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**RESEARCH ARTICLE**

# Investigating the Antiviral Effects of Silver Nanoparticles of *Portulaca oleracea* Against High Pathogenic Avian Influenza H5N1.

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## ABSTRACT

Avian influenza, commonly referred to as bird flu, is a highly contagious respiratory disease that affects millions of birds each year, leading to significant losses in poultry production and posing a risk to human health. The water extract from the leaves of Portulaca oleracea (WEP) has been shown to alleviate symptoms associated with influenza A virus (IAV) infections. However, the antiviral efficacy of WEP against IAV remains unclear. In this study, we utilized a safe, cost-effective, and environmentally friendly method to synthesize silver nanoparticles (Ag-NPs) from the leaf water extract of Portulaca oleracea. Our objective was to evaluate the cytotoxicity and antiviral properties of both WEP and Ag-NPs against High Pathogenic Avian Influenza (HPAI) H5N1 in the Madin Darby Canine Kidney (MDCK) cell line. We employed the crystal violet assay to assess the viability of MDCK cell line treated with the compounds under study followed by investigation of the anti-influenza activity against HPAI H5N1 with cytopathic effect (CPE) inhibition assay achieved via scoring the cytopathic effect under inverted microscope during the 72-hour incubation time, assested by crystal violet staining of the virus infected cells. The results indicated that the half-maximal cytotoxic concentration (CC50) for WEP and Ag-NPs was 66.98 µg/ml and 50.38 µg/ml, respectively. The half-maximal inhibitory concentration (IC50) values were 29.72 µg/ml for WEP and 10.78 µg/ml for Ag-NPs, leading to Selective Index (SI) values of 2.25 and 4.67, respectively. These findings displayed a moderate antiviral activity of both compounds against HPAI H5N1. Consequently, the previously reported alleviation of influenza symptoms may be attributed to the modulation of the host immune response rather than a direct antiviral effect against the influenza virus.

**Introduction**:

Avian Influenza Virus (AIV) is a significant pathogen that poses a serious threat, can infect many birds and mammals, as well as human. The causative agent of AIV is the Influenza A virus, classified within the Orthomyxoviridae family. This virus is characterized by its negative-sense, single-stranded RNA segmented genome [1]. Influenza A virus The HPAI H5N1 virus was first reported in 2006 and has since become endemic among domestic poultry populations and humans [4]. Its capability for zoonotic transmission—particularly from avian hosts to humans—highlights its potential for widespread outbreaks, resulting in severe disease and mortality, which carry significant economic and public health ramifications [5].

Influenza A viruses exhibits a remarkable ability to evolve rapidly through antigenic drift (mutations) and shift (reassortment of its segmented genome), resulting in the frequent emergence of new variants and subtypes. This rapid evolution poses a considerable challenge for the development of effective vaccines and antiviral treatments [6]. Although vaccination remains the cornerstone of prevention, there is an urgent need for antiviral therapies, especially as viral infections have caused millions of poultry deaths globally [7]. Seasonal influenza vaccines often fail to provide sufficient protection against drifted or pandemic strains due to their limited ability to match circulating viral variants [8].

Moreover, while vaccination may mitigate clinical symptoms, it does not completely prevent infection in vaccinated birds, allowing for the silent circulation of the virus. This silent spread poses a risk of outbreaks within poultry flocks and potential spillover to humans [9]. Therefore, there is an urgent demand for effective anti-influenza medications to overcome the limitations of current vaccination strategies [10].

Currently, FDA-approved antiviral drugs are categorized based on their mechanisms of action: Matrix protein 2 inhibitors (e.g., Amantadine and Rimantadine) that block viral entry, RdRp inhibitors (e.g., Baloxavir marboxil, marketed as Xofluza) that inhibit viral replication, and NA inhibitors (e.g., Zanamivir, Oseltamivir, and Peramivir) that prevent the progeny virus particles egress from the virus infected cells [11].

include many subtypes based on the antigenicity of glycoproteins, hemagglutinin (HA) spike and neuraminidase (NA) spike. $H_{1\ to\ 18}$ and $N_{1\ to\ 11}$ have been detected till now, with the exception of $H_{17}N_{10}$ and $H_{18}N_{11}$, which have been detected in bats. All other subtypes have been primarily detected in avian species, suggesting that birds act as the natural reservoirs for Influenza A viruses [2, 3].

*Portulaca oleracea* (*P. oleracea*), commonly known as purslane or Regla in Egypt, is a globally recognized weed species. Polysaccharides extracted from its aerial parts have shown inhibitory activities on the multiplication of herpes simplex virus type 2 (HSV-2) [12]. Additionally, the water extract of *P. oleracea* leaves (WEP) has demonstrated virucidal activity against H1N1 in vitro, exhibiting low inhibitory concentration (IC50) and a high selective index during the initial stages of infection [13].

Many researchers are interested in silver nanoparticles (Ag-NPs) because of the substantial impact they have had on many different scientific, medicinal, and industrial disciplines. Ag-NPs are known to be safe, but highly toxic to bacteria, fungus, and other microorganisms [14]. The antiviral properties of Ag-NPs have also been widely studied, revealing various mechanisms of action, including virucidal effects and interference with viral adsorption and endocytosis into host cells [15]. Through interactions with the viral envelope, receptors, or surface proteins, Ag-NPs can prevent the virus from attaching to host cells and causing cell harm. Nanoparticles may inhibit viral replication in host cells by interfering with nucleic acid (DNA or RNA) functions and disrupting fundamental enzymes required for viral replication [16]. Furthermore, infected cells may create reactive oxygen species (ROS) in response to NPs, which damages viral DNA, proteins, and lipid

membranes; this, in turn, hinders the virus's ability to replicate and infect cells [17]. Additionally, Ag-NPs may enhance the host immune response by influencing the production of interferons and proinflammatory cytokines, which the host uses to detect and respond to viral infections [18]. Because of this improved immune response, infected cells may be destroyed, and the spread of viruses can be controlled. Ag-NPs have the potential to impede apoptotic pathways, allowing the immune system additional time to fight off infections [18]. Green synthesis of Ag-NPs has an edge over the chemically and physically synthesized nanoparticles, including lower costs, absence of toxic byproducts, and environmentally friendly production pathways, while proving effective against numerous DNA and RNA viruses [19]. Many studies have documented the effectiveness of plant extracts in assembling Ag-NPs [20-27]. Because the NPs covered with different covering agents derived from plant extracts, the produced Ag-NPs with plant extracts exhibited greater activity compared to those with other biological materials [28] The phytochemical composition of *P. oleracea* is widely recognized for its abundance of polysaccharides, fatty acids, flavonoids, vitamins, minerals, and terpenoids [29] It is believed that these constituents are perfect for nanoparticle synthesis due to their strong reducing and capping properties. In addition, *P. oleracea* is widely spread and easily accessible. While there have been several studies looking into the green synthesis of Ag-NPs utilizing various plant extracts, relatively little has been done to bridge the gap between the therapeutic properties of *P. oleracea* and this goal.

This study aims to fill a gap in the literature through synthesizing Ag-NPs by the water extract of *P. oleracea* (WEP) and gather knowledge regarding environmentally friendly nanotechnology achieved with green fabrication of Ag-NPs followed by evaluating the cytotoxicity and antiviral activities of the WEP and the biosynthesized Ag-NPs from *P. oleracea* against HPAI H5N1 in the MDCK cell line.

## Materials and Methods

### *Avian Influenza Virus (H5N1)*

The avian influenza virus (H5N1) used in this study was kindly provided by Environmental virology lab, National Research Centre, Egypt. It was propagated in the Madin Darby Canine Kidney (MDCK) cell line. MDCK was cultured in Dulbecco's Modified Eagle Medium (DMEM) high-glucose medium supplemented with 10% fetal bovine serum, 0.1% antibiotic/antimycotic mixture (Gibco BRL, NY, USA), and trypsin-EDTA (Sigma-Aldrich, USA). The viral titer was calculated using the $TCID_{50}$ (Tissue Culture Infective Dose 50) method [30], yielding a titer of 1.5 x 10^5 TCID50.

### *Compounds*

Favipiravir was purchased from Sigma-Aldrich and utilized as a drug control.

### *Preparation of Water Extract from P. oleracea Leaves (WEP)*

Following the method described by Abdel-Rahman *et al.* [31], the leaves of plant were collected, washed with distalled water ($dH_2O$). After the leaves dried, they were finely ground into a powder that was mixed with $dH_2O$ at a ratio of 5 g per 100 mL followed by heating at 40°C with agitation (150-RPM) for 60 minutes. The water extract was obtained via centrifugation of the final mixture at at 10000RPM for 10 minutes then collection of supernatant.

### *Synthesis of Ag-NPs from P. oleracea*

As per the methodology outlined by Abdel-Rahman *et al.* [31], Ag-NPs were biosynthesized by dissolving 16.9 mg of silver nitrate ($AgNO_3$) in 80 mL of $dH_2O$. Subsequently, 20 mL of the previously prepared WEP was added to get Ag-NPs of a final concentration of 1 mM. The Ag-NPs solution was stirred for 1 hour (hr) at 150RPM, with adjusting the pH to 8 by gradually adding 1M sodium hydroxide (NaOH). The final mixture was then left at room temperature (RT) overnight to make sure that a complete reduction of the metal

precursor occured, the reduction was indicated by conversion of the intially colorless solution into yellowish-brown color.

***Characterization of Silver Nanoparticles (Ag-NPs)***

The characteristics of the synthesized silver nanoparticles were visualized with Transmission Electron Microscopy (TEM) JEOL JEM-2100, USA at the Electron Microscopy Unit, Mansoura University, Egypt. This analysis determined key factors as particle size, shape, and aggregation of the Ag-NPs prepared from the WEP. The TEM technique involves directing a concentrated beam through a highly thin specimen and its interfaces, which leads to the generation of an image. A carbon-coated copper grid was employed to prepare thin films of the synthesized Ag-NPs via appling 20 µL of the Ag-NPs solution to the copper grid. The slide was subsequently rinsed with few droplets of $dH_2O$ and stained with 1% uranyl acetate solution to improve contrast. After allowing a minute for preparation, the samples were placed on the grids for examintion .

***Determination of cytotoxicity of WEP, Ag-NPs and favipiravir using MDCK cells***

The toxicity of the investigated compounds was assessed using a modified crystal violet assay [32]. In microtiter plates, MDCK were distributed (2 x 10^4 cells/well) to achieve an initial confluency of 40%–50%. The microtiter plates were incubated at 37 °C and 5% $CO_2$. After 24-hr incubation, the media above cells were replaced with 100 µL of maintenance media containing serial two-fold dilutions of WEP, AgNPs and favipiravir (as a control drug) in triplicate (concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.8, 3.9, 1.95 and 1 µg/mL for each compound). Untreated MDCK monolayers served as cell controls.

At 72 hrs post-treatment, the media over cells were removed. Subsequently, the cells were washed with Phophate buffered saline (PBS) to get rid of detached dead cells then fixed with 100 µL/well of a 10% paraformaldehyde solution for 1 hr at RT. Subsequently, the cells were washed twice with tap water then removing of any water remnants from the wells via gentle tapping on absorbent material. The cells were stained with a 0.1% crystal violet (w/v) dye for 20 minutes. After washing and drying, the crystal violet stain was then dissolved with 100 µL of absolute methanol/well for 15 minutes. The optical density (OD) was measured using a spectrophotometer (BMG LabTech GmbH FLUOstar Omega, Ortenberg, Germany) at 570/620 nm. The 50% cytotoxic concentration (CC50) value for WEP, AgNPs, and favipiravir was defined as the concentration required to decrease cell viability by 50% and was calculated using GraphPad Prism software from the mean dose-response curves.

***Determination of antiviral activity of WEP, Ag-NPs and favipiravir:***

The antiviral activity of WEP, Ag-NPs and favipiravir was evaluated with a Cytopathic effect (CPE) inhibition assay [33], performed in 96-well microtiter plates. MDCK cells were grown in growth media with antibiotic/antimycotic mixture overnight with optimum density. A separate plate containing the same sets of twofold of the compounds (100 µL/well in triplicate) was prepared. The dilutions were then mixed with 100 µL/well of maintenance media containing a fixed titer of the virus, achieving a volume of 200 µL/well of the virus-compound mixture, which was incubated at 37°C for 1 hr. After incubation, the growth media over the MDCK monolayers were aspirated. After washing with 1x PBS. The MDCK cell monolayers were then inoculated with the virus-compound mixtures. While for the control drug, favipiravir, the monolayers were inoculated with 100 µL of the virus for 1 hr in incubator at 37 °C and 5% $CO_2$ to allow virus attechment followed by removal of the DMEM containing virus and replaced by 100 µL of DMEM containing favipiravir with different concentrations in triplicate. Cell control was included in the same plate with 200 µL of maintenance media above the cell, while virus control wells was

inoculated with maintenance media containing only the virus of fixed titer.

The antiviral effectiveness of the compounds was primarily assessed through microscopic analysis, complemented by crystal violet staining for more definitive results. During the incubation period at 37°C and 5% $CO_2$ for 72 hrs, CPE was monitored under an inverted microscope to track changes over time. To clarify the findings, the monolayers were fixed with 100 µL of a 4% paraformaldehyde solution for 20 minutes, washed twice with tap water, and stained with a 0.1% crystal violet (w/v) dye for 20 minutes at RT. After washing and drying, the crystal violet dye in each well was dissolved using 100 µL/well of absolute methanol, and the OD was measured using a spectrophotometer (BMG LabTech GmbH FLUOstar Omega, Ortenberg, Germany) at 570/620 nm. The inhibitory concentration 50% (IC50) was the concentration required to reduce the virus-induced CPE by 50%, relative to the virus control.

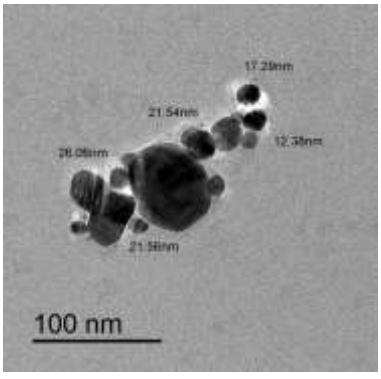
### *Calculation of IC50, CC50, and SI Values*

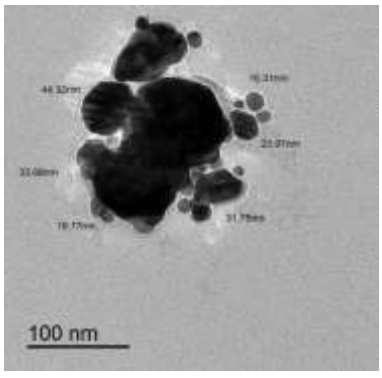
The determination of antiviral efficacy, represented by the 50% inhibitory concentration (IC50), involved plotting the OD of the dilutions of the compounds against their concentrations. The average viability of the cells relative to compound concentration was used to calculate the CC50 [34]. These values were obtained through non-linear regression analysis of transformed then normalized obtained data using GraphPad Prism software 5.01. The Selective Index (SI) was calculated as the ratio of CC50 to IC50.

## Results

### *Morphology analysis*

Figure 1 illustrates the transmission electron microscopy (TEM) images of green synthesized Ag-NPs, greater spatial resolution analysis was done on the samples (100 nm). It was found that the WEP could be used to produce Ag-NPs with diameters ranging from 11 - 44 nm, with an average size of 21.8 ± 5 nm with fine shadow surrounding the Ag-NPs indciating successful green synthesization of the nanoparticles. The morphology of the Ag-NPs is characterized by a blend of hexagonal and spherical-like structures. Analysis via TEM showed that most of the spherical nanoparticles are surrounded with a fine shadow indicating successful, with a particle size



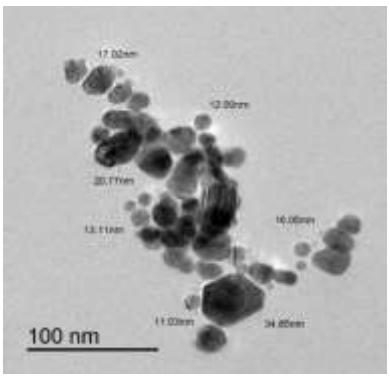



**Figure. 1 The transmission electron microscopy (TEM) images of Silver Nanoparticles (Ag-NPs) prepared from water extract of *Portualca oleraca***

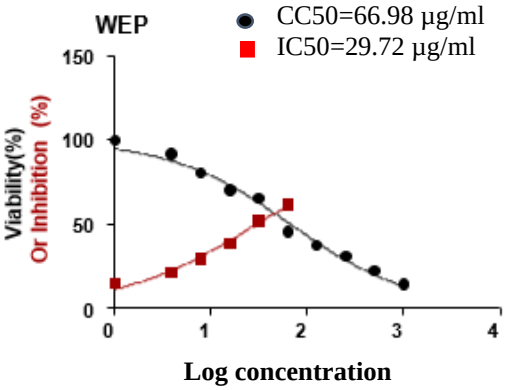
### *Determination of cytotoxicity of WEP, Ag-NPs and favipiravir using MDCK cells*

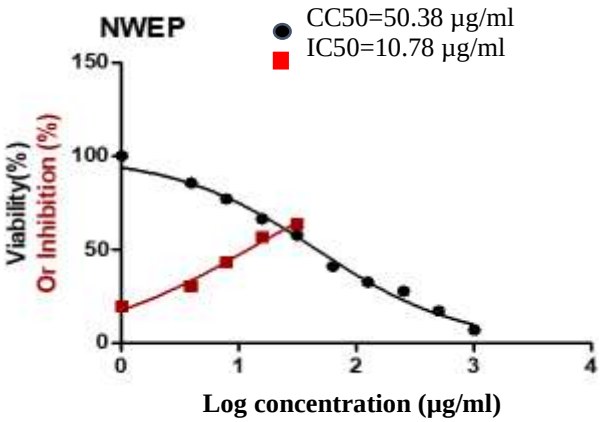
As illustrated in Table 1 and Figure 1, the water extract of *P. oleracea* (WEP) demonstrated moderate antiviral activity against H5N1. To enhance this inhibitory effect, we synthesized silver nanoparticles (Ag-NPs) using the water extract, as detailed in the Materials and Methods section. Initially, the toxicity of each compound was assessed on the MDCK cell line, a well-established model for studying influenza viruses [35].

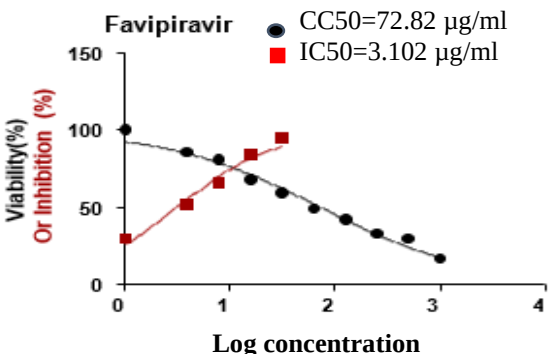
Cells were inocluated with twofold dilutions of WEP and Ag-NPs, and the cell viability was determined using the crystal violet assay. The CC50 values were 66.98 µg/mL for WEP and 50.38 µg/mL for Ag-NPs, compared to 72.82 µg/mL for favipiravir. The previous results indicate that WEP induces cytotoxicity at higher concentrations than Ag-NPs, suggesting that Ag-NPs exhibit a lower degree of cytotoxicity.

### *Determination of antiviral activity of WEP, Ag-NPs and favipiravir*

We evaluated the half-maximal inhibitory concentration (IC50) values against HPAI H5N1 in the MDCK cell line through a dose-response analysis. MDCK monolayers were treated with mixtures containing a constant TCID50 of HPAI H5N1 and two-fold serial dilutions of each compound, alongside the control drug, favipiravir. Our results demonstrated a dose-dependent antiviral activity against HPAI H5N1 for all tested compounds, with Ag-NPs showing a superior inhibitory effect compared to WEP. The IC50 value for Ag-NPs was 10.78 µg/mL versus 29.72 µg/mL for WEP, while favipiravir exhibited an IC50 value of 3.102 µg/mL (Figure 2). These findings indicate that Ag-NPs achieved a higher antiviral effect at lower concentrations in contrast with WEP. The SI values (CC50/IC50) for the investigated compounds and the control drug, favipiravir were as follows: WEP had an SI of 2.25, Ag-NPs had an SI of 4.67, and favipiravir exhibited a significantly higher SI of 23.47 (Table 1)



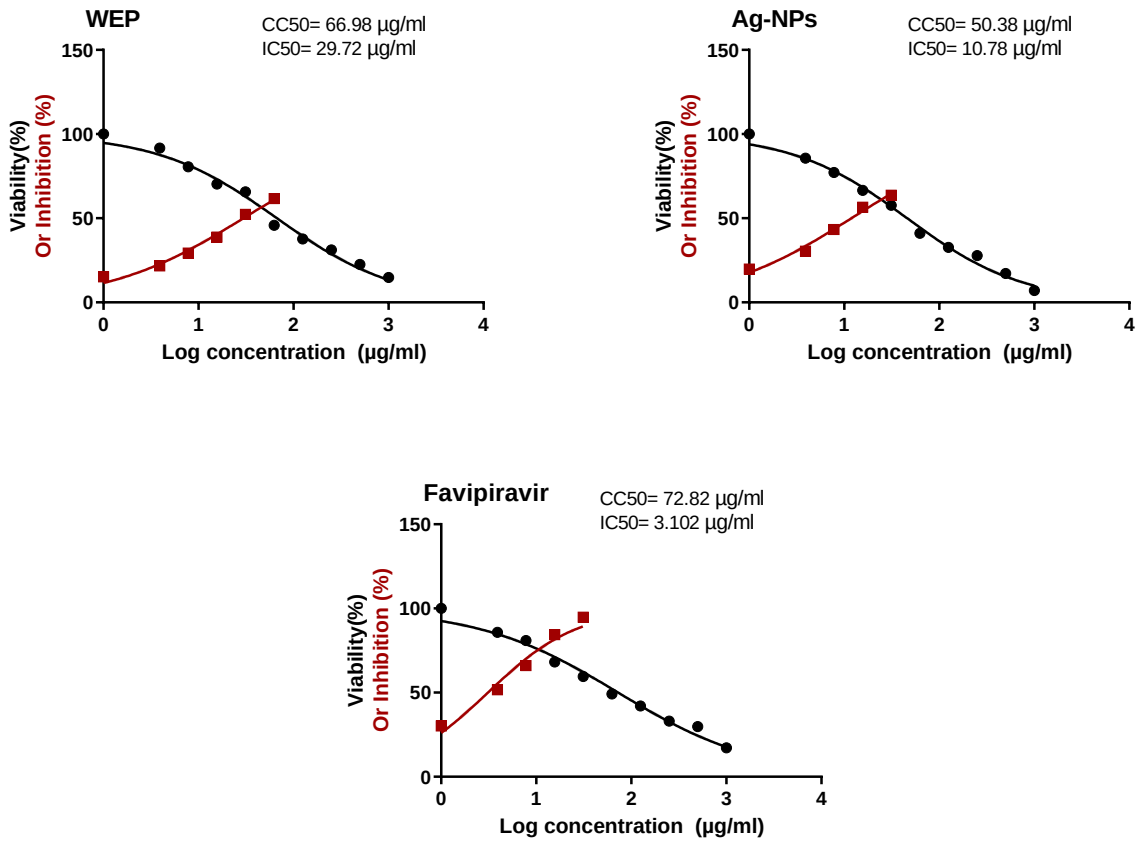



**Fig. 2. Determination of the half-maximal cytotoxic concentration ($CC_{50}$) and the half-maximal inhibitory concentration ($IC_{50}$) of WEP, Ag-NPs and Favipiravir against HPAI H5N1 in MDCK cells.**

**Table 1. The values of CC50[1], IC50[2], and SI[3] values of WEP[4], Ag-NPs[5], and Favipiravir against HPAI[6] H5N1 in MDCK[7] cells.**

| Compounds | CC50(µg/ml) | IC50 (µg/ml) | SI |
|---|---|---|---|
| WEP | 66.98 | 29.72 | 2.25 |
| Ag-NPs | 50.38 | 10.78 | 4.67 |
| Favipiravir | 72.82 | 3.102 | 23.47 |

[1] The half-maximal cytotoxic concentration
[2] The half-maximal inhibitory concentration
[3] Selective Index
[4] Water extract of Portulaca oleracea leaves
[5] Silver nanoparticles bio-synthesized with the water extract of portulaca oleracea leaves
[6] High Pathogenic Avian Influenza
[7] Madin Darby Canine Kidney

## Discussion

Avian Influenza Virus (AIV) presents significant threats to the poultry industry, as outbreaks of High Pathogenic Avian Influenza (HPAI) can lead to devastating losses in poultry populations, enforce trade restrictions, necessitate the culling of infected birds, and impose substantial financial burdens on farmers and the economy [36]. The emergence of influenza virus mutations and the development of resistance to existing antiviral treatments underscore the urgent need for new anti-influenza agents to combat potential future epidemics [10, 37]. Therefore, the present study was designed to screen *Portulaca oleracea*, commonly known as Regla, a traditional medicinal plant renowned for its therapeutic benefits against various diseases [38]. Particularly, we explored the antiviral properties of the water extract of its leaves for their therapeutic potential against HPAI H5N1.

The scientific community has increasingly recognized the value of medicinal plants and traditional medicine, which have demonstrated efficacy and are widely accepted by the public and expand the future anti-influenza regimens. *Portulaca oleracea* is noted for its health-promoting properties, including anti-inflammatory, antioxidant, and immunomodulatory effects [39, 40]. Previous clinical studies have shown that extracts from *P. oleracea* can significantly alleviate symptoms in patients with influenza. However, further investigations are required to elucidate the specific mechanism of action underlying its anti-influenza activity [13].

Based on the clinical studies, several *in vitro* studies investigated the antiviral activity of *P. oleracea* against different viruses. It was reported that the extract of the plant showed an antiviral effect against Hepatitis C virus (HCV) infection [41], Porcine Rotavirus [42], and HSV-2 [12]**.** A recent study reported that green-synthesized silver nanoparticles from the plant water extract showed high anti-hepatitis A virus and anti-Coxsackie Coxsackie B4 virus activities [31]**.** Regarding influenza virus, two previous studies investigated the anti-influenza A/WSN/1933 (H1N1) activity of the aerial part of the plant; one proved the potential activity of the water extract of it, indicated by a high selective index with an EC50 and CC50 of 220.1 µg/mL and 8067 µg/mL, respectively, achieving an SI of 36.6 when the MDCK cells were treated with media of pre-mixed virus and the water extract not at the post-infection phase nor with pre-treatment of cells with water extract [13], while the other study reported that the polysaccharides of the aerial part of the plant had no antiviral activity against influenza [12].

Driven by the previous evidence, our study aimed to focus on the anti-influenza $H_5N_1$ activity of the water extract of *P. oleracea* leaves (WEP) in the MDCK cell line followed by enhancing the antiviral activity by formulating silver nanoparticles (Ag-NPs) with non-toxic, cheap, and environmentally friendly green synthesis method that have many advantages over conventional chemical approaches [43]. Our evaluation was based on investigating the cytotoxicity (expressed as CC50) via the crystal violet assay and antiviral activity (expressed as IC50) via the CPE inhibition assay, and the relative values of CC50 and IC50 of each compound were expressed as SI that are compared with SI of the drug control, favipiravir.

Here, we investigated the virucidal mode of action of the tested compounds based on previous studies [13]. Virus-compounds mixtures of different dilutions incubated for 1 hr. at 37°C were inoculated in the MDCK cell line, and the virus-cells interaction was tracked during the incubation time for the progression of CPE, followed by crystal violet staining at the end of the 72-hour incubation time.

Our results indicated that WEP and Ag-NPs exhibited moderate virucidal activity with IC50 values of 29.72 µg/mL and 10.78 µg/mL, respectively, with corresponding CC50 values of 66.98 µg/mL and 50.38 µg/mL resulting in SI value of 2.25 and 4.67 compared to the SI of favipiravir, 23.47. These selective indices showed the enhanced antiviral activity of Ag-NPs coated with WEP, indicated by a lower IC50 value, meaning that Ag-NPs can exert their antiviral activity at lower concentrations.

Based on our results, we conclude that the difference in antiviral activity of *P. Oleracea* may be attributed to the susceptibility difference of the influenza virus from strain to another, the method of extraction, and the involvement of nanoparticles that can enhance the antiviral activity. Finally, there is a difference in the readout that is being used to evaluate the reduction of viral replication. The CPE inhibition assay employed in our study for determining antiviral activity; nonetheless, many researchers still rely on the plaque reduction assay to detect the reduction in viral titers. For this reason, it would be useful to establish standard methodologies for evaluating antiviral efficacy in order to facilitate more accurate comparisons [44].

Our findings showed that *P. Oleracea* possesses moderate direct antiviral activity against HPAI H5N1 in comparison to the drug control. Hence, the previous clinical studies that reported the anti-influenza activities may be attributed to the presence of multiple bioactive compounds within the plant [38, 41, 42]. These activities could result from the effect of a single compound or the synergistic interaction of several bioactive constituents, requiring further exploration in future studies. From another perspective, *P. Oleracea* has immunomodulatory characteristics [29] which have indirect antiviral action via boosting the host immune system.

The variations in cytotoxicity and antiviral activity may stem from the diverse chemical constituents in the plant extracts. Enhancing the antiviral properties of these native compounds through various chemical modifications could be an effective strategy. Additionally, it is suggested that these plant extracts could complement other antiviral therapies in a combinatorial treatment strategy.

## Conclusion

Our study underscores the potential of traditional medicinal plants in combating emerging HPAI H5N1 viruses. Our findings showed that *P. Oleracea* possesses moderate direct antiviral activity against HPAI H5N1 in comparison to the drug control. Hence, The antiviral activity of *Portulaca oleracea* may derive from its anti-inflammatory, antioxidant, and immunomodulatory phytochemicals, which play a critical role in reducing the pathogenicity of influenza, rather than exhibiting direct antiviral effects. In summary, further investigations are required to clarify the mechanisms of action of the bioactive molecules in Portulaca oleracea.

## Conflict of Interest

The authors declare that there are no conflicts of interest.

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**الملخص العربى**

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أنفلونزا الطيور، والتي يشار إليها عادة باسم إنفلونزا الطيور، هي مرض تنفسي شديد العدوى يصيب ملايين الطيور كل عام، مما يؤدي إلى خسائر كبيرة في إنتاج الدواجن ويشكل خطرًا على صحة الإنسان. وقد ثبت أن المستخلص المائي من أوراق نبات الرجلة يخفف الأعراض المرتبطة بعدوى فيروس الأنفلونزا من النوع A الوبائي. ومع ذلك، لا تزال فعالية المستخلص المائي ضد فيروس الأنفلونزا من النوع A الوبائي غير واضحة. في هذه الدراسة، استخدمنا طريقة آمنة وفعالة من حيث التكلفة وصديقة للبيئة لتصنيع جزيئات الفضة النانوية من المستخلص المائي من أوراق نبات الرجلة. وكان هدفنا هو تقييم السمية الخلوية والخصائص المضادة للفيروسات لكل من المستخلص المائي من أوراق نبات الرجلة وجزيئات الفضة النانوية ضد فيروس أنفلونزا الطيور عالي الضراوة H5N1 على خلايا MDCK. لقد استخدمنا اختبار الكريستال البنفسجي لتقييم مدى حيوية خلايا MDCK المعالج بالمركبات قيد الدراسة، متبوعًا بالتحقيق في النشاط المضاد للإنفلونزا شديدة الضراوةH5N1 مع اختبار تثبيط التأثير الخلوي (CPE) الذي تم تحقيقه من خلال تسجيل التأثير الخلوي تحت المجهر المقلوب خلال فترة الحضانة التي تبلغ 72 ساعة، وتم تقييمه عن طريق صبغ الكريستال البنفسجي للخلايا المصابة بالفيروس. أشارت النتائج إلى أن نصف التركيز السام للخلايا (CC50) للمستخلص المائي وجزيئات الفضة النانوية كان 66.98 ميكروجرام/مل و50.38 ميكروجرام/مل على التوالي. كانت قيم نصف التركيز المثبط للنشاط الفيروسي (IC50) هي 29.72 ميكروجرام/مل للمستخلص المائي و10.78 ميكروجرام/مل لجزيئات الفضة النانوية، مما أدى إلى قيم مؤشر انتقائي (SI) هي 2.25 و4.67 على التوالي. أظهرت هذه النتائج نشاطًا مضادًا للفيروسات متوسط المفعول لكلا المركبين ضد فيروس الإنفلونزا شديد الضراوة H5N1. وبالتالي، فإن تخفيف أعراض الأنفلونزا المذكور سابقا قد يُعزى إلى تغييرات في الاستجابة المناعية للعائل بدلاً من التأثير المضاد للفيروسات المباشر ضد فيروس الأنفلونزا.