Original Research Article **Pharmacognosy**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

PHYTOCHEMICAL COMPOSITION, IN VITRO ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF SEEDS OF *CONVOLVULUS ARVENSIS* LINN.

TAGHREED A. IBRAHIM [*1,2], ATEF A. EL-HELA [3], NEVEIN M. ABD ELHADY [3] AND NABIL M. ABO-ELFETOH [2]

[1]pharmacognosy department, college of pharmacy, king saud university,p.o. box 2457, riyadh 11451, saudi arabia.
[2]pharmacognosy department, faculty of pharmacy, cairo university, cairo, egypt.
[3]pharmacognosy department, faculty of pharmacy, al-azhar university, cairo, egypt.

ABSTRACT

In vitro antioxidant and cytotoxic activities of methanol extract of the seeds of *Convolvulus arvensis* Linn. and its successive fractions; chloroform, ethyl acetate and *n*-butanol were evaluated. Total phenolics and flavonoid contents of methanol extract and ethyl acetate fraction were estimated. The highest antioxidant activity was achieved in ethyl acetate fraction (120.3±0.55 mg GAE) followed by methanol extract (84.8±0.62 mg GAE). The ethyl acetate fraction showed the strongest DPPH and nitric oxide free radical scavenging activities (IC_{50} 28.16±0.45 and 51.92±1.45 for DPPH and nitric acid, respectively). Ethyl acetate fraction showed the highest cytotoxic effect against Hela cell line (IC_{50} 17.33±0.58). From ethyl acetate fraction seven compounds were isolated and identified; gallic acid (1), ferulic acid (2), chlorogenic acid (3), quercetin (4), apigenin-7-*O*-glucoside (5), quercetin-7-*O*-rhamnoside (6) and quercetin-3-*O*-rutinoside (7) using different spectroscopic techniques. Therefore, methanolic extract and the ethyl acetate fraction of the seeds of *Convolvulus arvensis* are recommended to be used as natural antioxidant and cytotoxic agents.

KEYWORDS: **Antioxidant, Convolvulus arvensis, Cytotoxic, seeds, phenolic acids, flavonoid**

TAGHREED A. IBRAHIM
Pharmacognosy Department, College of Pharmacy, King saud University, p.o box 2457, Riyadh 11451, Saudi Arabia.

INTRODUCTION

There is extensive evidence to implicate free radicals in the development of degenerative diseases[1,2]. Synthetic antioxidants have been proved to have undesirable side effects; therefore, there has been an increasing interest in the substitution of synthetic antioxidants by natural ones[3]. Plants are good sources of natural antioxidants and some of these have significant antioxidative properties[4]. In addition to, plant materials represent promising sources of anticancer agents with lower side effect as compared with chemical drugs[5]. Phenolic compounds are natural plant-derived substances that have positive antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory and antimicrobial activities[6-8]. The Convolvulaceae family includes a large number of important plants, widely distributed all over the world in different localities, some of them have the properties of treatment of many diseases such as headache, constipation, rheumatism, diabetes and skin diseases[9,10]. The different members of the Convolvulaceae family possess cytotoxic effects against a number of tumor cells. Methanolic extract of *Ipomoea aquatica* showed cytotoxic activity which might be attributed to a polyphenolic compound[11]. Whereas in other *Ipomoea* species such as *I. pes-caprae*, *I. stan* and *I. murucoids* lipophilic glycoside compounds were responsible for cytotoxic activity of non-polar extracts[12-14]. *Convolvulus pluricalus*, *C. althaoieds*, *C. pleuricauas* and *C. hystrix* showed potent antioxidant activities capable of scavenging free radicals in different experimental models[15-18]. Convolvulus arvensis is a wild plant native to Europe and Asia and widely distributed in Egypt. It is climbing or creeping herbaceous perennial plant growing to 0.5- 2 m high[19]. Cytotoxic and antioxidant activities of different extracts of aerial parts, leaves, stems and roots of *C. arvensis* were studied[20,21]. The antioxidant activity of *C. arvensis* extracts are mainly due to phenolic contents such as flavonoids, phenolic acids, tannins and phenolic diterpenes[22,23]. *C. arvensis* may be a promising source of anticancer agents[24]. Different extracts of *C. arvensis* affect tumor angiogenesis and immune cell function that stimulate immune cells[25]. Angiogenesis inhibitors derived from natural sources include flavonoids, sulphated carbohydrates and triterpenoids[26]. Literature reviews revealed that no study has been carried out on antioxidant and cytotoxic activities of seeds of *C. arvensis*. Therefore, this study was conducted to evaluate the antioxidant and cytotoxic effect of *C. arvensis* seeds and to isolate some biologically active constituents.

MATERIALS AND METHODS

Plant material

The seeds of *Convolvulus arvensis* were collected in July 2012 from the road sides, waste land of Tanta region, AL-Gharbia Governorate, Egypt. The collected plant material was authenticated by Dr. Abdu Marey, Professor of Plant Taxonomy, Department of Botany, Faculty of science, Al Azhar University, Cairo, Egypt. A voucher specimen was retained and deposited at Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt. The collected seeds were cleaned, dried under shade at room temperature, and powdered. The powdered plant was kept in dark container.

General experimental procedures

A Hitachi U-200 spectrometer was used for determination of total phenolics and total flavonoids content. ESI-MS was carried out on TSQ700 triple quadrupole instrument (Finningan, San Jose, CA, USA) [1]. H-NMR (500 MHz, DMSO-d_6) and ^{13}C-NMR (125 MHz, DMSO-d_6) were carried on Bruker 500 and JEOL-GX- spectrometer. UV spectra were investigated in methanol on a spectrophotometer (Milton Roy 601). Melting points were measured on a Kofler hot-stage apparatus. Silica gel 60 GF_{254} (Fluka, Buchs, Switzerland) was used for TLC. Sephadex LH-20 (25-100 µm, Sigma-Aldrich Chemie, Steinheim, Germany) and silica gel (70-230 mesh, Merck, Germany) were used for column chromatography. Whatmann No. 1 and 3 paper sheets (Whatmann, England) were used for paper chromatography. Spots were visualized by UV light (λ_{max} 254 nm & λ_{max} 365 nm), and spraying with different spray reagents; ethanolic $AlCl_3$ (2%) or 10% H_2SO_4 followed by heating.

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), Folin Ciocalteu's phenol reagent, ammonium molybidate, sodium nitroprusside, quercetin, and gallic acid were purchased from Sigma-Aldrich Chemie, Steinheim, Germany. Rutin, L- Ascorbic acid, Tri chloroacetic acid (TCA), sulfanilic acid, naphthylethylenediamine dihydrochloride, aluminum chloride and ferric chloride were procured from Fluka, Buchs, Switzerland. HPLC-grade solvents were obtained from Merck, Darmstadt, Germany. The other chemicals used were of the highest quality available.

Cell line

Hela cell line (Cervical cell line) was obtained frozen in liquid nitrogen (-180 ºC) from the American Type Culture Collection and were maintained in the National Cancer Institute, Cairo, Egypt, by serial sub-culturing. MTT assay was applied using Taxol (Sigma-Aldrich Chemie, Steinheim, Germany) as a positive control.

Extraction and isolation procedures

The powdered seeds of *Convolvulus arvensis* (500 g) were defatted with petroleum ether (60-80 °C), the dried marc was further extracted with 90% methanol at room temperature, the solvent was removed under reduced pressure to give (37.5 g) of methanol extract. The methanolic extract was dissolved in distilled water and successively fractionated with chloroform, ethyl acetate and *n*-butanol saturated with water[18]; the solvents were evaporated under reduced pressure to yield 4.0, 7.5 and 10.0 g, respectively.

Phytochemical analysis
Methanol extract, chloroform, ethyl acetate and *n*-butanol fractions were subjected to phytochemical screening using standard procedures[27,28]. Methanol extract and its successive fractions were subjected to TLC, paper and column chromatography using different mobile phases. Ethyl acetate fraction was subjected to UV scanning from 190- 400 nm using HPTLC.

Total antioxidant capacity
Methanol extract and its successive fractions were subjected to determination of total antioxidant capacity by the method described by Dasgupta and De[29]. The antioxidant capacity was expressed as the number of equivalents of gallic acid (mg GAE/g dry sample), a calibration curve of gallic acid concentration (µg/ml) against absorbance (nm) was established, [regression equation, y= 0.092+70.49; R^2= 0.745]. Results are presented in Table I.

DPPH radical-scavenging activity
The free radical scavenging activity of methanol extract and its successive fractions was determined using the method described by Braca et al[30]. The percent inhibition of activity was calculated as $[(A_o - A_e)/A_o] \times 100$ (A_o = absorbance without tested material; A_e = absorbance with tested material). IC_{50} values of the tested samples were compared with that of standard ascorbic acid, calibration curve was plotted using concentrations 5-80 µg/ml. The results were presented in Table II and Figure I.

Nitric oxide radical scavenging activity
For determining the nitric oxide radical scavenging activity[31,32]; two ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed, separately, with 0.5 ml of each of methanol extract, chloroform, ethyl acetate and *n*-butanol fractions at various concentrations (5-250 µg/ml). The mixtures were incubated at 25°C for 5 hr. Control experiments using equivalent amounts of buffer were conducted in an identical manner. From each of the incubated mixtures, 0.5 ml was taken out and added into 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. one ml naphthylethylenediamine dihydrochloride (0.1% w/v) was added followed by incubation at room temperature for 30 min before measuring the absorbance at 540 nm. Ascorbic acid is used as reference standard (5-80 µg/ml). Percentage inhibition was calculated by using the formula:
Percentage inhibition (%)= [Absorbance of control- Absorbance of test/ absorbance of control] X 100
The results were presented in Table III and Figures II& III.

In-vitro cytotoxicity assay
Cytotoxic effect of the methanol extract and its successive fractions against Hela cells was assayed colorimetrically, using 3-(4,5-dimethyl thiazol-2-yl)-2,5- diphenyl tetrazolium bromide (MTT) and compared with taxol as a positive control[33,34]. The negative control contained no sample or taxol while the positive control contained six concentrations of taxol from 64 to 2 µg/mL using two fold serial dilutions in DMSO. Absorbance was determined at 540 nm by an ELISA plate reader. Standard curve (absorbance against number of cells) for the cell line was plotted. Cell survival percentage was calculated based on the formula: Cell Survival %= A_t- A_b / A_c-A_b x 100At: Absorbance of tested concentration, Ab: Absorbance of blank, Ac: Absorbance of negative control. Percentage of cell survival in the negative control was assumed as 100. Results are presented in Table IV and Figure IV.

Determination of total phenol content
The total phenolic content in the methanol extract, chloroform, ethyl acetate and *n*-butanol fractions was determined spectrophotometrically using Folin-Ciocalteu reagent[35]. The total phenolic contents were expressed as gallic acid equivalents (GAE), in mg/g dry sample. Calibration curve of standard gallic acid concentration (µg/ml) against the absorbance (nm) was constructed, [regression equation, y= 6.026x + 0.039; R^2 = 0.998]. Concentration of total phenol content in the plant tested samples was calculated from the expression [C= c x (V/m)], where C, is the total phenolic content of tested samples in mg/g (GAE); c, is the concentration of gallic acid established from calibration curve (mg/g); V, is the volume of the extract (ml) and m is the weight of tested samples (g). The results are tabulated in Table I.

Determination of total flavonoids
Determination of the total flavonoid content in the methanol extract, chloroform, ethyl acetate and *n*-butanol fractions was done colorimetrically by using aluminum chloride solution[36]. Standard curve was constructed using different concentrations of rutin in methanol (six serial 2 fold dilution to give 100–3.2 µg /ml). Regression equation; y= 17.23x - 0.0582, R^2= 0.999. Concentration of total flavonoids was calculated as mg rutin equivalent (mg RE/g dry sample). The results are tabulated in Table I.

Statistical analysis
All experiments were run in triplicate and statistical analyses were performed using SPSS software. The results were given as means ± standard deviation. IC_{50} values were determined by interpolations.

Chromatographic separation of ethyl acetate extract
As ethyl acetate fraction showed the highest biological activity and proved to contain the highest phenolic and flavonoid content, it was subjected to chromatographic analysis. The ethyl acetate fraction (3 g) was subjected to

column chromatography (3 x 80 Cm) packed with silica gel (100 g, 70-230 mesh, Fluka). The elution was started with chloroform followed by a gradient mixture of methanol, till washing with 100% methanol. Factions (50 ml) were collected, concentrated and examined by TLC (*n*-butanol: acetic acid: water; 7:3:1 v/v/v) and PC using solvent system 15 % acetic acid. Fractions were pooled according to their chromatographic behavior into 15 fractions. Fraction 3 (350 mg, eluted with chloroform: methanol 70:30, v/v) was subjected to further chromatographic purification on Sephadex LH-20 column (20 g, 2 x 50 Cm) using methanol as eluent to yield compounds 1, 2 and 3. Fraction 9 eluted with chloroform: methanol 40:60 v/v (214 mg), contained a mixture of two major compounds, it was subjected to preparative paper chromatography (PPC, 3 MM and 15 % Acetic acid as eluent system) to give compounds 4 and 5. Fraction 12 (40 mg) and 13 (33 mg), eluted with chloroform: methanol 20: 80 v/v and 10: 90 v/v, respectively were separately purified on Sephadex LH-20 column (10 g, using 80 % MeOH and pure methanol as eluent) to give compounds 6 and 7, respectively.

Gallic acid (1)
140 mg, white amorphous powder, m.p. 260 ^{o}C, UV λ_{max} (MeOH): 215, 271 nm; ^{1}H-NMR (DMSO-d_6): 6.90 (1H, *s*, H-2,6); ^{13}C-NMR (DMSO-d_6): 121.2 (C-1), 109.7 (C-2 & C-6), 145.3 (C-3 & C-5), 138.4 (C-4), and 168.5 (C-7); ESI-MS (negative mode) m/z 169 [M-H].

Ferulic acid (2),
45 mg, white crystals, m.p. 228 ^{o}C, UV λ_{max} (MeOH): 219, 230 and 422 nm; ^{1}H-NMR (MeOD): 8.82 (1H, *s*, COOH), 7.50 (1H, *d*, *J* = 20 Hz H-C=C), 7.14 (1H, *d*, *J* = 2.5 Hz, H-a), 7.1(1H, *dd*, *J* = 2.5 & 2 Hz H-C), 6.80 (1H, *d*, *J* = 9 Hz, H-b), 6.20 (1H, *d*, *J* = 20 Hz, H-1), 3.81 (3H, *s*, OCH_3); ^{13}C-NMR (MeOD) δ 167.4 (C-9), 149.5(C-4), 146.6 (C-3), 144.7 (C-7), 127.5 (C-1), 123.6 (C-6), 117.0 (C-5), 112.5 (C-2), 56.78 (CH_3). MS: m/z 194, 151, 133, 105 and 77.

Chlorogenic acid (3),
67 mg, white crystals, m.p. 208 ^{o}C, UV λmax (MeOH): 325 nm; ^{1}H-NMR (DMSO-d_6): δ 12.20 (*s*, br, 1H, COOH), 9.50 (*s*, broad, 1H), 9.10 (*s*, broad, 1H), 7.40 (*d*, *j*= 15.0 Hz, 1H), 7.01 (*d*, *j*= 1.6 Hz, 1H), 6.93 (*dd*, *j*= 8.0 & 1.6 Hz, 1H), 6.74 (*d*, *j*= 8.0, 1H), 6.14 (*d*, *j*= 15.0, 1H), 5.22 (*m*, 1H), 4.82 (*d*, *j*=3.6, 1H), 4.72 (*s*, br, 1H), 3.76- 3.53 (*m*, 4H), 2.55- 1.80 (*m*, 4H); ^{13}C-NMR (DMSO-d_6): δ 175.0 (COOH), 165.9 (OCOCH=CH-), 149.6, 145.4 (CH=CH), 144.8, 126.0, 121.6, 116.0 (CH=CH); MS: m/z 354, 353, 191, 180, 135.

Quercetin (4),
53 mg, yellow needles, m.p. 314 °C, UV λmax (MeOH): 255,269 nm; ^{1}H-NMR (DMSO-d_6): δ 7.72 (1H, *d*, *J* = 2.5 Hz, H-2'), 7.60 (1H, *dd*, *J* = 8.5 & 2.5 Hz, H-6`), 6.85 (1H, *d*, *J* = 8.5 Hz, H-5'), 6.35 (1H, *d*, *J* = 2.5 Hz, H-6) and 6.15 (1H, *d*, *J* = 2.5 Hz, H-8); ^{13}C-NMR (DMSO-d_6): δ 177.2 (C- 4), 165.2 (C-7), 162.5 (C-5), 158.4 (C-2), 149.2 (C-9), 148.1 (C-4'), 146.1 (C-3'), 138.3 (C-3), 124.3 (C-6'), 121.6 (C-1'), 116.2 (C-2'), 115.2 (C-5'), 104.6 (C-10), 99.2 (C-6) and 94.4 (C-8); MS: m/z 302, 281, 273, 207, 95, 81.

Apigenin-7-O-glucoside (5),
30 mg, yellow powder, m.p. 216-218 ^{o}C, UV λmax (MeOH): 267, 329 nm; ^{1}H-NMR (DMSO-d_6): δ 12.50 (1H, *s*, C5-OH), 7.66 (2H, *d*, *J* = 8.5 Hz, H-2', H-6'), 6.84 (2H, *d*, *J* = 8.5 Hz, H-3', H-5'), 6.82 (1H, *d, J* = 2.0 Hz, H-6), 6.72 (1H, *d*, *J* = 2.0 Hz, H-8), 6.52 (1H, *s*, H-3), 4.78 (1H, *d*, *J* = 7.7 Hz, H-1"); ^{13}C-NMR: δ 181.53 (C-4), 164.20 (C-2), 163.85 (C-5), 162.10 (C-4'), 160,25 (C-9), 159.73 (C-7), 128.20 (C-2',C-6'), 123.40 (C-1'), 116.22 (C-3', C-5') 109.22 (C-10), 106.29 (C-3), 104.49 (C-6), 99.37 (C-8), 103.30 (C-1"), 77.90 (C-5"), 76.29 (C-3"), 74.34 (C-2"), 70.89 (C-4"), 61.65 (C-6"); MS: m/z 431, 269, 225, 251, 241, 197, 117, 107.

Quercetin-7-O-rhamnoside (6),
15 mg, yellow amorphous, UV λ_{max} (MeOH): 254, 266 nm; ^{1}H-NMR (DMSO-d_6): δ 7.2 (1H, *d*, *J* = 2 Hz, H-2`), 7.4 (1H, *dd*, *J* = 8.5 & 2.1 Hz, H-6`), 6.9 (1H, *d*, *J* = 8.5 Hz, H- 5`), 6.3 (1H, *d*, *J* = 2.5 Hz, H-8), 6.2 (1H, *d*, *J* = 2.5 Hz, H-6), 5.3 (1H, *d*, *J* = 2.5 Hz, H-1 rhamnose), 1.4 (3H, *d*, *J* = 6 Hz, CH_3) , sugar protons at δ 3.0 – 4.0; ^{13}C-NMR (DMSO-*d*6): δ 149.00 (C-2), 134.82 (C-3), 164.0 (C-4), 159 (C-5), 99.84 (C-6), 163.22 (C-7), 92.50 (C-8), 155.21 (C-9), 103.24, (C-10), 120.93 (C-1`), 114.85 (C-2`), 144.93 (C- 3`), 146.72 (C-4`), 115.68 (C-5`), 119.74 (C-6`), 100.74 (C-1``), 68.10 (C-2``), 70.35 (C-3``), 70.63 (C-4``), 75.28 (C-5``), and 17.66 (-CH3); MS: m/z 448, 286.

Quercetin-3-O-rutinoside (7),
16 mg, yellow crystals, m.p. 241-243 ^{o}C, UV λ_{max} (MeOH): 256, 290, 355 nm; ^{1}H-NMR (DMSO –d_6): δ 8.13 (1H, *d*, *J* = 2 Hz, H2`), 7.83 (1H, *d*, *J* = 8 Hz, H-6`), 6.93 (1H, *d*, *J* = 8 Hz, H-5`), 6.69 (1H, *d*, *J* = 2 Hz, H-8) , 6.4 (1H, *d*, *J* = 2 Hz, H-6), 5.17 (1H, *d*, *J* = 7.50 Hz, H-1``), 4.56 (1H, *d*, *J* = 1.3 Hz, H-1```), 3.80 (1H, *dd*, *J* = 10 *&* 2 Hz, H-6``), 3.64 (1H, *dd*, *J* = 3.5 Hz, H-2```), δ 3.45-3.88 (6H, *m*, sugar protons) and 1.25 (3H, *d*, *J* = 6 Hz, CH_3); ^{13}C-NMR (DMSO –d_6): δ ppm 177,00 (C-4) 164. 67 (C-7), 161.54 (C- 5), 158.95 (C-2), 148.40 (C-9), 148.22 (C-4'), 144.80 (C-3'), 134.33 (C-3), 123.10 (C-6'), 122.62 (C-1'), 116.30 (C-2'), 114.65 (C-5'), 104.21 (C- 10), 98.52 (C-6), 93.45 (C- 8), 102.32 (C-1``), 75.10 (C-2``), 78.12 (C-3``), 72.21 (C-4``), 77.20 (C-5``), 61.32 (C-6``), 100.13 (C-1```), 71.82 (C-2```), 71.35 (C-3```), 72.84 (C-4```), 70.10 (C-5```), 17.42 (C-6```); MS: m/z 610, 609, 301.

RESULTS AND DISCUSSION

Phytochemical analysis

HPTLC spectral analysis of ethyl acetate fraction showed absorbance peaks with λ_{max} of 263 nm, 251 nm, 234 nm which are characteristic absorbance of flavonoids. Phytochemical screening revealed the presence of flavonoids, phenolic acids, tannins, steroids and/or triterpenes, glycosides and alkaloids.

Determination of total antioxidant capacity

Total antioxidant capacity of the methanol extract of the seeds of *C. arvensis* and its successive fractions is expressed as the number of equivalents of gallic acid[37,38] . From Table I; ethyl acetate fraction showed highest antioxidant activity followed by methanol extract (120.3 and 84. 8 mg GAE/g dry sample, respectively), while chloroform fraction showed the least total antioxidant capacity (36.8 mg GAE/g dry sample).

Determination of DPPH radical-scavenging activity

DPPH is a free radical compound and has widely been used to test the free radical scavenging ability of various samples[39-41]. The different tested samples under investigation extinguish DPPH (Table II) in a dose-dependent manner: [y= 0.2193x + 37.044 (R^2 =0.931), y= 0.1887x + 12.122 (R^2 =0.974), y= 0.193x + 44.566 (R^2 =0.958) and y= 0.1953x + 24.13 (R^2=0.918)] ($p < 0.05$) for methanol extract, chloroform, ethyl acetate and *n*-butanol fractions, respectively (Figure I). IC_{50} values were found to be the least in ethyl acetate fraction (28.16 µg/ml), followed by methanol extract (59.1 µg/ml), while n-butanol and chloroform fractions showed the least IC_{50} (132.46 and 200.73 µg/ml, respectively). Ethyl acetate fraction showed significant DPPH scavenging activity when compared with IC_{50} of standard ascorbic acid (IC_{50}= 10.33 µg/ml), Table II and Figure III.

Determination of Nitric oxide radical scavenging activity

Nitrite radical scavenging assay was carried out on the methanol extract of the seeds of *C. arvensis* and its successive fractions from a concentration of 5-250 µg/mL. Percentage free radical scavenging was plotted against the concentration of the tested samples as shown in Table III and Figure II. The antioxidant activity increased with an increase in concentration of the tested samples [y= 0.2084x + 34.644 (R^2 = 0.888), y= 0.1578x + 13.977 (R^2= 0.983), y= 0.2373x + 37.679 (R^2=0.923) and y= 0.1886x + 23.689 (R^2= 0.946)] ($p < 0.05$) for methanol extract, chloroform, ethyl acetate and *n*-butanol fractions, respectively. Ethyl acetate fraction was the most potent with IC_{50} 57.6 µg/ml, followed by methanol extract 79.6 µg/ml while chloroform and butanol fractions showed the least potency IC_{50} 226.63 and 139.51 µg/mL, respectively. Both methanol extract and ethyl acetate fraction showed significant nitric oxide fraction scavenging effect when compared with standard ascorbic acid with IC_{50} 21.07 µg/ml (y= 1.7477x + 13.168; R^2= 0.996).The over mentioned results are in agreement with previous study carried on the leaves of *C. arvensis* which showed that methanol extract and ethyl acetate fraction were the most potent as free radical scavenging in DPPH and nitric acid models; [23] and to the results of the study carried on the ethanolic extract of *C. arvensis* root.[21] The present study proved that the methanol extract of the seeds of *C. arvensis* and ethyl acetate fraction are more potent as a free radical scavenging agent than that of leaves (IC_{50} 131.03 ± 2.46 and 43.21 ± 4.45 in methanol extract and ethyl acetate fraction, respectively) in DPPH model and in nitric oxide model (130.12 ± 2.46 and 57.5 ± 4.45 in methanol extract and ethyl acetate fraction, respectively).

Determination of In-vitro cytotoxicity assay

According to Figure IV and Table IV, ethyl acetate fraction showed the highest cytotoxic effect followed by methanol extract (IC_{50} = 17.33 ± 0.58 and 28.52 ± 1.28 µg/ml, respectively) whereas chloroform and *n*-butanol fractions showed the least cytotoxic effect against Hela cells (53.50 ± 0.76 and 41.56 ± 1.26 µg/ml, respectively). Methanol extract of the seeds of *C. arvensis* and its successive fractions inhibited tumor growth in a dose dependant manner [y= 1.812x + 83.688 (R^2 = 0.8058), y= 1.9135x + 83.152 (R^2= 0.9244), y= 0.7621x + 86.265 (R^2= 0.7383) and y= 0.8725x + 86.265 (R^2= 0.7383)], for methanol extract, chloroform, ethyl acetate and butanol fractions, respectively. Polyphenolic compounds are proved to be cytotoxic[11]. In this study, preliminary phytochemical screening proved the presence of phenolic compounds and flavonoids in methanol extract and ethyl acetate fraction. These results are in agreement with that in a previous study which proved a cytotoxic effect of ethanol extract of aerial part of *C. arvensis* on Jurkat cell line[42].

Determination of the Total Phenol Content

The total phenol content of the methanol extract of seeds of *C. arvensis* and its successive fractions was determined using the Folin-Ciocalteu reagent in comparison with standard gallic acid, and the result was expressed in terms of mg GAE/g of dry sample. Ethyl acetate fraction showed the highest amount of phenol content followed by methanol extract (425.26 and 236.11 mg GAE/g dry sample, respectively). *n*-butanol fraction proved the presence of 142.43 mg GAE/g dry sample while chloroform fraction illustrated the least phenol content (33.45 mg GAE/g dry sample).

Determination of Flavonoid Contents

Colorimetric estimation of the total flavonoids of methanol extract of seeds of *C. arvensis* and its successive fractions calculated based on rutin proved that ethyl acetate fraction contains the highest amount followed by methanol extract, *n*-butanol fraction and chloroform fraction (124.53, 94.01, 43.48 and 22.62 mg RE/ g dry sample, respectively). Results in Table I revealed that there is a positive correlation between the total antioxidant, DPPH and nitric oxide

radical-scavenging activities of the methanol extract of the seeds of *C. arvensis* and its successive fractions and their phenolic and flavonoid contents. These results were in full agreement with several studies which proved that there is a linear correlation between the total phenolic and flavonoid contents and total antioxidant capacity and DPPH and nitric oxide scavenging activities of many plants[43-45].

Identification of the isolated compounds

The phytochemical investigation of the ethyl acetate fraction of the methanol extract of the seeds of *C. arvensis* afforded three phenolic acids (1-3) and four flavonoids (4-7), they are gallic acid **(**1), ferulic acid (2), chlorogenic acid (**3**), quercetin (4), apigenin-7-*O*-glucoside **(**5), quercetin-7-*O*-rhamnoside (6) and quercetin-3-*O*-rutinoside (7). Their structure elucidation was carried out through R_f-values, color reactions and spectral investigations (UV, ^{1}HNMR, ^{13}CNMR and MS)[46-48]. Spectral data of the known compounds were in good accordance with those previously published[18, 46-51]. These compounds (Figure V), were isolated for the first time from the seeds of *C. arvensis* while Compounds 2, 4, 6 and 7 were previously isolated from *C. hystrix* leaves[18].The antioxidant and cytotoxic activities of the ethyl acetate fraction of the methanol extract of the seeds of *C. arvensis* might be attributed to its content of phenolic acids, as gallic, ferulic and chlorogenic acids were proved to have antioxidant activity[52-54]; and cytotoxic activity[52,55]; in addition to its content of flavonoids as quercetin, apigenin-7-*O*-glucoside, quercetin-7-*O*-rhamnoside and quercetin-3-*O*-rutinoside were proved to have antioxidant and cytotoxic activities[56-60] .

Table I

Total antioxidant capacity, phenol and flavonoid contents of the tested samples

Sample	Total antioxidant capacity (mg GAE/g dry sample)	IC_{50}		Total phenol content (mg GAE/g dry sample)	Total flavonoid content (mg RE/g dry sample)
		DPPH	Nitric oxide		
Methanol extract	84.8 ± 0.62	59.1 ± 1.46	73.69 ± 1.46	236.11 ± 1.06	94.01 ± 2.38
Chloroform fraction	36.8 ± 0.40	200.73 ± 1.67	228.28 ± 2.76	33.45 ± 0.43	22.62 ± 0.38
Ethyl acetate fraction	120.3 ± 0.55	28.16 ± 0.45	51.92 ± 1.45	425.26 ± 1.84	124.53 ± 2.66
n-butanol fraction	67.2 ± 0.39	132.46 ± 1.65	139.51 ± 2.30	142.43 ± 0.75	43.48 ± 1.47

Table II

Results of DPPH free radical scavenging activity of tested samples

Conc. (µg/ml)	Scavenging effect (%)				Ascorbic acid	
	Methanol extract	Fractions				
		Chloroform	Ethyl acetate	*n*-butanol	Concentration (µg/ml)	Scavenging effect (%)
5	33.2 ± 0.72	14.6 ± 0.47	40.5 ± 0.46	20.2 ± 0.83	5	38.3
50	45.5 ± 0.38	22.5 ± 0.44	55.6 ± 0.29	32.6 ± 0.62	10	53.8
100	67.7 ± 0.81	30.6 ± 0.52	68.4 ± 0.48	48.3 ± 0.77	20	62.2
150	74.2 ± 0.39	36.4 ± 0.80	76.6 ± 0.85	60.4 ± 0.39	30	78.6
200	80.4 ± 1.03	47.8 ± 0.39	82.5 ± 0. 79	64.5 ± 0.27	40	88.5
250	86.8 ± 1.29	63.3 ± 0.58	89.5 ± 0.41	66.2 ± 0.65	50	100

Values are Mean ± SD (n=3).

Table III

Results of nitric oxide free radical scavenging activity of tested samples

Conc. (µg/ml)	Scavenging effect (%)				Ascorbic acid	
	Methanol extract	Fractions				
		Chloroform	Ethyl acetate	*n*-butanol	Concentration (µg/ml)	Scavenging effect (%)
5	27.1 ± 0.07	15.8 ± 0.29	32.2 ± 0.51	20.5 ± 0.48	5	20.6
50	46.3 ± 0.52	22.5 ± 0.06	48.2 ± 0.08	33.2 ± 0.84	10	32.7
100	63.5 ± o.82	27.5 ± 0.64	70.6 ± 0.43	45.1 ± 0.37	20	48.4
150	72.7 ± 0.70	38.4 ± 0.69	78.4 ± 0.61	57.1 ± 0.88	30	62.9
200	75.8 ± o.48	43.2 ± 0.47	85.3 ± 0.50	63.4 ± 0.07	40	85.3
250	79.8 ± 0.44	55.6 ± 0.33	90.5 ± 0.84	65.2 ± 0.63	50	100

Values are Mean ± SD (n=3).

Table IV
Results of In-vitro cytotoxic effect of tested samples on Hela cells

Conc. (µg/ml)	Cell survival (%)				Taxol	
	Methanol extract	Fractions				
		Chloroform	Ethyl acetate	*n*-butanol	Concentration (µg/ml)	Cell survival (%)
1	94.3 ± 0.58	96.3 ± 1.03	92.6 ± 1.15	98.7 ± 0.58	2	80.2 ± 0.38
2.5	82.0 ± 0.32	91.1 ± 0.67	82.1 ± 0.65	83.5 ± 0.54	4	72.5 ± 0.42
5	77.5 ± 0.57	82.7 ± 0.48	68.5 ± 0.65	76.4 ± 0.72	8	58.5 ± 0.54
10	60.4 ± 0.48	77.3 ± 0.69	55.3 ± 0.37	70.2 ± 0.58	16	46.8 ± 0.35
20	52.4 ± 0.26	75.3 ± 0.37	37.7 ± 0.76	64.6 ± 0.39	32	37.1 ± 0.75
40	42.8 ± 0.75	62.1 ± 0.55	12.5 ± 0.36	55.7 ± 0.61	64	10.0 ± 0.07

Values are mean ± SD (n=3)

Figure I
Free radical scavenging effect of the tested samples by DPPH

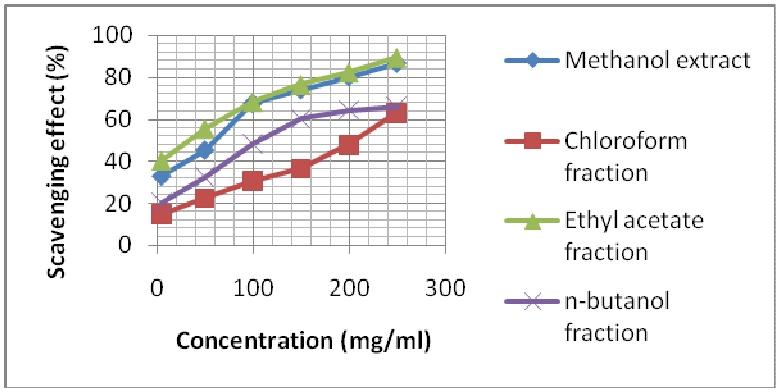


Figure II
Nitric oxide scavenging effect of the tested samples

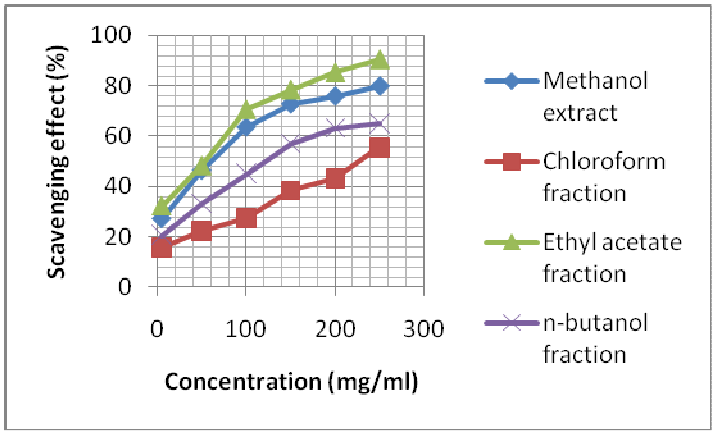


Figure III
Free radical scavenging effect of standard ascorbic acid by DPPH and nitric oxide

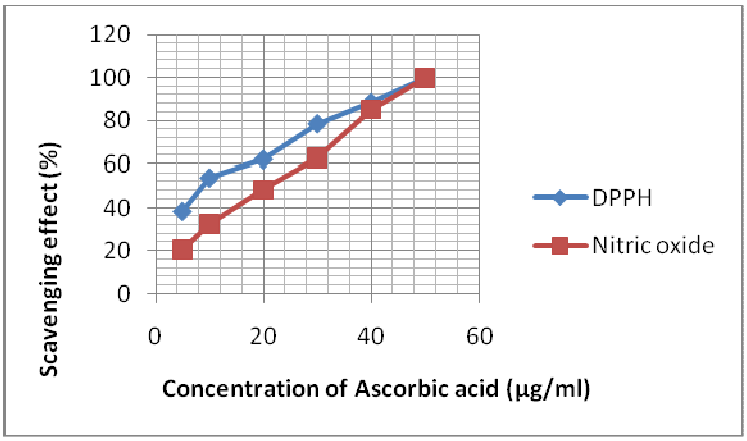


Figure IV
Results of in vitro cytotoxic effect of tested samples on Hela cells

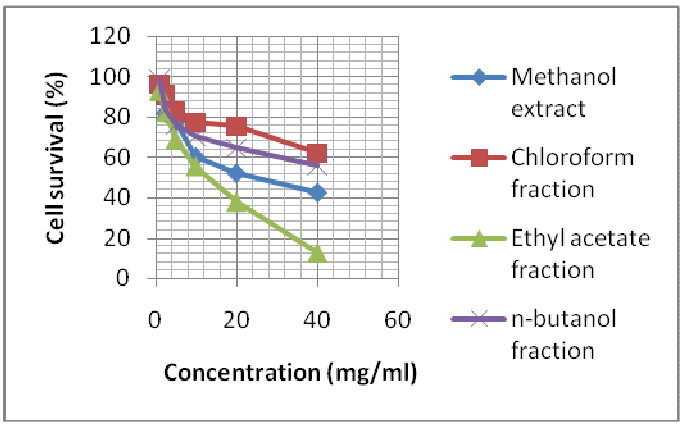
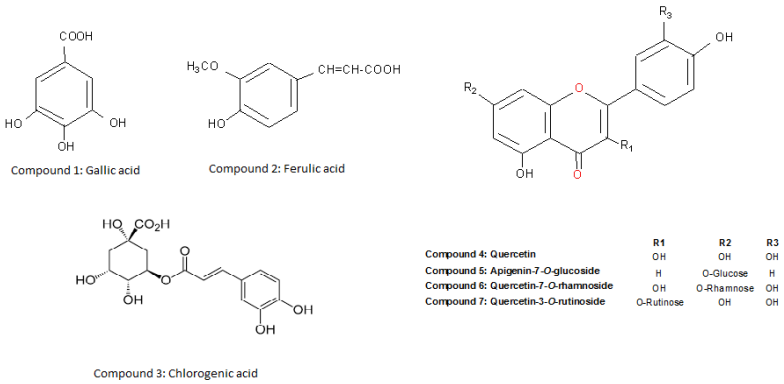


Figure V
Isolated compounds from ethyl acetate fraction of C. arvensis seeds



CONCLUSION

In conclusion, methanolic extract of *C. arvensis* seeds and its ethyl acetate fraction exhibited antioxidant and cytotoxic activities. These effects might be attributed to the detected and isolated phenolic acids and flavonoids. These results showed that *C. arvensis* seeds methanolic extract and ethyl acetate fraction could be considered as natural antioxidant with potential cytotoxic activity.

CONFLICT OF INTEREST
Conflict of interest declared none.

REFERENCES

1. Cross CE. Oxygen radicals and human diseases. Ann Intern Med. 1987;107:526-45.
2. Marx JL. Oxygen free radicals linked to many diseases. Science 1987;235:529-31.
3. Al-Soqeer A. Antioxidant activity and biological evaluation of hot-water extract of *Artemisia monosperma* and *Capparis spinosa* against lead contamination. Res J Bot. 2011;6:11-20.
4. Exarchou V, Nenadis N, Tsimidou M, Gerothanasssis IP, Troganis A, Boskou D. Antioxidant activities and phenolic composition of extracts from Greek oregano Greek sage and summer savory. J Agric and Food Chem. 2002;50:5294-5299.
5. Sadeghi-aliabadi H, Ghasemi N, Kohi M. Cytotoxic effect of *Convolvulus arvensis* extracts on human cancerous cell line. Res Pharm Sci. 2008;3(1):31-34.
6. Su XY, Wang Z, Liu J. *In vitro* and *in vivo* antioxidant activity of *Pinus koraiensis* seed extract containing phenolic compounds. Food Chem. 2009;117:681-86.
7. Caliskan O, Polat AA. Phytochemical and antioxidant properties of selected fig (*Ficus carica* L.) accessions from the Eastern Mediterranean region of Turkey. Sci. Hortic. 2011;128:473-78.
8. Anago E, Lagnika L, Gbenou J, Loko F, Moudachirou M, Sanni A. Antibacterial activity and phytochemical study of six medicinal plants used in Benin. Pak J Biol Sci. 2011;14(7):449-55.
9. Ibn Sina AH. Alkanun fil Tibb. Cairo: El Halaby Co.; 1968.
10. Al-Antaki D. Tazkarit Oli El-Albab. Cairo: El Halaby Co.; 1952.
11. Prasad KN, Ashok G, Rajhu C, Shivamurthy GR, Vijayan P, Aradhya SM. *In vitro* cytotoxic properties of *Ipomoea aquatica* leaf. Indian J Pharmcol. 2005;37(6):397-98.
12. Leon I, Enriquez RG, Gnecco D, Villarreal ML, Cortes DA, Reynold WF, et al. Isolation and characterization of five new tetrasaccharide glycosides from the roots of *Ipomoea stan* and their cytotoxic activity. J Nat Prod. 2004;67:1552-1556.
13. Leon I, Enriquez RG, Nieto DA, Alonso D, Reynolds WF, Aranda E, et al. Pentasaccharide glycosides from the roots of *Ipomoea murucoides*. J Nat Prod. 2005;68(8):1141-1146.
14. Pereda-Miranda R, Escalante-Sanchez E, Escobedo-Martinez C. characterization of lipophilic pentasaccharides from beach morning glory (*Ipomoea pes-caprae*). J Nat Prod. 2005;68(2):226-30.
15. Tawaha K, Alali FQ, Gharaibeh M, Mohammad M, El-Elimat T. Antioxidant activity and total phenolic content of selected Jordanian plant species. Food Chem. 2008;104:1372-1378.
16. Parihar MS, Hemnanit T. Phenolic antioxidants attenuate hippocampal neuronal cell damage against kainic acid induced excitotoxicity. J Biosci. 2003;28(1):121-28.
17. Dini I, Tenore JC, Dini A. New polyphenol derivative in *Ipomoea batatas* tubers and its antioxidant activity. J Agric Food Chem. 2006;54(23):8733-8737.
18. Donia AM, Alqasoumi SI, Awaad AS, Cracker L. Antioxidant activity of *Convolvulus hystrix* Vahl and chemical constituents. Pak J Pharm Sci. 2011;24(2):143-47.
19. Tackholm V. Students' flora of Egypt. 2nd Ed. Cairo: Cairo University press, 1974.
20. Al-Asady AA, Suker DK, Hassan KK. Cytotoxic and cytogenetic effects on rhabdomyosarcoma (RD) tumor cell line *in vitro*. J Med Plants Res. 2014;8(15):588-98.
21. Nurul AM, Maria GG, Luis J, Lluis F. The effect of *Convolvulus arvensis* dried extract as a potential antioxidant in food models. Antioxidants 2015;4(1):170-84.
22. Awaad A, Jaber N. Antioxidant natural plant. RPM. Ethnomed. Source Mech. 2010;27:1-35.
23. Thakral J, Sakshi B, Roopa A, Kalia N. Antioxidant potential fractionation from methanol extract of aerial parts of *Convolvulus arvensis* Linn. (Convolvulaceae). IJPSDR. 2010;2(3):219-23.
24. Thatte U, Bagadey S, Dahanukar S. Modulation of programmed cell death by medicinal plants. Cell Mol Biol. 2000;46(1):199-214.
25. Kidd P. The use of mushroom glucans and proteoglycans in cancer treatment. Altern Med Rev. 2000;5(1):4- 27.
26. Paper D. Natural products as angiogenesis inhibition. Planta Med. 1998;64:686-95.
27. Harborne JB. Phytochemical methods. 3rd Ed. London: Chapman and Hall, 1998.
28. Trease GE, Evans WC. Pharmacognosy. 15th Ed. Edinburgh: Saunders/Elsevier, 2002.
29. Dasgupta N, De B. Antioxidant activity of *Piper betle* L. leaf extract *in vitro*. Food Chem. 2004;88(2):219–24.
30. Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I. Antioxidant principles from *Bauhinia terapotensis*. J Nat Prod. 2001;64(7):892–95.
31. Balakrishnan N, Panda AB, Raj NR, Shrivastava A, Prathani R. The evaluation of nitric oxide scavenging activity of *Acalypha Indica* Linn root. Asian J Res Chem. 2009;2(2):148-50.
32. Garrat DC. The quantitative analysis of drugs. 3rd Ed. Japan: Chapman and Hall, 1964.
33. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65(1-2):55-63.
34. Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. Cancer Res. 1987;47(4):936-42.
35. Zhou K, Yu L. Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in Colorado. LWT Food Sci Technol. 2006;39(10):1155–1162.
36. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002;10:178–82.

37. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem. 1999;269(2):337–41.
38. Ruchi GM, Majekodunmi OF, Ramla A, Gouri BV, Hussain A, Saud SA. Antioxidant capacity of some edible and wound healing plants in Oman. Food Chem. 2007;101(2):465–70.
39. Cuvelier ME, Bondet V, Berset C. Behavior of phenolic antioxidants in a partitioned medium: structure–activity relationship. JAOCS. 2000;77(8):819–27.
40. Sakanaka S, Tachibana Y, Okada Y. Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). Food Chem. 2005;89(4):569–75.
41. Shimoji Y, Tamura Y, Nakamura Y, Nanda K, Nishidai S, Nishikawa Y, et al. Isolation and identification of DPPH radical scavenging compounds in kurosu (Japanese unpolished rice vinegar). J Agric Food Chem. 2002;50(22):6501–6503.
42. Mohammad S, Imran Q, Bashir A, Uzma S, Faiza N, Valerie S, et al. Cytotoxic effect of ethanol extract of *Convolvulus arvensis* L (Convolvulaceae) on lymphoblastic leukemia Jurkat cells. Trop J of Pharm Res. 2014;13(5):705-09.
43. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenol and other oxidative substrates and antioxidants by means of Folin-Ciocalteu reagent. Method. Enzymol. 1999;299:152-78.
44. Khodaie LK, Bamdad S, Delazar A, Nazemiyeh H. Antioxidant, total phenol and flavonoid contents of two *Pedicularis* L., species from Eastern Azerbaijan, Iran. Bioimpacts. 2012;2(1):47-53.
45. Jackson RG, Marianna RD, Amanda LG, Ana PD, Luciano AD, Ana SS, et al. Phenolic quantification and antioxidant activity of *Anaxagorea dolichocarpa* and *Duguetia chrysocarpa* (Annonaceae). IJPBS. 2011;2(4):367-74.
46. Mabry TJ, Markham KR, Thomas MB. The systematic identification of flavonoids. New York, Heidlberg, Berlin: Springer-Verlag, 1970.
47. Markham KR. Techniques of flavonoid identification. London: Academic Press, 1982.
48. Agrawal PK. Carbone-13 NMR of flavonoids. New York: Elsevier Science Publishing Co. Inc., 1989.
49. Markham KR, Geiger H. The flavonoids: Advances in research since1986. London: Harborne, J.B., Ed., Chapman & Hall, 1994.
50. Nedime D, Seckin O, Esra U, Yasar D, Mustafa K. The isolation of carboxylic acids from the flowers of *Delphinium formosum.* Turk J Chem. 2001;25:93-97.
51. Meshram G, Patil B, Yadav S, Shinde D. Isolation and characterization of gallic acid from *Terminalia bellerica* and its effect on carbohydrate regulatory system *in vitro*. IJRAP 2011;2(2):559-62.
52. Kanai S, Okano H. Mechanism of the protective effects of sumac gall extract and gallic acid on the progression of CCl4-induced acute liver injury in rats. Am J Chin Med. 1998;26(3-4):333-41.
53. Picone P, Nuzzo D, Di Carlo M. Ferulic acid: a natural antioxidant against oxidative stress induced by oligomeric A-beta on sea urchin embryo. Biol Bull. 2013;224(1)18-28.
54. Kadoma Y, Fujisawa S. A comparative study of the radical-scavenging activity of the phenolcarboxylic acids caffeic acid, p-coumaric acid, chlorogenic acid and ferulic acid, with or without 2-mercaptoethanol, a thiol, using the induction period method. Molecules. 2008;13(10):2488-99.
55. Chiung-Chi P, Charng-Cherng C, Hui-Er W, Chi-Huang C, Kuan-Chou C, Kuang-Yu C, et al. Cytotoxicity of ferulic acid on T24 cell line differentiated by different microenvironments. BioMed Research International 2013;2013:Article ID 579859.
56. José C, Luis O, Najeh M, Vanderlan da Silva B, Omar A, Francine A, et al. Antioxidant and cytotoxic studies for kaempferol, quercetin and isoquercitrin. Eclet. Quím. 2011;36(2):7-20.
57. Lamson DW, Brignall MS. Antioxidants and cancer, part 3: quercetin. Altern Med Rev. 2000;5(3):196-208.
58. Said A, Tundis R, Hawas UW, El-Kousy SM, Rashed K, Menichini F, et al. *In vitro* antioxidant and antiproliferative activities of flavonoids from *Ailanthus excelsa* (Roxb.) (Simaroubaceae) leaves. Z Naturforsch C. 2010;65(3-4):180-6.
59. Legault J, Perron T, Mshvildadze V, Girard-Lalancette K, Perron S, Laprise C, et al. Antioxidant and anti-inflammatory activities of quercetin 7-O-β-D-glucopyranoside from the leaves of Brasenia schreberi. J Med Food. 2011;14(10):127-34.
60. El-Readi MZ, Antioxidant and cytotoxic activities of the flavonoid rutin. JISAHN. 2015;1(1):10-240.