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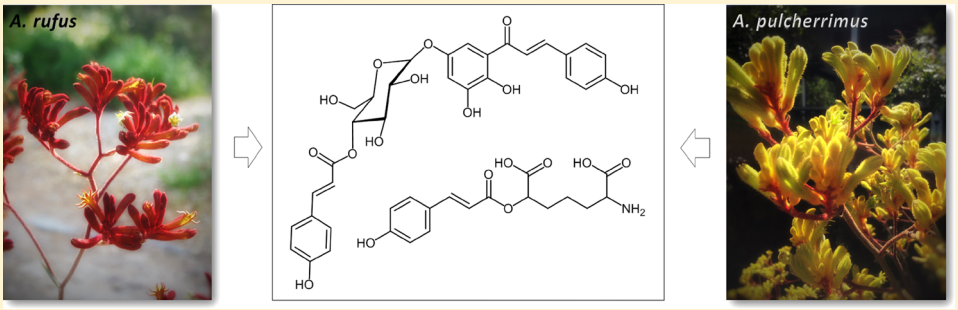
Phytochemical Studies on Two Australian *Anigozanthos* Plant Species

Rudi Hendra and Paul A. Keller*

School of Chemistry, University of Wollongong, Wollongong, NSW 2522, Australia

Supporting Information



ABSTRACT: Phytochemical studies of two Australian *Anigozanthos* (kangaroo paw) species, *A. rufus* and *A. pulcherrimus*, resulted in the identification of 13 secondary metabolites. 2-Amino-6-*O*-*p*-coumarylheptanedioic acid (**3**) and chalcone-5′-*O*-(4-*O*-*p*-coumaryl)-*O*-*β*-D-glucopyranoside (**12**) are reported as new compounds and are accompanied by nine flavonoids (**2**, **5**−**11**, **13**) and two anthocyanins (**1**, **4**). Compounds **1** and **4** were isolated as red solids from *A. rufus* and are likely responsible for the coloration of the flowers. Compounds **1**, **3**, and **6** showed weak antimicrobial activities against *Acinetobacter baumannii* ATCC 19606 at concentrations of 52.4, 94.9, and 53.9 μM, respectively.

The *Anigozanthos* genus is a member of the Hemodoraceae family with examples more commonly known as kangaroo paws. The genus contains nine species and is endemic to southwest Australia, where it is the floral emblem of the State of Western Australia.[1] The flowers have a variety of colors such as red, yellow, black, and red-green depending on the species, and their unique shape resembles a tubular structure coated with dense hairs that opens at the apex with six claw-like structures.[2,3]

Apart from the unique and attractive flowers, kangaroo paws possess tuberous roots that contain significant levels of starch and form part of the diet of the indigenous people in the Yellagonga region of Western Australia.[4] While there are no documented reports of members of the *Anigozanthos* genus as medicinal plants, four phenylpropanoids were isolated and their structures elucidated from the red root of *Anigozanthos rufus* in 1975; extracts from these roots were used as a dye by the indigenous people in the Northern Territory, Australia.[5,6] This characteristic class of secondary metabolites occurs in plant families such as Hemodoraceae, Ponederaiaceae, Strelitziaceae, and Musaceae;[7] for example, six phenylphenalenones were isolated from *Hemodorum simplex* (Hemodoraceae) and found to possess moderate cytotoxicity against the P388 murine leukemia cell line, with an IC_{50} value of >26−39 μM.[8] In a separate study, Hidalgo et al. reported the biosynthesis of phenylphenalenone from in vitro root cultures of *A. preissii* and isolated four phenylpropanoids, including 4′-methoxyanigorufone.[7] Similar compounds were isolated from the flowers of the black kangaroo paw (*Macropidia fuliginosa*) and showed low antibacterial activity against *Staphylococcus aureus* ATCC 25923.[7] Interestingly, despite the similar name, the black kangaroo paw belongs to a different genus from the rest of the kangaroo paw species.

We have an ongoing interest in colors from Nature, concentrating particularly on flowers that are endemic to Australia,[9] as well as any relationship between the isolated compounds and indigenous uses. Although the aforementioned studies investigated the roots of *Anigozanthos* sp. and its family, the flowers from these species have not yet undergone phytochemical investigation. Here, we report the extraction, isolation, and identification of compounds from the flowers of the red (*A. rufus*) and yellow (*A. pulcherrimus*) kangaroo paw and document the compounds responsible for the intense coloration present in the flowers. Further, we extrapolate correlations between the biological activities of some of the isolated compounds and known traditional medicinal uses within the Hemodoraceae family.

RESULTS AND DISCUSSION

Separate MeOH extractions of the flowers of *A. rufus* and *A. pulcherrimus* were concentrated and sequentially solvent-partitioned into *n*-hexane/MeOH, resulting in polar and nonpolar fractions. The chemical constituents from the nonpolar fraction were analyzed using GC-EIMS (Figure S42, Supporting Information). The polar fractions from both species were subjected to analytical RP-HPLC followed by semipreparative

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RP-HPLC, which resulted in the isolation of 13 compounds (Figure 1), 11 of which were known. The structures were

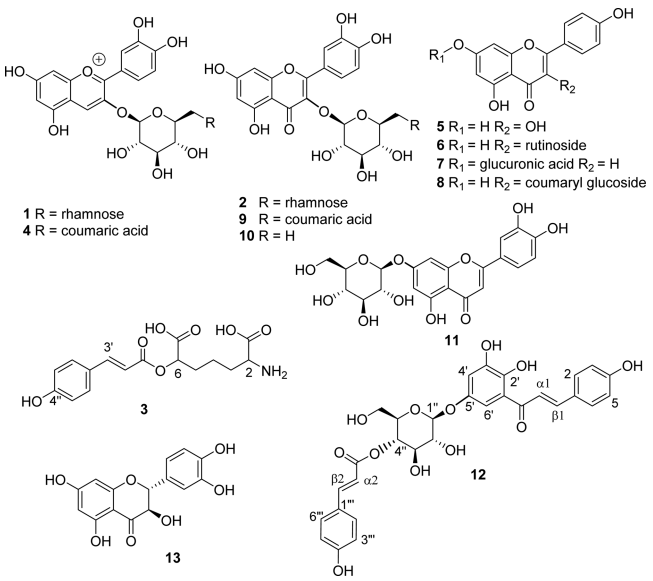

Figure 1. Molecular structures of compounds isolated from the flowers of *Anigozanthos* sp. (*A. rufus* and *A. pulcherrimus*). Compounds **2**, **3**, and **5**–**12** were isolated from both species. Compounds **1** and **4** were isolated from *A. rufus*, and compound **13** was isolated from *A. pulcherrimus*.

elucidated through extensive NMR analyses and by literature comparisons. The known compounds were identified as cyanidin-3-rutinoside (**1**),[10] quercetin-3-rutinoside (**2**),[11] cyanidin-3-*O*-(6-*O*-*p*-coumaryl)-*O*-*β*-D-glucopyranoside (**4**),[12] kaempferol (**5**),[11] kaempferol-3-*O*-rutinoside (**6**),[11] apigenin-7-*O*-*β*-D-glucopyranoside (**7**),[13] kaempferol-3-*O*-(6-*O*-*p*-coumaryl)-*O*-*β*-D-glucopyranoside (**8**),[14] quercetin-3-*O*-(6-*O*-*p*-coumaryl)-*O*-*β*-D-glucopyranoside (**9**),[15] quercetin-3-*O*-*β*-D-glucopyranoside (**10**),[11] luteolin-7-*O*-*β*-D-glucopyranoside (**11**),[16] and dihydroquercetin (**13**).[17] Compounds **3** and **12** (t_R = 10.36, and 16.99 min) are new and were isolated from both plants.

Compound **3** was isolated as a bright yellow solid with HRESIMS analysis indicating a peak at *m*/*z* 338.1249 ([M + H]$^+$), assigned to the molecular formula $C_{16}H_{20}NO_7$. The ^{1}H and ^{13}C NMR spectroscopic data are collated in Table 1.

The ^{13}C NMR spectroscopic analysis of **3** showed resonances at δ_C 168.5 (C-6), 171.9 (C-7), and 173.8 (C-1) assigned to three carbonyls, suggesting the presence of an ester and two hydroxy carbonyl functional groups. Analysis of the ^{1}H NMR data revealed two sets of doublets at δ_H 7.48 (H-2″/6″) and 6.82 (H-3″/5″) with coupling constants of 8.3 Hz, assigned as aromatic *ortho* hydrogen atoms, with supporting evidence from gCOSY analysis indicating a correlation between these protons. Two further doublets at δ_H 7.68 (H-3′) and 6.39 (H-2′), together with carbon resonances at δ_C 114.5 (C-2′) and 145.6 (C-3′), indicated the presence of a pair of *E*-olefinic protons with a coupling constant of 15.9 Hz. From the analysis of gHMBC data, a doublet at δ_H 7.48 (H-2″/6″) showed both a long-range correlation with the oxygenated aromatic carbon resonance at δ_C 161.4 (C-4″) and a correlation with the resonance at δ_C 145.6, which was assigned to the *E*-olefinic C-3′ (Figure 2).

The two shielded resonances at δ_H 5.10 and 3.99 and three methylene groups at δ_H 2.02, 1.93, and 1.69 were assigned as H-6, H-2, H-3, H-5, and H-4, respectively. Through gHMBC analysis, the carbonyl resonance at δ_C 171.9 (C-7) correlated with H-5, and the carbonyl resonance at δ_C 173.8 (C-1) correlated with H-3. Moreover, gHMBC spectroscopic analysis revealed a correlation between H-5 and carbon resonances at δ_C 168.6 and 22.2, assigned as C-1′ and C-4, respectively. Furthermore, a three-bond proton–carbon correlation between H-2 and the carbon resonance at δ_C 22.2 allowed its assignment to C-4. Therefore, compound **3** was assigned as the novel 2-amino-6-*O*-*p*-coumarylheptanedioic acid. Its specific rotation was determined as $[\alpha]_D^{25}$ 0 (*c* 0.13, MeOH), indicating the likely presence of a racemate.

Table 1. NMR Spectroscopic Data (500 MHz, Methanol-d_4) for Compound 3

position	δ_C, type	δ_H (*J* in Hz)	gCOSY	gHMBC
1	171.9, C			
2	53.9, CH	3.99, dd (8.8, 7.5)	3	4, 1[a]
3	31.9, CH_2	2.10–2.00, m	2, 4	1, 5
4	22.2, CH_2	1.75–1.50, m	3, 5	2, 6
5	31.3, CH_2	1.95–1.93, m	4, 6	3, 7
6	72.8, CH	5.10, dd (7.4, 4.9)		4, 7,[a] 1′
7	173.8, C			
1′	168.6, C			
2′	114.5, CH	6.39, d (15.9)	3′	1′[a], 1″
3′	145.6, CH	7.68, d (15.9)	2′	1′, 2″, 6″
1″	127.4, C			
2″	131.1, CH	7.48, d (8.3)	3″	3′, 4″, 6″
3″	116.9, CH	6.82, d (8.2)	2″	1″, 5″
4″	161.4, C			
5″	116.9, CH	6.82, d (8.2)	6″	1″, 3″
6″	131.1, CH	7.48, d (8.3)	5″	3′, 2″,4″

[a]Indicates weak signal.

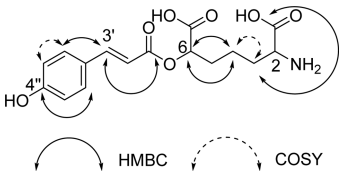

Figure 2. Key HMBC and COSY correlations for compound **3**.

Compound **12** was isolated as a yellow solid with HRESIMS analysis indicating a peak at *m*/*z* 603.1489 ([M + Na]$^+$), assigned to the molecular formula $C_{30}H_{28}O_{12}Na$. The ^{1}H and ^{13}C NMR spectroscopic analysis is collated in Table 2.

Analysis of the ^{1}H NMR spectra of **12** showed resonances at δ_H 6.03 (s) and 6.26 (s) assigned to H-4′ and H-6′ of ring A, respectively, which, via gHMBC analysis, correlated to a quaternary carbon resonance at δ_C 127.2, assigned to C-1′, and three oxygenated tertiary carbon resonances at δ_C 165.9, 161.4, and 161.1, assigned to C-3′, C-5′, and C-2′, respectively. Furthermore, the ^{1}H NMR spectrum revealed resonances at δ_H 7.62 (2H, d, *J* = 8.0 Hz) and 6.87 (2H, d, *J* = 8.0 Hz), assigned to H-2/6 and H-3/5 of ring B, respectively. This 1,4-disubstituted benzene ring contained a quaternary carbon C-1 and an oxygenated tertiary carbon C-4, which were assigned using gHMBC analysis to resonances at δ_C 128.3 and 161.8, respectively. A pair of *trans* olefinic proton resonances at δ 8.02 (1H, d, *J* = 15.0 Hz) and 7.68 (1H, d, *J* = 15.0 Hz) were assigned to H-*α* and H-*β*, respectively, and through gHMBC analysis and showed a proton–carbon correlation to the signals

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Table 2. NMR Spectroscopic Data (500 MHz, Methanol-d_4) for Compound 12[a]

position	δ_C, type	δ_H (J in Hz)	gCOSY	gHMBC
1	128.3, C			
2	131.9, CH	7.62, d (8.0)	3	4, β, 6
3	117.1, CH	6.87, d (8.0)	2	1, 5
4	161.8, C			
5	117.1, CH	6.87, d (8.0)	6	1, 3
6	131.8, CH	7.62, d (8.0)	5	4, β, 2
1′	127.2, C			
2′	161.1, C			
3′	165.9, C			
4′	98.7, CH	6.03, s		2′, 6′
5′	161.4, C			
6′	96.0, CH	6.26, s		4′, 2′, 194.8
α1	125.6, CH	8.02, d (15.0)	β1	1, 1″
β1	144.3, CH	7.68, d (15.0)	α1	2, 194.8
carbonyl	194.8, CO			
Glucosyl				
1″	101.7, CH	5.23, d (7.8)	2″	5′, 3″, 5″
2″	75.3, CH	3.69, dd (8.9, 7.8)	1″, 3″	4″
3″	76.3, CH	3.82, dd (9.5, 9.0)	2″, 4″	1″, 5″
4″	72.1, CH	5.02, dd (9.5, 8.5)	3″, 5″	168.7
5″	76.,7 CH	3.90, ddd (8.9, 6.5, 12.0)	4″, 6″	
6″	62.3, CH_2	3.72, dd (6.5, 1.2)	5″	
		3.62, dd (12.0, 6.5)		
Coumaryl				
1‴	125.8, C			
2‴	131.4, CH	7.49, d (8.0)	3‴	
3‴	117.0, CH	6.83, d (8.0)	2‴	
4‴	161.2, C			
5‴	117.0, CH	6.83, d (8.0)	6‴	
6‴	131.4, CH	7.49, d (8.0)	5‴	
α2	114.8, CH	6.36, d (15.0)	β2	
β2	147.6, CH	7.70, d (15.0)	α2	
carbonyl	168.7, CO			

[a] ^{1}H and ^{13}C NMR experiments were performed in methanol-d_4 at 500 and 125 MHz, respectively.

at δ_C 127.2, 128.3, and 194.8, assigned as C-1′, C-1, and the ketocarbonyl carbon, respectively (Figure 3).

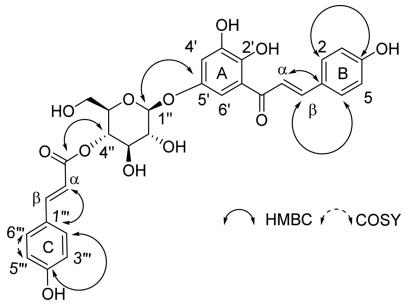

Figure 3. Key HMBC and COSY correlations for compound **12**.

A set of *ortho*-coupled aromatic resonances of ring C protons at δ_H 7.49 (2H, d, J = 8.0 Hz) and 6.83 (2H, d, J = 8.0 Hz) were assigned to H-2‴/H6‴ and H-3‴/H5‴, respectively, with gHMBC analysis showing correlations between these protons and the quaternary carbon resonance at δ_C 125.8 (C-1‴) and the oxygenated tertiary carbon resonance at δ_C 161.2 (C-4‴). Two further doublets at δ_H 7.49 (1H, d, J = 15.0 Hz) and 6.83 (1H, d, J = 15.0 Hz) were assigned to H-α′ and H-β′, respectively, and showed proton−carbon correlations to resonances at δ_C 125.8, 131.4, and 168.7, assigned to C-1‴, C-2‴/6‴, and the ester carbonyl carbon, respectively (Figure 3).

^{1}H NMR spectroscopic analysis showed the presence of sugar moieties with resonances at δ_H 5.23 (1H, d, J = 7.8 Hz), 5.02 (1H, dd, J = 9.8, 5.3 Hz), 3.90 (1H, t, J = 5.9 Hz), 3.82 (1H, dd, J = 9.8, 9.3 Hz), 3.72/3.62 (2H, dd, J = 12.6, 5.9 Hz), and 3.69 (1H, dd, J = 10.5, 8.6 Hz), assigned to H-1″, H-4″, H-5″, H-3″, H-6″, and H-2″, respectively. The proton sequences were confirmed by gCOSY and TOCSY through bond analysis and suggested a glucose moiety. In addition, acid hydrolysis of **12** indicated the presence of a β-D-glucopyranose moiety via HPLC comparison to authentic standards at a retention time of 8.33 min (Figure S35, Supporting Information). Further gHMBC analysis indicated a proton−carbon correlation from H-1″ to C-5′ and from H-4″ to the ester carbonyl carbon, confirming the link between ring A and glucose and from glucose to the cinnamoyl moiety, respectively (Figure 3). Therefore, **12** was proposed as the novel chalcone 5′-*O*-(4‴-*O*-*p*-coumaryl)-*O*-β-D-glucopyranose.

The HPLC chromatogram profile of flowers from both species showed the presence of similar compounds (**2**, **3**, **5**−**12**). Compound **2** (t_R = 10.07 min) was found be the major constituent from the polar extract of both flowers followed by compound **8** (t_R = 13.00 min). Furthermore, compound **2** was also found in the flowers of the black kangaroo paw (*M. fuliginosa*).[7] Compound **13** was found to be present only in *A. pulcherrimus*, with **1** and **4** (t_R = 9.62 and 10.88 min) found only in *A. rufus*. Compounds **1** and **4** were dark red solids, and analysis of their NMR data indicated the presence of anthocyanins, supported by ESIMS data analysis. Although phenalenone pigments are well known as red coloration in several species of the plant family Hemodoraceae,[6,18,19] anthocyanins **1** and **4** are reported here for the first time in this species.

Antibacterial Activity Studies. The MeOH extracts from both species and the isolated compounds (**1**−**13**) were screened for their antibacterial activities against five bacteria and two fungi at 32 μg/mL. Their activities were analyzed based on the ability of the sample to inhibit the growth of microorganisms equal to or more than 80% with a Z-score ≥ 2.5 (data in Supporting Information). All samples tested were inactive against all the tested microorganisms based on the above criteria. However, based on the percentage of growth inhibition, compounds **1**, **3**, and **6** showed weak inhibitory activity to *Acinetobacter baumannii* ATCC 19606 at concentrations of 52.4, 94.9, and 53.9 μM, respectively.

Plants from the family Hemodoraceae, which are found predominantly in Australia, have been reportedly used by the indigenous people for several purposes. Extracts of the bulbs from the genus *Hemodorum* have been used as a purgative, an ointment for snake bites, and coloring agents.[8] Recent studies reported that the MeOH and CH_2Cl_2 extracts from flowers of black kangaroo paw (*M. fuliginosa*) showed potential as antimicrobial agents against *Pseudomonas aeruginosa* and *Streptococcus pyogenes*.[18] Despite the lack of reports regarding medicinal usage from the genus *Anigozanthos*, especially from the flowers, it was found that the isolated compounds from both species possess various biological activities from previous studies. Flavonoid compounds were the major compounds isolated from both flowers, and these are well known to possess antimicrobial

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activities. Quercetin and quercetin glycosides (**2**, **9**, and **10**) have been reported to exhibit antioxidative, anticarcinogenic, anti-inflammatory, and vasodilating effects.[20]

Many studies report the benefit of kaempferol and its glucoside to reduce the risk of chronic disease. Kaempferols (**5**−**8**) have the ability to modulate a number of key elements in cellular signal transduction pathways linked to apoptosis, angiogenesis, inflammation, and metastasis.[21] Additionally, taxifolin (**13**) is believed to have therapeutic promise in treating cancer and cardiovascular and liver disease;[22] this study is the first to report its isolation from the *Aningozanthos* genus.

In summary, phytochemical studies led to the successful isolation of the major constituents from the flowers of two species from the *Aningozanthos* genus. Anthocyanins **1** and **4** from *A. rufus* flowers are reported for the first time, and additionally, the new compounds **3** and **12** were isolated and identified. An antimicrobial assay of the isolated compounds indicated they were inactive against the tested microorganisms.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Jasco P-2000 polarimeter. UV−visible spectra of samples diluted in MeOH were obtained using a Shimadzu UV-1601 UV−vis spectrophotometer. ECD spectra were recorded on a JASCO J-810 spectropolarimeter with a path length of 0.1 cm and concentration between 50 and 100 μM in MeOH. IR spectra were recorded with a Shimadzu IR Affinity-1 FT-IR spectrometer fitted with a 1.5 round diamond crystal. NMR spectra were recorded at 500 and 125.7 MHz, respectively, on a Varian Unity Inova-500 MHz spectrometer, controlled by Varian VNMR software (version 6.1 revision C). NMR spectra were acquired in methanol-d_4 with chemical shifts (δ) reported in parts per million (ppm) relative to methanol-d_4 (^{1}H: δ = 3.31; ^{13}C: δ = 49.2) (unless otherwise specified). Coupling constants (J) are reported in hertz (Hz). J values listed in ^{1}H NMR spectroscopic data refer to coupling between hydrogen nuclei. ESI mass spectra were obtained on an LCMS-2010 EV (Shimadzu). Samples were injected as a solution in methanol HPLC grade. HRESIMS were acquired on a Micromass QTOF2 Ultima spectrometer.

The HPLC profiles from both species were obtained using a Waters system (Waters 1525 pump, Waters 2487 detector, controlled by Breeze software v3.30) with a Symmetry C_{18} column (5 μm, 4.9 × 150 mm) and a Wakosil C-18 RS column (5 μm, 4.6 × 250 mm). All compounds were isolated by Preparative HPLC using a Waters prep-LC system (LC-600 controller, 2489 detector, LC150 pump, PD1 degasser) with a Waters reversed-phase OBD Sunfire C_{18} column (5 μm, 19 × 150 mm) protected with a Waters Sunfire C_{18} guard column (5 μm, 19 × 10 mm). The sugar moiety from compound **12** was identified by using an RP-HPLC (Shimadzu HPLC) system, coupled with an automatic sampler (Shimadzu SIL-10A XL) and a Sedere Sedex 60 LT evaporative light scattering detector (ELSD) with a Prevail Carbohydrates ES column (250 × 4.6 mm i.d.; Alltech). All analytical HPLC samples were filtered through Grace syringe filter PTFE 0.45 μm, 4 mm, and preparative HPLC samples were filtered through Grace syringe filter 0.45 μm, 30 mm. A Büchi rotary evaporator (R-114/200) with a high vacuum pump was used for evaporation of solvents under reduced pressure at 40 °C.

Plant Material. The red (*A. rufus*, voucher specimen Woll11570) and yellow (*A. pulcherrimus*, voucher specimen Woll11569) kangaroo paw flowers were collected in January 2015 from the campus grounds of the University of Wollongong, Australia (34°24′16.9″ S 150°52′38.2″ E). All the flowers were washed, freeze-dried, and stored in a refrigerator until analysis. Voucher specimens of both species are stored in the Janet Cosh Herbarium Reference Collection at the University of Wollongong.

Extraction and Isolation of Flowers. The freeze-dried flowers (70 g each) were crushed, suspended in MeOH (1.0 L), stirred for 24 h, and then filtered, and the filtrate was extracted with MeOH (3 × 1.0 L). The supernatants for each species were separately pooled and concentrated in vacuo to produce residues, 12.371 g (*A. rufus*) and 11.946 g (*A. pulcherrimus*). The residues were liquid−liquid extracted with *n*-hexane and MeOH, resulting in polar extracts (3.154 and 2.832 g, respectively). The nonpolar extracts were analyzed for their chemical constituents using GC-EIMS analysis. The MeOH fraction from *A. rufus* (400 mg) in MeOH (50 mL) was filtered through a Whatman syringe filter 0.45 μm, and the volume was reduced to 20 mL. The solution was injected (10 × 2.0 mL/injections) onto preparative HPLC with a gradient elution from 90% of solvent A (0.1% trifluoroacetic acid (TFA) in H_2O) to 50% of solvent B (0.1% TFA in MeCN) within 35 min. All isolates were manually trapped based on the chromatographic profile with the following retention times (min) for each compound (Figure S1, Supporting Information): **1** (22 mg, t_R = 9.62 min), **2** (90 mg, t_R = 10.07 min), **3** (17 mg, t_R = 10.36 min), **4** (21 mg, t_R = 10.88 min), **5** (5.5 mg, t_R = 12.09 min), **6** (10 mg, t_R = 12.28 min), **7** (12 mg, t_R = 12.54 min), **8** (14 mg, t_R = 13.00 min), **9** (8.5 mg, t_R = 13.59 min), **10** (11 mg, t_R = 14.47 min), **11** (8.0 mg, t_R = 14.47 min), and **12** (6.5 mg, t_R = 16.99 min). In an identical HPLC procedure, the MeOH fraction from *A. pulcherrimus* (400 mg) resulted in the isolation of compounds **2** (83 mg), **3** (12.5 mg), **5** (6.2 mg), **6** (8.3 mg), **7** (10.3 mg), **8** (11.5 mg), **9** (7.2 mg), **10** (10.4 mg), **11** (7.8 mg), **12** (7.5 mg), and **13** (4.0 mg, t_R = 14.47 min). All the isolates were freeze-dried, producing solid compounds for spectroscopic analysis.

Cyanidin-3-rutinoside (**1**): UV-active red solid; UV (MeOH) λ_{max} 280 (3.87); 519 (3.95) nm; LREIMS m/z 595 $[M + 1]^+$; HRESIMS m/z 595.1781 (calcd for $C_{27}H_{31}O_{15}$ 595.1752). All NMR data were identical to reported data.[10]

Quercetin-3-rutinoside (**2**): UV-active, pale yellow solid; LREIMS m/z: 633 $[M + Na]^+$, 609 $[M + H]^-$. All NMR data were identical to reported data.[11]

2-Amino-6-O-p-coumarylheptanedioic acid (**3**): UV-active, pale yellow, oily solid; $[\alpha]_D^{25}$ 0 (c 0.13, MeOH); IR [cm^{-1}] 3365 (br, m), 2944 (s), 831 (s), 1717 (s); ^{1}H NMR (500 MHz, methanol-d_4) and ^{13}C NMR (125 MHz, methanol-d_4) data, see Table 1; HRESIMS m/z 338.1240 (calcd for $C_{16}H_{20}NO_7$, 338.1240).

Cyanidin-3-O-(6-O-p-coumaryl)-O-β-D-glucopyranoside (**4**): UV-active, red solid; UV (MeOH) λ_{max} 282 (3.92); 313 (3.85); 523 (3.84) nm; LREIMS m/z 595 $[M + 1]^+$. All NMR data were identical to reported data.[12]

Kaempferol (**5**): UV-active, yellow solid; UV (MeOH) λ_{max} 228 (3.26); 267 (4.08); 366 (4.02) nm; LREIMS m/z 310 $[M + Na]^+$. All NMR data were identical to reported data.[11]

Kaempferol-3-O-rutinoside (**6**): UV-active, yellow solid; UV (MeOH) λ_{max} 225 (4.08); 268 (4.03); 350 (3.99) nm; LREIMS m/z: 595 $[M + H]^+$; HRESIMS m/z 617.1484 (calcd for $C_{27}H_{30}O_{15}Na$, 617.1482). All NMR data were identical to reported data.[11]

Apigenin-7-O-β-D-glucopyranoside (**7**): UV-active, yellow solid; LREIMS m/z 469 $[M + Na]^+$. All NMR data were identical to reported data.[13]

Kaempferol-3-O-(6-O-p-coumaryl)-O-β-D-glucopyranoside (**8**): UV-active, yellow solid; LREIMS m/z 617 $[M + Na]^+$. All NMR data were identical to reported data.[14]

Quercetin-3-O-(6-O-p-coumaryl)-O-β-D-glucopyranoside (**9**): UV-active, yellow solid; LREIMS m/z 609 $[M - H]^-$, 633 $[M + Na]^+$. All NMR data were identical to reported data.[15]

Quercetin-3-O-β-D-glucopyranoside (**10**): UV-active, yellow solid; UV (MeOH) λ_{max} 228 (4.14); 259 (4.12); 359 (4.07) nm; LREIMS m/z 465 $[M + 1]^+$. All NMR data were identical to reported data.[11]

Luteolin-7-O-β-D-glucoside (**11**): UV-active, yellow solid; LREIMS m/z 449 $[M + 1]^+$. All NMR data were identical to reported data.[16]

Chalcone-5′-O-(4-O-p-coumaryl)-O-β-D-glucoside (**12**): UV-active, yellow solid; $[\alpha]_D^{22}$ +12.8 (c 0.15, MeOH); IR [cm^{-1}] 3566 (br, m), 2360 (s), 1683 (s); ^{1}H NMR (500 MHz, methanol-d_4) and ^{13}C NMR (125 MHz, methanol-d_4) data, see Table 2; LREIMS, m/z 603 $[M + Na]^+$; HRESIMS m/z 603.1506 (calcd for $C_{30}H_{28}O_{12}Na$, 603.1478).

Dihydroquercetin (**13**): UV-active, light yellow solid; $[\alpha]_D^{25}$ +46 (c 0.15, MeOH) [lit.[23] $[\alpha]_D^{25}$ +46.2 (c 0.12, MeOH)]; LREIMS m/z 304 $[M - 1]^-$. All NMR data were identical to reported data.[17]

Acid Hydrolysis of Compound 12. A mixture of **12** (1.0 mg) in aqueous HCl (15%, 2.5 mL) was heated with stirring at 50 °C for 6 h. The solution was neutralized by the dropwise addition of NaOH (5%)

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with pH monitoring using universal indicator paper. This aqueous mixture was partitioned with EtOAc (2 × 2.5 mL), and the aqueous fraction evaporated under reduced pressure.[24] The residue was subjected to RP HPLC using a Carbohydrates ES column with ELSD and an isocratic flow (H_2O/MeCN, 20:80) for 20 min with a flow rate of 1.0 mL/min. The retention time of the fraction (8.3 min) corresponded to β-D-glucopyranose, as evidenced by a co-injection with a standard.

Antimicrobial Assay. Antimicrobial activities were conducted by the Community for Open Antimicrobial Drug Discovery, Queensland, Australia. The MeOH extracts from both species and isolated compounds were evaluated for their antimicrobial activities against seven microorganisms (*Staphylococcus aureus* ATCC 43300 (MRSA), *Eschericia coli* ATCC 25922 (FDA control strain), *Klebsiella pneumoniae* ATCC 700603 (MDA), *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 19606, *Candida albicans* ATCC 90028, and *Cryptococcus neoformans* ATCC 208821). All the samples were dissolved in DMSO at a concentration of 10 mg/mL. Each sample (5 μL) was plated in duplicate into a 384-well nonbinding surface plate for each strain. All bacteria were cultured in cation-adjusted Mueller Hinton broth at 37 °C overnight; then 45 μL was added to each well of the compound-containing plates, giving a cell density of 5×10^5 CFU/mL to give a final compound concentration of 32 μg/mL. All the plates were covered and incubated at 37 °C for 18 h. For the fungi strains, all the fungi were cultured for 3 days on yeast extract−peptone dextrose agar at 30 °C, and these stock suspensions were diluted with yeast nitrogen base broth to a final concentration of 2.5×10^5 CFU/mL. Then, 45 μL was added to each well of the compound-containing plates, giving a final compound concentration of 32 μg/mL, and incubated at 35 °C for 24 h. Inhibition of bacterial growth was determined by measuring absorbance at 600 nm using a Tecan M1000 Pro monochromator plate reader, while growth inhibition of the fungi was determined by measuring absorbance at 530 nm after the addition of resazurin (0.001% final concentration) and incubation at 35 °C for an additional 2 h. The absorbance was measured using a Biotek Synergy HTX plate reader. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (microorganisms without inhibitors) on the same plate. The significance of the value was determined by *Z*-scores, calculated using the average and standard deviation of the sample wells (no controls). Samples with an inhibition value above 80% and *Z*-score above 2.5 for either replicate were classified as active.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00063.

General procedures, HPLC profiles, GCMS, antimicrobial data, and NMR spectral data of compounds **1**−**13** including 2D NMR spectral data of compounds **3** and **12** (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: keller@uow.edu.au. Tel: +61 2 4221 4692. Fax: +61 2 4221 4287.

ORCID

Paul A. Keller: 0000-0003-4868-845X

Notes

The authors declare no competing financial interest.

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