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The cytotoxic activity of *Linum grandiflorum* leaves

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ABSTRACT

The $CHCl_3$ and MeOH fractions of the leaves of *Linum grandiflorum*, showed cytotoxic activity against EL_4 (Murine Leukemia) cell line with IC_{50} = 60 and 250 μg/mL respectively. Bioassay guided fractionation and isolation (BGFI) of the MeOH fraction resulted in the isolation of two new cytotoxic compounds **1** [luteolin 7-*O*-*α*-D-(6```-E-feruloyl)glucopyranosyl (1→2)-*β*-D-glucopyranoside] and **6** [2-[(3`-isopropoxy-*O*-*β*-D-glucopyranosyl)oxy]-2-methylbutane nitrile] against EL_4 with IC_{50} = 0.2 and 0.3 μM/mL respectively, together with **2** [luteolin 7-*O*-*β*-D-glucopyranoside], **3** [vicenin-1], **4** [vicenin-2], **5** [vicenin-3], **7** [linamarin], **8** [lotaustralin], **9** [neolinustatin], and **10** [butan-2-*O*-*β*-D-glucopyranoside] which showed cytotoxicity against EL_4 with IC_{50} = 0.2, 0.9, 0.8, 0.9, 0.4, 0.4, 0.2, 0.4 μM/mL respectively. BGFI of the $CHCl_3$ fraction revealed the isolation of three cytotoxic aryltetrahydronaphthalene-type lignans identified as **11** [podophyllotoxin], **12** [deoxypodophylotoxin] and **13** [5-methoxypodophyllotoxin] against EL_4 with IC_{50} = 0.2, 0.09 and 0.2 μM/mL respectively. The isolated compounds were structurally elucidated using 1D, 2D NMR, HR-ESI-MS, and HR-MALDI-MS techniques.

1. Introduction

It is well known that natural products have played an important role in the discovery of useful antitumor agents. Especially, clinically relevant anticancer drugs such as taxol, camptothecin, vinblastine and vincristine were discovered from higher plants. Nonetheless, as exemplified by the frequent morbidity and mortality associated with metastatic conditions, there still, clearly a need for the discovery of new agents with higher clinical efficacy [1]. The Medicinal herb *Linum grandiflorum* Desf., has been reported in the folk medicine for the treatment of various ailments such as laxative & expectorant, treatment of mental deficiencies in adults, gonorrhoea and relieves pain. The seeds exhibited analgesic, emollient, pectoral and resolving, while the flowers exhibited cardiotonic and nervine characterises. The crushed seed used for the treatment of ulceration and inflammations [2], and its mucilage was valuable for the remedy of coughs, cold and inflammation of urinary organs. Linum species have long history of folkloric-uses as antitumor agents for the treatment of different types of cancer such as inflamed tumours, glandular tumours.etc [3]. As part of our screening program for new anticancer agents from natural sources, the $CHCl_3$ and MeOH fractions of the leaves of *L. grandiflorum*, (Linaceae) were found to exhibit cytotoxic activity against EL_4 (Murine Leukemia) cell line. Bioassay-guided fractionation was undertaken using a standard high-flux anticancer-drug screening method which led to the isolation of five flavonoids, four cyanogenic glycosides and one alkyl glycoside from the MeOH fraction together with three aryltetrahydronaphthalene lignans isolated from the $CHCl_3$ fraction, which were all exhibited cytotoxic activity against EL_4 (Murine Leukemia) cell line.

2. Experimental

2.1. General

The Infra red (IR) spectra were obtained (KBr-DISK and/or FILM/NaCl) on a Perkin-Elmer 1720 Infrared Fourier Transform Spectrometer. Ultraviolet (UV) spectra were measured using SHIMADZU MPS-2000. ^1H, ^{13}C NMR, ^1H–^1H COSY, ^1H–^{13}C HETCOR, DEPT and DIFNOE spectra were obtained using a pulse sequence supplied from Varian VXR-Unity 300 and 200 MHz spectrometer (in DMSO-d_6). Chemical shifts were given in values (ppm) relative to trimethylsilane (TMS) as an internal reference. High-Resolution ESI-MS, using Nano-electrospray tandem (MS/MS) mass spectrometry on a hybrid quadrupole time-of-flight (Q-TOF) MS instrument equipped with Protana's Nano-ESI source for HRESI/MS and Nano-spray needles from Proxeon (Applied Biosystems/MDS Sciex) (QSTAR, prototype, PE-Sciex, Canada). Tandem (MS/MS) spectra were interpreted using the programs BioMultiView (PE Sciex, Canada) and GPMAW (Lighthouse Data, Denmark). Collision induced dissociation (CID) spectra were obtained using N_2 in the collision cell, collision energies between 30–40 eV (E_{lab}). ESI/MS first conducted in positive mode to obtain ionized molecular species, then tandem MS/MS spectra were obtained by CID of the $[M + H]^+$ ion [4,5]. The sequence ion notations have been used, e.g. $\mathbf{Y}^*$ ion corresponding to the loss of an internal dehydrated sugar residue. Subsequent charge-remote rearrangements take place resulting in the $\mathbf{Y_0}$ and $\mathbf{Y_1}$ ions [6,7]. High-Resolution MALDIMS spectrum was recorded on an IonSpec Fourier Transform Ion Cyclotron Resonance Mass Spectrometer. Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) Consists of L-6200 Intelligent

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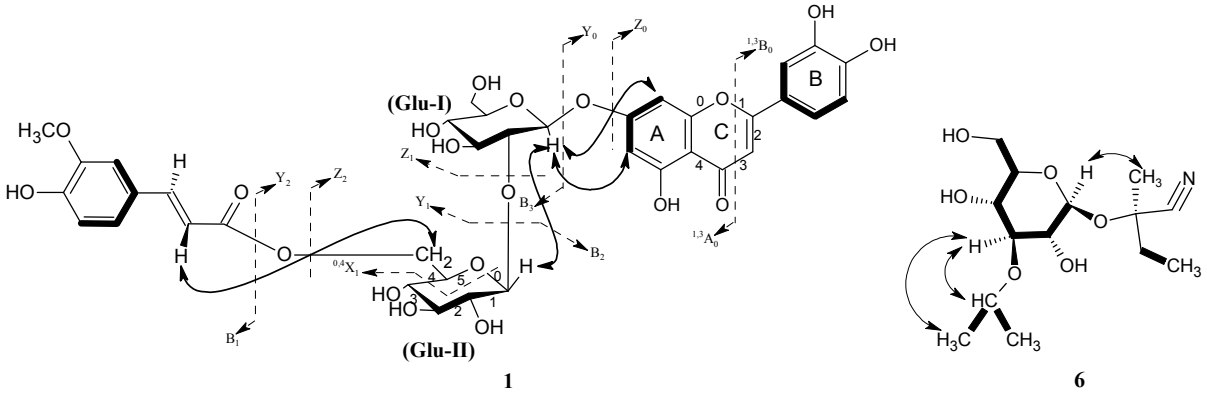

Figure 1. The ion nomenclature of **1** indicated by dotted arrows. NOEs are indicated by arrows (↔) and ^{1}H–^{1}H COSY correlations are indicated by bold bonds (**—**), for compounds **1** and **6**.

Pump (Merck-HITACHI) equipped with UV-VIS Detector SPD-10AV (SHIMADZU), the column used in HPLC separation is (20ϕ x 250 mm, Develosil ODS-HG-5, Nomura Chemicals). Samples of 8 mL volume each were injected into 10 mL loop (after prefiltration with Nylon Filter 0,45 μm). HPLC solvents used for all analyses were of grade M (Sigma-Aldrich chemie, UK) with ultra-pure water. All solvents used were of AR grade. TLC was carried out using 0.25 mm (Silica gel 60 F_{254}, Merck) and 0.5 mm (Kieselgel GF_{254}, Merck) for the analytical and preparative purposes, respectively.

2.2. Plant materials

The aerial parts (leaves and seeds) of *L. grandiflorum*, were collected in March 2006, from El-Orman garden – Giza – Egypt. It was kindly identified by Miss. Tressa Labib the Head of Specialists of Plant Taxonomy at the garden. A voucher specimen (No. 38) of the whole plant was deposited at the Herbarium of National Research Center (HNRC).

2.3. Extraction and isolation

The air-dried aerial parts [leaves (2.4 Kg) and seeds (184.46 g)] of *L. grandiflorum*, were extracted by Maceration process (5L x 5) followed by defatting with petroleum ether (1L x 3) and the residue dissolved in dist. H_2O and refractionated with $CHCl_3$ (2L x 3) to give (10.5 g) the residue which were concentrated till dryness and finally extracted with MeOH (3L x 3) to give (91.7 g). The MeOH fraction (15.5 g) was dissolved in MeOH–H_2O (100 mL, 1:1), filtered and subjected to fractionation using preparative ODS-HPLC at room temp, using solvent A = 1% HCOOH and Solvent B = 100% MeOH, with elution profile 90% A, 50% A, and finally with 100% B, monitoring at 340 nm, with flow rate 3 mL/min, accumulated three main fractions (**I, II**, and **III**), and subjected to TLC and UV examinations which revealed that fraction **II** (50% A) contains the main phenolics. Fraction **II** refractionated with prep. RP-HPLC at room temp, isocraticaly eluted with 60% A, monitoring at 280 nm, with 0.8 mL/min flow rate, resulted in the isolation of **1** (5 mg), **2** (12 mg), **3** (8 mg), **4** (15 mg), **5** (10 mg), **6** (10 mg), **7** (8 mg), **8** (12 mg), **9** (6 mg), and **10** (11 mg). The $CHCl_3$ fraction (10.5 g) was concentrated till dryness, then dissolved in CH_3CN–H_2O (100 mL, 1:1) and left over-night at r.t., then the supernatant was separated and examined with TLC for the presence of lignans, concentrated and refractionated using prep. RP-HPLC at room temp., isocraticaly eluted with MeOH-H_2O (20:80), monitoring at 280 nm, with 1.0 mL/min flow rate, resulted in the isolation of **11** (35 mg), **12** (44 mg) and **13** (13 mg).

2.3.1. Luteolin 7-O-α-D-(6```-E-feruloyl)glucopyranosyl (1→2)-β-D-glucopyranoside (1)

Pale yellow amorphous solid, with a molecular formula $C_{37}H_{38}O_{19}$ as determined by HR-ESI-MS (Positive mode) *m/z* = 809.1917 $[M+Na]^+$ (calcd. for $C_{37}H_{38}O_{19}Na$: 809.1905). UV λ_{max} (MeOH): 252, 269, 300(sh), 337; (NaOMe): 268, 390; ($AlCl_3$): 275, 300(sh), 325, 360(sh), 423; ($AlCl_3$/HCl): 277, 298(sh), 325, 385; (NaOAc): 265(sh), 269(sh), 335, 408; (NaOAc/H_3BO_3): 258, 296(sh), 335, 376. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 5.24 (1H, *d*, *J* = 7.32 Hz, H-1`` of Glc I), 3.58 (1H, br*d*, *J* = 8.0 Hz, H-2``), 3.78-3.90 (2H, *m*, H-6``), 5.18 (1H, *d*, *J* = 3.66 Hz, H-1``` of Glc II), 3.26 (1H, br*d*, *J* = 9.0 Hz, H-2```), 4.15-4.27 (2H, *m*, H-6```), 3.00-3.55 (6H, overlapped, H-3``, 4``, 5``, 3```, 4``` and 5```) 6.67 (1H, *s*, H-3), 6.46 (1H, *d*, *J* = 2.1 Hz, H-6), 6.74 (1H, *d*, *J* = 2.1 Hz, H-8), 7.41 (2H, br*d*, *J* = 8.5 Hz, H-2`, 6`), 6.95 (1H, *d*, *J* = 8.4 Hz, H-5`), 3.73 (3H, *s*, feruloyl-OMe), 7.12 (1H, *d*, *J* = 1.5 Hz, H-2````), 6.70 (1H, *d*, *J* = 8.1 Hz, H-5````), 6.89 (1H, br*d*, *J* = 8.1 Hz, H-6````), 6.37 (1H, *d*, *J* = 15.80 Hz, H-*α*), 7.45 (1H, *d*, *J* = 15.8 Hz, H-*β*); DIFNOE and COSY spectra: (see Figure 1); HRESIMS (Positive mode) *m/z* 809.1917 $[M+Na]^+$ (calcd for $C_{37}H_{38}O_{19}Na$, 809.1905) (see Figure 1 for the fragmentations). Acid hydrolysis of **1** by conc. H_2SO_4 released *α*-, *β*-glucose and luteolin, which were identified by PC and TLC as compared with the authentic samples. Alkaline hydrolysis of **1** gave ferulic acid which was identified by comparing with authentic sample [8].

2.3.2. 2-[(3`-isopropoxy-O-β-D-glucopyranosyl)oxy]-2-methylbutanenitrile (6)

Yellow amorphous; IR υ_{max} 3499, 2859, 2928, 2252, 1094, 1011 cm^{-1}; UV λ_{max} (MeOH): 274, 320 (sh); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.86 (3H, *t*, *J* = 7.61 Hz, CH_3-4), 1.14 (6H, *d*, *J* = 6.23 Hz, –CH=(2C*H*$_3$)), 1.27 (3H, *s*, CH_3), 1.46 (2H, *q*, *J* = 7.61 Hz, CH_2-3), 3.06 (1H, *dd*, *J* = 7.69 Hz, H-2`), 3.24 (1H, br*d*, *J* = 8.05 Hz, H-4`), 3.48 (1H, *m*, H-5`), 3.64 (2H, *m*, H-6`), 3.67 (1H, *sep*, *J* = 6.23 Hz, –C*H*=(2Me)), 4.29 (1H, *d*, *J* = 7.69 Hz, H-1`), 4.74 (1H, *t*, *J* = 9.16 Hz, H-3`); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ_C 121.5 (C≡N), 74.5 (C-2), 32.9 (C-3), 8.6 (C-4), 104.5 (C-1`), 74.6 (C-2`), 81.9 (C-3`), 71.9 (C-4`), 78.1 (C-5`), 62.6 (C-6`), 24.6 (CH_3-2), 73.8 (–*C*H=(2CH_3)), 22.4 (–CH=(2*C*H$_3$)); DIFNOE and COSY spectra: (see Figure 1); HRMALDIMS (Positive mode) *m/z* 326.1579 $[M+Na]^+$, (calcd for $C_{14}H_{25}NO_9Na$, 326.1575) *m/z* 303 $[M+H]^+$, *m/z* 284 $[M+Na-(:C(CH_3)_2)]^+$, *m/z* 611 $[2M+Na-H_2O]^+$.

2.4. Assay for cytotoxicity

The cell line EL_4 (Murine Lymphoma) was purchased from National Cancer Institute (NCI). A standard high-flux anticancer-drug screening method using Sulforhodamine B

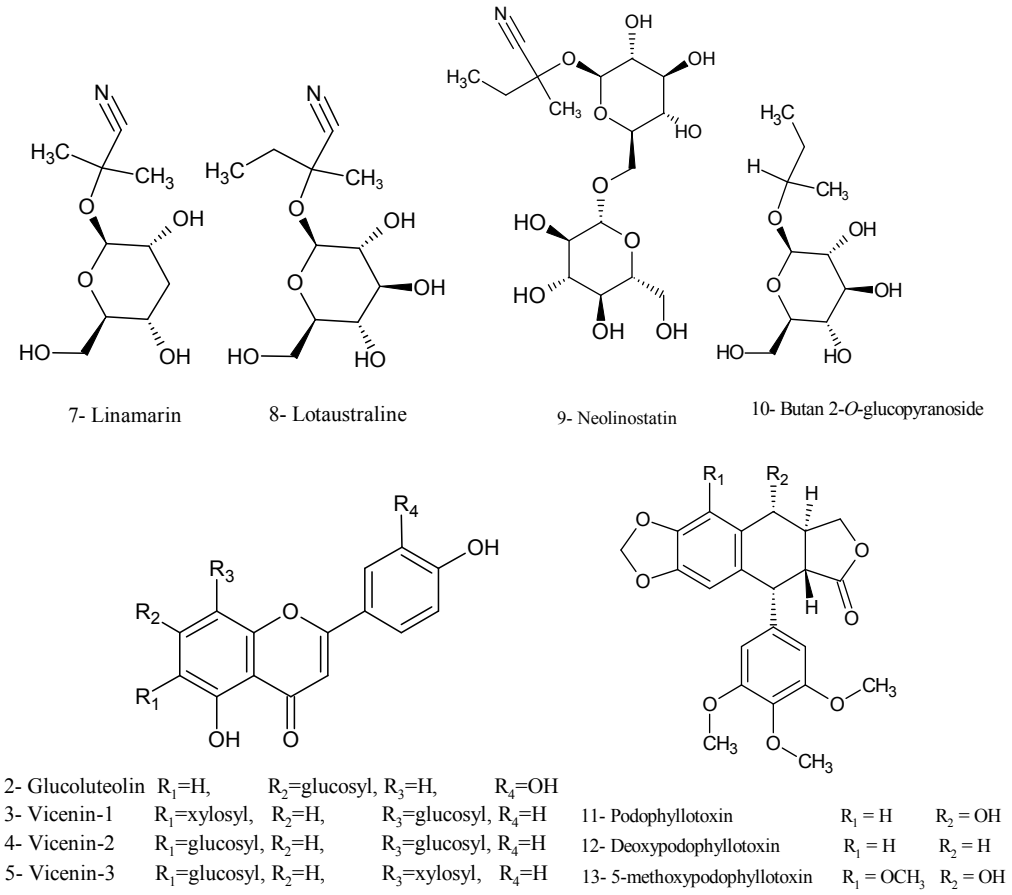

Figure 2. Structures of the isolated known compounds from *Linum grandiflorum*.

(SRB) assay, which permitted a simpler, faster, inexpensive and more sensitive than the MTT assay, provided better linearity with cell number, permitted the use of saturating dye concentrations, was less sensitive to environmental fluctuations, independent of intermediary metabolism, and provided a fixed end point that did not require a time-sensitive measurement of initial reaction velocity [9,10]. The method is suitable for ordinary laboratory purposes and for very large-scale applications, briefly; cancer cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the samples to allow attachment of the cell to the wall of the plate. Different concentrations of the samples under test (0, 1, 5, 10, 25, 50, 100, 250, 500, and 1000 μg/mL) were added to the cell monolayer, triplicate wells were prepared for each individual dose. The monolayer cells were incubated with the test samples at 37 ^{o}C for 48h in atmosphere of 5% CO_2. Cultures were fixed with trichloroacetic acid, then stained with sulforhodamine B and the colour intensity was measured at 490 nm by ELISA reader. All experiments were performed at least two times in triplicates. Thapsigargin (T-9033, >96%, Sigma) was used as positive potent cytotoxin with IC_{50} 1.9 ± 0.5 μM/mL. Data are given as IC_{50} (μM/mL) mean ± SEM from 4 different experiments [11].

3. Results and Discussion

For the screening of novel anti-leukemic agents with potent cytostatic activity and low cytotoxicity, this research was directed to the exploration of *L. grandiflorum*, which was not investigated before for its biological activity. A standard high-flux anticancer-drug screening method was used to evaluate the cytotoxicity of the MeOH and $CHCl_3$ fractions (Table 1), which revealed that both fractions have cytotoxic activity against EL_4 (Murine Leukemia) cell line with IC_{50} 60 and 250 μg/mL respectively, this prompts us to follow up the fractionation procedure in parallel with the bioassay activity of the isolated compounds; this resulted in the isolation of two novel compounds **1** and **6** (Figure 1), with IC_{50} 0.2, 0.3 μM/mL respectively, together with eight known compounds (Figure 2) structurally elucidated according to their spectral data in comparison with those in the literature and identified as **2** luteolin 7-*O*-β-D-glucoside [12], **3**–**5** vicenin-1, vicenin-2 and vicenin-3 [13], **7**–9 linamarin, lotaustralin and neolinustatin [14] and **10** butan-2-*O*-β-D-glucoside [15] (Figure 1) with IC_{50} 0.2, 0.9, 0.8, 0.9, 0.4, 0.4, 0.2, 0.4 μM/mL respectively. Furthermore, three aryltetrahydronaphthalene-type lignans **11**–**13** (Figure 2) were isolated from the $CHCl_3$ fraction and identified as podophyllotoxin, deoxypodophylotoxin and 5-methoxypodophyllotoxin [16,17] with IC_{50} 0.2, 0.09 and 0.2 μM/Ml, respectively.

Compound **1** was isolated as a pale yellow amorphous solid, with a molecular formula $C_{37}H_{38}O_{19}$ as determined by HR-ESI-MS (Positive mode) *m/z* = 809.1917 $[M+Na]^+$ (calcd. for $C_{37}H_{38}O_{19}Na$: 809.1905). Its UV spectrum was the same as compound **2**, with shift reagents suggested an occupied 7-OH group. ^{1}H-NMR spectrum showed a singlet signal at δ_H 6.67 (1H, *s*, H-3) characteristic for the flavone-type, a hydrogen-bonded hydroxyl proton at δ_H 12.95 (1H, br*s*, 5-OH), two anomeric protons of two hexose moieties at δ_H 5.24 (1H, *d*, *J* = 7.3 Hz, H-1`` of Glc I) and δ_H 5.18 (1H, *d*, *J* = 3.7 Hz, H-1``` of Glc II) characteristic for the β- and α-glucopyranoside as confirmed from the $^3J_{H\text{-}1,H\text{-}2}$ coupling constant [18]. The interglycosidic

Table 1. The cytotoxic activities of the isolated compounds.

Compounds	*	1	2	3	4	5	6	7	8	9	10	11	12	13
EL_4	1.9	0.2	0.2	0.9	0.8	0.9	0.3	0.4	0.4	0.2	0.4	0.2	0.09	0.2
IC_{50}	±	±	±	±	±	±	±	±	±	±	±	±	±	±
(μM/mL)	0.5	7.5	8.0	8.9	9.0	7.6	8.6	5.6	8.3	5.8	6.7	6.5	6.8	5.3

* Thapsigargin.

linkage as well as its attachment to the aglycone was established as shown in (Figure 1) according to the DIFNOE spectroscopy [19]. By the irradiation at δ_H 5.24 (Glc I) caused a strong negative NOEs at δ_H 5.18, δ_H 6.47 and δ_H 6.74 corresponding to H-1``` (Glc II), H-6 and H-8 respectively; confirmed the attachment of (Glc I) at 7-OH of luteolin, by the irradiation at δ_H 5.18 (Glc II) caused a strong negative NOE at δ_H 5.24 H-1`` (Glc I), indicating that, the interglycosidic linkage to be α-D-glucopyranosyl (1→2)-β-D-glucopyranoside. 2D COSY spectra showed a cross-peak of two doublets at δ_H 6.37 (1H, *d*, *J* = 15.8 Hz, H-α) and δ_H 7.45 (1H, *d*, *J* = 15.8 Hz, H-β) corresponding to AM system in the *trans*-configuration as indicated from the $^3J_{H\text{-}\alpha,H\text{-}\beta}$ coupling constant [18], the down-field shift of the methylene proton of (Glc II) at δ_H 4.15-4.27 (2H, *m*, 6-CH_2) assigned from $^1H-^1H$ COSY suggesting the attachment position of the *E*-feruloyl group at 6-CH_2OH of (Glc II), which was confirmed by the irradiation at δ_H 6.37 (H-α) resulted in a weak negative NOE at δ_H 4.15-4.27. From the above data Compound **1** suggested as luteolin 7-*O*-α-D-(6```-*E*-feruloyl)glucopyranosyl (1→2)-β-D-glucopyranoside. This proposed structure was confirmed by the analysis with nano-ESI-CID-MS/MS, it has been investigated that the interglycosidic linkage-type (1→2) or (1→6) can be concluded from the relative abundances of the $\mathbf{Y_0}$ and $\mathbf{Y_1}$ ions obtained from the protonated molecule and the relative abundance of the $\mathbf{Y^*}$ ion provide information on the nature of the aglycone and on the linkage position of the disaccharide [7,20-22]. The interglycosidic linkage and the aglycone part of compounds **1** can be characterized by CID of the molecular ion peak $[M+H]^+$ at *m/z* 787, it was found a protonated aglycone $\mathbf{Y_0}^+$ ion at *m/z* 287, the fragments at *m/z* 177 and 339 corresponding to feruloyl$^+$ and feruloylhexose$^+$ respectively [23], the weak abundance of the $\mathbf{Y^*}$ ion at *m/z* 625 confirmed the flavone type, where the high relative abundance of $\mathbf{Y_0}$ at *m/z* 287 compared with $\mathbf{Y_1}$ at *m/z* 449 ($\mathbf{Y_0} > \mathbf{Y_1}$) confirmed the interglycosidic linkage to be (1→2) agreed with DIFNOE spectra, it was suggested from the (DIFNOE) that the exact location of the *E*-feruloyl to be at the 6```-OH (Glc II), which was confirmed from the presence of the molecular ion $^{0,4}\mathbf{X_1}$ at *m/z* 321, this proved the attachment of *E*-feruloyl at the 6```-OH (Glc II) [24]. Hence; Compound **1** was confirmed to be luteolin 7-*O*-α-D-(6```-*E*-feruloyl)glucopyranosyl (1→2)-β-D-glucopyranoside [25].

Compound **6** was isolated as yellow amorphous solid; its molecular formula was determined to be $C_{14}H_{25}NO_6$ by HR-MALDI-MS (Positive mode) *m/z* = 326.1579 $[M+Na]^+$, (calcd. for $C_{14}H_{25}NO_9Na$: 326.1575). The ^1H- and ^{13}C-NMR spectra was the same as that of compound **8**, with extra three signals classified into; a doublet signal at δ_H 1.14 (6H, *d*, *J* = 6.2 Hz, 2C***H***$_3$ of the *iso*propoxy group) and ***C***H_3 of the *iso*propoxy group at δ_C 22.43, a multiplet signal at δ_H 3.67 (1H, *sep*, *J* = 6.2 Hz, C***H*** of the *iso*propoxy group) and ***C***H of the *iso*propoxy at δ_C 73.80, and a triplet signal at δ_H 4.74 (1H, *t*, *J* = 9.2 Hz, H-3`) and C-3`at δ_C 81.98. Analysis of ^1H-NMR and $^1H-^1H$ COSY spectra showed a cross-peak correlated the triplet methyl signal at δ_H 0.86 (3H, *t*, *J* = 7.6 Hz, CH_3-4) with a quartet methylene signal at δ_H 1.46 (2H, *q*, *J* = 7.6 Hz, CH_2-3), and a cross-peak correlated the doublet signal at δ_H 1.14 (6H, −CH=(2C***H***$_3$)) with the methene group at δ_H 3.67 (1H, −C***H***=(2CH_3)) corresponding to *iso*propyl group, the downfield shift of the methene group at δ_H 3.67 confirmed the form of *iso*propoxy group, which suggested to be attached to the 3`-OH of the glucose moiety as indicated by the downfield shift of H-3` at δ_H 4.74, and C-3` at $\delta_C$ 81.98, this was confirmed by the irradiation of the methene proton ($\delta_H$ 3.67) caused a strong NOEs enhancement at $\delta_H$ 4.74 and $\delta_H$ 1.14 corresponding to H-3` of glucose moiety and the doublet signal of the two methyls of the *iso*propoxy group, respectively. Hence; compound **6** was confirmed to be 2-[(3`-*iso*propoxy-*O*-β-D-glucopyranosyl)oxy]-2-methylbutanenitrile [25].

Several investigations showed that flavonoids inhibit tumour cell growth via the cessation of aerobic glycolysis by blocking membrane Na^+, K^+-ATPase of tumour cells [26], flavonoids appeared to be compounds of low toxicity and some of them apparently have antiproliferative activity against human tumour cells [27]. The chemopreventive activity of flavonoids may result from their ability to inhibit phase I and induce phase II carcinogen metabolizing enzymes that initiate carcinogenesis. They also inhibit the promotion stage of carcinogenesis by inhibiting oxygen radical-forming enzymes or enzymes that contribute to DNA synthesis or act as ATP mimics and inhibit protein kinases that contribute to proliferative signal transduction. Also, they may prevent tumor development by inducing tumor cell apoptosis by inhibiting DNA topoisomerase II and p53 down regulation or by causing mitochondrial toxicity, which initiates mitochondrial apoptosis [28]. Thus, some of the past studies support the antitumor cell effects of flavonoids being caused via DNA damage to tumour cells [26]. The limited understanding about a possible structure-activity relationship of flavonoids as anticancer agents could possibly because of the few or not appropriated flavonoids used in the previous studies. It was found that the presence of sugar moiety reduce the cytotoxic activity of the aglycone on tumor cell lines and this may be due to the fact that the hydrophilic nature of sugars or the increased volume of glycosides could interfere with the drug entering through the cellular membrane [29], this appeared clearly in our study as the IC_{50} against EL_4 of the mono-glucoside, if compared with the diglucosides **1** with IC_{50} against EL_4 is 0.2 μM/mL, which in turn more potent than the Di- C-glycoside flavonoids **3−5**. It can be concluded that the presence of the −H at C-3 as well as the C-4 oxo group is required for maximal biological activity of flavonoids. Both of the aromatic substituents and the keto-enol functionality can serve as targets for future structure activity relationship (SAR) studies of flavonoids [30]. Furthermore, the cyanogenic glycosides were used for the treatment of human neoplastic disease, its activity as anticancer may be due to the fact that these types of compounds liberate hydrocyanic acid (HCN) by hydrolysis, resulted in their potential danger as poisons to livestock as well as human [31].

Aryltetrahydronaphthalene-type lignans as podophyl lotoxin and its derivatives are important natural products in the armamentarium of antineoplastic agents. The biological assessment of podophyllotoxin was followed by discovery of its mode of action and culminated in the synthesis of the anticancer drugs etoposide and teniposide. The long journey from podophyllotoxin to etoposide and teniposide illustrates the fascinating development of clinically useful anticancer drugs from natural product prototypes through chemical modification. It is particularly distinctive that structural variation of podophyllotoxin caused a radical change in the mechanism of action. Today, several new podophyllotoxin analogs have emerged as potential anticancer drugs. Some recent literature contributions have provided comprehensive updates on various aspects of this compound class primary molecular mechanisms underlying the antineoplastic activities of podophyllotoxin analogs include preventing the assembly of tubulin into microtubules or inhibiting the catalytic activity of

DNA topoisomerase II, although other known and in some cases ambiguous mechanisms are also involved [32,33]. Thus, podophyllotoxin derivatives, etoposide and teniposide were reported to break DNA strands, which results in a cessation of the tumour cell proliferation at the G2 cell cycle [34].

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