Abstract

Plant-based drugs are in demand as they show better acceptability, better compatibility and fewer side effects. *Plumbago* is one of the important medicinal plants from the family Plumbaginaceae. The present research work is carried out with the plant *Plumbago auriculata* with blue coloured flowers. *Plumbago* contains a naphthaquinone compound plumbagin which is mainly present in the roots of the plant. Traditionally *Plumbago* is used in treatments of various diseases in Ayurveda and Siddha. Plumbagin exhibits many therapeutic activities like antimicrobial, antihyperglycemic, antioxidant, anticancer activity etc. Therapeutic potential of plumbagin has increased its demand in pharmaceutical market. Intervention of modern biotechnological approaches like plant tissue culture can elevate the *in vitro* production of plumbagin.

Plant tissue culture techniques like callus culture, suspension culture, adventitious root and hairy root culture were performed for the enhancement of plumbagin production from *P. auriculata*. Media for all cultures were standardised by supplying plant growth regulators like IAA, NAA, 2, 4-D and BAP. Plumbagin content was estimated qualitatively using TLC, HPLC and GCMS and quantitatively by using spectrophotometric analysis. Callus culture was performed using explants leaf, stem internodes and shoot apex. Cell suspension cultures were established using cultured callus. Elicitors like chitosan & pectin, cobalt chloride, methyl jasmonate and salicylic acid were added to the suspension culture for the enhancement of *in vitro* plumbagin production. Adventitious root culture was performed using different explants. Leaf explants were used for *A. rhizogenes* mediated hairy root transformation, and the experiments indicated unresponsiveness. Strong antibacterial activity of leaf extracts might be responsible for the failure of hairy root induction.

Results indicated that callus showed higher plumbagin content than leaf and stem, however it was less than that of the root. *In vitro* adventitious roots contained more plumbagin than that of the callus and *in vivo* roots. Plumbagin was estimated separately from spent media and biomass of cell suspension culture and effect of elicitors were checked. Maximum enhancement was observed in the media supplied with methyl jasmonate followed by salicylic acid. Scaling up of these suspension culture protocols with suggested elicitors can be used to develop a commercially viable method for the production of plumbagin.
Multi drug resistant (MDR) bacteria are major threat for the medical field today. In the present study, antibacterial activity of extracted plumbagin and plumbagin from Sigma (control) was checked using disc diffusion assay and MIC assay against five different MDR bacteria. The bacteria used were the MDR strains from clinical isolates of *E. coli*, *P. aeruginosa*, *S. aureus*, *K. pneumoniae* and *Citrobacter*. The results indicated that *S. aureus* and *Citrobacter* exhibited strong sensitivity against plumbagin. *K. pneumoniae* and *E. coli* showed moderate sensitivity whereas *P. aeruginosa* indicated resistance. These bacteria are responsible for the nosocomial infections. Current study results would suggest the development of hygiene maintenance products like sanitizer, hand wash and soaps using extracts of *P. auriculata* to keep patients free from hospital induced infections. Stable and biologically active antibacterial agents using plumbagin can be developed against MDR bacteria.

Anticancer activity of plumbagin was checked on three oral cancer cell lines using Sulphorhodamine B (SRB) assay. The results demonstrated that increasing concentration of plumbagin inhibited the growth of oral cancer cells. Growth was typically controlled by plumbagin at concentration of $10^{-7}$M. Total growth inhibition values and LC$_{50}$ values indicated plumbagin induced cell death in oral cancer cells. The present results suggest that, plumbagin can be used in design of lead molecules for development of anticancer agent against oral cancer.

Computer aided drug designing (CADD) saves the time and expense in the process of drug development. Plumbagin is known as an anticancer agent. Hydrazine compounds are reported for their therapeutic potential in cancer drug development. Considering these, molecular modelling of eight hydrazine derivatives of plumbagin was carried out in the present study. Molecular properties of these compounds were calculated using Molinspiration. Docking studies of the designed molecules were carried out using Autodock 4 to check the binding of ligand and NF-$\kappa$B. The molecular properties and docking results indicated that all the compounds including plumbagin and its eight hydrazine derivatives were bound in the cavity of p50 subunit of NF-$\kappa$B with good fit. Negative binding energies of designed molecules confirmed that the modification at C-4 with hydrazine moieties had not induced any major steric changes in the parent molecule. Results indicated that hydrazide conjugates of plumbagin were indeed the superior molecules in targeting NF-$\kappa$B. All derivatives showed good fit from which PLTSC and PLPHZ were considered as the best lead molecules for development of an anticancer agent.