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Antimicrobial and Antioxidant Potential of Pomegranate Extract-Mediated Zinc Oxide Nanoparticles

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Abstract

The increasing load of antimicrobial resistance and antioxidative-stress-induced conditions pose a significant challenge to the health of the general population, hence necessitating the survival of safe, effective and sustainable solutions to the traditionally used antimicrobial compounds. Zinc oxide nanoparticles (ZnO-NPs) were prepared using an ecologically friendly green method based on extracts of *Punica granatum* L., and more specifically the peel and aril fractions, which contain a high concentration of phenolic bioactive compounds. The phytochemicals obtained in the plant played the role of an inherent reducing and capping agent and helped in the formation of the nanoparticles in mild conditions without the use of toxic substances. The antioxidant activity was determined in the DPPH free radical scavenging assay, and the antibacterial activity was measured on Gram-positive and Gram-negative bacterial strains as representatives in the agar-well diffusion method. The formation of pomegranate phytochemicals functionalised ZnO nanoparticles was proved by spectroscopic analysis and confirmed the surface functionalisation. This data showed that the peel extract of the *P. granatum* had the strongest antioxidant activity, which was additionally enhanced when the peel extract was added to the ZnO-NPs. It was shown that ZnO-NPs intermediated through the peel exhibited significantly increased antibacterial effects as compared to crude extracts, and this highlights a higher bioavailability and contact with the microbes at the nanoscale. Moreover, when ZnO-NPs were utilised in combination, the antibacterial activities of the formulations showed synergistic behaviour, as opposed to their use as single formulation. Taken together, these results suggest that ZnO nanoparticles produced in the form of *P. granatum* containing multifunctional, environmentally friendly agents with high antioxidant and antibacterial activities, which thus pre-condition their potential use in biomedical and food-industry settings.

Keywords: *Punica granatum*; Nanoparticles of zinc oxide; Green synthesis; Antibacterial action; Antioxidant action; Phenolic compounds; Antimicrobial resistance

Introduction

Antimicrobial resistance (AMR) is nowadays among the most significant global health issues of the twenty-first century, and the number of oxidative-stress-related disorders is on the rise. The emergence of multidrug-resistant bacterial and fungal pathogens in small amounts is the main cause of increased infection periods, higher mortality and enormous economic cost to human beings globally (Lithi et al., 2025). Simultaneously, the generated overproduction of reactive oxygen species (ROS) and damaged antioxidant defense systems are the core of the pathogenesis of chronic inflammatory diseases, cancer, metabolic syndromes, and neurodegenerative disorders (Mushtaq et al., 2023). The overlap of microbial resistance and oxidative stress has heightened the efforts on alternative therapeutic and preservative methods that are safe, effective, environmentally sustainable, and pathway to deal with causes of microbial proliferation and oxidative damage.

There has been increased interest in metal-oxide nanoparticles as versatile agents that can overcome some of the drawbacks of the conventional antimicrobial agents. One of them is zinc oxide nanoparticles (ZnO-NPs), which has been widely researched due to their broad-spectrum antimicrobial activity, intrinsic antioxidant properties, ultraviolet-blocking properties, chemical stability, as well as relatively favourable biocompatibility (Mohamad Sukri et al., 2019; Ifeanyichukwu et al., 2020). It is because at the nano level, ZnO-NPs have a high ratio of surface volume and increased reactivity, which allows them to interact strongly with microbial cells. Such interactions entail destabilisation of cell-membrane integrity, generation of intracellular ROS, regulated release of Zn^{2+} ions,

and interference of vital biomolecules, including proteins and nucleic acids, and result in the inhibition of growth or death of microbial cells (Bouttier-Figueroa et al., 2024; Lithi et al., 2025). Besides antimicrobial, ZnO-NPs have also been found to control oxidative stress, which can be applied in biomedical, pharmaceutical, and food-related systems (Sarhadi et al., 2024).

Though these benefits are there, the synthesis route is decisive in defining the physicochemical behaviour, biological functionality, and safety of ZnO-NPs. The traditional physical and chemical methods of synthesis are usually associated with extreme reaction conditions, large amounts of energy, and poisonous reducing or stabilising reagents, which raise concerns of environmentally unfriendly sustainability and residual toxicity (Bouttier-Figueroa et al., 2024). These constraints limit the application of chemically prepared ZnO-NPs to the form of direct exposure with biological tissue or food matrices. Therefore, there has been an emergence of interest in green synthesis methods that allow synthesis of nanoparticles under mild conditions with fewer ecological and health hazards.

Green synthesis through plants has also become a green and green process of preparing metal and metal-oxide nanoparticles. The phytochemicals in this strategy act as reducing, capping and stabilising agents thus preventing the use of toxic chemicals (Ifeanyichukwu et al., 2020; Lithi et al., 2025). It is a straightforward approach that is cost-effective, scalable, and can be used with mild conditions of the reaction. Notably, the presence of phytochemical residues on the surface of nanoparticles may also add a new biological property, which in many cases leads to an antimicrobial and antioxidant effect and reduces cytotoxicity (Mushtaq et al., 2023; Hussien et al., 2025). It has been reported in several studies that ZnO-NPs produced through the green synthetic method have controlled particle size, characterised crystalline structure, and improved antimicrobial activity over synthetic counterparts produced through chemical methods (Mohamad Sukri et al., 2019; Fouda et al., 2023).

Punica granatum L. has become the subject of specific attention of plant sources in green synthesis because of its exceptionally high phytochemical composition. Pomegranate fruits especially the peel, are rich in phenolic compounds, including punicalagin, ellagic acid, and anthocyanins that are highly recognised as potent antioxidants as well as antimicrobials (Abdelmigid et al., 2022; Shaban et al., 2022). These phenolics have several hydroxyl and carbonyl functional groups, which enable zinc ion reduction and stabilisation of nanoparticles in the process of ZnO-NP. It has been reported that ZnO-NPs mediated by *P. granatum* peel extracts have a spherical or hexagonal morphology and particle sizes of the order of nanometres and that they possess significant antimicrobial effects against Gram-positive and Gram-negative bacteria and pathogenic fungi (Ifeanyichukwu et al., 2020; Fouda et al., 2023).

Fourier-transform infrared (FTIR) spectroscopic characterisation is always used to verify the use of pomegranate-derived phenolic compounds to nucleate, grow, and functionalise nanoparticles. Effective phytochemical capping is proven by the existence of typical hydroxyl and carbonyl vibrational bands on the surface of ZnO (Abdelmigid et al., 2022; Kokabi and Nejad Ebrahimi, 2020). These surface-associated phytochemicals, besides playing a structural role also play a significant role in determining the biological behaviour of the nanoparticles. The synthesised ZnO-NPs have proven to have stronger antimicrobial activity and antioxidation capacity than the crude extracts or the uncoupled ZnO nanoparticles, and the interaction between the metal-oxide core and the polyphenols of the plant extracts is synergistic (Hashem and El-Sayyad, 2023; Fouda et al., 2023).

In spite of significant advances in the green synthesis of ZnO-NPs using *P. granatum*, there are still significant research gaps. Nanoparticle preparation and initial biological screening are the centre of interest of numerous studies, but there have been minimal attempts to correlate the phytochemical composition with nanoparticle activity systematically and calculate the synergistic effect of nanoparticles and conventional antibiotics (Ifeanyichukwu et al., 2020; Shaban et al., 2022). It is important to address them to enhance reproducibility, optimisation of surface chemistry, and functional efficacy.

In this regard, the current research will focus on zinc oxide nanoparticles mediated by *Punica granatum* extracts and the overall assessment of their physicochemical, antioxidant and antimicrobial characteristics. The objectives are:
(i) to establish an environmentally friendly green synthesis method of ZnO-NPs with *P. granatum* peel and aril extracts;
(ii) to characterise the synthesised nanoparticles in terms of size, morphology, crystallinity and surface functional groups;
(iii) to comparatively evaluate the antioxidant and antimicrobial activities of plant extracts and their associated ZnO nanoparticles;
(iv) to evaluate enhanced and synergistic antibacterial actions due to associations of pomegranate-mediated ZnO-NPs.

This study aims at developing the rational design of plant-mediated ZnO nanoparticles to be used in sustainable biomedical and food-related applications by directly relating nanoparticle performance to phytochemical composition and analysing integration strategies based on combination.

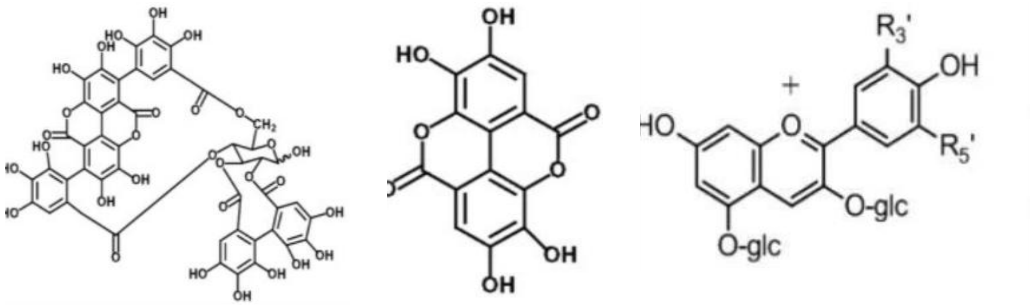
Literature Review: Bioactive Compounds, Mechanisms, and Green Synthesis of ZnO Nanoparticles Using Punica granatum Peel and Aril

The increasing occurrence rate of antimicrobial resistance (AMR) has become a central concern in the realm of public health, and it has a devastating effect on the efficacy of traditional antimicrobial therapy. The multidrug-resistant bacterial and fungal pathogens presently prolong the courses of illness, increase the cost of treatment, and raise the risk of mortality (Lithi et al., 2025). At the same time, such oxidative stress as the excess formation of reactive oxygen species (ROS) overwhelming endogenous antioxidant defences has been attributed to the pathogenesis of infectious diseases, as well as to a series of long-term diseases, such as oncological, cardiovascular, metabolic, and neurodegenerative (Mushtaq et al., 2023). These related issues have spurred rigorous academic investigation of novel antimicrobial modalities possessing simultaneous antioxidant activity and thus seeking to prevent microbial persistence and oxidative damage.

Medicinal plants have taken a leading position in the history of traditional medicine because of their enormous stores of bioactive secondary metabolites. The combination of phenolic acids, flavonoids, tannins, and alkaloids with other organic acids provides a range of biological activities, including antimicrobial, antioxidative, anti-inflammatory, and immunomodulatory activity. Although more than 2,50,000 vascular plant taxa have been described worldwide, a small fraction of this group has received a systematic phytochemical and pharmacological interrogation. Among the latter, *Punica granatum L*. has emerged as a paradigmatic species on numerous occasions due to its sizeable phenolic backbone and thoroughly reported therapeutic properties.

The better phenolic loading of *P. granatum,* particularly in its pericarp compared to the arils that are edible, is also delineated by extensive empirical evidence. The plant has potent antioxidant and antimicrobial activity that is supported by the synergy of such principal bioactive constituents as punicaealgin, ellagic acid, gallic acid, rutin, quercetin, and anthocyanins (Abdelmigid et al., 2022; Shaban et al., 2022). The antioxidative activity of pomegranate peel extracts has been documented to be stronger than a wide range of medicinal taxa as a result of high free-radical scavenging, metal-binding, and lipid peroxidation inhibition properties (Dali et al., 2025). These antioxidant capabilities are especially important in an infectious situation, where oxidative stress plays a key role in inducing tissue damage to the host body and promoting the survival of the pathogen.

In addition to their redox-regulatory merits, *P. granatum* extracts have wide-spectrum antimicrobial effects on Gram-positive and Gram-negative bacteria and opportunistic fungi. The multifaceted mechanisms of action have been explained by sows and colleagues, including the destruction of cellular membrane integrity, protein denaturation, suppression of nucleic acid synthesis, and inhibition of key metabolic enzymes (Ifeanyichukwu et al., 2020). The occurrence of multiple phenolics at the same time reduces the likelihood of resistance development and makes pomegranate-based formulations especially appealing candidates in the current search for the next antimicrobial approach.



(i) (ii) (iii)

Fig. 1. Bioactive compounds in Pomegranate: (i) Punicalagin(ii) Ellagic acid (iii) Anthocyanin

The intersection between plant-based therapeutics and nanotechnological innovations during the last 10 years has identified new opportunities to increase the effectiveness and uses of natural bioactive compounds. The metal oxide nanoparticles, particularly zinc oxide nanoparticles (ZnO-NPs), have attracted a lot of attention due to their unique physicochemical characteristics and versatile biology. ZnO-NPs have a strong antimicrobial effect, inherent antioxidant activity, ultraviolet absorption behaviour, chemical stability and a relatively positive biocompatibility profile (Mohamad Sukri et al., 2019; Ifeanyichukwu et al., 2020). At the nanoscale, the ZnO

particles develop a significantly increased surface area and reactivity and hence allow an enhanced interaction with the microbial cells.

Antimicrobial activity of ZnO-NPs is naturally multimodal, which includes the physical destruction of the membrane of microorganisms, the execution of intracellular reactive oxygen species (ROS), and the release of Zn^{2+} ions, as well as the interaction of these nanoparticles with key biomolecules – proteins, lipids, and nucleic acids (Bouttier-Figueroa et al., 2024). The result of these convergent activities is dysfunctional cellular activities and, eventually, the death of the microbes. Most importantly, the multimodal attack by ZnO-NPs suppresses the appearance of resistance compared to traditional antibiotics, which typically activate a single cellular room (Lithi et al., 2025). In addition to their antibacterial arsenal, ZnO-NPs have also been demonstrated to overcome the oxidative stress through free-radical scavenging and ROS control, supporting their future applications in the biomedical and food-industry applications (Sarhadi et al., 2024).

Despite such advantages, synthetic route used in the production of ZnO-NPs critically influences the particle characteristics including size, morphology, surface chemistry, biological activity, and safety. Traditional physicochemical processes often require elevated temperatures, markedly different pHs, and the use of toxic reducing or stabilising agents, which increases environmental sustainability-related issues and remaining toxicities (Bouttier contra, 2024). These disadvantages have prompted a growing desire to explore green synthesis approaches that can utilise biological resources in the development of nanoparticles using benign/mild conditions.

Plant-mediated green synthesis is a green and environmentally friendly method of producing metal and metal-oxide nanoparticles. Phytochemicals obtained by plants in this paradigm serve the functions of reducing agents, capping agents and stabilisers, thus eliminating the use of hazardous chemicals (Ifeanyichukwu et al., 2020; Lithi et al., 2025). The method is cheap, scalable and able to work in mild reaction conditions. Besides, the phytochemical residues left on the surface of nanoparticles may enhance biological performance, which generally results in increased antimicrobial and antioxidant activity and decreased cytotoxicity (Mushtaq et al., 2023; Hussien et al., 2025).

Punica granatum (pomegranate) is one of the botanical sources that have received specific interest in the context of the green synthesis due to the presence of amazingly rich phenolic profile. Various studies have managed to produce ZnO-NPs with pomegranate peel extracts with morphologies that are either of a spherical or a hexagonal shape and with diameters of between 10 and 80 nm (Ifeanyichukwu et al., 2020; Fouda et al., 2023). The presence of functional groups of hydroxyl and carbonyl on the nanoparticle surface is always demonstrated with the help of Fourier-transform infrared spectroscopy, which is a clear indication of successful capping and stabilisation of the nanoparticle with pomegranate-derived phenolics (Abdelmigid et al., 2022; Kokabi and Nejad Ebrahimi, 2020).
The ZnO-NPs that had been modified by pomegranate are more effective than crude plant extracts as well as chemically synthesised counterparts in biological terms. The synergistic effect between the production of ROS at ZnO, release of zinc ions, and the natural antimicrobial activity of surface-bound phenolics is associated with the enhanced antimicrobial activity (Fouda et al., 2023; Hashem and El-Sayyad, 2023). These nanoparticles have exhibited strong inhibitory action against clinically significant bacterial strains, such as Escherichia coli and Bacillus subtilis, and antifungal action against pathogenic moulds.

In addition to antimicrobial potency, ZnO-NPs mediated by pomegranate have better antioxidant activity compared to crude extracts. Conservation of polyphenolic compounds on the particle surface increases the electron-donating characteristics and free-radical scavenging ability, and thus the overall antioxidant behaviour (Dali et al., 2025). Such dual antimicrobial-antioxidant is especially beneficial in those applications where microbial control and oxidative stability are required, e.g., wound dressings, biomedical coatings, and food packaging materials. Combinatory strategies of matching plant-mediated ZnO-NPs to traditional antimicrobial agents have also been investigated recently. The synergies have been reported and have led to the enhancement of antibacterial activity and lower effective doses of antibiotics (Lithi et al., 2025). These approaches are viewed as promising in reducing the antimicrobial resistance through the reduction of selective pressure on the microbial population, and still retaining the therapeutic efficacy. Regardless of such enormous progress, existing literature concedes a number of gaps. Numerous studies mainly focus on nanoparticle production and primitive antimicrobial screening, providing in-depth comparisons between crude extracts and nanoparticles and their hybrids. Furthermore, there is a lack of extensive research to match phytochemical composition with biological activity through nanoparticles. It is urgent that these gap areas are addressed to realise rational design of the reproducible multifunctional nanomaterials with optimised antimicrobial and antioxidant properties.

To conclude, the existing literature is a strong indicator of the zinc oxide nanoparticles mediated by *Punica granatum* as an effective antimicrobial and antioxidant. However, additional combined research involving

phytochemical profiling, nanoparticle characterisation, and synergistic biological assessment is necessary to reap maximum advantages of them in combating antimicrobial resistance and oxidative stress-related issues.

Material and Methodology

Materials

Microbial Strains

The bacterial strains used

- *Escherichia coli*
- *Aspergillus niger*
- *Bacillus subtilis*
- *Unknown species* (Likely to be a strain
- of *Penicillium species*)

Table 1. Overview of *Punica granatum*–mediated ZnO nanoparticles

Plant part	Key bioactive compounds	Role in ZnO-NP synthesis	Biological activity reported	Selected references
Peel	Punicalagin, ellagic acid, gallic acid, flavonoids	Strong reducing and capping agents	High antibacterial and antioxidant activity	Ifeanyichukwu et al., 2020; Abdelmigid et al., 2022; Fouda et al., 2023
Aril/juice	Anthocyanins, ellagic acid	Mild reduction and surface functionalization	Moderate antioxidant and antibacterial activity	Shaban et al., 2022; Dali et al., 2025
Whole fruit extract	Mixed phenolics and organic acids	Combined reduction and stabilization	Broad-spectrum antimicrobial and antioxidant effects	Hashem and El-Sayyad, 2023
Peel-mediated ZnO-NPs (vs extract)	Surface-bound polyphenols	Enhanced nanoparticle stabilization	Superior activity compared to crude extracts	Fouda et al., 2023; Lithi et al., 2025

Culture media and growth conditions

Microbial culture media used in the study were:

a. Nutrient Broth (NB)
b. Nutrient Agar (NA)
c. Luria Bertani Broth (LB Broth)
d. Potato Dextrose Broth (PDB)
e. Potato Dextrose Agar (PDA)
f. Muller-Hinton Agar (MHA)

Growth Conditions for Microbial Cultures: Bacterial cultures were grown in Nutrient Broth (NB) and Luria-Bertani (LB) Broth at 37°C with agitation at 120 rpm for 16–24 hours. Nutrient Agar (NA) plates were incubated statically at 37°C for the same duration.

Fungal cultures were cultivated in Potato Dextrose Broth (PDB) at 25–27°C with continuous shaking at 120 rpm for 48–72 hours. Corresponding Potato Dextrose Agar (PDA) and Mueller-Hinton Agar (MHA) plates were incubated at 25–27°C for 48–72 hours under static conditions.

Antibiotics Used

Streptomycin Sulphate

Chemicals and Reagents used

- Crystal Violet Dye
- Gram's Iodine
- Methanol
- Ascorbic Acid
- Safranine Dye
- Immersion Oil
- Ethanol
- Distilled Water
- Lactophenol Cotton Blue Dye
- Zinc acetate
- DPPH(2,2-diphenyl-1-picrylhydrazyl)

All the chemicals used were of high-quality brands like HIMEDIA and CDH.

Table. 2. Characteristics of Natural Compounds

Sr. No.	Scientific name	Family	Common name	Part of the plant used	Active compound
1.	*Punica granatum*	Lythraceae	Pomegranate	Peel	Ellagic Acid, Gallic Acid, Rutin, Quercetin
				Arils	Punicalagin

Pomegranates were collected from the campus of Shri Ratanlal Kanwarlal Patni Girls' College, Kishangarh, Ajmer, Rajasthan, India.

(i) (ii)

Fig. 2. Plant parts used: (i) Immature Pomegranate fruit, (ii) Immature Pomegranate arils

Methods

Isolation of Microorganisms

Isolation of microorganisms was done by serial dilution of one gram of soil sample. Inoculation of 100 μlof each dilution was done on petri plates containing solidified PDA using the spread plate technique, and plates were kept in an incubator at 27°C for 48-72 hours.

Characterisation of Microorganisms

Gram staining and lactophenol cotton blue is staining were done to confirm the purity of microorganisms and their characterisation.

Gram Staining

Gram staining is a common technique used to differentiate bacteria as Gram-positive or Gram-negative based on differences in their cell wall structure. The process begins with the preparation of a bacterial smear. A small amount of bacterial culture is placed on a clean glass slide and spread into a thin layer. After air drying, the smear is heat-fixed by gently passing the slide through a flame, which kills the bacteria, adheres them to the slide, and preserves their structure.

The staining procedure then follows: crystal violet is applied as the primary stain and gently rinsed off with water after 60 seconds, followed by iodine to fix the dye. The slide is then decolourised with 95% alcohol or acetone for 60 seconds and counterstained with safranin for 30-40 seconds. Gram-positive bacteria appear purple, while Gram-negative bacteria appear pink when observed under the light microscope at 100X magnification (Tripathi and Sapra, 2020).

Lactophenol Cotton Blue Staining

Lactophenol Cotton Blue (LPCB) staining is a widely used technique in mycology for the microscopic examination of fungal structures. It combines staining and mounting in a single step, allowing efficient visualisation of fungi. The stain contains lactic acid to preserve morphology, phenol to kill and clear the cells and cotton blue dye, which stains chitin in the fungal cell wall to enhance contrast. A small portion of the fungal culture is placed into a drop of LPCB on a glass slide and covered with a coverslip. The slide is then examined under a light microscope, typically beginning at 10X and progressing to 40X for detailed observation. This method clearly reveals structures such as hyphae, spores, and conidia, aiding in fungal identification (Leck, 1999).

Preparation of Natural Compound Extract

A 50% solution of the plant extract was prepared by weighing 10 grams of fresh plant material, which was thoroughly washed and crushed using a blender. To the crushed material, 10ml of ethanol was added to form a paste-like mixture, which was then heated at 75°C for 30 minutes. After heating, the mixture was strained to separate the liquid extract, which was collected and stored at 4°C for further use.

Preparation of Antibiotic Extract:

Streptomycin sulphate was used as a standard antibiotic for the determination of antibacterial activity. A stock solution was prepared by dissolving 10 mg of streptomycin sulphate in 1 ml of ethanol.

Table. 3. Amount of obtained extracts

Sr. No.	Extract	extract obtained (ml)
1.	Immature *Punica granatum* peel	3
2.	Immature *Punica granatum* aril	4.2

Preparation of ZnO Nanoparticles

Based on the antimicrobial activity results, pomegranate peel, pomegranate granules, and cinnamon were each selected individually for the synthesis of zinc oxide (ZnO) nanoparticles. The plant materials were thoroughly washed with distilled water and air-dried for several days. Once completely dried, each was separately ground into a fine powder using a mortar and pestle. For each sample, 10 grams of the powdered material was boiled in 100 ml of distilled water for 30 minutes. The resulting extracts were filtered using Whatman filter paper and stored at 4°C for further use.

For the synthesis of ZnO nanoparticles, 4 grams of zinc acetate was dissolved in 50 ml of distilled water and stirred. To this solution, 10 mL of each plant extract (pomegranate peel and pomegranate granules) was added separately. Each mixture was stirred individually on a magnetic stirrer for 2 hours at 50°C. After stirring, the mixtures were transferred to centrifuge tubes and centrifuged at 5000 rpm for 10 minutes, repeated three times. The resulting precipitates from each extract were collected separately and air-dried in an oven overnight, yielding three distinct ZnO nanoparticle samples (Jiang et al., 2023).

A specific concentration of each ZnO nanoparticle sample was dispersed in 1ml of ethanol. This dispersion was then subjected to ultrasonic treatment using a sonicator for 30 minutes to ensure uniform distribution of nanoparticles within the liquid. The concentration of ZnO nanoparticles used in each case was determined based on the yield of the corresponding plant extract prepared earlier.

Determination of Antioxidant Activity of Plant Extracts

The antioxidant activity of the samples was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay (Gulcin and Alwasel, 2023). A 0.004% DPPH solution was prepared by dissolving 4 mg of DPPH in 100ml of methanol. The plant extracts were taken at varying concentrations, and each sample was diluted with methanol to a final volume of 2 ml. To each of these, 1 ml of the prepared DPPH methanolic solution was added. The mixtures were then incubated at room temperature for 30 minutes. Antioxidant activity was visually indicated by a change in colour, and the absorbance of each sample was measured at 517 nm using spectrophotometer. The percentage of radical scavenging activity (% RSA) of the samples was calculated using the following formula:

$$\text{Radical Scavenging Activity (\%} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where:

- A_0= Absorbance of the control (DPPH solution without sample),
- A_1= Absorbance of the sample (DPPH solution with plant extract)

Combination Activity

Combinations of natural compound extracts, ZnO nanoparticles, and standard antibiotics were tested to evaluate possible synergistic, antagonistic, or additive effects. The agar well diffusion method, as previously described for antimicrobial activity testing, was employed to assess the interaction effects of these combinations.

In this method, wells were created on agar plates previously inoculated with the test microorganisms. Each well was filled with a mixture of two different extracts, nanoparticles, or a combination of extract/nanoparticle with an antibiotic, prepared at varying concentrations. The plates were then incubated under appropriate conditions, and the zones of inhibition were measured to determine the nature of the interaction—synergistic (enhanced effect), antagonistic (reduced effect), or additive (combined individual effects). The Growth Inhibitory Index (GII) was calculated to assess the interaction between two antimicrobial agents when used in combination.

The GII was determined using the following formula:

$$\text{GII} = \frac{\text{Zone of Diameter of Inhibition (ZDI) in Combination}}{\text{Sum of ZDI of the Two Agents When Used Individually}}$$

Interpretation of GII values

GII > 0.5 → Synergistic effect
GII = 0.5 → Additive effect
GII < 0.5 → Antagonistic effect

For the study of combination therapy of extracts and extract with antibiotics, the amount of solution poured in wells was 25 µl for each of them.

Characterisation of Nanoparticles

FTIR and UV-VIS spectroscopy was used for the characterisation of the synthesised nanoparticles.

FTIR Spectroscopy

For FTIR analysis, samples were prepared by mixing each ZnO nanoparticle sample with potassium bromide (KBr). The mixtures were finely ground using a mortar and pestle to obtain a homogeneous blend. The resulting powder was then compressed into a thin pellet using a hydraulic press. This KBr pellet was placed into the sample holder of the FTIR instrument. The FTIR spectra were recorded to observe the specific vibrational frequencies corresponding to functional groups present in the sample. These characteristic peaks helped identify the biomolecules from the plant extracts responsible for capping, reduction, and stabilisation of the ZnO nanoparticles.

UV-VIS Spectroscopy

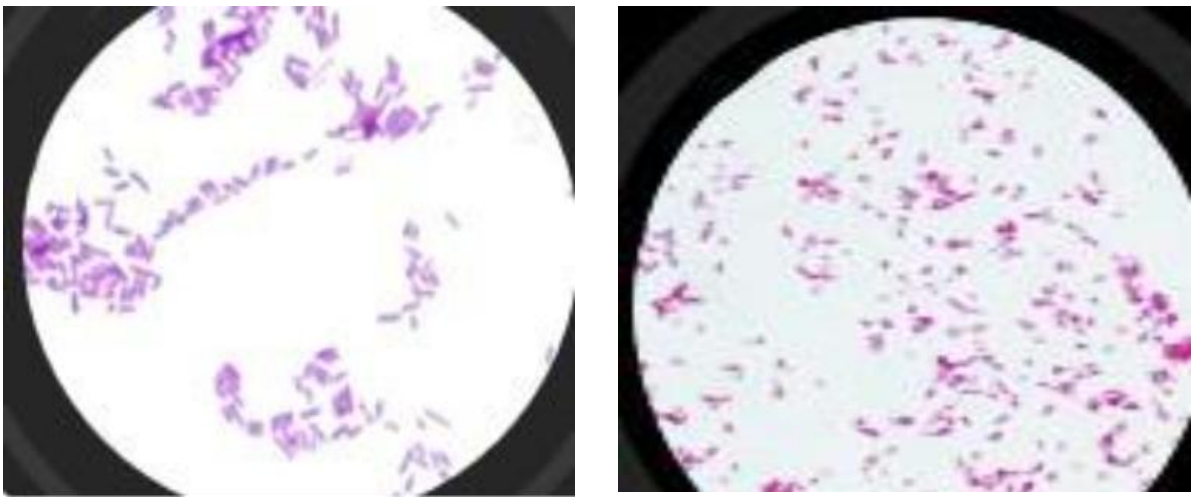
For UV–VIS spectroscopic analysis, the synthesised ZnO nanoparticles were dispersed in ethanol. Although the nanoparticles did not completely dissolve in the solvent, they formed a stable suspension. The suspension was then subjected to UV–VIS analysis to examine the optical properties of the nanoparticles.

Result

Characterisation of Microorganisms

Gram Staining

Bacillus subtilis strains purple in the Gram staining procedure, indicating their classification as gram-positive bacteria due to their thick cell walls. In contrast, *Escherichia coli* stains pink, characteristic of Gram-negative bacteria, as its thinner peptidoglycan layer and outer membrane do not retain the primary stain.



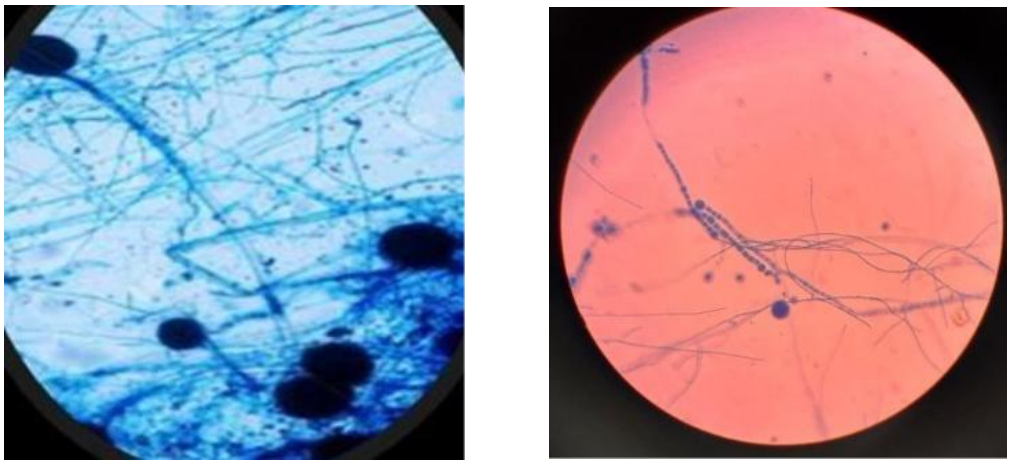
(i) (ii)

Fig. 3. Bacterial stains under a light microscope at 100X:

(i) Gram-positive- *Bacillus subtilis* (ii) Gram-negative – *Escherichia coli*

Lactophenol cotton blue staining

In Lactophenol cotton blue stained slide of *Aspergillus niger*, the conidial heads appear as dark, globose structures composed of long conidiophores ending in round vesicles. The slide of unknown fungus reveals well-defined septate hyphae, appearing as long, thin, branching filaments stained blue. Arising from the hyphae are conidiophores with small, round conidia forming in chains.

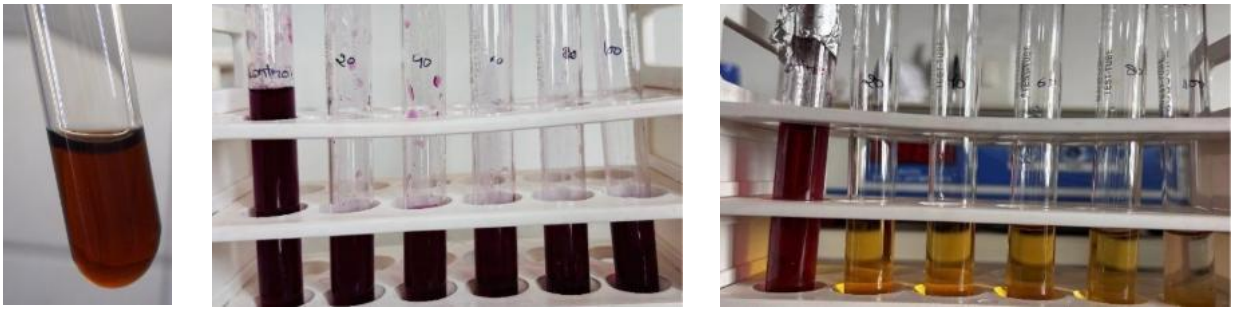


(i) (ii)

Fig. 4. Fungal strains under a light microscope at 40X:
(i) Aspergillus niger (ii) Unknown fungal strain

Antioxidant Activity of Plant Material Extracts via DPPH Assay

The antioxidant potential of pomegranate peel extract was assessed through the *2,2-diphenyl-1-picrylhydrazyl* (DPPH) radical scavenging assay. Following the addition of the extract to the DPPH solution and an incubation period, a distinct colour transition from deep violet to nearly colourless was observed, signifying effective free radical neutralisation. Absorbance values were recorded at 517 nm using a UV–Vis spectrophotometer, and the percentage of radical scavenging activity was subsequently calculated. The data revealed a concentration-dependent increase in antioxidant activity, with the highest scavenging effect of approximately 90% observed at a 100 µl extract concentration, indicating substantial antioxidant efficacy of the peel extract.



(i) (ii) (iii)

Fig.5. Antioxidant activity of pomegranate peel extract: (i) Peel extract, (ii) Peel extract after addition of DPPH solution, (iii) Peel extract after incubation for 30 minutes

Table 4. Absorbance and %RSA of Peel extract

Sr no.	Concentration of Peel extract (µl)	Absorbance of peel extract at 517nm	~ %RSA of peel extract
1.	20	0.040	64%
2.	40	0.030	71%
3.	60	0.025	77%
4.	80	0.020	82%
5.	100	0.011	90%

Synthesis and Characterisation of Nanoparticles

Nanoparticles were synthesised through a green synthesis approach employing *Punica granatum* peel extract, *Punica granatum* aril extract, and *Cinnamomum verum* bark extract as natural bio-reducing and capping agents, while zinc acetate was used as the metal precursor.

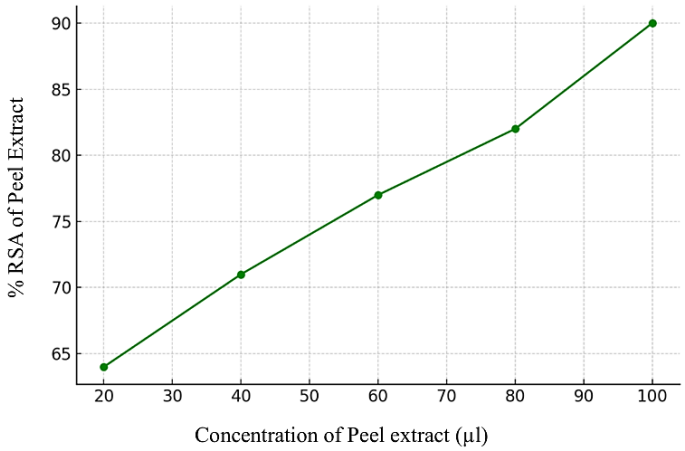


Fig. 6. % RSA of Peel Extract vs Concentration

Antioxidant activity of other extracts

Table. 5. Absorbance and %RSA of other extracts

Extract	Conc. of plant extract (µl)					Mean % RSA
	20	40	60	80	100	
Immature *Punica granatum* aril	0.048	0.039	0.031	0.022	0.016	~71%

Synthesis and Characterisation of Punica granatum Peel Nanoparticles

(i) (ii) (iii) (iv) (v)

Fig.7. Synthesis of Peel nanoparticles: (i) Dried immature pomegranate peel, (ii) Dried immature pomegranate peel powder, (iii) extract obtained by boiling the peel powder, (iv) Addition of zinc acetate and peel extract, (v) Peel-ZnO nanoparticles

FTIR Analysis of Peel ZnO Nanoparticles

The FTIR spectrum of zinc oxide (ZnO) nanoparticles synthesised using *Punica granatum* peel extract displays prominent absorption bands at 3435.94 cm^{-1}, 1572.65 cm^{-1}, 1424.45 cm^{-1}, 1347.10 cm^{-1}, 1193.56 cm^{-1}, and 1057.81 cm^{-1}, indicating the presence of functional groups such as hydroxyl (O–H), aromatic (C=C), and ether or alcohol (C–O) groups. These peaks confirm the involvement of phenolic compounds, flavonoids, and other phytochemicals from the peel extract as bio-reducing and stabilising agents during nanoparticle synthesis. The broad O–H stretch suggests strong hydrogen bonding, while the C=C and C–O stretches further support the presence of plant-derived compounds coating the nanoparticle surface. The broad O–H stretch suggests strong hydrogen bonding, while the C=C and C–O stretches further support the presence of plant-derived compounds coating the nanoparticle surface. Collectively, the spectrum confirms both the successful formation and biofunctional stabilisation of ZnO nanoparticle through a green synthesis route using pomegranate peel extract. Most importantly, the characteristic Zn–O stretching vibration observed in the region of 500–600 cm^{-1} confirms the successful formation of ZnO nanoparticles.

The UV–Visible spectrophotometric analysis of zinc oxide (ZnO) nanoparticles synthesised using *Punica granatum* (pomegranate) peel extract revealed distinct absorption peaks at 314.5 nm and 272.5 nm. The prominent peak at 314.5 nm is indicative of the characteristic excitonic absorption of ZnO nanoparticles, confirming their successful formation. The additional peak observed at 272.5 nm may correspond to residual phytochemicals from the peel extract, such as polyphenols or flavonoids, which likely act as natural capping and stabilising agents during the green synthesis process. These findings support the effective utilisation of pomegranate peel extract in the eco-

friendly synthesis of ZnO nanoparticles, with phytoconstituents contributing to both reduction and stabilisation mechanisms.

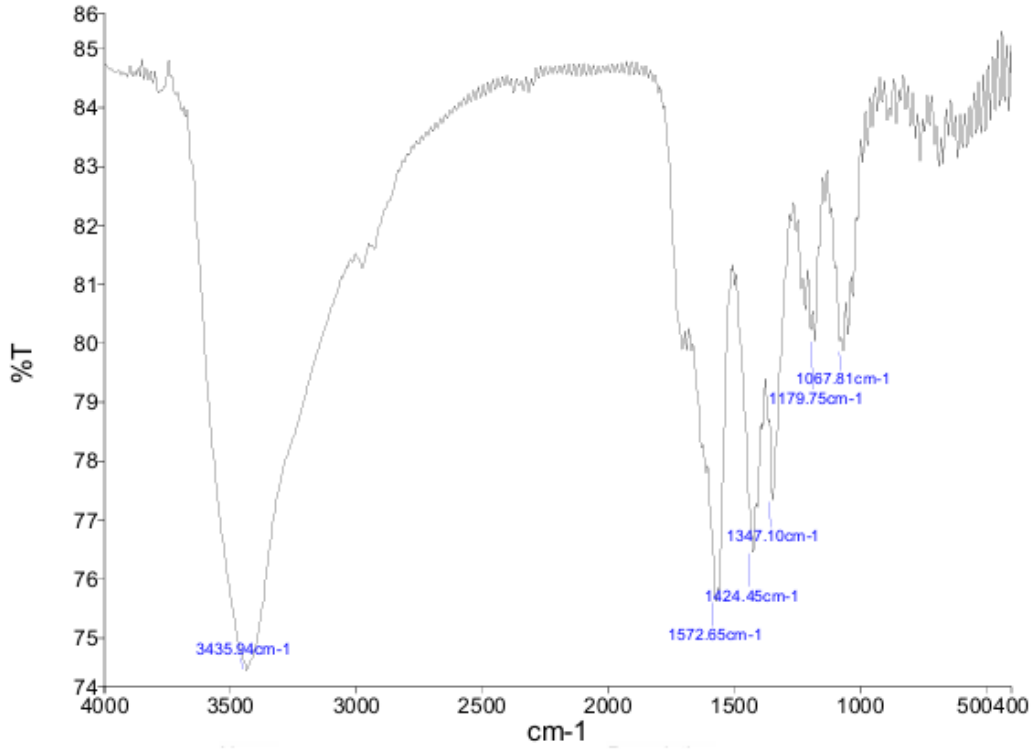


Fig.8. FTIR Analysis of Peel Nanoparticles

UV-Vis spectrophotometric analysis of Peel ZnO nanoparticles

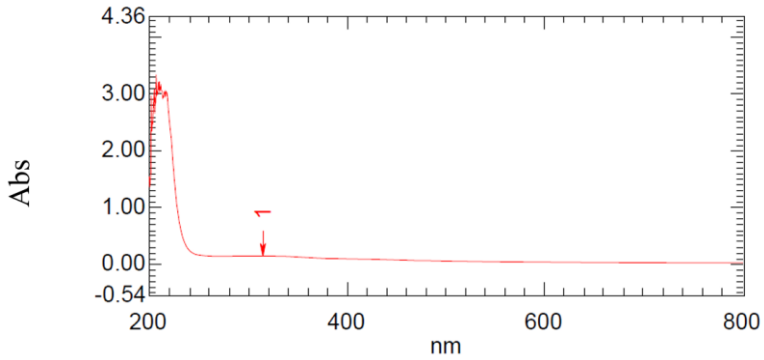


Fig. 9. UV-Vis spectrophotometric Analysis of Peel Nanoparticles

Synthesis and Characterisation of Punica granatum Aril Nanoparticles

(i) (ii) (iii) (iv) (v)

Fig. 10. Synthesis of aril nanoparticles: (i) Dried immature pomegranate aril, (ii) Dried immature pomegranate aril powder, (iii) extract obtained by boiling the aril powder, (iv) Addition of zinc acetate and aril extract, (v) Aril-ZnO nanoparticles

FTIR Analysis of Aril ZnO Nanoparticles:

The FTIR spectrum of zinc oxide (ZnO) nanoparticles synthesised using *Punica granatum* (pomegranate) aril extract reveals key functional groups involved in the green synthesis process. The broad absorption band at 3435.13 cm^{-1} corresponds to O–H stretching vibrations, indicating the presence of hydroxyl groups from phenolic or alcoholic compounds. A peak at 2974.58 cm^{-1} is assigned to C–H stretching, likely from aliphatic compounds present in the extract. The peaks at 1632.75 cm^{-1} and 1380.29 cm^{-1} correspond to C=O stretching (carbonyl groups) and C–H bending, respectively, suggesting the presence of organic acids or proteins. These biomolecules act as natural reducing and stabilising agents during nanoparticle formation.

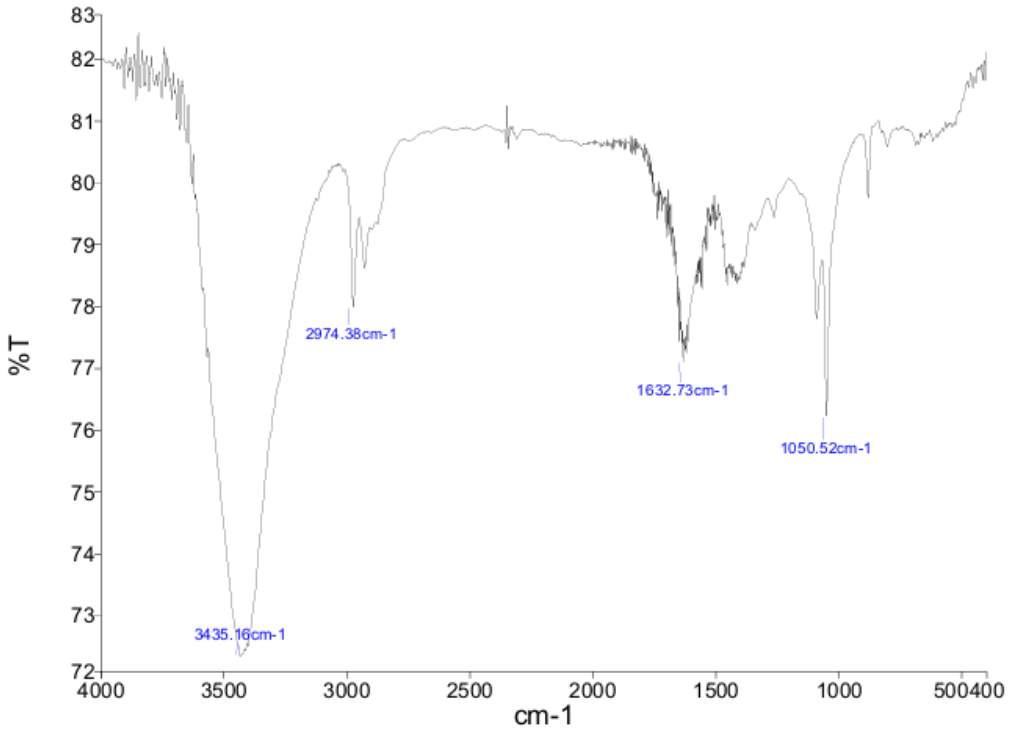


Fig.11. FTIR Analysis of Aril Nanoparticles

UV-Vis spectrophotometric analysis of Aril ZnO nanoparticles:

The UV-Vis spectrophotometric analysis of the Granule Pomegranate sample shows distinct absorption peaks between 264–285 nm, which are characteristic of organic compounds such as polyphenols, flavonoids, or other phytochemicals commonly found in pomegranate extracts. These peaks do not correspond to the typical absorption range of ZnO nanoparticles, which usually exhibit a strong absorption edge around 360–380 nm due to their wide bandgap. The absence of a clear ZnO-related peak suggests that either the nanoparticles are extremely small—resulting in a blue shift due to quantum confinement effects or present in very low concentrations, or their absorption is masked by the dominant signals from organic constituents.

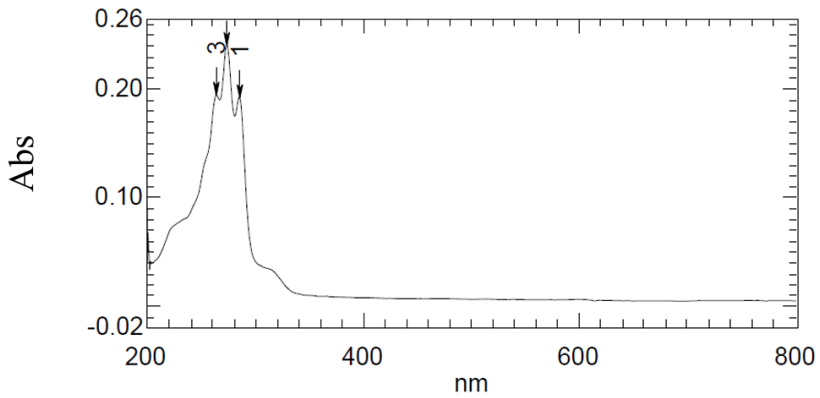
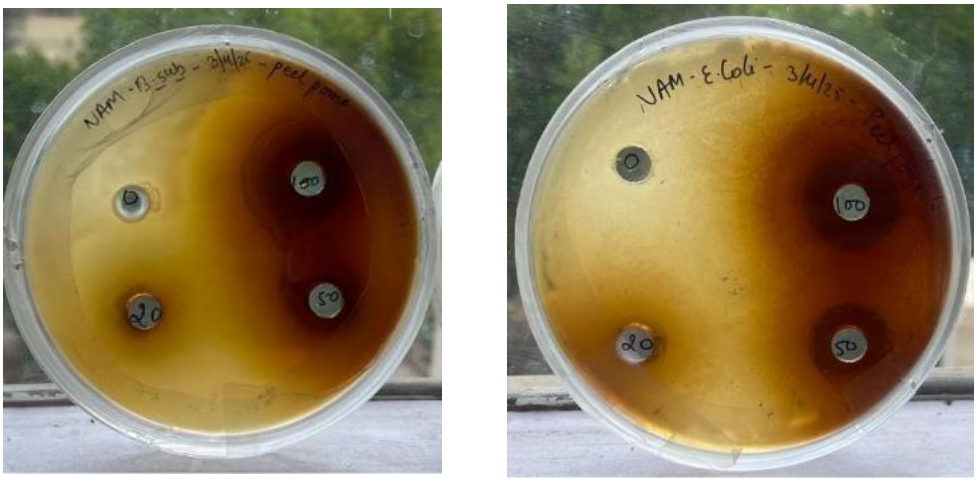


Fig.12. UV-Vis spectrophotometric Analysis of Aril Nanoparticles

Antibacterial activity of Plant material extracts

Antibacterial activity of the plant material extracts was evaluated by the agar well diffusion technique, employing sterile puncture tool to prepare wells of 6 mm diameter.

Antibacterial activity of pomegranate peel extract



(i) (ii)

Fig. 13. ZDI obtained from peel extract against: (i) *Bacillus subtilis (ii) E coli*

Peel extract showed ZDI of 38 mm and 26 mm at concentration of 50 μl against *Bacillus subtilis* and *E. coli*, respectively.

Antibacterial activity of pomegranate aril extract

(i) (ii)

Fig 14. ZDI obtained from aril extract against: (i) *Bacillus subtilis* (ii) *E coli*

Aril extract showed ZDI of 32 mm and 20 mm at concentration of 50 μl against *Bacillus subtilis and E. coli*, respectively.

Table 6. ZDI obtained (mm) from prepared extracts against Bacillus subtilis

Extract	Sample conc. (μl)		
	100	**50**	**20**
Immature *Punica granatum* peel	44	38	30
Immature *Punica granatum* aril	40	32	20

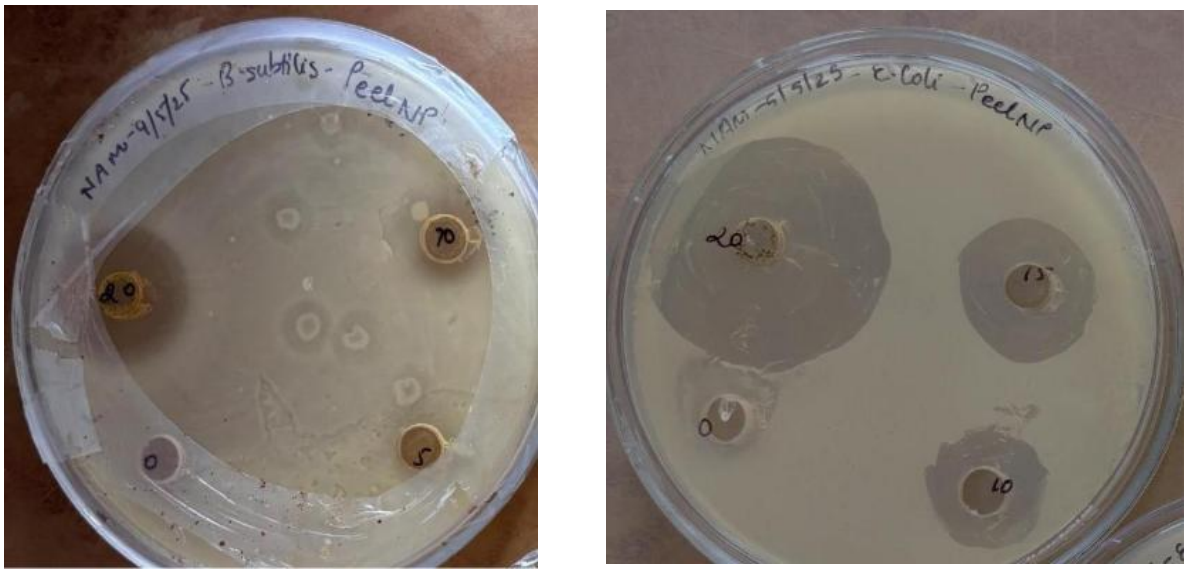
(Here **+** indicates small and improper ZDI formation

Table. 7. ZDI obtained (mm) from prepared extracts against E coli

Extract	Sample conc. (μl)		
	100	**50**	**20**
Immature *Punica granatum* peel	38	26	12
Immature *Punica granatum* aril	34	24	**+**

Antibacterial activity of Extract Nanoparticles

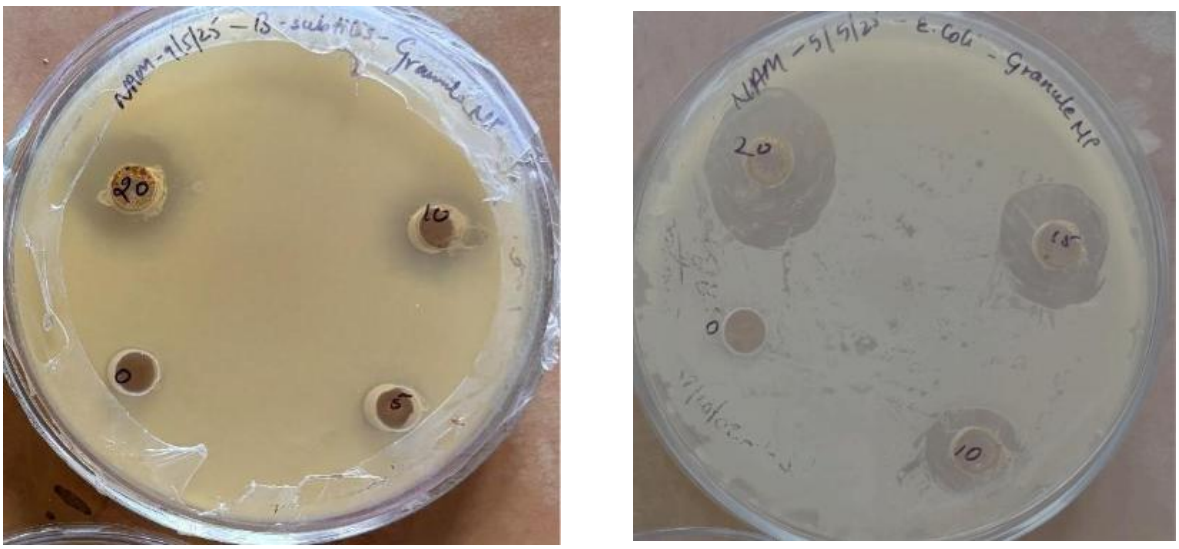
Antibacterial activity of Pomegranate peel Nanoparticles



(i) (ii)

Fig. 15. ZDI obtained from Pomegranate Peel Nanoparticles against:
(i) *Bacillus subtilis*, (ii) *E coli*

Antibacterial activity of Pomegranate Aril Nanoparticles



(i) (ii)

Fig. 16. ZDI obtained from pomegranate aril nanoparticles against:
(i) *Bacillus subtilis*, (ii) *E coli*

Table 8. ZDI obtained (mm) from prepared Nanoparticles against *Bacillus subtilis*

Nanoparticles	Sample conc. (µl)		
	20	**10**	**5**
Immature *Punica granatum* peel	30	20	12
Immature *Punica granatum* aril	38	34	26

Table 9. ZDI obtained (mm) from prepared Nanoparticles against *E coli*

Nanoparticles	Sample conc. (µl)		
	20	**10**	**5**
Immature *Punica granatum* peel	44	30	18
Immature *Punica granatum* aril	36	30	20

Antibacterial activity of Combination of Extracts

(i) (ii)

Fig. 17. ZDI obtained from combination of Peel and Aril extracts of *P granatum* against: (i) *Bacillus subtilis*, (ii) *E coli*

A synergistic effect was shown by the combination of peel and aril extracts of *P. granatum* against both *Bacillus subtilis and E. coli*.

Table. 10. ZDI obtained (mm) from combination of extracts against *B subtilis* and *E coli*

Plant Extract 1	**Plant Extract 2**	**ZDI (mm)**	
		B subtilis	***E coli***
Immature *P granatum* aril	Immature *P granatum* peel	28	20

Antibacterial activity of Combination of Nanoparticles

(i) (ii)

Fig. 18. ZDI obtained from combination of Peel and Aril Nanoparticles of *P granatum* against: (i) *Bacillus subtilis*, (ii) *E. Coli*

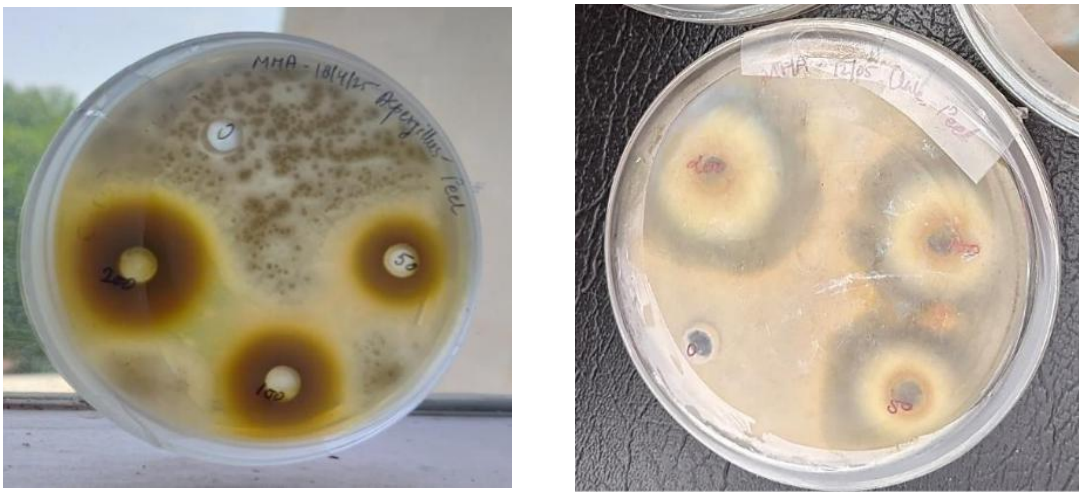
A synergistic effect was shown by the combination of nanoparticles against *Bacillus subtilis and E. coli.*

Table 11. ZDI obtained (mm) against Antibacterial Activity of Combination of plant extract nanoparticles

Plant Nanoparticle 1	**Plant Nanoparticle 2**	**ZDI (mm)**							
		B. subtilis				***E. coli***			
		10 + 10	**10 + 5**	**5 + 10**	**5 + 5**	**10 + 10**	**10 + 5**	**5 + 10**	**5 + 5**
Immature *Punica granatum* aril	Immature *Punica granatum* peel	32	21	22	14	36	22	22	18

Antifungal activity of Pomegranate extracts

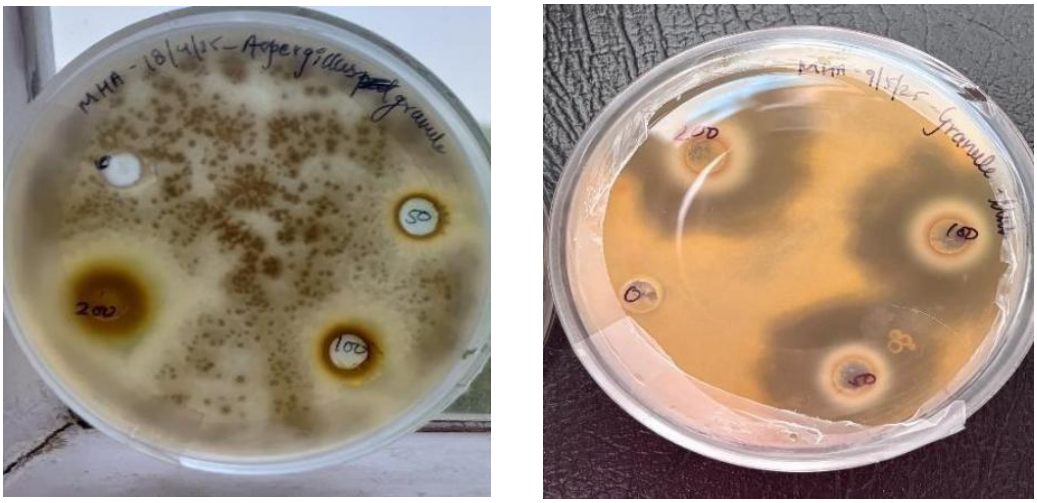
Antifungal activity of Pomegranate peel extract



(i) (ii)

Fig. 19. ZDI obtained from peel extract against: (i) *A niger*, (ii) Unknown fungal strain

Antifungal activity of Pomegranate aril extract



(i) (ii)

Fig. 20. ZDI obtained from aril extract against: (i) *A niger*, (ii) Unknown fungal strain

Table. 12. ZDI obtained (mm) from prepared extracts against *A. niger* fungal strain

Extract	Sample conc. (µl)		
	200	100	50
Immature *Punica granatum* peel	36	28	24
Immature *Punica granatum* aril	20	12	8

Table 13. ZDI obtained (mm) from prepared extracts against unknown fungal strain

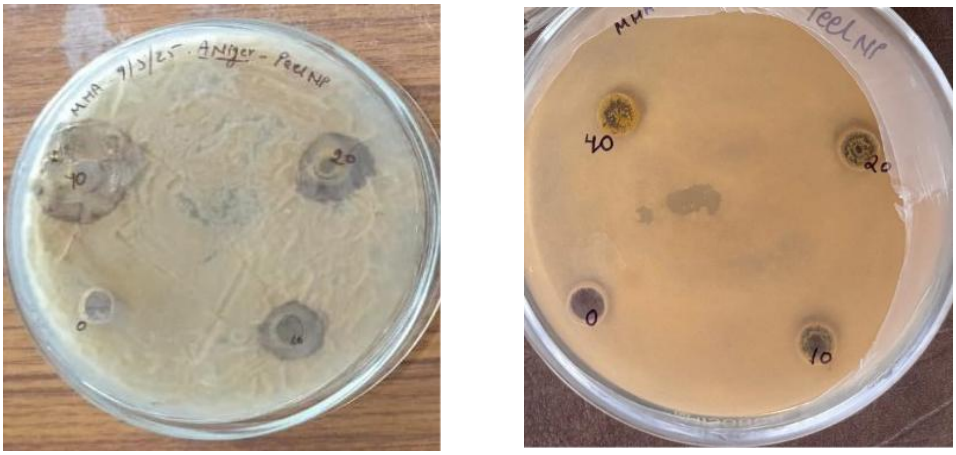
Extract	Sample conc (µl)		
	200	100	50
Immature *Punica granatum* peel	42	38	32
Immature *Punica granatum* aril	38	34	28

Table 14. ZDI (mm) obtained from Plant extract Nanoparticles against *Aspergillus niger*

Nanoparticles	Sample conc. (µl)		
	40	20	10
Immature *Punica granatum* peel	38	18	14
Immature *Punica granatum* aril	22	16	12

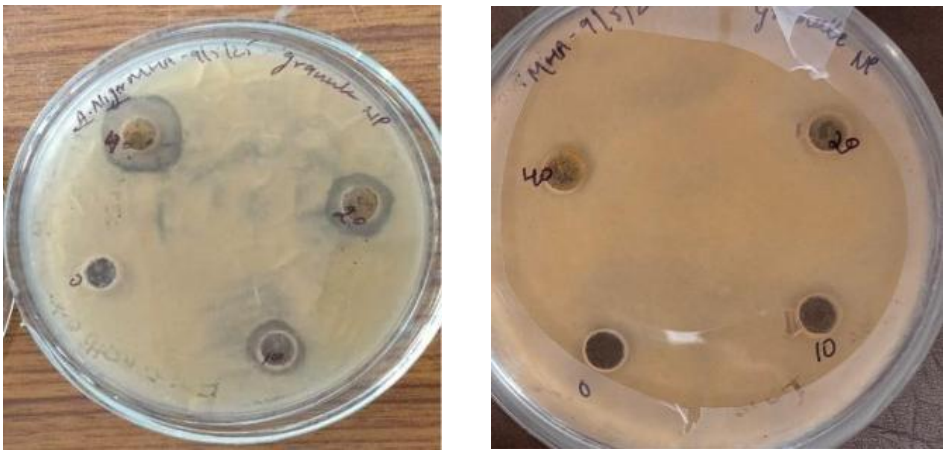
Antifungal activity of Plant extract Nanoparticles

Antifungal activity of Pomegranate peel Nanoparticles



(i) (ii)

Fig. 21. ZDI obtained from peel Nanoparticles of *P. granatum* against: (i) *A. niger*, (ii) Unknown fungal strain*Antifungal activity of Pomegranate aril Nanoparticles*



(i) (ii)

Fig. 22. ZDI obtained from aril Nanoparticles of *P. granatum* against: (i) *A. niger*, (ii) Unknown fungal strain

Table 15. ZDI (mm) obtained from Plant extract Nanoparticles against Unknown fungal strain

Nanoparticles	Sample conc. (µl)		
	40	20	10
Immature *Punica granatum* peel	+		
Immature *Punica granatum* aril	6		

Here measurement of 6 mm indicates No Antimicrobial activity, and '+' indicates small and improper ZDI Formation.

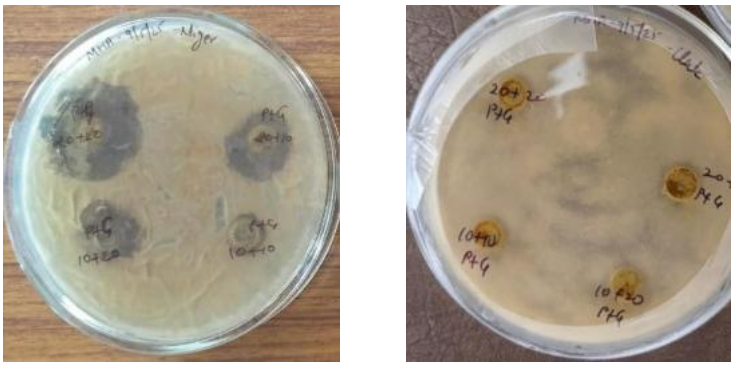
Discussion

This study defines the enhanced antioxidant and antimicrobial activity of zinc oxide nanoparticles mediated by *Punica granatum*, produced through green procedures, and, therefore, highlights the potential of plant nanomaterials, deployed as a viable alternative to traditional antimicrobials. With the global epidemic of antimicrobial resistance, these nanoconstructions offer multi-targeted systems that combine phytochemical bioactivity with nanoscale physicochemical impact, which helps to reduce chances of resistance emergence.

Among the considered samples, the peel extract of *P. granatum* had the strongest antioxidant activity, which can be explained by the fact that its phenolic content was the highest. As is always observed in comparative studies, pomegranate peel is found to contain significantly higher levels of hydrolysable tannin – specifically punica galagin and ellagic acid – compared to other fruit tissues, which explained its superior ability to scavenge free radicals (Singh et al., 2018; Ranjha et al., 2021). The dose-dependent DPPH scavenging presented in this article is

consistent with the current findings indicating that phenolic concentration is indirectly correlated with phenol antioxidant activity in pomegranate peel extracts (Magangana et al., 2020).

Antifungal activity of combination of Nanoparticles



(i) (ii)

Fig. 23. ZDI obtained from combination of Nanoparticles of Peel and aril of *P. granatum* against: (i)*A. niger,* (ii)Unknown fungal strain

Table 16. ZDI (mm) obtained from Antifungal Activity of Combination of Plant Extracts Nanoparticles

Plant Nanoparticle	**Plant Nanoparticle**	***Aspergillus niger***				**Unknown fungal strain**			
1	**2**	**20 +**	**20 +**	**10 +**	**10 +**	**20 +**	**20 +**	**10 +**	**10 +**
		20	**10**	**20**	**10**	**20**	**10**	**20**	**10**
Immature *Punica granatum* aril	Immature *Punica granatum* peel	30	22	20	12	+			

(Here measurement of **+** indicates small and improper ZDI Formation.)

Antibacterial results also support the superiority of peel over the aril extracts whereby there is greater inhibition of Bacillus subtilis and Escherichia coli. Concordant patterns have been observed in pre-studies where peel-derived extracts have always shown greater and stronger antibacterial action than juice or seed extracts as a result of higher tannin and flavonoid contents (Valero-Mendoza et al., 2022). The observed relative increased vulnerability of Gram-positive bacteria is also in line with previous studies, which explain this phenomenon by the cell-wall architecture differences which predispose Gram-positive organisms to polyphenolic compounds (Bukhari et al., 2021).

One of the key results of this study is the significant enhancement of antibacterial potential after the green synthesis of ZnO nanoparticles with the help of the *P. granatum* extracts. Peel-based ZnO nanoparticles also had significantly larger regions of inhibition compared to the crude extracts, which is an example of improved antimicrobial activity at the nanoscale. Similar improvements have been reported in previous studies involving the use of pomegranate as a nanocap to ZnO and silver nanoparticles, which depicts phytochemical capping as an intensifier of membrane disruption and intracellular damage (Chavan, 2022). This enhancement has been greatly attributed to the concerted effect of ZnO-induced reactive oxygen species generation and availability of surface-bound polyphenols that contribute to microbial adhesion and penetration.
These observations are further supported by spectroscopic examination, including FTIR and UV spectroscopy, which entails the presence of hydroxyl and carbonyl functional groups in the stabilisation of ZnO nanoparticles as observed in other ZnO nanoparticles using plants (Huang et al., 2021). The stronger ZnO-related spectral characteristics of peel-derived nanoparticles, compared to aril-derived ones, indicate that the stronger the phenolic density of the nanoparticle, the easier it is formed. A similar trend is observed in comparative studies of green synthesis that uses phenolic-rich plant matrices (Lithi et al., 2025).

The interesting aspect of the work is the evidence of synergistic antibacterial effects in case of using pomegranate extracts or their ZnO nanoparticles in combination with a conventional antibiotic. Parallel synergistic interactions between plant-mediated metal-oxide nanoparticles and antibiotics have been described, in which nanoparticles improve permeability of the membrane and uptake of the antibiotic, after which the effects increase with lower doses (Vanlalveni et al., 2021). These results are also particularly relevant to the control of antimicrobial resistance because they imply a possible method to reduce the use of antibiotics without affecting treatment.

In addition to the antimicrobial aspect of pomegranate as a ZnO nanoparticle, the antioxidant activity of the ZnO nanoparticles mediated by pomegranate highlights the multifunctionality of ZnO nanoparticles. Previously, it has been hypothesised that phytochemically capped ZnO nanoparticles have the potential to selectively induce oxidative stress in the cells of microorganisms but retain antioxidant activity in non-target systems (Hosseini et al., 2022). Such dual functionality makes these nanomaterials more viable in terms of its application in medical and food-related applications.

Overall, the results of the present study are solidly consistent with the available literature on comparative studies, and at the same time, the work can contribute to the current body of knowledge by demonstrating the excellent performance of *Punica granatum* peel-mediated ZnO nanoparticles compared to crude extracts and aril-based systems. The sustainability and translational applicability of the technology are further enhanced by the virtuosity of pomegranate peel as a green nanomaterial raw material.

Conclusion and Future Perspective

The current study clearly shows that nanoparticles of zinc oxide synthesized by the green method using *Punica granatum* extracts have a great antioxidant and antibacterial activity. The results are clearly shown that the most bioactive plant constituent was pomegranate peel fraction which exhibited better free-radical scavenging and antimicrobial activity as compared to the aril extracts and other botanical materials tested. This enhanced performance can be explained by the fact that the phenolic constituents (especially hydrolysable tannins and other polyphenols) are very abundant, and they play an essential role in the biological activity as well as the nucleation of the nanoparticles.

The fact that pomegranate extracts are successful in the biosynthesis of ZnO nanoparticles confirms that plant metabolites can be used as effective reducing and stabilising agents under environmentally friendly conditions. The spectroscopic studies confirmed the creation of the nanoparticles and functional groups that are attached to the surface, which demonstrates successful phytochemical capping. The resulting nanoparticles had significant superiority in antibacterial activity as compared to their respective crude extracts, which further portrays the benefits of the nanoscale formulations to facilitate the level of interaction, surface reactivity, and general biological accessibility of the interaction by the microbes.

The multidomesticity of the ZnO nanoparticles mediated by pomegranate is highlighted by the broad-spectrum antibacterial action against Gram-positive and Gram-negative bacteria. The improved antimicrobial effects can be attributed to the combination of various effects, such as higher contact with microbial membranes, production of oxidative stress, and ZnO synergy with surface-bound phytochemicals. Notably, the synergistic potentiation that is realised when these nanoparticles are used in combination with conventional antibiotics indicates their possible use as antibiotic adjuvants, where reduced doses of drugs are used without compromising the treatment effects and they may also help curb the proliferation of antimicrobial resistance.

Besides the antimicrobial effects, the great antioxidant effect as depicted by pomegranate-mediated ZnO nanoparticles demonstrates the twofold functionality of the compound. The oxidative protection with antimicrobial activity is especially helpful with this kind of application where the redox balance is to be controlled, e.g., biomedical preparations, preservative food systems, and agricultural interventions. The use of pomegranate peel, which is a rich by-product in the agro-industry, also supports the sustainability, economic viability and environmental applicability of this green synthesis approach.

Translational perspective wise, the results present numerous opportunities to research and practise. Future studies must examine the in vivo safety, biocompatibility, as well as the pharmacokinetics, of pomegranate-mediated ZnO nanoparticles. Biomedical and agricultural use that requires approval by the regulatory authorities needs detailed toxicity testing and long-term exposure testing. Moreover, synthesis parameters and scale-up plans will be of key importance to the industrial implementation. Their use can be further enhanced through exploration of development of formulations, surface functionalisation and incorporation into the coating or packaging materials or therapeutic delivery systems. In general, the combination of ancient plant-based materials and the application of nanotechnology is a promising and sustainable approach to deal with current issues in antimicrobial resistance and oxidative-stress-related applications.

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