



Phytochemistry and Cytotoxicity of *Asphodelus aestivus* Brot., Growing in Libya

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Abstract

Background: *Aesphodelus aestivus* Brot. (Family: Asphodelaceae) is a Libyan medicinal plant that has been used in traditional medicine for treating various human ailments, especially inflammatory conditions, burns and wounds. The cytotoxic activities of the *n*-hexane, dichloromethane (DCM), and methanol (MeOH) extracts from the leaves and tubers of this species were tested against five human cancer cell lines.

Methods: The MTT assay was used to test cytotoxicity. Extracts from leaves and tubers were evaluated for their effects on cancer cell viability. The selectivity index (SI) was calculated using human normal prostate cells (PNT2). Seven compounds, including flavonoids and anthraquinones, were isolated and structurally characterized using different chromatographic (vacuum liquid chromatography (VLC) and preparative HPLC) and spectroscopic (UV, NMR and MS) techniques. The isolated compounds **2**, **4**, **5**, and **7** were tested for cytotoxicity against the prostate cancer (PC3) cell line.

Results: Tubers exhibited higher cytotoxicity than leaves. The DCM tuber extract showed potent activity against A549 and PC3 cell lines with IC₅₀ values of 16 and 19 µg/mL, respectively. The MeOH and *n*-hexane extracts demonstrated no cytotoxicity in the tested cell lines. Leaves exhibited moderate cytotoxicity against HepG2 and A549, with IC₅₀ values of 70 and 90 µg/mL, respectively. The tuber extract showed a high selectivity index (SI = 26) for PC3 cells over normal prostate cells. Seven compounds were isolated from the various fractions of *A. aestivus*, two flavonoids, quercetin 7-*O*-rhamnoside (**1**) and luteolin (**2**), a ferulate derivative, *p*-hydroxy-phenethyl *trans*-ferulate (**3**), and four anthraquinones, chrysophanol anthrone (**4**), chrysophanol-10,10'-bianthrone (**5**), aloe-emodin (**6**) and C- α -rhamnopyranosyl bianthracene-9, 9', 10 (10'H)-trione glycoside (**7**). Compounds **1**, **3**, **4**, **5** and **7** were isolated for the first time from *A. aestivus*. Among isolated compounds, the bianthracene-trione (compound **7**) displayed significant cytotoxicity against PC3 (IC₅₀ = 62.0 µM), comparable to the reference drug paclitaxel (IC₅₀ = 57.9 µM).

Conclusion: The DCM extract of *A. aestivus* tubers demonstrated potent and selective cytotoxic activity, particularly against lung and prostate cancer cell lines. Compound **7**, a bianthracene-trione, exhibited promising activity comparable to paclitaxel. These findings highlight the therapeutic potential of *A. aestivus* for cancer treatment.

Introduction

Aesphodelus aestivus Brot., also known as White Asphodel or Gamon, belongs to the Asphodelaceae family, and is a common spring-flowering geophyte that is widely distributed across the Southern Alps to the western Balkans, as well as in meadows and heathland in central

Spain.^{1,2} It is also found in Africa, primarily in Libyan territory. Traditionally, *A. aestivus* has been used for both culinary and medicinal purposes. The tubers and roots of the plant have been used to treat conditions such as hemorrhoids, nephritis, burns, wounds, and some skin diseases.¹ They are also used in the eastern Mediterranean

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region of Libya to treat arthritis, rheumatic diseases, and skin diseases.²

Previous studies on the tubers of *Asphodelus* species have shown the presence of anthranoides, flavonoids, and triterpenes.³ However, a survey by Çalıř *et al.*⁴ on the fresh leaves of *A. aestivus* found additional compounds including flavone C-glycosides, anthranoides, adenosine nucleoside, phenylalanine and tryptophan, and chlorogenic acid.

The cytotoxic effects of *A. aestivus* extracts on MCF-7 breast cancer cells were studied using trypan blue exclusion assay, comet assay, and Hoechst propidium iodide double staining.¹ The study found that the cytotoxic and apoptotic effects of the extracts varied depending on the type of extract used. Previous research has shown that the key anthraquinones in rhubarb, such as emodin, aloe-emodin, and rhein, can suppress the growth and spread of various cancer cells.⁵

The literature survey showed limited cytotoxic studies on the Libyan plant *A. aestivus*. Therefore, in this study, we report on the cytotoxicity of the extracts, fractions and isolated compounds from both the leaves and tubers of *A. aestivus* growing in Libya against five human cancer cell lines: breast cancer (MCF7), liver hepatocellular carcinoma (HEPG2), lung cancer (A549), prostate cancer (PC3) and urinary bladder cancer (EJ-138).

Methods

Plant materials

A. aestivus leaves and tubers were collected from Tarhona city, Libya, in 2014. The plant specimens were identified based on the Flora of Libya at the Faculty of Science Herbarium, University of Tripoli-Libya, and a sample with voucher number D6857302 was deposited there. The leaves and tubers were air-dried, ground into powder, and stored in tightly closed amber containers.

Extraction

The leaves (150 g) and tubers (159.26 g) of *A. aestivus* were extracted using a Soxhlet apparatus successively with *n*-hexane, DCM, and MeOH, 800 mL each, in a ten-cycle process at temperatures close to the boiling point of each solvent. All extracts were filtered and evaporated to dryness in a rotary evaporator (Cole-Parmer) and stored at 4°C.

Fractionation techniques

Vacuum liquid chromatography (VLC)

The DCM crude extract of *A. aestivus* tubers (AETD) was separated into various fractions using vacuum liquid chromatography (VLC) on silica gel 60 GF 254 (Merck).⁶ The VLC was applied two times using two different mobile systems; Firstly, VLC1 (1.855 g) eluting with *n*-hexane-ethyl acetate mixtures of increasing polarity to yield seven fractions: F1: 2.4 mg, F2: 2.2 mg, F3: 10.8 mg, F4: 10.0 mg, F5: 160.0 mg, F6: 1272.3 mg, F7: 110 mg. Secondly, VLC2 (200 mg) starting elution with 100% *n*-hexane, 50-100% DCM/*n* in hexane, then increasing polarity with MeOH to yield seven fractions: F1: 4.8 mg, F2: 2.7 mg, F3: 3.0 mg, F4: 140 mg, F5: 11.0 mg, F6: 4.0 mg and F7: 9.8 mg.

Solid-phase extraction (SPE)

A portion of the dried MeOH extract of *A. aestivus* leaves (AELM) (1.8 g) was re-suspended in 10% MeOH in water and subjected to solid-phase extraction (SPE) using a cartridge (20 g) pre-packed with reversed-phase silica C₁₈ (ODS). A step gradient was applied starting with 20% MeOH in water, then 50%, 80% and 100% MeOH in water (200 mL each).⁶ Four fractions were collected: F1: 1043.3 mg, F2: 51.2 mg, F3: 35.3 mg and F4: 44.3 mg. All fractions were dried using a rotary evaporator and a freeze-dryer.

Isolation of compounds from *A. aestivus*

The MeOH extract of *A. aestivus* leaves AELM

Isolation of compounds from the SPE fractions 2, 3 and 4 of AELM utilised semi-preparative HPLC technique (Agilent) employing a 30-100% gradient of MeOH in water for 30 min with a volume of injection of 200 µL and a flow rate of 2 mL/min. Two flavonoids were isolated: quercetin 7-*O*-rhamnoside (**1**, 2.9 mg, $t_R = 10.3$ min) from SPE fraction 2, luteolin (**2**, 5.7 mg, $t_R = 22$ min) from SPE fraction 3, and (**2**, 1.8 mg, $t_R = 19.5$ min) from SPE fraction 4.

The MeOH extract of *A. aestivus* tubers AETM

The total MeOH extract (AETM) (1.33 g) was fractionated every 5 min by preparative reversed-phase HPLC using a gradient solvent system 30-100% MeOH in water and a flow rate of 10 mL/min. Six fractions [F1= 14 mg (0-5 min), F2= 12.3 mg (5-10 min), F3 = 80.3 mg (10-15 min), F4 = 53.6 mg (15-20 min), F5 = 24.2 mg (20-25 min), F6 = 69 mg (25-31.8 min)] were obtained and allowed to dry.

HPLC-MS screening of AETM fraction 4

AETM fraction 4 exhibited the most interesting ¹H NMR spectrum and HPLC (10-100% ACN/water gradient) chromatogram, so it was analysed by HPLC-MS to detect the molecular weights of the major metabolites, and thus to tentatively identify aloe-emodin 271 [M+H]⁺ and aloe-emodin acetate 312 [M+H]⁺, chrysophanol (peak) 255 [M+H]⁺, chrysophanol-10,10'-bianthrone 479 [M+H]⁺, and *p*-hydroxy transferulate 315 [M+H]⁺.

The DCM extract of *A. aestivus* leaves AELD

The total DCM extract (AELDM) (500 mg) was separated by preparative reversed-phase HPLC using a gradient solvent system 30-100% ACN in water and a flow rate of 10 mL/min. This led to the isolation of *p*-hydroxy-phenethyl *trans*-ferulate (**3**, 1 mg, $t_R = 25.2$ min) and aloe-emodin anthrone (**6**, 1.4 mg, $t_R = 35.3$ min).

The DCM extract of *A. aestivus* tubers AETD

Two pure compounds were completely crystallized from the VLC fractions 4 (VLC 1) and 3 (VLC 2) as follows: chrysophanol-10, 10'-bianthrone (**5**, 10.8 mg, $R_f = 9.5$) and chrysophanol anthrone (**4**, 3 mg, $R_f = 9.0$).

Separation of the active DCM VLC (2) Fraction 4

The active DCM fraction 4 purified from VLC (2) (140 mg) of *A. aestivus* tubers was isolated by prep-HPLC using

a gradient solvent system 50-100% ACN in water. This afforded two major compounds: C- α -rhamnopyranosyl bianthracene-9, 9', 10 (10'H)-trione glycoside (7, 1.2 mg, $t_R = 23.1$ min) and chrysophanol-10, 10'-bianthrone (5, 2 mg, $t_R = 30.2$ min).

MTT assay

The cytotoxicity of the *n*-hexane, DCM and MeOH extracts of *A. aestivus* leaves and tubers were evaluated against various human cancer cell lines, such as breast cancer (MCF7), liver hepatocellular carcinoma (HEPG2), lung cancer (A549), prostate cancer (PC3) and urinary bladder cancer (EJ-138), using the MTT assay.¹⁵ The cytotoxicity of the fractions from these extracts and isolated compounds were also evaluated against the PC3 cell lines. The cells were seeded into 24-well plates (5×10^4 cells/well) and incubated under 5% CO₂ and 95% humidity at 37°C for 24 h. The different extracts, fractions or isolated compounds were diluted in a medium containing DMSO (0.01% (v/v) (including the negative control), to achieve extract concentrations of (0, 0.8, 4, 20, 100 and 500 $\mu\text{g/mL}$) or isolated fraction concentrations of (0, 0.4, 2, 10, 50 and 250 $\mu\text{g/mL}$). These doses were then used to treat the cells for 24 h before assessment using the MTT assay following the standard protocol. Briefly, each treatment was removed from the 24-well plates of cells and replaced with MTT solution [0.5 mg/mL MTT in medium (1 mL/well)]. The cells were then incubated under 5% CO₂ and 95% humidity at 37°C for 2 h. The MTT solution was then removed from each well of cells and replaced with isopropanol (0.5 mL/well) to lyse the cells and to release and solubilise the blue formazan product. The absorbance reading (540 nm) of lysates from treated cells in each well on each occasion was recorded and was expressed as a percentage of the mean value of the control absorbance on each occasion. The results for each treatment and dose were derived from at least 12 wells ($n \geq 12$) from three or more separate occasions. To determine IC₅₀ values, data were presented in EXCEL graphs with the dose value ($\mu\text{g/mL}$) on the X-axis on a log scale and the % viability compared to control on the Y-axis. The IC₅₀ values are determined from the trend line of the data points.

Statistical analysis

All experiments were carried out in triplicate on separate occasions. Data were expressed as means with a \pm standard error of the mean. One-way ANOVA of multiple comparisons was applied (between control and different concentrations) using Dunnett's multiple comparison tests (GraphPad Prism version 7.04). The differences between groups were considered statistically non-significant (ns) at a P value ≥ 0.05 , significant (*) at a P value < 0.05 (0.01-0.05), very significant (**) at a P value < 0.01 (0.001 to 0.01), highly significant at a P value < 0.0001 (0.0001-0.001). The results were mean values \pm standard error of the mean derived from $n \geq 12$ on three separate occasions.

The isolated compounds from *A. aestivus*

Quercetin 7-O-rhamnoside (1)

Amorphous white powder, UV (MeOH) λ_{max} : 272, 357

nm, the ESIMS m/z 447 [M-H]⁻, corresponding to the molecular formula C₂₁H₂₀O₁₁ (Figure 1). ¹³C NMR (75 MHz, CD₃OD) 149.7 (C-2), 130.1 (C-3), 182.6 (C-4), 160.6 (C-5), 100.3 (C-6), 164.9 (C-7), 93.8 (C-8), 157.4 (C-9), 102.6 (C-10), 122.2 (C-1'), 112.8 (C-2'), 145.9 (C-3'), 145.6 (C-4'), 115.4 (C-5'), 118.9 (C-6'), 100.3 (C-1'' anomeric carbon of sugar); 61.4 (C-2''), 70.4 (C-3''), 71.2 (C-4''), 73.9 (C-5'') and 39.0 (-CH₃). All data were comparable with the published data.⁷

Luteolin (2)

Yellow crystals, UV (MeOH) λ_{max} : 256, 270 (sh) and 348 nm, the ESIMS m/z 287 [M+H]⁺, corresponding to the molecular formula C₁₅H₁₀O₆ (Figure 1). The ¹H NMR (600 MHz, CD₃OD) data showed signals at δ 6.56 (s, 1H, H-3), 6.46 (d, J = 2.0 Hz, 1H, H-6), 6.23 (d, J = 2.0 Hz, 1H, H-8), 7.42 (d, J = 2.4 Hz, 1H, H-2'), 6.92 (d, J = 8.4 Hz, 1H, H-5'), and 7.41 (dd, J = 8.4, 2.4 Hz, 1H, H-6') and ¹³C NMR (150 MHz, CD₃OD) data showed peaks at δ 165.0 (C-2), 102.5 (C-3), 182.5 (C-4), 161.8 (C-5), 98.8 (C-6), 164.7 (C-7), 93.6 (C-8), 158.0 (C-9), 103.9 (C-10), 119.8 (C-1'), 112.8 (C-2'), 145.7 (C-3'), 149.6 (C-4'), 115.4 (C-5'), and 122.3 (C-6'). All data were comparable with the published data.^{8,9}

p-Hydroxy-phenethyl trans-ferulate (3)

White amorphous powder, UV (MeOH) λ_{max} : 318 nm, the ESIMS m/z 315 [M+H]⁺, significant peaks at m/z 193 [M+H]⁺ *p*-hydroxy phenethyl moiety and 120 [M+H]⁺ feruloyl moiety indicating that the molecular formula could be C₁₈H₁₈O₅ (Figure 1). The ¹H NMR (600 MHz, CD₃OD) data showed signals at δ 7.14 (d, J = 1.7 Hz, 1H, H-2), 6.80 (d, J = 8.2 Hz, 1H, H-5), 7.04 (dd, J = 8.2, 1.7 Hz, 1H, H-6), 7.45 (d, J = 15.7 Hz, 1H, H-7), 6.42 (d, J = 15.6 Hz, 1H, H-8), 7.07 (d, J = 8.0 Hz, 2H, H-2' and H-6'), 6.74 (d, J = 8.5 Hz, 2H, H-3' and H-5'), 3.48 (t, J = 7.3 Hz, 2H, H-1''), 2.77 (t, J = 7.6 Hz, 2H, H-2''), and 3.91 (s, 3H, -OCH₃) and ¹³C NMR (150 MHz, CD₃OD) data showed peaks at δ 131.5 (C-1), 111.7 (C-2), 149.4 (C-3), 150.1 (C-4), 116.4 (C-5), 123.4 (C-6), 142.1 (C-7), 118.9 (C-8), 169.5 (C-9), 130.9 (C-2' and C-6'), 157.4 (C-4'), 115.4 (C-3' and C-5'), 42.7 (C-1''), 36.0 (C-2''), and 56.6 (-OCH₃). All data were comparable with the published data.¹⁰

Chrysophanol anthrone (4)

Orange red crystals, the ESIMS m/z 255 [M+H]⁺, corresponding to the molecular formula C₁₅H₁₀O₄ (Figure 1) The ¹H NMR (600 MHz, CDCl₃) data showed chemical shifts at δ 7.10 (s, 1H, H-2), 7.69 (s, 1H, H-4), 7.67 (d, J = 7.4 Hz, 1H, H-5), 7.83 (dd, J = 1.2, 7.5 Hz, 1H, H-6), 7.30 (dd, J = 1.14, 8.3 Hz, 1H, H-7), 2.47 (s, 3H, 3-OCH₃), 12.00 (s, 1H, 8-OH), and 12.10 (s, 1H, 1-OH). The DEPT data (150 MHz, CDCl₃) showed signals at δ 162.8 (C-1), 121.4 (C-2), 149.3 (C-3), 120.0 (C-4), 119.9 (C-5), 137.0 (C-6), 124.6 (C-7), 162.8 (C-8), 193.5 (C-9), 179.5 (C-10), and 22.3 (3-CH₃). All data were consistent with published reports and confirmed the structure of Chrysophanol anthrone (4).¹¹

Chrysophanol-10, 10'-bianthrone (5)

Brownish red crystals, the ESIMS m/z 477 [M+H]⁺, confirmed the molecular formula C₃₀H₂₂O₆ (Figure 1). The

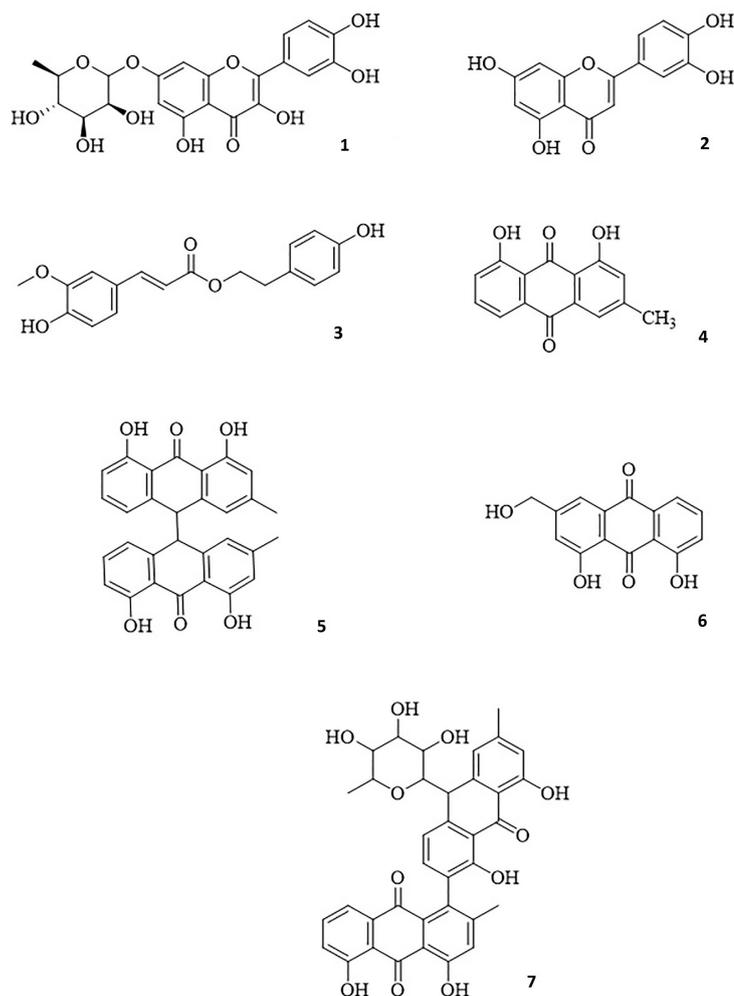


Figure 1. Structure of isolated compounds from the Libyan plant *Aesphodelus aestivus*.

^1H NMR (600 MHz, CDCl_3) data revealed signals at δ 6.70 (s, 1H, H-2 and H-2'), 7.10 (s, 1H, H-4 and H-4'), 7.67 (d, $J = 7.4$ Hz, 1H, H-5 and H-5'), 7.83 (dd, $J = 1.2, 7.5$ Hz, 1H, H-6 and H-6'), 7.29 (dd, $J = 1.14, 8.3$ Hz, 1H, H-7 and H-7'), 6.42 (d, $J = 15.6$ Hz, 1H, H-8 and H-8'), 4.41 (s, 1H, H-10 and H-10'), 1.27 (s, 3H, 3-CH₃ and 3'-CH₃), 12.00 (s, 1H, 8-OH), and 12.20 (s, 1H, 1-OH) and DEPT NMR (150 MHz, CDCl_3) showed signals at δ 162.4 (C-1 and C-1'), 116.0 (C-2 and C-2'), 148.0 (C-3 and C-3'), 120.0 (C-4 and C-4'), 120.8 (C-5 and C-5'), 137.0 (C-6 and C-6'), 115.5 (C-7 and C-7'), 162.7 (C-8 and C-8'), 193.6 (C-9 and C-9'), 56.4 (C-10 and C-10'), and 22.3 (C-3-CH₃ and C-3'-CH₃). All data were consistent with the published literature and confirmed the structure of Chrysophanol-10,10'-bianthrone (5).¹²

Aloe-emodin anthrone (6)

Orange powder, the ESIMS m/z 273 $[\text{M}+\text{H}]^+$, corresponded to the molecular formula $\text{C}_{15}\text{H}_{10}\text{O}_5$ (Figure 1) The ^1H NMR (600 MHz, CD_3OD) data showed δ 7.35 (d, $J = 1.02$ Hz, 1H, H-1), 7.34 (d, $J = 1.02$ Hz, 1H, H-3), 7.82 (br s, 1H, H-6), 7.77 (t, 1H, H-7), 7.82 (br s, 1H, H-8) and the ^{13}C NMR (150 MHz, CD_3OD) revealed δ 121.0 (C-1), 155.0 (C-2), 124.0 (C-3), 164.9 (C-4), 166.4 (C-5), 124.0 (C-6), 136.9

(C-7), 117.2 (C-8), 182.3 (C-9), 187.5 (C-10). All data were comparable with the published data.¹³

C- α -Rhamnopyranosyl bi-anthrone-9, 9', 10 (10'H)-trione glycoside (7)

Brownish-yellow powder, UV (MeOH) λ_{max} : 224 nm, 256, 368 and 440 nm. The ESIMS m/z 637 $[\text{M}-\text{H}]^+$, corresponded to the molecular formula $\text{C}_{36}\text{H}_{30}\text{O}_{11}$ (Figure 1) The ^1H NMR (600 MHz, CD_3OD) data showed δ 7.34 (s, H-2), 7.27 (dd, $J = 7.0, 10.0$ Hz, H-5), 7.67 (t, $J = 8.0$ Hz, H-6), 7.58 (d, $J = 8.0$ Hz, H-7), 2.44 (s, H-11), 6.75 (s, H-2), 7.27 (dd, $J = 7.0, 10.0$ Hz, H-5), 7.26 (d, $J = 9.0$ Hz, H-6), 6.94 (s, H-4), 4.58 (H-10), 2.15 (s, H-11), 3.96 (dd, $J = 3.1, 8.8$ Hz, H-2), 4.04 (dd, $J = 3.4, 8.5$ Hz, H-3), 3.91 (t, $J = 2.9$ Hz, H-4), 3.39 (dd, $J = 2.4, 6.2$ Hz, H-5), 1.07 (d, $J = 6.5$ Hz, H-6''-12) and the ^{13}C NMR (150 MHz, CD_3OD) data revealed peaks at δ 125.6 (C-2), 151.5 (C-3), 129.3 (C-4), 124.5 (C-5), 136.8 (C-6), 121.6 (C-7), 163.0 (C-8), 196.2 (C-9), 184.3 (C-10), 20.8 (C-11), 162.4 (C-1), 115.5 (C-2), 149.3 (C-3), 120.5 (C-4), 115.5 (C-5), 134.5 (C-6), 134.3 (C-7), 160.7 (C-8), 194.9 (C-9), 45.2 (C-10), 20.0 (C-11'), 80.1 (C-2), 47.5 (C-5). All data were comparable with the published data.¹⁴

Table 1. The IC₅₀ (µg/mL) of *n*-hexane, dichloromethane and Methanol extracts of both *A. aestivus* leaves and tubers on the selection of five human cancer cell lines using the MTT assay.

Cell type	IC ₅₀ values (µg/mL) (AEL)				IC ₅₀ values c(µg/mL) (AET)	
	AELH	AELD	AELM	AETH	AETD	AETM
EJ138	> 100	> 100	> 100	> 100	> 100	> 100
Hep G2	> 100	70 ± 0.62	> 100	> 100	> 100	> 100
A549	> 100	90 ± 1.25	> 100	> 100	16 ± 0.76	> 100
MCF7	> 100	> 100	> 100	> 100	> 100	> 100
PC3	> 100	> 100	> 100	80 ± 0.94	19 ± 1.04	> 100

Values greater than 100 µg/mL were considered as non-cytotoxic.¹⁶

The results were mean values ± standard error of the mean derived from n ≥ 12 from three separate occasions.

AELH: *A. aestivus* leaves hexane extract. AELD: *A. aestivus* leaves dichloromethane extract. AELM: *A. aestivus* leaves methanol extract. AETH: *A. aestivus* tubers hexane extract. AETD: *A. aestivus* tubers dichloromethane extract. AETM: *A. aestivus* tubers methanol extract.

Results and Discussion

Previous research on various species of the Asphodelaceae family revealed the presence of flavonoids (luteolin, isovitexin, and isoorientin), phenolic acids, and some anthraquinones, mainly in the aerial parts of these plants. On the other hand, the roots have been found to contain anthraquinone derivatives like aloe-emodin, chrysophanol, and triterpenoids.¹⁷

This study tested the cytotoxicity of *A. aestivus* using the MTT assay on five human cancer cell lines (A549, EJ138, HepG2, MCF7 and PC3). Thus, this is the first time that the cytotoxicity of this plant has been evaluated on these cell lines, with only one previous report on its cytotoxicity against the breast cancer cell line MCF7¹. The results showed that the tuber extracts of *A. aestivus* had a higher cytotoxicity level than the leaves (Values greater than 100 µg/mL were considered as non-cytotoxic).¹⁷ The DCM tuber extract showed high cytotoxicity against the human lung carcinoma A549 and prostate cancer PC3 cell lines with IC₅₀ values of 16 and 19 µg/mL, respectively (Table 1), while the MeOH and *n*-hexane extracts showed no cytotoxicity. The leaves showed weak cytotoxicity against the human liver hepatocellular carcinoma HepG2 and human lung carcinoma A549 with IC₅₀ values of 70 and 90 µg/mL, respectively (Table 1). It is also of interest that the prostate cancer cells (PC3) were more sensitive to *A. aestivus* tubers than normal prostate cells (PNT2) with a high selective index (SI = 26) (Table 2) which indicates the safety of *A. aestivus* on normal cells. This selectivity may be due to the presence of anthraquinones as reported by Huang *et al.*⁵. The degree of selectivity of the extracts or compounds can be expressed by its selectivity index (SI) value. The selectivity index (SI) = The IC₅₀ (µg/mL) of extracts against the normal cells divided by the IC₅₀ (µg/mL) of extracts against the cancer cells. Selectivity value higher than 2 gives a selective toxicity towards cancer cells.^{18,19}

The cytotoxicity of the DCM fractions obtained from both VLC chromatographic fractions of *A. aestivus* tubers was tested against the most sensitive cancer cell line (human prostate cancer cell line PC3). The highest activity was found in the DCM F4 fraction obtained from VLC (2) with an IC₅₀ value of 21 µg/mL. The IC₅₀ values for the different DCM fractions of *A. aestivus* tubers on PC3 are listed in Tables 3 and 4.

Anthraquinones are bioactive compounds derived

Table 2. The IC₅₀ (µg/mL) values for *A. aestivus* tubers dichloromethane extract on PC3 and PNT2 cells and the selectivity index (SI) using the normal human prostate cells (PNT2).

Cell type	IC ₅₀ values (µg/mL) (AETD)
PC3	19 ± 1.04
PNT2	500 ± 0.81
Selectivity index	26

Selectivity index (SI) = The IC₅₀ (µg/mL) of extracts against the normal cells divided by the IC₅₀ (µg/mL) of extracts against the cancer cells, where IC₅₀ is the concentration required to kill 50% of the cell population.¹⁷

Table 3. The IC₅₀ (µg/mL) of *A. aestivus* tubers dichloromethane fractions from VLC (1) on PC3 (human prostate cancer cell line) using the MTT assay.

Fraction	F1	F2	F3	F4	F5+ F6+ F7
IC ₅₀ µg/mL	>100	>100	>100	>100	73 ± 0.52

All experiments were carried out in triplicate on separate occasions. Data were expressed as means ± standard error of the mean derived from n ≥ 12 from three separate occasions.

from plants such as Aloe, Asphodelus, Purslane, Rhubarb, and Senna.²⁰ They have been shown to have various biological properties such as antibacterial, antifungal, gastrointestinal protection, and renal protection. More recently, their potential as anticancer agents have gained interest, especially among specific anthraquinones like emodin, aloe-emodin, and rhein.⁵ These compounds exert their anti-cancer effects by targeting various molecular pathways and processes. They influence angiogenesis, apoptotic mechanisms, autophagy, and gene expression in synthesis, repair, and damage response. Furthermore, they regulate cell cycle processes, epithelial-mesenchymal transition (EMT) markers, epigenetic factors, heat shock responses, inflammation, oxidative stress, metastasis-related proteins, microRNAs, protein synthesis, and markers of proliferation or stem-like behaviour.

Advances in understanding the tumour microenvironment and the molecular mechanisms provide valuable opportunities to discover and develop new natural anthracene-based compounds. These findings not only enhance the repertoire of therapeutic options but also offer potential strategies for cancer prevention.²¹

In this study, four anthraquinones were isolated from *A. aestivus* plant; three were found in the tubers (obtained

Table 4. The IC₅₀ (µg/mL) of *A. aestivus* tubers dichloromethane fractions from VLC (2) on PC3 (human prostate cancer cell line) using the MTT assay.

Fraction	F1	F2	F3	F4	F5	F6	F7
IC ₅₀ µg/mL	>100	>100	>100	21 ± 1.56	98 ± 0.67	>100	>100

All experiments were carried out in triplicate on separate occasions.

Data were expressed as means ± standard error of the mean derived from n ≥ 12 from three separate occasions.

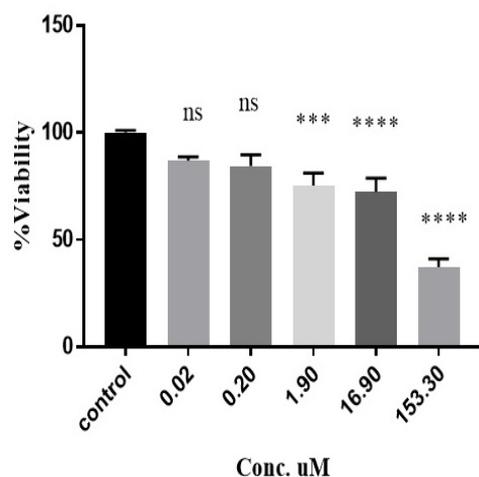
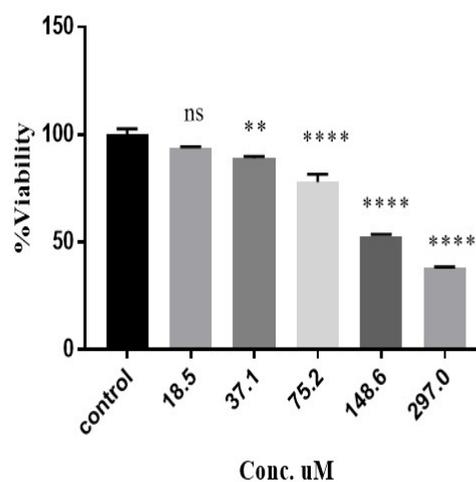
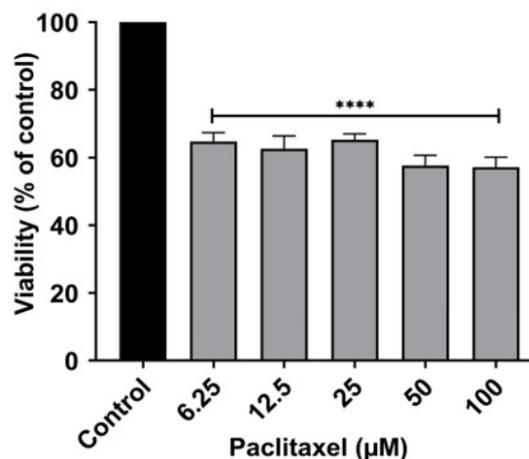
Table 5. The IC₅₀ (µM) of different isolated compounds from *A. aestivus* tubers and leaves on prostate (PC3) cancer cells using the MTT assay.

Pure compound	IC ₅₀ (µM)
Luteolin	201 ± 1.10
C-α-rhamnopyranosyl bianthracene-trione glycoside	62 ± 1.61
Chrysophanol anthrone	No activity*
Chrysophanol bianthrone	No activity*
Paclitaxel (Taxol)	57.86 ± 9.8

*No activity at the highest concentration of 500 µM

from VLC fractions 3 and 4) and included chrysophanol anthrone (4), chrysophanol-10,10'-bianthrone (5), and C-α-rhamnopyranosyl bianthracene-9,9',10 (10'H)-trione glycoside (7). The fourth anthraquinone was found in the leaves (obtained from DCM extract) and was aloemodin (6). This was the first report on the occurrence of compounds 4, 5, and 7 in *A. aestivus*¹⁶. However, compound 7 had previously been isolated from *A. ramosus* tubers¹⁴ and chrysophanol 1-O-gentiobioside was reported only in *A. aestivus*⁴ while chrysophanol anthrone (4) was isolated only in this study.

The three compounds 4, 5 and 7 isolated from the VLC tuber fractions of *A. aestivus* tubers and compound 2 from the leaves were tested against prostate cancer cells (PC3) (Table 5). The bianthracene-trione 7 showed good cytotoxicity with an IC₅₀ value of 62 µM (Figure 2), which is a new finding and might contribute to the high potency of the fraction (IC₅₀ value = 21 µg/mL). In contrast, the flavonoid luteolin 2 (Figure 3) showed weak cytotoxicity

**Figure 2.** The cytotoxic activity of C-α-rhamnopyranosyl bianthracene-9,9',10 (10'H)-trione glycoside (7) against the prostate cancer cells (PC3).**Figure 3.** The cytotoxic activity of luteolin (2) against the prostate cancer cell line (PC3).**Figure 4.** The cytotoxic activity of Paclitaxel (Taxol) against the prostate cancer cells (PC3).

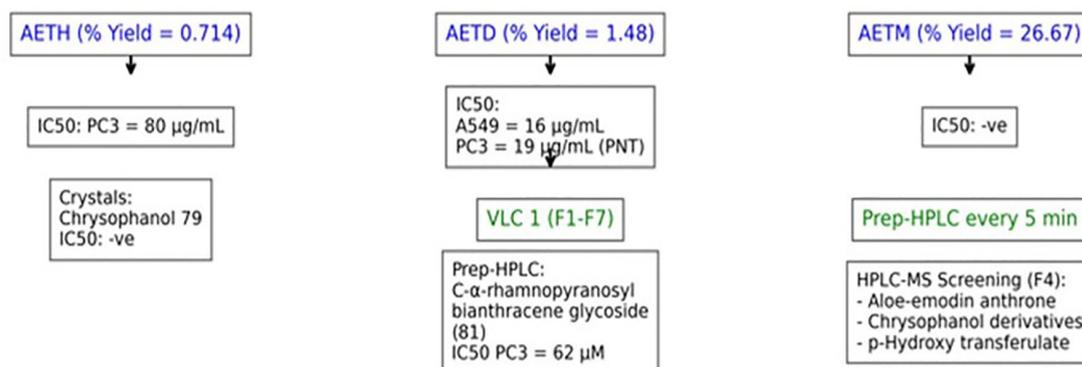


Figure 5. A summary of the extraction yield, isolation techniques, the purified compounds obtained from *A. aestivus* tubers and their IC_{50} s.

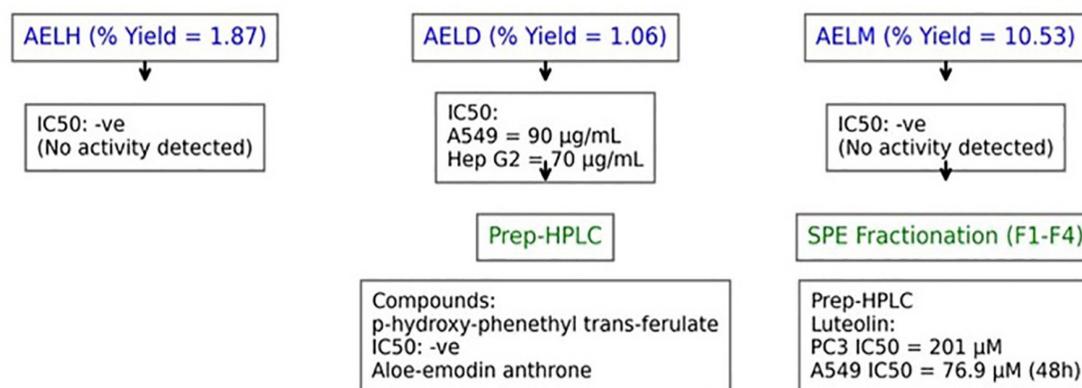


Figure 6. A summary of the extraction yields, isolation techniques, the purified compounds obtained from *A. aestivus* leaves and their IC_{50} s. AELH: *A. aestivus* leaves hexane extract. AELD: *A. aestivus* leaves dichloromethane extract. AELM: *A. aestivus* leaves methanol extract. AETH: *A. aestivus* tubers hexane extract. AETD: *A. aestivus* tubers dichloromethane extract. AETM: *A. aestivus* tubers methanol extract.

with IC_{50} value of 201.0 μ M. This was compared with the reference Paclitaxel (IC_{50} 57.9 μ M) (Figure 4).

However, compounds 4 and 5 did not show any cytotoxicity. A concise summary of the extraction yield, the methods used for isolation, the pure compounds isolated and their IC_{50} s are illustrated in Figures 4 and 5. Previous studies reported weak cytotoxic activity for chrysophanol (4), which has a similar chemical structure to the cytotoxic anthraquinones emodin and aloe-emodin. Chrysophanol has also been reported to have antiproliferative effects against mouse lymphocytic leukemia cells and human colon and breast cancer cells, but its effects on human leukemia cells were not consistent. It has also been previously reported that chrysophanol (4) has weak cytotoxic activity⁵, and it shares a very similar chemical structure with cytotoxic emodin and aloe-emodin anthraquinones.

In addition, chrysophanol has been reported as an antitumor agent acting against the growth and proliferation of breast cancer MCF-7 and MDA-MB-231 cells, lung cancer A549 cells and murine leukemic L1210 cells.²²

Another study revealed, the antiproliferative effects of anthraquinones including chrysophanol in human colon cancer cells (SW620).²³

Conclusion

The current study supports the traditional use of *A. aestivus* for the treatment of tumours. The results suggest that the cytotoxicity is higher in the tubers than in the leaves. The observed toxicity in the tubers was contributed to by various cytotoxic anthraquinones e.g., C- α -rhamnopyranosyl bianthracene-9,9',10 (10'H)-trione glycoside (7), while the cytotoxic activity of the leaves was due to the presence of cytotoxic flavonoids e.g., quercetin rhamnoside (1) and luteolin (2). However, further investigations on the mechanism of action of cytotoxic anthraquinone C- α -rhamnopyranosyl bianthracene-9,9',10 (10'H)-trione glycoside (7) are crucial for developing targeted cancer therapies. Additionally, Structure-activity relationship (SAR) studies could guide the development of more potent derivatives.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Data

Supplementary data (NMR spectra) are available at <https://doi.org/10.34172/PS.025.40814>.

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