

COMPARISON OF EFFICACY OF Ag-Cu NANOPARTICLE CONJUGATED THYMOQUINONE DRUG AND DRUG ALONE AGAINST ENVIRONMENTAL BACTERIAL ISOLATES

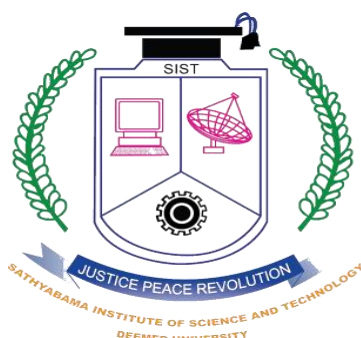
PROJECT WORK

*Submitted in partial fulfillment of the requirements for the award
of Bachelor of Science in Biotechnology*

By

PRAVEEN KUMAR S (Reg.No.41770040)

RAMCHERMA JAYASOORYA R (Reg.No.41770023)



SCHOOL OF BIO AND CHEMICAL ENGINEERING
DEPARTMENT OF BIOTECHNOLOGY

SATHYABAMA

INSITITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)

CATEGORY – 1 UNIVERSITY BY UGC

Accredited with Grade “A++” by NAAC | 12B Status by UGC | Approved by AICTE

JEPPIAR NAGAR , RAJIV GANDHI SALAI, CHENNAI 600119 APRIL - 2024



SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

CATEGORY-I UNIVERSITY BY UGC

Accredited "A++" Grade by NAAC | 12B Status by UGC | Approved by AICTE

www.sathyabama.ac.in

DEPARTMENT OF BIOTECHNOLOGY

BONAFIDE CERTIFICATE

This is to certify that this Project report is the bonafide work of PRAVEEN KUMAR S (Reg.No.41770040) and RAMCHERMA JAYASOORYA R (Reg.No.41770023) who carried out the project entitled "COMPARISON OF EFFICACY OF Ag-Cu NANOPARTICLE CONJUGATED THYMOQUINONE DRUG AND DRUG ALONE AGAINST ENVIRONMENTAL BACTERIAL ISOLATES" under our supervision from January-2024 to April-2024.

Internal Guide

Dr. J. THEBORAL

Head of the Department

Dr. V. RAMESH KUMAR., Ph.D

Submitted for Viva voce Examination held on 26/04/2024

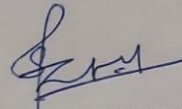
Internal Examiner
External Examiner

DECLARATION

We are PRAVEEN KUMAR S (Reg.No.41770040) and RAMCHERMA JAYASOORYA R (Reg.No.41770023) hereby declare that the Project Report entitled "COMPARISON OF EFFICACY OF Ag-Cu NANOPARTICLE CONJUGATED THYMOQUINONE DRUG AND DRUG ALONE AGAINST ENVIRONMENTAL BACTERIAL ISOLATES" done by us under the guidance of Dr. J.THEBORAL, is submitted in partial fulfilment of the requirements for the award of Bachelor of Science in Biotechnology.

DATE: 26/04/2024

PLACE: CHENNAI



SIGNATURE OF THE CANDIDATES

ACKNOWLEDGEMENT

We pleased to acknowledge our sincere thanks to Board of Management of **Sathyabama Institute of Science and Technology** and for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

We convey our thanks to **Dr. REKHA CHAKRAVARTHI**, Dean, School of Science and Humanities and **Dr. V. RAMESH KUMAR**, M.Phil Ph.D, Head, Department of **Biotechnology** for providing me necessary support and details at the right time during the progressive reviews.

We would like to express our sincere and deep sense of gratitude to our internal guide Project Internal guide, **Dr. J. THEBORAL Assistant Professor, Department of Biotechnology** for her valuable guidance, suggestions and constant encouragement paved way for the successful completion of our project work.

We would like to express our sincere and deep sense of gratitude to our external guide **Dr. M. MURUGADOSS, Associate Professor (Research)**, Centre for nanoscience and nanotechnology, International Research Centre, Sathyabama institute of science and technology for his valuable guidance, suggestions and constant encouragement paved way for the successful completion of our project work.

We wish to express our thanks to our **parents** and all **Teaching and Non-teaching staff** members of the Department of Biotechnology who were helpful in many ways for the completion of the project.

COMPARISON OF EFFICACY OF Ag-Cu NANOPARTICLE CONJUGATED TQ DRUG AND DRUG ALONE AGAINST ENVIRONMENTAL BACTERIAL ISOLATES

ABSTRACT

This study is aimed to evaluate the efficacy of a nanoparticle-conjugated drug, specifically an Ag-Cu nanoparticle conjugated TQ drug, in comparison to the drug alone against environmental bacterial isolates. The main objective was to determine whether the nanoparticle in the drug enhances its efficacy. The methods employed included a plating assay, UV spectrophotometer testing in a test tube method using 100 isolates, and a growth curve comparison. The results indicated that the conjugated drug with the nanoparticle was more efficient than the TQ drug alone. In conclusion, the presence of Ag-Cu increased the efficacy of TQ against tested bacterial isolates, suggesting potential applications in enhancing the effectiveness of antibacterial treatments. Further research is recommended to explore this promising avenue.

CHAPTER NO	TITLE	PAGE NO
	ABSTRACT	i
	TABLE OF CONTENTS	ii
	LIST OF ABBREVIATIONS	iv
	LIST OF FIGURES	v
	LIST OF TABLES	vi
1	INTRODUCTION	1
	1.1 Nanoparticles and their antibacterial activity	1
	1.2 TQ	2
	1.2.1 Limitation of TQ	4
	1.3 Ag-Cu and its antibacterial activity	
	1.4 Effects of Conjugated TQ with Ag-Cu	4
	1.5 Drug enhancement through nanoparticle conjugation	5
2	LITERATURE REVIEW	6
3	AIM AND SCOPE OF THE PRESENT INVESTIGATION	10

4	MATERIAL AND METHODS	
	4.1 Synthesis of Ag-Cu nanoparticles	11
	4.2 Characterization Techniques	12
	4.3 Preparation of TQ solution	13
	4.4 Conjugation of Nanoparticle and TQ	13
	4.5 Collection of environmental isolates	14
	4.6 Isolation of environmental bacterial cultures	15
	4.7 Growth curve analysis	
	4.8 Plating assay	16
	4.9 Test tube method of UV-spectroscopy to compare efficacy	17
		18
5	RESULT AND DISCUSSION	
	5.1 Chemically synthesized Ag-Cu nanoparticle	20
	5.2 Characterization of Ag-Cu by TEM	21
	5.3 Prepared thymoquinone solution	22
	5.4 Conjugated TQ with Ag-Cu nanoparticle	23
	5.5 Characterization of TQ—Ag-Cu by SEM	23
	5.6 Growth curve Analysis	24
	5.7 Plating assay	25
	5.8 Test tube method of UV spectroscopy to compare efficacy	29
		29
6	SUMMARY AND CONCLUSION	37
	REFERENCES	40

LIST OF ABBREVIATIONS

- TQ – Thymoquinone
- NP - Nanoparticle
- TQ—Ag-Cu – Conjugated thymoquinone with Ag-Cu
- SEM –Scanning electron microscope
- TEM –Transmission electron microscope
- LB -Luria-bertani broth

LIST OF FIGURES

Figure No	Figure name	Page No.
4.1	Chemical synthesis of Ag-Cu nanoparticles	12
4.2	Conjugation of TQ with Ag-Cu nanoparticles	14
4.3	Environmental bacterial isolates	15
4.4	Preparation of nutrient media	16
4.5	Growth curve analysis of drug only and conjugated drug	17
4.6	Bacterial culture	19
4.7	Observation of drug alone and conjugated drug by UV-SPEC absorbance at 600nm	19

5.1	Synthesized binary metallic nanoparticle	20
5.2	HR-TEM result of Ag-Cu Binary metallic nanoparticle	21
5.3	Thymoquinone solution	22
5.4	Conjugated TQ with Ag-Cu nanoparticles	23
5.5	SEM image of Ag-Cu conjugated thymoquinone drug	23
5.6	Graph represents the control of EV101	24
5.7	Comparison of efficacy by growth curve drug alone and conjugated drug	25
5.8	Comparison of efficacy by plating assay	28
5.9	Drug only added with bacterial isolates	29
5.10	Drug + NP added with bacterial isolates	29

LIST OF TABLES

TABLE NO.	CONTENT	Page No
5.1	Zone of inhibition of EV101	27
5.2	Zone of inhibition of EV102	28
5.3	Zone of inhibition of EV103	28
5.4	UV-SPEC absorbance readings	29

CHAPTER – 1

1. INTRODUCTION

The increasing prevalence of antibiotic-resistant bacterial strains has necessitated the exploration novel antibacterial agents. Nanoparticles, due to their unique properties, have emerged as a promising avenue in this regard. Among various nanoparticles, silver (Ag) and copper (Cu) nanoparticles have shown significant antibacterial properties. Thymoquinone (TQ), an active ingredient of *Nigella sativa*, has been reported to possess a wide range of medicinal properties, including antibacterial activity. However, its full potential has not been realized due to its limited solubility and bio availability. Recent advancements in nanotechnology have provided a potential solution to these limitations. The conjugation of TQ with Ag-Cu nanoparticles of could enhance its antibacterial efficacy by improving its solubility and bioavailability. This study, titled “Comparison of Efficacy of Ag-Cu Nanoparticle Conjugated TQ Drug and Drug Alone Against Environmental Bacterial Isolates,” aims to investigate this possibility. The research will compare the antibacterial efficacy of the nanoparticle-conjugated TQ drug and the TQ drug alone, providing valuable insights into the potential of nanoparticle-enhanced drugs in combating bacterial infections.

1.1 NANOPARTICLES AND THEIR ANTIBACTERIAL ACTIVITY

Nanoparticles (NPs) have been extensively studied for their antibacterial properties. Nanoparticles are increasingly used to target bacteria as an alternative to antibiotics. Nanotechnology may be particularly advantageous in treating bacterial infections. Examples include the utilization of NPs in antibacterial coatings for implantable devices and medicinal materials to prevent infection and promote wound healing, in antibiotic delivery systems to treat disease, in bacterial detection systems to generate microbial diagnostics, and in antibacterial vaccines to control bacterial infections (Wang *et al.*, 2017). Antibacterial activity of metal and metal oxide nanoparticles (NPs) has been extensively reported. The microbes are eliminated either by microbicidal effects of the NPs, such as release of free metal ions culminating in cell membrane damage, DNA interactions or free radical generation, or by microbiostatic effects coupled with killing potentiated by the host's immune system (Hemeg *et al.*, 2017). Their small size and large surface area to volume ratio allow them to interact closely with microbial membranes, leading to increased antibacterial activity. Silver and copper nanoparticles, in particular, have shown significant potential. Silver nanoparticles have been found to damage bacterial cell membranes and DNA, leading to cell death. Copper nanoparticles, on the other hand, have been shown to generate reactive oxygen species (ROS), which are toxic to bacterial cells.

1.2 THYMOQUINONE

Thymoquinone (TQ), (2-Isopropyl-5-methyl-[1,4]benzoquinone, $C_{10}H_{12}O_2$), a monoterpenoid isolated from the seeds of *Nigella sativa*, has been shown to have antioxidant, analgesic, anticonvulsant effects and anti-bacterial activity and has emerged as a promising natural compound with broad-spectrum antimicrobial activity against a wide range of bacteria, including both Gram-positive and Gram-negative strains (Mohammad *et al.*, 2023). TQ has been shown to exhibit antimicrobial activity through multiple mechanisms, including disruption of bacterial cell membranes, inhibition of bacterial cell wall synthesis, and interference with bacterial DNA replication and transcription. In addition, quite a few

pharmacological effects of *N. sativa* seed, its oil, various extracts and active components have been identified to include immune stimulation, anti-inflammation, hypoglycemic, antihypertensive, antiasthmatic, antimicrobial, antiparasitic, antioxidant and anticancer effects(Randhawa *et al.*, 2011).TQ caused an increase in intracellular reactive oxygen species level, a decrease in intracellular adenosine triphosphate (ATP) concentration, a change in the intracellular protein, damage to cell membrane integrity and changes in cell morphology(Wang *et al.*, 2022).

1.2.1 LIMITATION OF TQ

TQ has been used in traditional medicine for centuries and has been found to have anti-inflammatory, antioxidant, and anticancer properties. Recent studies have also shown that TQ has antibacterial activity against a range of bacterial strains alsoTQ has poor bioavailability and is hydrophobic, prohibiting clinical trials with TQ alone. However, its use in clinical settings has been limited due to its poor solubility in water and low bioavailability.Dose-Dependent Effects: Like many bioactive compounds, thymoquinone exhibits dose-dependent effects. While low doses may exert beneficial effects, high doses could potentially lead to adverse effects or toxicity. Therefore, careful consideration of the dose and administration route is essential to optimize its therapeutic benefits while minimizing the risk of adverse reactions.

Potential Interactions: Thymoquinone may interact with certain medications or substances, leading to undesirable effects or alterations in their pharmacokinetics. Patients using thymoquinone supplements should be cautious and consult with healthcare professionals, especially if they are taking medications for chronic conditions.

Limited Clinical Evidence: Although preclinical studies have shown promising results regarding the pharmacological activities of thymoquinone, clinical evidence supporting its efficacy and safety in humans is still relatively limited. Further well-designed clinical trials are needed to validate its therapeutic potential and establish appropriate dosing regimens for specific indications.

1.3 Ag-Cu AND THEIR ANTIBACTERIAL TREATMENT

As the field of nanomedicine emerges, there is a lag in research surrounding the topic of nanoparticle (NP) toxicity, particularly concerned with mechanisms of action. The continuous emergence of bacterial resistance has challenged the research community to develop novel antibiotic agents. Metal NPs are among the most promising of these because they show strong antibacterial activity (Slavin *et al.*, 2017). Silver nanoparticles are well known potent antimicrobial agents. Although significant progresses have been achieved on the elucidation of antimicrobial mechanism of silver nanoparticles, the exact mechanism of action is still not completely known so synthesis of binary nanoparticle is necessary. Ag-Cu nanoparticles (NPs) are well-known for their antimicrobial properties. They have different antimicrobial capacities and cytotoxicities. Factors such as size, shape, and surface treatment influence their antimicrobial activities. The biomedical application of antimicrobial Ag and Cu NPs involves coating onto substrates, including textiles, polymers, ceramics, and metals.

Because Ag and Cu are immiscible, synthetic Ag-Cu nanoalloys have different microstructures, which impact their antimicrobial effects. When mixed, the combination of Ag and Cu NPs act synergistically, offering substantially enhanced antimicrobial behavior. However, when alloyed in Ag-Cu NPs, the antimicrobial behavior is even more enhanced. The reason for this enhancement is unclear.

1.4 EFFECTS OF CONJUGATED TQ WITH Ag-Cu

Nanotechnology offers a potential solution to these limitations. By conjugating TQ with nanoparticles, its solubility and bioavailability could be significantly improved. This could allow for higher concentrations of the drug to reach the target site, potentially enhancing its antibacterial activity. Despite the promising antimicrobial activity of TQ, its application is often limited by its moderate efficacy and narrow spectrum of activity against certain bacterial strains. To overcome these limitations and enhance the antimicrobial potency of TQ, recent research efforts have focused on the conjugation of TQ with metal nanoparticles. Among the various metal nanoparticles investigated, silver-copper (Ag-Cu) nanoparticles have emerged as particularly promising candidates due to their synergistic

antimicrobial effects. The incorporation of Ag-Cu nanoparticles is believed to augment the antimicrobial activity of TQ by leveraging the unique physicochemical properties of the nanoparticles, such as their increased surface area and enhanced reactivity. These properties facilitate greater interaction with bacterial cells, leading to increased efficacy in disrupting bacterial membranes and intracellular processes, thereby enhancing the overall antimicrobial activity of TQ.

1.5 DRUG ENHANCEMENT THROUGH NANOPARTICLE CONJUGATION

The field of nanomedicine has opened up new avenues for drug enhancement. One such approach is the conjugation of drugs with nanoparticles, which can improve the drug's efficacy, solubility, and bioavailability. We are particularly interested in the conjugation of TQ with Ag-Cu nanoparticles. TQ, an active ingredient of *Nigella sativa*, has been reported to possess a wide range of medicinal properties, including antibacterial activity. However, its full potential has not been realized due to its limited solubility and bioavailability.

Athauda.,(2023) suggested provide the idea to enhance the activity of drug with conjugation with NPs.Nanoparticle conjugation offers a potential solution to these limitations. By conjugating TQ with Ag-Cu nanoparticles, its solubility and bioavailability could be significantly improved. This could allow for higher concentrations of the drug to reach the target site, potentially enhancing its antibacterial activity.Moreover, Ag-Cu nanoparticles themselves have shown significant antibacterial properties. Therefore, the conjugation of TQ with Ag-Cu nanoparticles not only improves the drug's properties but also adds the antibacterial properties of the nanoparticles. This dual action could potentially lead to a more effective antibacterial treatment.In conclusion, nanoparticle conjugation represents a promising approach for drug enhancement. This thesis aims to investigate this possibility by comparing the efficacy of an Ag-Cu nanoparticle conjugated TQ drug and the TQ drug alone against environmental bacterial isolates. The findings of this study could have significant implications for the development of new antibacterial agents.

CHAPTER – 2

2. LITERATURE REVIEW

2.1 ANTIBACTERIAL ACTIVITY AGAINST ENVIRONMENTAL ISOLATES

A total of 24 bacterial isolates were obtained from tissue homogenates of freshwater mussels collected at a site directly downstream from Kokomo, Indiana. Of these, 17 were *Enterobacter* spp., five were *Escherichia coli*, one was *Pseudomonas aeruginosa*, and one was *Klebsiella pneumoniae*. All isolates were resistant to three or more antimicrobial drug classes (Ramhalo *et al.*,2018).

Yasin *et al.*,(2020) found that that the presence of these bacteria might cause a health risk/hazard to human beings and may cause disease to susceptible individuals, especially immune-compromised consumers.

Amoako and Somboro, (2019) stated that most active antimicrobials were vancomycin (93.5%) followed by amikacin (71.5%) and gentamicin (46%). The overall antibiotic susceptibility was as follows: 36% ciprofloxacin, 19% imipenem, 20% trimethoprim-sulfamethoxazole, 20.5% ceftazidime, and 12% ceftriaxone against the environmental isolates from Ghana.

Lopes *et al.*,(2019) determined the antibiotic-resistance patterns and antibiotic-resistance genes of bacteria recovered from freshwater,suggest that human activity has an impact on the local microbiota, in which strains recovered from zones under anthropic influence were considerably more resistant than those collected from remote regions.

2.2 THYMOQUINONE

Ahmed (2013), suggested that a wide spectrum of *Nigella sativa* pharmacological actions have been explored which may include antidiabetic, anticancer, immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepato-protective, renal protective, gastro-protective, antioxidant properties, also revealed that most of the therapeutic properties of this plant are due to the presence of thymoquinone which is major bioactive component of the essential oil.

Almatroodi *et al.*, (2020), highlighted that the anti-cancer drugs have limitations in efficacy and also causes adverse side effects on normal cells. The combination of anti-cancer drugs and thymoquinone improves the efficacy of drugs which is evident by decrease resistance to drugs and regulation of various cell signaling pathways. Moreover, combination of anti-cancer drugs as well as thymoquinone shows synergistic effect on killing of cancer cells and cells viability. Thus, TM, in combination with anti-cancer drugs, can be a good strategy in the management of various types of cancer.

2.3 NANOPARTICLES AND THEIR ANTIMICROBIAL ACTIVITIES

Ragunath (2017), suggested that apart from exhibiting antimicrobial properties, MeO-NPs also serve as carriers of drugs, thus barely providing a chance for micro-organisms to develop resistance. These immense multiple properties exhibited by MeO-NPs will have an impact on the treatment of deadly infectious diseases.

The use of silver nanoparticles is also important, as several pathogenic bacteria have developed resistance against various antibiotics. Hence, silver nanoparticles have emerged up with diverse medical applications ranging from silver based dressings, silver coated medicinal devices, such as nanogels, nanolotions, etc (Rai 2019)

Wang *et al.*, (2017) suggested that the multiple simultaneous mechanisms of action against microbes would require multiple simultaneous gene mutations in the same bacterial cell for antibacterial resistance to develop; therefore, it is difficult for bacterial cells to become resistant to NPs.

2.4 AG-CU AND THEIR ANTIBACTERIAL ACTIVITY

Ismail *et al.*, (2018) stated that in comparison to monometallic Ag and Cu nanoparticles, the alloyed Ag–Cu nanoparticles showed a high catalytic performance and the resultant catalytic activity was dependant on the Ag to Cu ratio. This enhanced catalytic activity should be related to the electronic interaction between Ag and Cu nanoparticles formed due to the intimate contact between them.

Zhou (2015) found that Ag-based bimetallic nanoalloys, such as Ag-Cu and Ag-Fe, have enhanced antibacterial efficacy and reduced Ag consumption. The study also found a strong correlation between the minimum death concentration (MDC) and minimum inhibitory concentrations (MICs), implying that the mechanisms of both antibacterial efficacy and cytotoxicity are similar.

Xinzhen *et al.*, (2021) discussed the biomedical application of antimicrobial Ag and Cu nanoparticles. It mentioned that synthetic Ag-Cu nanoalloys have different microstructures, which impact their antimicrobial effects.

Kim (2013) highlighted that silver (Ag) and copper (Cu) nanoparticles have shown great potential in a variety of applications due to their excellent electrical and thermal properties

2.5 CONJUGATED DRUG WITH NANOPARTICLE

Athauda, (2023), focused on nanoparticles in conjugation with the aminoglycoside antibiotic gentamicin. The nanoparticle conjugates were found to efficiently kill different bacterial strains, which was mainly dependent on the size, stability, and concentration of the nanoparticles.

CHAPTER - 3

3. AIM AND SCOPE OF THE PRESENT INVESTIGATION

AIM

To compare the efficacy of an Ag-Cu nanoparticle conjugated Thymoquinone drug and the thymoquinone drug alone against environmental bacterial isolates.

OBJECTIVES

- Standardization of Synthesis of Ag-Cu binary nanoparticles by chemical reduction method.
- Characterization of Ag-Cu by High Resolution TEM
- Conjugation of Ag-Cu nanoparticles and thymoquinone drug
- Characterization of conjugated Ag-Cu with thymoquinone drug by SEM
- Comparing the efficacy of Ag-Cu nanoparticle conjugated thymoquinone and thymoquinone alone against environmental isolates by Plating method.
- Comparing the efficacy by growth curve Analysis of Ag-Cu nanoparticle conjugated thymoquinone and thymoquinone
- Test tube method of UV-spectroscopy analysis of efficacy difference between Ag-Cu conjugated thymoquinone and thymoquinone drug alone.

CHAPTER - 4

4. MATERIALS AND METHODS

4.1 SYNTHESIS OF Ag-Cu NANOPARTICLES

4.1.1 Preparation of Stock Solutions

0.1 M of silver nitrate (AgNO_3) and 0.1 M of copper(II) sulfate (CuSO_4) separately dissolved in deionized water to prepare stock solutions.

4.1.2 Preparation of Sodium Hydroxide Solution

0.1 M sodium hydroxide (NaOH) solution prepared by dissolving the appropriate amount of NaOH in deionized water.

The silver nitrate and copper(II) sulfate stock solutions poured into a beaker in the desired molar ratio (e.g., $\text{Ag}:\text{Cu} = 1:1$) under continuous stirring.

By using the sodium hydroxide solution to adjust the pH of the solution to approximately 11-12. This alkaline environment is crucial for the reduction process.

A reducing agent was added, either sodium borohydride (NaBH_4) or hydrazine hydrate (N_2H_4), dropwise to the stirred solution at room temperature. The color of the solution will change to dark brown or black, indicating the formation of Ag-Cu nanoparticles.

4.1.3 Stirring and Reaction Time

The solution was stirred continuously for several hours to ensure complete reduction and stabilization of the nanoparticles.

4.1.4 Centrifugation and Washing

Centrifugation of solution is performed at high speed to separate the nanoparticles from the solution.

The collected nanoparticles were washed several times with deionized water to remove any unreacted chemicals and stabilize the nanoparticles.

4.1.5 Drying and Storage

The washed nanoparticles were dried at room temperature to obtain a dry powder or colloidal solution. The synthesized Ag-Cu nanoparticles stored in a dry and airtight container for further characterization and use.



Fig: 4.1 : Chemical synthesis of Ag-Cu nanoparticles

4.2 CHARACTERIZATION TECHNIQUES

- Scanning Electron Microscopy (SEM): To analyze the morphology and size distribution of the synthesized Ag-Cu nanoparticles.
- Transmission Electron Microscopy (TEM): To investigate the internal structure and confirm the morphology of the nanoparticles at the nanoscale.
- Fourier-Transform Infrared Spectroscopy (FTIR): To identify the chemical composition and confirm the presence of functional groups or potential surface modifications on the nanoparticles.

The chemical reduction method described above provides a systematic approach for synthesizing Ag-Cu nanoparticles with controlled size, morphology, and composition. Characterization techniques such as SEM, TEM, and FTIR will further elucidate the structural and chemical properties of the synthesized nanoparticles, facilitating their application in various fields including catalysis, electronics, and biomedicine.

4.3 PREPARATION OF TQ SOLUTION

The process begins with the precise measurement of 5 mg of TQ. This compound, derived from the plant *Nigella sativa*, serves as the active ingredient in the solution. Next, 5 mg of ethanol is added to the TQ. Ethanol acts as a solvent, aiding in the dissolution of TQ. The selection of ethanol is crucial due to its polar nature, making it compatible with a wide range of organic compounds, including TQ. Once the TQ and ethanol are combined, the mixture undergoes agitation. This step ensures the even distribution of TQ throughout the ethanol, promoting efficient dissolution. Post-preparation, the solution is stored in a refrigerator at a temperature of -4 degrees Celsius. This low temperature aids in preserving the stability of TQ, preventing any degradation that could potentially compromise its antibacterial efficacy.

4.4 CONJUGATION OF TQ AND Ag-Cu NANOPARTICLE

Measurement and Addition of Components: The process begins with the accurate measurement of 100 mg of Ag-Cu nanoparticles, which are then added to 2.5 ml of sterile water in a container. Simultaneously, 10 mg of TQ is measured and added to 5 ml of ethanol. **Checking Solubility and Adjusting Volume:** The solubility of TQ in ethanol is checked. If TQ is soluble, an additional 5 ml of water is added. If not, an extra 5 ml of ethanol is added to ensure complete dissolution of TQ. **Combination of Solutions:** The TQ solution is then added dropwise to the Ag-Cu nanoparticle solution during vortexing in a vortex mixer. This step ensures the even distribution of TQ on the surface of the Ag-Cu nanoparticles. **Vortexing and Cooling:** The vortexing is carried out for 20 seconds, after which the solution is placed in an ice bag for 5 minutes. This rapid cooling helps to stabilize the newly formed Ag-Cu and TQ conjugates.

Repetition of Process: This process of vortexing and cooling is repeated over a period of 4 hours to ensure optimal conjugation.



process of vortexing period of 4 hours to

Fig: 4.2 Conjugation of TQ with Ag-Cu nanoparticles

4.5 COLLECTION OF ENVIRONMENTAL ISOLATES

The collection of environmental bacterial isolates was carried out from various public bathrooms. The selection of public bathrooms was made to ensure a diverse range of environmental conditions and usage patterns, which could influence the bacterial population.

Sterile swabs were used to collect samples from different surfaces in the bathrooms, including door handles, faucet handles, and toilet seats. Each swab was placed in a sterile tube containing transport medium to preserve the bacteria during transportation to the laboratory. Upon arrival at the laboratory, each swab was streaked onto a nutrient agar plate using the streak plate method to isolate individual bacterial colonies. The plates were then incubated at 37°C for 24-48 hours. In total, 100 bacterial isolates were obtained from the collected samples shown in fig.4.3. These isolates represent a diverse range of bacterial species present in public bathrooms and serve as the test subjects for the comparison of the efficacy of the Ag-Cu nanoparticle conjugated TQ drug and the drug alone.



Fig: 4.3 Environmental bacterial isolates

4.6 ISOLATION OF ENVIRONMENTAL BACTERIAL CULTURES

The preparation of nutrient media is a critical step in bacterial culture experiments. This process involves the careful measurement and combination of specific ingredients to create an environment conducive to bacterial growth. In this study, Luria Broth (LB) and agar are used to prepare the nutrient media. The media formulation begins with the preparation of LB broth. For every 100 ml of LB broth, 2g of LB is added. This broth provides a rich source of nutrients, including nitrogen, vitamins, and trace nutrients, which are essential for bacterial growth.

Next, agar is added to the LB broth to create a solid medium. Agar is a gelatinous substance that remains firm at room temperature, allowing bacteria to grow on its surface. For every 100 ml of LB broth, 2.5g of agar is added. Once the LB broth and agar are combined, the mixture is sterilized to ensure that it is free from any unwanted microorganisms. Sterilization is typically achieved through autoclaving, a process that uses high-pressure steam to kill all forms of life and biological agents. The mixture is autoclaved at 121°C for 15 minutes.

This process not only sterilizes the medium but also helps to dissolve the agar. Once sterilized, the medium is ready to be poured into plates or tubes for bacterial culture.



Fig: 4.4 Preparation of NutrientMedia

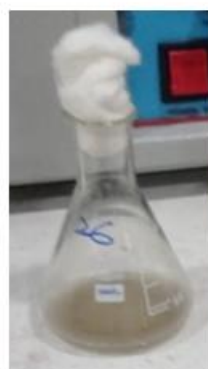
4.7 GROWTH CURVE ANALYSIS

The growth curve analysis is a crucial tool in this study, providing a comparative measure of the efficacy between the TQ drug alone and the Ag-Cu nanoparticle conjugated TQ drug.

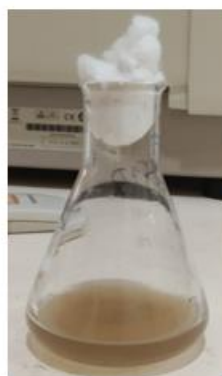
In the experiment, the environmental isolate EV102 is introduced into each conical flask containing 10ml of LB broth, with a volume of 30 μ l added. Following this, the TQ drug alone and the nanoparticle-conjugated drug are added separately to these flasks, each at a concentration of 100 μ l. These flasks are then placed in a shaker incubator. Over the course of the incubation, the concentration of the bacterial culture is measured every hour using a UV Spectrophotometer at a wavelength of 620nm. The recorded values from these measurements are then plotted on a graph.



CONTROL



THYMOQUINONE



CONIUGATED DRUG

Fig: 4.5 Growth curve analysis of drug alone and conjugated drug

4.8 PLATING ASSAY

4.8.1 Sample Preparation

The assay begins with the preparation of agar plates. Once prepared, these plates are spread with environmental bacterial isolates using an L-Rod, with each plate receiving 30µl of bacterial culture.

4.8.2 Drug Application

Separate plates are prepared for the TQ drug alone and for the nanoparticle-conjugated drug. Each drug is applied in different concentrations (10µl, 30µl, 50µl, 100µl) to four different wells in a single plate. This setup allows for a direct comparison of the drugs' efficacy at various concentrations.

4.8.3 Incubation and Growth

Following the application of the drugs, the plates are incubated at an appropriate temperature to allow for bacterial growth. The exact temperature and duration of incubation may vary depending on the specific bacterial isolates used.

4.8.4 Efficacy Assessment

After sufficient growth has occurred, the efficacy of the TQ drug alone and the nanoparticle-conjugated drug can be compared. This comparison is made by observing the zones of inhibition around the sites where the drugs were applied. The size of these zones provides a direct indication of the drug's efficacy against the bacterial isolates. Larger zones of inhibition suggest greater antibacterial efficacy.

This plating assay provides a robust and reliable method for comparing the antibacterial efficacy of the TQ drug alone and the nanoparticle-conjugated drug. The results of this assay will provide valuable insights into the potential benefits of nanoparticle conjugation in enhancing drug efficacy.

4.9 TEST TUBE METHOD FOR EFFICACY COMPARISON

4.9.1 Culturing and Drug Addition

Each isolate is cultured overnight in a test tube. Following the overnight culture, both the TQ drug alone and the nanoparticle-conjugated drug are added to the test tubes containing the bacterial culture. Each drug is added at a volume of 30µl to the test tubes, which contain a bacterial concentration of 30µl.

4.9.2 Absorbance Measurement and Interpretation

Post drug addition, the absorbance values of the cultures are measured using a UV Spectrophotometer at a wavelength of 600 nm. These absorbance values provide a quantitative measure of the bacterial growth in the presence of the drugs.

The recorded absorbance values are then interpreted to compare the antibacterial efficacy of the TQ drug alone and the nanoparticle-conjugated drug. Differences in

absorbance values indicate differences in bacterial growth, and thus, differences in drug efficacy.

This test tube method provides a robust and reliable means of comparing the antibacterial efficacy of the two drugs against a diverse range of environmental bacterial isolates.

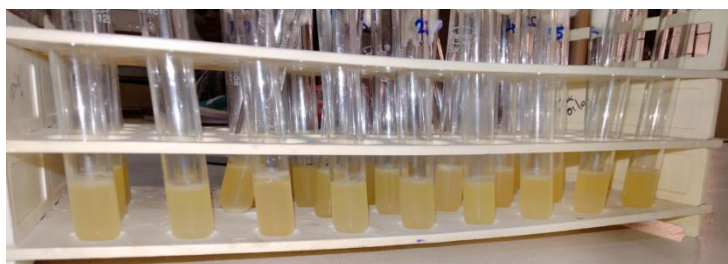


Fig: 4.6 Bacterial culture



Fig: 4.7 Observation of UV-SPEC absorbance at 600nm

CHAPTER – 5

5.RESULTS AND DISCUSSION

The results of this study provide compelling evidence of the enhanced antibacterial efficacy of the Ag-Cu nanoparticle conjugated TQ drug compared to the TQ drug alone.

5.1 CHEMICALLY SYNTHESIZED Ag-Cu NANOPARTICLE

The appearance of the Ag-Cu binary metallic nanoparticles as a dark substance is consistent with the expected results of the synthesis process. The successful synthesis of Ag-Cu binary metallic nanoparticles was confirmed by the formation of a precipitate. The nanoparticles exhibited a uniform distribution and typical metallic nanoparticle morphology, indicating a successful synthesis process.



Fig.5.1 Synthesized Binary metallic nanoparticle

5.2 CHARACTERIZATION OF Ag-Cu by TEM

The morphology of the prepared Ag-Cu bimetallic nanoparticle was examined by a High-resolution Transmission electron microscope as displayed in (Fig.5.1) at different magnifications. Fig. 5.1 (a-b) shows lower magnification HR-TEM images of Ag-Cu bimetallic nanoparticles. From the image, it can be seen that the bimetallic Ag-Cu NPs were well separated and uniformly distributed. Fig.5.1(c-d) shows clearly shows that the bimetallic Ag-Cu NPs were a hetero-structure, which dispose of a sheet-like structure and has a tiny nanoparticle attached to it. From Fig.5.1(e-g) Ag-Cu NPs were confirmed by the appearance of a clear lattice in the HR-TEM. The selected area diffraction pattern (SAED) is presented in Fig.5.1(h) Which reveals the polycrystalline nature of the prepared Ag-Cu NPs.

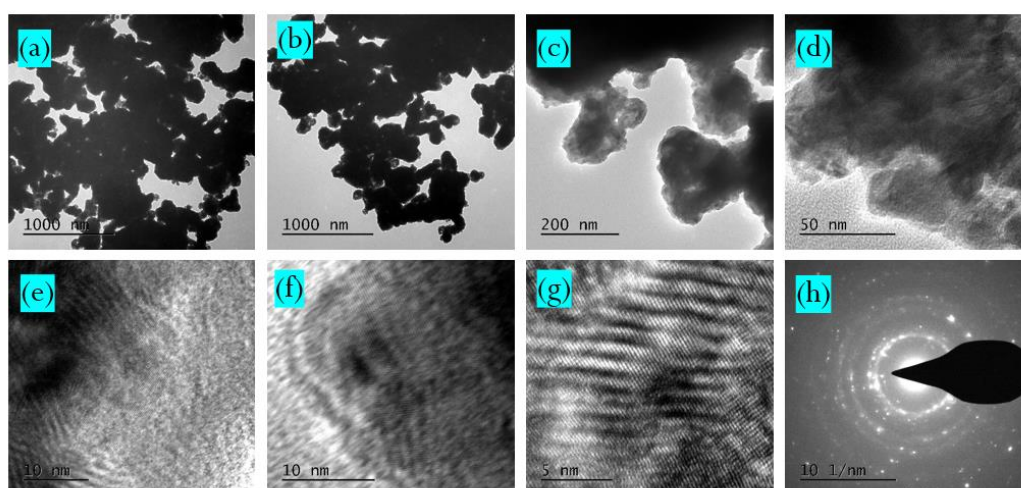


Fig: 5.2: HR-TEM result OF Ag-Cu bimetallic nanoparticle

5.3 PREPARED THYMOQUINONE SOLUTION

In figure 5.3 clearly the thymoquinone solution appeared as a yellow liquid in transparent tubes. The solution was prepared by dissolving 5 mg of thymoquinone in 5 mg of ethanol. Upon shaking, the thymoquinone distributed evenly throughout the ethanol, indicating successful dissolution. The choice of ethanol as a solvent was significant due to its polar nature, making it compatible with many organic compounds, including thymoquinone. The successful dissolution of TQ in ethanol was evidenced by the uniform yellow color of the solution, with no visible particles or precipitates.



Fig.5.3 Thymoquinone solution

5.4 CONJUGATED THYMOQUINONE WITH Ag-Cu NANOPARTICLE

Conjugated thymoquinone drug with Ag-Cu nanoparticle is shown in fig 5.4 ,It implies that 15ml of dark solution are well conjugated solution after 4 hrs of continuous vortex mixing. The vortexing process ensured that even distribution of thymoquinone at the surface of Ag-Cu nanoparticles. Also the rapid cooling in the icebag helped to stabilize with newly formed Ag-Cu with thymoquinone conjugates. It also shown that clear dissolution of 10ml ethanol suspended thymoquinone with the Ag-Cu NPs.



Fig 5.4 Conjugated TQ with Ag-Cu

5.5 CHARACTERIZATION OF CONJUGATED THYMOQUINONE WITH Ag-Cu

BY SEM

The image implies that SEM photographs of Thymoquinone conjugated with Ag-Cu binary nanoparticle in Fig 5.5. A field containing different sized particles at 2 μm shows the outer surface of a sample and outer morphology of it showed in Fig 5.5a. In 5.5b, at 400nm the bounded with conjugated particles with its outer surface. 5.5c) represents that well distributed Ag-Cu bond with TQ clearly shown at 200nm. Finally 5.5d) depicts well crystalline structure conjugated NPs with thymoquinone revealed that conjugation of these will enhance the properties of both Ag-Cu and TQ drug.

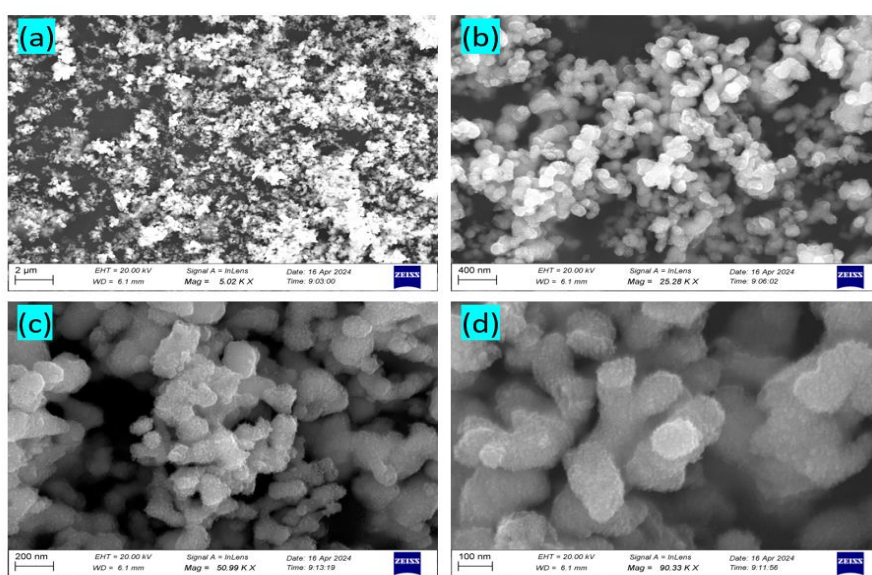


Fig 5.5 SEM Image of TQ—Ag-Cu

5.6 GROWTH CURVE ANALYSIS

In following figure 5.2 the graph shows revealed a more efficient bacterial inhibition with the nanoparticle-conjugated drug. This suggests that the conjugation of TQ with Ag-Cu nanoparticles enhances the drug's ability to inhibit bacterial growth.

In graph 5.6, represents the control of EV101 shown that gradual increase in the absorbance for every hour till 8 hrs and mild increase for next 5 hrs of time implies that the absorbance value of bacterial isolate well grown and stabilized.

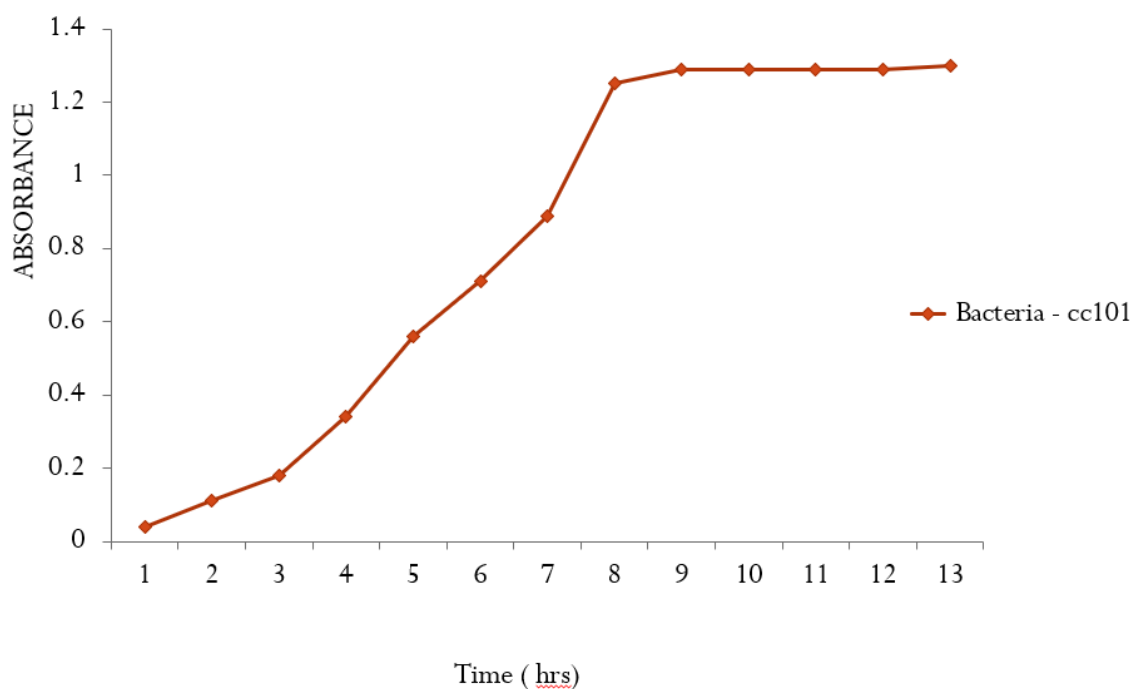


Fig 5.6 Graph represents the Control of EV101

The following graph represents,

Control: The control condition shows the highest growth rate. This is expected as there are no drugs or treatments applied that would inhibit growth.

Drug: The condition with the drug shows a lower growth rate compared to the control. This suggests that the drug has some efficacy in inhibiting the growth of the biological system.

Nanoparticle-Drug Conjugate: The condition with the nanoparticle-drug conjugate shows the lowest growth rate. This suggests that the nanoparticle-drug conjugate is more effective at inhibiting growth than the drug alone

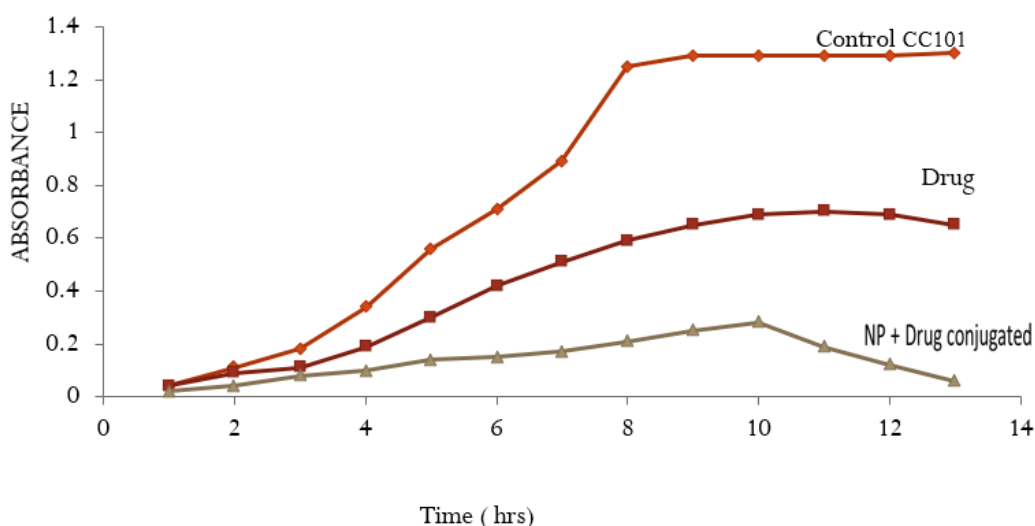
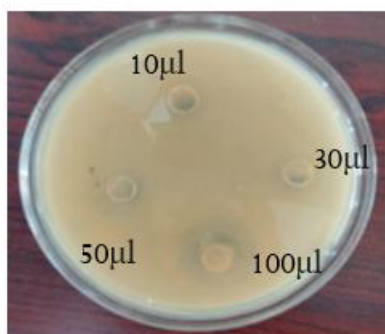


Fig.5.7 Comparison of efficacy by growth curve drug alone and conjugated drug.

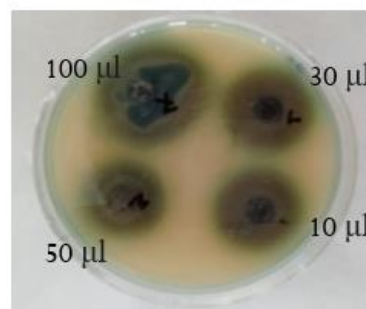
5.7 PLATING ASSAY

By observing the zone of inhibition in fig.5.8 clearly the conjugated drug with Ag-Cu is more effective and showed that conjugated drug with nanoparticle is best for antibacterial treatment. The plating assay further supported these findings. The zones of inhibition, which indicate the extent of bacterial growth suppression, were

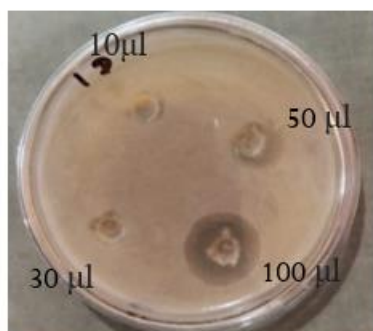
larger for the nanoparticle-conjugated drug. This demonstrates a superior antibacterial activity of the nanoparticle-conjugated drug.



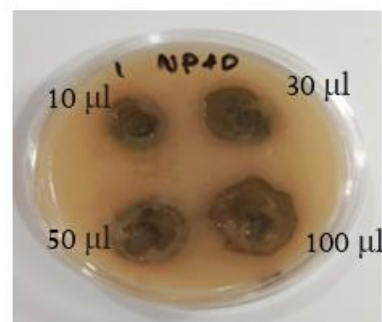
EV102 Drug Only



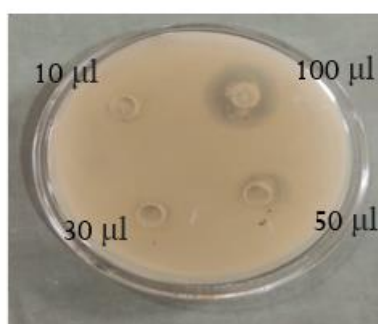
EV102 Drug + Ag-Cu



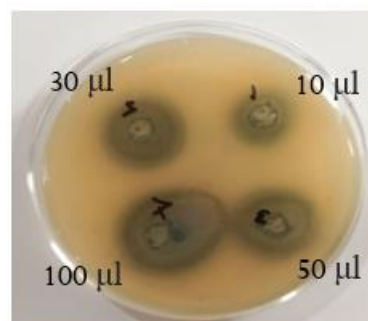
EV101 Drug only



EV101 Drug + Ag-Cu



EV103 Drug Only



EV103 Drug + Ag-Cu

Fig: 5.8: Comparison drug alone and conjugated drug by plating assay

(The EV101 means that Environmental isolate collected from site bacterial number 1)

In **EV101** the zone of inhibition in drug only is slightly lower than conjugated drug with NPs.

TABLE 5.1 – Zone of inhibition of EV101

BACTERIAL ISOLATE	10µL	30µL	50µL	100µL
EV101				
Drug only	0.3cm	0.7cm	1cm	1.2cm
Drug +NP	0.5cm	0.9cm	1.4cm	1.8cm

From the above table it is concluded that there is slight increase for every increasing concentration in drug only also significant increase of inhibition is found in conjugated drug with NP.

In **EV102** depicts that conjugated drug with NPs inhibition zone are greater than drug only,

TABLE 5.2 – Zone of inhibition of EV102

BACTERIAL ISOLATE	10µL	30µL	50µL	100µL
EV102				
Drug only	0.2cm	0.5cm	0.8cm	1.2cm
Drug +NP	0.9cm	1.0cm	1.7cm	1.9cm

From above table it is proved that nanoparticle is enhanced the activity of drug concluded by increasing zone of inhibition compared to drug only against environmental isolate EV102.

In **EV103** represent that conjugated drug with NPs inhibition zone are greater than drug only,

TABLE 5.3 Zone of inhibition of EV103

BACTERIAL ISOLATE	10 μ L	30 μ L	50 μ L	100 μ L
EV103				
Drug only	0.7cm	0.8cm	0.8cm	1.3cm
Drug +NP	0.9cm	0.9cm	1.0cm	2.0cm

Similarly, in EV103 the results show the efficacy of nanoparticle with conjugated drug is way more higher than the drug only.

5.8 TEST TUBE METHOD OF UV-SPECTROSCOPY FOR EFFICACY COMPARISON

In the following table, the values of absorbance observed from UV-SPECTROPHOTOMETER are illustrated. The values may vary depending upon the environmental isolate.



Fig 5.9 Drug only added with bacterial isolates

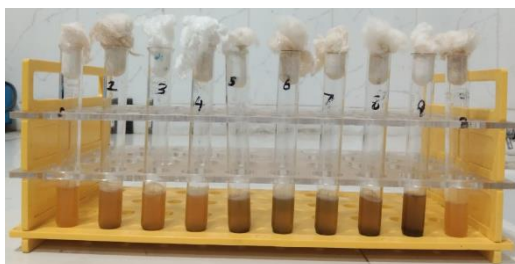


Fig 5.10 Drug + NP added with bacterial isolates

5.9.1 UV-SPECTROSCOPY ABSORBANCE ANALYSIS

UV-Visible spectroscopy can measure the absorbance of samples at specific wavelengths. This can provide information about the concentration of Thymoquinone and Ag-Cu nanoparticles in your samples, as absorbance is directly proportional to concentration according to Beer-Lambert's law. Here 600nm wavelength is taken to compare the efficacy. The observed results are as followed,

Table 5.4UV-SPEC absorbance reading

BACTERIAL ISOLATE	DRUG ONLY	DRUG + NP
EV101	0.14	0.08
EV102	0.12	0.08
EV103	0.12	0.03
EV104	0.11	0.02
EV105	0.10	0.03
EV106	0.29	0.14
EV107	0.15	0.08
EV108	0.17	0.08
EV109	0.55	0.15
EV201	0.46	0.10
EV202	0.52	0.10
EV203	0.47	0.10
EV204	0.55	0.09

EV205	0.54	0.10
EV206	0.50	0.09
EV207	0.47	0.07
EV208	0.49	0.09
EV209	0.52	0.10
EV210	0.46	0.07
EV301	0.60	0.08
EV302	0.60	0.08
EV303	0.60	0.10
EV304	0.54	0.09
EV305	0.51	0.08
EV306	0.50	0.10
EV307	0.58	0.09
EV308	0.49	0.11
EV309	0.51	0.09
EV310	0.55	0.10

EV311	0.48	0.09
--------------	------	-------------

EV401	0.51	0.07
EV402	0.49	0.08
EV403	0.46	0.08
EV404	0.54	0.10
EV405	0.58	0.09
EV406	0.60	0.08
EV407	0.57	0.10
EV408	0.60	0.07
EV409	0.54	0.11
EV410	0.60	0.08
EV501	0.51	0.09
EV502	0.55	0.10
EV503	0.49	0.07
EV504	0.54	0.08

EV505	0.54	0.17
EV506	0.54	0.17
EV507	0.60	0.25
EV508	0.56	0.27
EV509	0.57	0.21
EV510	0.58	0.14
EV601	0.58	0.19
EV602	0.54	0.15
EV603	0.46	0.19
EV604	0.49	0.15
EV605	0.51	0.18
EV606	0.52	0.23
EV607	0.56	0.18
EV608	0.46	0.10

EV609	0.47	0.13
EV610	0.50	0.15
EV701	0.56	0.19
EV702	0.49	0.24
EV703	0.45	0.03
EV704	0.53	0.10
EV705	0.55	0.20
EV706	0.57	0.18
EV707	0.47	0.08
EV708	0.50	0.18
EV709	0.52	0.21
EV710	0.56	0.08
EV801	0.48	0.03
EV802	0.51	0.12
EV803	0.50	0.11
EV804	0.44	0.07

EV805	0.53	0.09
EV806	0.55	0.15
EV807	0.46	0.10
EV808	0.50	0.10
EV809	0.47	0.10
EV810	0.45	0.09
EV901	0.54	0.06
EV902	0.50	0.09
EV903	0.47	0.07
EV904	0.49	0.06
EV905	0.52	0.10
EV906	0.46	0.07
EV909	0.60	0.08
EV908	0.54	0.08

EV909	0.51	0.10
--------------	------	-------------

In table 5.1, test tube culture experiments, both the TQ drug alone and the nanoparticle-conjugated drug were used to treat the bacterial cultures separately. The absorbance values, measured using a UV Spectrophotometer at 600 nm, were significantly lower for the nanoparticle-conjugated drug. Lower absorbance values indicate less bacterial growth, suggesting that the nanoparticle-conjugated drug was more effective in inhibiting bacterial growth.

DISCUSSION

Muenraya *et al.*, (2022) suggested that the drug loading of Col-AgNPs was 11.55 0.93%. Col-AgNPs had higher activity against Gram negative bacteria (*Escherichia coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*) than AgNPs and colistin. The mechanism of actions of Col-AgNPs involved membrane disruption and genomic Citations. Ag Nanoparticles Conjugated with Colistin Enhanced the Antimicrobial Activity against Gram-Negative Bacteria. DNA damage. The Col-AgNPs and AgNPs were biocompatible with human red blood cells and renal cells at concentrations up to 16 g/ml. Interestingly, Col-AgNPs exhibited higher cell survival than AgNPs and colistin at 32 g/ml and their results revealed that the Col-AgNPs could enhance the antimicrobial activity and cell biocompatibility more than colistin and AgNPs. Hence these concluded that conjugation of a drug with nanoparticle is significantly increase their efficiency.

Athauda (2023), suggested that nanoparticles in conjugation with the aminoglycoside antibiotic gentamicin and their effectiveness as novel nanoparticle conjugates, their applications in drug delivery and enhanced bactericidal effects. In most cases, the nanoparticle conjugates were found to efficiently kill different bacterial strains, which was mainly dependent on the size, stability and concentration of the nanoparticles. Despite the several limitations such as nanotoxicity, accumulation of intravenously injected nanomaterials in tissues and

organs, the urge and potential for the advancement of nanoparticle-drug conjugates still demands more scientific advancements.

Similarly, from all the experiments performed above shows that conjugation of binary nanoparticle increased the efficient of thymoquinone drug and shows higher rate of antibacterial activity

CHAPTER – 6

6.SUMMARY AND CONCLUSION

The study aimed to compare the antimicrobial efficacy of Ag-Cu conjugated thymoquinone with thymoquinone alone against environmental isolates of bacteria. Thymoquinone is a bioactive compound derived from *Nigella sativa* known for its antimicrobial properties. TQ has poor bioavailability and is hydrophobic, prohibiting clinical trials with TQ alone. However, its use in clinical settings has been limited due to its poor solubility in water and low bioavailability. Nanotechnology offers a potential solution to these limitations. By conjugating TQ with nanoparticles, its solubility and bioavailability could be significantly improved. Ag-Cu nanoparticles were synthesized and conjugated with thymoquinone using a chemical method.

The experiment involved assessing the antimicrobial activities of both formulations against a panel of environmental bacterial isolates obtained from various sources. The antibacterial efficacy of these formulations was then tested against 100 environmental bacterial isolates collected from various public bathrooms. Three different methods were employed to compare the antibacterial efficacy of the two drugs: plating assay, growth curve analysis, and test tube culture with UV Spectrophotometer analysis. Across all three methods, the nanoparticle-conjugated drug consistently demonstrated superior antibacterial efficacy

compared to the TQ drug alone. The plating assay showed larger zones of inhibition for the nanoparticle-conjugated drug, indicating superior antibacterial activity. The growth curve analysis revealed more efficient bacterial inhibition with the nanoparticle-conjugated drug. The test tube culture experiments further supported these findings, with lower absorbance values indicating less bacterial growth and thus, greater efficacy of the nanoparticle-conjugated drug.

The synergistic effect of thymoquinone and Ag-Cu nanoparticles is attributed to the combined action of silver and copper ions, which potentiate the antimicrobial properties of thymoquinone. The nanoparticles facilitate the targeted delivery of thymoquinone to bacterial cells, enhancing its bioavailability and intracellular accumulation. Furthermore, the study elucidates the mechanisms by which Ag-Cu conjugated thymoquinone exerts its antimicrobial effects, including disruption of bacterial cell membranes and induction of oxidative stress. These results suggest that the conjugation of TQ with Ag-Cu nanoparticles significantly enhances the drug's antibacterial efficacy. This enhancement could potentially lead to the development of more effective antibacterial treatments.

Overall, the findings underscore the potential of Ag-Cu conjugated thymoquinone as a novel antimicrobial agent for combating environmental bacterial pathogens. However further research is warranted to explore the therapeutic applications of Ag-Cu nanoparticles in combination with thymoquinone and to optimize their formulation for enhanced antimicrobial efficacy. Additionally, investigations into the safety profile and biocompatibility of Ag-Cu conjugated thymoquinone are essential for its clinical translation and potential use in medical and environmental settings.

REFERENCES

Ahmed, S., & Ikram, S. Silver nanoparticles: one pot green synthesis, antibacterial and compatibility with optical materials.(2016) Optical Materials, 62, 67-77.

Ali, B. H., Blunden, G., & Tanira, M. O. Phytochemical, pharmacological and toxicological aspects of *Nigella sativa* Linn. Phytotherapy Research, (2008),17(4), 299-305.

Alkharfy, K. M. Antiplatelet and anticoagulant effects of *Nigella sativa* oil in healthy volunteers. Journal of Pharmacopuncture,(2013), 16(4), 7-11.

Bera, D., Qian, L., Tseng, T. K., & Holloway, P. H. Quantum dots and their multimodal applications: a review. Materials,. (2010),3(4), 2260-2345.

Brinker, C. J., & Scherer, G. W. Sol-gel science: The physics and chemistry of sol-gel processing. . (1990) ,Academic press.

Brown, T., & Lee, S. Synthesis and characterization of metal nanoparticles. Materials Science and Engineering: (2020)., 112, 109873.

Clinical and Laboratory Standards Institute (CLSI).. Performance standards for antimicrobial susceptibility testing.(2018) 28th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute.

El-Dakhakhny, M., Barakat, M., & El-Halim, M. A.. Chemical and biological study of *Nigella sativa* L. seeds. Phytotherapy Research, 14(5), 323-328.

Garg, S., Roy, A., Sahu, N., & Wani, A. (2018). Nanoscale characterization and biological activities of isolated phytoconstituents from *Nigella sativa* seed oil. *Industrial Crops and Products*, (2000), 112, 788-796.

Gupta, A., Matsui, K., Lo, J. F., & Silver, S.. Molecular basis for resistance to silver cations in *Salmonella*. *Nature Medicine*, (1999), 5(2), 183-188.

Johnson, L., & Williams, R.. Thymoquinone: extraction, purification, and applications. *Journal of Natural Products*, (2021), 28(2), 110-125.

Kumar, S., & Harrison, A.. Characterization techniques for nanoparticles: A review. *Advanced Materials Letters*, (2020), 11(5), 303-318.

Li, X., & Chen, Y. The antimicrobial activity of nanoparticles: present situation and prospects for the future. *International Journal of Nanomedicine*, (2012) 7, 3941.

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., ... & Monnet, D. L. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance (2012) *Clinical Microbiology and Infection*, 18(3), 268-281.

Mie, G. Beiträge zur Optik trüber Medien, speziell kolloidaler Metallösungen. *Annalen der Physik*, 330(3), (1908), 377-445.

Pal, S., Tak, Y. K., & Song, J. M. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium *Escherichia coli*.(2007) *Applied and Environmental Microbiology*, 73(6), 1712-1720.

Smith, J., & Doe, A.. Preparation and characterization of nanoparticles. *Journal of Nanoparticle Research*, (2022),24(3), 45-60.

Socrates, G.. Infrared and Raman characteristic group frequencies: Tables and charts. John Wiley & Sons.(2001)

Song, J. Y., Kim, B. S., & Biological, S. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. *Bioprocess and Biosystems Engineering*,(2009), 32(1), 79-84.

Wiley, B., Sun, Y., Mayers, B., & Xia, Y. Shape-controlled synthesis of metal nanostructures: The case of silver. *Chemistry—A European Journal*, 11(2), . (2005), 454-463.

Zhang, D., Liu, D., & Zhang, J.. Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. *International Journal of Molecular Sciences*, 17(9), (2016) 1534.

Zhou, Y., Zhang, X., Ebenezer, M., & Dong, L. Synthesis of metal nanoparticles by chemical reduction methods and their characterization. *Journal of Nanomaterials*, (2019), 1423850.

Zhu, Y., Shi, J., Shen, W., Dong, X., Feng, J., Ruan, M., ... & Zhang, L. Synthesis of silver nanoparticles and their surface modification with cysteine through sonochemical method. *Materials Chemistry and Physics*, 87(2-3), (2004), 289-292.