Phenolic and Antibacterial Constituents of *Vahlia capensis*

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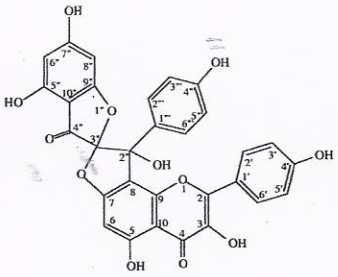
Abstract: **The *n*-butanol fraction of *Vahlia capensis* yielded kaempferol, quercetin, afzelin, astragalin, quercitrin, isoquercitrin, rutin, gallic acid, chiro-inositol, dulcitol, and a novel biflavonoid, VC-15B (vahlia biflavone). The compounds were identified using 1D and 2D NMR techniques and FABMS. Vahlia biflavone and gallic acid were isolated, using bioassay-guided procedure and identified as the antibacterial components. Both compounds showed activity against Gram positive *Staphylococcus aureus* and *Bacillus subtilis*. Vahlia biflavone gave MIC values of 15.3 μg/ml and 30.6 μg/ml against *S. aureus* and *B. subtilis*, respectively while gallic acid gave a value of 71.3 μg/ml for both organisms.**

In an earlier communication (1) we reported components from the non-active fractions of *Vahlia capensis*, a medicinal plant that has been used widely in Botswana (2) and also in Zimbabwe (3) to treat eye infections. Here we report on a novel biflavone (VC-15B) and other phenolic and antibacterial components of *V. capensis*.

FABMS of VC-15B (vahlia biflavone, $C_{30}H_{18}O_{12}$) gave a molecular ion at 571 $(M + H)^+$ but EIMS gave the highest ion at *m/z* 418. The ^{13}C-NMR data (Table **1**) showed thirty carbons with chemical shifts characteristic of flavonoids, the number of carbons suggesting a biflavonoid. The ^{1}H-NMR (Table **1**) showed the presence of two 4-oxyphenyl groups [6.80, 7.36 (each 2H, d, J = 8.8 Hz), 6.92, 7.40 (each 2H, d, J = 8.7 Hz)]; a 2,4,6-trioxyphenyl [6.07 and 6.11 (each 1H, d, J = 2.3 Hz)], and a further 3-substituted 2,4,6-trioxyphenyl group (6.47, 1H, s).

These four fragments were further confirmed by the HC-direct and HMBC correlations (Table **2**), which enabled twenty-five carbons to be assigned unambiguously. Of the remaining unassigned quaternary carbons the signal at δ_C 137.0 suggests a C-3 carbon of a flavonol (4, 5) while those at δ_C 176.9 and 186.3 were assigned to C-4 and C-4″, respectively. These signals represent, respectively, carbonyls on an unsaturated and a saturated C ring of a flavonoid nucleus (6). The carbon at 152.8 was assigned to C-9 of a kaempferol moiety [HMBC not possible because the H-8 is replaced by a quaternary carbon at δ_C 108.3]. The HMBC data unambiguously confirmed that this kaempferol moiety was substituted at C-8 and not C-6. The carbon at δ_C 103.8 was assigned to C-3″ by analogy to an almost identically substituted carbon in podoverine C reported in the literature (7). The EIMS gave two strong ions at *m/z* 286 and 285 which together with the NMR data confirmed the presence of a kaempferol nucleus. These data are consistent with either of the two structures **1** and **2**. Structure **2** is more preferred on stability aspects and from the ease with which one can rationalise the observed fragment ions in the mass spectrum of the natural product. While biflavonoids are common in plants, spirobiflavonoids are rare with very few cited in the literature (8, 9). There are also very few biflavonoids with 7, 8→2″, 3″ furan linkage (9). Both of these biflavonoids are reported from the genus *Daphne* (Thymelaeaceae) (8, 9).



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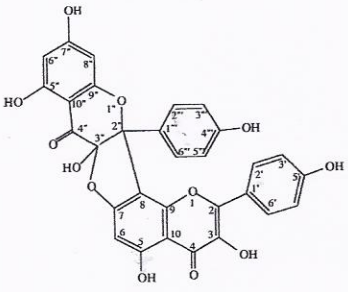

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Table 1 $^{13}C^{*}$ and $^{1}H^{+}$ Data for VC 15B.

Carbon	^{13}C (δ_C)	^{1}H (δ_H)
2	147.8	
3	137.0	
4	176.9	
5	165.0	13.2 br s (chelated OH)
6	95.5	6.47 s
7	167.1	
8	108.3	
9	152.8	
10	105.9	
1′	122.8	
2′	130.7	7.36 d (J = 8.8 Hz)
3′	116.3	6.80 d (J = 8.8 Hz)
4′	160.4	
5′	116.3	6.80 d (J = 8.8 Hz)
6′	130.7	7.36 d (J = 8.8 Hz)
2″	93.4	
3″	103.8	
4″	186.3	
5″	165.6	13.2 br s (chelated OH)
6″	97.9	6.07 d (J = 2.3 Hz)
7″	170.2	
8″	96.2	6.11 d (J = 2.3 Hz)
9″	164.0	
10″	99.9	
1‴	126.0	
2‴	130.3	7.40 d (J = 8.7 Hz)
3‴	115.9	6.92 d (J = 8.7 Hz)
4‴	159.0	
5‴	115.9	6.92 d (J = 8.7 Hz)
6‴	130.3	7.40 d (J = 8.7 Hz)

* Measured at 100 MHz in acetone-d_6.
+ Measured at 400 MHz in acetone-d_6.
^{1}H and ^{13}C chemical shifts were referenced to the residual solvent signals (in ppm).

Table 2 HC-direct and HMBC correlations for VC 15B.

Proton	Carbon		
	HC-direct	HMBC	HMBC
1H	^{1}J	^{2}J	^{3}J
6.07 d	97.9 d	165.6 s, 170.2 s	96.2 s, 99.9 s
6.11 d	96.2 d	164.0 s, 170.2 s	97.9 s, 99.9s
6.47 s	95.5 d	165.0 s, 167.1 s	105.9 s, 108.3s
6.80 d	116.3 d		122.8s , 160.4 s
7.36 d	130.6 d	116.3 d	147.8 s, 160.4 s
6.92 d	115.9 d	159.0 s	126.0 s
7.40 d	130.3 d		93.4 s, 159.0 s

Antibacterial activity for vahlia biflavone and gallic acid are summarized in Table **3**. Although the crude water extract of *V. capensis* did not show activity against Gram-negative bacteria it gave reasonable activity against Gram-positive bacteria. VC-15B was fairly active against *S. aureus* and *B. subtilis* and though not as potent as chloramphenicol, it was active enough to justify the use of *V. capensis* in folklore medicine.

Materials and Methods

Plant material: *V. capensis* (L. f) Thunb. ssp *vulgaris* Bridson were collected in Serowe, Botswana in February 1991 and identified by Inga Hedberg (voucher No. VC RM 362, National Herbarium, Gaborone, Botswana).

Table 3 Antibacterial activities of *Vahlia capensis*, its active constituents and chloramphenicol (for comparison).

Organism	compound	Loading (μg/disc)	Inhibition zone (mm)
B. subtilis	chloramphenicol	10	17
	chloramphenicol	25	21
	chloramphenicol	50	25
	crude water extract	200	10
	VC 15B	50	12
	gallic acid	50	10
S. aureus	chloramphenicol	10	18
	chloramphenicol	25	20
	chloramphenicol	50	23
	crude water extract	200	11
	VC 15B	50	14
	gallic acid	50	10
	compound	MIC in μg/ml	
B. subtilis	VC 15B	30.6	
	gallic acid	71.3	
	chloramphenicol	0.1	
S. aureus	VC 15B	15.3	
	gallic acid	71.3	
	chloramphenicol	0.1	

Powdered plant (1 kg) was extracted with cold water, concentrated, then freeze-dried to give 150 g residue. This was tested for antibacterial activity, at a loading of 200 and 1000 μg/disc (10, 11). The crude water extract (100 g) was dissolved in 500 ml distilled water and was partitioned successively with 5 × 200 ml aliquots each of *n*-hexane, EtOAc and *n*-BuOH. The extracts were evaporated in vacuo to yield 6 g, 10 g, and 63 g crude fractions, respectively. The *n*-BuOH fraction (40 g) was preadsorbed on silica gel and applied on 200 g silica gel VLC column. Elution was done sequentially with *n*-hexane/EtOAc, EtOAc, EtOAc/CH_3OH, where 5% increments of the more polar solvent were made, the last fraction was eluted with a 1 : 1 EtOAc/CH_3OH mixture. Forty fractions (each 150 ml) were collected. Fractions 1–6 yielded triterpenes and coumarins and are reported elsewhere (1). Fractions 7–9 (4.0 g) were purified on a polyamide column (100 g) by successive elution with H_2O, H_2O/CH_3OH (1 : 1), CH_3OH, CH_3OH/CH_3COCH_3 (1 : 1), and CH_3COCH_3. Twenty fractions (150 ml) were collected, the first seven fractions contained non-polar compounds (TLC) and were not investigated further. Fractions 8–20 were analysed by TLC (in $CHCl_3/CH_3OH$, 4 : 1 or 7 : 3) and similar fractions were pooled and passed through Sephadex LH-20 (10 g) using a 1 : 1 $CHCl_3/CH_3OH$ mixture. Two compounds crystallized out of the polyamide column (Fr. 8–10) and these were recrystallized from a 9 : 1 CH_3OH/H_2O mixture to yield dulcitol (180 mg) and inositol (200 mg). Final purification of fractions 11–20 was done on HPLC using a semi-preparative column (RP 18, CH_3OH/H_2O, 60 : 40 or 50 : 50, flow rate 6 ml/min). Isolated compounds were kaempferol (5 mg), quercetin (8 mg), afzelin (30 mg), astragalin (38 mg), isoquercitrin (30 mg), quercitrin (30 mg), rutin (15 mg), gallic acid (100 mg), and VC 15B (30 mg). The isolated compounds were identified using spectroscopic methods and by comparison with authentic samples.

Vahlia biflavone: VC 15B: brown amophous powder, $C_{30}H_{18}O_{12}$, m.p. 220 °C (dec) $[\alpha]_D$: −6.33 (*c* 0.18, CH_3OH]. UV (MeOH): λ_{max} = 268, 303, 372; (+ NaOMe) 280, 338, 428; (+ NaOAc) 250, 340 sh, 380 sh; (+ NaOAc/H_3BO_3) 270, 340, 380. IR (KBr): υ_{max}: 3411, 2924, 1637, 1609, 1512, 1458, 1272, 1154, 828 cm^{-1}: ^{1}H-NMR and ^{13}C-NMR (see Table 1). EIMS (rel. int.): *m/z* = 418 $[C_{23}H_{14}O_8]^+$ (41), 389 $[C_{22}H_{13}O_7]^+$ (26), 286 $[C_{15}H_{10}O_6]^+$ (28), 285 $[C_{15}H_9O_6]^+$ (31), 152 $[C_7H_4O_4]^+$ (11), 126 $[C_6H_6O_3]^+$ (100), 121 $[C_7H_5O_2]^+$ (59), 94 $[C_6H_6O]^+$ (20), 85 $[C_5H_5O_2]^+$ (25), 83 $[C_5H_4O_2]^+$ (23), 77 $[C_6H_5]^+$ (30). FABMS: *m/z* = 571 $(M + H)^+$ $[C_{30}H_{18}O_{12}]$, 525, 481, 441, 419, 413, 286, 285, 277, 207, 187, 183, 171, 153, 127, 126.

Microorganisms and antibacterial assay: The procedure employed has been described elsewhere (12).

Preparation of material for preliminary testing: A 50 mg/ml stock solution of the crude water extract was made. Filter paper discs containing 200 and 500 μg were prepared and dried at room temperature before use. A 10 mg/ml methanolic solution of each of the pure compounds was prepared and aliquots of 1–10 μl of this were applied to discs giving a loading of 10–100 μg/disc.

Minimum inhibitory concentration (MIC) assay: (10, 11). Test solutions, 350 ml each, 0.1–100 mg/ml, were made up in methanol. Chloramphenicol standards were also made up in the same concentration range. To each microtitre well were pipetted 290 μl of broth and 10 μl of the test solution were added. Bacterial slopes were taken up in 20 ml of double strength nutrient broth and 50 μl of this bacterial cell suspension were added to each well. Controls were run with methanol and bacteria, and broth and bacteria, only. All tests were each done in triplicate. The plates were then incubated at 37 °C for 18 hours. The MIC value was determined from the data.

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