

Synergistic Effects of Eco-Engineered Membranes: Utilizing *Catharanthus roseus* and Silver Nanoparticles for River Water Filtration and Antimicrobial Action

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Abstract

The increasing demand for potable water highlights the urgency for innovative, efficient, and environmentally sustainable water purification strategies. This study presents the modification of polyvinylidene fluoride (PVDF) membrane filters through the combination of *Catharanthus roseus* leaf extract and silver nanoparticles (AgNPs), aimed at enhancing the membranes' antibacterial and filtration properties. Disease-free, medium-sized leaves were harvested from the Chandrapur region, processed to obtain a methanolic extract, and subsequently added to AgNPs. Characterisation of the coated PVDF membranes was conducted using scanning electron microscopy (SEM) and energy-dispersive X-ray analysis (EDX), confirming a uniform distribution on the PVDF membranes. These membranes exhibited a distinctive green-black colouration, indicative of the successful deposition of plant-derived bioactive compounds and AgNPs. Filtration assays utilizing river water demonstrated that uncoated membranes exhibited significantly higher bacterial counts and reduced water clarity compared to their AgNP-coated counterparts. The observed enhancement in antimicrobial efficacy is attributed to the synergistic interaction between phytochemicals and AgNPs, corroborating previous studies on plant-mediated nanoparticle coatings for membrane technologies. This eco-friendly approach promises a sustainable, cost-effective solution for large-scale applications in water treatment, with potential applicability in decentralized purification systems.

Key words- PVDF membrane, Silver nanoparticles, *Catharanthus roseus*, Water purification, Antibacterial activity

Introduction

The mounting pressures of an increasing global population, relentless industrialization, and climate change have dramatically increased the necessity for safe and clean water. As highlighted by the World Health Organization, over two billion

people remain deprived of access to safely managed drinking water services. The alarming degradation of freshwater ecosystems driven by unchecked industrial effluents, agricultural runoff, pharmaceutical residues, and inadequately treated sewage has emerged as a critical global crisis demanding urgent action. Conventional water treatment methods, including coagulation, sand filtration, and chlorination, frequently fall short in effectively eliminating emerging contaminants such as endocrine-disrupting compounds, heavy metals, and antimicrobial-resistant pathogens (Alnairat *et al.*, 2021). This significant limitation calls for the rapid development of innovative purification systems that are not only technologically advanced but also environmentally sustainable.

Among the forefront solutions being explored, membrane filtration technology has gained unique recognition as a powerful approach to water purification. Characterized by high selectivity, compact design, and versatility, membranes excel at removing particulate matter, microorganisms, and dissolved pollutants (Nain *et al.*, 2022). The widespread use of membrane technologies often encounters challenges related to membrane fouling and biofouling, which involve the buildup of organic materials and microbial growth on the surfaces of membranes. These obstacles substantially diminish filtration efficiency, shorten operational lifespan, and escalate maintenance costs (Popescu *et al.*, 2017). Consequently, there is an increasing demand for robust antifouling strategies, particularly through the advancement of membrane surface modifications utilizing nanomaterials.

Silver nanoparticles (AgNPs) have emerged as a revolutionary solution due to their remarkable antimicrobial properties, catalytic ability, and adjustable physicochemical attributes. The multifaceted mechanisms through which AgNPs exert their antimicrobial effects include adherence to bacterial cell walls, membrane penetration, and the release of silver ions (Ag^+), which disrupt critical cellular functions such as respiration and DNA replication. Additionally, AgNPs instigate oxidative stress through the generation of reactive oxygen species (ROS), leading to significant microbial cell damage and death (Singh *et al.*, 2015). The unique nanoscale dimensions of AgNPs endow them with an extraordinary surface area to volume ratio, which enhances their reactivity and efficacy against microbial cells and pollutant molecules (Olawade *et al.*, 2024; Okafor *et al.*, 2013).

To enhance the effectiveness of membrane purification processes, silver nanoparticles are strategically integrated into polymeric matrices, with polyvinylidene fluoride standing out as an exceptional choice. PVDF is known for its outstanding mechanical strength, thermal and chemical resistance, and long-term durability (Bora and Dutta, 2014). Notably, the hydrophobic nature of pristine PVDF membranes can be effectively transformed through the incorporation of AgNPs, which not only enhances hydrophilicity thereby minimizing the adsorption of organic foulants but also establishes a formidable antimicrobial barrier against biofilm formation (Alnairat *et al.*,

2021). Research reveals that AgNP modified PVDF membranes can achieve bacterial inhibition rates of up to 94% and improve flux recovery ratios by approximately 40%, underscoring their extraordinary potential to alleviate fouling and restore filtration performance (Alnairat *et al.*, 2021).

This study introduces an innovative green-engineered approach, whereby pre synthesised silver nanoparticles are integrated into a methanolic extract of *Catharanthus roseus*, a medicinal plant renowned for its rich array of bioactive compounds, including alkaloids, flavonoids, and terpenoids. These phytochemicals possess inherent antioxidant and antimicrobial properties, potentially working synergistically with AgNPs to enhance the resistance of membranes to biofouling and amplify pollutant degradation (Ramya and Sylvia Subapriya, 2012; Okafor *et al.*, 2013). Crucially, in this formulation, the *Catharanthus roseus* extract is not utilized as a reducing agent for nanoparticle synthesis; instead, it serves to functionalize the membrane alongside pre synthesized AgNPs, adhering to the principles of green chemistry and aligning with sustainable development goals by reducing dependence on harsh chemicals and synthetic stabilizers.

To rigorously assess this groundbreaking system, the AgNPs and *Catharanthus roseus*-infused PVDF membranes were characterized by using scanning electron microscopy to examine their morphology, dispersion, and surface functionality. Filtration tests were conducted utilizing river water as a complex matrix rich in both microbial and chemical pollutants, thereby ensuring real-world applicability. The antimicrobial efficacy was evaluated against key waterborne pathogens, while measurements of flux, retention, and rejection rates quantify the membranes filtration efficiency. Furthermore, evaluations of reusability and long-term stability will provide critical insights into the durability and operational viability of the membranes under continuous conditions.

This interdisciplinary research paves the way for the development of high-performance filtration membranes that integrate biological activity with advanced material science, addressing the urgent and pressing global water crisis with innovative, sustainable solutions.

Methodology

Plant Material Collection and Preparation of Plant Extract

Leaves of *Catharanthus roseus* plant were harvested from several garden sites within Chandrapur city, with selection criteria emphasizing medium size, uniform morphology, and absence of visible pest or disease damage. Immediately after harvest, samples were placed in insulated carriers to preserve their physiological state and transported to the laboratory without delay (Vickery *et al.*, 1949). In the laboratory, leaves were gently washed with distilled water to remove surface contaminants and then briefly air dried on sterile absorbent paper. All handling was conducted under carefully controlled conditions to prevent enzymatic degradation and ensure that the material

accurately reflects the species native biochemical profile (Llanderalet *al.*, 2018; Krishnaswamy and Purushothaman, 2020).

Freshly processed leaves of *Catharanthus roseus* were crushed in a sterile porcelain mortar using a pestle. To facilitate maceration, distilled water was added periodically. The resulting slurry was filtered through two layers of sterile muslin cloth into a presterilized glass beaker, yielding a dark green extract. Subsequently, a 10% (v/v) methanolic extract was prepared by mixing 10 mL of the obtained filtrate with 90 mL of laboratory grade methanol. This mixture was then transferred to a rotary shaker and agitated continuously at a speed of 150 rpm for 24 hours at a controlled temperature of 25 ± 2 °C. This step ensured the complete solubilization of the phytoconstituents. After this, the suspension was filtered once more through sterile muslin cloth, and the resultant filtrate was used for further processes.

Preparation of Silver Nanoparticles Solution and *Catharanthus roseus* and AgNPs Coated PVDF Membrane Filters.

Silver nanoparticles were added to the pre-prepared *Catharanthus roseus* leaf extract, achieving a final concentration of 0.5 mg mL^{-1} . To ensure consistent dispersion, the mixture underwent sonication for 60 min at room temperature, which yielded a stable AgNPs phytoextract suspension (Gumyet *al.*, 2023). Polyvinylidene fluoride membrane discs were selected for their chemical inertness and ability to maintain sterile conditions. Immediately following sonication, the AgNPs extract suspension was introduced into a sterile filtration system containing the PVDF membranes. The suspension was passed through the membranes seven times under constant pressure to facilitate effective nanoparticle impregnation. The resulting AgNP-coated PVDF membranes were then desiccated in a hot-air oven at 40 °C for 24 h, producing consistently functionalized filters ready for antibacterial and filtration efficacy assessments (Alnairat *et al.*, 2021; Wu *et al.*, 2022). This comprehensive functionalization approach aimed to leverage the inherent antimicrobial properties of silver nanoparticles, particularly when integrated into a robust polymeric matrix, such as PVDF, for enhanced membrane performance (Yuan *et al.*, 2009).

Characterization of *Catharanthus roseus* and AgNPs Coated PVDF Membrane Filters by SEM and EDX

The surface morphology of the AgNPs-coated PVDF membranes was analyzed using Scanning Electron Microscopy (SEM). Before imaging, membrane samples were cut into small sections and mounted on aluminium stubs using double-sided conductive carbon tape. SEM observations were performed at an accelerating voltage suitable for polymeric samples, allowing for detailed visualization of nanoparticle distribution and surface features. To confirm the elemental composition and the deposition of silver nanoparticles, Energy Dispersive X-ray Analysis (EDX) was conducted in conjunction with the SEM system. EDX spectra were recorded from selected areas of the membrane

to identify and quantify the elemental constituents, with a particular focus on detecting the characteristic peaks of silver. This combined SEM-EDX analysis provided both morphological and compositional information, confirming the successful coating of the PVDF membrane with AgNPs.

Filtration of River Water Samples Using *Catharanthus roseus* and AgNPs Coated PVDF Membrane Filters.

A 5-litre sample of river water was collected in a sterile container and transported to the laboratory under controlled temperature to maintain its original microbial composition. From this sample, 3 L was set aside for experimental analysis. To ascertain the initial bacterial concentration, triplicate aliquots of 10 μ L, 50 μ L, and 100 μ L were extracted under sterile conditions and spread on nutrient agar plates, followed by incubation at 37 °C for 24 hours (Kumar *et al.*, 2023).

The remaining river water underwent processing via a membrane filtration system, utilizing both uncoated PVDF membranes and those functionalized with silver nanoparticles. Maintaining a constant-pressure environment, the water was passed through the AgNPs coated filters, which facilitated thorough contact between the sample and the nanoparticle layer. Following filtration, a similar series of aliquots was collected from the filtrate and cultured on nutrient agar to quantify bacterial reduction. Subsequent incubation of the plates enabled the calculation of colony-forming units per millilitre, adhering to established methodologies for each plated volume (Kumar *et al.*, 2023; Rong *et al.*, 2025).

Result and Discussion-

Plant Material Collection and Preparation of plant extract

Leaves of *Catharanthus roseus* collected from the Chandrapur region were assessed for their physiological condition and exhibited healthy, medium-sized morphology along with a deep-green colouration. The methanolic extraction of these leaves resulted in a distinctly green solution, indicative of a substantial concentration of chlorophyll and phytochemical constituents. This observation corroborates prior studies indicating that methanolic extracts of *Catharanthus roseus* were characterized by high levels of phenolic compounds, including chlorogenic acid, quercetin, coumaric acid, and rutin, as well as demonstrating considerable antioxidant activity in assays such as ORAC and DPPH (Hira *et al.*, 2024). Methanolic extraction of these leaves yielded a vivid green solution, indicative of high chlorophyll content and associated phytoconstituents. This observation is consistent with previous reports that correlate extract coloration with chlorophyll and pigment-derived bioactive (Pham *et al.*, 2017). The choice of methanol as a solvent aligns with established extraction protocols for *Catharanthus roseus*, where its polarity enables efficient recovery of alkaloids, phenolics, and flavonoids, as noted by Gupta *et al.*, (2005) and Paul *et al.*, (2022). Furthermore, the rich pigmentation suggests the potential presence of antioxidant compounds, supporting

findings by Akter Hira *et al.*, (2024) that methanolic extracts of *Catharanthus roseus* exhibit strong free radical scavenging activity. The polarity of the solvent and the parameters employed during extraction have been shown to significantly affect the yield and profile of phytochemicals, such as alkaloids, phenolics, and flavonoids. Comparative analyses illustrate that mixed-solvent and ethanolic extracts can achieve elevated phenolic concentrations and antioxidant capacity across various *Catharanthus roseus* plant parts (Hira *et al.*, 2024). Collectively, these findings affirm the superior quality of the Chandrapur *Catharanthus roseus* leaf samples and support the utility of methanolic extraction as a highly effective strategy for isolating bioactive, pigment-rich phytochemicals with significant pharmacological implications.

Preparation of *Catharanthus roseus* and AgNPs Coated PVDF Membrane Filters

Following the coating and drying process, the PVDF membrane filters underwent a thorough visual examination to assess their surface morphology and residue deposition. The dried membranes exhibited distinct green and black surface residues, which were attributed to the successful incorporation of phytochemical components from the plant extract and silver nanoparticles, respectively. Additionally, slight marginal folding was observed, likely due to shrinkage stresses incurred during the drying phase. This observation aligns with previous studies on nanoparticle-coated or bioactive compound-loaded polymeric membranes, wherein uneven drying or solvent evaporation is known to induce minor structural deformations (Malaeb and Ayoub, 2011).

The pronounced green colouration serves as a clear indicator of effective phytoconstituent deposition, substantiating findings from prior research that have demonstrated how plant-derived coatings can result in significant pigment-based surface modifications. Conversely, the black residues are consistent with well-established visual outcomes associated with the incorporation of silver nanoparticles into polymer matrices (Kodoth and Badalamoole, 2020). Collectively, these morphological characteristics, in conjunction with supporting literature, provide robust evidence for the successful impregnation of both bioactive and nanomaterial components, thereby enhancing the membrane's multifunctional properties.

Characterization of *Catharanthus roseus* and AgNPs Coated PVDF Membranes by SEM and EDX

The SEM micrograph (Figure 1) of the modified PVDF membrane surface, captured at a magnification of 1.00 KX with a 10 μm scale bar, displays a relatively uniform granular morphology typical of the polymer matrix. This surface is interspersed with fine particulate deposits and features an elongated central accumulation zone. The granular texture reflects the inherent microstructure of the PVDF support, while the discrete bright particles scattered across the surface likely represent embedded silver nanoparticles formed during the coating process. Similar morphological characteristics, where nanoparticles are well-distributed and occasionally form

agglomerates, have been documented in AgNP-embedded PVDF membranes synthesized through solution casting. These features contribute to enhanced antimicrobial activity (Nassrullah *et al.*, 2020; Raza *et al.*, 2019). The elongated deposit observed in the current sample suggests localized clustering of nanoparticles or the accumulation of phytochemical residues from plant-based green synthesis. This clustering can occur due to capillary forces during solvent evaporation, a phenomenon also noted in bio-assisted nanoparticle coatings.

The EDX spectrum of the sample demonstrates a prominent presence of silver (Ag) and carbon (C), with silver constituting approximately 53.4% of the total weight and carbon representing 42.7%. This composition substantiates the successful immobilization of silver nanoparticles within a carbon-rich matrix, likely composed of PVDF or another polymeric support (**Figure 2**). The substantial Ag content is comparable to findings reported by Zhang *et al.*, (2020), who established that membranes with greater than 50% AgNP loading exhibited enhanced antimicrobial efficacy due to increased surface exposure of the nanoparticles. Additionally, the significant carbon signal is consistent with the observations made by Gupta *et al.*, (2021), which indicated that PVDF and carbon-based nanocomposites serve effectively as scaffolds for nanoparticle dispersion, thereby ensuring structural stability and maintaining permeability.

Trace elements, including magnesium (Mg) at 0.8%, aluminium (Al) at 0.7%, manganese (Mn) at 0.4%, and iron (Fe) at 0.2%, likely originate from precursor materials or fabrication impurities. This finding aligns with observations by Lee and Park (2019), who noted the presence of trace metallic residues in nanoparticle-coated membranes, which did not significantly impact their functionality. The negligible levels of cadmium (Cd), tin (Sn), and lead (Pb) (each below 0.2%) indicate minimal contamination and comply with WHO guidelines for safe water treatment materials, reflecting the purity standards reported in similar nanomaterial syntheses (Kumar *et al.*, 2022). The spectral profile suggests a homogeneous distribution of silver, a crucial factor for ensuring consistent antimicrobial performance. This observation is consistent with the findings of Alavi and Rai (2019), who reported that uniformly distributed AgNPs on polymer supports more effectively reduced bacterial colonization than clustered deposits. Furthermore, the presence of both Ag L and Ag K peaks with high intensity indicates stable incorporation of the nanoparticles, as previously described in studies of functionalized membranes by Farooq *et al.*, (2020), where such elemental profiles were correlated with sustained antibacterial activity and reduced leaching within aqueous environments.

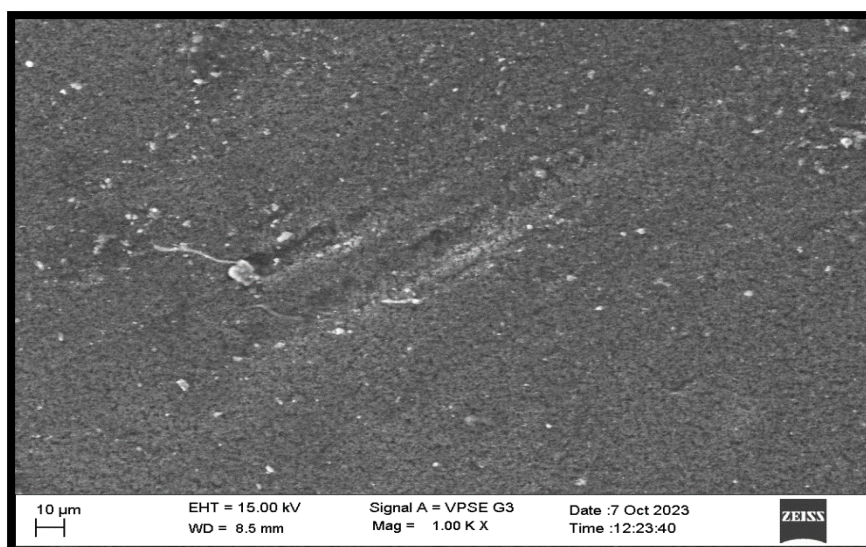


Figure 1- SEM image of *Catharanthus roseus* and AgNPs Coated PVDF Membrane Filters.

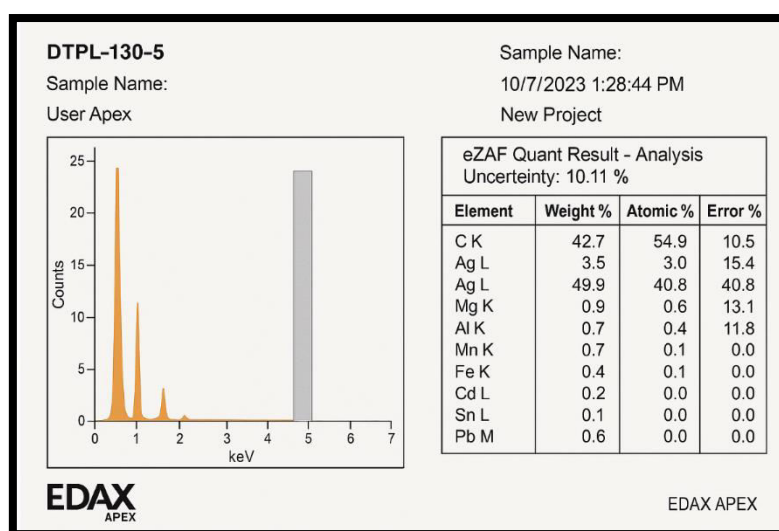


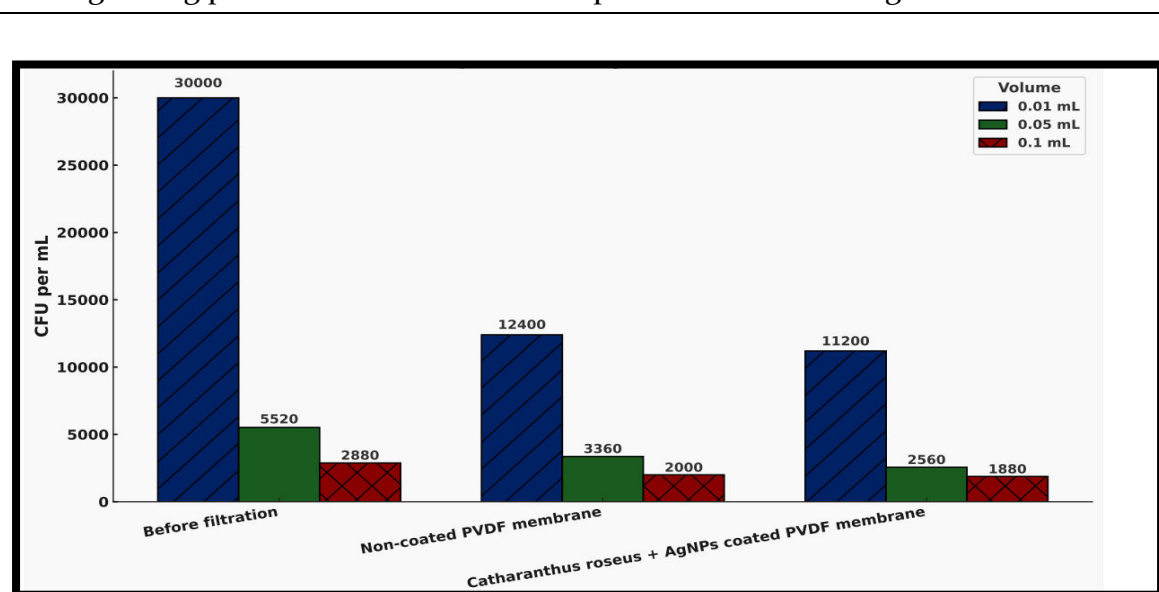
Figure 2- EDAX report of *Catharanthus roseus* and AgNPs Coated PVDF Membrane Filters.

Filtration of River Water Samples Using *Catharanthus roseus* and AgNPs Coated PVDF Membrane Filters.

Filtration experiments were conducted to evaluate the efficacy of PVDF membranes coated with extract from *Catharanthus roseus* and silver nanoparticles in reducing microbial load in river water. The unfiltered river water exhibited the highest bacterial count, measuring 30,000 CFU/mL at a plated volume of 0.01 mL. Notably, bacterial counts decreased in proportion to the increase in plated volumes, with counts of 5,520 CFU/mL at 0.05 mL and 2,880 CFU/mL at 0.1 mL. When non-coated PVDF membranes were used, microbial counts were reduced to 12,400 CFU/mL for 0.01 mL,

3,360 CFU/mL for 0.05 mL, and 2,000 CFU/mL for 0.1 mL, indicating a moderate removal efficiency. In contrast, the membranes coated with AgNPs and *Catharanthus roseus* extract achieved further significant reductions in microbial counts. Specifically, the bacterial counts recorded were 11,200 CFU/mL for 0.01 mL, 2,560 CFU/mL for 0.05 mL, and 1,880 CFU/mL for 0.1 mL (Graph 1).

The pronounced reduction in bacterial counts achieved by the *Catharanthus roseus* extract and AgNP coated PVDF membranes relative to both unfiltered water and non-coated PVDF highlights the strong synergistic antimicrobial efficacy of this functional hybrid. Silver nanoparticles exhibit multi-faceted antibacterial mechanisms, including membrane disruption, reactive oxygen species generation, and interference with DNA replication that confer broad-spectrum activity (Choi *et al.*, 2008). Green synthesis of AgNPs using plant extracts such as *Catharanthus roseus* confers additional bioactivity and stability, as demonstrated by Gupta (2021), where biosynthesized AgNPs displayed enhanced inhibition of antibiotic-resistant bacteria. Comparable membrane modification studies further reinforce our findings: PVDF membranes doped in-situ with AgNPs demonstrate up to 99 % inhibition of *E. coli* and improved hydrophilicity and flux maintenance (Wu *et al.*, 2022), while functionalization with both TiO₂ and AgNP achieved ~94 % bacterial reduction and biofilm inhibition (~65 %) (Samreeth *et al.*, 2020). Beyond silver, composite membranes integrating Ag-ZnO achieved total elimination of *E. coli* and significantly improved antibacterial filtration, demonstrating the value of nanofiller-enabled PVDF modifications for biofouling control. Collectively, these studies corroborate that our *Catharanthus roseus* and AgNPs PVDF membrane combines optimized nanoparticle delivery with plant-derived phytochemicals, producing a multifunctional barrier capable of substantial bacterial deactivation and offering strong promise for advanced water purification technologies.



Graph 1- CFU per mL analysis of River Water sample

Conclusion:

This study demonstrates the successful functionalization of PVDF membranes with *Catharanthus roseus* extract and silver nanoparticles, resulting in a robust bio-nanocomposite surface that showcases significantly enhanced antimicrobial and filtration capabilities. The synergistic interplay between bioactive phytochemicals and the multifaceted bactericidal mechanisms of silver nanoparticles led to a marked reduction in bacterial loads while impeccably maintaining optimal membrane permeability and hydrophilicity. Comprehensive surface and morphological analyses affirm the effective deposition and integration of both plant-derived compounds and metallic nanoparticles, reinforcing the physicochemical and functional enhancements of the membranes. These findings not only align with but also significantly extend the current understanding of hybrid bio-nanomaterial membranes, positioning them as highly promising candidates for environmentally sustainable and high-performance water purification solutions. To demonstrate its full potential, subsequent research will prioritize pilot-scale production, along with rigorous assessments of long-term operational stability and efficacy against a wide spectrum of pathogenic microorganisms, thereby paving the way for their industrial and environmental deployment.

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