Prospective of biosynthesized \textit{L.sativum} oil/PEG/Ag-MgO bionanocomposite film for its antibacterial and anticancer potential

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\textbf{A B S T R A C T}

A substantial interest has been manifested in utilizing oil/metal oxide hybrid bionanocomposite, especially organic/inorganic to design different biomedical applications. The present study reports the synthesis, characterization, antibacterial and anticancer properties of biogenic silver nanoparticles (AgNPs) and \textit{L.sativum} oil/PEG/Ag-MgO bionanocomposite. The fabricated AgNPs and \textit{L.sativum} oil/PEG/Ag-MgO bionanocomposite were characterized by employing different spectroscopic (UV, FTIR, XRD) and microscopic (TEM, SEM) techniques. The particle size analysis showed that the mean size of 16.32 nm for AgNPs and 13.45 nm \textit{L.sativum} oil/PEG/Ag-MgO, indicating the excellent dispersion of Ag-MgO nanoparticles in the PEG–\textit{L.sativum} oil matrix. The antimicrobial activity of AgNPs and polymeric bionanocomposite was investigated against two pathogenic bacteria. The highest antibacterial effect was observed for polymeric bionanocomposite towards Gram-positive \textit{Staphylococcus aureus} (27 mm) and Gram-negative \textit{Escherichia coli} (25 mm) at 40 \textmu{g} well\textsuperscript{-1}. The bionanocomposite completely vanished the bacterial growth (100%) at 80 \textmu{g} mL\textsuperscript{-1} concentrations. Moreover, the AgNPs and polymeric bionanocomposite was evaluated for anticancer activity against human cervical cancer cells (HeLa cells) at different doses (50, 250, 500, and 1000 \textmu{g} mL\textsuperscript{-1}). The results showed polymeric bionanocomposite was stronger in inducing the HeLa cancer cell death than AgNPs. Overall, the fabricated \textit{L.sativum} oil/PEG/Ag-MgO bionanocomposite serve as a potential antimicrobial and anticancer agent and could be used in the development of novel drugs and health care products in near future.

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1. Introduction

Bionanocomposites have emerged as an advanced group of nano-sized materials obtained from the combination of biopolymers such as polysaccharides, nucleic acid, proteins, and essential oils with inorganic fillers at nanometric scale (Mousa et al., 2016). They possess the synergism between the exceptional features of inorganic fillers (excellent mechanical strength, high thermal stability, optical behavior, etc.) with those of the biopolymeric matrix (biodegradability, biocompatibility, etc.). The resultant bionanocomposites exhibit improved thermal, optical, mechanical, magnetic, photoelectronic and biological properties (Zia et al., 2020). These biohybrid finds application in various fields including; food packaging (Zubair and Ullah, 2020), biomedical materials (Ismail and Razali (2020)), drug delivery systems (Patwekar, 2016), agriculture sector (Zhang et al., 2020), sensing and electronic materials (Burrs et al., 2015; Liu et al., 2019).

Recently, the functionalizations of renewable resources-derived polymers via incorporation of metallic nanoparticles have become an area of huge interest, where innate features of nanoparticles are bestowed into the polymer matrix (Hanisch et al., 2011). The resultant environmental benign bionanocomposite combines both the characteristics, superiority of low-dimensional organic layers and wide surface area of nanoparticles, thus creating a broad spectrum of applications in manufacturing and science (George et al., 2018). These bionanocomposites are multifaceted, significantly biodegradable, and their polymer can be obtained from the vast diversity of sustainable precursors, including oxygen-rich and
L. sativum seed oil (LSO) is one of the important oil and widely found across the world. It is comprised of balanced amounts of polyunsaturated fatty acids (46.8%) and monounsaturated fatty acid (37.6%). LSO is relatively stable oil owing to its high content of natural antioxidant components such as tocopherols carotenoids and phytoestrogens (Diwaker et al., 2010). The oil has been reported to possess various biological activities including, antimicrobial, antioxidant, anticancer (Alqahtani et al., 2019), analgesic, anti-inflammatory, antiulcer, antipyretic (Al-Yahya et al., 1994), antihypertensive (Maghrani et al., 2005), diuretic (Patel et al., 2009), nephroprotective (Yadav, 2010), antiasthmatic (Panja and Mehta, 2006), hepatoprotective and hypoglycemic (Abuelgasim et al., 2008) properties. The oil has also shown synergistic effects by inhibiting the levels of thromboxane B2 and platelet aggregation in lung and spleen tissues in Wistar rats (Raghavendra and Akhilender Naidu, 2011). Recently, an oil-based polymeric material has grabbed immense attention and extensive research work is being carried out to manufacture these biopolymeric materials owing better chemical and physical properties (Irme and Pučinkszky, 2013). Several biodegradable polymers such as polyurethane, polystyrene, polyethylene glycol, polyvinyl alcohol and polyvinyl alcohol have been used to prepare these oil-based biopolymers (Song et al., 2018; De Conto et al., 2020). The oil-based polymeric materials or coatings have shown a vast range of desired potential such as biocompatibility (Arevalo et al., 2018), moderate hydrophilicity (Bai et al., 2017), optical transparency (Ahmad et al., 2014), biomaterials (as antimicrobial surfaces and biocompatible) (Matharu et al., 2018), pharmaceutical industry (as coatings for medicine) (Vasile, 2018) and electronics (as superior layers in organic and hybrid devices) (Bazaka et al., 2011). Among various polymers, polyethylene glycol (PEG) is the most biocompatible hydrophilic polymer, synthetic polymer that has shown many biomedical and chemical applications due to its non-toxic and high solubility nature. It is used in various pharmaceutical ointments, creams, binding and dispersing agent, coatings non-toxic and environment friendly in nature that can be readily used in pharmaceutical industry (Cai et al., 2018). However, the aforementioned applications are largely due to the controlled size, morphology and structure because of its variations in chemical and physical properties. Hence, to prepare MgO nanomaterials with diverse competence, it is necessary to modify size, morphology and natural chemistry as the above-stated applications arise on the surface of nanoparticles (Jayapriya et al., 2018). Thus, the fabrication of nanomaterials with different morphologies provides reaction specificity due to the formation of different surface atoms and crystallographic facets. In recent times, various reports on MgO-metal based nanomaterials are documented in the literature which is actively being used for multiple purposes (Liong et al., 2008). Several studies have shown that metal/metal oxide expressed better antibacterial effects towards many Gram-positive and Gram-negative bacteria (Abudula et al., 2020). Thus, the present study aims to develop oil based polymeric Ag/MgO biocomposite to improve the surface morphology of polymeric bionanocomposite for the up-gradation of its biological and physio-chemical potential. Silver nanoparticle (AgNPs) is well known for its extensive scope of antimicrobial, anticancer, antioxidant, anti-inflammatory and catalytic activities (Jayapriya et al., 2019; Gomathi et al., 2020; Reddy et al., 2015). AgNPs has expressed fascinating biological properties and at very low dose it is lethal to pathogens, but safe to human beings (Reddy et al., 2015). Several approaches have been applied to prepare metal/metal oxide nonanocomposites with different morphologies such as bimetallic alloys (Navya et al., 2019), core-shells (Chiozzi and Rossi, 2020), nanosheets (Pazmiño-Durán et al., 2001), nanorattle (Wu et al., 2011), nanotubes (Tripathi et al., 2017) and yolk-shell (Moon, 2020) etc. The fabrication of these nanostructures involves various physiochemical procedures that have been generated hazardous byproducts, rigorous energy and harsh environmental conditions. Consequently, there is an increase demand for the development of a lucrative, environmentally friendly approach that gives a superior platform to synthesize the Ag/ MgO bionanocomposite with a well-defined morphology that can serve as a potential candidate to resolve varied problems with multifunctional properties (Jayapriya et al., 2020; Ayinde et al., 2018).

Considering the unique individual properties of L. sativum seed oil, PEG and Ag/MgO nanoparticles, we herein report the fabrication and characterization of of L. sativum oil/PEG bionanocomposite decorated with Ag/MgO nanoparticles. The pre-synthesised bionanocomposite was tested for antibacterial and anticancer potential. To the best our knowledge, this is the first study on the synergy between L. sativum oil and Ag/MgO nanoparticles in an eco-friendly coating context.

2. Materials and methods

2.1. Chemicals and reagents

Ethanol (96%), Petroleum ether (40–60 °C), Dimethyl sulfoxide (DMSO, ≥99.9%) Silver nitrate (AgNO3, ACS reagent, ≥99%), Polyethylene glycol-2000 (PEG-2000), Magnesium nitrate hexahydrate (Mg(NO3)2·6H2O, ≥98%) 2.5% glutaraldehyde, Paraformaldehyde,
Anhydrous sodium sulfate (Na2SO4, >99%), Anhydrous sodium hydroxide (NaOH, ≥98.0%), 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazole bromide (MTT), Dulbecco’s Modified Eagle’s Medium (DMEM), Fetal bovine serum (FBS), Antibiotic antitoxicic solution, phosphate buffer solution (PBS, pH 7.4), Ethylenediaminetetraacetic acid (EDTA), β-Nicotinamide adenine dinucleotide (NADH), Sodium pyruvate (≥99%), Proteinase K. Ethidium bromide solution, Agarose gel, were acquired from Sigma-Aldrich. The Nutrient agar broth was supplied by HiMedia.

2.2. Botanical sample and oil extraction

The seeds of L. sativum were purchased from a local supermarket of Riyadh, Saudi Arabia and taxonomically identified by Prof. Dr. Mohamed Yousef, at College of Pharmacy, King Saud University. The seeds were screened manually and the best ones were chosen. The collected seeds were dried, powered in an electric grinder and oil was extracted with petroleum ether for 24 h in a Soxhlet apparatus as per method described earlier (Khan et al., 2016). The oil was freed from petroleum ether by evaporation on rotary at ± 25 °C under reduced pressure. To determine the total oil content, 12.0 g triplicates of powdered seeds were extracted in a Soxhlet apparatus for 24 h. After the removal of petroleum ether the weights of residual oil were calculated. The obtained oil was dried over anhydrous sodium sulfate, transferred in capped glass vials and stored in refrigerator at 4 °C.

2.3. Analysis of L. Sativum oil

Chemical composition analyses of the essential oils of L. sativum was conducted on GC–MS/FID (QP2010 Ultra, Shimadzu Corporation, Kyoto, Japan), equipped with an AOC-20i Autosampler (Shimadzu). Rtx®-5MS Restek fused silica capillary column (5%-diphenyl–95%-dimethyl polysiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 μm) was used for the separations of components. Helium (99.999%) was applied as the carrier gas at a constant flow rate of 0.7 mL/min. A 0.5 μL of injection volume with a split ratio of 1:10 was employed. The GC oven temperature was programmed as follows: start from 50 °C (isothermal for 10 min), then rising with 4 °C/min up to 210 °C, followed by an increase up to 250 °C at 5 °C/min rate.

A detector splitting system with split flow ratio 1:4 (MS:FID) was employed to acquire the simultaneous MS and FID data. Two restrictor tubes with 0.62 mm × 0.15 mm i.d and 0.74 mm × 0.22 mm m i.d. dimensions were used to connect the splitter to the MS and FID detector, respectively. The MS data were obtained in the full scan mode (m/z of 40–350) at a 0.3 scan/s scan rate, using the electron ionization (EI) with 70 eV electron energy. The injector and ion-source and FID temperatures were maintained at 250 °C. Hydrogen, helium and air at a flow rate of 30, 30 and 300 mL/min, respectively, served as a gas supply for the FID. Each component was quantified by the normalization percentage of the peak area. The concentrations of each component were calculated from the GC peak areas without the correction factor and were arranged in the order of GC elution. The retention index and retention times were obtained by injecting a linear hydrocarbon mixture (C7–C30) under similar conditions. The components of oil were identified by comparing retention times, retention indexes and the mass spectra with those already reported in literature (Adams, 2007) and computerized matching of the acquired mass spectra with those stored in the NBS 54 K, NIST107, NIST21, and WILEY 8 mass spectral libraries of the GC–MS data system (Linstrom and Mallard, 2001).

2.4. Synthesis of silver nanoparticles using L. Sativum seed extract

The aqueous extract of L. sativum seed was prepared by taking 1:10 (w/v) proportion of a finely powdered seeds and double distilled H2O. The aforementioned mixture was boiled under reflux conditions at for 30 min at 60 °C. Then, the extract was decanted and filtered using Whatman No. 1 filter paper to separate the suspended solid particles. The obtained filtrate was stored at 4 °C and used as a reducing and capping mediator for the fabrication of nanocomposite. 60 mL of the filtrate of the L. sativum seed was added to 90 mL of 1 mM silver nitrate (AgNO3) in 250 mL conical flasks at the ratio of 2: 3 v/v for the reduction of Ag+ to Ag0. The reaction mixture was then placed on a turntable domestic microwave oven and subjected to irradiation for different time intervals of 20, 40, 60, 80, and 100 s at a power of 700 W and frequency of 2450 MHz to complete bio-reduction. The preparation of silver nanoparticles (AgNPs) was monitored using UV–Vis Spectrophotometer over the wavelength range of 220–600 nm. Time-resolved absorption spectra of the UV–Vis spectroscopy were used in monitoring the periodic bioprocess growth kinetics of AgNPs through color variation. The prepared nanoparticle was used in subsequent experiments.

2.5. Synthesis of L. Sativum oil/PEG/Ag-MgO bionanocomposite

The L. sativum oil/PEG/Ag-MgO bionanocomposite was prepared through a one-pot process in L. sativum oil solution. Around 20 mL of the L. sativum oil and 10 g of polyethylene glycol-2000 (PEG 2000) was dissolved in 100 mL of ethanol and 100 mL of distilled water separately with continuous stirring at room temperature. Equal volumes of L. sativum oil (50 mL) and PEG-2000 (50 mL) were thoroughly mixed. After complete dissolution, 20 mL of 0.1 M magnesium nitrate hexahydrate (Mg(NO3)2.6H2O) with 2 mL of 0.1 M. NaOH solution was added drop wise to react with L. sativum oil/PEG solution in an aqueous bath system at 80 °C under gentle stirring. Stirring was continued for 2 h until a complete precipitation was achieved. After reaction was performed and the cream solid product was appeared, an aqueous mixture of L. Sativum seed: 1 mm AgNO3 (2:3 v/v%) was added into the above mentioned suspension, and the reaction mixture was subjected to controlled ultrasonic irradiation at 100% amplitude (0.5 cycles) for 30 min. The product obtained was centrifuged for 10 min at 2500 rpm, filtered, and washed with ethanol to remove extra L. sativum oil and finally freeze dried.

2.6. Characterization of silver nanoparticles and L. Sativum oil/PEG/Ag-MgO bionanocomposite

AgNPs and L. sativum oil/PEG/Ag-MgO bionanocomposite have been characterized by using spectroscopic techniques such as ultraviolet–visible (UV–vis), Fourier transform infrared (FTIR), and X-ray diffraction (XRD). UV–vis spectra of green synthesized AgNPs and Ag-MgO/L. sativum oil bionanocomposite have been carried out on Ultrospec 2100-Biochrom spectrophotometer, (Biochrom Ltd, Cambium, Cambridge, UK). FT-IR of the pre-synthesized nanoparticles and bionanocomposite spectra have been recorded on a PerkinElmer FT-IR spectrophotometer (PerkinElmer Ltd, Yokohama, Japan). XRD measurements of biosynthesized samples were performed on EMPREAN PAN analytical diffractometer (Malvern Panalytical B.V., Eindhoven, Netherlands) employed with a Cu-Kα (λ = 1.5406 Å) radiation source. The morphology and size of AgNPs and bionanocomposites were studied using scanning electron microscopy (SEM, JSM-6390LV, JOEL Ltd, USA) and transmission electron microscope (TEM, JEM-3100F, JEOL Ltd, USA). Energy-dispersive X-ray spectroscopy (EDX, RONTEC’s EDX system, Model
QuanTax 200, Germany) was performed to evaluate the components of the biosynthesized samples.

2.7. Antibacterial activity

2.7.1. Bacterial cell viability in the presence of L. Sativum oil/PEG/Ag-MgO bionanocomposite

The biosynthesized AgNPs and L. sativum oil/PEG/Ag-MgO bionanocomposite were tested for the bactericidal effect against Staphylococcus aureus (ATCC 25922) and Escherichia coli (ATCC 25922). Both the bacteria S. aureus and E. coli were procured from the Microbiology department of the King Khalid hospital (KKH) Riyadh, Saudi Arabia. The pure bacterial cultures were sub-cultured on nutrient agar broth under the optimum conditions with overnight shaking in an incubator. The growth of bacteria was determined by the turbidity of culture as a qualitative measurement and further confirmed by culture plate testing to measure the viability in the presence and absence of test samples. The rate of bacterial growth was determined by adding different concentrations (5, 10, 20, 40, 80 and 160 \( \mu \)g/mL) of the test sample to the liquid medium and incubated with the bacteria at 37 °C. Bacterial cultures without test samples were incubated in the similar medium under optimum growth conditions were chosen as control. Whereas, same concentrations of test sample without the bacteria in separate media were applied as blank controls to account for the optical interference by the light-scattering effects of the test sample. 1 mL of overnight incubated bacterial culture was served to inoculate the 100 mL of secondary culture (nutrient broth) with different doses of the test sample. The bacterial culture was incubated at 37 °C and the optical density was recorded every hour using UV–vis spectrophotometer (Evolution 20, Thermo Fisher Scientific). 0.1 mL of culture was taken from each treated flask and poured over the surface of media and incubated at 37 °C for 12 h. The number of colonies that appeared on the surface of the media was counted as an indicator of the number of bacteria. The result obtained was used to calculate the minimum inhibitory concentration and maximum bactericidal concentration of applied bionanocomposite.

2.7.2. Determination of antibacterial potential of AgNPs and bionanocomposite by zone inhibition assay

The antibacterial effects of the biosynthesized bionanocomposite against S. aureus and E. coli were tested. A fresh culture of both the microorganisms was sub-cultured in nutrient broth in conical flask and incubated for 12 h at 35 °C on an orbital shaker at 120 rpm. Also nutrient agar plates were prepared separately and inoculated with 100 mL of fresh culture of each bacterium (S. aureus and E. coli) by spread plate procedure. A sterilized steel borer was used to prepare 8 mm well on these inoculated plates. Each plate well was then filled with 100 mg of pre-synthesised bionanocomposite suspension and incubated for 12 h at 35 °C. After overnight incubation, the zone formation was noticed around the loaded material.

2.7.3. Effect of bionanocomposite on bacterial cell morphology

The cellular morphological changes of both the bacterial strains were examined by SEM before and after the treatment with different concentrations of bionanocomposite. The bacterial cells were fixed on glass slides with primary fixative reagents (2.5% glutaraldehyde, 0.1 mL paraformaldehyde in sodium cacodylate buffer) and incubated at 4 °C for 1 h. After 1 h incubation, bacterial strains were subjected to centrifugation at 1200 rpm for 5 min. Bacterial cell samples were washed thrice with Millipore water. Different concentrations of ethanol (5%, 15%, 25%, 35%, 45%, 55%, 65%, 80% and 100%) in water were used to dehydrate the bacterial cell samples. These dehydrated samples were dried in oven at 40 °C. A thin layer carbon tape was used to place dried bacterial cells on SEM stub and coated with a sputter (K575-Sputter Coater Emitech, Kent, UK). Finally, the sub-mounted cell samples were examined by SEM at low voltage (JOEL, Ltd, Tokyo, Japan).

2.8. Anticancer activity

2.8.1. MTT cell viability assay

In vitro cytotoxicity evaluation was performed in human cervical cancer cells (HeLa cells) using MTT (3-(4, 5- dimethylthiazol-2-yl)-2,5-diphenyltetrazole iodide) assay (Al-Qubaisi et al., 2011). Briefly, HeLa cells were seeded in 96 well plates (1 x 10\(^4\) cells/well) in Dulbecco’s Modified Eagle’s Medium (DMEM) containing 10% fetal bovine serum and 1% antibiotic antimycotic solution in a humidified incubator at 37 °C with continuous flow of 5% CO\(_2\). Then cell were treated with different concentrations (50, 250, 500, and 1000 \( \mu \)g/mL) of test sample and incubated at 37 °C for 24 h. After that, freshly prepared MTT (0.5 mg/mL) was added and incubated for 4 h at 37 °C. After the exposure period, the MTT containing medium was discarded from the cells and washed with 400 \( \mu \)L of phosphate buffer solution (PBS, pH 7.4). After washing, the obtained crystal was dissolved in 400 \( \mu \)L of dimethyl sulfoxide (DMSO) and mixed thoroughly. Microplate reader was used to measure the cell viability at the absorbance of 570 nm. The percentage of viability was calculated by using the following equation:

\[
\text{Cell viability(%) = } \frac{Abs_{570nm\text{sample}}}{Abs_{570nm\text{control}}} \times 100
\]

2.8.2. Lactate dehydrogenase (LDH) cell cytotoxicity assay

The evaluation of cytotoxicity by LDH assay was performed by obeying previously described method with slight modification (Zhang and Wang, 2013). Briefly, 6 well plates was seeded with 2 x 10\(^5\) HeLa cells per well and incubated at 37 °C for 24 h in a CO\(_2\) humidified incubator until the cells attained confluent growth. After 24 h incubation, the cells were treated with different doses (50, 250, 500, and 1000 \( \mu \)g/mL) of test samples and positive control (quercetin), followed by 24 h incubation at 37 °C. After the exposure, 100 \( \mu \)L of supernatant from each well was taken and mixed with 2 mL of Tris-EDTA-NADH buffer and incubated for another 30 min at 37 °C. After this, freshly prepared sodium pyruvate (400 \( \mu \)L) was added to the reaction mixture and cell viability was measured at 340 nm after every 15 sec for 3 min.

2.8.3. Acidine Organge/ ethidium bromide (AO/EB) staining

The formation of apoptotic body and nuclear changes in the HeLa cells was monitored under the fluorescence microscope (Pajaniaridje et al., 2014). The nanoparticles treated and untreated HeLa cells were harvested and washed with PBS. 20 \( \mu \)L of cell suspension (0.5 x 10\(^5\) cell mL\(^{-1}\)) were stained with 1 \( \mu \)L of AO/EB (1:1) and incubated for 5 min. After incubation, the residual stain was removed from the cells by washing several times with PBS and observed under the fluorescence microscope.

2.8.4. Estimation of ROS generation

DCFH-DA staining was performed to measure the production of ROS in HeLa cells by obeying the previously described method (Jiang and Li, 2014) with little modification. Briefly, the HeLa cells (2 x 10\(^5\) cell mL\(^{-1}\)) were seeded in RPMI 1640 medium containing confocal glass bottom dish and allowed to attach the HeLa cells. After 24 h, PBS was used to wash the cells and the medium was replaced with nanoparticles incorporated medium (IC\(_{50}\) concentration) and incubated for 24 h at room temperature. Afterwards, cold PBS was applied to wash the cells again and stained with 50 \( \mu \)M of DCFH-DA. The DCFH-DA treated cells were then incubated at 37 °C.
for 30 min. Finally, the incubated cells were washed with PBS and preserved in 1 mL of PBS. Fluorescence microscope was used to assess the ROS production at 488 nm and 530 nm of excitation and emission wavelengths, respectively.

2.8.5. Morphology of cellular nucleus

The morphology of cell nucleus was studied by applying DAPI staining according to the earlier described protocol (Pajamiradje et al., 2014) with slight modifications. Briefly, RPMI 1640 medium containing confocal glass bottom dish was used to grow the HeLa cells and then cells after 24 h cells were washed with PBS. After washing, the HeLa cells were exposed to nanomaterial samples (IC50 concentration) for 24 h. After 24 h treatments, the cells were washed again with PBS and fixed in formaldehyde (4%) at room temperature for 4 min. Finally, 300 μL of DAPI (300 nM) solution was put on the cells at ambient temperature for 5 min under dark conditions and washed again with PBS multiple times to get rid of residual dye. The cells were observed at 358 nm excitation and 461 nm emission wavelengths under the fluorescence microscope.

2.8.6. DNA fragmentation assay

The DNA fragmentation was performed by following Baharara et al. method with little modification (Baharara et al., 2015). The bionanocomposite treated cells were collected and washed thrice with PBS at 4 °C. After washing, centrifugation of cells was done at 3500 rpm for 5 min. The pellets suspended with DNA lysis buffer were collected and incubated for 60 min on an ice. After this, 20 μL of RNase (20 mgmL−1) was added to the pellet suspension and incubated at 37 °C for 1 h. Then, this pellet suspension was treated with 20 μL of Proteinase K (20 mgmL−1) and centrifuged for 15 min at 10,000 rpm at 4 °C. The supernatant was aspirated and transferred to micro-centrifuge sterile tubes. Finally, the addition of 0.15 M NaCl and ice cold absolute ethanol resulted in precipitation of RNAse (20 mgmL−1) the HeLa cells were exposed to nanomaterial samples (IC50 concentration) for 24 h. After 24 h treatments, the cells were washed again with PBS and fixed in formaldehyde (4%) at room temperature for 4 min. Finally, 300 μL of DAPI (300 nM) solution was put on the cells at ambient temperature for 5 min under dark conditions and washed again with PBS multiple times to get rid of residual dye. The cells were observed at 358 nm excitation and 461 nm emission wavelengths under the fluorescence microscope.

3. Results

3.1. Identification of chemical constituents of L. Sativum oil by GC–MS

The solvent Soxhlet extraction of L. sativum seeds led to the isolation of light yellow colored oil with 72.6% yield. GC–MS analysis was performed to determine the chemical components of oil and 25 different compounds were detected, accounting for 94.6% of the total oil composition. The identified compounds with their retention time (RI) and relative content in the oil are summarized in Table 1. The major effective components found in L. sativum oil were monounsaturated and polysaturated fatty acids. 7,10-Hexadecadienoic acid (35.82%) and 11-octadecenoic acid (18.32%) was the most abundant fatty acids detected in the oil. Other prominent fatty acid components present were behenic acid (11.95%), stearic acid (6.23%) and 7,10,13-hexadecatrienoic acid (5.65%), with small amounts of 15-tetracosenoic acid, hexadecanoic acid, heoceneosaic acid, 9,12-hexadecadienoic acid, 10-octadecenoic acid, eicosanoic acid, tetraocanoic acid, 9,12-octadecadienoic acid and tetradecanoic acid (Fig. 1, Table 1).

3.2. Physicochemical and morphological characterization of AgNPs and L. Sativum oil/PEG/ Ag-MgO/ bionanocomposite

3.2.1. UV–Visible analysis

UV–Vis absorbance spectra of pre-synthesised AgNPs and bionanocomposite have been displayed in Fig. 2 and Fig. 3. The successful bioreduction preparation of AgNPs by aqueous extract of L. sativum seed was indicated by the color change of the reaction mixture from light yellow to dark brown at different time intervals (0–100 sec) under microwave irradiation (Fig. 2). A characteristic surface plasmon vibration band between 410 and 450 nm were observed for AgNPs at approximately 30 s during the reaction nucleation and onset of growth. The intensity of the absorption peak increases with the increase in irradiation time, resulting in overlapping of plasmon bands and the formation of more AgNPs with same sizes which might be due to a stabilizing effect of bioactive components present in L. sativum seed extract. The peak intensity suggested that L. sativum seed extract increased the bioreduction of Ag+ with the persistent production of AgNPs up to 100 sec with slight difference in absorption maxima. Thus, the morphology of the nanoparticles is affected by plasmon surface resonance, due to the mutual vibration of electrons of metal nanoparticles in resonance with light wave serving as the basis for measuring adsorption of the material onto the surface of metal nanoparticles (Anandalakshmi et al., 2016). Moreover, the synthesis of nanoparticles by use of microwave provides a rapid, even heating, and eco-friendly approach, offering a uniform nucleation and growth conditions for the nanoparticles within in a short time span unlike the conventional synthesis (Meng et al., 2016).

As shown in Fig. 3, two distinct peaks were observed in the UV–Vis spectrum of L. sativum oil/PEG/Ag-MgO/ bionanocomposite. An intense prominent absorption band at 290 nm characteristic of MgO in the lower UV-region along with a broad absorption band at 382 nm associated with AgNPs. The obtained result revealed that the incorporation of MgO onto the Ag layer has resulted in a blue shift from 445 nm to 382 nm in the peak of AgNPs. The shift in the UV-peak is attributed to its particle size that depends on nucleation and growth mechanism based on interband transition or the excitation of plasmon resonance, aggregation of Ag-MgO nanoparticle and interaction of organic constituents with silver and magnesium oxide (Cai et al., 2017). This clearly indicates that the optical property of polymeric Ag-MgO bionanocomposite resulted in reduction of particle size. Furthermore, ultrasound exposure affected the size of bionanocomposite due to the cavitation collapse process leading to inter-particle collision and affecting the physicochemical features of the bionanocomposite. It has been also established that the availability and impacts of the secondary metabolites in L. sativum seed extract and oil were responsible for the bio-reduction rate leading to the production of bionanocomposite.

3.2.2. FTIR analysis

The functional groups of different phytochemicals present in the L. sativum seed extract and oil responsible for the bioreduction and stabilization of AgNPs and polymeric bionanocomposite were identified between 4000 and 500 cm−1 regions of FTIR. Fig. 4 displayed a comparative FTIR spectrum of as-prepared AgNPs and bionanocomposite from the aqueous L. sativum seed extract and oil, respectively. A prominent band centered at 3434 cm−1 were observed in the FTIR spectrum of AgNPs due to the stretching vibrations of hydroxyl (−OH), carboxyl (COOH), amide (−NH) functional groups of various phytochemicals found in the seed extract in the form of alcohols, carboxylic acids, amines or amides, ethers and esters (Anandalakshmi et al., 2016). However, the intensity of these peaks was shifted to a higher and broader frequency range of 3440–3222 cm−1 upon binding
with MgO in *L. sativum* oil/PEG/Ag-MgO/ bionanocomposite. Two sharp peaks at 2898 cm\(^{-1}\) and 2787 cm\(^{-1}\) were observed in AgNPs which was completely missing in the bionanocomposite spectrum could be assigned to symmetric and asymmetric C-H stretching vibrations of methyl (–CH\(_3\)), methylene (–C\(_2\)H\(_2\)) and methoxy (–OCH\(_3\)) groups (Fig. 4b) (Li and Barron, 2010). A vibration band at 1723 cm\(^{-1}\) was noticed for C-O in AgNPs which was shifted to 1674 cm\(^{-1}\) in bionanocomposite, indicating the presence of carboxylate ion and an amide group in the bionanocomposite. Various sharp characteristic peaks between 1525 and 1365 cm\(^{-1}\) were observed in both the spectra which were assigned to the asymmetric stretching (C-N), aromatic rings (–C–C–) in addition to the bending vibration of the methyl (–CH\(_3\)) and methylene groups of fatty acids. The stretching vibration bands at 1238, 1156, and 1032 cm\(^{-1}\) were identified for C-O, C-O-C and C-O, respectively. The appearance of absorption band at 568 cm\(^{-1}\) wavelength indicates the Mg-O-Mg deformation of the Mg-O (Fig. 4b) (Balamurugan et al., 2014). Thus, the appearance and interaction of the above mentioned bands in *L. sativum* extract and oil clearly suggest that the bioactive constituents serve as reducing and stabilizing agents for the biogenic synthesis of *L. sativum* oil/PEG/Ag-MgO/ bionanocomposite.

Table 1

| Peak No | Compound | Retention time (Rt) | Area | Area % |
|--------|----------|-------------------|------|--------|
| 1      | Methyl palmitate | 10.786 | 8785 | 0.07   |
| 2      | Methyl stearate   | 15.627 | 88,668 | 0.05 |
| 3      | Capric acid methyl ester | 16.207 | 2,588,259 | 0.21 |
| 4      | Methyl linoleate | 17.158 | 7821 | 0.11   |
| 5      | 8-Nonynoic acid, methyl ester | 17.890 | 1,827,628 | 0.06 |
| 6      | Behenic acid, methyl ester | 18.602 | 234,224,872 | 11.95 |
| 7      | Eicosanoic acid, methyl ester | 19.482 | 2,178,102 | 1.12 |
| 8      | 9,12-Hexadecadienoic acid, methyl ester | 19.898 | 28,254,212 | 2.51 |
| 9      | 7,10-Hexadecadienoic acid, methyl ester | 20.273 | 1,041,148,423 | 5.36 |
| 10     | 7,10,13-Hexadecatrienoic acid, methyl ester | 20.587 | 225,428,643 | 5.56 |
| 11     | Stearic acid, methyl ester | 20.892 | 98,167,478 | 6.23 |
| 12     | 9,12-Octadecadienoic acid, methyl ester | 20.990 | 10,278,217 | 0.78 |
| 13     | Tetradecanoic acid, 12-methyl-, methyl ester | 21.278 | 1,675,895 | 0.72 |
| 14     | 11-Octadecenoic acid, methyl ester | 21.982 | 352,024,326 | 18.32 |
| 15     | Hexadecanoic acid, 15-methyl-, methyl ester | 22.531 | 103,093,332 | 3.54 |
| 16     | 7-Octadecenoic acid, methyl ester | 23.422 | 3,625,642 | 1.14 |
| 17     | 15-Tetraicosenoic acid, methyl ester | 23.685 | 134,871,375 | 4.36 |
| 18     | Heneicosanoic acid, methyl ester | 24.152 | 32,721,254 | 2.15 |
| 19     | 13-Docosenoic acid, methyl ester | 24.672 | 2,531,462 | 0.65 |
| 20     | Triacontanoic acid, methyl ester | 24.960 | 2,625,763 | 0.21 |
| 21     | 10-Octadecenoic acid, methyl ester | 25.016 | 33,219,152 | 1.25 |
| 22     | Tetraicosanoic acid, methyl ester | 25.784 | 20,624,212 | 0.96 |
| 23     | Oleic acid, methyl ester | 26.335 | 2,371,814 | 0.25 |
| 24     | Methyl behenate | 26.642 | 94,216 | 0.11 |
| 25     | Methyl erucate | 27.264 | 1680 | 0.07 |

Fig. 1. GC chromatogram of *L. sativum* oil, chemical constituents.
3.2.3. X-ray diffraction (XRD) analysis

The X-ray diffraction with Cu-Kα radiation (\( \lambda = 1540 \text{ Å} \)) over a range of 20 to 90° was used to confirm the synthesis of AgNPs and dispersion of \( L. \text{sativum} \) oil and Ag-MgO in the PEG polymeric solution of bionanocomposite. The voltage and current applied were 30 kV and 50 mA, respectively. A broad peak appearance for plain PEG in XRD spectrum suggested its amorphous nature (Fig. 5a) (Zhao et al., 2019). However, \( L. \text{sativum} \) oil exhibited a high intensity broad peak near \( ~30° \) (Fig. 5a). The XRD pattern revealed that the biosynthesized AgNPs cubical in structure. The bionanocomposite displayed major consistent predominant reflection peaks at 2θ values ranged between 20 and 90° indexed differently to (111), (200), (220), (311), and (222) allotted to cubical planes of Ag (JCPDS 96–901-2962), along with an extended reflection peak at (420) associated with the cubical phase system of MgO (JCPDS 96–901-3264), which has shifted to a higher 2θ value due to deposited Ag-MgO plane lattice interaction (Fig. 5c). Also, Ag-Mg planer lattice (JCPDS 96–150-9064) was detected, confirming the bionanocomposite nature of the material possessing the cubic crystal lattice network.

3.2.4. Surface morphology

Electron microscopy was used to visualize the morphological and topographical micrograph of biosynthesized AgNPs and polymeric bionanocomposite. Fig. 6a–6h showed the surface morphology of PEG, \( L. \text{sativum} \) oil, AgNPs and \( L. \text{sativum} \) oil/PEG/Ag-MgO bionanocomposite. PEG has displayed a smooth face with small diameter at 10000 × and 30000 × magnifications (Fig. 6a and 6b). The images of the plain \( L. \text{sativum} \) oil confirmed that the oil droplets were distorted and homogeneously distributed with irregular edges and small pores on coagulant masses at 10000 × and 30000 × (Fig. 6c and 6d). The SEM images of AgNPs showed uniformly well dispersed spherical nanoparticles (Fig. 6e and 6f), whereas the SEM image of fabricated bionanocomposite image displayed the distribution of Ag-MgONPs in the polymeric matrix with particle size 13.45 nm at the same magnification (Fig. 6g and 6h). The agglomeration observed in the bionanocomposite may be due the aggregation, polarity, electrostatic attraction and attachment orientation of Ag and MgO nuclei which is clearly presented in Fig. 7. In addition, the presence of high amounts of organic moieties serving as the reducing, capping and stabilizing agents in the \( L. \text{sativum} \) seed extract and oil may also result in the aggregation (Tamilselvi et al., 2013).

Further, the formation of AgNPs and bionanocomposite was substantiated using TEM. Fig. 8 depicts the shape, particle size distribution and morphology of biosynthesized AgNPs and polymeric bionanocomposite through the TEM images. The TEM images of AgNPs and bionanocomposite revealed that the particles were dispersed uniformly and spherical in shape with particle size of...
16.32 nm and 13.45 nm, respectively (Fig. 8a-c). The distribution and stabilization of the embedded-layered core–shell is due to the interaction between Ag and MgO in the pre-synthesized polymeric bionanocomposite. The soft and smooth surface of the fabricated \textit{L. sativum} oil /PEG/Ag-MgO bionanocomposite can be hypothesized by the appearance of Ag-MgONPs, and revealed the...
successful use of *L. sativum* oil with PEG for the preparation of the suggested bionanocomposite. The results showed that the small size and spherical shape of the nanoparticles were well in agreement with the above mentioned shape of the UV–visible plasmon resonance band that the increase in intensity exposure resulted in conversion of larger particle sizes to smaller sizes during the cavi-
tation process.

3.3. Effect of AgNPs and bionanocomposite on the growth inhibition of bacteria

The effect of *L. sativum* oil, AgNPs and pre-synthesised bionanocomposite on the growth of *S. aureus* and *E. coli* was evaluated at different doses by the broth-dilution as well as by plating on agar plates. The appearance of clear zone of inhibition of varying diameters against the test samples with polymeric bionanocomposite showing a wider antibacterial effect on the both bacterial strains. The bionanocomposite displayed a higher level of antibacterial effect on *S. aureus* and *E. coli* with zone of inhibition 27 nm and 24 nm, respectively, in comparison with *L. sativum* oil, and AgNPs (Table 2). The results obtained revealed that increasing concentrations of bionanocomposite had a potential inhibitory effect on the growth of both the bacterial strains (Fig. 9A). The *S. aureus* displayed more prominent bacteriostatic effects as compared to *E. coli*. It was noticed that the treatment of 40 mgmL⁻¹ of bionanocomposite inhibited the 80% of *S. aureus* and 75% of *E. coli* growth. However, MIC was observed at 80 mgmL⁻¹, the growth of *S. aureus* completely vanished, whereas more than 95% growth of *E. coli* was also inhibited at the similar concentration. The MIC and MBC of the bionanocomposite ranged from 40 to 80 µgL⁻¹ and 80–160 µgL⁻¹, respectively, against *S. aureus* and *E. coli*. The bactericidal effect of the bionanocomposite might be attributed to many factors. The inactivation mechanism of action of nano-
materials generally towards microorganisms has been a major debate due to the continuous emergence of bacterial resistance (Slavin et al., 2017). However, various studies have suggested that the antibacterial susceptibility and mechanism of nanoparticles, not only depend on the type of bacteria and cell wall structure, but also on the cellular enzymes, contact action and biochemical events involved in production of intercellular reactive oxygen spe-
cies (ROS) (Ahluluwalia et al., 2018; Leung et al., 2014; Zhu et al., 2016). Other responsible factors that may be associated with the death of bacteria could be sizes, surface area and structure of the bionanocomposite. The small sized particle with large surface area possesses strong affinity for interaction, exerting strong bactericidal potential as compared to those larger particles (Raghupathi et al. 2011). Thus, the promising antibacterial activity exhibited by *L. sativum* oil/PEG/Ag-MgO bionanocomposite likely is due the small size and deep penetration of Ag and MgO ions released from *L. sativum* oil/PEG/Ag-MgO bionanocomposite on the adsorbent surface and their interaction with microbial genome and cellular enzymes leading to disruption of the bacterial cell membrane. Furthermore, it has been observed that reducing agents and stabilizers used in preparation of nanomaterials also play decisive roles in determining the antibacterial potential of bionanocomposite (Garmasheva et al., 2016). Thus, the essential oil used in the in the synthesis of *L. sativum* oil/PEG/Ag-MgO bionanocomposite may have contributed to the bactericidal properties due to the presence of different phytochemical components in the *L. sativum* oil (Alqhtaani et al., 2019).

### 3.4. Antibacterial activity of bionanocomposite determined by zone of inhibition assay

The biosynthesized bionanocomposite exhibited excellent antibacterial activity towards *S. aureus* and *E. coli*. The zone of inhibition assessments was carried on solid nutrient agar plates. Each agar plate has a well filled with bionanocomposite, which dispersed into the surrounding media and prevented the growth of bacteria in a zone around the well. At 40 mg/well bionanocomposite concentration, an excellent inhibition zone at 27 nm and 24 nm were observed against *S. aureus* and *E. coli*, respectively (Fig. 9B). The bionanocomposite release and diffuse Ag and MgO ions into the surrounding media, which interact with the inoculated bacteria and significantly stop the bacterial growth around the each well and a clear halo was developed.

### 3.5. Effect of AgNPs and bionanocomposite on morphology of *S. Aureus* and *E. Coli*

The effect of the AgNPs and bionanocomposite on the cellular morphology of *S. aureus* and *E. coli* was explored by SEM. The SEM images of *S. aureus* and *E. coli* showed partial damage and distortion of bacterial cells on treatment with 60 µgL⁻¹ of bionanocomposite (Fig. 10). Images at different magnifications (50,000 to 10,000 ranges) were captured using a 15 kV voltage. Fig. 10a-c and Fig. 10d-f shows cells of *S. aureus* and *E. coli*, respectively. The treatment of AgNPs and bionanocomposite has changed the shape and size of selected bacteria due to nanoparticles coating on the surface bacterial cells. However, the distortion bacterial cells were more pronounced in bionanocomposite treated cells. It was hypothesized that the presence of Ag-Mgo nanoparticles on the surface of bionanocomposite make bacterial cells extremely stressed probably due to an enhanced interaction between bionanocomposite and lipid components of the bacterial cell membrane. This interaction may result in the production of free radical species, which can destroy the transport system of membrane and affect the metabolism and growth of bacterial cell. Previous studies have shown that protoplasmic inclusions and leakage of bacterial cell depends upon the amount of interaction with Ag-
MgO naomaterials (Ayinde et al., 2018).

### 3.6. Anticancer activity of AgNPs and bionanocomposite

The toxicity potential of the synthesized AgNPs and bio-
nanocomposite on human cervical cancer cells was investigated towards HeLa cell line. The cancer cells were exposed to different concentrations of AgNPs and bionanocomposite for 24 h and 48 h. After treatments, the toxicity effects of AgNPs and bio-
nanocomposite was assessed by applying the MTT assay and LDH leakage assay. The MTT results revealed that the AgNPs and bio-
nanocomposite reduced the percentage of cell viability dose and time dependently (Fig. 11A). The inhibitory concentrations (IC₅₀), calculated for AgNPs and polymeric bionanocomposite were

| Table 2 | Antibacterial activity of *L. stavitum* oil, AgNPs and bionanocomposite. |
|---------|---------------------------------|
| Method  | Test samples | MIC (µgL⁻¹) | Zone of inhibition (mm) |
| Broth-dilution assay | *L. stavitum* oil | 120 | *S. aureus* 9.23 ± 0.82 |
|         | AgNPs | 80 | 11.65 ± 0.65 |
|         | Bionanocomposite | 40 | 18.32 ± 0.98 |
|         |         |       | 27.01 ± 0.58 |
|         |         |       | 24.45 ± 1.16 |
|         |         |       | 14.26 ± 0.98 |
|         |         |       | 11.65 ± 0.65 |
|         |         |       | 27.01 ± 0.58 |
|         |         |       | 24.45 ± 1.16 |
The results illustrated the polymeric bionanocomposite induced more cell death than AgNPs due to the presence of magnesium oxide ions which are reported as a cytotoxic agent towards many cancer cell lines including MCF-7, HT 29, Hep2 cells (Amina et al., 2020; Saravanakumar and Wang, 2019). Furthermore, many studies have

**Table 3**

Comparison between the biological activity of the pre-synthesized *L.sativum* oil/PEG/Ag-MgO bionanocomposite film and previously published Ag-MgO based nanocomposites.

| Name of nanocomposite | Source | Biological activity | Microorganism and cancer cell lines | Effective concentration µg/mL<sup>1</sup> | References |
|------------------------|--------|---------------------|----------------------------------|------------------------------------------|------------|
| Ag-MgO/nanohydroxyapatite (Ag-MgOnHaP) | Ag, MgO, rGO and *C. paradisi* peel extracts | Antibacterial | *E.coli*, *K. pneumonia.* | 30–60 | (Ayinde et al., 2018) |
| Ag-MgO Nanocomposite | Ag, MgO, and peel extract of *Citrus paradisi* | Antibacterial | *E.coli* | 20–40 | (Ayinde et al., 2018) |
| cauliflower like Ag/MgO nanocomposite | Ag, MgO and cauliflower extract | Antibacterial, anticancer, and catalytic | *E.coli*, *S. aureus*, AS-49 cancer cells | 60 | (Jayapriya et al., 2019) |
| *L.sativum* oil/PEG/Ag-MgO bionanocomposite | Ag, MgO, PEG, and *L.sativum* oil | Antibacterial Anticancer | *S. aureus*, *E. coli* human cervical cancer cells (HeLa) | 40 | Present work |

220.35 µg mL<sup>−1</sup> and 135 µg mL<sup>−1</sup>, respectively. The results illustrated the polymeric bionanocomposite induced more cell death than AgNPs due to the presence of magnesium oxide ions which are reported as a cytotoxic agent towards many cancer cell lines including MCF-7, HT 29, Hep2 cells (Amina et al., 2020; Saravanakumar and Wang, 2019). Furthermore, many studies have
shown that the silver-doped nanoparticles can easily penetrate the cell membrane and induce the free radicals, production of ROS and trigger the damage of the nucleus (Jayapriya et al., 2020). The morphological changes in the cell and membrane damage were observed under phase contrast microscopic analysis. The obtained results revealed a typical epithelial morphology with high density cell population in the untreated group, whereas chromatin condensation, rounding and shrinkage of cells, membrane blebbing and formation of apoptotic body with less cell population was noticed in AgNPs and bionanocomposite treated cells. These morphological changes might occur due the caspase cascade activation wherein the poly (ADP-ribose) polymerase (PARP) substrate needed for DNA repair mechanism would be cleaved. The cellular absorption of nanoparticles by endocytosis would trigger the production of ROS and actuate the apoptotic pathway and eventually resulting in cell death.

Lactate dehydrogenase (LDH) assay is one of the most commonly applied procedure to examine the cellular or tissue damage based on the level of LDH content in the extracellular medium. LDH is a cytoplasmic soluble enzyme, which is quickly released into the extracellular medium due to cell membrane damage. Thus, the estimation of LDH level is considered as an indicator for the cell toxicity. The cytotoxic effect of the AgNPs and bionanocomposite was further validated by the LDH leakage assay. The results showed that the LDH leakage percentage is concentration dependent in both AgNPs and bionanocomposite treated cells, epitomizing the cell death. However, the bionanocomposite exerted more pronounced cytotoxic effect as compare to AgNPs (Fig. 11B), which could be due to the combined effect of Ag and MgO ions. The aforementioned results were in agreement with the previous report that AgNPs and Ag-MgONPs treatment would permeabilize the cell wall, causing the LDH leakage in lung cancer cells, resulting in cell death (Oh et al., 2014; Saravanakumar and Wang, 2019).

3.6.1. Morphological change observations by using DAPI and acridine orange (AO) staining and bright field microscopy

The fluorescent stains such as DAPI, ethidium bromide (EB) AO label DNA and Hoechst, permit easy visualization of the nucleus in the cells. Herein, the effect of IC_{50} concentrations of these two nanomaterials was analyzed by AO/EB staining, ROS production, followed by nucleus damage by DAPI staining. The AO fluorescent dye can stain both alive and dead cells, but EB dye can stain only dead cells that have lost membrane integrity. However, the AO/EB stains were applied to observe the morphological changes occurred in treated and untreated cells. Whereas, the DAPI staining was performed to monitor the morphological changes induced by AgNPs and bionanocomposite within the nucleus. The DAPI quickly binds to double-stranded DNA groove and the DAPI fluorescence increases 20 fold approximately. For this purpose, HeLa cells were exposed to IC_{50} concentrations of Ag-NPs and polymeric bionanocomposite. The results showed that the treatment of polymeric bionanocomposite induces more apoptosis necrosis (Fig. 11Ca-f), the ROS generation (Fig. 11Cg-i), and the damage of cellular and nuclear membrane (Fig. 11Ci-l) than the AgNPs. The most identified morphological changes noticed in the treated groups were cell shrinkage, cytoplasmic condensation, and nuclear chromatin aggregation. As shown in Fig. 11Bi-l, the AgNPs and bionanocomposite caused morphological alteration and chromatin condensation in the nucleus of HeLa cells, illustrating the fact that the apoptotic effect of AgNPs and bionanocomposite completely dependent on the production of ROS, which in turn has led to the oxidative stress mediated cell death in HeLa cancer cells. After 24 h, the apoptotic cell percentage of Ag-NPs and bionanocomposite (50, 250, 500 and 1000 μg/mL) was examined against the HeLa cell line. It is illustrated through the results that with the increase in concentration of nanomaterials, their apoptotic effect significantly increases as well. The bionanocomposite showed 100% apoptotic effect at 500 μg/mL, whereas AgNPs exhibited 86% apoptosis effect on the HeLa cells at 1000 μg/mL concentrations. The proportion of late late apoptotic (yellow)/necrotic (red) cancer cells significantly increased (1% – 45%), while as the number of live cells dramatically decreased (Fig. 11C). In the DNA fragmentation assay, a fragmented DNA with laddering pattern was noticed in the treated cells, which is considered as an indication of apoptosis. Whereas untreated cells displayed single DNA laddering band (Fig. 11D).

4. Discussion

The biosynthesis of L. sativum oil/PEG/Ag-MgO bionanocomposite was eco-friendly and did not require hazardous chemical compounds, high temperatures, or high pressures. The biosynthesized
polymeric bionanocomposite expressed higher antimicrobial effects in comparison with unalloyed AgNPs and pure *L. sativum* oil. A previous study has shown that AgNPs exhibited more toxic effects towards Gram-positive (*S. aureus*) and Gram-negative (*E. coli* and *P. aeruginosa*) bacteria. They concluded that the responsible factor for the toxicity difference of AgNPs was a differentiation in their cell membrane. Also the results confirmed that *E. coli* and *P. aeruginosa* displayed identical sensitivity at equal doses of AgNPs. The enhanced antimicrobial property of biosynthesized bionanocomposite might be due to its spherical shape, large surface area, and surface to volume ratio. Additionally, the efficacy of polymeric bionanocomposite increases with the increase in concentration of bionanocomposite. Qais et al (2019) showed that the increasing concentration of silver nanoparticles, the antibacterial properties of nanoparticles towards *S. aureus* and *E. coli* increases. Another study conducted by Ydollahi et al (2016) showed that the antibacterial performance (MIC, MBC) of silver nanoparticles against *S. aureus* and *E. coli* were 100 and 150 μg/mL, respectively. However, the results of our study showed that the biosynthesized polymeric bionanocomposite and silver nanoparticles exerted antibacterial effects against *S. aureus* at a lower concentration of 40 μg/mL and 80 μg/mL, respectively. The higher antibacterial potential of polymeric bionanocomposite is supposedly due to existence of Ag, MgO ions and bioactive constituents, making an advantage compared to the silver nanoparticle. Moreover, the antibacterial action of AgNPs can possibly be attributed to the chemical interaction between lipid bilayer or proteins of bacterial membrane and hydrogen peroxide that induce oxidative stress and damage to proteins and nucleic acids. The AgNPs also penetrate into the cytoplasm and interact with protein and DNA causing cell death. Furthermore, AgNPs can release Ag⁺ ions, causing further damage to the cell. However, the penetration of bionanocomposite into the bacterial cells, release of Ag⁺ and Mg²⁺ ions, increases the intracellular accumulation of nanoparticles, thus destroying the bacterial cells. According to the above results, improvement antibacterial of binanocomposite is due smaller size of Ag-Mgo with large the surface area and higher concentration of surface defects. SEM and TEM images revealed the spherical shape of nanoparticles, which releases Ag⁺ and Mg²⁺ ions is involved in the bacterial growth inhibition. Our findings suggested that bionanocomposite treated bacterial cells resulted in elevation of ROS generation (Fig. 12 a). The treatment of *S. aureus* with bionanocomposite showed higher production of ROS compared to *E. coli* that could be explained by the different potentials that are applied to change the surface polarity of Gram-positive and Gram-negative bacteria. The ROS production resulted in bacterial cell membrane disruption, denaturation of protein and other macromolecules damage of bacteria that ultimately leading to cell death. The toxic effect of biosynthesized polymeric bionanocomposite on HeLa cervical cancer cells starts at 50 μg/mL, exhibited the more toxic effect than AgNPs biosynthesized by *L. Sativum* seed extract. The bionanocomposite destroyed 100% of HeLa cancer cells at 500 μg/mL concentrations, whereas ~57% of cancerous cells remained alive after treatment with the same concentration of AgNPs. This means that bionanocomposite eliminate cancerous cells twice as higher than AgNPs at similar concentrations. Our results were almost close to earlier findings that reported a 50% decrease in HeLa and MCF-7 cancer cells after treatment with AgNPs at a 27.79 μg/mL concentration (Abdel-Fattah and Ali, 2018). Bethu et al. (2018) reported that AgNPs in nano-sized dimensions damage the DNA through oxidative stress and lipid peroxidation in human ovarian carcinoma (SKOV3) and human lung adenocarcinoma (A549). They showed that nanostructured substances can be applied as a drug carrier, reducing the non-specific toxicity of strong anticancer agents. Thus nanomaterials with the small size possess unique physiochemical features that provide superior platform of exceptional interaction with proteins, lipids, and nucleic acids present on the surface and within the cell body. Hence, the obtained results revealed that the smaller size of bionanocomposite compared to AgNPs actively triggers apoptotic cell death in HeLa cancer cell line. Moreover, in Ag-Mgo nanoparticles, a part of Mg²⁺ ions are substituted by Ag + ions, maintaining the overall neutral charge and oxygen vacancies are created. Also, combination of Ag can make resistance of electron-transfer of MgO decreases, thereby accelerating the electron transfer to the surface to produce \( \text{O}_2 \) by reduction of single electron of adsorbed oxygen on the oxygen vacancy. In brief, the elevation in ROS production and contact interaction between bionanocomposite and cancer cells leads to enhancement of anticancer activity of bionanocomposite (Fig. 12b). Moreover, the presence of different bioactive components of seed oil play an important role in antibacterial and anticancer properties of bionanocomposite. Thus, the excellent antibacterial and anticancer profile of *L. sativum* oil/PEG/Ag-MgO bionanocomposite is the result of synergy between *L. sativum* oil.

![Fig. 12. Schematic (a) antibacterial and (b) anti-cancer mechanisms of bionanocomposite.](image-url)
components, PEG and Ag-MgO nanoparticles used in its biosynthesis. The possible mechanism of AgNPs and polymeric bionanocomposite behind the above stated biological properties may be due to free radical species (ROS) that induces oxidative stress which results in the formation of hydroxyl radicals (.OH), superoxide anions (O2−) and dissolution of cations (Liou and Storz, 2010). Also penetration of these nanoparticles displays cell membrane dissolution that results in inhibition of enzyme activity, DNA synthesis, and upsetting the energy transduction (Podder et al., 2018).

5. Conclusion

The present research work demonstrated the characterization, antibacterial and cytotoxicity activity of the biogenic AgNPs L. sativum oil/PEG-Ag-MgO bionanocomposite. The high throughput characterization of biomaterial revealed the successful fabrication of polymeric bionanocomposite. Particularly characterization analysis of XRD and TEM results strongly evidenced the formation of a polymeric L. sativum oil bionanocomposite enriched with 13.45 nm of silver-magnesium oxide nanoparticles (Ag-MgO NPs). The biosynthesized nanomaterials were tested for biological activities including antibacterial and anticancer effects. The outcome of the results showed that both the nanomaterials were sensitive towards the tested S. aureus and E. coli. However, the bionanocomposite showed strong antibacterial effects against both the strains at a minimum concentration. Furthermore, the anticancer activity against the HeLa cancer cell lines also confirmed that bionanocomposite induced cell death through the enhanced production of ROS followed by cellular and nucleus damage. Conclusively, the results of the study establish that the biogenic synthesis of polymeric bionanocomposite from natural oil, polymer and nanometals leads to the formation pure nanomaterial with antimicrobial and anticancer properties. The bionanocomposite has the potential for the development into promising antibacterial and anticancer drugs for the treatments of multidrug resistance bacteria and cancer.

Data Availability

The data used to support the findings of this study are included within the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author’s contributions

M.A.: Designed the study; performed the experiment; Draft manuscript. N.M.A.M.: Data interpretation. G.A.A.: Performed biological study. A.A.: Funding Acquisition. A.E.: Revise the manuscript. M.A.A.: Data compilation. All authors reviewed the manuscript.

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