring A had two hydroxyl groups in addition to a hydroxylated prenyl unit. The presence of a prenyl unit at either C-6 or C-8 of ring A was shown to influence the chemical shift of the chelated proton (Bojase et al., 2001a, Shirataki et al., 1999), in this case the chelated proton was relatively deshielded (δ 13.23) and also C-6 resonated downfield (δ 111.7) confirmed the assignment of a hydroxylated prenyl unit at C-6. This was further confirmed by the HMBC correlations of the protons at δ 3.27 (H-1″) with C-6, C-7, and C-5 (δ 159.7) of the isoflavone skeleton. Compound **4** is an isomer of **1**, first isolated by Fukai et al. from the aerial parts of *Glycyrrhiza uralensis* and trivially named gancaonin C. Compound **1** was thus identified as 5,7,4′-trihydroxy-6-[1-hydroxy-2-methylbuten-2-yl]isoflavone and named isogacaonin C.

Compound **2** was isolated as a brown paste, whose EI-MS spectrum showed the molecular ion peak m/z 270 consistent with the molecular formula $C_{16}H_{14}O_4$. The UV and ^{1}H NMR spectra were suggestive of an isoflav-3-ene skeleton by showing absorption maxima at 323 nm and characteristic ^{1}H NMR signals at δ 5.01 (2H, *br s*) and 6. 62 (1H, *br s*) due to the two H-2 and H-4 protons respectively. The ^{1}H NMR spectrum revealed further the presence of two ABD spin systems, the first one comprised of protons at δ 6.95 (1H, *d*, *J* = 8.1 Hz), 6.43 (1H, *dd*, *J* = 2.3, 8.1 Hz) and 6.34 (1H, *d*, *J* = 2.3 Hz) assignable to H-5, H-6 and H-8 of ring A respectively. The assignments were confirmed by their key HMBC correlations, notably between a proton resonating at δ 6.95 and C-4 (δ 120.9), C-7 (δ 158.3), C-8 (δ 102.9) and C-9 (δ 156.0), and that between a proton at δ 6.34 and C-6 (δ 108.8), C-7, C-9 & C-10 (δ 116.5). The second ABD spin system was comprised of protons at δ 6.51 (1H, *d*, *J* = 2.2 Hz), 7.21 (1H, *dd*, *J* = 2.2, 8.3 Hz) and δ 6.47 (1H, *d*, *J* = 8.0 Hz), assignable to H-3′, H-5′ and H-6′ of ring B respectively. The assignment was confirmed by the HMBC correlations of the proton at δ 6.47 (H-6′) with C-1′ (δ 118.8), C-2′ (δ 158.3), C-4′ (δ 160.1), C-5′ (δ

129.4) and C-3 (δ 128.7). The ^{1}H NMR spectrum further showed a singlet signal at δ 3.75 (3H, *s*) typical of the methoxy protons and those showed HMBC correlations with C-4′ confirming the placement of the methoxy group at C-4′. Thus compound **2** was identified as 7,2′-dihydroxy-4′-methoxyisoflav-3-ene and named bolusanthin III.

Compound **3** was obtained as brown solid with the mp 178–180 °C, and the EI-MS spectrum showed the molecular ion peak at m/z 256 consistent with the molecular formula $C_{15}H_{12}O_4$. The ^{1}H and COSY NMR spectrum indicated the presence of two ABD spin systems, the first one comprised of protons resonating at δ 7.39 (1H, *d*, *J* = 8.3 Hz), 6.80 (1H, *dd*, *J* = 2.1, 8.3 Hz) and 7.00 (1H, *dd*, *J* = 0.9, 8.1 Hz) while the second one consisted of protons resonating at δ 7.81 (1H, *dd*, *J* = 0.9, 8.1 Hz), 6.62 (1H, *br d*, *J* = 1.6 Hz) and δ 6.59 (1H, *d*, *J* = 2.4 Hz). The ^{13}C NMR spectrum gave fourteen non equivalent carbon signals implying the presence of a 6-2-6 system indicative of an arylbenzofuran skeleton. This was further confirmed by the ^{1}H NMR which showed a one proton doublet signal at δ 7.20 (1H, *d*, *J* = 0.9 Hz) which was assignable to H-3, and the magnitude of the coupling constant is indicative of an extended W-coupling or zigzag coupling with either protons H-7 (δ 7.00) and/or proton H-6′ (δ 7.81). Thus on the basis of their HMBC correlations, protons of the first spin system (δ 7.39, 6.80 and 7.00) were assigned to H-4, H-5 and H-7 in ring A while those of the second spin system (δ 7.81, 6.62 and 6.59) were assigned to H-3′, H-5′ and H-6′ in ring B respectively. The ^{1}H NMR spectrum further revealed the presence of the singlet signal at δ 3.75 (3H, *s*) being a typical signal of methoxy protons and these showed HMBC correlations with C-4′ (δ 160.7) thus confirming the assignment of the methoxy group at C-4′. From the data above compound **3** was identified as 6,6′-dihydroxy-4′-methoxy-2-aryl-benzofuran and named bolusanthin IV.

2.1. Antimicrobial and DPPH radical scavenging activity

The isolated compounds were screened for antimicrobial activity using the TLC bioautography technique (Rahalison et al., 1991). It was generally observed that, prenylated isoflavones showed higher activity

1: R_1 = 4″-hydroxylated prenyl unit
   R_2 = H
4: R_1 = H
   R_2 = 4″-hydroxylated prenyl unit

2

3
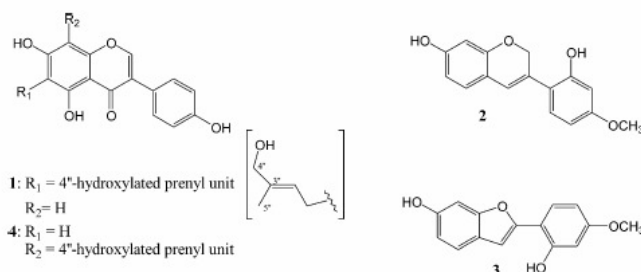
than non-prenylated ones (see Table 3). Comparing **8** and **9** it seems 6-prenylation enhances activity against both Gram-negative and Gram-positive bacteria while activity against these organisms goes down with 8-prenylation. The activity against fungi seem to be virtually identical for both isomers. What is more interesting however is that when one of the methyl groups of a prenyl unit is converted to a hydroxymethyl and put at either 6 or 8 position (**1** and **4**) the activity profile of these compounds remain virtually the same. However, other classes of new isolated flavonoids, **2** (an isoflav-3-ene) & **3** (a 2-arylbenzofuran) demonstrated a relatively moderate activity against the test organisms in the bioassay. This observation is consistent with our previous observation of 2-arylbenzofurans exhibiting good antimicrobial activity (Wanjala et al., 2002). The DPPH radical scavenging properties of the isolated compounds (Table 3) seem to indicate that for isoflavonoids, prenylation in A-ring did not seem to have any effect, and that both 3′ and 4′-hydroxylation was important for activity while the presence or absence of 5-hydroxylation did not seem to affect activity. Compound **2** (2-arylbenzofuran) showed higher free radical scavenging ability than ascorbic acid, a well known standard radical scavenger. Compound **3** (an isoflav-3-ene) was also a moderate radical scavenger. It is noteworthy to mention that **2**, **3** and **6** bleached the DPPH immediately suggesting that they could be classified as fast kinetic antioxidants (Brand-Williams et al., 1995).

3. Experimental

3.1. General experimental procedure

Melting point; Stuart Scientific (SMP1) melting point apparatus; UV: Shimadzu UV-2101PC spectrophotometer. The 1D {^{1}H (300 MHz), ^{13}C (75.5 MHz), DEPT} and the 2D (COSY, HMQC, HMBC) spectra acquired on Bruker Avance DPX 300 spectrometer and referenced to residual solvent signal. HMBC experiments were run using the standard pulse sequence. MS: EI on Finnigan MAT SSQ 7000 single quadrupole instrument. Column chromatography silica gel 60 particles size 0.04–0.063 mm for column chromatography (Merck); Sephadex LH-20 (Sigma); Preparative TLC-silica gel 60 PF$_{254+366}$ for preparative thin layer chromatography (Merck); Analytical TLC:- TLC silica gel 60-F$_{254}$ precoated alumina sheets (Merck) and visualized using UV (254 and 366 nm) and vanillin-sulphuric acid spray.

3.2. Plant material

The root wood of *Bolusanthus speciosus* was collected from Mapoka Village in the North East District, Botswana in August 1997. Dr. L.M. Turton identified the plant and a voucher specimen (B 0897) was deposited at the University of Botswana Herbarium.

3.3. Extractions and isolation

Air-dried and pulverized root wood (3 kg) was sequentially soaked in chloroform, ethyl acetate and methanol. The chloroform and ethyl acetate extract were combined to yield 108 g of brown residue. The methanolic extract contained some free sugars and other uninteresting highly polar materials and hence was not investigated further. Part (80 g) of the crude combined extract obtained was adsorbed in silica gel PF 60 and later packed into a big column (58 cm×5 cm) for chromatographic elution. The column was eluted using a series of solvent systems; 100% *n*-hexane, *n*-hexane/ CHCl$_3$ (8:2, 6:4) and then *n*-hexane/CHCl$_3$/EtOAc (5:4:1, 4:4:2, 2:4:4) and subsequently with CHCl$_3$/ EtOAc (6:4, 4:6) to 100% EtOAc giving five fractions BSR 1/4, 5/9, 10/17, 18/26 and 27/36. Fractions BSR 1/4 contained mainly fatty materials and was not investigated further. Fraction BSR 5/9 was applied on Sephadex LH-20 column [CHCl$_3$/MeOH (1:1)] giving two sub fractions one from which 7-hydroxy-4′-methoxyisoflavone **5** (206 mg) (Selenge et al., 1986) crystallized out and the other on prep TLC [×2, *n*-hexane/CHCl$_3$/acetone (7:2:1)] yielded 7,3′-dihydroxy-4′-methoxyisoflavone **6** (34 mg) (Selenge et al., 1986) and 5,7,4′-trihydroxyisoflavone (genistein) **7** (27 mg) (Asres et al., 1985).

Fraction BSR 10/17 was subjected to silica gel chromatography using silica gel PF 60 and eluted using *n*-hexane/CHCl$_3$ (6:4, to 1:1) and finally 100% CHCl$_3$, yielding three main fractions BSR″ 1/6, 7/12 and 13/17. Fraction BSR″ 1/6 afforded the same compounds isolated before from fraction BSR 5/9 (i.e. **5**, **6**, **7**). Fraction BSR″ 7/12 was loaded on Sephadex LH-20 (1:1 CHCl$_3$/ MeOH) and preparative TLC [*n*-hexane/CHCl$_3$/acetone (6:3:1)] on resulting fractions gave 5,7,4′-trihydroxy-6-γ,γ-dimethylallylisoflavone (wighteone) **8** (40 mg) (Ingham et al., 1990) and 5,7,4′-trihydroxy-8-γ,γ-dimethylallylisoflavone (lupiwighteone) **9** (35 mg) (Fukai et al., 1989). Using the same solvent system as above fraction BSR″ 13/17 was subjected to multiple development prep TLC (×5) that yielded two compounds, 5,7,4′-trihydroxy-6-[1-hydroxy-2-methylbuten-2-yl]isoflavone **1** (68 mg) (isogancaonin C) and 5,7,4′-trihydroxy-8-[1-hydroxy-2-methylbuten-2-yl]isoflavone (gancaonin C) **4** (56 mg) (Fukai et al., 1989). Fraction BSR 18/26 was subjected to Sephadex LH-20 and prep TLC to give 3-hydroxy-9-methoxy[6aR,11aR]pterocarpan (medicarpan) **10** (58 mg) (Ingham et al., 1990), 5,4′-dihydroxy-6,6-dimethyl-4,5-dehydropyrano-[2,3:7,8]isoflavone (derrone) **11** (8.5 mg) (Chibber et al., 1980), 7,2′-dihydroxy-4′-methoxy-isoflav-3-ene **2** (68 mg) and 6,6′-dihydroxy-4′-methoxy-2-arylbenzofuran **3** (31 mg). Work on fraction BSR 27/ 36 gave more of compounds **2** and **3**.

3.4. Physical and spectroscopic data

3.4.1. 5,7,4'-Trihydroxy-6-[1-hydroxy-2-methylbuten-2-yl]isoflavone (isogancaonin C) 1

Creamy solid mp 259–260 °C. UV λ_{max} (MeOH) nm (log ϵ_{max}) 267 (3.19), 213 (3.17), +NaOMe 267, 214, +AlCl$_3$ 277, 221, +AlCl$_3$/HCl 279, 218, +NaOAc 274, 217, +NaOAc/H$_3$BO$_3$ 276, 221. IR (KBr) ν_{max} (cm^{-1}) 3281, 2923, 1647, 1619. EI-MS m/z 354 [M]$^+$ (100), (MF C$_{20}$H$_{18}$O$_6$.) ^{1}H and ^{13}C NMR data in DMSO-d_6 (see Table 1).

3.4.2. 7,2'-Dihydroxy-4'-methoxyisoflav-3-ene (bolusanthin III) 2

Brown paste, UV λ_{max} (MeOH) nm (log ϵ_{max}) 323 (1.73), 207 (2.06), +NaOMe 333, 311, +AlCl$_3$ 323, 206, +AlCl$_3$/HCl 318, 205, +NaOAc 321, 216, +NaOAc/H$_3$BO$_3$ 326, 209. IR (KBr) ν_{max} (cm^{-1}) 3380, 2840, 1615. EI-MS m/z 270 [M]$^+$ (100). (MF C$_{16}$H$_{14}$O$_4$), ^{1}H and ^{13}C NMR data in acetone-d_6 (Table 2).

3.4.3. 6,6'-Dihydroxy-4'-methoxy-2-arylbenzofuran (bolusanthin IV) 3

Brown solid with a mp 178–180 °C, UV λ_{max} (MeOH) nm (log ϵ_{max}) 335 (2.53), 283 (2.13),

+NaOMe 334, 281, +AlCl$_3$ 335, 281, +NaOAc 334, 281, NaOAc/H$_3$BO$_3$ 335, 282, IR (KBr) ν_{max} (cm^{-1}) 3456, 1618. EI-MS m/z 256 [M]$^+$ (rel. int.). (MF C$_{15}$H$_{12}$O$_4$), ^{1}H and ^{13}C NMR in acetone-d_6 (see Table 2).

3.5. Antibacterial and antifungal assays

A TLC bioautography procedure (Rahalison et al., 1991) was used. Exact procedure is as previously reported (Bojase et al., 2002; Wanjala et al., 2002).

6. 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$_5$O$_6$, 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, 2000; Takao et al., 1994).

Table 1
^{1}H and ^{13}C NMR data for compounds **1** and **4** in DMSO-d_6

Position	1		4	
	δ_H	δ_C	δ_H	δ_C
2	8.29, 1H, s	153.5	8.16, 1H, s	154.1
3		123.0		123.9
4		181.0		181.7
5		159.7		161.7
6		111.7	6.31, 1H, s	100.9
7		162.9		165.0
8	6.45, 1H, s	92.0		106.9
9		156.2		156.7
10		105.0		105.2
1'		122.2		123.8
2'	7.36, 1H, dd, (8.6, 2.0 Hz)	129.9	7.46, 1H, br d, (8.4 Hz)	130.5
3'	6.82, 1H, dd, (8.6, 2.0 Hz)	114.8	6.88, 1H, br d, (8.3 Hz)	115.2
4'		158.2		158.6
5'	6.82, 1H, dd, (8.6, 2.0 Hz)	114.8	6.88, 1H, br d, (8.3 Hz)	115.2
6'	7.36, 1H, dd, (8.6, 2.0 Hz)	129.9	7.46, 1H, br d, (8.4 Hz)	130.5
1''	3.27, 2H, d, (7.1 Hz)	20.2	3.53, 2H, d, (7.1 Hz)	21.3
2''	5.39, 1H, t, (7.2 Hz)	120.7	5.36, 1H, t, (7.2 Hz)	125.8
3''		136.1		136.4
4''	3.75, 2H, s	66.0	4.26, 2H, s	61.0
5''	1.72, 3H, s	14.4	1.74, 3H, s	21.6

Assignments were confirmed by COSY, HMQC, HMBC and DEPT 135.

Table 2
^{1}H and ^{13}C NMR data for compounds **2** and **3** in acetone-d_6

Position	2		3	
	δ_H	δ_C	δ_H	δ_C
2	5.01, 2H, brs	68.2		152.1
3		128.7	7.20, 1H, d, (0.9 Hz)	104.0
4	6.62, 1H, brs	120.9	7.39, 1H, d, (8.3 Hz)	121.1
5	6.95, 1H, d, (8.1 Hz)	127.9	6.80, 1H, dd, (8.3, 2.1 Hz)	112.2
6	6.43, 1H, dd, (8.1, 2.3 Hz)	108.8		155.1
7		158.3	7.00, 1H, dd, (2.1, 0.9 Hz)	97.7
8	6.34, 1H, d, (2.3 Hz)	102.9		155.6
9		156.0		122.8
10		116.5	–	
1'		118.8	111.5	
2'		158.3	7.81, 1H, dd, (8.1, 0.9 Hz)	127.3
3'	6.51, 1H, d, (2.0 Hz)	102.1	6.62, 1H, br d, (1.6 Hz)	103.2
4'		160.7		160.9
5'	7.21, 1H, dd, (8.3, 2.2 Hz)	129.4	6.59, 1H, d, (2.4 Hz)	105.9
6'	6.47, 1H, d, (6.0 Hz)	105.7		155.5
OCH$_3$	3.75, 3H, s	54.9	3.80, 3H, s	55.0

Assignments were confirmed by COSY, HMQC, HMBC and DEPT 135.

Table 3
Bioactive compounds isolated from the root wood of *Bolusanthus speciosus*

Compound	Test organism (minimum inhibitory amount of compound in μg)				DPPH assays	
	E. coli	*B. subtilis*	*S. aureus*	*C. mycoderma*	TLC (μg)	IC_{50} (μg/ml)
1	0.10	0.05	0.05	0.05	0.5	650
2	1.00	0.50	0.50	0.05	0.1*	11*
3	0.50	0.05	0.01	0.05	0.1*	29*
4	0.10	0.05	0.05	0.05	0.5	610
5	50.0	50.0	10.0	10.0	0.5	960
6	NA	NA	NA	0.1	0.5*	150
7	NT	NT	NT	NT	1.0	1810
8	0.05	0.01	0.01	0.05	1.0	2100
9	10.0	0.50	0.5	0.05	1.0	670
10	NA	100.0	10.0	1.00	1.0	1100
11	100.0	20.0	0.10	1.00	NT	NT
Reference compound(s)	Chl 0.001	Chl 0.0001	Chl 0.0001	Miconazole 0.0001	Qu < 0.05 Ga < 0.05 Aa < 0.10	Qu 7 Ga 4 Aa 18

NA = not active, NT = not tested, * = reacted instantaneously, chl = chloramphenicol, Qu = quercetin, Ga = gallic acid, Aa = ascorbic acid.

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 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 is used as the IC_{50} value of the compound (Naik et al., 2003). The samples were done in triplicate.

Acknowledgements

References

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

Asres, K., Phillipson, J.D., Mascagani, P., 1986. Alkaloids of *Bolusanthus speciosus*. Phytochemistry 25, 1449–1452.

Bojase, G., Wanjala, C.C.W., Majinda, R.R.T., 2001a. Flavonoids from the stem bark of *Bolusanthus speciosus*. Phytochemistry 56, 837–841.

Bojase, G., Wanjala, C.C.W., Majinda, R.R.T., 2001b. Two new isoflavonoids from *Bolusanthus speciosus*. Bull. Chem. Soc. Ethiop. 15, 131–136.

Bojase, G., Wanjala, C.C.W., Gashe, B.A., Majinda, R.R.T., 2002. Antimicrobial flavonoids from *Bolusanthus speciosus*. Planta Medica 68, 615–620.

Brand-Williams, W., Cuvelier, M.E., Besert, C., 1995. Use of a free radical method to evaluate antioxidant activity. Lebensm.-Wiss. u. Technol. 28, 25–30.

Chibber, S.L., Sharma, R.P., 1980. Derrone and new isoflavanones from *Derris robusta* seeds. Phytochemistry 19, 1857–1859.

Cuendet, M., Hostettmann, K., Potterat, O., 1997. Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. Helvetica Chimica Acta 80, 1144–1152.

Cuendet, M., Potterat, O., Salvi, A., Testa, B., Hostettmann, K., 2000. A stilbene and dihydrochalcones with radical scavenging activities from *Loiseleuria procumbens*. Phytochemistry 54, 871–874.

Fabian, A., Germishhuizen, G., 1997. Wild Flowers of Northern Southern Africa. Fernwood Press, Vlaeberg.

Fukai, T., Wang, Q.-H., Nomura, T., 1989. Four new prenylated flavonoids from the aerial parts of *Glycyrrhiza uralensis*. Heterocycles 29, 1369–1378.

Ingham, J.L., 1990. Systematic aspects of phytoalexin formation within tribe Phaseolaceae of the Leguminosae (subfamily Papilioniodeae). Biochem. Syst. Ecol. 18, 329–343.

Mabry, T.J., Markham, K.R.. In: Harborne, J.B., Mabry, T.J., Mabry, H. (Eds.), The Flavonoids, Part 1. Academic Press, New York, London, p. 81.

Naik, G.H., Priyadarsini, K.I., Satav, J.G., Banavalikar, M.M., Sohoni, D.P., Biyani, M.K., Mohan, H., 2003. Comparative antioxidant activity of individual herbal components used in Ayuverdic medicine. Phytochemistry 63, 97–104.

Palgrave, K.C., 1977. Trees of Southern Africa. Struik, Cape Town, p. 287.

Rahalison, L., Hamburger, M., Hostettmann, K., Monod, M., Frenk E, A., 1991. Bioautographic agar overlay method for the detection of antifungal compounds from higher plants. Phytochemical Analysis 2, 199–203.

Selenge, D., Batsuren, D., Batirov, E.K., Malikov, V.M., 1986. Chemical studies of Mongolian flora-isoflavones from *Ammopiptanthus mongolicus*. Khim. Prir. Soedin. 2, 242–244.

Shirataki, Y., Matsuoka, S., Komatsu, M., Ohyama, M., Tanaka, T., Iinuma, M., 1999. Four isoflavanones from the roots of *Sophora tetraptera*. Phytochemistry 50, 695–701.

Takao, T., Kitatani, F., Wanatabe, N., Yagi, A., Sakata, K., 1994. A

simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish. Biosci. Biotech. Biochem. 58, 1780–1783.

Van Wyk, B., Van Wyk, P., 1998. Field Guide to Trees of Southern Africa. Struik Publishers, Cape Town, pp 390, 512.

Venter, F., Venter, J.-A., 1996. Making the Most of Indigenous Trees. Briza Publications, Pretoria.

Wanjala, C.C.W., Juma, B.F., Bojase, G., Gashe, B.A., Majinda, R.R.T., 2002. Erythrinaline alkaloids and antimicrobial flavonoids from *Erythrina latissima*. Planta Medica 68, 640–642.