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Phytochemical constituents of the butanol fraction of *Arum palaestinum* Boiss.: cytotoxic and antiviral screening

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Abstract

The present study aims to investigate the phytochemical constituents of the butanol soluble fraction of *Arum palaestinum* aqueous methanol extract, by using different chromatographic techniques. The isolated compounds were identified by chemical and physical methods. The *in vitro* cytotoxic activity of the aqueous methanol extract, butanol fraction and the isolated compounds were investigated against four cell lines MCF7, HepG2, Hep2 and HeLa, while the antiviral activity was evaluated against H5N1 virus. Five compounds; vitexin**,** isovitexin**,** isoorientin**,** chrysoeriol 7-*O*-neohesperidoside**,** chrysoeriol 7-*O*-(*β*-apiosyl)-*β*-glucopyranoside were isolated from the butanol fraction of *A. palaestinum*. The aqueous methanol extract exhibited high antiproliferative activity with IC_{50} 17.8, 14.2, 23.6 and 23.9 (µg/ml) against MCF7, HepG2, Hep2 and HeLa cell lines, respectively. The butanol fraction also revealed significant activity against MCF7 and HepG2 cell lines with IC_{50} 19.6 and 16.4 (µg/ml), respectively. Moreover, chrysoeriol-7-*O*-neohesperidoside showed moderate activity against Hep2 cell line (IC_{50} 37.8 µM). The aqueous methanol extract showed remarkable inhibition against H5N1 virus (65% at concentration 100 µg/µl) in comparison with that of the butanol fraction. In conclusion, two chrysoeriol-*O*-glycosides were isolated for the first time from *A. palaestinum*. Also, this considered the first report on the antiviral screening of that species.

1. Introduction

Arum palaestinum Boiss., also called Black Calla, is a perennial herbaceous plant from subfamily Aroideae (family: Araceae). It is native to Western Asia, Northern Africa, Europe and widely distributed in Mediterranean region [1]. In Palestine, *A. palaestinum* was reported to be the most edible wild plant and also the common used medicinal plant among cancer patients [2,3]. Traditionally, it has been used for treatment of different diseases such as stomach parasitic worms, coughing with phlegm, constipation, acne, hemorrhoids and prostate disorders [2,4]. The plant is also used to cure various chronic diseases including atherosclerosis, diabetes, cancers, hypertension, stomach sourness, and toxicity [5-8].

Many studies were carried out on the screening of *A. palaestiunm* extracts and/or their constituents against different carcinoma cell lines and estimate their antioxidant activity [3, 9-14]. Dessoky *et al.* [1], Afifi *et al.* [15] and Farid *et al.* [8,16] reported the flavonoid constituents of *A. palaestinum* as luteolin, chrysoeriol, vitexin, isovitexin, vicenin II, orientin, isoorientin and 3,6,8-trimethoxy-5,7,3',4'-tetrahydroxy flavone. *A. palaestinum* also contains isovanillin, linolenic acid and *β*-sitosterol [17]. Additionally, the phytochemical profiling of *A. palaestinum* leaves using liquid chromatography–tandem mass spectrometry led to the detection of numerous metabolites including flavonoids, phenolic acids, terpenoids, iridoids and amino acids [18]. The present study is our ongoing research on *A. palaestinum* and aims to investigate the chemical constituents of the butanol fraction in addition to evaluate its anticancer and antiviral activities. To best of our knowledge, the antiviral activity of the investigated plant was not evaluated before.

2. Experimental

2.1. *Plant collection*

A. palaestinum Boiss. was collected from its wild habitats in Bergesh protected area, Irbid, Jordan (latitude: 32° 25′ 43.1682″ N and longitude: 35° 46′ 47.0094″ E) in February 2012 by Dr. Ahmed El-Oqlah, Department of Biological Sciences, Yarmouk University, Jordan.

2.2. *Extraction and isolation*

The aerial parts of *A. palaestinum* were air dried and ground, the obtained powder (1450 g) was extracted at room temperature with 3:1 methanol- water until exhaustion for an interval of six days. The aqueous methanol extract was evaporated under reduced pressure to obtain a residue of 302 g. The extract was dissolved in distilled water (500 mL) then fractionated by using diethyl ether (44 g), dichloromethane (26 g), ethyl acetate (40 g), butanol (75 g), methanol (56 g) and water (58 g) according to their polarity. The butanol fraction was further subjected to Whatman 3 mm paper chromatography by using butanol: acetic acid: water, 5:4:1 (BAW). Final purification of the isolated compounds was carried out on Sephadex LH-20 column [19] and afforded individual compounds; **1** (21 mg), **2** (3.5 mg), **3** (2.7 mg), **4** (20 mg), and **5** (10 mg). The other fractions were previously subjected for biological and chemical investigations [8,16].

2.3. *Structure elucidation of the isolated compounds*

The identification of compounds was determined by R_f values, colour reactions, UV spectrometry, 1D, 2D-NMR spectroscopy [19,23]. The 1D and 2D NMR (^{1}H and ^{13}C NMR, COSY, HSQC, HMBC and ROESY) spectra were recorded on a Bruker Avance II+ 600 NMR spectrometer with operating frequency 600 MHz (^{1}H) and 150 MHz (^{13}C), using the residual solvent signal (δ 3.31/49.05 and 2.50/39.52 in ^{1}H/^{13}C for CD_3OD and DMSO, respectively) as a reference. LC-ESI-MS analysis system consists of HPLC (Waters Alliance 2695) and mass spectrometry (Waters 3100). Spectra were recorded in the ESI negative mode between 50-1000 m/z. Sugars obtained by acid hydrolysis of the flavonoid glycosides (2N HCl, 100^{o}C, 60 min) were identified by PC using BBPW (Benzene: n-Butane: Pyridine: Water; 1:5:3:3), with a standard sugar mixture. The dried chromatograms were visualized by aniline phthalate reagent. The sugar spots were observed in daylight [19]. The R_f values of tested sugars were compared with those of reference sugars. The present work deals with the isolation and characterization of five compounds identified as vitexin **(1)**, isovitexin **(2)**, isoorientin **(3)**, chrysoeriol 7-*O*-neohesperidoside **(4)**, chrysoeriol 7-*O*-(*β*-apiosyl)-*β*-glucopyranoside **(5)**. The data of the isolated compounds were listed below:

Vitexin **(1)**

Yellow powder, R_f 0.38 BAW. UV λmax; (MeOH) 271, 337; (+NaOMe) 279, 330sh, 398; (+$AlCl_3$) 279, 305, 349, 350, 385; (+$AlCl_3$/HCl) 280, 302, 343, 382; (+NaOAc) 276, 305, 361; (+NaOAc/H_3BO_3) 273, 336.^{1}H-NMR (DMSO-d_6): δ 4.71 (1H, d, *J*=9.2 Hz; H-1")**,** 5.97 (1H, s; H-6), 6.59 (1H, s; H-3), 6.87 (2H, d, *J*= 8.4 Hz; H-3', H-5'), 7.96 (2H, d, *J*= 8.4 Hz; H-2', H-6') [24,25].

Isovitexin **(2)**

Yellow powder, R_f 0.38 BAW. UV λmax; (MeOH) 269, 331; (+NaOMe) 279, 327 sh, 394; (+$AlCl_3$) 276, 303, 346, 382; (+$AlCl_3$/HCl) 277, 303, 343, 381; (+NaOAc) 279, 389; (+NaOAc/H_3BO_3) 270, 319, 345. ^{1}H-NMR (DMSO-d_6): 4.6 (1H, d, *J*= 9.3 Hz; H-1"), 6.46 (1H, d, *J*= 2.0 Hz; H-8), δ 6.75 (1H, s; H-3), 6.8 (2H, d, *J*= 8.7 Hz; H-3', H-5'), δ 7.8 (2H, d, *J*= 8.7 Hz; H-2', H-6') [25].

Isoorientin **(3)**

Yellow powder, R_f 0.34 BAW. UV λmax; (MeOH) 258, 270sh, 350; (+NaOMe) 268, 335, 407; $AlCl_3$: 275, 300sh, 423; (+$AlCl_3$/HCl) 263, 278sh, 299sh, 362, 389; (+NaOAc) 273, 325sh, 379; (+NaOAc/H_3BO_3) 263, 377, 440. ^{1}H-NMR (DMSO-d_6): δ 4.5 (1H, d, *J*= 9.6 Hz; H-1")**,** δ 6.4 (1H, d, *J*= 1.8 Hz; H-8), δ 6.6 (1H, s; H-3), δ 6.84 (2H, d, *J*= 6.8 Hz; H-5'), δ 7.37 (1H, dd, *J*= 2.5 Hz; H-2'), δ 7.41 (1H, dd, *J*= 2.5, 8.6 Hz; H-6') [26].

Chrysoeriol -7-*O*-neohesperidoside **(4)**

Yellow powder, R_f 0.42 BAW. UV/Vis λmax; (MeOH) 253, 267sh, 345; (+NaOMe) 245,262, 305, 390; (+$AlCl_3$) 272, 300sh, 353, 390; (+$AlCl_3$/HCl) 276, 303, 352, 390; (+NaOAc) 250,266,349; (+NaOAc/H_3BO_3) 250, 266, 346. ^{1}H NMR (CD_3OD): 1.33 (d, *J*= 6.1 Hz, H-6'"), 3.40 (2H, m, H-4" and H-4'"), 3.54 (1H, m, H-5"), 3.62 (1H, m, H-2'"), 3.64 (1H, m, H-3"), 3.69 (1H, m, H-2"), 3.71 (1H, m, H-6b"), 3.92 (1H, m, H-6a"), 3.94

(1H, m, H-5'''), 3.95 (3H, s, OCH3), 3.96 (1H, m, H-3'''), 5.20 (1H, d, *J*= 7.5 Hz, H-1''), 5.29 (1H, d, *J*= 2.0 Hz, H-1'''), 6.66 (1H, s, H-3), 6.45 (1H, d, *J*= 2.0 Hz, H-6), 6.82 (1H, d, *J*= 2.0 Hz, H-8), 6.88 (1H, d, *J*= 8.4 Hz, H-5'), 7.48 (1H, d, *J*= 2.2 Hz, H-2'), 7.54 (1H, dd, *J*= 8.4, 2.2 Hz, H-6'). ^{13}C NMR (CD3OD): 16.8 (C-6'''), 54.9 (C-OMe), 60.8 (C-6''), 68.6 (C-5'''), 69.8 (C-4''), 70.5 (C-3'''), 70.6 (C-2'''), 72.2 (C-4'''), 76.8 (C-5''), 77.5 (C-3''), 77.6 (C-2''), 94.3 (C-8), 98.2 (C-1''), 99.5 (C-6), 100.9 (C-1'''), 102.1 (C-3), 105.4 (C-10), 109.0 (C-2'), 115.8 (C-5'), 120.1 (C-1'), 120.8 (C-6'), 148.7 (C-3'), 160.2 (C-5), 162.8 (C-72), 165.5 (C-2), 182.5 (C-4) [27, 28]. HMBC correlations: H-3: (C-2, C-4, C-10); H-5': (C-6', C-3'); OCH_3: (C-3'); H-1'': (C-7); H-1''': (C-3'''or C-2'''); H-4''': (C-3''', C-5'''); H-6''': (C-5''', C-4'''). ROESY correlations: H-6: (H-1'', H-6''', H-5'''); H-8: (H-1'', H-5'', H-6''', H-5'''); H-2': (H-3, OCH_3); H-6': (H-5', H-3); H-5': (H-6'); H-1'': (H-6, H-8, H-5'',H-3''); H-6''': (H-4''') [29]. The negative-ion ESIMS showed a pseudo-molecular ion peak at *m/z* 606.97 $[M-H]^-$, consequent to the chemical formula $C_{28}H_{31}O_{15}$.

Chrysoeriol 7-*O*-(*β*-apiosyl)-*β*-glucopyranoside **(5)**
Yellow powder, R_f 0.40 BAW. UV/Vis λmax; (MeOH) 253, 267sh, 346; (+NaOMe) 245, 262, 304, 389; (+$AlCl_3$) 272, 300sh, 352, 390; (+$AlCl_3$/HCl) 276, 302, 352, 389; (+NaOAc) 250, 267, 350; (+NaOAc/H_3BO_3) 250, 266, 346. ^{1}H NMR (DMSO-d_6): 3.95 (3H, s, OCH_3), 5.09 (1H, d, *J*= 7.5 Hz, H-1''), 5.39 (1H, d, *J*= 1.7 Hz, H-1'''), 6.67 (1H, s, H-3), 6.46 (1H, d, *J*= 2.0 Hz, H-6), 6.84 (1H, d, *J*= 2.0 Hz, H-8), 6.90 (1H, d, *J*= 8.5 Hz, H-5'), 7.47 (1H, d, *J*= 2.5 Hz, H-2'), 7.52 (1H, dd, *J*= 2.5, 8.5 Hz, H-6').

2.4. *Cell culture and in vitro anticancer activity*
Human tumor cell lines; epidermal carcinoma of larynx (Hep2), cervix (HeLa), liver (HepG2) and breast (MCF7) were obtained from the American Type Culture Collection (ATCC, Minisota, U.S.A.). The cell lines were maintained by serial sub-culturing at the National Cancer Institute, Cairo, Egypt. The samples were prepared by dissolving Stock solution in dimethylsulfoxide (DMSO) at a concentration 100 mM and stored at -20°C. The cytotoxic screening of the aqueous methanol extract, butanol fraction and pure compounds were carried out using Sulphorhodamine-B (SRB) assay and followed the method reported by Vichai and Kirtikara [20]. SRB is a bright pink aminoxanthrene dye with two sulphonic groups. It is a protein stain that binds to the amino–groups of intracellular proteins under mildly acidic conditions to provide a sensitive index of cellular protein content. Cells were seeded in 96-well microtiter plates at initial concentration $3x10^3$ cell/well in a 150 µl fresh medium for 24 hrs before treatment with the extract to allow attachment of cells to the wall of the plate. Different concentrations of the extracts (0, 5, 12.5, 25 and 50 µg/ml) and compound (**4**) (0, 5, 12.5, 25 and 50 µM) were separately added to the cell monolayer in triplicate. Monolayer cells were incubated with the compounds for 48 hrs at 37°C and in an atmosphere of 5% CO_2. After 48 hrs, cells were fixed, washed and stained with SRB. The excess stain was washed with acetic acid and attached stain was recovered with *tris*-EDTA buffer. Colour intensity was measured at 570 nm with an ELISA reader. The relation between surviving fraction and drug concentration was plotted to obtain the survival curve of each tumor cell line as compared with Doxorubcin; the control anticancer drug. The IC_{50} values (the concentrations of drug required to produce 50% inhibition of cell growth) were calculated.

2.5. *Antiviral activity against H5N1 virus*
2.5.1. *Cells and virus*
Madin-Darby Canine Kidney (MDCK) cells were maintained in the Center of Scientific Excellence for influenza viruses at the National Research Center. The cells were propagated till confluence in multiwell plates. The highly pathogenic avian influenza (HPAI) virus A/Chicken/Egypt/M7217B/2013 (H5N1) used in this study was isolated from the infected chickens in Egypt in 2013 and characterized at immunologic and molecular levels.

2.5.2. *Preparation of extracts for bioassay*
Stock solutions of the tested extracts were dissolved as 0.1 g in 1 ml of 10% DMSO in deionized water. The prepared extract solutions were used for both cytotoxicity and antiviral bioassays.
2.5.3. *MTT cytotoxicity assay (TC_{50})*
The MTT cytotoxicity assay was carried out with the same method described by Salem *et al*. [21] with minor modification. Briefly, the cells were seeded in 96 well-plates (100 µl/well at a density of 3×10^5 cells/ml) and incubated for 24 hrs at 37°C in 5% CO_2. After 24 hrs, cells were treated with various concentrations of the tested compounds in triplicates. After further 24 hrs, the supernatant was discarded and cell monolayers were washed 3

times with sterile phosphate buffer saline (PBS). MTT solution (20 µl of 5 mg/ml stock solution) was added to each well and incubated at 37 °C for 4 hrs followed by medium aspiration. In each well, the formed formazan crystals were dissolved with 200 µl of acidified isopropanol (0.04 M HCl in absolute isopropanol= 0.073 ml HCL in 50 ml isopropanol). Absorbance of formazan solutions were measured at λmax 540 nm with 620 nm as a reference wavelength using a multi-well plate reader. The percentage of cytotoxicity compared to the untreated cells was determined with the following equation: % Cytotoxicity = (Absorbance of cell without treatment – Absorbance of cell with treatment)/Absorbance of cell without treatment × 100.
The plot of % cytotoxicity versus sample concentration was used to calculate the concentration which exhibited 50% cytotoxicity (TC_{50}).

2.5.4. *Plaque reduction assay*
Anti-H5N1 activity of the aqueous methanol extract and butanol fraction was investigated by plaque reduction assay with confluent 24 hrs old monolayer of MDCK cells. The assay was carried out according to the method described by Hayden *et al.* [22] in a six-well plate where MDCK cells (10^5cells/ml) were cultivated for 24 hrs at 37^{o}C. A/CHICKEN/7217B/1/2013 (H5N1) virus was diluted to give 10^5 PFU/ well, mixed with the safe concentration of the tested compounds, and incubated for 30 minutes at 37^{o}C before being added to the cells. Growth medium was removed from the cell culture plates and virus-Cpd or virus-extract and Virus-oseltamivir mixtures were inoculated (100 µl/well). After 1 hr contact time for virus adsorption, 3 ml of DMEM supplemented with 2% agarose containing the virus-Cpd or virus-extract and Virus-oseltamivir mixtures was added onto the cell monolayer, plates were left to solidify and incubated at 37^{o}C till formation of viral plaques (3 to 4 days). Formalin (10%) was added for two hours then plates were stained with 0.1% crystal violet in distilled water. Control wells were included where untreated virus was incubated with MDCK cells and finally plaques were counted and percentage reduction in plaques formation in comparison to control wells was recorded as following: % inhibition= viral count (untreated) - viral count (treated)/viral count (untreated)x100.

3. Results and discussion

3.1. *Flavonoids identification*
Five compounds were isolated and identified as vitexin **(1)**, isovitexin **(2)**, isoorientin **(3)**, chrysoeriol 7-*O*-neohesperidoside **(4)** and chrysoeriol 7-*O*-(*β*-apiosyl)-*β*-glucopyranoside **(5) (**Figure 1). Through our previous report [8,16], compounds **1**- **3** were also separated and identified from ethyl acetate and diethyl ether fractions of *A. palaestinum*, while compounds **4**&**5** are firstly isolated from *A. palaestinum*.

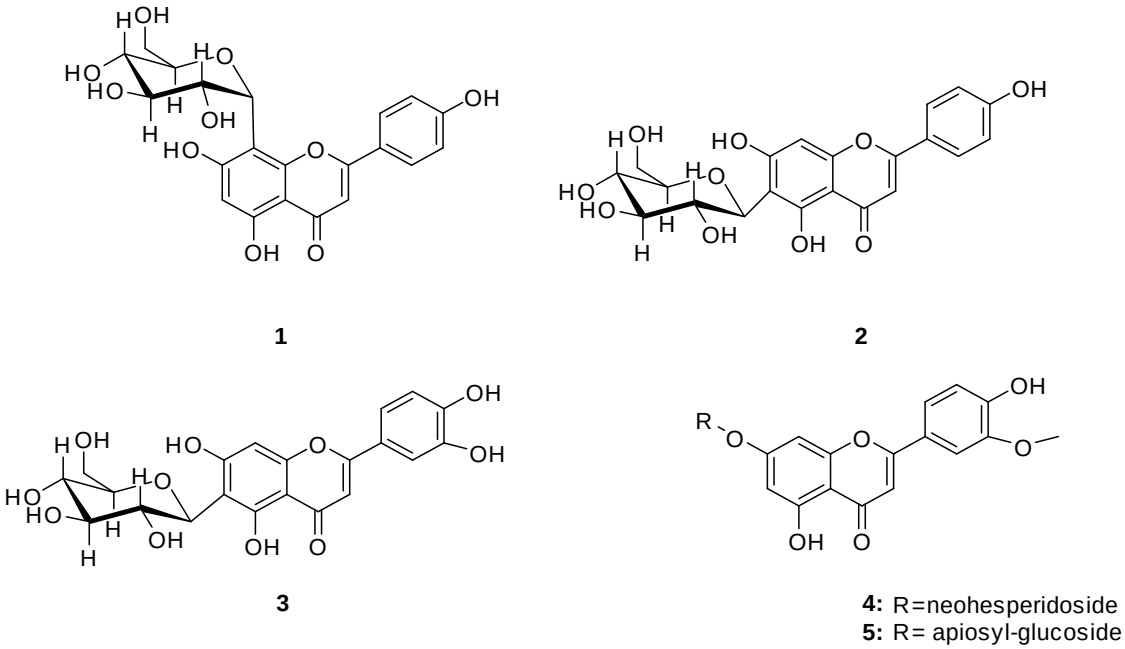


Figure 1: Chemical structures of the isolated flavonoids

Compound **4** was isolated as yellow powder. The UV data indicated a flavone nucleus with a free hydroxyl groups at C-5 and C-4' position, while those at C-7 and C-3' are substituted [19, 23]. Complete acid hydrolysis

yielded chrysoeriol (UV & Co-PC) as well as glucose and rhamnose as sugar moieties (Co-PC). The negative-ion ESIMS showed a pseudo-molecular ion peak at *m/z* 606.97 $[M-H]^-$, consequent to the chemical formula $C_{28}H_{31}O_{15}$.The ^{1}H NMR spectrum showed two anomeric signals at δ 5.20 (1H, d, *J* = 7.5 Hz, H-1") and δ 5.29 (IH, *J* = 2.0 Hz, H-1'"), confirming the *β*-glucopyranoside and *α*-rhamnopyranoside moieties, respectively. The signal of H-6' appeared as doublet of doublets at δ 7.54 (*J*= 2.2, 8.4 Hz) and that of H-2' appeared as doublet at δ 7.48 (*J*= 2.2 Hz). Another doublet at δ 6.88 (*J*= 8.4 Hz) was assigned for H-5'. In addition, the spectrum showed a singlet at δ 6.66 assignable for H-3, and two proton doublets at δ 6.82 and 6.45, each with *J*= 2.0 Hz, describable to H-8 and H-6, respectively which indicates that position 7 is substituted. The singlet at δ 3.95 was assigned for -OCH_3 group [32]. The ^{13}CNMR data of the aglycone were in agreement with a 7-substituted of chrysoeriol moiety while the ^{13}C NMR peaks of the glycoside part could be assigned to α-rhamnopyranosyl-(1→2)-*β*-glucopyranoside (neohesperidoside moiety). The HMBC shows a correlation of H-1"-glucose with C-7 of the aglycone and H-1'"-rhamnose with H-2"-glucose. Further, in ROESY spectrum there are correlations of H-6 and H-8 with methyl group from rhamnosyl part and with H-1" from glucose [29]. Therefore, compound **4** was identified as chrysoeriol 7-*O*-neohesperidoside which was isolated before from *Morinda morindoides* [27] and only identified by ^{1}H, ^{13}C NMR but contradicted with the present study in the position of the anomeric sugars proton which proved by full characterization by HMBC, HSQC, COSY and ROESY while the spectroscopic data are in a good agreement with other study on apigenin 7-*O*-neohesperidoside isolated from *Selaginella moellendorffii* Hieron [29].

Compound **5** are isolated as yellow powder. The UV data indicated a flavone nucleus with a free hydroxyl groups at C-5 and C-4' position, while those at C-7 and C-3' are substituted [19]. Complete acid hydrolysis yielded chrysoeriol aglycone (UV & Co-PC), glucose and apiose as sugar moieties (Co-PC). The ^{1}H NMR spectrum showed two anomeric signals at δ 5.09 (d, *J*=7.5 Hz, H-1"), 5.39 (d, *J*=1.7 Hz, H-1'"), indicating the *β*-configuration of glucopyranose and apiose moieties, respectively [32]. The signal of H-6' appeared at δ 7.52 (*J*=2.5, 8.5 Hz) and that of H-2' appeared at δ 7.47 (*J*= 2.5 Hz). The doublet at δ 6.90 (*J*= 8.5 Hz) was assigned for H-5'. In addition, the spectrum showed a singlet at δ 6.67 assignable for H-3, and two proton doublets at δ 6.84 and 6.46, each with *J*= 2.0 Hz due to meta-coupling, assigned to H-8 and H-6, respectively. Therefore, compound **5** was identified as chrysoeriol 7-*O*-(*β*-apiosyl)-*β*-glucopyranoside.

3.2. *Cell culture and in vitro anticancer activity*

A. palaestinum aqueous methanol extract exhibited obviously high antiproliferative activity compared with the regular regimen for cancer treatment in which doxorubicin was used as a protocol with IC_{50} 17.8, 14.2, 23.6 and 23.9 (µg/ml) against MCF7, HepG2, Hep2 and HeLa cell lines, respectively. Also, butanol fraction revealed strong activity against MCF7, HepG2 cell lines with IC_{50} 19.6, 16.4 (µg/ml), respectively and moderate activity against the other cell lines. In our previous studies on the same plant, compounds **1-3** were isolated from the diethyl ether and ethyl acetate extracts and showed moderate anticancer activity using the same procedure [8, 16]. Compound **4** showed antitumor activity against HeLa, HepG2 and MCF7 with IC_{50} 75.2, 57.9, 71.9 (µM), respectively and moderate activity against Hep2 cell line with IC_{50} 37.8 µM (Figure 2), however the insufficient amount of compound **5** makes us incapable of completing its anticancer screening. The previous studies showed that vitexin, isovitexin and isoorientin have antimutagenic activity on different cell lines [8,16,30,31]. To best of our knowledge, this is the first report of the anticancer activity of chrysoeriol 7-*O*-neohesperidoside **(4)**.

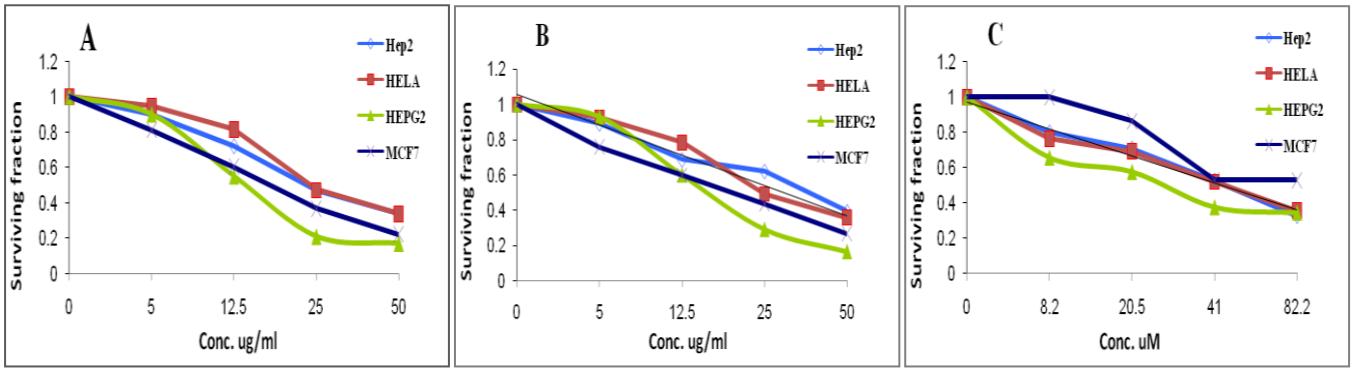


Figure 2: Cytotoxic screening of *A. palaestinum* on different carcinoma cell lines using SRB assay; (A) Aqueous methanol extract, (B) Butanol fraction, (C) Chrysoeriol-7-*O*-neohesperidoside.

3.3. *Antiviral activity against H5N1 virus*

3.3.1. *MTT Cytotoxicity assay*

The cytotoxicity of the aqueous methanol extract and butanol fraction was determined using MTT assay and plotted as shown in Figure 3. The TC_{50} was found to be 262.0 and 269.5 µg/µl, respectively. The MTT assay for antiviral activity detected living cells and the signal generated is contingent on the degree of cells activation and can be used to measure cytotoxicity activation or proliferation and this study consider the first report on the antiviral activity of *A. palaestinum.*

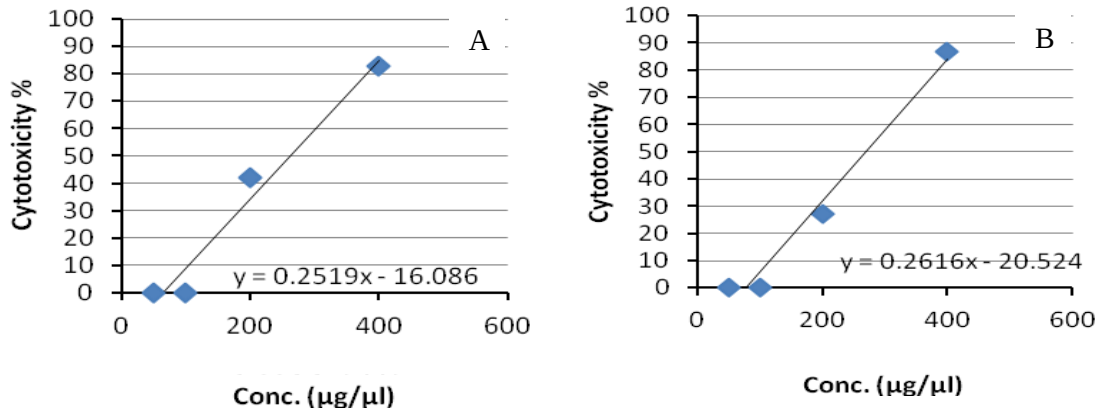


Figure 3: Cytotoxicity of *A. palaestinum* on MDCK cell line using MTT assay; (A) Aqueous methanol extract, (B) butanol fraction.

3.3.2. *Plaque assay method*

This assay confirmed to be a dependable and fast method for determining 50% inhibitory concentrations that correlated well with achievable drug concentrations and the results of clinical studies. The concentration of 100 µg/µl of the aqueous methanol extract showed remarkable inhibition against H5N1 virus (65%) while the butanol fraction showed a weak inhibition (18%). The preliminary studies determined that the number of plaques in the plaque assay method depend on the duration of the adsorption period and the presence or absence of drug [22].

Conclusions

The phytochemical analysis of the butanol fraction led to the isolation of five compounds; two of them were isolated for the first time from *A. palaestinum.* The butanol fraction revealed significant activity against MCF7 and HepG2 cell lines with IC_{50} 19.6 and 16.4 (µg/ml), respectively. Moreover, chrysoeriol-7-*O* neohesperidoside showed moderate activity against Hep2 cell line (IC_{50} 37.8 µM). On the other hand, the aqueous methanol extract of *A. palestinum* showed remarkable antiviral activity against the highly pathogenic avian influenza H5N1virus.The biological and phytochemical study on different species of *Arum* species is worth of further investigations.

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