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# Anticancer, antimicrobial potential of silver nanoparticles biosynthesized from *Clarias batrachus*, *Oreochromis mossambicus* epidermal mucus on A431 human epidermoid carcinoma cells and nosocomial microbes

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**Abstract**
Nanomedicine crafted from biogenic methods has revolutionized the healthcare sector for their remarkable applications in devising consumer goods for biomedical, semi-healthcare and para-pharmaceutical industries. Silver nanoparticles have prominent dominance over other metallic nanoparticles chiefly due to their antimicrobial and antiproliferative properties. The incessant rise in the cases of skin cancer and nosocomial infection poses a great threat to public welfare thus search for biogenic antibiotics becomes a paramount step towards its prevention. This study presents an eco-friendly, scalable method for mediating the formation of silver nanoparticles from hitherto unexplored *Clarias batrachus* (Catfish), *Oreochromis mossambicus* (Tilapia) skin mucus. The preliminary assessment of the extracted epidermal mucus from both the fishes involves partial purification of the samples before utilizing it for silver nanoparticle synthesis. Molecular characterization of mucus samples was done through UV-Visible Spectroscopy, FTIR Spectroscopy, SEM, TEM which further verified the genesis of silver nanoparticles and provided functional insights into its morphology and distribution. Antimicrobial efficacy assessment of the resultant samples was done by employing an agar cup diffusion assay for the selected nosocomial microbes and accordingly ZOI, MIC, MBC and MFC were determined. MTT assay (3-[4,5-dimethylthiazol-2-yl] 2,5- diphenyltetrazolium bromide) was executed for evaluating the cytotoxic ability of mucus derived Ag-NPs on A431 cells and IC50 value was recorded. Overall, the most potent antimicrobial activity was displayed by Catfish NPs while Tilapia NPs showcases the highest anticancer activity by demonstrating a cytotoxicity of 98% towards A431 cells. This research will aid in adopting a green sustainable alternative approach towards organic-inorganic hybrid nanomedicine formulations and its implementation for tackling skin cancer & Infectious nosocomial diseases.

**Keywords:** A431 cells, catfish, epidermal mucus, nosocomial infection, silver nanoparticles and tilapia

## 1. Introduction
Skin cancer prevalence and its related mortality has escalated in recent years with the incidence rate of 3-5% per year among all malignancies was fundamentally due to its poor detection in the early stage, misdiagnosis as well as underreporting (Wang *et al*. 2021) [1]. After a series of genetic altercations in a single cell these complex heterogenous groups of cancer arise that can be broadly classified into two major types: melanoma skin cancer (MSC) and non-melanoma skin cancers (NMSCs) with the latter one further divided into two subtypes namely basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Human epidermoid carcinoma i.e. A431 cells belong to squamous cell carcinoma (SCC) that is a fastest-growing cancer associated with malignancy of epidermal keratinocytes due to exposure of carcinogens or prolonged UV rays. Current trends of the 21st century have displayed that besides skin cancer, nosocomial infection is also rising at an alarming rate affecting more than 100 million patients globally (Taye *et al*. 2023) [2] along with emergence of new and renewed infectious disease of multidrug resistant pathogens poses menace to public health. Failure of chemotherapy accompanied with misuse of drugs have contributed to rapid generation of

resistance in microbes together with heightened malignancy additionally prolonged hospital stay, poor early diagnosis has resulted into the rise of morbidity & mortality. In this context, nanotechnology or nanoengineering, i.e. an emerging discipline of multifactorial nanoscience that comprises of manipulating elements at nanoscale range, one-billionth or $10^{-9}$ m, were found to be a forerunner and indispensable tool in addressing these new age diseases.

Silver nanoparticles have become a major exponent in the nanomedicine field as these multifaceted microparticles possess higher surface to volume ratio (35-45%) as compared to their bulk materials that induce a quantum effect on the nanostructure thus leading to radical modification in their size, distribution, shape which subsequently enhances their size- dependent antimicrobial and antiproliferative capabilities (Abbas *et al*. 2024) [3]. However, biogenic methods are often limited to plants, microbial extract and their uncontrolled usage has led to biodiversity loss of many medicinal herbs thus exploring other biological resources for nanoparticle crafting becomes a crucial step towards conservation (Mane *et al*. 2021) [4]. Fish mucus remains a novel, unexplored reservoir of amino acids, glycoproteins, lipids that can serve as a potent reductant and stabilizing agents in designing nanomaterials along with being advantageous by not sacrificing and safely returning the fish to nature. *Clarias batrachus* (Catfish), *Oreochromis mossambicus* (Tilapia) are commercial edible fishes of Indian market that are characterizes by their inherent capabilities of secreting a lot of mucus from their epidermal goblet cells that forms a biochemical matrix around the fish which act as a dynamic physicochemical barrier against invading microorganisms as well as imparts distinct lubricating qualities. Fish epidermal mucus is an integral part of innate immunity that also operates as a first line of defense by regular sloughing off its mucus layer consisting predominantly of entrapped opportunistic pathogens and contains repertoire of biochemical molecules. Those broad-spectrum active biomolecules exhibit antimicrobial activities by producing lysozyme, proteases, immuno-globulin, complement proteins, antimicrobial peptides (AMPs), saturated fatty acids (SFA), lectins, C-reactive protein (CRP), alkaline phosphatases (ALP) etc. that can assist in serving the role of bio-reductant /stabilizers in metal nanoparticle consolidation.

In the present study epidermal mucus is sourced from Catfish, Tilapia as a novel biomaterial for biogenic synthesis of silver bio nanoparticles. In-vitro investigation on the antiproliferative and antimicrobial effects of the formulated bio-nanocomposite can help in achieving sustainable development by enhancing the circular economy, providing an effective green alternative to nanostructure fabrication along with bioprospecting the applications of it in combating skin cancer and nosocomial infection.

## 2. Materials and Methods

### 2.1 Specimen collection and maintenance

Live healthy *Clarias batrachus* (27-32 ± 0.5cm,180-221 ± 0.8g), *Oreochromis mossambicus* (12-18 ± 0.5cm, 250-276 ± 0.4g) were randomly sampled and procured from local commercial fish market plus from fish farms near Thane, Mumbai regions respectively with no earlier history of contamination. Prior to selection, fish's morphological features like fins, eyes, gills, texture, lesion free body were examined carefully, and Tilapia were captured using seine net (25mm) in between 7 to 10 am from the farms. After the collection, fishes were transported in plastic buckets filled with dechlorinated water, portable air pump and immediately brought back to the Aquaculture technology laboratory, Department of Life Sciences, University of Mumbai. Fishes were acclimatized by introducing them to glass aquariums (120 L) equipped with conditioned water, an aeration system for 14 days and 25% of water was replaced with fresh dechlorinated water on every alternate day to maintain hygienic conditions additionally they were fed twice daily with commercial food consisting of 32% crude protein.

#### 2.1.2 Epidermal mucus collection and preservation

Catfish (n=15), Tilapia (n=15) were starved for dietary stress till 24h prior to mucus collection that enhances overall mucus production. Utilizing a sterile plastic spatula the mucus was scarped from the dorso-lateral surface of the fishes although mucus extraction from the ventral cutaneous region was avoided for preventing the contamination by urine-genital, intestinal excretions and was stored at -80 °C for further molecular analysis.

#### 2.1.3 Partial purification of mucus extract for Ag-NPs synthesis

Collected mucus samples were processed for partial protein purification by ammonium sulphate precipitation for concentrating bioactive molecules in it which was followed by dialysis. Solid ammonium sulphate was gradually added to the extract to reach a final saturation of about 80% a level sufficient for protein precipitation and was allowed to incubate at 4°C overnight. The obtained samples were centrifuged at 15,000 rpm for 20 minutes at 4°C and the resulting protein pellets were resuspended in phosphate-buffered saline (PBS). To remove excess ammonium sulfate and other small molecules, the resuspended protein solution was dialyzed against 20 mM Tris-HCl buffer (pH 7.4) containing 50 mM NaCl, for retaining the proteins of interest. Samples were placed in dialysis tube of 6 cm x 15 mm (molecular cut off 10-12 KDa) at 4 °C with Tris-HCl buffer, NaCl for ensuring the thorough desalting that is succeeded by the collection and storage of the purified, concentrated protein samples at -20° for the synthesis of Ag-NPs.

### 2.2 Biochemical analysis of Catfish & Tilapia skin mucus

Estimation of total protein, carbohydrate and lipid content in crude skin mucus of *Clarias batrachus*, *Oreochromis mossambicus* was determined by Bradford method, Carrol *et al*. and Folch *et al*. method.

### 2.3 Biosynthesis of epidermal mucus silver nanoparticles (EM-AgNPs)

For synthesizing the epidermal mucus mediated bio-silver nanoparticles from *Clarias batrachus*, *Oreochromis mossambicus*, the partially purified protein mucus extract (100 ml) was diluted with 284 mM ascorbic acid, 66 mM polyvinyl pyrrolidone, and 35 mM $AgNO_3$ in a 1:10 v/v ratio. The initial pH of the diluted mucus was approximately 7, but after including ascorbic acid and polyvinyl pyrrolidone, the pH dropped to 4. Both samples were stirred at 250 rpm for 45 minutes subsequently followed by a brief exposure to sunlight and within 2-5 minutes the color of the dispersion mixture changes from gray to brown ensuring the formation of silver nanoparticles. Once the distinct color shift appears the process was stopped, this transition was ascribed due to unique size

and shape- dependent Surface Plasmon Resonance of the silver nanoparticles.

#### 2.3.1 Physico-chemical characterization of EM-AgNPs

For UV-visible spectroscopy study, Shimadzu UV 1780 spectrophotometer was preheated, stabilizes along with resuspension of the EM-AgNPs in sterile distilled water and the resultant spectral scan was recorded typically in the range of 200-400 nm against distilled water as baseline solution. Information about functional groups of epidermal mucus which played a role in molecular interaction by reducing, stabilizing and capping the formulated nanoparticles were identified by Fourier Transform Infra-Red (FTIR) spectroscopy (Shimadzu IR Prestige- 21, FTIR). After preheating and stabilizing the spectrometer, a small amount of the samples was placed on a clean NaCl pellet and was gently pressed onto by another NaCl pellet for the uniform spread. The infrared absorption spectra analysis operates in the range of 400-4000 cm−1 along with 16 accumulations and measurement parameters of 8 cm−1 resolution. Biosynthesized silver nanoparticles surface morphology was examined by Environmental SEM (ESEM) and its elemental composition profiling was elucidated by Energy dispersive X-ray spectroscopy (EDS). Colloidal solution of the silver nanoparticles was produced by dispersing it in the distilled water and placing a drop of it on a clean, carbon-coated SEM grid additionally allowing the sample to air-dry at room temperature for the formation of thin, uniform layer of nanoparticles on the substrate. Prepared SEM grid was loaded into the ESEM chamber with the pressure of 1-10 Torr, temperature 0-20°C and low accelerating voltage, typically 5-20 kV for minimizing sample charging. The size, shape and distribution of the resultant EM-AgNPs were determined by using TEM. A 200 kV acceleration voltage was applied for obtaining high-resolution images of the silver nanoparticles.

### 2.4 Biological assays

#### 2.4.1 Culturing of Microbial strains

Nosocomial microbes comprising both of Gram-positive bacteria namely *Staphylococcus aureus* MTCC 1430, *Staphylococcus epidermidis* MTCC 3382, *Enterococcus faecalis* MTCC 2729, Gram- negative bacteria *Serratia marcescens* MTCC 97, *Pseudomonas aeruginosa* MTCC 1943, *Escherichia coli* MTCC 1610, *Klebsiella pneumoniae* MTCC 2653 and fungus *Trichophyton rubrum* MTCC 7859, *Candida albicans* MTCC 1637 were procured from CSIR-IMTECH, Chandigarh. Bacterial strains were inoculated in sterile Muller Hinton (MH) broth (HiMedia), and fungal strains in sterile Sabouraud Dextrose (SAB) broth (HiMedia) respectively.

#### 2.4.2 Antimicrobial and minimum inhibitory concentration assay of epidermal mucus-silver nanoparticles

Agar cup diffusion was employed for screening the antimicrobial potential of the constructed silver nanoparticles. Microbial inoculum in their respective nutrient media was incubated at 37°C for 24 hours and on solidified agar plates, four wells of 6mm were drilled into via a sterile cork borer-one well was marked as a positive control with 100 µl of 20 µg/ml Cefoperazone sulbactam (1:1) for bacteria, Ketoconazole for fungus respectively, another well serve as a negative control comprises of 100 µl of sterile distilled water and the remaining wells were loaded with 100 µl of test samples. Plates were incubated at 37°C for 24, 48 hours for bacterial and fungal strains followed by measurement of zone of inhibition for each plate (Patel M *et al*. 2020) [9]. Resazurin Microtiter Assay (REMA) was applied for determining the Minimum Inhibitory Concentration (MIC) of EM-AgNPs against varied microbes as per Reham *et al*., 2013. The MBC/MFC values of the EM-AgNPs were determined by broth dilution that includes subculturing of the samples representing the MIC and at least one more concentrated dilution on freshly prepared agar plate for determining the viability of the organism.

#### 2.4.3 Cytotoxicity assay

Human epidermoid carcinoma (A431 cells) were acquired from National Center for Cell Science (NCCS), Pune and maintained in T25 flasks with 10% fetal bovine serum (FBS) containing Dulbecco's Modified Eagle Medium (DMEM).Cell suspension was created by treating the adherent monolayer with trypsin-ethylenediaminetetraacetic acid (EDTA) and hemocytometer was use to establish a seeded density of $1 \times 10^5$ cells /ml. 96 well plates were seeded with 0.05 Million cells that were allowed to incubated overnight in a $CO_2$ incubator at 37°C with 5% $CO_2$, 95% air in a humidified atmosphere for facilitating cell adhesion. After observing the presence of fully confluent cells under the microscope, cell treatment was done by synthesized Catfish, Tilapia EM-AgNPs at different dose such as1,2,4,5,10,20,30 & 40 µg/ml followed by 48h incubation at 37°C, 5% $CO_2$ and fluorouracil was used as a positive control. Cytotoxicity assay was performed by applying a yellow, water-soluble tetrazolium salt, 3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide (MTT) for enumerating cellular viability which is directly correlated to the amount of formazan produced by succinate-dehydrogenase a mitochondrial enzyme. Following incubation, 10µL of 5mg/mL MTT-phosphate-buffered saline (PBS) was added to the wells and incubated for 4 hours for the formation of reduced MTT or formazan crystals. The media was then discarded and the formazan crystals were dissolved by adding 100µL of DMSO. Shake the whole mixture for five minutes at 150 rpm for the proper action of solvent on the formazan crystals and finally, the optical density of the cells was measured by microplate reader at 595 nm absorbance. $IC_{50}$ values were calculated from the graphs created GraphPad Prism software. Percent cytotoxicity of EM-AgNPs were measured by using equations such as-

% Cell Viability = (Sample's mean O.D/ Control group's mean O.D) × 100

% Cytotoxicity = 100- % cell viability

#### 2.4.4 Statistical analysis

All tests were conducted in triplicates, accordingly mean and standard deviation were calculated. The statistical significance of the data was assessed through two-way ANOVA for anticancer data and one way ANOVA followed by Tukey's HSD for antimicrobial sets at a significance limit of 0.01, 0.05 respectively in GraphPad Prism software.

## 3. Results and Discussion

Biochemical quantification from the epidermal mucus of *Clarias batrachus*, *Oreochromis mossambicus* reveals that the total protein content as 23.91±0.23 µg/ mL, 38.26 ±0.29 µg/

mL; 59.8±0.5 µg/ mL,42.8±0.2 µg/ mL was the total carbohydrate estimation and about 0.33±0.01 mg/mL, 0.42±0.004 mg/mL lipid was present in skin mucus respectively. This highlights that both fish's cutaneous mucus is rich in biomolecules that act as an external protective chemical barrier or blanket to the invasive pathogens.

### 3.1 UV-Visible Spectroscopy of Catfish & Tilapia EM-AgNPs

Preliminary assessment was conducted for examining the optical properties of the synthesized silver nanoparticles and the visual colour change from gray to brown of the reaction mixture further ensure the successful formation of the nanoparticles. The spectrum exhibits a distinct absorption peak in the range of 250-300 nm for *Clarias batrachus* and a surface plasmon peak of 270 to 320 for *Oreochromis mossambicus* that is coherent with the study of Tasleem *et al*. 2024 [11] as they also obtain maximum absorption peak for Ag nanoparticles in the range of 190-300 nm. Tilapia EM-AgNPs displayed a broad nature of peak (Fig 1) due to high protein content in their skin mucus which initiates varied growth or nucleation of formed nanostructure leading to heterogeneity, polydispersity in size distribution and is also directly related to the concentration of silver ions being reduced, capped or stabilized by biomolecules. Additionally, the absence of significant absorption beyond 400 nm suggests minimal agglomeration as larger nanocomposite exhibits broad peak at higher wavelength.

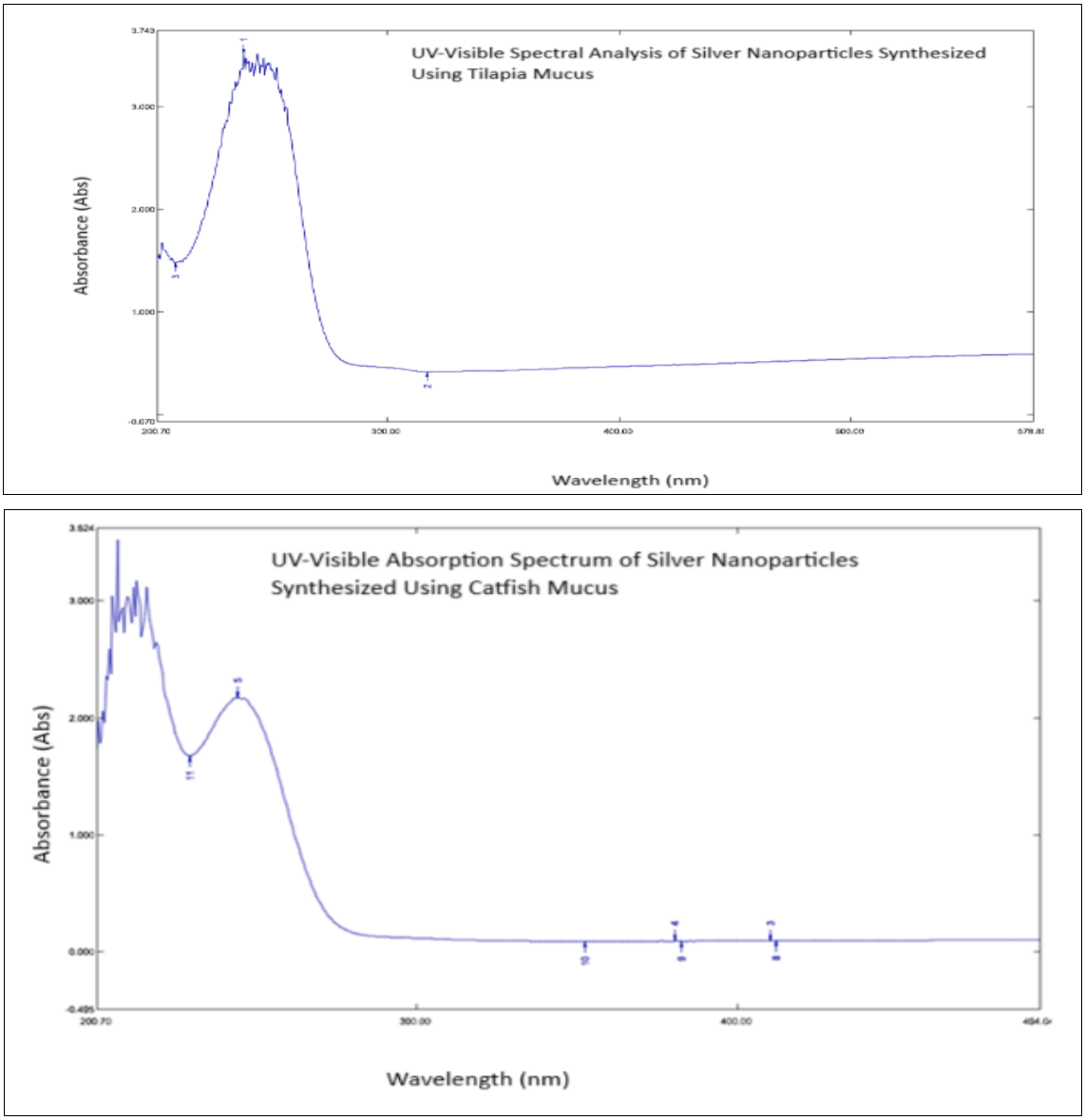
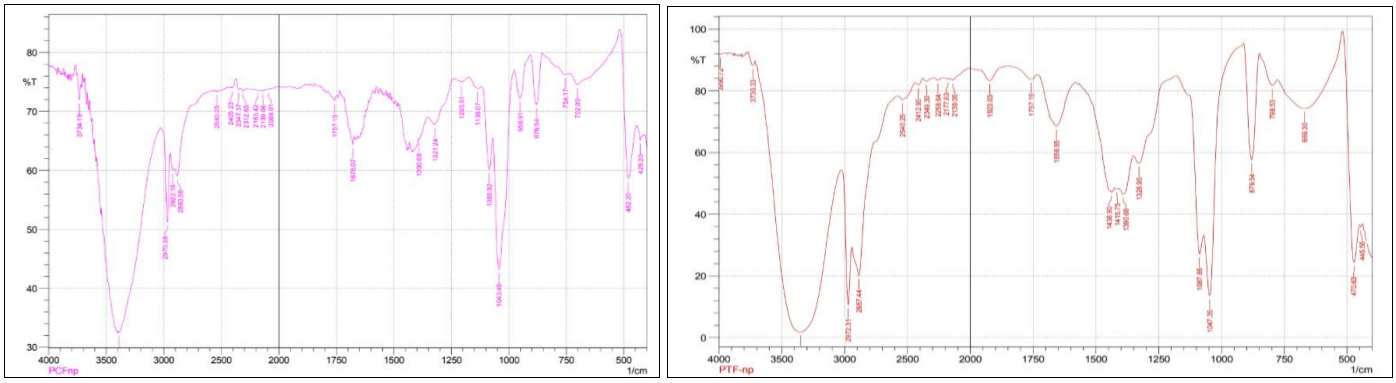



**Fig 1:** UV-visible spectroscopy of EM-AgNPs

### 3.2 FTIR Spectroscopy

FTIR provides a molecular insight into the identification of functional groups or metabolites present in the mucus which played a major role in the biogenic synthesis of Ag nanoparticles by acting as a potent capping or reducing agent. Spectral profile of Catfish EM-AgNPs (Fig 2a) exhibits a broad peak at 3390-3734 $cm^{-1}$ correspond to O-H, N-H stretching suggesting the presence of alcohol and aliphatic primary amine respectively. 2970 $cm^{-1}$,2883 $cm^{-1}$ indicates N-H stretching of amine salts whereas 1757-1678$cm^{-1}$ peaks represent C=O secondary, tertiary amine and ester groups. Numerous bands in the region of 2405-2088 $cm^{-1}$implies combination of functional groups such as carbonyl, alkenes showcasing lipid molecule involvement in nanoparticle reduction. C-H bending of aldehyde & alkane is displayed in the 1390-1321 $cm^{-1}$ peaks, 1085-1205 $cm^{-1}$ denotes C-O & C-N stretching of amines, Carbohydrate- based compounds presence is highlighted by 1043 $cm^{-1}$ peak furthermore

additional peaks at 700-800 $cm^{-1}$ are attributed due to C-H bending of aromatic rings. *Oreochromis mossambicus* EM-AgNPs (Fig 2b) reveals a distinct broad absorption band at 3350 $cm^{-1}$ for N-H stretching which indicates the presence of secondary amine, proteins in Tilapia skin mucus that actively reduced and stabilized Ag nanoparticles. The peaks at 2972 $cm^{-1}$, 2887 $cm^{-1}$ correspond to C-H stretching of alkanes that may originate from fatty acids or lipid-based components subsequently peaks at1656 $cm^{-1}$,1438 $cm^{-1}$ are attributed to amide I and amide II vibrations respectively confirming the presence of proteins or peptides. Anhydride C-O groups are illustrated by the peak at 1047 $cm^{-1}$ that marks the participation of polysaccharides or glycoproteins in the nanoparticle formation process. Additionally, the peaks observed in the 600-800 $cm^{-1}$ region can be assigned to C-H bending and metal-oxygen (Ag-O) vibrations, confirming the successful formation of silver nanoparticles.

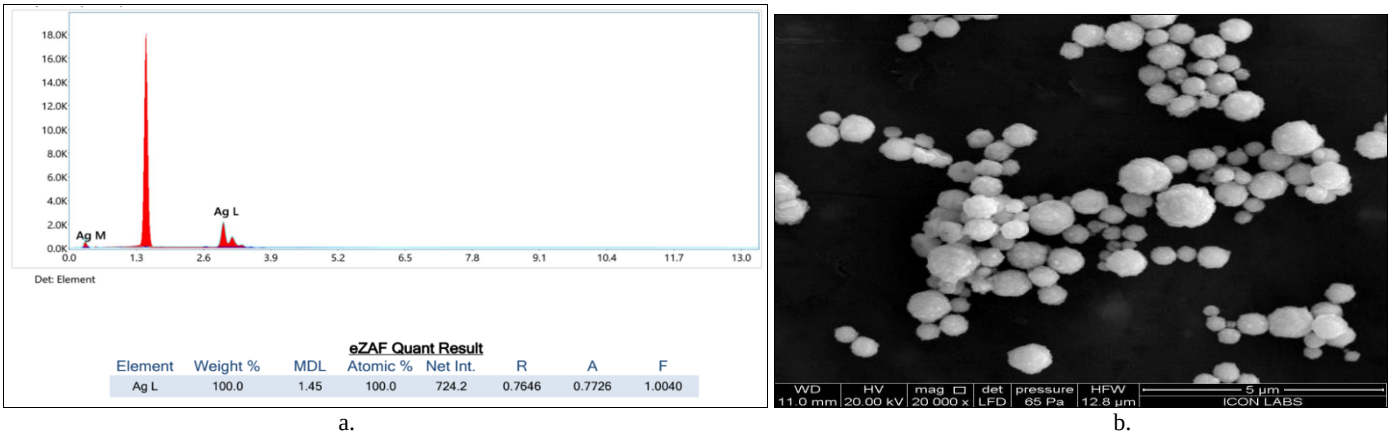



a. b.

**Fig 2:** FTIR analysis a. *Clarias batrachus* EM-AgNPs b. *Oreochromis mossambicus* EM-AgNPs

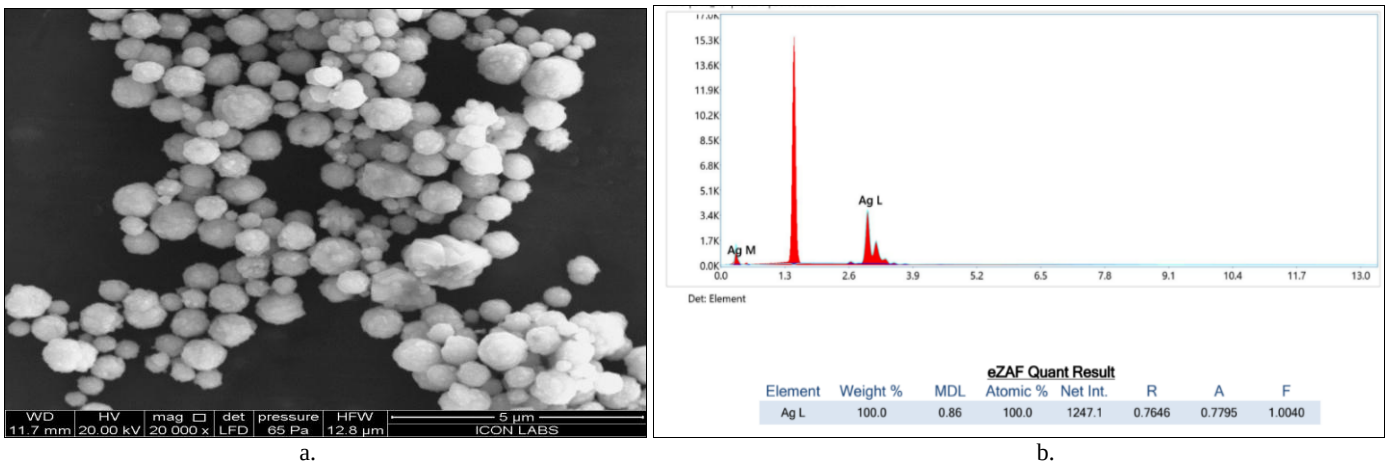
### 3.3 ESEM with EDS analysis

A detailed morphological evaluation along with the elemental composition examination was done for Catfish (Fig 3a & b) & Tilapia (Fig 4 a & b) Ag nanoparticles. The ESEM images predominantly display polydisperse, stable and polycrystalline spherical shape ultrafine AgNPs that ranges <100 nm are produced from both fishes cutaneous mucus thus ensuring the role of biomolecules in bio-capping & bio-stabilizing of AgNPs. EDS assessment discloses the presence of silver by two specific peaks pertinent to signals at 1.3 keV (Ag M) and 2.6 keV (Ag L) furthermore eZAF Quant Result demonstrates that silver constitutes 100% by weight & atomic percentage infers a high purity level of the synthesized nanoparticles with no detectable impurities.




a. b.

**Fig 3:** SEM micrograph of Catfish EM-AgNPs a. ESEM b. EDS

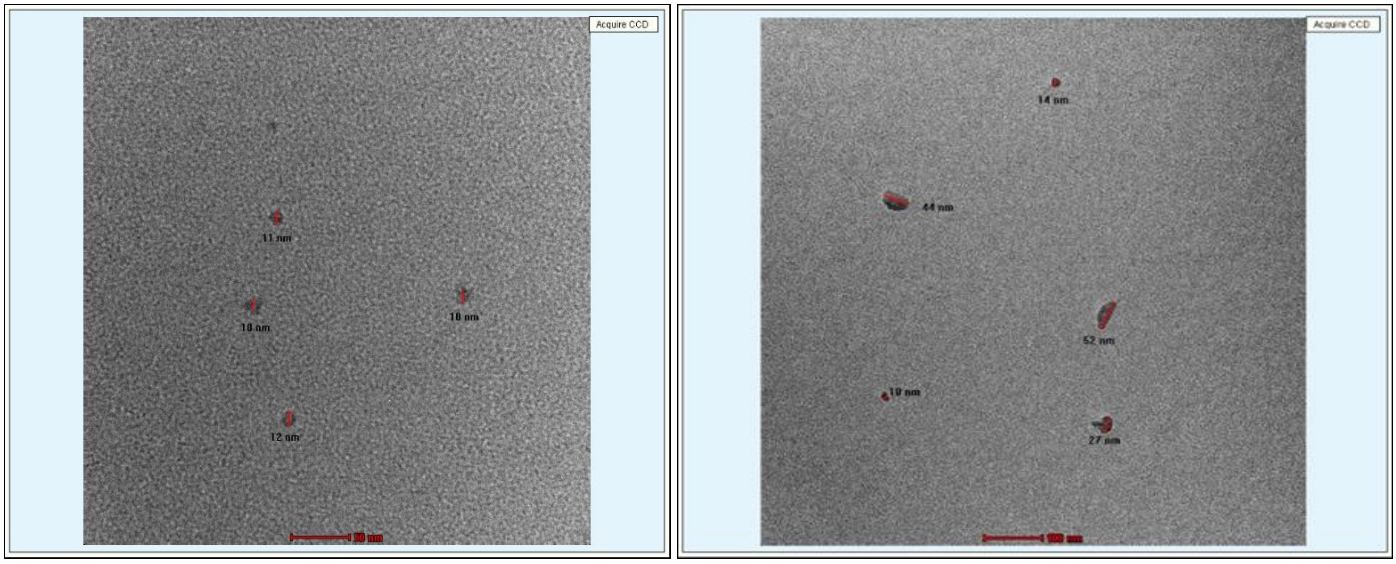



a. b.

**Fig 4:** Tilapia EM-AgNPs SEM micrograph a. ESEM b. EDS

### 3.4 TEM analysis

Catfish biosynthesized EM-AgNPs were in the size range of 10 to 12 nm (Fig 5.a) whereas 10 to 52 nm was the observed size distribution for Tilapia epidermal mucus- mediated Ag nanoparticle (Fig 5.b). Occurrence of polydisperse distribution in Tilapia Ag nanoparticles was attributed to the heterogenous nucleation process where different biomolecular interaction between mucus organic compounds influenced the rate of reduction and stabilization result into the synthesis of larger nanoparticles with size of 44 & 52 nm. Other studies (Alabssawy *et al*. 2024; Mane *et al*. 2021) [12, 4] have also reported the utilization of mucus as a bio-extract for the reduction $Ag^{+}$ ions to $Ag^{0}$ in AgNPs and their size limit also corresponds to our findings.

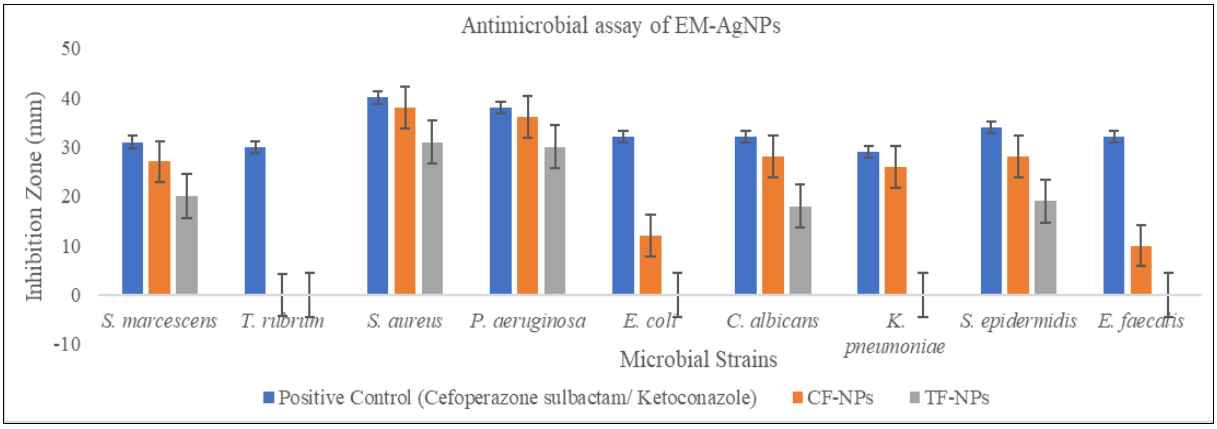



a. b.

**Fig 5:** TEM images a. *Clarias batrachus* EM-AgNPs b. *Oreochromis mossambicus* EM-AgNPs

### 3.5 Antimicrobial activity

Numerous reports in literature have preferred the green synthesis of metallic nanoparticles over chemical, physical methods from eclectic resources like phytogenic, microbial or fungal (Mendes *et al*. 2022) [15] and its supremacy in nanomaterial fabrication mainly owing to their inherent antimicrobial, cytotoxic potential. Catfish Ag nanoparticles exhibited a strong inhibition ability towards the tested microorganisms when compared to Tilapia Ag nanoparticles (Fig 6). The decreasing order of sensitivity of Catfish EM-AgNPs are *S. aureus* (38 mm) > *P. aeruginosa* (36 mm) > *C. albicans* (28 mm, *S. epidermidis* (28 mm) > *S. marcescens* (27 mm) > *K. pneumoniae* (26 mm) > *E. coli* (12 mm) > *E. faecalis* (10 mm) however Tilapia Ag nanoparticles displayed inhibitory actions towards *S. aureus* (31 mm) > *P. aeruginosa* (30 mm) > *S. marcescens* (20 mm) > , *S. epidermidis* (19 mm) > *C. albicans* (18 mm) only, although *Trichophyton rubrum* was insensitive to any treatments, which addresses the encountering of crucial challenges while treating fungal dermatophytes. The observed MIC (Fig 7) and MBC (Fig 8) value for Catfish Ag nanoparticles discloses an exceptionally low value of 0.195 mg/mL, 0.78 mg/mL for *S. marcescens*, *P. aeruginosa* & *E. faecalis* while 0.78mg/mL was the lowest MIC/MBC value recorded for *P. aeruginosa* corresponding to Tilapia EM-AgNPs antibacterial effects which is concurrent with the ease of Ag nanoparticle in delimiting the growth of Gram negative pathogens.




* $P<0.05$ shows a statistically significant difference in the ZOI caused by AgNPs from both the fishes epidermal mucus

**Fig 6:** Antimicrobial activity of EM-AgNPs against some nosocomial microbes

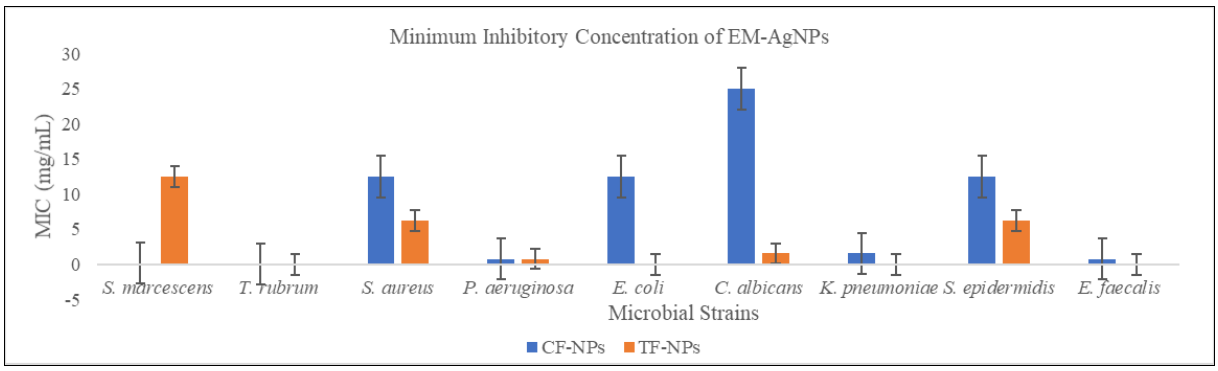



**Fig 7:** Evaluation of MIC value of EM-AgNPs

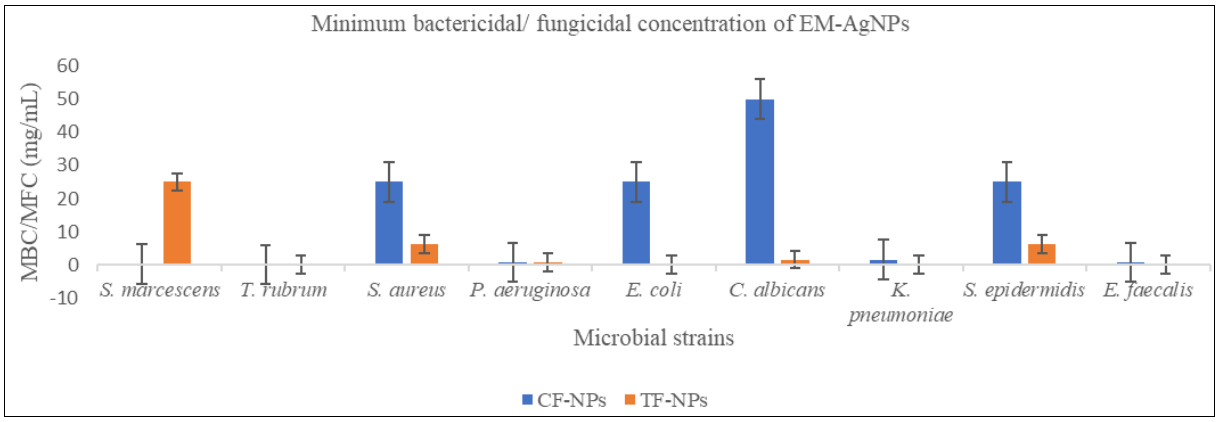



**Fig 8:** Assessment of MBC/MFC value of EM-AgNPs

### 3.6 Anticancer activity

Determination of *in vitro* cytotoxicity effects of *Clarias batrachus* & *Oreochromis mossambicus* EM-AgNPs on A431 cells (human epidermoid carcinoma) were elucidated by MTT assay at varied concentration such as-1, 2, 4, 5, 10, 20, 30 & 40 µg/ml (Fig 9). It was observed that Tilapia AgNPs significantly increases cell inhibition from 22.92% at 1 µg/ml to a maximum of 98.29% at 40 µg/ml with 2.542 µg/ml $IC_{50}$ value as compared to 5.651 µg/ml, 1.01µg/ml $IC_{50}$ corresponds to 92.98% and 93.83% of antiproliferative ability of Catfish AgNPs, Fluorouracil respectively. Two-way ANOVA showed a statistically significant interaction between fish source (Catfish or Tilapia) and AgNPs concentration on A431cell viability ($p < 0.0001$ and $p = 0.0176$, respectively).The analysis revealed a strong dose-dependent effect which demonstrates that anticancer activity of AgNPs increases proportionately to its quantity along with 92.74%, 2.876% total variation on cell viability accounting from AgNPs concentration and its respective fish mucus source. Our findings illustrate that if aquatic resources are exploited for the extraction of bioactive compounds, concerning mainly to the crafting of silver bionanomaterials will be more advantageous than the phytogenic synthesis of AgNPs (Kamalanathan *et al*. 2024) [16] due to their enhanced cytotoxic activity towards limiting the uncontrolled growth of A431 cells.

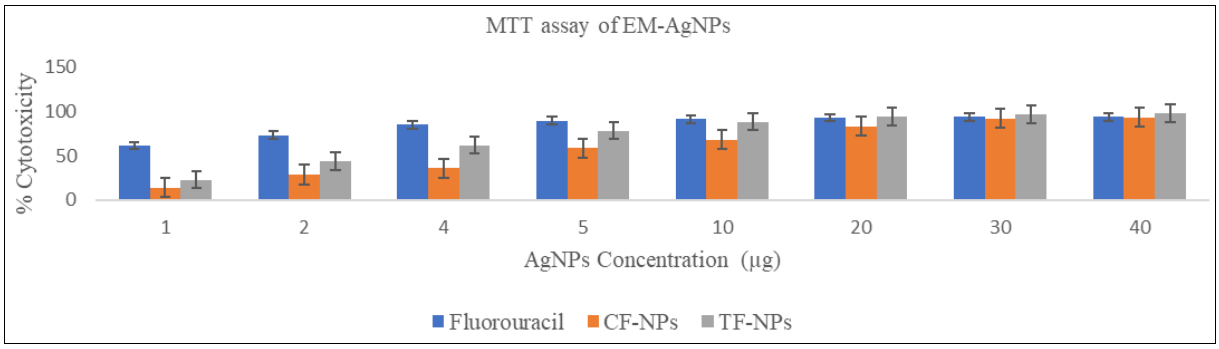



**Fig 9:** MTT assay of Catfish and Tilapia EM-AgNPs

### 4. Conclusion

This study probes into the synthesis of aquatic mucogenic derived-silver nanocomposite from hitherto overlooked *Clarias batrachus*, *Oreochromis mossambicus* cutaneous secretion that is a repository of essential micro or macromolecules that can facilitate the nanoparticulate conformation by acting as a bio-reductant or bio-stabilizers. Both fish EM-AgNPs are a blue alternative to conventional metallic nanoparticles by being eco-friendly, scalable and cost-effective entity and exhibits broad spectrum antimicrobial activity against a wide range of nosocomial pathogens. Tilapia EM-AgNPs displayed a remarkable

inhibition of 98.29% as compared to 92.98% of Catfish Ag nanoparticles towards skin cancer (A431 cells) which clearly suggest that their mucus can be used in formulating anticancer ointments (lotions, creams & gels) for topical applications, moreover additional clinical trials will be needed for its proper implementation. This research can provide an opportunity for scientists in bioprospecting the value or utility of mucosal fluid that is often looked as an unutilized product in fish processing industries for the construction of nanostructures that can boost regenerative economy and further purification of novel molecules from it, can aid in the formation of new therapeutic medication for curing cancer as well as harmful nosocomial infection.

## 5. Acknowledgments

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## 6. Conflict of Interest

The author(s) declares no potential competing interest.

## 7. Ethics statement

No ethical approval is required for this research.

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