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New Epoxy Megastigmane glucoside from *Dactyloctenium aegyptium* L.P.Beauv Wild (Crowfootgrass)

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Abstract

Phytochemical investigation of *Dactyloctenium aegyptium* L. Wild herbrevealed the isolation and identification of three new compounds5-hydroxypyrimidine- 2,4 (3H,5H)- dione [6],6'Glyceryl asysgangoside[8],and 2 amino, 2 methyl,(5,6 di hydroxymethyl), 1,4 dioxane [11]were isolated for the first time from nature in addition tonine known compounds, P.hydroxy benzaldhyde[1], tricin [2],P.hydroxy benzoic acid[3], vanillic acid [4], β-sitosterol-3-O-β-D-glucoside [5], asysgangoside[7] adenine[9], uridine[10] and sucrose[12]The structural elucidations of isolated compounds were established on the basis of UV,IR, NMR and MS spectral analyses. Compounds 7 was isolated for the first time from the family. The n-hexane, ethyl acetate and n-butanol fractions of *Dactyloctenium aegyptium* L. showed significant activities against antiviral, antimicrobial and cytotoxic activities. The ethyl acetate fraction appeared to be the most active one.

Keywords: *Dactyloctenium aegyptium*, Asysgangoside, Glyceryl Asysgangoside, Uridine, Dioxane, Cytotoxicity.

INTRODUCTION

The grass family Poaceae. is the fourth largest family of the floweringplants. It includes about 700-800 genera and 11000-13000 species distributed worldwide [1-2]. Poaceae are also one of the most ecologically and economically important plant families [3]. All the cereals and millets are cultivated grasses. Sugarcane, the main source of sugar around the world is also a cultivatedgrass species. Besides, grasses constitute the main source of forage and fodder for livestock. Apart from food and fodder several grasses are used to extract of aromatic oils .and scents [4-7]. Grasses also comprise the main source of green cover of our lawns and landscape for tourism and sports. Also, their use in handicraft and cottage industry is well known. Some plants of poaceae used in folk medicine for hypertension, ant diabetic, anti-inflammatory, anthelmintic, astringent, antiulcer, diuretic and antioxidant [8-9]. 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 [10]. *Dactyloctenium aegyptium* L. Wild, 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 [11]. Grains of *Dactyloctenium aegyptium* L. are given to mother after childbirth suffering from bellyache. Decoction of seeds is used as alleviator of pains in the region of kidney. The herbaceous parts are applied externally for the cure of ulcers [12]. The whole plant is used in a decoction to remedy lumbago [13-14]. Ethno botanical studies on plants with medicinal and anti-bacterial properties [15-17] and anti diabetic [18]. *Dactyloctenium aegyptium* L. Wild, belongs to Poaceae (Graminae) family, is perennial, weeds in fields and a long channels, which represented in flora of Egypt by two species, represented under the local name as qadam el Ghorab [19-20]. Chemical study of *Dactyloctenium aegyptium* revealed the presence of Cynogenic glycosides and oxalic acid have been reported [21]. It worth noting that nothing was reported about phytochemical and biological investigation of *Dactyloctenium aegyptium* L. Wild Therefore, phytochemical and biological investigation of the different extracts of *Dactyloctenium aegyptium* L. are very important.

MATERIALS AND METHODS

Experimental

General experimental procedures. UV spectra were determined with Pye Unicam spp. 1750 spectrophotometer. ESIMS was carried out on a XEVO TQD triple quadruple instrument (Waters Corporation, Milford, MA01757, USA) mass spectrometer. EIMS was carried on Scan EIMS-TIC, VG-ZAB-HF, X-mass (158.64, 800.00) mass spectrometer (VG Analytical, Inc.). The ^{1}H- and ^{13}C NMR measurements were obtained with a Bruker Avance III (400) NMR spectrometer operating at 400 MHz (for ^{1}H) and 100 MHz (for ^{13}C) in *CD_3OD* or DMSO-*d_6* solution, and chemical shifts were expressed in δ (ppm) with reference to TMS, and coupling constant (*J*) in Hertz. ^{13}C multiplicities were determined by the DEPT pulse sequence (135^{o}C). 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 was carried out on percolated silica gel 60 F_{254} (Merck) plates. Developed chromatograms were visualized by spraying with 1% vanillin-H_2SO_4, followed by heating at 100 ^{0}C for 5 min, or spraying with ammonia or aluminum chloride solutions.

Plant material

Dactyloctenium aegyptium L. 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 Abdo Marey Prof. of taxonomy, Botany Dept. Faculty of Science Al-Azhar University, Cairo, Egypt.A voucher specimen has been deposited in the Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

Extraction and isolation

Air-dried powdered aerial parts of *Dactyloctenium aegyptium* L (2 kg) were subjected to exhaustive extraction with 70% ethanol (8Lx3). The combined ethanolic extracts were concentrated under vacuum at 40^{o}C to dryness. The concentrated ethanolic extract (100g) was suspended in distilled water (800 ml) and partitioned successively with *n*-hexane, ethyl acetate and *n*-butanol to give 13gm, 5g 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 (50mg), B (280mg), C (50mg), D (150mg), E (135mg). Fraction A was re chromatographed on Si gel CC eluted with *n*-hexane:ethyl acetate 90:10 to 80:20 to give compound **1** (5mg). The B fraction was chromatographed on a Sephadex LH-20 column eluted with CH_2Cl_2:MeOH 50:50 to give compounds **2** (11mg) and sub fraction B-1 (40mg). The sub fraction **B**-1 was further subjected to solid phase extraction (RP-C_{18}) using 50:50-60:40 MeOH:water system to obtain compound **3** (12mg). Fractions C was subjected to Sephadex LH-20 columns eluted with MeOH to afford compounds **4** (5mg)Fractions D (150mg)was subjected to Sephadex LH-20 columns eluted with MeOH to afford compounds **5** (9mg) Fraction E was subjected to Sephadex LH-20 columns eluted with MeOH to afford compound **6** (9mg). 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 (550mg), B (300 mg), C (1.7g), D (3g) and E (2.2g). Fraction A was chromatographed on Si gel CC eluted with CH_2Cl_2:MeOH 95:5 to give three sub fractions of A-3 (300mg), A-2 (250mg) and A-3 (430mg). The sub fraction A-3 was purified on a Sephadex LH-20 eluted with MeOH to obtain compound **7** (20mg) and **8** (31mg). 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 **9** (10mg) Fraction C (1.7g), was repeatedly chromatographed on silica gel columns eluted with CH_2Cl_2:MeOH 95:10 to 80:20 and subjected to Sephadex LH-20 columns eluted with MeOH to afford compounds **10** (10mg) and compound **11** respectively.

P-hydroxybenzaldhyde [1]: Yellow to tan powderwith m.p =112 to 116 °C IR (KBr) Vmax 3450, 3000, 2850,2650, 2550, 1660, 1590, 1510, 1450;^{1}H NMR (*CD_3OD*, 400 MHz) δ8.08 (1H, d, J = 8Hz.H-2) 6.83 (1H , d , J= 8 Hz,H-3), 6.83 (1H , d , J= 8 Hz,H-5), 8.08 (1H , d, J= 8 Hz,H-6), 9.74 (1H , S,H-7) ^{13}C NMR (*CD_3OD*, 100 MHz) δ130.03 (C-1), 127.02 (C-2,C-6), 116.71(C-3,C-5), 165.86(C-4),194.79 (C-7). EIMS121(M-1).

Tricin [2] 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_3OD*, 400 MHz) δ7.33(2H, s, H-2`, 6`), 6.98 (1H, s, H-3), 6.57 (1H, d, *J*=1.6 Hz, H-8), 6.21 (1H, d, *J*=1.7 Hz, H-6), 3.89 (6H, s, 3`, 5`-OMe); ^{13}C NMR (*DMSO-d_6*, 100 MHz) δ182.26 (C-4), 164.19 (C-2), 164.58 (C-7), 161.79 (C-5), 157.81 (C-9), 148.62(C-3`,5`), 140.24(C-4`), 120.86 (C-1`), 104.71 (C-2`, 6`), 104.00 (C-3), 104.16 (C-10), 99.33 (C-6), 94.71 (C-8), 56.80 (11, 12-OMe); EIMS *m\z* 330 [M]$^{+}$.

P-hydroxy benzoic acid [3] White solid; IR (KBr) Vmax 3450, 3000, 2850,2650, 2550, 1660, 1590, 1510, 1450; ^{1}H NMR (400 MHz, *CD_3OD*) δ 7.81 (1H, d,H2-H8), 6.75(1H, d,H3-H5); ^{13}C NMR (100 MHz, CD_3OD) δ170.14(-COOH), 161.7 (C-1), 116.08(C-3,5), 163.22(C-4),132.95(C-2,6); ESIMS m/z 137 (M-H).

Vanillic acid [4] White solid; IR (KBr) Vmax 3484, 3097, 1680,1597, 1522, 1434, 1298, 1284, 1238, 1203, 1028, 757cm-1; ^{1}H NMR (400 MHz, *CD_3OD*) δ 9.85 (1H, s, 4-OH), δ 3.82 (3H, s, 8-OCH3), δ 7.49 (1H, dd, J=8.7, 1.6 Hz, H-6), δ 6.77 (1H, d, J=8.4Hz,H-5), δ 7.49 (1H, d, H-2); ^{13}C NMR (100 MHz, *CD_3OD*)δ 170.14 (C7), 152.60 (C-4), 148.16 (C-3), 125.04 (C-6), 113.75 (C-2), 122.85 (C-1), 115.75 (C-5), 56.29 (8-OCH3); ESIMS m/z 167 (M-H).

β-Sitosterol 3-*O*-*β*-D-glucopyranose [**5**] White flakes; IR (KBr) υ_{max} 3432, 1634 cm^{-1}; ^{1}H 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]$^{+}$.

5-hydroxypyrimidine- 2,4 (3H,5H)- dione[6] Yellowish amorphous powder (9 mg),^{1}H NMR (400 MHz, *DMSO-d6*) δ8.06(1H,s,H-3), 5.2 (1H,d,J8Hz, H-5),δ7.01 (1H,d,J8Hz,H-6),δ 10.6(1H,S,H-7)^{13}C NMR (100 MHz, *DMSO-d_6*) δ157.84(C-2), 174.11 (C-4), 63.89 (C-5), 157.17 (C-6), EIMS m/z 128.

Asysgangoside [7] Amorphous powder, ^{1}H NMR (*CD_3OD*, 400 MHz) δ2.20 (1H, dd, J = 18.0, 2.4 Hz,H-2a)δ 2.60 (1H, dd, J = 18.0, 2.9 Hz,H-2b)**,**δ 2.35 (1H, dd, J = 17.6, 2.4 Hz,H-4a),δ 2.77 (1H, d, J = 17.6 Hz,H-4b),δ 6.25 (1H, d, J = 16Hz,H-7)**,** 5.95 (1H, dd, J = 15.5, 6.4 Hz,H-8)**,** 4.52 (1H, dq, J = 6.6, 6.4 Hz,H-9), 1.22 (3H, d, J = 6.6 Hz,H-10)**,** 3.53 (1H, d, J = 7.6 Hz,H-11a), 3.89 (1H, dd, J = 7.6, 2.9 Hz,H-11b)**,** 0.98 (3H, s,H-12)**,** 1.16 (3H, s,H13), 4.22 (1H, d, J = 7.8 Hz,H1')**,** 3.20 (1H-H2') 3.20 (1H,H3'), 3.23 (1H)a 3.55 (1H, dd, J = 12.0, 5.4 Hz,H-6'a), 3.85 (1H, br d, J = 12.0 Hz,H-6'b).^{13}C NMR (100 MHz, CD_3OD) δ 46.68 (C-1), 53.27 (C-2), 211.02 (C-3), 53.97 (C-4), 87.18 (C-5), 82.62 (C-6), 129.34 (C-7), 136.95(C-8), 74.23(C-9), 22.43(C-10), 78.24 (C-11), 15.8(C12), 19.12(C-13), 101.49(C-1'), 73.2(C-2'), 78.39(C-3'),

71.1(C-4'), 78.34(C-5'),62.88(C-6'): positive ESI MS, m/z: [M+H]– 402.1798 (calc. for $C_{19}H_{29}O_9$).

6' glycerylasysgangoside [8] Amorphous powder, ^{1}H NMR (*CD_3OD*, 400 MHz) δ 2.20 (1H, dd, J = 18.0, 2.4 Hz,H-2a) δ 2.60 (1H, dd, J = 18.0, 2.9 Hz,H-2b), δ 2.35 (1H, dd, J = 17.6, 2.4 Hz,H-4a), δ 2.77 (1H, d, J = 17.6 Hz,H-4b), δ 6.25 (1H, d, J = 16Hz,H-7), 5.95 (1H, dd, J = 15.5, 6.4 Hz,H-8), 4.52 (1H, dq, J = 6.6, 6.4 Hz,H-9), 1.22 (3H, d, J = 6.6 Hz,H-10), 3.53 (1H, d, J = 7.6 Hz,H-11a), 3.89 (1H, dd, J = 7.6, 2.9 Hz,H-11b), 0.98 (3H, s,H-12), 1.16 (3H, s,H13), 4.22 (1H, d, J = 7.8 Hz,H1'), 3.20 (1H-H2') 3.20 (1H,H3'), 3.23 (1H)a 3.55 (1H, dd, J = 12.0, 5.4 Hz,H-6'a), 3.85 (1H, br d, J = 12.0 Hz,H-6'b),3.65(1H,m,H1"),3.42(2H,dd,J12,6Hz,H2"),3.47(2H,dd,J12,6Hz,H3")^{13}C NMR (100 MHz, CD_3OD)45.96 (C-1), 53.67 (C-2), 211.73 (C-3), 54.43 (C-4), 87.92 (C-5), 82.96 (C-6), 129.46 (C-7),137.1(C-8), 74.93(C-9), 22.79(C-10), 78.53 (C-11), 16.18(C12), 20.02(C-13), 101.1(C-1'), 73.2(C-2'), 78.74(C-3'), 71.1(C-4'), 78.81(C-5'),69.86(C-6').δ 74.23 (C-1"),δ64.86 (C-2"),δ64.86 (C-3"): postive ESI MS, m/z: [M+H]– 476.1798 (calc. for $C_{22}H_{35}O_{11}$.

Adenine [9] Yellowish amorphous powder,^{1}H NMR (*CD_3OD*, 400 MHz) δ8.20 (S,H-2), 8.14 (S,H-8) ^{13}C NMR (100.6 MHz, CD_3OD)153.09 (C-2), 140.45 (C-8). EI /mss , m/z: 135 (calc. for C_5 H_5 N_5 (M) ,108(M-NCH) 81 (M- N2C2H2) , 66 (M- N2C2H2 + NH).

Uridine [10] Yellowish amorphous powder; IR (KBr) Vmax 3450, 3000,1660, 1590, 1510, 1450; ^{1}H NMR (*CD_3OD*, 400 MHz) δ5.6 (1H , d, J = 8 Hz,H5) 7.9 (1H, d, J = 8 Hz,H-6), 5.8 (1H, d, J = 4.4 Hz,H1'), 3.3 m (1H,H-2')4.1 m (1H.H-3'), 4.2 m (1H,H-4;) 3.7 (2H , dd , J= 12 , 2.8 Hz)3.8 (2H , dd, J = 12 , 2.7 Hz)^{13}C NMR (100 MHz, CD_3OD)δ 166.18 (C-4), 152.63 (C-2), 142.62 (C-6), 102.49 (C-5),90.82(C-1'),86.23 (C-4'), 75.8 (C-2'), 71.25 (C-3'),61.99 (C-5'). EI /MS, m/z 244 with (calc. for C_9 H_{12} N_2 O_9 (M) , 112, 113 for uracil and 133 for ribose moiety respectively .

2 amino, 2 methyl, (5,6 di hydroxymethyl), 1,4 dioxane [11] Yellowish amorphous powder(10 mg). IR (KBr) Vmax 1597, 1657, 640, 1400, 3350 cm^{-1} . ^{1}H NMR (*CD_3OD*, 400 MHz) δ3.34 (1H , d , J = 11.2 Hz,H-3a), 3.42 (1H , d , J = 11 .4 Hz,H-3b), 3.50 (1H, m,H-5), 3.55(1H, m,H-6), 3.42 (1H , d , J = 2 Hz,H-7a), 3.47 (1H , dd , J = 2 , 10 Hz,H-7b), 3.421 (1H, d, J = 4.8 Hz,H-8) ,3.47 (1H , dd, J= 4.8 , 2 Hz,H-8), 1.01 (3H , S).^{13}C NMR (100.6 MHz, CD_3OD)δ 74.86 (C-2), 68.36 (C-3), 76 .11(C-5), 73 .88(C-6), 64.38 (C-7), 63.76 (C-8), 19.65 (C-9). EI /MS, m/z 179.24 with (calc. for C_7 H_{16} O_4 N_1 (M+2H).

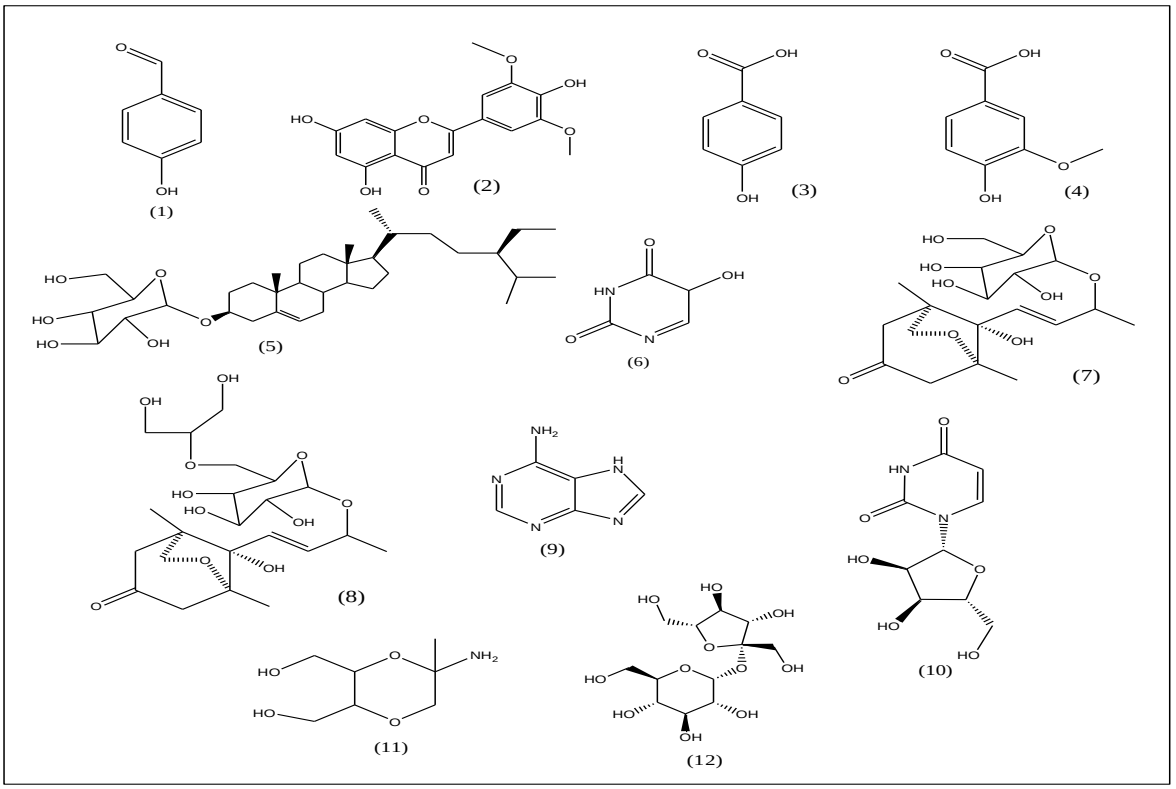

Figure 1: Isolated compounds [1-12]

Antiviral assay :

The screening antiviral assay system using cytopathic effect inhibition assay at the Regional Center for Mycology and Biotechnology (RCMB) at Al- Azhar University. This assay was selected to show specific inhibition of a biologic function, i.e., cytopathic effect (CPE) in susceptible mammalian cells [11]. In brief, monolayers of 10,000 vero cells adhered at the bottom of the wells in a 96-well microtiter plate incubated for 24h at 37ºC in a humidified incubator with 5%CO_2. The plates were washed with fresh DMEM and challenged with 10^4 herpes simplex type 2 virus doses and simultaneously the cultures were treated with two-fold serial dilutions of tested compoundin fresh maintenance medium and incubated at 37ºC for two days. An infection control as well as untreated vero cells control was made in the absence of tested compound. Six wells were used for each concentration of the tested compound. Every 24 h the observation under the inverted microscope was made until the virus in the control wells showed complete viral-induce cytopathic effects (CPE). Antiviral activity was determined by the inhibition of cytopathic effect compared to control, *i.e.*, the protection offered by the tested compound to the cells was scored [22]. The monolayers were fixed with formalin then stained with a 0.1% crystal violet solution and digital photos were taken using Olympus inverted microscope Model CKX41. Three independent experiments

were assessed each containing four replicates per treatment. Acyclovir, which is clinically used for the treatment of herpetic viral disease, was used as a positive control under this assay system [22].

Antimicrobial assay

Antimicrobial activities of *n*-hexane, ethyl acetate and *n*-butanol fractions of *Dactyloctenium aegyptium* L herb were investigated in *vitro* against different bacteria and fungi using the diffusion agar technique according to Bauer *et al*., 1966 [23]. The following bacterial strains were employed in the screening: Gram-positive bacteria; *Staphylococcus aurous*(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 RegionalCenter for Mycology and Biotechnology antimicrobial unit (RCMB), Al-AzharUniversity.

Cytotoxicity assay

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 [24-25]**.** 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 AND DISCUSSION

Dactyloctenium aegyptium L herb 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 purification using several chromatographic techniques (repeated silica gel, RP-18 and sephadex LH-20 columns) and solid phase extraction (SPE) to afford twelve compounds [**1**-**12**] (Fig. 1) for the first time from the plant and identified by comparison of their spectroscopic data with the corresponding literature. From these extracts and by using combined chromatographic separations, 8 known compounds were isolated (Fig. 1).Their structures were elucidated using physicochemical and spectroscopic methods. The isolated metabolites were identified P.hydroxy benzaldhyde[1], tricin [2], parahydroxy benzoic acid[3], vanillic acid [4], *β*-sitosterol-3-*O*-*β*-*D*-glucoside [5], asysgangoside[7]adenine[9], uridine[10] [26-37].

Compound 6 was isolated as yellowish amorphous powder (9 mg), one spot on TLC with R_f value 0.654 using {mobile system, DCM : MeOH :H_2O, 80:20:2}, give a yellow color with ammonia and intensity of color was increased after spraying with vanillin/H_2SO_4 and heating at 110 ^{0}C.

EI /MS analysis showed mol Ion peak at 128 consists with an empirical formula C_4 H_4 N_2 O_3 (M) and 113 (M- OH). and the fragment Ion peak at 85 {M - (CH2 OH)}, thus the 85 exhibit the remaining uracil ($C_2HN_2O_2$). Farther fragmentation gives ion peak at 58 indicating loss of (HCN).

^{1}HNMR showed the presence of two protons with coupling at δ 5.22 (1H,d, 6.8 Hz, CH-5)and δ 7.01 (1H,d, 6.4 ,CH-6). also showed a singlet signal at δ 8.058 (1H,S) was assigned as NH Proton , and the hydroxyl proton H7 which was attached to C5 also showed significant hydrogen bonding with carbonyl group C4 make this proton appears mostly downfield at δ 10.606 (1H, S, C5-OH).

^{13}CNMR showed 4 peaks ,two quaternary carbons at δc 174.11 (C4), δc 157.84 (C2) and two methine carbons at δc 157.17 (C6) and 63.89 (C5).

From the above data compound 6 is suggested to be 5-hydroxypyrimidine- 2,4 (3H,5H)- dione. This is suggestion was confirmed by direct comparison the data with the literature.

It was isolated for the first time from nature.

Compound 8 yellowish amorphous powder (31 mg), have the same structure of compound 7 (asysgangoside) with few differences, which confirmed by the molecular formula determined as $C_{22}H_{35}O_{11}$ by ESI/MS mass spectrometric analysis. The ^{1}H and ^{13}C NMR spectroscopic data showed the presence of a *B*-glucopyranosyl unit from the anomeric proton signal at 4.25 ppm (1H, d, J = 7.8 Hz) and from the carbon signals atδc 102.9, 78.1, 77.8, 74.9, 71.3 and C-6 appear at 69.86 indicate that C-6 is substituted. The substitution was confirmed by further investigation of ^{1}H-NMR, APT and HMBC.ESI/MS analysis showed pseudo molecular ion peak at 477 (M +1).

^{1}H-NMR for the Megastigmane skeleton (aglycone) showed two singlet methyl peaks at 0.89, 1.16 ppm and doublet methyl peak at 1.24 ppm indicates it's coupling with proton at C9, both of H2, H4 protons showed two different chemical shift was confirmed by HSQC as H2 showed two peaks first one for H2a at 2.20 ppm (1H, dd, J = 18, 2.4 Hz) and second one for H2b at 2.60 ppm (1H, dd, J = 18, 2.9 Hz), also H4 showed two peaks first for H4a at 2.35ppm (1H, dd, J = 17.6, 2.4 Hz) and second one for H4b at 2.65 ppm (1H, d, J= 17.6 Hz), as well as H11 protons showed 2 correlations was confirmed by HSQC first for H11a at 3.55ppm (1H, d, J = 7.6 Hz) and the second for H11b at 3.8ppm (1H, dd, J = 7.6, 2.9 HZ), while H9 proton showed peak at 4.55ppm (1H, dq, J = 6.6 , 6.4 Hz) and for two olefinic protons H7,H8 when H7 showed peakat 6.32 ppm (1H, d, J = 16 Hz), H8 showed peak at5.93 ppm (1H, dd, J = 16, 6.4 Hz) due to correlation coupling with H9. (for glucose moiety) spectrum showed significant anomeric proton at 4.25ppm (1H, d, J = 7.8 Hz)from this *J*-Value*B*-form configuration of glucose was confirmed ,H6 two protons showed 2 correlation confirmed by HSQC 1st for H6a proton at 3.55ppm (1H, brd J = 12 Hz) and second for H6b proton at 3.75ppm (1H , dd, J = 12, 6 Hz) and others H2,H3,H4 and H5 showed overlapped peaks at 3.1 -3.25ppm due to protons equivalency, (for glycerol moiety) H1" showed peak at 3.65 (1H.,3.65 m) and each of H2", H3" showed doublet of doublet at 3.42 ppm,3.47 ppm respectively.

APT test showed the presence of three methyl (16.18 ppm, 20.02 ppm, 22.79 ppm), nine methine (72.07 ppm, 74.93 ppm,74.23 ppm, 75.41 ppm, 78.74 ppm 78.81 ppm, 101.1ppm, 129.46 ppm and 137.74 ppm), seven methylene (53.67 ppm, 54.43 ppm, 69.86ppm, 78.53 ppm, 64.84, 64.84) and four quaternary carbon (45.96 ppm, 82.96 ppm, 87.92 ppm and 211.73 ppm).

From the above data (*^{1}HNMR, APT ,ESIMASS and* HMBC) compound 8 is suggested to be6' glyceryl asysgangoside**.**The furanoid ring suggestion was confirmed by HMBC correlation between H11 and C5 also the other HMBC correlation has been confirmed The absolute configuration of sugar moiety was determined to be D-form by treating the residue of a sugar fraction with L-cycteine methyl ester in pyridine after hydrolysis of this compound with crude hesperidinase to provide the thiazolidine derivatives of sugars, and then comparison of the Rf values with the standard samples of thiazolidine derivative of D-glucose was made.

It was isolated for the first time from nature.

This suggestion also was based on HMBC correlation similar to compound 7 (Asysgangoside) when compared with literature,in addition to the glycerol moiety and it's substitution on C6' glucose of Asysgangoside was confirmed by 1D-Spectrum

(APT test) when chemical shift of C6' was downfield from 62.88 ppm to 69.86 ppm indicating it's substitution with glycerol moiety while, 2D-Spectrum (HMBC) confirmed glycerol moiety correlation to 6' of glucose as H6'b of glucose at 3.75 ppm showed HMBC correlation with C1" (74.23 ppm) of glycerol and H6'a showed correlation with both of C2", C3" at 64.86 ppm. also H1" of glycerol at 3.65 ppm showed HMBC correlation with C5 of glucose moiety at 78.74 ppm[38-45].

Compound 11 Compound 11 was isolated as yellowish amorphous powder (10 mg), one spot on TLC with Rf value 0.295 using {mobile system , DCM : MeOH :H_2O, 80:20:2}, stained red after spraying with vanillin/H_2SO_4 and give rose color changed immediately to violet with ninhydrin ,indicating the presence of an amino group [46].

ESI /MS analysis shows pseudo mol. Ion peak at 179.24 consistent with an empirical formula C_7 H_{16} O_4 N_1 (M+2H), fragment ion peak at 144 (M-2OH) due to [charge site initiated cleavage] and 113{M-(CH3 + NH2)}.

IR spectrum showed strong bending vibration at 1597 $cm^{-1}$to 1657 cm^{-1} Indicate presence of Primary amide NH2 in solid state with KBr in addition to very broad band at 640 cm^{-1}, as well as sharp peak for C-N had been detected at 1400 cm^{-1}.and two small sharp bands at 3580 cm^{-1} due to amino group which overlapped with OH group [47].

^{1}H-NMR showed a singlet signal at δ 1.13 (3H,S, CH3-9) , also the two characteristic Diastereotopic Protons showing germinal coupling at 3.34 ppm (1H,d,11.2 Hz, H3a), and 3.42 ppm (1H, d, 11.4 Hz, H3b), the two protons of each methylene groups H7, H8 showing doublet and doublet of doublet, for H7a, H7b showed coupling at 3.42 ppm (1H,dd, 2Hz-10Hz, H7a), and 3.47 ppm (1H,d, 2Hz, H7b), for H8a,H8b protons showing coupling at 3.47 ppm (1H,d, 4.8Hz, H8a), and 3.67 ppm (1H,dd, 4.8 Hz, 2 Hz, H8b) respectively, each of the two protons at H5, H6 showing multiplet at 3.52 ppm and 3.55 ppm respectively .

^{13}C-NMR showed 7 peaks (C9 at 19.65 ppm), (C2 at 74.86 ppm), (C3 at 68.36 ppm), (C5 at 76.11 ppm), (C6 at 73.88 ppm), (C7 at 64.38 ppm) and (C8 at 63.76 ppm).

APT test showed the presence of one methyl (19.65 ppm), two methine (73.88 ppm,76.11ppm), three methylene (62.76 ppm, 64.38 ppm and 68.36) and one quaternary carbon (74.86 ppm). From the above data compound 11 is suggested to be {2 amino, 2 methyl, (5,6 di hydroxymethyl), 1,4 dioxane**}** this suggestion is confirmed by HMBC correlations where methyl protons at 1.13 ppm shows HMBC correlation at 68.36 ppm(C3) and 74.86 ppm(C2) respectively, also the two methylene protons at C3 -68.36 ppm shows HSQC correlations at two position 3.34 ppm for H3a and 3.42 ppm for H3b was confirmed by HSQC spectrum ,the proton of H3a at 3.34 ppm showed HMBC correlation at 19.65 ppm (C9), 74.86 ppm (C2) and 76.11 ppm (C5), the proton of H3b at 3.42 ppm showed the same HMBC correlations of H3a with C9, C2 and C5 respectively , the two protons of H7 also showing HSQC at two position first one for H7a at 3.42 ppm (the same position of H3b protons at HSQC), H7a protons showed HMBC correlations at 63.76 ppm (C8) and also at 73.88 ppm (C6), and another one for H7b at 3.47 ppm which also showed the similar correlation of H7a with C8 and C6 respectively, the two methylene protons of H8 also have two correlations at HSQC at two positions, first one for H8a at 3.47 ppm (the same position of H7b protons which confirmed by HSQC) 7, H8a proton showed HMBC correlation with C5 at 76.11 ppm and C8 at 63.76 ppm ,and the second one for H8b at 3.67 ppm which showed HMBC correlation with C5 at 76.11 ppm 7, the H5 proton showed HSQC correlation at 3.52 ppm ,this proton showed HMBC correlations at 63.76 ppm (C8) and 68.36 ppm (C3), and finally the H6 proton's position had been detected by its HSQC Correlation at 3.55 ppm and it's HMBC correlations with C7 at 64.38 ppm. The structure elucidation was based on (^{1}HNMR, ^{13}CNMR, APT, MASS, HMQC AND HMBC) data confirming this compound is **{**2 Amino, 2 Methyl, (5,6 di hydroxymethyl), 1,4 Dioxane **}**.

It was isolated for the first time from nature .Also it was noting in literature that dioxane dione was previously isolated from nature [48].

The ethyl acetate showed weak antiviral activity ,*n*-butanol extracts of *Dactyloctenium aegyptium* showed moderate antiviral effects against HAV-10 and and HSV-1. The *n*-hexane extract showed strong antiviral activity against all viruses tested (Table 1). The ethyl acetate and *n*-butanol extracts of *Dactyloctenium aegyptium* demonstrated variable antimicrobial activity against most of the specific organisms tested (Table 2). 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 *Dactyloctenium aegyptium* were the most active extracts as cytotoxic agents against the tested cell lines with values of IC_{50} from 6.1 to 9.6µg/ml compared to that of *n*-butanol (Table 3).

Table 1: Antiviral activity of *Dactyloctenium aegyptium* (L). using CPE inhibition assay

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

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

Table 2: Antimicrobial activity of *Dactyloctenium aegyptium* (L). using agar diffusion method

Organisms	**Diameter of inhibition zone (mm)**			
	n-hexane	Ethyl acetate	*n*-butanol	Standards
Fungi				Amphotericin
Aspergillus fumigatus (RCMB 02564	NA	16.2±0.44	NA	22.9±0.44

Table 3: Summarizes the cytotoxic activity of the extract obtained from *Dactyloctenium aegyptium* (L). using vinblastine as standard reference

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	20.97	31.89	5.41	8.65	23.72	4.73	37.42	57.89	14.98
50	34.18	45.78	8.97	15.81	36.94	8.08	56.89	71.94	37.84
25	47.52	58.62	12.54	31.86	52.83	11.93	70.61	88.42	69.51
12.5	73.06	71.83	23.33	42.57	68.17	20.41	82.98	91.23	85.72
6.25	87.42	87.91	47.68	63.07	84.54	27.68	90.35	96.86	92.34
3.125	92.83	92.48	65.86	75.22	91.81	38.52	96.17	99.17	98.19
1.56	95.47	98.05	73.91	84.31	96.97	51.27	98.96	100	100
0.78	98.91	100	82.16	91.54	98.46	69.74	100	100	100
0	100	100	100	100	100	100	100	100	100
IC_{50}	23.8 µg	41.8 µg	5.85 µg	10.2 µg	29.5 µg	1.72 µg	67.5 µg	50 µg	40.4 µg

Table 4: Evaluation of cytotoxicity of ethyl acetate, n-butanol and hexane fractions from *Dactyloctenium aegyptium* against MCF-7, HCT-116 and HepG-2 cell lines

Sample code		Ethyl acetate fraction			*n*-butanol fraction				Hexane fraction			
	Cell line	MCF-7	HCT-116	HepG - 2	Cell line	MCF-7	HCT-116	HepG-2	Cell line	MCF-7	HCT-116	HepG-2
	Sample conc.(µg)	1.72	29.5	10.2	Sample conc.(µ)	40.4	50	67.5	Sample conc.(µg)	5.85	41.8	23.8

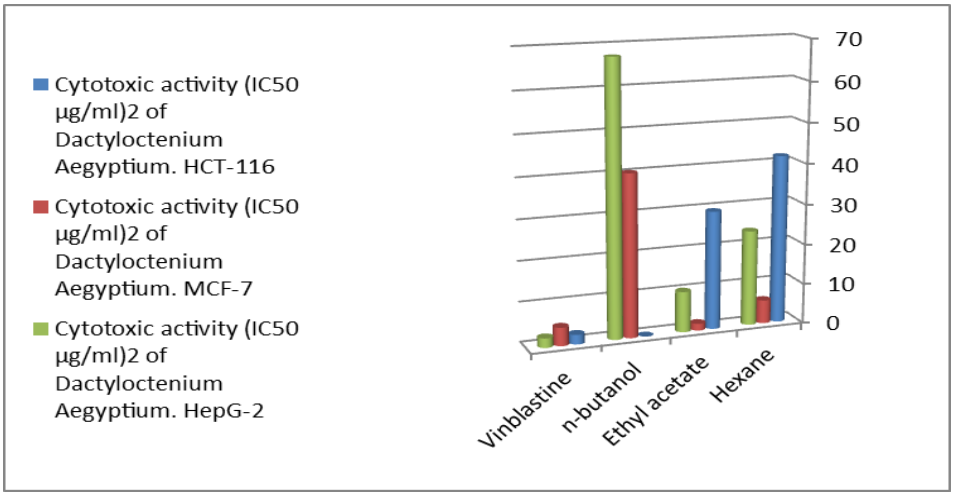

Figure 2: Cytotoxicity of different fractions from *Dactyloctenium aegyptium* against MCF-7, HCT-116 and HepG-2 cell lines

This study provides an evidence for the strong cytotoxic activity of the ethyl acetate and *n*-hexane extracts of *Dactyloctenium aegyptium* (Table 3). In addition to the highest antimicrobial activity of the ethyl acetate extract of the plant against *C. albicans* and *E. coli* (Table 2) that could be considered a valuable medicinal plant species. The higher activities of the ethyl acetate extract may due to the flavonoid contents which were reported previously. Additional studies are needed to identify the constituents of *n*-hexane extract that are responsible for its higher activity. Evaluation of cytotoxicity of ethyl acetate, n-butanol and hexane fractions from *Dactyloctenium aegyptium* against MCF-7, HCT-116 and HepG-2 cell lines showed in Table 4 and Figure 2.

CONCLUSION

Dactyloctenium aegyptium (L). wild herb afford 12 compounds for the first time from the plant as no reported data was published on it. Comp(7) was isolated for the first time from the poaceae family, Comp(6), (8) and (11) were isolated for the first time from nature, Comp (9) and (10) only isolated from only one plant all over the family was named as *Hygroryza aristata* [49], while the remaining compounds were isolated for the first time from this species the biological study provides an evidence for the strong cytotoxic and antimicrobial activity of ethyl acetate extract of *Dactyloctenium aegyptium.* In addition to the highest antiviral activity of hexane extract and moderate antiviral activity of n-butanol extract and from the previous results this is plant could be considered a valuable medicinal plant species. So, additional studies are needed to identify the constituents of these extracts.

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