Evaluation of the *in vitro* anti-inflammatory potential of biogenically synthesized Silver/Silver Oxide nanoparticles utilizing pumpkin agricultural byproducts

The abundant bioactive compounds and anti-inflammatory metabolites in pumpkins have prompted increasing research interest in utilizing cucurbit residues to derive *in vitro* anti-inflammatory agents. The present study investigates and compares the anti-inflammatory potential of Ag/Ag_2O nanoparticles (NPs) synthesized using pumpkin peels (PP), seeds (PS), and leaves (PL). Ag/Ag_2O NPs were synthesized using aqueous extracts of pumpkin byproducts under varying conditions, including the concentration of $AgNO_3$ precursor, extract-to-metal ion solution ratio, irradiation method (solar, microwave, UV), and incubation time. Biosynthesized Ag/Ag_2O NPs were characterized by UV-visible spectrophotometry, FTIR, SEM, TEM, and XRD analysis. Anti-inflammatory activity was assessed through egg albumin denaturation and human red blood cell membrane stabilization assays. The activity was compared to standard anti-inflammatory drugs ibuprofen and aspirin (100-1000 ppm) respectively. The biogenic Ag/Ag_2O NPs synthesized under optimum conditions exhibited characteristic surface plasmon resonance peaks ranging from 436-450 nm in UV-vis spectrophotometry, confirming NP formation. FTIR spectroscopy revealed the functional groups in the plant extracts involved in NP synthesis. SEM imaging showed agglomerated spherical morphologies of the NPs. TEM analysis indicated particle sizes ranging from 7-10 nm. XRD patterns confirmed the face-centered cubic crystalline structure of the Ag/Ag_2O NPs. The PP-mediated Ag/Ag_2O NPs exhibited significantly higher ($p<0.05$) anti-inflammatory activity compared to ibuprofen in the egg albumin denaturation assay, with IC_{50} values of 478 ppm and 598 ppm, respectively, while the PL-mediated Ag/Ag_2O NPs demonstrated significantly higher membrane stabilization activity compared to aspirin with IC_{50} values of 419 ppm and 452 µg/mL, respectively. In both assays, the anti-inflammatory activity of the plant extracts alone was very low compared to Ag/Ag_2O NPs. The Ag/Ag_2O NPs exhibited significantly enhanced anti-inflammatory activities over their corresponding plant extracts and standard drugs, demonstrating their potential as novel anti-inflammatory agents for biomedical applications.

Keywords: Ag/Ag_2O nanoparticles, Anti-inflammatory, Byproducts, Green synthesis, Pumpkin.