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Fish Mucus Mediated Synthesis of Silver Nanoparticles from *Piractus brachypomus*

Graphical abstract

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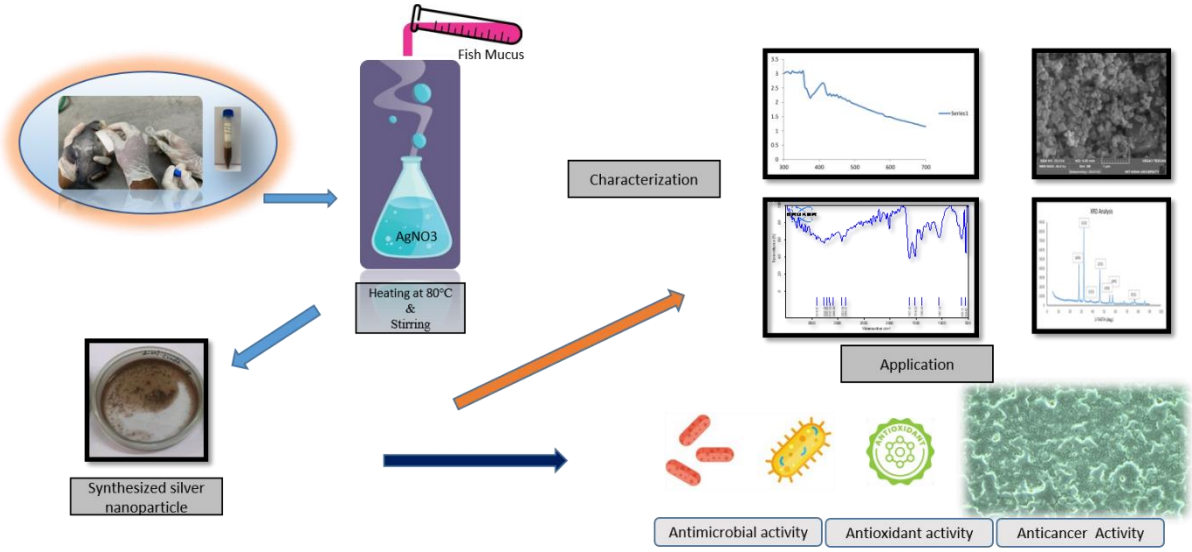

Abstract

Nanomedicine is been a breakthrough for years due to its high efficiency in bactericidal property, antioxidant and cancer therapeutics. Silver Nanoparticle (AgNPs) being the safest metal was utilized in this biosynthesis method to synthesize AgNPs utilizing *Piractus brachypomus* skin mucus, a biomolecule abundant source for the conversion of nanoparticle. The converted nanoparticle was characterized using UV spectrometric analysis exhibit a peak at 410nm, FTIR and XRD found properties corresponds to AgNPs, FESEM-EDAX identified average size of AgNPs as 35.303±23.43nm, EDAX confirmed silver through secondary diffraction. The highest bactericidal zone was 16mm against *Salmonella* spp., and antifungal activity was evident against *Candida albicans* and *Dermatophytes* spp. The DPPH exhibit a significant antioxidant activity of 49.32% at 10µL. The cytotoxicity analysis revealed 88% cytotoxic effect at 100µL exhibiting IC_{50} of 58.05 µg/mL, and a CC_{50} of 434µg/mL on Vero cells, yielding 7.5 selectivity index lead AgNPs, a significant candidate for biomedical application.

Keywords: antimicrobial, anti-cancerous, antioxidant, fish mucus, HeLa, AgNPs.

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Introduction

The significance of nanotechnology among various field interests' global researchers towards material science and biotechnology. To minimize the adverse effects associated with chemical and physical methods, there is a growing preference for eco-friendly nanoparticle synthesis using microbes and plant extracts [1-6]. Green synthesis of the nanoparticle is determined to be more noteworthy than physical and chemical techniques because of its friendliness to the environment, economic viability, and ease of scaling up for production. Many significant studies have proved that AgNPs are next-generation antibacterial agents [7-9]. Nanoparticles are small particles of nanometer length, considered between 1-100 nm. Various types of nanoparticles are available, such as silver, copper, zinc, gold, etc. Silver is known for its safe nature, a non-toxic inorganic antibacterial agent that can eradicate 650 different types of pathogenic bacteria, according to Holt [8]. Metal nanoparticles, such as AgNPs(AgNPs), are widely utilized across various sector due to their unique properties including optical, chemical, and physical, which makes nano silver particles useful for target-specific drug delivery. Cancer nanomedicine is rapidly advancing due to its unique features, such as small size, customizable shape, selective binding, and enhanced permeability, which allow targeted delivery of insoluble drugs to distant tumor sites. With no side effects nanotechnology serves as an efficient instrument for diagnostics and cancer therapy such as ovarian, breast, and non-small cell lung tumors with very less side effects than other therapies [9, 10]. The abundance and extended half-life of nanoparticle leads to low toxicity towards healthy cells which penetrates between the skin surfaces [11] NPs can cross cell membranes, interact with biological systems, and remain in circulation longer [12]. The size range between 10-100nm shows better cancer treatment with upgraded drug delivery and enhanced penetrability and retaining EPR effect [13]. The uptake of AgNPs into bacteria and living cell leads to propagation of free radicals or silver ions which causes DNA damage that kills cancer cells by break the mitochondrial respiratory chain, and inhibits ATP production [14]. AgNPs are also used in the engineering and materials sciences for the construction of sensors and other components [15]. AgNPs selectivity target and destroy cancer cells while sparing healthy cells [16]. The HeLa cell line was created in 1951 and named for Henrietta Lacks, a cervical cancer patient. The creation of a human continuous cell line offers an accepted model for research on human cellular and molecular biology as well as cancer pathology [17]. The HeLa cell has been examined and utilized during the past ten years in research on cancer, gene expression and alteration, gene profiling, etc [18]. It was discovered that cervical cancer was the fourth most frequent malignancy among women because of its high incidence, and many drugs have been synthesized for better solutions. AgNPs' lethal dose (LD50) against HeLa cells was determined to be 300 µg/ml in one study, demonstrating the cytotoxicity of the material against cancer cells [19]. The popular native American fish known as red-bellied pacu, or *Piaractus brachypomus*, was first introduced to India in West Bengal and is currently available in several states, including Andhra Pradesh, Kerala, and the Northeast [20, 21]. One new prospective species for industrial aquaculture has been suggested to be Pacu [22]. Large-scale fish farming enterprises are more vulnerable to infections from a variety of diseases. Fish, on the other hand, have a variety of unique and robust defense mechanisms that protect them from these pathogenic infections, with fish skin mucus serving as the first line of physical defense [23]. Fish mucus plays a vital role in innate immunity, containing proteins and enzymes such as lysozyme, immunoglobulin, complement proteins, lectins, CRP, transferrin, and antimicrobial peptides [24,25]. Fish mucus possess lipids, carbohydrates and metabolites which maintains immune regulations. The sticky nature is mucin with glycoprotein, which possess fatty acids, SFA, MUFA and PUFA [26]. Excess mucus from fish is released during the harvest which can be converted into significant purposes. The study improves the biosynthesis of silver nanoparticles using fish skin mucus to assess the biological potential. The produced AgNPs were characterized through analytical

techniques and microbicidal efficiency against selected human pathogens. Further the study explores the antioxidant and cytotoxic potential against Hela cancer cell lines.

The objective is to determine the applicability of these nanoparticles in antimicrobial and anticancer treatments and contribute to the growing body of research on biologically synthesized nanoparticles for biomedical applications.

Methodology

Collection of Fish Mucus

Live Piaractus brachypomus (300–500 g) were procured from "Frontier Aquaculture" and maintained in 100 L aerated glass tanks with regular water exchange. Fish were acclimated on commercial feed and monitored daily. Skin mucus was gently scraped without anaesthesia after washing the fish to remove surface debris. The collected mucus was stored at –20 °C in a deep freezer (Thermo Scientific Forma 900 series) to prevent microbial contamination and protein denaturation [27].

Materials

Nutrient Agar (NA), Mueller Hinton Agar (MHA), and Sabouraud Dextrose Agar (SDA) (HiMedia, Mumbai) were used for culturing bacterial and fungal strains. Silver nitrate ($AgNO_3$; HiMedia) served as the nanoparticle precursor. Dimethyl sulfoxide (DMSO; ISOCHEM, Chennai) was used as solvent for test preparations. Methanol (Qualigens) and 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich) were used in antioxidant assays. Dulbecco's Modified Eagle Medium (DMEM) with fetal bovine serum (FBS), penicillin, and streptomycin (HiMedia) supported HeLa cell culture. Phosphate-buffered saline (PBS) was prepared with analytical-grade reagents.

Synthesis of AgNPs

A 0.1 M $AgNO_3$ solution was prepared in distilled water. Fish mucus (1–3 mL) was added as a reducing and stabilizing agent to 100 mL of $AgNO_3$ and heated at 80 °C for 3 h until the solution turned brownish-black, indicating nanoparticle formation. The precipitate was centrifuged at 8000 rpm for 10 min at 4 °C, washed with distilled water, and dried at 100 °C for 12 h. The resulting AgNPs were scraped, powdered, and stored in sterile containers.

2.4.Characterization

The biosynthesised AgNPs were characterised using multiple analytical techniques to confirm their formation, understand their structure, morphology, and elemental composition.

2.4.1 UV-visible spectroscopy

UV-Visible spectral analysis was conducted using a Motras Scientific UV-Visible True Double Beam Spectrophotometer. The colloidal AgNP suspension was scanned between 300 and 700 nm at room temperature. The characteristic surface plasmon resonance (SPR) peak of AgNPs was used to confirm their synthesis. A quartz cuvette with a path length of 1 cm was used, and distilled water served as a blank.

2.4.2. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was performed using a Bruker Alpha-Platinum FTIR spectrometer at IIT Madras. The dried AgNP sample was ground with potassium bromide (KBr) and pressed into a pellet. Spectra were recorded over the wavelength range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1}. This analysis helped identify the functional groups responsible for the reduction and stabilization (capping) of nanoparticles.

2.4.3. X-ray diffraction (*XRD)*

XRD analysis was carried out using a Bruker D8 Discover powder X-ray diffractometer (IIT Madras, Chennai). The dried nanoparticle sample was evenly spread onto a sample holder. The scan was performed in the 2θ range of 20°-80°, with a step size of 0.02° and scanning rate of 1°/min, using Cu-Kα radiation (λ = 1.5406 Å). The crystalline nature and phase identification of the synthesized nanoparticles were determined based on the diffraction peaks.

2.4.4. Field Emission Scanning Electron Microscopy and Energy-dispersive X-ray (FESEM-EDAX)

The surface morphology and particle size of AgNPs were examined using FESEM (VEGA3 TESCAN) at the Madras Institute of Technology. The nanoparticles were mounted on carbon-coated stubs and sputter-coated with a thin layer of gold before testing. Energy-Dispersive X-ray (EDAX) analysis was performed in conjunction with FESEM to determine the elemental composition of the synthesized nanoparticles. The presence of elemental silver confirmed the successful synthesis.

Antimicrobial Assay

The antimicrobial activity of AgNPs synthesized from fish mucus was tested using the agar well diffusion method. Gram-negative (*Klebsiella* spp*., Salmonella spp., E. coli* MTCC 043*, Pseudomonas* spp.) and Gram-positive (*Staphylococcus aureus, S. haemolyticus*) bacteria were cultured on MHA, while fungal strains (*Candida albicans, Aspergillus niger, Dermatophytes* spp.) were cultured on SDA. AgNP suspensions (25–100 µL, 100 µg/mL) were loaded into wells and incubated at 37 °C for 24 h (bacteria) and 28 °C for 48 h (fungi). Inhibition zones were measured in millimetres [28].

Antioxidant (DPPH) Assay

The free radical scavenging ability of AgNPs was tested by mixing 2 mL of 0.4 mM DPPH with AgNP samples (20–100 µL, 100 µg/mL) in methanol. After 15 min incubation at room temperature, absorbance was read at 517 nm [29].

Equation (1)

Radical scavenging activity/Inhibition (%) = (ABS control-ABS sample/ABS control) *100

MTT Cytotoxicity Assay

MTT Cytotoxicity Assay HeLa and Vero cells (NCCS, Pune) were cultured in DMEM with 10% FBS and antibiotics at 37 °C, 5% CO_2. The cell lines were further tested with AgNPs (10 µg/mL, 20-100 µL) for 24h. Post treatment, 20 µL of 5mg/ml MTT was mixed and incubated for 2h. Formazan crystals were mixed into 100 µL DMSO, and absorbance was observed at 570nm[30].

Equation (2) Cell viability (%) = (Absorbance of sample / Absorbance of control) × 100. 2.8.

Statistical Analysis

Was performed using SPSS statistical software (version 26), ANOVA and Tukey's post hoc test was used to perform comparing group menans. Microsoft excel and R software were used to construct graphical illustrations.

Results and Discussion

3.1.UV-VIS spectrophotometry

The preliminary indication for the synthesis of that AgNPs, when the reaction mixture turned from pale white to brown after three hours of heating at 80°C while being stirred regularly. The reduction of silver ions (Ag^+) to metallic silver (Ag^0) is indicated by this visual change. The 1 mL sample showed the best nanoparticle synthesis out of all the various

volumes of Piaractus brachypomus mucus extract that were tested. UV-visible spectroscopy verified this, showing a clear surface plasmon resonance (SPR) spike at 410 nm (Figure 1), a spectral range commonly linked to AgNPs (400–500 nm) [31]. These findings unambiguously show that AgNPs were successfully biosynthesised, which is in line with the distinctive SPR profiles that have been documented for silver nanostructures.

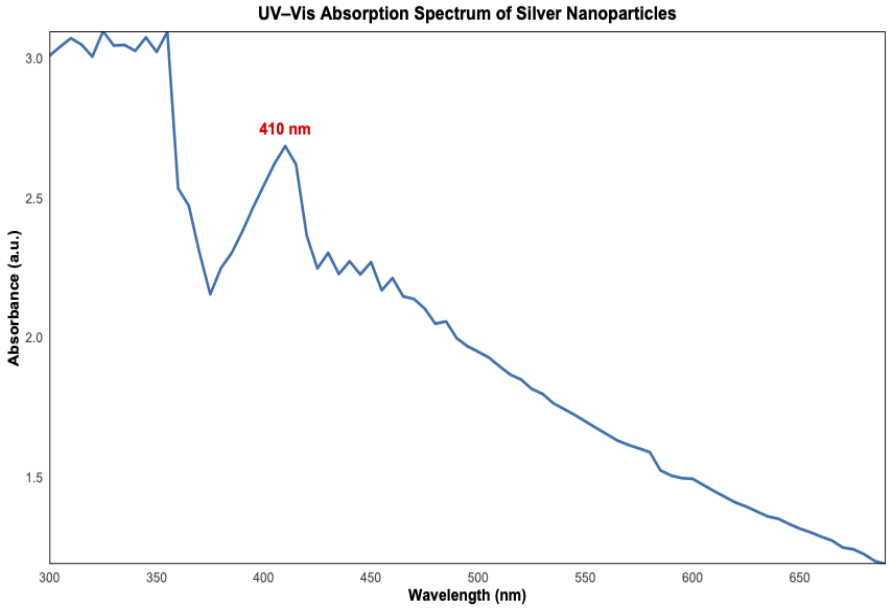

Figure 1: UV-VIS spectrometry of fish mucus-mediated synthesized AgNPs.

3.2. Fourier Transform Infrared Spectroscopy (FTIR) analysis

Analysis using Fourier Transform Infrared Spectroscopy (FTIR) Major functional groups involved in the reduction and stabilization process were identified by FTIR analysis of the AgNPs derived from Piaractus brachypomus mucus.

Prominent peaks were observed at 548.82 cm^{-1} (C–Br), 628.12 cm^{-1} (C–Cl), 1057.28 cm^{-1} (C–O–C), 1393 cm^{-1} (CH_3 bending), 1519.26 cm^{-1} (NO_2 stretching), and 1627.65 cm^{-1} (C=C), indicating the presence of alkyl halides, ether linkages, and nitro compounds (Figure 2). The C–H and CH_2 stretching vibrations from aliphatic chains are represented by peaks at 2856.37 and 2923.56 cm^{-1}, whereas aromatic C–H groups can be detected by a peak at 3085.09 cm^{-1}. N–H and O–H stretching from amino and hydroxyl groups, most likely derived from proteins, polyphenols, and carbohydrates found in fish mucus, has been verified by broad peaks at 3167.33, 3207.85, 3262.59, and 3410.07 cm^{-1} [28]. Similar peaks around 2927, 1631, and 1383 cm^{-1}, which indicate the presence of C–H, C=O/N–H, and C–C/C–N functional groups, were reported in previous investigations [32–34], which confirms the role of biomolecules in the synthesis and stabilization of nanoparticles. The crystalline nature of the nanoparticle was further identified using XRD analysis.

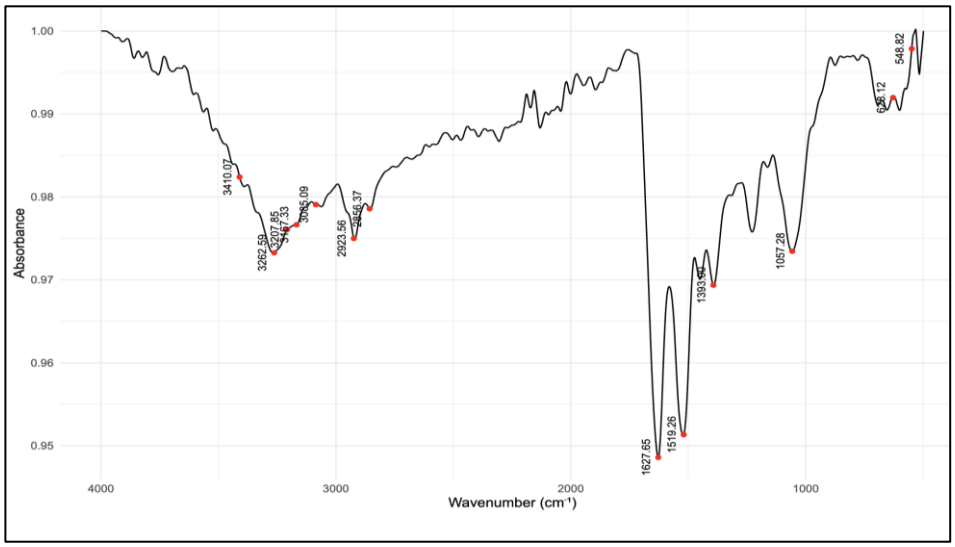

Figure 2: FTIR of fish mucus-mediated synthesis of silver nanoparticles

3.3.X-ray diffraction (XRD) analysis

The XRD pattern of AgNPs made from fish mucus was examined. The XRD displays the corresponding peaks at 27.91°, 32.34°, 38.23°, 46.30°, 54.88°, 57.54°, 67.57°, 76.82°, and 85.69°. Standard AgNPs are often used to evaluate and validate the synthesis of silver nanoparticles, and the JCPDS diffraction card 04-0783 corresponds to the silver nanoparticles. The standard states that the peaks produced by biosynthesis have planar values (111), (200), (220), and (311). AgNPsexistence is confirmed by comparing the planar value obtained by XRD data with a normal JCPDS diffraction card. The values (220), (122), (111), (231), (331), (241), and (311) are associated with the peaks as shown in Figure 3. The obtained peaks match the silver nanoparticle, indicating that it is a bio-synthesized and crystalline particle [35, 36]. The structural analysis of the synthesised AgNPs was carried out using FESEM.

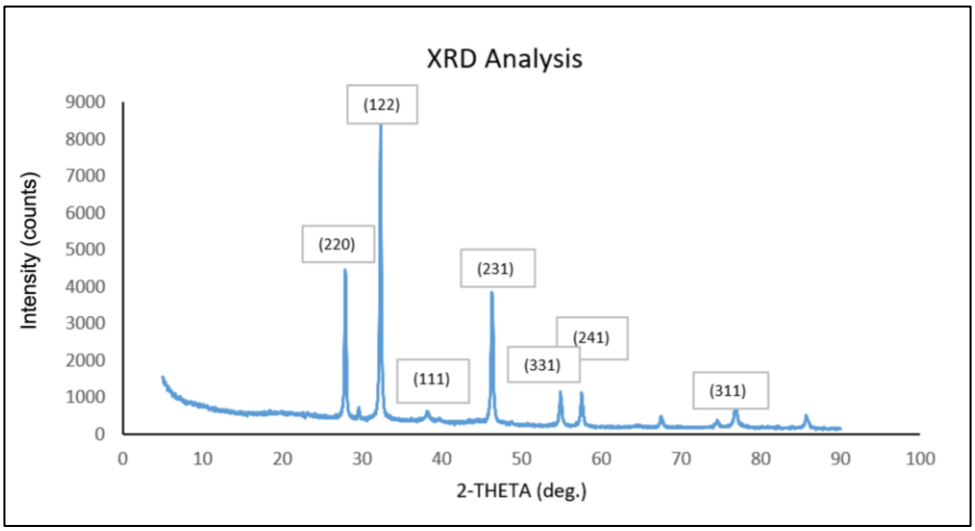

Figure 3: XRD analysis of fish mucus-mediated synthesised AgNPs

3.4. Field Emission Scanning Electron Microscopy and Energy-dispersive X-ray (FESEM-EDAX) analysis

The morphology of the biosynthesized AgNPs was analyzed using Field Emission Scanning Electron Microscopy (FESEM). Images captured at magnifications corresponding to 1 µm, 2 µm, and 10 µm scales revealed predominantly spherical nanoparticles, with an

average size of 35.303 ± 23.43 nm (analysed using Image J), Figure 4(a-c). The presence and elemental composition of silver were further confirmed through Energy Dispersive X-ray Analysis (EDAX), which showed strong signals corresponding to elemental silver, along with other contaminants from fish mucus and scales, indicating successful nanoparticle formation. The FESEM-EDAX characterization results are comparable between the current and prior investigations of fish mucus and other green synthesis generated using various precursors for the creation of nanoparticles [37, 38]. The results support earlier findings on nanoparticle formation using fish mucus as a biological agent. According to a previous investigation, 3 mL of fish mucus was used to synthesize copper nanoparticles, which had an average size of 82.38 ± 8.42 nm [27]. However, the current study used just 1 mL of mucus to form AgNPs. The particle size and morphology may be influenced by variables like temperature, pH, salt concentration, and the biochemical makeup of mucus, especially amino acids, lectins, and other organic compounds.

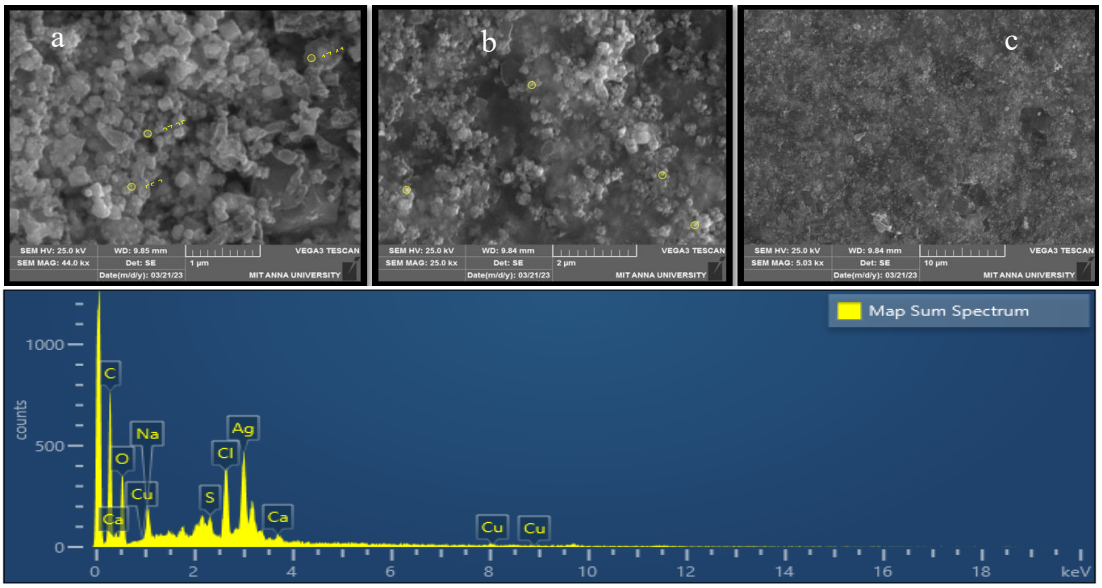

Figure 4: (a) FESEM image of AgNPs mediated by fish mucus at 1 µm magnification, displaying the size distribution and shape of the nanoparticles. (b) A 2 µm magnification FESEM image showing nanoparticle aggregation and spatial dispersion. (c) A lower-resolution FESEM image showing surface coverage and uniformity at a magnification of 10 µm.

The presence of nanoparticles is indicated by strong Ag peaks in the EDAX spectrum, whereas biomolecules from fish mucus that function as reducing and capping agents are reflected by C, O, Na, Cl, S, and Ca peaks. Cu peaks originate from the support grid.

3.5. Conversion of AgNPs.

A variety of substances found in fish mucus contribute to the transformation of silver nitrate into silver nanoparticles. The FTIR analysis, as reported by [39,40], indicates that the formation and stabilization of AgNPs are attributed to C=O stretching, N–H bending, and C–N stretching vibrations from protein functional groups commonly found in fish mucus. Biomolecules such as alkaloids, proteins/enzymes, amino acids, alcohols, and polysaccharides [24,25] act as reducing agents for silver ions, which supports the formation of AgNPs in solution [41]. An additional factor in the reduction activity and AgNO3's transformation into AgNPs is the soluble amino acids found in fish mucus [27].

3.6. Anti-microbial activity

The antibacterial and antifungal activities of biosynthesized AgNPs from *Piaractus brachypomus* skin mucus were evaluated against both Gram-positive and Gram-negative pathogens using the agar well diffusion method Figure 5a-e. A 100 µg/mL AgNP suspension was tested at varying volumes (25 µL, 50 µL, 75 µL, and 100 µL) against *Klebsiella* spp., *Salmonella* spp., *Escherichia coli* (MTCC No. 043), *Pseudomonas* spp., *Staphylococcus aureus*, and *Staphylococcus haemolyticus*. Inhibition zones were observed at 75 µL and 100 µL, particularly notable against *Pseudomonas* spp., *E. coli*, and *Salmonella* spp. (Table 2). Similarly, antifungal activity against *Candida albicans*, *Aspergillus niger*, and *Dermatophytes* spp. showed concentration-dependent inhibition at higher volumes. This shows that the inhibition activity enhances with an increase in concentration. The optimum concentration for antimicrobial activity is found to be 100 µL. According to standard testing conditions, the maximum inhibition zone of 16 mm against Salmonella spp. is similar to that attained by first-line antibiotics like ampicillin and ciprofloxacin. But when compared, conventional antibiotics often exhibit bactericidal effects at lower concentrations that are more reliable. AgNPs nevertheless have further advantages, such as longer-lasting activity, less harmful and slowed resistance development. The mechanism of inhibition is due to the attachment of AgNPs to bacterial cell walls, resulting in a breakdown of the integrity of the membrane, and dispensing silver ions (Ag^{+}) that generate reactive oxygen species (ROS), denature proteins, and block with DNA replication [42,43]. The Oxidative stress and eventually microbial cell death are the outcomes of these combined effects. By enhancing membrane permeability, promoting greater intracellular formation, and even reviving antibiotic activity against resistant bacterial strains, AgNPs have also been demonstrated to improve the effectiveness of conventional antibiotics [44–46]. AgNPs may not have the same pronounced potency as conventional antibiotics, but the biosynthesized nanoparticles have useful additional qualities. They are promising candidates for future combination therapies or as sustainable alternatives in cases of antibiotic resistance because of their broad-spectrum antimicrobial activity, biocompatibility, and ability to work in combination with current medications.

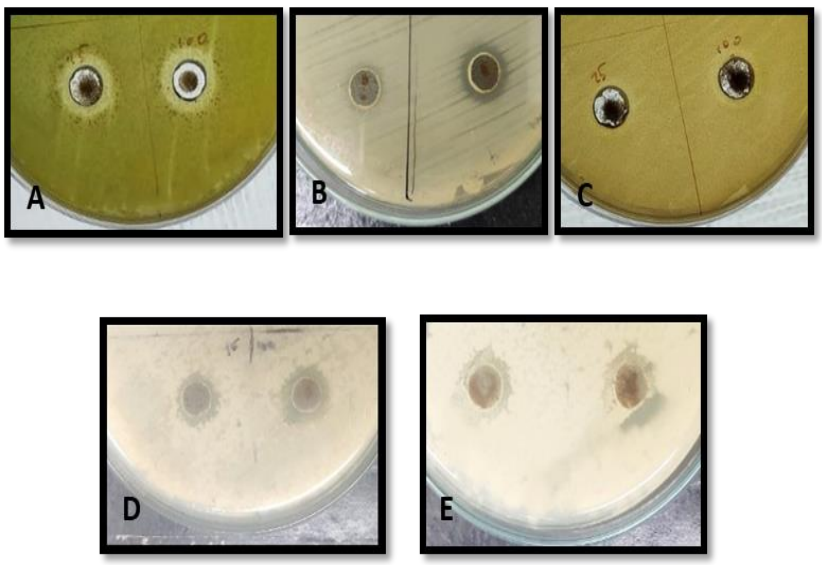

Figure 5: Antimicrobial activity of fish mucus-mediated synthesized AgNPs against bacteria (a) *P. aeruginosa,* (b) *E. coli,* (c) *Salmonella* spp.*,* and antifungal against fungi (d) *C. albicans,* (e) *Dermatophytes* spp.

Table 2: Zone of inhibition of AgNPs against bacteria and fungi

BACTERIAL SPECIES	Concentration			
	25 µL	**50 µL**	**75µL**	**100 µL**
Pseudomonas. spp.,	-	-	5mm	5mm
Escherichia coli	-	-	-	5mm
Salmonella. spp.,	-	-	-	5mm
FUNGAL SPECIES	**Concentration**			
	25 µL	**50 µL**	**75µL**	**100 µL**
Candida albicans	-	-	5 mm	5 mm
Dermatophytes spp.,	-	-	5 mm	5 mm

3.7. Antioxidant activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, which measures the capacity to scavenge free radicals, was used to evaluate the antioxidant potential of the synthesized AgNPs. At volumes 20, 40, 60, 80, and 100 µL, a 100 µg/mL AgNPs suspension was tested, and the absorbance at 517 nm was measured. According to the results, absorbance decreased concentration-dependently from 0.602 to 0.413, which was consistent with rising inhibition percentages of 26.13%, 30.42%, 36.44%, 41.59%, and 49.32% (Figure 6). At higher concentrations, this trend suggests increased antioxidant activity. Tukey's HSD test further validated significant differences at higher volumes ($p < 0.01$), and statistical analysis using one-way ANOVA confirmed the significance of this dose-dependent response ($p < 0.05$). The scavenging potential is still noteworthy even though the maximum inhibition (49.32%) was less than that recorded for some plant-based AgNPs (e.g., 62.33%). Fish mucus biomolecules like proteins and polyphenols, which aid in the stabilization and reduction of AgNPs, are probably what drive the antioxidant activity. Differences in mucus composition between species may also affect the effectiveness of radical scavenging [47-49].

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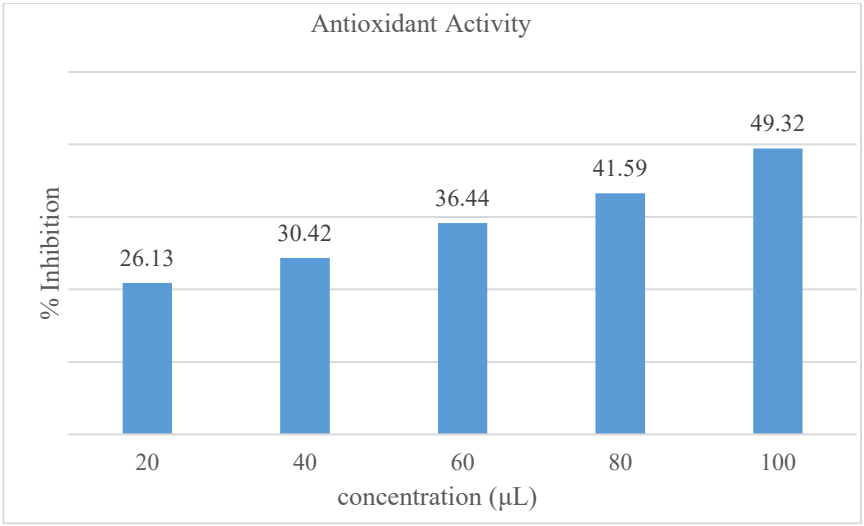

Figure 6: Anti-oxidant property of fish mucus-mediated synthesized AgNPs

3.8. Anti-cancerous activity (MTT assay)

MTT assay for anti-cancer activity The MTT assay was used to check the in in vitro anticancer effect of AgNPs made from Piaractus brachypomus skin mucus against HeLa

cells. Cells were incubated for 24 hours after being treated with different volumes (20–100 µL) of a 100 µg/mL AgNP suspension of cells.

A dose-dependent reduction in cell viability was observed, with average absorbance values of 0.885 (control), 0.824 (20 µL), 0.6515 (40 µL), 0.458 (60 µL), 0.2065 (80 µL), and 0.11 (100 µL), as shown in Figure 7. The test was carried out thrice to ensure the data is correct and for more accuracy. The corresponding relative viability percentages were 93.10%, 73.61%, 51.75%, 23.33%, and 12.42%, as presented in Table 3. The maximum cytotoxic effect was seen at 100 µL concentration, which was found to be the optimum concentration, while only cell viability remained 12%, indicating potential for strong anticancer property. The calculated IC_{50} (half-maximal inhibitory concentration) value was 58.05 µg/mL. Cytotoxicity assessment on normal Vero cells showed a CC_{50} of 434 µg/mL, resulting in a selectivity index (SI) of 7.5, underlining specific toxicity toward cancer cells while leaving normal cells unaffected [16]. This anticancer activity may be linked to the activity of AgNPs, causing several intracellular effects, including the generation of reactive oxygen species (ROS), disruption of mitochondrial membrane, and initiation of apoptosis via DNA fragmentation and inhibition of pivotal pathways that are required for cell growth. Further down, their nanoscale size and surface bioactivity enhance cellular uptake and interaction. Additionally, previous studies have highlighted and reported the therapeutic effects of AgNPs by detecting their anti-angiogenic and anti-metastatic effects of similar biogenic AgNPs [50-52]. Statistical analysis using ANOVA ($p< 0.05$) and Tukey's HSD test ($p < 0.01$) confirmed significant, dose-dependent cytotoxic effects. When compared with AgNPs synthesized from plant sources like *Quisqualis indica* or black beans, the fish mucus-derived AgNPs demonstrated comparable cytotoxic efficacy, establishing them as a viable and eco-friendly alternative [53]. Furthermore, although conventional chemotherapeutics often display IC_{50} values in the lower range of 1-10 µg/mL, the higher CC_{50} Figure 8 observed for the biosynthesized AgNPs (434 µg/mL) suggests a favourable therapeutic window with reduced toxicity toward normal cells, an important limitation of traditional agents. Altogether, these findings suggest that fish mucus-mediated AgNPs may serve as promising adjuncts or alternatives in anticancer therapies.

Table 3: Anti-cancerous activity of fish mucus-mediated synthesized AgNPs against the HeLa cell line

Concentration (100µg\mL)	Absorbance I	Absorbance II	Absorbance III	Mean	SD	Cell viability (%)
Control	0.879	0.891	0.885	0.885	0.006	100
20	0.82	0.828	0.824	0.824	0.004	93.1073446
40	0.645	0.658	0.6515	0.6515	0.0065	73.6158192
60	0.466	0.45	0.458	0.458	0.008	51.7514124
80	0.201	0.212	0.2065	0.2065	0.0055	23.3333333
100	0.103	0.117	0.11	0.11	0.007	12.4293785

Table 4: Cytotoxicity activity of fish mucus-mediated synthesized AgNPs against the HeLa cell line

Concentration (100µg\mL)	Absorbance I	Absorbance II	Absorbance III	Mean	SD	Cell viability (%)
Control	0.846	0.848	0.846	0.846667	0.0011547	100
20	0.774	0.778	0.769	0.773667	0.00450925	91.37795
40	0.742	0.751	0.748	0.747	0.00458258	88.22835
60	0.705	0.704	0.711	0.706667	0.00378594	83.46457
80	0.675	0.677	0.654	0.668667	0.01274101	78.97638
100	0.665	0.664	0.662	0.663	0.00152753	78.30709

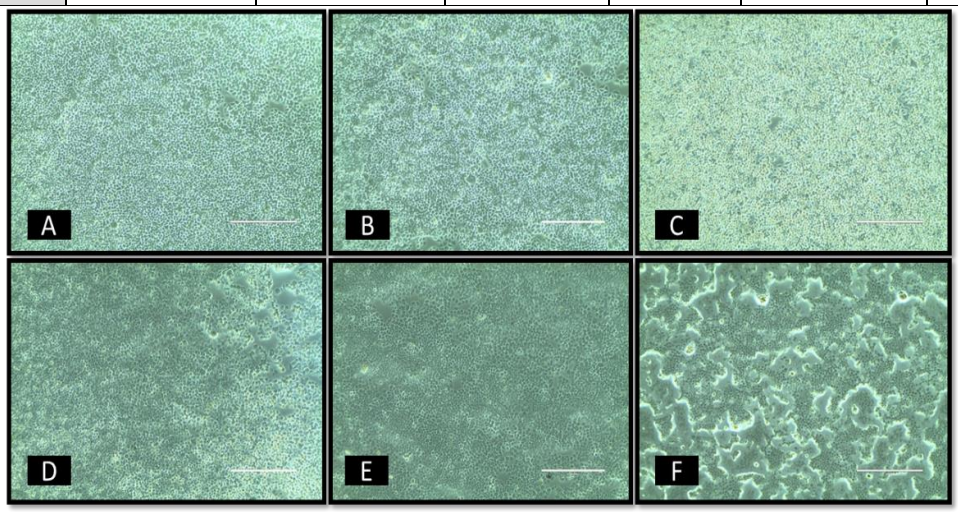

Figure 7: Observation of Anti-cancerous activity (MTT assay) of fish mucus-mediated synthesized AgNPs against HeLa cell line, with a scale bar of 100µm. (A) Control (untreated), (B-F) Influence of 20µL, 40µL, 60µL, 80µL, 100µL AgNPs on HeLa cell line.

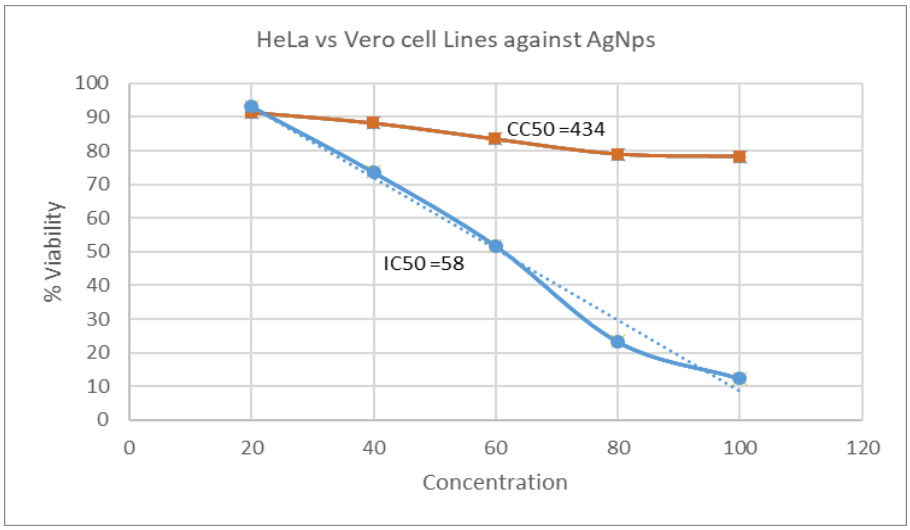

Figure 8: IC50 (Inhibitory Concentration 50%) and CC50 (Cytotoxic Concentration 50%) values of HeLa and Vero cell lines of fish mucus-mediated synthesized silver nanoparticle

Conclusion

This study successfully demonstrated the biosynthesis of AgNPs using the skin mucus of *Piaractus brachypomus*. The synthesized AgNPs exhibited stable, spherical morphology with an average size of 35.303±23.43nm, as confirmed by UV-Vis, FTIR, XRD, and FESEM-

EDAX analyses. These nanoparticles showed considerable antibacterial and antifungal activity against both Gram-positive and Gram-negative pathogens, as well as dermatophytes and *Candida albicans*. AgNPs showed moderate antioxidant activity and potent anticancer effects against HeLa cells (88% inhibition, SI = 7.5), indicating high therapeutic potential with low normal cell toxicity. The above findings using fish skin mucus provide an effective biological medium for the synthesis of AgNPs with promising biomedical properties. Additionally, the nanoparticles can be further explored for their potential application in aquaculture as a nano-based strategy to improve disease resistance in fish, offering a sustainable alternative to conventional antimicrobial treatments

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Conflict of interest

The authors declare they have no conflicts of interest

Author contribution

The authors Samson Lijoseraj C and T. Sathish Kumar worked on Conceptualization. Samson Lijoseraj C, Hari Prasad R and Magdalene Hannah E worked on Methodology. Data curation, Writing-Original draft were done by Samson Lijoseraj C, Premina S, Candace Crystal Arulraj and Hari Prasad R. Investigation, Supervision, and Validation were carried out by T. Sathish Kumar. Writing- Reviewing and editing was done by Samson Lijoseraj C, Premina S and T. Sathish Kumar

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References

[1] H. Duman, F. Eker, E. Akdaşçi, A. M. Witkowska, M. Bechelany, and Karav, "Silver nanoparticles: A comprehensive review of synthesis methods and chemical and physical properties," *Nanomaterials*, vol. 14, no. 18, pp. 1527, 2024 https://doi.org/10.3390/nano14181527

[2] W. F. Al-Malki and N. S. Alharbi, "Ficus Plant-Mediated Silver Nanoparticles: Synthesis, Optimization, Characterization, and Biomedical Applications," *Journal of Pure and Applied Microbiology*, vol. 19, no. 1, pp. 74-99, Mar. 2025, https://doi.org/10.22207/jpam.19.1.58

[3] M. A. M. Oliveira, M. L. Calado, S. F. C. Guerreiro, J. R. Dias, M. J. Campos, P. G. Murray, and S. C. Novais, "Improved biosynthesis and characteristics of silver nanoparticles using marine endophytic fungi exposed to hypo-osmotic stress," *Scientific Reports*, vol. 15, no. 1, 2025, https://doi.org/10.1038/s41598-025-98978-x

[4] V. N. Kalpana, B. A. S. Kataru, N. Sravani, T. Vigneshwari, A. Panneerselvam, and V. Rajeswari, "Biosynthesis of zinc oxide nanoparticles using culture filtrates of Aspergillus niger: Antimicrobial textiles and dye degradation studies*, "OpenNano*, vol. 3, pp. 48-55, 2018, https://doi.org/10.1016/j.onano.2018.06.001

[5] S. Vijayaram, H. Razafindralambo, Y. Sun, S. Vasantharaj, H. Ghafarifarsani, S. H. Hoseinifar, and M. Raeeszadeh, "Applications of green synthesized metal nanoparticles — a review," *Biological Trace Element Research*, vol. 202, no. 1, pp. 360–386, 2023, https://doi.org/10.1007/s12011-023-03645-9

[6] H. Singh and T. Sharma, "Green synthesis and evaluation of silver nanoparticles using Cyperus rotundus," *International Journal of Medical Sciences and Pharma Research*, vol. 7, no. 3, pp. 1–7, 2021, https://doi.org/10.22270/ijmspr.v7i3.26

[7] R. Tripathi and V. Chourasia, "Formulation and characterization of silver nanoparticles incorporated gel of ethanolic extract of Celastrus orbiculatus for effective antimicrobial effect," *International Journal of Environmental Sciences*, vol. 11, no. 7s, pp. 796–807, 2025, https://doi.org/10.64252/z29y0t64

[8] I. V. Esarev and I. Ott, "Modulation of the mechanism of action of antibacterial silver N-heterocyclic carbene complexes by variation of the halide ligand," *RSC Advances*, vol. 15, no. 3, pp. 1782–1791, 2025, https://doi.org/10.1039/D4RA08093A

[9] S. Ahmed, M. Ahmad, B. L. Swami, and S. Ikram, "A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise," *Journal of Advanced Research*, vol. 7, no. 1, pp. 17–28, Mar. 2015, https://doi.org/10.1016/j.jare.2015.02.007

[10] D. Mundekkad and W. C. Cho, "Nanoparticles in clinical translation for cancer therapy," *International Journal of Molecular Sciences*, vol. 23, no. 3, pp. 1685, Feb. 2022, https://doi.org/10.3390/ijms23031685

[11] H. I. O. Gomes, C. S. M. Martins, and J. A. V. Prior, "Silver nanoparticles as carriers of anticancer drugs for efficient target treatment of cancer cells," *Nanomaterials*, vol. 11, no. 4, pp. 964, Apr. 2021, https://doi.org/10.3390/nano11040964

[12] N. Alrushaid, F. A. Khan, E. A. Al-Suhaimi, and A. Elaissari, "Nanotechnology in cancer diagnosis and treatment," *Pharmaceutics*, vol. 15, no. 3, p. 1025, Mar. 2023, https://doi.org/10.3390/pharmaceutics15031025

[13] Y. Yao, Y. Zhou, J. Liu, X. Feng, X. Wei, L. Chen, Y. Fu, and Y. Luo, "Nanoparticle-based drug delivery in cancer therapy and its role in overcoming drug resistance," *Frontiers in Molecular Biosciences*, vol. 7, Aug. 2020, https://doi.org/10.3389/fmolb.2020.00193

[14] M. Moond, S. Singh, S. Sangwan, R. Devi, and R. Beniwal, "Green synthesis and applications of silver nanoparticles: A systematic review," *AATCC Journal of Research*, vol. 9, no. 6, pp. 272–285, 2022, https://doi.org/10.1177/24723444221119847

[15] H. I. O. Gomes, C. S. M. Martins, and J. A. V. Prior, "Silver nanoparticles as carriers of anticancer drugs for efficient target treatment of cancer cells," *Nanomaterials*, vol. 11, no. 4, p. 964, Apr. 2021, https://doi.org/10.3390/nano11040964

[16] C. Gomathi, S. R. X. Rajarathinam, A. M. Sadiq, and S. Rajeshkumar, "Anticancer activity of silver nanoparticles synthesized using aqueous fruit shell extract of *Tamarindus indica* on MCF-7 human breast cancer cell line," *Journal of Drug Delivery Science and Technology*, vol. 55, p. 101376, Nov. 2019, https://doi.org/10.1016/j.jddst.2019.101376

[17] P. Mirabelli, L. Coppola, and M. Salvatore, "Cancer cell lines are useful model systems for medical research," *Cancers*, vol. 11, no. 8, p. 1098, Aug. 2019, https://doi.org/10.3390/cancers11081098

[18] J. J. M. Landry, P. T, T. Rausch, T. Zichner, M. M. Tekkedil, A. M. Stütz, A. Jauch, R. S. Aiyar, G. Pau, N. Delhomme, J. Gagneur, J. O. Korbel, W. Huber, and L. M. Steinmetz, "The genomic and transcriptomic landscape of a HELA cell line," *G3 Genes Genomes Genetics*, vol. 3, no.8, pp. 1213–1224. Mar. 2013, https://doi.org/10.1534/g3.113.005777

"The genomic and transcriptomic landscape of a HeLa cell line," *G3 Genes Genomes Genetics*, vol. 3, no. 8, pp. 1213–1224, Mar. 2013, https://doi.org/10.1534/g3.113.005777

[19] N. Korkmaz, Y. Ceylan, R. İmamoğlu, D. Kısa, F. Şen, and A. Karadağ, "Eco-friendly biogenic silver nanoparticles: synthesis, characterization and biological applications," *International Journal of Environmental Science and Technology*, 2024, https://doi.org/10.1007/s13762-024-05860-w

[20] L. N. Murthy, B. M. Rao, K. K. Asha, and M. M. Prasad, "Nutritional composition, product development, shelf-life evaluation and quality assessment of pacu Piaractus brachypomus

(Cuvier, 1818)," *Indian Journal of Fisheries*, Mar. 27, 2015, https://epubs.icar.org.in/index.php/IJF/article/view/25069

[21] J. P. Duarah, M. Mall, and S. C. Phukan, "Exotic fish culture in pond aquaculture system: Study of Rupchanda (Piaractus brachypomus) culture in Assam, India," *Cogent Business & Management,* vol. 10, no. 3, 2023, https://doi.org/10.1080/23311975.2023.2257830

[22] M. Mukherjee, S. Das, and A. K. Borah, "Successful breeding and seed production of pacu (Piaractus brachypomus) in captivity," *Fishing Chimes*, vol. 32, pp. 57–60, 2012.

[23] S. Wang, Y. Wang, J. Ma, Y. Ding, and S. Zhang, "Phosvitin plays a critical role in the immunity of zebrafish embryos via acting as a pattern recognition receptor and an antimicrobial effector," *Journal of Biological Chemistry*, vol. 286, no. 25, pp. 22653–22664, Apr. 2011, https://doi.org/10.1074/jbc.M111.247635

[24] A. Arasu, V. Kumaresan, A. Sathyamoorthi, R. Palanisamy, N. Prabha, P. Bhatt, A. Roy, M. K. Thirumalai, A. J. Gnanam, M. Pasupuleti, K. Marimuthu, and J. Arockiaraj, "Fish lily type lectin-1 contains β-prism architecture: Immunological characterization," *Molecular Immunology*, vol. 56, no. 4, pp. 497–506, 2013. https://doi.org/10.1016/j.molimm.2013.06.020

[25] F. Tiralongo, G. Messina, B. M. Lombardo, L. Longhitano, G. L. Volti, and D. Tibullo, "Skin mucus of marine fish as a source for the development of antimicrobial agents," *Frontiers in Marine Science*, vol. 7, 2020, https://doi.org/10.3389/fmars.2020.541853

[26] S. Dash, S. K. Das, J. Samal, and H. N. Thatoi, "Epidermal mucus, a major determinant in fish health: a review," *Iranian Journal of Veterinary Research*, vol. 9, no.2, pp. 72-81, 2018, https://doi.org/10.22099/ijvr.2018.4849

[27] G. Chinnadurai, R. Subramanian, and M. Ahamed, "Fish mucus mediated biosynthesis of copper oxide nanoparticles: spectral characterization, morphology and biological activity," *Materials Research Express*, vol. 7, no. 12, p. 125012, Nov. 2020, https://doi.org/10.1088/2053-1591/abcee7

[28] E. Urnukhsaikhan, B.-E. Bold, A. Gunbileg, N. Sukhbaatar, and T. Mishig-Ochir, "Antibacterial activity and characteristics of silver nanoparticles biosynthesized from *Carduus crispus*," *Scientific Reports*, vol. 11, no. 1, Oct. 2021, https://doi.org/10.1038/s41598-021-00520-2

[29] E. S. Al-Sheddi, N. N. Farshori, M. M. Al-Oqail, S. M. Al-Massarani, Q. Saquib, R. Wahab, J. Musarrat, A. A. Al-Khedhairy, and M. A. Siddiqui, "Anticancer potential of green synthesized silver nanoparticles using extract of *Nepeta deflersiana* against human cervical cancer cells (HeLa)," *Bioinorganic Chemistry and Applications*, vol. 2018, pp. 1–12, 2018. https://doi.org/10.1155/2018/9390784

[30] K. Jemal, B. V. Sandeep, and S. Pola, "Synthesis, characterization, and evaluation of the antibacterial activity of *Allophylus serratus* leaf and leaf-derived callus extracts mediated silver nanoparticles," *Journal of Nanomaterials*, vol. 2017, pp. 1–11, Jan. 2017, https://doi.org/10.1155/2017/4213275

[31] S. Marimuthu, A. Rahuman, S. Rajakumar, C. Santhoshkumar, A. Kirthi, and G. Jayaseelan, "Evaluation of green synthesized silver nanoparticles against parasites," *Parasitology Research*, vol. 108, no. 6, pp. 1541–1549, Dec. 2010, https://doi.org/10.1007/s00436-010-2212-4

[32] Y. S., R. S., K. K., and N. S., "Investigation of functional groups in epidermal mucus of endemic Bagridae fishes of Western Ghats," *International Journal of Fisheries and Aquatic Studies*, vol. 10, no. 1, pp. 112–115, Jan. 2022, https://doi.org/10.22271/fish.2022.v10.i1b.2627

[33] M. J. Bose, B. Velmurugan, D. Poornima, V. Vineetha, N. Samima, and A. A. Banu, "Antimicrobial studies on epidermal mucus of fish *Anabas testudineus*," *Journal of Advanced Zoology*, vol. 43, no. 1, 2022, https://doi.org/10.17762/jaz.v44i3.1048

[34] M. Goudarzi, N. Mir, M. Mousavi-Kamazani, S. Bagheri, and M. Salavati-Niasari, "Biosynthesis and characterization of silver nanoparticles prepared from two novel natural precursors by facile thermal decomposition methods," *Scientific Reports*, vol. 6, no. 1, Sep. 2016, https://doi.org/10.1038/srep32539

[35] U. Ramaswamy, D. Mukundan, A. Sreekumar, and V. Mani, "Green synthesis and characterization of silver nanoparticles using aqueous whole plant extract of *Vernonia cinerea* L. and its biological activities," *Materials Today: Proceedings*, vol. 2, no. 9, pp. 4600–4608, 2015, https://doi.org/10.1016/j.matpr.2015.10.080

[36] N. Reddy, H. Li, T. Hou, Z. Bethu, Z. Ren, and Z. Zhang, "Phytosynthesis of silver nanoparticles using *Perilla frutescens* leaf extract: Characterization and evaluation of antibacterial, antioxidant, and anticancer activities," *International Journal of Nanomedicine*, vol. 16, pp. 15–29, Jan. 2021, https://doi.org/10.2147/IJN.S265003

[37] A. Hussain and S. G. Sachan, "Fish epidermal mucus as a source of diverse therapeutical compounds," *International Journal of Peptide Research and Therapeutics,* vol. 29, no. 3, 2023, https://doi.org/10.1007/s10989-023-10505-6

[38] A. Panáček, M. Smékalová, M. Kilianová, R. Prucek, K. Bogdanová, R. Večeřová, M. Kolář, M. Havrdová, G. Płaza, J. Chojniak, R. Zbořil, and L. Kvítek, "Strong and nonspecific synergistic antibacterial efficiency of antibiotics combined with silver nanoparticles at very low concentrations showing no cytotoxic effect," *Molecules*, vol. 21, no. 1, p. 26, 2015. https://doi.org/10.3390/molecules21010026

[39] J. K. Patra and K.-H. Baek, "Antibacterial activity and synergistic antibacterial potential of biosynthesized silver nanoparticles against foodborne pathogenic bacteria along with its anticandidal and antioxidant effects," *Frontiers in Microbiology*, vol. 8, Feb. 2017, https://doi.org/10.3389/fmicb.2017.00167

[40] K. Roy, C. K. Sarkar, and C. K. Ghosh, "Plant-mediated synthesis of silver nanoparticles using parsley (*Petroselinum crispum*) leaf extract: spectral analysis of the particles and antibacterial study," *Applied Nanoscience*, vol. 5, no. 8, pp. 945–951, Dec. 2014, https://doi.org/10.1007/s13204-014-0393-3

[41] S. R. Kitimu, E. K. Mutitu, H. L. Maghenda, J. M. Mutuku, P. G. Kareru, and A. M. Mwachiro, "Anti-angiogenic and anti-metastatic effects of biogenic silver nanoparticles synthesized using *Azadirachta indica*," *Advances in Bioscience and Biotechnology*, vol. 13, no. 4, pp. 188–206, Jan. 2022, https://doi.org/10.4236/abb.2022.134010

[42] E. Urnukhsaikhan, B.-E. Bold, A. Gunbileg, N. Sukhbaatar, and T. Mishig-Ochir, "Antibacterial activity and characteristics of silver nanoparticles biosynthesized from *Carduus crispus*," *Scientific Reports*, vol. 11, no. 1, Oct. 2021, https://doi.org/10.1038/s41598-021-00520-2

[43] M. Saravanan, S. Arokiyaraj, T. Lakshmi, and A. Pugazhendhi, "Synthesis of silver nanoparticles from *Phenerochaete chrysosporium* (MTCC-787) and their antibacterial activity against human pathogenic bacteria," *Microbial Pathogenesis*, vol. 117, pp. 68–72, Feb. 2018, https://doi.org/10.1016/j.micpath.2018.02.008

[44] T. C. Dakal, A. Kumar, R. S. Majumdar, and V. Yadav, "Mechanistic basis of antimicrobial actions of silver nanoparticles," *Frontiers in Microbiology*, vol. 7, Nov. 2016, https://doi.org/10.3389/fmicb.2016.01831

[45] A. Menichetti, A. Mavridi-Printezi, D. Mordini, and M. Montalti, "Effect of size, shape and surface functionalization on the antibacterial activity of silver nanoparticles," *Journal of Functional Biomaterials*, vol. 14, no. 5, p. 244, Apr. 2023, https://doi.org/10.3390/jfb14050244

[46] D. McShan, Y. Zhang, H. Deng, P. C. Ray, and H. Yu, "Synergistic antibacterial effect of silver nanoparticles combined with ineffective antibiotics on drug resistant *Salmonella typhimurium* DT104," *Journal of Environmental Science and Health, Part C*, vol. 33, no. 3, pp. 369–384, Jun. 2015, https://doi.org/10.1080/10590501.2015.1055165

[47] H. Deng, D. McShan, Y. Zhang, S. S. Sinha, Z. Arslan, P. C. Ray, and H. Yu, "Mechanistic study of the synergistic antibacterial activity of combined silver nanoparticles and common antibiotics," *Environmental Science & Technology*, vol. 50, no. 16, pp. 8840–8848, 2016. https://doi.org/10.1021/acs.est.6b00998

[48] S. Iravani, H. Korbekandi, S. V. Mirmohammadi, and B. Zolfaghari, "Synthesis of silver nanoparticles: Chemical, physical and biological methods," *International Scholarly Research Notices*, Dec. 2014, https://pubmed.ncbi.nlm.nih.gov/26339255/

[49] A. Akintola, B. Kehinde, P. Ayoola, A. Adewoyin, O. Adedosu, J. Ajayi, and S. Ogunsona, "Antioxidant properties of silver nanoparticles biosynthesized from methanolic leaf extract of *Blighia sapida*," *IOP Conference Series: Materials Science and Engineering*, vol. 805, no. 1, p. 012004, 2020. https://doi.org/10.1088/1757-899X/805/1/012004

[50] F. Khuda, M. Jamil, A. a. K. Khalil, R. Ullah, N. Ullah, F. Naureen, M. Abbas, M. S. Khan, S. Ali, H.M.U. Farooqi, and M. Ahn "Assessment of antioxidant and cytotoxic potential of silver nanoparticles synthesized from root extract of *Reynoutria japonica* Houtt," *Arabian Journal of Chemistry*, vol. 15, no. 12, pp. 104327, Oct. 2022, https://doi.org/10.1016/j.arabjc.2022.104327

[51] P. C. Nagajyothi, P. Muthuraman, T. V. M. Sreekanth, D. H. Kim, and J. Shim, "Green synthesis: In-vitro anticancer activity of copper oxide nanoparticles against human cervical carcinoma cells," *Arabian Journal of Chemistry*, vol. 10, no. 2, pp. 215–225, Feb. 2016, https://doi.org/10.1016/j.arabjc.2016.01.011

[52] G. Rajakumar, T. Gomathi, M. Thiruvengadam, V. D. Rajeswari, V. N. Kalpana, and I.-M. Chung, "Evaluation of anti-cholinesterase, antibacterial and cytotoxic activities of green synthesized silver nanoparticles from *Millettia pinnata* flower extract," *Microbial Pathogenesis*, vol. 103, pp. 123–128, Dec. 2016, https://doi.org/10.1016/j.micpath.2016.12.019

[53] R. Mukhopadhyay, J. Kazi, and M. C. Debnath, "Synthesis and characterization of copper nanoparticles stabilized with *Quisqualis indica* extract: Evaluation of its cytotoxicity and apoptosis in B16F10 melanoma cells," *Biomedicine & Pharmacotherapy*, vol. 97, pp. 1373–1385, Dec. 2017, https://doi.org/10.1016/j.biopha.2017.10.167