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Phytochemical investigation and biological evaluation of *Dichanthium annulatum*(Forrsk)

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Abstract

Phytochemical investigation of *Dichanthium annulatum* (Forrsk), herbs revealed the isolation and identification of two pairs of lignoflavone derivatives stereoisomers; an epimers of tricin 4`-O-(threo-β-guaiacylglyceryl) ether (Salcolin A) and tricin 4`-O-(erythro-β-guaiacylglyceryl) ether (Salcolin B) (1) and an epimer of tricin 4`-O-[threo-β-guaiacyl-(7``-O-methyl-9``-O-acetyl)-glyceryl] ether and tricin 4`-O-[erythro-β-guaiacyl-(7``-O-methyl-9``-O-acetyl)-glyceryl] ether (2), one flavone; tricin (3), two flavone glycosides; tricin 7-O-β-D-glucopyranoside (4) and tricin 7-O-neohesperidoside (5), one flavone C-glucoside; isoorientin (6), one phenolic acid; p-coumaric acid (7), one lignan; 4-ketopinoresinol (8) and two sterols; stigmasterol (9) and β-sitosterol-3-O-β-D-glucoside (10). The structural elucidations of isolated compounds were established on the basis of NMR and MS spectral analyses. All isolated compounds and biological activities for different extracts, n-hexane, ethyl acetate and n-butanol fractions (antiviral, antimicrobial and cytotoxic activities) of *Dichanthium annulatum* were done for the first time.

Keywords: *Dichanthium annulatum*, Poaceae, Lignoflavone, Lignan, Cytotoxicity.

Introduction

In recent years, an increased interest in the Phytochemistry of family Poaceae (Graminaea) has been motivated by the discovery of several phenolic compounds as Lignans, flavonoids and flavolignan[1, 2]. Some plants of poaceae used in folk medicine for hypertension, antidiabetic, anti-inflammatory, anthelmintic, astringent, antiulcer, diuretic and antioxidant [3, 4]. Poaceae contains a very wide range of constituents as foodstuffs, starches, sugar and secondary metabolites such as "volatile oils, alkaloid, saponins, cyanogenetic substances, phenolic acids, flavonoids and terpenoids [5]. *Dichanthium annulatum* (Forrsk), belongs to Poaceae (Graminae) family, is perennial, densely tufted grasses, with rhizomiferous main stems. It is native to tropical Asia, the Middle East, and parts of Africa. It has been introduced to many other parts of the world for cultivation, and it has become naturalized in some places, such as Australia [6]. It wothnoting that nothing was reported about phytochemical and biological investigation of *Dichanthium annulatum* (Forrsk),. Therefore, phytochemical and biological investigations of the different extracts of *Dichanthium annulatum* are very important.

Materials and methods

Plant material

Dichanthium annulatum herb was collected from the surroundings of faculty of pharmacy Al-Azhar University, Cairo, Egypt, in Jun 2013. The plant was kindly identified by Professor AbdoMarey Prof. of taxonomy, Botany Dept. Faculty of Science Al-Azhar University, Cairo, Egypt. A specimen of *Dichanthiumannulatum* herb has been deposited in Pharmacognosy dept., Faculty of pharmacy, Al-Azhar University, Cairo, Eygpt.

General experimental procedures

UV spectra were determined with PyeUnicam spp. 1750 spectrophotometer. ESIMS was carried out on a XEVO TQD triple quadruple instrument (Waters Corporation, Milford, MA 01757, USA) mass spectrometer. The ^{1}H- and ^{13}C NMR measurements were obtained with a Bruker Avance III (400)

NMRspectrometer operating at 400 MHz (for 1H) and 100 MHz (for 13C) in CD3OD or DMSO-d6 solution, and chemical shifts were expressed in δ (ppm) with reference to TMS, and coupling constant (*J*) in Hertz. EIMS was carried on Scan EIMS-TIC, mass (158VG-ZAB-HF, X-.64, 800.00) mass spectrometer (VG Analytical, Inc.). Si gel (Si gel 60, Merck) and Sephadex LH-20 (Pharmacia) were used for open column chromatography. Solid phase extraction was performed on SPE-C_{18} cartridges (A Strata column, Phenomenex, USA). TLC in this work was carried out on silica gel 60 F_{254} (Merck) plates which precoated. Chromatograms were visualized, by spraying with ammonia or aluminum chloride solutions, or spraying reagent (1% vanillin/H_2SO_4), followed by heating at 100 ^{0}C for 5 minutes.

Extraction and isolation

Air-dried powdered aerial parts of *Dichanthium annulatum* (1.5kg) were subjected to exhaustive extraction with 70% ethanol (7Lx3). The combined ethanolic extracts were concentrated under vacuum at 40°C to dryness. The concentrated ethanolic extract (100g) was suspended in distilled water (500 ml) and partitioned successively with *n*-hexane, ethyl acetate and *n*-butanol to give 14gm, 10g and 18g, respectively. The ethyl acetate extract was subjected to a silica gel column eluted with *n*-hexane:ethyl acetate 95:5 to 10:90 to obtain six fractions of A (500mg), B (700mg), C (450mg), D (850mg), E (900g) and F (800mg). Fraction B was re chromatographed on Si gel CC eluted with *n*-hexane:ethyl acetate 90:10 to 80:20 to give compound **9** (45mg). The D fraction was chromatographed on a Sephadex LH-20 column eluted with CH_2Cl_2:MeOH 50:50 to give compounds **7** (20mg) and sub fraction D-1 (90mg). The sub fraction D-1 was further subjected to solid phase extraction (RP-C_{18}) using 50:50-60:40 MeOH:water system to obtain compound **8** (25mg). Fractions E and F were separately subjected to Sephadex LH-20 columns eluted with MeOH to afford compounds **3** (50mg) and **2** (12mg) from fraction E and compounds **1** (8mg) and **10** (30mg) from fraction E. The *n*-butanol fraction was subjected to VLC eluted with CH_2Cl_2:MeOH 95:5 to 30:70 to give five fractions of A (1.5g), B (2g), C (1.7g), D (3g) and E (2.2g). Fraction A was rechromatographed on Si gel CC eluted with CH_2Cl_2:MeOH 95:5 to give three sub fractions of A-1 (300mg), A-2 (250mg) and A-3 (430mg). The sub fraction A-2 was purified on a Sephadex LH-20 eluted with MeOH to obtain compound **4** (20mg). Fraction B was repeatedly chromatographed on silica gel columns eluted with CH_2Cl_2:MeOH 95:5 to 80:20 and Sephadex LH-20 eluted with MeOH to afford compounds **6** (40mg) and **5** (30mg), respectively.

Antiviral assays

The screening antiviral activities of *n*-hexane, ethyl acetate and *n*-butanol fractions of *Dichanthium annulatum* herb using cytopathic effect inhibition assay which was reported in literature[7-9].

Antimicrobial assays

Antimicrobial activities of *n*-hexane, ethyl acetate and *n*-butanol fractions of *Dichanthium annulatum* were investigated in *vitro* against different bacteria and fungi using the diffusion agar technique [10].The following bacterial strains were employed in the screening: Gram-positive bacteria; *Staphylococcus aureus* (RCMB 010028) and*Bacillis subtilis* (RCMB 010067), Gram-negative bacteria; *Escherichia coli* (RCMB 010052) and *Pseudomonas aeruginosa* (RCMB 010043). As fungal strains *Aspergillus fumigates* (RCMB 02568) and *Candida albicans* (RCMB 05031). Ampicillin, Gentamycin and Amphotericin B were used as reference drugs. The microbial species are environmental and clinically pathogenic microorganisms obtained from Regional Center for Mycology and Biotechnology antimicrobial unit (RCMB), Al-Azhar University.

Cytotoxicity assays

The cytotoxicity of the *n*-hexane, ethyl acetate and *n*-butanol fractions were tested against three human tumor cell lines; Hepatocellular carcinoma cells (HepG-2), Colon carcinoma cells (HCT-116) and Breast carcinoma cells (MCF-7). The cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were grown on Roswell Park Memorial Institute (RPMI) 1640 medium (Nissui Pharm. Co., Ltd., Tokyo, Japan) supplemented with 10% inactivated fetal calf serum and 50µg/mL gentamycin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO_2 and were sub cultured two to three times a week. The cytotoxic activity was determined by using cell viability assay method as described previously [11, 12]. The experiments were performed in triplicates and the percentage of cell viability was calculated as the mean absorbance of control cells/mean absorbance of treated cells. Concentration-response curves were prepared and the IC_{50} values were determined.

Results

[Compound **1**] Salcolin A and B Yellow amorphous powder; UV λ_{max} (MeOH) nm: 271, 287, 331, 338 nm; IR υ_{max} (KBr) cm^{-1}: 3424, 2945, 2890, 2840, 1655, 1660, 1592, 1497, cm^{-1}; ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) and ^{13}C NMR (DMSO-*d*$_6$,100 MHz) see table 1; EIMS *m\z* 526 [M]$^+$.

[Compound **2**] Tricin 4`-*O*-[threo-β-guaiacyl-(7``-*O*-methyl-9``-*O*-acetyl)-glyceryl] ether and tricin 4`-*O*-[erythro-β-guaiacyl-(7``-*O*-methyl-9``-*O*-acetyl)-glyceryl] ether :Yellow amorphous solid; UV λ_{max} (MeOH) nm: 272, 287, 320, 335 nm; IR υ_{max} (KBr) cm^{-1}: 3410, 2940, 2890, 1735, 1655, 1620, 1595, 1498, cm^{-1}; ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) and ^{13}C NMR (DMSO-*d*$_6$,100 MHz) see table 1; EIMS *m\z* 582 [M]$^+$.

[Compound **3**] Tricin: Yellow needles; UV λ_{max} (MeOH) nm: 245, 268, 299sh, 349, (NaOMe) nm: 263, 274sh, 330, 415, ($AlCl_3$) nm: 257sh, 277, 304, 365sh, 395, ($AlCl_3$/HCl) nm: 258sh, 277, 303, 358, 387, (NaOAc) nm: 264, 276sh, 320, 413, (NaOAc/boric acid) nm: 269, 304sh, 349; IR υ_{max} (KBr) cm^{-1}: 3340 (OH), 1655 (CO), 1614, 1505 (C=C aromatic); ^{1}H NMR (*CD$_3$OD*, 400 MHz) δ 7.18 (2H, s, H-2`, 6`), 6.59 (1H, s, H-3), 6.43 (1H, d, *J*=1.6 Hz, H-8), 6.19 (1H, d, *J*=1.7 Hz, H-6), 3.94 (6H, s, 3`, 5`-OMe); ^{13}C NMR (*CD$_3$OD*, 100 MHz) δ 182.97 (C-4), 165.17 (C-2), 165.09 (C-7), 162.50 (C-5), 158.65 (C-9), 149.00 (C-3`, 5`), 140.59 (C-4`), 121.91 (C-1`), 104.71 (C-2`, 6`), 104.17 (C-3, 10), 99.51 (C-6), 94.61 (C-8), 56.53 (3`, 5`-OMe); EIMS *m\z* 330 [M]$^+$.

[Compound **4**] Tricin 7-*O*-*β*-*D*-glucopyranoside: Yellow amorphous powder; UV λ_{max} (MeOH) nm: 208, 255, 272, 349, (NaOMe) nm: 263, 299sh, 412, ($AlCl_3$) nm: 272, 295sh, 330, 396, ($AlCl_3$/HCl) nm: 272, 294sh, 358, 388, (NaOAc) nm: 259, 266sh, 364, 410, (NaOAc/boric acid) nm: 258, 350; IR υ_{max} (KBr) cm^{-1}: 3420 (OH), 1653 (CO), 1618, 1499 (C=C aromatic); ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) aglyconδ 12.99 (5-OH), 7.36 (2H, s, H-2`, 6`), 7.06 (1H, s, H-3), 6.93 (1H, brs, H-8), 6.46 (1H, brs, H-6), 3.88 (6H, s, 3`, 5`-OMe), sugar δ 5.05 (1H, d, *J*=7.1 Hz, H-1``), 3.72 (1H, m, H-6``a), 3.42 (1H, m, H-6``b), 3.32 (1H, t, *J*=9.6 Hz, H-3``), 3.29 (1H, m, H-5``), 3.27 (1H, t, *J*=8.6 Hz, H-2``), 3.15 (1H, d, *J*=8.7 Hz, H-2``); ^{13}C NMR (DMSO-*d*$_6$, 100 MHz) aglyconδ 182.49 (C-4), 164.65 (C-2), 163.46 (C-7), 161.55 (C-5), 157.35 (C-9), 148.75 (C-3`, 5`), 141.50 (C-4`), 121.0 (C-1`), 105.83 (C-10), 105.00 (C-2`, 6`), 104.10 (C-3), 99.97 (C-6), 95.75 (C-8), 56.83

(3`, 5`-OMe), sugar δ 100.61 (C-1``), 77.82 (C-5``), 76.95 (C-3``), 73.62 (C-2``), 70.12 (C-4``), 61.11 (C-6``); ESIMS (positive mode) *m\z* 493 $[M+H]^+$, 515 $[M+Na]^+$, 331 $[aglycon+H]^+$, 353 $[aglycon+Na]^+$; ESIMS (negative mode) *m\z* 491 $[M-H]^-$, 329 $[aglycon-H]^-$.

[Compound **5**] Tricin 7-*O*-neohesperidoside: Yellow amorphous powder; UV λ_{max} (MeOH) nm: 208, 250, 271, 352, (NaOMe) nm: 260, 300, 415, ($AlCl_3$) nm: 275, 299sh, 329, 400, ($AlCl_3$/HCl) nm: 275, 295sh, 357, 389, (NaOAc) nm: 259, 266sh, 364, 412, (NaOAc/boric acid) nm: 256, 352; IR υ_{max} (KBr) cm^{-1}: 3385 (OH), 1662 (CO), 1605, 1498 (C=C aromatic); ^1H NMR (DMSO-d_6, 400 MHz) aglyconδ 12.98 (5-OH), 7.34 (2H, s, H-2`, 6`), 7.06 (1H, s, H-3), 6.89 (1H, brs, H-8), 6.37 (1H, brs, H-6), 3.88 (6H, s, 3`, 5`-OMe), sugar δ 5.21 (1H, d, *J*=7.1 Hz, H-1``), 5.13 (1H, brs, H-1```), 1.20 (3H, d, *J*=6.1 Hz, H-6```); ^{13}C NMR (DMSO-d_6, 100 MHz) aglyconδ 182.47 (C-4), 164.5 (C-2), 163.15 (C-7), 161.9 (C-5), 157.39 (C-9), 148.75 (C-3`, 5`), 140.3 (C-4`), 120.6 (C-1`), 105.9 (C-10), 104.98 (C-2`, 6`), 104.4 (C-3), 99.6 (C-6), 95.38 (C-8), 56.81 (3`, 5`-OMe), sugar δ 101.14 (C-1```), 100.9 (C-1``), 77.70 (C-2``, 5``), 76.9 (C-3``), 72.33 (C-4``), 70.87 (C-3```, 4```), 70.25 (C-2```), 68.8 (C-5```), 60.99 (C-6``); ESIMS (positive mode) *m\z* 661 $[M+Na]^+$; ESIMS (negative mode) *m\z* 637 $[M-H]^-$, 329 $[aglycon-H]^-$.

[Compound **6**] Isoorientin: Yellow amorphous powder; UV λ_{max} (MeOH) nm: 240sh, 257, 270, 350, (NaOMe) nm: 265, 277, 335, 405, ($AlCl_3$) nm: 277, 300sh, 334, 426, ($AlCl_3$/HCl) nm: 265sh, 278, 294, 360, 387, (NaOAc) nm: 276, 324, 395, (NaOAc/boric acid) nm: 265, 376, 430; IR υ_{max} (KBr) cm^{-1}: 3400 (OH), 1658 (CO), 1585, 1510 (C=C aromatic); ^1H NMR (DMSO-d_6, 400 MHz) aglyconδ 13.56 (5-OH), 7.43 (1H, brs, H-2`), 7.40 (1H, brs, H-6`), 6.92 (1H, d, *J*= 8.1 Hz, H-5`), 6.6 (1H, s, H-3), 6.58 (1H, brs, H-8), sugar δ 4.58 (1H, d, *J*=9.1 Hz, H-1``), 4.06 (1H, t, *J*=9.0 Hz, H-1```), 3.68 (1H, d, *J*=11.4 Hz, H-6``a), 3.42 (1H, m, H-6``b), 3.28 (1H, t, *J*=8.9 Hz, H-3``), 3.21 (1H, t, *J*=8.5 Hz, H-4``), 3.15 (1H, m, H-5``),$^{13}C$ NMR (DMSO-$d_6$, 100 MHz) aglyconδ 182.29 (C-4), 164.08 (C-2, C-7), 161.09 (C-5), 156.63 (C-9), 150.25 (C-4`), 146.26 (C-3`), 121.79 (C-1`), 119.35 (C-6`), 116.61 (C-5`), 113.74 (C-2`), 103.71 (C-10), 103.16 (C-3), 109.30 (C-6), 94.04 (C-8), sugar δ 82.02 (C-5``), 79.45 (C-3``), 73.48 (C-1``), 71.06 (C-2``), 70.59 (C-4``), 61.92 (C-6``); ESIMS (positive mode) *m\z* 471 $[M+Na]^+$; ESIMS (negative mode) *m\z* 447 $[M-H]^-$, 285 $[aglycon-H]^-$.

[Compond**7**] *p*-coumaric acid: Colorless needles, UV λ_{max} (MeOH) nm: 310; IR υ_{max} (KBr) cm^{-1}: 3420 (OH), 3100-2500 (OH), 1675 (CO), 1620, 1600, 1515 (C=C); ^1H NMR (*CD_3OD*, 400 MHz) δ 7.53 (1H, d, *J*= 16.0 Hz, H-β), 7.31 (2H, d, *J*= 8.4 Hz, H-2, H-6), 6.68 (2H, d, *J*= 8.4 Hz, H-3, H-5), 6.23 (1H, d, *J*= 15.8 Hz, H-α);^{13}C NMR (*CD_3OD*, 100 MHz) δ 167.66 (CO), 159.90 (C-4), 145.59 (C-β), 129.88 (C-2, 6), 125.73 (C-1), 115.47 (C-3, 5), 113.43 (C-α); EIMS *m\z* 164 $[M]^+$.

.[Compound **8**] 4-ketopinoresinol: Amorphous powder; UV λ_{max} (MeOH) nm: 232, 280, 285sh, IR υ_{max} (KBr) cm^{-1}: 3400 (OH), 1760 (CO), 1605, 1515 (C=C aromatic); ^1H NMR (*$CDCl_3$*, 400 MHz) δ 6.93-6.78 (6H, m, H-2`, 2``, 5`, 5``, 6`, 6``), 5.34 (1H, d, *J*= 3.8 Hz, H-6), 5.33 (1H, d, *J*= 4.1 Hz, H-2), 4.33 (1H, dd, *J*= 9.3, 6.8 Hz, H-8b), 4.03 (1H, dd, *J*= 9.4, 4.6 Hz, H-8a), 3.89 (6H, s, 3`, 3``-OCH_3), 3.46 (1H, dd, *J*= 9.2, 3.8 Hz, H-5), 3.24 (1H, m, H-1); ^{13}C NMR (*$CDCl_3$*, 100 MHz) δ 177.13 (C-4), 147.09, 146.82 (C-4`, 4``), 146.21, 145.48 (C-3`, 3``), 132.42, 131.22 (C-1`, 1``), 118.54, 118.16 (C-6`, 6``), 114.87, 114.57 (C-5`, 5``), 108.27, 107.95 (C-2`, 2``), 84.78 (C-6), 83.51 (C-2), 72.83 (C-8), 56.22, 56.16 (3`, 3``-OCH_3), 53.45 (C-1), 50.10 (C-5); EIMS *m\z* 372 $[M]^+$.

[Compound **9**] Stigmasterol: White crystalline needles;IR υ_{max} (KBr) cm^{-1}:3380 (OH), 2942 and 2870 (aliphatic CH), 1643 (C=C); ^1H NMR (*$CDCl_3$*, 400 MHz) δ 5.28 (1H, d, *J*= 4.6 Hz, H-6), 5.09 (1H, dd, *J*=15.1, 8.6 Hz, H-22), 4.95 (1H, dd, *J*=15.1, 8.6 Hz, H-23),3.45 (1H, m, H-3), 0.95 (3H, d, *J*=7.4 Hz, Me-21), 0.93 (3H, s, Me-19), 0.80 (3H, t, *J*=6.4 Hz, Me-29), 0.77 (3H, d, *J*=7.4 Hz, Me-26), 0.75 (3H, d, *J*=7.4 Hz, Me-27),0.60 (3H, s, Me-18); ^{13}C NMR (*$CDCl_3$*,100.0 MHz) δ 140.73 (C-5), 138.32 (C-22), 129.28 (C-23), 121.73 (C-6), 71.84 (C-3), 56.77 (C-14), 56.12 (C-17), 51.25 (C-24), 50.14 (C-9), 42.33 (C-13), 42.25 (C-4), 40.50 (C-20), 39.78 (C-12), 37.26 (C-1), 36.51 (C-10), 33.95 (C-2), 31.93 (C-7), 31.91 (C-8), 31.62 (C-25), 28.93 (C-16), 26.09 (C-28), 24.73 (C-15), 22.70 (C-21), 21.09 (C-26), 21.22 (C-11), 19.82 (C-27), 19.39 (C-19), 12.25 (C-18), 11.86 (C-29); EIMS m\z 412 $[M]^+$.

[Compound **10**] β-Sitosterol 3-*O*-β-D-glucopyranose: White amorphous powder; IR (KBr) υ_{max} 3432, 1634 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 0.63 (s, Me-18), 0.76 (d, *J*=6.5 Hz, Me-27), 0.78 (t, *J*=7.0 Hz, Me-29), 0.80 (d, *J*=6.5 Hz, Me-26),0.87 (d, *J*=6.5 Hz, Me-21), 0.93 (s, Me-19), 3.02-3.39 (m, H-2`-H-5`), 3.41 (m, H-6`b), 3.44 (m, H-3), 3.61 (dd, *J*=10.7, 5.8 Hz, H-6`a), 4.18 (d, *J*=6.5 Hz, H-1`), 5.30 (m, H-6); ESI-MS m\z 577 $[M+H]^+$.

Acid hydrolysis of compounds 4 and 5.

A 5 mg of each compound was refluxed with 2M HCl in MeOH (5 mL) for 6 h. at 80°. The reaction mixture was evaporated, and the hydrolysate after dilution with H_2O (10 ml) was extracted with $CHCl_3$ (3 x 20 ml). The $CHCl_3$ extracts were evaporated to afford the aglycones, which were identified as Tricin from compounds **4 and 5** (by ^1H-NMR &^{13}C-NMR).The aqueous layer was neutralized with 2 N KOH solution and concentrated to 1 ml under reduced pressure (50°C) and compared with standard sugars by TLC (adsorbent: Silica gel; solvent system: ($CHCl_3$–MeOH–H_2O: 30: 12: 4), 9 ml of lower layer and 1 ml of HOAc). From compound **4**, the sugars were identified as glucose. From compounds **5**, the sugars were glucose and rhamnose.

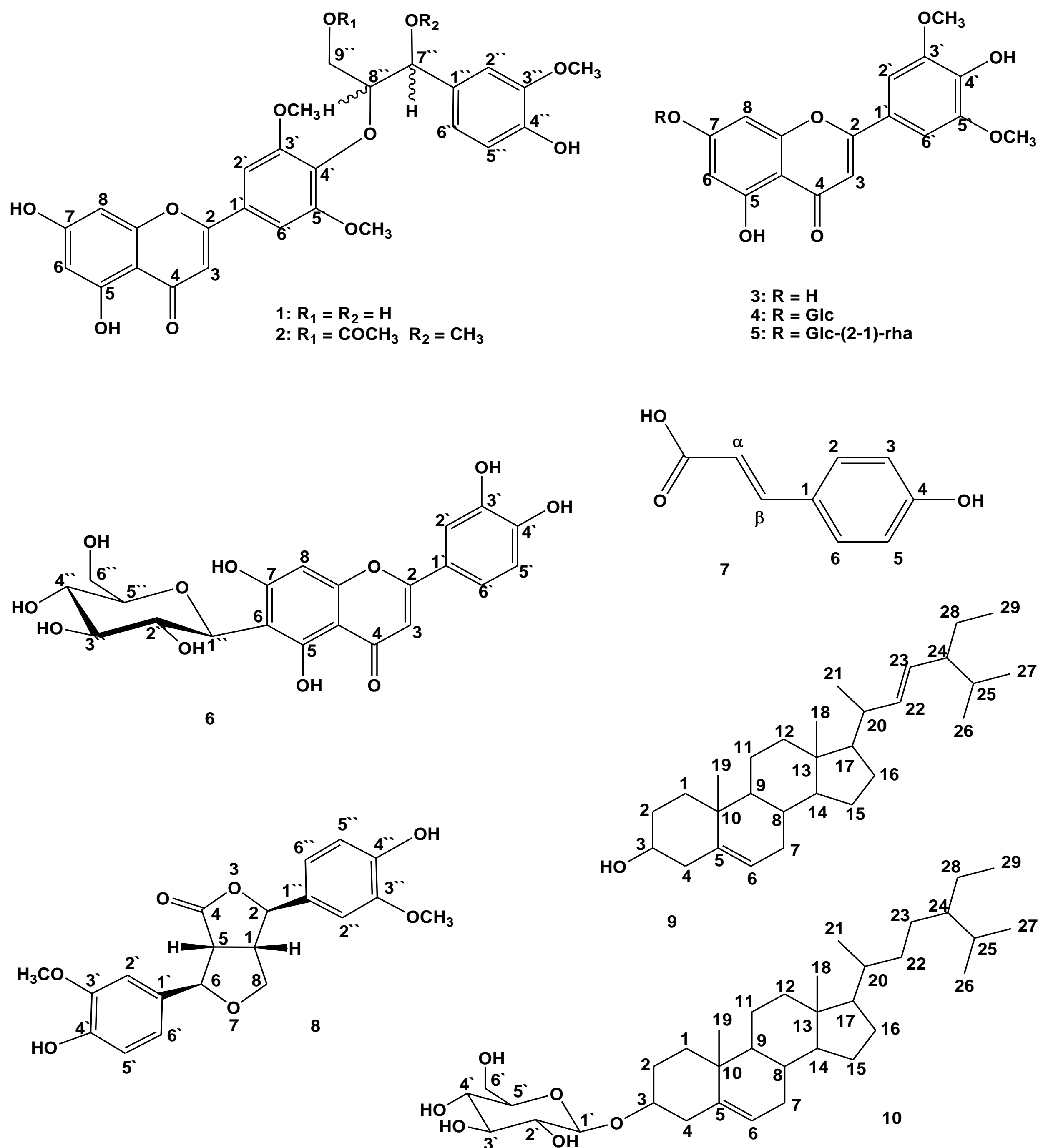

Figure 1: Structure of compounds (1-10)

Table 1: ^{1}H and ^{13}C NMR data of compounds 1 and 2 (DMSO-d_6, 400 and 100 MHz).

Position	1		2	
	^{1}H (*J* in Hz)	^{13}C	^{1}H (*J* in Hz)	^{13}C
2	-	163.07	-	163.0
3	6.98, s	105.16	7.06 brs	105.10
4	-	181.93	-	181.87
5	-	161.80	-	161.40 (t) 161.12 (e)
6	6.14, s	100.0	6.21, brs	99.01 (t) 98.99 (e)
7	-	167.23	-	167.15
8	6.47, s	95.10	6.57, brs	94.35 (t) 94.37 (e)
9	-	158.04	-	158.19
10	-	103.42	-	103.79
1`	-	125.99	-	128.89
2`	7.29, s	104.60	7.30, s (t) 7.32, s (e)	103.86
3`	-	153.40 (t)	-	152.83 (t)

		153.36 (e)		152.62 (e)
4`	-	140.21 (t) 139.77 (e)	-	140.04
5`	-	153.40(t) 153.36 (e)	-	152.83 (t) 152.62 (e)
6`	7.29, s	104.60	7.30, s (t) 7.32, s (e)	103.86
1``	-	133.45 (t) 133.68 (e)	-	132.19
2``	6.93, brs	111.44	6.74, d, 2.3 (t) 6.76, d, 2.3 (e)	111.27 (t) 110.88 (e)
3``	-	147.37 (t) 147.46 (e)	-	146.35 (t) 146.13 (e)
4``	-	145.88 (t) 145.85 (e)	-	147.46 (t)148.55 (e)
5``	6.68, d, 8.1	115.14	6.71, d, 8.8	115.10
6``	6.80, d, 8.1 (t) 6.75, d, 8.0 (e)	119.65 (t) 119.84 (e)	6.84, dd, 8.2, 2.3	119.95
7``	4.85, d, 4.8 (t) 4.81, d, 4.9 (e)	72.07 (t) 72.62 (e)	4.45, d, 6.2 (t) 4.50, brs (e)	83.82
8``	4.25, q, 4.7 (t) 4.35, q, 3.8	87.47 (t) 86.96 (e)	4.52, m	82.20 (t) 82.53 (e)
9``a  9``b	3.25, dd, 11.4, 4.7 (t) 3.50, dd, 11.6, 3.2(e) 3.64, dd, 11.5, 4.5 (t) 3.73, m (e)	60.87 (t) 60.60 (e)	4.02, dd, 11.6, 2.7 (t), 4.14, dd, 11.6, 2.1 (e) 4.30, dd, 11.8, 6.7 (t), 4.41, m, (e)	65.43
3`, 5`-OMe	3.86 (t) 3.88 s (e)	56.84	3.85, s (t) 3.87, s (e)	56.22 (t) 56.27 (e)
3``-OMe	3.74, s (t) 3.75, s (e)	55.98	3.75, s (t) 3.74, s (e)	56.79
7``-OMe	-	-	3.20 (t) 3.08 (e)	55.54 (t) 55.61 (e)
5-OH	12.84, brs	-	12.80, brs	-
$CO\underline{CH_3}$	-	-	1.91, s (t) 1.82, s (e)	20.43 (t) 20.46 (e)
$\underline{CO}CH_3$	-	-	-	170.08

t = threo, e = erythro

Table 2: Antiviral activity of *Dichanthium annulatum* using CPE inhibition assay:

Plant extract	HAV-10	HSV-1	HSV-2
n-hexane	-ve	-ve	-ve
Ethyl acetate	+	-ve	-ve
n-butanol	+	-ve	-ve

+: Weak antiviral effect; -ev: No antiviral activity.

Table 3: Antimicrobial activity of *Dichanthium annulatum* using agar diffusion method:

Organisms	Diameter of inhibition zone (mm)			
	***n*-hexane**	**Ethyl acetate**	***n*-butanol**	**Standards**
Fungi				Amphotericin
A. fumigatus	NA	16.2±0.44	NA	22.9±0.44
C. albicans	NA	20.9±0.25	NA	21.4±0.25
Gram +ve				Ampicillin
S. aureus	NA	16.9±0.58	15.4±0.25	28.9±0.14
B. subtilis	NA	20.9±0.25	17.9±0.37	28.3±0.37
Gram –ve				Gentamycin
P. aeruginosa	NA	15.2±0.58	11.1±0.37	20.3±0.37
E. coli	NA	17.1±0.25	12.6±0.63	21.4±0.25

Well diameter: 6.0 mm …. …. (100µl was tested), Sample concentration (20mg/ml),

NA: No activity, data are expressed in the form of mean ± Standard deviation.

Table 4: Cytotoxicity of *Dichanthium annulatum* against Hepatocellular carcinoma cells (HepG-2), Colon carcinoma cells (HCT-116) and Breast carcinoma cells (MCF-7):

Sample conc. (µg)	Viability %								
	n-hexane			Ethyl acetate			*n*-butanol		
	HepG-2	HCT-116	MCF-7	HepG-2	HCT-116	MCF-7	HepG-2	HCT-116	MCF-7
100	11.04	13.65	8.63	12.68	7.08	5.94	19.47	43.28	69.71
50	20.43	28.72	15.44	22.96	16.89	10.16	34.28	64.51	86.05
25	29.17	36.21	27.78	36.78	30.46	21.59	48.96	78.92	92.62
12.5	42.49	48.90	39.15	45.69	41.23	29.74	69.82	86.34	97.43
6.25	78.81	61.78	60.63	60.26	54.81	43.38	78.14	93.18	99.84
3.125	96.28	78.94	74.92	73.32	69.82	68.42	84.02	98.46	100
1.56	100	86.29	89.31	81.94	80.37	79.04	92.17	100	100
0.78	100	91.48	94.24	92.18	86.14	88.96	98.23	100	100
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
IC_{50} (µg)	11.2	12.0	9.34	10.7	8.46	5.42	24.4	84.2	> 50

Discussion

Dichanthium annulatum aerial parts were extracted with alcohol and the dried alcoholic extract was suspended in water and fractionated with *n*-hexane, ethyl acetate and *n*-butanol. The ethyl acetate and *n*-butanol fractions were separately subjected to subsequent purificationusing several chromatographic techniques (repeated silica gel, RP-18 and sephadex LH-20 columns) and solid phase extraction (SPE) to afford ten compounds (1-10) [Fig. 1] for the first time from the plant and identified by comparison of their spectroscopic data with the corresponding literature values as two flavonolignans; an epimers of tricin 4`-*O*-(threo-β-guaiacylglyceryl) ether (Salcolin A) and tricin 4`-*O*-(erythro-β-guaiacylglyceryl) ether (Salcolin B) [1, 13, 14] and an epimer of tricin 4`-*O*-[threo-β-guaiacyl-(7``-*O*-methyl-9``-*O*-acetyl)-glyceryl] ether and tricin 4`-*O*-[erythro-β-guaiacyl-(7``-*O*-methyl-9``-*O*-acetyl)-glyceryl] ether [14],tricin[1, 14, 15], tricin 7-*O*-*β*-*D*-glucopyranoside [1, 15],tricin 7-*O*-neohesperidoside [1, 15], isoorientin[1, 16, 17], *p*-coumaric acid [18], 4-ketopinoresinol [19],stigmasterol [20] and β-sitosteryl-3-*O*-*β*-glucopyranoside [21, 22].

The ethyl acetate and *n*-butanol extracts of *Dichanthium annulatum* showed weak antiviral effects against HAV-10 and showed no activity against HSV-1 and HSV-2. The *n*-hexane extract showed no antiviral activity against all viruses tested (Table 2). The ethyl acetate and *n*-butanol extracts of *Dichanthium annulatum* demonstrated variable antimicrobial activity against most of the specific organisms tested (Table 3). The ethyl acetate extract was the most active against *C. albicans* and *E. coli* compared to that of *n*-butanol. The *n*-hexane showed no antimicrobial activity against all microorganisms tested. The ethyl acetate and *n*-hexane of *Dichanthium annulatum* were the most active extracts as cytotoxic agents against the tested cell lines with values of IC_{50} from 5.42 to 12.0µg/ml compared to that of *n*-butanol (Table 4).

Conclusion

Dichanthium annulatum aerial parts afford ten compounds for the first time from the plant. This study provides an evidence for the strong cytotoxic activity of the ethyl acetate and *n*-hexane extracts of *Dichanthium annulatum*. In addition to the highest antimicrobial activity of the ethyl acetate extract ofthe plant against *C. albicans* and *E. coli* that could be considered a valuable medicinal plant species. The higher activities of the ethyl acetate extract may due to the flavonolignan and flavonoid contents which were reported previously. Additionalstudies are needed to identify the constituents of *n*-hexane extract that are responsible for its higher activity.

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