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# Bioactive Ingredients and Anti-influenza (H5N1), Anticancer, and Antioxidant Properties of *Urtica urens* L.

Alaa A. Gaafar [1], Sami I. Ali [1,*], Omnia Kutkat [2], Ahmed M. Kandeil[2], Salwa M. El-Hallouty[3]

*[1]Plant Biochemistry Department, National Research Centre, Dokki, Giza, Egypt. P.O. 12622, ID: 60014618. [2]Center of Scientific Excellence for Influenza Viruses, Water Pollution Research, National Research Centre, Dokki, Giza, Egypt. P.O. 12622, ID: 60014618. [3]Pharmacognosy Department, National Research Centre, Dokki, Giza, Egypt. P.O. 12622, ID: 60014618.*

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**Abstract**

This study aims to emphasize the bioactive ingredients, antioxidant, anticancer, and anti-influenza (H5N1) effects of different extracts of *Urtica urens* L.. Three different extracts were prepared successively based on polarity yielding diethyl ether (DE), ethyl acetate (EA), and methanol (ME) extracts. GC-MS analysis revealed cyclopentasiloxane-decamethyl, quinine-1,1'-dioxide, and carotene as the prominent compounds in DE extract. The three extracts contain adequate amounts of total phenolics, total flavonoids, and total tannins and the superior amounts were detected in EA extract. Hesperidin, apigenin-6-arabinose-8-galactose, pyrogallol, and ferulic acid were the predominant individual phenolics identified and quantified in EA and ME extracts by HPLC, with a higher concentration in EA than in ME. The EA extract exhibited the highest antioxidant activity with $IC_{50}$ 306.15±2.27 and 103.32±0.34 µg/ml for DPPH and ABTS assays, respectively and $EC_{50}$ 352.50±2.50 µg/ml for reducing power assay. The DE and EA extracts have an efficient cytotoxicity effect against breast cancer cells (MCF-7) with $IC_{50}$ values 28.9 and 48.58 µg/ml and colon cancer cells (HCT116) with $IC_{50}$ values 39.81 and 41.21µg/ml, respectively. This is the first report regarding the antiviral effect of DE and ME extracts of *U. urens* against the avian influenza H5N1 virus. The DE exhibited the highest antiviral activity through the inhibition of H5N1 propagation with $IC_{50}$ value 125 µg/ml. The results of the mode of action against H5N1 virus showed the potent virucidal effect of DE extract with 80% inhibition of viral propagation. The DE extract showed a limited effect on H5N1 viral replication and viral adsorption process. The DE extract of *Urtica urens* exhibited the highest anticancer and anti-influenza (H5N1) effects besides adequate antioxidant activity and is recommended for future use in the pharmaceutical applications for H5N1 and cancer (breast and colon) treatment.

**Keywords:** anticancer; antioxidant; avian influenza; H5N1; phenolic profile; *Urtica urens*.

## 1. Introduction

Currently, pharmaceutical industries depend on plant bioactive ingredients as a primer source of many drugs. Bioactive secondary metabolites of different medicinal plants including phenolic compounds, coumarins, flavonoids, alkaloids, terpenes, and tannins possess an array of beneficial biological properties to human health. Medicinal plants are one of the main sources of effective, safe, and economical therapeutics in several diseases (Mohamed *et al.,* 2016; Ali *et al.,* 2018).

Viral diseases infecting humans and animals cause serious health and economic issues. The prevention and the controlling of such viral diseases are so important to human health, and enormous research has been assigned to antiviral measures especially from natural products (Lee *et al.,* 2018). A variety of studies described the antiviral action of plant-derived polyphenols on herpes simplex virus (Moradi *et al.,* 2016), and rotavirus Wa, adenovirus type 7, and coxsackievirus B4 (Mohamed *et al.,* 2015). Worldwide, infection with influenza A virus is one of the serious problems that threaten human health and several animal species. Global seasonal influenza epidemics are responsible for 291,000- 646,000 deaths annually, depending on the virulence and transmissibility of the circulating viral strain (Sah *et al.,* 2019). Influenza A virus cannot be controlled due to its high level of genes mutation. Consequently, the avian influenza virus can be invasive for the human population at any time causing a considerable pandemic (Yang *et al.,* 2013). The high ability of the highly pathogenic avian influenza H5N1virus to infect and kill humans, besides the growing resistance to the currently used anti-influenza drugs such as oseltamivir and zanamivir, made the need to find new, natural, safe, and effective antiviral drugs against influenza virus crucial (Lee *et al.,* 2018). The antiviral effects of a number of compounds (gossypol, procyanidin B2-di-gallate, and Poly-galloylglucose) isolated from different medicinal plants against influenza viruses were reported (Lee *et al.,* 2018; Yang *et al.,* 2013; Derksen *et al.,* 2014; Ge *et al.,* 2014).

Cancer is a set of diseases provoked through uncontrolled cell life cycle producing abnormal and

* Corresponding author e-mail: samiali.nrc@gmail.com.

uncontrolled cell growth. It is one of the leading diseases that threaten human life worldwide, causing high levels of mortality more than coronary heart diseases or all strokes. Chemotherapy, hormone therapy, radiotherapy, and surgery are the present treatments of cancer. However, every one of these traditional treatments has very bad side effects (Islam *et al.,* 2018). The high death prevalence and the severe side effects of anticancer drugs motivated the scientists to explore safe and more efficient anticancer agents with minor side effects from natural sources. Recently, a wide array of plant-derived compounds was discovered to treat cancer, including vincristine isolated from the leaves of *Catharanthus roseus*, paclitaxel, isolated from the bark of *Taxus brevifolia*, besides many other compounds derived from different medicinal plants (Velde *et al.,* 2017; Seca and Pinto, 2018).

Oxidative stress, resulting from the imbalance of oxidative molecules and antioxidant substances in the body, causes the degeneration of tissues and cellular macromolecules. It induces inflammation, cancer, early aging, diabetes, and cardiovascular and neurodegenerative diseases, besides other negative issues that affect human health (Elnakish *et al.,* 2013). The utilization of plant-derived compounds as safe and more effective antioxidant agents was recommended through many investigations owing to its ability to neutralize and scavenge the harmful reactive oxygen species thus minimize oxidative harm of body tissues and avoid several diseases (Ali *et al.,* 2018; Gaafar *et al.,* 2018).

*Urtica urens* L. (stinging nettle), a perennial herb of family Urticaceae, is a native wild flowering plant that grows widely in North Africa, Europe, Asia, and North America. It is famous by the stinging hairs that line its stem, leaves, and flowers, and provoke irritation to the skin (Fu *et al.,* 2006). The general ancient therapeutic usage of stinging nettle especially in ancient Egyptians is the remedy of rheumatism, lumbago, muscular paralysis, and arthritis (Upton, 2013). The recent studies reported that Urtica spp. has antiviral, antiulcer, anti-inflammatory, antioxidant, anticancer, antimicrobial, analgesic, and immunomodulatory activities (Akbay *et al.,* 2003; Manganelli *et al.,* 2005; Upton, 2013; D'Abrosca *et al.,* 2019). The different categories of essential therapeutic phytoconstituent existing in Urtica plant are terpenoids (Ganber and Spiteller, 1995), flavonol glycosides including kaempferol-3-o-glucoside, and -3-O-rutinoside; quercetin-3-O-glucoside, and -3-O-rutinoside, isorhamnetin-3-O-glucoside, -3-O-rutinoside, and -3-O-neohesperidoside (Akbay *et al.,* 2003), and phenolic acids such as chlorogenic and caffeoyl malic acid (Pinelli *et al.,* 2008).

Preceding *in vitro* studies reported the cytotoxic effect of hydro-alcohol crud extracts of Urtica spp. in different cancer cell lines, including breast (Fattahi *et al.,* 2013), non-small cell lung cancer (NSCLC) (D'Abrosca *et al.,* 2019), and prostate cancers (Durak *et al.,* 2004). In addition, the antiviral effect of aqueous extract of Urtica spp. against the feline immunodeficiency virus (FIV) infection was reported (Manganelli *et al.,* 2005). Hitherto, to the best of our information, no prior investigations have studied the therapeutic potential effects of different extracts of *U. urens* against avian influenza H5N1 virus, breast, and colon cancer cells, along with its antioxidant activity. Thus, this study aims to investigate the potential anti-influenza (H5N1), anticancer, and antioxidant activities of diethyl ether, ethyl acetate, and methanol extracts of *U. urens*. Besides, this study is also set to identify the main bioactive secondary metabolites of these extracts to explore their potential contribution to such biological activities.

## 2. Materials and Methods

### *2.1. Plant material*

The aerial part of *U. urens* was collected from the agricultural cultivated fields from El Sharkia government during February-2018. DR. Sameh Hussein identified the plant sample and deposited it in the Herbarium of the National Research Centre under the registered number 18975. Plant material was air-dried, then pulverized using a cutting mill.

### *2.2. Preparation of extracts*

The pulverized plant material (100 g) was soaked with diethyl ether (1000 ml, 3 times) in 2000 ml conical flask and kept on an orbital shaker (Stuart, England) at 160 rpm at room temperature for 24 h. Then, the extract was filtered through filter paper Whatman No.1 to get the diethyl ether filtrate. This step was repeated twice, and the pooled filtrates of diethyl ether were concentrated using a vacuum rotary evaporator (Heidolph Unimax 2010, Germany) at 40°C to dryness giving diethyl ether extract (DE). After diethyl ether extraction, plant residue was dried and re-extracted with ethyl acetate (1000 ml, 3 times) following the same steps to give ethyl acetate extract (EA). Then plant residue after ethyl acetate extraction was dried and re-extracted with methanol (1000 ml, 3 times) following the same steps to give methanol extract (ME). The dried crude extracts were re-dissolved in dimethyl sulfoxide (DMSO) for further analysis.

### *2.3. Phytochemical analysis*

#### *2.3.1. GC mass spectrometry (GC-MS) analysis*

The chemical profile of DE extract of *U. urens* was carried out using Thermo Scientific Capillary Gas Chromatography (model Trace GCULTRA) directly coupled to ISQ Single Quadruple MS and equipped with TG-5MS nonpolar 5% phenyl methylpolysiloxane capillary column (30 m × 0.25 mm ID × 0.25 μm). The operating condition of GC oven temperature was maintained as initial temperature 40$^{o}$C for 3 min, programmed rate 5$^{o}$C/min up to final temperature 280$^{o}$C with isotherm for 5 min. For GC-MS detection, an electronionization system with ionization energy of 70 eV was used. Helium was used as a carrier gas at a constant flow rate of 1.0 ml/min. Ten microliters of DE extract (1 mg/ml) were injected automatically in the splitless mode. Detection was performed in the full scan mode from 40 to 500 m/z. The quantification of the components was based on the total number of fragments (total ion count) of the metabolites as detected by the mass spectrometer. The identification of the chemical components was carried out based on the retention time of each component (Rt) compared with those of Wiley9 and NIST08 mass spectral libraries.

*2.3.2. Total phenolic content*

The total phenolic (TP) content was determined in DE, EA, and ME extracts of *U. urens* by Folin Ciocalteu reagent assay (Singleton and Rossi, 1965). A suitable aliquot (1 ml) of each extract was added to a 25 ml volumetric flask, containing 9 ml of distilled water. One milliliter of Folin Ciocalteu phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7 % $Na_2CO_3$ solution was added to the mixture. The solution was diluted to 25 ml with distilled water and mixed. After incubation for 90 min at room temperature, the absorbance was determined at 750 nm with a spectrophotometer (Unicum UV 300) against prepared reagent as blank. A total phenolic content in the sample was expressed as mg Gallic acid equivalents (GAE)/g dry weight. All samples were analyzed in triplicate.

*2.3.3. Total flavonoid content*

The aluminum chloride method was used for the determination of total flavonoid (TF) content in DE, EA, and ME extracts of *U. urens* (Zhishen *et al.,* 1999). One milliliter of each extract was added to a 10 ml volumetric flask containing 4 ml of distilled water. To the flask, 0.3 ml 5 % $NaNO_2$ was added and after 5 min 0.3 ml 10 %, $AlCl_3$ was added. At 6th min, 2 ml of 1M NaOH were added and the total volume was made up to 10 ml with distilled water. The solution was mixed well, and the absorbance was measured against prepared reagent blank at 510 nm by using spectrophotometer (Unicum UV 300). The total flavonoid in the sample was expressed as mg quercetin equivalents (QE)/ g dry weight. All samples were analyzed in triplicate.

*2.3.4. Total tannin content*

Total tannin (TT) of DE, EA, and ME extracts of *U. urens* was measured using the Folin-Ciocalteu reagent methods (Polshettiwar *et al.,* 2007). One milliliter of each extract was added to 7.5 ml distilled water then add 0.5 ml of Folin reagent and 1 ml of 35% sodium carbonate solution. The volume was made up for 10 ml with distilled water and absorbance was measured against prepared blank reagent (all the reagents with 1 ml methanol instead of the extract) at 775 nm by using a spectrophotometer (Unicom UV 300, England). Total tannins in samples were expressed as mg tannic acid equivalent (TAE)/g dry weight.

*2.3.5. HPLC analysis of individual phenolic and flavonoid compounds*

The dried crude EA and ME extracts (10 mg) were dissolved in 2 ml methanol HPLC spectral grade by vortex mixing for fifteen minutes. The extract was filtrated through a 0.2µm Millipore membrane filter. The phenolic and flavonoid compounds were identified by HPLC (Agilent Technologies 1100 series, Germany), equipped with a quaternary pump (G131A model). The separation was achieved on ODS reversed-phase column (C18, 25×0.46 cm i.d. 5 µm, Netherlands). The injection volume (35 µl) was carried out with an auto sampling injector. The column temperature was maintained at 35°C. Gradient phenolic compounds' separation was carried out with an aqueous formic acid solution 0.1 % (A) and methanol (B) as a mobile phase at a flow rate of 0.3 ml/min following the method of (Goupy *et al.,* 1999). In addition, the flavonoid compounds' separation was carried out with 50 mM $H_3PO_4$, pH 2.5 (solution A) and acetonitrile (solution B) as a mobile phase at a flow rate of 0.7 ml/min as described by Mattila *et al.* (2000). Elutes were monitored using a UV detector set at 280 nm for the phenolic acids, and at 330 nm for flavonoids. Chromatographic peaks were identified by comparing the retention times with the respective retention times of known standard reference material. Phenolic acids and flavonoid compounds concentration were calculated by comparing its peak areas with the peak areas of used standards (with known concentration) based on the data analysis of Hewlett Packard software. Phenolic acids and flavonoid compounds were expressed as µg/g, DW.

*2.4. Antioxidant capacity*

*2.4.1. DPPH• radical scavenging assay*

The $DPPH^{\bullet}$ (0.1 mM) in methyl alcohol was prepared and 0.5 ml of this solution was added to 1 ml of DE, EA, and ME extracts of *U. urens* at different concentrations (100, 200, 300, and 400 µg/ml). The mixture was shaken vigorously and was allowed to stand at room temperature in the dark for thirty minutes. Butylhydroxytoluene (BHT, Sigma Aldrich, St. Louis, MO, USA) was used as a positive control, whereas the negative control contained the entire reaction reagent minus the extract. Then, the absorbance was measured at 515 nm against DMSO (Chu *et al.,* 2000). The capacity to scavenge the $DPPH^{\bullet}$ radical was calculated using the following equation:

$$DPPH^{\bullet} \text{ scavenging effect (\%)} = (A_c - A_s / A_c) \times 100$$

Where $A_c$ is the absorbance of the control reaction and $A_s$ is the absorbance in the presence of the plant extracts. The results were expressed as $IC_{50}$ (the concentration (µg/ml) of the plant extract that scavenges 50 % of $DPPH^{\bullet}$ radical).

*2.4.2. ABTS•+ antioxidant assay*

Briefly, $ABTS^{\bullet+}$ was dissolved in double distilled water to 7.4 mM concentration, and potassium persulphate was added to a concentration of 2.6 mM. The working solution was prepared by mixing the two stock solutions in equal quantities. They were allowed to react for 12-16 hours at room temperature in the dark. The solution was then diluted by mixing 1 ml of the $ABTS^{\bullet+}$ solution with 60 ml of methanol to obtainabsorbance of 1.1 ± 0.02 at 734 nm using the spectrophotometer (Arnao *et al.,* 2001). The DE, EA, and ME extracts of *U. urens* (150 µl) at different concentrations (50, 100, 150, and 200 µg/ml) were allowed to react with 2850 µl of the freshly prepared $ABTS^{\bullet+}$ solution for two hours in the dark at room temperature. Then the absorbance was recorded at 734 nm. Trolox was used as a positive control. $ABTS^{\bullet+}$ scavenging activity (%) was calculated using the equation:

$$ABTS^{\bullet+} \text{ scavenging (\%)} = (A_0 - A_1) / A_0) \times 100$$

Where $A_0$ is the $ABTS^{\bullet+}$ absorbance of the control (the entire reaction reagent minus the sample), and $A_1$ is the $ABTS^{\bullet+}$ absorbance in the presence of the sample. The results were expressed as $IC_{50}$ (the concentration (µg/ml) of the plant extract that scavenges 50 % of $ABTS^{\bullet+}$ radical).

*2.4.3. Reducing power assay*

The reducing power was assayed as described by Kuda *et al.* (2005). One milliliter of DE, EA, and ME extracts of *U. urens* at different concentrations (50, 100, 150, and 200

µg/ml) was mixed with 2.5 ml of phosphate buffer (50 mM, pH 7.0) and 2.5 ml of 1 % potassium ferricyanide. The mixture was then incubated at 50°C for twenty minutes. After the addition of 2.5 ml of trichloroacetic acid (10 %) to the mixture, centrifugation at 3000 rpm for ten minutes was performed. Finally, 1.25 ml from the supernatant was mixed with 1.25 ml of distilled water and 0.25 ml $FeCl_3$ solution (0.1 %, w/v). The absorbance was measured spectrophotometrically at 700 nm. BHT was used as positive control. The results were expressed as $EC_{50}$ (the concentration (µg/ml) of the plant extract that provided the reading of 0.5 absorbance at 700 nm).

*2.5. Cytotoxic activity*

*2.5.1. Cell lines and culture conditions*

Two human carcinoma cell lines obtained from the Karolinska Institute, Stockholm, Sweden viz human breast cancer cells (MCF-7) and human colon cancer cells (HCT116) were used in this assay. The MCF-7 and HCT116 cancer cells were maintained in DMEM medium (LonzaBiowahittkar, Belgium). Media were supplemented with 1% antibiotic-antimycotic mixture (10,000 Uml-1 potassium penicillin, 10,000 µg/ml streptomycin sulphate, 25 µg/ml amphotericin B and 1% L-glutamine).

*2.5.2. MTT assay*

Cell viability was investigated using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] assay as published previously by Mosmann (1983). Cell lines were incubated in an incubator with 5% $CO_2$ at 37 °C (Sheldon, TC2323, Cornelius, OR, USA). Cells were plated into 96-well microplates at a concentration of $10^4$ cells/well and allowed to stand for 24 h. The medium was aspirated, and fresh medium (without serum) was added to the cells with different concentrations (1.25-100 µg/ml) of DE, EA, and ME extracts of *U. urens* dissolved in DMSO. After 48h incubation, the medium was aspirated and 40 µl MTT solution (2.5 µg/ml DMSO) was added to each well and incubated for further 4 h. The final concentration of DMSO was less than 0.2%. The formazan crystals formed were dissolved and the reaction was stopped by adding 200 µl of 10% sodiumdodecyl sulfate (SDS) to each well for overnight at 37°C. The amount of formazan produced was measured at 595 nm with a reference wavelength of 620 nm as a background using a microplate reader (Bio-Rad Laboratories, model3350, USA). For the untreated cells (negative control), medium was added instead of the tested extracts. A positive control Adrinamycin® (doxorubicin) was used as a known cytotoxic natural agent giving 100% inhibition. Data were expressed as growth inhibition (%) using the following formula:

$$\text{Growth inhibition (\%)} = 100 - (A_{sample}/A_{control}) \times 100.$$

where; $A_{sample}$ is the absorbance of treated cells with extract, and $A_{control}$ is the absorbance of untreated cells. The results were expressed as $IC_{50}$ (the concentration (µg/ml) of the plant extract that inhibits 50% of cancer cell growth).

*2.6. Antiviral activity*

*2.6.1. MTT assay*

To determine $TC_{50}$ value for each extract, MTT assay was applied for Madin Darby Canine Kidney (MDCK) cells as previously described by Mossman (1983) with minor modification. Briefly, MDCK cells were cultured in 96 well-plates (100 µl/well at a density of $3\times10^5$ cells/ml) and incubated for 24 h at 37°C in 5% $CO_2$. After 24 h, cells were treated with serially diluted extracts ranged from 100 - 800 µg/ml: in triplicates. After further 24 h, the supernatant was discarded, and cell monolayers were washed with sterile phosphate buffer saline (PBS) 3 times and MTT solution (20 µl of 5 mg/ml stock solution) was added to each well and incubated at 37 °C for 4 h 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). The absorbance of formazan solutions was measured at $\lambda_{max}$ 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.

$$\% \text{ Cytotoxicity} = (A_c - A_s/A_c) \times 100$$

Where; $A_c$: Absorbance of cells without treatment, and $A_s$: Absorbance of cells with treatment

Data were expressed as $EC_{50}$ that represents the concentration of extract causing 50% cytotoxicity against MDCK cells corresponding to the control (without treatment).

*2.6.2. Plaque reduction assay*

The antiviral activities of DE, EA, and ME extracts of *U. urens* were determined by plaque reduction assay (Schuhmacher *et al.*, 2003). Briefly, MDCK cells were seeded in 6 well-culture plates ($10^5$cells/ml) and incubated for 24 h at 37°C in 5% $CO_2$. Previously titrated A/chicken/Egypt/B13825A/2017 (H5N1) virus was diluted to optimal virus dilution, which gave countable plaques/well, and mixed with the safe concentration of each tested extracts of *U. urens*. The virus was incubated for an hour at 37°C before being added to cells. The medium was removed from the 6-well cell culture plates and virus-compound mixtures inoculated in duplicate. After 1 h contact time for virus adsorption, 3 ml of DMEM supplemented with 2% agarose, 1% antibiotic antimycotic mixture, and 4% bovine serum albumin (BSA, Sigma) were added to the cell monolayer. The plates were left to solidify and incubated at 37°C until the formation of viral plaques (3 days). Formalin (10%) was added to each well for 1 h and the over layer removed. Fixed cells were stained with 0.1% crystal violet in distilled water. The untreated virus was included in each plate as a control. Finally, plaques were counted and the percentage reduction in virus count was recorded as follows:

% inhibition= viral count (untreated) - viral count (treated)/viral count (untreated) x 100

Data were expressed as $IC_{50}$ that represents the concentration of extract causing 50% inhibition on the propagation of H5N1 virus corresponding to the control (without extract).

The selective index per extract was determined from the ratio of $EC_{50}/IC_{50}$

*2.6.3. Mode of antiviral activity*

*2.6.3.1. Viral replication*

The assay was carried out in a 6 well plate where MDCK cells were cultivated ($10^5$ cell/ml) for 24 h at 37°C. The virus was diluted to give $10^1$ to $10^2$ PFU/ well and

applied directly to the cultured cells and incubated for 1 hour at 37°C; unabsorbed viral particles were removed by washing cells three successive times by supplements free-medium. The effective plant extract was applied at different concentrations, after 1hour of contact time; 3 ml of DMEM medium supplemented with 2% agarose was added to the cell monolayer. Plates were left to solidify and incubated at 37°C till appearance of viral plaques. Cell monolayers were fixed in 10% formalin solution for 2 h and stained with crystal violet (Schuhmacher *et al.,* 2003). Control wells were included where MDCK cells were incubated with the virus, and finally plaques were counted and percentage reduction in plaques formation in comparison to control wells was recorded as mentioned above.

*2.6.3.2. Viral adsorption*

MDCK cells were cultivated in a 6 well plate ($10^5$cell/ml) for 24 h at 37 °C. The effective plant extract was applied at different concentrations in 200 µl medium without supplements and co-incubated with the cells for 2 h at 4 °C. The unabsorbed extract was removed by washing cells three successive times with supplements free-medium then A/chicken/Egypt/B13825A/2017 (H5N1) virus diluted to give 10 to 100 PFU/ well was co-incubated with the pretreated cells for 1 h followed by adding 3 ml DMEM supplemented with 2% agarose (Schuhmacher *et al.,* 2003). Plates were left to solidify then incubated at 37 °C to allow formation of viral plaques, fixed and stained as above mentioned to calculate percentage reduction in plaques formation in comparison to control wells where untreated MDCK cells were directly infected with influenza virus (H5N1).

*2.6.3.3. Virucidal*

The assay was carried out in a 6 well plate where MDCK cells were cultivated ($10^5$ cell/ml) for 24 h at 37 °C. A volume of 200 µl serum-free DMEM containing 10 to 100 PFU forming was added to the concentration of the effective plant extract, after 1-hour incubation, the mixture was diluted using serum-free medium 3 times each 10-fold that still allows existence of viral particles to grow on MDCK cells but leaves nearly no extract and 100 µl of each dilution were added to the MDCK cell monolayer. After 1h contact time, DMEM over layer was added to cell monolayer (Schuhmacher *et al.,* 2003). Plates were left to solidify then incubated at 37 °C to allow the formation of viral plaques, fixed and stained as above mentioned to calculate percentage reduction in plaques formation in comparison to control wells where the cell was infected with virus that was not pretreated with the tested extract.

*2.7. Statistical analysis*

All tests were conducted in triplicate. Data are reported as means ± standard deviation (SD).

Analysis of variance and significant differences among means were tested by one-way ANOVA using the COSTAT computer package. The least significant difference (LSD) at $P \leq 0.05$ level was calculated.

## 3. Results and Discussion

Recently, the therapeutic use of plant bio-active ingredients in complementary and alternative medicine for diverse disease treatment is now increasing worldwide. These natural bioactive components may act as new effective drugs for different kinds of harmful and mortal diseases or serve as an initial synthesizer for developing and designing new drugs for such diseases (Tringali, 2011). Different extracts of *U. urens* investigated in this study showed different bioactive ingredients including volatile compounds, phenolic acids and flavonoid compounds with different biological activities including antioxidants, anticancer and antiviral activities.

*3.1. The GC-MS analysis of DE extract*

The GC-MS analysis of DE extract showed the presence of different volatile bioactive components of different chemical categories (Table 1). Oxygenated compounds are the most category identified in DE extract including cyclopentasiloxane-decamethyl (CAS) (4.58%), dodecamethyl-cyclohexasiloxane (4.37%), and cycloheptasiloxane-tetradecamethyl (3.54%). Nitrogen-containing compounds were identified also in DE extract with an adequate percent including quinine-1,1'-dioxide (4.73%), 19,20-Didehydroyohimbinone (2.47%), and papaveroline (1.86%). Besides, hydrocarbon compounds (carotene, 1.05%) were identified in DE extract by GC-MS (Table 1). These results are in consensus with the results of Dar *et al.* (2013) who identified several volatile bioactive components including octadecan-1-ol, 4,6-di-tert-butyl-m-cresol, 2,4-ditertbutylphenol, n-cetane, dodecane, and other compounds in the hexane extract of *U. dioica* collected from India using GC-MS analysis. 19,20-Didehydroyohimbinone, identified in this study, was isolated from different species of genus Aspidosperma and is one of the β-carboline alkaloids that possess antimicrobial and antitumor effects (Layne *et al.,* 2015). Correspondingly, papaveroline, identified in this study, is an alkaloid compound isolated hitherto from opium, and earlier studies have confirmed its phosphodiesterase inhibition effects (Castellani and Zagaria, 1978). β-carotene the major hydrocarbon compound identified in DE extract of *U. urens* in this study was formerly reported in different Urtica species with its antioxidant activity (Upton, 2013).

**Table 1.** GC-MS analysis of diethyl ether (DE) extract of *U. urens.*

| No. | RT | Compound Name | Area % | Molecular Formula |
|---|---|---|---|---|
| 1 | 9.09 | Papaveroline | 1.86 | $C_{16}H_{13}NO_4$ |
| 2 | 9.14 | 19,20-Didehydroyohimbinone | 2.47 | $C_{21}H_{22}N_2O_3$ |
| 3 | 9.22 | 3-Hydroxyandrosta-5,7,9(11)-trien-17-one | 1.99 | $C_{19}H_{24}O_2$ |
| 4 | 9.71 | Ethyl 3,7,12-trihydroxycholan-24-oate | 0.95 | $C_{26}H_{44}O_5$ |
| 5 | 13.67 | Cyclopentasiloxane, decamethyl (CAS) | 4.58 | $C_{10}H_{30}O_5Si_5$ |
| 6 | 16.40 | 3,5-Androstadien-17-one oxime | 0.95 | $C_{19}H_{27}NO$ |
| 7 | 17.36 | 7,8-Epoxylanostan-11-ol,3-acetoxy | 1.06 | $C_{32}H_{54}O_4$ |
| 8 | 18.37 | Dodecamethylcyclohexasiloxane | 4.37 | $C_{12}H_{36}O_6Si_6$ |
| 9 | 19.68 | Pregn-4-ene-3,11,20-trione,6,17,21-tris-[(trimethylsilyl)oxy],3,20-bis (Omethyloxime), (6á) | 1.95 | $C_{32}H_{58}N_2O_6Si_3$ |
| 10 | 20.53 | Quinine 1,1'-dioxide | 4.73 | $C_{20}H_{24}N_2O_4$ |
| 11 | 22.63 | Cycloheptasiloxane, tetradecamethyl | 3.54 | $C_{14}H_{42}O_7Si_7$ |
| 12 | 25.67 | Penta-2,4-dien-1-one,5-dimethylamino-1-[5-(4-dimethylamino) buta-1,3-dienyl-2-thienyl]- | 1.05 | $C_{17}H_{22}N_2OS$ |
| 13 | 26.22 | 5-[(2,4-Dinitrophenyl) hydrazono] pentan-2-ol | 1.03 | $C_{11}H_{14}N_4O_5$ |
| 14 | 26.46 | 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyl-octasiloxane | 2.38 | $C_{16}H_{50}O_7Si_8$ |
| 15 | 29.76 | 4-(4-Chlorophenyl)-2-(cyclopropyl)-6-[4-benzylpiperazinyl-1-yl] benzonitrile | 1.51 | $C_{27}H_{26}ClN_3$ |
| 16 | 33.23 | Plectaniaxanthin | 0.99 | $C_{40}H_{56}O_2$ |
| 17 | 35.33 | Quercetin 7,3',4'-trimethoxy | 1.07 | $C_{18}H_{16}O_7$ |
| 18 | 36.53 | 2,15-Heptadecadiene,9-(ethoxymethyl)- | 1.17 | $C_{20}H_{38}O$ |
| 19 | 37.73 | Acouenoside A | 0.98 | $C_{30}H_{46}O_9$ |
| 20 | 41.20 | Docosahexaenoic acid, 1,2,3-propanetriyl ester | 0.96 | $C_{69}H_{98}O_6$ |
| 21 | 42.05 | 5á-Cholestan-3-one, cyclic ethylene acetal | 1.36 | $C_{29}H_{50}O_2$ |
| 22 | 47.00 | Phorbol 12,13,20-triacetate | 1.59 | $C_{26}H_{34}O_9$ |
| 23 | 48.07 | Carotene | 1.05 | $C_{40}H_{56}$ |
| 24 | 48.53 | Ethyl isoallocholate | 1.66 | $C_{26}H_{44}O_5$ |
| 25 | 49.76 | (14á)3,19-Epoxyandrosta-5,7-diene,4,4-dimethyl-3-methoxy-17-methylthiomethoxy | 1.26 | $C_{24}H_{36}O_3S$ |

### *3.2. Total phenolic (TP), total flavonoid (TF), and total tannins (TT) contents*

The successive extraction of *U. urens* using polar gradient solvents of diethyl ether, ethyl acetate, and methanol showed a significant effect on the contents of TP, TF, and TT (Figure 1). Consistent with Folin-Ciocalteu test results, the highest amounts of TP and TT were detected in EA (160. 63±1.38 and 92.75±1.13 mg/g) followed by ME (68.10±0.63 and 42.56±0.83 mg/g), then DE (25.71±0.95 and 16.59±0.55mg/g), respectively. Correspondingly, the results of Aluminum chloride assay showed that the elevated amount of TF was detected in EA (51.67±0.37 mg/g), compared to ME (20.37±0.55mg/g), and DE (9.63±0.62mg/g) as presented in Figure 1. The contents of TP, TF, and TT of EA extract in this study were superior to the TP, TF, and TT contents of organic solvent extracts of different Urtica species from other countries (Mzid *et al.,* 2017; Zekovic *et al.,* 2017). This high content of TP, TF, and TT in EA extract is probably a result of the successive extraction of *U. urens* using polar gradient solvents that increased the liberation of more chemical compounds within different solvent fractions. A number of former studies reported the positive effect of successive extraction by polar gradient solvents on the high content of different bioactive compounds in different medicinal plants (Mohamed *et al.,* 2016; Zlotek *et al.,* 2016; Zekovic *et al.,* 2017).

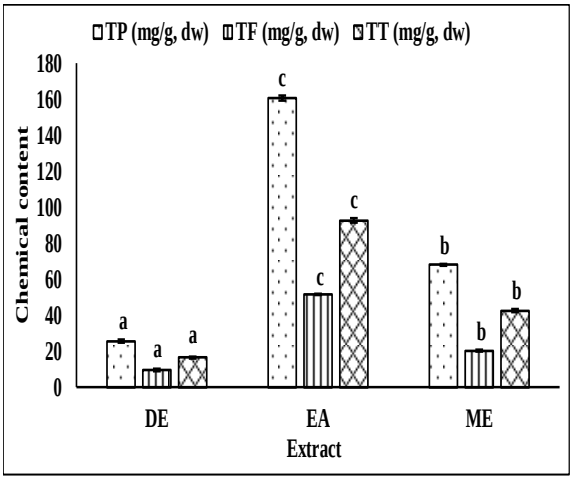


**Figure 1.** Content of total phenolic compounds (TP mg/g), total flavonoids (TF mg/g), and total tannins (TT mg/g) of diethyl ether (DE), ethyl acetate (EA), and methanol (ME) extracts of *U. urens.* Results are mean values ± standard deviations (n=3). Category bars with different letters are significantly different ($p \leq 0.05$).

### *3.3. HPLC identification and quantification of individual phenolics*

Thirty-four individual phenolics were identified and quantified by HPLC in EA and ME extracts of *U. urens* (Table 2). Depending on the chemical structure of phenolic compounds, the identified phenolic compounds were corresponding to six groups namely benzoic acid

derivatives (6 compounds), cinnamic acid derivatives (7 compounds), flavonols (5 compounds), flavanone (4 compounds), flavones (7 compounds), and other phenols (5 compounds) as presented in Table 2. Excepting of gallic acid, benzoic acid, and luteolin-7-glucose, which were identified only in ME extract while all the other individual phenolics were identified in both EA and ME, with a higher concentration in EA than in ME (Table 2). This might be due to the more liberal of phenolic compounds after successive extraction and the more recovery effect of ethyl acetate on the phenolic individuals in *U. urens*. The positive effect of used solvent in the content of total phenolics and phenolic individuals was previously reported (Zlotek *et al.*, 2016; Zekovic *et al.*, 2017). As revealed in Table 2, benzoic acid derivatives (0.82 mg/g vanillic acid and 0.22 mg/g p-OH-benzoic acid), cinnamic acid derivatives (1.14 mg/g ferulic acid and 0.59 mg/g chlorogenic acid), flavonols (1.10 mg/g quercetin and 0.79 mg/g kaempferol), flavanone (6.54 mg/g hesperidin and 1.72 mg/g naringin), flavones (5.56 mg/g apigenin-6-arabinose-8-galactose and 1.11 mg/g acacetin-7-O-rutinoside), and other phenolics (1.25 mg/g pyrogallol and 0.97 mg/g caffeine) were the predominant individual phenolics identified in EA extract. The HPLC phenolic profile of *U. urens* in this study is in good accord with earlier results of Otles and Yalcin (2012) and Zekovic *et al.* (2017); they identified various phenolic compounds and flavonoids in *U. dioica* including gallic acid, ferulic acid, protocatechuic acid, kaempferol, quercetin, apigenin, naringenin, catechin, luteolin-7-O-glycoside, rutin, quercitrin, and other phenolics. Other findings identified and quantified varied contents of phenolic compounds and flavonoids in different extracts of Urtica spp. from different countries (Carvalho *et al.*, 2017; Mzid *et al.*, 2017; Vajic *et al.*, 2018). In this context, the contents of chlorogenic acid (15.3±0.21 mg/g) and rutin (6.01±0.09 mg/g) in *U. dioica* (Vajic *et al.*, 2018) were greater than their contents in *U. urens* in this study, while the contents of ferulic acid (1.14 mg/g), kaempferol (0.79 mg/g), quercetin (1.10 mg/g), apigenin (0.35 mg/g), and naringenin (0.62 mg/g) in *U. urens* were greater than their contents in *U. dioica* reported by Zekovic *et al.* (2017). This variation could be caused by varying geographical and ecological growing conditions where the plant has been collected (Hegazy *et al.*, 2019).

**Table 2.** HPLC identification and quantification of phenolic compounds of ethyl acetate (EA), and methanol (ME) extracts of *U. urens.*

| Phenolic compound | Concentration (mg/g, extract) | | Phenolic compound | Concentration (mg/g, extract) | |
|---|---|---|---|---|---|
| | E A | ME | | EA | ME |
| Benzoic acid derivatives | | | flavanone | | |
| Gallic acid | - | 0.11 | Narengin | 1.72 | 0.20 |
| Protocatchuic acid | 0.21 | 0.18 | Hesperidin | 6.54 | 5.95 |
| *p*- OH- benzoic acid | 0.22 | - | Naringenin | 0.62 | 0.08 |
| 4-Amino-benzoic acid | 0.12 | 0.04 | Hespirtin | 0.29 | 0.02 |
| Benzoic acid | - | 0.35 | Flavones | | |
| Vanillic acid | 0.82 | 0.28 | Apigenin-6-arabinose-8- galactose | 5.56 | 5.60 |
| Cinnamic acid derivatives | | | Apigenin-6-rhamnose 8- glucose | 0.08 | 0.03 |
| Chlorogenic acid | 0.59 | 0.15 | Luteolin-7-glucose | - | 0.39 |
| Caffeic acid | 0.04 | 0.02 | Apigenin-7-glucose | 0.15 | 0.01 |
| Rosmarinic acid | 0.06 | 0.04 | Apigenin-7-O-neohespiroside | 0.85 | 0.20 |
| Ferulic acid | 1.14 | 0.10 | Acacetin-7-O-rutinoside | 1.11 | 0.39 |
| Iso- Ferulic acid | 0.14 | 0.04 | Apigenin | 0.35 | 0.02 |
| *α*- Coumaric acid | 0.05 | 0.03 | Other simple phenols | | |
| 3,4,5-Methoxy-cinnamic acid | 0.24 | 0.11 | Pyrogallol | 1.25 | 4.90 |
| Flavonols | | | Caffeine | 0.97 | 0.06 |
| Quercetin | 1.10 | 0.39 | Coumarin | 0.57 | 0.07 |
| Kaempferol | 0.79 | 0.24 | Oleuropin | 0.54 | 0.22 |
| Catechein | 0.69 | 0.25 | Catechol | 0.04 | 0.06 |
| Quercetrin | 0.49 | 0.05 | | | |
| Rutin | 0.31 | 0.05 | | | |

### *3.4. Antioxidant activity*

The antioxidant activity of DE, EA and ME extracts of *U. urens* was assessed using DPPH, ABTS, and reducing power assays. The results are given in Table 3 and expressed as $IC_{50}$ µg/ml for DPPH and ABTS and as $EC_{50}$ µg/ml for reducing power. Consistent with DPPH, ABTS, and reducing power assays data, the EA extract exhibited the highest activity with $IC_{50}$ 306.15±2.27 and 103.32±0.34 µg/ml for DPPH and ABTS assays, respectively and $EC_{50}$ 352.50±2.50 µg/ml for reducing power assay followed by DE then ME; however, EA extract showed lower antioxidant activity than the antioxidant standards Trolox and BHT (Table 3). These results are in consensus with hitherto published findings stated the antioxidant activity of Urtica spp. using DPPH, ABTS and reducing power assays (Gulçin *et al.*, 2004; Otles and Yalcin, 2012; Carvalho *et al.*, 2017; Zekovic *et al.*, 2017). The high antioxidant activity of EA extract in this study might be owing to its high content of TP, TF, TT, and individual phenolics and flavonoids when compared to DE and ME extracts as presented in Figure 1 and Table 2. Numerous studies reported the association of TP, TF, TT, and individual phenolics and flavonoids with the antioxidant activities of different plants (Ibrahim *et al.*, 2015; Gaafar *et al.*, 2016; Ali *et al.*, 2018; Gaafar *et al.*, 2018). A significant variation among antioxidant activity of Urtica spp. extracts has been reported based on the used

solvent. Water extract of *U. dioica* exhibited reducing power activity ($EC_{50}$ of 110 mg/ml) and DPPH scavenging activity (37%) at 60 µg/ml concertation (Gulçin *et al.,* 2004). The 50% aqueous ethanol extract of *U. dioica* exhibited DPPH scavenging activity (2.89±0.33 Trolox equivalents) and ABTS scavenging activity (2.60±0.14 Trolox equivalents) as descried by Carvalho *et al.* (2017). Besides, the 80% hydroalcoholic extract of *U. dioica* and its sub-fractions petroleum ether, ethyl acetate, n-butanol and water revealed DPPH scavenging activity with $IC_{50}$ values 140, 215.96, 78.99, 168.24 and 302.90 µg/ml, respectively (Joshi *et al.*, 2015).

**Table 3.** Antioxidant activity: DPPH ($IC_{50}$ µg/ml), ABTS ($IC_{50}$ µg/ml), and reducing power ($EC_{50}$ µg/ml) of diethyl ether (DE), ethyl acetate (EA), and methanol (ME) extracts of *U. urens.*

| Extracts | $IC_{50}$ µg/ml | | $EC_{50}$ µg/ml |
|---|---|---|---|
| | DPPH | ABTS | Reducing Power |
| DE | $378.52^{c}$ ± 6.40 | $113.91^{c}$± 1.26 | $382.67^{b}$±0.58 |
| EA | $306.15^{b}$ ± 2.27 | $103.32^{b}$ ± 0.34 | $352.50^{c}$±2.50 |
| ME | $696.21^{d}$ ± 2.18 | $220.68^{d}$ ± 0.12 | $678.33^{d}$±1.44 |
| BHT | $3.30^{a}$ ± 0.18 | - | $3.29^{a}$ ± 0.14 |
| Trolox | - | $4.83^{a}$±0.01 | - |

Results are mean values ± standard deviations (n=3). Means followed by the different letters in a column are significantly different ($p \leq 0.05$). Trolox and Butylated hydroxytoluene (BHT) was used as standard.

### *3.5. Anticancer activity*

The MTT cytotoxicity assay data illustrated in Figure 2 indicated that DE and EA extracts have an efficient cytotoxicity effect against breast cancer cells (MCF-7) with $IC_{50}$ values 28.9 and 48.58 µg/ml and colon cancer cells (HCT116) with $IC_{50}$ values 39.81 and 41.21µg/ml, respectively as compared with ME extract. The present results are in accord with a number of previous studies indicated the cytotoxic effect of Urtica plant against different human cancer cells including breast (MCF-7) (Abu-Dahab and Afifi, 2007; Fattahi *et al.*, 2013), human colon cancer (HCT-116) and human prostate cancer (PC3) (Mohammadi *et al.*, 2016), human colon cancer (HT29) and human gastric cancer (MKN45) (Ghasemi *et al.*, 2016), prostate (Durak *et al.*, 2004), non-small cell lung cancer (NSCLC) cell lines (H460, H1299, A549 and H322) (D'Abrosca *et al.*, 2019), and human rhabdomyosarcoma (RD), human cervix carcinoma-HeLa derivative (Hep2c) and murine fibroblast (L2OB) (Zekovic *et al.*, 2017). Compared with the available results, the cytotoxic effect ($IC_{50}$ values 28.9 and 48.58 µg/ml) of DE and EA extracts respectively, of *U. urens* in this study against breast cancer cells (MCF-7) is higher than that ($IC_{50}$ value 2 mg/ml) of aqueous extract of *U. dioica* (Fattahi *et al.*, 2013), and higher than that of ethanol extract of *U. dioica* that caused about 6.88 ± 6.88 % growth inhibition of breast cancer cells (MCF-7) at 50 µg/ml concentration (Abu-Dahab and Afifi, 2007). Conversely, the cytotoxic effect ($IC_{50}$ values 39.81 and 41.21 µg/ml) of DE and EA extracts respectively, of *U. urens* in this study against colon cancer cells (HCT116) is lower than that ($IC_{50}$ value 23.61 µg/ml) of dichloromethane extract of *U. dioica* (Mohammadi *et al.*, 2016), also is lower than that ($IC_{50}$ value 24.7 µg /ml) of ethanol extract of *U. dioica* roots against HT29 human colon cancer (Ghasemi *et al.*, 2016). The cytotoxic effect of the DE extract against MCF-7 and HCT116 cancer cells in this study could be owing to its richness of oxygenated and nitrogen-containing compounds (Table 1). Related to our findings D'Abrosca *et al.* (2019) attributed the antiproliferative effect of the *U. dioica* extract against small cell lung cancer (NSCLC) cell lines (H460, H1299, A549 and H322) to the presence of oxylipins as oxygenated compounds. Also, 19, 20-Didehydroyohimbinone, identified in DE extract (Table 1), is one of the β-carboline alkaloids that possess antimicrobial and antitumor effects (Layne *et al.*, 2015). Besides, the high cytotoxic effects of EA extract against MCF-7 and HCT116 cancer cells in these findings are parallel to its highest content of total phenolic, total flavonoids and total tannins (Figure 1) and its richness of individual phenolic acids and flavonoid compounds (Table 2), as well as to its highest antioxidant activities (Table 3). Thus, its cytotoxic properties could be owing to the presence of different phenolic acids and flavonoid compounds. This is in consensus with Konrad *et al.* (2000) who attributed the cytotoxic effect of *U. dioica* extract against prostate cancer cells to the presence of caffeic malic acid, caffeic acid, chlorogenic acid, and quercetin. A number of previous findings attributed the anticancer activities of different plant extracts to their high contents of total phenolic and total flavonoids besides different individuals of phenolics (Fattahi *et al.*, 2013; El Baz *et al.*, 2015; Gaafar *et al.*, 2018).

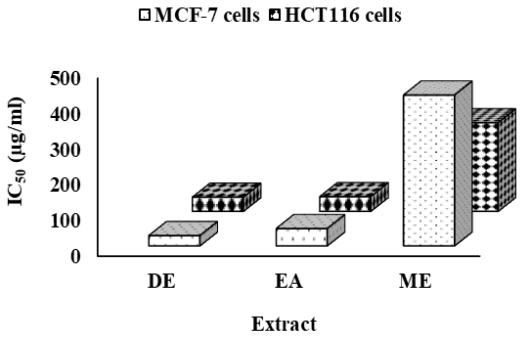


**Figure 2.** Cytotoxicity ($IC_{50}$, µg/ml) of diethyl ether (DE), ethyl acetate (EA), and methanol (ME) extracts of *U. urens* against breast cancer cells (MCF-7) and colon cancer cells (HCT116).

### *3.6. Antiviral activity*

The cytotoxicity effect of DE, EA and ME extracts of *U. urens* against MDCK cells was detected by MTT assay. The data illustrated in Table 4 indicated that EA extract showed a cytotoxic effect against MDCK with $TC_{50}$ value 54.49 µg/ml, while DE and ME were low toxic against MDCK with $TC_{50}$ values 299.59 and 395.86 µg/ml, respectively. Due to the uppermost cytotoxicity effect of EA against MDCK cells, the antiviral activity of EA was not tested. The DE exhibited the highest antiviral activity through the inhibition of H5N1 propagation with $IC_{50}$ value 125 µg/ml and selective index 2.397 as compared to ME with $IC_{50}$ value 1333.33 µg/ml and selective index 0.297 (Table 4). To the best of our knowledge, this is the first report regarding the antiviral effect of DE and ME extracts of *U. urens* against the highly pathogenic avian influenza H5N1 virus. By studying the mode of action of DE extract of *U. urens*, the results showed the potent virucidal effect of DE extract with 80% inhibition of viral propagation. The DE extract showed a limited effect on

viral replication and viral adsorption process with 25 and 45% inhibition of viral replication and viral adsorption, respectively. The antiviral effect of aqueous extract of *U. dioica* against the feline immunodeficiency virus (FIV) through the inhibition of FIV-induced syncytium formation was reported at different concentrations (0.5-1µg/ml) with maximum inhibition level of 84% (Manganelli *et al.,* 2005). The N-acetylglucosamine-specific lectin from *U. dioica* showed antiviral effect against human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV2), cytomegalovirus (CMV), respiratory syncytial virus (RSV), and influenza A (H3N2) virus through cytopathic induction at an $EC_{50}$ ranging from 0.3 to 9 µg/ml (Balzarini *et al.,* 1992). The potent anti-influenza (H5N1) effect of DE and ME extracts of *U. urens* in this study is in consensus with previous studies that reported the anti-influenza (H5N1) effect of different plant extracts (Gaafar *et al.,* 2015; Nagy *et al.,* 2018). The anti-influenza (H5N1) effect of DE and ME extracts of *U. urens* could be attributed to different phenolic compounds in ME extract and the high content of oxygenated and nitrogen-containing compounds such as terpenoids and alkaloids as presented in Tables 1 and 2. The recent study of Sadati *et al.* (2019) indicated the antiviral effect of different flavonoids: hispidulin, quercetin, luteolin, catechin, naringenin, kaempferol, vitexin, and chrysin against influenza virus (H1N1). These flavonoids may efficiently block the neuraminidase (NA), an enzyme able to break the glycoside bonds and empowers the viral invasion through the host cell membrane, active site as compared to oseltamivir (Sadati *et al.,* 2019).

**Table 4.** Cytotoxicity ($TC_{50}$, µg/ml) against Madin Darby Canine Kidney cells (MDCK), and H5N1 propagation inhibition ($IC_{50}$, µg/ml) of diethyl ether (DE), ethyl acetate (EA), and methanol (ME) extracts of *U. urens.*

| Extract | Cytotoxicity ($TC_{50}$, µg/ml) | Viral inhibition ($IC_{50}$, µg/ml) | Selective index |
|---|---|---|---|
| DE | 299.59 | 125 | 2.397 |
| EA | 54.49 | NT | NT |
| ME | 395.86 | 1333.33 | 0.297 |

$TC_{50}$: represents the concentration of extract causing 50% cell toxicity against Madin Darby Canine Kidney (MDCK) corresponding to the control (without extract); $IC_{50}$: represents the concentration of extract causing 50% inhibition on propagation of influenza virus H5N1corresponding to the control (without extract) by using Plaque reduction assay; NT: not tested due to the high toxicity against MDCK.

## 4. Conclusion

These results emphasize that bioactive ingredients-rich extracts from the Egyptian plant *Urtica urens* show a potent effect as antioxidant, anticancer, and anti-influenza (H5N1). These results support the positive effect of the successive extraction using polar gradient solvents on the chemical contents and biological activities of *U. urens.* The highest amounts of total phenolics, total flavonoids, and total tannins, along with higher concentrations of phenolic individuals were detected in EA extract compared to ME extract. The EA extract exhibited the highest DPPH, ABTS, and reducing power antioxidant activity. The DE and EA extracts have an efficient cytotoxicity effect against breast cancer cells (MCF-7) and colon cancer cells (HCT116). The DE exhibited the highest antiviral activity against the extremely pathogenic avian influenza H5N1 virus through the inhibition of H5N1 propagation, replication, and viral adsorption process. This study provides a new effective and bioactive chemical scaffolds from the Egyptian plant *U. urens* that might help in the development of new effective anti-cancer and antiviral bioactive compounds. Extra studies are required to isolate and identify the chemical structures of bioactive individuals responsible for the anticancer and anti-influenza effects and to elucidate the molecular mechanisms in both *in vitro* and *in vivo* models underlying the anticancer and anti-influenza (H5N1) activities.

## Conflict of interest

The authors declare that they have no conflict of interest.

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