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# Flavonoids and *ent*-labdane diterpenoids from *Andrographis paniculata* and their antiplatelet aggregatory and vasorelaxing effects

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Two new flavones, designated as andropaniculosin A (**1**) and andropaniculoside A (**2**), and 30 known compounds were isolated as a result of detailed chemical examination on the whole plants of *Andrographis paniculata*. Their structures have been elucidated mainly by 1D and 2D NMR, and MS spectroscopic methods. Among them, four flavonoids showed potent inhibition of collagen, arachidonic acid, thrombin, and platelet activation factor induced platelet aggregation. Furthermore, a diterpenoid demonstrated moderate vasorelaxing effect in isolated rat thoracic aorta.

*Keywords*: *Andrographis paniculata*; Acanthaceae; Andropaniculosin A; Andropaniculoside A; Anti-platelet aggregation; Vasorelaxing effect

## 1. Introduction

*Andrographis paniculata* Nees (Acanthaceae) is an erect herb found widely in subtropical Asia, Southeast Asia, India and China. It has a considerable medicinal reputation in traditional Chinese and Ayurvedic medicine where it is used as medicine against a variety of diseases including cold, fever, snakebite, diarrhoea, dyspepsia, dysentery, respiratory infections and malaria [1,2]. *Andrographis* extract and some of its diterpene lactones are claimed to be antidiabetic, anti-inflammatory, immunostimulant, hepatoprotective, antimalarial, analgesic, antipyretic, and antiulcerogenic [3]. *A. paniculata* is also reported to contain flavones and flavone glycosides [4].

Considering the fact that this medicinal plant is included as an active ingredient in several herbal preparations, we have undertaken the detailed chemical examination of the methanol

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extract of *A. paniculata* collected from Taiwan. As a result, two new flavones, andropaniculosin A (**1**) and andropaniculoside A (**2**), and 30 known compounds were isolated and characterised. Evaluation of their vasorelaxing and antiplatelet aggregation properties is also presented.

## 2. Results and discussion

The methanol extract of *A. paniculata* was suspended in $H_2O$ and the $H_2O$ solubles were partitioned with $CHCl_3$. The successive purification of $CHCl_3$, $H_2O$ soluble extracts and $H_2O$ insolubles by a combination of conventional chromatographic techniques afforded thirty-two compounds, among which two compounds (**1** and **2**), were new.

Andropaniculosin A (**1**), obtained as yellow crystalline solid, mp 178–180°C, gave the molecular formula $C_{18}H_{16}O_7$ from the pseudomolecular ion peak at *m/z* 345.0974 in the HRESI-MS. The UV spectrum of **1** showed absorption maxima at 273 and 362 nm, characteristic of the flavone series with 5,7,8-trioxygenation [5]. The IR spectrum exhibited two strong absorption bands at 3200 and 1647 $cm^{-1}$, corresponding to hydroxyl and carbonyl functions, respectively. The existence of a typical signal at $\delta$ 12.65 of the chelated hydroxyl in the $^1H$ NMR spectrum and the bathochromic shift observed in UV spectrum on addition of $AlCl_3$ + HCl suggested the presence of the hydroxyl group at C-5. The $^1H$ NMR spectrum of **1** showed signals for three aromatic methoxyl groups at $\delta$ 3.94, 3.91, and 3.89 and one sharp proton signal at $\delta$ 7.09 characteristic of C-3 proton of 2′-oxygenated flavone [6]. The other sharp singlet at $\delta$ 6.42 was attributed to H-6 of 5,7,8-trioxygenated flavone, which was further substantiated by its NOE correlation with OH-5 [7]. The $^1H$ NMR spectrum also showed a typical ABX system for three aromatic protons at $\delta$ 6.93 (1H, d, $J = 9.2$ Hz, H-3′), 6.99 (1H, dd, $J = 9.2$, 3.2 Hz, H-4′) and 7.47 (1H, d, $J = 3.2$ Hz, H-6′), characteristic of a 2′,5′-dioxygenated flavone [8] (table 1). The substitution pattern of B-ring (ABX-type) was concluded to be 2′-methoxy and 5′-hydroxy type by NOESY spectrum which showed a NOE correlation between a methoxyl at $\delta$ 3.89 and an aromatic proton at $\delta$ 6.93 (H-3′). The second methoxyl group at $\delta$ 3.94 was placed at C-7 on the basis of its NOE cross-peak with H-6, and thus the remaining methoxyl at $\delta$ 3.91 was connected to C-8. Finally, these assignments were confirmed by the ESI-MS analysis, which showed a molecular ion at *m/z* 345 and the diagnostic peaks of *retro*-Diels Alder cleavage of C-ring at *m/z* 197 and 149, indicating the presence of a hydroxyl and two methoxyls in ring A and a hydroxyl and a methoxyl in ring B. From this spectral evidence, the structure of **1** was elucidated as 5,5′-dihydroxy-7,8,2′-trimethoxyflavone and named andropaniculosin A (figure 1).

Andropaniculoside A (**2**), isolated as an optically active yellow crystalline solid, showed $[M + H]^+$ peak at *m/z* 477.1399 in its HRESI-MS consistent with the molecular formula $C_{23}H_{24}O_{11}$ which was further supported by its NMR spectra (table 1). The UV spectrum of **2** in methanol with absorption maxima at 264 and 338 nm was similar to those of flavones with 5,7,8-trioxygenation [5]. The IR spectrum of **2**, apart from hydroxyl (3400 $cm^{-1}$) and carbonyl (1634 $cm^{-1}$) absorption bands, showed an additional C—O absorption at 1043 $cm^{-1}$ indicating the presence of glycoside linkage [9]. No chelated hydroxyl signal in $^1H$ NMR spectrum of **2** and no bathochromic shift of UV maxima with $AlCl_3$ + HCl indicated the absence of free hydroxyl group at C-5. The $^1H$ NMR spectrum exhibited signals for two methoxyl groups at $\delta$ 3.95 and 4.01. One sharp proton singlet at $\delta$ 7.12 was attributed to H-6 of 5,7,8-trioxygenated A-ring [7]. This was further substantiated by its HSQC

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Table 1. ^{1}H NMR and ^{13}C NMR spectral data for compounds **1** and **2** (ppm, *J* in Hz).

| | ***1*** *($CDCl_3$)* | ***2*** *($CD_3OD$)* | |
|---|---|---|---|
| *Position* | $\delta_H$ | $\delta_H$ | $\delta_c$ |
| 2 | | | 164.6 |
| 3 | 7.09 s | 7.91 s | 110.4 |
| 4 | | | 178.3 |
| 5 | | | 152.8 |
| 6 | 6.42 s | 7.12 s | 101.6 |
| 7 | | | 162.9 |
| 8 | | | 133.6 |
| 9 | | | 157.9 |
| 10 | | | 110.4 |
| 1′ | | | 119.0 |
| 2′ | | | 171.3 |
| 3′ | 6.93 d (9.2) | 6.75 dd (8.0, 1.1) | 123.4 |
| 4′ | 6.99 dd (9.2, 3.2) | 7.15 td (8.0, 1.1) | 133.6 |
| 5′ | | 6.56 td (8.0, 1.1) | 114.9 |
| 6′ | 7.47 d (3.2) | 7.89 dd (8.0, 1.1) | 128.1 |
| 1″ | | 4.83 d (7.8) | 105.8 |
| 2″ | | 3.61 m | 74.8 |
| 3″ | | 3.50 m | 78.8 |
| 4″ | | 3.38 m | 71.6 |
| 5″ | | 3.50 m | 77.5 |
| 6″ | | 3.72 dd (11.9, 5.5)<br>3.99 dd (11.9, 5.5) | 62.8 |
| OH-5 | 12.65 s | | |
| OH-5′ | 7.16 s | | |
| $OCH_3$-7 | 3.94 s | 4.01 s | 56.9 |
| $OCH_3$-8 | 3.91 s | 3.95 s | 61.9 |
| $OCH_3$-2′ | 3.89 s | | |

connectivity with a carbon at $\delta$ 101.6 (C-6) and HMBC correlation with C-5 ($\delta$ 152.8). In the HSQC spectrum, a singlet at $\delta$ 7.91 correlating with C-3 ($\delta$ 110.4) was ascribed to H-3 of a 2′-oxygenated flavone [6]. In UV spectrum of **2**, addition of NaOAc did not cause any bathochromic shift in band II, but a bathochromic shift of 54 nm was observed in band I absorption indicating the presence of free hydroxyl group at C-2′, not at C-7 [6]. A methoxyl group at $\delta$ 4.01 was placed at C-7 because it displayed a HMBC correlation with C-7 and a NOESY cross-peak with H-6. The second methoxyl at $\delta$ 3.95 was placed at C-8, as its carbon resonated at $\delta$ 61.9, a chemical shift characteristic of a di-*ortho* substituted methoxy group [10]. The remaining signals in the aromatic region of ^{1}H NMR spectrum appeared as a typical ABCD system at $\delta$ 6.75 (1H, dd, $J = 8.0$, 1.1 Hz), 7.15 (1H, td, $J = 8.0$, 1.1 Hz),

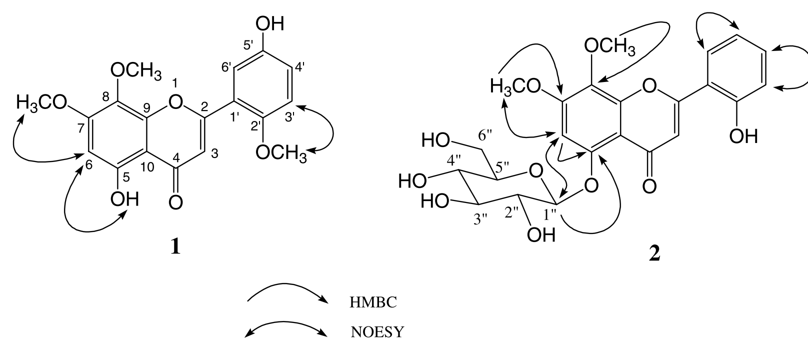


Figure 1. Significant NOESY and HMBC correlations of compounds **1** and **2**.

6.56 (1H, td, $J = 8.0$, 1.1 Hz) and 7.89 (1H, dd, $J = 8.0$, 1.1 Hz), which were assigned for H-3′, 4′, 5′, and 6′, respectively, of the B-ring of flavone [10]. In addition, in the $^{1}$H NMR and $^{13}$C NMR spectra of **2**, an anomeric proton signal at $\delta$ 4.83 (1H, d, $J = 7.8$ Hz, H-1″) and a set of carbon signals ($\delta_C$ 74.8, 78.8, 71.6, 77.5, and 62.8) due to sugar moiety including an anomeric carbon signal at $\delta$ 105.8 inferred the presence of a β-glucopyranosyl moiety in the molecule (figure 1).

Acid hydrolysis of **2** with 2 N HCl afforded glucose and skullcapflavone I [11]. Presence of a chelated hydroxyl group in aglycone and not in the glycoside indicated that the glucose moiety must be attached to C-5. The 5-*O*-glycosylation in **2** was also revealed by the upfield shifts of 4.1 and 2.8 ppm for C-4 and C-5 and downfield shifts of 5.8 and 4.5 ppm for C-6 and C-7 resonances [12]. Finally, the site of glycosylation was confirmed by HMBC correlation between H-1″ and C-5, and a NOE correlation between H-1″ and H-6. Thus, compound **2** was defined as 2′-hydroxy-7,8-dimethoxyflavone-5-*O*-β-D-glucopyranoside and named andropaniculoside A.

Among 30 known isolates, seven *ent*-labdane diterpenoids: andrographolide (**3**), isoandrographolide (**4**), 14-deoxy-11,12-dihydroandrographolide (**5**), deoxyandrographiside (**6**), neoandrographolide (**7**), 14-deoxy-12-methoxyandrographolide (**8**), 14-deoxyandrographolide (**9**), ten flavonoids: apigenin-7-*O*-β-D-methylglucuronide (**10**), isoswertisin (**11**), cosmosiin (**12**), 7-*O*-methylwogonin (**13**), skullcapflavone I (**14**), quercetin (**15**), apigenin (**16**), (−)-onysilin (**17**), 5-hydroxy-7,8,2′,5′-tetramethoxyflavone (**18**), scutellarin-6-*O*-β-D-glucoside-7-methyl ether (**19**), and two quinic acid derivatives: 3,4-dicaffeoylquinic acid (**20**), and methyl-3,4-dicaffeoylquinate (**21**) were significant (figure 2).

Some isolates of the present study were examined for their inhibitory effects on aggregation of washed rabbit platelets stimulated by agonists, thrombin (Thr), arachidonic acid (AA), collagen (Col), and platelet activating factor (PAF). Among the tested, four compounds **13**, **16**, **17**, and **20** significantly inhibited platelet aggregation but to various degrees. Apigenin (**16**) and onysilin (**17**) were the most effective inhibitors, with little difference between them. At 100 μM concentration **17** caused 68.1 ± 3.4%, 100%, 83.7 ± 2.9%, and 84.1 ± 7.6% inhibition of aggregation induced by Col, Thr, AA and PAF, respectively. Even at very low concentrations (50, 20, 10, and 5 μM) **17** exhibited excellent inhibitory (100%, 96.6%, 66.3%, and 38.7%) activity against AA-induced platelet aggregation. Compound **16** demonstrated 91.8% and 90.5% inhibition rate against AA and Col inducers at 50 μM, but not active against Thr and PAF (table 2). From these findings, compounds **16** and **17** appear to be promising antiplatelet aggregatory agents and deserve further investigation.

Recently, 14-deoxyandrographolide and 14-deoxy-11,12-didehydro-andrographolide demonstrated vasorelaxing action in isolated rat aorta and cardiovascular activity in anaesthetised rat and isolated right atria, respectively [13]. Thus, some of the isolates of present study were examined for their vasorelaxing properties in isolated rat thoracic aorta. A diterpenoid **5** depressed markedly the contractions induced by $Ca^{2+}$ (1.9 mM) in high $K^{+}$ (80 mM) medium, but not active against the phasic and tonic contractions caused by norepinephrine (3 μM) (table 3). It has been reported that high $K^{+}$ induced contraction in vascular smooth muscle is mediated by an increase in $Ca^{2+}$ influx through voltage-dependent $Ca^{2+}$ channels. Since **5** inhibited the $Ca^{2+}$-dependent contractions in high $K^{+}$ medium, it may be a blocker of voltage-dependent $Ca^{2+}$ channels.

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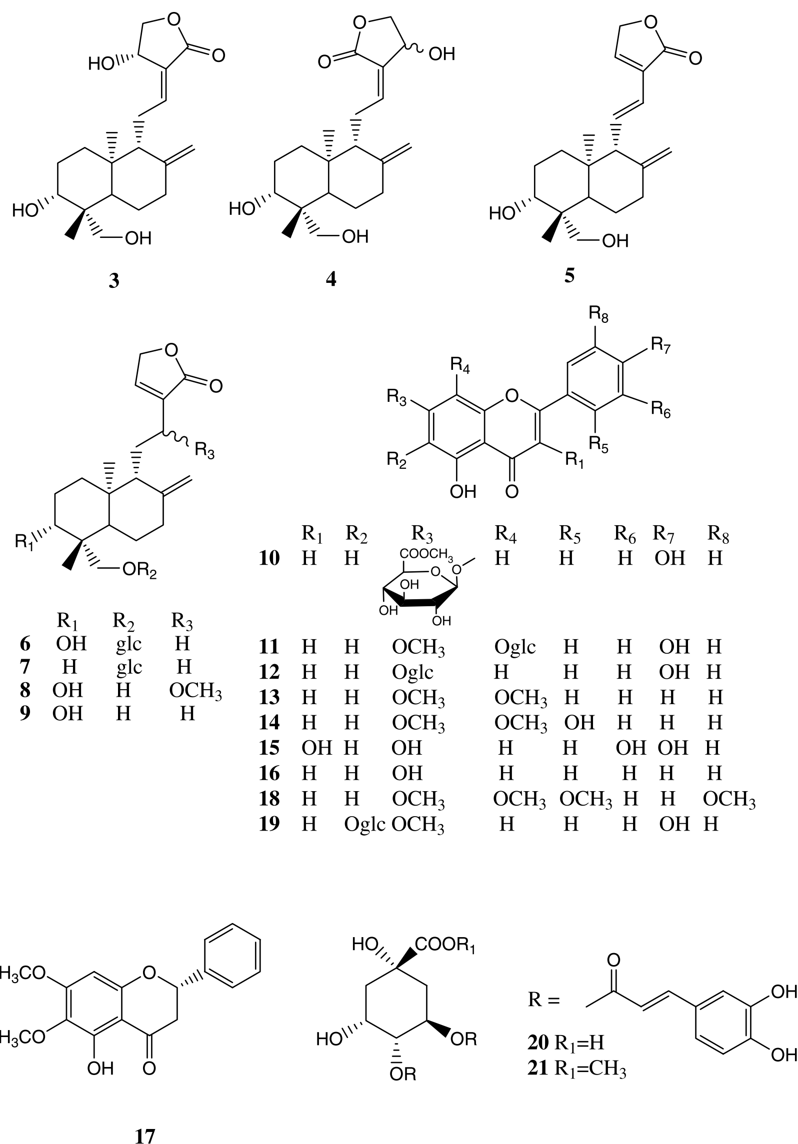


Figure 2. Structures of compounds **3–21**.

## 3. Experimental

### *3.1 General experimental procedures*

Melting points were determined on Yanaco MP-S3 micro-melting point apparatus without correction. Optical rotations were measured on a Jasco DIP-370 polarimeter. UV spectra were taken on a Hitachi UV-3210 spectrophotometer. IR spectra were recorded on a Shimadzu IR Prestige-21 spectrophotometer as KBr discs. $^{1}$H NMR, $^{13}$C NMR, COSY, HMQC, HMBC, and NOESY spectra were recorded on the Bruker Avance-300 NMR spectrometer, using tetramethylsilane (TMS) as the internal standard. Standard pulse sequences and parameters were used for the NMR experiments and all chemical shifts were

Table 2. The antiplatelet aggregation activities of isolated compounds.

| | | *Inducer* | | | |
|---|---|---|---|---|---|
| *Compound* | *Conc. (μg/ml)* | *Thr 0.1 U/ml* *0.0 ± 0.1* | *AA 10 μM* *0.0 ± 1* | *Col 10 μM/μl* *0.0 ± 0.6* | *PAF 2 ng/ml* *0.0 ± 1.5* |
| **4** | 100 | 0.66 ± 0.33 | 35.3 ± 8.5 | 6.4 ± 2.4 | 20.3 ± 5.7 |
| **5** | 100 | 3.2 ± 1.4 | 22.7 ± 8.9 | 16.0 ± 7.8 | 48.1 ± 7.2 |
| **6** | 100 | 0.4 ± 0.5 | 7.7 ± 1.3 | 1.2 ± 0.8 | 3.4 ± 1.1 |
| **7** | 100 | 0.7 ± 0.6 | 4.5 ± 1.3 | 1.2 ± 0.7 | 2.9 ± 1.2 |
| **8** | 100 | 2.0 ± 1.2 | 4.0 ± 1.3 | 9.4 ± 1.9 | 11.0 ± 4.2 |
| **10** | 100 | 2.5 ± 1.1 | 8.7 ± 0.5 | 0.2 ± 1.3 | 12.9 ± 8.1 |
| **11** | 100 | 0.4 ± 0.7 | 5.9 ± 1.1 | 1.5 ± 1.1 | 3.3 ± 2.0 |
| **12** | 100 | 1.3 ± 0.8 | 22.3 ± 4.8 | 3.5 ± 0.1 | 2.6 ± 0.5 |
| **13** | 100 | 11.1 ± 1.8 | 12.8 ± 4.0 | 61.4 ± 19.0 | 25.2 ± 4.0 |
| **16** | 50 | 1.1 ± 0.6 | 91.8 ± 7.0 | 90.5 ± 2.1 | 5.7 ± 3.3 |
| | 20 | | 5.4 ± 1.8 | | |
| **17** | 100 | 68.1 ± 3.4 | 100.0 ± 0.0 | 83.7 ± 2.9 | 84.1 ± 7.6 |
| | 50 | | 100.0 ± 0.0 | | |
| | 20 | | 96.6 ± 2.9 | | |
| | 10 | | 66.3 ± 9.9 | | |
| | 5 | | 38.7 ± 12.7 | | |
| | 2 | | 4.5 ± 2.5 | | |
| **20** | 100 | 11.5 ± 2.1 | 37.7 ± 9.6 | 58.8 ± 14.8 | 32.2 ± 2.3 |

Effect of compounds on the aggregation of washed rabbit platelets. Platelets preincubated with compounds or DMSO (0.5%, control) at 37°C for 3 min, induced by thrombin (Thr), arachidonic acid (AA), collagen (Col), and platelet activating factor (PAF) (% inhibition). Values are mean ± S.E.M. ($n = 1$–3).

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reported in parts per million (ppm, $\delta$). All the low and high-resolution mass spectra were obtained on a JEOL JMS-700 spectrometer. TLC was conducted on precoated Kieselgel 60 F 254 plates (Merck) and the spots were detected either by examining the plates under a UV lamp or by treating the plates with a 10% methanolic solution of sulphuric acid followed by heating at 110°C.

### 3.2 Plant material

The whole plants of *Andrographis paniculata* Nees (Acanthaceae) were collected from Tainan, Taiwan, in June 2000. The plant material was identified and authenticated by Professor C.S. Kuoh, Department of Life Sciences, National Cheng Kung University,

Table 3. Effect of isolated compounds from *A. paniculata* on high $K^+$- and $Ca^{2+}$- induced and norepinephrine-induced contraction of rat thoracic aorta.

| | | | *Norepinephrine* | |
|---|---|---|---|---|
| *Compound* | *Conc. (μg/ml)* | *$K^+$ (80 mM) + $Ca^{2+}$ (1.9 mM)* | *Phasic* | *Tonic* |
| **4** | 50 | 74.5 ± 2.4 | 94.5 ± 3.9 | 87.2 ± 2.4 |
| **5** | 50 | 46.3 ± 5.2 | 93.4 ± 4.7 | 81.6 ± 0.2 |
| | 15 | 80.7 ± 9.9 | N | N |
| **6** | 50 | 110.1 ± 0.4 | 94.1 ± 0.2 | 98.9 ± 0.8 |
| **7** | 50 | 98.3 ± 1.2 | 112.9 ± 2.7 | 114.6 ± 0.2 |
| **10** | 50 | 110.9 ± 3.2 | 119.3 ± 2.7 | 114.6 ± 5.7 |
| **11** | 50 | 100.0 ± 0 | 110.3 ± 2.8 | 105.9 ± 2.5 |
| **12** | 50 | 112.4 ± 7.0 | 110.2 ± 2.2 | 97.5 ± 6.3 |

Rat aorta rings preincubated with components or DMSO (0.5%, control) at 37°C for 15 min, then inducer added. Values are mean ± S.E.M. ($n = 3$–8). N, no test.

Tainan, Taiwan. A voucher specimen (TSWu 20000111) has been deposited in the herbarium of the National Cheng Kung University.

### *3.3 Extraction and isolation*

Whole plant material of *A. paniculata* (11.5 kg) was shade dried, ground and extracted with methanol (10 L × 6) under reflux for 8 h, and filtered to give residue. The combined filtrate was concentrated under reduced pressure to obtain a dark green crude extract (2.35 kg), which was suspended in $H_2O$. The suspension was then treated with $CHCl_3$, to give, after removal of the solvent, $CHCl_3$ soluble and $H_2O$ soluble residues, and insoluble residue. The $CHCl_3$ soluble residue (700 g) was chromatographed over a silica gel column, which was developed by gradient elution with benzene and increasing concentrations of EtOAc to afford seven fractions. Fraction 4 gave tetracosyl ferulate (20.4 mg) when subjected to silica gel column chromatography eluting with *n*-hexane/EtOAc (12:1). Chromatography of fraction 5 on silica gel column by eluting with benzene/EtOAc (19:1) led to the isolation of compound **17** (9.7 mg). Separation of fraction 6 by silica gel column chromatography with *n*-hexane/$Me_2CO$ (9:1) afforded β-sitosterol (100 mg). Fraction 7 was subjected to a series of silica gel column chromatographic separations using $CHCl_3$/MeOH. Final purifications of the resulting fractions were achieved through preparative TLC on silica gel ($CHCl_3$/MeOH, 9:1) to obtain pure compounds **4** (17.3 mg), **5** (208 mg), **8** (5.0 mg), **9** (2.1 mg), **16** (2.0 mg), methyl ferulate (5.4 mg), methyl vanillate (1.5 mg), and methyl caffeate (0.8 mg).

The $H_2O$ soluble residue (1.5 kg) was subjected to Diaion HP-20 column chromatography eluting with increasing concentrations of MeOH in $H_2O$ to give seven fractions. Chromatography of fraction 4 over Sephadex LH-20 eluting with a mixture of $H_2O$/MeOH and purification by preparative TLC on silica gel with $CHCl_3$/MeOH/$H_2O$ (9:1:0.1) gave caffeic acid (1.2 mg) and *p*-hydroxybenzoic acid (1.4 mg). Fraction 5 gave ferulic acid (8.1 mg) when purified by Sephadex LH-20 chromatography eluting with a mixture of $H_2O$/MeOH. Chromatography of fraction 6 on Sephadex LH-20 column eluting with a mixture of $H_2O$/MeOH followed by a series of column chromatography separations over silica gel led to the isolation of **10** (43 mg), **12** (8.7 mg), **15** (1.2 mg), **20** (31 mg), **21** (38 mg), and vanillic acid (2.6 mg). Separation of fraction 7 by repeated column chromatography over silica gel using $CHCl_3$/MeOH gradients followed by purification with preparative TLC on silica gel with $CHCl_3$/MeOH/$H_2O$ (19:1:0.1, 9:1:0.1, 5:1:0.1) yielded compounds **1** (0.8 mg), **2** (11 mg), **3** (10 mg), **6** (305 mg), **11** (39 mg), **13** (11 mg), **14** (17 mg), **18** (1.0 mg), **19** (3.0 mg), cinnamic acid (16 mg), and adipic acid (30 mg). The insoluble residue was subjected to silica gel column chromatography using a $CHCl_3$/MeOH gradient system to give four fractions. The purification of fraction 2 with silica gel column chromatography by eluting with $CHCl_3$/MeOH (49:1) afforded methyl caffeate (2.3 mg). Separation of fraction 3 over silica gel column using $CHCl_3$/MeOH (19:1) yielded compound **7** (310 mg). Fraction 4 was further separated by silica gel column chromatography with $CHCl_3$/MeOH (19:1) to give **3** (135 mg) and **13** (11 mg).

**3.3.1 Andropaniculosin A (1)**. $C_{18}H_{16}O_7$, yellow crystals, mp 178–180°C; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 362 (3.11), 273 (4.01); UV $\lambda_{max}$ (MeOH + $AlCl_3$/HCl) nm (log $\varepsilon$): 368, 273; IR (KBr) $\nu_{max}$ ($cm^{-1}$): 3200 (OH), 2927, 1647 (C=O), 1549, 1439, 1216; ESI-MS/MS (positive mode) *m/z*: 345 $[M + H]^+$(2), 330 $[M + H - Me]^+$(10),

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317 [M + H − CO]$^+$(15), 315 [M + H − 2Me]$^+$(8), 300 [M + H − 3Me]$^+$(100), 197 [A$^+$] (11), 149 [B$^+$] (12); HRESI-MS found *m/z*: 345.0974 [M + H]$^+$ (calcd for $C_{18}H_{17}O_7$, 345.0974).

**3.3.2 Andropaniculoside A (2)**. $C_{23}H_{24}O_{11}$, $[\alpha]_D^{25}$ + 155.5 (*c* 0.01, MeOH), yellow crystals, mp 274–276°C; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 338 (2.71), 264 (4.51); UV $\lambda_{max}$ (MeOH + NaOAc) nm (log $\varepsilon$): 392, 262; IR (KBr) $\nu_{max}$ (cm$^{-1}$): 3400(OH), 2927, 1634, 1603, 1450, 1043; HRESI-MS *m/z*: 477.1399 [M + H]$^+$ (calcd for $C_{23}H_{25}O_{11}$, 477.1397).

### *3.4 Acid hydrolysis of 2*

Compound **2** (5 mg) was refluxed at 100°C for 1 h with 2 N HCl in MeOH (10 ml). The acid hydrolysate was extracted with EtOAc and evaporated to dryness to yield a yellow amorphous solid, which was purified by recrystallisation from MeOH to afford compound **14** (3 mg), identified by mp, UV, IR, $^1$H NMR and $^{13}$C NMR spectral analyses, while the sugar in the aqueous layer was identified as glucose by co-paper chromatography. (*n*-BuOH/AcOH/$H_2O$, 4:1:5, $R_f$ 0.18, aniline phthalate spray).

### *3.5 Antiplatelet aggregatory and vasorelaxing activity assays*

Assays of the antiplatelet aggregatory and vasorelaxing activities of isolates were done according to the procedures of Teng and coworkers [14,15].

## Acknowledgements

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