DOI: 10.69946/ojms/2026.04.01.04, pp(27-39)

The Effectiveness of Banana Peels and Copper Oxide Nanoparticles in Inhibiting Staphylococcus aureus and Klebsiella pneumoniae

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Abstract

Objective: This study aimed to evaluate the antibacterial activity of banana peel extract (BPE) and green-synthesized copper oxide nanoparticles (CuO-NPs) against *Staphylococcus aureus* and *Klebsiella pneumoniae*, and to investigate their synergistic effects.
Methods: Banana peel extract was prepared using Soxhlet extraction, while CuO nanoparticles were synthesized using plant-derived bioactive compounds as natural reducing and stabilizing agents. The synthesized nanoparticles were characterized using UV–Vis and FTIR spectroscopy. GC–MS analysis was performed to identify the major bioactive compounds in the extract. Antibacterial activity and synergistic interactions were assessed using minimum inhibitory concentration (MIC) and checkerboard assays.
Results: UV–Vis analysis confirmed CuO nanoparticle formation with an absorption peak at 971 nm, while FTIR analysis revealed functional groups involved in nanoparticle stabilization. GC–MS analysis identified several antioxidant and reducing compounds in banana peel extract. The combined use of BPE and CuO-NPs showed enhanced antibacterial activity, particularly against *S. aureus*, with a strong synergistic effect (FICI = 0.375). A lower antibacterial effect was observed against *K. pneumoniae*.
Conclusion: The findings suggest that banana peel-mediated green synthesis of CuO nanoparticles is an eco-friendly and promising strategy for future antimicrobial and biomedical applications.

Keywords: Copper oxide nanoparticles; banana peel extract; green synthesis; antibacterial activity; synergistic effect

1. Introduction

Bacterial resistance to antibiotics has emerged as one of the most significant health determinants in this modern society, a challenge to the efficacy of modern medicine and the quality of health care treatment for the community- and hospital-dwelling population worldwide [1]. The worldwide report finds that drug-resistant infections caused 1.27 million deaths annually in 2019 and is expected to increase to 10 million in 2050 if new, highly aggressive, and potent therapeutic options are not established [2].

As a result, resistance towards antibiotics, which bacteria have developed well-established resistance mechanisms, has expanded significantly in recent years towards the discovery of novel-based antimicrobial antibiotics with several-task mechanisms of action [3]. Banana peels and other

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agricultural wastes are a common yet under-utilized source of bioactive compounds. Banana peels contain many phenolic compounds and antioxidants, which have a wide range of biological applications [4]. These bioactive ingredients have been shown to possess prominent antioxidant and antimicrobial properties, making banana peel extract (BPE) a possible natural candidate for further research on novel therapeutic agents [5].

Concurrently, nanotechnology has changed the field of antimicrobial research, via metallic nanoparticles, with their favorable properties due to nanometer size (less than 100 nm) and high surface space to volume ratio. These features improved cellular interactions at the microbial level and enhanced antimicrobial activity [6]. Among these materials, copper oxide nanoparticles (CuO-NPs) have been widely studied in recent years owing to their superior and fast bactericidal activity compared with other products on the market. Recent reports suggested that CuO-NPs exert their antibacterial effects via several concurrent mechanisms, including lessening the likelihood of the development of resistance, as opposed to agents that are aimed at a singular cellular route. These mechanisms consist of adhesion of a microorganism to the bacterial cell surface, membrane disruption, copper ion release, and induction of reactive oxygen species (ROS), leading to oxidative stress on cellular proteins, lipids, and/or genetic material [7].

Oxidative stress induced by ROS is considered one of the most well-established mechanisms of antibacterial action obtained from metallic nanoparticles [8]. Reactive oxygen species may induce widespread oxidative damage in bacterial cells by inducing lipid peroxidation of their membranes and causing membrane destabilization and loss of structure. Furthermore, ROS can promote protein oxidation and therefore, modify protein structural and functional aspects, as well as oxidative damage in DNA that can disrupt the replication, transcription and cell division of cells [9]. Despite the notable progress in the investigation of plant extracts and metallic nanoparticles as antibacterial agents, comparisons between these two strategies regarding their cellular mechanisms of action are so far confined, especially with respect to the structural damage patterns in bacterial cells. Plant extracts such as banana peel extract for example, are described as having a multifaceted composition containing bioactive compounds that may act synergistically.

In comparison, metallic nanoparticles, such as copper oxide (CuO) nanoparticles, exert both physical and chemical mechanisms, such as surface interactions, ion release, and significant induction of oxidative stress. This fundamental difference in antibacterial mechanisms may lead to differences in their damage pattern, the rate, and types of cell-like damage against the bacterial cells [10]. Thus, the current study investigates the antibacterial activity of banana peel extract (BPE) and copper oxide nanoparticles (CuO-NPs) by observing their inhibition on bacterial growth and studying cellular and structural related mechanisms. This comparative This comparative study aims to elucidate cellular damage patterns between a multi-component natural extract and a multi-mechanistic nanomaterial to facilitate the advancement of an effective and durable antibacterial approach.

2. Materials and Methods

2.1 Sample Collection

Banana fruits were collected from the central market of Baqubah District, Diyala Governorate, Iraq, between June and October, for the purpose of obtaining banana peels used in this study.

DOI: 10.69946/ojms/2026.04.01.04, pp(27-39)

2.2. Sample Preparation

The banana peels were washed with distilled sterile water to remove dust and surface impurities and then air-dried at room temperature (25 ± 2 °C) in a dark environment to prevent photodegradation of bioactive compounds. The peels were then cut into small pieces after drying and ground by an electric mill to get a fine banana peel powder. This procedure not only increases the surface area but also provides a better solvent interface, as shown in Fig. 1, to facilitate the extraction process.

Fig. 1 Drying and grinding stages of banana peels.

2.3. Preparation of the Extraction Using a Soxhlet Apparatus

The samples were weighed using a precise electronic balance. Subsequently, 50 g of the dried sample was placed into a thimble made of Whatman No. 1 filter paper and securely sealed. The extraction process was carried out using a Soxhlet apparatus with 400 mL of methanol as the extraction solvent for a duration of 6 h. After extraction, the obtained extract was allowed to cool and then concentrated using a rotary evaporator for 7 min to obtain a final volume of 50 mL, as illustrated in Fig. 2.

Fig. 2 Soxhlet Extraction of Banana Peels and Concentration of the Extract Using a Rotary Evaporator

Each extract was evaluated individually in combination with the nanoparticle using the checkerboard assay. For each extract, a 7 × 7 concentration matrix was prepared (7 concentrations of the extract × 7 concentrations of the nanoparticle), starting from 1024 µg/mL and followed by two-fold serial dilutions (1024, 512, 256, 128, 64, 32, and 16 µg/mL), as illustrated in Figs. 3 and 4.

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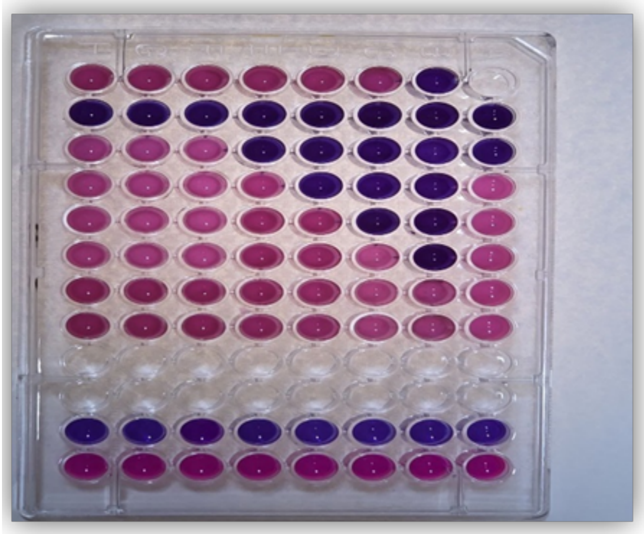

Fig. 3 Antibacterial activity of banana peel extracts and copper oxide nanoparticles against Klebsiella pneumoniae using the checkerboard assay

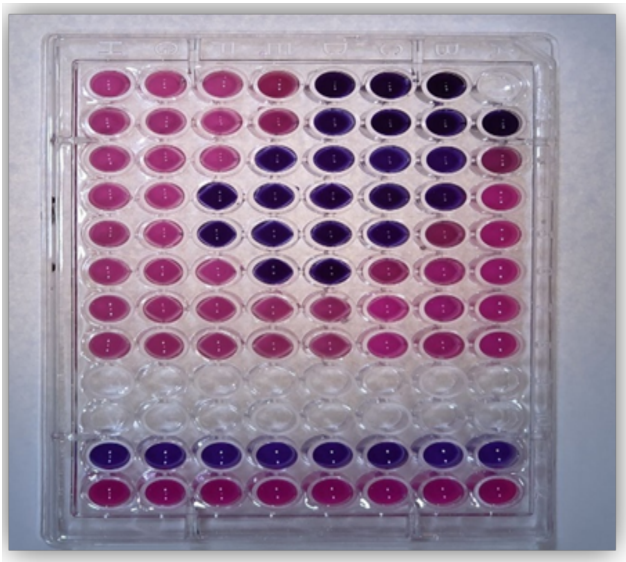

Fig. 4 Antibacterial activity of banana peel extract and copper oxide nanoparticles against Staphylococcus aureus using the checkerboard assay

2.4. Chemical Analysis

Chemical analyses were performed to determine the chemical composition of banana peel extract and copper oxide nanoparticles. GC–MS analysis was performed using an Agilent 7890B gas chromatograph coupled with a 5977A mass selective detector (Agilent Technologies, USA). at the Laboratories Department, Basra Oil Company, Iraq. The oven temperature program was set to begin at 40 °C and maintained for 5 min, followed by a temperature ramp of 10 °C/min to 300 °C. Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. An injection volume of 1 µL was applied for each extract. Compound identification was performed using the Mass Hunter software in conjunction with the National Institute of Standards and Technology (NIST) mass spectral library.

2.5. Experimental Design

Each extract was evaluated individually and in combination with the nanoparticles using a checkerboard assay. For each extract, a 7 × 7 concentration matrix was prepared (7 concentrations of

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the extract × 7 concentrations of the nanoparticle), starting at 1024 µg/mL and employing two-fold serial dilutions as follows: 1024, 512, 256, 128, 64, 32, and 16 µg/mL, as illustrated in Figs. 3 and 4

2.6. Preparation of Stock and Working Solutions

2.6.1. Preparation of the Extract Stock Solution

Each extract stock solution was prepared at a concentration equivalent to twice the highest final concentration required for the assay (2×), i.e., 2048 µg/mL, using an appropriate solvent. When necessary, solutions were sterilized or clarified, for example, by filtration in the case of clear solutions.

2.6.2. Preparation of the Stock Nanomaterial Solution

The nanomaterial was prepared at a concentration twice the highest final concentration (2×), i.e., 2048 µg/mL Ultrasonic mixing was used for a short period to minimize clumping and agglomeration.

2.7. Preparation of the Bacterial Inoculum

1. From a freshly prepared overnight bacterial culture, a bacterial suspension was prepared in saline or PBS.
2. The turbidity was adjusted to a standard value (typically approximately 0.5 McFarland standard), then diluted in broth to obtain a ready-to-use inoculum that yielded a final concentration in the well of approximately 5×10^5 CFU/mL.

2.8. Plate Layout

Extract (Factor 1): the highest concentration was placed in well B1, followed by serial dilution down to H1. Nanomaterial (Factor 2): the highest concentration was placed in well A2, then decreasing horizontally to A8. The interaction matrix ranged from wells B2 to H8. The remaining columns can be designated for controls. Concentrations used were 1024, 512, 256, 128, 64, 32, and 16 µg/mL as shown in Table 5.

Table 1: Checkerboard assay layout for evaluating the interaction between banana peel extract (E) and copper oxide nanoparticles (N).

Row\Col	1	2	3	4	5	6	7	8	NC	PC
A		N1024	N512	N256	N128	N64	N32	N16	■	·
B	E1024	E1024 + N1024	E1024 + N512	E1024 + N256	E1024 + N128	E1024 + N64	E1024 + N32	E1024 + N16	■	·
C	E512	E512 + N1024	E512 + N512	E512 + N256	E512 + N128	E512 + N64	E512 + N32	E512 + N16	■	·
D	E256	E256 + N1024	E256 + N512	E256 + N256	E256 + N128	E256 + N64	E256 + N32	E256 + N16	■	·
E	E128	E128 + N1024	E128 + N512	E128 + N256	E128 + N128	E128 + N64	E128 + N32	E128 + N16	■	·
F	E64	E64 + N1024	E64 + N512	E64 + N256	E64 + N128	E64 + N64	E64 + N32	E64 + N16	■	·

DOI: 10.69946/ojms/2026.04.01.04, pp(27-39)

G	E32	E32 + N1024	E32 + N512	E32 + N256	E32 + N128	E32 + N64	E32 + N32	E32 + N16	■	·
H	E16	E16 + N1024	E16 + N512	E16 + N256	E16 + N128	E16 + N64	E16 + N32	E16 + N16	■	·

2.9. Steps for preparing a checkerboard test per board/per extract

1. Distribute the broth in the wells so that the final volumes are equal (typically 100–200 µL per well).
2. Prepare nanomaterial dilutions across row A from column 2 to 8 (dual dilution).
3. Prepare extract dilutions across column 1 from row B to H (dual dilution).
4. Construct the blending matrix (B2–H8) by combining the extract dilution (vertically) with the nanomaterial dilution (horizontally), maintaining the desired final concentrations.
5. Add the bacterial inoculum to all test wells (extract alone, nanomaterial alone, and blending wells).
6. Include controls (preferably in unused columns 11–12):

- Growth control: inoculum + broth without agents (column 12).
- Sterilization control: broth only without inoculum (column 11). 7. Cover the plate to reduce evaporation, and then incubate at 35–37°C for 18–24 hours.

2.10. Reading Results and Interpreting Outcomes

- Visual Interpretation: Turbidity/sediment indicates growth, while clarity indicates inhibition.
- Rizazol: Color change indicates metabolic activity (growth).

2.11. Data Analysis

Calculation of FIC and FICI

- FIC of Extract = MIC of Extract in Mixture / MIC of Extract alone
- FIC of Nanomaterial = MIC of Nanomaterial in Mixture / MIC of Nanomaterial alone
- FICI = FIC of Extract + FIC of Nanomaterial

3. Results and Discussion

3.1 Results

3.1.1 Characterization of copper oxide nanoparticles (CuO NPs)

3.1.1.1 Ultraviolet-Vis (UV-Vis) analysis

Table 2 showed that the ultraviolet–visible (UV–Vis) spectrum of the biosynthesized copper oxide nanoparticles exhibited an absorption peak at a wavelength of 971 nm, with an extension in the absorption range at 1086 nm, as shown in Fig. 5. High absorbance values were also recorded at certain wavelengths.

Table 2: Recorded absorption peaks of copper oxide nanoparticles (CuO NPs) using ultraviolet–visible (UV–Vis) spectroscopy

Technique	Peak / Range	Interpretation
UV–Vis	971 nm	Formation of CuO nanoparticles
UV–Vis	1086 nm	Extended nanoscale absorption

DOI: 10.69946/ojms/2026.04.01.04, pp(27-39)

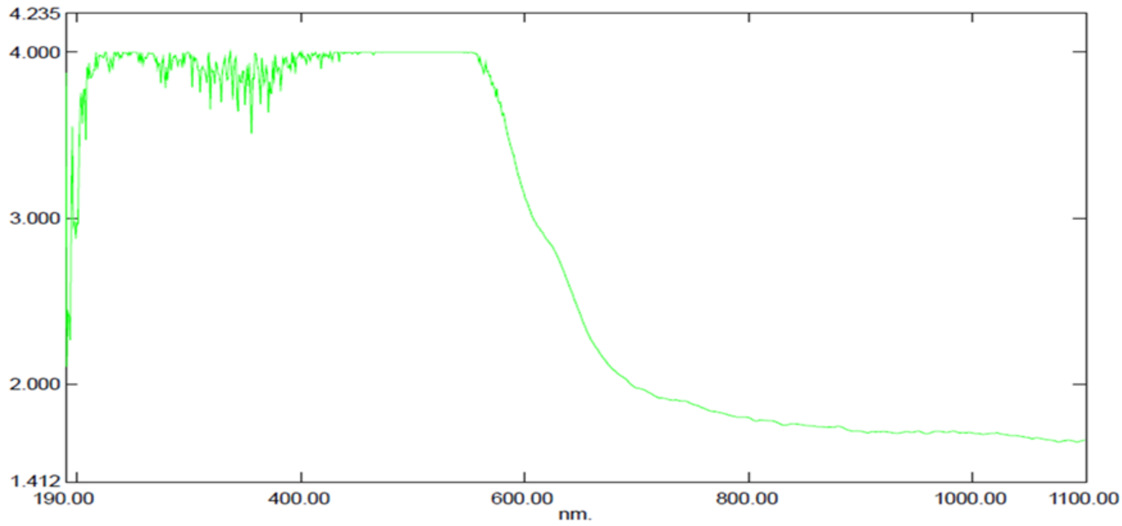


Fig. 5 UV-Vis spectrum of copper oxide nanoparticles (CuO NPs) prepared using banana peel extract

3.1.1.2. Infrared Spectroscopy (FTIR) Analysis

Table 3 showed that the Fourier transform infrared (FTIR) spectrum recorded absorption bands at 3300–3500 cm^{-1}, 2920–2850 cm^{-1}, and 1600–1700 cm^{-1}, in addition to a band at 1000–1100 cm^{-1}, as shown in Fig. 6.

Table 3: Recorded absorption bands in the Fourier transform infrared (FTIR) spectrum of copper oxide nanoparticles (CuO NPs)

Technique	Peak / Range	Interpretation
FTIR	3300–3500 cm^{-1}	O–H stretching vibration
FTIR	2920–2850 cm^{-1}	C–H stretching vibration
FTIR	1600–1700 cm^{-1}	C=O stretching vibration
FTIR	1000–1100 cm^{-1}	C–O / C–O–C stretching vibration

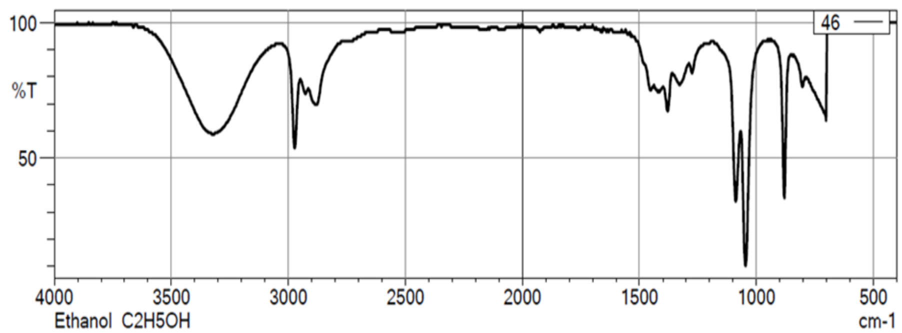


Fig. 6 FTIR spectrum of banana peel extract used in the biosynthesis of copper oxide nanoparticles.

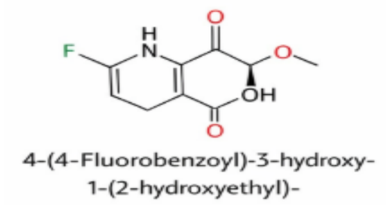
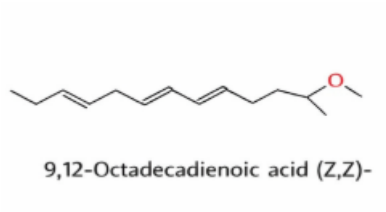
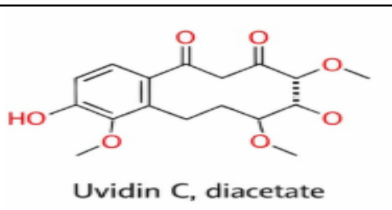
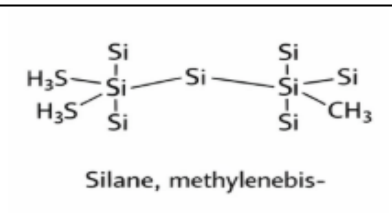
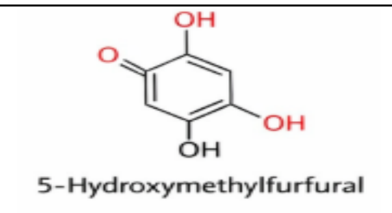
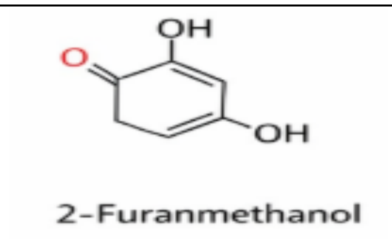
3.1.2 GC–MS analysis results of banana peel extract

Table 4 showed the presence of 33 chemical compounds with different retention times in the banana peel extract, as shown in Fig. 7. The results also showed that among the compounds with the highest area percentages were: 4H-Pyran-4-one with a retention time of 12.559 min and an area percentage of 23.63%, 9,12-Octadecadienoic acid with a retention time of 23.666 min and an area percentage of

DOI: 10.69946/ojms/2026.04.01.04, pp(27-39)

16.71%, and 4-(4-Fluorobenzoyl)-3-hydroxy-1-(2-hydroxyethyl)- with a retention time of 33.414 min and an area percentage of 17.50%.

Table 4: Major chemical compounds identified in banana peel extract using GC–MS analysis.

compound name	RT (min)	Area %	Chemical composition
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	12.559	23.63	
4-(4-Fluorobenzoyl)-3-hydroxy-1-(2-hydroxyethyl)-	33.414	17.50	
9,12-Octadecadienoic acid (Z,Z)-	23.666	16.71	
Uvidin C, diacetate	33.238	5.68	
Silane, methylenebis-	5.567	5.47	
5-Hydroxymethylfurfural	13.761	3.54	
2-Furanmethanol	6.626	3.30	

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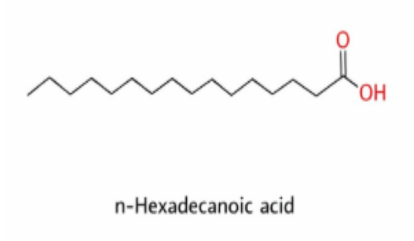
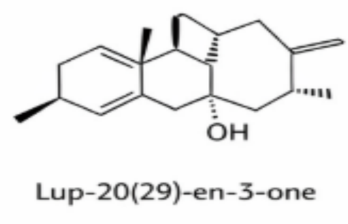
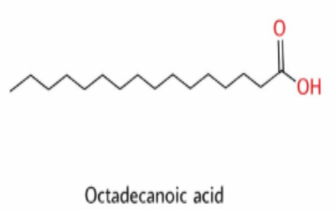
compound name	RT (min)	Area %	Chemical composition
n-Hexadecanoic acid	21.962	2.99	
Lup-20(29)-en-3-one	33.645	1.55	
Octadecanoic acid	23.829	1.41	

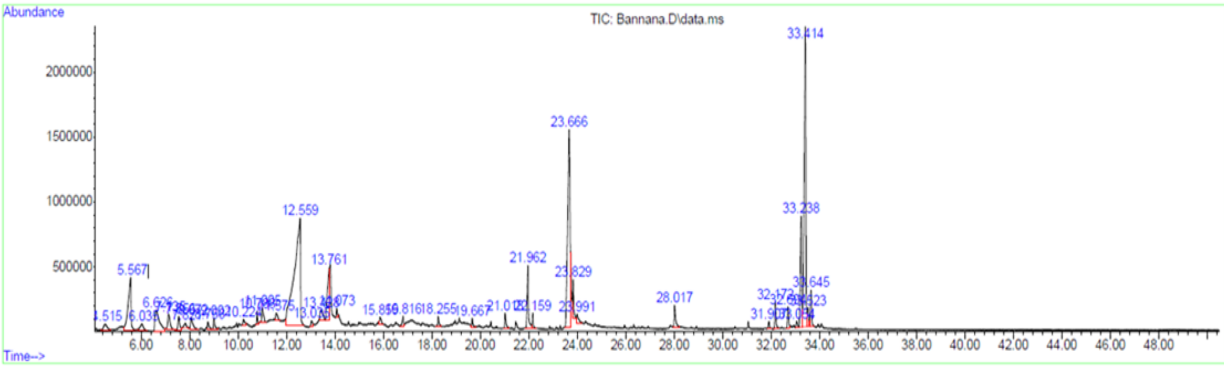


Fig. 7 Total ion chromatogram (TIC) for GC–MS analysis of banana peel extract.

3.1.3 Minimum Inhibitory Concentration (MIC)

Table 5 presents the minimum inhibitory concentration (MIC) values of banana peel extract and copper oxide nanoparticles (CuO NPs) against the tested bacterial strains. The results showed that the MIC value against S. aureus was 256 µg/mL for the extract and 1024 µg/mL for CuO NPs. Similarly, the MIC value against K. pneumoniae was 1024 µg/mL for the extract and 512 µg/mL for CuO NPs.

Table 6: MIC values for individual factors (µg/mL)

bacteria	MIC extract	MIC CuO NPs
S. aureus	256	1024
K. pneumoniae	1024	512

DOI: 10.69946/ojms/2026.04.01.04, pp(27-39)

3.1.4 Fractional Inhibitory Concentration Index (FICI)

Table 7 presents the fractional inhibitory concentration index (FICI) values for the tested bacterial strains. The results showed that the FICI value for S. aureus was 0.375, whereas the FICI value for K. pneumoniae was 0.75.

Table 7: FICI results and synergistic effect

bacteria	MIC(N)	MIC(E)	FICI	Interpretation
S. aureus	1024	256	0.375	Synergy
K. pneumoniae	512	1024	0.75	Lack of Synergy

3.2 Discussion

Green synthesis using banana peel extract successfully produced copper oxide nanoparticles, as confirmed by UV–Vis spectroscopy. The absorption peak at 971 nm can be considered direct evidence of the formation of nanoparticles and supports previous reports on the green synthesis of metal nanoparticles [11]. Thus, the location and intensity of this peak corroborate the inherent electronic characteristics of copper oxide nanostructures, indicating the formation of relatively stable nanoparticles [12]. Furthermore, by the broadening of the absorption band, there is a fairly homogeneous particle size distribution, which is desirable for possible future applications. Previous studies on plant-based synthesis have reported unique optical properties and improved size uniformity in the nanoparticles, so that they may benefit better in antibacterial properties [13].

Compared with conventional chemical synthesis methods, the biological approach applied herein has shown superior stability and homogeneity [14] of the nanoparticles produced. Additionally, the optical features demonstrated are reliable for nanoparticle creation that have been reported as good and constitute an early indication of structure stability [15]. FTIR analysis provided further evidence of hydroxyl (–OH) and carbonyl (C=O) functional groups. A typical phenolic, alcoholic, and carbohydrate molecule-based functional group is responsible for a two-fold action on the synthesized materials; they provide a reducing agent for copper ions and stabilize nanoparticles to prevent their aggregation [16].

Previous studies have shown, in earlier reports [17], that an organic capping layer has been formed around the nanoparticle in this study. It should be emphasized that the biochemical nature of the plant extract significantly influences the efficiency of the green synthesis [18]. The organic phase on the surface of the nanoparticle not only leads to structural stability, but also may increase the biological and functional characteristics of the prepared nanoparticles [19]. Banana peel extract (PEC) is characterized by the diversity of phytochemicals as indicated by GC–MS analysis. It was mainly composed of 4H-Pyran-4-one (23.63%) and 9,12-Octadecadienoic acid (16.71%). These compounds have known reducing and antioxidative properties [20]. Such chemically diverse constituents probably contributed to the efficient reduction and stabilization processes, which may be why the newly synthesized NPs had good quality and yield properties.

In reference [21], the authors showed that the phytochemical profile of plant extracts directly affected the size and stability of NPs. The diversity shown by banana peel extract may have facilitated a synergistic effect of reduction mechanisms and stabilization mechanisms, with a positive impact on the quality and application potential of the nanoparticles [22]. Based on antibacterial

DOI: 10.69946/ojms/2026.04.01.04, pp(27-39)

activity, the microorganisms studied showed some significant differences between the investigated microorganisms. Banana peel extract was more potent against S. aureus (MIC = 256 µg/mL) compared to a higher concentration (1024 µg/mL) being needed to antagonize K. pneumoniae. Such a difference could be due to the structural distinction between Gram-positive and Gram-negative bacteria. In contrast, CuO nanoparticles exhibited greater activity against K. pneumoniae (512 µg/mL) compared with S.aureus (1024 µg/mL), revealing that the action of metallic nanoparticles is different from phytochemical compounds [23]. When the extract was co-administered with the nanoparticles, a significant combination synergism against

S. aureus was reported with FICI = 0.375, thereby indicating that combined treatment not only improves antibacterial action on the sole components but also enhances the activity of bioactive compounds. The increase in such performance may be attributed to phenolic compounds acting as antagonists against bacterial defence systems, which further enables penetration of nanoparticles into the host cell as well as intracellular activity. In contrast, the synergistic effect against K. pneumoniae was not as potent (FICI = 0.75), and this could be attributed to the outer membrane containing lipopolysaccharide, which restricts permeability [24]. CuO nanoparticles primarily induce the generation of reactive oxygen species (ROS), resulting in oxidative stress among bacterial cells [25], acting as a potential antibacterial mechanism. This damage includes damaging the cellular membranes as well as proteins and nucleic acids, ultimately leading to bacterial cell death.

4. Conclusion

The copper oxide nanoparticles (CuO NPs) synthesized during this study were successfully synthesized using banana peel extract via an environmentally friendly green synthesis approach Nanoparticle formation was confirmed by the appearance of a characteristic absorption peak at 971 nm with the help of UV–Vis analysis and plant-derived functional groups that contributed to the reduction of copper ions and stabilization of the nanoparticles as confirmed by FTIR analysis Bioactive compounds of banana peel extract enhance biosynthesis efficiency and biological activities of CuO nanoparticles characterized by GC–MS analysis. The results also revealed antibacterial performance and a definite synergistic action against Staphylococcus aureus while limited effectiveness was found against Klebsiella pneumoniae which could be explained possibly by different cell wall structure. These results show that the green synthesis of CuO nanoparticles from plant waste constitutes a feasible and environmentally friendly way to proceed with green CuO nanomaterial with the potential for medical and environmental purposes. More experimental investigations are indicated for biocompatibility and widespread application.

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