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Title: Screening of Medicinal Plants for the synthesis of silver nanoparticles and their application as antimicrobial agents

Nanotechnology has acquired pace due to its ability of modifying metals ions into their nano range, which dramatically changes their chemical, physical and optical properties. Silver nanoparticles have been proved a potential antimicrobial agent. Recently, the use of silver nanoparticles (AgNPs) has been greatly enhanced,due to the development of antibiotic resistance against several pathogenic bacteria. The silver nanoparticles have been widely employed in biomedical industry as coatings in dressings, in medicinal devices and in the form of nanogels in cosmetics and lotions, etc.There are well established protocols for the preparation of silver nanoparticles can be broadly classified into physical, chemical and biological protocols. The physical and chemical processes often involve high temperatures/pressure for the reaction and the use of hazardous chemicals. Therefore, the research in synthesis of nanoparticles by biological methods is gaining importance. Plant extracts are considered cost-effective, environment friendly and efficient alternative for the large-scale synthesis of nanoparticles. The study involves, the synthesis of silver nanoparticles using 21 different plant extracts having medicinal properties' Selection of the plants was made as they based on their cultivation and easy availability.The optimization of various parameters and characterization of biosynthesized silver nanoparticles were done using UV- Vis spectroscopy. The $\lambda_{max}$of nanoparticles synthesized by different plant extracts varied and ranged between 400 to 468 nm that correspond to the "plasmon absorbance" of silver nanoparticles. This is further confirmed by differential light scattering. Silver nanoparticles are known to have antimicrobial properties therefore the synthesized nanoparticles were used to study their effect against *Escherichia coli* and *Candida albicans.* Among the 21 different plants selected for the study only 11 plants shows antibacterial activity against *E.coli* whereas 3 of them shows both antibacterial and antifungal activity against *E.coli* and *C. albicans* respectively. The two most promising *Ocimum sanctum* and *Artemisia annua* mediated silver nanoparticles were studied in

detailed. Alcoholic extract of Tulsi leaves was used as a reducing and stabilizing agent for the synthesis of silver nanoparticles (AgNPs). The biosynthesized AgNPs were characterized using UV-VIS, Transmission Electron Microscopy (TEM), X-ray Diffraction (XRD) and Fourier transform infrared (FTIR) analysis. The antifungal activity of the AgNPs was evaluated for opportunistic human fungal pathogens *Candida albicans*, *Candida glabrata* and *Candida tropicalis* by determining minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC) and Disk diffusion assay. Further, to assess the pathogenicity, proteinase and phospholipase assays were performed.TEM analysis of treated *Candida* cells reveal that the AgNPs may be exerting antifungal activity by disrupting the cell membrane structure and integrity. Cytotoxicity of AgNPs was checked by performing haemolytic assay and MTT ssay.

Further *Artemisia annua* leaf extract was used to synthesized fluorescent silver nanoparticles. The synthesized fluorescent silver nanoparticles were characterized by UV-VIS, fluorescence spectroscopy TEM, EDX, XRD, and FTIR. The antimicrobial activity of the nanoparticles against gram negative, gram positive bacterial strains and opportunistic human fungal pathogens was studied. TEM analysis of treated bacterial cells was done, which reveals that the silver nanoparticles exert antibacterial activity by disrupting the cell membrane structure and integrity. The antifungal activity was tested against 3 Candida strains, with minimum inhibitory concentrations (MICs) varying from 80 to 120 μgmL^{-1}. The in vitro effect of silver nanoparticles on growth, sterol content, proteinase secretion, phospholipase and germ tube induction was investigated for 3 standard strains *Candida albicans* ATCC 90028, *Candida tropicalis* ATCC 750 and *Candida glabrata* ATCC 90030. The biosynthesized silver nanoparticles were biocompatible which were confirmed by checking the cytotoxicity against human erythrocytes and MTT. The effect on protein expression of *Artemisia annua* mediated AgNPs on *E.coli* was investigated by Label free quantification.The results clearly show that they inhibit the expression of enzyme and protein. Further specific unique proteins were identified in control and treatment samples having 114 exclusive proteins for control and 11 exclusive proteins were treatment specific. The current study may contributes to the development of efficient antibacterial nanoparticles.