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Original Article

CHROMATOGRAPHIC ISOLATION OF *ALLIUM CEPA* (SSP. RED ONION) AND ITS CYTOTOXIC ACTIVITY AGAINST HUMAN LIVER CARCINOMA CELL LINES (HEPG2)

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ABSTRACT

Objective: The present study aimed to find out cytotoxic agent from *Allium cepa* L. (ssp. red onion) family *Alliaceae* as well as chromatographic isolation of its active constituents.

Methods: The defatted methanol extract of *Allium cepa* (ssp. red onion) was fractionated with chloroform, ethyl acetate and n-butanol. Each of ethyl acetate and butanolic fraction was separately submitted to chromatographic isolation using different chromatographic techniques such as column chromatography (CC), thin layer chromatography (TLC) and paper chromatography (PC). The structures of the isolated compounds were elucidated through spectroscopic analysis.

Results: Four compounds were isolated from ethyl acetate fraction and identified as kaempferol (1), quercetin (2) isorhamnetin (3) and isorhamentin-4`-*O*-β- D-glucopyranoside (4) whereas two compounds were isolated and identified from the butanolic fraction as α -D-glucopyranose (5), β-D-glucopyranosyl- α- D-glucopyranoside (6). The cytotoxic activity of defatted methanolic extract of *A. cepa* (ssp red onion), ethyl acetate and butanolic fractions against human liver carcinoma cell line (HepG2) was IC_{50} = 10.9, 6.08 and 9.95 µg/ ml respectively. Also, compounds 4 isolated from ethyl acetate fraction and compound 6 isolated from butanolic fraction showed cytotoxic activity (IC_{50} = 11.90 and 22.0 µg/ ml) respectively.

Conclusion: It was appeared that the ethyl acetate fraction was the most active due to it contain high amount of phenolic compounds.

Keywords: *Allium cepa*, Cytotoxic, Human liver carcinoma cell lines HepG2, Chromatographic isolation.

INTRODUCTION

Cancer is a complex disease involving numerous tempospatial changes in cell physiology, which ultimately lead to malignant tumors. Abnormal cell growth is the biological endpoint of the disease. Tumor cell invasion of surrounding tissues and distant organs is the primary cause of morbidity and mortality for most cancer patients [1]. Hepatocellular cancer (HCC) is the fifth most common cause of cancer and the third leading cause of cancer-related deaths worldwide [2]. The main risk factors for HCC, chronic infections of hepatitis B virus (HBV), hepatitis C virus (HCV), aflatoxin, alcohol, smoking as well as other dietary agents are the most important in humans [3].

The plant kingdom represents an enormous reservoir of biologically active phytochemicals with various chemical structures and protective properties. These phytochemicals are often secondary metabolites in the plants and including alkaloids, steroids, flavonoids, terpenoids, tannins, and other groups. Many of these groups have scavenging activity for free radicals therefore; they can be reducing the tissue injury [4-6]. Now several drugs used in medicine are of plant origin, therefore much current research devoted to phytochemical of the plants [7].

The *Allium* (family *Alliaceae*) has over 700 members each of them has special tastes, forms and colors. They have several pharmacological and biological properties such as anti-bacterial, anti-fungal and anti-inflammatory activities [8]. Many of these biological effects are related to the volatile sulfur compounds which are responsible of their characteristic pungent aroma and taste. However, these compounds are unstable and give rise to transformation products. For this reason, recent attention has been focused on polar compounds that are more stable to cooking and to the storage. Among these compounds, sapogenins, saponins, and flavonoids are the main classes found in *Allium* family [9]. In this study the defatted methanolic extract of *A. cepa* (ssp. red anion) was fractionated using different organic solvent as $CHCl_3$, EtOAc and n-BuOH. Each of the ethyl acetate and n-butanol fractions was submitted to chromatographic isolation and evaluated as cytotoxic agent against Hep-G2 cell lines.

MATERIALS AND METHODS

General experimental procedures

Melting points were determined on an electrothermal apparatus (Electrothermal 9200). The nuclear magnetic resonance as ^{1}H-NMR and ^{13}CNMR spectra were recorded on a Brucker Avance- 500 and JEOL GX-spectrometer (500 MHz for 1H and 125 MHz for 13C). The chemical shifts were expressed in δ (ppm) with TMS as reference and coupling constant (*J*) in Hertz. UV spectra (λ_{max}) of the isolated compounds were measured in methanol before and after addition of different reagents on Camspec M550 UV/Vis spectrophotometer (UK). Silica gel 60 (70-230 mesh, Merck) and Sephadex LH-20 (25-100 µm, Sigma) were used for column chromatography. Analytical and preparative thin layer chromatographies (TLC) were performed on silica gel GF254 pre-coated plates (Merck). Paper chromatography (PC) was carried out on Whatmann No. 1 and No. 3 paper sheets. Spots were visualized by absorption of UV radiation and spraying with ethanolic $AlCl_3$ (2%), $FeCl_3$ (1%) and 20% H_2SO_4 followed by heating for flavonoids compounds and sugars.

Chemicals

Aluminum chloride, Sodium acetate, Boric acid and Sodium methoxide were purchased from Merck (Darmstadt, Germany), Quercetin, kaempferol and isorhamnetin, were purchased from Sigma Aldrich (Milan, Italy) and all solvents are analytical grade.

Plant Materials

The bulbs of *Allium cepa* (ssp. red onion) were collected from El-Sharkia governorate, Egypt in March 2012. The collected plant was kindly identified by Prof. Dr. Waffa Amer, Professor of plant

taxonomy, Faculty of Science, Cairo University. Voucher specimen of the plant was deposited at laboratory of medicinal chemistry, Theodor Bilharz Research Institute, Giza, Egypt. Then the plant materials were submitted to extraction process.

Extraction and Fractionation

Fresh bulbs of *Allium cepa* (ssp. red onion) (4 Kg) were cut to small pieces then grinded with electric mixer to be ready for extraction process. The pieces of plant bulbs were extracted with pure methanol on cold several times till exhaustion. The solvent was distilled using rotatory evaporator till dryness to give dried extract 290 g. The dried methanolic extract (250 g) was defatted with petroleum ether. The defatted methanolic extract was successively extracted with aqueous chloroform, ethyl acetate and n-butanol. The obtained different fractions were evaporated under reduced pressure to dryness using rotatory evaporator. The ethyl acetate and butanolic extracts were kept for chromatographic separation process.

Chromatographic Separation of EtOAc Extract

Ethyl acetate extract of *A. cepa* (ssp. red onion) (5.50 g) was subjected to column chromatography (3×60 cm) packed with silica gel 60 (70-230 mesh, Merck) as stationary phase. Elution started with petroleum ether, petroleum ether/ $CHCl_3$ mixture, $CHCl_3$ and then $CHCl_3$/ MeOH gradient and ending with pure methanol. Fractions 250 ml each were collected, concentrated and examined by TLC silica gel using solvent systems; Benzene: MeOH (9:1 and 8:2), $CHCl_3$: MeOH (9:1 and 8:2) and $CHCl_3$: MeOH: H_2O (7: 3: 0.5) and on PC using solvent system 15% AcOH and 4: 1: 5 BuOH: AcOH: H_2O (BAW). Chromatograms were visualized under UV before and after spraying with $AlCl_3$ (2%), $FeCl_3$ (1%) and 20% H_2SO_4. Four major fractions (I-IV) were obtained. Fraction (I) eluted with pure $CHCl_3$ was concentrated and purified on sephadex LH-20 column (2×50 cm) using H_2O/ MeOH as eluent and the resulted fractions were examined by paper chromatography Whatmman No (1) using solvent system 15% AcOH to give compound **1**. Fraction II eluted with $CHCl_3$ - MeOH (98: 2) and re-chromatographed over sephadex LH-20 column using H_2O/ MeOH as eluent and then purified on preparative paper chromatography Whatmman No (3) using solvent system 15% AcOH to give compound **2**. Fraction III eluted by 95% $CHCl_3$ was subjected to sub- column sephadex LH-20 and H_2O/ MeOH as eluent to give compound No 3 and 4.

Compound 1 was obtained as yellow powder, its m.p. 273-278 ˚C, R_f values= 0.036 (15% Acetic acid, PC), 0.68 (n-butanol, acetic acid, water, 4:1:5, PC). UV λ_{max} (MeOH) 255, 268^{sh} and 368; (MeOH+NaOMe) 275, 318,420; (MeOH + $AlCl_3$) 265, 303 ^{sh}, 352 ^{sh} 423; (MeOH + $AlCl_3$ + HCl) 266, 303^{sh}, 354 ^{sh}, 424; (MeOH + NaOAc) 268, 301 ^{sh}, 386; (MeOH + NaOAc + H_3BO_3)262, 298 ^{sh}, 373.

Compound 2 was obtained as yellow powder, m.p. 315-317 ˚C, R_f values= 0.051 (15% AcOH, and 0.60 (n-BuOH: AcOH: H_2O; 4: 1: 5, PC). UV λ_{max} (MeOH) 256, 268 ^{sh}, 372; (MeOH+NaOMe) 273, 330,415; (MeOH + $AlCl_3$) 275, 303 ^{sh}, 455; (MeOH + $AlCl_3$ + HCl) 269, 354 ^{sh}, 427; (MeOH + NaOAc) 275, 320, 392; (MeOH + NaOAc + H_3BO_3) 261, 303 ^{sh}, 388.

Compound 3 was obtained as yellowish brown powder, m.p. 306-308 ˚C, R_f values= 0.10 (15% AcOH, and 0.56 (n-BuOH: AcOH: H_2O; 4: 1: 5, PC). UV λ_{max} (MeOH) 254, 268 ^{sh}, 303 ^{sh}, 369; (MeOH+NaOMe) 270, 327, 430; (MeOH + $AlCl_3$) 268, 304 ^{sh}, 428; (MeOH + $AlCl_3$ + HCl) 266, 302 ^{sh}, 427; (MeOH + NaOAc) 274, 325 ^{sh}, 390; (MeOH + NaOAc + H_3BO_3) 257, 275 ^{sh}, 377.

Compound 4 was isolated as yellow powder, m.p. 242-244 ˚C, R_f value= 0.155 (15% AcOH, PC), 0.50 (n-BuOH: AcOH: H_2O; 4:1:5, PC) and 0.48 (n-BuOH: MeOH: H_2O; 4: 1: 1, TLC). UV λ_{max} (MeOH) 254, 303 ^{sh}, 365; (MeOH+NaOMe) 264, 402; (MeOH + $AlCl_3$) 263, 305 ^{sh}, 423; (MeOH + $AlCl_3$ + HCl) 260, 302 ^{sh}, 420; (MeOH + NaOAc) 272, 316 ^{sh}, 386; (MeOH + NaOAc + H_3BO_3) 257, 275 ^{sh}, 368. The ^{1}H-NMR δ; 12.39 **(**1H, s, 5-OH), 9.52 (1H, s, 3-OH), 8.99 for (1H, s, OH), 7.66 (1H, d, J= 2.0 Hz, H-2'), 7.59 (1H, dd, J= 8.6 and 2.1 Hz, H-6'), 7.21 (1H, d, J= 8.6 Hz, H-5'), 6.41 (1H, d, J= 2.0 Hz, H-8), 6.16 (1H, d, J= 2.0 Hz, H-6), 5.15 (1H, d, J= 6.7 Hz, H-1'', Glc) and 3.70 (3H, s, OCH3). ^{13}C-NMR δ: 176.57 (C-4), 164.61 (C-7), 161.22 (C-5), 156.74 (C-9), 147.29 (C-2), 146.84 (C-3`), 146.40(C-4`), 136.95 (C-3), 125.61 (C-1`), 120.04 (C-6`), 116.27 (C-5`),115.64 (C-2`),103.59 (C-10), 101.82(C-1``), 98.78 (C-6), 98.04 (C-8), 77.79(C-3``), 76.44 (C-5``), 73.77 (C-2``),70.40 (C-4``), 69.72 (C-6``), 61.19 (3`-OCH_3).

Chromatographic Separation of n-BuOH Extract

Butanolic extract of *Allium cepa* (ssp. red onion) (30 g) was subjected to column chromatography (6×120 cm) packed with silica gel 60 (70-230 mesh, Merck) as stationary phase. Elution started with pure CHCl3 and then $CHCl_3$/ MeOH gradient and ending with pure methanol. Fractions 250 ml were collected, concentrated and examined by TLC silica gel using solvent systems; Benzene: MeOH (9:1 and 8:2), $CHCl_3$: MeOH (7: 3) and $CHCl_3$: MeOH: H_2O (7: 3: 0.5). The TLC of each fraction was visualized under UV before and after spraying with 20% H_2SO_4. Also each fraction was examined by paper chromatography Whatmann No 1 using different solvent systems, 15% AcOH and 4: 1: 5 BuOH: AcOH: H_2O (BAW). The paper chromatography (PC) was visualized under UV before and after spraying with $FeCl_3$ (1%) and $AlCl_3$ (2%). Three major fractions were obtained. Fraction I eluted with 90% $CHCl_3$/MeOH were concentrated and purified with preparative TLC using solvent system $CHCl_3$: MeOH (7: 3) to give compound **5**. Fraction III eluted by 75 % $CHCl_3$/MeOH were collected and submitted to further purification on sub column silica gel (2× 50 cm) with gradient mobile phase $CHCl_3$ the $CHCl_3$/ MeOH to give compound **6.**

Compound 5 was obtained as a white amorphous powder, its m.p. 160-162 ˚C, R_f values= 0.45 ($CHCl_3$: MeOH: H_2O; 65: 35: 5, TLC), 0.74 (n-BuOH: MeOH: H_2O, 4:1:0.5, TLC). ^{1}H-NMR δ: 6.18(1H, d, J= 3.8 Hz, H1), 4.85 (1H, d, H6), 4.75 (1 H, dd, H5), 4.63 (1H, d, H3), 4.75 (1 H, dd, H5), 4.36 (1 H, d, H4). ^{13}C-NMR δ: 92.70 (C-1), 73.55(C-3), 72.82 (C-2), 72.44 (C-5), 71.01 (C-4), 61.67 (C-6).

Compound 6 was obtained as a white crystals, its m.p. 196-198 ˚C, R_f values= 0.50 ($CHCl_3$: MeOH; 7: 3, TLC), 0.66 ($CHCl_3$: MeOH: H_2O; 70: 30: 5, TLC) and 0.68 (n-BuOH: MeOH: H_2O, 4: 1: 0.5, TLC). ^{1}H-NMR δ: 5.18 (1H, d, J= 3.6 Hz, H1), 4.803 (1H, d, J= 8 Hz, H1`), 4.50 (1H, d, H6`), 4.41 (1 H, d, H3), 4.37 (1H, dd, H5), 3.83 (1H, d, H6), 3.62 (1 H, d, H2), 3.49 (1 H, d, H3`), 3.44 (1 H, d, H4), 3.43 (1 H, dd, H5`), 3.40 (1 H, d, H4`), 3.35 (1 H, d, H2`). ^{13}C NMR δ: 104.48 (C-1`), 92.22 (C-1), 83.0 (C-2), 77.44 (C-5`), 74.70 (C-3`), 73.30 (C-2`), 72.09 (C-3), 70.27 (C-5), 62.60 (C-4), 62.51 (C-4`), 60.94 (C-6), 60.94 (C-6`).

Cytotoxic Assay

The defatted methanolic extract of *A. cepa* (ssp. red onion) and their fractions (EtOAc and n-BuOH) were investigated toward cytotoxic assay of human liver carcinoma cell line (HepG2). This part was carried out at National Cancer Institute, Cairo, Egypt, according to protocol of **Skehan *et al.,* 1990** [10]. This is a colorimetric assay that estimates cell number indirectly by staining total cellular protein with the dye sulphorhodamine-B (SRB). Cells were seeded in 96-well microtiter plates at a concentration of 5×10^4-10^5 cell/well in a fresh medium and left to attach to the plates for 24 h. For each sample, different concentrations (0, 5, 12.5, 25, and 50 μg/mL) were added to 100 μL wells. Wells were completed to total of 200 μL volume/ well using fresh medium and incubated for 48 h at 37°C in 5% CO_2. Following 48h treatment, the cells were fixed with 50 μL cold 50% trichloroacetic acid for 1 h at 4°C. Wells were washed 5 times with distilled water and stained for 30min at room temperature with 50μL 0.4% SRB dissolved in 1% acetic acid. The plates were air-dried, and the dye was solubilized with 100 μL/well of 10mM tris base (pH 10.5) for 5min on a shaker and optical density (O.D.) of each well was measured spectrophotometrically at 564 nm with an ELIZA microplate reader (Meter tech. Σ 960, USA). The experiment was repeated 3 times. The percentage of cell survival was calculated according to the following equation:

Survival fraction (%) = [O.D. of treated cell/O.D. of control cells] × 100.

Statistical analysis

All experimental were carried out in triplicate, and statistical analysis were performed using SPSS (13) software and Microsoft Excel program.

RESULTS AND DISSCUSION

Compound 1 was obtained as yellow powder, its m.p 273-278 °C, R_f values 0.036 (15% Acetic acid, PC), 0.68 (n-butanol, acetic acid, water, 4:1:5, PC). UV spectrum of methanolic solution of the compound **1** showed characteristic bands of flavonol structure at 255, 268^{sh} and 368 nm. Bathochromic shift of band I (52 nm) was observed with NaOMe indicating the presence of free OH at 4`. Also, bathochromic shift of band II (20 nm) was observed indicating the presence of free 5 and 7 OH. Bathochromic shift of band II with $AlCl_3$ (10 nm) reflected the presence of free OH at position 5. Also, bathochromic shift of band II (13 nm) after addition of NaOAc indicated the presence of OH group in position 7. Addition of NaOAc+H_3BO_3 led to small shift of band I (5 nm) indicating the absence of OH group in position 3`. Compound **1** showed no depression in m.p. when mixed with authentic kaempferol. Its UV data was in good agreement with that of kaempferol [11, 12]. By comparing its m.p., Co-PC behavior with authentic sample of kaempferol. Thus compound **1** was identified as **kaempferol**.

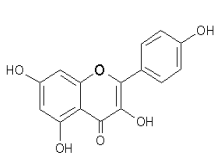
Compound 2 was obtained as yellow powder, m.p. 315-317 °C, R_f values 0.051 (15% AcOH, and 0.60 (n-BuOH: AcOH: H_2O; 4: 1: 5, PC). UV spectrum of the compound in methanol showed the presence of major absorption bands at 256, 268 ^{sh}, 372 which confirmed the flavonol structure. Observation of bathochromic shift in both bands I and band II with NaOMe indicated the presence of free 7-OH, 5-OH, 3`OH and 4`OH groups. There are strong bathochromic shift in band I with $AlCl_3$ (73 nm) reflected the presence of *O*-dihydroxy group in B-ring at 3` and 4` positions bathochromic shift of band II with (19 nm) indicated of free 5-OH group Hypsochromic shift in band I with $AlCl_3$/ HCl (28 nm) indicated the presence of 3`, 4`OH groups. Bathochromic shift (16 nm) in band I with NaOAc/H_3BO_3 also indicated the presence of *O*-dihydroxy groups in B-ring at 3` and 4` positions [13, 14]. By comparing its m.p., Co-PC behavior with authentic sample of quercetin. Thus compound **2** was identified as **quercetin.**

Compound 3 was obtained as yellowish brown powder, m.p. 306-308 °C, R_f values 0.10 (15% AcOH, and 0.56 (n-BuOH: AcOH: H_2O; 4: 1: 5, PC). UV spectrum of compound 3 in methanol showed the presence of major absorption bands at 254, 268 ^{sh}, 303 ^{sh}, 369nm which confirmed the flavonol structure. Observation of bathochromic shift in band II with NaOMe indicated the presence of free 7-OH and 5-OH group. Bathochromic shift in band I with $AlCl_3$ (61 nm) reflected the presence of free OH group in B-ring at 3` or 4` positions and band II with (14 nm) indicated of free 5-OH group. A very small hypsochromic shift in band I with $AlCl_3$/HCl (1 nm) indicated the presence only free OH at 3`or 4`position. There is a limited Bathochromic shift (8 nm) in band I with NaOAc/H_3BO_3 also indicated the absence of *O*-dihydroxy groups in B-ring at 3` and 4` positions [13, 15]. By comparing their m.p., Co-PC behavior with authentic sample of isorhamnetin. Thus compound **3** was identified as **isorhamnetin.**

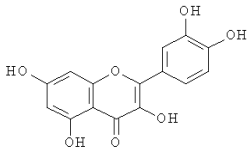
Compound 4 was isolated as yellow powder, UV spectrum exhibited characteristic absorption bands of flavonoid structure at 254, 303 ^{sh} and 365 nm [13, 16]. Bathochromic shift in band II (10 nm) was clear by addition NaOMe. This reflected the presence of free OH at C-7. Moderate bathochromic shift (58 nm) in band I by addition of $AlCl_3$ reflected the absence of free OH groups at position 3` and 4`. Also no hypsochromic shift with $AlCl_3$/ HCl was observed which means that 3` and 4` positions are substituted. Bathochromatic shift was obtained by band I (21nm) with NaOAc indicating the presence of substituted groups at position 3` and 4` [13]. The ^{1}H-NMR spectrum showed sharp singlet was appeared at δ 12.39 for (1H, s, 5-OH), 9.52 for (1H, s, OH) and 8.99 for (1H, s, OH). Other protons of the aglycone moiety was appeared at δ 7.66 (1H, d, J= 2 Hz), 7.59 (1 H, d, J=8.6 Hz), 7.21 (1H, d, J= 8.6 Hz), 6.41 (1H, d, J=2.1 Hz) and 6.16 (1 H, d, J=2 Hz) corresponding to H-2`, H-6`, H-5` and H-8 and H-6 respectively. Also the anomric proton signal was appeared at δ 5.15 (1 H, d, J= 6.7 Hz, H-1`` Glc) and methoxy group signal at δ 3.70 (s, 3H**)** [13, 17, 18]. $^{13}$C-NMR showed characteristic peak of (C-4) at δ 176.57, 101.82 for (C-1``) of anomric carbon of glucose unit and 61.19 for 3`-$OCH_3$ group. This confirmed that compound **4** was identified as **isorhamnetin - 4`-*O*-β-D- glucopyranoside.**

Compound 5 was obtained as a white amorphous powder. The ^{1}H-NMR spectrum showed the presence of six peaks in region δ 4.3-6.18 this area of sugar moiety. There are a sharp doublet peak was appeared at δ 6.18 for (1H, d, J= 3.8 Hz, H1 anomric proton). Other protons were appeared at δ 4.85 (1H, d, H6), 4.75 (1 H, dd, H5), 4.63 (1H, d, H3), 4.44 (1H, d, H2) and 4.36 (1 H, d, H4) these signals of D-glucose unit**.** In the carbon spectra of gluco-oligosaccharides three chemical shift regions for the signals are usually seen resulting from the anomeric (90–105 ppm), methane (69–83 ppm), and methylene carbons (61–69 ppm) [19, 20]. ^{13}C-NMR of compound **5** showed the presence a single peak of anomric carbon (C1) at δ 92.70 of D-Glucose and other five carbon atoms at δ 73.55, 72.82, 72.44, 71.01 and 61.67 for C3, C2, C5, C4 and C6 respectively. The conformational space of α and β -D- glucose has been widely studied with experimental and theoretical methods [19]. In vacuo the α - anomer is more stable than the β-anomer because of the anomric effect, but in aqueous solution the α / β ratio is reversed due to solvent effects and hydrogen bonding [21]. Therefore from ^{1}H, ^{13}C NMR data and co-TLC compound **5** was identified as **α -D - glucopyranos.** This is the first time to isolate this free glucose from *A. cepa* (ssp. red onion).

Compound 6 was obtained as white crystals. The ^{1}H-NMR spectrum showed the presence of peaks in region δ 3.344- 5.185 this area of sugar moiety. There are a doublet peak was appeared at δ 5.185 for (1H, d, J= 3.6 Hz, H1 anomric proton of α-D-glucose unit) and another peak appeared at δ 4.803 for (1H, d, J= 8 Hz, H1` anomric proton of another β-D-glucose unit). The signals of the anomeric protons in the non-reducing unit of the α - or β -disaccharides were close to each other whereas those of the reducing unit at α - and β - equilibrium were well separated [19]. The ^{1}HNMR signals indicate the presence of two D- glucose units**.** The^{13}C-NMR data of compound 6 showed the presence of peak at δ 104.48 of anomric carbon (C1) of β-D-Glucose, carbon atom at δ 92.22 of anomric carbon (C1) of α -D-Glucose. It was observed that the α -linked anomeric carbon of the non-reducing end resonates in the region 97.0-104.6 ppm, while the β –linked non-reducing anomeric carbon resonates at 98.4-103.5 ppm [19, 20]. From reported data, ^{1}H and ^{13}C NMR data compound 6 was identified as **β -D- glucopyranosyl (1→2) - α -D – glucopyranoside.** This is the first time to isolate this dimer of glucose from *A. cepa* (ssp. red onion).



Compound1



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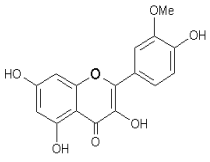

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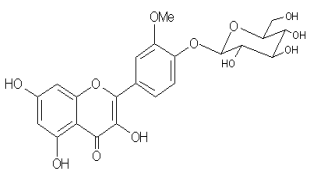

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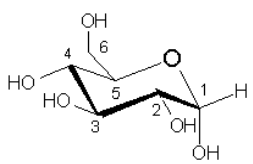
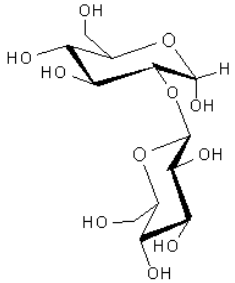


Compound 5



Compound 6

Cytotoxic Assay

Many investigations indicate that phenolic compounds are of great value in preventing the onset and progression of cancer. They are toxic to cancer cells, but are not toxic or are less toxic to normal cells. So it plays an important role in the prevention of cancer [22, 23]. In present study, ethyl acetate fraction which derived from the defatted methanolic extracts of *A. cepa* (ssp. red onion) is the most active extract (IC_{50} = 6.08 µg/ ml) against human liver carcinoma cell lines HepG2 as shown in figure (1) and its activity is nearby doxorubicin (IC_{50} = 4.0 µg/ ml) which used as cytotoxic standard. The butanolic fraction and defatted methanolic extract have IC_{50} = 9.95 and 10.9 µg/ ml respectively lower than ethyl acetate fraction. Compound 4 (isorhamnetin-4`-*O*-β-D-gloucopyranoside) which isolated from EtOAc fraction has IC_{50} = 11.9 µg/ ml and compound 6 (β-D-glucopyranosyl- α- D-glucopyranoside) which isolated from n-BuOH fraction has IC_{50} = 22.0 µg/ ml. According to the National Cancer Institute guideline, an extract and/or a compound with IC_{50} values < 20 µg/mL is considered active on hepatocellular carcinoma cell line [24, 25]. These results indicated and confirmed that phenolic compounds especially flavonoids have high cytotoxic activity and it depend on the number of hydroxylation and glycosylation of phenolic compounds as reported by Kathrin *et al.,* 2007 [26] who stated that there is inverse correlation between the cytotoxicity and the number of hydroxyl phenolic groups of flavonoids.

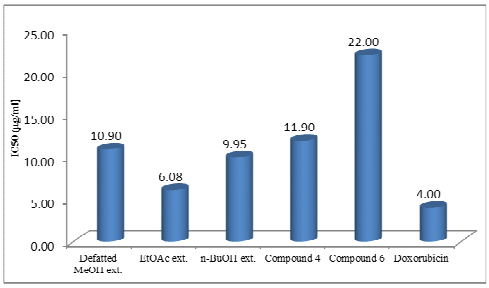


Fig. 1: Cytotoxic activity of defatted methanolic extract of *A. cepa* (ssp. red onion), derived fractions and its pure isolated compounds against Hep G2 cell line.

CONCLUSION

The present study showed the cytotoxic activity of defatted methanolic extract of *Allium cepa* (ssp. red onion) and is derived fractions (EtOAc and n-BuOH) as well as chromatographic isolation of their active chemical constituents. The results indicated that the two fractions contain phenolic and free sugars which have cytotoxic properties against Hep G2 cell line.

CONFLICT OF INTERESTS

Declared None

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