Antiviral Activity of Zinc Oxide Nanoparticles Mediated by *Plumbago indica* L. Extract Against Herpes Simplex Virus Type 1 (HSV-1)

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**Introduction:** *Plumbago indica* L. is considered a valuable source in the Plumbaginaceae family for various types of active compound such as alkaloids, phenolics and saponins. To promote the usage of *P. indica* in the bionanotechnology field, zinc oxide nanoparticles (ZnONPs) were biosynthesized by using its alcoholic extract. The inhibitory effects of ZnONPs and the plant extract were also evaluated against HSV-1.

**Methods:** ZnONPs were described by the following techniques, UV–visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), zeta potential, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and x-ray diffraction (XRD). The phenolic and flavonoid contents of *P. indica* extract, which are accountable for bioreduction, formation and stabilization of the nanoparticles, were analyzed by HPLC technique. The antiviral assessment was implemented on both agents by using Vero cell lines.

**Results:** DLS revealed that the average size of ZnONPs was 32.58 ± 7.98 nm and the zeta potential was –20.8 mV. The observation of TEM analysis revealed that the particle size of ZnONPs varied from 2.56 to 8.83 nm. The XRD analysis verified the existence of pure crystals of hexagonal shapes of nanoparticles of ZnO with a main average size of 35.28 nm that is approximating to the values of particle size acquired by SEM analysis (19.64 and 23.21 nm). The HPLC analysis of *P. indica* ethanolic extract showed that gallic acid, chlorogenic acid and rutin were the major compounds, with concentrations equal to 8203.99, 2965.95 and 1144.99 µg/g, respectively. Regarding the antiviral assessment, the synthesized uncalcinated ZnONPs were found to exhibit a promising activity against HSV-1, with CC50 and IC50 values equal to 43.96 ± 1.39 and 23.17 ± 2.29 µg/mL, respectively.

**Conclusion:** The green synthesized ZnONPs are considered promising adjuvants to enhance the efficacy of HSV-1 drugs.

**Keywords:** ZnO nanoparticles, *Plumbago indica* L., HPLC analysis, antiviral activity and green synthesis

**Introduction**

The nanoparticles of metal oxide possess valuable physical, chemical, and biological properties and are considered a promising tool used in various applications.1 In the process of nanoparticle synthetization, numerous side effects are arising due to the usage of toxic chemicals acting as reductive and capping agents. However, the utilization of numerous natural extracts, particularly plant extracts, in the metal oxide nanoparticles synthesis has received attention. This is because the natural approach is time saving and ecofriendly compared to the other conventional methods; also, the occurrence of several bioactive phytochemicals assists in the...
reduction and stabilization of nanomaterials. Recently, scientists have recognized the importance of zinc oxide (ZnO) due to its plentiful applications and unique properties. The nanoparticles containing ZnO have an important role in avoidance of the aquatic and dependent ecosystem imbalances arising from organic contaminants such as dye fragments. ZnONPs synthesized by Artocarpus heterophyllus leaves were effective in degradation of Congo red dye. ZnO nanorods produced by Calotropis gigantea were proved to act as a photo catalyst in the degradation process of titan yellow dye. Also, the ZnONPs application at concentration of 100 μg mL⁻¹ protects plants from tobacco mosaic virus infection.

Reviewing previous reports, ZnONPs were successfully synthesized from numerous natural extracts as Aloe vera, Passiflora caerulea and Azadirachta indica. Furthermore, ZnONPs have received much attention and are presently applied in numerous fields such as packaging and food additives. Among other types of nanoparticle, ZnONPs become absorbed easily by the biological tissues and have a good biocompatibility with human cells more than zinc metal. Additionally, ZnONPs demonstrate an antiviral activity against many viruses such as various types of respiratory viruses and herpes viruses, including SARS-CoV-2. There are several mechanisms for the antiviral activity of ZnONPs, such as prevention of viral entry, viral replication and spreading to organs, which can eventually trigger reactive oxygen species leading to oxidative injury and viral death. Zinc containing compounds revealed antiviral activity against numerous viruses by different mechanisms such as physical processes including attachment to virus, inhibition of virus infection, and uncoating. Also, these compounds showed activity by biological mechanisms such as inhibition of viral polymerases and protease enzymes.

Generally, Zn is an important component that occurs in human tissue such as the bone, brain, muscle and skin. This vital element is also involved in various enzyme processes such as metabolism and protein and nucleic acid biosynthesis.

It is worth noting that the US Food and Drug Administration (FDA) categorized ZnO as a nontoxic substance. Therefore, ZnONPs are allowed to be applied in numerous biomedical activities. ZnONPs are characterized by their low toxicity and cost effectiveness and therefore can be applied in numerous therapeutic fields, such as wound healing and drug delivery, and for anticancer, antidiabetic, antibacterial and anti-inflammatory purposes.

Plumbago indica L. belongs to the largest genus of flowering plant in the family Plumbaginaceae. This plant, previously known as P. rosea, can grow in diverse regions of Southeast Asia, Europe, Malaysia, Indonesia, Africa, China and India. P. indica contains various types of constituent, such as apigenin, kaempferol, luteolin, plumbaginol, myricetin tetra methyl ether, amelopsin tetra-methyl ether, Plumbagin-5-O-α-L-Rhamnopyranoside, campesterol, β-sitosterol and stigmasterol. Among numerous Plumbago species, P. indica contains a high amount of plumbagin. Numerous pharmacological activities for plumbagin were reported, such as antioxidant, antimicrobial, and anticancer activities.

The interactions between nanoparticles and biological targets have revolutionized the field of drug development. Selenium NPs play important roles in various medical applications such as anticancer, antitumor, antibacterial and antiviral agents. They boost drug and gene delivery and show synergistic properties. The micro and nano ZnO particles exhibit highly promising prophylactic agents against HSV-1 and PEGylation of these nanoparticles causes reduction of the cytotoxic effect and enhances their antiviral efficacy. Numerous metal NPs were successfully ecofriendly synthesized by different Plumbago species and found to have various biological activities. Sliver NPs were previously green-synthesized using various Plumbago species such as P. indica, P. auriculata, and P. zeylanica and proved to possess antioxidant, antitumor, antibacterial, larvicidal and antitubercular properties. P. zeylanica was used to synthesize selenium, copper, silver, gold, and bimetallic (silver and gold) NPs. These nanoparticles were found to exhibit antioxidant, antibacterial, antimicrobial, antimicrobial and antibiofilm effects. Highly stable ZnONPs were also obtained from P. zeylanica extract and were found to have antibacterial activity against Staphylococcus aureus and Salmonella typhimurium. ZnONPs synthesized from P. auriculata alcoholic extract demonstrated an antiviral effect against avian metapneumovirus subtype B.

Most of the investigations done on the antimicrobial activity of ZnONPs focused on the antibacterial effects and there are limited studies dealing with interaction between ZnONPs or P. indica extract and viruses.

The current study aimed to inspect the effect of ZnONPs produced by alcoholic extract of P. indica against HSV-1. Furthermore, the phytochemical constituents present in this extract are characterized by HPLC technique.
Materials and Methods

Plant Material

Flowering aerial parts of *P. indica* were procured from EL-MAZHAR botanical garden, Giza, Egypt. The plant was kindly authenticated by Engineering Therease Labib Consultant for plant identification at EL-Orman botanical garden, Giza and Dr. Mohamed El Gebaly, Botany Taxonomist at National Research Centre Herbarium, Dokki, Giza. Voucher specimen (numbered 19062020) was reserved at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University. *P. indica* flowering aerial parts were air-dried, coarsely powdered and preserved in tightly closed amber-colored glass containers at room temperature. This research was performed in accordance with the guidelines of occupational health and safety committee at National Research Centre, Giza, Egypt and accepted by scientific research ethics committee of Faculty of Pharmacy, Cairo University, Giza, Egypt.

Extraction of Plant Active Ingredients

The air-dried powder of the flowering aerial parts of *P. indica* (100 g) were successfully extracted by maceration technique with 90% ethanol (250 mL × 3). The obtained extract was evaporated using Buchi Rotary Evaporators under reduced pressure till dryness (2.5 g.).

HPLC Analysis

HPLC analysis was performed by means of an Agilent 1260 series. The separation column was Eclipse C18 column (4.6 mm x 250 mm i.d., 5 μm). The mobile phase was comprised of (A) water and (B) 0.05% trifluoroacetic acid in acetonitrile using a flow rate of 1 mL/min. The mobile phase was automatic flow in a linear gradient as follows: 0 min (82% A and 18% B); 0–5 min (80% A and 20% B); 5–12 min (60% A and 40% B) and 12–16 min (82% A and 18% B). The detector used was multi-wavelength and adjusted at 280 nm. The injection volume was 10 μL for the standard and extract solutions. The column temperature was set at 35 °C.

Green Synthesis of Zinc Oxide Nanoparticles

ZnONPs were synthesized from the alcoholic extract of the flowering aerial parts of *P. indica* by a method illustrated previously by Attaia et al. with slight modification in which *P. indica* dried extract (1 g) was dissolved in ethanol (100 mL) reacted with zinc acetate (10 g) dissolved in doubly distilled water (1000 mL) and heated in water bath for 20 min at 100 °C. Ammonium hydroxide (a few drops) was added to the reactant media to maintain pH at 12, in which ZnONPs precipitate is created. The mixture was set for 60 minutes for complete reduction of zinc acetate to ZnONPs. The obtained ZnONPs was centrifuged at 4000 rpm, followed by washing in bi-distilled water (twice) and ethanol (twice) to yield white pellets of nanoparticles upon freeze drying.

Characterization of Metal Nanoparticles

The ZnONPs were initially analyzed by means of UV-1601 (Shimadzu Corporation, Japan) UV–visible spectroscopy ranging between 200 and 600 nm. Then, FTIR analysis using attenuated total reflectance mode by a Jasco FTIR 4100 spectrophotometer (Japan) was applied to categorize the functional groups and various phytochemical compounds responsible for formation and stabilization of the nanoparticles. Dynamic light scattering (DLS) analysis obtained by a Zetasizer (HT Laser, ZEN3600, Malvern Instruments, Malvern, UK) was applied to investigate particle size and zeta potential of the nanoparticles. Morphology and particle size of ZnONPs mediated by *P. indica* extract was determined by TEM (JEOL-JEM-1011, Japan) and FE-SEM (Mira3 Tescan). A few drops from the suspension of ZnO nanoparticles were applied to a carbon-coated copper grid and the solvent was evaporated at room temperature prior to recording the images. The powdered sample was also subjected to CuKα1-X Ray diffractometer radiation (λ = 1.5406 Å) operating at 40 kV and 30 mA with 2θ ranging from 20–90° to verify the occurrence of ZnO crystals and define their structure and size.

Evaluation of the Antiviral Activity of ZnONPs and *P. indica*

The antiviral assessment of ZnONPs and *P. indica* extract was performed by plaque inhibition assay using Vero cells (ATCC, Manassas, VA, USA) acquired from the kidneys of African green monkey, Herpes Simplex Virus type 1 (EA2387, Hopital PitiéSalpêtrière, France) and acyclovir as standard reference drug. The proliferation of the Vero cells line and the preparation of the virus stock were performed according to El-Toumy et al.37 and stored at −70°C until used. Virus titrations were calculated from cytopathogenicity and directly related to the 50% of
infectious doses per milliliter ($2 \times 10^{7.4}$ ID$_{50}$/mL) according to the Reed and Muench dilution method.\(^{38}\)

**Cytotoxicity Assay**

The cytotoxicity of the investigated agents was assessed by neutral red dye-uptake technique as previously reported.\(^{39}\) Briefly, the test compounds and acyclovir were diluted in 0.1% dimethyl sulfoxide (DMSO). Stock solutions at a concentration of 200 µg/mL were prepared by 0.1% DMSO. Vero cell monolayers cultivated with investigated agents or the positive control (two-fold serial dilutions each) were incubated for 72 h at 37°C in 5% CO$_2$ using Eagle’s MEM containing 8% FCS. After incubation, the alterations in cellular morphology were visualized by means of an inverted optical microscope for determination of MNTC (maximum non-toxic concentrations). The CC$_{50}$ values (concentrations of each agent essential for reduction of cell viability by 50%) were computed by comparing with the untreated cells. The cytotoxicity of compounds active against HSV-1 was also assessed in triplicate to obtain statistically relevant data for final calculations.

**Anti-herpes Simplex Virus Type 1 (HSV-1) Assay**

Each well in a 96-well plate containing Vero cellular suspension (100 µL, $3.5 \times 10^5$ cells/mL) and investigated agents (50 µL) was infected with viral suspension (50 µL) at MOI (multiplicity of infection) of 0.001 ID$_{50}$/cells. The plate was then incubated for 3 days at 37°C in 5% CO$_2$ without changing the media. Blanks for virus and cell were run simultaneously. After the viral growth, the cultures were stained with neutral red dye (50 µL, 0.15% in saline, pH 5.5) and incubated for 45 min at 37°C.\(^{40}\) Phosphate buffered saline (pH 7.2) was used for removing excess dye while the elution of the dye attached in the viable cells was performed using citrate ethanol buffer (100 µL/well). The anti-herpetic compound acyclovir was used with concentrations ranging from 0.5 to 5 µg/mL. The cell culture monolayer was completely destroyed after shaking the plate for 20 min. The absorbance (OD) of each well was recorded using a multichannel spectrophotometer at 540 nm. The OD was directly proportional to the percentages of viable cells that were inversely related to the cytopathic effect (CPE) ratio. The values of CC$_{50}$ and IC$_{50}$ of all the investigated agents were computed from the regression straight line of each assay that was obtained on the basis of virus controls (100% CPE) and cell controls (0% CPE).\(^{41}\) The percentage of protection (%P) was calculated by the following formula: 

$$\text{noP} = \frac{(A1) - (A2)}{(A3) - (A2)} \times 100$$

where (A1) is the absorbance of the test sample, (A2) is the absorbance of the virus-infected control and (A3) is the absorbance of the mock-infected control. The ratio of (A2) to (A3) is expressed as “% of control”.

**Statistical Analysis**

GraphPad PRISM software V.5 was used for calculations and statistical analysis. The values of mean and standard deviations (SDs) for each experiment were calculated. ANOVA followed by Tukey’s multiple comparison test was used to obtained the significance between the tested agents and the positive control.

**Results and Discussion**

**HPLC Analysis of Phenolic Compounds**

HPLC analysis (Figure 1) revealed the occurrence of 15 compounds in the alcoholic extract of the aerial parts of *P. indica*. The HPLC analysis of this extract showed that gallic acid, chlorogenic acid and rutin were the major compounds, with concentrations equal to 8203.99, 2965.95 and 1144.99 µg/g, respectively (Table 1). The rest of the compounds were characterized as catechin, methyl gallate, caffeic acid, syringic acid, rutin, ellagic acid, coumaric acid, vanillin, ferulic acid, naringenin, taxifolin and cinnamic acid and are reported here for the first time. Kaempferol was previously reported as kaempferol–3–O–rhamnoside from *P. indica* L. flowers.\(^{42}\) To the best of our knowledge, this is the first approach for identification of phenolic compounds using the HPLC technique.

**ZnO Nanoparticle Formation**

*P. indica* L. alcoholic extract reacted with zinc acetate solution in hot water bath at 80°C. Addition of ammonium hydroxide to the reaction mixture precipitated ZnONPs (Figure 2).\(^{36}\)

**ZnONPs Characterizations**

**UV Analysis**

A maximum absorption peak of ZnONPs synthesized from *P. indica* extract was found at 368 nm (Figure 3), indicating the ZnONPs formation.\(^{43}\) The broadness of absorption could be attributed to the transition of the electronic cloud on the overall skeleton of the ZnONPs.\(^{44}\) At a nano scale, ZnO has
shorter wavelengths than those found in standard ZnO, which is in parallel with the studies supporting the shorter wavelengths of material oxides.45,46 The absorption of the ZnONPs in the UV region proves that the nanoparticles could be incorporated in various medical applications such as antiseptic ointments or sunscreen protectors.47

**FT-IR Analysis of ZnONPs and P. indica L**

The FTIR was analyzed in the range of 400 to 4000 cm$^{-1}$ to identify the different functional groups present in the nanoparticles and P. indica extract. Regarding ZnONPs (Figure 4A), an intense absorption peak at 3457 cm$^{-1}$ indicated the presence of the OH group responsible for the water adsorption on the ZnNP surface. Bands observed at 2925 and 2859 cm$^{-1}$ related to the stretching vibration while the band existing at 913 cm$^{-1}$ was assigned to the bending vibration of alkane groups. Absorption peaked at 1084 cm$^{-1}$ signifying the occurrence of CO group of ethers, carboxylic acid esters and alcohols. The representative peaks of the ZnONPs were assigned at 524 and 493 cm$^{-1}$. FTIR spectrum for P. indica extract (Figure 4B) displayed a significant
peak at 3317 cm\(^{-1}\), indicating the existence of phenolic or alcoholic OH groups. Medium bands observed at 2931 and 2830 cm\(^{-1}\) revealed the incidence of alkane groups. The noticeable peaks at 1623, 1391 and 1334 cm\(^{-1}\) in the alcoholic extract indicated the existence of cyclic C–C or C=O stretch of polyphenolic compounds shifted to 1609 cm\(^{-1}\) in ZnONPs as a result of the bond between the polyphenols and NPs. The intense peak acquired at 1019 cm\(^{-1}\) proved the presence of alkane groups. The moderate bond that appeared at 730 cm\(^{-1}\) and the weak peaks at 894 cm\(^{-1}\) represented the alkenes (=C–H) group.

Dynamic Light Scattering and Zeta Potential
The size distribution image (DLS) of green synthesized form of ZnONPs is shown in Figure 5A. The detected distribution of ZnONPs size varied from 15 to 94 nm. This nanoparticle’s estimated average particle size distribution was 32.58 ± 7.98 nm. The stability of ZnONPs can be estimated by the zeta potential value. ZnONPs can be confirmed as stable colloidal solution when the absolute zeta potential is more than 30 mV. The value of ZnONPs’ zeta potential produced by \(P.\) indica was demonstrated as a clear peak at –20.8 mV (Figure 5B), signifying that the biosynthesized ZnONPs were negatively charged and moderately distributed in the medium.

Transmission Electron Microscope (TEM) and Scanning Electron Microscopy (SEM) Analyses
Low and high resolution transmission electron microscope (TEM) analysis showed the existence of ZnONPs in hexagonal shape and their corresponding range of particle size was between 2.56 and 8.83 nm (Figure 6A). The surface morphology of the obtained ZnONPs was visualized by scanning electron microscopy (SEM). The obtained image showed that most ZnONPs varied from spherical to hexagonal, with the particle diameter ranging from 19.64 to 23.21 nm (Figure 6B). SEM analysis illustrated the morphology and size of ZnONPs that was dispersed moderately in the medium. The relatively larger sizes observed by SEM were due to the smaller nanoparticle agglomeration.

XRD Analysis
The investigation of the structural features of ZnONPs was established through X-ray diffraction (XRD) (Figure 7). ZnONPs mediated by \(P.\) indica extract presented peaks with 20 values recognized at 31.799\(^{\circ}\), 34.448\(^{\circ}\), 36.271\(^{\circ}\), 47.581\(^{\circ}\), 56.641\(^{\circ}\), 62.902\(^{\circ}\), 67.981\(^{\circ}\), 68.98\(^{\circ}\), and 72.751\(^{\circ}\) corresponding to (100), (002), (101), (102), (110), (103), (200), (112), (201),

| No. | Compound         | RT*  | RRT* | Area   | Conc. (µg/g) |
|-----|------------------|------|------|--------|--------------|
| 1   | Gallic acid      | 3.034| 0.43 | 2725.95| 8203.99      |
| 2   | Chlorogenic acid | 3.806| 0.54 | 1159.31| 2965.95      |
| 3   | Catechin         | 4.195| 0.59 | 193.11 | 792.42       |
| 4   | Methyl gallate   | 4.826| 0.68 | 36.21  | 16.83        |
| 5   | Coffeic acid     | 5.313| 0.75 | 313.07 | 458.24       |
| 6   | Syringic acid    | 5.879| 0.83 | 120.66 | 180.82       |
| 7   | Rutin            | 7.056| 1.00 | 245.62 | 1144.99      |
| 8   | Ellagic acid     | 7.693| 1.09 | 404.02 | 925.77       |
| 9   | Coumaric acid    | 8.188| 1.16 | 192.09 | 111.81       |
| 10  | Vanillin         | 8.88 | 1.26 | 508.81 | 391.65       |
| 11  | Ferulic acid     | 9.476| 1.34 | 154.96 | 185.20       |
| 12  | Naringenin       | 10.074| 1.43 | 278.66 | 527.48       |
| 13  | Taxifolin        | 11.896| 1.69 | 9.87  | 24.58        |
| 14  | Cinnamic acid    | 13.266| 1.88 | 66.36  | 22.94        |
| 15  | Kaempferol       | 14.128| 2.00 | 404.00 | 542.65       |

Abbreviations: *RT, retention time; RRT, relative retention time to rutin.

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Table 1 Polyphenolic Compounds of \(P.\) Indica L. Aerial Parts Identified by HPLC
Figure 2 Schematic demonstration for the green synthesized ZnONPs mediated by the alcoholic extract of the aerial parts of *P. indica*.

Figure 3 UV spectrum of biosynthesized ZnO nanoparticles.
Furthermore, ZnONPs were considered impurity-free as there were no abnormal XRD peaks other than ZnONPs peaks. These peaks are reported in crystal system of hexagonal phase with a space group of P63mc and reference code of (01-089-0510). The average crystal size of ZnONPs was estimated to be 35.28 nm, very similar to SEM and DLS results.

\[ \text{Figure 4} \text{ FTIR spectra of (A) the nanoparticles of ZnO; (B) the alcoholic extract of the} \text{ aerial parts of} \ P. \text{ indica.} \]
To calculate the crystal size, Scherrer’s equation was applied:

\[
\text{Crystal size} = \left( \frac{0.9 \times \lambda}{d \cos \Theta} \right) = \frac{0.9 \times 0.154060}{\text{Rad}}
\]

where; \(\Theta = 2\theta/2\), \(d\) = the full width at half maximum intensity of the peak (in Rad), \(\lambda = 0.154060\) nm.

Cell Viability and Antiviral Activity Results of \(P. \text{indica}\) L. Extract and ZnONPs Concentrations (\(\mu g/mL\))

HSV-1 and HSV-2 are two types of the herpes viridae family related to orolabial and genital infections. The in vivo studies recommended that Zn salts can be effective against the viral infection.\(^{52,53}\) The first trial for employing nanoparticles as a candidates for an anti-HSV agent showed that a notable antiviral activity against HSV-1 in Vero cell line was observed in silver and gold NPs coated with mercapto ethane sulfonate.\(^{54,55}\) ZnO micro-NPs covered with multiple nanoscopic spikes are considered a virostatic agent against HSV-1, while ZnO tetrapod micro-nanostructures is regarded as a prophylactic compound for the prevention of HSV-2 infection.\(^{25,55}\)

The screening of the antiviral activities of \(P. \text{indica}\) L. extract and ZnONPs obtained by in vitro cell viability revealed that both agents displayed moderate inhibition. The observed medium percentages of cell destruction on Vero cell line were \(CC_{50} = 70.58 \pm 4.32\) and \(43.96 \pm 1.39\) \(\mu g/mL\), respectively. \(P. \text{indica}\) L. and ZnONPs exhibited anti-herpetic activity, with \(IC_{50} = 54.62 \pm 1.53\) and \(23.1667\) \(\mu g/mL\), respectively, for MOI of \(0.001 \text{ ID}_{50}/\text{cells}\). These results revealed that ZnONPs have better activity than the plant extract (Table 2). The antiviral activity of ZnONPs against HSV-1 could be due to direct interaction with the virus particles leading to trapping the virions followed by blocking the viral entrance into target cells. The selective inhibitory effect of Zn ions on viral

Figure 5 (A) DLS of biosynthesized zinc oxide nanoparticles; (B) zeta potential of biosynthesized zinc oxide nanoparticles.
Figure 6 (A) TEM analysis of ZnONPs; (B) SEM analysis of ZnONPs.

Figure 7 XRD analysis of ZnONPs.
DNA polymerase leading to inhibition of HSV replication might be an alternative mechanism. The antiviral activity of *P. indica* extract could be due to the active ingredients such as kaempferol that were previously found to have an antiviral property.

In comparison to acyclovir, which provided 100% total protection against HSV-1 at 1 μg/mL, both *P. indica* extract and ZnONPs could be considered promising adjuvants to enhance the efficacy of HSV-1 drugs.

**Conclusion**

In this work, ZnONPs were successfully green synthesized by means of *P. indica* alcoholic extract. The resultant nanoparticles were characterized using DLS, zeta potential, UV–visible spectroscopy, FTIR, XRD, SEM and TEM. The antiviral activity of the ZnONPs was examined on herpes simplex virus – type 1. Our findings exposed that zinc oxide nanoparticles synthesized from this extract might contribute to the search for new agents against HSV-1 or adjuvant with the standard antiviral drug, acyclovir.

**Acknowledgments**

The authors acknowledge Dr. Sedki Sedik Hasan Ahmed the associate Professor of English Language and Literature, Department of Foreign Languages, Faculty of Arts, Taif University for the English editing of the article.

**Funding**

This research funded by the Taif University Researchers Supporting Project number (TURSP-2020/81), Taif University, Taif, Saudi Arabia.

**Disclosure**

The authors declare no conflicts of interest in this work.

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**Table 2** Anti-Herpetic (HSV-1) Activity of *P. Indica* Extract and ZnO Nanoparticles

| Species | Zovirax (Positive Control) | *P. indica* | ZnO Nanoparticles |
|---------|---------------------------|-------------|-------------------|
| Mean ± SD | CC<sub>50</sub> >200 ± 0.2 | I<sub>C<sub>50</sub></sub> 0.5 ± 0.4 | CC<sub>50</sub> 70.58 ± 4.32 | I<sub>C<sub>50</sub></sub> 43.96 ± 1.39 |
|         | CC<sub>50</sub> 54.6 ± 1.53 |             | CC<sub>50</sub> 43.96 ± 1.39 |             |

**Notes:** *Values are expressed as μg/mL. Mean ± SD. **Significantly different from positive control (Zovirax) group at p<0.01.*

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