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Research Article

# Antibacterial Effects of Grape Seed Extract and Zinc Oxide Nanoparticles against Selected Pathogenic Bacteria

**Entissar Razzaq Ibrahim**

*Horticulture Department, College of Agricultural Engineering Sciences, Raparin University, Iraq.*

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**ABSTRACT**

The increasing prevalence of antibiotic-resistant bacteria has stimulated interest in plant-derived antimicrobials and nanomaterials as alternative control strategies. This study investigated the biochemical composition and antibacterial potential of grape seed extract (GSE), alone and in combination with zinc oxide nanoparticles (ZnO-NPs), against selected Gram-positive and Gram-negative pathogenic bacteria. Fourier transform infrared (FTIR) spectroscopy revealed the presence of diverse bioactive functional groups in GSE, including phenolics, flavonoids, tannins, glycosides, aromatic compounds, and fatty acids. These compounds are known to contribute to antimicrobial activity through mechanisms such as membrane disruption, protein denaturation, enzyme inhibition, and metal ion chelation. The identification of carbonyl and ester groups further indicated the presence of phenolic esters, supporting both antimicrobial efficacy and the potential of GSE to act as a stabilizing and capping agent for ZnO-NPs. Antibacterial activity, evaluated using agar diffusion assays, demonstrated that GSE exhibited strong broad-spectrum inhibition, particularly against Gram-negative bacteria, with maximum inhibition zones observed for *Pseudomonas aeruginosa* (35 mm) and *Proteus mirabilis* (32 mm). ZnO-NPs also showed notable antibacterial activity, especially against *Enterococcus faecalis* (22 mm). However, the combined treatment (GSE + ZnO-NPs) displayed moderate effects and did not consistently surpass GSE alone. Statistical analysis confirmed significant differences among treatments for selected bacterial strains. In conclusion, GSE possesses potent antibacterial activity attributable to its rich phytochemical profile, while ZnO-NPs contribute through oxidative and membrane-targeting mechanisms. Although their combination showed limited synergism, GSE alone emerges as a promising natural antimicrobial agent with potential applications in controlling pathogenic bacteria.

*Corresponding Author: Entissar Razzaq Ibrahim*
*Email: entissar.razzaq@uor.edu.krd*
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## Introduction

The emergence and spread of antibiotic resistant bacteria is a serious health problem globally, with immense consequences on the efficacy of traditional antimicrobial interventions. Besides antimicrobial resistance, the widespread application of synthetic antibiotics is commonly linked to adverse side effects and environmental issues, which has further fueled the quest to find safer, sustainable, and biologically derived alternatives. In this regard, natural antimicrobials

derived in the form of plant-based products, especially agro-industrial by-products have become the focus of growing interest. With a well-established antimicrobial and antioxidant activity of their bioactive metabolites, fruit and vegetable residues are promising to be used as novel antimicrobial formulations (Garcia-Montalvo et al., 2022; Karastergiou et al., 2024).

Among plant-based bioactive compounds, grape seed extract (GSE), a by-product of *Vitis vinifera* seed, is one of the most promising natural antimicrobial agents that can be used as a substitute to synthetic products since it contains a high concentration of polyphenolic compounds, such as catechins, epicatechins, and oligomeric proanthocyanidins. These substances have powerful antioxidant properties, as well as broad-spectrum antimicrobial action against Gram-positive and Gram-negative microbes (Georgiev et al., 2014). GSE has been shown to have antimicrobial activity against a broad spectrum of pathogenic microorganisms, such as methicillin-resistant *Staphylococcus aureus* (MRSA), with reductions of about 2.9-4.0 $\log_{10}$ CFU $ml^{-1}$ observed following brief exposure durations (Rodríguez-Pérez et al., 2019; Unusan, 2020). The mechanisms involved are complex and involve interference with bacterial cell membrane, prevention of microbial adhesion and biofilm formation, disrupting enzymes, and binding of vital metal ions. Such multifaceted action mechanisms decrease the chances of developing resistance and increase its therapeutic possibilities.

Recent reports have also established the applicability of GSE in highly developed biomaterials. As an example, Tovar-Carrillo et al. (2024) found out that GSE-enriched hydrogels not only display sustained antibacterial activity but also have good biocompatibility. Likewise, Ivanov and Godjevargova (2024) emphasized the application of grape-based extracts in antimicrobial polymer films in food packaging, which showed their potential in prolonging the shelf life of food and food safety. These results highlight the multifunctionality of GSE as an antimicrobial agent both medically and industrially.

Simultaneously, nanotechnology has paved the way to develop highly effective antimicrobial agents (Nazir et al., 2019; Shahbaz et al., 2023; Jamil et al., 2025; Hussain et al., 2026). Among different types of nanomaterials, zinc oxide nanoparticles (ZnO-NPs) have gained widespread interest in the recent past because of their exceptional physicochemical characteristics such as high surface area, stability, and broad-spectrum antimicrobial activity.

ZnO-NPs have strong antibacterial effects, which can be attributed to a variety of different mechanisms, including the production of reactive oxygen species (ROS), the release of $Zn^{+2}$ ions, and direct contact with microbial cell membranes, leading to membrane damage, intracellular contents leakage, protein denaturation, and DNA disruption (Krishnamoorthy et al., 2014). Recent studies by Reda et al. (2024) also affirm that ZnO-based nanomaterials have a broad-spectrum antimicrobial effect, especially against multidrug-resistant pathogens. Additionally, developments in green nanotechnology have shown that plant extracts can also be used as reducing and stabilizing agents in the synthesis of nanoparticles in an environmentally friendly manner. According to Hussien (2023), biosynthesized ZnO-NPs made with plant extracts have increased antimicrobial activity because of synergistic actions of phytochemicals and nanoparticles. Likewise, Donmez and Keyvan, (2024) revealed that ZnO-NPs prepared by grape seed extract performed better in terms of antibacterial activity than the traditional methods. More recently, Mazhar et al. (2024) found that functionalized ZnO-NPs have high antibacterial properties against resistant bacterial strains, which further prove their potential in overcoming the antimicrobial resistance. Furthermore, novel research has developed nanocomposite systems that incorporate grape seed-based biomolecules and ZnO-NPs, leading to better stability and antimicrobial activity in their applications in food preservation and biomedical areas (Ubaid and Saini, 2025).

Considering the increasing danger of antibiotic resistance, the combination or comparative analysis of natural bioactive compounds and nanomaterials can be a potential and innovative approach (Khan et al., 2021; Jabbar et al., 2022; Mehak et al., 2026). GSE offers a biocompatible, biodegradable and plant-based antimicrobial source with multiple modes of action while ZnO-NPs offer potent and rapid bactericidal effects through physicochemical interactions.

Thus, the objectives of the present study include to determine and compare the antimicrobial activity of grape seed extract (*Vitis vinifera*) and zinc oxide nanoparticles (ZnO-NPs) against selected pathogenic bacteria, as well as to evaluate their prospects as alternative or complementary agents to manage the microbial infection in the medical, food, and agricultural context.

## Materials and Methods

### Bacterial strains and ZnO nanoparticles

In the current study, gram-positive and gram-negative bacterial strains were used to determine antimicrobial activity. Gram positive bacteria used in the evaluation were *Enterococcus faecalis*, *Staphylococcus aureus* and *Streptococci* spp., while Gram negative bacteria consisted of *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. The bacterial strains were procured from the Department of Biotechnology, University of Baghdad from already identified and characterized cultures.

A commercially available colloidal suspension of zinc oxide nanoparticles (ZnO NPs) was acquired from Sigma-Aldrich (USA). The nanoparticles were of the size of about 35 nm on average as per the specifications of the manufacturer and were provided in different concentrations. The nanoparticles provided by the company were already characterized; no further physicochemical characterization using XRD, SEM, or DLS analyses was done before their application in the study.

### Preparation of grape seed extract and bacterial inoculum

The grape seeds (*Vitis vinifera*) were well rinsed in tap water, to remove impurities, and then dried in air. Seeds were then dried in the oven at 40°C over 10 h to remove remaining moisture. Dried seeds thus obtained were pulverized with the help of electric grinder.

To extract the bioactive compounds, 100 g of grape seed powder was added to 360 ml of 95% methanol and continuously shaken at room temperature for 24 h to enhance the release of bioactive compounds. Whatman No. 1 filter paper was used to filter the mixture to get a clear filtrate. A rotary evaporator was used to remove methanol at low pressure at 30℃. The obtained aqueous solution was then dried under the lyophilization condition (freeze-drying) to have a stable fine powder without organic solvent residues, as proposed by Tarek et al. (2025). The dried extract was kept at 4°C until further use.

Bacterial inocula were made by picking off single colonies of freshly grown cultures on the suitable agar medium for the particular bacterial strain. These colonies were cultured in the Mueller-Hinton broth and incubated at 37°C for 18-24 h. Before antimicrobial assays, the turbidity of the bacterial suspension was adjusted to 0.5 McFarland standard (estimated 1.5 × 108 CFU/ml) with the help of sterile 0.85% sodium chloride solution.

### Antibacterial activity assay

The agar well diffusion method was used to determine the antibacterial activities of grape seed extract (GSE) and ZnO nanoparticles singly and in combination, using the protocol of Valgas et al. (2007). Using sterilized cotton gauzes, uniform bacterial suspensions were inoculated on the Mueller-Hinton agar plates.

The 5-mm-diameter wells were punched into the agar under sterilized conditions and to each well, 50 µl of the test solutions were applied. GSE, dissolved in distilled water, was assessed at a concentration of 200 mg/ml whereas ZnO nanoparticles were tested at a dose of 80 mg/ml. For the preparation of combined treatment (1:1, v/v), GSE and ZnO nanoparticle suspensions at equal volumes were mixed to get final concentrations of 100 mg/ml and 40 mg/ml for GSE and ZnO NPs respectively.

Agar Plates were incubated in aerobic conditions for 24 h at 37°C. To evaluate antibacterial activity, the diameter of the inhibition zones (mm) around the well was used.

A modified broth microdilution method in 96-well microplates was used to determine the minimum inhibitory concentration (MIC) of GSE and ZnO nanoparticles, as described by Lutfi et al. (2024). GSE (200 to 0.2 mg/ml) and ZnO nanoparticles (80 to 0.78 mg/ml) were diluted in serial dilution. Plates, after inoculation, were incubated with standardized bacterial suspensions for 18-24 h at 37°C. The lowest concentration which completely suppressed visible bacterial growth was defined as MIC values. All the bioassays were performed in triplicate. To ascertain assay validity, a negative control that contained sterile distilled water was used.

### Statistical analysis

Each bacterial species was individually analyzed statistically. The experiments were conducted in groups of 3 (n = 3), and the average with standard deviation (SD) was used to present the results of diameters of inhibition zones. Differences among treatments (grape seed extract, ZnO nanoparticles, and 1:1 v/v mixture) were determined using one-way ANOVA. In situations where the differences were found to be significant, the Tukey Honestly Significant Difference (HSD) post hoc test was used to make pair wise comparisons. A p-value of less than 0.05 was taken to be significant.

## Results and Discussion

### Fourier transform infrared spectroscopy (FTIR) analysis

The antimicrobial effect of grape seed extract (GSE) is mostly credited to its high concentration of bioactive phytochemicals such as phenolic compounds, tannins, flavonoids, organic acids, aromatic compounds, and

glycosides. These substances easily penetrate into the medium and have an inhibitory effect on the growth of the microbes. The antimicrobial activity of GSE depends on a number of parameters, including its phenolic contents, acidity, and the interactions between its phytochemicals with bacterial cell membranes.

Among the key active mechanisms of significant importance is the capacity of polyphenols and tannins to interact with and destabilize the cell walls and membranes of bacteria, which eventually cause the death of cells. There are also other modes of actions such as protein denaturation, chelation of metal ions, and prevention of the activity of important microbial enzymes (Alhasyimi et al., 2017).

FTIR analysis showed the presence of various functional groups in GSE including phenolics, tannins, flavonoids, aromatic compounds, lipids, and glycosidic constituents as shown in Table 1. These results confirm the suggested antimicrobial and antioxidant effects and modes of action of grape seed extract.

These secondary metabolites found in GSE also allow it to serve as a useful capping and stabilizing agent when used with zinc oxide nanoparticles due to its complex biochemical nature. This kind of interaction improves the antibacterial effectiveness of the combined system. Several strong peaks in the FTIR spectrum can be detected (Figure 1), corresponding to these functional groups, which confirm the presence of high concentrations of flavonoids, polyphenols, tannins, and traces of fatty acids in the sample. These compounds are well known to have high biological activities.

Table 1. FTIR characterization of functional groups in grape seed extract.

| No. of waves/$cm^{-1}$ | structures | Functional Group |
|---|---|---|
| 3334 $cm^{-1}$ | Broad O–H stretching vibration | phenolics, tannins, and flavonoids |
| 3010–2856 $cm^{-1}$ | C–H stretching of aliphatic chains | Indicating the presence of lipids and fatty acids |
| 1744 $cm^{-1}$ | Sharp C=O stretching band | Ester and carbonyl groups are present in phenolic esters and natural oils. |
| 1611–1518 $cm^{-1}$ | Aromatic C=C stretching vibration | flavonoids and proanthocyanidins, the main polyphenolic constituents of grape seeds |
| 1454–1367 $cm^{-1}$ | C–H bending of methyl and methylene | Carbohydrates and lignins |
| 1315–1223 $cm^{-1}$ | – C–O stretching | phenols, tannins, and flavonoid glycosides |
| 1155–1024 $cm^{-1}$ | Strong C–O–C and C–O bands | Indicating polysaccharides, glycosides, organic acids, and ether linkages. |
| 989–718 $cm^{-1}$ | Fingerprint region peaks aromatic ring bending and C–H deformation vibrations | Confirming the presence of aromatic compounds. |

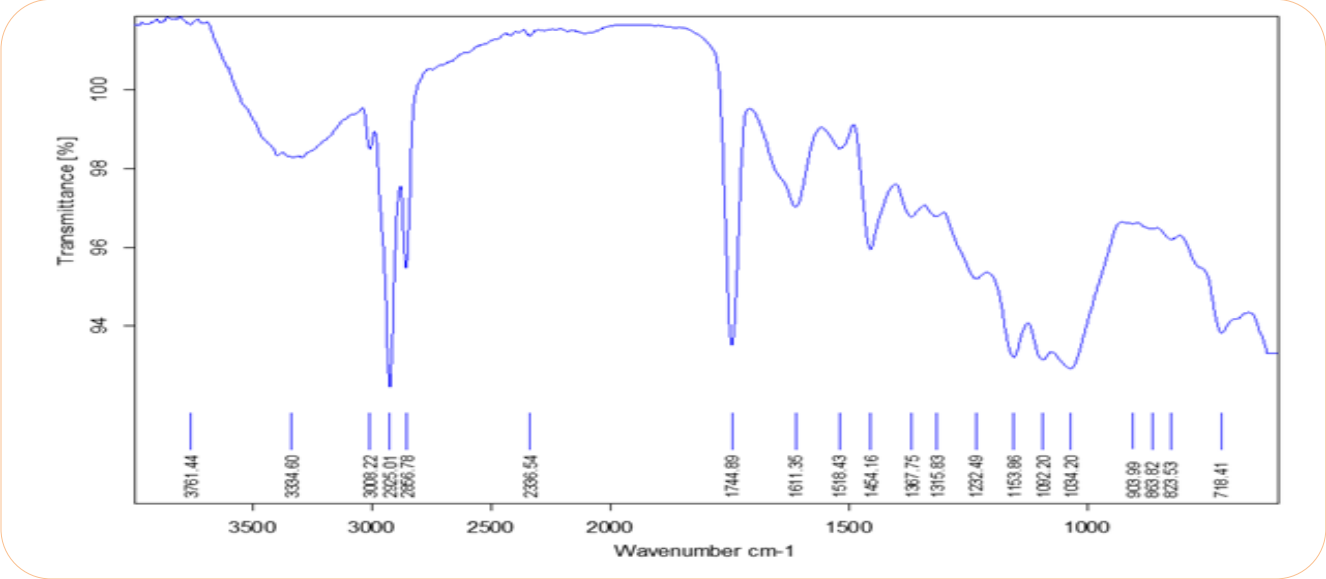



Figure 1. FTIR spectrum of grape seed extract.

It is also documented that polyphenols and tannins can react with microbial cell walls, and disrupt vital cellular processes. The presence of carbonyl and ester functional groups further indicates the presence of bioactive phenolic esters which play a significant role in antibacterial activity. These functional groups can be used to enhance the stabilization of nanoparticles and foster a stronger antimicrobial interaction when used in combination with ZnO nanoparticles.

Thus, according to FTIR results, the main compounds that contribute to the antibacterial properties of GSE are flavonoids, phenolic acids, polyphenols (e.g., proanthocyanidins), fatty acids, tannins, and glycosidic derivatives. These findings are in tandem with previous studies that reported GSE to be a strong natural antimicrobial agent (Yilmaz and Toledo, 2004).

**Effects of grape seed extract on bacterial growth**

The antimicrobial effectiveness of GSE and ZnO NPs was tested against clinically pathogenic Gram-negative and Gram-positive bacteria, i.e. *E. coli*, *P. mirabilis*, *P. aeruginosa*, and *S. aureus*, *Streptococcus* spp., and *E. faecalis*. MICs of GSE and ZnO NPs were determined following agar diffusion assay. Antibacterial activity was measured in the form of zones of inhibition. The results are displayed in Figure 2 and presented in Table 2.

Table 2. Inhibition zone diameters (mm) of grape seed extract (GSE), ZnO nanoparticles (ZnO-NPs), and their combination against tested bacteria (mean ± SD; n = 3).

| Bacterium | GSE (200 mg/mL) Mean ± SD | ZnO-NPs (80 mg/mL) Mean ± SD | Mixture (1:1 v/v) Mean ± SD | One-way ANOVA p |
|---|---|---|---|---|
| *S. aureus* | 17.50 ± 0.50 | 14.83 ± 0.29 | 16.67 ± 0.58 | 0.001214 |
| *Streptococcus* spp. | 14.00 ± 1.00 | 12.50 ± 0.50 | 13.00 ± 0.00 | 0.072338 |
| *E. faecalis* | 20.50 ± 0.50 | 21.67 ± 0.58 | 21.33 ± 0.58 | 0.096282 |
| *E. coli* | 31.00 ± 1.00 | 30.33 ± 0.58 | 29.67 ± 0.58 | 0.171468 |
| *P. mirabilis* | 32.33 ± 0.58 | 30.00 ± 1.00 | 30.67 ± 2.52 | 0.260781 |
| *P. aeruginosa* | 35.00 ± 1.00 | 30.00 ± 1.00 | 32.00 ± 1.73 | 0.009042 |

GSE showed a strong antimicrobial activity against all the tested bacteria especially against *P. aeruginosa* and *P. mirabilis* producing colony diameters of 35 mm and 32 mm respectively. ZnO nanoparticles were also found to have significant antibacterial properties particularly against *E. faecalis* giving a colony diameter of 22 mm. The combination treatment (1:1, v/v) was moderately active but in most of the cases, it was not better than GSE alone treatment.

The HSD post-hoc test (adjusted p-values) showed that in *S. aureus*, both the GSE and the 1:1 mixture had significantly bigger inhibition zones than ZnO-NPs ($p = 0.0011$ and $p = 0.0074$, respectively). On the other hand, no significant difference was found between GSE and the mixture ($p = 0.1565$). GSE was significantly different in case of *P. aeruginosa* compared to ZnO NPs ($p = 0.0076$) but when GSE and the mixture were compared, no significant difference was found.

The 1:1 (v/v) mixture was made by adding equal amounts of GSE (200 mg/ml) and ZnO-NPs (80 mg/ml), which were used to obtain the final concentration of 100 mg/ml of GSE and 40 mg/ml of ZnO-NPs. In general, the patterns of inhibition were species-specific, larger zones were generally observed with Gram-negative bacteria.

GSE was found to show strong broad-spectrum antibacterial effects especially against Gram-negative bacteria (*P. aeruginosa*, *P. mirabilis*, and *E. coli*). This effect can be ascribed to its richness in anthocyanins, flavonoids, polyphenols, and tannins, which are known to destabilize bacterial cell walls, inhibit the synthesis of nucleic acids and precipitate proteins (Daglia, 2012). The strong inhibitory effect of *P. aeruginosa* is of special interest, since this bacterium has inherent resistance mechanisms including efflux pumps and biofilm formation. Extracts rich in polyphenols have been found to counter quorum sensing, thus decreasing bacterial virulence and increasing their vulnerability (Fischer et al., 2014; Nain et al., 2022).

Conversely, ZnO nanoparticles were found to have an antibacterial effect mainly by the following mechanisms: the production of reactive oxygen species (ROS), the peroxidation of lipids of the bacterial cell membrane, release of $Zn^{+2}$, and direct contact with bacterial cell walls (Hadi et al., 2020). The susceptibility of gram-positive bacteria to the metal oxide nanoparticles is because of their thick peptidoglycan layer, which enables the bacteria to interact with the nanoparticles of ZnO by electrostatic forces. This is why a very strong inhibitory effect is observed against *E. faecalis* (Ibraheem and Ibraheem, 2015).

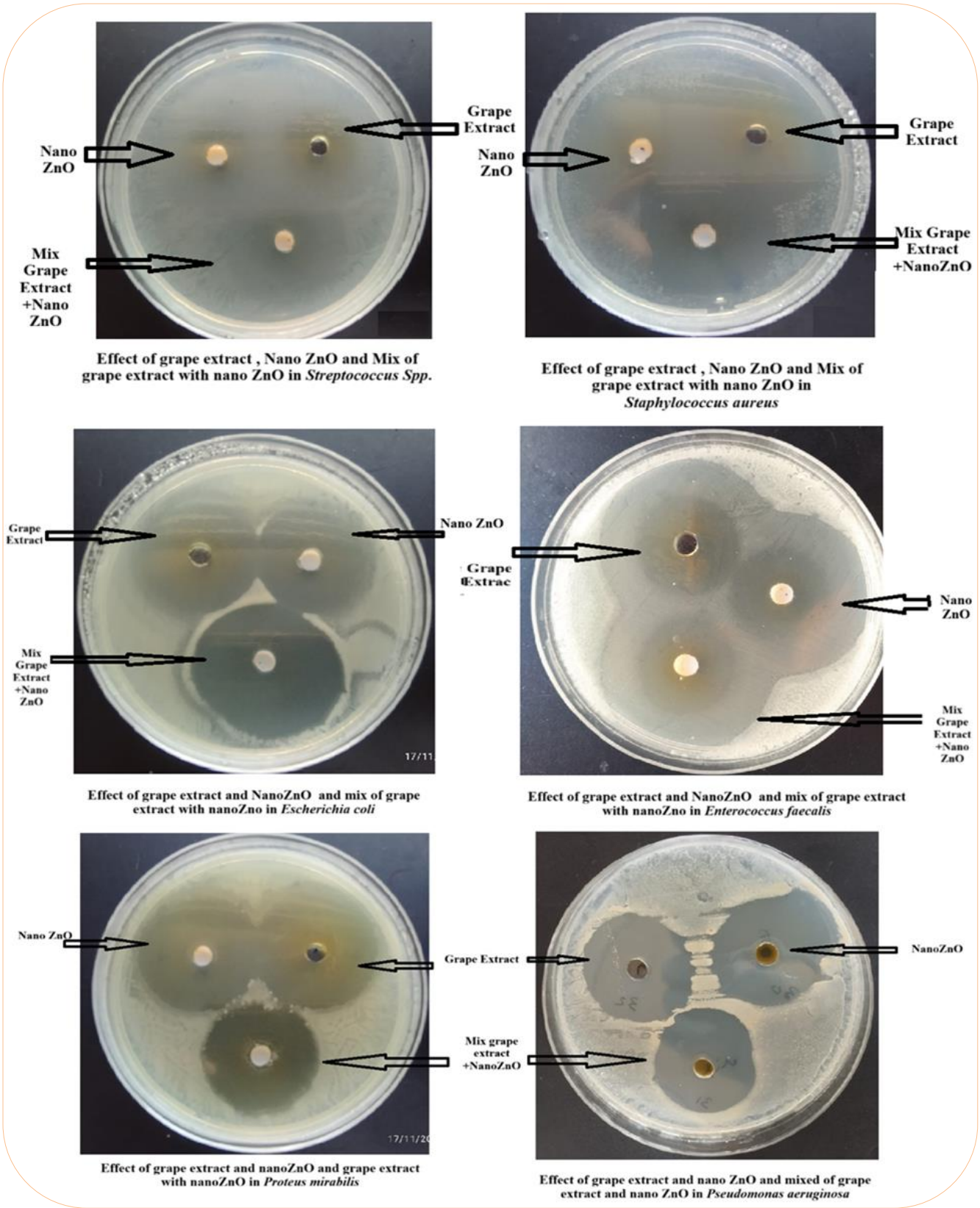



Figure 2. Comparative antibacterial activity of grape seed extract, zinc oxide nanoparticles, and their combination against selected pathogenic bacteria.

The synergistic effect was not clearly observed in the combined treatment (1:1, v/v) but only showed moderate antibacterial activity. This might be attributed to the dilution of active phytochemicals, possible agglomeration of nanoparticles when organic compounds are present, and the antioxidant activity of phenolics that could decrease the production of ROS by ZnO nanoparticles. However, the blend retained significant inhibitory properties, especially against *P. mirabilis* and *P. aeruginosa*, which is indicative of partial rather than actual synergy (Iravani, 2011).

Gram-positive bacteria (*S. aureus*, *Streptococcus* spp. and *E. faecalis*) had inconsistent yet typically high responses to all treatments. Nevertheless, Gram-negative bacteria were more susceptible to GSE, probably because of the possibility of polyphenols to penetrate outer membrane and disrupt biofilm development, as well as the capacity of metal nanoparticles to affect membrane proteins. These results are in agreement with the earlier research which has shown strong antibacterial effects of phenolic-rich extracts against *E. coli* and *P. aeruginosa* (Boban et al., 2010).

## Conclusion

The FTIR study revealed that the grape seed extract contains a large amount of bioactive functional groups, mainly flavonoids, phenolics, tannins, and glycosidic compounds, which mainly contribute to its high antimicrobial potential. These phytochemicals have antibacterial effects by exerting various modes of actions such as cell membrane disruption, protein denaturation, enzyme inhibition and disrupting the microbial metabolic pathways. The antimicrobial testing showed that GSE has broad-spectrum activity and strong antimicrobial efficacy against Gram-negative bacteria, including *P. aeruginosa* and *P. mirabilis*. Even though ZnO nanoparticles also showed strong antibacterial activity, especially against *E. faecalis*, the combined use (GSE + ZnO NPs) failed to demonstrate clear synergism and was mostly less effective when GSE was applied alone. In general, the results indicate that GSE is a powerful natural antimicrobial agent, the effectiveness of which is highly associated with its phytochemical structure.

## Recommendations

The optimization of the ratio and formulation of GSE and ZnO nanoparticles to experience the real synergistic effects and prevent the potential antagonistic effects should be the focus of the future research. More sophisticated characterization methods and mechanistic investigations are suggested to gain a deeper insight into the interaction of the nanoparticles with the phytochemicals. Moreover, there is need of *in vivo* assessments and toxicity testing to confirm applicability of GSE-based formulations in practice and safety. The development of nano-encapsulation or targeted delivery systems could also contribute to the stability and antimicrobial effectiveness of GSE. Lastly, the possible employment of GSE as a green substitute of synthetic antimicrobials in medical, agricultural and food preservation systems should be further studied.

## Author's Contributions

The author conceived and designed the study, performed the laboratory experiments, collected and analyzed the data, supervised the research process, and drafted and revised the manuscript. The author reviewed and approved the final version of the manuscript.

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This research did not receive any grant from funding agencies.

## Conflict of Interest

The authors declare no conflict of interest.

## Sustainable Development Goals Targeted

SDG 3: Good Health and Well-being
SDG 9: Industry, Innovation and Infrastructure
SDG 12: Responsible Consumption and Production

## Policy Addressed

1. Antimicrobial Resistance (AMR) Control Policy
2. Food Safety and Preservation Policy

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