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ROLE OF *MEDICAGO SATIVA* L. MEDIATED BIOLOGICAL SYNTHESIS OF AU AND AG NANOPARTICLES IN ANTICANCER APPLICATIONS

Due to their unique physicochemical properties, silver (Ag) and gold (Au) nanoparticles (NPs) have significant potential in medicine. However, "bottom-up" chemical synthesis often involves reagents toxic to healthy human cells, triggering oxidative stress-mediated apoptosis and DNA damage. Biosynthesis using plant extracts has become a promising, eco-friendly alternative. In this process, plant biomolecules—including polyphenols, flavonoids, and terpenoids—act as both reducing and capping agents, obtaining nanoparticles that are generally non-toxic and safer for diverse biomedical applications.

Keywords: *Medicago sativa* L., green synthesis, metal nanoparticles, cytotoxicity

Medicago sativa L. is a rich source of phytochemicals, such as alkaloids, amino acids, carotenes, coumarins, and flavonoids. These biomolecules provide the extract with strong anticancer properties. During synthesis, the extract donates electrons to metal ions (Ag^{+} or Au^{3}), reducing them into neutral atoms. These atoms assemble into controlled nanostructures during nucleation and growth phases, until biomolecules cover the particles (capping), stabilizing them, and imparting the plant's biological characteristics.

In this study, Ag and Au NPs were biologically synthesized using callus cell extracts from two *Medicago sativa* L. genotypes, ‘Kometa’ and ‘La Bella Campagnola’, with $AgNO_3$ and $HAuCl_4$ as precursors. Successful biosynthesis was confirmed with Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS), and zeta potential analysis. UV–Vis spectroscopy confirmed highly successful AgNP formation for both Alfalfa genotypes, displaying intense and broad surface plasmon resonance (SPR) absorption bands between 350–600 nm. On the other hand samples exposed to $HAuCl_4$ showed very low synthesis activity, with only minor elevations near 540 nm, indicating restricted gold reduction and low nanoparticle concentration.

To complement these findings, Transmission Electron Microscopy (TEM) was utilized to visualize the precise physical morphology and structural traits of the nanoparticles, revealing the successful green synthesis of spherical silver (AgNPs) and gold (AuNPs) nanoparticles using *Medicago sativa* L. callus extracts. Nanoparticle sizes were genotype- and precursor-dependent: 'Kometa' extracts obtained AuNPs of 3–7 nm and AgNPs of 1–20 nm, while 'La Bella Campagnola' produced AuNPs of 1–12 nm and highly uniform AgNPs of 1–5 nm. Although minor clustering occurred, agglomerate sizes remained under 20 nm. The plant

biomolecules performed a dual role as both reducing and capping agents, resulting in stable, bio-functionalized particles with high potential for biomedical applications. Additionally, Dynamic Light Scattering (DLS) and zeta potential analyses were performed to evaluate the hydrodynamic diameter and colloidal stability of the particles in suspension. DLS analysis revealed genotype-dependent variations in hydrodynamic diameter (Z-average), PDI, and zeta potential. Under $HAuCl_4$ treatment, 'La Bella Campagnola' exhibited a significant size reduction (to 159.93 nm) and improved homogeneity (PDI decreased to 0.49–0.30), indicating efficient biomolecular capping and stabilization.

On the other hand, 'Kometa' under $HAuCl_4$ showed uncontrolled particle growth (Z-average increased to 806.80 nm) and high heterogeneity (PDI up to 0.91) due to natural stabilizers. Both genotypes showed stable and smaller average sizes (174–216 nm) under $AgNO_3$ treatment. Zeta potential shifts further confirmed precursor-specific alterations in surface charge, highlighting that 'La Bella Campagnola' possesses a superior intrinsic stabilization capacity. Together, the combined UV-Vis, TEM, and DLS data provided definitive structural proof of the successful biological synthesis and stabilization of the silver nanostructures by the callus culture extracts.

To evaluate anticancer activity, the resulting extracts and NPs solutions were applied to glioblastoma (T98), liver cancer (C3A), and osteosarcoma (MG-63) cell lines. In vitro cytotoxicity revealed genotype, precursor, and dose-dependent effects on cell viability. Biosynthesized gold nanoparticles (AuNPs) from the 'Kometa' genotype at low concentrations (1% and 5%) significantly stimulated cellular activity/viability in C3A and MG-63 lines compared to controls, whereas 'La Bella Campagnola' AuNPs exhibited minimal interaction and failed to inhibit metabolic activity.

In contrast, biosynthesized silver nanoparticles (AgNPs) from both genotypes demonstrated dose-dependent cytotoxicity. At the maximum concentration of 20%, AgNPs induced a critical, statistically significant drop in cell viability across nearly all tested models (C3A, T-98, and 'Kometa'-treated MG-63), outperforming all other groups. Notably, the MG-63 cell line demonstrated greater overall resistance to the extracts and nanoparticles than the more sensitive C3A and T-98 lines, highlighting the cell-line-specific efficacy of these green-synthesized nanostructures.

To monitor the real-time effect on cancer cells, an incubation monitoring system (CM30) was utilized to measure cell confluency and cell count. Real-time monitoring with the CM30 system revealed cell line- and precursor-dependent variations in confluency and cell count. In MG-63 osteosarcoma cells, silver (Ag) treatments induced a dramatic antiproliferative effect (suppressing confluency to <2%), whereas gold (Au) formulations maintained cellular viability. A different pattern was observed in C3A hepatic cells; while the negative control group maintained a lower baseline confluency (~9%), treatment with Ag nanoparticles resulted in a gradual, moderate tolerance (~10–13%), while Au treatments supported steady cell growth.

On the other hand, T-98 glioblastoma cells exhibited the highest overall proliferation potential and distinct sensitivity profiles. In this line, the introduction of Ag nanoparticles completely halted expansion, reducing confluency to ~1–3%. In contrast, treatment with Au nanoparticles, particularly 'La Bella Campagnola Au', supported aggressive cell growth, with confluency rising to ~51%, even exceeding the negative control levels.

These findings highlight that while biomolecule-capped silver nanoparticles consistently exert potent cytotoxic effects across all lines, gold nanoparticles preserve or enhance real-time cell proliferation, heavily influenced by the intrinsic characteristics of each specific cancer cell type. Laser-Induced Breakdown Spectroscopy (LIBS) was employed for elemental and structural analysis. The LIBS analysis confirmed that silver and gold nanoparticles were successfully formed in both plant extracts, showing clear peaks for silver

(Ag I at 328.069 and 338.289 nm) and gold (Au I at 242.795 nm). However, the 'Kometa' gold samples also contained a lot of silver, while the 'La Bella Campagnola' matrix had high levels of calcium and magnesium that reduced the background signal when nanoparticles were added. Understanding how these NPs affect tumor cell division is crucial for evaluating their future therapeutic potential.

Future work should focus on identifying the exact plant molecules involved in this green synthesis, since the current method did not fully separate the organic layers. It is also important to study whether the anticancer effects come from the metal core itself or from the plant shell surrounding the particles. The novelty of this research lies in the low-cost and eco-friendly creation of small, round nanoparticles using M. sativa callus cultures. It proves that genetic differences between plant genotypes—like the high calcium and magnesium levels in 'La Bella Campagnola' or the silver-gold mixing in 'Kometa'—directly affect how stable the nanoparticles are and how well they work against cancer cells.