Eco-friendly and superficial approach for synthesis of silver nanoparticles using aqueous extract of *Nigella sativa* and *Piper nigrum L* seeds for evaluation of their antibacterial, antiviral, and anticancer activities a focus study on its impact on seed germination and seedling growth of *Vicia faba* and *Zea mays*

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Received: 11 September 2020
Revised: 22 September 2020
Accepted: 22 September 2020
Published: 4 January 2021

Egyptian Pharmaceutical Journal 2020, 19:401–413

Background
Silver nanoparticles (AgNPs) have fascinated extensive interest owing to their potential applications, especially in biomedicine.
Materials and methods
Through this investigation, AgNPs were green synthesized from AgNO**3** using *Nigella sativa* and *Piper nigrum* aqueous seed extracts as a reducing agent. Biosynthesized AgNPs were characterized using transmission electron microscopy, Fourier-transform infrared spectroscopy, zeta potential, and dynamic light scattering. Antibacterial, antiviral, and anticancer activities of AgNPs were studied. Furthermore, the effect of biosynthesized AgNPs on seed germination and seedling growth of *Vicia faba* and *Zea mays* was determined.
Results and conclusion
Results evoked the ability of *N. sativa* and *P. nigrum* aqueous seed extracts to build up AgNPs in spherical shape with average size of 20 and 50 nm, respectively, by using transmission electron microscopy analysis. The results indicate the ability of *N. sativa* and *P. nigrum* AgNPs to inhibit both tested gram-positive and gram-negative bacteria. It is conceivable from the results that AgNPs of the *N. sativa and P. nigrum* are markedly effective against herpes simplex virus-1 in terms of decreased viral load and mortality, exhibiting 83.23 and 94.54% of antiviral activity, respectively. Furthermore, in-vitro studies of *N. sativa* and *P. nigrum* AgNPs against hepatocellular carcinoma have shown good cytotoxic effect, with IC50 values equal to 7.12 and 4.98 μg/ml, respectively. AgNPs exert a promoting action on seed germination percentage and seedling growth of both *Vicia faba* and *Zea mays*. This study indicated an economical, simple, and efficient ecofriendly technique using aqueous seed extracts of *N. sativa* and *P. nigrum L.* for synthesis of AgNPs and confirmed that green AgNPs are safer.

Keywords:
AgNPs, anticancer and seed germination, antiviral, *Nigella sativa*, *Piper nigrum* L

Egypt Pharmaceut J 19:401–413

1687-4315

Introduction

Natural products afford researchers with an effective alternative for designing novel therapeutic agents against a variety of pathogens. Nanotechnology has currently exhibited a vital role as an exceptional multidisciplinary field that extensively employs materials at a nanoscale, targeting to provide new application potentials in numerous fields, covering medicine, chemistry, biotechnology, physics, etc. [1–4]. Silver nanoparticles (AgNPs) might be effective against pathogenic microbes. The high surface area of AgNPs is responsible for their potent antimicrobial action in contrast to the bulk form of silver and that is because of the superior contact with microorganisms. AgNPs are effective against a wide range of gram-positive and gram-negative bacteria [5]. Nanotechnology applications are currently in use, particularly in agricultural systems [6]. Main properties of AgNPs in plants were the size of the particles, and the smaller the particles the larger the surface area, which could result in higher cellular uptake [7]. In commercial agriculture, rapid and uniform seed germination and seedling emergence are important determinants of successful stand establishment. Some studies [8–10] have documented that plants, plant products, bacteria, fungi, algae, and yeast are

available natural sources for green synthesis of nanoparticles. AgNPs have been reported to be synthesized from different parts of some herbal plants like *Piper longum* [11], *Piper nigrum* [12] and *Plumeria rubra* [13]. *Artocarpus heterophyllus* [14], *Strychnos potatorum* [15], *Foeniculum vulgare* [16], *Silybum marianum* [17], and *Syzygium cumin* [18]. Black pepper (*P. nigrum* L.) is an important healthy food stuff owing to its antioxidant and antimicrobial activity and gastro-protective modules [19]. *Nigella sativa* L. (Ranunculaceae), generally referred to as black seed or black cumin, has been used for therapeutic purposes for several centuries. It is originally native to south eastern Asia [20]. *N. sativa* also known as black seed contains numerous active compounds like flavonoids, terpenoids, proteins, and alkaloids [21]. Cancer is the second primary cause of mortality worldwide and is responsible for a proposed 9.6 million deaths in 2018 [22]. Hepatocellular carcinoma is the sixth most common cancer but the second primary cause of cancer-mediated death [23,24]. Herpes simplex virus-1 (HSV1) and herpes simplex virus 2 (HSV-2) are alpha herpesviruses, causing more than 4 billion human infections, which can be visible as oral and genital ulcerations, neonatal disease, herpetic keratitis, and encephalitis [25,26]. In view of these, the current study was performed to monitor the antibacterial, antiviral, and anticancer efficacy of biosynthesized AgNPs of *N. sativa* and *P. nigrum* L seed extracts and assess their effect on seed germination and seedling growth of *Vicia faba* and *Zea mays*.

Materials and methods

Regarding plant material collection, the seeds of *N. sativa* and *P. nigrum* L were obtained from an authorized agricultural market in Egypt.

Extraction procedure

Overall, 150 g of each of *N. sativa* and *P. nigrum* seeds was grinded separately into fine powder. Then, extraction was done in 200 ml of sterilized distilled water for 24 h and filtered using Whatman No. 1 filter paper (Merck), particle retention of 11 µm. It was then left to concentrate, dried into powdered form at 40°C in the oven, and then stored at 4°C for further experiments. All laboratory works were performed in Botany and Microbiology Department, Faculty of Science, Al-Azhar University (Girls Branch) according to the guidelines and regulation ethics of the institute.

Biogenic synthesis of AgNPs using aqueous seed extracts

Silver nitrate was used as a precursor for the synthesis of AgNPs. A solution of silver nitrate (1 mmol/l) was prepared by dissolving 0.017 g of the compound in 100 ml of distilled water. Then, 1 ml of aqueous extract of each of *N. sativa* and *P. nigrum* was added separately to 99 ml of silver nitrate solution. The solution was subjected to heating at 70°C for 30 min, which produced brown to brownish-red color, according to the method described by Amin *et al.* and Kumar *et al.* [27,28].

Characterization of silver nanoparticles

The resultant AgNPs were characterized using Fourier-transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), zeta potential, and transmission electron microscopy (TEM). All characterization steps were carried out at the Egyptian Petroleum Research Institute- Nanotechnology Center, Cairo, Egypt. Chemical structure of functional groups of AgNPs was analyzed using Fourier-transform infrared spectroscopy (ATR-FTIR, JASCO FTIR 4100 spectrometer) over a wide range of 4000–400 cm^{-1}. For the size distribution of AgNPs, the DLS technique using Malvern instruments Nano Series HT was used. Zeta potential was determined using ZETASIZER Nano Series HT Malvern. Morphological characteristics of biosynthesized AgNPs including size and shape were investigated using a TEM using Jeol 2100 high resolution (Musashino, Akishima, Tokyo, Japan).

Determination of the antibacterial activity of biosynthesized AgNPs

Six human pathogenic bacteria were kindly obtained from the Central Water Quality Laboratory of Greater Cairo Water Company, Cairo, Egypt. Gram-positive bacteria used were *Bacillus megaterium*, *Bacillus subtilis* SK09, and *Staphylococcus aureus* ATCC 6538, whereas gram-negative bacteria were *Escherichia coli* ATCC 11775, *Klebsiella oxytoca*, and *Pseudomonas aeruginosa* ATCC 27853. The antibacterial activity of the AgNPs was quantified by the paper disk diffusion method, wherein 24-h cultures of different pathogens were inoculated onto nutrient agar plates, then 20 µl of each AgNP extract was loaded on each paper disk of 6-mm diameter, allowed to stand for 30 min, and finally incubated at 37°C for 18–24 h. At the end of incubation period, the diameter of inhibition zones was measured and recorded as mm.

Determination of antiviral activity of biosynthesized AgNPs

This step was carried out at Science Way for Scientific Researches and Consultations, Cairo, Egypt. All

viruses were obtained from Microbiology Department, Faculty of Medicine Al-Azhar University (Girls Branch).

Determination of samples cytotoxicity on Vero cell

Various concentrations of AgNPs under investigation were prepared. The medium used for the growth was decanted from 96-well microtiter plates, and then later, a confluent sheet of Vero cell was produced. Washing of the cell monolayer was done twice using wash media. Double-fold dilutions of the tested samples were made in DMEM. Then, 0.1 ml of each dilution was tested in different wells, leaving three wells as control, receiving only maintenance medium. The plate was incubated at 37°C for 48 h and examined regularly. Any physical signs of cell toxicity like loss of the monolayer, rounding, shrinkage, or cell granulation were noted. Approximately 5 mg/ml in PBS of MTT solution was prepared (Bio Basic Canada Inc., founded in Toronto, Canada - Headquartered in Markham, Ontario and Amherst, New York). Each well was filled with 20-µl MTT solution. MTT was thoroughly mixed with the media by shaking at 150 rpm for 5 min. Incubation was run at 37°C, 5% CO_2, for 4 hours. The plate was dried on paper towels to get rid of remains. The metabolic product (Formazan) was re-suspended in 200 µl DMSO, shacked at 150 rpm for 5 min, to allow mixing of formazan with the solvent. OD was recorded at 560 nm; background absorbance was subtracted at 620 nm. The maximum nontoxic concentration (MNTC) of each extract was also estimated.

Antiviral assay (MTT assay protocol)

Overall, 10 000 cells were plated in 200-µl media per well in a 96-well plate. In the plate, three empty wells served as blank controls. Incubation was carried out at 37°C, 5% CO_2, incubator for one night to allow attaching the cells to the wells. Incubation of equal volume (1:1 v/v) of nonlethal dilution of the tested sample and the viral suspension for one hour was carried out. Then, 100 µl from viral/sample suspension was added, placed on a shaking table at 150 rpm for 5 minm and then incubated at 37°C in 5% CO_2 incubator for one day to allow the virus to do the effect. Overall, 2 ml or more of MTT solution per 96-well plate at 5 mg/ml in PBS was added and shacken at 150 rpm for 5 min, to allow mixing the MTT with the media and then incubated for 1–5 h at 37°C, 5% CO_2 incubator. The plate was dried on paper towels to get rid of remains. The metabolic product (Formazan) was re-suspended in 200 µl of DMSO, shacken at 150 rpm for 5 min, to allow mixing the formazan with the solvent. OD was recorded at 560 nm and subtracts background at 620 nm. The maximum non- MNTC of each extract was also estimated.

In-vitro cytotoxic effect of biosynthesized AgNPs on HepG2 cell

The cytotoxicity was determined *in vitro* against the HepG2 cell line at the Department of Virology and Tissue Culture, Faculty of Medicine, Al-Azhar University, Cairo, Egypt.

Cell culture

HepG2 cell line was maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-anti-mycotic solution. The cells were grown to confluence at 37°C and 5% CO_2 atmosphere. All experiments were performed in tissue culture flasks unless otherwise stated. The cells were seeded onto the plates at a density of 1×10^6cells per well and incubated for 24 h before the experiments. The cells were incubated in fresh medium containing different concentrations of AgNPs *N. sativa* and *P. nigrum* extracts separately. Dilutions of AgNPs of *N. sativa* and *P. nigrum* seed extracts used for HepG2 cell line were 0.5, 1, 2, 5, and 10 µg/ml concentrations. In-vitro assay of cytotoxic activity (MTT protocol) was carried out according to Mossman [29].

Effect of Nigella sativa and Piper nigrum AgNPs (80 ppm) on seed germination and seedling growth of Vicia faba and Zea mays

Seeds from the *V. faba* and *Z. mays* were obtained from an authorized agricultural market in Egypt. Overall, 20 selected seeds or grains were placed on filter papers inside a petri-dish 9 cm in diameter to allow germination, and 15–20 ml of the extract (or tap water in the case of the control) was added. The germinated seeds were recorded daily for a period of 15 days. In addition, the growth of the radicles and the plumules was recorded for 7 days, starting from the first day of emergence.

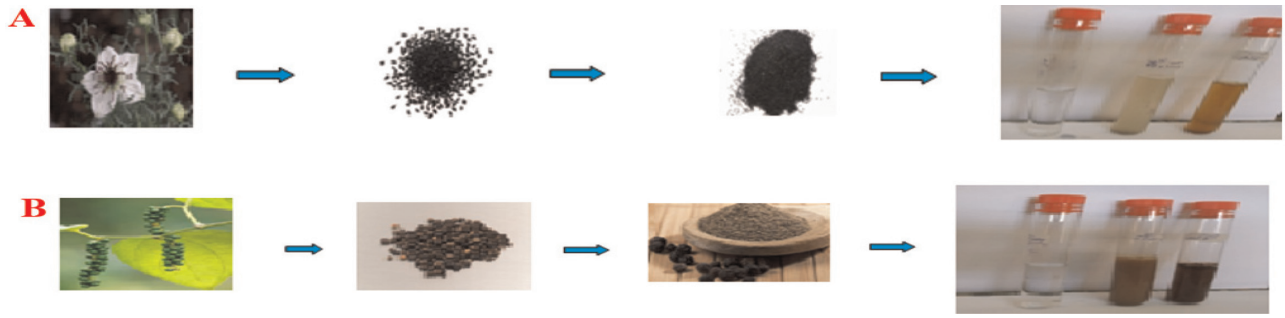
Statistical analysis

The values are expressed as mean±SD. All data (three replicates) were subjected to analysis of variance of one-way by using SPSS ver. 16.0 (IBM software, USA). We took a level of significance at *P* value of 0.05, as our desired level of significance.

Results

Biogenic synthesis of AgNPs was firstly recognized by visual inspection. The appearance of a dark brown color in the reaction flasks informs the formation of AgNPs [30] (Fig. 1a and b).

Figure 1



(a) The color change of plant extracts after addition of silver nitrate to *Nigella sativa* seeds. (b) The color change of plant extracts after addition of silver nitrate to *Piper nigrum* L seeds.

The FTIR spectroscopic analysis of the *N. sativa* AgNPs is shown in Fig. 2a. The obtained FTIR spectrums showed intense absorption peaks appearing at 3448, 1637, 1541, 1384, and 667 cm^{-1}. The band at 3448 cm^{-1} clarified alcoholic O-H stretching and 1637 cm^{-1} clarified the alkynyl C<td: glyph name=″dbnd″/>C stretching manner in the aromatic compounds, which indicates the existence of aromatic compounds such as flavonoids. Moreover, the presence of a band at 1541 cm^{-1} indicated nitro compound N-O stretching, whereas the band at 1384 cm^{-1} revealed O-H phenol bending. Moreover, the band at 667 cm^{-1} showed C-Br stretching of a halo compound. In addition, FTIR bands observed owing to AgNPs produced by *P. nigrum* L aqueous seed extract were recorded from 497 to 3911 cm^{-1} (Fig. 2b). The bands at 3911, 3892, 3886, 3875, 3848, 3827 3811, 3765, 3740, and 3453 cm^{-1} were a definite indicator of alcoholic O-H stretching. Precisely, new bands at 2089 and 1634 cm^{-1} that appeared in FTIR spectrum of AgNPs produced by *P. nigrum* L may correspond to iso-thiocyanate and alkynes owing to N=C=S stretching and C&9552;C stretching, respectively. However, the band that occurred at 1457 and 666 cm^{-1} corresponds to the C-H bending alkane and C-Br stretching halo compound, respectively.

The structural morphology and crystallinity of biologically synthesized AgNPs were further confirmed by TEM. The TEM micrograph of synthesized AgNPs was observed in spherical shape without agglomeration, and the average size was 20 and 50 nm under different magnifications for *N. sativa* and *P. nigrum*, respectively (Fig. 2c and d).

Size distribution analysis was carried out by the aid of a DLS in aqueous solution. It was found that the average size of *N. sativa* AgNPs was 63.99 nm, whereas the average size of *P. nigrum* AgNPs was 57.31 nm (Fig. 2e and f).

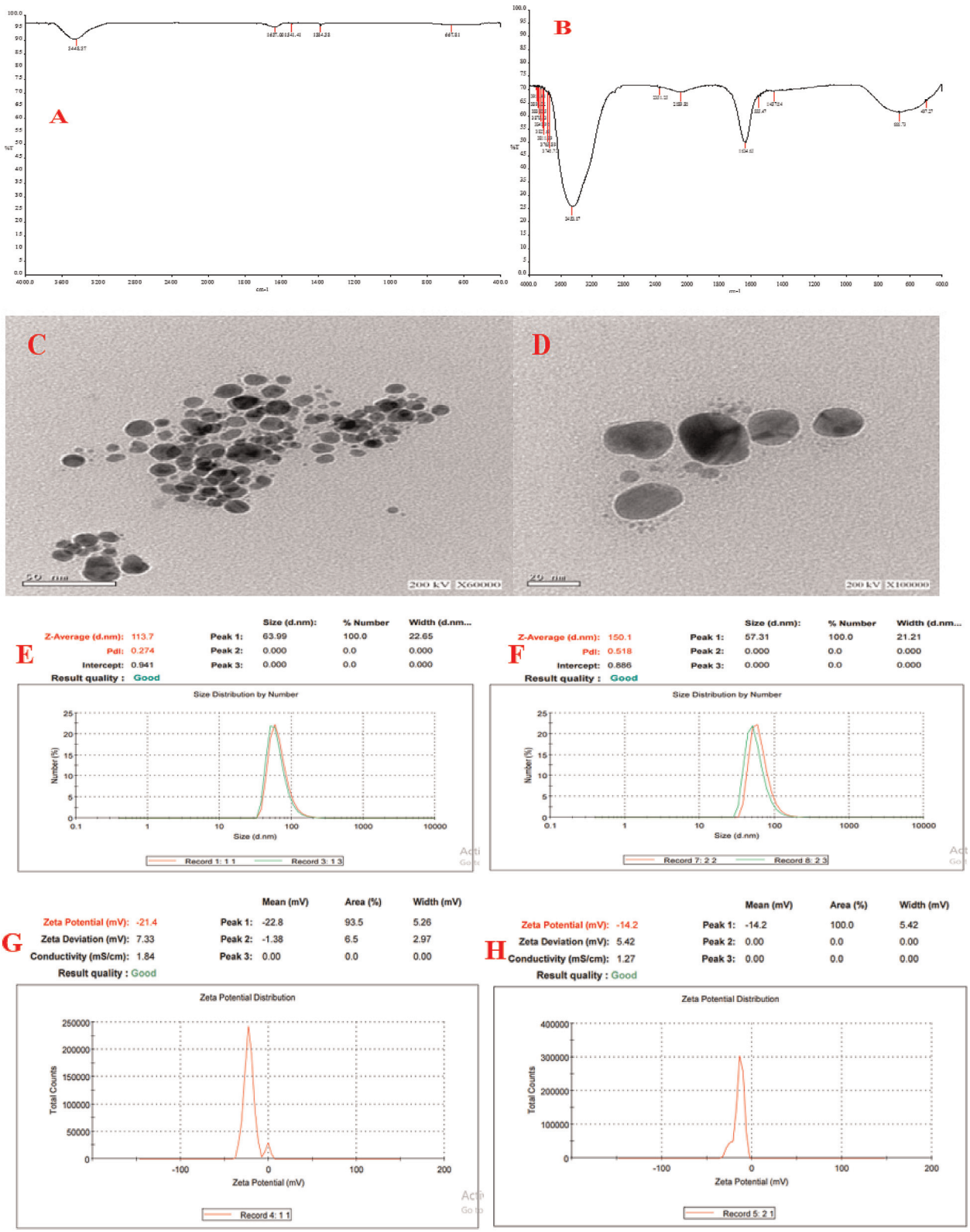
Zeta potential was carried out using photon correlation spectroscopy, and the value of *N. sativa* AgNPs and *P. nigrum* AgNPs was found to be −21.4 mV and −14.2, respectively (Fig. 2g and h). The preferred AgNPs gave a negative zeta potential value, with a non-significant difference among them.

Antibacterial activity of AgNPs

Bioactivity of AgNPs synthesized by *N. sativa* and *P. nigrum* aqueous seed extracts was evaluated against pathogenic gram-positive bacteria (*Bacillus megaterium*, *Bacillus subtilis* SK09, and *Staphylococcus aureus* ATCC 6538) and gram-negative bacteria (*Escherichia coli* ATCC 11775, *Klebsiella oxytoca,* and *Pseudomonas aeruginosa* ATCC 27853), at 20 µl concentrations. The results proclaimed that, biologically synthesized AgNPs from both plant extracts under study have antibacterial activity against all tested bacterial strains (Table 1 and Fig. 3).

Interestingly, the antiviral activity of the *N. sativa* and *P. nigrum* AgNPs was explored against HSV1, as shown in Fig. 4a and b, respectively, in comparison with HSV1 action on Vero cells (Fig. 4c). The HSV1 was found to be more sensitive to AgNPs *N. sativa* and *P. nigrum*. Based on the current data, AgNPs of *N. sativa* and *P. nigrum* apply 83.23 and 94.54% inhibition of plaque of HSV1 (Table 2) when the extract was applied to the Vero cells 2 hrs. before the virus infection. The anti-HSV activity might be owing to the binding of phytochemical compounds in AgNPs of *N. sativa* and *P. nigrum* with the protein coat of the virus and inhibit the absorption of a virus into the Vero cells. Interestingly, AgNPs *N. sativa* and *P. nigrum* seed extract demonstrated antiretroviral activity, and it could be used as a promising candidate for new and potent antiviral herbal preparation with fewer adverse effects. The MNTC was 3.286 and 3.033% for AgNPs *N. sativa* and *P. nigrum* seeds extract, respectively, against HSV1.

Figure 2



Fourier-transform infrared spectroscopy analysis of AgNPs synthesized using (a) *Nigella sativa* aqueous seed extract. (b) *Piper nigrum* L aqueous seed extract. (c) Transmission electron microscopy image of biosynthesized AgNPs of *Nigella. sativa* aqueous seed extract with diameters of 50 nm (magnification power 6000×). (d) Transmission electron microscopy image of biosynthesized AgNPs of *Piper nigrum* aqueous seed extract with diameters of 20 nm (magnification power 10000×). (e) Size distribution report by number of AgNPs synthesized by *Nigella sativa* seed. (f) Size distribution report by number of AgNPs synthesized by *Piper nigrum* seed aqueous extract. (g) Zeta potential distribution of AgNPs synthesized by *Nigella sativa* seed. (h) Zeta potential distribution of AgNPs synthesized by *Piper nigrum* L seed aqueous extract. AgNP, silver nanoparticle.

Cytotoxic effect of AgNPs synthesized by *N. sativa* and *P. nigrum* extracts has been investigated toward HepG2 cell line. The variation in morphological nature of HepG2 cell line is the initial sign shown after exposure to AgNPs of both *N. sativa* and *P. nigrum* seed extract. As the concentrations of AgNPs of both plant extracts increased, cell viability decreased, and cytotoxicity increased. Cell survival rate and cytotoxicity effect are shown in Table 3. The results revealed that there were significant morphological changes, which are characteristic features of apoptotic cells, such as loss of membrane integrity, cell shrinkage, reduced cell density, and granulation when compared with control cell line, which has a well-ordered cytoskeleton. Data also affirmed that the IC_{50} values of AgNPs *N. sativa* and *P. nigrum* were 7.12 and 4.98 µg/ml respectively.

Table 1 Inhibition zone (mm) of *Nigella sativa* and *Piper nigrum* AgNPs against various pathogenic bacteria

Bacterial strain	Mean inhibition zone (mm) of *Nigella sativa* AgNPs	Mean inhibition zone (mm) of *Piper nigrum* AgNPs
Bacillus megaterium	13.2±0.152	23.1±0.153
Staphylococcus aureus ATCC 6538	7.03±0.058	8±0.000
Bacillus subtilis SK09	9±0.0000	9.2±0.057
Pseudomonas aeruginosa ATCC 27853	8.9±0.058	9±0.000
Klebsiella oxytoca	7.4±0.000	7.9±0.057
Escherichia coli ATCC 11775	10±0.000	10±0.057

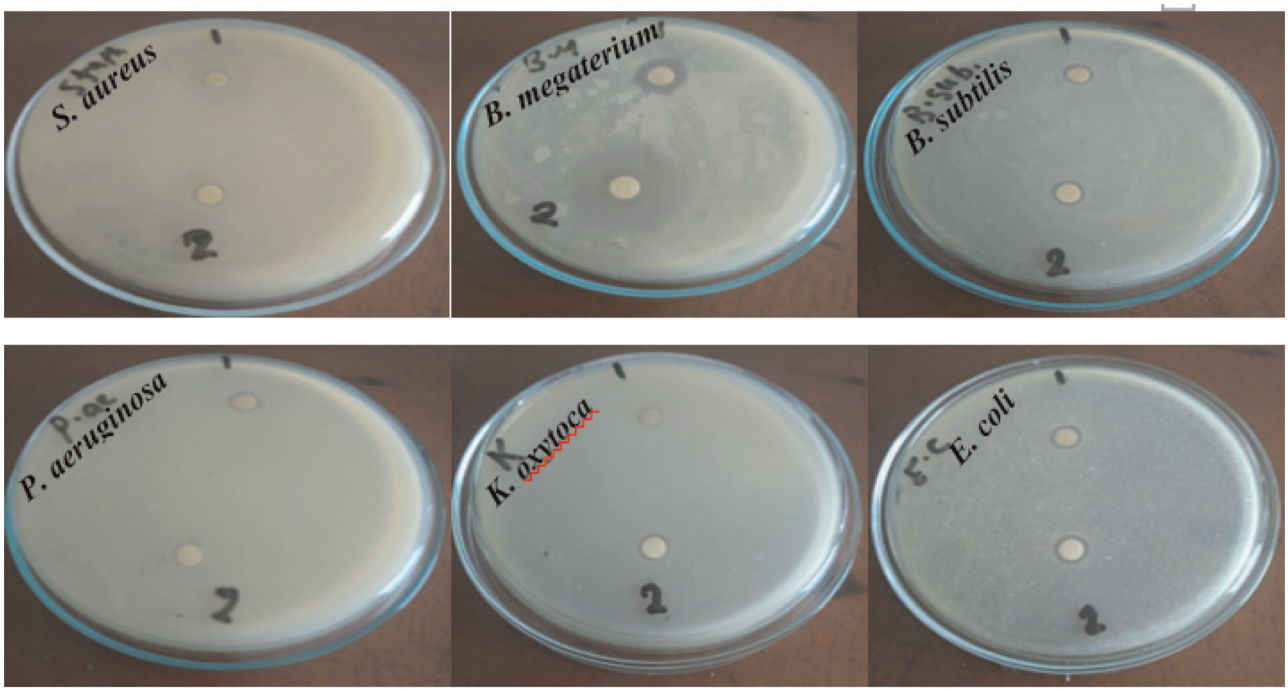
AgNP, silver nanoparticle.

Effect of Nigella sativa and Piper nigrum AgNPs (80 ppm) on seed germination percentage and seedling growth of Vicia faba and Zea mays

N. sativa and *P. nigrum* AgNPs are prepared to explore their effects on seed germination percentage and seedling growth of *V. faba* and *Z. mays*. Significant results regarding seeds germination percentage, radicle length, and plumule length were observed for all seeds as compared with control.

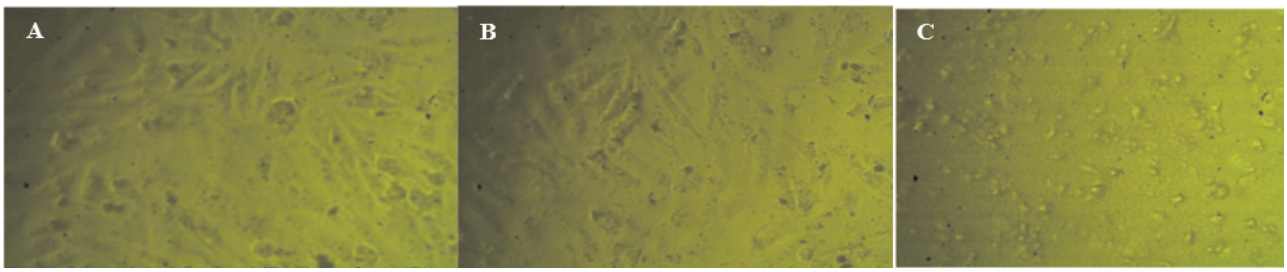
The data presented in Fig. 5a and b indicated that when the seeds of studied plants were soaked in 80 ppm AgNPs for different times, seed germination percentage of *V. faba* and *Z. mays* increased, and the increment was proportional to the soaking time used as compared with the control. The highest germination percentage (90%) was obtained at 90 min, but at 120 min, the seed germination percentage of *V. faba* and *Z. mays* decreased to 4% and 70%, respectively. Moreover, the effects of different soaking times (30, 60, 90, and 120 min) of *N. sativa* nanoparticles extract on shoot and root length of *V. faba* and *Z. mays*

Figure 3



Antibacterial activity of biosynthesized AgNPs, where (1) is *Nigella sativa* aqueous seeds extract (2) *Piper nigrum* aqueous seeds extract (20 µg/ml). AgNP, silver nanoparticle.

Figure 4



(a) Antiviral effect of AgNPs of *Nigella sativa* on HSV1. (b) Antiviral effect of AgNPs of *Piper nigrum* on HSV1. (c) Effect of HSV1 on Vero cells. Irregular out line and showing cytoplasmic projections, cytoplasmic vacuolation, nuclear membrane begins to disintegrate, some nuclei are difficult to be seen, the cytoplasm appears as mottled, lamps diffuse mass distributed throughout the cytosol with dense lysosomes and myelin figure. AgNP, silver nanoparticle; HSV, herpes simplex virus.

Table 2 Percentages of antiviral effect of AgNPs of *Nigella sativa* and *Piper nigrum* seed extract against HSVI

Test	Dilution no.	OD			Mean OD	Viability	Toxicity	Viral activity %	Antiviral effect %
Control Vero cells	–	0.251	0.293	0.278	0.274	100	0	–	–
HSV I	–	0.103	0.119	0.105	0.109	39.7810	60.2189	100	0
Nigella sativa AgNPs	5	0.246	0.238	0.255	0.24633	89.9026	10.0973	16.7676	83.23
Piper nigrum AgNPs	5	0.263	0.259	0.273	0.265	96.7153	3.28467	5.45454	94.54

AgNP, silver nanoparticle; OD, optical density.

Table 3 Cytotoxic effect of AgNPs of *Nigella sativa* and *Piper nigrum* seed extract against human hepatocellular carcinoma (HepG2) cell line

ID	Concentration (μg/ml)	OD			Mean OD	STE	Viability %	Toxicity %	IC50 μg/ml
HepG2 (control cell)	1 : 2	0.356	0.341	0.368	0.355	0.00781	100	0	Dil
Nigella sativa AgNPs	1	0.021	0.018	0.019	0.019333	0.000882	5.44600939	94.55399061	
	2	0.02	0.02	0.017	0.019	0.001	5.352112676	94.64788732	
	3	0.023	0.021	0.025	0.023	0.001155	6.478873239	93.52112676	7.12
	4	0.036	0.024	0.035	0.031667	0.003844	8.920187793	91.07981221	
	5	0.045	0.052	0.066	0.054333	0.006173	15.30516432	84.69483568	
	6	0.089	0.096	0.072	0.085667	0.007126	24.1314554	75.8685446	
	7	0.148	0.136	0.151	0.145	0.004583	40.84507042	59.15492958	
	8	0.267	0.25	0.286	0.267667	0.010398	75.39906103	24.60093897	
Piper nigrum AgNPs	1	0.018	0.02	0.019	0.019	0.000577	5.352112676	94.64788732	
	2	0.024	0.016	0.023	0.021	0.002517	5.915492958	94.08450704	4.98
	3	0.028	0.022	0.023	0.024333	0.001856	6.854460094	93.14553991	
	4	0.086	0.092	0.084	0.087333	0.002404	24.60093897	75.39906103	
	5	0.142	0.136	0.158	0.145333	0.006566	40.93896714	59.06103286	
	6	0.321	0.336	0.324	0.327	0.004583	92.11267606	7.887323944	
	7	0.362	0.351	0.352	0.355	0.003512	100	0	
	8	0.355	0.348	0.362	0.355	0.004041	100	0	

AgNP, silver nanoparticle; OD, optical density.

seeds were evaluated at the seventh day from germination. The data revealed that shoot and root lengths of studied plants were significantly increased at 30 and 60 min, and at 90 and 120 min, shoot and root length decreased slightly.

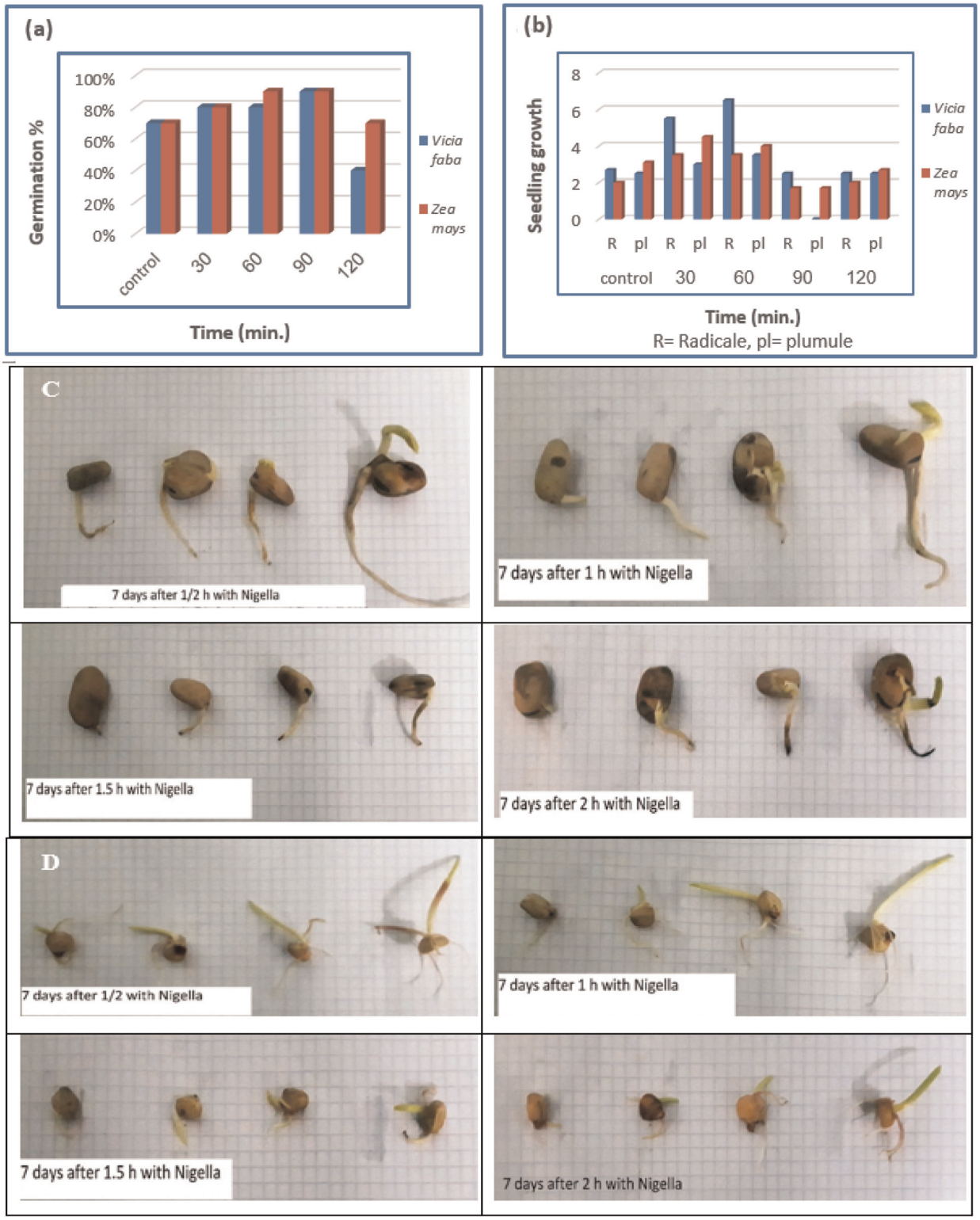
Effect of Piper nigrum AgNPs (80 ppm) on seed germination percentage and seedling growth of V. faba and Z. mays

The results from Fig. 6a and b revealed that *P. nigrum* AgNPs promoted seed germination percentage in *V. faba* and *Z. mays* at all soaking time as compared with control to give maximum value of 100% with *V. faba* and 90% with *Z. mays* at 120 min The length of radicle and plumule of *V. faba and Z. mays* increased gradually with increase in the soaking time till 60 min, and after this time, length of radicle and plumule of two studied plants decreased sharply.

Discussion

The aim of this study was to evaluate the antibacterial, antiviral, and anticancer efficacy of biosynthesized AgNPs of *N. sativa* and *P. nigrum*

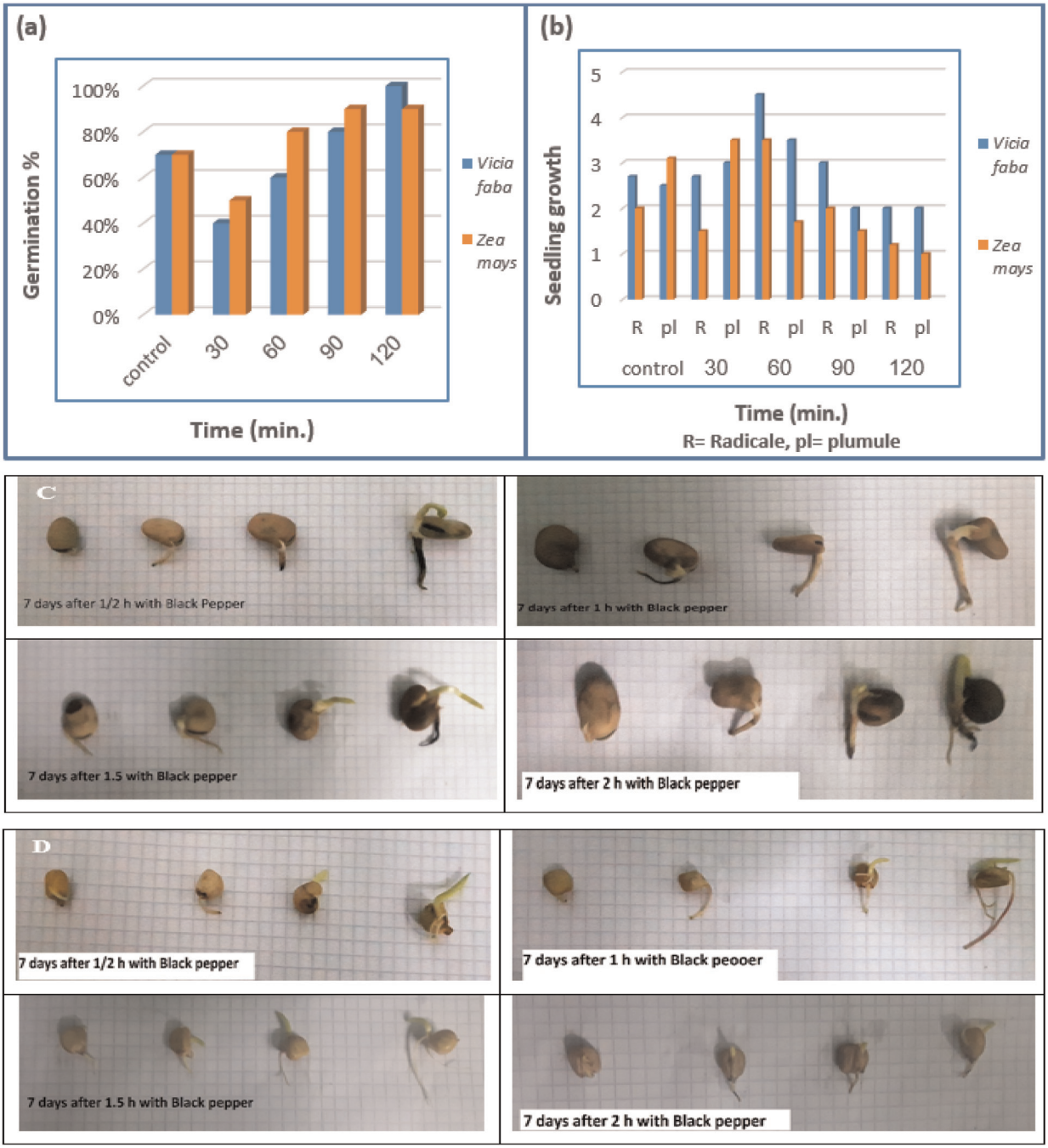
Figure 5



(a) Effect of *Nigella sativa* AgNPs (80 ppm) on seeds germination percentage of *V. faba* and *Z. mays*. (b) Effect of *Nigella sativa* AgNPs (80 ppm) on seedling growth of *V. faba* and *Z. mays*. (c) Effect of *Nigella sativa* AgNPs (80 ppm) on seedling growth of *V. faba*. (d) Effect of *Nigella sativa* AgNPs (80 ppm) on seedling growth of *Z. mays*. AgNP, silver nanoparticle.

on seed germination and seedling growth of *Vicia faba* and *Zea mays*. First, biological synthesis of AgNPs was determined by a dark brown color appearance. Several reports detailed that the biosynthesis of Ag^{+} is owing to the electron shuttle quinines and reducing agents such as enzymes [31,32]. This was confirmed by TEM for the occasioned AgNPs which was found to be spherical in shape and ranging from 20 and 50 nm in size under different magnifications.

Figure 6



(a) Effect of *Piper nigrum* AgNPs (80 ppm) on seed germination percentage of *V. faba* and *Z. mays.* (b) Effect of *Piper nigrum* AgNPs (80 ppm) on seedling growth of *V. faba* and *Z. mays*. (c) Effect of *Piper nigrum* AgNPs (80 ppm) on seedling growth of *V. faba.* (d) Effect of *Piper nigrum* AgNPs (80 ppm) on seedling growth of *Z. mays.* AgNP, silver nanoparticle.

Additionally, FTIR spectrum analysis confirmed the biosynthesized AgNPs of *N. sativa* and *P. nigrum,* exhibiting prominent peaks, which were assigned to amides, alkynes, alkane, phenol, and alcohol groups as the most efficient groups. A parallel inspection is recorded in the biological synthesis of AgNPs using seeds extract of *Artocarpus heterophyllus* Lam [14]. These results were in complete agreement with Khan *et al.* and Mukesh Kumar *et al.* [20,33]. Groups present in the bioactive compounds were determined by FTIR analysis. The functional groups were easily identified through the standard IR functional data report [34–36].

Size distribution analysis and zeta potential were carried out. As previously reported by Parmar *et al.* [37], nanoparticles with elevated zeta potential values are extremely electrically stable and might resist aggregation.

The antibacterial activity of *N. sativa* and *P. nigrum* AgNPs was explored against some pathogenic gram-positive and gram-negative bacteria, and the results indicated that biologically synthesized AgNPs from both plant extracts have antibacterial activity against all tested bacterial strains.

The study conducted by Gardea-Torresday *et al.* [38] reported that biogenic synthesis of metal nanoparticles could be a conventional method, and application of plant extracts incorporates a new-fangled consciousness to manage the disease, besides being safe and having no toxic effects. Previous studies indicated the antibacterial activity of AgNPs by its affection on the bacterial cell wall, or the creation of free radicals [39,40]. The silver ions released from AgNPs may play a critical role in the antibacterial activity owing to the interaction of silver ion with the thiol groups of enzymes [41]. Additionally, it was publicized that the antibacterial activity of AgNPs was size and shape dependent. Hasan *et al.* and Abdallah *et al.* [42,43]. documented that thymoquinone extracted from seeds of *N. sativa* showed broader spectrum activities against many strains of gram-positive and gram-negative bacteria, including *Bacillus*, *Listeria*, *Enterococcus*, *Micrococcus*, *Staphylococcus*, *Pseudomonas*, *Escherichia*, and *Salmonella* and stopping the bacterial biofilm formation.

The results also showed strong abilities of AgNPs synthesized from *N. sativa* and *P. nigrum* in controlling the activity of HSV1 applying 83.23 and 94.54% inhibition of plaque of HSV1, respectively. The MNTC was 3.286 and 3.033% for AgNPs *N. sativa* and *P. nigrum* seeds extract, respectively, against HSV1. Several studies recorded that the antiviral activity of AgNPs against numerous types of viruses is owing to direct binding of the AgNPs with glycoproteins of viral envelope, in that way inhibiting penetration of virus into the host cell [44–47]. The effect of the size of AgNPs on antiviral activity was usually observed, suggesting spatial restriction of binding between virions and AgNPs [44,45].

Our results also, proved that, *N. sativa* and *P. nigrum* AgNPs have potent antitumor activity toward HepG2 cell line, and the IC_{50} value were 7.12 and 4.98 µg/ml for *N. sativa* and *P. nigrum* AgNPs, respectively. MTT method is an accurate colorimetric assay method assigned for precisely estimation of both cytotoxicity assays and number of viabilities in the cell proliferation. The results also evoked that, the mortality of HepG2 cell line was dose dependent when exposed to various concentrations of AgNPs of both *N. sativa* and *P. nigrum*. Numerous studies documented that AgNPs are cytotoxic, and their cytotoxicity is dependent on size and dose. Moreover, surface modifications of AgNPs can radically alter the toxicity [48,49]. In addition, there has been a grand attention in the use of natural compounds for cancer treatment. Many medicinal herbs have anticancer properties [50]. Consequently, the synergistic effect of herbal extract and AgNPs can be a promising strategy for cancer therapy.

Moreover, Black cumin seed extract and seed oil were reported to reduce the viability of human lung cancer, inhibiting proliferation, migration, and invasion of lung cancer cells [51,52] and has antiproliferative and proapoptotic effects in breast cancer [53–55] and inhibiting cell proliferation in liver cancers [56,57]. In recent times, various studies showed anticancer activities of piperine, due to free-radical scavenging activity. Piperine could be supportive in chemoprevention and controlling the progression of tumor growth and inhibiting propagation and survival of various cancerous cell lines by modulating the progression of cell cycle and exhibiting anti-apoptotic activity [19,58]. As documented by Jie *et al.* [59], several spices are potential sources for prevention and treatment of cancers, such as *N. sativa* (black cumin) and *P. nigrum* (black pepper) which contained several important bioactive compounds. Several studies [60–62] showed the activity of black pepper to inhibit proliferation, growth, and motility of breast cancer, whereas Yaffe *et al.* and Yaffe *et al.* [63,64] reported its activity in impairing cell cycle progression and inducing apoptosis in colorectal cancer.

By applying AgNPs of *N. sativa* on seeds of studied plants for different times, it was noticed that seed germination percentage of *V. faba* and *Z. mays* increased, and the highest germination percentage (90%) was obtained at 90 min, but at 120 min, the seed germination percentage of *V. faba* and *Z. mays* decreased to 40 and 70%, respectively. The data also revealed that shoot and root lengths of studied plants were significantly increased at 30 and 60 min, and at 90 and 120 min, shoot and root length decreased slightly. These results are in agreement with studies that indicated AgNPs had a positive role in the promotion of plant growth in *Brassica juncea* [65], *Panicum virgatum*, *Phytolacca Americana* [66], *Phaseolus vulgaris*, and *Z. mays* [67]. Seed germination was positively affected by treatment

with AgNPs in *Boswellia ovalifoliolata* plant. Savithramma *et al.* and Qian *et al.* [68,69] reported that AgNPs may increase or inhibit growth of plants depending on AgNPs dosage, and exposure to specific concentrations of AgNPs could enhance plant growth compared with nonexposed plants, whereas higher and lower concentrations affect plant growth negatively.

Interestingly, *P. nigrum* AgNPs endorsed seed germination percentage in *V. faba* and *Z. mays* at all soaking time as compared with control to give maximum value of 100% with *V. faba* and 90% with *Z. mays* at 120 min. Same results were obtained by Savithramma *et al.* [68], who found that AgNPs significantly increased germination value of seeds of *Boswellia ovailifoliolata* plant, but increased germination time. It is probably that nanoparticles could penetrate into the seed coat and exert a helpful effect on the process of seed germination. Based on the studies on nanoparticles effect on seed germination mechanism, it could be stated that nanoparticles might have helped the water absorption by the seeds; increase nitrate reductase enzyme; increase seed abilities of absorbing and utilizing water and fertilizer [70]; reduced antioxidant stress by reducing H_2O_2, superoxide radicals, and malonyl dialdehyde content; and increasing superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase, and catalase activities, which result in improve seed germination in some plant species [71]. In contrast, Abdel-Azmeen and Elsayed [72] reported that seed germination was not affected by exposure to AgNPs in *V. faba*. The effect of AgNPs of *N. sativa* and *P P. nigrum* on seedling growth of the two studied plants was not related to its effect on germination parameters. AgNPs had toxic effect on seedling root length of two studied plants at 90 and 120 soaking time in AgNPs. On the contrary, Salama [67] mentioned that corn and common bean root length mean values decreased with exposure to concentration higher than 60 ppm of AgNPs and increased with AgNPs concentrations less than this concentration. Watermelon and zucchini plants root length values enhanced in response to AgNPs treatment for *Vicia faba* [72]. AgNPs had a positive effect on *Pennisetum glaucum* plant, which its seed germination promoted in response to AgNPs while seedling root elongation was inhibited [73]. This is also consistent with previous studies that report NPs had less effect on seed germination than seedling growth [74]. This may be explained by the protective effect of the seed coat according to Wierzbicka and Obidzinska [75].

Conclusion

The present study described an ecofriendly and simple method to synthesize AgNPs by *N. sativa* and *P. nigrum* seed extracts. The green synthesized AgNPs were characterized using FTIR spectroscopy, DLS, and TEM analysis. The synthesized AgNPs using seed extract of *N. sativa* and *P. nigrum* proved antibacterial activity against the examined bacterial strains and exhibited a significant cytotoxic effect on HPG2 cell lines. Interestingly, stronger antiviral activity was generally observed, and it could be used as a promising candidate for new and potent antiviral herbal preparation with fewer adverse effects. Moreover, it exerts a promoting action on seed germination percentage and seedling growth of *Vicia faba* and *Zea mays*.

Author Contribution

All authors designed the experiments, analyzed experimental data, performed the experiments, provided the chemicals and prepared the figures and tables; Amira Y. Mahfouz and Asmaa M. Radwan wrote the manuscript text, Ghadir E. Daigham and Abeer A. Mohamed reviewed the manuscript.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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