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RESEARCH PAPER

Antioxidant and Antibacterial Profiling of Pomegranate-pericarp Extract Functionalized-zinc Oxide Nanocomposite

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Abstract With the advancement in green nanotechnology, considerable attention is being given to the synthesis of different kinds of nanomaterials for biological applications. In this study, zinc oxide nanocomposites (ZnO NPs) were synthesized using *Punica granatum* L. (Pomegranate) pericarp ethanolic extract (PE) by the chemical precipitation method. The prepared ZnO NPs showed a characteristic peak at 270 nm in the UV-Vis spectrophotometer and chemical bond stretching in the Fourier transforms infrared spectroscopy (FT-IR) spectra, indicated the formation of PE-functionalized zinc oxide nanocomposite (PE-ZnO NPs). The SEM results showed agglomerated PE-ZnO NPs of a spherical shape with an average size of 80-100 nm. Moreover, biological assessment of the PE-ZnO NPs revealed significant scavenging activity in DPPH (116.5%) and $ABTS^{+}$ (95.2%) radical assay methods, and substantial antibacterial activity against *Bacillus cereus*, *Bacillus licheniformis*, and *Escherichia coli*. Furthermore, PE-ZnO NPs showed about 96.3% of cell viability for human HaCaT cells at the maximum concentration (100 μg/mL), marked as a reliable bioactive agent. Therefore, the developed PE-ZnO NPs were elucidated with substantial ROS scavenger and non-antibiotic antibacterial agent and hence, can be applied in respective biological applications.

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1. Introduction

Nanotechnology is an emergent study area of advanced science and technology because of its several multifunctional applications. Nanoparticles (NPs) refer to particles with about 100 nm and less than one-tenth of a million meters, multiple morphologies, and other physicochemical properties [1]. The NPs are used in various applications in many fields; for example, medicine, environment, agriculture, cosmetics, and diagnostic fields [2]. NPs are classified into four types: polymer, carbon, metal and metal-oxide, and other inorganic substances. Amongst the multiple forms of NPs, metal-oxide NPs have been used more frequently on humans and biological applications because of less toxicity [3].

Among the various metal oxides: zinc-oxide nanoparticles (ZnO NPs) is widely used in many application, including environmental remediation, piezoelectric, electronics, sensor, solar energy conversion, drug delivery, light-emitting diode, cosmetics, and antimicrobial products [4]. Moreover, the ZnO NPs have many advantages as a modern drug delivery system because of easy fabrication, low production cost, tunable structure, high drug-loading capability, biocompatibility, controlled drug-release ability, and target delivery [5,6]. The ZnO NPs are also being used to produce a white appearance and UV-blocking material in textiles [7]. Besides, the ZnO NPs can easily engage on the bacterial cell wall and lead to membrane damage [8]. In recent years, ZnO NPs are also applied as external antibacterial agents in food packaging, preparation of ointments, cloth fabrics, lotion, and mouthwash to avert microbial load [9].

Nano cosmetics are cosmetics in a formulation in which bio nanoparticles or structures such as zinc oxide nanoparticles of about 100 nm can be used as raw materials [10]. In addition, nanomaterials are used to develop high-value-added cosmetics that increase skin health promotion effects by blocking fine dust, ultraviolet rays, infrared rays, and harmful rays or chemicals from outside [11]. In this context, the ZnO NPs have been used in cosmetics, such as sunscreens, to protect the skin from harmful radiations [12]. Moreover, the food drug administration (FDA, USA) accepts ZnO as a GRAS (Generally Recognized As Safe) material compared to another metal nanomaterial [13].

The sunscreen comes in a range of colors, including colorless and powder. Materials such as aminobenzoic acid, acid salicylates, cinnamates, and benzoic acid are used in the structure of the sunscreens. Recent trends in cosmetics ingredients use plant-derived natural compounds. For these natural-oriented trends in cosmetics, natural preservatives mainly used plant extracts that have antioxidant, antibacterial, and photoprotective effects [14]. Recently, green synthesis of ZnO NPs has been reported using orange fruit and peel extracts for antibacterial activities [15-17]. Since fruits have abundant beneficial phytochemicals, including alkaloids, flavonoids, terpenoids, carotenoids, and tannins, plant fruits can be used as nanocomposite agents to synthesize NPs materials [18].

Punica granatum L. (Pomegranate) is an edible fruit that belongs to the family Punicaceae (now in the *Lythraceae* family) [19]. Pomegranate fruit is rich in natural polyphenols, flavonoids, and proanthocyanidins, and commonly consumed as nutritional food, for therapeutic activities, promoting health, and high anti-oxidant function [20]. The pomegranate seeds have been recognized to scavenge the reactive oxygen species (ROS), as it contains vitamin C, vitamin K, folic acid, and polyphenol-rich bioactive compounds. Furthermore, pomegranate fruit pericarp mainly contains potent bioactive compounds, such as gallagic, ellagic acid, ellagitannins, punicalagin, anthocyanins delphinidin, pelargonidin, and luteolin [21,22], while pomegranate fruit peel was estimated to mainly contain 25-28% of the polyphenols with gallic acid and tannins for scavenging the ROS (reactive oxygen species) [23]. Moreover, many studies reported that pomegranate seed juices and peel extracts are potentially beneficial for treating diabetes [24], obesity [25], cardiovascular disease [26,27], and inflammation [28,29]. In addition, pomegranate fruit extract has anti-cancer properties and alternative therapy for prostate cancer [30,31]. In particular, the primary substance of the polyphenol of pomegranate fruit has antimicrobial action against *Klebsiella pneumonia*, *Escherichia coli*, and *Pseudomonas aeruginosa* [32]. Hence, we synthesized novel PE-ZnO NPs with pomegranate ethanol extract (PE) for antioxidant and antibacterial activities as well as studied for the cytotoxicity *in vitro* using human keratinocyte cell lines.

2. Material and Methods

2.1. Chemicals

Folin-Ciocalteu, 1,1-diphenyl-2-picryl hydrazyl (DPPH), 2, 2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and Methyl thiazolyl diphenyl-tetrazolium bromide (MTT) were obtained from Sigma Aldrich CO. (St. Louis, USA) and Duksan (Ansan, Korea). The fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), and trypsin-disodium ethylenediamine tetra-acetic acid (EDTA) were procured from Welgene (Gyeongsan, Korea). Phosphate buffered saline (PBS) and penicillin (100 units/mL)/streptomycin (100 μg/mL) were obtained from HyClone (Logan, USA). All other chemicals and reagents of analytical grade were used in this present study.

2.2. Ethanol extract of pomegranate pericarp

The healthy pomegranate fruits were harvested at the Yeungnam University farm in Gyeongsan, Korea and washed with tap water. Next, the pomegranate fruits were cut manually, and pericarps were dried at room temperature; then, grounded using an electronic grinder to a coarse powder and stored in an incubator at 4°C. To prepare a sample, 50 g of grounded pericarp was soaked in 500 mL of 70% ethanol and stored at room temperature for 48 h. After this, the samples were centrifuged at 8000 × *g* for 10 min, the collected supernatant was filtered with 0.4 μm of filter paper (Whatman No.1) and dried by employing a rotary evaporator at 60°C (N-1000, Eyela, Tokyo, Japan). At the end of the extraction, the collected dried extract was stored in sterile bottles in the incubator at 4°C. The pooled pomegranate extract (PE) was then used for the analysis of phenolics, antioxidant, and antibacterial activities.

2.3. Determination of total polyphenolic and flavonoid content

Initially, the collected PE was assessed for the total phenolic content using the Folin-Ciocalteu reagents [33]. Briefly, 1 g test sample (PE) was mixed with 10 mL of distilled water and centrifugation at 240 × *g* for 10 min. The collected supernatant was filtered with Whatman No.1, and the filtrate was further centrifuged at 8000 × *g* for 10 min. Next, 200 μL PE was mixed with 670 μL distilled water followed by the addition of 600 μL sodium carbonate (2%) and 30 μL of Folin-Ciocalteu (50%) reagent. The complete reaction mixture was incubated at room temperature for 30 min and measured for absorbance at 765 nm using a UV-Vis spectrophotometer (Optizen

2120UV, Mecasys, Daejeon, Korea). Besides, gallic acid (Sigma Aldrich Co., St. Louis, USA) was used as standard phenol to plot a calibration curve ($y = 0.0003x + 0.1094$, $R^2 = 0.9989$) for the calculation of the total phenolic content in PE and expressed by gallic acid equivalents (mg GAE/g extract).

Moreover, the total flavonoid content was also measured using the previously reported method [33]. Briefly, 200 μL of the collected PE was mixed with a solution mixture of 790 μL distilled water, 10% aluminum chloride hexahydrate (AlCl3), 30 μL 1M potassium acetate (CH_3COOK), and 450 μL 95% ethanol and mixed well. The reaction mixture was incubated at room temperature for 30 min and measured for absorbance at 510 nm using a UV/Vis spectrophotometer (Optizen 2120UV, Mecasys, Daejeon, Korea). Also, quercetin was employed as a standard flavonoid to plot a standard curve ($y = 0.0024x + 0.0055$, R2 = 0.9976) for the calculation of the total flavonoid concentration in PE and expressed as quercetin equivalents (mg QE/g extract).

2.4. Synthesis and characterization of PE-ZnO NPs

The synthesis of pomegranate-pericarp extract functionalized-zinc oxide nanocomposite (PE-ZnO NPs) was carried out using the chemical precipitation method. Briefly, 0.2 g of PE was dissolved in 200 mL of deionized water and amended with 1 mL of 1 M zinc nitrate (Sigma Aldrich), incubated under shaking conditions at room temperature. Next, 500 mL of 2 M of sodium borohydride was added dropwise to the above solution under vigorous shaking conditions at room temperature for 4 h. Finally, synthesized PE-ZnO NPs were harvested from the solution using centrifugation at 2,500 × *g* under room temperature and washed thrice with deionized water by centrifugation. The synthesized NPs were then dried in the incubator at 40°C and used in further experimental analysis. Under similar conditions, only ZnO NPs were also prepared and used as a reference control for the PE-ZnO NPs.

The synthesized PE-ZnO NPs were examined for the nanocomposite formation by monitoring the metallic plasmonic peaks in the UV-Vis spectra calculated using a UV/Vis spectrophotometer (Optizen 2120UV, Mecasys, Daejeon, Korea). Moreover, the functionalization of PE on the ZnO NPs was also evaluated in terms of transmittance (400 to 4,000 cm^{-1} range) using the Fourier Transform-Infrared spectrometer (FT-IR, PerkinElmer, Inc., Waltham, USA). Next, the morphology of the synthesized PE-ZnO NPs was also visualized using Scanning Electron Microscope (SEM), where 10 μL of 0.2% (w/v) of PE-ZnO NPs solution was dropped on the carbon tape and air-dried. Finally, the samples were coated with platinum (Pt) and visualized under SEM S4800, Hitachi, Ltd.

2.5. $ABTS^{+}$ and DPPH scavenging activity measurement

The antioxidant activity of the synthesized PE-ZnO NPs was monitored by $ABTS^{+}$ free radical scavenging method as reported earlier [34]. Herein, 7 mM ABTS and 2.5 mM potassium persulfate were mixed in equal volume and stored in dark under ambient temperature for 24 h to form $ABTS^{+}$ radicals. Next, this solution was diluted 2 times with ethanol and measured for absorbance at 734 nm using an ELISA reader (InfiniteTM F200, Männedorf, Switzerland). To monitor the scavenging activity of PE-ZnO NPs, equal volume (150 μL) of ABTS solution and the 0.2% test sample was mixed well, allowed to react for 6 min at room temperature, and measured for the absorbance at 734 nm using an ELISA reader (InfiniteTM F200, Männedorf, Switzerland).

The DPPH free radical scavenging activity was also assessed for the PE-ZnO NPs as previously reported [35]. Briefly, 200 μL of the 0.2% PE-ZnO NPs was mixed with 100 μL of 0.2 mM DPPH solution in ethanol, allowed to react for 30 min at room temperature, and measured for absorbance at 517 nm using ELISA reader (Infinite™ F200, Männedorf, Switzerland). Besides, ethanol was treated instead of DPPH or $ABTS^{+}$ as negative control and ascorbic acid was employed as a positive control. The scavenging activity of the test sample was calculated using Eq. 1.

$$\text{Scavenging rate (\%)} = \frac{\text{OD(control)} - \text{OD(sample)}}{\text{OD(control)}} \times 100\% \quad (1)$$

Further, the PE and ZnO NPs were also considered under similar experimental conditions for the $ABTS^{+}$ and DPPH scavenging activity as reference controls against the PE-ZnO NPs.

2.6. Cell culture

Human keratinocytes (HaCaT) cell line was obtained from American type culture collection (ATCC) Manassas, VA, USA. The HaCaT cells were grown and maintained in Dulbecco's Modified Eagle Medium (DMEM) amended with 10% FBS, penicillin (100 units/mL)/streptomycin 100 μg/mL) under 5% CO_2 atmosphere at 37ºC in a CO_2 incubator. The cell lines were cultured with undersupply and subculture was performed at 2-3 days intervals.

2.7. Cytotoxicity and proliferation test

The cytotoxicity and proliferation inhibition induced by the synthesized PE-ZnO NPs were also assessed by MTT assay. Briefly, the HaCaT cells were harvested (1×10^4 cells/well) and seeded in 96 well cell culture plates and incubated with growth for 24 h. Following, old medium was replaced with a new growth medium amended with

various concentrations (25, 50, and 100 mg/mL) of PE, ZnO NPs, and PE-ZnO NPs and further incubated under 5% CO_2 atmosphere at 37ºC for 48 h in a CO_2 incubator. Following, the cell Titer 96®AQueous One Solution Cell Proliferation Assay Kit (Promega, USA) was employed to monitor the effect on sample treatment on cell proliferation. Herein, all the medium from the treated cells was replaced with fresh 100 µL of DMEM followed by the addition of 20 µL of MTT solution and gently mixed. This reaction mixture was then incubated under a 5% CO_2 atmosphere at 37ºC in a CO_2 incubator and measured the absorbance at 490 nm by employing an ELISA reader (InfiniteTM F200, Männedorf, Switzerland). An untreated cell culture set was employed as a control.

2.8. Antimicrobial activity

Bacterial strains, *i.e*., *Bacillus licheniformis* (ATCC 1458), *Bacillus cereus* (ATCC 14579), and *E. coli* (ATCC 15597), were purchased from the ATCC (American Type Culture Collection, Manassas, Virginia, USA) and used for the antibacterial activity assay with experimental samples, *i.e*., synthesized PE-ZnO NPs, ZnO NPs, and PE (0.1 and 1.0 mg/mL), against Tetracycline as a positive control. Herein, 10 µL of each bacterial culture was inoculated from broth culture in 10 mL LB (Luria-Bertani) media and incubated for 12 h under shaking condition (120 rpm) at 37ºC. Following bacterial cells were harvested and washed with PBS (pH 6.8) to obtain 10^4 cells/mL. Next, these cells were inoculated in fresh LB media amended with various concentrations of sample and further incubated under shaking conditions at 37ºC for 12 h. After that, treated bacterial cultures were serially diluted (10^{-5}-10^{-7}) and gently speeded on the LB agar media plates, incubated at 32ºC for 12 h. Finally, colonies appeared on the agar plates were counted to calculate the antibacterial activity induced by experimental samples.

2.9. Statistical analysis

All data was calculated as Mean ± S.D values using SPSS package (SPSS, USA). Statistical analysis of the experimental data made the significance of the experimental group relative to the control group as student's t-test, and when the value of $p < 0.05$.

3. Results and Discussion

In this study, pomegranate pericarp extracts functionalized ZnO NPs (PE-ZnO NPs) were synthesized, characterized, and examined for biological properties. It was hypothesized that bioactive ingredients in PE, *i.e*., polyphenols and flavonoids, can be decorated on the growing clusters of Zn^{+2} ions in presence of a reducing agents to form PE-ZnO NPs. The bioactive compounds profiling revealed considerable content of polyphenols and flavonoids in PE which can be used in the synthesis of PE-ZnO NPs.

3.1. Total polyphenolic content assay of PE extracts

The PE fruit is enriched with bioactive polyphenols and is commonly consumed in large quantities around the world as health functional foods [36]. However, pomegranate pericarp is usually discarded as fruit waste. Therefore, in this study, pomegranate pericarp was evaluated for the total bioactive content and used in the synthesis of PE-ZnO NPs. Initially, the bioactive compounds in the PE were extracted using 70% ethanol and analyzed for the total phenolic and flavonoid contents. Herein, PE demonstrated significant quantities of polyphenol (363.04 ± 0.10 mg GAE/g) and flavonoid (20.17 ± 0.01 mg QE/g) (Table 1). Therefore, the PE sample may be considered for biological functions by comparison to quercetin, which is a physiologically active substance [18]. Moreover, recent studies have also reported high content of polyphenolic compounds, including punicalagin isomer, anthocyanins (cyanidin, delphinidin, and pelargonidin 3-glucoside and 3,5-diglucoside), ellagic acid, flavonoids, phenolic acids, and other polyphenolic substances in ethanol extract of PE [37]. Due to excellent polarity, ethanol fraction can elute a higher amount of phenolic and flavonoid contents from the extract, and these results were also supported by our finding as reported previously [38,39].

3.2. Physical properties of PE-ZnO NPs

The synthesis of PE-ZnO NPs was performed from PE and zinc nitrate as an ingredient by chemical precipitation method and the formation of nanocomposite material was examined using UV-Vis spectra (Fig. 1A). The PE shows maximum absorbance at 310 and 380 nm, respectively while PE-ZnO NPs showed at broad peaks in the region of

Table 1. Total polyphenolic and flavonoids contents of *Punica granatum* extraction

Extracts	Total Polyphenol (mg GAE[#]/extract g)	Total flavonoid (mg QE[##]/extract g)
Punica granatum	363.04 ± 0.10[a]	20.17 ± 0.01[a]

[#]GAE: gallic acid equivalents; [##]QE: quercetin equivalents. [a]Mean ± SD (n = 3).

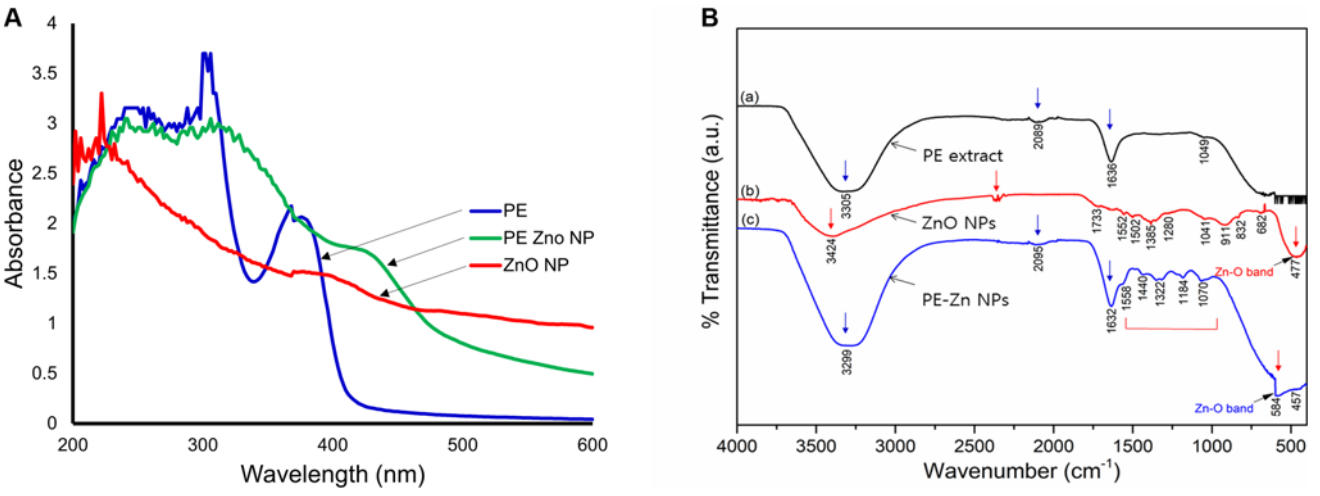

Fig. 1. (A) UV absorption spectrum of PE, ZnO NPs, and PE-ZnO NPs. (B) The FT-IR spectra analysis of PE, ZnO NPs, and PE-ZnNP.

250-480 nm which overlaps with the UV-Vis spectra of ZnO NPs as reported previously [40].

Furthermore, FT-IR spectroscopy was performed to identify the functional groups related to PE, ZnO NPs, and PE-ZnO NPs (Fig. 1B). The PE shows a characteristic transmission infrared wavelength band of 3,305 cm^{-1} for carboxylic acid O-H strain, 2,089 cm^{-1} to aromatic C-H folded, 1,636 cm^{-1} for aromatic ring vibration, and 1,049 cm^{-1} for primary alcohol C-O stretching of the functional molecules. Likewise, transmission spectrum peaks at 3,424, 1,733, 1,552, 1,502, 911, and 1,280 cm^{-1} indicated the formation of ZnO NPs. Analysis of the PE-ZnO NPs showed peaks at 3,299 and 2,095 cm^{-1} for O-H stretch (phenols) and N=C=S stretch (isothiocyanate) regions, respectively. Also, the bands at 1,632 and 1,558 cm^{-1} correspond to amine I & II regions, 1,440 cm^{-1} for C-C stretch (aromatics), 1,322 cm^{-1} N-O symmetric stretch (nitro compounds), 1,184 cm^{-1} for C-N stretch (aliphatic amines), 1,070 cm^{-1} for =C-H bond (alkenes), indicated the functionalization of ZnO NPs (strong bands at 584-457 cm^{-1})

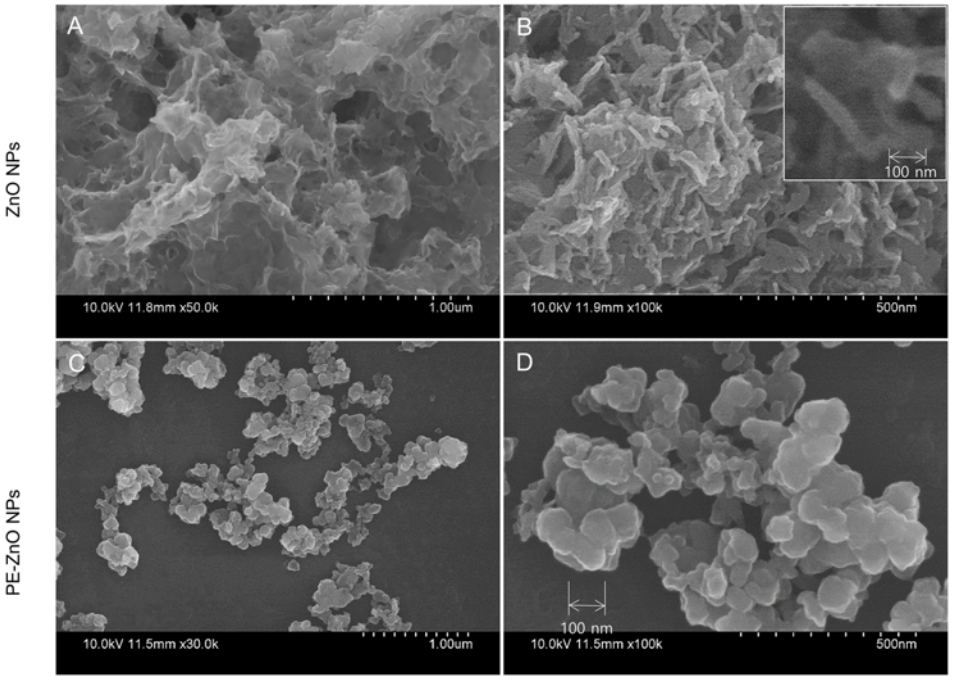

Fig. 2. (A and B) This is a schematic diagram showing the shape of ZnO NPs. (C and D) Pomegranate-zinc oxide nanoparticles (PE-ZnO NPs) measured by SEM.

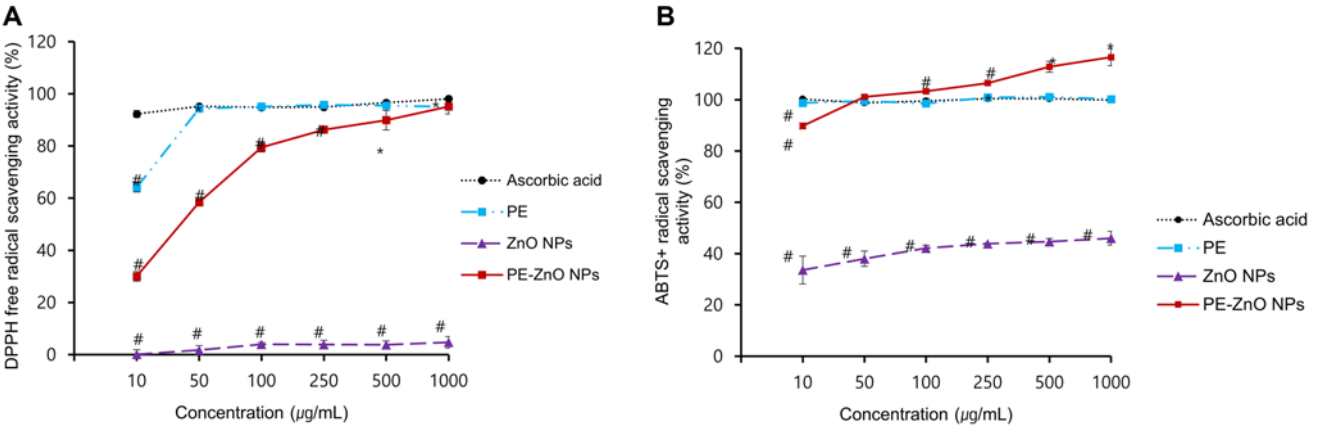

Fig. 3. (A and B) DPPH and $ABTS^{\cdot+}$ radical scavenging activity of PE, ZnO NPs, and ZnO-PE NPs. Results are the means ± SD, $^{*}p < 0.05$, $^{\#}p < 0.005$ compared with ascorbic acid (AA).

with bioactive compounds in PE. To note, ZnO NPs show a broad transmittance peak at 477 cm^{-1} while characteristic peaks at 585-450 cm^{-1} noted in the PE-ZnO NPs are not present in PE (Fig. 1B). Based on FT-IR results, functionalization of PE with ZnO NPs was successfully established. In the previous reports, ZnO NPs were fabricated using extracts of *Rubus fairholmianus* [18] and *Tectona grandis (L.)* [41]. Therefore, collected results demonstrate that bioactive compounds in the PE can be used in the functionalization of ZnO NPs.

Moreover, SEM imaging was applied to characterize the formed nanostructures in PE-ZnO NPs by comparison to ZnO NPs. Fig. 2A shows the ZnO NPs as sheets and foam-like nanostructures in the range of 20-100 nm thickness (Fig. 2A and 2B). However, the PE-ZnO NPs are noted as clusters of nanoplates with varied size in the range of 80-100 nm (Fig. 2C and 2D). Recently, the biosynthesis of ZnO-NPs using *Pongamia pinata*, *Plectranthus amboinicus*, and *Vitex negundo* also showed agglomerated NPs of different sizes in the range of 80-100 nm [42-44].

3.3. *In vitro* antioxidant activity of PE and PE-ZnO NPs

PE and PE-ZnO NPs showed an enhanced $ABTS^{+}$ and DPPH radical scavenging activity in a concentration-dependent fashion (Fig. 3A). The percentage scavenging activity of PE and PE-ZnO NPs (1,000 μg/mL) were 95.1 and 95.2%, respectively. $ABTS^{+}$ and DPPH assays are widely applied as indicators to assess the antioxidant activity of diverse plant extracts via hydrogen donating antioxidants [45]. As a result of measuring $ABTS^{+}$ radical scavenging activity, the PE-ZnO NPs (50 and 1,000 μg/mL) exhibited a yield of 101.1 and 116.5%, respectively by comparison to PE and ZnO NPs, which showed the better scavenging ability of PE-ZnO NPs against $ABTS^{+}$ (Fig. 3B). Hence, the functionalization of ZnO NPs with polyphenol compounds present in PE in ZnO (PE-ZnO) was assumed to enhance the $ABTS^{+}$ radical scavenging activity.

3.4. Cytotoxicity and proliferation rate of PE-ZnO NPs in HaCaT cells

A cytotoxicity activity using MTT assay to find the nontoxic concentration of PE-ZnO NPs in HaCaT cells line. Results showed that ZnO NPs reduced the cell number by 27.2% at 100 μg/mL concentration by comparison to the control. The result showed that PE and PE-ZnO NPs at 100 μg/mL concentration have 91.3 and 96.3% of cell viability, respectively. These results indicated that PE and PE-ZnO NPs are not shown significant toxicity to HaCaT cells (Fig. 4). The ZnO NPs presented photocatalytic toxicity and cytotoxicity in mammalian cells by photo induced effects of noble metal-doped ZnO NPs to produced ROS under UV and visible light [46]. However, the PE-ZnO NPs are not cytotoxic to HaCaT cells, it can be explained

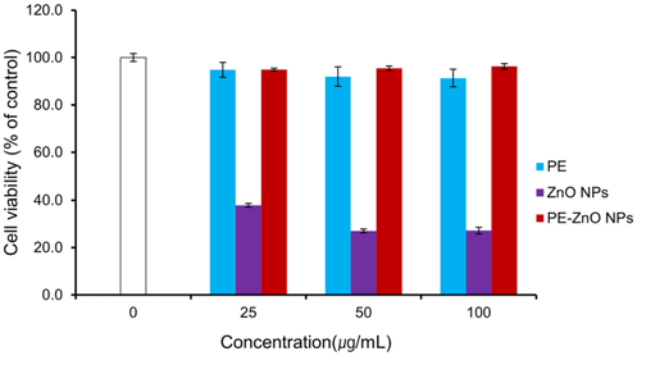

Fig. 4. Effect of PE, ZnO NPs, and PE-ZnO NPs on human skin epithelial keratinocytes (HaCaT) cells viability. The cells were treated with different concentrations of PE-ZnO NPs (25, 50, and 100 μg/mL) for 24 h and then assessed for cell viability by MTT assay.

that the synthesized PE-ZnO NPs decorated with phyto-components present in PE, which could reduce the cell toxicity than that of ZnO NPs (Fig. 4).

3.5. *In vitro* antimicrobial activity of PE-ZnO NPs

Although several species of the *Bacillus* and *Escherichia* genus are useful as probiotics, food fermentations, and industrial applications but many of the species from the same genus are also known as pathogens [47,48]. Of note, *B. cereus*, gram-positive, facultative anaerobic and spore-forming soil, plant, and food bacteria have been noted for causing. foodborne illnesses, severe nausea, vomiting, and diarrhea [49]. Similarly, *B. licheniformis* is also a Gram-positive and mesophilic bacterium and commonly found in the soil. It is a human pathogen causing skin infections with degradation of β-keratin [50]. Moreover, *E. coli* is a gram-negative, facultative anaerobic, rod-shaped, coliform bacterium, which commonly found in the gut of mammals. Although, most of the *E. coli* strains are non-pathogenic bacteria, but some strains of *E. coli* strains, such as *E. coli* O157:H7, are known to cause serious food poisoning [51]. Therefore, anti-microbial activity of PE-ZnO NPs against such pathogenic bacteria projected the potential and prospective application of nano-materials as non-antibiotic drugs. Therefore, in this study, we tested the antibacterial activity of PE and PE-ZnO NPs at various percentages against *B. cereus*, *B. licheniformis*, and *E. coli* by colony-forming unit (CFU) methods.

Herein, the CFU of *B. cereus* was reduced to 6×10^5 CFU when treated with PE-ZnO NPs (0.1 and 1.0 mg/mL) by comparison to 100×10^5 CFU/mL of non-treated control, 12×10^5 CFU/mL of PE (0.1 and 1.0 mg/mL) and 6×10^5 CFU/mL of ZnO NPs (0.1 and 1.0 mg/mL) as depicted in Fig. 5. The treatments with PE-ZnO NPs (1.0 mg/mL) also showed substantial reduction in the growth of *B. cereus* (Fig. 5A and 5B). Moreover, there was no colony formation in *B. licheniformis* and *E. coli* treated with 0.1 and 1.0 mg/mL of PE-ZnO NPs (Fig. 5C-F). Therefore, the synthesized

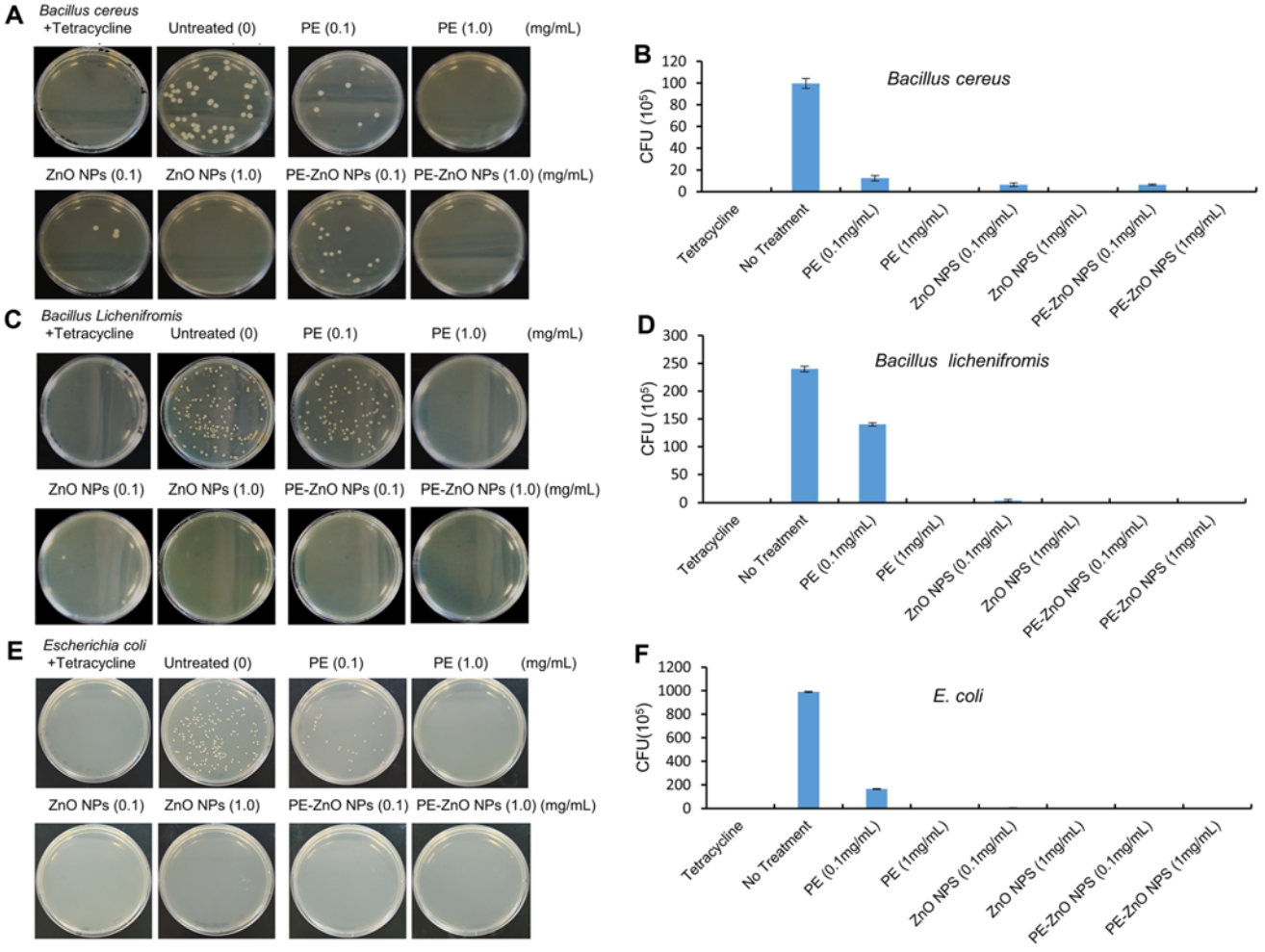

Fig. 5. The antibacterial efficiency of the PE, ZnO NPs, and PE-ZnO NPs against *Bacillus cereus*, *Bacillus licheniformis*, and *Escherichia coli.* The colony-forming units (CFU/mL) of PE, ZnO NPs, and PE-ZnO NPs.

PE-ZnO NPs were elucidated with potential antimicrobial activity. Recently, several studies reported that ZnO NPs effectively inhibit bacterial growth [43]. The ZnO NPs streamlines their access via the microbial cell membrane, and hence, to inactivate the protein and bacteria growth [44]. Among the several antimicrobial mechanisms, ZnO NPs are known to produce ROS and hydrogen peroxides (H_2O_2). These chemical species can easily inactivate the DNA replication and induce damage in the bacterial cell membrane, resulting in the demolition of bacteria [45]. Bacterial cell degradation proposed of noble metal-doped ZnO NPs for generating ROS for killing bacterial cells. Hence, the synthesized PE-ZnO NPs were suggested to exhibit superior anti-microbial activity via causing DNA instability and destruction of the bacterial cell membrane. Furthermore, PE-ZnO NPs enhanced the ROS eruption with polyphenolics, leading to antibacterial effects.

Recently, antibiotic resistance microbes (ARM), including multi-drug-resistant (MDR) bacteria, have been increasing globally due to an increased prescription of antibiotic drugs specifically in the COVID-19 pandemic [52]. Therefore, since the PE-ZnO NPs exhibited a wide range of antimicrobial activities, these novel NPs can be used as multifunctional antimicrobial agents function as non-antibiotic drugs. In addition, the PE-ZnO NPs have multifunctional properties with strong ROS scavengers due to the composition of metallic Zn ions and bioactive compounds.

4. Conclusion

In this study, we developed and synthesized the novel organic-ZnO NPs by functionalizing the bioactive compounds in the pomegranate pericarp ethanol extracts (PE) with ZnO NPs. Furthermore, the PE-ZnO NPs exhibited strong radical scavenging activity, suggesting the PE-ZnO NPs would be useful antioxidant agent against ROS-mediated dysfunctions in human cells. Altogether, the synthesized PE-ZnO NPs are organic and metal-based nanoparticles and valuable as multi-target antimicrobial agents in cosmetics and pharmaceuticals.

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Disclosure

The authors declare no conflicts of interest.
No ethical approval or informed consent was required for this study.

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