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Comparative Study of Antibacterial and Antibiofilm Activities of Seed Extracts and Their Phytofabricated Silver Nanoparticles Against Ocular Bacterial Isolates

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KEYWORDS

Phyto fabricated, MIC, antibiofilm, plant-based therapeutics, *Citrobacte*r, Ophthalmic applications, Contact lens

ABSTRACT:

Ocular infections, especially those related to contact lens usage, are often caused by biofilm-forming bacteria that display resistance to conventional antibiotics. This study investigates the antibacterial and antibiofilm potential of seed extracts—*Coriandrum sativum* (coriander), *Piper nigrum* (white pepper), and Triphala—and their Phyto fabricated silver nanoparticles (AgNPs) against *Pseudomonas aeruginosa* and *Citrobacter sp*., two key ocular pathogens. Clinical samples were collected from infected eyes, contact lenses, and their cases after obtaining informed consent. Bacterial strains were isolated and identified using morphological, cultural, and biochemical methods. Their biofilm-forming abilities were assessed quantitatively. Crude seed extracts were prepared and evaluated for antibacterial efficacy using the agar well diffusion and MIC methods. Silver nanoparticles were synthesized using green synthesis protocols and characterized through UV-Vis spectroscopy and SEM analysis. The plant extracts exhibited inhibition zones of 22 mm and 18 mm against *Pseudomonas aeruginosa* and *Citrobacter sp*., respectively. Phyto fabricated AgNPs demonstrated significantly enhanced activity, showing inhibition zones of 25 mm and 20 mm at an MIC of 0.156 mg/mL, outperforming the standard antibiotic moxifloxacin (18 mm and 17 mm respectively). This highlights the potential of AgNPs in overcoming antibiotic resistance and disrupting bacterial biofilms. In conclusion, the Phyto fabricated silver nanoparticles showed superior antibacterial and antibiofilm properties compared to both crude extracts and conventional antibiotics. These findings underscore the promise of integrating plant-based therapeutics with nanotechnology for effective management of ocular infections. Further exploration into mechanism of action and cytocompatibility is recommended to translate this research into ophthalmic applications.

1. Introduction

The presence of microorganisms on contact lenses (CL) is associated with infiltrative keratitis (IK) an inflammatory condition, and microbial keratitis (MK) an eye infection that might ultimately lead to vision loss Visual impairment (VI), a global concern that is likely to escalate with prolonged life expectancies, has gained increasing attention in the realm of eye care. Globally, an estimated 43.3 million people worldwide suffer from blindness and 295 million people have moderate and severe vision impairment in 2020[1]. Major bacterial pathogens, including *Staphylococcus aureus, Haemophilus influenzae, Streptococcus pneumoniae*, and *Pseudomonas aeruginosa*, are responsible for these infections. *Staphylococcus aureus* is one of the most common causes of conjunctivitis and more severe infections like keratitis, often linked to trauma, surgery, or contact lens use. A significant challenge in treating ocular infections is the rise of antimicrobial resistance (AMR)[2]. AMR has emerged as a major global public health threat, leading to treatment failures, increased morbidity, and higher healthcare costs[3]. The inappropriate use and overuse of antibiotics have significantly contributed to the development and spread of resistant strains in ophthalmology[4]. Infection is predominantly triggered by biofilm formation. It is projected that more than 60% of all bacterial infections are associated with biofilms[5]. The complex biofilm architecture defends the bacterial cells within them from antibiotics, antiseptics, soaps and detergents, physical shear forces and the host immune system [6]. Clinically significant bacteria known as ESKAPE, which stands for *Enterococcus faecalis*, *Staphylococcus aureus*, *Klebsiella pneumoniae, Acinetobacter baumannii,*

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Pseudomonas aeruginosa, and Enterobacter spp., have come under increased attention in recent years due to their ability to form biofilms on medical devices. It has also been observed that biofilm growth has been subjected to a variety of ophthalmology-related materials and equipment [7]. The variability in the biofilm composition as well as tolerance versus the antimicrobial drugs commonly used in conventional therapies suggest the need for multi-targeted or combinational therapies aimed at the eradication of biofilms; furthermore, polymicrobial biofilms represent a further concern that needs to be addressed. These phytochemicals have displayed themselves as future drugs and knowledge that is more scientific in this regard is essential [8]. Even though there are numerous successful examples of phytochemicals combating antibiotic resistant infections, the translational success or commercial application of such phytochemicals is very low and needs a drastic improvement. Hence, there is the utmost need for fast-track research, clinical approval and application of these phytochemicals to battle against the MDR associated clinical complications.

2. Methods

2.1 Data Collection -The aim of this study was to analyze the bacterial micro biota found in Eyes, contact lens and cases. All participants were advised to submit the lens case as it is, whether it was dry or wet as we aimed to sample the case as last used.

2.2 Isolation Of Optmetric Bacterial Isolates- The Contact Lens, infected eye's conjunctiva and Contact lens case were wiped using a sterile cotton swab that was moistened with distilled water and swiped in a circular motion, ensuring that the entire inside of the compartment was swabbed. The swab was immediately placed in a nutrient-rich broth and incubated at 37°C for 24-48 hours [9]. The inoculations were cultured on nutrient agar, and then incubated aerobically at 37 C for 24 hrs. All culture media were prepared following the manufacturer's instructions and sterilized by autoclaving at 121 °C for 20 minutes [10].

2.3 VIRULENCE FACTOR DETECTION OF ISOLATED BACTERIAL ISOLATES

2.3.1 ENZYMATIC ACTIVITY:

2.3.1.1 Protease Activity Assay by Skim Milk Agar Plate Assay-Protease activity of bacterial isolates was determined using the skim milk agar plate method. Skim milk agar was prepared by adding 1% (w/v) skim milk powder to nutrient agar..

2.3.1.2 Lipase Activity Assay-Lipase production was assessed using tributyrin agar. The medium was prepared by incorporating 1% (v/v) tributyrin oil into nutrient agar, using Tween 80

2.3.1.3 DNase Activity Assay-To evaluate DNase activity, DNase agar containing 0.01% methyl green was used.

2.4 Biofilm Formation Assessment By Crystal Violet Assay-A qualitative assessment of biofilm formation was determined as TSBglu (10mL) was inoculated with loopful of microorganism from overnight culture plates and incubated for 24 hours at 37°C. The tubes were decanted and washed with PBS (pH 7.3) and dried. Dried tubes were stained with crystal violet (0.1%). Excess stain was removed and tubes were washed with deionized water [11]. After washing three times with PBS, the bacteria biofilm was incubated with a mixture of 80% ethanol and 20% acetone (4:1) for 10 min. Absorbance was measured at 570 nm [12]. Biofilm production was classified as negative, weak, moderate, and strong based on the cutoff value, calculated according to the following formula, using the optical density (OD) values [13].

2.5 IDENTIFICATION OF POTENT BACTERIAL ISOLATES

2.5.1 Morphological characteristics- Colony morphology of the bacterial isolates was observed by streaking them onto nutrient agar plates and incubating at 37°C for 24 to 48 hours.

2.5.2 Staining- Gram staining, negative staining, endospore, flagella staining capsule staining was performed

2.5.3Catalase and Oxidase Tests- The catalase and oxidase tests were conducted as preliminary

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biochemical assays to distinguish between major bacterial groups.

2.5.4IMViC Tests (Indole, Methyl Red, Voges-Proskauer, Citrate)-The IMViC test series was used primarily to differentiate members of the Enterobacteriaceae family.

2.5.5Urease, TSI, and Nitrate Reduction Tests- Urease activity was tested using Christensen's urea agar slants. Isolates were streaked and incubated at 37°C for 24 hours. Nitrate reduction was tested in nitrate broth by adding sulfanilic acid and α-naphthylamine after incubation.

2.6 Extraction From Organic And Inorganic Solvent- Extraction of a phytochemical from the plant material is mainly dependent on the type of solvent used. Extraction was done for Coriander seeds, white pepper and triphala powder using both organic and inorganic solvents.

2.7 PHYTOCHEMICAL SCREENING

Phytochemicals can be separated from the plant material by various extraction techniques. . Identification of Phytoconstituents in the plant material helps to predict the potential pharmacological activity of that plant [14].

2.8 PREPARATION OF SILVER NANOPARTICLES

The preparation of silver nanoparticles was carried out using coriander seeds, white pepper, and Triphala powder as reducing and stabilizing agents. Initially, 10 grams each of coriander seeds, white pepper, and Triphala powder were separately washed thoroughly and ground into a fine powder using a clean mortar and pestle. For the aqueous extract, 10 grams of each powder was boiled separately in 100 mL of distilled water for 10–15 minutes and allowed to cool to room temperature. The resulting mixtures were filtered through Whatman No. 1 filter paper to obtain clear extracts.To synthesize silver nanoparticles, 10 mL of each plant extract was mixed with 90 mL of 1 mM silver nitrate ($AgNO_3$) solution in separate conical flasks. The reaction mixtures were incubated for 24 hours and then centrifuged at 10,000 rpm for 15 minutes. The resulting pellets were washed three times with distilled water and dried at 60°C to obtain the silver nanoparticles in powdered form. The synthesized nanoparticles were then subjected to further characterization using UV-Vis spectroscopy, SEM to confirm their formation and study their properties.

2.9 ANTIMICROBIAL SUSCEPTIBILITY TESTING (AST)

2.9.1 WELL DIFFUSION METHOD- Extracts of coriander seeds, white pepper and triphla powder were tested for their antibacterial activity by agar well diffusion method Well-diffusion of agar method was applied to determine antimicrobial activities. Muller Hilton agar (MHA) plates were wiped with sterile (cotton swabs) 12-hour old broths cultured from the respective bacteria. Wells were made in each plate using a sterile cork drill. Approximately 100 µl of plant solvent extract was added using sterile syringe into the wells and allowed to infuse at room temperature for 2 h. Pure distilled water was used as solvent control in the antimicrobial susceptibility test for all three different extracts. All dishes were incubated at 37 °C for 24h. The diameter of the (mm) inhibition zone was measured [15].

2.9.2 MIC – BROTH MICRODILUTION METHOD Solvent Extract with maximum zone size was selected for Minimum inhibitory concentration (MIC), which was determined for extracts of coriander seeds, white pepper and Triphala powder against two biofilm forming bacterial isolates, *Pseudomonas aeruginosa* and NB7. Broth microdilution method was used for this. Different concentrations of extracts 1%, 2% and 3% (w/v) were used. Sterile distilled water was used as negative control The lowest concentration inhibiting bacterial growth was considered the MIC valueThe broth microdilution method was carried out according to the CLSI guideline [16]. Different concentrations of extract 1%, 2% and 3% (w/v) were prepared for Coriander seeds; white pepper and triphala powder.100 µL of both nutrient broth and extract were added. 20 µL of standardized bacterial suspension was used for inoculation.

2.1. Well Diffusion Assay For Antibacterial Activity Of Plant Extracts And Silver Nanoparticle Against Antibiotic - The antibacterial activity of selected plant extracts was evaluated using the agar well diffusion method, following the guidelines of the Clinical and Laboratory Standards Institute[17]and modifications

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reported in recent literature[18]. Mueller-Hinton Agar (MHA) plates were prepared and seeded with standardized bacterial inocula (0.5 McFarland standard, ~1.5 × 10^8 CFU/mL) using a sterile swab.

3. Results

The present study involved the collection, isolation, and morphological characterization of bacterial isolates obtained from various ophthalmic-associated sources, including contact lenses, contact lens cases, spectacles, and clinical samples from Jaipur. A total of 26 isolates were studied, and the data offers insight into the possible microbial contamination risks associated with vision-care products and clinical environments.

3.1 Distribution of Isolates and Source Analysis

Out of the 19 field isolates (N1–N19), the majority were derived from contact lenses and their storage cases, highlighting them as primary vectors for microbial contamination. Only a few isolates originated from the eye surface (N3, N4) and spectacles (N5, N6). These findings are consistent with literature indicating that improper lens hygiene and storage conditions significantly contribute to ocular infections such as microbial keratitis[19].

Figure 1. **Collection of Eye conjunctiva Swab Sample for bacteriological Analysis.**-The seven isolates (NB1–NB7) represent clinical or environmental samples and help provide a comparison between hospital-sourced bacteria and field strains. This distinction is critical, as hospital isolates often display greater resistance and more robust biofilm-forming potential[20].

3.2 Colony morphological characteristics- Morphological analysis revealed diverse phenotypic traits among the isolates, which may reflect species-level diversity or different stages of growth: Colour: Predominantly white and pale-yellow colonies were observed, with occasional neon green and yellow colonies (e.g., N1, N2, N7, and N10). These may indicate species like *Pseudomonas*, *Staphylococcus*, or *Citrobacter*, which are commonly associated with contact lens-related infections [21]. Notably, undulate and curled margins—observed in isolates like N3, N7, N10, N16, and N17—are often correlated with biofilm-producing strains, which pose greater clinical challenges

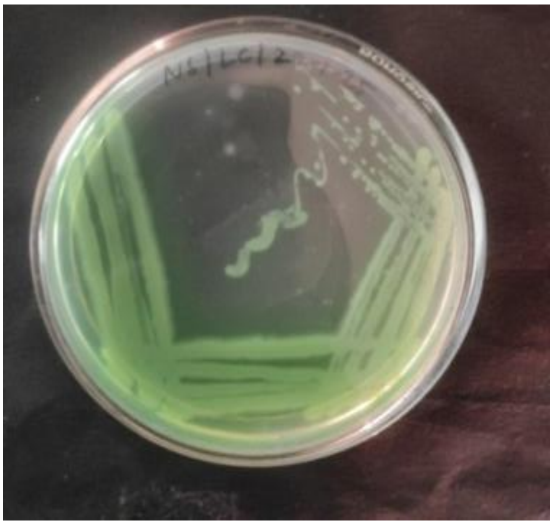

Figure 2.**Colony Morphology of white, offwhite and Neon Green Pigmented Bacteria Isolated from Ocular Swab on Nutrient Agar**

The image shows bacterial colonies exhibiting a neon green pigment grown on nutrient agar. The colonies appear flat with an entire margin, characteristic of isolates coded as N1/N2 based on colony morphology records. The uniform pigmentation and smooth colony edge suggest the presence of a pigment-producing ocular bacterium, possibly *Pseudomonas species*, commonly associated with ocular infections **[22].**

3.2 Clinical Relevance and Implications- Moreover, isolates (NB1–NB7)—which showed more uniform morphology, particularly white raised colonies with entire margins—could represent clinical strains adapted to controlled environments, but potentially more resilient in terms of antimicrobial resistance profiles..

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Code	Test Tube method	OD	Biofilm Strength	Protease Qualitative activity	DNAase activity	Lipase activity
N1	Present	1.45	Very Strong	+	+	+
N2	Present	0.42	Moderate	+	-	-
N3	Present	0.45	Moderate	-	-	+
N4	Present	1.06	Very Strong	-	-	+
N5	Absent	0.58	None	-	-	-
N6	Present	0.36	Moderate	-	-	+
N7	Present	1.41	Very Strong	+	+	+
N8	Present	1.31	Very Strong	+	+	+
N9	Present	0.89	Strong	+	+	
N10	Present	0.78	Strong	-	+	+
N11	Present	0.23	Moderate	-	+	-
N12	Present	0.56	none	-	-	-
N13	Present	1.02	Very Strong	+	+	+
N14	Present	0.70	Strong	-	+	+
N15	Absent	0.45	None	-	-	-
N16	Absent	0.78	None	-	-	-
N17	Absent	0.26	None	-	-	-
N18	Absent	0.05	None	-	-	-
N19	Absent	0.56	Moderate	+	-	-
NB1	Present	0.55	Moderate	-	-	-
NB2	Present	0.64	Strong	-	-	-
NB3	Present	0.62	Strong	-	-	-
NB4	Absent	-	None	-	-	-
NB5	Present	1.0	Very Strong	-	+	+
NB6	Present	0.43	Moderate	-	-	-
NB7	Present	1.43	Very Strong	-	-	-

Table.1. Biofilm Formation and Extracellular Enzymatic Activities of Ocular Isolates.

Biofilm Category	No. of Samples (26)	Percentage (%)
None	7	26.92%
Moderate	8	30.76%
Strong	5	19.23%
Very Strong	6	23.07%

Table 2 .Distribution of Biofilm Formation Strength among Ocular Bacterial Isolates

This table presents the categorization of 26 bacterial isolates based on their biofilm-forming ability, determined by optical density measurements. Isolates were classified into four categories: None (7 isolates, 26.92%), Moderate (8 isolates, 30.76%), Strong (5 isolates, 19.23%), and Very Strong (6 isolates, 23.07%). The results indicate that a significant proportion (73.08%) of the isolates exhibited some degree of biofilm formation, highlighting the potential for persistent colonization and infection in ocular environments.

3.3 Biofilm Strength and Enzyme Activities

Isolates show a clear correlation between biofilm biomass (as measured by OD via tube method) and enzyme activity profiles: Very Strong (OD ≥ 1.0): N1, N4, N7, N8, N13, NB5 – most of them exhibit ≥2 enzymes positive (+). Strong to Moderate isolates (OD 0.42–0.99) have mixed enzyme profiles (1–2 positives). None or weak biofilm (OD < 0.6 or absent in test tube) largely lack enzyme activity. This aligns with previous studies where a positive correlation between lipase and biofilm ($r = 0.195$, $p = 0.10$), but no correlation for protease—matching your moderate associations for lipase but weaker links for protease.

3.4 Role of Hydrolytic Enzymes in Biofilm Formation and Stability: Evidence from Lipase, Protease, and DNase Activity- The present study highlights the significant role of lipase as a biofilm-enhancing factor. Among the six isolates categorized as "Very Strong" biofilm producers, five exhibited notable lipase activity, establishing a strong correlation between lipase production and increased biofilm biomass. Conversely, isolates lacking lipase activity generally displayed lower optical density (OD) values (<1.0), suggesting a diminished biofilm-forming capacity. These findings align with recent literature emphasizing the utility of anti-biofilm enzyme cocktails, where lipase, in combination with protease and cellulase, effectively disrupts the extracellular polymeric substances (EPS) matrix. This reinforces the conclusion that lipase contributes not only to the establishment but also to the structural integrity of biofilms. In addition to lipase, the presence of protease and DNase was evaluated for their roles in biofilm dynamics. Notably, half of the "Very Strong" biofilm producers (3 out of 6) demonstrated the presence of both protease and DNase. This dual enzymatic activity suggests that, beyond their established function in biofilm dispersion through degradation of proteins and extracellular DNA, these enzymes may also facilitate EPS remodeling during biofilm development. Supporting this, previous studies have shown that treatments combining DNase I and proteinase K lead to substantial disruption of biofilm architecture and reduction in overall biomass. Some strong biofilm-forming isolates (e.g., N4 and NB5) lacked detectable protease and DNase activity, indicating that other EPS components—such as lipids and polysaccharides—may play compensatory roles. This observation aligns with current understanding that biofilm matrices are multifactorial and compositionally diverse, as highlighted in recent reviews on bacterial biofilms. Overall, the data support the hypothesis that biofilm formation is a multifaceted process involving multiple enzymatic and structural factors, with lipase emerging as a key contributor in the present study.

3.5 Test Tube OD Method Validity

The OD-based tube assay is widely used to quantify biofilm:It correlates with quantification techniques in studies using DNase and proteases.OD thresholds (OD 0.1–1.4) in your data reliably differentiate moderate to very strong biofilm producers.

3.6 Correlation between Enzyme Expression and Biofilm Robustness in Moderate and Weak Producers

A closer examination of moderate biofilm-producing isolates (e.g., N2, N3, N6, and N11) reveals a pattern of partial enzyme expression. Specifically, isolate N2 exhibited protease activity, while N3, N6, and N11 were positive for lipase alone. The limited enzymatic repertoire in these isolates is associated with a moderate level of biofilm formation, suggesting a less complex and structurally weaker EPS matrix compared to multi-enzyme producers. Furthermore, isolates that showed no detectable enzymatic activity (protease, DNase, or lipase) consistently demonstrated minimal or negligible biofilm formation, as indicated by low OD values (<0.6). This strongly supports the hypothesis that extracellular enzymes play a critical role in initiating and maintaining stable EPS structures, reinforcing their importance in biofilm physiology. Isolates exhibiting oxidative metabolism, catalase and oxidase positivity, and a combination of indole negativity, starch hydrolysis, and citrate utilization were consistent with *Pseudomonas spp*. Gram-positive isolates displaying starch hydrolysis and sporulation suggest a Bacillus-like

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identity, characteristic of environmental saprophytes. Additionally, one isolate matched the biochemical profile of *Citrobacter spp*., showing traits typical of the Enterobacteriaceae family with distinguishable IMViC test outcomes. These identifications offer further insight into the ecological roles and potential pathogenicity of the isolates in relation to their enzymatic and biofilm-forming capabilities.

Phytochemical-Coriander Seed	**(Methanolic Extract)**	**(Ethanol Extract)**	**(Water Extract)**
Alkaloids	+	+	+
Carbohydrate	+	+	+
Saponins	+	–	+
Protein	+	+	–
Phenolic Compounds	+	–	-

Phytochemical-Piper nigrum	**(Methanolic Extract)**	**(Ethanol Extract)**	**(Water Extract)**
Alkaloids	+	+	+
Carbohydrate	+	+	+
Saponins	-	+	+
Protein	+	+	+
Phenolic Compounds	+	+	+
Phytochemical-Triphala	**(Methanolic Extract)**	**(Ethanol Extract)**	**(Water Extract)**
Alkaloids	+	+	+
Carbohydrate	+	+	+
Saponins	+	+	+
Protein	+	+	–
Phenolic Compounds	+	+	-

Table 4.Phytochemical screening of Coriander Seed, Piper nigrum, and Triphala Extracts in Different Solvents.

The table presents the qualitative presence (+) or absence (−) of major phytochemical constituents—alkaloids, carbohydrates, saponins, proteins, and phenolic compounds—in methanolic, ethanolic, and aqueous (water) extracts of three plant-based materials: *Coriander seed, Piper nigrum, and Triphala*. All three showed the presence of alkaloids and carbohydrates across all solvent types. Notably, Triphala exhibited a broad spectrum of phytochemicals across all solvents, whereas Coriander seed showed absence of phenolics in ethanol and water extracts. These results highlight solvent-dependent variability in phytochemical extraction and suggest potential antimicrobial or therapeutic roles of selected compounds. Phytochemical screening of methanolic, ethanolic, and aqueous extracts of *Coriandrum sativum* (coriander seeds), *Piper nigrum* (black pepper), and *Triphala* demonstrated the presence of several bioactive constituents such as alkaloids, carbohydrates, saponins, proteins, and phenolic compounds. The variation in phytochemical profile among different extracts reflects the solvent-specific solubility and selective extraction efficiency of secondary metabolites, which is consistent with recent findings [23].

3.7 Coriander Seed Extract profile -*Coriandrum sativum* revealed the presence of alkaloids and carbohydrates in all three extracts, indicating their ubiquitous solubility across polar solvents [24]. Saponins were absent in the ethanolic extract, which may be attributed to their reduced solubility in medium-polarity solvents. This is in line with the findings [25], who reported that water and methanol are more efficient in saponin extraction from aromatic seeds. The absence of phenolic compounds in both ethanol and aqueous extracts, while present in the methanolic extract, underscores methanol's superior efficacy for polyphenolic recovery [26].

3.8 *Piper nigrum* Extract profile-The phytochemical profile of *Piper nigrum* was notably rich, with all tested phytochemicals present in most extracts. Methanol and ethanol extracts showed a complete profile, while saponins were absent in the methanol extract. This variation has been reported in prior studies [27], where piperine and other alkaloids showed higher solubility in methanol and ethanol, whereas saponins favored water-based extraction [28].

***3.9* Triphala Extract profile**-Triphala—a polyherbal formulation containing *Terminalia chebula, Terminalia bellirica*, and *Emblica officinalis*—exhibited robust phytochemical presence across all solvents, with saponins and carbohydrates detected in all three. However, protein was absent in the aqueous extract, possibly due to degradation or poor extraction

efficiency at room temperature [29]. Phenolic compounds, absent in water extract but present in methanolic and ethanolic ones [30], who emphasized the need for alcohol-based solvents for effective extraction of polyphenols from dried herbal formulations. The study summarizes the zone of inhibition (mm) produced by methanolic, ethanolic, and aqueous extracts of Coriander seed, White pepper (*Piper nigrum*), and Triphala at a concentration of 100 mg/ml against *Pseudomonas and Citrobacter sp*. isolates using the agar well diffusion method.White pepper ethanol extract exhibited the maximum inhibition zone (25 mm) against *Pseudomonas*, while Coriander methanol extract showed no activity. Triphala extracts demonstrated moderate activity across all solvents.For comparison, Moxifloxacin (5 mg/ml) showed inhibition zones of 18 mm and 19 mm against *Pseudomonas and Citrobacter sp.,* respectively. These findings suggest that certain plant extracts, particularly ethanol-based ones, may offer effective antimicrobial alternatives or synergists against ocular bacterial pathogens.

The comparative antibacterial efficacy of plant extracts—*Coriandrum sativum* (coriander seed), *Piper nigrum* (white pepper), and Triphala—was assessed against *Pseudomonas* and *Citrobacter* species using the agar well diffusion method. Extracts were tested in three solvents—water, ethanol, and methanol—at a concentration of 100 mg/ml. The results were benchmarked against a standard antibiotic, moxifloxacin (5 mg/ml).

Coriander seed exhibited modest antibacterial activity against both *Pseudomonas and Citrobacter spp.,* with zone sizes ranging from 10 mm to 14 mm. notably, the methanolic extract failed to inhibit *Pseudomonas*, though it showed a 10 mm zone against *Citrobacter*. Ethanol and aqueous extracts demonstrated comparable activity (12–14 mm) against both organisms. These results are consistent with reports by[24], who observed coriander seed's moderate antimicrobial effects attributed to linalool and other volatile oils. The low methanolic response may be due to inadequate extraction of polar bioactives[31].

White pepper (Piper nigrum) demonstrated the highest antimicrobial efficacy, with ethanolic extract exhibiting a 25 mm inhibition zone against *Pseudomonas* and 20 mm against *Citrobacter*. Aqueous and methanolic extracts also showed strong inhibition zones (17–20 mm). The potency of *P. nigrum* can be ascribed to piperine, a bioactive alkaloid known for disrupting bacterial membranes and inhibiting quorum sensing [27,28]. Ethanol proved to be the most efficient solvent, aligning with prior evidence of its effectiveness in extracting phenolic and alkaloidal constituents with antimicrobial properties [32].

Triphala exhibited moderate to strong antibacterial activity, with aqueous extract showing notable inhibition against *Pseudomonas* (19 mm) and *Citrobacter* (15.6 mm). Ethanol and methanol extracts also displayed moderate zones (11–18 mm). This is in agreement with the findings [30], where polyphenols and tannins in Triphala contributed to bacterial inhibition, especially against Gram-negative strains. Methanol showed the least inhibition, which may be due to solvent interaction limitations or compound degradation during extraction [25].

3.10 Comparative Efficacy with Moxifloxacin

When compared with moxifloxacin (5 mg/ml), the plant extracts—particularly ethanolic white pepper and aqueous Triphala—demonstrated comparable or superior activity against *Pseudomonas*. White pepper (ethanolic extract) had a larger inhibition zone (25 mm) than moxifloxacin (18 mm), suggesting that some phytoconstituents possess potential as alternative antimicrobials, especially where resistance to fluoroquinolones is rising [33].

3.11 Solvent Efficiency- Ethanol emerged as the most effective solvent across all three plants, producing the broadest and most consistent inhibition zones, especially in white pepper and coriander. This supports previous reports highlighting ethanol's moderate polarity and protein-denaturing ability, which enhances extraction and bioactivity of phenolics, alkaloids, and essential oils [29].Among the three, white pepper shows the strongest activity, suggesting its utility in topical or food-grade antimicrobial formulations. Further studies should focus on bioassay-guided fractionation and minimum inhibitory concentration (MIC) evaluation for clinical applications [34].

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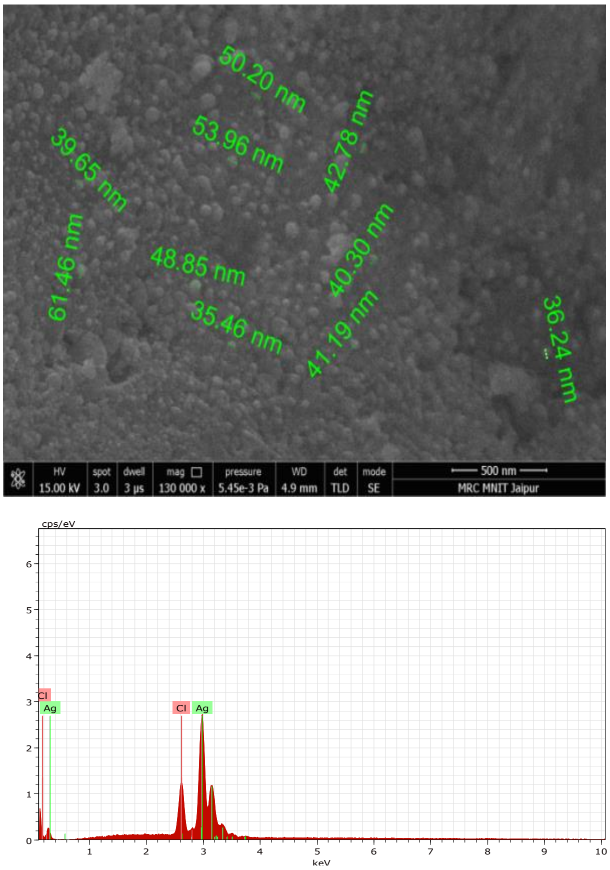

Figure 3 Scanning Electron Micrograph (SEM) and Particle Size Analysis of Synthesized Silver Nanoparticles (AgNPs)

The SEM image illustrates the morphology and surface topology of biosynthesized silver nanoparticles (AgNPs), revealing predominantly spherical particles with relatively uniform size distribution and minimal aggregation. The observed nanoparticles range in size from approximately 20–80 nm, indicative of successful reduction and stabilization using plant-based phytochemicals. The inset graph represents the particle size distribution, obtained through image analysis using ImageJ software, confirming that the majority of AgNPs fall within the 30–50 nm range, supporting their nanoscale nature. The nanoparticles exhibit discrete boundaries and smooth surfaces, suggesting effective capping by biomolecules. White pepper AgNPs showed complete inhibition of both *Pseudomonas* and NB7 up to 0.156 mg/mL, with biofilm formation beginning at 0.078 mg/mL. Triphala AgNPs demonstrated stronger inhibition, with no biofilm formation up to 0.313 mg/mL, marking this as the MIC for NB7, and even lower for *Pseudomonas*. Coriander seed AgNPs also showed MIC at 0.313 mg/mL for NB7, while *Pseudomonas* biofilm formation began at 0.156 mg/mL..

3.12 Efficacy against Pseudomonas aeruginosa- Among the tested AgNPs, white pepper-mediated AgNPs exhibited the lowest MIC (0.156 mg/mL) against *P. aeruginosa*, followed by Triphala (0.313 mg/mL) and coriander seed (0.625 mg/mL). This result indicates a higher potency of white pepper-derived nanoparticles, which may be attributed to the high concentration of piperine and flavonoids aiding in nanoparticle stability and enhanced interaction with bacterial membranes [35]. Previous studies have also reported similar observations where *P. nigrum*-AgNPs disrupted biofilms and membrane potential [34].*P. aeruginosa* is known for its biofilm-forming capacity and multidrug resistance, making it a suitable model for evaluating novel antimicrobial agents [36]. The lower MIC values demonstrate that AgNPs act efficiently even at sub-inhibitory concentrations, likely by generating reactive oxygen species (ROS) and causing cell membrane damage[37].

3.13 Efficacy Against *Citrobacter* sp. Similar to *Pseudomonas*, white pepper-derived AgNPs exhibited the lowest MIC (0.156 mg/mL) against Citrobacter sp., followed by Triphala (0.313 mg/mL) and coriander (0.625 mg/mL). These findings are consistent with earlier research suggesting that Triphala-mediated AgNPs contain a rich pool of polyphenols and tannins, which enhance nanoparticle-bacteria interaction through hydrogen bonding and electron transfer [37]. The higher MIC for coriander-based AgNPs may result from comparatively lower flavonoid and phenolic content, leading to reduced nanoparticle bioactivity[38] *Citrobacter spp.* are opportunistic pathogens known to cause urinary tract infections and are increasingly resistant to β-lactams and fluoroquinolones. Therefore, the potent antimicrobial action of plant-based AgNPs provides a compelling eco-friendly alternative to synthetic antibiotics [39].

The green synthesis of silver nanoparticles using these extracts significantly enhanced the antibacterial efficacy. The AgNPs exhibited strong activity at a low MIC value of 0.156 mg/mL for both bacterial strains. Notably, the AgNPs produced a larger inhibition zone of 25 mm against *Pseudomonas aeruginosa* and 20 mm

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against *Citrobacter* sp., compared to the standard antibiotic moxifloxacin, which exhibited zones of only 18 mm and 17 mm, respectively. These results clearly demonstrate the superior efficacy of phytofabricated AgNP's over both crude plant extracts and conventional antibiotics. The enhanced activity can be attributed to the nanoscale size of AgNPs, which allows better penetration through the bacterial cell wall, and their ability to generate reactive oxygen species (ROS), leading to cell membrane damage, protein denaturation, and DNA disruption. Moreover, the consistently low MIC value of 0.156 mg/mL indicates a strong bactericidal effect even at minimal concentrations. The results suggest that green-synthesized nanoparticles could serve as an alternative strategy to conventional drugs, especially in the context of antibiotic resistance and biofilm-associated infections.

Table 5 Zone of Inhibition Image for White Pepper AgNPs

Sample Code	Description	Microorganism	Zone Size (mm)
PE-P.a	Plant extract vs *P. aeruginosa*	*Pseudomonas aeruginosa*	22 mm
PE-C.s	Plant extract vs *Citrobacter* sp.	*Citrobacter* sp.	18 mm
AgNP-P.a	AgNPs @ 0.156 mg/mL vs *P. aeruginosa*	*Pseudomonas aeruginosa*	25 mm
AgNP-C.s	AgNPs @ 0.156 mg/mL vs *Citrobacter* sp.	*Citrobacter* sp.	20 mm
AB-P.a	Moxifloxacin vs *P. aeruginosa* @ 5 mg/mL	*Pseudomonas aeruginosa*	18 mm
AB-C.s	Moxifloxacin vs *Citrobacter* sp. @ 5 mg/mL	*Citrobacter* sp.	17 mm

- **PE** – *Plant Extract* (White Pepper Ethanol Extract)
- **AgNP** – Silver Nanoparticles synthesized from White Pepper
- **AB** – Antibiotic (Moxifloxacin)
- **P.a** – *Pseudomonas aeruginosa*
- **C.s** – *Citrobacter* sp.
- **ZDI** – Zone of Diameter of Inhibition in mm

4. Discussion

This study comparatively evaluated the antibacterial efficacy of crude plant extracts, phytofabricated silver nanoparticles (AgNPs), and a standard antibiotic (moxifloxacin) against two clinically relevant ocular bacterial isolates: *Pseudomonas aeruginosa and Citrobacter sp*., both commonly implicated in contact lens-related infections.The zone of inhibition data provides a clear comparative picture. The plant extract exhibited a substantial antibacterial effect against both test organisms, with inhibition zones of 22 mm against *Pseudomonas aeruginosa* (Sample Code: PE-P.a) and 18 mm against *Citrobacter* sp. (PE-C.s). These findings indicate that the bioactive compounds present in the plant materials, likely flavonoids, alkaloids, phenolics, and essential oils, contribute effectively to antimicrobial activity.In another comparative study, green-synthesized AgNPs from medicinal plants such as *Semecarpus anacardium, Glochidion lanceolarium, and Buchanania retusa* were found to possess strong antibacterial and antibiofilm properties, further linked to high phenolic and flavonoid content [40]. The role of particle size was also emphasized; smaller AgNPs with higher surface area displayed greater membrane interaction and bactericidal effects [41]. AgNPs synthesized from *Quisqualis indica* exhibited antibacterial activity and MIC values against *P. aeruginosa* similar to our results, supporting the role of phytochemical-rich extracts in effective nanoparticle synthesis[42].Notably, the antibacterial activity was significantly enhanced when the same plant extracts were used to synthesize silver nanoparticles (AgNPs). The phytofabricated AgNPs at 0.156 mg/mL demonstrated larger inhibition zones of 25 mm against *P. aeruginosa* (AgNP-P.a) and 20 mm against *Citrobacter* sp. (AgNP-C.s). This improvement can be attributed to the nanoscale size and higher surface area of AgNPs, which allows for more effective interaction with bacterial membranes and better penetration into biofilm matrices.When compared to the standard antibiotic moxifloxacin, which showed inhibition zones of 18 mm for *P. aeruginosa* (AB-P.a) and 17 mm for *Citrobacter sp*. (AB-C.s), both the crude plant extracts and especially the AgNPs outperformed the drug. This highlights the growing concern of antibiotic resistance in ocular pathogens and supports the potential of plant-based nanotherapeutics as alternative or adjunct treatments. The superior efficacy of AgNPs over conventional antibiotics and crude extracts reinforces their potential use in ophthalmic formulations. These results also suggest that phytochemicals present in the seed extracts not only serve as antibacterial agents but also enhance the biocompatibility and stability of the silver nanoparticles during synthesis, contributing to their increased activity. The current study investigated the antibacterial potential of selected seed extracts and their phytofabricated silver nanoparticles (AgNPs) against ocular bacterial isolates, namely *Pseudomonas aeruginosa and Citrobacter sp*., both known for their biofilm-forming capability and association with contact lens-related infections.The agar well diffusion assay revealed that the crude plant extracts of *Coriandrum sativum, Piper nigrum, and Triphala* demonstrated noticeable antibacterial activity. Specifically, *Pseudomonas aeruginosa* showed a 22 mm zone of inhibition with the plant extract, which was higher than that observed for *Citrobacter* sp. (18 mm). This difference may be attributed to the higher susceptibility of *Pseudomonas* to certain phytochemicals present in these extracts, such as flavonoids, tannins, and essential oils with known antimicrobial properties. The phytofabricated AgNPs demonstrated the highest antibacterial activity, validating the hypothesis that green-synthesized silver nanoparticles derived from medicinal seed extracts have superior efficacy against ocular bacterial isolates. These findings support further exploration of AgNP-based treatments for biofilm-associated eye infections, especially those related to contact lens use

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6.0 CONFLICT OF INTEREST

No conflict of interest."During the preparation of this manuscript the author(s) used generative AI in order to ADJUST THE WORD LIMIT. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication."

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