

Ellagic acid-Wrapped gold nanoparticles: Evaluation of antimicrobial, antioxidant, anti-inflammatory, and cytotoxicity effects of KB cells

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Abstract

The green synthesis of metal nanoparticles using plant-based molecules is gaining attention for its potential in biomedical applications. The aim of present study the eco-friendly synthesis of gold nanoparticles (AuNPs) using ellagic acid, a natural antioxidant, which acted as both reducing and capping agent. The successful synthesized ellagic acid-coated AuNPs (EA-AuNPs) was indicated by a colour change to dark brown and confirmed through UV-visible spectroscopy with a peak at 587.5 nm. Transmission electron microscopy (TEM) revealed long rod-shaped nanoparticles ranging from 50 to 70 nm in size, and energy-dispersive X-ray spectroscopy (EDX) confirmed the presence of Au, C, Cl, and N elements. Zeta potential analysis showed a stable surface charge of -17.7 mV. EA-AuNPs exhibited strong antibacterial activity, including a 37.26 ± 0.9 mm inhibition zone against *Klebsiella pneumoniae* at 100 µg/mL and minimum inhibitory concentration (MIC) values below 1 µg/mL against *Staphylococcus aureus*. The nanoparticles also demonstrated potent antioxidant activity, achieving 91% and 89% radical scavenging in DPPH and ABTS assays, respectively. Anti-inflammatory testing showed 78% membrane stabilization at 200 µg/mL. Moreover, cytotoxicity studies revealed that EA-AuNPs had an IC_{50} of 59.58 µg/mL against oral epidermoid carcinoma (KB) cells. In-silico analysis also demonstrated a promising binding affinity of -7.3 kcal/mol with *Candida albicans*, suggesting notable antifungal properties. The present study highlights the multi-functional therapeutic potential of EA-AuNPs in oral health care, offering a biocompatible approach to combat microbial infections, oxidative stress, inflammation, and oral cancer. The integration of both *in-vitro* and *in-silico* results supports their future application in dental and oral medicine.

Keywords: Eco-friendly, Ellagic acid, Gold nanoparticles, Oral cancer properties, Antibacterial

1.Introduction

Nanotechnology is a transformative field that has significantly impacted drug discovery and development [1]. Through nanoscale fabrication of materials (1-100 nm), researchers may produce nanoparticles with specific properties that improve delivery of medicines, effectiveness, and safety[2]. Nanotechnology has revolutionized various fields, including medicine, electronics, and environmental science[3]. One of the most promising and sustainable approaches in nanotechnology is plant-based synthesis, which leverages the natural biochemical processes of plants to create nanoparticles [4]. This method has gained significant attention due to its eco-friendly nature, cost-effectiveness, and potential for large-scale production. As with any emerging technology, there are concerns about the potential toxicity and long-term environmental impact of nanoparticles [5]. Regulatory frameworks are being developed to ensure the safe use of nanotechnology in various applications [6]. Nanotechnology allows for the creation of multifunctional nanoparticles that can perform multiple tasks simultaneously, such as imaging, diagnosis, and therapy [7]. This integration of functionalities can improve the efficiency of treatment and provide real-time monitoring of therapeutic outcomes. Nanotechnology can help address the growing problem of drug resistance, particularly in cancer and infectious diseases [8]. Nanomaterials have garnered considerable attention in recent years due to their unique physicochemical properties, including high surface area-to-volume ratio, enhanced reactivity, and tuneable morphology. Among these, metallic nanoparticles, particularly AuNPs stand out for their excellent biocompatibility, stability, and ease of surface functionalization, making them highly valuable in biomedical, pharmaceutical, and diagnostic applications [8].

Antimicrobial resistance (AMR) is most critical public health challenges globally and in India in 2024 [9]. In India, widespread misuse and overuse of antibiotics, inadequate infection control practices, and limited access to new antimicrobial agents [10] [11]. This situation is particularly alarming in multi drug resistant patients, who are highly susceptible to infections due to their weakened immune systems, frequent hospitalizations, and aggressive treatment [12]. AMR convergence presents two threats: more difficult-to-treat infections that result in longer hospital stays, higher medical expenses, and more deaths [13], [14]. As India continues to grapple with growing AMR problem, there is an urgent need for coordinated efforts in research, policy-making, and healthcare practice to address this dual challenge, ensuring that effective treatment options remain available for bacterial infections [15]. Thus, to tackle this highly alarming issue, the World Health Organization (WHO) is urging on scientists worldwide

to perform studies and develop new antimicrobial agents, especially drugs against bacteria [16]. It is a severe global crisis, with pathogens increasingly developing resistance to commonly used antibiotics, rendering many standard treatments ineffective. Novel combinations based on various types of nanoparticles (NPs) with varying sizes, shapes, and diverse antimicrobial properties have become possible due to recent developments in nanotechnology [17].

Cancer is one of the most common diseases, characterized by unregulated cell division that results in malignant tumours able to infecting nearby tissues and migrating through the body via the lymphatic system or blood circulation [18]. It is currently accountable for 8 million deaths and 14 million new cases each year, which makes it the second most common cause of mortality worldwide [19]. Oral cancer is the sixth fastest-growing kind of cancer worldwide, accounting for 275,000 new cases annually. It is a serious health concern [20]. Heavy drinking and tobacco use are responsible for almost 80% of cases of mouth cancer. Current chemotherapeutic treatments for a variety of malignancies are frequently found to have systemic organ toxicity, negative side effects, and decreased efficacy because of resistant tumour drugs [21]. To reduce the sickness and its related deaths, the attention of anticancer drugs was moved to novel sources with minimal side effects and better efficacy. One of the most notable and rapidly expanding fields of the present decade is nanotechnology. Because of its enhanced performance, nanotechnology is gaining more attention in the fields of biomaterials, nano-electronics, nano-medicines and medicinal textiles [22], [23].

Many medicinal plants are used in the industrial production of herbal products and bioactive substances for medicines and nutritional supplements [24]. Flavonoids, alkaloids, organic acids, volatile oils, polysaccharides, saponins, anthraquinones, and terpenoids belong to the natural molecules that during the past several years have demonstrated potential in combating against bacterial and viral diseases [25], [26]. Bioactive secondary metabolites found in plants have the potential to be anticancer drugs with a broad range of medicinal applications [27]. Ellagic acid (EA) is a naturally present phenolic antioxidant agent. It consists of four hydroxyl rings bonded together groups, while the hydrophilic portion is represented by two lactone rings. Fruits and nuts that are rich in ellagic acid (EA) contain pomegranates, walnuts, blueberries, raspberries, strawberries, and grapes. This molecule has a wide range of biological actions, such as antiviral, anti-mutagenic, anti-carcinogenic, and antioxidant properties [28]. EA is commonly used as a dietary supplement to treat disorders including cancer, cardiovascular diseases, and other health issues. It is easily available in a variety of forms, including liquids, powders, and capsules [29]. The environmentally friendly production of gold nanoparticles

(AuNPs) has become a viable substitute for conventional chemical and physical processes, which frequently call for unsafe molecules and adverse conditions [30]. Green synthesis influences biological materials such as plant extracts, bacteria, fungi, and biomolecules to produce nanoparticles in an environmentally friendly manner [31]. They are considered the biological agents that have the strongest capability to produce a diversity of biological compounds, such as proteins, carbohydrates, phenols, alkaloids, saponins, and flavonoids, all of which can play a critical role in the reduction of Au ions into AuNPs [32]. Gold nanoparticles (AuNPs) are well-known for their therapeutic activity, such as antioxidant, antibacterial, antifungal, anticancer, and wound-healing properties [33], [34], [35]. Its excellent biocompatibility, low reactivity, ideal stability, and low toxicity in biological systems have earned them widespread recognition. AuNPs also have unique optical, electrical, scattering, and absorption characteristics, as well as a high surface-to-volume ratio [36]. It can be utilized in many sectors, such as electronics, sensing, environmental monitoring, treatments, and biology, due to their easily developed surface function and adjustable plasmon resonance properties. This method is not only eco-friendly but also offers the other advantage of producing biocompatible nanoparticles suitable for various biomedical applications [37].

Ellagic acid (EA), a natural polyphenol found in fruits and vegetables, is known for its strong antioxidant, antimicrobial, and anticancer properties [38]. The integration of EA in the synthesis of AuNPs not only enhances the therapeutic efficacy of the resulting nanomaterials but also ensures biocompatibility and environmental sustainability. The aim of current study describes the first report on the synthesis of AuNPs mediated by ellagic acid and their synthesized EA-AuNPs were spectral characterized using UV-Vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), Field emission scanning electron microscopy (FE-SEM), High-resolution transmission electron microscopy (HR-TEM), Energy-dispersive X-ray spectroscopy (EDX), Dynamic light scattering (DLS), and Zeta potential analysis, confirming their optimal physical and physicochemical properties. Further, bio-synthesized nanoparticles tested antimicrobial activity against oral and clinical pathogens. Additionally, *in-silico* screening was evaluated using selected pathogens. Furthermore, the EA-AuNPs tested anticancer potential against KB oral cancer cells.

2. Materials and methods

2.1. Materials used

Gold chloride LR ($\text{HAuCl}_4 \cdot x\text{H}_2\text{O}$, 99.9%) is purchased from Sigma-Aldrich, Methanol, Dimethyl sulfoxide (DMSO) and Ethanol absolute ($\text{C}_2\text{H}_5\text{OH}$, EtOH) was procured from Central Drug House (P) LTD. 95%). Mueller-Hinton Agar, Sodium hydroxide procured from Hi-Media, Mumbai. The biological test was carried out using sterile water overall. All glassware and Petri plates were properly cleaned with distilled water and concentrated nitric acid (HNO_3) before to drying in a hot air oven.

2.2. Biosynthesis of EA-AuNPs

The eco-friendly synthesis of ellagic acid-mediated gold nanoparticles (EA-AuNPs) was conducted followed with slight modification. Initially, 1.51 mg of ellagic acid were precisely weighed using an analytical balance and dissolved in 5 mL of methanol to prepare the base solution. Subsequently, 12.13 mg of auric (III) chloride, equivalent to a 1mM concentration, was accurately measured and introduced into the ellagic acid-methanol solution. This mixture was then subjected to magnetic stirred at an exact temperature of 28°C , with a stirring speed maintained at 900 rpm for 1 h. The progress of the reaction was monitored visually, with a noticeable colour change from colourless to dark brown, indicating the successful formation of ellagic acid-mediated gold nanoparticles. This shift in colour signifies the reduction of gold ions and the subsequent stabilization of the nanoparticles by ellagic acid, confirming the effective synthesis of EA-AuNPs. After the synthesis was finished, the reaction solution was centrifuged for 15 min at 12,000 rpm and the dark particles collected were cleaned with 70% ethanol to remove impurities from the final outcome. A dark particle was formed after six hours of drying at 60°C in a closed oven.

2.3. Spectral analysis of EA-AuNPs

2.3.1. UV Vis spectroscopy

Ellagic acid-mediated AuNPs were characterized using UV-Vis spectroscopy to monitor nanoparticle formation and stability [39]. The sample was scanned within the 200–800 nm range, where the characteristic surface plasmon resonance (SPR) peak of gold nanoparticles typically appears between 500–550 nm, indicating successful synthesis. The intensity and position of the SPR peak were linked to particle size and dispersion [40]. To track gold ion bio-depletion, 2–3 mL samples were periodically collected and scanned using UV-vis spectroscopy (Jasco Instruments). If the solution darkened, the samples were diluted with 2 mL of double-distilled water before scanning. The UV-Vis measurements were conducted using a Jasco Instruments double-beam spectrophotometer with a 1 nm resolution.

2.3.2. FTIR analysis

A small quantity of synthesized EA-AuNPs is mixed with a suitable matrix, such as potassium bromide (KBr), to form a pellet (1:9 ratio, W/W). This mixture is then placed in the sample holder of the (PerkinElmer Spectrum IR) FTIR spectrometer [41]. The instrument is calibrated, and the sample is scanned over a precise wavelength range from 4000 to 400 cm^{-1} . The resulting spectrum shows absorption peaks corresponding to various functional groups present in the EA-AuNPs and any stabilizing agents used in their synthesis. By analysing these peaks, one can identify the chemical bonds and functional groups involved in the formation and stabilization of the EA-AuNPs [42].

2.3.3. SEM and EDX analysis

First, a small aliquot of the synthesized EA-AuNPs is placed on a clean and conductive substrate, such as a silicon wafer or a copper grid, and allowed to dry completely to avoid aggregation. The sample is then coated with a thin layer of conductive material, like gold or carbon, if it is non-conductive, to enhance imaging quality. Once prepared, the sample is loaded into the SEM chamber, where it is subjected to a high-energy electron beam, generating high-resolution images that reveal the morphology, size, and distribution of the EA-AuNPs [43]. For EDX analysis, the same SEM setup is used (ZEISS Sigma); the electron beam excites the sample, causing it to emit characteristic X-rays, which are collected to provide qualitative and quantitative elemental analysis of the EA-AuNPs. This combined approach allows for detailed insights into the size, shape, and elemental configuration of the nanoparticles.

2.3.4. TEM analysis

TEM analysis of AuNPs is a technique for assessing their size, shape, and physical properties at the nanoscale. The synthesized AuNPs are first dispersed in a suitable solvent and then dropped onto a copper grid coated with a thin film of carbon, allowing the sample to dry. Once prepared, the grid is placed in the TEM instrument, such as the JEOL JEM-2100. The TEM operates by transmitting a high-energy electron beam through the sample (100 eV), which scatters and forms an image based on the contrast between the AuNPs and the background. This process provides high-resolution images that reveal the morphology, crystalline structure, and particle size distribution of the EA-AuNPs [44]. Additionally, the diffraction pattern obtained during the analysis can be used to confirm the crystallinity and phase of the nanoparticles, offering valuable insights into their properties for various applications.

2.3.5. XRD analysis

X-ray diffraction (XRD) analysis is performed to determine the crystalline structure of EA-AuNPs. The EA-AuNPs material was analyzed using Bruker's D-8 advanced powder XRD (Bruker, Germany) in the two theta degrees (10° – 70°) range, with a step diameter of 0.02 and a scanning speed of 10.00° per minute. The power supply approximately 40 kV and the current were 50 mA. The well-dried EA-AuNPs powder was placed in the sample holder and spectra were collected [45]. The characteristic diffraction peaks corresponding to the face-centred cubic (FCC) structure of gold appear in the XRD pattern, allowing determination of crystal size using the Debye-Scherrer equation.

$$\text{Crystalline size (D)} = \frac{k\lambda}{\beta \cos \theta} \quad \text{Eq. 1}$$

2.3.6. Dynamic light scattering and Zeta potential

DLS and Zeta potential analysis of gold nanoparticles (AuNPs) are essential techniques for evaluating their size distribution and surface charge, which are crucial for stability and reactivity [46]. To prepare the sample for DLS, a small volume of synthesized AuNPs is diluted with a suitable solvent, typically double-distilled water or a buffer, to achieve a concentration that prevents multiple scattering effects. The diluted sample is then transferred to a clean cuvette, ensuring that no bubbles are present, as they can interfere with the measurements. For DLS analysis, the cuvette is placed in a DLS instrument, such as a (Anton Paar) Zetasizer, which measures the intensity of scattered light as the nanoparticles move in the solution, providing information on their size distribution. Following DLS, the same diluted sample is used for Zeta potential analysis. The sample is loaded into a Zeta potential cell, where an electric field is applied to measure the electrophoretic mobility of the particles, which is then transformed into Zeta potential using the Smoluchowski equation [47].

2.4. Biological application

2.4.1. Collection of bacterial strains

Current study investigated seven different human pathogenic microorganisms. The four Gram-positive bacterial strains included *Enterococcus faecalis* (ATCC 15753), *Streptococcus mutans* (ATCC 735668), *Klebsiella pneumoniae* (ATCC 700603), and *Staphylococcus aureus* (MTCC 737). Additionally, two Gram-negative bacterial strains were examined: *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922), and one for fungi *Candida albicans* (MTCC 183).

2.4.2. Antimicrobial assay

The antibacterial property of EA-AuNPs against human pathogens was evaluated using the agar well diffusion technique. The agar well diffusion method was used to test the antibacterial properties of the Mueller Hinton agar (MHA) dishes while maintaining bacterial strains in the nutrient agar medium[48]. After autoclaving the nutritional agar medium for 15 min at 121 °C, 20 mL of the sterilized MHA medium were transferred into each sterile Petri plates and allowed to solidify in the laminar airflow hood's aseptic condition. After that, 100 µL of bacterial (1.5×10^8 cell) culture was inoculated onto spread plates. The inoculation is produced from a broth solution that is grown overnight and diluted to the 0.5 McFarland turbidity level. A sterile metallic cork borer was used to form 6 mm holes under aseptic conditions. Different doses (50 and 100 µg/mL) were obtained by dissolving the EA-AuNPs and the negative control (solvent only) in 1% aqueous DMSO. A micropipette was used to gently load these solutions into the appropriate wells. The positive control was amoxicillin (4 mg/mL). For 12 h, the MHA plates containing the sample loaded wells were maintained in a bacteriological incubator at 37 °C or 25°C for fungus. A scale was used to evaluate and describe the zone of growth inhibition in mm [49].

2.4.3. Minimum inhibitory concentration (MIC)

The MIC assay was used to determine the minimal quantity of EA-AuNPs essential to inhibit the growth of the microorganisms *E. faecalis*, *E. coli*, *S. aureus*, *S. mutans*, *C. albicans*, *P. aeruginosa*, and *K. pneumoniae* [50]. A sterile microtiter plate was prepared, with each well containing varying concentrations of EA-AuNPs starting from 0.5, 1, 2.5, 5, 7.5, 10, 12.5, 15, 25, 50, 100, and 200 µg/mL. To each well, an additional 100 µL of inoculum was added, consisting of a uniform suspension of the test microorganisms at a concentration of approximately 1×10^7 CFU/mL [51]. After being kept at 37 °C for 24 h and 25°C for fungi). After incubation, the optical density (O.D.) of bacteria growth was estimated at 600 nm [52]. The MIC was calculated as the lowest concentration of EA-AuNPs that totally inhibited observable bacterial growth, as demonstrated by the absence of colony or turbidity in the well [50]. The only 1% DMSO is used as a negative control.

2.5. In-silico studies

2.5.1. Selection and preparation of proteins and ligand

In-silico studies offer a rapid, cost-effective, and reliable method to predict and validate molecular interactions between bioactive compounds and biological targets [53]. This approach complements the in-vitro findings by providing molecular-level insights into how the nanoparticles may exert their therapeutic effects. By integrating computational analysis, we enhance our understanding of target-specific interactions, help identify possible binding sites, and support the experimental results with mechanistic validation. Thus, the in-silico study serves as a valuable tool to substantiate the biological efficacy of EA-AuNPs and to guide further development of nanoparticle-based therapeutics. Proteins used in the study were retrieved from the RCSB Protein Data Bank (<https://www.rcsb.org/>), ensuring high structural accuracy with resolutions of 3 Å or better. The selected proteins included the Structure of *S. aureus* PstA with a resolution of 2.60 Å, The Crystal Structure of smu.1704 from *S. mutans* UA159 with a resolution of 2.20 Å, the Crystal Structure of *C. albicans* protein farnesyltransferase binary complex with the isoprenoid farnesyl diphosphate at 2.70 Å, a conserved protein of unknown function from *Enterococcus faecalis* V583 with a resolution of 2.50 Å, a 6-pyruvoyltetrahydropterin synthase homologue from *Escherichia coli* at 2.93 Å, an enoyl-CoA hydratase/isomerase family protein from *P. aeruginosa* at 2.50 Å, and the structure of *K. pneumoniae* MrkH in its apo state with the highest resolution of 1.95 Å. The ligand, Ellagic acid, was obtained from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) in 3D SDF format. With a molecular formula of C₁₄H₆O₈, the ligand provided a detailed structural basis for subsequent binding interaction analysis.

2.5.2. Molecular docking

The PyRx program was employed to carry out molecular docking. The Open Babel GUI program was used for transforming the selected ligand from its native SDF file to PDB format. The proteins, earlier in PDB format, were imported into PyRx as macromolecules. A grid box was then created around the protein, maximizing coverage to ensure accurate docking. The docking score was subsequently predicted and compared with the standard antibiotic amoxicillin to evaluate the ligand's potential efficacy.

2.5.3. Ligand-receptor interaction

The binding of a biological receptor, such as a protein, to a ligand, such as a substrate or potential drugs, is known as a receptor-ligand interaction. These interactions are crucial for deciphering the mechanisms of action in drugs, enzymes, and other biomolecules. In this study, Biovia Discovery Studio 2021 was used to model these interactions, specifically focusing on

the binding of ellagic acid with dental proteins. Both 3D and 2D structures of the interactions were generated and saved as image files, detailing key features such as bond lengths, bond types, and involved amino acids, offering a comprehensive visualization of the molecular dynamics at play.

2.5.4. *In silico* pharmacological property analysis

SwissADME is a powerful tool that predicts the Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) profiles of tiny molecules using advanced computational models and procedures. The program uses Quantitative Structure-Activity Relationship (QSAR) methods and machine learning methods to predict important ADMET parameters after receiving the chemical makeup or SMILES of a ligand. These consist of oral absorption, tissue distribution, metabolic stability, elimination, and possible safety endpoints. The tool provides detailed estimates for each parameter, accompanied by confidence scores, enabling researchers to evaluate the drug-likeness and safety profiles of compounds during the drug detection and development process. Furthermore, the oral toxicity of the phytochemicals was evaluated using the ProTox-II webserver (http://tox.charite.de/protox_II). This platform estimates the median lethal dose (LD₅₀), assigns a toxicity classification, and provides prediction accuracy and average similarity as standard outputs. For the analysis, the SMILES strings of the ligands were entered into the webserver for assessment.

2.6. Antioxidant properties

2.6.1. DPPH scavenging test

The DPPH test evaluates an antioxidant's potential to eliminate the DPPH radical, a stable radical with an unpaired electron, by donating hydrogen molecules or electrons [54]. The antioxidant activity of the EA-AuNPs was assessed using this technique at four different levels: 25, 50, 75, and 100 µg/mL. A 0.1 mM DPPH solution was prepared by dissolving the radical in ethanol [55]. For the assay, 100 µL of EA-AuNPs at each concentration was combined with 900 µL of the DPPH solution. The mixture was kept in darkness chamber for 30 min to prevent any light-induced reactions [56]. Following incubation, the absorbance was measured at 517 nm using a UV-visible spectrometer, enabling the evaluation of the antioxidant activity of the EA-AuNPs. The absorption was thoroughly investigated through the application of a defined formula.

$$\text{Scavenging activity/ inhibition percentage} = (A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}) \times 100$$

2.6.2. ABTS assay

The ABTS assay evaluates an antioxidant's capacity to neutralize ABTS free radicals, which are generated when ABTS is oxidized by potassium peroxodisulfate [57]. A solution with a concentration of potassium peroxodisulfate (2.45 mM) and ABTS (7 mM) was added, and the combination was allowed to stand at room temperature in dark conditions for 16 h in order to produce ABTS radical cations. After that, ethanol was added to the obtained ABTS solution until its absorbance at 734 nm became 0.70 ± 0.02 [58]. EA-AuNPs were produced at different doses (25, 50, 75, and 100 $\mu\text{g/mL}$). For the assay, 100 μL of each EA-AuNPs concentration was added to 900 μL of the diluted ABTS solution. The mixture was kept in the dark at room temperature for 6 min to prevent any light-induced reactions [59]. After incubation, a UV-Vis spectrophotometer was used to determine the solution's wavelength at 734 nm, which revealed information on the EA-AuNPs antioxidant capacity. The absorption was evaluated using a specific formula.

$$\text{Scavenging capacity/ percentage of inhibition} = (A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}) \times 100$$

2.7. Anti-inflammatory properties

2.7.1. Albumin denaturation inhibition

The albumin denaturation prevention technique was used to determine AuNPs' anti-inflammatory effects. 0.45 mL of bovine serum albumin (5% aqueous solution) and 0.05 mL of distilled water were added to the reaction mixture, and 1 N HCl was utilized to gradually precise the pH. Various concentrations of the EA-AuNPs were incorporated into the mixture, which was subsequently incubated at 37°C for 20 min. Following this, the mixture was heated to 57°C for 5 min. After cooling, 2.5 mL of PBS was added to the mixture and a spectrophotometer was applied to detect the turbidity at 600 nm[60]. Calculate the % reduction in protein breakdown employing the equation below:

$$\text{Percentage inhibition (\%)} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

2.7.2. Assay of HRBC membrane stabilization

Lysosomal enzymes, released during inflammation, are responsible for various diseases and are linked to both acute and chronic inflammation when active extracellularly. To ensure the effectiveness of nonsteroidal medications, it is crucial to either stabilize the lysosomal membrane or inhibit these enzymes [61]. In this study, EA-AuNPs were tested individually

with HRBC (human red blood cell) solutions at concentrations from 50, 100, 150 to 200 µg/mL. Blood samples from healthy individuals were mixed with sterile Alsever's solution and centrifuged at 3500 rpm. The cells were cleaned using an isosaline solution and a 10% v/v saline suspension. 1 mL of phosphate buffer, 2 mL of hyposaline, and 0.5 mL of 10% HRBC solution were utilized for each assessment. The test setup included aspirin as a standard, synthesized AuNPs at varying concentrations (50–200 µg/0.5 mL in normal saline), and double-distilled water as a control to induce 100% haemolysis (instead of hyposaline) [62]. After incubating the samples at 37°C for 30 min, followed by centrifugation at 3000 rpm for 20 min, the haemoglobin content in each test AuNPs was measured using spectrophotometry at 560 nm [63]. The amounts of haemolysis and protection are calculated by the following formulas:

$$\text{Inhibition percentage (\%)} = (\text{Abs}_{\text{Control}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{Control}} \times 100$$

2.8. Cytotoxicity study

2.8.1. Collection and maintenance of cells

The KB cells used in this study were sourced from the National Centre for Cell Science in Pune, India. The cultured in Gibco™ DMEM, supplemented with maximum glucose levels and sodium bicarbonate to maintain optimal growth conditions. The medium was further enriched by adding 100 U/mL of streptomycin and penicillin (Sigma, St. Louis, MO, USA) and 10% fetal bovine serum (FBS) from Hi-Media, Mumbai, India. The cells were incubated at 37°C in a Thermo Scientific (USA) incubator, under a humidified atmosphere with 5% CO₂ to ensure ideal growth [64]. For cell culture and maintenance, T25 flasks with a surface area of 25 cm² were used, initially seeding 0.2×10^6 cells per flask to promote cell division. The process began with 805 confluent T25 flasks. For experimental purposes, cells were sown in 96-well plates at a thickness of 5.0×10^3 cells per well, ensuring the appropriate number of cells for the study's evaluations [65].

2.8.2. MTT assay

Human epithelial cancer cells were grown in Dulbecco's Modified Eagle's Medium (DMEM), which has been modified with 1% penicillin-streptomycin and 10% fetal bovine serum (FBS)[66]. The cultures were kept at 37°C in a moistened incubator with 5% CO₂ to ensure optimal growth conditions. Once the cells reached 70–80% confluency, they were exposed to various concentrations of EA-AuNPs for 24 h. The MTT test was used to measure cell viability.

This approach involved removing the media and washing the cells with phosphate-buffered saline (PBS). Following the addition of an MTT solution (5 mg/mL in PBS) to each well, the dishes were incubated for 3 h at 37°C. Following incubation, the formazan crystals were dispersed in dimethyl sulfoxide (DMSO) and the MTT mixture was aspirated. A microplate reader was employed to determine absorbance at 570 nm, providing a numerical value for cell viability [67]. The following formula was used to determine the viability % :

$$\text{Percentage viability} = (\text{Sample OD}/\text{Control OD}) \times 100$$

2.9. Statistical analysis

All experiments were conducted in triplicate, and the data are presented as mean $\pm$ standard deviation (n=3, SD). Statistical analyses were performed using one-way analysis of variance (ANOVA) to assess significant differences among treatment groups. Post hoc comparisons were carried out using Duncan's Multiple Range Test (DMRT) to determine statistically significant differences between the control and EA-AuNP-treated groups. A significance level of $p \leq 0.05$ was considered statistically significant. Distinct lowercase superscript letters in figures and tables denote significantly different means among groups.

3. Result and Discussion

3.1. Spectral analysis of EA-AuNPs

3.1.1. UV-visible spectroscopy

The visual observation of the vials (Fig.1A-C) shows a development in colour from pale yellow to dark brown, which is consistent with the formation of gold nanoparticles. The UV-Vis spectrum and the distinct SPR peak at 587.5 nm confirm the successfully synthesized EA-AuNPs (Fig.1D). Pure ellagic acid has a distinctive absorption peak at 255 nm in the UV-Vis absorption wavelengths, demonstrating its unique electronic transition profile (Fig.1E). The sharpness and position of the SPR peak indicate well-dispersed and stable nanoparticles, while the colour changes observed in the samples further confirm the formation of gold nanoparticles. Ellagic acid acts as both a reducing and stabilizing agent in the synthesis of AuNPs, converting gold ions (Au^{3+}) to elemental gold (Au^0) [68]. The presence of ellagic acid likely prevents excessive aggregation, as demonstrated by the relatively sharp and well-defined SPR peak. During this reduction process, ellagic acid simultaneously acts as a capping agent, stabilizing the newly formed nanoparticles and preventing their aggregation. This dual role of ellagic acid in reduction and stabilization is crucial for obtaining uniformly sized and stable gold

nanoparticles, which are desirable for applications in drug delivery, diagnostics, and therapeutic interventions. Similarly, Das et al. [69] reported the synthesis AuNPs using *Curcuma*, which was confirmed by an evident surface plasmon resonance (SPR) band with a sharp peak at about 550 nm, demonstrating the effective synthesis of nanoparticles. Likewise, Muniyappan et al. [70] found that the produced AuNPs combined with curcumin had a UV spectrum at 542 nm .

3.1.2. Fourier transform infrared spectroscopy (FTIR)

FTIR is a powerful tool for determining the functional groups existing in both pure compounds and nanoparticles [71]. In the case of ellagic acid-capped AuNPs, the FTIR spectrum reveals critical shifts in the vibrational frequencies of functional groups, indicating the interaction between the nanoparticles and ellagic acid (Fig.2A). For ellagic acid synthesized AuNPs, the O-H stretching in the alcohol group appears at 3453 cm^{-1} , and the O-H stretching of the carboxylic acid group is observed at 3046 cm^{-1} . These peaks suggest a possible involvement of hydroxyl and carboxyl groups in the capping and stabilization of the AuNPs [72]. The strong C=O stretching at 1684 cm^{-1} , related to the conjugated acid group, further highlights the interaction between the nanoparticles and the carboxyl groups of ellagic acid. In the pattern region, the C-H bending at 1448 cm^{-1} (alkane), C-F stretching at 1175 cm^{-1} (fluoro compound), and C-O stretching at 1059 cm^{-1} (primary alcohol) show notable shifts, suggesting that these functional groups are affected by the binding to the gold nanoparticles. Additional peaks at 847 cm^{-1} (C-Cl stretching) and 570 cm^{-1} (C-Br stretching), indicative of halo compounds, further confirm the structural modifications caused by nanoparticle formation.

In contrast, the FTIR spectrum of pure ellagic acid shows different functional group frequencies (Fig.2B). The O-H stretching for carboxylic acids occurs at 3069 cm^{-1} , and the C=O stretching of conjugated acids is observed at 1692 cm^{-1} . An additional C=O stretching related to δ -lactam appears at 1616 cm^{-1} , while the N-H bending of amine is detected at 1581 cm^{-1} . These peaks are notably shifted or diminished in the nanoparticle spectrum, indicating the participation of these groups in the formation of the AuNPs. Other key features in ellagic acid, such as C-F stretching at 1330 cm^{-1} (fluoro compound), C-O stretching at 1110 cm^{-1} (secondary alcohol), and S=O stretching at 1052 cm^{-1} (sulfoxide), also differ from the corresponding peaks in the ellagic acid AuNPs spectrum. The shifts in functional groups when ellagic acid is capped onto gold nanoparticles suggest a strong interaction, particularly through the hydroxyl and carboxyl groups, which may play a role in stabilizing the nanoparticles. This functional group alteration

can influence the biological activity of the nanoparticles, potentially enhancing properties such as antioxidant, antibacterial, and anticancer effects [49]. The FTIR results imply that the nanoparticle formation leads to a functional shift, altering the chemical environment around key groups, which in turn may modulate the overall bioavailability and efficacy of ellagic acid in biomedical applications. Thus, the functional group modifications upon nanoparticle formation not only indicate successful capping but also open the possibility for enhanced therapeutic applications due to altered chemical reactivity. Safwat et al. [73] found that stretching vibrations of EGCG resulted in absorption bands in the EGCG-AuNPs spectrum at 3335.9 and 1691.3 cm^{-1} , as well as several bands in the 1400-1628.2 cm^{-1} range. These bands attest to EGCG's successful loading onto the surface of AuNPs. Likewise, Fathy et al. [74] reported that gallic acid-conjugated gold nanoparticles showed a characteristic C=O stretching at 1637.27 cm^{-1} , confirming effective conjugation by the phenolic OH vibration observed at 3440.38 cm^{-1} . Similarly, Sulaiman et al. [75] reported that the FTIR spectra of Hesperidin-mediated AuNPs demonstrated OH symmetric stretching at 3454.62 and 3273.31 cm^{-1} , signifying the presence of alcohol. Likewise, Rajendran et al. [48] investigated the FTIR spectra of AuNPs based on apigenin revealed a prominent band at 3422 cm^{-1} , signifying the –OH stretch of phenolic groups.

3.1.3. XRD analysis

The XRD analysis of ellagic acid-capped gold nanoparticles EA-AuNPs reveals several distinct peaks, which correspond to the crystalline structure of gold. The crystalline nature of EA-AuNPs was confirmed using X-ray diffraction (XRD) analysis. The diffraction peaks observed at $2\theta = 38.29^\circ$, 44.45° , 64.75° , and 77.63° correspond to the (111), (200), (220), and (311) planes, respectively, which are characteristic of the face-centered cubic (fcc) structure of AuNPs, as indexed to JCPDS file no. 04-0784 (Fig.3). The sharp and narrow peaks further suggest high crystallinity of the synthesized nanoparticles. No additional peaks corresponding to impurities were detected, confirming the phase purity of the EA-AuNPs. Furthermore, Das et al. [69] reported that the XRD pattern of the synthesized AuNPs exhibited four prominent Bragg reflections at (111), (200), (220), and (311), which were indexed to the face-centered cubic (fcc) structure of gold crystal planes. Additionally, Yang et al. [76] reported that AuNPs synthesized using *Nicotiana plumbaginifolia* showed XRD peaks at 38.00° , 44.10° , 63.75° , and 77.10° , corresponding to the (111), (200), (220), and (311) reflections of the face-centered cubic (FCC) assembly of metallic gold, as referenced by JCPDS no. 04-0784.

3.1.4. SEM-EDAX analysis

The morphology of EA-AuNPs was analysed using SEM, which revealed that the nanoparticles were predominantly long rod-like and irregularly shaped. The particles appeared agglomerated to some extent, but distinct rod-like structures were visible (Fig.4A&B). The effective dispersion of the nanoparticles, as observed in the SEM images, highlights the role of Ellagic acid in preventing particle agglomeration. Similarly, Sulaiman et al. [75] reported that the FESEM image showed AuNPs with a relatively sphere-shaped shape and a width between 15 to 30 nm. The EDX analysis of Ellagic acid-mediated EA-AuNPs provided significant insights into their elemental composition and the success of the nanoparticle formation. The key element, gold (Au), was detected at 14.19% by weight, confirming the presence of gold in the sample (Fig.4C). The atomic percentage of gold was lower (1.51%), which is expected due to gold's heavier atomic weight relative to the other elements present. The detection of gold indicates that the reduction of gold ions by Ellagic acid was successful, leading to the formation of Au nanoparticles. Oxygen (O) was present in high amounts, with a weight percentage of 39.23% and an atomic percentage of 51.37%. This suggests that oxygen-containing functional groups from Ellagic acid, such as hydroxyl and carbonyl groups, were involved in the reduction and stabilization of the gold nanoparticles. These oxygen atoms likely contribute to the surface coating of the nanoparticles, preventing agglomeration and maintaining nanoparticle stability. Additionally, sodium (Na) was found at 36.87% by weight and 33.61% by atomic percentage, likely originating from the buffer solution used during the synthesis. Sodium may also play a role in stabilizing the nanoparticles in solution. Carbon (C) was detected at 6.74% by weight, which points to the organic nature of Ellagic acid and its presence on the surface of the nanoparticles. This organic layer formed by carbon-containing molecules from Ellagic acid acts as a stabilizing shell around the gold nanoparticles, providing biocompatibility and functionality. Chlorine (Cl) was present in smaller quantities (2.96% by weight), possibly from reagents or the buffer solution used during the reaction process. The elemental mapping further corroborated these findings by showing the spatial distribution of these elements across the sample. The mapping revealed clear areas of gold concentration, confirming the localized presence of gold nanoparticles. The distribution of oxygen and carbon around the nanoparticles supports the notion that the Ellagic acid molecules are coating the nanoparticles, which helps in stabilizing them and prevents particle aggregation [77]. The Na and Cl elements were more dispersed, likely reflecting their presence in the surrounding medium rather than being directly involved in the nanoparticle structure. Likewise, Sulaiman et al. [75] stated that EDAX analysis

of Hsp-AuNPs indicated gold (64.65%) as the main element, with carbon and oxygen levels indicating hesperidin loaded.

3.1.5. TEM analysis

The present study TEM analysis showing a heterogeneous size distribution ranging from 50 to 70 nm. The particles exhibited varying shapes, including long rods and ovals. This variation may be attributed to the concentration of ellagic acid, reaction conditions, and kinetics of nucleation and growth during synthesis (Fig. 5A). Likewise, Sulaiman et al., [75] found the TEM images revealed that Hesperidin-AuNPs had a rod and spherical shape, crystalline nature, smooth surface, and a diameter ranging from 10 to 50 nm. Furthermore, Das et al. [69] reported that TEM analysis of the optimized AuNPs synthesized using *Curcuma* revealed nanoparticles with both spherical and hexagonal shapes. Likewise, Wanderson et al. [78] reported that AuNPs synthesized using coffee extract had a median width of 14.90 ± 3.02 nm, as determined by analysing TEM images of 1000 nanoparticles, which is revealed a predominant spherical shape NPs. Additionally, Radadi et al., [79] demonstrated the TEM images of *C. myrrh*-mediated AuNPs showed ranging in size from 1.68 to 7.86 nm, with a spherical shape and no aggregation.

3.1.6. DLS and Zeta potential

The DLS and zeta potential analysis of EA-AuNPs provide valuable insights into their size distribution, stability, and outward charge. The DLS results show that the hydrodynamic dimension of the EA-AuNPs is 74 nm, with a mean intensity of 331.9 kcounts/s, indicating the median size of the particles in a liquid medium (Fig.6A). The polydispersity index (PDI) is 14.5%, suggesting a relatively uniform size distribution with minimal aggregation. The absolute intensity is recorded at 621,449.1 kcounts/s, further confirming the presence of stable, well-dispersed nanoparticles. The diffusion coefficient is $0.1 \mu\text{m}^2/\text{s}$, reflecting the particle's mobility in the medium, while the intercept (g12) of 0.7463 indicates good signal quality. The transmittance of 23.1% and a baseline of 1.041 suggest some absorption or scattering of light, which is typical for nanoparticle suspensions. Similarly, Yang et al. [76] reported that AuNPs synthesized using *Nicotiana plumbaginifolia* were analysed by DLS, which revealed a maximum particle size of 68.69 nm and a zeta potential of -47.92 mV, indicating good colloidal stability. In our present study, Zeta potential measurements reveal a mean zeta potential of -17.7 mV, with a distribution peak at -19.9 mV, indicating moderately stable nanoparticles in solution (Fig.6B). This negative surface charge suggests that the EA-AuNPs have sufficient electrostatic repulsion to prevent significant aggregation, which is crucial for maintaining

stability in colloidal systems. The mean intensity during the zeta potential measurement is 743.6 kcounts/s, and the standard deviation is 0.9 mV, showing consistent results with minimal variation. The electrophoretic mobility of the nanoparticles is measured at $-0.8666 \mu\text{m}^2\text{cm/Vs}$, indicating their movement in an electric field. Additionally, the conductivity of the sample is 3.919 mS/cm, while the transmittance is 57.9%, which is higher than in the DLS measurements, likely due to changes in the light scattering properties during zeta potential analysis. According to Sulaiman et al., the Hsp-AuNPs were further characterized using a zetasizer to measure particle size and zeta potential. The nanoparticles averaged around 40 nm in size, with a zeta potential of -29.40 mV , indicating good stability in solution according to established guidelines [75]. Similarly, Isa et al. [80] reported that AuNPs synthesized using *Artocarpus odoratissimus* peel extract showed a zeta potential of -43.42 mV for the extract, which decreased to -35.74 mV after AuNP formation. Despite the reduction, the zeta potential remained above -30 mV , indicating that the synthesized AuNPs were stable. Additionally, Vorobyova et al. [81] reported that the zeta potential values for AuNPs synthesized using B-U and CI-U plum extracts were -46.9 mV and -44.7 mV , respectively, indicating good stability of the synthesized nanoparticles.

3.2. Biological applications of EA-AuNPs

3.2.1. Antimicrobial activity

The antibacterial properties of EA-AuNPs were determined against various bacterial strains (*E. faecalis*, *E. coli*, *S. aureus*, *S. mutans*, *C. albicans*, *P. aeruginosa*, and *K. pneumoniae*) using the well diffusion approach (Fig.7). The results, demonstrated the mean zone of inhibition (mm) with standard deviation (SD), indicate that EA-AuNPs exhibit notable antibacterial activity, with a concentration-dependent increase observed across all strains (Fig.8). At 50 $\mu\text{g/mL}$, EA-AuNPs produced a zone of inhibition (ZOI) of $7.3 \pm 0.7 \text{ mm}$ against *E. faecalis*, $33.1 \pm 0.5 \text{ mm}$ against *E. coli*, $31.87 \pm 0.2 \text{ mm}$ against *S. aureus*, $6.62 \pm 0.3 \text{ mm}$ against *S. mutans*, $14.96 \pm 0.7 \text{ mm}$ against *C. albicans*, $30.1 \pm 0.9 \text{ mm}$ against *P. aeruginosa*, and $34.33 \pm 0.1 \text{ mm}$ against *K. pneumoniae*. At 100 $\mu\text{g/mL}$, the ZOI increased to $7.8 \pm 0.4 \text{ mm}$ for *E. faecalis*, $33.9 \pm 0.8 \text{ mm}$ for *E. coli*, $35.4 \pm 0.4 \text{ mm}$ for *S. aureus*, $6.98 \pm 0.7 \text{ mm}$ for *S. mutans*, $22.28 \pm 0.6 \text{ mm}$ for *C. albicans*, $31.98 \pm 0.6 \text{ mm}$ for *P. aeruginosa*, and $37.26 \pm 0.9 \text{ mm}$ for *K. pneumoniae*. In comparison to the positive control, which yielded ZOI of $6.01 \pm 0.6 \text{ mm}$ for *E. faecalis*, $33.6 \pm 0.8 \text{ mm}$ for *E. coli*, $33.57 \pm 0.2 \text{ mm}$ for *S. aureus*, $7.2 \pm 0.4 \text{ mm}$ for *S. mutans*, $21.6 \pm 0.3 \text{ mm}$ for *C. albicans*, $29.8 \pm 0.3 \text{ mm}$ for *P. aeruginosa*, and $36.77 \pm 0.5 \text{ mm}$ for *K.*

pneumoniae, EA-AuNPs demonstrated higher activity, especially at higher concentrations. The negative control, 1% DMSO, had no inhibitory effect on the tested bacteria. The present results show that EA-AuNPs exhibit significant antibacterial activity, with varying ZOI depending on the bacterial strains. The activity was particularly noticeable against *E. coli*, *S. aureus*, and *K. pneumoniae*, with ZOI exceeding 35 mm at 100 µg/mL, indicating strong efficacy. Notably, *C. albicans* and *P. aeruginosa* also showed considerable sensitivity to EA-AuNPs, with substantial inhibition observed at both 50 µg/mL and 100 µg/mL. The concentration-dependent increase in inhibition zones suggests that higher doses of EA-AuNPs improve bacterial killing efficacy. While the positive control displayed consistent antibacterial effects, EA-AuNPs showed competitive or enhanced activity, particularly at 100 µg/mL, highlighting their potential as an effective antibacterial agent. The combination of ellagic acid and AuNPs enhances the antibacterial properties, likely due to the improved interaction between nanoparticles and bacterial cell membranes, leading to increased bacterial cell disruption. The findings indicate that because of their small size, the nanomaterials may have bind to the surface of the bacterial pathogens, released more ions, and spread in the surrounding environment of the bacterial cell wall, possibly disrupting the cell membrane and causing ROS production [82] [96]. Therefore, it is possible that the nanomaterials entered the bacterial cells and produced oxidative stress, cellular membrane damage, DNA and bacterial protein damage, or cell lysis [83]. Likewise, Radadi et al., [79] investigated the ZOI for *C. myrrh*-mediated AuNPs against *B. subtilis*, *E. coli*, *S. aureus*, and *P. aeruginosa*. Similarly, Anwar et al., [84] reported strong antibacterial activity against *S. aureus*. Additionally, Alizadeh et al., [85] found that *Crocus caspius* mediated AuNPs exhibit maximum ZOI against *S. aureus* and *K. pneumoniae*. Furthermore, Fouda et al., [86] examined *Zingiber officinale* coated AuNPs exhibits antimicrobial properties against *E. coli*, and *P. aeruginosa*. Additionally, Rosales et al., [87] investigated *Capsicum chinense* derived AuNPs exhibits significant antibacterial properties against *E. faecalis*. TEM analysis revealed that EA-AuNPs exhibited sizes within the 50–70 nm range. This nanoscale size enhances cellular uptake via endocytosis, allowing for effective interaction with bacterial membranes and intracellular targets in cancer cells. Smaller particles also exhibit a higher surface-to-volume ratio, which increases the availability of active sites for interaction with reactive oxygen species (ROS), biomolecules, and cellular membranes, thus contributing to heightened antibacterial and antioxidant activities. Table 1 and 2. provided an overview of the different phyto-derived gold nanoparticles (AuNPs) that were produced utilizing different bioactive substances and biomedical applications.

3.3. Minimum inhibitory concentration (MIC)

The MIC results for EA-AuNPs against various microbial strains, including *E. coli*, *S. aureus*, *S. mutans*, *P. aeruginosa*, *E. faecalis*, *C. albicans*, and *K. pneumoniae*, reveal a concentration-dependent inhibition effect. Positive control (PC) values ranged from 0.7 to 1.9 (O.D), showing strong inhibition at lower concentrations compared to the negative control (NC), which ranged from 1.1 to 1.7 (O.D values) (Supplementary Fig.1). At lower EA-AuNPs concentrations (0.5–2.5 µg/mL), inhibition was minimal, with MIC values generally above 1.0 (O.D) for most strains, indicating limited antimicrobial activity. However, as the concentration increased to 10 µg/mL, noticeable inhibition started across all strains, with MIC values ranging between 0.7 and 1.0 (O.D), particularly active against *E. coli* and *S. aureus*. Similarly, Azmy et al., [88] reported that *A. platensis* mediated AuNPs exhibits MIC against *S. pneumoniae*. Higher concentrations (25–100 µg/mL) demonstrated enhanced efficacy, with MIC values dropping to 0.1–0.3 (O.D) for most strains, indicating strong inhibition and suggesting that EA-AuNPs exhibit significant antimicrobial activity in a dose-dependent manner. At the maximum tested concentration (200 µg/mL), consistent inhibition was observed, achieving MIC values as low as 0.1(O.D) for nearly all strains, underscoring EA-AuNPs potential as an antimicrobial agent across diverse microbial species. Similarly, Silvero et al., [89] demonstrated amoxicillin mediated AuNPs exhibits strong inhibition against *S. aureus*. Furthermore, Almutleb et al., [90] investigated in *Nilavembu choornam* synthesized AuNPs which demonstrated inhibition against *K. pneumoniae*, *S. aureus*, *P. aeruginosa*, and *E. faecalis*. Furthermore, AuNPs biosynthesised from *Allium ampeloprasum* leaf extract shown good efficacy against both Gram-ve (*E. coli*, *P. aeruginosa*) and Gram +ve (*S. aureus*, *B. subtilis*) bacteria [91]. For our present study, the possible mechanism of action of EA-AuNPs against bacterial strains involves several key processes. Initially, EA-AuNPs adsorb onto the bacterial cell surface due to their high surface area-to-volume ratio and the presence of functional groups in ellagic acid, disrupting normal physiological functions. This interaction increases membrane permeability, leading to uncontrolled influx and efflux of substances, ultimately resulting in cell lysis [92]. Furthermore, EA-AuNPs generate reactive oxygen species (ROS) within the bacterial cells, causing oxidative stress that damages lipids, proteins, and DNA, contributing to cell death. Additionally, EA-AuNPs inhibit biofilm development by interfering with bacterial quorum sensing, while ellagic acid enhances the antibacterial effects through its own ability to bind to bacterial DNA, inhibiting replication and transcription [93]. The nanoparticles improve the bioavailability and stability of ellagic acid, confirming efficient delivery of active compounds

to target bacterial cells[99]. The moderately negative zeta potential of EA-AuNPs indicates good colloidal stability and potential electrostatic interaction with positively charged regions of bacterial membranes or cancer cell surfaces. This negative charge may disrupt membrane integrity in pathogens, leading to leakage of intracellular contents. Additionally, a stable zeta potential prevents agglomeration, ensuring consistent biological interaction and enhanced distribution at the cellular level[101].

3.4. *In silico* docking studies

3.4.1. Molecular docking and Ligand receptor interaction

Ellagic acid (EA) demonstrated prominent binding affinities with several targeted microbial proteins, suggesting its potential as an effective antimicrobial agent. In *S. aureus* PstA protein (PDB ID: 4WK3), the binding affinity was -5.0 kcal/mol, with interactions involving MET, LEU, GLN, ALA, TRP, ILE, and HOH. These interactions suggest that EA may disrupt cell membrane integrity or transport mechanisms, weakening the bacterial defences. For *S. mutans* (PDB ID: 3L7W), the binding affinity was slightly stronger at -5.6 kcal/mol, with involvement of MET, VAL, ALA, and ILE, possibly interfering with bacterial metabolic functions critical for growth and virulence. EA showed its strongest binding to *C. albicans* farnesyltransferase (PDB ID: 4YDE), with a binding affinity of -7.3 kcal/mol. The 3D interactions are shown in (Fig.9). This interaction, involving TYR, GLN, SER, ALA, PHE, VAL, ARG, and HOH, suggests that EA inhibits protein farnesylation, a key step in fungal protein maturation and membrane anchoring, thus impairing fungal growth and survival (Fig.10). In *E. faecalis* (PDB ID: 3CLQ), the binding affinity was lower at -3.7 kcal/mol, with ILE, LEU, and ALA interactions, indicating that its antimicrobial effect on this pathogen may be weaker or involve less critical targets. Against *E. coli* (PDB ID: 3QN9), EA had a binding affinity of -5.7 kcal/mol, forming interactions with GLU, GLY, LYS, THR, ILE, and ASN, potentially affecting bacterial enzyme activity and metabolic pathways. In *P. aeruginosa* (PDB ID: 3OT6), the binding affinity was -5.5 kcal/mol, with interactions involving PHE, ALA, VAL, ILE, SER, GLU, and HIS, suggesting disruption of fatty acid metabolism essential for bacterial growth. In *K. pneumoniae* (PDB ID: 5KEC), EA bound with a -5.6 kcal/mol affinity, interacting with THR, HOH, PRO, and ASN, likely affecting regulatory pathways involved in bacterial virulence. Similarly, Azmy et al., [88] reported *A. platensis* ligands exhibited binding affinity to *S. pneumoniae* topoisomerase IV.

3.4.2. *In silico* pharmacological property analysis of ellagic acid

Ellagic acid exhibits promising potential as an orally bioavailable compound, with favourable physicochemical properties and pharmacokinetics (Supplementary Fig.2A). It has a molecular weight of 302.19 g/mol, a moderate lipophilicity (Log Po/w 1.00), and is classified as soluble across different prediction models, indicating good absorption. The high GI absorption suggests effective oral bioavailability, although the compound's large topological polar surface area (TPSA) of 141.34 Å² may limit its membrane permeability, particularly through the blood-brain barrier (BBB), which it is predicted not to cross. Additionally, it is not a P-glycoprotein substrate, meaning it is less likely to be effluxed from cells (Supplementary Fig.2B), a positive trait for intracellular retention. However, Ellagic acid inhibits CYP1A2, signalling a potential for drug-drug interactions, but does not inhibit other critical enzymes like CYP3A4, reducing concerns over broader metabolic interactions. Despite these advantages, certain aspects of drug-likeness warrant caution. It adheres to Lipinski's rule of five without violations, but violations in Ghose, Veber, and Egan filters, due to the high TPSA, suggest challenges in cell membrane permeability. Additionally, medicinal chemistry flags one PAINS alert (catechol) and multiple Brenk alerts, indicating the presence of substructures that could lead to toxicity or promiscuous binding. Nonetheless, the compound's bioavailability score of 0.55 and lead-likeness point to its potential in drug discovery, although the synthetic accessibility score of 3.17 suggests moderate complexity in synthesis.

Additionally, The ProTox-II analysis of ellagic acid yielded a predicted LD₅₀ of 2991 mg/kg, placing it in Toxicity Class 4, indicating low toxicity. With an average similarity score of 83.33% and a prediction accuracy of 70.97%, the results suggest moderate reliability in the predictions. Specific organ toxicity assessments identified ellagic acid as inactive for hepatotoxicity and neurotoxicity, with probabilities of 0.83 and 0.91, respectively, suggesting it poses minimal risk to the liver and nervous system (Table 3). However, it showed potential activity for nephrotoxicity and respiratory toxicity, with probabilities of 0.58 and 0.84, respectively, indicating a higher likelihood of adverse effects on kidney and respiratory functions. It was also predicted as inactive for cardiotoxicity (probability of 0.76), suggesting it has a low risk of heart toxicity[97]. In terms of broader toxicity endpoints, ellagic acid was predicted to be carcinogenic with a probability of 0.59, suggesting a moderate likelihood of carcinogenicity (Table 4). However, it was found to be inactive for immunotoxicity, mutagenicity, and cytotoxicity, with probabilities of 0.81, 0.84, and 0.90, respectively, indicating a low risk in these areas. The compound was predicted to cross the blood-brain barrier (BBB) with a probability of 0.60, which may suggest some potential for neurological

effects if present in the brain. Additional predictions indicate active clinical toxicity (0.56) and nutritional toxicity (0.71), highlighting possible risks if used at high concentrations or over prolonged periods.

3.5. Antioxidant activity

3.5.1. DPPH assay

The DPPH assay outcomes determine a concentration-dependent increase in the antioxidant properties of EA-AuNPs compared to Ellagic acid alone. At 25 µg/mL, EA-AuNPs exhibited 41% radical scavenging properties, which was significantly higher than EA (27%) but lower than the standard ascorbic acid (64%) (Fig.11A). As the dose increased to 50 µg/mL, the scavenging activity of EA-AuNPs rose to 60%, surpassing EA (38%) and approaching that of ascorbic acid (75%). At 75 µg/mL, EA-AuNPs showed even better activity at 83%, well above EA (57%) and ascorbic acid (92%). Finally, at 100 µg/mL, EA-AuNPs reached 91% scavenging activity, significantly higher than EA (69%) and almost equivalent to ascorbic acid (99%). The results show that the formation of AuNPs enhances the antioxidant potential of EA, likely due to the improved electron-donating ability and surface interactions facilitated by the nanoparticles. EA-AuNPs demonstrated superior activity across all concentrations, with the most significant effect seen at higher doses, where they nearly matched the antioxidant power of ascorbic acid. This suggests that EA-AuNPs could serve as an effective antioxidant agent, with potential applications in oxidative stress-related therapies. Similarly, Patra et al., [102] reported papaya peel biosynthesized AuNPs exhibit antioxidant potential in DPPH and ABTS. Likewise, Malik et al., [103] demonstrated DPPH antioxidant activity assay for aloe vera, Honey, and *Gymnema sylvestre* leaf extract AuNPs showed notable free radical scavenging potential.

3.5.2. ABTS assay

The ABTS assay outcomes showed the antioxidant potential of Ellagic acid (EA) and synthesized EA-AuNPs at different concentrations (25, 50, 75, and 100 µg/mL) compared to the standard ascorbic acid (Fig.11B). At 25 µg/mL, EA-AuNPs demonstrated a radical scavenging activity of 57%, significantly higher than EA alone (29%) but lesser than ascorbic acid (69%). At 50 µg/mL, EA-AuNPs showed an increased activity of 69%, outperforming EA (40%) and closely related to the standard (77%). The trend continued at 75 µg/mL, with EA-AuNPs showing 77% activity, which was higher than EA (59%) but still below ascorbic acid (89%). At the maximum doses of 100 µg/mL, EA-AuNPs achieved 89% scavenging activity,

significantly higher than EA (70%) and ascorbic acid's showed scavenging activity of 99%. The obtained data indicate that EA-AuNPs exhibit superior antioxidant activity compared to EA alone, with a clear dose-dependent increase in scavenging potential. Likewise, Ngungeni et al., [104] demonstrated *Avocado* seed derived AuNPs which exhibits notable free radical scavenging activity. The better activity of EA-AuNPs is likely due to the enhanced bioavailability and surface reactivity provided by the AuNPs, allowing for more effective neutralization of free radicals. While EA alone shows moderate antioxidant capacity, its combination with AuNPs significantly enhances its ability to quench radicals, particularly at higher concentrations. The results suggest that EA-AuNPs offer a promising antioxidant profile, comparable to the standard ascorbic acid, especially at concentrations of 75 µg/mL and above. Similarly, Phukan et al., [105] investigated the Onion peel assisted AuNPs exhibit remarkable antioxidant potential against ascorbic acid.

3.6. Anti-inflammatory activity

3.6.1. Protein denaturation assay

The albumin denaturation assay results highlight the anti-inflammatory potential of Ellagic acid (EA) and synthesized EA-AuNPs at various amounts of 50, 100, 150, and 200 µg/mL, compared to the standard drug aspirin (**Fig.12A**). At 50 µg/mL, EA-AuNPs exhibited 15.22% inhibition of albumin denaturation, which was higher than EA alone (8%) but lower than aspirin (22.84%). At 100 µg/mL, the inhibition by EA-AuNPs increased to 27.3%, significantly surpassing EA (14%) but still below the standard (38.27%). The trend continued at 150 µg/mL, where EA-AuNPs showed 48.6% inhibition, more than double that of EA (23%) but lower than aspirin (67.4%). At 200 µg/mL, EA-AuNPs achieved 73.3% inhibition, considerably higher than EA (34%) and approaching the inhibition seen with aspirin (88.2%). The results demonstrate that EA-AuNPs possess enhanced anti-inflammatory properties compared to EA alone, with a dose-dependent increase in inhibition of protein denaturation. The significant improvement in activity when EA is combined with AuNPs indicates that the nanoparticles enhance the stability and efficacy of EA, allowing for better interaction with albumin and improved inhibition of denaturation. While EA alone shows moderate anti-inflammatory potential, its combination with AuNPs significantly boosts its effectiveness, particularly at higher concentrations. Although EA-AuNPs do not reach the inhibitory levels of aspirin, they show substantial promise as an alternative anti-inflammatory agent with enhanced bioactivity. According to Phukan et al., [106] demonstrated anti-inflammatory activity in onion peel-

derived gold nano bioconjugates which shown powerful anti-inflammatory activity. Also, in another study, Phukan et al., [105] examined onion peel driven AuNPs which exhibits anti-inflammatory potential in ABTS assay at maximum concentration.

3.5.2. Membrane stabilization assay

The membrane stabilization assay results reveal the anti-inflammatory potential of Ellagic Acid (EA), AuNPs (Gold nanoparticles), and their combination (EA-AuNPs) compared to aspirin as the standard (Fig.12B). At 50 µg/mL, EA-AuNPs showed 22% membrane stabilization, which was higher than EA alone (12%) but lower than aspirin (28%). As the concentration increased to 100 µg/mL, EA-AuNPs exhibited 34% stabilization, significantly surpassing EA (18%) but still below aspirin (39%). At 150 µg/mL, the stabilization effect of EA-AuNPs increased to 46%, nearly double that of EA (26%) but lower than aspirin (70%). At the highest concentration of 200 µg/mL, EA-AuNPs demonstrated 78% membrane stabilization, markedly higher than EA (38%) and approaching the activity of aspirin (89%). These results indicate that EA-AuNPs exhibit enhanced anti-inflammatory effects compared to EA alone, with a dose-dependent improvement in membrane stabilization. The presence of AuNPs enhances the protective ability of EA against hypotonicity-induced lysis of red blood cells, indicating better membrane stabilization and anti-inflammatory potential. While EA alone shows moderate membrane-stabilizing effects, its combination with AuNPs significantly boosts its efficacy, particularly at higher concentrations. Although EA-AuNPs do not achieve the same level of activity as aspirin, they demonstrate promising anti-inflammatory properties that suggest their potential use as a therapeutic agent for conditions involving inflammation. Earlier, Situmorang et al., [107] examined *Cassia alata*-mediated AuNPs shows anti-inflammatory potential in DPPH and ABTS assays. Likewise, Eltahir et al., [108] reported for AuNPs synthesized from liquiritin and other phenolics from *Glycyrrhiza glabra* shows good anti-inflammatory potential in *in vitro* assays. Ellagic acid serves both as a reducing and capping agent, imparting antioxidant and anti-inflammatory bioactivity. The polyphenolic groups present on the nanoparticle surface scavenge free radicals and stabilize membranes, supporting the high radical scavenging activity observed (91% in DPPH, 89% in ABTS assays) and membrane protection (78% stabilization in anti-inflammatory assay). This functionalization also aids in selective toxicity towards cancerous cells while sparing normal ones.

3.6. Anticancer activity

3.6.1. MTT assay

KB cells are a human epithelial cell line initially derived from an epidermoid carcinoma of the oral cavity, making them a suitable model for studying oral cancer [109]. In cancer treatment, inhibiting cell proliferation is a positive result, as it indicates that the growth of cancer cells is being slowed or stopped. This can ultimately contribute to tumour regression and enhance patient outcomes [110]. Oral cancer ranks as the 6th most common cancer globally, with over 370,000 new cases diagnosed each year [111]. The disease has a high mortality rate due to late-stage diagnosis and limited therapeutic options [112]. Oral cancer denotes a significant public health task in India, accounting for about 30% of all cancers in the country, largely due to the high prevalence of tobacco use, including smokeless tobacco. India has one of the highest rates of oral cancer globally, with an predictable 77,000 new cases and 52,000 deaths annually. Men, particularly from lower socioeconomic backgrounds, are at a higher risk, and early detection remains a challenge due to limited healthcare resources [113].

The MTT assay results of Ellagic acid-AuNPs (EA-AuNPs) against KB oral cancer cells demonstrate a dose-dependent cytotoxic effect. In this experiment, various doses of EA-AuNPs ranging from 25 to 100 µg/mL were evaluated for their anticancer activity, as shown in the microscopic images and cell viability bar graph. The microscopic images (Fig.13A-E) reveal a progressive reduction in cell density and increased cell death as the concentration of EA-AuNPs increases. In the control group (Fig.13A), cells are dense and healthy, indicating high cell viability. At 25 µg/mL (Fig.13B), there is slight inhibition of cell growth, with some cellular shrinkage. As the concentration increases to 50 µg/mL (Fig.13C) and 75 µg/mL (Fig.13D), a notable decrease in cell viability and clustering of dead cells is observed. At 100 µg/mL (Fig.13E), the majority of cells appear rounded and detached, indicating significant cytotoxicity. Similarly, Matic et al., [114] reported that anticancer properties of luteolin capped Au nanoparticles showed cytotoxic effects of human cervical adenocarcinoma cells. The cell viability data (Fig.13F) quantifies this trend. At 25, 50, 75, and 100 µg/mL concentrations, cell viability is approximately 92.99%, 74.73%, 47.71%, and 13.46%, respectively. This is reflected in the calculated IC₅₀ value of 59.58 µg/mL, with an R² value of 0.988, indicating a strong fit of the data to the inhibitory model. These values suggest that EA-AuNPs effectively inhibit KB oral cancer cell proliferation at higher concentrations. Likewise, Ahmad et al. [115] found that *Quercus infectoria* tannins have anti-proliferative and apoptotic inducing effects on oral cancer KB cell lines, with an IC₅₀ value of 76.82 µg/mL. The anticancer properties of EA-AuNPs are likely due to a combination of mechanisms. Ellagic acid is known to induce apoptosis in cancer cells by activating caspase pathways, generating reactive oxygen species (ROS), and disrupting mitochondrial function. Likewise, Marunganathan et al. [116] revealed that zinc oxide nanoparticles coated with κ-

carrageenan caused oral cancer to suffer apoptosis. The AuNPs enable cellular uptake, enhancing the bioavailability and intracellular accumulation of ellagic acid in cancer cells [117]. The nanoparticles may also promote additional ROS generation, causing oxidative stress that leads to DNA damage, ultimately triggering cell death pathways[94]. This work provides important information to develop nanotherapeutics to treat severe cancer. Rod-shaped nanoparticles are known to possess distinct biological behavior compared to spherical counterparts. Their anisotropic geometry can enhance membrane penetration and prolong cellular retention, particularly in cancer cells, potentially explaining the observed cytotoxic efficacy (IC₅₀ of 59.58 µg/mL on KB cells) [95]. The rod shape also facilitates greater surface contact with bacterial cells, contributing to the strong antibacterial effects (e.g., 37.26 ± 0.9 mm inhibition against *K. pneumoniae* at 100 µg/mL). The cumulative effect of size-dependent cellular uptake, surface-charge-induced membrane disruption, and ellagic acid-mediated surface functionality collectively enhances biological interactions[98]. These physicochemical traits synergize to improve ROS modulation, bacterial membrane disintegration, cancer cell apoptosis induction, and inflammatory mediator inhibition.

4. Conclusion

The innovative use of ellagic acid as both a capping and reducing agent in the green synthesis of AuNPs has demonstrated promising multi-therapeutic potential. The outcomes revealed significant antibacterial, antioxidant, anti-inflammatory, and anticancer activities of EA-AuNPs, underscoring their relevance for oral healthcare applications. The successful synthesis of EA-AuNPs is confirmed by the UV-Vis spectroscopic examination, which proves the synthesis of stable gold nanoparticles with a clear surface plasmon resonance (SPR) peak at 587.5 nm. FE-SEM examination showed that the average size of the particles ranged from 10 to 50 nm, and the morphology was long and rod-shaped. The produced EA-AuNPs shown remarkable antibacterial activity, especially against *K. pneumoniae*, *S. aureus*, and *E. coli*. At a dosage of 100 µg/mL, zones of inhibition reached 35 mm, demonstrating their significant antimicrobial efficacy. Furthermore, on the human oral cancer KB cell line, in vitro anticancer tests demonstrated a strong antiproliferative effects. This study thus offers a promising platform for the development of biocompatible, nanoparticle-based treatments tailored to address specific clinical challenges, particularly in antimicrobial and cancer therapeutics.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Availability of data and materials

This published publication contains all of the data obtained or analysed during this investigation.

CRedit authorship contribution statement

CR, MS and MI: organised the study, carried out the tests, divided up the chemicals, examined the equipment, and composed the report. CR, SM and IN: conceptualization, methodology, and CK, SM, MI performed the antibacterial experiments. Writing, editing, and reviewing for SM and CR. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The studies described in this publication could not have been influenced by any known conflicting financial interests or close relationships of the authors.

Data availability

Data will be made available on request.

5. References

- [1] B. Wang *et al.*, "Current advance of nanotechnology in diagnosis and treatment for malignant tumors," *Signal Transduct. Target. Ther.*, vol. 9, no. 1, p. 200, Aug. 2024, doi: 10.1038/s41392-024-01889-y.
- [2] K. A. Altammar, "A review on nanoparticles: characteristics, synthesis, applications, and challenges," *Front. Microbiol.*, vol. 14, p. 1155622, Apr. 2023, doi: 10.3389/fmicb.2023.1155622.

- [3] B. Elzein, "Nano Revolution: "Tiny tech, big impact: How nanotechnology is driving SDGs progress"," *Heliyon*, vol. 10, no. 10, p. e31393, May 2024, doi: 10.1016/j.heliyon.2024.e31393.
- [4] D. Gupta, A. Boora, A. Thakur, and T. K. Gupta, "Green and sustainable synthesis of nanomaterials: Recent advancements and limitations," *Environ. Res.*, vol. 231, p. 116316, Aug. 2023, doi: 10.1016/j.envres.2023.116316.
- [5] S. Kazemi *et al.*, "Recent advances in green synthesized nanoparticles: from production to application," *Mater. Today Sustain.*, vol. 24, p. 100500, Dec. 2023, doi: 10.1016/j.mtsust.2023.100500.
- [6] R. Kumari *et al.*, "Regulation and safety measures for nanotechnology-based agri-products," *Front. Genome Ed.*, vol. 5, p. 1200987, Jun. 2023, doi: 10.3389/fgeed.2023.1200987.
- [7] S. Gupta, R. Kaur, A. Bhardwaj, and D. Parashar, "Multifunctional Nanomaterials: Recent Advancements in Cancer Therapeutics and Vaccines," *Indian J. Microbiol.*, May 2024, doi: 10.1007/s12088-024-01274-x.
- [8] P. Garg, J. Malhotra, P. Kulkarni, D. Horne, R. Salgia, and S. S. Singhal, "Emerging Therapeutic Strategies to Overcome Drug Resistance in Cancer Cells," *Cancers*, vol. 16, no. 13, p. 2478, Jul. 2024, doi: 10.3390/cancers16132478.
- [9] S. K. Ahmed *et al.*, "Antimicrobial resistance: Impacts, challenges, and future prospects," *J. Med. Surg. Public Health*, vol. 2, p. 100081, Apr. 2024, doi: 10.1016/j.glmedi.2024.100081.
- [10] C. A. Michael, D. Dominey-Howes, and M. Labbate, "The antimicrobial resistance crisis: causes, consequences, and management," *Front. Public Health*, vol. 2, p. 145, 2014, doi: 10.3389/fpubh.2014.00145.
- [11] A. Delgado and A. K. Guddati, "Infections in Hospitalized Cancer Patients," *World J. Oncol.*, vol. 12, no. 6, pp. 195–205, Dec. 2021, doi: 10.14740/wjon1410.
- [12] C. Ragavendran, M. Imath, C. Kamaraj, I. Nakouti, and S. Manoharadas, "Eco-friendly synthesis of betanin-conjugated zinc oxide nanoparticles: antimicrobial efficacy and apoptotic pathway activation in oral cancer cells," *Mol. Biol. Rep.*, vol. 51, no. 1, p. 1128, Dec. 2024, doi: 10.1007/s11033-024-10039-0.
- [13] A. S. Danielsen *et al.*, "Clinical outcomes of antimicrobial resistance in cancer patients: a systematic review of multivariable models," *BMC Infect. Dis.*, vol. 23, no. 1, p. 247, Apr. 2023, doi: 10.1186/s12879-023-08182-3.
- [14] M. Imath, V. Sathyanathan, S. Velayudam, and R. A. M. J. Nachiya, "Variability of Mycobacterium tuberculosis lipid body content in sputum and its implications for treatment response," *Lancet Microbe*, p. 101024, Nov. 2024, doi: 10.1016/j.lanmic.2024.101024.
- [15] M. Imath, J. Giri, F. Mohammad, and C. Ragavendran, "Eco-friendly synthesis of ZnO nanoparticles fabricated using *Fioria vitifolia* L. and their biomedical potentials," *Microb. Pathog.*, vol. 199, p. 107139, Feb. 2025, doi: 10.1016/j.micpath.2024.107139.
- [16] G. Muteeb, M. T. Rehman, M. Shahwan, and M. Aatif, "Origin of Antibiotics and Antibiotic Resistance, and Their Impacts on Drug Development: A Narrative Review," *Pharmaceuticals*, vol. 16, no. 11, p. 1615, Nov. 2023, doi: 10.3390/ph16111615.
- [17] E. Sánchez-López *et al.*, "Metal-Based Nanoparticles as Antimicrobial Agents: An Overview," *Nanomaterials*, vol. 10, no. 2, p. 292, Feb. 2020, doi: 10.3390/nano10020292.
- [18] K. Shanmugam, S. Sellappan, T. A. Alahmadi, H. S. Almoallim, N. Natarajan, and V. P. Veeraraghavan, "Green synthesized zinc oxide nanoparticles from *Cinnamomum verum* bark extract inhibited cell growth and induced caspase-mediated apoptosis in oral cancer KB cells," *J. Drug Deliv. Sci. Technol.*, vol. 74, p. 103577, Aug. 2022, doi: 10.1016/j.jddst.2022.103577.
- [19] F. Bray, J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre, and A. Jemal, "Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries," *CA. Cancer J. Clin.*, vol. 68, no. 6, pp. 394–424, Nov. 2018, doi: 10.3322/caac.21492.
- [20] A. Ramasubramanian, P. Ramani, B. Kannan, and P. Arumugam, "High expression of novel biomarker TBRG4 promotes the progression and invasion of oral squamous cell carcinoma," *J. Oral Pathol. Med.*, vol. 52, no. 8, pp. 738–745, Sep. 2023, doi: 10.1111/jop.13470.

- [21] F. Davodabadi *et al.*, "Cancer chemotherapy resistance: Mechanisms and recent breakthrough in targeted drug delivery," *Eur. J. Pharmacol.*, vol. 958, p. 176013, Nov. 2023, doi: 10.1016/j.ejphar.2023.176013.
- [22] M. O. Hussein and A. S. Abdulhameed, "Design of Bionanomaterial of Chitosan Carbohydrate Polymer Compositated with Broccoli Extract and Zinc Oxide Nanoparticles: Anticancer Activity in Human Osteosarcoma," *Appl. Biochem. Biotechnol.*, Oct. 2024, doi: 10.1007/s12010-024-05066-3.
- [23] S. Montazersaheb *et al.*, "Green-synthesized silver nanoparticles from peel extract of pumpkin as a potent radiosensitizer against triple-negative breast cancer (TNBC)," *Cancer Nanotechnol.*, vol. 15, no. 1, p. 47, Dec. 2024, doi: 10.1186/s12645-024-00285-z.
- [24] S. T. Anwer *et al.*, "Synthesis of Silver Nano Particles Using Myricetin and the In-Vitro Assessment of Anti-Colorectal Cancer Activity: In-Silico Integration," *Int. J. Mol. Sci.*, vol. 23, no. 19, p. 11024, Sep. 2022, doi: 10.3390/ijms231911024.
- [25] Zhou, J., Zhou, L., Chen, Z., Sun, J., Guo, X., Wang, H.,... Zhang, X. (2025). Remineralization and bacterial inhibition of early enamel caries surfaces by carboxymethyl chitosan lysozyme nanogels loaded with antibacterial drugs. *Journal of Dentistry*, 152, 105489. doi: <https://doi.org/10.1016/j.jdent.2024.105489>
- [26] Cheng, Y., Wang, L., Zhang, S., Jian, W., Zeng, B., Liang, L.,... Deng, Z. (2024). The Investigation of Nfkb Inhibitors to Block Cell Proliferation in OSCC Cells Lines. *Current Medicinal Chemistry*. doi: 10.2174/0109298673309489240816063313
- [27] D. Bharathi, R. Ranjithkumar, J. G. T. Nandagopal, S. Djearamane, J. Lee, and L. S. Wong, "Green synthesis of chitosan/silver nanocomposite using kaempferol for triple negative breast cancer therapy and antibacterial activity," *Environ. Res.*, vol. 238, p. 117109, Dec. 2023, doi: 10.1016/j.envres.2023.117109.
- [28] V. Arulmozhi, K. Pandian, and S. Mirunalini, "Ellagic acid encapsulated chitosan nanoparticles for drug delivery system in human oral cancer cell line (KB)," *Colloids Surf. B Biointerfaces*, vol. 110, pp. 313–320, Oct. 2013, doi: 10.1016/j.colsurfb.2013.03.039.
- [29] X. Yu *et al.*, "Ellagic acid-enhanced biocompatibility and bioactivity in multilayer core-shell gold nanoparticles for ameliorating myocardial infarction injury," *J. Nanobiotechnology*, vol. 22, no. 1, p. 554, Sep. 2024, doi: 10.1186/s12951-024-02796-8.
- [30] P. B. Santhosh, J. Genova, and H. Chamati, "Green Synthesis of Gold Nanoparticles: An Eco-Friendly Approach," *Chemistry*, vol. 4, no. 2, pp. 345–369, Apr. 2022, doi: 10.3390/chemistry4020026.
- [31] C. Pechyen, B. Tangnorawich, S. Toommee, R. Marks, and Y. Parcharoen, "Green synthesis of metal nanoparticles, characterization, and biosensing applications," *Sens. Int.*, vol. 5, p. 100287, 2024, doi: 10.1016/j.sintl.2024.100287.
- [32] S. H. Gebre, "Bio-inspired Synthesis of Metal and Metal Oxide Nanoparticles: The Key Role of Phytochemicals," *J. Clust. Sci.*, vol. 34, no. 2, pp. 665–704, Mar. 2023, doi: 10.1007/s10876-022-02276-9.
- [33] T. Patil, R. Gambhir, A. Vibhute, and A. P. Tiwari, "Gold Nanoparticles: Synthesis Methods, Functionalization and Biological Applications," *J. Clust. Sci.*, vol. 34, no. 2, pp. 705–725, Mar. 2023, doi: 10.1007/s10876-022-02287-6.
- [34] B. A. Fadel, B. H. Elwakil, E. E. Fawzy, M. M. Shaaban, and Z. A. Olama, "Nanoemulsion of Lavandula angustifolia Essential Oil/Gold Nanoparticles: Antibacterial Effect against Multidrug-Resistant Wound-Causing Bacteria," *Molecules*, vol. 28, no. 19, p. 6988, Oct. 2023, doi: 10.3390/molecules28196988.
- [35] T. Mgijima *et al.*, "Wound healing effects of biogenic gold nanoparticles synthesized using red wine extracts," *Artif. Cells Nanomedicine Biotechnol.*, vol. 52, no. 1, pp. 399–410, Dec. 2024, doi: 10.1080/21691401.2024.2383583.

- [36] R. Eivazzadeh-Keihan, Z. Saadatidizaji, M. Mahdavi, A. Maleki, M. Irani, and I. Zare, "Recent advances in gold nanoparticles-based biosensors for tuberculosis determination," *Talanta*, vol. 275, p. 126099, Aug. 2024, doi: 10.1016/j.talanta.2024.126099.
- [37] S. Ekrikaya *et al.*, "Investigation of ellagic acid rich-berry extracts directed silver nanoparticles synthesis and their antimicrobial properties with potential mechanisms towards *Enterococcus faecalis* and *Candida albicans*," *J. Biotechnol.*, vol. 341, pp. 155–162, Nov. 2021, doi: 10.1016/j.jbiotec.2021.09.020.
- [38] A. Gupta, A. K. Singh, R. Kumar, S. Jamieson, A. K. Pandey, and A. Bishayee, "Neuroprotective Potential of Ellagic Acid: A Critical Review," *Adv. Nutr. Bethesda Md*, vol. 12, no. 4, pp. 1211–1238, Jul. 2021, doi: 10.1093/advances/nmab007.
- [39] S. Sangwan and R. Seth, "Synthesis, Characterization and Stability of Gold Nanoparticles (AuNPs) in Different Buffer Systems," *J. Clust. Sci.*, vol. 33, no. 2, pp. 749–764, Mar. 2022, doi: 10.1007/s10876-020-01956-8.
- [40] C. E. A. Botteon *et al.*, "Biosynthesis and characterization of gold nanoparticles using Brazilian red propolis and evaluation of its antimicrobial and anticancer activities," *Sci. Rep.*, vol. 11, no. 1, p. 1974, Jan. 2021, doi: 10.1038/s41598-021-81281-w.
- [41] A. Kassem *et al.*, "Applications of Fourier Transform-Infrared spectroscopy in microbial cell biology and environmental microbiology: advances, challenges, and future perspectives," *Front. Microbiol.*, vol. 14, p. 1304081, Nov. 2023, doi: 10.3389/fmicb.2023.1304081.
- [42] Y. Gong, X. Chen, and W. Wu, "Application of fourier transform infrared (FTIR) spectroscopy in sample preparation: Material characterization and mechanism investigation," *Adv. Sample Prep.*, vol. 11, p. 100122, Aug. 2024, doi: 10.1016/j.sampre.2024.100122.
- [43] B. J. Inkson, "Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) for materials characterization," in *Materials Characterization Using Nondestructive Evaluation (NDE) Methods*, Elsevier, 2016, pp. 17–43. doi: 10.1016/B978-0-08-100040-3.00002-X.
- [44] S. S. Al-Mafarjy, N. Suardi, N. M. Ahmed, D. Kernain, H. Hisham Alkatib, and M. A. Dheyab, "Green synthesis of gold nanoparticles from *Coleus scutellarioides* (L.) Benth leaves and assessment of anticancer and antioxidant properties," *Inorg. Chem. Commun.*, vol. 161, p. 112052, Mar. 2024, doi: 10.1016/j.inoche.2024.112052.
- [45] D. Kiani, "X-Ray Diffraction (XRD)," in *Springer Handbook of Advanced Catalyst Characterization*, I. E. Wachs and M. A. Bañares, Eds., in Springer Handbooks. , Cham: Springer International Publishing, 2023, pp. 519–539. doi: 10.1007/978-3-031-07125-6_25.
- [46] F. Babick, "Dynamic light scattering (DLS)," in *Characterization of Nanoparticles*, Elsevier, 2020, pp. 137–172. doi: 10.1016/B978-0-12-814182-3.00010-9.
- [47] A. Serrano-Lotina, R. Portela, P. Baeza, V. Alcolea-Rodriguez, M. Villarroel, and P. Ávila, "Zeta potential as a tool for functional materials development," *Catal. Today*, vol. 423, p. 113862, Nov. 2023, doi: 10.1016/j.cattod.2022.08.004.
- [48] Q. Yu, J. Li, Y. Zhang, Y. Wang, L. Liu, and M. Li, "Inhibition of gold nanoparticles (AuNPs) on pathogenic biofilm formation and invasion to host cells," *Sci. Rep.*, vol. 6, no. 1, p. 26667, May 2016, doi: 10.1038/srep26667.
- [49] A. Timoszyk and R. Grochowalska, "Mechanism and Antibacterial Activity of Gold Nanoparticles (AuNPs) Functionalized with Natural Compounds from Plants," *Pharmaceutics*, vol. 14, no. 12, p. 2599, Nov. 2022, doi: 10.3390/pharmaceutics14122599.
- [50] B. Kowalska-Krochmal and R. Dudek-Wicher, "The Minimum Inhibitory Concentration of Antibiotics: Methods, Interpretation, Clinical Relevance," *Pathogens*, vol. 10, no. 2, p. 165, Feb. 2021, doi: 10.3390/pathogens10020165.
- [51] I. Wiegand, K. Hilpert, and R. E. W. Hancock, "Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances," *Nat. Protoc.*, vol. 3, no. 2, pp. 163–175, Feb. 2008, doi: 10.1038/nprot.2007.521.

- [52] T. J. Hossain, "Methods for screening and evaluation of antimicrobial activity: A review of protocols, advantages, and limitations," *Eur. J. Microbiol. Immunol.*, vol. 14, no. 2, pp. 97–115, May 2024, doi: 10.1556/1886.2024.00035.
- [53] M. Roney and M. F. F. Mohd Aluwi, "The importance of in-silico studies in drug discovery," *Intell. Pharm.*, vol. 2, no. 4, pp. 578–579, Aug. 2024, doi: 10.1016/j.ipha.2024.01.010.
- [54] S. Baliyan *et al.*, "Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of *Ficus religiosa*," *Molecules*, vol. 27, no. 4, p. 1326, Feb. 2022, doi: 10.3390/molecules27041326.
- [55] J. La, M.-J. Kim, and J. Lee, "Evaluation of solvent effects on the DPPH reactivity for determining the antioxidant activity in oil matrix," *Food Sci. Biotechnol.*, vol. 30, no. 3, pp. 367–375, Mar. 2021, doi: 10.1007/s10068-020-00874-9.
- [56] M. Imath *et al.*, "Fioria vitifolia-mediated silver nanoparticles: Eco-friendly synthesis and biomedical potential," *J. Water Process Eng.*, vol. 66, p. 106020, Sep. 2024, doi: 10.1016/j.jwpe.2024.106020.
- [57] M. Imath, J. Giri, F. Mohammad, C. Ragavendran, J. Al-Tamimi, and H. Ebaid, "Eco-friendly synthesis of ZnO nanoparticles fabricated using Fioria vitifolia L. and their Biomedical Potentials," *Microb. Pathog.*, p. 107139, Nov. 2024, doi: 10.1016/j.micpath.2024.107139.
- [58] I. G. Munteanu and C. Apetrei, "Analytical Methods Used in Determining Antioxidant Activity: A Review," *Int. J. Mol. Sci.*, vol. 22, no. 7, p. 3380, Mar. 2021, doi: 10.3390/ijms22073380.
- [59] N. Bibi Sadeer, D. Montesano, S. Albrizio, G. Zengin, and M. F. Mahomoodally, "The Versatility of Antioxidant Assays in Food Science and Safety—Chemistry, Applications, Strengths, and Limitations," *Antioxidants*, vol. 9, no. 8, p. 709, Aug. 2020, doi: 10.3390/antiox9080709.
- [60] M. Islam *et al.*, "Phytochemical screening and evaluation of antioxidant, anti-inflammatory, antimicrobial, and membrane-stabilizing activities of different fractional extracts of *Grewia nervosa* (Lour.) Panigrahi," *Food Biosci.*, vol. 54, p. 102933, Aug. 2023, doi: 10.1016/j.fbio.2023.102933.
- [61] M. Cao, X. Luo, K. Wu, and X. He, "Targeting lysosomes in human disease: from basic research to clinical applications," *Signal Transduct. Target. Ther.*, vol. 6, no. 1, p. 379, Nov. 2021, doi: 10.1038/s41392-021-00778-y.
- [62] T. Fujita *et al.*, "Anti-inflammatory effect of gold nanoparticles supported on metal oxides," *Sci. Rep.*, vol. 11, no. 1, p. 23129, Nov. 2021, doi: 10.1038/s41598-021-02419-4.
- [63] H. Agarwal, A. Nakara, and V. K. Shanmugam, "Anti-inflammatory mechanism of various metal and metal oxide nanoparticles synthesized using plant extracts: A review," *Biomed. Pharmacother.*, vol. 109, pp. 2561–2572, Jan. 2019, doi: 10.1016/j.biopha.2018.11.116.
- [64] P. Rathinaraj, K. Lee, S.-Y. Park, and I.-K. Kang, "Targeted images of KB cells using folate-conjugated gold nanoparticles," *Nanoscale Res. Lett.*, vol. 10, no. 1, p. 5, Dec. 2015, doi: 10.1186/s11671-014-0725-y.
- [65] M. Ghasemi, T. Turnbull, S. Sebastian, and I. Kempson, "The MTT Assay: Utility, Limitations, Pitfalls, and Interpretation in Bulk and Single-Cell Analysis," *Int. J. Mol. Sci.*, vol. 22, no. 23, p. 12827, Nov. 2021, doi: 10.3390/ijms222312827.
- [66] M. Kus-Liśkiewicz, P. Fickers, and I. Ben Tahar, "Biocompatibility and Cytotoxicity of Gold Nanoparticles: Recent Advances in Methodologies and Regulations," *Int. J. Mol. Sci.*, vol. 22, no. 20, p. 10952, Oct. 2021, doi: 10.3390/ijms222010952.
- [67] M.-H. Moosavy, M. De La Guardia, A. Mokhtarzadeh, S. A. Khatibi, N. Hosseinzadeh, and N. Hajipour, "Green synthesis, characterization, and biological evaluation of gold and silver nanoparticles using *Mentha spicata* essential oil," *Sci. Rep.*, vol. 13, no. 1, p. 7230, May 2023, doi: 10.1038/s41598-023-33632-y.
- [68] K. Thamilchelvan, C. Ragavendran, D. Kamalanathan, R. Rajendiran, T. Cherian, and G. Malafaia, "In vitro somatic embryo productions from *Curculigo orchoides* derived gold nanoparticles: Synthesis, characterization, its biomedical applications, and their eco-friendly approaches to

- degradation of methylene blue under solar light irradiations," *Environ. Res.*, vol. 216, p. 114774, Jan. 2023, doi: 10.1016/j.envres.2022.114774.
- [69] A. K. Das, M. Borah, J. J. Kalita, and U. Bora, "Cytotoxic potential of Curcuma caesia rhizome extract and derived gold nanoparticles in targeting breast cancer cell lines," *Sci. Rep.*, vol. 14, no. 1, p. 17223, Jul. 2024, doi: 10.1038/s41598-024-66175-x.
- [70] N. Muniyappan, M. Pandeewaran, and A. Amalraj, "Green synthesis of gold nanoparticles using Curcuma pseudomontana isolated curcumin: Its characterization, antimicrobial, antioxidant and anti-inflammatory activities," *Environ. Chem. Ecotoxicol.*, vol. 3, pp. 117–124, 2021, doi: 10.1016/j.enceco.2021.01.002.
- [71] C. N. Andoh, F. Attiogbe, N. O. Bonsu Ackerson, M. Antwi, and K. Adu-Boahen, "Fourier Transform Infrared Spectroscopy: An analytical technique for microplastic identification and quantification," *Infrared Phys. Technol.*, vol. 136, p. 105070, Jan. 2024, doi: 10.1016/j.infrared.2023.105070.
- [72] M. Shin, S. Yang, H. W. Kwak, and K. H. Lee, "Synthesis of gold nanoparticles using silk sericin as a green reducing and capping agent," *Eur. Polym. J.*, vol. 164, p. 110960, Feb. 2022, doi: 10.1016/j.eurpolymj.2021.110960.
- [73] M. A. Safwat, B. A. Kandil, M. A. Elblbesy, G. M. Soliman, and N. E. Eleraky, "Epigallocatechin-3-Gallate-Loaded Gold Nanoparticles: Preparation and Evaluation of Anticancer Efficacy in Ehrlich Tumor-Bearing Mice," *Pharmaceuticals*, vol. 13, no. 9, p. 254, Sep. 2020, doi: 10.3390/ph13090254.
- [74] M. M. Fathy *et al.*, "An insight into synthesis and antitumor activity of citrate and gallate stabilizing gold nanospheres," *Sci. Rep.*, vol. 13, no. 1, p. 2749, Feb. 2023, doi: 10.1038/s41598-023-29821-4.
- [75] G. M. Sulaiman, H. M. Waheeb, M. S. Jabir, S. H. Khazaal, Y. H. Dewir, and Y. Naidoo, "Hesperidin Loaded on Gold Nanoparticles as a Drug Delivery System for a Successful Biocompatible, Anti-Cancer, Anti-Inflammatory and Phagocytosis Inducer Model," *Sci. Rep.*, vol. 10, no. 1, p. 9362, Jun. 2020, doi: 10.1038/s41598-020-66419-6.
- [76] X. Yang, Y. Bao, X. Zhou, H. Zhu, and J. Gao, "Green chemistry-based synthesis of gold nanoparticles using Nicotiana glauca and their anticancer properties against cervical cancer," *Arab. J. Chem.*, p. 106029, Oct. 2024, doi: 10.1016/j.arabjc.2024.106029.
- [77] N. Suganthi, V. Sri Ramkumar, A. Pugazhendhi, G. Benelli, and G. Archunan, "Biogenic synthesis of gold nanoparticles from Terminalia arjuna bark extract: assessment of safety aspects and neuroprotective potential via antioxidant, anticholinesterase, and anti-amyloidogenic effects," *Environ. Sci. Pollut. Res.*, vol. 25, no. 11, pp. 10418–10433, Apr. 2018, doi: 10.1007/s11356-017-9789-4.
- [78] W. J. Keijok *et al.*, "Controlled biosynthesis of gold nanoparticles with Coffea arabica using factorial design," *Sci. Rep.*, vol. 9, no. 1, p. 16019, Nov. 2019, doi: 10.1038/s41598-019-52496-9.
- [79] N. S. Al-Radadi, "Single-step green synthesis of gold conjugated polyphenol nanoparticle using extracts of Saudi's myrrh: Their characterization, molecular docking and essential biological applications," *Saudi Pharm. J.*, vol. 30, no. 9, pp. 1215–1242, Sep. 2022, doi: 10.1016/j.jsps.2022.06.028.
- [80] E. D. Mohamed Isa *et al.*, "Green synthesis of gold nanoparticles via Artocarpus odoratissimus peel extract for potential applications of optical filter and catalytic degradation," *J. King Saud Univ. - Sci.*, vol. 36, no. 6, p. 103209, Jul. 2024, doi: 10.1016/j.jksus.2024.103209.
- [81] V. Vorobyova, M. Skiba, K. Vinnichuk, and G. Vasyliov, "Synthesis of gold nanoparticles using plum waste extract with green solvents," *Sustain. Chem. Environ.*, vol. 6, p. 100086, Jun. 2024, doi: 10.1016/j.scenv.2024.100086.
- [82] A. Rosyidah *et al.*, "Eco-friendly synthesis of gold nanoparticles by marine microalgae Synechococcus mooringangae: Characterization, antimicrobial, and antioxidant properties," *Kuwait J. Sci.*, vol. 51, no. 2, p. 100194, Apr. 2024, doi: 10.1016/j.kjs.2024.100194.

- [83] A. Parthiban *et al.*, "Green synthesis of gold nanoparticles using quercetin biomolecule from mangrove plant, *Ceriops tagal*: Assessment of antiproliferative properties, cellular uptake and DFT studies," *J. Mol. Struct.*, vol. 1272, p. 134167, Jan. 2023, doi: 10.1016/j.molstruc.2022.134167.
- [84] S. Anwar, S. Altaf, M. S. U. R. Babar, B. Aslam, H. Muzaffar, and A. Iftikhar, "Camellia-sinensis- and Cocos-nucifera-Derived Gold Nanoparticles for Treatment of Infections Caused by Antibiotic-Resistant *Staphylococcus aureus*," in *IOCN 2023*, MDPI, May 2023, p. 67. doi: 10.3390/IOCN2023-14469.
- [85] S. R. Alizadeh, P. Biparva, H. R. Goli, B. A. Khan, and M. A. Ebrahimzadeh, "Green Synthesis of AuNPs by *Crocus caspius*—Investigation of Catalytic Degradation of Organic Pollutants, Their Cytotoxicity, and Antimicrobial Activity," *Catalysts*, vol. 13, no. 1, p. 63, Dec. 2022, doi: 10.3390/catal13010063.
- [86] A. Fouda *et al.*, "Green Synthesis of Gold Nanoparticles by Aqueous Extract of *Zingiber officinale*: Characterization and Insight into Antimicrobial, Antioxidant, and In Vitro Cytotoxic Activities," *Appl. Sci.*, vol. 12, no. 24, p. 12879, Dec. 2022, doi: 10.3390/app122412879.
- [87] D. A. Lomeli-Rosales *et al.*, "Green Synthesis of Gold and Silver Nanoparticles Using Leaf Extract of *Capsicum chinense* Plant," *Molecules*, vol. 27, no. 5, p. 1692, Mar. 2022, doi: 10.3390/molecules27051692.
- [88] L. Azmy *et al.*, "Antimicrobial Activity of *Arthrospira platensis*-Mediated Gold Nanoparticles against *Streptococcus pneumoniae*: A Metabolomic and Docking Study," *Int. J. Mol. Sci.*, vol. 25, no. 18, p. 10090, Sep. 2024, doi: 10.3390/ijms251810090.
- [89] M. J. Silvero C. *et al.*, "Selective Photoinduced Antibacterial Activity of Amoxicillin-Coated Gold Nanoparticles: From One-Step Synthesis to in Vivo Cytocompatibility," *ACS Omega*, vol. 3, no. 1, pp. 1220–1230, Jan. 2018, doi: 10.1021/acsomega.7b01779.
- [90] E. S. Almutleb, S. Ramachandran, A. A. Khan, G. A. El-Hiti, and S. A. Alanazi, "Synergistic Effect of Nilavembu Choornam–Gold Nanoparticles on Antibiotic-Resistant Bacterial Susceptibility and Contact Lens Contamination-Associated Infectious Pathogenicity," *Int. J. Mol. Sci.*, vol. 25, no. 4, p. 2115, Feb. 2024, doi: 10.3390/ijms25042115.
- [91] A. Hatipoğlu, "Rapid green synthesis of gold nanoparticles: synthesis, characterization and antimicrobial activities : Rapid green synthesis of gold nanoparticles," *Prog. Nutr.*, vol. 23, no. 3, p. e2021242, Oct. 2021, doi: 10.23751/pn.v23i3.11988.
- [92] A. Rajendran, "Antibacterial Properties and Mechanism of Gold Nanoparticles Obtained from *Pergularia Daemia* Leaf Extract," *J. Nanomedicine Res.*, vol. 6, no. 1, Aug. 2017, doi: 10.15406/jnmr.2017.06.00146.
- [93] C. Shen *et al.*, "Kinetically regulated one-pot synthesis of cationic gold nanoparticles and their size-dependent antibacterial mechanism," *J. Mater. Sci. Technol.*, vol. 162, pp. 145–156, Nov. 2023, doi: 10.1016/j.jmst.2023.03.061.
- [94] Z. Sharifiaghdam, S. M. Amini, F. Dalouchi, A. B. Behrooz, and Y. Azizi, "Apigenin-coated gold nanoparticles as a cardioprotective strategy against doxorubicin-induced cardiotoxicity in male rats via reducing apoptosis," *Heliyon*, vol. 9, no. 3, p. e14024, Mar. 2023, doi: 10.1016/j.heliyon.2023.e14024.
- [95] A. Salama *et al.*, "Curcumin-loaded gold nanoparticles with enhanced antibacterial efficacy and wound healing properties in diabetic rats," *Int. J. Pharm.*, vol. 666, p. 124761, Dec. 2024, doi: 10.1016/j.ijpharm.2024.124761.
- [96] Z. Dong *et al.*, "NIR-triggered tea polyphenol-modified gold nanoparticles-loaded hydrogel treats periodontitis by inhibiting bacteria and inducing bone regeneration," *Mater. Des.*, vol. 225, p. 111487, Jan. 2023, doi: 10.1016/j.matdes.2022.111487.
- [97] M. J. Kim, S. U. Rehman, F. U. Amin, and M. O. Kim, "Enhanced neuroprotection of anthocyanin-loaded PEG-gold nanoparticles against A β 1-42-induced neuroinflammation and neurodegeneration via the NF-KB/JNK/GSK3 β signaling pathway," *Nanomedicine Nanotechnol. Biol. Med.*, vol. 13, no. 8, pp. 2533–2544, Nov. 2017, doi: 10.1016/j.nano.2017.06.022.

- [98] F. G. Milanezi *et al.*, "Antioxidant, antimicrobial and cytotoxic activities of gold nanoparticles capped with quercetin," *Saudi Pharm. J.*, vol. 27, no. 7, pp. 968–974, Nov. 2019, doi: 10.1016/j.jsps.2019.07.005.
- [99] N. S. Al-Radadi, "Single-step green synthesis of gold conjugated polyphenol nanoparticle using extracts of Saudi's myrrh: Their characterization, molecular docking and essential biological applications," *Saudi Pharm. J.*, vol. 30, no. 9, pp. 1215–1242, Sep. 2022, doi: 10.1016/j.jsps.2022.06.028.
- [100] A. Salama *et al.*, "Curcumin-loaded gold nanoparticles with enhanced antibacterial efficacy and wound healing properties in diabetic rats," *Int. J. Pharm.*, vol. 666, p. 124761, Dec. 2024, doi: 10.1016/j.ijpharm.2024.124761.
- [101] D.-Y. Kim, M. Kim, S. Shinde, J.-S. Sung, and G. Ghodake, "Cytotoxicity and antibacterial assessment of gallic acid capped gold nanoparticles," *Colloids Surf. B Biointerfaces*, vol. 149, pp. 162–167, Jan. 2017, doi: 10.1016/j.colsurfb.2016.10.017.
- [102] J. K. Patra, H.-S. Shin, I.-J. Yang, L. T. H. Nguyen, and G. Das, "Sustainable Utilization of Food Biowaste (Papaya Peel) Extract for Gold Nanoparticle Biosynthesis and Investigation of Its Multi-Functional Potentials," *Antioxidants*, vol. 13, no. 5, p. 581, May 2024, doi: 10.3390/antiox13050581.
- [103] S. Malik *et al.*, "Cytotoxicity Study of Gold Nanoparticle Synthesis Using *Aloe vera* , Honey, and *Gymnema sylvestre* Leaf Extract," *ACS Omega*, vol. 8, no. 7, pp. 6325–6336, Feb. 2023, doi: 10.1021/acsomega.2c06491.
- [104] Y. Ngungeni *et al.*, "Anticancer, Antioxidant, and Catalytic Activities of Green Synthesized Gold Nanoparticles Using Avocado Seed Aqueous Extract," *ACS Omega*, vol. 8, no. 29, pp. 26088–26101, Jul. 2023, doi: 10.1021/acsomega.3c02260.
- [105] K. Phukan, R. Devi, and D. Chowdhury, "Green Synthesis of Gold Nano-bioconjugates from Onion Peel Extract and Evaluation of Their Antioxidant, Anti-inflammatory, and Cytotoxic Studies," *ACS Omega*, vol. 6, no. 28, pp. 17811–17823, Jul. 2021, doi: 10.1021/acsomega.1c00861.
- [106] K. Phukan, R. Devi, and D. Chowdhury, "Insights into Anti-Inflammatory Activity and Internalization Pathway of Onion Peel-Derived Gold Nano Bioconjugates in RAW 264.7 Macrophages," *ACS Omega*, vol. 7, no. 9, pp. 7606–7615, Mar. 2022, doi: 10.1021/acsomega.1c06131.
- [107] V. C. Situmorang *et al.*, "Microwave-assisted green synthesis of Cassia alata-mediated gold nanoparticles and evaluation of its antioxidant, anti-inflammatory, and antibacterial activities," *Biomass Convers. Biorefinery*, Jul. 2024, doi: 10.1007/s13399-024-05950-2.
- [108] A. O. E. Eltahir, K. L. Lategan, O. M. David, E. J. Pool, R. C. Luckay, and A. A. Hussein, "Green Synthesis of Gold Nanoparticles Using Liquiritin and Other Phenolics from *Glycyrrhiza glabra* and Their Anti-Inflammatory Activity," *J. Funct. Biomater.*, vol. 15, no. 4, p. 95, Apr. 2024, doi: 10.3390/jfb15040095.
- [109] V. R. Pradeep, S. Menaka, V. Suresh, and S. Jayaraman, "Anticancer Effects of Rosmarinus officinalis Leaf Extract on KB Cell Lines," *Cureus*, Feb. 2024, doi: 10.7759/cureus.54031.
- [110] S. Samal *et al.*, "Exploring the anticancer and antioxidant potential of gold nanoparticles synthesized from *Pterocarpus marsupium* bark extract against oral squamous cell carcinoma," *Artif. Cells Nanomedicine Biotechnol.*, vol. 52, no. 1, pp. 512–528, Dec. 2024, doi: 10.1080/21691401.2024.2416951.
- [111] C. Ragavendran, M. Imath, C. Kamaraj, I. Nakouti, and S. Manoharadas, "Eco-friendly synthesis of betanin-conjugated zinc oxide nanoparticles: antimicrobial efficacy and apoptotic pathway activation in oral cancer cells," *Mol. Biol. Rep.*, vol. 51, no. 1, p. 1128, Dec. 2024, doi: 10.1007/s11033-024-10039-0.
- [112] Y. Tan *et al.*, "Oral squamous cell carcinomas: state of the field and emerging directions," *Int. J. Oral Sci.*, vol. 15, no. 1, p. 44, Sep. 2023, doi: 10.1038/s41368-023-00249-w.

- [113] D. Khanna *et al.*, "Prevalence of Oral Potentially Malignant Lesions, Tobacco use, and Effect of Cessation Strategies among Solid Waste Management workers in Northern India: a pre-post intervention study," *BMC Oral Health*, vol. 24, no. 1, p. 1292, Oct. 2024, doi: 10.1186/s12903-024-05087-8.
- [114] I. Z. Matić, A. Mraković, Z. Rakočević, M. Stojković, V. B. Pavlović, and T. Momić, "Anticancer effect of novel luteolin capped gold nanoparticles selectively cytotoxic towards human cervical adenocarcinoma HeLa cells: An in vitro approach," *J. Trace Elem. Med. Biol.*, vol. 80, p. 127286, Dec. 2023, doi: 10.1016/j.jtemb.2023.127286.
- [115] F. Ahmad, K. M. Surapaneni, B. Kamaraj, and B. Kamaraj, "Anti-proliferative activity and apoptotic induction of tannins extracted from *Quercus infectoria* on oral cancer KB cell lines," *J. Appl. Pharm. Sci.*, 2024, doi: 10.7324/JAPS.2024.174009.
- [116] V. Marunganathan *et al.*, "Marine-derived κ-carrageenan-coated zinc oxide nanoparticles for targeted drug delivery and apoptosis induction in oral cancer," *Mol. Biol. Rep.*, vol. 51, no. 1, p. 89, Dec. 2024, doi: 10.1007/s11033-023-09146-1.
- [117] R. Geetha, T. Ashokkumar, S. Tamilselvan, K. Govindaraju, M. Sadiq, and G. Singaravelu, "Green synthesis of gold nanoparticles and their anticancer activity," *Cancer Nanotechnol.*, vol. 4, no. 4–5, pp. 91–98, Aug. 2013, doi: 10.1007/s12645-013-0040-9.