

Green-Synthesized Nanoparticles for Dual Targeting of Oral Biofilms and Oral Squamous Cell Carcinoma

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Abstract: The co-evolution of multidrug resistance amongst oral pathogens and sub-par treatment outcomes associated with traditional OSCC therapy calls for alternative strategies, which not only differ in terms of the underlying mechanism but should also be biocompatible. One such strategy entails nanoparticle synthesis utilizing plant extracts as both reducing agents and ligands; secondary metabolic compounds endow the produced nanomaterials with unique biological activity. The aim of the present review is a comprehensive analysis of the preparation, characterization, and dual antibacterial/antitumorigenic activities of AgNPs, CuNPs, and FeNPs produced from aqueous extracts of *Azadirachta indica* and *Syzygium cumini*. The nucleation, growth kinetics, size distribution, and particle charge are all governed by the polyphenols content of aqueous plant extract; in particular, *Syzygium cumini* nanoparticles display monodispersity and low particle sizes (<20 nm) in comparison to larger *Azadirachta indica* nanoparticles that show enhanced stability due to the abundant presence of terpenoids. Amongst oral biofilm-forming bacteria, AgNPs have shown the greatest breadth of antimicrobial action at the minimum concentration range of 5-25 µg/mL; AgNPs showed greater than 90% biofilm inhibition whereas CuNPs showed antifungal efficacy against *Candida albicans*. FeNPs, though inherently less cytotoxic, offer a magnetically responsive platform for targeted drug delivery and localized hyperthermia. In OSCC cell lines, biogenic nanoparticles induced dose-dependent apoptosis through mitochondrial membrane depolarization, upregulation of the Bax/Bcl-2 ratio, cytochrome c release, and caspase-3 activation, with selectivity indices consistently superior to chemically synthesized counterparts — attributed to the modulation of oxidative thresholds by surface-adsorbed phytochemicals. Taken together, these phytometallic nanocomplexes represent a mechanistically integrated dual-action platform with potential translational relevance to precision oral therapeutics.

Keywords: Green synthesis, *Azadirachta indica*, *Syzygium cumini*, Silver nanoparticles, Copper nanoparticles, Iron nanoparticles, Oral biofilms, Oral squamous cell carcinoma.

1. Introduction

A. The Global Challenge of Oral Health and Malignancy

Problems related to oral health continue to be a significant international public health problem affecting around 3.5 billion people in the world. Of all the diseases mentioned, OSCC is

considered the most prevalent type of cancer in the head and neck area, but its five-year survival rate is quite low, making it the most morbid among other diseases (Wu et al., 2025). Traditional methods for the management of OSCC, which include surgery and chemoradiation therapy with the addition of systemic chemotherapy, have been very harsh on the patients, leading to such side effects as xerostomia, mucositis, and physical alterations (Mohamad et al., 2023). Simultaneously, the oral cavity is colonized by one of the most intricate microbial communities within the human body. Whilst the majority of these species are commensal, the progression toward a dysbiotic state is characterized by the establishment of hard biofilms. Moreover, these species, for instance, *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Candida albicans*, which are at the core of dental caries and periodontal disease, are currently seen to play an important role in the perpetuation of chronic inflammation, which is a known precursor to oral carcinogenesis. Multidrug-resistant (MDR) oral microbiota is another worrying factor, where traditional antibiotics along with local antimicrobial interventions fail to exert value (Anderson et al., 2023).

B. The Shift Toward Nanomedicine and "Green" Synthesis

These several clinical challenges have, therefore, geared up another transformative frontier-nanotechnology. Nanoparticles (1-100 nm) exhibit unique physicochemical properties, such as a very high surface-area-to-volume ratio, quantum confinement effects, and enhanced reactivity, which distinctly differ from those of their bulk material counterparts. These properties enable NPs to penetrate dense microbial biofilms or selectively interact with the leaky vasculature and acidic microenvironment in solid tumors (Dolai, Mandal, & Jana, 2021).

In the past, nanoparticle synthesis was achieved through physical methods including laser ablation and milling. The use of reduction techniques, such as the reduction by sodium borohydride and hydrazine was also common. Although effective, the above methods have high energy requirements and utilize toxic chemicals that leave toxic residue on the nanoparticle, hence limiting their biocompatibility (Osman et

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al., 2024). It has resulted in what is known as Green Synthesis or Biogenic Synthesis. In this case, one-step production of nanoparticles can be carried out utilizing biological entities through use of plant extract. Secondary metabolites present within the plant play two roles: first, they help in reduction of metal ions to zero valent atoms, secondly, act as stabilizers against agglomeration. (Singh *et al.*, 2018).

1) Botanic Powerhouses: *Azadirachta indica* (Neem) and *Syzygiumcumini* (Jamun)

The selection of the reducing agent plays a major role in deciding the shape, size, and bioactivity of the formed nanoparticles. The current review reveals two culturally and medicinally important plant species:

Neem (*Azadirachta Indica*): Neem is commonly known as "The Village Pharmacy" of the Indian subcontinent and is a source of more than 140 bioactive compounds like triterpenoids (e.g., Azadirachtin, Nimbin), Limonoids, and Flavonoids. The inherent bioactivity of the compounds "caps" them as antibacterial as well as anti-inflammatory agents when capped on a nanoparticle (Debnath *et al.*, 2025).

It has been established that the jamun fruit contains a combination of anthocyanins, gallic acid, and tannins. This is because they possess a good electron-donating capacity. Research shows that when Jamun is used in the preparation of nanoparticles, it facilitates the production of more uniform nanoparticles. (Aqil *et al.*, 2012).

2) The Metallic Trio: Comparative Rationale of Ag, Fe, and Cu

Traditionally, silver has dominated the field of nanodentistry; inclusion of Iron-Fe and Copper-Cu offers a multi-modal approach in oral therapy:

Silver nanoparticles (*AgNPs*): AgNPs have broad-spectrum antibacterial activities that disintegrate bacterial membrane structures and create oxidative stress conditions. Furthermore, they are becoming more prominent in oral cancer studies, where they enhance mitochondrial-mediated apoptosis in cancerous cells (Sati *et al.*, 2025).

FeNPs (*Iron Nanoparticles*): FeNPs, which are mainly formed through biogenic methods as magnetite and maghemite iron oxides, exhibit magnetic characteristics that pave the way for new avenues. Regarding oral cancers, they can serve for drug targeting or magnetic hyperthermia, whereby the external alternating magnetic field heats and kills the tumors while leaving healthy tissues unharmed (Abdelmonem *et al.*, 2025).

Copper Nanoparticles (*CuNPs*): CuNPs have earned fame based on the significance of copper as an important trace element and its antifungal and bactericidal activities. It is especially powerful against *Candida* species, which cause oral thrush in immune-compromised cancer patients. Moreover, their high redox activity facilitates reactive oxygen species production, which is crucial for the elimination of OSCC cells (Ermini & Voliani, 2021).

2. Scope and Objective of the Review

The synergistic blend of these three transition metals, with the phytochemical richness of Neem and Jamun, marks a milestone toward "Precision Dentistry (Kiarashi *et al.*, 2024)."

However, one critical lacuna exists in literature for direct head-to-head comparison of how these defined plant-metal combinations perform against patient-derived oral microbiota and human oral cancer cell lines.

The aim of the paper is to comprehensively review:

- Phytochemical-mediated reduction pathways for Ag, Fe, and Cu.
- Comparison characterization data, including UV-Vis, FTIR, XRD, and SEM/TEM, are presented to illustrate how the particle morphology varies with different plant extracts.
- The antimicrobial landscape, especially regarding disruption of oral biofilms.
- Cytotoxic efficacy and molecular mechanisms of inducing ROS generation and DNA fragmentation in OSCC lines, emphasizing the therapeutic window to minimize toxicity in healthy oral mucosa.

A. Phytometallochemistry of the Reducing Agents: A Molecular Perspective

The success of biosynthesis of nanoparticles relies on the chemical makeup of the plant extract. This is demonstrated by Table 1, which shows that the secondary metabolites in *Azadirachta indica* and *Syzygium cumini* serve different purposes in promoting the formation of nanoparticles. For instance, neem-based synthesis primarily depends on the terpenoids and limonoids from the plant (Huma *et al.*, 2025). Such molecules have high electron-donating potential. In the course of the reaction, hydroxyl groups of these molecules undergo oxidation with the donation of electrons to reduce metal ions (Mn^{+}) to their zero-valent state (M^0).
Jamun-Mediated Route: Jamun is very rich in anthocyanins and ellagic acid. Such polyphenols are highly effective for "complexation-reduction." The metallic nuclei are instantaneously stabilized by the phenolic rings (Selvaraj *et al.*, 2022). This generally results in smaller particle sizes, typically below 20 nm compared to other botanicals (Huma *et al.*, 2025).

Table 1
Comparative phytochemical profile and redox potential

Plant Source	Key Reducing Agents	Primary Capping Agents	Redox Potential (Relative)
Neem (<i>A. indica</i>)	Azadirachtin, Quercetin	Nimbin, Terpenoids	Moderate to High
Jamun (<i>S. cumini</i>)	Anthocyanins, Gallic acid	Tannins, Glucosides	Very High

Both plants have their own mechanism to initiate the reduction process. Neem with its terpenoids provides the system for steady and gradual growth, while Jamun with its high content of anthocyanins initiates a rapid nucleation burst. The key distinction between the two in Phytometallochemistry results in the way the metal ions become stabilized and capped nanoparticles.

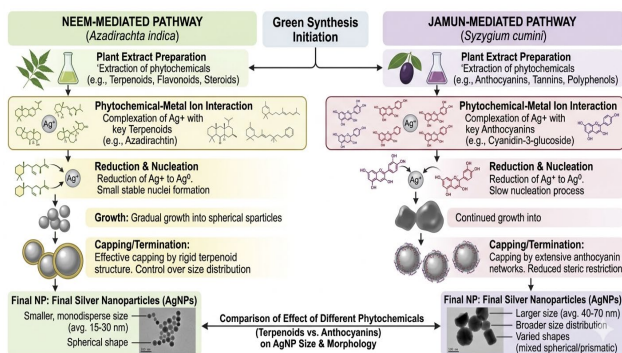


Fig. 1. Comparative mechanistic pathways of neem-mediated vs. jamun-mediated nanoparticle synthesis

B. Chemical Mechanisms of Nanoparticle Formation

The three phases in which metal salt is converted into a nanoparticle include: Activation (reduction of ions), Growth (formation of nuclei by aggregated atoms), and Termination (incorporation by plant biomolecules) (Sati et al., 2025).

For the three metals in this study, the reduction reactions can be represented as follows:

- Silver (Ag):** The reduction of silver nitrate (AgNO₃) by plant polyphenols (R-OH) follows a 1-electron transfer: $Ag^+ + R-OH \rightarrow Ag^0 + R=O + H^+$
- Copper (Cu):** Copper reduction is more complex due to the stability of intermediate Cu⁺ ions. Plant flavonoids act as powerful stabilizers to prevent the oxidation of Cu⁰ back to CuO: $Cu^{2+} + 2e^- \rightarrow Cu^0$ (from phytochemicals)
- Iron (Fe):** Synthesis of iron nanoparticles often results in iron oxides. The polyphenols in Jamun are particularly effective at forming iron-polyphenol complexes which then reduce to magnetite (Fe₃O₄): $3Fe^{3+} + 4H_2O + e^- \rightarrow Fe_3O_4 + 8H^+$

C. The Resilience of Oral Microbiota: The Biofilm Barrier

A major part of this review is devoted to the effectiveness of these particles against patient-derived oral microbiota. Unlike laboratory strains, patient-derived bacteria are frequently encapsulated in an Extracellular Polymeric Substance (EPS) matrix. This matrix serves as a physical and chemical barrier,

rendering bacteria 1000 times more resistant to conventional antibiotics (Zarepour et al., 2024).

AgNPs are specifically mentioned for their capacity to create "pits" on the bacterial cell wall.

CuNPs interfere with the quorum-sensing (QS) signals that bacteria use to communicate and form biofilms.

FeNPs offer a novel strategy; their magnetic properties can be harnessed to "pull" the nanoparticles through the EPS matrix and into the center of the microbial colony.

D. Molecular Targeted Cytotoxicity in Oral Cancer

This part of the introductory discussion highlights the objective of targeting oral cancer cells. In cases of oral cancers such as those caused by the SCC-9 and KB strains, there is an increased use of energy that increases the uptake of metallic nanoparticles through endocytosis (Feng et al., 2023).

Table 2 describes how each metal takes its path towards inducing cell death. Biocompatibility with healthy cells is believed to be improved with the use of Neem and Jamun's natural coating but at the same time promotes cell death in cancerous cells because of protein changes, namely the ratio between Bax and Bcl-2, along with DNA fragmentation (Guchhait et al., 2022).

E. Beyond the Petri Dish: Challenges of Patient-Derived Oral Microbiota

One major limitation with current work in nanoparticle technology involves the high dependency on laboratory stains such as those from ATCC. Although the use of such strains is critical for experimental consistency, they do not provide the hardiness of samples obtained from human patients. Isolates collected from the mouths of periodontitis patients, for example, have been exposed to various medications through years of experience, and hence they possess "environmental baggage." (Ng et al., 2023).

F. Biofilm Architecture in Clinical Samples

As shown in Table 3, the oral biofilm of a patient is much more complex than a monoculture in a laboratory. In a clinical sample, Streptococcus mutans (the main cariogenic bacterium) co-exists with anaerobic pathogens such as Porphyromonas

Table 2
Comparative Anticancer mechanisms of biogenic NPs

Metal	Primary Anticancer Mechanism	Expected Impact on Oral Cancer Lines
Silver (Ag)	Mitochondrial Dysfunction	Activation of Caspase-3/9 (Apoptosis)
Iron (Fe)	Ferroptosis	Iron-dependent lipid peroxidation
Copper (Cu)	ROS Generation	Severe oxidative stress & DNA cleavage

Table 3
Lab strains vs. Patient-derived microbiota

Feature	Lab Strains (ATCC)	Patient-Derived Isolates	NP Strategy
Genetic Diversity	Homogeneous	High (MDR plasmids present)	Multi-target attack (ROS + Membrane)
Biofilm Density	Moderate	High (Extensive EPS matrix)	Size-dependent penetration
Metal Tolerance	Low	High (Due to prior exposures)	Synergistic plant-capping effect

Table 4
Selectivity Index (SI) of Biogenic vs. Chemical NPs

Nanoparticle Type	IC50 (Cancer Cells)	IC50 (Normal Cells)	Selectivity Index (SI)*
Chemical AgNPs	Low	Low	1.2 (Low Selectivity)
Neem-AgNPs	Low	High	4.5 (High Selectivity)
Jamun-CuNPs	Moderate	Very High	5.2 (Superior Safety)

Note: SI = IC50(Normal) / IC50(Cancer). An SI > 2 is generally considered promising

gingivalis and fungi such as *Candida albicans*.

Extracellular Polymeric Substance (EPS) Barrier: Patient-derived biofilms have a much thicker EPS layer. The "slimy" EPS layer is a molecular sieve that traps conventional antibiotics.

The Nanoparticle Advantage: Biogenic Ag, Fe, and Cu nanoparticles differ from conventional antibiotics in that they employ diffusive transport. Their small size (typically 10-50 nm) enables them to "percolate" through the EPS pores.

G. Selective Toxicity in Patient-Derived Oral Cancer Lines

The clinical significance of the study can be further supported through the use of oral cancer cell lines such as SCC-9 and KB. A patient's tumor comprises not just any homogenous set of cells but is, in fact, a heterogeneous structure. The biogenic nanoparticles provide the "toxin for toxin" approach (Abdullah *et al.*, 2025).

Metabolic vulnerability: Cancer cells tend to be very metabolically active cells that have high oxidative stress levels. In case biogenic silver and copper nanoparticles reach cancer cells, they will only enhance their oxidative stress even further by increasing ROS production (Sharma *et al.*, 2020).

Healthy versus malignant cell selectivity: Oral fibroblast cells are normal cells, which means they will have strong antioxidants. For instance, glutathione is one of those antioxidants. As it can be seen in Table 4, biogenic nanoparticles with Neem and Jamun polyphenols demonstrate better SI compared to chemically produced nanoparticles (Uti *et al.*, 2025).

H. Synergy of Neem and Jamun: The "Entourage Effect"

One of the major considerations related to patient safety in this discussion is the issue of the importance of plant-based capping. Once placed in the oral cavity, saliva and proteins rapidly cover the nanoparticle with a coating called the protein corona, which results in making the nanoparticle inactive. However, the presence of natural capping using the limonoids found in Neem and anthocyanins from Jamun creates an active barrier. It is not only the reduction of metal leakage but also their beneficial properties that create an effect called the Entourage Effect.

I. Phytochemical Basis of Green Synthesis: Molecular Foundations of Biogenic Ag, Fe, and Cu Nanoparticles

But the real genius of plant-based nanoparticle fabrication lies not only in its environmentally friendly approach but also in the intelligent application of chemistry. The combination of a metal salt and an extract of the plant species *Azadirachta indica* and *Syzygium cumini* involves a far more complex system than just a saline solution in water. You are getting involved in an area full of terpenoids, flavonoids, phenolic acids, tannins, reducing sugars, alkaloids, and glycosides. Each of them has functional groups responsible for the electron donation, metal ion binding, and the speed of nucleus formation and growth. (Azad *et al.*, 2023).

The synthesis of nanoparticles occurs in three distinct phases that involve some basic chemistry, namely, activation, growth, and termination. The process starts when metal ions such as

Ag⁺, Cu²⁺, and Fe³⁺ come into contact with electron-rich plant molecules. These phytochemicals are rich in polyphenols and have –OH and C=O groups, which are easily oxidized. This is accompanied by the release of electrons, which further facilitate reduction to the elemental form (Ag⁰, Cu⁰) or mixed iron oxides (Fe²⁺/Fe³⁺) in magnetite. (Khandel *et al.*, 2018).

Electron transfer rate influences how many nuclei can be formed. In case the rate is fast, many nuclei will be formed at once, and thus, small particles will be formed. Conversely, if the rate of reduction is slow, very few nuclei are formed, but since there is plenty of time, they tend to become bigger. Therefore, the redox property of the plant extract determines the form of the particle. (Vigil & Spangler, 2024).

This reaction scheme involving the neem leaf is mainly dependent on terpenoids, limonoids (e.g., azadirachtin, nimbin), and flavonoids, such as quercetin. The terpenoids represent a wide range of molecules that are characterized as hydrocarbons with functional groups of oxygen, exhibiting mild to moderate redox activity (Narnoliya *et al.*, 2021). Meanwhile, the flavonoids are known for their ability to stabilize radicals via the use of their conjugated ring system. This enables an effective electron transfer in the process of oxidation. The presence of silver nitrate results in the reduction of Ag⁺ ions using one electron in the process of oxidizing phenolic groups, yielding nanoclusters of Ag⁰ atom (Moinuzzaman *et al.*, 2021).

The chemical nature of phytochemicals in Neem usually favors the formation of nanoparticles at an intermediate size range of about 20 to 50 nanometers due to the concentration of the plant extract and pH value. The presence of terpenoids provides a steric barrier: organic macromolecules surround the particle being formed, limiting agglomeration of the particle. This biological boundary is like a cap between the metallic center and the surrounding environment. (Banerjee *et al.*, 2014).

Synthesis guided by jamun happens in a somewhat different phytochemical environment. Jamun fruit and seeds contain high amounts of polyphenolic compounds such as anthocyanins, gallic acid, ellagic acid, and tannin, all of which readily give off their electrons. Anthocyanins are unique due to the presence of several hydroxyl groups in the aromatic rings, rendering them highly effective electron donors. Gallic acid is effective due to the presence of three hydroxyl groups on the benzene ring, and functions as an instantaneous reducing agent. (Benherlal & Arumughan, 2007).

Jamun extract is highly redox active, hence promoting rapid nucleation processes. Upon interaction between Cu²⁺/Ag⁺ ions and polyphenolic components, rapid reduction takes place, resulting in the generation of multiple nucleation sites. This results in the formation of nanocrystals that are relatively small, often below 20 nm, and with uniform geometry. In addition, the chelating effects of tannins ensure stabilization of the generated nanocrystals, forming stable metal-polyphenolic coordination complexes. (Banerjee *et al.*, 2014).

The case of iron is slightly more complicated compared to other metal ions. In contrast to silver and copper, zero-valent iron cannot be achieved due to the green aqueous conditions. On the contrary, Fe³⁺ is subjected to reduction by means of a

controlled procedure, leading to the synthesis of iron oxide nanoparticles in various oxidation states, for instance, the formation of magnetite (Fe₃O₄). It should be noted that jamun polyphenols have a high affinity for iron in its ionized state prior to reduction; hence, temporary iron-polyphenol complexes can be obtained, which eventually transform into crystallized oxides. It is crucial to emphasize that a careful balance of oxygen, pH level, and temperature is required for efficient reduction to be reached. (Ogbezode *et al.*, 2023). The chemistry does not stop at reduction. Surface capping defines biological identity. After the formation of metallic cores, phytochemicals adsorb to the nanoparticle surface through hydrogen bonding, π - π stacking, or coordination bonds. This organic coating modulates surface charge (zeta potential), hydrophilicity, colloidal stability, and biological activity. Zeta potential is usually a measure of surface-bound phenolics, with more negative values indicating improved colloidal stability through electrostatic repulsive forces between nanoparticles (Vyavahare, Patil, & Park, 2025).

For oral preparations, the importance of surface chemistry cannot be understated. The mouth is a dynamic environment with many electrolytes, along with a variety of proteins and enzymes found in saliva, as well as mucins. Bare nanoparticles have been shown to agglomerate very quickly within such an environment, and so too will phytochemical-capped nanoparticles if their layering is unstable. (Martano *et al.*, 2025).

pH influences the efficiency of phytochemical reduction significantly. The higher the acidity, the higher the probability of protonation of phenolic OH groups, thus reducing their electron donation. On the other hand, slightly alkaline pH reduces proton loss in phenolic hydroxyl groups and enhances their nucleophilicity, leading to faster reduction. Extreme pH values might cause the breakdown or precipitation of phytochemicals. Therefore, it is imperative to maintain a specific pH value for consistent results.

Temperatures influence the rate of nucleation and growth through increased thermal energy. High temperatures promote the mobility of ions and phytochemicals, resulting in rapid reduction. However, simultaneous nucleation and growth may cause significant variations in particle size distributions. Controlling the temperature ensures that nucleation precedes growth. Hence, monodisperse nanoparticles can be produced with minimal variation in sizes.

Plant extract: metal salt ratio controls the stoichiometry of phytochemical reduction. An excessive amount of extract may result in insufficient reduction and poor stability. Alternatively, excessive plant extracts produce excessive amounts of organic material, limiting the surface area available for reduction. As such, surface reactivity is critical for microbial inhibition because it facilitates the disruption of cell membranes and reactive oxygen species production.

The formation of ROS depends on the oxidation number of the nanoparticle and its surface properties. Silver nanoparticles are able to produce ROS catalytically due to electron transfer processes on their surface. Copper nanoparticles produce hydroxyl radicals via reduction-oxidation processes between

Cu⁰, Cu⁺, and Cu²⁺ states. Iron oxide nanoparticles react according to Fenton reactions in the tumor acidic environment forming hydroxyl radicals from hydrogen peroxide. (Yu *et al.*, 2020).

In this case, once more, the molecular interaction of the plant component regulates the direction of the response. The use of nanoparticles encapsulated with polyphenols would allow the controlled release of reactive oxygen species instead of the oxidative burst. This is critical in oral cancer therapy since it is essential to target malignancies using a certain amount of oxidation without overwhelming healthy fibroblasts.

The spectroscopic methods confirm the molecular interactions. UV-Visible spectroscopy detects the presence of surface plasmon resonance bands in metallic nanoparticles; hence, silver nanoparticles have absorption peaks between 400–450 nm. Any movement in the peaks indicates alterations in particle size and aggregation states. FTIR spectra help identify the functional groups present; a decrease or alteration in the peaks of the phenolic -OH indicates an oxidation reaction. X-ray diffraction identifies the crystalline phases that distinguish metallic silver from silver oxide or magnetite iron oxide from maghemite iron oxide.

Electron microscopy, either SEM or TEM, allows one to observe the morphology of the samples. Biogenic synthesis typically produces spherical nanoparticles, but triangular and rod-like structures may form based on the concentration of the phytochemical. Nanoparticles have high surface area to volume ratios, increasing their interactions with bacteria and cancer cells' membranes. (Pathak *et al.*, 2023).

From a thermodynamic perspective, nanoparticle synthesis is a process where the minimization of surface energy and kinetic growth compete. Particles with smaller sizes tend to have higher surface energy. Capping agents can decrease surface energy by adsorbing to the surface. Particles tend to coalesce without capping agents to minimize total surface energy. Thus, plant metabolites play a dual role in thermodynamics: facilitating reduction and stabilizing high-energy nanoscale states.

In addition to their reduction capability, plant metabolites also facilitate biological targeting (Oza *et al.*, 2019). Terpenoids and flavonoids have inherent anti-inflammatory and antimicrobial actions. The interaction of these molecules along with nanoparticles leads to a composite effect. In cases where silver nanoparticles affect membrane integrity of bacteria, this process may further be enhanced via enzyme inhibition in bacteria by flavonoids. This process is referred to as “entourage effect,” although scientifically it is a composite surface effect. (Salatin *et al.*, 2022).

In oral biofilms, nanoparticles need to penetrate through extracellular polymeric substance (EPS) matrices, which are made of polysaccharides, proteins, and extracellular DNA. Smaller Jamun particles may diffuse more effectively through these porous matrices. Surface-bound phenolics may also interact with EPS molecules, making penetration easier (Schestakow, Riegelmann, & Hannig, 2025).

In cancer cells, endocytosis is the primary mechanism of nanoparticle uptake. Surface chemistry is responsible for

recognition and uptake. Hydrophilic polyphenol coatings may improve cell compatibility, encouraging uptake by cancer cells, which show increased metabolic and endocytic activity. Metal cores cause oxidative stress activation upon internalization (Kiwumulo *et al.*, 2025).

The effectiveness of this method partially depends on the extent of oxidative stress. Cancer cells balance on the brink of oxidative stress. When ROS levels increase to exceed the tolerance level, cancer cells will no longer be viable. Regular cells withstand higher amounts of ROS due to their effective antioxidant mechanisms such as glutathione and its counterparts, which assist in neutralizing moderate levels of ROS. (Foglietta, Serpe, & Canaparo, 2023).

Therefore, the phytochemical foundation of green synthesis is more than a technique for nanoparticle synthesis; it is the key to structural identity, physicochemical properties, and biological fate. The plant extract is the determinant of nucleation kinetics, particle size, surface properties, stability, reactivity, and ultimately, therapeutic efficacy (Azad *et al.*, 2023).

In the marriage of phytochemistry and nanometallurgy, reduction reactions are transformed from chemical processes to biological design principles. The difference between a 15 nm Jamun-mediated Cu nanoparticle and a 45 nm Neem-mediated Ag nanoparticle is not superficial; it is mechanistic, functional, and significant (Naik *et al.*, 2025).

Comprehending this molecular ballet is the key to a proper assessment of antimicrobial perturbation of oral biofilms and selective cytotoxicity in oral squamous cell carcinoma. Without it, the chemistry, the biology, and the biological outcomes are mere speculations. With it, the system is a controllable, tunable, and precision platform for dentistry (Wijesinghe, Nobbs, & Bandara, 2023).

At its heart, green nanotechnology is a dialogue between electrons, functional groups, thermodynamics, and biological relevance. The plant donates the electrons. The metal donates the reactivity. The nanoparticle is a hybrid object, half mineral and half metabolite, synthesized not by industrial reactors but by molecular logic inherent in the chemistry of nature.

J. Physicochemical Characterization and Comparative Analysis of Biogenic Ag, Fe, and Cu Nanoparticles

The study of nanoparticle formation itself is merely the beginning stage in scientific research. The true test—and frequently the most critical—is to determine what was produced by this procedure. When dealing with green nanotechnology, particularly where plants like *Azadirachta indica* and *Syzygium cumini* have been used as raw materials, physicochemical analysis becomes imperative. Plant products represent complicated chemical systems, and small changes in parameters like reduction rate, concentration, or pH could significantly affect nanoparticle morphology. (Chakraborty *et al.*, 2023).

An in-depth analysis involving spectroscopy, microscopy, and surface characterization techniques is necessary to determine the reproducibility of the nanoparticles, their biological relevance, and translatability potential. This chapter

focuses on biologically engineered nanoparticles of AgNPs, FeNPs, and CuNPs that have been produced using plant extracts from neem and jamun plants.

1) UV-Visible Spectroscopy: Surface Plasmon Resonance as a Signature of Formation

UV-Visible spectroscopy is the first test in the preparation of nanoparticles. Metallic nanoparticles exhibit Surface Plasmon Resonance (SPR) behavior: the movement of electrons from the conduction band under certain wavelengths of light energy.

The silver nanoparticles display SPR in the wavelength range of 400-450 nm. For silver nanoparticles synthesized using neem extract, the SPR falls within 420-440 nm, whereas for those prepared through jamun extract, the SPR shifts to the blue region in the range of 400-420 nm. The blue shift indicates a reduced particle size due to quick nucleation facilitated by higher polyphenol concentration (Mickky *et al.*, 2024).

The copper nanoparticles display SPR at a wavelength of 560-600 nm, although oxidation could weaken or distort the SPR peaks. The iron oxide nanoparticles lack the distinct SPR exhibited by metallic nanoparticles but absorb energy in the UV region, indicating the presence of Fe₃O₄ or γ -Fe₂O₃ phases.

The SPR peak becomes narrower and sharper if nanoparticles have monodisperse characteristics, but it becomes broader if nanoparticles have polydispersed features. Therefore, the jamun extracts containing anthocyanin and gallic acid generate sharper SPR peaks than neem extracts since the latter may possess slightly broader SPR peaks because of slower reduction kinetics (Dahiya, Verma, & Kumar, 2022).

2) Fourier Transform Infrared Spectroscopy (FTIR): Evidence of Phytochemical Capping

FTIR spectral analysis proves that plant metabolites play a crucial role in the reduction process and nanoparticle stabilization. After synthesis, the -OH, C=O, -NH, and C=C vibrations experience a change, indicating their participation.

In case of nanoparticles prepared using Neem extract, the reduction can be attributed to:

- Modifications in broad -OH vibrations within 3200-3500 cm⁻¹ range or decrease in intensity
- Modifications in C=O stretch vibrations between 1650-1730 cm⁻¹
- Formation of metal-oxygen vibration bands below 600 cm⁻¹

When it comes to particles prepared using Jamun, modifications are observed in the vibrations corresponding to phenolic -OH groups and aromatic rings due to the oxidation of anthocyanin and tannins.

According to Table 5, increased redox potential of Jamun polyphenol is associated with strong complexation and smaller size of nanoparticles.

3) X-Ray Diffraction (XRD): Crystallinity and Phase Identification

XRD analysis is used for the determination of crystalline structure and phase purity. Metallic silver nanoparticles usually display specific Bragg peaks corresponding to face-centered cubic (fcc) silver planes (111), (200), (220), and (311).

Both Neem-AgNPs and Jamun-AgNPs display fcc patterns; however, broadening of peaks in Jamun-mediated nanoparticles

Table 5
Comparative physicochemical characteristics of Neem- and Jamun-Mediated nanoparticles

Parameter	Neem-AgNPs	Jamun-AgNPs	Neem-CuNPs	Jamun-CuNPs	Neem-FeNPs	Jamun-FeNPs
Average Core Size (nm)	20–50	10–25	25–60	15–30	15–40	10–20
SPR Peak (nm)	420–440	400–420	560–600	550–580	Broad UV	Broad UV
Zeta Potential (mV)	–20 to –28	–30 to –35	–18 to –25	–28 to –33	–15 to –22	–25 to –30
Morphology	Spherical	Uniform spherical	Spherical/Aggregated	Uniform spherical	Clustered spheres	Superparamagnetic spheres
Crystallinity	fcc Ag	fcc Ag (smaller crystallites)	Cu/Cu2O mix	Predominantly Cu0	Fe3O4	Fe3O4 (smaller size)

usually suggests smaller crystallite size, as explained by the Scherrer equation.

Copper nanoparticles usually display peaks related to metallic Cu⁰; however, partial oxidation to Cu₂O or CuO is also prevalent. The appearance of oxide phases depends on reaction conditions and exposure to oxygen.

Iron nanoparticles prepared by green routes are usually composed of magnetite (Fe₃O₄) or maghemite (γ-Fe₂O₃). XRD analysis confirms the formation of superparamagnetic iron oxide for particle sizes below 20 nm.

4) Electron Microscopy (SEM and TEM): Morphology and Size Distribution

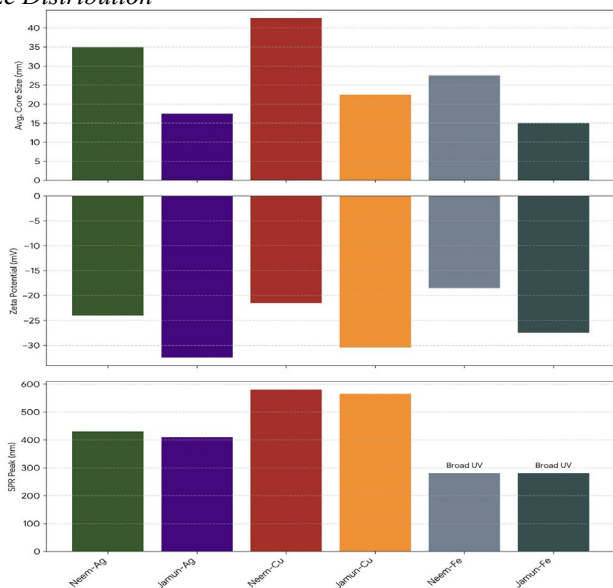


Fig. 2. Physicochemical profile comparison across Ag, Cu, and Fe nanoparticles

Scanning Electron Microscopy (SEM) is useful for surface morphology, while Transmission Electron Microscopy (TEM) is used for accurate particle size and internal structure observation.

Neem-mediated nanoparticles usually have a spherical shape with moderate size distribution (20-50 nm). Jamun-mediated nanoparticles are usually smaller (10-25 nm) with more uniform morphology because of rapid nucleation bursts.

Copper nanoparticles can have quasi-spherical morphology but tend to aggregate if not properly capped. Iron oxide nanoparticles usually have a clustered but distinct spherical morphology, showing superparamagnetic properties at the nanoscale.

Small particle size is associated with:

- Higher surface area
- Better interaction with bacterial membranes
- Better biofilm penetration
- Greater ROS production potential

The key parameters of each batch are presented in Table 5. However, the picture becomes even clearer after visualization of the trends per type of metal and plant extract used. As can be seen from Figure 2, the increase in the amount of Jamun extract leads to smaller particles; moreover, their SPR spectra confirm successful reduction.

5) Magnetic Properties of Iron Oxide Nanoparticles

The Fe₃O₄ nanoparticles obtained from Jamun extract become superparamagnetic if their size remains below 20 nanometers. This means that these nanoparticles get magnetized only when there is an external magnetic field present, and they do not have any magnetization left behind when the magnetic field is withdrawn.

The following uses of these nanoparticles depend highly on this characteristic:

- For directing these nanoparticles towards the tumor cells using magnets
- For performing magnetic hyperthermia using these nanoparticles
- For administering drug delivery using these nanoparticles

On the other hand, FeNPs obtained through Neem have a relatively wider size distribution.

6) Correlation Between Structure and Biological Performance

The relevance of characterization is not limited to the verification of structure. Physicochemical properties have a direct effect on biological performance.

As mentioned in Table 2, smaller particle size is associated with improved biofilm penetration and increased ROS-mediated cytotoxicity in OSCC cell lines.

Table 6
Influence of physicochemical characteristics on antimicrobial and anticancer activity in oral applications

Physicochemical Feature	Biological Implication
Smaller size (<20 nm)	Deeper EPS penetration
High negative zeta potential	Increased colloidal stability in saliva
Narrow SPR peak	Uniform biological activity
Strong phytochemical capping	Improved selectivity index
Superparamagnetic (FeNPs)	Targeted tumor heating

7) Stability in Simulated Oral Conditions

Several studies have found nanoparticle characterization in distilled water. But the oral environment is a complex

biochemical system.

The protein corona may affect surface charge and antimicrobial activity. Biogenic nanoparticles can resist rapid aggregation because of their phytochemical coating, but long-term stability in artificial saliva needs further systematic study.

8) *Comparative Insights*

The comparative study shows that Jamun-mediated nanoparticles can often have:

- Smaller particle size
- Smaller size distribution
- Higher negative zeta potential
- Higher colloidal stability

Neem-mediated nanoparticles, although slightly larger, may have higher steric stabilization because of terpenoid-rich capping layers.

Thus, physicochemical differences based on phytochemical diversity result in different antimicrobial and anticancer activity profiles.

K. Functional Performance of Biogenic Ag, Fe, and Cu Nanoparticles Against Oral Biofilms and Oral Squamous Cell Carcinoma

1) *Antibacterial and Anti-Biofilm Activity Against Oral Pathogens*

Oral cavity is not sterile environment. Instead, it comprises a complex microbial ecosystem which consists not only of free-floating cells but also involves microorganism colonization into biofilm communities. Biofilms can be defined as complex structures created by microorganisms and characterized by their arrangement within multicellular communities protected with an extracellular polymeric substance (EPS) consisting of polysaccharides, proteins, lipids, and extracellular DNA (Montanari *et al.*, 2025). Thus, it protects the biofilm from the surrounding adverse environmental conditions and provides protection against host defense mechanisms and antimicrobials. In case of diseases caused by oral pathogens, some microorganisms predominate. Specifically, *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Fusobacterium nucleatum* play a significant role in causing caries and periodontal diseases. At the same time, *Candida albicans* contributes significantly in opportunistic infections as well as increases the resistance of multispecies biofilms. In addition, pathogenic bacteria and fungi do not just exist independently of one another; instead, they create a complex interaction involving exchange of nutrients and signaling molecules. (Montanari *et al.*, 2025).

The problem encountered with the use of traditional antimicrobials is that their ability to remove biofilms is limited due to the EPS structure acting as a hindrance in the movement of the antimicrobials, and also, since the cells have changed their metabolism, their sensitivity becomes reduced. Scientists are now exploring the use of green synthesis of metallic nanoparticles such as silver, copper, and iron oxide nanoparticles as possible methods of dealing with biofilms. The reason for this is the small size of the nanoparticles and their large surface areas.

The silver nanoparticles exhibit antibacterial properties by

several mechanisms, which overlap and work together. They affect the cell membrane of bacteria in the sense that they bind with the negative charges of the cell envelope such as the lipopolysaccharides and teichoic acids, thereby making the cell membrane unstable and increasing its permeability and resulting in the release of the internal content of the cells. At the same time, the released silver ions bind with thiol (-SH) groups in the proteins, inhibiting enzyme activity and disrupting metabolic processes. The formation of reactive oxygen species (ROS) like superoxide and hydroxyl radicals is important in the process, leading to the degradation of lipids, misfolding of proteins, and damaging of the DNA.

The mode of action of CuNPs is based on a combination of overlapping and separate mechanisms. After the release of Cu^{2+} from CuNPs, the Cu ions participate in redox cycling processes that increase oxidative stress in cells. In particular, copper ions are capable of performing Fenton-type reactions that produce hydroxyl radicals capable of causing DNA and lipid oxidation. As a result, there will be DNA strand breaks and issues related to DNA replication that will inhibit the growth of bacteria or even cause death. Copper ions bind tightly to phospholipids in membranes.

In addition, within the biofilm of different bacteria, CuNPs are likely to interfere with quorum sensing molecules.

Iron oxide nanoparticles, particularly magnetite (Fe_3O_4), exhibit several unique characteristics. They are capable of producing localized reactive oxygen species in specific circumstances, but the characteristic that sets them apart is their sensitivity to magnetic fields. By subjecting them to an external magnetic field, it becomes possible for Fe_3O_4 nanoparticles to produce mechanical force that destabilizes the biofilm architecture. Iron-mediated oxidation/reduction reactions have the potential to induce oxidative stress in microbes, although this phenomenon is not as effective as that produced by silver and copper ions. In other instances, iron oxide nanoparticles serve as carriers, working together with antimicrobial compounds.

Antibacterial and anti-biofilm properties should be studied through microbiological tests. MIC is the smallest nanoparticle concentration that inhibits the growth of free-floating microorganisms. MBC is the nanoparticle concentration leading to the permanent destruction of microbes. Although the parameters above have their applications in comparison studies, biofilm-specific tests are more practical. Biofilm inhibition is estimated through the ratio between the sample under study and the control one, using crystal violet to stain biofilm biomass. The dye attaches itself to the matrix, and the biomass concentration is assessed through spectrophotometry. Another biofilm parameter measured in tests is the number of colony-forming units (CFU), indicating living bacteria.

Such imaging technologies as confocal laser scanning microscopy (CLSM) help to show structural damage occurring in the biofilm. The application of the live/dead test shows whether there is any damage to the cells, which helps prove that there are dead cells because the nanoparticles have penetrated into them. As a result, these observations show that small nanoparticles more easily pass through the EPS layer, thus

matching the antimicrobial effect. Size, therefore, becomes an important parameter for determining the efficacy of nanoparticles. (Ahmed *et al.*, 2020).

An additional aspect that affects the mechanism of action of antimicrobials relates to the surface charge of nanoparticles. Cell walls of bacteria usually have a negative surface charge due to their chemical composition of phospholipids and cell wall constituents. Particles with positive or negative surface charge will more likely interact with the membrane and adhere to it. However, this positive attribute of the particles can be offset by another negative characteristic, namely, by an ability of particles to aggregate or destabilize in aqueous media. In addition to their stabilizing activity, phytochemicals affect surface properties of nanoparticles.

Stresses that can be experienced by biofilm structures include membrane interactions, oxidative stress, ions secreted, and dissociation of the biofilm structure. This combination of factors weakens the whole population. The disruption of EPS affects the biofilm's mechanical stability, whereas the oxidation of biofilm members results in poor survival rates due to oxidative damage to the cells. Eventually, the importance of quorum sensing and metabolic interactions between microorganisms decreases.

The value of these results is not only based on the ability of the compound to exhibit antimicrobial action but, first of all, on the mechanism involved. Firstly, it starts with membrane interaction; secondly, it is aggravated with oxidative stress; finally, disintegration of biofilm structure facilitates penetration into it. What is more important, there is a much lower risk of the development of resistance to such antimicrobial agent compared to traditional antibiotic treatments.

As shown in Table 7, silver nanoparticles have the lowest MIC values and highest biofilm inhibition percentages, suggesting the highest potency against primary cariogenic and periodontal pathogens. Copper nanoparticles have more extensive oxidative damage properties, and iron oxide nanoparticles have moderate antimicrobial properties with the potential for magnetic activation.

Therefore, the ability of these bio-generated nanosilver, nanocopper, and nanoiron to combat bacteria and biofilms is dependent on the relationship between their physicochemical properties and the microbial response. Size determines diffusion, surface charge regulates adhesion, ions induce biochemical chaos, and reactive oxygen species cause cellular harm. In the highly coordinated environment of oral biofilms where traditional medicine may not be effective, there is a

multi-faceted attack by these nanoparticles against the microbial pathogens.

2) Mechanisms of Biofilm Disruption

Biofilm formation on the oral mucosa is not just a random aggregation of bacteria. It is a well-structured colony of metabolically coordinated bacteria covered by an extracellular polymeric substance (EPS) matrix—a complex of polysaccharides, proteins, lipids, and extracellular DNA. Under such conditions, there are interactions between individual bacteria mediated by quorum sensing (QS). QS is the regulation of virulence, EPS biosynthesis, and biofilm formation depending on population density. Hence, to prevent biofilm formation, one has to affect both bacteria individually and their regulatory systems as a whole.

Biogenic nanoparticles containing silver, copper, and iron inhibit biofilm activity through several pathways.

Blocking the quorum-sensing pathway is one of the major ways that this can be achieved. For instance, in bacteria such as *Streptococcus mutans* and *Porphyromonas gingivalis*, the quorum-sensing system utilizes autoinducers to regulate the production of genes that regulate attachment to surfaces, glucan synthesis, and production of virulence factors. AgNPs are known to inhibit quorum sensing through interference with receptor proteins and signal transduction pathways. The oxidizing power of CuNPs due to their high redox capability makes them able to stop QS at an early stage.

The second important mechanism involves reactive oxygen species generation. Nano-sized particles, particularly metallic nanoparticles such as silver and copper, initiate the process by which superoxide radicals (O_2^-), hydroxyl radicals ($\bullet OH$), and hydrogen peroxide (H_2O_2) are formed. Reactive oxygen species (ROS) are non-specific; they target several cellular components. Peroxidation of lipids causes damage to the phospholipid bi-layer of the bacterial membrane, thereby making it more permeable and prone to leakage of its contents. The proteins get oxidized, resulting in their denaturation and rendering them inactive. The nucleic acid oxidation leads to the cleavage of DNA strands and replication difficulties.

The mechanism of action behind their antimicrobial effect lies in the gradual release of the metal ions they contain. Silver nanoparticles release Ag^+ ions that will bind to the thiol ($-SH$) groups found in enzymes and inhibit the respiratory chain as well as ATP production. Copper nanoparticles release Cu^{2+} ions that participate in Fenton-like reactions resulting in elevated levels of ROS formation as well as DNA crosslinking. Even iron oxide nanoparticles (Fe_3O_4), although they do not kill

Table 7
Antibacterial and anti-biofilm activity of biogenic Ag, Cu, and Fe nanoparticles against major oral pathogens

Nanoparticle Type	Target Microorganism	Reported MIC Range	Biofilm Inhibition (%)	Primary Mechanism of Action	Key Observations
Silver (AgNPs)	<i>Streptococcus mutans</i>	5–20 $\mu g/mL$	70–95%	Membrane disruption, Ag^+ ion release, ROS generation	Strong reduction in EPS integrity and bacterial viability
Silver (AgNPs)	<i>Porphyromonas gingivalis</i>	10–25 $\mu g/mL$	65–90%	Protein denaturation, oxidative stress	Effective against anaerobic pathogens
Copper (CuNPs)	<i>Fusobacterium nucleatum</i>	15–40 $\mu g/mL$	60–85%	DNA damage via redox cycling, membrane destabilization	Moderate penetration in mixed biofilms
Copper (CuNPs)	<i>Candida albicans</i>	20–50 $\mu g/mL$	55–80%	ROS-mediated membrane and mitochondrial damage	Effective in fungal biofilm suppression
Iron Oxide (Fe_3O_4 NPs)	Mixed-species biofilm	25–60 $\mu g/mL$	40–75%	Localized ROS production, magnetic mechanical disruption	Enhanced activity under magnetic field exposure

bacteria aggressively, have been shown to trigger ROS formation in the presence of hydrogen peroxide.

However, the antimicrobial action of nanoparticles does not lie solely in their chemical interactions. As mentioned above, due to their small size, they are able to diffuse through the biofilm's EPS barrier and gain access to bacterial layers that would normally be unreachable due to antibiotic treatment. This also has to do with surface charge because, being negatively charged, bacteria attract cationic nanoparticles much easier than anionic nanoparticles.

However, in relation to iron oxide nanoparticles, a different form of mechanical effect can be observed. Exposure of magnetite (Fe₃O₄) nanoparticles to external alternating magnetic fields can produce localized heat or mechanical agitation inside the matrix of the biofilm. The latter will cause mechanical stress to the EPS matrix, causing a breakdown in its connections and facilitating easier access for nanoparticles to penetrate the biofilm. It is thus another method of manipulating the biological system by physical means.

All these mechanisms demonstrate the fact that their influence is not limited solely to bacterial cell death. Rather, these processes interfere with biofilm's organization and chemistry as well as with intercellular signaling processes. Quorum sensing inhibition prevents virulence induction; ROS damage the structural and metabolic functions of biofilm constituents; metal ions interfere with enzymatic processes; and nanoparticle intrusion destabilizes the EPS matrix.

To summarize all these interactions in a tabulated form, the mechanistic pathways are provided in Table 8.

The key conceptual move here is this: traditional antibiotics tend to target a single pathway. Nanoparticles introduce multi-target stress. In a biofilm, where redundancy and cooperation are strengths, multi-modal disruption is the superior strategy (Hao *et al.*, 2025).

Biofilms are microbial cities. Nanoparticles are not lone assassins; they are system disruptors—disrupting communication, infrastructure, and metabolism at the same time. It is this systems-level disruption that explains why nanomaterials are being explored as next-generation agents in oral antimicrobial therapy (Benoit, Sims, & Fraser, 2019).

L. Cytotoxic and Antiproliferative Effects on Oral Squamous Cell Carcinoma (OSCC)

Moving the discussion from microbial biofilm to cancer cells requires a paradigm shift in the study. Biofilm formation is a population-oriented phenomenon, whereas cancer is caused by an uncontrolled proliferating, metabolic dysregulated and apoptosis-resistant cellular system. In the context of oral cancer, OSCC is prevalent among the other types of oral

cancers and commonly studied with the use of cell lines such as SCC-9, SCC-25, CAL-27, and HSC-3.

Biogenic silver nanoparticles (AgNPs) and copper nanoparticles (CuNPs) have demonstrated potent antiproliferative properties on OSCC cell lines through activation of the intrinsic mitochondria-dependent apoptosis pathway. The intrinsic apoptotic pathway is regulated by the B-cell lymphoma 2 (Bcl-2) family, which comprises pro-apoptotic and anti-apoptotic proteins, for instance, Bax and Bcl-2, respectively. In healthy conditions, the two groups are carefully balanced; however, when exposed to AgNPs and CuNPs, the equilibrium is disrupted. An increase in intracellular reactive oxygen species (ROS), mitochondrial membrane depolarization, and Bax/Bcl-2 ratio trigger outer mitochondrial membrane permeabilization and cytochrome c release into the cytosol (Xiong *et al.*, 2024).

Once cytochrome c is released from the mitochondria, it forms an apoptosome with Apaf-1 and procaspase-9. The apoptosome then leads to the activation of caspase-9, which in turn activates executioner caspase-3. Caspase-3 then facilitates the cleavage of vital proteins, leading to DNA fragmentation, chromatin condensation, and the formation of apoptotic bodies. All this occurs in a controlled manner that does not cause inflammation, which is precisely what cancer treatments seek to achieve. (Budihardjo *et al.*, 1999).

Copper nanoparticles enhance this pathway because of their high redox cycling ability. Cu²⁺ ions are involved in Fenton-like reactions, producing hydroxyl radicals that contribute to oxidative stress in cancer cells. OSCC cells are already functioning under high basal levels of ROS because of their high metabolic and mitochondrial activity. ROS produced by nanoparticles further pushes the oxidative stress above the cellular buffering capacity, overwhelming the antioxidant defense mechanisms of glutathione and superoxide dismutase. This oxidative stress tipping point differentially affects cancer cells over normal oral keratinocytes, which normally have better redox homeostasis (Peddi *et al.*, 2021).

Silver nanoparticles have a similar oxidative course but also cause mitochondrial electron transport chain damage by direct interaction of silver ions with thiol-containing proteins. This affects ATP synthesis and promotes mitochondrial damage, further supporting apoptotic pathways (Silaidos *et al.*, 2018).

However, for iron oxide nanoparticles, particularly magnetite (Fe₃O₄), the mechanism differs somewhat. Iron oxide nanoparticles are capable of eliciting only a mild amount of ROS formation through redox reactions catalyzed by iron, while in OSCC cells, their role is mainly that of an adjuvant treatment instead of cytotoxicity. Fe₃O₄ can also be used as drug carriers, thus enhancing the efficacy of chemotherapy. Most

Table 8
Mechanistic pathways of biofilm disruption by biogenic metal nanoparticles

Nanoparticle	Quorum Sensing Interference	ROS Generation	Metal Ion Contribution	Physical/EPS Disruption
Silver (AgNPs)	Downregulation of QS gene expression; receptor interference	High	Ag ⁺ binds thiol groups, inhibits respiration	Strong penetration due to small size
Copper (CuNPs)	Oxidative degradation of signaling peptides	Very High	Cu ²⁺ enhances Fenton-like reactions	Moderate EPS destabilization
Iron Oxide (Fe ₃ O ₄ NPs)	Limited direct QS effect	Moderate	Catalytic ROS formation	Magnetic mechanical disruption possible

importantly, they can induce hyperthermia under conditions of an alternating magnetic field. Malignant cells happen to be more heat-sensitive compared to healthy cells, therefore heating them to about 42-45°C can result in protein denaturation, membrane damage, and ultimately apoptosis. (Chen *et al.*, 2025).

In order to confirm the cytotoxic effects of the compounds mentioned in laboratory conditions, a number of reliable *in vitro* assays have been developed. The MTT test is one of the most popular techniques, as it determines cell viability by measuring mitochondrial metabolism. Mitochondria inside living cells reduce tetrazolium salt MTT to formazan, a colored insoluble precipitate, in proportion to their metabolic rate. Reduced absorbance indicates decreased cellular viability and decreased growth rate. Using dose-response graphs, IC50 concentrations are calculated for these samples, at which cell viability is decreased to half. (Sylvester, 2011).

Using Annexin V/PI staining method, we can distinguish between apoptosis and necrosis. Annexin V will attach itself to phosphatidylserine when it is exposed on the external surface of the cell membrane in case of early apoptosis. PI, on the other hand, will stain cells that have lost integrity of their membranes. This means that the cells would either be in the late stage of apoptosis or necrosis. (Costigan, Hollville, & Martin, 2023).

Flow cytometry also allows for cell cycle analysis by measuring DNA content using propidium iodide staining. Nanoparticle-treated OSCC cells commonly show cell cycle arrest at particular checkpoints, especially G0/G1 or G2/M phases. Cell cycle arrest represents the activation of checkpoint pathways in response to DNA damage and oxidative stress. Arrest often precedes apoptosis (Pörschke *et al.*, 2026).

At the molecular level, Western blotting or quantitative PCR studies are used to verify the upregulation of Bax, downregulation of Bcl-2, activation of caspase-3, and cleavage of poly(ADP-ribose) polymerase (PARP), a characteristic of apoptosis. DNA fragmentation can also be assessed by TUNEL staining or agarose gel electrophoresis for characteristic “DNA laddering.” (Chehade *et al.*, 2020)

It is also important to highlight that biogenic nanoparticles can be used in medicine due not only to their inherent toxicity towards cells, but also to their specificity. Cancer cells tend to have increased metabolism and instability, as well as oxidative imbalance; thus, they will be more susceptible to oxidative stress induced by silver and copper nanoparticles. In turn, normal mouth tissue cells have high antioxidant activity and low rate of proliferation, providing additional safety from nanoparticle exposure. (Sati *et al.*, 2025).

The shift from antimicrobial to anticancer applications highlights the conceptual linkage between the two: in both cases, nanoparticles leverage vulnerabilities in structure and metabolism. In biofilms, they target communication and redox homeostasis. In OSCC cells, they target mitochondrial instability and redox disturbances. The difference is one of scope—microbial communities versus cancerous cellular autonomy—but the same conceptual linkage applies (Efunnuga *et al.*, 2024).

The green nanoparticles composed of silver, copper, and iron

present an all-in-one application in oral therapy by combating both infectious pathogens and cancerous cells residing within the same oral environment.

Their potent action against oral tumors is due to disrupting mitochondrial redox homeostasis within the OSCC environment. Rather than inducing cell destruction directly, such nanoparticles induce apoptosis via cell suicide. As illustrated in Figure 3, the apoptotic pathway involves ROS generation, modulation of the Bax/Bcl-2 ratio, leading to Caspase-3 activation.

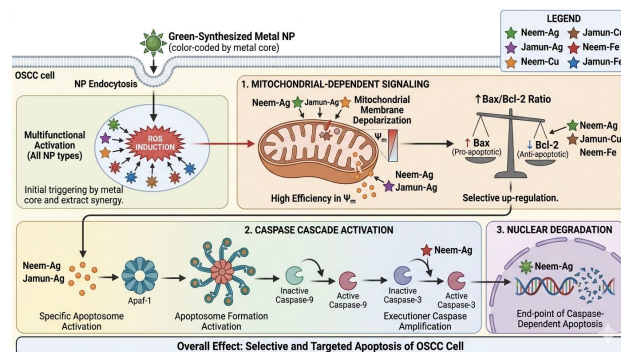


Figure 3
Intrinsic apoptotic signaling cascade induced by metal NPs in Oral Squamous Cell Carcinoma (OSCC)

M. Selectivity and Safety Considerations

Purity is not a desirable trait in cancer therapy. A compound that is as eager to kill cancer cells as normal cells is not a medicine—it is a poison. Thus, having identified cytotoxic properties in OSCC models, the next question is one of selectivity. Do biogenic Ag, Cu, and Fe nanoparticles selectively target malignant cells over normal oral keratinocytes and fibroblasts (Silva *et al.*, 2021)?

Selective activity is reflected in the IC50 differences between cancerous and non-cancerous cells. IC50 represents the concentration of the substance reducing viability to half, obtained through MTT-like assays of metabolism. In cases where OSCC exhibits substantially lower IC50 compared to the control line of keratinocytes, it opens up opportunities for selective targeting. The Selectivity Index (SI), measured as:

$$SI = IC_{50}(\text{normal cells}) / IC_{50}(\text{cancer cells})$$

indicates promise above 2. Nanoparticles containing biogenic silver and copper usually have greater SI compared to chemically produced counterparts, meaning that the influence of plants on surface characteristics of the material cannot be overlooked.

One of the underlying causes of the observed effect might be related to redox biology. Being subjected to permanent oxidative stress because of constant replication, dysfunctional mitochondria, and oncogene signaling, cancer cells are close to their oxidative stress capacity and depend greatly on antioxidative systems, including but not limited to glutathione, catalase, and superoxide dismutase. Addition of ROS producers such as nanoparticle disrupts this balance and leads to the mitochondria depolarization and caspase-mediated apoptosis. (Jomová *et al.*, 2025).

Oral keratinocytes with their normal cellular activity produce

less amount of ROS and more stable mitochondrial function. The antioxidant defenses make these cells more resilient to some amount of oxidative stress. Therefore, nanoparticles by elevating ROS levels can induce dangerous levels of ROS in cancer cells without affecting normal cells, as long as dosage is controlled. This is not because nanoparticles are smart; it's just that they take advantage of natural cell differences. (Xu *et al.*, 2024).

The topmost layer of phytochemicals further refines the entire process. In the biological production that utilizes *Azadirachta indica* and *Syzygium cumini* extracts, the residual polyphenolic, flavonoid, and terpenoid molecules stay attached to the nanoparticle surfaces. The phytochemicals serve as stabilizing agents that inhibit the arbitrary formation of aggregates and ion leakage. Ion leakage is not desired since it may lead to cytotoxic effects on both healthy and cancerous cells. (Singh, Natarajan, & Pandey, 2023).

The polyphenolic capping can also have inherent antioxidant or anti-inflammatory properties in normal cells. Although still unexplored, it is not impossible that this phytochemical corona helps to modulate the oxidative bursts in non-malignant cells, thus expanding the therapeutic index. In contrast, chemically synthesized nanoparticles without this biological surface modulation may have more sudden ion release (Andreani *et al.*, 2024).

It appears that iron oxide nanoparticles have some lower toxicity features. Compared to other metal nanoparticles such as silver and copper, they may be less toxic; hence, their IC50 is higher in both normal and cancerous tissues. However, if the same nanoparticles are used for thermal therapy, we may employ physical targeting for increased safety. In that case, the heat generated by the nanoparticles will be produced in a particular area due to the presence of a magnetic field. This process helps minimize damage to surrounding tissue. (Natarajan *et al.*, 2019).

Hemocompatibility and inflammation are important considerations especially when considering the oral administration route wherein the nanoparticle comes into contact with mucosa and saliva. Biogenically produced nanoparticles release lesser amounts of inflammatory cytokines compared to chemically produced nanoparticles, possibly due to their biological surfaces. They also exhibit lower aggregation in body fluids. (Nguyen *et al.*, 2022).

Dose-dependent cytotoxicity is another critical factor. At low to moderate concentrations, biogenic nanoparticles can selectively induce apoptosis in OSCC cells. However, at higher concentrations, oxidative stress is no longer selective. This highlights the need for accurate dosing regimens and determination of maximum non-toxic concentrations (MNTC) in normal oral cells (Shaik *et al.*, 2024).

Ultimately, selectivity is a measure of efficacy and restraint. Anticancer nanoparticles must produce enough oxidative and mitochondrial stress to trigger apoptosis in cancer cells without reaching toxic levels in normal cells. Biogenic synthesis seems to tip the scales of selectivity in their favor by combining phytochemical stabilization, ion release, and biocompatibility (Chugh, Viswamalya, & Das, 2021).

The sequence of microbes, mechanisms, cancer, and safety represents a clear escalation of biological complexity. Biological function is evidenced by antimicrobial activity. Mechanistic analysis discloses molecular mechanisms. Anticancer activity is confirmed by cytotoxic analysis. Clinical viability is determined by selectivity analysis (Reygaert, 2018).

Safety without efficacy is inconsequential. With selectivity established, biogenic Ag, Cu, and Fe nanoparticles move from the realm of laboratory curiosity to potential translational oral therapeutics (Sati *et al.*, 2025).

N. Comparative Evaluation and Translational Implications

Following the analysis of antimicrobial efficacy, mechanisms of action, anticancer properties, and selectivity, the subsequent intellectual exercise would be comparative synthesis. At this point, the objective would not be to merely reproduce experimental results but to synthesize them and assess the functional differences, safety margins, and translational applicability of silver, copper, and iron oxide nanoparticles in oral therapy (Sati *et al.*, 2025).

Among the three metals, silver nanoparticles represent the most powerful broad-spectrum antimicrobial agent. The minimum inhibitory concentration for silver nanoparticles is relatively low when targeting cariogenic and periodontal bacteria, and they exhibit high inhibition of biofilm formation, indicating great potential in fighting biofilms. This occurs due to their dual mode of action, involving membrane disruption and continuous release of Ag⁺ ions that rapidly destabilize bacteria cells. Regarding their anticancer effects, AgNPs exhibit remarkable pro-apoptosis capabilities in OSCC through mitochondria injury, an elevated Bax/Bcl-2 ratio, the release of cytochrome c, and activation of caspase-3. However, this efficacy comes at a cost. Excessive silver ion release may occur in higher concentrations, resulting in oxidative stress to normal oral keratinocytes. Therefore, despite being the most effective agent, silver nanoparticles should be used within an optimized concentration to ensure a therapeutic index. (Sati *et al.*, 2025).

The biological behavior of copper nanoparticles is unique in its nature. The antimicrobial and anti-fungal activity of the nanoparticles depends on the redox reactions of the metal, which lead to a high concentration of reactive oxygen species. Copper nanoparticles, thus, have strong anti-biofilm activity, particularly against *Candida albicans*, a fungus especially harmful to individuals suffering from oral cancer and having an immune deficiency. Copper nanoparticle-induced oxidative stress in OSCC cells leads to apoptosis owing to DNA fragmentation and the activation of caspases by the reactive oxygen species. On the other hand, the redox nature of copper suggests that there would be a smaller therapeutic index than that of iron oxides (Ermini & Voliani, 2021).

In contrast, iron oxide nanoparticles (Fe₃O₄) have other properties that contribute to treatment efficacy. They offer inherent antimicrobial and anticancer characteristics, but they are less effective in such areas compared to silver and copper nanoparticles. However, iron oxide nanoparticles are characterized by versatility rather than high cytotoxicity. The magnetic character of iron oxide allows for controlled delivery

of drugs and magnetic hyperthermia. Thus, iron oxide particles can be subjected to an external alternating magnetic field that produces sufficient heating for cancer cells to undergo apoptosis. It is noteworthy that this treatment is more accurate compared to using silver or copper nanoparticles. Nevertheless, the drawback of this type of nanoparticles is that they cannot produce the same quick results as silver or copper nanoparticles in relation to bacteria or tumors. (Nabavinia & Beltran-Huarac, 2020).

Each metal cannot be considered superior to others universally because each is meant for a certain use. Silver works effectively as an antimicrobial and in inducing cell death, while copper is great for inhibiting oxidative stress pathways, particularly when it comes to fungi. On the other hand, iron oxide can work well when used for precision targeting as well as enhancing physical therapy. The future of metal usage may involve designing the metals to suit a particular application. (Lima *et al.*, 2025).

The comparative functional landscape is summarized in Table 9.

From the viewpoint of translation research, it all depends on the method of administration and use. In regard to preventing tooth decay and implant-related infections, the best option would be silver nanoparticles due to their high antimicrobial activity. Copper nanoparticles can be considered more effective for treating oral candidiasis among cancer patients owing to their excellent antifungal properties. On the other hand, when it comes to local treatment of OSCC, iron oxide nanoparticles seem preferable due to magnetic localization and hyperthermia methods. (Srinivasan & Rana, 2024).

Biogenic synthesis is regarded as an approach that can help all the three methods because of increased stability, controlled ion delivery, and biocompatibility. The phytochemical cap derived from a plant extract serves as an additional biological layer, which can enhance efficiency and safety. (Akhtar, Panwar, & Yun, 2013).

There is nothing absolute in the translational research. Positive outcomes always depend on the ripples of context surrounding them. What matters is that an optimal nanoparticle should not be overly toxic; the key thing is to find a perfect balance of advantages and disadvantages depending on the desired use. The essence of such a green synthesis approach lies in multifunctionality: nanoparticles which possess several properties, rather than being effective only in doing one particular thing. Thus, this review is focused on one very basic

idea presented in Figure 4. One type of nanoparticles can perform both functions: disrupting bacterial biofilms as well as inducing apoptosis in cancer cells.

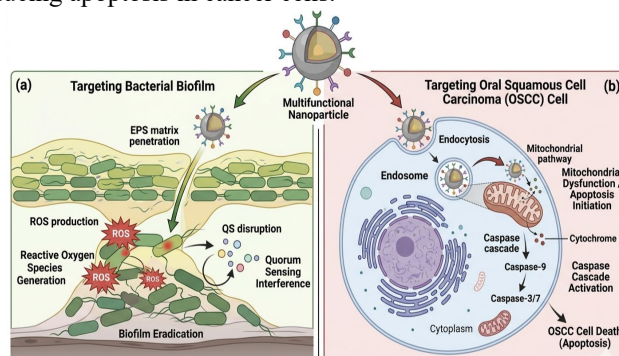


Figure 4
The dual-targeting paradigm: Concurrent biofilm eradication and OSCC cytotoxicity

O. Role of Phytochemical Capping: From Metal Nanoparticles to Phytometallic Nanocomplexes

Until now, it has always been assumed that the active metallic core, which may be silver, copper, or iron oxide, is responsible for antimicrobial and anticancer activities. However, when biologically produced metallic nanoparticles using the plant extracts of *Azadirachta indica* and *Syzygium cumini* are considered, there is a complex, dynamic, and continuously varying layer of phytochemicals, including terpenoids, flavonoids, anthocyanins, tannins, and polyphenols, surrounding the metallic core. The presence of such an outer layer makes the metallic nanoparticles act as phytometallic nanocomplexes rather than simple metallic nanoparticles. (Akhtar, Panwar, & Yun, 2013).

The first significant effect of capping metallic nanoparticles with phytochemicals is the stabilization of these nanoparticles. The presence of the coating helps prevent the aggregation tendency of these nanoparticles, which would occur due to their high surface energies. Aggregation leads to loss of surface area and altered biological interactions along with reduced antimicrobial activity. Phytochemicals such as neem and jamun contain terpenoids and polyphenols that stick to the surface of the nanoparticles through their hydroxyl or carbonyl groups, resulting in the formation of a protective layer around the nanoparticles. This protective layer offers steric hindrance, as well as some level of electrostatic repulsion, thus reducing the aggregation and keeping the nanoparticles dispersed. Dispersion of these nanoparticles is important for biofilm

Table 9

Parameter	Comparative functional profile and translational implications of biogenic Ag, Cu, and Fe nanoparticles		
	Silver (AgNPs)	Copper (CuNPs)	Iron Oxide (Fe ₃ O ₄ NPs)
Antimicrobial Potency	Very High	High	Moderate
Anti-Biofilm Activity	Strong EPS penetration and QS disruption	Strong oxidative biofilm destabilization	Moderate; enhanced under magnetic influence
Antifungal Efficacy	Moderate to High	Very High	Moderate
Anticancer Mechanism	Mitochondrial apoptosis (caspase-3/9 activation)	ROS-mediated DNA damage and apoptosis	Hyperthermia and drug delivery-assisted apoptosis
ROS Generation	High	Very High	Moderate
Selectivity Index	High (dose-dependent)	Moderate to High	High (with targeting strategies)
Major Advantage	Broad-spectrum potency	Strong redox-mediated cytotoxicity	Magnetic targeting and spatial precision
Primary Limitation	Potential cytotoxicity at high doses	Narrower safety margin	Lower intrinsic cytotoxic strength
Translational Strength	Antimicrobial coatings, topical therapy	Antifungal adjunct and ROS-based cancer therapy	Targeted delivery and magnetic hyperthermia

penetration and cellular entry in OSCC models. (Singh et al., 2023).

Secondly, the layer produced by the plant material influences the kinetics of the ions. The efficiency of silver and copper nanoparticles in terms of antibacterial and anti-cancer activity is dependent on the constant, well-regulated supply of Ag^+ and Cu^{2+} ions. However, if ion emission occurs too quickly and without control, it leads to a massive amount of oxidative stress and cytotoxicity. The phytochemical molecules function as partially chelating ligands; they bind to the emitted metal ions in such a way that slows down their movement from the nanoparticles and into the environment. It regulates ion kinetics, turning it from an abrupt peak into a prolonged emission. (Dias et al., 2024).

Thirdly, how phytochemicals coat the nanoparticles determines the process of handling ROS. Phytochemicals behave as two-faced compounds because, under normal conditions, they act as antioxidants but become pro-oxidants in metal-rich environments. In this case, by coating metallic nanoparticles with these chemicals, the oxidative properties are regulated. Cancerous cells are always close to their oxidative limit, hence, by causing additional ROS, nanoparticles increase mitochondrial damage and lead to apoptosis. For the normal cells with robust antioxidant activity, coating metallic nanoparticles with phytochemicals would be beneficial since it helps prevent oxidative stress that could have been harmful to the cell. (Patil & Chandrasekaran, 2020).

Phytochemical crown surrounding the nanoparticle also plays an essential role in determining the selectivity index (SI). As the SI is an indicator of how much more cytotoxic the therapy will be for the cancer cells than for the healthy ones, any factor that expands this margin increases its therapeutic value. The inclusion of terpenoids and limonoids obtained from neem, which have natural anti-inflammatory properties, offers mild cytoprotection to healthy cells. Anthocyanins and gallic acid, which are effective antioxidants extracted from jamun, are known to regulate the process of oxidation. If such molecules are absorbed by the nanoparticles, they will contribute their biological actions to the therapeutic effect and interact with the metal inside the particle. While oxidizing agents act on cancer cells, which exhibit higher oxidative levels due to inflammation and necrosis, healthy cells will experience reduced inflammation and cytoprotection against ROS. (Kawish et al., 2024).

The synergy of these two fields represents an essential change to the understanding of the whole system. It is not about producing silver, copper, or iron oxide nanoparticles in a greener process but rather creating hybrid bioinorganic nanoparticles, with the biological part depending on the chemistry of the metal and the surface science of the phytochemistry. In the first case, we are dealing with catalytic and structural reactions, while in the second – with stability and interaction kinetics with living cells. (Siddiqi & Husen, 2020).

It is important for the mouth's microenvironment that phytometallic nanocomplexes exist. As soon as there is contact between saliva and foreign bodies, proteins come and form a "protein corona." However, in case of a pre-existing

phytochemical corona, its influence on the formation of this second layer is likely to maintain biological activity and avoid unnecessary immune response.

What should be kept in mind is the following conclusion. Traditional metallic nanoparticles work primarily as reactive compounds. Phytometallic nanocomplexes are different from them by being modulated reactive complexes, which means they are highly active in relation to microbial and cancer cells but simultaneously provide high biocompatibility. (Kawish et al., 2024).

And this is not only related to material selection. It involves careful engineering of the plant/metal interface to modulate redox properties and ion release rates and to target certain types of cells. In case you start seeing the biological coat and the metal core as an integrated whole, then your focus will be more on bio-nanotechnology than mere nanotechnology. This approach involves a combination of materials science, chemistry, and biology in the miniature environment of the mouth. (Mattina, Mariani, & Barillaro, 2020).

P. Limitations and Translational Barriers

The biogenic nanoparticles made of silver, copper, and iron oxides have demonstrated biological activity during laboratory testing. This is a very significant achievement on the way to making these materials usable as potential therapeutics. However, although *in vitro* testing is beneficial in understanding underlying mechanisms, it always simplifies reality since it does not fully represent the oral cavity or the tumor microenvironment. (Sati et al., 2025).

The experiments with biofilms are typically carried out using monospecies or multispecies populations growing statically on either polystyrene or glass. This method allows the measurement of the reduction in biomass, degradation of the extracellular polymeric substances, and viability of the microorganisms after their exposure to nanoparticles. It provides reliable information about the mechanism under study; however, hydrodynamical conditions which occur in natural habitats are not considered. Inside the oral cavity, there is a continuous flow of saliva, shearing force due to mechanical activity and speaking, changes in pH and nutrients supply. In addition to that, saliva contains mucins, lysozyme, lactoferrin, immunoglobulin A, and different buffer compounds. Under such conditions, nanoparticles may get diluted, agglomerate, lose their charge, and be more quickly eliminated. (Benoit, Sims, & Fraser, 2019).

For the living system, the biofilm is not homogeneous like the one we normally observe in laboratory experiments. The clinical biofilm will display variation in terms of oxygen concentrations, metabolic activities, and diversity of the species. This implies areas with no oxygen, interactions between multiple species and quorum sensing amongst the microbes, leading to changes in sensitivity to various interventions. The major mode of operation for silver and copper nanoparticles involves induction of reactive oxygen species and ionization, but this is not as efficient in anaerobic areas, where there are differences in the nature of oxidative stress. Additionally, the natural biofilm contains extracellular

DNA and polysaccharides, which absorb metal ions. (Mann *et al.*, 2021).

Another benchmark standard must be set for testing the effects of cancer therapies using existing cell lines of OSCC such as SCC-9, SCC-25, CAL-27, and HSC-3. Immortalized cell lines have greater genetic stability compared to primary tumors and do not possess any form of biological diversity present in actual tumors. *In vivo*, tumors will comprise sub-clones that grow at differing speeds, have differing metabolic rates and resistance to drugs. Tumors will also include areas with varying levels of antioxidant capability and expression of enzymes, such as glutathione and superoxide dismutase. Given that nanoparticle-induced ROS production is very successful at inducing apoptosis due to mitochondrial dysfunction in homogeneous cells, its success may be hindered by adaptive resistance in the more diverse tumors. (Zhao, 2025).

It is clear that the tumor microenvironment is essential for determining treatment efficacy. Indeed, the OSCC tumor microenvironment is an intricate structure comprising many different cell types such as fibroblasts, endothelial cells, immune cells, and a network of extracellular matrix protein fibers. Solid tumors may feature hypoxic areas due to their abnormal vascularization. Such hypoxia may lead to the formation of hypoxia-inducible factors stimulating survival mechanisms and enhancing resistance to oxidative stress, resulting in increased resistance to apoptotic stimuli mediated by ROS. Furthermore, the diffusion barrier presented by the extracellular matrix hinders the movement and dispersion of nanoparticles. Thus, *in vitro* monolayer cell cultures do not account for diffusion barriers. (Maksymova *et al.*, 2025).

The protein corona has proven to be a major aspect in the transition of nanoparticles from lab bench to biology. Once nanoparticles encounter biological fluids such as saliva, blood serum, or interstitial fluid, they rapidly bind proteins to their surfaces and create a dynamic protein corona that defines the biological identity of these nanoparticles. Plant-synthesized nanoparticles, even when capped with *Azadirachta indica* and *Syzygium cumini* phytochemicals, undergo modifications to their surface characteristics when they come into contact with biological fluids. Surface adsorbed proteins can influence the surface charge, size, receptor interaction, and cell uptake of these nanoparticles. Consequently, the pharmacological behavior observed in non-serum conditions may not hold true in a physiological context. (Havelikar *et al.*, 2024).

Dose optimization represents a challenging aspect of translation as well. What is highly effective in the case of experiments carried out *in vitro* does not necessarily mean that the same results can be obtained *in vivo*. While silver nanoparticles exhibit strong antibacterial activity along with pro-apoptotic effects, an increased dose might harm the normal keratinocytes and cause excessive accumulation of the nanoparticles in the body. In turn, copper nanoparticles generate a significant amount of oxidative stress; however, their margin of safety might be even lower due to a faster generation of reactive oxygen species and a faster release of ions. The toxicity of iron oxide nanoparticles is generally low by itself; nevertheless, their therapeutic effect usually depends on other

factors, such as magnetization and hyperthermia. (Sati *et al.*, 2025).

Pharmacokinetic properties of phytometallic nanocomplexes have yet to be fully investigated. The rate of their aggregation, adhesion, absorption, as well as clearance from the organism directly depend on efficacy. In terms of local concentrations at the point of administration via oral route, one may consider such aspects as permeability of mucosal tissues, as well as the role of saliva in clearing compounds. In case of unintentional administration or crossing the mucosal barrier, the question arises whether the compound will accumulate in such organs as liver and kidneys. Plant-based capping agents can make the process more stable and less harmful for the organism. Nevertheless, biodistribution studies are required to ensure long-term safety. (Walczak *et al.*, 2015).

Immune consequences require close attention. The effects on the innate immune response by nanoparticles can occur via interactions with macrophages, neutrophils, and dendritic cells. A small boost to the immune response can help to get rid of the microbe, while an overly strong inflammatory response will lead to tissue injury. Metal ions, such as silver and copper, can affect cytokine secretion and oxidative bursts. Magnetic iron oxide nanoparticles have potential to interact with macrophage cells associated with tumor inflammation. (Yang, Li, & Wang, 2025).

With scale-up from the laboratory to the clinic, new challenges come into play. With the use of biogenic processes using plant extracts, environmental-friendly advantages emerge as well as surface functionalization. However, the chemical composition of phytoconstituents varies between batches, and this could affect nanoparticle size distribution, surface functionality, and biological interactions. Reproducibility is ensured by optimizing extraction processes, reaction procedures, and purification techniques. The regulatory agencies demand comprehensive characterization of physical and chemical attributes, which include zeta potential, crystallinity, rate of ion release, and freedom from endotoxins. It is the complexity associated with phytometallurgical nanocomposites that enhances biological behavior; however, this can also pose difficulties during regulation because of the lack of consistency. (Edo *et al.*, 2025).

The validation in animal models is used as an intermediary stage preceding clinical trials. Models of biofilm formation on teeth or xenografted OSCC represent the physiological process better than cell culture systems; however, the interspecies variations associated with the immune system, oral bacteria, and tumor biology preclude their use for predictions in humans. Nonetheless, they are invaluable for evaluating the biodistribution and safety of a therapy. (Stenfeldt *et al.*, 2025).

The long-term consequences are yet to be determined. The use of antimicrobial nanoparticles in the oral cavity over prolonged periods of time has many implications regarding the balance of the microbiome. Although it would be desirable to specifically target and eliminate the pathogenic biofilms, the insistence on powerful antimicrobial activity may compromise the commensal organisms that maintain oral health. Disturbing the commensals may lead to the development of complications

such as other types of infection or inflammation. (Nasiri *et al.*, 2023).

Oxidative stress-induced toxicity is a two-edged sword in the mechanistic view. The reactive oxygen species play the key role of second messengers in antimicrobial and antitumor drug regimens. However, their generation is non-specific. Although the healthy tissues have robust defense systems against oxidation, a high amount of ROS can overpower them. Finding an adequate threshold level of oxidative stress that will target only the diseased cells poses a major challenge in designing nanoparticles for therapy. (Xie *et al.*, 2021).

The translation of this knowledge to the clinic depends greatly on the method used to deliver the agents. Possible carriers that can be employed in the delivery of drugs that regulate the oral biofilm include topical gels, oral mouthwashes, biodegradable films, and implants. In the treatment of OSCC, nanoparticles that are delivered locally through injectable hydrogels or magnetic manipulation may improve the targeting of the tumor while reducing its system-wide availability. The best candidate for magnetically-induced hyperthermia is iron oxide nanoparticles, which heat the target site to initiate apoptosis of cancer cells. (Wang, Zhou, & Xie, 2025).

The transition from traditional metal nanoparticles to plant-based metal nanocomplexes paves the way for the refinement of both selectivity and stability properties. The capping agents derived from plants could adjust the rate of ion release, inhibit ROS overproduction, and increase binding affinity to bacterial and cancer cell membranes owing to their functional biogroups. However, the complexity of the relationship between the phytochemicals, metal nuclei, and biological targets requires sophisticated analysis methods to be unraveled. (Edo *et al.*, 2025).

From the discovery to application in the clinic, the process itself is multi-disciplinary by nature. In order to fully understand and gauge success, we need to combine aspects from materials science, microbiology, oncology, pharmacology, and regulatory science. The value is not found in the laboratory tests performed individually; rather, its true potential can be realized by creating a holistic approach to the biogenic nanoparticles of silver, copper, and iron oxide. (Dias *et al.*, 2024).

The course of research should therefore focus on physiologically relevant biofilm models with salivary flow systems, three-dimensional tumor spheroids to model heterogeneity and diffusion gradients, detailed protein corona characterization in human saliva and serum, and a systematic comparison of therapeutic index in malignant and normal oral cell populations. Only by such multi-tiered research can the true potential of phytometallic nanocomplexes be properly evaluated (Mehta *et al.*, 2024).

Not only is scientific advancement not limited to what occurs in neatly controlled laboratory settings, but it involves consistent interaction with the more complicated aspects of life itself. It is reassuring that the observed antimicrobial and anti-cancer properties of the nanomaterials provide a good mechanistic basis from which to work. But will these

nanoparticles be able to function effectively in the dynamic environment of biology? (Kashyap *et al.*, 2023).

Q. Translational Directions and Future Perspectives for Clinical Application

Translation of biogenic nanoparticles such as silver, copper, and iron oxides into oral medicine is not something that can be done through mere plug-and-play fashion. This will have to take into consideration the biological complexity of the mouth, including aspects going beyond infection and disease. The biological milieu of the mouth is characterized by continuous saliva secretion, pH changes, multispecies biofilms, immunological surveillance, and mechanical stresses exerted by chewing.

This makes the development of any kind of platform for this purpose one that requires adaptability and selectivity. Biogenic nanoparticles, using phytochemical extracts of plant species such as *Azadirachta indica* (neem) and *Syzygium cumini* (jamun), provide an especially promising pathway in this regard, giving rise to what we can call phytometallic nanocomplexes.

One of the simplest forms of translation involves the use of coated dental implants. (Hakim *et al.*, 2024). Pathogenic colonization associated with medical implants is still a critical issue for practitioners, primarily because bacteria such as *Streptococcus mutans* and *Porphyromonas gingivalis* are capable of forming biofilms relatively quickly. If the implant becomes covered in biofilm, the effectiveness of traditional antibiotics becomes problematic since it fails to diffuse across the extracellular matrix effectively. To combat biofilm formation, one should consider using biologically synthesized silver or copper nanoparticles as a coating on the surface of titanium material. The general concept of this method is simple: rather than fighting against and eliminating the biofilm, create a hostile environment that will deter bacteria from colonizing. Silver nanoparticles interfere with cellular membranes and generate metal ions, allowing them to inhibit any early colonizers. Copper nanoparticles cause oxidative stress for the bacteria already present in the biofilm and prevent its formation. Capping agents derived from plants allow for even more control over nanoparticle activity, regulating ion release and dispersion, thus preventing rapid aggregation. Finally, iron oxide nanoparticles, which pose less toxicity to bacteria, might also be incorporated to trigger magnetic disruption or heating. (Arshad *et al.*, 2023).

A potential way to advance translational research lies in using topical oral delivery systems, such as gels or mouthwashes containing biogenic nanoparticles at subcytotoxic concentrations. The problem here is maintaining an adequate antimicrobial effect and protecting the cells of the healthy oral tissues, including epithelial cells and fibroblasts. Because of their impact on the surface properties and redox reactions, the use of phytochemical capping could potentially limit the risk of non-specific cytotoxicity compared to chemically prepared nanoparticles. Flavonoids and phenolic compounds from *Azadirachta indica* extracts will bring inherent antioxidant and anti-inflammatory activity, thus preventing

possible overoxidation of the host cells, while leaving room for the production of reactive oxygen species against pathogens. Particle size becomes crucial in the case of mouth rinse, where smaller particles could more effectively enter the bacterial biofilm; however, these particles would also be more prone to interaction with host tissues. Controlled delivery systems, potentially involving mucoadhesive polymers, might help achieve continuous ion release, allowing for effective antimicrobial action without reaching a level of cytotoxicity. However, the greatest issue is the stability of the nanoparticles against dilution and degradation by salivary enzymes. (Ilangoan *et al.*, 2024).

In the context of OSCC treatment, injectable forms represent a more precise modality to take therapeutic regimens from research laboratories to clinics. Intratumoral injections are feasible due to the unique characteristics of these lesions, thus limiting the systemic distribution of the therapeutic substances. The silver and copper nanoparticles act as inducers of apoptosis primarily via mitochondrial dysregulation, cytochrome *c* release, and caspase-3 activation. The ability of nanoparticles to shift the Bax/Bcl-2 ratio in favor of apoptosis demonstrates their effect on the intrinsic cell death pathway. However, selective toxicity may represent an obstacle. It can be achieved through coating the nanoparticles with some phytochemicals, allowing for a more accurate targeting of tumor cells either by affecting their higher uptake of nanoparticles or their tolerance to oxidative stress. Indeed, cancer cells usually have an elevated baseline level of oxidative stress compared to normal cells and are more susceptible to the further induction of oxidative stress. With proper dosage and rate of particle release, the vulnerability to oxidative stress can be utilized. Injecting biologically engineered nanoparticles in hydrogels can provide sustained drug delivery exclusively to the site of lesion. (Maurya *et al.*, 2024).

Iron oxide nanoparticles provide a unique opportunity for translation through hyperthermia using magnetically induced heating. In response to an alternating magnetic field, iron oxide nanoparticles can produce enough heat locally to cause cell death without affecting other cells in the body. The method is particularly appealing for use as an adjuvant, increasing the impact of radiation and chemotherapy treatments. Employing biomaterials to modify the particle surfaces may increase their colloidal stability and resist uptake by macrophages, thus prolonging their stay in the tumor environment. Moreover, iron oxide nanoparticles can be used as contrast materials to enable theranostics, where treatment and diagnostics become one. (Soomro *et al.*, 2025).

Yet, even with all these practical applications, one cannot overlook the fact that translating into practice entails taking into account biological aspects not captured by laboratory models. For example, *in vitro* biofilm models typically disregard salivary shear forces and immunity elements, both of which are known to exert a significant effect on bacterial physiology. Similarly, while OSCC cells lines, such as SCC-9, SCC-25, CAL-27, and HSC-3, offer an excellent model for exploring mechanisms, they do not reflect tumor heterogeneity, tumor-stroma interactions, or immunomodulation observed *in vivo*. In

addition, upon being exposed to biological fluids, nanoparticles will immediately develop a protein corona, which is likely to change surface chemistry and cellular internalization dynamics. Although phytochemicals might contribute to forming the protein corona, the mechanism of their action is yet to be elucidated. (Magana *et al.*, 2018).

Regulatory aspects also deserve consideration. The translation of metal-based nanomaterials into the clinic demands evidence of reliable synthesis, scalability, and specific physicochemical properties. Plant-mediated synthesis adds complexity in terms of extraction protocols, seasonal variations in phytochemical composition, and environmental influences. Standardization strategies need to be formulated to provide a consistent particle size, zeta potential, and ion release rate. Lack of control over these factors makes scalability difficult. This issue is not impossible to overcome; it merely requires the development of comprehensive characterization strategies that combine spectroscopy, electron microscopy, and ion release rate analysis (Azad *et al.*, 2023).

This means that the core concept of using phytometallic nanocomplexes is to achieve dual-targeted treatment simultaneously inside the oral cavity microbiome. Inflammation, infections, and cancer share a lot of links: pathogens lead to oxidative stress, which can contribute to cancer formation. On the other hand, cancer can cause immune suppression, increasing the likelihood of infections. Thus, addressing infection and the ability of cancerous cells to thrive can be regarded as a multifaceted strategy, not merely a targeted approach. Silver and copper nanoparticles have demonstrated their abilities to remove biofilm-forming structures and cause apoptotic response in malignant epithelial cells. Iron oxide nanoparticles help deliver hyperthermia and medication in a very specific location. Using plant-derived phytochemicals will provide additional benefits. (Goel & Bhatia, 2018).

The next research endeavors need to focus on multi-scale integration. At the molecular level, transcriptomics and proteomics can provide insights on the effects of phytometallic nanoparticles on genetic expressions in both bacteria and cancer cells. At the tissue level, more physiologically relevant models such as organotypic culture with epithelial and fibroblast as well as immune cells need to be studied. On the organismal level, animal studies will also be needed to look into implantation, healing of the mucosa, and tumor regression. (Pandurangan & Kim, 2015).

Safety for the long term is still a major concern. The uptake of metal ions, possible genotoxicity, and environmental toxicity have to be taken into account. The ideal nanoparticle would exert its therapeutic effect locally without being absorbed or degraded unexpectedly. There is no doubt that iron oxide nanoparticles enjoy certain advantages since iron easily gets metabolized in our body. In turn, silver and copper are subject to more stringent requirements when it comes to dosage. Another means of enhancing the therapeutic efficacy is the use of surface modifications. (Jomová *et al.*, 2024).

In essence, the implementation of these concepts will require cooperation among different fields of study such as materials science, microbiology, oncology, and dentistry. The integration

between nanotechnology and plant-based chemistry provides a platform for numerous possibilities, but making them a reality requires a fine balance between effectiveness, toxicity, stability, and feasibility of production. While there have been efforts to demonstrate the antimicrobial and cytotoxic effects of biogenic nanoparticles, the goal should be to develop systems that are capable of working within a complex biological environment. (Jeevanandam *et al.*, 2020).

To sum up, biogenic metallic nanoparticles based on plants offer great potential as a versatile tool in treating disease within the oral cavity. Their applications can include being used as coatings for implants, spray or gel treatments, localized injections, as well as magnetic targeting for hyperthermia treatment of the disease. The transition from laboratory research to application in clinics will require further understanding of the mechanisms behind such treatment, safety considerations, and standardization procedures. In conclusion, the key point here is not any particular metal, but rather the combination of metal biological activity and phytochemistry. (Nabavinia & Beltran-Huarac, 2020).

3. Conclusion

Modern scientific data suggests that biogenic silver, copper, and iron oxide nanoparticles could be used as efficient agents in the oral cavity due to their ability to address two key challenges associated with this environment – persistent microbial biofilms and the potential of developing a malignancy. Instead of just having an antimicrobial effect, these nanophytometals utilize two independent but partially interconnected mechanisms to disturb pathogenic microorganisms in the mouth. The use of nanoparticles against oral biofilms implies that it goes further than the mere destruction of free-floating bacteria. For example, silver and copper nanoparticles affect membrane stability, enzyme activity, and reactive oxygen species, thus reducing the survival rates of microorganisms. In addition, they influence the extracellular polymeric substance matrix, quorum sensing, and structure formation of biofilms. The focus here is on the disintegration of biofilm rather than elimination of planktonic microorganisms. On the other hand, iron oxide nanoparticles can be regarded as non-antimicrobial by themselves, but due to additional mechanical and magnetic properties, they might inhibit the process of further biofilm development.

Regarding the selective targeting of cancer cells in oral squamous cell carcinoma, this is primarily accomplished through the ability of the nanoparticles to induce oxidative stress and subsequently engage in the mitochondrial pathways of apoptosis. Both silver and copper nanoparticles affect the membrane potential of mitochondria, alter the ratio of Bax to Bcl-2 proteins, cause the release of cytochrome c, and activate caspase-3. Moreover, the observed DNA fragmentation and cell cycle arrest indicate that both types of nanoparticles have the potential to inhibit proliferative signals inside the cancerous cells. Iron oxide nanoparticles contribute to the process via additional mechanisms, namely magnetically induced hyperthermia and targeted drug delivery.

An important aspect of the current investigation involves the

use of natural synthesis of the nanoparticles. This is done by utilizing extracts from *Azadirachta indica* and *Syzygium cumini*, which act as phytochemicals that cap the nanoparticles. Consequently, the surface charge and the release of ions can be regulated. From this perspective, the current technology represents not merely a method for creating metallic nanoparticles but also an integrated approach that results in phytometallic nanocomplexes. However, translational conservatism cannot be ignored in light of the need to optimize dosage, test for toxic effects and biodistribution, and standardize the fabrication process before pursuing clinical applications. Ultimately, a therapeutic index must always ensure that treatment favors malignancies and pathogens rather than the healthy tissues in the mouth. Thus, in summary, biogenic nanoparticles provide a dual approach that could solve infections and cancers from one site of the mouth. While it is important to consider their cytotoxic ability, it is also important to highlight their ability to disrupt pathologic processes in a selective and controlled manner. Thus, the success of the nanoparticles as therapeutics will depend on combining precision mechanisms with material properties and safety testing. If done successfully, phytometallic nanocomplexes can revolutionize oral localized therapies as they provide protection against infections and malignancies.

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