ABSTRACT

Rauvolfia tetraphylla L. is a medicinal plant belongs to Apocynaceae, notorious for its pharmacological properties and folk medicine since ancient period. It became threats due to habitat destruction and over-exploitation by ethnic people and pharmaceutical industries. This study focused on conservation of *R. tetraphylla*, on account of lowest germination percentage and to explore micropropagation techniques to mitigate these challenges and investigated seed germination, callus formation from various explants, direct organogenesis, and somatic embryogenesis. The seed coat is hard it prevents the seed germination, to overcome the seed dormancy and the seeds were pre-treated with 4℃ for 48 hours. The germination of 83.33% was achieved by precut seed coats at one end. Callus formation was induced from leaf, node, internode, and root explants using different hormone combinations. Regeneration of *R. tetraphylla* through *in vitro* propagation both direct and indirect regeneration. Somatic embryogenesis provides insights into the plant regeneration in *R. tetraphylla*, offering valuable contributions to tissue culture protocols and conservation efforts for the endangered medicinal plant.

Since, ancient times the extracts of *Rauvolfia* are still excessively used for skin issues and wound healing as traditional medicine. Wound healing property was systematically investigated the antimicrobial, antioxidant, anti-inflammatory, cytotoxicity, angiogenesis, and wound-healing properties of ethyl acetate and methanol extracts from *R. tetraphylla* leaves and fruits. The wound-healing was confirmed by both *in vitro* and *in vivo* studies. The *in vitro* scratch assay, representing the rapid cell migration was obtained at 100 µg/mL of all the four extracts revealed the potential of wound healing of the *R. tetraphylla*. *In vivo* studies carried out in Zebra fish models proven that the efficacy of wound-healing potential. The caudal fin incisional wounds were treated with ethyl acetate and methanol extracts exhibited accelerated regeneration and normal morphology was achieved on the 21st day. Histological studies evaluated that rapid cellular regeneration compared with control group lesser than the treated group at 100 µg/mL extracts. This study elucidated that the significance of wound healing property of *R. tetraphylla* it emphasis the future drug discovery.

Wound healing property of *R. tetraphylla* was enhanced in the presence of alkaloids. Hence, the Tryptophan decarboxylase (TDC) is responsible for the production of alkaloids.

Tryptophan decarboxylase (TDC, EC 4.1.1.28) gene facilitates the conversion of tryptophan to tryptamine. A new gene encoding TDC was identified from the alkaloid producing plant *R. tetraphylla* by transcriptome analysis, termed as *RtTDC*. It contains 1,500 base pair which encodes an open reading frame for 499-amino-acid polypeptide with molecular mass of 55729.29 kDa and isoelectric point of 5.37. Multiple sequence alignment and phylogenetic tree analysis showed the closest similarity (95.3 %) with the TDC from the *Rauvolfia verticillata*. This enzyme has property of recombinant tryptophan decarboxylase from *R. tetraphylla* was characterized. The potential activity of tryptophan decarboxylase specific to L-tryptophan may contribute to the biosynthesis of indole alkaloids in *R. tetraphylla*. The finding of tryptophan metabolites in *R. tetraphylla* plants is a novel report, lead to hypothesize the existence of TDC enzymatic activity, from which aromatic amino acid decarboxylases is formed. These results support the *in-silico* annotation of the examined protein sequences of *R. tetraphylla* as TDC and suggest the involvement of TDC enzymatic activity in this plant. Molecular modeling of the TDC gene evidencing the reliability, stability and the structural similarities of the *R. tetraphylla* TDC gene with *R. verticillata* TDC gene. The L-tryptophan used as ligand in docking analysis to verify the TDC gene enzymatic activity for synthesis of Indole alkaloids. High performance liquid chromatography data analyses of *RtTDC* catalyzed reaction mixture confirmed the catalytically decarboxylative activity of *RtTDC*.

Bioactive compounds identified through GCMS and LCMS analysis were subjected to molecular docking studies suggested that potential pharmacological implications of *R. tetraphylla* in drug design and development. *In silico* docking studies were accomplished using three different protein structures (PDB IDs: 6Y8M, 6B8Y, 1GEN) with various ligands selected for their potential in wound healing applications. The binding affinities of the ligands were evaluated, and their interactions with amino acid residues within the protein structures were analysed. Though, the present study suggested that further research is required to investigate the of *R. tetraphylla* bioactive compounds to promote the formulation of wound healing drug, intensive clinical trial to prove efficiency of *R. tetraphylla* and as a good candidate for wound healing, anticancer drug.